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Cover

Nepenthes distillatoria L.

"The pitchers of the leaves . . . supply water that is clear, pleasant soothing and cold, for necessary human use: so that sometimes six or eight of them hold as much water as can well quench a single person's thirst with the greatest delight."

—Linnaeus: Flora Zeylanica (1747)

ILLUSTRATION BY I. WICKRAMASURIYA
PROCEEDINGS
of the
INTERNATIONAL SYMPOSIUM
on
MEDICINAL PLANTS

Organized by
THE GOVERNMENT OF CEYLON
and
THE SOUTH ASIA SCIENCE COOPERATION OFFICE
OF UNESCO
and
held in
KANDY, CEYLON

15th to 18th December, 1964

Editor
S. R. KOTTEGODA

1966

GOVERNMENT PRESS, COLOMBO, CEYLON
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THE INAUGURAL ADDRESS

by

THE HON. AL-HAJ BADIUDDIN MAHMUD
Minister of Health, Government of Ceylon

Mr. Chairman, Distinguished Delegates, Ladies & Gentlemen,

I am honoured by your invitation to deliver the Inaugural Address at this International Symposium on Medicinal Plants. Let me first welcome the foreign delegates to this beautiful island of ours, and I am glad indeed that Ceylon was selected as a venue for the Second International Symposium. I hope your short stay in this island will prove to be enjoyable and profitable, and that the mild and equitable climate of Kandy will be conducive to your deliberations.

The value of international co-operation and co-ordination of research on Medicinal Plants was realised at the First International Symposium on Plant Resources of the Middle East held in Peshawar, Pakistan, in 1960. There it was decided to hold regular symposia in different countries in South East Asia on this subject. UNESCO, which values highly the sharing of technical knowledge and the pooling of resources of various countries for the furtherance of science, has come forward to sponsor this Symposium as well.

The history of medicine is long and eventful. Man’s fight against disease and death started with the dawn of history. Starting with instinctive cures adopted by animals, medical science gathered momentum while it was based on observations and experiments, analysis and inference and the transmission of this stimulative experience from generation to generation. The inquisitive spirit of man made for progress in diagnosis with improved tools, and in cure, both by attacking the disease and by helping to develop resistance to it within the body. In course of time the authority of Hippocrates and gallant overall spirit of inquisitiveness had slowed down, and so too the progress of medical science. But the revival of the spirit of inquiry by Leonardo da Vinci and his successors has enabled steady progress to be made in the art and science of healing. But the most spectacular advances in medicine have been made in the 20th century. This has become possible because medicine during this era came to be placed firmly on a scientific basis. The basic sciences like physics and chemistry helped in a more thorough understanding of the various diseases and enabled a systematic attack to be made on them. Drawing from such diverse disciplines as biochemistry, bacteriology, nuclear and radiation physics, medicine has made phenomenal progress in the last few decades. The present symposium is an attempt to evaluate the progress made by the application of modern sciences to the study of medicinal plants of our regions — plants that have afforded the drugs which form the basis of our ancient system of ayurvedic medicine. The great physician of ancient times Charaka said, “The science of life can never be complete. The entire world consists of teachers for the wise and enemies to the fools”.

Let us therefore dwell deeply into the study of medicinal plants and herbs and use this study in full measure for the future progress of medicine. Plants have been used in medicine for centuries in this part of the world. However, the curative properties of plants and prescriptions for their use were known to a select few only,
as there was no facility for mass production of the ola leaf books on which texts were written at that time. Though oral traditions introduced elements of distortion in some instances, by and large the methods of preparation of medicines from plants have remained unchanged over the centuries. The approach of the indigenous system of medicine was empirical, and the restoration of the health of the patient was adequate. Modern science uses other techniques for evaluating the therapeutic efficacy of plants. Scientists wish to know the causes of the diseases, the active principle in the medicine which cures the disease and the method by which it attacks the disease. Such detailed problems will not only prove the efficacy of the medicine, but also open the way to new uses for medicine. For example, *Rauwolfia serpentina* was used in ayurveda as an antidote for poison and as a cure for insanity. Modern scientific research has resulted in the isolation from this plant alone of many therapeutically valuable substances. Furthermore, the study of the chemical constitution of these substances has made possible the syntheses of similar substances which display the same or enhanced therapeutic qualities. However, it is possible that isolation of the active principles and the exclusive use of these as drugs thereby rejecting the rest of the plant may cause undesirable side effects. Ayurveda and allied systems used the whole plant and not only certain elements in the plants. Modern scientific research, I am sure would be able to identify the other elements in the plants which combat the side effects and prepare a composite drug which will be free from side effects. There are also great advantages in the use of pure compounds as drugs instead of plant extracts. Proper control of dosage could be maintained, storage and transportation becomes simple problems than when dealing with whole plants. Preparation of the drugs in a form which lends itself of facile administration and assimilation becomes possible.

Besides published books, oral tradition has also handed down in certain families a knowledge of the medicinal value of plants and this knowledge is kept a close secret. By careful inquiry these cures will have to be sorted out, evaluated by modern methods and made available for the use of everybody with the aid of modern scientific methods. Thus the task before us is enormous—cataloguing of medicinal plants and diseases for which they are used, evaluating their therapeutic properties and isolating and studying the active principles within them; large scale production of these drugs either by extraction from plants or by synthesizing the active compounds—all these need much painstaking research and ample organization.

The financial resources and the scientific personnel needed for this work will not be within the capacity of any single country. I would, therefore, urge for your consideration the setting up of an international institute to undertake research on medicinal plants with the support of all the countries which have met here today. We would welcome such an institute to be located here. The collective effort of all of us should go a long way towards making available to the whole world the essence of time-tested cures found in various Asian systems of medicine. Efforts by philanthropic foundations and pharmaceutical companies on similar lines either by themselves or in collaboration with the proposed international institute or with the existing research institutes in the countries of our region would be welcome as this is an enormous task.

Let me thank you once again for the kind invitation to me to deliver this Inaugural Address and wish you all success in your deliberations.
ADDRESS OF WELCOME

by

N. WIMALASENA Esq.
Acting Mayor of Kandy, Ceylon

Mr. Chairman, Hon’ble Minister, Ladies & Gentlemen,
I must apologize to you that the Mayor of Kandy is not here this morning to welcome you. He is away from the island and that is the reason for his absence.

As the senior member of the Municipal Council of Kandy and as the last year’s Mayor, perhaps, I have been requested to come here and say a few words of welcome. I can tell you that we in Kandy are really proud and we are very happy indeed to welcome you to our city. And I consider it a singular privilege accorded to me to speak a few words of welcome on this occasion.

I feel it is fitting that you have selected Kandy as the venue for your Symposium because you will be meeting under the shadow of the Temple of the Tooth venerated by millions the world over. And, furthermore, most of you might be knowing that Kandy is situated in the centre of a belt of luxuriant tropical vegetation, wherein you see a variety of medicinal herbs if you care to get about in the surrounding countryside. As the Minister said awhile ago, it is of great significance to all of us in countries like Ceylon, India and the neighbouring countries that you have gathered together in such large numbers from various countries for this Symposium.

Our system of medicine had been based in Ceylon and in the South East Asian regions mainly on herbs and our people have been served for centuries by our physicians by the use of these herbs. It is rather sad that no adequate scientific research or study of these herbs had been made right up to recent times and we should really thank the UNESCO for starting these series of Symposia way back at least in 1960. To my mind it appears that the lack of a true scientifically based research effort may be primarily the reason for the static position of ayurveda when compared to the dynamic progress made by the western systems of medicine. But I am sure with your deliberations and efforts and the research that you are engaged in now ayurveda will be able to take its place side by side with the western systems of medicine in the service of mankind.

It may interest you perhaps if I tell you that we in the Kandy Municipal Council had pioneered a project for the cultivation of medicinal herbs on a sizable extent of land up on the hills about 1½ miles away from here and this herbarium is serving our needs in the various ayurvedic dispensaries that we run for the benefit of our ratepayers. If you are interested, our officers, I am sure, will be only too glad to take you there.
Once again I say that we are really proud that you are gathered in Kandy and we are indeed happy to welcome you most heartily. We wish that your deliberations will be crowned with success and that you will carry away with you pleasant memories of your stay in this island.

We must also thank the Hon'ble Ministers and the Government for sponsoring this Symposium here in Ceylon and also the officers for the excellent arrangements they have made; and also the delegates of the various countries who have come here at great inconvenience to participate in this Symposium.

I hope that your deliberations will be really of lasting benefit to mankind.
ADDRESS BY UNESCO REPRESENTATIVE

DR. A. G. EVSTAFIEV

Director, UNESCO South Asia Science Co-operation Office, New Delhi, India

Mr. Chairman, Honourable Minister, Mr. Wimalasena and Friends,

It is a privilege and a pleasure for me to be present among the distinguished scientists who have gathered here from different parts of the world to discuss the various problems of medicinal plants. This Symposium which has been organized under the joint auspices of the Government of Ceylon and UNESCO is the second of kind in South Asia. The first international Symposium on Plant Resources of the Middle East and Rauwolfia for the Pharmaceutical Industry was held at Peshawar, Pakistan. After the success of the first symposium a resolution was passed to hold similar symposia on Medicinal Plants at regular intervals in the countries of this region since the subject of medicinal plants is one of great importance to countries of South East Asia. There is wealth of medicinal flora in these regions which has been used for purposes of healing for many centuries.

The methods of modern science have only comparatively recently been applied in order to exploit these hidden resources for the benefit of all mankind. Even so, many therapeutically valuable drugs have been obtained from plants in recent times by the application of the newer scientific techniques.

The principal aim of this symposium is to bring together groups of scientists of the various countries interested in different specialized fields of work relating to the botanical, chemical and pharmacological aspects of studies in the medicinal plants of the region. I consider this occasion to be another step forward in our efforts towards a fuller exploitation of the natural resources and I am very happy that in this endeavour the scientists of the South Asian countries, having similar problems, as well as those from other advanced countries, have gathered together to find solutions to the common problems which are engaging their minds.

UNESCO is the only Specialized Agency of the United Nations which, by virtue of its Constitution, has a general and continuing responsibility for the promotion of international scientific co-operation.

As a fundamental part of this responsibility, one of UNESCO's primary duties is to promote and develop international action by organizing symposia, seminars, courses etc. in the basic sciences: physics, chemistry and biology. This includes assistance in the evaluation and standardization of essential research methods and furthering theoretical knowledge, since such activities are the basis for all scientific progress.

I extend my heartiest welcome to the international experts who have kindly accepted our invitation to attend this symposium and deliver lectures in their specialized fields of knowledge. I also welcome the scientists who have gathered here as guests
of UNESCO or as delegates of their own governments, universities and institutions. I do hope that they will find this meeting useful and will exchange their views through discussion and personal contacts.

I am particularly grateful to the Government of Ceylon for acting as host to this Symposium and providing such facilities as have been needed for its successful organizations. I look forward for further collaboration in organizing symposia, seminars in Ceylon in the field of science and technology which will considerably help in the development of science for human progress.

I would also like to extend my grateful thanks to the members of the Organizing Committee for their constant help in organizing this symposium.

Before I conclude, I would once again mention how greatly UNESCO appreciates the joint efforts of scientists of different countries towards the solution of their common problems. I do hope that the scientific deliberations which will take place during the course of this Symposium will yield valuable results and will be of mutual benefit and interest.

*THANK YOU.*
ADDRESS BY THE CHAIRMAN, SYMPOSIUM ORGANIZING COMMITTEE

DR. D. B. GUNASEKARA
Director, Medical Research Institute, Colombo 8, Ceylon

Hon. Minister, Your Worship, Dr. Evstafiev, Distinguished Delegates and Guests.

It is indeed a great honour and privilege that this Symposium on Medicinal Plants sponsored by the UNESCO and the Government of Ceylon is being held this week in this historical city of Kandy in Ceylon. It has been mentioned earlier this morning that this is the second Symposium of its kind—the first was held in Peshawar in Pakistan in 1960 and it is our good fortune that our turn has come early.

I have great pleasure in extending a warm and cordial welcome to all our distinguished delegates and participants both from abroad and from our country.

I wish to extend a special word of welcome to all our distinguished guests and representatives of international organisations here today. I must also welcome and thank all the other guests who have kindly come here to join us in this inauguration ceremony.

I should specially mention and thank our Minister of Health, Hon. Al Haj Badiuddin Mahmud for having so graciously consented to address us and inaugurate this Symposium today. He has always evinced a great interest in Ayurveda and so it is fitting that he should inaugurate a Symposium where the subject is Medicinal Plants which are daily in use and are of much value to practitioners in this system of medicine.

We are sorry that His Worship the Mayor of Kandy is not able to be present here today owing to unforeseen circumstances. We sincerely thank Mr. N. Wimalasena the acting Mayor, for representing him at short notice. And we are grateful for all the hospitable arrangements he has made for this Symposium.

Finally, I must express our gratitude to UNESCO for initiating and sponsoring this Symposium and giving us an opportunity of meeting so many eminent scientists from other countries.

Symposia of the kind that we are about to hold are of much benefit in more respects than one. First of all they enable well-known scientists of many countries to come to know each other and their work. Thus it is far easier for them to discuss their common problems and to appreciate each other’s views. There is moreover an opportunity to learn of the latest developments in techniques and other knowledge in a particular field of work, that otherwise one may come to learn of much later and be behind the times because of the rapid advance of modern science. And, besides all this there is the chance to learn at first hand of the kind of organisation and set-up of scientific work in various countries and also to get to know something of their cultures and ways of life. These advantages cannot be over-estimated and therefore it behoves us to encourage the holding of symposia and to provide every opportunity for scientists to attend them.
There is another way by which a symposium can lead to a most useful result and that is by creating and stimulating an interest in a not so well-known subject and thus attract others into that particular field of study and thereby help to develop and expand the work being done.

It would appear that this is particularly applicable to studies of medicinal plants. Although much has been already done, it is a field of study that is not so widely engaged in and popular as are many other fields of study. One may wonder whether this was due to the fact that scientists schooled in modern rigorous scientific methods, had not paid much attention to medicinal plants as they are mostly used in Ayurveda and other indigenous systems of medicine where the concepts of health and disease are so different from those of the Western or allopathic system of medicine. But although the difference is there it need never discourage research into medicinal plants because one has to remember that there is a factor common to both systems and that is that they both have one objective—that is the cure of disease and the alleviation of suffering arising from ill-health.

The concepts of disease may be different in the two systems but it is the remedy we are concerned with and this can be independent of the concepts and still yield the desired results. One has only to remember the great interest that was aroused when the pharmacological actions of reserpine isolated from Rauwolfia serpentina were made known to the world.

It is a matter for congratulation that we have here today thirty two eminent scientists from abroad. They come from twelve countries. What is more interesting is that they represent many different specialities. As such I am confident that when they have pooled their knowledge at this Symposium their deliberations should be most fruitful. I wish them all success.

May I take this opportunity to thank the members of the Organising Committee and the Reception Committee who have put in so much hard work in making arrangements for this Symposium to be a success. And I must also thank the many other helpers who have assisted the Committee in their work. They have all worked as a team and we are very grateful to them.

THANK YOU.
BOTANY
MEDICINAL PLANTS OF
GARHWAL-HIMALAYAS

A Preliminary Study

BY

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There are some mountains and valleys in Garhwal-Himalayas which are considered amongst the most beautiful in the world for their vivid and abundant vegetation. Bhyundhar valley has earned the name 'Valley of Flowers' all over the world. Besides, there are several hills unique in their floral beauty, in the Alpine meadows, situated from 10,000 ft. to 14,000 ft. above the sea level, which can also be distinguished as the 'Valley of Flowers' in the real sense of the word.

The early botanists who made collections in this area have not examined the medicinal uses of these plants.

Taking into consideration the importance of medicinal flora, the area was explored by the author to survey the general flora and gather information regarding their uses among the local inhabitants. Several excursions were undertaken to the higher peaks of Garhwal-Himalayas. The specimens collected were photographed in their natural habitat.

The first botanical tour by the author was undertaken in June, 1957. The route taken was from Rishikesh to Devprayag, Rudraprayag, Guptakashi and Kedarnath. From Rudraprayag the road proceeds along the river Mandakini. It covers a distance of more than 80 kms before it meets the Alakananda river in Rudraprayag. On the return from Kedarnath the author climbed Tungnath via Ukhimath and Devriatatal. After visiting Badrinath (3,000 m.) and Bhyundhar valley the journey ended in Hardwar. The mountains rising up to the height of 14,000 ft. were visited in this journey.

In the second trip the author went to Virahital (6,000 ft.) via Chamoli along the stream Virahiganga in May, 1958. Here it was experienced that this was too early for studying and photographing the flora of upper Garhwal. The plant life on the open slopes was dormant. The land was dry and grassless.

In October-November, 1963, the author proceeded along the route of Bhilanganga river from Tehri and climbed up to 13,000 ft. above the sea level, 10 miles beyond Panvali on way to Kedarnath via Triyuginarayana. Bhagirathi valley right up to
Gangotri shrine was also surveyed. This valley forms a large part of Uttarkashi Forest Division which lies to the north and north-west of Tehri Division. The river Bhagirathi springs from Gomukha glacier situated at an altitude of about 3,100 m. which is about 25 kilometers beyond Gangotri. It joins with Bhilangana river at Tehri and with Alakananda river at Devprayaga. From this confluence the river is named Ganga.

The influence of Himalayan climatic conditions was clearly visible on vegetation. Most of the herbs were frozen to death. It was difficult to collect specimens with flowers.

The fruits of various important medicinal plants from different genera such as *Rose, Aconitum, Allium, Juniperus*, etc., were fully ripe and seeds ready for harvesting. This was the proper time for study and collection of seeds of alpine herbs.
Botanical collection work and study of the vegetation in the valleys of Yamuna and Bhilangana rivers was undertaken in July, 1964. The Panvali area was revisited and medicinal plant wealth was explored. The actual collection work was carried out in this trip. Specimens of important medicinal herbs such as katuki (Picrorhiza), jatamansi (Nardostachys) vana karkata (Podophyllum), darubaridra (Berberis), salampanja (Orchis) and many others were collected. A plant of Podophyllum with extraordinary big rhizomes believed to be more than 25 years old was also dug out in Panvali. It was a rare find in this area. The entrie meadow of Panvali was full of primulas, potentillas, poppies and other alpine flowers.

Since most of these herbs of higher and interior Himalayas bloom in rainy season the month of July proved to be a suitable time for plant study. However, it was felt that the month of August would also prove valuable for the collection and study of some of the other species of Alpine medicinal flora such as aconite and Saussurea which bloom in that month.

**Source of Information**

Information regarding properties and various uses of medicinal plants of Garhwal Himalayas was obtained from the following sources:—

(a) Local physicians practising indigenous systems of medicine
(b) Shepherds and cowherds
(c) Hill women
(d) Gujars, (nomadic tribes from Kathuvin)
(e) Migrant Nepalese porters
(f) Adivasis, primitive tribes from Garhwal-Himalayas
(g) Mendicants (Sadhus or holy men).

It is possible that some of the information might have not been previously recorded. It is necessary that the chemical, pharmacological and clinical study of these claims should be undertaken to assess the value of these plants which are being employed in remote parts of Garhwal in the treatment of various ailments from time immemorial.

**Climate**

The climate of the area under survey varies considerably in different places depending upon altitude and situation of the site. The area receives annually a rain fall between 1660 mm. and 2190 mm. The rainy season starts in June and extends till September. Secondary rains occur in the latter half of December and continue now and then till the end of January. Bhundhar valley (11,000 ft.) and other hills of this height are covered with snow in winter. The area upward from 6,000 ft. elevation above the sea level gets periodical snow fall between December and March. The snow continues to lie for several weeks, and in places situated above 10,000 ft. the snow remains for over five months. Seeds, bulbs, rhizomes, tubers and roots of the species of Aconitum, Allium, Picrorhiza kurroa, Nardostachys jatamansi, Bergenia, Rheum, etc., remain buried and dormant under snow for about six months. The minimum temperature falls down to 6.6°C. The active life of vegetation is resumed with the melting of snows.
The plant collections made during these visits amount to nearly 1,500 specimens comprising about 300 species representing Dicots, Monocots and Pteridophytes.

The specimens collected in these tours were deposited in the Herbarium of the Ministry of Health, Government of India, New Delhi.

Suggestions

The Garhwal-Himalayas alone can supply annually several million rupees worth of crude plant drugs required for use in India. But it is observed that the supply is decreasing every year, due to the following reasons:

(a) indiscriminate exploitation of medicinal flora,
(b) clearing of forest areas for agricultural purposes,
(c) wanton exploitation of medicinal plants for non-medical purposes such as fuel, timber, fodder; and
(d) heavy grazing by cattle.

It is therefore, suggested that:
(i) the collection of plants should be systematized,
(ii) drug-farms should be established for cultivation of important medicinal herbs,
(iii) large-scale experimental cultivation of these medicinal plants which are at present being imported from outside should be undertaken,
(iv) the rules and regulations of the Forest Department with regard to grazing and cutting of medicinal trees should be made stricter.
FOLKLORE OF MEDICINAL PLANTS OF THE BHAGIRATHI VALLEY (HIMALAYAS)

BY

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Importance of the area

Among the various regions in the Himalayas of traditional importance, the Bhagirathi Valley is regarded as the most sacred. It is considered as a heaven of potent medicinal herbs. The valley lies entirely within the Himalayan ranges and consists of a series of ridges separated by narrow valleys. The ridges run mainly from North-East to South-West. With the holy shrine, the Gangotri, this valley forms a large part of the Uttarkashi forest division. The river Bhagirathi rises from Gomukh which is situated 25 kilometres beyond Gangotri at an altitude of about 4,100 m. In its further course it is joined by the river Alakananda at Deopravadhe, and in its further course it is named as Ganga.

Shepherds are often seen, in these areas, carrying bundles of drugs for domestic use. These have been seen to contain atis (Aconitum heterophyllum), meetha (Aconitum falconeri), archa (Rheum sp.) and other local plants of medicinal value.

While the forest flora of the area is predominantly coniferous, gregarious forest of Deodar, Kaily (blue pine) and spruce cover great tracts between 1,800-3,600 m. Most of the ridges with steep gradients are devoid of tree growth due to prevailing dry conditions. As the area consists mainly of rocks, xerophytic conditions have been further accentuated. Meadows of alpine type are however seen on some high ridges.

In addition to trees and shrubs of medicinal importance a wide range of annual and perennial herbs of traditional value await investigation.

Itinerary of the present collection

The present paper deals with the collection of plants and folklore of Himalayan flora in the region of the Bhagirathi valley. The uses mentioned here are not as found recorded in any other literature on the subject whether ancient or modern. Investigation on these lines can open a vista for systematic utilization of Himalayan medicinal flora.

This collection is a result of tours undertaken during the year 1963, in the months of July, October and November. Among the places visited are Dharasu, Uttarkashi, Bhatwari, Gangnani, Sukkhi, Harshit, Bhaironghati, Gangotri, and Rudra-ganghati. The altitudes thus covered range from 1,000 to 3,400 m.
The identified specimens of plants mentioned in this paper are deposited in the Herbarium, Survey of Medicinal Plants, Gurukul Kangri University, Hardwar, India

**Aconitum falconeri** Stapf.

Bikh, bis, meetha.

A biennial herb with paired tuberous roots, 40-60 cm. high, root-tuber conical or cylindrical, 8 cm. long, 2 cm. diam., brown externally and on fracture, slightly horny on drying, bitter and strong giving a burning sensation; flowers bluish.

Alpine and sub-alpine Himalaya: Garhwal in moist and shady places; Bhagirathi valley: Raithal, Sukkhi at 2,400-2,700 m. elevation.

Fl. September; fr. October.

**Local Uses:**

The root is soaked in cow’s urine for a week, then washed, dried and powdered. The powder (0.4 gm.) is administered internally in acute non-malarious fever and also in diarrhoea (mixed with *Piper nigrum*). Externally too dry root boiled in ghee is used for branding rheumatic joints. Aconite leaves are used to improve the flavour of country liquor.

The roots are collected during autumn in large quantities. Annually about 20,000 kilos. of roots can be collected.

**Aconite** is being exported from Bombay market in large quantities. Cultivation is suggested. Suitable localities for cultivation are Kushkalyani (Bhatwari), Raithal, Sukkhi, and Harsil.

**Aconitum heterophyllum** Wall.

Atis, ativisha.

A biennial herb with paired tuberous roots, the daughter roots cylindrical or cylindrically oblong or conical, 2-5 cm. long, 0·5-1·2cm. diam., pure white on fracture.

Alpine and sub-alpine Himalaya Bhagirathi valley: Raithal, Sukkhi and Harsil at 2,400-2,700 m. in open grassy meadows.


**Local Uses:**

The root is administered internally with water for relief of abdominal pain and in fever and diarrhoea, especially of infants.

The roots are collected during autumn. Annual collection from the area is about 800 kilos. Cultivation is suggested. Suitable places for cultivation are as given for **Aconitum falconeri** Stapf.

**Actaea spicata** L.

Banberry.

Mamira.
A perennial herb with a horizontal rootstock, 70-90cm. in height with white flowers and black ovoid fruits.

Temperate Himalayas from Hazara to Bhutan.
Collected on way from Gangotri to Rudra-ganga valley 3,300 m. under forest cover.

Local Uses:
The root is considered by local Sadhus (mendicants) to be *Mamira*, and is used by them for cure of eye diseases. The root is rubbed into a paste with water and applied as collyrium.

This is not being collected at present. Supply is limited.

*Allium victorialis* L.
Pangri.
A bulbous herb.
Temperate Himalayas: Gangotri to Rudra-ganga valley, 3,400 m. elevation.

Local Uses:
The bulbs and the leaves are used as vegetables and are considered a general tonic.
Not collected for the market. Supply is limited.

*Artemisia sacrorum* Ledeb.
Tanga-tulsi, chamriya.
A strongly aromatic herb, 60-80 cm. high with brownish-purple flowers.
Temperate Himalayas from 2,500 m. to 3,000 m. elevation in inner ranges. Harsil Gangotri, 3,100 m. common and abundant.
Fl. September-October; fr. November.

Local Uses:
The leaf is administered internally for relief of abdominal pain.
Not collected at present; can be economically collected in large quantities.

*Artemisia vestita* Wall.
Kundja, chamriya.
An aromatic hoary herb with purplish flowers.
Temperate Himalayas from 1,000-3,500 m. Harsil, 2,500 m. Gangotri, 3,100 m. elevation.
Fl. September-October; fr. November.
Local Uses:
The crushed leaves are employed externally as haemostatic.
Not collected. Available abundantly.

Artemisia vulgaris L.
Kundja, magadanna, nagadamni.
An aromatic herb with brownish yellow heads.
Throughout Sub-Himalayan and Himalayan regions. Gangnani to Bhairongnati, 2,700 m. elevation; common weed.
Fl. August-October.

Local Uses:
The crushed leaves are used as haemostatic application. The leaves are also used externally to allay the burning sensation in conjunctivitis.
Supply abundant.

Astragalus candolleanus Royle.
Rudravanti, rudanti.
A perennial, low, rigid, decumbent shrub, 30–60 cm. high.
Temperate Himalayas 3,000–4,000 m. elevation. Gangnani 3,000 m., common in open places.
Fl. July-August; fr. October-November.

Local Uses:
The root powder or decoction is used internally in tuberculosis, skin diseases, cough, and for purifying the blood. The whole plant is dried in shade and crushed and taken with a mixture of ghee and honey followed by milk after an hour. Treatment is to be continued for six months, with restriction of salt in the diet during the period. The drug is recommended for clinical and pharmacological screening. There is a specific demand for it among the Sadhus for its curative properties in tuberculosis conditions.

Supply is limited from Gangnani area. Cultivation will be required if there is a demand.

Betula utilis D. Don.
Bhoj, bhojapatra.
A tree with a brownish-white papery bark.
Temperate Himalayas: Kashmir to Bhutan, 2,000–4,000 m. elevation in the alpine zone.
Fr. April-May; fr. August-October.
Local Uses:
The bark mixed with ghee and the gum of *Boswellia serrata* Roxb., is employed as a fumigant to produce postpartum contraction of the uterus.

A fungus found in the tree is used as a tonic for cattle.

Supply abundant.

**Berberis lycium** Royle.
Kingor, dar-hald, daruharidra.

An erect, rigid, spiny shrub, 2–3 m. tall with yellow flowers and bluish violet berries.

Outer Himalayas from Kashmir to Garhwal 1,000–3,000 m. elevation. Common in open dry places between Uttarkashi and Bhatwari.

Local Uses:
Rasaut, a popular medicine for eye diseases, is the solid extract prepared from the root. A decoction of the roots along with other ingredients is also used in menorrhagia. Ripe fruits are edible and considered useful in stomach disorders.

A local pharmacy prepares a medicine for piles using the root extract mixed with other ingredients.

Supply abundant.

**Calotropis procera** (Ait.) R. Br.
Ak. madar.

A laticiferous shrub with pink flowers.

Drier and hot parts of India. Common in and around Dharasu, and Uttarkashi.

Fl. March-May ; Fr. June.

Local Uses:
Leaf bud mixed with molasses administered internally. Oil from the seeds is used as a geriatric tonic. Green copra in which the latex has been cooked is charred in a closed vessel, is powdered and is administered internally in asthma. Leaves mixed with other ingredients have been found useful in gastric disorders.

Supply from hilly areas is limited, but good quantities can be collected from the plains.

**Cedrus deodar** (Roxb. ex Lam.) G. Don.
Deodar, devadaru, amarkashtha.

A large gregarious tree with horizontal branches and branchlets; female cones 10–13 cm. long.

Temperate Himalayas from Kashmir to Garhwal 1,000–3,500 m. elevation. Harsil to Bhaironghati, 2,650 m. elevation.
Male cones September-October; female cones ripen in October-November after a year of flowering.

Local Uses:
Oil extracted from the wood is applied on rheumatic joints; the oil is also used as a remedy for ulcers and eruptions, and as an effective insect repellant.
Supply is abundant.

Centella asiatica (L.) Urban.
Brahmi, naandukparni.
A creeping herb with reniform leaves.
Throughout India. Uttarkashi in moist places.
Fl. April-May.

Local Uses:
It is considered one of the best local remedies for diarrhoea in children. Fresh leaf-juice, slightly warmed, is given in doses of one spoonful thrice a day. Rice gruel prepared with it is also employed.

Collected during spring, dried in shade. Not commercially collected in the area as the main supply is obtained nearer the plains at Dehra Dun and Hardwar.

Cissampelos pareira L.
Parhi, jaljamni, patha, ambashtha.
A dioecious twiner with yellow-green flowers and red fruits.
Throughout tropical and sub-tropical India. Common between Uttarkashi and Bhatwari.

Local Uses:
The decoction of the root is a useful internal remedy for colic and intestinal disorders. The powdered plant mixed with black pepper and pippali is administered with cold water in diarrhoea, and is considered very effective in diarrhoea of children. Leaves are applied externally in pururific complaints.

The plant is not collected from the area, but a good quantity may be obtained. Elsewhere, the collection is done in autumn.

Ephedra gerardiana (Wall.) Stapf.
Tutgantha.
A small decumbent shrub.
Temperate Himalayas in the inner region.
Local Uses:

The dried stem is powdered and give as snuff for headache.

Sporadic and not gregarious, collection in good quantity not possible. Collection made in autumn in Punjab and Kashmir. May be grown in areas above Harsil.

Euphorbia pilosa Linn.

Chopala.

A small glabrous herb with stout tuberous roots.

Temperate Himalayas: Bhatwari, Raithal and Sukkhi, common.

Flowers in rainy season; fruit ripens in winter season.

Local Uses:

Root is purgative. An inch long piece causes a single motion, and two inch piece causes two motions. Stem is emetic. The root and stem, when used together, cause purgation and vomiting.

Plant is sporadic, large scale collection is possible when cultivated.

Ficus cumia Buch-Ham.

Bhedu, kharpatra.

A small tree in fruit throughout the year.

Outer Himalayan range. Valley along the sub-Himalayan tract, common.

Local Uses:

Green stem is scorched in fire, applied on bites of tiger, to avoid septic formation. Common in villages. Collection is possible in good quantity.

Geranium wallichianum Sweet.

Lal-jarhi.

A herb, 30-60 cm. high with pink flowers 3-6 cm. diameter.

Kashmir to Kumaon above 1,500 m., between Harsil and Bhaironghatti. Frequent in shady places.

Fl. July.

Local Uses:

The roots are applied on cuts and bruises, and are commonly carried by every shepherd.

Not collected, collection possible in good quantity.


**Heracleum pinnatum** C. B. Clarke.

Gandhrayan.

A perennial herb with long aromatic roots and white flowers; fruits broadly winged.

Temperate Himalayas in Alpine zone. Gangotri to Rudra-ganga valley, 3,100m. elevation.

Fl. July-August; fr. October-November.

**Local Uses:**

The paste of the root is applied externally as antidote to snake-bite (Russel’s viper) and scorpion sting. Locally it is much used. Every shepherd has been found to keep this drug.

Not very common, cultivation is indicated.

**Lilium polyphyllum** D. Don.

Kandmul.

A herb with dry fruits.

Temperate Himalayas. Gangotri to Rudra-ganga valley, 3,300 m. elevation.

Fr. November.

**Local Uses:**

The roasted bulbs are taken as a general tonic by Sadhus, and forms an important constituent of the well known Ashtvarga employed in geriatrics.

Sporadic, regeneration hampered due to over-exploitation and suppression by weeds. Cultivation urgently indicated as there is future possibility of extinction of the species.

**Mallotus philippensis** (Lam.) Muell.-Arg.

Raini, ruina, kamila, kampillak.

A small evergreen tree with yellow flowers. Fruits covered with crimson powder.

Throughout tropical India. Frequent at Bhatwari.

Fl. September-November; fr. March-May.

**Local Uses:**

The decoction of the bark is given to relieve abdominal pain. The red powder on the pericarp is used as a vermicide and given to cattle with whey (milk). Seed is anthelmintic.

A nurse-plant for the sal in the lower hills. Any quantity could be collected.
**Myrica nagi** Thunb.
Kaphal, katphal.
An evergreen dioecious moderate sized tree; fruit reddish, tubercled.
Sub-tropical Himalayas, Punjab eastwards, 1,000 to 2,000 m. elevation; Khasia hills in Assam. Bhatwari occasional.
Fl. October-December; fr. May-June.

*Local Uses:*
The ripe fruits are edible. The dry bark is used internally in menorrhagia and headache. In cough and bronchitis a paste of the bark is applied on the chest as an antiphlogistic.
Sporadic. May be cultivated as a road-side plant.

**Nardostachys jatamansi** DC.
Masi, mansi, jatamansi.
A perennial herb with aromatic bunches of roots and bluish white flowers.
Alpine Himalayas 4,000–5,000 m. Sukhki, Harsil, etc. in open grassy hill cliffs.
Fl. August-September; fr. October.

*Local Uses:*
Dried base of the stem (jatamansi) is employed in incense along with other ingredients. Root paste removes blemishes on facial skin. Hair oil is flavoured by the aromatic root which is supposed to be hair tonic. Root paste useful in hiccough and in piles.

It is collected from the area in large quantities for sale.
Plants grown over rocks, well covered with soil, yield a better root system. Plants grown on garden soil do not yield good fibrous roots.

**Paonia emodi** Wall.
Chandola, udsalep, mamekh.
A stout glabrous perennial herb about one meter high; flowers white, fruits green, seeds red.
Temperate Himalayas 2,000–4,000 m. elevation. Collected from Gangotri, 2,400 m. in thickets, generally near villages; sometimes in large patches.

*Local Uses:*
The roots are applied on cuts. Root powder is also applied in foul ulcers to kill maggots. Leaves and young shoots are cooked and eaten as vegetable to cure dysentry.
Gregarious in places. Collection in good quantity is possible. It should be collected after the fruits ripen.
**Picrorhiza kurroa** Royle ex Benth.

Karu, katuki.

A perennial herb with an elongate, stout, creeping, bitter root-stock and blue flowers.

Alpine Himalayas from Kashmir to Sikkhim, 3,000–5,000 m. elevation. Harsil, Raithal and Sukkhi. Common in open grassy meadows.

Fl. June-July; fr. September-October.

**Local Uses:**

The root extract in the form of powder is purgative and is used to relieve abdominal pain, powdered root stops the spasm of asthma.

Collection in good quantity is possible, but it is desirable that crop should be collected in two years rotation. Collection is made in August-September.

**Pyrus pashia** Buch.-Ham.

Mehal, mol.

A large shrub or moderate-sized deciduous tree.

Temperate Himalayas, up to 350 m. elevation. Common between Uttarkashi and Harsil.

Fl. March-April; fr. September-October.

**Local Uses:**

The over-ripe fruit is eaten. The juice of the unripe fruit is used as an application for corneal ulcer in animals.

Common all along the villages.

**Prunus puddum** Rosb.

Payan, padam, padmak, padmakashta.

A moderate-sized tree with pink flowers and red fruits.

Temperate Himalayas. Common between Uttarkashi and Bhatwari.


**Local Uses:**

The ripe fruit is eaten. The twigs are used in the preparation of hair-oil and oil for massage. The paste of the bark is applied over the forehead for hemicrania and is also used as a plaster for fractures and dislocations. Kernels are used as a remedy for lithiasis.

Common throughout the area in and around villages.
Rheum webbianum Royle.

Archa.
A tall herb with large leaves; petiole acidic.
Alpine Himalayas from Nepal to Kashmir, 3,000–5,000 m. elevation. Along the banks of Rudra-ganga.

Local Uses:
The roots are applied externally on cuts and wounds, and administered internally to cattle as digestive and restorative. Much used locally. Every shepherd carries this herb.

Not common. Cultivation indicated in the areas above Harsil. Collection is made during autumn.

Rhododendron arboreum Sm.

Brans.
An evergreen tree with dark-green leaves and large, showy, scarlet flowers.
Temperate Himalayas 1,500–4,000 m. elevation. Common associate of oak-forest, Gangnani, at 1,800 m. elevation.
Fl. March-April; fr. October-December.

Local Uses:
The flowers are eaten raw, sometimes made into sauce. The petals are used as expectorant.

Collection possible in March in good quantity.

Rhus parviflora Roxb.

Tungla, raitung.
A large unarmed shrub, 1–3 m. high, bearing trifoliolate leaves and orange or brown fruits.
Outer Himalayas Sutlej to Nepal 700 m. to 2,000 m. elevation, also in Pachmarhi Hills in Godavari district.
Collected between Uttarkashi and Bhatwari.

Local Uses:
Old pale leaves are smoked with tobacco. Leaf paste is applied to cuts and bruises. Gregarious. Not collected.
**Roscoea purpurea Sm.**

Saleb.

A herb with tuberous roots and purple flowers.

Temperate Himalayas 1,500–2,500 m. elevation. Gangnani, 2,000 m. elevation.

Fl. July-August.

**Local Uses:**

The root is used as a restorative and tonic in weakness and sold as the Saleb of commerce.

Collected during autumn. Good quantity could be collected from the area.

**Rubia cordifolia L.**

Lachkura, manjeet, manjishtha.

A large herbaceous perennial climber covered with small minute white prickles, flowers yellow.

Hill districts of India up to 3,999 m. elevation and southward. Bhaironghati, 2,700 m. elevation; common in hedges.

Fl. July-September; fr. October-November.

**Local Uses:**

The roots are used internally in menorrhagia, and externally applied along with vinegar on pimples.

Collection possible from all the areas of the locality. Collection during autumn.

**Rumex hastatus D. Don.**

Amelu, ameldo.

A perennial plant; leaves acidic; flowers very showy in early winter.

Throughout Bhagirathi valley on exposed rocks and dry stony hill sides.

**Local Uses:**

Root juice mixed with other ingredients is used in intestinal disorders. Leaves are acidic and are eaten as sour dish. Decoction of the plant is used in the purification of the metals.

Gregarious. No collection is made.

**Thalictrum reniforme Wall.**

Mamira.

A perennial herb; leaflets mostly orbicular, 5–6 cm. across, coarsely crenate; flowers white.
Temperate Himalayas 3,000–4,000 m. elevation. On the way from Gangotri to Rudra-ganga valley along the banks of Rudra-ganga, 3,400 m. under forest cover, in association with *Actaea spicata* L.


**Local Uses:**

The roots are considered useful as collyrium in eye diseases. The root mixed with antimony is made into a local preparation known as ‘Mamira Surma’ and is in great demand on the hills.

Alpine Himalayas. Collection during autumn.

**Thymus serpyllum** L.

Banajwain.

An aromatic herb with pink flowers.

Temperate Himalayas from Kashmir to Kumaon 2,000–5,000 m. elevation
gangnani and Harsil, 2,400 m. elevation.

*Fl*. May-October; fr. November.

**Local Uses:**

The powdered flowers are mixed with Gur and used as vermicide.

No collection is made.

**Viburnum cotinifolium** D. Don.

Bansura.

A large shrub with red fruits.

Kashmir to Kumaon in open places on the rather drier exposures. Gangotri to Rudra-ganga valley, 3,300 m. elevation.


**Local Uses:**

The bark is used for menorrhagia. It is collected for the preparation of a uterine tonic.

Collection is made in spring. Bark is removed from the lopped off stems, dried in the sun, packed in gunny bags and carted to Dehra Dun Market.

**Vitex negundo** L.

Simali, shemalu, nirgundi.

A large shrub with 3 to 5 foliate leaves and bluish flowers.

Throughout *India*, common between Bhatwari and Nakuri.

*Fl*. June-August; fr. in the cold season.
Local Uses:
The leaves are used internally in diarrhoea of infants; externally applied on contusions, sprains and dislocations.
Not collected, but may be collected in any quantity. Collection is made in all seasons.

Withania somnifera (L.) Dunal.
Asgand, ashvagandha.
An erect herb with greenish fruits.
Drier parts of India ascending to 1,700 m. elevation in Himalayas. Dharasu, 900 m. elevation, in waste places.
Fl. July-September, sometimes throughout the year.

Local Uses:
An application of root-paste relieves the glandular swellings of bubonic plague. Fine powder of the root is given with water or milk as an alterative in pulmonary tuberculosis.
Not collected. In indigenous system the drug is collected from cultivated plants.

Woodfordia fruticosa (L.) Kurz.
Dhaura, dhai, dhataki.
A large shrub with long spreading branches, 1-3 m. high; leaves opposite; flowers scarlet.
Throughout India up to 1,500 m. elevation. Between Uttarkashi and Bhatwari, common.
Fl. February-April; fr. April-June.

Local Uses:
The flowers mixed with sugar candy is administered internally for seminal weakness, and menorrhagia. It is also used internally in children's diarrhoea.
Very little collection is made.
STUDIES ON *Rauwolfia serpentina* Benth
IN SOUTH-EAST ASIA

BY

P. D. VARADARAJAN

Associate Director, Botany,
Sarabhai Chemicals Research
Institute, Ahmedabad.

*Rauwolfia serpentina* Benth. is distributed over a wide area in South-East Asia under varied geographic and climatic conditions mainly represented in Ceylon, India, Burma, Thailand and East Indies. The botanical and pharmacological aspects of the species have been dealt with by Monachino (1954) and Woodson *et al.* (1957). In India, *Rauwolfia serpentina* is distributed in a natural state along the lower elevations of Himalayas extending to Sikkim, Khasia Hills and Assam; in the Deccan peninsula along the Western Ghats ascending up to 2,000 feet—Hooker (1882). Data on the climate and soil conditions of *Rauwolfia serpentina* in India have been worked out (Varadarajan, 1963).

During an intensive survey of this species in Ceylon, India, Burma and Thailand certain striking differences and similarities in their morphology and growth forms were noted. Later, it was also found that there were significant variations in the chemical composition of roots—(Wiley, Personal Communication). The above observations prompted the author to undertake an extensive collection of both living specimens (vivarium) and herbarium materials from the said regions for further investigation. This paper has been presented in 4 parts.

**Table 1** showing the comparative data of the morphology of *R. serpentina* from Ceylon, India, Burma and Thailand. Figures represent linear measurements in mm.

<table>
<thead>
<tr>
<th></th>
<th>Ceylon</th>
<th>India</th>
<th>Burma</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internode</td>
<td>41</td>
<td>27</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>Leaf : Length</td>
<td>70</td>
<td>117</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Width</td>
<td>33</td>
<td>39</td>
<td>61</td>
<td>46</td>
</tr>
<tr>
<td>No. of veins in pairs</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Petiole</td>
<td>8</td>
<td>12</td>
<td>17</td>
<td>14</td>
</tr>
</tbody>
</table>

**Flower Parts:**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Peduncle</td>
<td>28</td>
<td>44</td>
<td>61</td>
<td>54</td>
</tr>
<tr>
<td>Pedicel</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Sepal</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corolla tube</td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Fruit</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Colour of corolla tube</td>
<td>pink</td>
<td>pink</td>
<td>red</td>
<td>red</td>
</tr>
</tbody>
</table>
TABLE 2 showing the statistical analysis of leaf size (area) of *R. serpentina* in Ceylon, India, Burma and Thailand.

<table>
<thead>
<tr>
<th></th>
<th>Ceylon</th>
<th>India</th>
<th>Burma</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value of leaf area :</td>
<td>34</td>
<td>50</td>
<td>126</td>
<td>61</td>
</tr>
<tr>
<td>Calculated F value : 6.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F value from tables : at 5% level—3.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 1% level—4.94.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance by F test :</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Critical difference :</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conclusions :</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ceylon</th>
<th>India</th>
<th>Burma</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>50</td>
<td>126</td>
<td>61</td>
<td></td>
</tr>
</tbody>
</table>

(I) Morphology

Linear measurements of both the vegetative and reproductive organs of the shoot system were taken from at least 50 specimens from each country which are once again distributed over a wide territory and are presented in Table I.

The root system is similar in all the collections while there are certain variations in the shoot system. The entire collection apparently falls into two groups, namely, Ceylon-India and Burma-Thailand. The shoot system of the former group has a tendency to branch and grows as tall as 75 cm. while in the later group the shoots are short, seldom branch freely and hardly grow up to 25 cm. in height. Among the Burma-Thailand group, the Burmese collections can be easily distinguished from that of Thailand by their significantly large leaf blade (Table 2).

(II) Growth forms

The indication of the presence of 2 morphological groups of plants in *Rauwolfia serpentina* has led the author to investigate into the growth rates of the different collections from Ceylon, India, Burma and Thailand under identical conditions of controlled soil, temperature, humidity and light in glass houses.

Growth measurements were confined to current year’s shoot of about 2-3 year old plants selected at random from the vast collections in the greenhouse. Growth of the shoot after production of the first inflorescence is sympodial. Generally during one growing season 5-8 nodes are produced before the shoot ends in an inflorescence. Invariably the lower two or three nodes of the shoot were so close together that the length of the 5 internodes from the terminal node bearing the inflorescence in basipetal succession was taken as a criterion representing the elongation of the current year’s shoot.
The analysis of the data showed significant differences in growth rates and the entire population falls into two groups, namely, (1) Ceylon-India group, which comprises of tall plants with elongated internodes having an average growth rate of about 20 cms. per growing season; and (2) Burma-Thailand group comprising of short plants with condensed internodes having an average growth rate of about 10 cm. (Table 3).

TABLE 3 showing the rate of growth in cm. of *R. serpentina* from Ceylon, India, Burma and Thailand (figures represent total of 5 internodes) and the statistical analysis of the data

| Mean values of growth rates of *R. serpentina* : | 29—Ceylon ; 25—India ; 10—Burma ; 5—Thailand |
| Statistical analysis : | |
| Calculated F value : | 30.7 |
| F value from tables : | at 5% level—2.62 |
| | at 1% level—3.83 |
| Significance by F test : | Yes |
| Critical difference at 1% level—9 |
| Conclusions : | |

<table>
<thead>
<tr>
<th>Ceylon</th>
<th>India</th>
<th>Burma</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>25</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

(III) Anatomy

Normally response to environmental conditions is manifested in the shoot and root system as adaptations which can be correlated with the internal anatomy. Therefore, it was found necessary to investigate into the anatomical details. Studies were confined to cross sections of stem and root only.

Stem

A cross section revealed a typical dicot stem after normal secondary thickening. Inner phloem was present which is common to many members of Apocynaceae. Laticiferous tubes were abundant in the inner cortex. Stone cells (sclereids) were absent in *Rauwolfia serpentina*—a distinguishing feature from the rest of the species of *Rauwolfia*.

The general anatomy of the stem was similar in all specimens, namely, Ceylon, India, Burma and Thailand.

Root

A cross section revealed a typical dicot root after normal secondary thickening. The root was well protected by cork tissue composed of 5 to 7 concentric layers of rectangular or squarish cells which were radially arranged. The phellogen was quite prominent. The cortex was often a thin layer followed by the crushed elements of the primary phloem. The primary phloem led to the secondary phloem which becomes gradually many cells in thickness as the cambium widens during secondary
thickening. Well defined rays wedge in between the phloem elements and continue through the xylem elements down to the core. Phloem sclereids were absent in this species, which are normally present in other species of Rauwolfia.

The secondary xylem or wood of Rauwolfia is of greatest practical and theoretical interest and shows strong correlation with the habit of the species (Woodson et al., 1957). The tracheary elements are composed of vessels, tracheids and apotracheal parenchyma. The xylem elements in the roots of Ceylon and India regions are comparatively more abundant than that of Burma and Thailand, which is occasioned by the fact that in the less woody forms, the wedges of tracheary cells become increasingly narrow and farther apart due to the remarkable expansion of the rays. The vessels are diffuse and disposed of in radial rows of elements of variable diameter, up to a maximum of 40 μ. Because of their narrow lumina, often times it is difficult to distinguish them from other tracheary cells in cross section. Starch is present in varying degrees in all cells of both phloem and xylem with the exception of isolated sieve cells of phloem and the commonly uniseriate and interrupted tracheary wedges of the xylem (Woodson et al., 1957).

(IV) Pollen morphology

To get a better understanding of the two groups within the population a study of pollen morphology of the entire collection was undertaken, since the pollen grains include very definite characters of a species.

All the spores examined were almost identical in shape irrespective of the country of origin.

Spores of Rauwolfia serpentina—Description

Polarity : Sub-isopolar.
Symmetry : Asymmetrical.
Apertures : Angulaperturate, 3 colporate with operculate pores.
Shape : Sub-oblate (longest axis—equatorial).
Size : Spores large (50–100μ) sporae magnae.
Sporoderm : Sexine fused ; inner exine surface uneven.
Sexine pattern : Smooth.
LO Analysis : Areolate.

It was found that the differences in the size of spores were not statistically significant and the entire population collected from Ceylon, India, Burma and Thailand fall under the same species.
TABLE 4 showing the pollen measurements of *R. serpentina* from Ceylon, India, Burma and Thailand and the statistical analysis of the data.

<table>
<thead>
<tr>
<th></th>
<th>Ceylon</th>
<th>India</th>
<th>Burma</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value of pollen</td>
<td>81µ</td>
<td>81µ</td>
<td>81µ</td>
<td>78µ</td>
</tr>
</tbody>
</table>

Calculated F value: 0.35
F value from tables: at 5% level—more than 216
at 1% level—more than 5,403

Significance by F test: No
Critical difference: 8

Conclusion:

<table>
<thead>
<tr>
<th></th>
<th>Ceylon</th>
<th>India</th>
<th>Burma</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>81µ</td>
<td>81µ</td>
<td>81µ</td>
<td>78µ</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The genus *Rauwolfia* seems to have originated from Central African regions and migrated on either side of the Continent (MacFarlane, 1933). The Lamurian Land apparently reached the southern and eastern parts of Ceylon which received abundant supplies of evolving species, one of which was *Rauwolfia serpentina*.

Further migration of this species from Ceylon extended in two directions, namely, (1) through the Western Ghats to the lower Himalayan regions and continued eastward into Burma and Thailand, and (2) from Ceylon into Sumatra, Java, Borneo and thence to New Guinea. It was evident that during this migration, a gradual transition from an arborescent to a shrubby habit (or even as an under shrub) has taken place. This fact is particularly evidenced from our collections of *Rauwolfia serpentina* from Ceylon, India, Burma and Thailand, which cover about 3 million sq. miles representing a wide range of geographical and climatic variations (Table 5).

**TABLE 5 showing the mean annual rainfall and temperature of Ceylon, India, Burma and Thailand.**

<table>
<thead>
<tr>
<th></th>
<th>Ceylon</th>
<th>India</th>
<th>Burma</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall in inches</td>
<td>80</td>
<td>120</td>
<td>160</td>
<td>80</td>
</tr>
<tr>
<td>Temperature in°F</td>
<td>80</td>
<td>80</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Temperature in°C</td>
<td>32</td>
<td>32</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>


A perusal of the data presented shows that the mean annual rainfall increases from 80° to 160° in Ceylon, India and Burma respectively and drops to 80° in Thailand. Whereas the mean annual temperature shows a decreasing temperature gradient from Ceylon to Thailand, through India and Burma from 32° C–20° C. The profound influence of temperature as a limiting factor in growth rate within a range...
of 0°C-35°C has been demonstrated by Vant Hoff (1938) that an increase of every 10°C doubles the rate of growth of many plant species. In the light of these findings and in relation to our data presented earlier, the affinities between the populations of different regions are discussed under the following headings:

(1) Morphology
(2) Growth characteristics
(3) Anatomy
(4) Chemical analysis of roots
(5) Pollen morphology.

1. Morphology

A perusal of the data presented earlier under this head shows that great variations in shoot length (internode) and leaf area (size) were evident. The collections of Ceylon-India origin have a tall and vigorously growing shoot, normally as high as 75 cms. while that of Burma-Thailand have a stunted shoot, condensed internodes and seldom grow more than 25 cm. in height. However, data on area of the leaves of Ceylon, India and Thailand fall in one group within statistical limits while that of Burma stands out and can easily be distinguished from the first group by the significantly large lamina.

It would be logical to assume that the species after migrating from Ceylon to India, Burma and Thailand, have adapted themselves to their new surroundings both edaphic as well as climatic during the course of the migration. Thus we find the progressive dwarfing of the shoot system from Ceylon to Thailand while the size of the lamina bears direct correlation to the rainfall.

The perceptible variations observed in the vegetative organs were not found in the inflorescence, except the colour of corolla tube which is pink in Ceylon-India group and red in Burma-Thailand.

2. Growth characteristics

The data presented earlier under this head showed that the entire population of Rauwolfia serpentina from Ceylon, India, Burma and Thailand falls into two statistically distinct groups, namely, (1) the tall specimens from Ceylon and India, whose elongation in one growing season ranges from 20-40 cm. and (2) the short specimens of Burma and Thailand, which seldom grow beyond 15 cm. in height.

The fact that the two groups of plants have retained their individuality even when grown under strictly controlled conditions of soil, moisture, temperature and light period shows that the species had undergone certain genetic readjustments during their migration and resettlement in the four regions and consequently have condensed into two groups distinct from each other in physiology and growth habits.
3. Anatomy

Further confirmatory evidence regarding the possible existence of the distinct groups noted in _Rauwolfia serpentina_ can be sought from a study of the comparative anatomy of the roots. As the stem is short lived and herbaceous in nature there is no significant difference in its structure among all the members investigated.

However, a close scrutiny of the anatomy of roots of the entire collection delimits two distinct tendencies in the disposition of secondary xylem and rays. The modifications of secondary xylem of _Rauwolfia_ which we have been discussing, are, of course, in reality modifications of the vascular cambium. Since each cell of the secondary xylem has its counterpart in the secondary phloem through the origin of both from a common cambial initial, it follows that the modification of the phloem in response to the subherbaceous tendency, which we have been able to discern, may be interpreted as precisely comparable to that of the xylem. Thus, in the Ceylon-India group the tracheary elements are continuous from the centre to the periphery and are separated by uniseriate or multiseriate rays composed of only 2-3 rows of cells. Whereas the Burma-Thailand group shows a scanty and often discontinuously disposed radial tracheary elements separated by wide rays of parenchyma invariably in many rows. The storage of starch grains is relatively more abundant in the latter group. Woodson et al. (1957) question as to whether the anatomical modifications which have been discussed are _per se_ products of the subherbaceous tendency or are more directly the products of purely physiological tendencies such as storage. Further, they note that the expansion of the rays is not invariably associated with storage of starch and protein, based on extensive study of the genus _Rauwolfia_ comprising of very many species. But the present investigation, which is based on an intensive study extending over 3,000,000 sq. miles and confined to a single species, does throw more light on Woodson et al.'s open question.

The conspicuous and large proportion of tracheary elements and phloem present in the roots of Ceylon-India group of plants are indeed a challenge to their vigorously growing shoots possessed of great capacity of axillary branching demanding large quantities of water and nutrients from the soil. The dwarf and less vigorous shoot system possessed of a greater photosynthetic capacity owing to their large sized leaves of the Burma-Thailand group, presents a modified root anatomy meeting the altered demands of their shoot system, namely, a larger proportion of storage tissue replacing the tracheary elements in the roots, thus shifting the main role of conduction found in Ceylon-India group to that of storage in Burma-Thailand group.
4. Chemical analysis

The findings of Wiley* from the chemical analysis of roots from Ceylon, India, Burma and Thailand, for their total alkaloids, both qualitative and quantitative, revealed that similarities were found among Ceylon and India on one hand and Burma and Thailand on the other, presumably the result of the altered growth habits and physiological anatomy of the tall and dwarf groups of Rauwolfia serpentina.

5. Pollen analysis

Earlier it was observed that the floral parts irrespective of their place of collection did not show variations in size or shape excepting the colour of corolla tube which was pale pink and red in the Ceylon-India and Burma-Thailand groups respectively.

Since the pollen grains include definite characters of identification of a species, further clue to the species problem of Rauwolfia serpentina was sought from palynological studies. Pollen grains from all the four regions showed similar morphological characters with minor variations in their size only.

However, these differences were not significant statistically (Table 4), thereby confirming once again our earlier findings that the entire population from Ceylon, India, Burma and Thailand belongs to the same species.

Summing up our findings, the sympodial nature of the shoot system and similarities in structure and of measurements of leaf shape, floral parts and the pollen grains within statistical limits and absence of sclereids in the entire population bring the entire population under one species, namely Rauwolfia serpentina. Yet the physiologically irreversible characters like the leaf size, differences in growth rates, colour of corolla tube, variations in quality and quantity of tracheary elements, phloem and the rays in the root, and the chemical analysis of roots demand a reconsideration of the species. Therefore, a new variety under R. serpentina, R. serpentina var. foggiana var. nov. has been established. This variety differs from R. serpentina in shoot height, nature of shoot system and flower, growth habits, anatomy of root and distribution.

Key to Species

A. Shoot system tall, generally 50-75 cm., internodes long, 2-7-4-1 cm., stem freely branching; leaves 7-0-16-5 cm. long and 3-3-3-9 cm. broad; corolla tube pale pink in colour and seasonal growth of stem ranging from 20-40 cm.

Distribution—Ceylon and India

---var. serpentina.

B. Shoot system dwarf, less than 30 cm., internodes condensed, 1-5-2-4 cm., stem seldom branched; leaves larger than the previous group, 13-0-15-0 long and 4-6-6-1 cm. broad, corolla tube red in colour and seasonal growth of stem ranging from 5-15 cm. only.

Distribution—Burma and Thailand

---var. foggiana.

* Personal Communication from Mr. Wiley, The Squibb Institute for Medical Research, New Brunswick, N.J.
R. serpentina (L) Benth. ex Kurz. var. foggiana Varadarajan var. nov. which has now been proposed is named after my teacher Professor John M. Fogg, Jr., Director, The Morris Arboretum, Philadelphia Pa, U.S.A., in appreciation of his extensive contribution to Plant Taxonomy and systematic botany.

Acknowledgement

The author wishes to express his deep gratitude and thanks to Prof. John M. Fogg, Jr., Director, The Morris Arboretum, University of Pennsylvania, U.S.A., for keeping his entire collection of Rauwolfia serpentina both herbarium and vivarium specimens at the author's disposal and for his constant encouragement and valuable criticisms throughout the course of this work. To Olin Mathieson Chemical Corporation, New York, U.S.A., for a research grant and to the University of Pennsylvania for affording excellent facilities for work.

REFERENCES

DRUG RESOURCES OF WEST PAKISTAN

BY

M. IKRAM and S. A. WARSNI

North Regional Laboratories,
Pakistan Council of Scientific and
Industrial Research, Peshawar
W. Pakistan

Well over one thousand plants with established medicinal properties in the indigenous systems of medicine, and a good number of British Pharmacopoeial drugs, occur in West Pakistan. The majority of these plants grow in the northern hilly regions. In spite of the comparative abundance and easy availability of medicinal plants, the pharmaceutical industry in the country has not made much headway, chiefly because the trade in crude drugs is still in a chaotic state and hardly any attempt appears to have been made, so far, to organise it on scientific lines. There are no well established firms specialising in the collection and supply of drugs. The methods of collection and processing are very crude and no grading, based on the actual chemical analysis, is done at any stage. Because of the inferior quality of drugs and unrestricted adulteration, as they reach the market, the pharmaceutical industry has been compelled to import drugs, or preferably concentrates, wherever available, for the preparation of galenicals and other proprietary preparations.

For the scientific organisation and development of the pharmaceutical industry, it was imperative that all available information should be collected regarding the requirements of raw materials of the industry followed by a survey of the drug resources of the country. Immediately after establishment, the Indigenous Drugs Division of the North Regional Laboratories started the survey of the crude drug requirements of the pharmaceutical industry of the country.

The total of about fifty factories working in West Pakistan, at that time, primarily produced pharmaceutical products in dosage forms of tablets, capsules, injections, proprietary preparations, extracts and tinctures, etc. It was, however, significant that the bulk of the total investment by the industry was concentrated in factories engaged on packing penicillin, streptomycin, and tabletting or repacking ready-made imported materials. Kurram Chemical Works, Rawalpindi, and Marker Alkaloids, Quetta, were and still are the only two basic industrial concerns mainly producing santonin and ephedrine from Artemisia and Ephedra available in the country. This survey also brought into light the very interesting fact that quite a few drugs, although abundantly available in the country, were not being fully utilised by the industry. Table I shows the average annual consumption of crude drugs by the West Pakistan factories based on figures for 1954–58.

From Table I, it may be seen that many of the pharmacopoeial drugs, e.g. Cassiae fistulae fructus, Filicis rhizome, Herba menthae viridis, Iridis rhizoma, Junperi fructus, Lini semina, Psyllium semen, Ricini semina, Sennae folium and Sennae fructus, are
**Table 1**

<table>
<thead>
<tr>
<th>Name of the Drugs</th>
<th>Average annual consumption in maunds</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ephedra</td>
<td>26,000</td>
<td>Used by Marker Alkaloids, Quetta and also exported</td>
</tr>
<tr>
<td>2. Glycyrrhiza radix</td>
<td>25,000</td>
<td>Imported</td>
</tr>
<tr>
<td>3. Santonica</td>
<td>2,000</td>
<td>Used by Kurram Chemical Works, Rawalpindi</td>
</tr>
<tr>
<td>4. Acaciae gummi</td>
<td>365</td>
<td>Major part imported</td>
</tr>
<tr>
<td>5. Hyoscyamii folia</td>
<td>350</td>
<td>Imported—Small quantities available in local markets</td>
</tr>
<tr>
<td>6. Aurantii amari cortex</td>
<td>125</td>
<td>*</td>
</tr>
<tr>
<td>7. Olea resin a pini</td>
<td>125 Galls.</td>
<td>*</td>
</tr>
<tr>
<td>8. Senegae radix</td>
<td>105</td>
<td>*</td>
</tr>
<tr>
<td>9. Zingiberis rhizoma</td>
<td>100</td>
<td>Imported, seldom purchased from local markets</td>
</tr>
<tr>
<td>10. Belladonnae radix</td>
<td>90</td>
<td>*</td>
</tr>
<tr>
<td>11. Gentianae radix</td>
<td>85</td>
<td>Mostly imported</td>
</tr>
<tr>
<td>12. Belladonnae herba</td>
<td>80</td>
<td>*</td>
</tr>
<tr>
<td>13. Stramonii folia</td>
<td>80</td>
<td>*</td>
</tr>
<tr>
<td>14. Valerianae rhizome</td>
<td>65</td>
<td>*</td>
</tr>
<tr>
<td>15. Anethi fructus</td>
<td>55</td>
<td>*</td>
</tr>
<tr>
<td>16. Colchi cornus</td>
<td>50</td>
<td>*</td>
</tr>
<tr>
<td>17. Anisi fructus</td>
<td>45</td>
<td>Imported</td>
</tr>
<tr>
<td>18. Cauri fructus</td>
<td>40</td>
<td>*</td>
</tr>
<tr>
<td>19. Rhei rhizoma</td>
<td>40</td>
<td>*</td>
</tr>
<tr>
<td>20. Sarsaparillae radix</td>
<td>40</td>
<td>Imported</td>
</tr>
<tr>
<td>21. Catechu</td>
<td>35</td>
<td>Imported</td>
</tr>
<tr>
<td>22. Lemonis cortex</td>
<td>30</td>
<td>*</td>
</tr>
<tr>
<td>23. Coriandri fructus</td>
<td>25</td>
<td>*</td>
</tr>
<tr>
<td>24. Isphagula semina</td>
<td>25</td>
<td>*</td>
</tr>
<tr>
<td>25. Colocynthides fructus</td>
<td>20</td>
<td>*</td>
</tr>
<tr>
<td>26. Herba menthae piperitae</td>
<td>20</td>
<td>*(Most of the requirements are imported in the form of extracts).</td>
</tr>
<tr>
<td>27. Aconiti radix</td>
<td>15</td>
<td>Imported</td>
</tr>
<tr>
<td>28. Aloe</td>
<td>15</td>
<td>Imported</td>
</tr>
<tr>
<td>29. Colchici semen</td>
<td>15</td>
<td>*</td>
</tr>
<tr>
<td>30. Opium</td>
<td>15</td>
<td>Imported</td>
</tr>
<tr>
<td>31. Foeniculi fructus</td>
<td>15</td>
<td>*</td>
</tr>
<tr>
<td>32. Podophylli rhizome</td>
<td>15</td>
<td>*</td>
</tr>
<tr>
<td>33. Curcumae rhizoma</td>
<td>2</td>
<td>*</td>
</tr>
</tbody>
</table>

* Entire requirements met within the country.
not being utilised at all by the industry, although abundantly available. Many of
the drugs, e.g. Aconiti radix, Belladonnae radix, Gentianae radix and Scillae bulbus,
have been imported although they are available in the country. The remaining
ones are also not utilized properly although large quantities are available.

In the second phase, a general survey of the medicinal flora of West Pakistan,
including Swat Valley, Dir, Chitral and parts of Azad Kashmir was undertaken,
followed by a more detailed assessment of their extent of distribution. The information
collected is contained in various publications sent out from the Laboratories.

Medicinal plants were collected from all over West Pakistan. The collected
samples, particularly those with established economic value were analysed for their
active constituents. Some of the results are reproduced in Table II. A phyto-
chemical survey of alkaloidal-bearing plants of North-Western parts of West
Pakistan and standardisation of some local aromatic herbs and drugs was also
undertaken to facilitate the grading of locally produced material.

Side by side with this work, some work was also done on the separation and
estimation of alkaloids using the technique of non-aqueous titration and thin-layer
chromatography. The method of residual non-aqueous titration was successfully
employed for the estimation of atropine, papaverine, serpentine, ajmaline, ajmal-
cine, etc. The method was also extended to the estimation of weakly basic or acidic
substances which cannot normally be titrated with water as solvent, e.g. thiamine
hydrochloride, pyridoxine hydrochloride, nicotinic acid, p-aminobenzoic acid, etc.

A method was also developed for the estimation of total Solanaceous alkaloids
by the technique of thin-layer chromatography as atropine, hyoscyamine and hyos-
cine are important alkaloids and are used extensively in the pharmaceutical industry.

The crude alkaloidal extract of the sample prepared in the usual way was applied
to the layer of alumina and irrigated with methyl alcohol. The absorbent material
corresponding to the spot of alkaloid was extracted out with chloroform and was
estimated by acidimetry.

All this information proved very useful in answering queries from the pharmaceu-
tical industry. From the collected data, it was possible to tell the locality where
a particular drug was available and give a fair idea of its active constituents.

After the completion of the survey work, some plants were selected for detailed
chemical studies on the basis of their alleged efficacy either in the folklore medicine
or their importance in the Unani Materia Medica. As most of the alkaloids are
pharmacologically active, and while the bitters have equally good reputation as
a cure of different ailments in the indigenous systems of medicine, bitter and alkaloidal-
bearing plants were preferred for detailed investigation. The first plant to be exami-
ned was Adhatoda vasica from which vasicine, vacicinone and betaine have already
been isolated.

This plant occurs abundantly in the northern hilly regions of West Pakistan
and is used as a popular remedy for cough, cold, asthma, etc. Vasicine is broncho-
constrictor, whereas vasicinone was found to have broncho-dilator effect. It was
later found that vasicinone is formed as a result of aerial oxidation of vasicine.
<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Locality</th>
<th>Part Analysed</th>
<th>Per cent total alkaloids as Hyoscyamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Local sample</td>
</tr>
<tr>
<td><em>Atropa acuminata</em></td>
<td>Shahid Pani (Bhugar Mung Valley)</td>
<td>Roots</td>
<td>0.67</td>
</tr>
<tr>
<td>Royle Ex Lindley</td>
<td>Darband (Indus Kohistan)</td>
<td>Leaves and flowering tops</td>
<td>0.60</td>
</tr>
<tr>
<td>do</td>
<td>Market sample</td>
<td>Roots</td>
<td>0.54</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>Baragali (Hazara)</td>
<td>Leaves</td>
<td>0.29</td>
</tr>
<tr>
<td>Linn.</td>
<td></td>
<td>Seeds</td>
<td>0.29</td>
</tr>
<tr>
<td>do</td>
<td>Abbottabad</td>
<td>Leaves and flowering tops</td>
<td>0.23</td>
</tr>
<tr>
<td>do</td>
<td>Abbottabad</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Datura alba</em></td>
<td>Peshawar</td>
<td>Leaves</td>
<td>0.25-3</td>
</tr>
<tr>
<td>Nees</td>
<td></td>
<td>Seeds</td>
<td>0.24</td>
</tr>
<tr>
<td>do</td>
<td>Peshawar</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Datura fastuosa</em></td>
<td>Experimental Farm, North Regional Labs. Peshawar</td>
<td>Leaves</td>
<td>0.112-0.120</td>
</tr>
<tr>
<td>Linn.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Locality</th>
<th>Part Analysed</th>
<th>Resin percent</th>
<th>Resin percent</th>
<th>Podophyllotoxin percent</th>
<th>Podophyllotoxin percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Local sample</td>
<td>In foreign species</td>
<td>Local sample</td>
<td>In foreign species</td>
</tr>
<tr>
<td><em>Podophyllum emodi</em></td>
<td>Kaghan Valley</td>
<td>Rootlets &amp; Rhizomes</td>
<td>10.9-11.5</td>
<td>4.17-5.2</td>
<td>3.9-4.0</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>P. peltatum</em></td>
<td></td>
<td><em>P. peltatum</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(U.S.A.)</td>
<td></td>
<td>(U.S.A.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9-11.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>P. emodi</em></td>
<td></td>
<td><em>P. emodi</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(India)</td>
<td></td>
<td>(India)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

34
Working with fresh chopped leaves of the drug, Siddiqui et al (C.S.I.R. Laboratories) reported the presence of vasicinone and betaine only but no vasicinone. This finding almost confirmed the contention that vasicinone is formed during drying and storage of the material. These results were in accordance with the earlier experience that even if shade dried and stored plant material was used, appreciable quantities of dark resinous matter were encountered, which was difficult to remove, making the isolation of pure crystalline compounds far too difficult. While extracts of fresh material were far too easy to handle, and it was possible to affect higher recoveries of the various constituents and involved fewer steps. To avoid the complications arising out of enzymatic activity, aerial oxidation or later fermentative spoilage likely to happen during drying and storage, only fresh material was used for these investigations and put in the suitable extracting solvent within hours of collection.

The method of isolation employed by Siddiqui and his co-workers was: alcoholic extract of fresh coarsely chopped leaves was concentrated under reduced pressure until free from alcohol. The extract was diluted with water. Fatty matter and chlorophyll were removed by extraction with petroleum ether. Vasicine was isolated as hydroiodide by the addition of potassium iodide. The potassium bismuth iodide complex obtained from the mother liquor was decomposed and finally treated with silver chloride and identified as betaine.

As the plant grows abundantly in the low-lying areas of the neighbouring Khyber Agency, the plant was taken up for further examination to assess the effect of climatic and other environmental factors on the alkaloidal constituents. Fresh flowering tops (blooming stages) were collected early in April, cut into half inch bits and repeatedly percolated with 95% alcohol. The combined extracts were first concentrated in a cyclone evaporator to a point where chlorophyll and fatty matter began to separate out, then allowed to stand overnight in the cold and passed through a wad of cotton. The filtrate was once or twice extracted with petroleum ether and finally concentrated under reduced pressure to a thick syrupy consistency. The sugars and the carbohydrates were precipitated out by the addition of 98% ethyl alcohol. The supernatant layer was decanted off and the thick syrupy residue exhaustively extracted with absolute alcohol. The alcoholic extract after complete removal of the solvent was taken up in an excess of 10% aqueous ammonia. On standing overnight, the bulk of the alkaloids crystallised out and were removed by centrifuging or filtration. The final mother liquor was finally extracted with chloroform and the solid residue obtained was taken up with the earlier crops of crystalline precipitates and divided into chloroform soluble and insoluble fractions.
Another batch of the tops (seeding stage) was collected by the end of May and similarly processed. The results are given in Table III. The chloroform soluble fraction was found to contain only vasicine, identified from the melting point of the free base and its derivatives.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Materials</th>
<th>Flowering stage</th>
<th>Seeding stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weight of the drug on moisture free basis</td>
<td>5.5 kg.</td>
<td>5.9 kg.</td>
</tr>
<tr>
<td>2</td>
<td>Weight of crystalline precipitate</td>
<td>40.59 g.</td>
<td>25.30 g.</td>
</tr>
<tr>
<td>3</td>
<td>Weight of residue from chloroform extracts</td>
<td>32.24 g.</td>
<td>47.10 gm.</td>
</tr>
<tr>
<td>4</td>
<td>Yield of chloroform soluble fraction</td>
<td>35.34 g. (0.64 percent)</td>
<td>52.1 g. (0.88 percent)</td>
</tr>
<tr>
<td>5</td>
<td>Yield of chloroform insoluble fraction</td>
<td>37.49 g. (0.68 percent)</td>
<td>20.3 g. (0.34 percent)</td>
</tr>
</tbody>
</table>

The chloroform-insoluble fraction, an entirely new base, was isolated and has been provisionally named vasicinine, the name originally proposed for betaine before its identification. It was insoluble in benzene, ether, pet. ether, and diffusely soluble in moist ethyl acetate or hot dilute alcohol, from which it crystallised in colourless rectangular plates (m.p. 246-48° decomposition). On the basis of analytical results, the molecular formula of the base was found to be C_{11}H_{12}O_{2}N_{2}. The base is soluble in dilute mineral acids and alkali. It reduces Tollén's reagent and also decolourizes alkaline potassium permanganate solution. On adding a few drops of concentrated nitric acid to a solution of the base in concentrated sulphuric acid, a deep orange colour is produced whereas vasicine gives a yellow colour. This base and vasicine both gave positive tests for tertiary amine. The hydrochlorides prepared by the usual methods both in aqueous and non-aqueous meJia gave the same salt, (m.p. 258-260° decompo.) which analyses as monohydrochloride proving it to be mono-acidic base with one tertiary nitrogen. The crystalline sulphate melted at 190-200° (decomposition) and the crystalline picrate at 207-210° (decomposition).

**Paper chromatography**

The free base, being sparingly soluble in organic solvents, gave very faint colour with Dragendorff's reagent. The chloride or the acetate developed prominent brown spots. The acetate was, therefore, used for spotting on Whatman No. 1, chromatographic paper and irrigated with N-butanol – acetic acid – water (10 : 1 : 5), descending. The Rf value of the new base was found to be 0.43 while that of vasicine under the same conditions was 0.51.
This work also revealed that the amount of vasicine is minimum at the blooming stage, but increases with seed formation. On the other hand, the quantity of vasicine, maximum at the flowering stage, decreases with seed formation. The molecular formula of vasicine (C_{11}H_{16}O_{2}N_{2}) contains an oxygen atom in excess of the vasicine formula indicating the possibility of its close structural similarity with vasicine.

Roots of *Berberis ceratophyllum* or *Berberis lycium*, growing wild around Murree hills and in the neighbouring ‘Gallies’ was subsequently taken up for chemical investigation.

Concentrated semi-solid extracts of the roots, called Rasaut, is a very highly esteemed drug in the indigenous medicine as a bitter tonic and febrifuge. Mixed with opium or lime-juice, it is painted over eye-lids in acute conjunctivitis and in chronic opthalmia. It is also considered useful in the enlargement of liver and spleen and in the treatment of gastric and duodenal ulcers, as also in chronic diarrhoea and piles. Crude powdered roots taken with milk, are reputed to relieve painful conditions of joints, caused by rheumatism or rheumatoid arthritis, and also muscular pains.

The various species of Berberis are not easily distinguished from one another and considerable ambiguity still exists, especially as most of them are known by the same vernacular names and possess identical properties. Moreover, the species multiply by natural hybridisation.

The five varieties of *Berberis vulgaris* Linn, as given in the flora of British India are now considered to be distinct species. *Berberis vulgaris* Linn of Europe does not occur in the sub-continent. The isolation of umbellatine from *Berberis umbellata* and *Berberis lycium* was first reported by Chatterjee from the air-dried stem bark of the two plants. Chatterjee reported a close resemblance between umbellatine and berberine. The near ultra violet absorption spectra of the two bases were found to have curves of marked similarity. The salts were also prepared and characterised.

In view of the medicinal importance of the plant and the comparatively little work done, *Berberis ceratophyllum* roots were taken for detailed chemical examination. This work was also expected to indicate if the plant was correctly identified by the Indian worker.

For this work, fresh roots of *Berberis ceratophyllum* were collected, under the personal supervision of a systematic botanist, washed well, and then within hours of collection, were put in alcohol, after cutting them into small bits with a sharp axe. The results were according to expectations, and we could easily isolate three alkaloids—one colourless and two yellow. The colourless base being soluble in Ethyl acetate could be isolated as the free base by basifying the crude aqueous extract with ammonia and extracting with ethyl acetate. The two yellow bases were isolated as the sparingly soluble hydrochloride and hydro-iodide on saturating the mother liquor with sodium chloride or potassium iodide.
The white base is crystallised from ethyl alcohol m.p. 300–305 (dec.) \( [\alpha]_D^{28} + 137 \) in methyl alcohol. It is insoluble in water, petroleum ether, ether, alkali and soluble in all basic solvents. So far no crystalline derivative could be obtained. The colour reactions of white base are as follows:

1. Conc. nitric acid—Very light brown colour.
2. To alkaloid solution in sulphuric acid added a crystal of Pot. dichromate—Pale yellow colour changes into reddish brown.
3. Frohde’s reagent—Deep violet colour ultimately changing into light violet.
4. Mandelin’s reagent—Bluish green colour.

Yellow base obtained as hydrochloride.

This base was prepared by dissolving the hydrochloride in water: acetone mixture and basifying with 10 per cent sod. hydroxide. It was crystallised from ethyl acetate in lemon yellow rectangular shaped crystals m.p. 164–165° (dec.). It is optically inactive and gives the following reactions:

1. conc. nitric acid—Light brown colour changing into orange.
2. Frohde’s reagent—Light yellow colour changing into dark yellow.
3. Mandelin’s reagent—Bluish colour changing into dark brown.
4. To the base dissolved in 0.1 ml. of 10 per cent sulphuric acid added 2 ml. of pure sulphuric acid in an evaporating dish. Then 0.1 ml. of 5 per cent solution of gallic acid in ethanol is added and the mixture warmed on water bath—Light green colour.
5. To the alkaloid solution in conc. sulphuric acid added a crystal of pot. dichromate—Dark brown colour.

It failed to give the specific colour test of berberine or umbellatine, i.e. to the base dissolved in dil. hydrochloric acid, added a drop of bromine water and a drop of hydrochloric acid—No colour.

This does not appear to be umbellatine as reported by Chatterjee because its physical properties, i.e. m.p., solubility and colour reactions are quite different from umbellatine. This base has the same m.p. and solubility as berberine. As the m.p.’s. of berberine and its salts are indefinite and consequently of little value for characterisation, the point needs to be investigated further to arrive at a definite conclusion. Further work to characterise this base, as well as the third yellow base is in progress.

*Erythraea kamosissima* is a small shrub containing a bitter glycoside; it belongs to the family Gentianaceae. It grows on damp soil and bears numerous small pink flowers; the plant is very common in Swat and in the plains round-about Rawalpindi. The plant has great local reputation as a remedy for excessive menstrual
bleeding or post-abortive haemorrhage. Powdered whole plant, given orally, effectively stops nasal bleeding and one or two doses repeated at weekly intervals almost completely checks this tendency.

The bitter glycosidal principle contained in the plant was suspected to be responsible for the medicinal properties of the drug.

The alcoholic extract of the plant was concentrated in a cyclone evaporator and the semi-solid residue treated with petroleum ether to remove chlorophyll and the fatty matter. The light brown residue, thus obtained, was dissolved in methanol. On the gradual addition of acetone followed by some di-ethyl ether, bulk of the sugars separated out.

At this point, a very interesting fact was observed that the bitter principle could almost be completely precipitated by treatment of its methyl alcoholic solution with methyl alcoholic caustic soda. This precipitate after a few washings with methanol, to remove excess of alkali, and on standing for some time in a mixture of acetone and ether, gave a brown powder which could be separated by filtration. It is freely soluble in water and very bitter in taste. Pharmacological and chemical work on the "bitter" is in progress.

Fagonia cretica is another plant being investigated. It is a small shrub, widely distributed throughout West Pakistan and belongs to the family of Zygophyllaceae. The aqueous decoction of leaves and young twigs is popularly considered to be a blood purifier and is given for the treatment of skin lesions (boils and abscesses), particularly among children. Of late, the plant has assumed greater importance and is claimed to cure tumors and check the growth of cancer in its early stages. It is also stated to be an astringent and a febrifuge and to cure any disorders arising from poisoning.

As a result of the work, so far carried out, we have been able to isolate the bitter principle (as an amorphous light brown powder), which is insoluble in most of the organic solvents, but soluble in ethyl and methyl alcohols and disperses easily in water giving a colloidal solution. This complex, on treatment with alcoholic hydrochloric acid, gives a crystalline product (m. p. 305–307°), difficultly soluble in methyl or ethyl alcohol and crystallises out of these solvents on slow evaporation.

In view of the insolubility of this complex in immiscible inorganic solvents, removal of the sugar ballast presented considerable difficulties. The primary aim, however, was to isolate the bitter principle, as it occurs in the plant, for pharmacological investigations and later clinical trials.

The semi-solid residue obtained on removal of the solvent was taken up in methyl alcohol and exhaustively extracted with petroleum ether until the chlorophyll and the fatty matter was completely removed and a light brown alcoholic solution was obtained. This was partially concentrated and treated with acetone and a little ether.
The brown semi-solid precipitate which separated out was now taken up in absolute ethyl alcohol, followed by the addition of acetone and ether. The process was repeated until the precipitated sugars were no longer soluble in absolute ethyl alcohol, and were not bitter in taste. The light yellow filtrate was again concentrated and similarly treated until the free sugars were almost completely removed.

The semi-solid residue, finally obtained, was repeatedly macerated with ethyl acetate. The bitter principle was obtained as a light brown powder on standing for some time in the same solvent. This complex has to be subjected to pharmacological tests and later clinical trials to assess the medicinal properties claimed for the drug.
THE DISTRIBUTION OF
INDIGENOUS AND
NATURALIZED
MEDICINAL PLANTS OF
CEYLON

BY

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AND
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From very early times the use of herbal medicines developed in Ceylon as in
other parts of the world. The early inhabitants seem to have brought with them
systems of medicine which were then prevalent in India and adapted or modified
them to suit the local conditions. Even today the large majority of the drug plants
used in Ceylon are the same as those currently used in the neighbouring mainland.
The realization of this fact may have given rise to the popular legend that certain
forested hills, e.g. Doluwakanda and Rumassalakanda, from which drug plants
are often collected, are only fragments of a part of the Himalayas that was carried
over to Ceylon by the mythical monkey-king, Hanuman, to provide drugs for the
wounded in the Rama-Ravana battle.

A full and accurate enumeration of the indigenous medicinal plants is made very
difficult by the fact that the same species often has a plurality of popular names and
that in different regions different species are given the same name. This has caused
much confusion even in the published works. Further, in many instances, superf-
icially similar plants are used in place of the correct, medicinal plants. For example,
Euphorbia tirucalli L. is sometimes used in place of Sarcostemma brunonianum
Wight & Arn. as Muwa-kiriya and Indigofera enneaphylla L. in place of Tribulus
terrestris L. as nerench. Of the plants that have been recorded as being of medicinal
use by Attygalle (1917), Roberts (1931) and Trimen (1893–1900), we have therefore
taken into consideration only the species which have been accurately identified
botanically. Secondly, no complete survey of the distribution of the Ceylon drug
plants has been carried out as yet, and the present account is based on the available
data from general botanical collections.

The natural regions of Ceylon

Ceylon has a total area of about 25,000 square miles and its maximum length and
breadth are approximately 270 miles and 140 miles respectively. The south-central
part of the Island is mountainous and has an elevation ranging from 3,000–8,000 ft. This region is surrounded by an upland belt of 1,000 to 3,000 ft. The coastal area forms a plain which is most extensive on the northern and eastern sides.

The average temperature in the lowlands is about 80° F but the central highlands are much cooler and have a somewhat temperate climate.

The northern and the eastern plains have a seasonal rainfall. The extreme northwest and the southeast of this region receive only 25–50 ins. per annum and are referred to as the Arid Zones. The rest of this region—the Dry Zone—gets a rainfall of 50–75 ins. annually.

In the southwestern lowlands and the central highlands the rainfall ranges from 75–200 ins. per annum. The lowlands and the hilly areas up to about 3,000 ft. is referred to as the low country wet zone and the central highlands above this elevation form the montane zone.

**Plant distribution**

The present distribution of the indigenous species is largely determined by the tolerances exhibited by the individual species to external physical conditions, and by their ability to grow successfully in competition with other organisms in the particular area. In general, species with narrow tolerances or those with limited powers of competition would be restricted to smaller areas, whilst those with wide tolerances or which are more aggressive would have wider distributions.

In Ceylon about 515 species of indigenous plants are used as sources of medicinal drugs. In addition, a large number of exotics which have been introduced to the Island are used in medicine. Of these a few, even though they are very early introductions, e.g. *Tamarindus indica* L. and *Aegle marmelos* (L) Corr., have not become fully naturalized and are found only in cultivation or as escapes from cultivation in or near gardens or in abandoned cultivations. Others, e.g. *Sesamum indicum* L., *Anacardium occidentale* L., *Mimosa pudica* L., *Ageratum conyzoides* L. etc., have become fully naturalized in the Island. The naturalized plants which are now used in medicine number about 35, making a total of 550. Their distribution in Ceylon is as follows.

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arid Zone</td>
<td>2</td>
</tr>
<tr>
<td>Arid and Dry Zones</td>
<td>7</td>
</tr>
<tr>
<td>Dry Zone</td>
<td>148</td>
</tr>
<tr>
<td>Dry Zone and Low Wet Zone</td>
<td>189</td>
</tr>
<tr>
<td>Low Country Wet Zone</td>
<td>108</td>
</tr>
<tr>
<td>Low Wet Zone and Montane Zone</td>
<td>40</td>
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<tr>
<td>Montane Zone</td>
<td>25</td>
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<tr>
<td>Present in all Regions</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>550</strong></td>
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</tbody>
</table>
Of the two species confined to the arid zone, one, Grewia tenax (Forsk.) Fiori, is rather common within the area but the other, Cassia italica (Mill.) Lam. ex Sturd., is somewhat rare but is said to be frequent near Jaffna. These two and some of the species common to both dry and arid zones, e.g. Salvadora persica L. and Aloe vera var. littoralis L., are plants from the Arabian-Iranian desert regions and they seem to reach their eastern limits of distribution in Ceylon.

Among the dry zone species are several which are commonly cultivated in gardens, e.g. Baselia alba L., Clitoria ternatea L., Cassia fistula L., Murraya koenigii (L.) Spreng., Thespesia populnea (L.) Soland., Azadirachta indica A. Juss., and Cissus quadrangularis L. Their absence in the wet lowlands is probably due to their inability to compete successfully with faster growing species but under cultivation when competition is eliminated they can be very easily grown in that area.

A very large number of the medicinal plants are present in the dry and wet lowlands. This is partly due to the large extent of this area and partly to the very wide range of habitats within the region. Of the species present here, those with wide tolerances or are aggressive are present in both dry and wet zones. They include aquatics, e.g. Pistia stratiotes L., Nymphaea lotus L., N. stellata Willd. etc.; garden weeds, e.g. Alternanthera sessilis (L) R. Br., Boerhavia diffusa L., Alysicarpus vaginalis (L.) DC., Mimosa pudica L. etc.; sandy seashore plants, e.g. Hydrophyllax maritima L.f. and Ipomoea pes-caprae (L) R. Br.; mangrove plants, e.g. Avicennia officinalis L., Exoecaria agallocha L., and Luminitzeria racemosa Willd., and forest species like Hydnocarpus venenata Gaertn. Those less tolerant of dry conditions are confined to the wet zone lowlands. Among them are the endemics Nepenthes distillatoria L., Dipterocarpus zeylanicus Thw. and Dipterocarpus glandulosus Thw., and some important drug plants of commerce, e.g. Cinnamomum zeylanicum Bl. and Rauwolfia serpentina (L) Benth ex Kurz.

Moisture-demanding species with a relatively wide temperature tolerance are common to the wet zone lowlands and the montane zone. These include the orchids Anaeuctochilus regalis Bl. and Zeuxine regia (Lindl.) Trim. which grow on the forest floors up to an elevation of about 5,000 ft.

The plants confined to the montane zone are generally those which cannot withstand high temperatures and it is often difficult if not impossible to grow most of them in the lowlands even under cultivation conditions. Among some of the interesting drug plants here are the root parasite Balanophora indica (Arn.) Wedd., the stem parasite Cuscuta reflexa Roxb., which is often found growing on Strobilanthes spp., and the local "Wintergreen" plant—Gaultheria rudis Stapf.

Lastly we have the group which is most aggressive and has the widest tolerances, e.g. Panicum repens L., Amaranthus spinosus L., Cassia occidentalis L., Oxalis corniculata L., Centella asiatica (L.) Urb., Leucas zeylanica (L.) Benth., Emilia sonchifolia (L.) DC. etc. These have a wide distribution in the island and are among the most troublesome weeds on wastelands and cultivated ground.
Fire as a factor determining distribution

In the savannas between the central highlands and the dry eastern plains the ground layer is composed mainly of coarse tussock grasses, e.g. *Imperata cylindrica var. major* C. E. Hubb., *Cymbopogon nardus* (L.) Rendle, *Sorghum nitidum* (Vahl) Pers. etc. These grasses are burnt periodically by cattle graziers and the fires destroy most trees and shrubs. Only twisted gnarled trees belonging to fire resistant species, e.g. *Careya arborea* Roxb., *Phyllanthus emblica* L., *Terminalia chebula* Retz., *Terminalia bellirica* (Gaertn.) Roxb. etc., survive the fires and as a result they dominate the vegetation of these areas. In areas which have escaped fires for some time other shrubs and trees are usually found. These forests have been interpreted by some as plantations of medicinal plants whilst others have interpreted them as natural climaxes. The available evidence, however, indicates that they are only seral communities maintained by the periodical fires (Holmes, 1951).

Potential sources of other drug plants

The total number of indigenous flowering plants in the flora of Ceylon is about 3,000, and of these 515 are used as drug plants. This means that about 17 per cent. of the local species are used. Most of the species used, however, are the non-endemics, i.e. those not confined to the Island. If the endemics, i.e. the species which are found only in Ceylon, are taken into consideration the proportion is very much smaller. We have in all about 860 endemic species. Of them only 36 are now used as drug plants. This represents a percentage of only about 4.2 as against 17 for the total indigenous flora. This difference becomes very clear if we consider the proportion of endemics and non-endemics used in medicine from families which have a high proportion of endemics.

<table>
<thead>
<tr>
<th>Family</th>
<th>Endemics</th>
<th>Non-endemics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No. of spp. in Ceylon</td>
<td>No. of spp. used in medicine</td>
</tr>
<tr>
<td>Lauraceae</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Rutaceae</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>Anacardiaceae</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Guttiferae</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Melastomaceae</td>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td>Sapotaceae</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Symplocaceae</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td>82</td>
<td>4</td>
</tr>
</tbody>
</table>

There are two possible explanations. One is that endemics may be poorer in alkaloids etc. when compared to the non-endemics. The other is that here in Ceylon we have been using the species which had been found to be of use in ancient times
in the neighbouring mainland with only very rare additions or substitutions from the local flora. Of these the first seems improbable and the second seems to be the more likely explanation.

To settle this question and at the same time to discover any hidden resources of drugs routine testing for alkaloids etc. should be undertaken at least with all species in the medicinally more important families and genera. Before such studies can be completed there is a danger that many endemics may disappear with the present large-scale clearing up of forests. In the past our vegetation has been subject to much disturbance by man and at present we have hardly any virgin forests except for small patches in the Adam's Peak Wilderness, Sinharaja forest, Knuckles ridge and a few other localities (Abeywickrama, 1956). Already some species, which were present a century or two ago, appear to have become extinct or are on the verge of extinction, and early steps should be taken to give protection to what we still have with us.

It is said that Jivaka (who subsequently became the Royal Physician during Buddha's time) on his completing a seven-year medical course at Taxila was given the following problem at his Final Examination: “Take this spade and seek around Takkasila, a yojana on every side, and whatever plant you see, which is not medicinal, bring it to me.” Jivaka examined all the plants in the specified area and returned to the teacher without any plants and replied that he had not been able to find anything which had no medicinal properties (Mookerji, 1947).

Let us therefore try to preserve our wild plants at least in a few selected areas till at some future date some other Jivaka finds what their uses may be.

REFERENCES


STUDIES IN
THE CULTIVATION OF
SOME RARE
INDIGENOUS
DRUG PLANTS

BY

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Several species of rare medicinal plants were grown in the Campus of the East
Regional Laboratories, Pakistan Council of Scientific and Industrial Research
and also in the private gardens of the author. This paper mentions only of those
species which have been brought under cultivation successfully and grown on a
large scale.

They are as follows:—

(1) Aristolochia indica Linn.
(2) Catharanthus roseus (Linn.) G. Don.
(3) Abrus precatorius Linn.
(4) Acorus calamus Linn.
(5) Caesalpinia bonducella Flan.

1. Aristolochia indica Linn

A climber which flowers in May and June, fruits in February and March.

Medicinal Uses: The root is bitter. It is regarded in indigenous medicine as an
astringent, febrifuge, tonic, and aphrodisiac. It is used in remittent fever and various
other diseases and regarded as an antidote to snake poison. In Malaya, leaf and
leaf juice are used against snake poisoning.

It is also regarded as efficacious against cholera and used in abortion and teething
in children. There is another species named A. bracteata Lamk which is mostly
used as anthelmintic and treatment for scabies.

The plants were located in the jungles, fruits bagged and seeds collected and
stored for germination later on. The seeds germinated in 2 weeks, and seedlings
transplanted in 6 weeks and trained on to bamboo platforms. The climbers flower
in September and fruit in February-May.
The seeds were collected from the cultivated plants in the same manner as described earlier. For commercial production of the drug, the seeds are sown in June in well prepared, 100 ft. long beds, seedlings thinned and trained on to bamboo platforms.

Roots were harvested on three occasions. Firstly, when the plants were about to flower, i.e., in September; secondly, when the fruits were fully mature, i.e., in November and thirdly, after the mature fruits were harvested in May.

The average weight of the roots for each plant was 1·5 oz. to 2·7 oz., in the first harvest; 1·9 to 2·8 oz., in the 2nd harvest; and 1·7 to 2·5 oz., in the third harvest.

The roots of only a few plants were harvested, but the rest of the plants were allowed to remain. The aerial parts of the plants withered and dried in June. With rains in June and July, shoots sprouted and grew quite vigorously. They were staked to supports and trained on to bamboo platforms as before.

The weight of the roots of 2 year old plants were determined as before and they recorded an increase in weight, viz.: 3 oz. to 5 oz. in the first harvest, 4 oz. to 6 oz. in the 2nd harvest and 5 to 7 oz. in the third harvest.

The result showed that the root yield increased more than 100 per cent. when the plants were allowed to grow for two years.

Per acre yield is calculated as 4,000 to 5,000 lbs. from two year old plants.

2. Catharanthus roseus (Linn.) G. Don (≡ Vinca rosea Linn.)

As the fruits are capsules and dehiscence shedding their seeds the immature pods were tied and harvested when fully mature. In this way about ½ oz. of seeds were collected from several plants in the first year. In the next season, in the beginning of monsoons seeds beds were prepared to raise seedlings in May, and seeds sown. The seed germinated within a fortnight and the germination was over 80 per cent. The seedlings were transplanted at the end of July in rows 2½ feet apart, the individual plants 1½ feet apart in the row. The plants were ready for harvesting in January and February.

It has been calculated that per acre yield was somewhere from 2,000 to 3,000 lbs.

3. Abrus precatorius Linn.

The plant is a slender perennial twiner with compound leaves bearing small leaflets and small reddish white flowers in small racemes. The fruits long and broad. The pods ripen in December and January and dehisc in February and March and are conspicuous by their red seeds with black spots.

It is regarded as a poisonous plant. The root and leaves are sweetish. The fruits are bitter, acrid, aphrodisiac, tonic, toxic, removes biliousness, useful in eye diseases; cures leucoderma, itching, skin disease. The root and leaves have the same properties as the fruit and in addition they cure fevers, head complaints, asthma, thirst, tuberculous glands, caries of the teeth according to Ayurveda. According to Yunani, the fruit is acrid with a bad taste, tonic to the brain and the body, aphrodisiac, harmful to old men.
The root is considered as eryectie. The watery extract is useful in relieving obstinate coughs. The root is employed as a substitute for liquorice.

The seeds taken internally by women as a contraceptive as they disturb the uterine functions. The plant was cultivated experimentally in 1961 and the procedure adopted was the same as for Aristolochia indica. Being deciduous with maturity of the fruits, the vines should be carefully harvested and stacked on a threshing floor and allowed to dry in the sun for a few days. Seeds are separated from the vines by beating with sticks, cleaned and stored in a cool, dry place.

The roots left behind in soil after harvesting the vine, shoot out again with the monsoon rains.

It has been calculated that from one acre, yield to be found 3,000 lb. to 5,000 lb. of roots.

4. Acorus calamus Linn.

Aromatic marsh herb with a stout, creeping, ginger-like sympodial rhizome. Leaves distichous 15-30 cm. long, 1·8-3·7 cm. broad.

*Medicinal Use*: Mostly the use is to cure indigestion. It is bitter aromatic, tonic, antispasmoic, anthelmintic. In certain ailments acorus has been pronounced as invaluable and more efficaceous than ipecacuanha, specially in vomiting. So in those ailments where use of ipecacuanha is necessary, the substitution of ipecacuanha by acorus have given better results. The cultivation of acorus requires marshy conditions. If the condition is not obtained naturally, then the condition may be created by irrigation.

A marshy area in the campus of Government Regional laboratories was brought under cultivation. Small shoots were planted 3 feet apart in rows.

The rhizome can be harvested after one year of growth, but for better and higher yield, it should be harvested after two to three years growth.

The yield has been calculated at 150 to 200 maunds per acre at one year growth.

5. Caesalpinia bonducella Flan.

According to modern nomenclature it is Caesalpinia crispa Linn. It is a straggling perennial climbing shrub with prickles on the stems, branches and even on fruits. It is commonly found on road side jungles, and the jungle growths along the railway lines and deciduous in spring.

The seeds, roots and leaves are used medicinally. The seeds are anthelmintic, leaves, roots and seeds are tonic and febrifuge.
Considerable amounts of chemical investigations have been done in the East Regional Laboratories and several important chemical constituents of the plant and seeds have been determined.

Some seeds were collected and sown along the wall of the campus at about 10" apart, two to three seeds in one hole prepared for the purpose. The seeds germinated with the rains in June and the plants flowered and fruited in 18 months.

It is calculated that about 20 lb. seeds may be obtained from a single plant. The seeds are hard and can be stored in bags.
POTENTIAL USE OF
PLANT TISSUE CULTURES
IN INVESTIGATIONS OF
BIOSYNTHESIS OF
PLANT CONSTITUENTS
OF MEDICINAL VALUE

BY

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LITERATURE concerning plant tissue culture has been well documented by Riker and Hildebrandt (1958) Gautheret (1959), Hildebrandt (1962) and White (1963) and in the research publications of a host of other workers. In the context of the present symposium, however, it would not be out of place to summarize its present status and future possibilities by way of introducing the subject.

During the past two decades, with refinements in technique and advancement in knowledge concerning nutritional and other requirements for a wide variety of excised organs and tissues, plant tissue culture can be said to have developed on a broader front. It is now possible to cultivate practically any part of the plant aseptically and under controlled conditions: thus root, stem, leaf, endosperm, embryo, pollen, etc., could be grown as de-differentiated tissue masses known as callus and maintained for long through periodic sub-cultures.

Three modern developments in culture methods, viz. (1) the initiation of suspension cultures in liquid media, (2) isolation of single cell clones and (3) the carboy culture system of mass cultivation of tissue chiefly through the efforts of such workers as Tulecke, Nickell, Steward, Staba and others have improved the prospect of utilising tissue cultures in studies concerned with morphogenetic and metabolic problems at the organ, cellular and sub-cellular levels.

The purpose of the present paper is to set a framework for the possible large-scale utilisation of plant tissue cultures of aromatic and medicinal plants, as a potential tool in investigations of biosynthesis of natural products in vitro.
Initiation and types of cultures

The methods of induction of callus growth of an explanted organ are now fairly well-defined and present no special problems. They have led to the establishment of tissue strains belonging to different categories. Tumor tissues such as those caused by the crown-gall bacterium differ from the auxin-induced callus ('normal' type) in their physiology and nutrition. They possess the capacity for continued rapid growth and unregulated proliferation without exogenous IAA or other auxins. Virus tumor tissues are derived from the neoplastic growth induced on the host plant by an insect-vectored virus and are all pathological. Genetic tumors arise spontaneously during certain species crosses as when Nicottiana glauca is crossed with N. langsdorffii, perhaps as a result of nuclear or cytoplasmic incompatibility. Belonging to the category of the 'normal' callus, are the habituated tissues—tissues that can grow without auxin but originating by prolonged cultivation in vitro in the presence of an auxin and represent self-proliferating mutant tissues.

Basic microbiological techniques have now been adopted for growing plant tissue as suspension cultures. (Tulecke & Nickell 1959, Wang & Staba 1963). It is a means of growing plant tissues on a large scale under controlled environmental factors, useful in studies of basic cell functions. High growth values have been obtained from liquid shake cultures in the case of rose, Lilium, holly and Ginkgo tissues for a 4-week growth period. Melchers and Englemann (1955) report the production of 3.7 gm. of tissue for a 20 mg. inoculum in 95 days for carrot tissue in liquid culture. With suitable modifications of the design for temperature control, aeration, composition of the medium and with the use of antibiotics for bacterial prophylaxis, higher yields can be expected.

Recent investigations have shown that normal organs and callus tissues can biosynthesize natural products such as alkaloids. For instance root callus of Atropa have yielded atropine in economically feasible quantities (West & Mika, 1957) and crown-gall tumors of Datura contained almost 500 per cent. more hyoscine than normal roots and up to 300 per cent. more tropane alkaloid than the entire plant (Klein, 1960). Nicotine and anabasine have been isolated from root organ cultures of tobacco (Dawson, 1942; Solt et al., 1960). Investigations to determine whether or not tissue cultures of Digitalis can biosynthesize cardiac glycosides and of the various mints, useful aromatic constituents are under way (Staba, 1962 and Staba and Lamba, 1963). Similar studies have been reported with the leaf of Agave toumeyana for sapogenins (Weinstein et al., 1959) and stem tissue cultures of the Cactus Trichocereus spachianus for the alkaloid, candicine (Steinhart 1962).

Investigations at the AEET

In vitro propagation of callus tissues of excised plant parts of some aromatic and medicinal plants has been taken up at the Atomic Energy Establishment Trombay, Bombay, with the two-fold object of (1) investigating problems concerned with morphogenesis and (2) exploring the feasibility of utilising tissue cultures in the study of biosynthesis of plant constituents.
Two genera, viz., *Mentha* and *Ocimum* of the family Labiatae, known to contain aromatic constituents, have been chosen as source materials. *Ocimum kilimandscharicum* Guerke is known to produce camphor *in vivo*. The plants were grown from seeds* and living stock materials maintained at the Field Experiment Station, Trombay for use as needed.

Callus cultures were prepared from stem segments containing a node and planted on a modified White's medium—coconut milk (10–15 per cent. v/v) and incorporating 2, 4-dichlorophenoxyacetic acid (2, 4-D) in a wide range of concentrations. Rapid callus growth was obtained on a basal medium to which coconut milk (10 per cent. v/v) and 2, 4-D (2 ppm) were added, and kept in diffuse light at a temperature of 26° ± 1°C.

Influence of gibberellic acid, kinetin, adenine and indolyl-acetic acid, separately and in combinations on the growth and proliferative capacity of the tissue explants was investigated. It has not been possible to replace coconut milk in the medium. Preliminary studies have indicated the possibilities of obtaining higher yields in shake cultures using cell suspensions of the extremely friable callus tissue.

Stems and leaves of *Ocimum* were analysed for camphor content and found to contain about 100 mg. per 1 gm. of dry weight. Since the initial tests with the callus tissue of the same grown on solid media could not confirm the presence of camphor, the medium on which the callus was grown, was fortified with dextrose and sucrose at 2 and 5 per cent. (w/v) levels and substances like sodium acetate (1,000 ppm) and and mevalonic acid lactone (3,000 ppm), in order to obviate possible precursor deficiencies. Analysis of the solvent-extracted distillate of the callus tissue by chromatographic methods is in progress. Similar studies involving the use of cell suspensions using the carboy system of large-scale cultivation are also contemplated.

Tissue cultures of *M. arvensis*, *Rauwolfia canescens*, *R. serpentina*, *Peganum harmala* etc. have been started and show promise of large-scale cultivation. Recently successful growth *in vitro* of root cultures of *Rauwolfia serpentina* Benth. has been reported in the laboratory of the National Botanical Gardens, Lucknow, India (Mitra and Kaul, 1964) for examining the presence of reserpine.

It must be noted, however, that capacity to increased proliferation *in vitro*, does not mean greater ability of the tissue system to synthesize natural products. To what extent manipulation of the chemical and physical environment in which the tissue is grown, can augment or regulate the ability of the tissue to biosynthesize useful compounds, remains to be explored and remains a challenging problem. Since the tissues of higher plants *in vitro* are not similar to the parent tissue from

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* Obtained through the courtesy of the Regional Research Laboratory, Jammu.
which they are derived (Tulecke et al., 1962), the problem is one of channelling the
in vitro system in its biosynthetic capacity to perform specific functions in a sophisti-
cated predictable manner. That this is feasible is indicated in experiments with
guayule stem tissue cultures which have shown a 3-fold increase of rubber content
grown on agar media in the presence of acetone, sodium acetate, B-methyl crotonic
acid etc. (Arreguin & Bonner, 1950).

Recently it has been definitely shown that nicotine could be isolated from cell
cultures of Nicotiana tabacum var. virginica, both at the Pharmaceutical Division
of Akers Research Laboratory of the Imperial Chemical Industries Ltd., and at the
Department of Biochemistry, University of Leeds (Speake et al., 1964). For an
initial tissue inoculum representing 30 mg. dry weight in suspension in 100 ml. of the
media, the final tissue dry weight was 600 mg. per culture for 4-5 week growth
period. The tissue as well as the supernatant liquid, solid callus and sometimes the
agar substrate all contained nicotine. Highest nicotine levels were obtained by
them to the extent of 7 μg/mg. dry weight of tissue.

Root, stem and leaf tissues have shown ability to synthesize the alkaloid.

Information on the specific effects produced in response to irradiation, photoperiod
and growth substances might prove useful in later studies and are contemplated at
the AEET, for which facilities are available.

Utilisation of mixed cell cultures of unrelated plants in the production of both
simple and complex compounds is also of considerable import and is on the
programme. It is speculated that in vitro systems of plant tissues might prove an
important source for the isolation of active principles of primary origin and of
intermediates, knowledge of which may be useful in the understanding of the biogen-
essis of these constituents. Staba (1963) speculates that pigment containing plant
suspension cultures may produce isoprene structures useful as precursor material,
vitamins, flavinoids or chlorophyll.

With advances in our knowledge of the metabolic control of growth and differenti-
tation, "There is", as Street (1963) has remarked, "a strong possibility that
the pharmaceutical and allied industries will be able to utilize on a large scale
the biosynthetic properties of such plant cultures". It is not mere babblings of
fancy to hope that in the next few years, plant tissue culture would find practical
applications in the realm of industry, with a better control on in vitro systems
genetically and physiologically.
REFERENCES


PRODUCTION OF MEDICINAL PLANTS IN INDIA

BY

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Central Indian Medicinal Plants Organisation, Council of Scientific and Industrial Research, India

INDIA imports crude drugs of vegetable origin and their preparations to the value of about Rs. 4,000,000 every year, and to save this foreign-exchange, attention is being given to their large scale production. India possesses climatic conditions varying from the torrid to the frigid zone, and it embraces vast tracts of tropical plains, temperate hills and valleys, irrigated soil, moist and dry climate and cheap labour. For the above reasons, acclimatisation is considered possible for many exotic plants and successful efforts have also been made in this direction.

The Government of India, with the help of State Governments, had been encouraging research on medicinal plants for a long time, so that their commercial production may be taken up. The Indian Council of Agricultural Research, under the Ministry of Food & Agriculture, Government of India, had been giving grants to different State Governments, private individuals and scientific institutions for research on medicinal plants, and the results obtained so far, have been very encouraging. The lines of investigation taken up by these researchers are enumerated below:

(1) Determination of sowing time by germination trials.
(2) Comparison of direct sowing and vegetative propagation.
(3) Effect of climatic factors on active principles.
(4) Soil conditions, which give maximum yield and active principle contents.
(5) Effect of hormones, fertilizers and manures on growth and active principles.
(6) Determination of best period of harvesting.
(7) Cytogenetical studies for improvement of the plants.
(8) Spacing experiments to determine the best yield.
With the establishment of the Central Indian Medicinal Plants Organisation, under the Council of Scientific & Industrial Research, in 1959, activities in the field of large scale production of medicinal plants have been taken up. At present, the following farms, under this Organisation, are doing this work:—

(i) Bangalore .. 74 acres
(ii) Haldwani .. 300 acres
(iii) Jammu and Kashmir .. 4,500 acres
(iv) Assam .. 1,000 acres
(v) Coorg .. 250 acres
(vi) Kerala .. 300 acres

The cultivation of medicinal plants is somewhat different from that of agricultural crops. It is often found that a plant growing luxuriously does not possess the required percentage of active principles, and hence, does not have any commercial value. The following factors need active consideration for achieving success in the production of these plants:—

(1) Edaphic factors .. i.e., soil conditions which affect the active principle content.

(2) Climatic factors .. The same plants growing under different altitudes and consequently subjected to varied rainfall, temperature, light, humidity, wind velocity, etc., show great variation in active principle content.

(3) Methods of propagation .. Different methods of propagation as raising from seeds, cuttings, etc., as well as spacing in the field, brings also variation in yield and active principles.

(4) Collection and harvesting .. There are no hard and fast rules regarding collection and harvesting, but the following general principles are to be usually observed:—

(i) Roots, rhizomes and barks are collected in late autumn or early spring when the vegetative growth has ceased.

(ii) Leaves and flowering tops are collected about the time of development of flowers and before the maturing of fruit and seed, because the photosynthetic activities are maximum at this time and the active principle content is also high.

(iii) Flowers are collected prior to or just about the time of pollination.

(iv) Fruits are collected when fully grown, but unripe.
(v) Seeds are collected when fully matured, and if possible, before the fruits have opened.

The mode of harvesting varies with each drug according to its pharmaceutical requirement. Seedlike fruits, as coriander, fennel, anethum, etc., are harvested a little before they are fully ripe for retaining a fresh and bright appearance.

(5) Drying

The object of drying is removal of sufficient moisture, so that it may develop keeping qualities. It also prevents fungal attacks and controls certain chemical and enzymatic changes, which are brought about by excess of moisture. Under natural conditions drugs may be dried under sun or shade, according to the nature of active principle content, but more success is achieved in commercial driers, where the temperature and flow of the air are regulated. Certain drugs like digitalis require a specific temperature for drying.

(6) Garbling

The final stage in preparation of a drug is garbling, which means removal of extraneous matter as well as other unwanted parts of the plants, dirt and other adulterants.

(7) Packing

Different kinds of packings are to be followed in case of different drugs for protection against insects, fungus and moisture, so as to prevent deterioration.

(8) Storage and Preservation

Specifications for storage and preservation are also to be specified for certain plants. In case of digitalis and ergot, which deteriorate in presence of moisture, the insertion of a suitable dehydrating substance in the container is recommended, care being taken that it does not come in contact with the drug and the container is kept air-tight. In general, the ideal temperature for preservation of drugs is refrigeration conditions or low temperature.
Researches on the foregoing points have been or are being carried out for finding out the requirements in respect of each medicinal plant, whose cultivation is undertaken. Important findings in the field of researches carried on a number of medicinal plants in India are listed below.

1. **Acorus calamus** Linn. (Fam. Araceae)

   It is a semi-aquatic annual with much branched rhizome, which is emetic, nauseant, antispasmodic and carminative.

   The plant has been grown successfully at Rongo (Darjeeling District) and Cinchona Plantations (Annamalais). Two strains of this plant, viz.: Darjeeling (Merik) and Kashmir, were tried. Both the varieties thrived well under marshy conditions at lower altitudes. The plants are propagated from rhizomes. The total volatile oil content in the dried sample was found to be 6·5 to 9·0 per cent. The rhizomes are more or less immune to attack of insects due to the pungent nature of oil.

   There is a good demand for the rhizomes, which sells at about Re. 1 per kg. for fresh rhizomes.

2. **Aloes** (Fam. Liliaceae)

   It is the solid residue obtained by evaporating the liquid, which drains from the leaves cut from the various species of aloes, viz.: *Aloe ferox* Mill., *A. vera* Willd. and *A. perriyi* Baker. It is largely used as a cathartic and in much demand. It has been grown successfully in Madras, Mysore and throughout the table land of Central India, and now found naturally in dry and poor soil. Large quantities of aloes are imported into India and the cultivation of this plant on commercial scale needs active consideration.

3. **Ammi majus** L., *A. visnaga* L. (Fam. Umbelliferae)

   These plants are used in the treatment of leucoderma in Egypt as well as in pharmaceutical preparations for its active content khellin. It has recently come into prominence for treatment in angina pectoris and bronchial asthma.

   The plant has been acclimatised in India and grown at Rongo. 50 per cent. of imported seeds have germinated in about 3 week's time. It flowers in about 4½ to 5 months. Flowering in the first year was scarce, but plants raised in the 2nd year from seeds obtained from acclimatised plants flowered profusely. The plant has also been grown successfully as an ornamental plant in gardens of Bengal, Madras and Mysore.

   At Chakrata, Dehra Dun, experiments have shown that direct planting of the seeds in the field gives better results than when seedlings are transplanted. It was also observed that October sowing is better than that of November, and that close spacings of one foot × one foot or 1½ ft. × 1 ft. are better than wider spacing 2 ft. × 2 ft. It can be raised under forest conditions and is not damaged by cattle, insects, birds or other pests.
4. **Anethum graveolens**. (Fam. Umbelliferae) **Peucedanum graveolens** Benth. (Fam. Umbelliferae)

It is an annual herb cultivated in England and Germany. The plant has been grown successfully in Jammu (Jammu and Kashmir State) and experimentally at Lucknow and other places of U. P. as a winter crop.

It has been found that a pound of seed is required for planting 1 acre. The most favourable soil is a well prepared loam, but the plants grow well in any garden soil. It is best to collect the seeds when they are not fully ripe but mature as the seeds fall to the garden if allowed to ripen fully. About 500 lbs. of seeds are obtained from 1 acre.

In the Indian Agricultural Research Institute, Delhi, it was observed that the day to flower varied from 60-213, yield of seeds per plant varied from 0.2 to 70 gm and the essential oil content could be improved by selection to 5-4 per cent., normal yield being 1 to 4 per cent.

5. **Anacyclus pyrethrum** D. C. (Fam. Compositae)

The seeds of this plant are considered stimulant sialagogue and used in rheumatism. It contains essential oil, pellitorine or pyrethin. It is indigenous to North Africa and has been successfully introduced in India. It is being cultivated in Gujrat on a small scale.

6. **Anthemis nobilis** Linn. (Fam. Compositae)

This plant is a native of Europe but has been acclimatised in India and is grown in the Punjab. The flowers are carminative and also used in dysmenorrhoea and hysteria. An infusion of the same is also used as an anthelmintic.

7. **Artemisia maritima** L., **A. brevifolia** Wall. (Fam. Compositae)

The plants yield santonin and have been cultivated successfully in Dehra Dun. At Kathian, about 28 miles from Chakrata, having rainfall of 50 inches **A. brevifolia** has been raised on both forest and agricultural land. The plants were cultivated by transplanting seedlings and they continue to grow for 7 years or more. It was found that samples collected from plants during summer have more santonin content than those collected in autumn at the time of flowering and fruiting.

8. **Atropa belladonna** Linn., **A. acuminata** Linn., (Fam. Solanaceae)

These plants have been tried in several places in India. At Chakrata, Dehra Dun the following results were noted:

(i) Alkaloid content in leaves is highest at the primordial bud stage and decreases as the development of flower progresses.

(ii) Leaves when dried in shade gave better yield than those dried in the sun.

(iii) The roots of the plant, dug in December, when the plants are dormant, have more alkaloid content than those dug in March when the plants have resumed growth.
At Rongo (Darjeeling dt.) the following points were observed:—

(i) Seeds germinated within 15–18 days, and 50 per cent. germination was effected.
(ii) Manuring with leaf-mould and cow-dung (3 : 1), at the rate of 2 lb. per
     plant with a top dressing of mustard cakes of 2 oz. per plant gave a good
     yield.

In Kashmir the plants of *A. belladonna* have been grown at higher altitudes, whereas *A. acuminata* has been grown as a winter crop in Jammu province at an altitude of 1,000 ft. The plants have been raised from seeds as well as root and shoot cuttings. The plant required a good amount of water but water logging is considered injurious. The yield has been reported to be 500 lb. of herb (dry weight) per acre. The alkaloid content increases the second year onwards. A natural hybrid of *A. belladonna* *A. acuminata* has been developed where the alkaloid content is intermediate between the two.

9. *Carum carvi* L. (Fam. Umbelliferae)

   It is an European plant of the Umbelliferae family but has been successfully grown
   in India in many places.

   Preliminary cultural experiments have shown that it can grow. —ll at Chakrata,
   Dehra Dun. The fruits gave a yield of 4·85 per cent. of oil which is above I.P.
   standard. Seeds were sown in April, seedlings transplanted in June-July and they
   flowered in April having been buried in the snow during the winter months. About
   1½ lb. of dried fruits were collected in June.

10. *Cassia angustifolia* Vahl (Tinnevelley senna) (Fam Leguminosae)

    It has been cultivated with success in Tinnevelly and Madurai districts in S. India,
    at Poona and in Udampur district in Kashmir. The plant thrives well in places
    having temperature 50–95°F and annual rainfall 35–40 inches.

    Large quantities of this plant were exported from South India. The leaves and
    pods of plants cultivated in different parts of India came up to B. P. Standard.

11. *Cephaelis ipecacuanha* (Brot.) A. Rich (Rubiaceae)

    It is a native of Brazil but it has been introduced in India with great success in
    Mungpoo (Darjeeling District) and Nongpoh (Assam). The dried roots of the plant
    are now sold at Rs. 51 per lb.

    The plants grow well in areas where the climate is very humid, the annual rainfall
    about 90–100 inches and a minimum temperature of 50°F in winter and maximum
    of 100°F in summer. The soil should be sandy loam, rich in humus, potash, magnesia
    and lime. About 600 lb. of dry root are obtained from an area of 1 acre from a good
    plantation. The plants are cultivated throughout in sheds in Kamras (covered plots)
    about 12 ft. × 3 ft. or 6 ft. × 6 ft.
The germination of Ipecac seeds is somewhat difficult. It was found that lime water treatment for 45 hours, gave the best results. Treatment with hydrogen peroxide for 96 hours was next best and treatment with hydrogen peroxide for 168 hours and 10 per cent. sulphuric acid was third in order. Magnesium sulphate with lime water also gave good results. It was found that greater the periodicity of treatment and lower the concentration, the higher is the percentage of germination.

The plants grow best between 1,000 and 2,000 ft. in a tropical rain forest while those grown at 3,000 ft. show slow growth and those at 5,000 become stunted.

Results of researches on this plant have been published by Dr. Biswas in brochure "Ipecac cultivation in India"—I.C.A.R. publication No. 1, 1958, New Delhi.

12. Chenopodium ambrosiodes Linn. (Fam. Chenopodiaceae)

Russian strain of this plant has been acclimatised at Rongo. Ascaridole content has been found higher than standard.

The plants grown in Assam did not possess the requisite ascaridole content.

Oil of chenopodium is well known for its anthelmintic activity but in recent years due to discovery of several other anthelmintic drugs, its demand has fallen and the production is not being done on large scale.

13. Chrysanthemum cinerariofolium Vis. (Compositae)—Pyrethrum

The flowers of this plant are the best vegetable insecticide known to-day. It is grown in large scale in many parts of the world and Kenya occupies the top position to-day. The plants have been grown successfully in Mayurbhanj (Orissa), Nilgiris and Assam, but on commercial scale it is now grown in Kashmir at altitudes between 5,000–8,000 ft.

It has been tried out in Rongo (Darjeeling district) and Chakrata (Dehra Dun) but without success.

The plant does not require very rich soil and can thrive well on lands which are not suitable for other crops. Drying of flowers could be done under sun for 3 days and then kept under shade. The pyrethrum content was about 1·02 per cent.

14. Colchicum luteum Baker (Fam. Liliaceae)

This plant is considered a good substitute for C. autumnale which is official in B.P. and is a good remedy for gout. The plant grows in forests of Kashmir and Chamba. The corms are collected in the spring when the plants flower. After the snow melts, this is the first plant in the meadow to flower, but the period of flowering is short. The corms after collection need curing by fumigation for preservation against attacks of insects and fungus.

No large scale cultivation has been undertaken but it can grow well in Himachal Pradesh and Kashmir.
15. *Datura fastuosa* Linn., *D. stramonium* Linn. (Fam. Solanaceae)

These plants have been grown successfully in different parts of India and can grow on soils which are not suited for other crops. They grow well in Bengal, Madras, Mysore and other places in the plains. It is reported that the plants become richer in alkaloids when grown at higher altitudes.

In Rongo, plants grown at higher altitudes could not stand frost whereas in lower elevations three crops were obtained in between spring and summer, before the rains and in autumn. Experiments showed that the plant *D. stramonium* can be best grown as an annual, to be sown in autumn and harvested in late spring.

Plants of *D. innoxia* have been grown with success on a large scale in Bangalore. Its seeds contain scopolamine.

16. *Derris elliptica* (Roxb.) Benth., & *D. malaccensis* Prain (Leguminosae)

The plants have been introduced in Dehra Dun and are multiplied from stem cuttings. It is well known insecticide containing retenone and its large-scale production is under consideration.

17. *Digitalis purpurea* Linn., *D. lanata* Linn., (Scrophulariaceae)

The plants have been cultivated successfully in different parts of India. Digitalis leaves are mainly used as heart tonics, *D. purpurea* has been produced successfully in Kashmir, Himachal Pradesh, Nilgiris, Rongo, Chakrata and other places. The plant grows well at altitudes of 5,000 ft. or so but it has also been grown well at lower altitudes.

At Chakrata it was observed that plants grown from seeds supplied by Kew Gardens, England, showed requisite potencies, but those grown from Kashmir seeds did not have requisite content. The plants are raised from seeds, and then transplanted after about a month’s time. At Rongo the plants showed better growth of foliage under partial shade. The plant has acclimatized well at Rongo and in Nilgiris. The glycoside content from Nilgiri plants is fairly higher than those of other places.

*D. lanata* has also been grown successfully at Rongo, Nilgiris and Kashmir. At Chakrata, it also grew well and its potency was satisfactory. The seeds germinate within 9 to 10 days but percentage of germination was not so satisfactory. It has been observed that many seeds are attacked by fungus and this results in the death of the plants in very early stage.

18. *Dioscorea deltoides* Wall. (Fam. Dioscoreaceae)

The plant has lately come into prominence due to its diosgenin content which is considered an important source for preparation of cortisone. The plant grows under natural conditions in Himachal Pradesh, Kulu (Punjab) and Kashmir. It is a climber and grows in forests at altitudes of 5,000–8,000 ft.

*D. przeri* and *D. glauca*, which also contain diosgenin have been grown successfully at Rongo.

The plants have been multiplied by cuttings at Chakrata.
19. *Duboisia myoporoides* R. Br. (Fam. Solanaceae)

These Australian plants which are sources of important mydriatic alkaloids have been experimentally cultivated in Dehra Dun. It is still in the experimental stage.

20. *Ephedra gerardiana* Wall. (Fam. Gnetaceae)

Plants have been successfully grown from seeds at Chakrata. Alkaloid contents of female plants were found much higher than male plants. Seeds were germinated in April and transplanting done in July. Transplanting of splits also gave good result.

It grows under natural conditions in North West and Sikkim Himalayas but its production on a large scale has not been undertaken as yet.

21. *Claviceps purpurea*—Ergot

The sclerotia of the fungus *Claviceps purpurea*, growing in the ovary of *Secale cereale* (Rye) is known as ergot.

It has been produced successfully in W. Bengal, Madras and Jammu. It was also produced in Nilgiris and Assam.

Rongo, at present, is the chief producer of ergot. It is highly valued in medicine as an oxytocic or uterine tonic.

It was found in Rongo that (i) the optimum sowing date is mid-October for best yield, (ii) that lower elevation (1,600–2,700 ft.) is best suitable for ergot yield and (iii) equal proportion of water and rye seed in the preparation of sterilized rye, gave the best growth and sporulation of the fungus.

About 400 lbs. of ergot annually has been produced at Rongo.

22. *Gentiana lutea* Linn. (Fam. Gentianaceae)

This has been grown successfully in Madras Govt. Cinchona Plantation in Nilgiris. Gentian is used as a bitter. Its cultivation has not been extended due to its limited demand in the market.

23. *Glycyrrhiza glabra* Linn. (Licorice). (Fam. Leguminosae)

Preparations of licorice go into the manufacture of cough medicines and cigarettes, as it has the property of concealing any acid or nauseating taste. The plant can grow well in river valleys in a warm climate and requires a deep and moderately rich loamy soil. Its large-scale cultivation has been undertaken by the Forest Department, Punjab, with collaboration from the Council of Scientific and Industrial Research.

24. *Hyoscyamus niger* Linn. H. muticus Linn. (Fam. Solanaceae)

The plants have been grown successfully in many places in India. It grows in the Himalayas at altitudes of 5,000 ft. or above but has also been grown in the plains.
It is produced commercially in Kashmir, Himachal Pradesh, Saharanpur, Punjab. *H. muticus* contain a higher percentage of alkaloids and it is being experimentally cultivated in Kashmir, Saharanpur, Bangalore and other places.

At Rongo, the seeds germinated within 9–10 days. It was found that most seedlings could not withstand transplanting probably due to injury to tap roots, and it is advisable to allow them to grow in the seed bed to attain a fair height before transplanting. *H. muticus* gave out better vegetative growth and proved to be more resistant than *H. niger*.

At Chakrata, it was found that the plant does better when sown direct than as a transplanted crop. Seeds are collected in August, sown in December, germinated in March and the plants grew well during summer when irrigation was done once a week. The plants of *H. muticus* did not flourish well at this place.

25. *Humulus lupulus* Lir. n. (Hops). (Fam. Moraceae)

This is required in the beer industry. It is a climber which has been successfully grown in Himachal Pradesh. It is still in experimental stage.


Production of *Mentha arvensis* has been undertaken on a commercial scale in Jammu. About 600 acres are at present under this plant and further expansion programme to 1,500 acres is in progress. It grows well in the plains and requires a good amount of water. It contains about 70–80 per cent of menthol and an acre of crop is reported to give a return of Rs. 1,500–2,000 annually. Other production programmes are in progress in U. P. and South India.

*M. piperita* is also being grown in many places but on a smaller scale as compared to *M. arvensis*. It has been experimented with success at Chakrata and Rongo.

27. *Ocimum kilimandscharicum* Guerke (Fam. Labiatae)

This plant has been considered a good source of camphor and can be grown on different types of land. It does not need any special attention and grows as a weed in many waste places.

Its production was taken up by many forest departments but the yield has been found to be uneconomic as it cannot compare with the market rate of synthetic camphor.


It is a tropical plant and its roots are considered to be sedative and useful in hypertension. Due to recent discovery of reserpine in this plant which is a very good tranquillizer, the plant has come into great prominence.

It grew well in different parts of India under natural conditions but ruthless exploitation has destroyed this natural source. It is now being cultivated under Directorate of Agriculture, Kerala, where a plantation 600 acres has been raised.
It is also being cultivated by the Central Indian Medicinal Plants Organisation in its different centres. The following observations have been noted.

(1) The plant can be propagated from seeds, root cuttings, stem cuttings and even from leaf cuttings.

(2) The germination of seeds is low varying usually from 10 to 50 per cent. When placed in water some seeds float whereas others sink to the bottom. The seeds which float do not usually germinate. From root and stem cuttings 75 per cent propagation is assured.

(3) The plant grows well in soils which are not too sandy or clayey. Application of farmyard manure gives good result.

(4) Plants raised from seeds show better root development than those propagated from cuttings.

(5) Best season for sowing the seeds is May.

(6) In plants grown at Rongo, total alkaloids were found to be from 1.424 to 2.078 per cent with reserpine content 0.61 to 0.106.

*R. canescens* is a much harder and vigorous plant than *R. serpentina*. It has been grown with success at Rongo, West Bengal plains, U. P. and many others parts of India. The cultivation procedure is the same as that of *R. serpentina*. Total alkaloids of the roots were found to be 1.550 per cent and reserpine group of alkaloids 0.1527 per cent against the U. S. P. limit of 0.07 per cent.

The plants have also been experimented on at Chakrata and Madras Government Cinchona Plantation.

29. *Plantago ovata* Forsk. (Fam. Plantaginaceae)

This plant is found growing in the plains of Punjab and is cultivated in U. P., Bengal, Mysore and South India. The seeds of this plant are boat shaped and contain mucilage for which they are used in dysentery, diarrhoea and other irritative conditions of the gastro-intestinal tract.

Experiments have shown that it prefers a loamy soil, normal irrigation, and application of farmyard manure for production of a good crop.

30. *Podophyllum hexandrum* Royle

*Podophyllum emodi* Wall. (Fam. Berberidaceae)

This plant has recently come into prominence due to the discovery of an active principle which is found to be effective in some types of cancer. Some foreign firms have showed great interest in the cultivation of this plant and with collaboration of Central and State Governments, Punjab Himachal Pradesh and some other places. The plant is found under natural conditions in high altitudes 6,000-9,000 ft. and they remain dormant under snow during the winter months. When the snow melts, the plants produce vegetative growth and come to flowering and fruiting in about 4 to 5 months' time. The pods are red and attract the attention of birds and these are also eaten by local people.
Vegetative propagation by planting rhizome cuttings was resorted to at Chakrata and this was found satisfactory. It was also observed that the drug could be successfully exploited commercially by planting the youngest top parts of the rhizome bearing leaf buds and this gave better results both in the percentage of sprouting and in the growth of the sprouts.

Seeds also collected in June-July were immediately sown with the fruit pulp. These remained dormant for a period of 9 months but with approach of spring the snow melted away, and the seeds started germinating in April. The germination was about 45 per cent and was completed within 3 weeks.

Chemical analysis showed that resin content was about 8.0 per cent.


The bark of this plant is known as *Cascara sagrada* and it is in great demand as a purgative. The plant has been successfully introduced at Dehra Dun and 20 of these are surviving. Nine more plants have been raised by vegetative propagation. Flowering and fruiting has not been good and Dehra Dun may not be an ideal place for this plant.

It is a small tree 20 to 30 ft. high. The trees are reported to grow better in clay loam soil than in either sand or clay. Propagation from seed is easy, but the seeds should be sown fresh as soon as they are gathered. If the trees are pruned properly, a crop of bark may be harvested each year without killing the whole tree. Ageing is said to improve the quality of bark.

It is proposed to grow the plants along the bank of streams which are clayey loam soils in U. P., Punjab and other places.

32. **Saussurea lappa** (Kunth). (Fam. Compositae).

The plant has been cultivated commercially in Lahaul and Spitti (Punjab). The demand of the roots of this plant which is mainly consumed in China has fallen recently. The price of roots which were about Rs. 225 for 40 kg. has now fallen to Rs. 100 or so. Unless a good market is assured its commercial production has to be curtailed.

Originally the plants were raised in Shansha Government farm on 2 acres. Later on, experiments were started in Keylong, another farm at a distance of 15 miles from the original place. The following points were observed.

1. Plants raised from Lahaul seeds (i.e. acclimatised plants) gave better results than those raised from Kashmiri seeds.
2. The plants put in rows 4 inches apart gave a good yield.
3. There was no appreciable difference in time and broadcast sowing.
4. About 20 lb. of seeds are required per acre.
5. Seeds sown in November yielded better results than those shown in April.

The plant has also been successfully grown in Kashmir on a small scale.
33. Strophanthus kombe Oliver. (Fam. Apocynaceae).

The plant yields an important cardiac tonic drug and India imports large quantities of its preparations every year.

It has been experimentally grown in India Botanic Gardens, Calcutta on loamy soil, but no effort has been made for its large-scale cultivation.

The plant is proposed to be grown in the drier regions of South India.

34. Urginea maritima (Linn.) Baker (Fam. Liliaceae)

The bulbs of this species, used as expectorant and in some heart diseases, were procured from Italy and experimented at Dedra Dun.

A few of the bulbs were sent to Nilgiris for experimental trial. The plants did not flower in 15 months time, whereas another strain from Anamallais (acclimatised plants) flowered within 8 months.

35. Valeriana officinalis Linn., V. wallichii D.C. (Fam. Valerianaceae)

The plants have been grown successfully in the temperate Himalayas by the Forest Departments of different States. V. wallichii grows in those areas under natural conditions. It contains essential oil from 0.5 to 0.9 per cent and the drug has been found to be useful in the treatment of shell shock.

Its large-scale production is under consideration.
SAPONINS
AND SAPOGENINS OF
Panax ginseng AND
SOME RELATED PLANTS

BY

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GINSENG was employed for many centuries in China, Korea and Japan as an important
drug. The first scientific study on ginseng was reported by Garrique (1854) who
isolated a saponin named panaquilon from American ginseng (Panax quinquefolium).
Some early Japanese workers, Asahina (1906), Kono (1915) and Kotake (1930)
and their co-workers also claimed to have isolated the saponin prosapogenin, or
the sapogenin from ginseng, which, however, was not obtained in a pure state.

Recently several papers concerning this drug have appeared both in the chemical
and pharmacological fields. Horhammer, Wagner and Lay (1961) and Wagner-
Jouregg et al. (1962), (1963); have reported on sapogenins of ginseng while Eliakov,
Strierna, Charlin and Kochetkov (1962) isolated the saponins named panaxosides
A and B. Lin (1961) also reported the isolation of oleanolic acid and a new sapogenin
named ginsengenin. However, none of these workers have reached precise con-
clusions as to the chemical constituents of ginseng.

The pharmacological actions of ginseng were also studied, or are being studied

For past several years, Shibata and coworkers, (1962 a, b; 1963 a, b, c; 1964)
have been engaged in the study of the chemical structures of Ginseng saponins
and their sapogenins. They employed ginseng which is obtainable in the Japanese
drug market as the material of investigation.
The drug was extracted with methanol and the methanolic extracts were treated as follows:

The crude neutral saponin of ginseng was fractionated into acetone-soluble and less soluble portions.

The ginseng saponin which is less-soluble in acetone was hydrolysed with HCl to obtain panaxadiol. \( C_{30}H_{50}O_8 \) m.p. 250, \((\alpha)D^{18.5} + 1.0\). The structure of panaxadiol has been suggested to be a tetracyclic triterpene by the following reactions converting it into isotirucalleny1 acetate.

The trimethyltetrahydropyran ring of panaxadiol was proved by the mass-spectrum which gave a base peak at \( M/e \ 127 \).
It has been found that panaxiadol is an artifact which was formed during the process of acid-hydrolysis of ginseng saponin. A ring closure of the side chain occurred to form a trimethyltetrahydropryran ring by the action of acid.

A genuine sapogenin of ginseng has been named protopanaxadiol.

Hydrogenated ginseng saponin afforded on hydrolysis dihydropotopanaxadiol. Prosapogenin was obtained from ginseng saponin by treatment with 0.7 per cent sulphuric acid in aqueous methanol and on refluxing this prosapogenin with 7 per cent hydrochloric acid in aqueous ethanol panaxadiol was afforded whereas on treating
prosapogenin with concentrated hydrochloric acid at room temperature, an unstable chlorine containing compound was obtained, this was converted into protopanaxadiol by the action of diethylaniline in xylene.

Protopanaxadiol was converted into panaxadiol by the ring closure of the side chain, when it was refluxed with dilute hydrochloric acid for 1.5 hrs. The position and the configuration of a hydroxyl of panaxadiol was proved by the following conversion. A hydroxyl of panaxadiol is strongly hydrogen bonded as it shows a concentration independent OH band in the I.R. spectrum. The hydroxyl is converted into ketone on oxidation and then on reduction with lithium aluminium hydride it affords the epi-hydroxyl. Such a stereochemical conversion can only be explained by the location of the hydroxyl at 12 position of panaxadiol. The 12 keto compound yields panaxadiol and 12 epi-panaxadiol on treatment with sodium and propanol.

This would also suggest that the ring conversion does not take place at the C/D ring juncture during the process of such reactions.

The stereochemistry of protopanaxadiol has been established by the correlation of dihydroprotopanaxadiol with betulafoliane-triol. The configuration of betulafolienetriol was established under the correlation with dammarane-diol II, whose stereochemistry was fully elucidated by Ourisson and his co-workers.

Thus, it has been concluded that dihydroprotopanaxadiol differs from betulafolianetriol only in the configurations at 3-OH and 20-OH.
From the acetone-soluble saponin of ginseng we obtained on hydrolysis a homologue of panaxadiol which possesses one more hydroxyl in its molecule. We proposed that it be named as panaxatriol (Shibata et al., 1964).

As the properties of panaxatriol is similar to panaxadiol and in regard to biogenetical view point, it seemed quite probable to assume that panaxatriol would possess the same stereo-chemical carbon skeleton of panaxadiol.
Panaxatriol: M.p. 238 - 238.5° (from C6H6)
(α)D +14.2° (CHCl3)
Calcd. for C30H38O4: C 75.58  H 11.00
Found: 75.84  10.94
75.65  10.84

Diacetate: M.p. 268 - 269° (from MeOH)
(α)D +24.9° (CHCl3)
Calcd. for C34H46O6: C 72.82  H 10.06
Found: 73.09  10.04

The mass-spectrum of panaxatriol showed the base peak of m/e 127, which suggested the presence of trimethyltetrahydroprpyran ring as given by panaxadiol. This would indicate the absence of a new hydroxyl in this part of the structure.

Of the three hydroxyls of panaxatriol, one is strongly hindered and intramolecularly hydrogen bonded to show a resistance against acetylation. This would suggest that the hydroxyl is located in the 12 position as it occurs in panaxadiol. Another hydroxyl is regarded to be partially hindered for the following reason, though it can be acetylated. A diketonic compound which was prepared by the oxidation of panaxatriol with chromic acid in pyridine yielded only a monosemicarbazone whose infra-red spectrum showed the presence of a 6-membered ring ketone (1,720 cm in CCl4).
The diketone gave no strong absorption band in the UV-spectrum which indicated the absence of 1,2- or 1,3- diketonic system. Thus the possibility of the new hydroxyl being located in the ring A at the position of 1 or 2, can be ruled out. The other possibility of presence of a 2 ketol system in the diketonic compound was also eliminated, because it gave no positive reaction with triphenyl-tetrazolium chloride. This can eliminate the possibility of the presence of the third hydroxyl in the ring C at the 11 position. Consequently, it becomes most probable that the new hydroxyl would be located in the ring B of panaxatriol at position 6 or 7.

On Wolff-Kishner reduction, one of the carbonyls of the diketone was reduced to afford a monoketonic compound, C_{30}H_{50}O_{3} m.p. 199° IR in CS_{2} CO1714 cm^{-1} The N.M.R. spectrum CDCl_{3} of this compound showed AB type doublet at 7.40 and 8.20 (1H each) and a singlet at 7.91 (IH). These signals disappeared when the monoketonic compound was deuterised, using NaOCH_{3} in CD_{3} OD-D_{2}O. This means that three reactive hydrogen atoms would exist at the α : α' positions of the carbenyl in the monoketonic compound to form a \( \text{C} = \text{C} - \text{C} \) system. Such a system can only be represented by the 6 position in the ring B.

The 6 carbonyl in zeorinone and zeorininone is also strongly hindered to show a resistance to ketonic reagents, and to the Wolff-Kishner reduction.

The O. R. D. curve of these compounds showed a similar negative Cotton effect as that given by the monoketonic compound derived from panaxatriol. These facts also supported the above conclusion regarding the location of the new hydroxyl in the 6 position of ring B.
As the 6α-(equatorial) hydroxyl of zeorin is readily acetylated, whereas the 6 (axial) hydroxyl of epi-zeorin shows a resistance to acetylation, the configuration of the 6 hydroxyl of panaxatriol which was not readily acetylated can be assigned to α-equatorial.

The structure of panaxatriol is now put forward. But in regard to the occurrence of panaxadiol as an artefact the genuine sapogenin corresponding to panaxatriol would exist in the form of protopanaxatriol.

The neutral saponin fraction of the methanolic extracts of ginseng gave 10 spots on the thin layer chromatogram. These saponins were tentatively named, according to the sequence of Rf values from the bottom to the top, as ginsenosides Rx (x = a to f, g1, g2, g3, and h).

Of these compounds, ginsenoside Rf might possibly be a prosapogenin of ginsenosides Rb, Re, Rg, and Rf, which was formed secondarily during the process of preparation.

On saponification, it has been confirmed that the sapogenin of ginsenosides Rb, Re, Rg, and Rf is protopanaxadiol, and that of ginsenoside Rg is protopanaxatriol.

The sugar part of ginseng saponin has been studied only in the case of ginsenoside Rb. It has been found that some molecules of D-glucose are attached to the 3 and 20 hydroxyls of protopanaxadiol.
Thin Layer Chromatograms of Ginseng saponins (ginsenosides-R)
on Silica gel G.
Solvent A: n-BuOH:AcOH:H₂O
( 4 : 1 : 5 )
(upper layer)
Solvent B: CHCl₃:MeOH
( 4 : 1 )

This was proved by the hydrolysis of fully methylated and hydrogenated ginsenoside Rb to yield dihydroprotopanaxadiol-12-methyl ether.

Prosapogenin, C₄₂H₇₂O₁₃, m.p. 264°, which was obtained by refluxing ginsenoside Rb in 50 per cent acetic acid was fully methylated with methyl iodide and sodium hydride in dimethyl sulfoxide, and the methanolysis products of this methylated prosapogenins was examined by gas-chromatography. The result showed that 1, 3, 4, 6-tetramethyl-D-glucose and 1, 2, 3, 4, 6-pentamethyl-D-glucose were formed by the methanolysis. Therefore the sugar portion which attaches at the 3 hydroxyl of the genin has now been proved to be Kojibiose (2α-D-Glucopyranosyl-D-glycopyranose) or its 1-epimer.

The original plant of Chinese San-chi ginseng has to be discussed.

In the earlier literature, the original plant of San-chi ginseng which is cultivated or grows wild in Southern part of China (Yun-Nan, Kwan-Si, and Su-Twan) has been reported as being Aralia (Panax) bipinnatifidus Seem or Panax pseudoginseng Wall.
A specimen of the fresh rhizome and root of *Panax pseudoginseng* was kindly supplied to us from Prof. H. Hara, Department of Botany, University of Tokyo, who collected it in Toglu, Shikkim in 1964.

A comparison of the thin layer chromatograms has been made using the extracts of the root of *Panax pseudoginseng* and those of the root and the rhizome of *Panax japonicus* C. A. Mayer, which is of Japanese origin.

**Thin Layer Chromatograms of Saponins**

![Chromatograms](image)

It can be concluded that ginseng (Korean ginseng), American Ginseng, and San-chi ginseng belong to a group containing saponins of the tetracyclic triterpenoid dammarane series as the dominant principles while the root and the rhizome of *Panax pseudoginseng* and *Panax japonicus* belong to another group in which oleanolic acid saponins are contained in a dominant proportion.

It should be noted that San-chi ginseng contains all the main saponins of Korean ginseng, in particular, ginsenoside Rb and Rg1 in a dominant proportion, while American ginseng contains largely ginsenosides Rb and Rg3. The aglycone of ginsenoside Rg3 is not identical with any known ginseng sapogenins.

A comparative study on saponins has been carried out using American Ginseng (the root of *Panax quinquefolium*) and San-chi ginseng of South Chinese origin, as the materials.
The thin layer chromatograms of these preparations are shown below:

![Thin Layer Chromatograms of Saponins](image)

Consequently, the original plant of San-chi ginseng cannot be *Panax pseudo ginseng*, and according to Prof. Hara's opinion it would probably be *Panax notoginseng* Binkill (*Aralia quinquefolius var. notoginseng* Rinkill). It has not yet been completely established, but if we could obtain the specimen of *Panax notoginseng*, it will not be so difficult to identify the original plant of San-chi ginseng by microchemical methods.

**Acknowledgements**

We are grateful to Dr. T. S. Mills, National Gallery, London, Prof. Emeritus M. Kotake, Osaka City University, and Dr. N. Seiter, Max-Planck Inst. für hirn forschung, Marburg for kindly supplying us the authentic samples; to Prof. G. Ourisson, University of Strasbourg for communications and discussions.

We wish to thank Dr. H. R. Ryhage—Karolinska Institute, Stockholm, for measurement of mass spectra.

Our thanks are also due to Research Laboratory of Takeda Pharmaceutical Industry Co., Ltd. Osaka for supplying materials and Yakurikenkynkai and Ministry of Education of Japan for grants.
REFERENCES


Note.—After the completion of our work on panaxatriol, we have been aware of the appearance of a report on ginseng saponins and sapogenins published by G. B. Eliakov, L. I. Strigina, N. I. Uvarova, V. E. Vaskovsky, A. K. Dzieneko and N. K. Kochetkov (*Tetrahedron Letters No. 48*, 3591 (1964), who obtained a sapogenin from panaxosides A, B, and C and named it panaxatriol, C_{30}H_{44}O. (OH)_{3}, m.p. 225–227°, (α)D_{+} 18, 18. The fundamental carbon skeleton of panaxatriol was proved by Russian workers as same as that of panaxadiol, and the position of the third hydroxyl was assigned to be located at (C23) in the tetrahydropryane ring. However this should not have happened in panaxatriol, if the compound obtained by Russian workers is identical with our compound, since the panaxatriol gave a basic peak at m/e 127 in the mass-spectrum as given by panaxadiol suggesting this part of structure is same in panaxatriol and panaxadiol. Further evidence for the presence of the third hydroxyl in the 6 position in ring B was provided by the lower shift of the n.m.r. signal of the gem-dimethyl in the ring A of panaxatriol in comparison with that of panaxadiol.
5-HYDROXY
7-METHOXYFLAVANONE
AND 5-METHOXY
7-HYDROXYFLAVANONE
FROM Boesenbergia pandurata

BY
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Chulalongkorn University,
Bangkok, Thailand

Boesenbergia pandurata Roxb. is listed (Holttum, 1950) as a genus of the family Zingiberaceae. Formerly this plant was treated as Kaempferia pandurata Roxb. and Gastrochilus panduratus Ridl., respectively. This plant is found predominantly in many countries in the tropical zone, especially in Indonesia, India, Malaya, China and Thailand. Boesenbergia pandurata is commonly known by the name of 'Kachai' in Thailand and 'Kunchi' in Malaya. Fresh roots of pandurata serve as a spice in food, and particularly in medicine. They were used for coughs, stomach troubles, and various diseases of women, and externally for ringworm (Ridley, 1897; Heyne, 1927). A dextrorotatory yellow oil has been obtained from the roots of this plant. Methyl cinnamate and cineole have been identified (Ultee, 1927). The present paper reports the evidence identifying the structure of two flavanones from the dried powdered roots.

Concentration of a petroleum ether extract solution and an acetone extract solution of the dried powdered roots gave two crystalline compounds, m.p. 100-101° and 221-222°, respectively. The analysis of these compounds fitted best with the same molecular formula C_{16}H_{18}O_{4}. They did not contain a C-CH_{3} grouping. The presence of the methoxyl grouping from each of them was established by a Zeisel methoxy determination. These two compounds gave a positive flavone test (Shinoda, 1928) and yielded the same methyl ether derivative. The identity of methyl ether derivatives from both of them was confirmed by infrared spectra and mixed melting point.

On hydrolysis with 10 per cent sodium hydroxide solution the methyl ether derivatives of both parent compounds yielded benzaldehyde and 2-hydroxy-4,6-dimethoxyacetophenone. The latter was confirmed by synthesis from phloroglucinol. From these results it seemed clear that the methyl ether derivative was 5,7-dimethoxyflavanone (I) and the synthesis confirmed this.
The compound, m.p. 100-101°, gave a positive ferric chloride test, suggesting a phenolic hydroxyl group. The existence of an absorption peak in the infra-red spectrum of this compound at 3500cm⁻¹ indicated the presence of a hydrogen bonded —OH function (Bellamy, 1959). The chemical and physical properties of this compound pointed to the structure 5-hydroxy-7-methoxy flavanone (II). Since the compound, m.p. 221-222°, is an isomer of II, its structure would be 5-methoxy—7-hydroxyflavanone (III).

These two flavanones have been isolated from other plants (Rao and Seshadri, 1946; Kimura, 1940) and their names are pinostrobin and alpinetin, respectively. The annexed table confirms the identity of the products from the two different sources as shown below:

<table>
<thead>
<tr>
<th></th>
<th>Pinostrobin</th>
<th>Compound II</th>
<th>Alpinetin</th>
<th>Compound III</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.p.</td>
<td>101-102°</td>
<td>100-101°</td>
<td>223°</td>
<td>221-222°</td>
</tr>
<tr>
<td>Acetate (m.p.)</td>
<td>147°</td>
<td>147-148°</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Me-ether (m.p.)</td>
<td>146°</td>
<td>145-146°</td>
<td>143°</td>
<td>145-146°</td>
</tr>
</tbody>
</table>

EXPERIMENTAL PART

General

Melting points are uncorrected and were taken on a Fisher-Johns hot stage. Infra-red spectra were made by means of a Perkin Elmer Infracord spectrophotometer model 137E. Petroleum ether refers to the fraction boiling between 40-80°. Microanalyses were carried out by Dr. K. W. Zimmermann and associates, C. S. I. R. O., Carlton, Victoria, Australia.

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Extraction and Separation of Components

(a) 5-Hydroxy-7-methoxyflavanone

The dried roots of *Boesenbergia pandurata* were ground to a powder. The powder (3 kg) was steeped in petroleum ether (10 L) for 7 days at room temperature and then filtered. The extraction was repeated twice with fresh petroleum ether. The combined filtrates were evaporated to 800 ml. and allowed to stand overnight. A crude solid was obtained. The solid was collected and evaporation of the filtrate almost to dryness on the steam bath left a brownish oily residue (20g). The solid residue was washed with a small portion of petroleum ether. After several recrystallizations from petroleum ether-acetone (10 : 1) small white needles of 5-hydroxy-7-methoxyflavanone, m.p. 100–101° (9g) were obtained. (Found : C, 71.37; H, 5.34; OMe, 11.04. \( \text{C}_{16}\text{H}_{14}\text{O}_4 \) requires : C, 71.10; H, 5.22; OMe, 11.48 per cent).

Nujol
Infrared spectrum : \( \sqrt{\text{max}} \) 3450 (OH), 1640 (C = C).

Color reactions : 5-Hydroxy-7-methoxyflavanone gave a violet color with ferric chloride. The flavone test with methanol, magnesium and hydrochloric acid resulted an orange-red color. With concentrated nitric acid a blue color was obtained.

(b) 5-Methoxy-7-hydroxyflavanone

The extracted ground roots from petroleum ether were next steeped in acetone (10 L) for 7 days before filtration. This extraction was also repeated with another fresh portion of acetone. The same procedure as for 5-hydroxy-7-methoxyflavanone (above) was used in the case of the acetone extractions of the extracted ground roots. Finally, a crude solid (6 g) and brownish oily residue (18 g) were obtained. After several recrystallizations from ethanol, white needles of 5-methoxy-7-hydroxyflavanone, (3 g) m.p. 221–222°, were collected. (Found : C, 70.90; H, 5.30; OMe 11.40. \( \text{C}_{16}\text{H}_{14}\text{O}_4 \) requires : C, 71.10; H, 5.22; OMe 11.48 per cent).

Nujol 3590 and 3450 (OH), 1640 (C = C).
Infrared spectrum : \( \sqrt{\text{max}} \)

Color reactions : The compound did not give a ferric chloride test, but the flavone test and concentrated nitric acid test were similar to 5-hydroxy-7-methoxyflavanone.

Acetylation of 5-hydroxy-7-methoxyflavanone

Prepared by the acetyl chloride method, the mono-acetyl derivative separated from alcohol in white needles, m.p. 147–148° (lit. m.p. 147°). (Found ; C, 69.20; H, 5.27 Ac, 14.5. Calc. for \( \text{C}_{16}\text{H}_{12}\text{O}_4 \text{Ac} \), C, 69.25; H, 5.16; Ac, 13.78 per cent).

Methylation of 5-hydroxy-7-methoxyflavanone and 5-methoxy-7-hydroxyflavanone

By the methyl sulfate-potassium carbonate method, the compounds give methyl ether derivatives. The two methyl ether derivatives were identical in all respects (m.p., I. R.) with each other. A mixture of the two melted at 145–146°, unpressed.
Found: C, 72.08; H, 5.79; OMe, 21.51%. Cals. for C_{17}H_{16}O_{4} from
5-hydroxy-7-methoxyflavanone; C, 71.81; H, 5.67; OMe, 21.83%.

Found: C, 71.70; H, 5.80; OMe, 21.50%. Cals. for C_{17}H_{16}O_{4} from
5-methoxy-7-hydroxyflavanone; C, 71.81; H, 5.67; OMe, 21.83%.

Hydrolysis of 5, 7-dimethoxyflavanone

5, 7-Dimethoxyflavanone from both of 5-hydroxy-7-methoxyflavanone and 5-methoxy-7-hydroxyflavanone was hydrolyzed with 10 per cent. sodium hydroxide in ethanol solution. The same products, 2-hydroxy-4, 6-dimethoxyacetophenone and benzaldehyde, were obtained. The following procedure was used.

A solution of 1 g. in ethanol (40 ml) and 10 per cent. sodium hydroxide (150 ml.) was subjected to steam distillation for two hours. The distillate was shaken with ether (3 times with 100 ml. portions). The combined ether solution was washed with water, dried over anhydrous sodium sulfate, and the ether was removed in a rotating evaporator at room temperature. The residue, which gave a benzaldehyde odor, was treated with a solution of 2,4-dinitrophenylhydrazine sulfate. The resulting hydrazone, m.p. 234° was identical in all respects with hydrazone of benzaldehyde.

The remainder of the steam distillate was allowed to cool and the solution acidified with 6 N hydrochloric acid. The product was isolated as a white solid. Recrystallization from a small amount of petroleum ether gave white, fine needles (0.4 g.) m.p. 80-81°. This compound was found to be 2-hydroxy-4, 6-dimethoxyacetophenone, since the spectra of the compound and that of a synthetic specimen were identical. A mixture of the two melted at 80-81°, undepressed.

Synthesis of phloroacetophenone

Phloroglucinol was converted to phloroacetophenone by the method of Gulati, Seth and Venkataraman (1957).

Synthesis of 2-Hydroxy-4-6-dimethoxyacetophenone

Methylation of phloroacetophenone (5 g.) in boiling acetone (50 ml.) with dimethyl sulfate (10 g.) and potassium carbonate (30 g.) for 10 hrs., furnished 2-hydroxy-4, 6-dimethoxyacetophenone, forming white needles (2.8 g.) m.p. 80-81° from petroleum ether (b.p. 40-80°).

The spectra of this synthetic compound and the hydrolyzed products of methyl ether of natural specimens were identical.

Synthesis of 5, 7-dimethoxyflavanone

5, 7-Dimethoxyflavanone was prepared by the method of Kimura (1940) 2-hydroxy
4, 6-dimethoxyacetophenone (2 g.) on heating with benzaldehyde (1.1 g.) in 10
per cent. sodium hydroxide solution (20 ml.) at 60-70° for 2 hrs. gave 2-hydroxy-4,
6-dimethoxychalcone (1.2 g.), m.p. 89-90°. Refluxing the above compound in hydrochloric acid (2N, 20 ml.) for 30 hrs. gave 5, 7-dimethoxyflavanone (1 g.) m.p. 145-146°.

The spectra of products from the synthesis and methyl ether derivatives of natural 5-hydroxy-7-methoxyflavanone and 5-methoxy-7-hydraxyflavanone were identical. A mixture of the three melted at 145-146°, undepressed.

Acknowledgements

The authors wish to thank Professor K. Suvatabandhu for his suggestions regarding the botanical identity of the plant. They are also indebted to Dr. K. E. Jewer for absorption spectrophotometric measurements and to Dr. Kamchron Manunapichu for many helpful discussions and suggestions.

REFERENCES

A NEW APORPHINE
ALKALOID, NEOLITSINE,
FROM THE
HONG KONG SPECIES,
*Neolitsea pulchella*

BY

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Miss W. H. HUI
AND
S. N. LOO

*Department of Chemistry,*
*University of Hong Kong,*
*Hong Kong*

**Neolitsine**, C$_{18}$H$_{27}$O$_4$N, was isolated from the alcohol extract of the leaves of *Neolitsea pulchella* and has structure (I).

The ultraviolet absorption curve of neolitsine suggested that it was an aporphine. Since it gave a positive Labat test and did not contain phenolic or methoxyl groups, it was concluded that there were two methylenedioxy-groups whose presence was also detected by characteristic infrared bands at 2760 and 940 cm$^{-1}$. The molecular weight was shown to be 323 by mass spectrometry; and intense (M-1) base peak and an (M-43) peak typical of aporphines with an N-methyl function were observed (Sharma and Shuarchyk, 1964) and the presence of this group was confirmed in the n.m.r. spectrum.

The optical rotatory dispersion curve corresponded with that of a 1, 2, 9, 10-tetrasubstituted aporphine with absolute configuration as shown in (I) (Djerassi *et al.*, 1962). Thus the methylenedioxy-groups were fixed in the 1, 2- and 9-10-positions; this was confirmed by reference to the ultraviolet spectrum in ethanol which showed maxima at 310 and 284 m$\mu$ ($\log \epsilon$ 4·11 and 3·9) characteristic of a 1, 2, 9, 10-tetrasubstituted aporphine (Djerassi *et al.*, 1962). In the n.m.r. spectrum the methylenedioxy-groups appeared as two doublets centred at $\tau$ 4·04, 4·17 (1, 2- substituted group), (Shamma and Shuarohyk, 1964) and a singlet at $\tau$ 4·05 (9-10-substituted group). Signals for the three aromatic hydrogens were at $\tau$ 2·43 (11-H) and 3·32, 3·57 (3- and 8- H's). The N-Me group was represented by a signal 7·53.
Acknowledgements

The authors thank the Tropical Products Institute, the D. S. I. R., for financial assistance, the University of Stanford for physical measurements and Mr. H. C. Tang of the Government Herbarium, Hong Kong for identification of plant material.

REFERENCES

CHROMATOGRAPHIC SCREENING FOR ALKALOIDS AND INDOLE COMPOUNDS OF ONE HUNDRED SPECIES OF TAIWAN FRESH MEDICINAL PLANTS

BY

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Medical College, National
Taiwan University, Taiwan

Introduction

For several thousand years, the Chinese people have used plants in the treatment of snake-bite, paralysis, trauma, gastric indigestion, various blood diseases, tumours, paediatric convulsion, etc. It has been shown that some Chinese plants have the property of being able to change behaviour, perhaps these might have contained, among other things, certain analgesics to relieve the suffering of the patient. We were anxious to know the cause of such striking physiological actions which would open the door for the discovery of new drugs.

Our interest in the study of fresh Chinese medicinal plants had been furthered by the fact that there was no record of chemical analysis of its constituents available while the explanation of its pharmacological action involved was either insufficient or unreliable from a modern scientific viewpoint. Therefore we wanted to fill up this blank field of knowledge which we felt could be of considerable significance.
Hence the chromatographic screening of alkaloids and indole compounds of one hundred species of fresh medicinal plants was carried out and recorded as follows:

**LIST OF HUNDRED PLANTS**

<table>
<thead>
<tr>
<th>Part Used</th>
<th>Botanical Origin</th>
<th>Time of Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole plant</td>
<td>Acanthaceae : <em>Hypoestes purpurea</em>, R. Brown</td>
<td>June 22, 1963</td>
</tr>
<tr>
<td>Leaf, branch, flower</td>
<td>Aquifoliaceae : <em>Ilex asperrula</em> Champ</td>
<td>March 15, 1962</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Araceae : <em>Pothos seemannii</em>, Schott</td>
<td>June 22, 1963</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Araceae : <em>Alocasia macrorrhiza</em> (L) Schott</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, branch, fruit</td>
<td>Araliaceae : <em>Heptapleurum octophyllum</em> Benth</td>
<td>March 9, 1962</td>
</tr>
<tr>
<td>Stem, leaf</td>
<td>Araliaceae : <em>Aralia bipinnata</em> Blanco</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Berberidaceae : <em>Podophyllum pleianthum</em> Hance</td>
<td>April 1, 1963</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Betulaceae : <em>Alnus formosana</em> (Burkill) Makino</td>
<td>April 1, 1962</td>
</tr>
<tr>
<td>Leaf, branch, fruit</td>
<td>Bixaceae : <em>Bixa orellana</em> L.</td>
<td>March 22, 1962</td>
</tr>
<tr>
<td>Leaf, branch, flower</td>
<td>Boraginaceae : <em>Ehretia resinosa</em>, Hance</td>
<td>May 22, 1962</td>
</tr>
<tr>
<td>Leaf, branch, fruit</td>
<td>Camelliaceae : <em>Eurya acuminata</em> DC</td>
<td>May 11, 1962</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Camelliaceae : <em>Eurya suzukii</em>, Yamanata</td>
<td>May 11, 1962</td>
</tr>
<tr>
<td>Leaf, branch, flower</td>
<td>Camelliaceae : <em>Tristylium ochraceum</em> (DC) Merrill</td>
<td>May 11, 1962</td>
</tr>
<tr>
<td>Leaf, branch, flower</td>
<td>Caprifoliaceae : <em>Viburnum luzonicum</em> Rolfe</td>
<td>April 1, 1962</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Cephalotaxaceae : <em>Pinus luchuensis</em> Mayer</td>
<td>March 15, 1962</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Chloranthaceae : <em>Chloranthus glabra</em> (Thumb) Makino</td>
<td>May 11, 1962</td>
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<tr>
<td>Whole plant</td>
<td>Compositae : <em>Pluchea indica</em>, Less</td>
<td>April 29, 1962</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Compositae : <em>Gnaphalium indicum</em>, Linn</td>
<td>March 11, 1962</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Ebenaceae : <em>Diospyros eriantha</em> Champ</td>
<td>April 29, 1962</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Elaeocarpaceae : <em>Sloanea dasyarpa</em>, Hemsli</td>
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</tr>
<tr>
<td>Part Used</td>
<td>Botanical Origin</td>
<td>Time of Collection</td>
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<td>-------------------------------------------</td>
<td>--------------------</td>
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<td>April 11, 1963</td>
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<tr>
<td>Leaf, branch, fruit</td>
<td>Euphobiacae : <em>Glochidion fortunei</em>, Hance</td>
<td>March 15, 1962</td>
</tr>
<tr>
<td>Stem, leaf</td>
<td>Fagaceae : <em>Quercus glauca</em> Thunb</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Gesneriaceae : <em>Trichosporum acuminatum</em> Kuntze</td>
<td>Jan. 22, 1963</td>
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<td>Leaf, branch</td>
<td>Lauraceae : <em>Lindera communis</em> Hemsl</td>
<td>March 18, 1962</td>
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<tr>
<td>Leaf, branch</td>
<td>Lauraceae : <em>Beilschmiedia erythrophaolia</em>, Hayata</td>
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<tr>
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<td>Lauraceae : <em>Actinodaphne pedicellata</em>, Hayata</td>
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</tr>
<tr>
<td>Leaf, stem</td>
<td>Lauraceae : <em>Neolitsea koristii</em></td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, bark, fruit</td>
<td>Leguminosae : <em>Tamarindus indica</em>, L.</td>
<td>Sept. 22, 1962</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Liliaceae : <em>Paris formosana</em></td>
<td>March 11, 1962</td>
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<tr>
<td>Whole plant</td>
<td>Liliaceae : <em>Dianella ensifolia</em>, (Linn) DC</td>
<td>March 15, 1962</td>
</tr>
<tr>
<td>Rhizome</td>
<td>Polygonaceae : <em>Polygonum chinense</em> L.</td>
<td>May 6, 1962</td>
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<td>Whole plant</td>
<td>Loganiaceae : <em>Buddleja asiatica</em>, Lour</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Menispermaceae : <em>Stephanica japonica</em>, (Thunb.) Miers</td>
<td>March 15, 1962</td>
</tr>
<tr>
<td>Stem</td>
<td>Moraceae : <em>Ficus caronata</em>, Reinw</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Moraceae : <em>Ficus vaccinoides</em>, Hemel et King</td>
<td>May 11, 1962</td>
</tr>
<tr>
<td>Branch</td>
<td>Myrsinaceae : <em>Ardista crispa</em> DC</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Myrsinaceae : <em>Ardista villosa</em>, Mez.</td>
<td>April 11, 1962</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Myrtaceae : <em>Eucalyptus robusta</em> Sm.</td>
<td>March 15, 1962</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Oleaceae : <em>Fraxinus griffithii</em>, C. B. Clarke</td>
<td>May 22, 1962</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Phylolacaceae : <em>Phytolacca acinosa</em>, Roxb. var esculenta, Maxim</td>
<td>March 11, 1962</td>
</tr>
<tr>
<td>Part Used</td>
<td>Botanical Origin</td>
<td>Time of Collection</td>
</tr>
<tr>
<td>--------------</td>
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<td>--------------------</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Polygonaceae : <em>Polygonum chinense</em> L.</td>
<td>May 6, 1962</td>
</tr>
<tr>
<td></td>
<td><em>tenifolia</em> Makino</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>Polypodiaceae : <em>Asplenium nidus</em> L.</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Polypodiaceae : <em>Microsorium membranaceum</em> (Don.) Ching</td>
<td>March 11, 1962</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Polypodiaceae : <em>Dryopteris subdecipiens</em>, Hayata</td>
<td>June 10, 1962</td>
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<td>Whole plant</td>
<td>Polypodiaceae : <em>Rumora aristata</em>, (Forat.) Ching</td>
<td>March 9, 1963</td>
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<tr>
<td>Leaf, branch</td>
<td>Proteaceae : <em>Grevillea robusta</em> A. Gunn.</td>
<td>March 22, 1962</td>
</tr>
<tr>
<td>Stem, leaf</td>
<td>Rosaceae : <em>Rubus sievchoe</em>, Hance</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, branch, flower</td>
<td>Rosaceae : <em>Prunus phaeosticta</em>, (Hance) Maxim.</td>
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<tr>
<td>Whole plant</td>
<td>Rubiaceae : <em>Psychotria serpens</em> L.</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Branch</td>
<td>Rubiaceae : <em>Ourouparia formosana</em> Hayata</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Sapindaceae : <em>Koelreuteria formosana</em>, Hayata</td>
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</tr>
<tr>
<td>Leaf, branch</td>
<td>Saxifragaceae : <em>Deutzia taiwanesis</em>, (Maximow), Schneid</td>
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<tr>
<td>Whole plant</td>
<td>Selaginellaceae : <em>Selaginella springiana</em>, V. A. V. Rosenb</td>
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<tr>
<td>Leaf, branch, flower</td>
<td>Staphylaceae : <em>Eucaphis japonica</em> Dippel</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, stem</td>
<td>Styracaceae : <em>Styrax formosanum</em> Matsum</td>
<td>Jan. 22, 1963</td>
</tr>
<tr>
<td>Leaf, branch</td>
<td>Taxodiaceae : <em>Cryptomeria japonica</em> D. Don</td>
<td>March 4, 1962</td>
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<td>Branch</td>
<td>Ulmaceae : <em>Celtis formosana</em> Hayata</td>
<td>Jan. 22, 1963</td>
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<tr>
<td>Stem, leaf</td>
<td>Urticaceae : <em>Pellionia scabra</em> Benth</td>
<td>Jan. 22, 1963</td>
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</table>
**EXPERIMENTAL SCHEME**

**Procedure:**

200-500 g. Fresh Plant (cut to pieces)

- + twice its volume of 95% alcohol
- Mix in a Waring blender
- Filter

---

Filtrate Residue

- Dried below 60°C

Dried Extract + acetic acid (10 ml. of 5%)

- Warm on water bath for one hour
- Filter

---

Filtrate Residue

- + 28% NH₃ test solution to pH 9.2

Transferred in separating funnel

- extracted with CHCl₃ (3 times with portions of 10, 10, and 5 ml. respectively)
- concentrated to 5 ml.

Spot 1 drop on Whatman chromatography paper 1

Set in B. A. W. Solvent (Butanol : Acetic acid : Water 4 : 1 : 1)

- for 18 hours, 24 hours in winter

Dried the Chromatogram in air

Spray the following reagents for alkaloids

(i) Dragendorff’s reagent

(ii) Iodoplatinate solution

(iii) Iodine test solution

Spray with Ehrlich reagent for indole compounds
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<td></td>
<td>Color</td>
<td>Rf Value</td>
<td>Color</td>
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<tr>
<td>1</td>
<td>Acanthaceae: <em>Hypoestes purpurea</em>, R. Brown</td>
<td>Pink</td>
<td>0.29</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purple</td>
<td>0.35</td>
<td>—</td>
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<tr>
<td>2</td>
<td>Anacardiaceae: <em>Rhus javaeac</em> Linn var roxburgii Rchd. et Wils</td>
<td>No reaction</td>
<td>—</td>
<td>Purple</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Yellow</td>
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<tr>
<td>3</td>
<td>Apocynaceae: <em>Ecdysanthera rosea</em>. Hoc’ &amp; Aru.</td>
<td>Purple</td>
<td>0.13</td>
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<td>—</td>
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<tr>
<td>4</td>
<td>Aquifoliaceae: <em>Ilex asprella</em> Champ</td>
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<tr>
<td>5</td>
<td>Araceae: <em>Pothos seemanni</em>, Schott</td>
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<td>7</td>
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<td>8</td>
<td>Araliaceae: <em>Heptapleurum octophyllum</em> Benth</td>
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<td>9</td>
<td>Araliaceae: <em>Fatsia japonica</em> Decne et Planch</td>
<td>No reaction</td>
<td>Greenish Blue</td>
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<td>10</td>
<td>Araliaceae: <em>Aralia bipinata</em> Blanco</td>
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<td>Color</td>
<td>Concentration</td>
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<td>11</td>
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<td>12</td>
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<td>Berberidaceae: Podophyllum pleianthum Hance</td>
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<td>Camelliaceae: Eurya acuminata DC</td>
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<td>Camelliaceae: Eurya japonica, Thunb</td>
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<td>Camelliaceae: Tristylium ochraceum (DC.) Merrill</td>
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<td>21</td>
<td>Caprifoliaceae: Viburnum luzonicum Rolfe</td>
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<td>22</td>
<td>Cephalotaxaceae: Pinus luchuensis Mayer</td>
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<td>23</td>
<td>Chloranthaceae: Chloranthus glabra (Thumb) Makino</td>
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<td>24</td>
<td>Compositae: Pluchea indica, Less</td>
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<td>25</td>
<td>Compositae: Gnaphalium indicum, Linn</td>
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<td>Dilleniaceae: Saurauia tristyila, DC. Ohl.nami Finet &amp; Gagn.</td>
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</table>
**Table of the Color Reaction for the Alkaloids Contained in One Hundred Species of Medicinal Plants and their Rf Value—contd.**

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<td>Rf Value</td>
<td>Color</td>
<td>Rf. Value</td>
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<td>Ebenaceae: <em>Diospyros eriantha</em> champ</td>
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<td>28</td>
<td>Elaeocarpaceae: <em>Sloanea dasycarpa</em>, HemsI</td>
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<td>29</td>
<td>Ericaceae: <em>Vaccinium wrightii</em>, A. Gr.</td>
<td>Purplish orange</td>
<td>0·32</td>
<td>Light Orange</td>
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<td>30</td>
<td>Ericaceae: <em>Vaccinium donianum</em> Wight var. <em>hancehouense</em> Matsuda</td>
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<td>0·31</td>
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<td>Ericaceae: <em>Vaccinium randaiense</em>, Hayata</td>
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<td>32</td>
<td>Euphorbiaceae: <em>Euphorbia thymifolia</em> Burm</td>
<td>Purple</td>
<td>0·25</td>
<td>Purple</td>
<td>0·14</td>
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<td>Euphorbiaceae: <em>Glochidion hongkongense</em>, Muell. Arg.</td>
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<td>36</td>
<td>Fagaceae: <em>Quercus glauca</em> Thunb</td>
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<td>37</td>
<td>Flacourtiaeceae: <em>Hydnocarpus anthelmintica</em> Pierr</td>
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<td>Blue purple</td>
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<td>Presence</td>
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<td>Gesneriaceae</td>
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<td>Juglandaceae</td>
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<td>Beilschniedia erythrophloia Hayata</td>
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<td>Banhina championi Benth</td>
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<td>Dianella eritofilia, (Linn.) Dc.</td>
<td>Purplish red</td>
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### Table of the Color Reaction for the Alkaloids Contained in One Hundred Species of Medicinal Plants and their Rf Value—contd.

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<td>Color</td>
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<td>Moraceae: <em>Ficus caronata</em>, Reuw</td>
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<td>55</td>
<td>Moraceae: <em>Ficus vaccinioides</em>, Hemel et King</td>
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<td>Moraceae: <em>Ficus g gibbos</em>, Blume</td>
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<td>57</td>
<td>Myrsinaceae: <em>Maes p formosana</em>, Mez.</td>
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<td>58</td>
<td>Myrsinaceae: <em>Ardpia sieboldii</em>, Miq.</td>
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<td>59</td>
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<td>0.11</td>
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<td>0.40, 0.24</td>
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<td>61</td>
<td>Myrtaceae: <em>Eucalp i robusta</em> Sm.</td>
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<td>62</td>
<td>Oleaceae: <em>Fraxinus griffithii</em>, C. B. Clarke</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No reaction</td>
</tr>
<tr>
<td>63</td>
<td>Palmae: <em>Arega euglori</em> Bece</td>
<td>No reaction</td>
<td></td>
<td>No reaction</td>
</tr>
<tr>
<td>64</td>
<td>Pandananaceae: <em>Pandaman graamnifoliol Kurz.</em></td>
<td>Purple</td>
<td>0.23</td>
<td>Grayish blue</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yellow</td>
</tr>
<tr>
<td>65</td>
<td>Phytolaccaceae: <em>Phytolacca acinosa</em>, Roxb. var esculenta, Maxim.</td>
<td>Purple</td>
<td>0.24</td>
<td>No reaction</td>
</tr>
<tr>
<td>No</td>
<td>Pine family: <em>Tsuga chinensis</em>, Prinzel var <em>formosana</em> (Hauta sub. SP)</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>----</td>
<td>---------------------------------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>67</td>
<td>Pittosporaceae: <em>Pittosporum tobira</em> Ait var. <em>calvecins ohwi</em></td>
<td>Purple 0.38</td>
<td>Yellow 0.28</td>
<td>No reaction</td>
</tr>
<tr>
<td>68</td>
<td>Piperaceae: <em>Piper kadsura</em>, ohwi</td>
<td>Purple 0.14</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>69</td>
<td>Polypodiaceae: <em>Drimosoria chinensis</em>, J. Sm. var. <em>tenui-folia makino</em></td>
<td>Orange 0.34</td>
<td>Brown 0.16</td>
<td>No reaction</td>
</tr>
<tr>
<td>70</td>
<td>Brown 0.78</td>
<td>Brown 0.21</td>
<td>No reaction</td>
<td></td>
</tr>
<tr>
<td>Polypodiaceae: <em>Asplenium nidus</em> L.</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>Polypodiaceae: <em>Microsorium membranaceum</em> (Don.) Ching</td>
<td>Orange 0.20</td>
<td>Purple 0.19</td>
<td>No reaction</td>
</tr>
<tr>
<td>72</td>
<td>Brown 0.28</td>
<td>Purple 0.93</td>
<td>No reaction</td>
<td></td>
</tr>
<tr>
<td>Polypodiaceae: <em>Dryopteris subdecipiens</em>, Hayata</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>Polypodiaceae: <em>Rumora aristata</em> (Forst.) Ching</td>
<td>Yellow 0.11</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>74</td>
<td>Polypodiaceae: <em>Macadamia ternifolia</em>, F. Muell.</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>75</td>
<td>Proteaceae: <em>Grevillea robusta</em> A. Gunn</td>
<td>Yellow 0.10</td>
<td>Brown 0.23</td>
<td>No reaction</td>
</tr>
<tr>
<td>76</td>
<td>Yellow 0.34</td>
<td>Yellowish brown 0.46</td>
<td>No reaction</td>
<td></td>
</tr>
<tr>
<td>Primulaceae: <em>Lysimachia decurrens</em>, Forst. f.</td>
<td>No reaction</td>
<td>Yellow 0.58</td>
<td>Brown 0.22</td>
<td>No reaction</td>
</tr>
<tr>
<td>77</td>
<td>Rhamnaceae: <em>Rhamnus formosana</em> Matsum</td>
<td>Greenish yellow 0.70</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>78</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>Rosaceae: <em>Rubus swinhoe</em> Hance</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
</tbody>
</table>
### Table of the Colour Reaction for the Alkaloids Contained in One Hundred Species of Medicinal Plants and their Rf Value

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Color</td>
<td>Rf Value</td>
<td>Alcoholic Iodine T. S. Color</td>
</tr>
<tr>
<td>80</td>
<td>Rosaceae : <em>Prunus phaeosticta</em>, (Hance) Maxim.</td>
<td>Blue</td>
<td>0.35</td>
<td>No reaction</td>
</tr>
<tr>
<td>81</td>
<td>Rubiaceae : <em>Psychotria serpens</em> L.</td>
<td>Orange</td>
<td>0.32</td>
<td>No reaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orange</td>
<td>0.50</td>
<td>No reaction</td>
</tr>
<tr>
<td>82</td>
<td>Rubiaceae : <em>Mussaenda parviflora</em> Miq.</td>
<td>Purple</td>
<td>0.26</td>
<td>No reaction</td>
</tr>
<tr>
<td>83</td>
<td>Rubiaceae : <em>Ourouparia formosana</em> Hayata</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>84</td>
<td>Rubiaceae : <em>Morinda citrifolia</em>, L.</td>
<td>Purple</td>
<td>0.25</td>
<td>No reaction</td>
</tr>
<tr>
<td>85</td>
<td>Rutaceae : <em>Evodia glauca</em> Miq.</td>
<td>Reddish orange</td>
<td>0.44</td>
<td>No reaction</td>
</tr>
<tr>
<td>86</td>
<td>Sapindaceae : <em>Koelreuteria formosana</em>, Hayata</td>
<td>No reaction</td>
<td>Purple</td>
<td>0.46</td>
</tr>
<tr>
<td>87</td>
<td>Saxifragaceae : <em>Deutzia tawainesis</em>, (Maximow), Schneid</td>
<td>Purple</td>
<td>0.21</td>
<td>No reaction</td>
</tr>
<tr>
<td>88</td>
<td>Selaginellaceae : <em>Selaginella delicatula</em> (Desv) Alston</td>
<td>Orange</td>
<td>0.28</td>
<td>No reaction</td>
</tr>
<tr>
<td>89</td>
<td>Staphyleaceae : <em>Turpinia formosana</em> Nakai</td>
<td>No reaction</td>
<td>Yellowish brown</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yellowish brown</td>
<td>0.95</td>
</tr>
<tr>
<td>90</td>
<td>Moraceae : <em>Ficus gibbosa</em> Bl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>Styracaceae : <em>Styrax formosarum</em> Matsum</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>92</td>
<td>Taxodiaceae : <em>Taxodium distichum</em>, Rich</td>
<td>No reaction</td>
<td>No reaction</td>
<td>No reaction</td>
</tr>
<tr>
<td>93</td>
<td>Taxodiaceae : <em>Cryptomeria japonica</em> D. Don.</td>
<td>Tan.</td>
<td>0.09</td>
<td>No reaction</td>
</tr>
<tr>
<td>94</td>
<td>Trochodendraceae : <em>Trochedendron uralioides</em>, S. et Z.</td>
<td>No reaction</td>
<td>Purple</td>
<td>0.14</td>
</tr>
<tr>
<td>95</td>
<td>Ulmaceae : <em>Celtis formosana</em> Hayata</td>
<td>Purple</td>
<td>0.26</td>
<td>—</td>
</tr>
<tr>
<td>96</td>
<td>Urticaceae : <em>Elatostema lineolatum</em>, Forst. var. major, Thwait</td>
<td>Purple</td>
<td>0.33</td>
<td>Purple</td>
</tr>
<tr>
<td>97</td>
<td>Urticaceae : <em>Pellionia scabra</em> Benth.</td>
<td>Purple</td>
<td>0.29</td>
<td>0.17</td>
</tr>
<tr>
<td>98</td>
<td>Urticaceae : <em>Debregeasia edulis</em>, Wedd.</td>
<td>Pink</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>99</td>
<td>Verbenaceae : <em>Callicarpa pilosissima</em> Maxim</td>
<td>Purple</td>
<td>0.22</td>
<td>—</td>
</tr>
<tr>
<td>100</td>
<td>Verbenaceae : <em>Vitex quinata</em> (Lam) F. N. Will</td>
<td>Purple</td>
<td>0.24</td>
<td>0.50</td>
</tr>
</tbody>
</table>
### Table of Fresh Medicinal Plants Among One Hundred Species Investigated which Had Positive Reaction to Ehrlich's Reagent Sprayed

<table>
<thead>
<tr>
<th>Origin</th>
<th>Solvent B. A. W.</th>
<th>Ehrlich's R.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Rf. Value</td>
</tr>
<tr>
<td>Apocynaceae: <em>Ecdysanthera rosea</em>, Hook et Arn.</td>
<td>Purple</td>
<td>0.16</td>
</tr>
<tr>
<td>Araceae: <em>Alocasia macrorrhiza</em> (L) Schott</td>
<td>Purple to blue</td>
<td>0.42</td>
</tr>
<tr>
<td>Araliaceae: <em>Heptapleurum octophyllum</em> Benth</td>
<td>Purple</td>
<td>0.28</td>
</tr>
<tr>
<td>Asclepiadaceae: <em>Hoya carnosa</em>, R. Brown</td>
<td>Purple</td>
<td>0.52</td>
</tr>
<tr>
<td>Bixaceae (<em>Bixa orellana</em> L.)</td>
<td>Purple</td>
<td>0.44</td>
</tr>
<tr>
<td>Camelliaeae: <em>Eurya suzukii</em> Yamanata</td>
<td>Grayish maroon</td>
<td>0.10</td>
</tr>
<tr>
<td>Caprifoliaceae: <em>Viburnum luzonicum</em> Rolfe</td>
<td>Green</td>
<td>0.27</td>
</tr>
<tr>
<td>Ericaceae: <em>Vaccinium wrightii</em>, A. Gr.</td>
<td>Black</td>
<td>0.49</td>
</tr>
<tr>
<td>Ericaceae: <em>Vaccinium donlanum</em>, Wight var. <em>hangchouense</em> Matsuda</td>
<td>Purple</td>
<td>0.14</td>
</tr>
<tr>
<td>Betulaceae: <em>Alnus formosana</em> (Burkili) Makino</td>
<td>Purple</td>
<td>0.09</td>
</tr>
<tr>
<td>Ericaceae: <em>Vaccinium randalense</em>, Hayata</td>
<td>Blue</td>
<td>0.50</td>
</tr>
<tr>
<td>Euphorbiaceae: <em>Glochidion fortunei</em>, Hance</td>
<td>Blue</td>
<td>0.22</td>
</tr>
<tr>
<td>Flacourtiaceae: <em>Hydnocarpus anghelminica</em>, Pierr</td>
<td>Purplish maroon</td>
<td>0.25</td>
</tr>
<tr>
<td>Liliaceae: <em>Paris formosana</em> Mez.</td>
<td>Blue</td>
<td>0.76</td>
</tr>
<tr>
<td>Liliaceae: <em>Dianella ensifolia</em>, (Linn) DC.</td>
<td>Purplish blue</td>
<td>0.15</td>
</tr>
<tr>
<td>Moraceae: <em>Ficus caronata</em>, Reinw</td>
<td>Blue</td>
<td>0.09</td>
</tr>
<tr>
<td>Moraceae: <em>Ficus vaccinioides</em>, Hemel et King</td>
<td>Purple</td>
<td>0.21</td>
</tr>
<tr>
<td>Phytolaccaceae: <em>Phytolacca acinosa</em>, Roxb. var. <em>esculenta</em>, Maxim.</td>
<td>Blue</td>
<td>0.55</td>
</tr>
<tr>
<td>Pinaceae: <em>Tsuga chinensis</em>, Pritzel</td>
<td>Purple</td>
<td>0.39</td>
</tr>
<tr>
<td>Polypodiaceae: <em>Odontosoria chinensis</em>, J. Sm. var. <em>tenuifolia</em> Makino</td>
<td>Blue</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.76</td>
</tr>
</tbody>
</table>
### TABLE OF FRESH MEDICINAL PLANTS AMONG ONE HUNDRED SPECIES INVESTIGATED WHICH HAD POSITIVE REACTION TO EHRLICH’S REAGENT SPRAYED—contd.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Solvent B. A. W.</th>
<th>Ehrlich's R.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Rf. Value</td>
</tr>
<tr>
<td>Polypodiaceae: <em>Davallia mariesii</em> Moore</td>
<td>Blue</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>Polypodiaceae: <em>Asplenium nidus</em> L.</td>
<td>Purple blue</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.74</td>
</tr>
<tr>
<td>Polypodiaceae: <em>Dryopteris subdecipiens</em>, Hayata</td>
<td>Purple</td>
<td>0.14</td>
</tr>
<tr>
<td>Polypodiaceae: <em>Asplenium nidus</em> L.</td>
<td>Purple blue</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Purple</td>
<td>0.74</td>
</tr>
<tr>
<td>Primulaceae: <em>Lysimachia decurrens</em>, Forst. f.</td>
<td>Purple</td>
<td>0.63</td>
</tr>
<tr>
<td>Saxifragaceae: <em>Deutzia taiwanensis</em>, (Maxinow), Schneid</td>
<td>Blue</td>
<td>0.45</td>
</tr>
<tr>
<td>Taxodiaceae: <em>Cryptomeria japonica</em> D. Don.</td>
<td>Blue</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>Ulmaceae: <em>Celtis formosana</em> Hayata</td>
<td>Purple</td>
<td>0.43</td>
</tr>
<tr>
<td>Urticaceae: <em>Pellionia scabra</em> Benth</td>
<td>Blue</td>
<td>0.50</td>
</tr>
<tr>
<td>Verbenaceae: <em>Callicarpa pilosissima</em> Max.</td>
<td>Purple</td>
<td>0.69</td>
</tr>
</tbody>
</table>

### Summary

1. The medicinal plants were collected from the high mountains in Taiwan. These species belong to sixty families.

2. Paper chromatography indicated that sixty species contained alkaloids. The following alkaloidal reagents were used in preparing the chromatograms:—
   
   (a) Dragendorff's reagent
   
   (b) Alcoholic iodine T. S.
   
   (c) Iodo-platinate T. S.
3. Indole compounds were indicated in twenty-nine species sprayed with Ehrlich's reagent.

4. The chemical constituents of the wild plants differ from the cultivated species.

5. Isolation of crystals from the plant (No. 2040) a member of the Liliaceae family, yielded two crystalline compounds with melting points 116-117°C and 107-109°C.

6. Screening of anti-tumourous tests for plant extracts of all species had been carried out at the National Cancer Institute in the U. S. A.

7. In our laboratory the presence of saponins had been proved in all of the plant extracts which were effective in the anti-tumourous test.

8. There were nine fresh plants containing both the alkaloids and the indole compounds simultaneously.
HAYATIN AND OTHER
ALKALOIDS OF
Cissampelos pareira Linn

BY

S. BHATTACHARJII
AND
M. L. DHAR

Central Drug Research
Institute, Lucknow, India.

Cissampelos pareira Linn. (N. O. Menispermaceae) is a reputed drug in Ayurvedic medicine. A specimen of the plant of South American origin was examined by Wiggers (1840) who reported the presence of an amorphous alkaloid, cissampeline (pelosine). Schötz (1896) established that pelosine was identical with crystalline l-bebeereine, isolated earlier from Nectandra rodioei (Maclagan, 1843) and later found to be present in a commercial drug powder "Bebeerinum purum", in tube curare (Boehm, R., 1897) and in several species of Chondrodendron.

The roots of Cissampelos pareira are very rich in alkaloids. The total content of alkaloids and their composition can vary considerably according to the region from which the roots are collected. We have examined (Bhattacharjii, Sharma and Dhar, 1956) roots from Kashmir and Uttar Pradesh states of India; the former give 1·8% of total bases and do not contain any l-bebeereine, while the Uttar Pradesh roots have an alkaloid content of 1·2%, of which l-bebeereine constitutes one-third. Again, the yield of hayatin, the alkaloid of special interest to us, is also variable, ranging from 0·25% in Kashmir roots (one sample gave as high as 0·6) to 0·1 in U. P. roots, while Kupchan et al. (1960) in U. S. A. reported only 0·005% from Madras roots, which again contained l-bebeereine as the major constituent.

The powdered roots are extracted with rectified spirit and the extract concentrated under reduced pressure to about one-fifth its volume. On keeping the concentrate in the cold, d-quercitol crystallises out. The concentrate is now defatted with petroleum ether and gradually diluted with 5 per cent. acetic acid until dark, non-basic material does not separate out any more. The clear supernatant is basified with dilute ammonia, the precipitated alkaloids again dissolved in dilute acetic acid, re-precipitated with ammonia and filtered (yield 1·8 per cent.). The ammoniacal filtrate still contain water-soluble, quarternary bases.

The total alkaloid precipitate is a complex mixture of closely related bases and the separation of individual alkaloids involves a step by step process of solvent fractionation and chromatography. The mixture of bases is first exhaustively extracted with ether (fraction A). The ether-insoluble material is triturated with cold sodium hydroxide solution (0·5 per cent.) to remove dark impurities and the alkali-insoluble fraction is washed free of alkali giving a clean, white product. The latter is refluxed
Fig. 1. Isolation and fractionation of total bases
Isolation and fractionation of the total bases

successively with benzene (fraction B) and pure chloroform (fraction C). The chloroform-insoluble fraction is dissolved in a minimum quantity of dilute hydrochloric acid (1:4) with slight warming, and on keeping for a few hours a fairly insoluble base hydrochloride separates out. This hydrochloride mixture is divided into two fractions; one more soluble in hot ethanol (D) and the other less soluble (E).

Separation and characterisation of individual alkaloids

1. Cycleanine.—The ether-soluble fraction (A) is dissolved in benzene with slight warming, leaving an insoluble residue. The benzene solution is chromatographed over alumina. The benzene eluates, on concentration, give needles, C_{35}H_{32}N_{2}O_{6}, m.p. 272-273°, (α)_{D} -25·6° (alcohol); methiodide, m.p. 300-301° (dec.), identified as cycleanine (O,O-dimethyl isochondrod endrime).

2. Hayatidine.—Subsequent elution of the column with benzene containing 1 per cent. methanol gives another crystalline base, m.p. 165-170°, which after chromatography in chloroform over alumina and repeated crystallisation from methanol furnished cubes, m.p. 179-180°, (α)_{D} -109° pyridine, C_{35}H_{34}N_{2}O_{4}; methiodide, m.p. 258-259° (dec.) hydrochloride (powder), m.p. 271 (dec.); ultraviolet absorption spectrum similar to that of l-beberine.

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It is soluble in chloroform and pyridine, less so in alcohol methanol and acetone, and insoluble in ether, benzene and petroleum ether. It does not give any colour with FeCl₃, does not couple with p-nitrobenzene diazonium chloride and the Millon's test is negative. There is no free hydroxyl group. It appears to be a new base and has been named hayatidine.

3. *Hayatin*.—The benzene soluble portion (B) consists almost entirely of hayatin. It is purified by chromatography over alumina and elution with pure chloroform C₁₇H₉₀N₂O₄, m.p. 231-232°, (α)D⁻3° (alcohol), ultra-violet spectrum similar to that of bebeerine, max. at 280 m.p. hydrochloride, m.p. 252-253° (dec.); sulphate, 250-252° (dec.); dimethiodide, 262-263° (dec.); O-methyl dimethiodide, 250-251° (dec).

4. *Hayatin*.—The more soluble hydrochloride fraction (D) is recrystallised from water and then methanol to give needles, m.p. 265-268° (dec).

The hydrochloride is dissolved in methanol and the solution made just alkaline with dilute ammonia when hayatin crystallizes out as colourless, prismatic rods, m.p. 292-295° (dec.). Recrystallisation from pyridine-methanol gives a m.p. 298-303° (dec.), (α)D⁰ (pyridine or HCl). C₃₈H₃₈N₂O₆, ultra-violet max. at 279 m.p.; hydrochloride m.p. 265° (dec.); dimethiodide, 281° (dec.); dimethochloride, 306° (dec.); O, O-dimethyl dimethiodide, 271-292° (dec.).

5. *Orange base*.—The less soluble hydrochloride fraction (E) is recrystallized from water and then propanol, in yellow needles, shrinking at about 195°, melting 237°, (α)D⁻17-5° (H₂O). It gives a single spot on TLC on silica gel. Basification of the hydrochloride solution with sodium bicarbonate gives orange coloured needles, m.p. 183-4° C (dec.).

In addition to these crystalline bases there is much amorphous material which is still under investigation.

Kupchan *et al.* (1960) have reported the presence of *d*-isochondrodendrine also from the Madras roots.

All the alkaloids that have been mentioned up to now are water insoluble bases. In addition, a number of water-soluble bases have been isolated by Khare and Srivastava of the Lucknow University (personal communication) from the ammoniacal filtrate remaining after the precipitation of the tertiary bases. These were precipitated as the water-insoluble reineckates, followed by conversion to the sulphate and then to the chloride. The individual bases were separated as the chlorides by partition chromatography on cellulose, the solvent being ether-methyl ethyl ketone (1 : 5) saturated with 1 per cent. aqueous HCl. Three of these bases have been crystallised and characterised.


*Cissamin chloride*, C₃₆H₂₈N⁺O₅·Cl⁻·½H₂O, m.p., 215-220° (α)D -129° (methanol) picrate, m.p. 157-158°.
Pareirin chloride C$_{34}$H$_{36}$N$^+$O$_4$Cl$^-$ (hygroscopic), shrinks 88°, melts 100-105° (α)$_D$ +120° (methanol), perchlorate, m.p. 110-120°, (α)$_D$ +112° (methanol).

Structure of hayatin

Analysis of hayatin (I) conforms to the empirical formula C$_{16}$H$_{14}$NO$_3$.

Because of its very sparing solubility in organic solvents and in camphor it was not possible to determine its molecular weight. However, the fact that it occurs along with beberine, known to be a bisbenzylisoquinoline alkaloid, and has an ultraviolet absorption spectrum practically identical with that of the latter, suggested that this base may also be a bisbenzylisoquinoline and accordingly should have the molecular formula C$_{36}$H$_{38}$N$_2$O$_6$. The infra-red absorption bands at 3525 and 1225 cm$^{-1}$ suggested the presence of OH and $\equiv$ C-O (aryl ether) groups respectively.

Two out of the six oxygen atoms in the molecule are present as methoxy groups. The base does not give any colouration with ferric chloride and does not dissolve in aqueous sodium hydroxide but its solution in methanolic potassium hydroxide indicates the presence of a phenolic group. Complete methylation with methyl iodide in methanolic alkali furnishes a dimethyl hayatin dimethiodide (II) which established the presence of two OH groups in the molecule. Thus four oxygens are accounted for. Hayatin does not contain any carbonyl or methylenedioxy groups; hence, the remaining two oxygen atoms would appear to be present as ether functions as is the case in other bisbenzylisoquinoline alkaloids. The base contains two N-CH$_3$ groups and the tertiary nature of both nitrogen atoms is established by the nitrous acid test and the formation of a dimethiodide.

Now, chemical evidence for the skeletal structure of the molecule was obtained by a two-stage Hofmann degradation of dimethyl hayatin dimethiodide (II), which gave trimethylamine, identified through its aurichloride, and a non-nitrogenous product, C$_{36}$H$_{38}$O$_4$, m.p. 198-199°, which was found to be identical with O-methyl beberilenine (III) of known structure, obtained by the analogous degradation of dimethyl beberine dimethiodide. This confirmed the bisbenzyltetrahydricoisoquinoline structure for the base and established that the orientation of the oxygen atoms in hayatin was the same as in beberine.

There remained only the determination of the relative positions of the two methoxyl and the two hydroxyl groups. Hayatin gives a positive Millon reaction; according to King (Manske and Holmes, 1954), this would indicate that one of the OH groups must be located either at 4' of the benzyl residue or at 7 of the isoquinoline. But this test alone is not sufficient to decide the position of both the hydroxyls. It was therefore necessary to mark the free OH groups in hayatin by conversion to the corresponding diethyl ether and then to degrade the molecule and locate the marked OH (now OEt) in the smaller degraded units.

Hayatin dimethiodide was ethylated with ethyl iodide in presence of sodium ethoxide and the product (IV) was subjected to two stage Hofmann degradation to give a non-nitrogenous product (V) m.p. 171-172°, C$_{38}$H$_{40}$O$_4$. This was oxidised
with potassium permanganate in acetone solution when two isomeric acids, C_{18}H_{18}O_{9}, were obtained. One of the acids, m.p. 248°, did not depress the m.p. of a sample of 2-methoxy-2'-ethoxy-4, 5, 5'-tricarboxydiethyl ether (VI). The other acid, m.p. 186° could either be 2-methoxy-3-ethoxy-5, 6, 4'-tricarboxydiethyl ether (VII) or its 3-methoxy-2-ethoxy isomer (XII). Our acid depressed the m.p. of an authentic sample of the latter compound (XII), and the infra-red spectra of the two although very similar (as would be expected) were not identical. Hence our acid would be the 2-methoxy-3-ethoxy-isomer (VII). Since the position of the ethoxy groups in these acids would depend on the position of the two OH groups in the parent hayatin, it is quite clear that in hayatin the OH groups are in positions 6 and 4'.

As a sample of the 2-methoxy-3-ethoxy acid was not available for direct comparison its synthesis has been undertaken by the following route:—

![Chemical diagram]

Fig. 3

**Structure of hayatinin**

Hayatinin, C_{37}H_{40}N_{2}O_{6}, (VIII) contains three methoxyl and two N-CH_{3} groups. Methylation with methyl iodide in methanol gives a dimethiodide indicating the tertiary character of both nitrogen atoms. Further methylation of the dimethiodide with methyl iodide in presence of sodium methoxide gives the corresponding monomethyl ether, thereby showing the presence of a single hydroxyl group. Thus four of the six oxygen atoms in the molecule are accounted for. Tests for carbonyl and methylene-dioxy functions were negative; the remaining two oxygen atoms may therefore be present as ether linkages.
The molecular formula of the base, and the resemblance of its ultra-violet absorption spectrum with that of bebeerine would suggest a bisbenzylisoquinoline structure. A negative test for the diphenylene dioxide ring (blue colour with conc. H₂SO₄-HNO₃) indicates that hayatinin does not belong to the menisarine-trilobine group of bisbenzylisoquinoline alkaloids.

Fig. 4

The procedure followed for the determination of its structure is analogous to that employed in case of hayatin. Two-stage Hofmann degradation of O-methyl hayatinin dimethiodide (IX) gives the same non-nitrogenous product (III) m.p. 197-198°, as was obtained both from bebeerine and hayatin, thus establishing that hayatinin also belongs to the bebeerine group in respect of its skeletal structure and the orientation of the six oxygen atoms.

The position of the single hydroxy group was established entirely on the basis of two colour tests and then confirmed by degradative experiments. Hayatinin gives a positive Millon reaction and therefore as stated before the OH group could only be situated either at positions 7 or 4'. It is evident that the location of a hydroxyl at position 7 would not lead to coupling of hayatinin with diazonium salts, but in the event of its presence at 4', coupling would be possible. This was verified by
trying the coupling reaction with a number of benzylisoquinoline bases of established structure: it was found that alkaloids having a OH at 4' couple readily whereas those with OH in the 7 position do not.

**Table I—Reaction of Benzy1tetrahydroisoquinoline Alkaloids with p-Nitrobenzene Diazonium Chloride**

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Position of OH</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beberine</td>
<td>7, 4'</td>
<td>Purple</td>
</tr>
<tr>
<td>N-Methyl cocolaurine</td>
<td>7, 4'</td>
<td>Purple</td>
</tr>
<tr>
<td>1-(4'-Hydroxybenzyl)—6, 7—dimethoxy-N-methyl-tetrahydroisoquinoline</td>
<td>4'</td>
<td>Reddish purple</td>
</tr>
<tr>
<td>Isochondrodendrine</td>
<td>7, 7</td>
<td>Yellow</td>
</tr>
<tr>
<td>Cycleanine</td>
<td>Completely methylated</td>
<td>Yellow</td>
</tr>
<tr>
<td>Phaeanthine</td>
<td>Completely methylated</td>
<td>Yellow</td>
</tr>
<tr>
<td>Hayatinin</td>
<td>7 or 4'</td>
<td>Yellow</td>
</tr>
<tr>
<td>Hayatin</td>
<td>6, 4'</td>
<td>Purple</td>
</tr>
</tbody>
</table>

Since hayatinin does not couple with p-nitrobenzene diazonium chloride, the single hydroxyl must be located at 7.

Confirmation was obtained by marking the OH by ethylation. The resulting mono-ethyl hayatinin dimethiodide (X), on two stage Hofmann degradation, gives a non-nitrogenous product (XI). C_{27}H_{34}O_8, which is oxidised with KMnO_4 in acetone to two crystalline acids: (i) C_{18}H_{18}O_9. H_2O, m.p. 192° which was identified through mixed m.p. determination with an authentic sample as 3-methoxy-2-ethoxy-5, 6,4'-tricarboxydi phenyl ether (XII) and (ii) C_{17}H_{14}O_9. H_2O, m.p. 262–264° which was likewise identified as 2, 2-dimethoxy-4, 5, 5'-tricarboxydi phenyl ether (XIII). The formation of these acids is only possible if the ethoxy group (and hence the OH group) in hayatinin is in position 7.

**Thin layer chromatography**

With a view to the rapid identification of the individual alkaloids in the total bases the thin layer chromatographic behaviour of the pure bases isolated by us, as well as other available benzylisoquinoline alkaloids (sixteen in all), has been studied (Bhatnagar and Bhattacharji, 1965) in four solvents system. But with these solvents it is not possible to obtain a complete resolution of the natural base mixture in *Cissampelos* roots.
Table II—Rf values (× 100) of Bisbenzylisoquinoline Alkaloids on Thin-layer Chromatograph on Kieselgel G.

(Solvent systems: (I) methanol-chloroform (1 : 5); (II) chloroform-diethylamine (9 : 1); (III) benzene-ethyl acetate-diethylamine (7 : 2 : 1); and (IV) dimethylformamide-diethylamine-ethyl acetate-thanol (1 : 1 : 6 : 12)

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>No. of free OH groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Methyl coclaurine*</td>
<td>2</td>
<td>66</td>
<td>82</td>
<td>64</td>
<td>70</td>
</tr>
<tr>
<td>Laudanosine*</td>
<td>—</td>
<td>88</td>
<td>93</td>
<td>88</td>
<td>81</td>
</tr>
<tr>
<td>Papaverine*</td>
<td>—</td>
<td>91</td>
<td>93</td>
<td>84</td>
<td>83</td>
</tr>
<tr>
<td>Dauricine†</td>
<td>1</td>
<td>66</td>
<td>82</td>
<td>64</td>
<td>70</td>
</tr>
<tr>
<td>Magnolone†</td>
<td>3</td>
<td>38</td>
<td>26</td>
<td>21</td>
<td>68</td>
</tr>
<tr>
<td>Berbamine‡</td>
<td>1</td>
<td>61</td>
<td>71</td>
<td>70</td>
<td>74</td>
</tr>
<tr>
<td>Isotetrandine‡</td>
<td>—</td>
<td>58</td>
<td>71</td>
<td>65</td>
<td>72</td>
</tr>
<tr>
<td>Phaeanthine‡</td>
<td>—</td>
<td>85</td>
<td>86</td>
<td>89</td>
<td>72</td>
</tr>
<tr>
<td>Cepharanthine‡</td>
<td>—</td>
<td>82</td>
<td>86</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td>Isochondodendrine‡</td>
<td>2</td>
<td>52</td>
<td>58</td>
<td>38</td>
<td>60</td>
</tr>
<tr>
<td>Cycleanine‡</td>
<td>—</td>
<td>78</td>
<td>87</td>
<td>89</td>
<td>77</td>
</tr>
<tr>
<td>Beberine‡</td>
<td>2</td>
<td>65</td>
<td>66</td>
<td>47</td>
<td>70</td>
</tr>
<tr>
<td>Hayatin†</td>
<td>2</td>
<td>64</td>
<td>68</td>
<td>44</td>
<td>78</td>
</tr>
<tr>
<td>Hayatinin†</td>
<td>1</td>
<td>74</td>
<td>88</td>
<td>66</td>
<td>73</td>
</tr>
<tr>
<td>Isotrilocine‡</td>
<td>—</td>
<td>94</td>
<td>86</td>
<td>86</td>
<td>77</td>
</tr>
<tr>
<td>Hayatinin††</td>
<td>—</td>
<td>76</td>
<td>87</td>
<td>69</td>
<td>76</td>
</tr>
</tbody>
</table>

* Benzylisoquinoline bases.
† Bases containing 1 diphenylether linkage.
‡ Bases containing 2 diphenylether linkages.
§ Base containing 3 diphenylether linkages.
‖ No. of diphenylether linkages not known.

Acknowledgements

This paper presents work carried out jointly with our colleagues Dr. V. N. Sharma (now in National Botanic Gardens, Lucknow), Dr. A. C. Roy, Dr. T. R. Rajagopalan (now in the Delhi University, Delhi) and Mr. A. K. Bhatnagar who have been associated with this problem at different periods.

REFERENCES

Academic Press.
Wiggers, A. (1840), Annalen., 33, 81.
VOACAMINE AND
VOBTUSINE FROM
Voacanga megacarpa
Merr.

BY

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The investigations of Janot and Goutarel (1955 a, 1955 b, 1956), of La Barre and co-workers (1955 a, 1955 b, 1956) on Voacanga africana Stapf; and of other investigators (Renner 1957, 1959; Stauffacher and Seebeck 1958; Rao 1958; Prins and Renner 1961) which led to the isolation of many alkaloids prompted us to undertake chemical studies on Philippine species of Voacanga. Further interest has been created by the report on the cardiovascular activity exhibited by alkaloids of Voacanga species (Quevaviller and Blanpin 1958; La Barre 1960.)

At present, several Voacanga alkaloids belonging to two types are known: (a) the monomeric indoles exemplified by voacangine (Ia), voacristine (Ib), vobasine (IIa) and vobasolin (IIb), and (b) the bisindolie dimers, represented by voacamine (IIia) and voacorine (IIib)
In a previous study we have dealt with the alkaloids of *Voacanga globosa* (Blanco) Merr. (Santos *et al.*, 1961). We are now reporting on the alkaloids of *Voacanga megacarpa* Merr. (Quisumbing and Merrill, 1928). The following scheme shows the method used which led to the isolation of two crystalline alkaloids.
Powdered and dried bark (10.3 kg.) of *Voacanga megacarpa* Merr.

- macerated and percolated with methanol, concentrated under reduced pressure and dried

**Concentrated dried extract**
- extracted with pet. either in Soxhlet

**Pet. ether extract**
- Residue
  - extracted with chloroform in Soxhlet

**Chloroform extract**
- Residue
  - concentrated, evaporated to dryness, incorporated with Supercel, exhausted with 1% HCl
  - Acid extract
    - alkalinified with ammonia, extracted with ether

**Ether extract**
- washed, passed through Na₂SO₄
- ether recovered, extract concentrated

- Crystals (Alkaloid I)
  - m.p. 286°
- Mother liquor
  - dried, taken up with benzene, purified over neutral alumina
  - Benzene recovered, extract concentrated and dried
  - Dried, concentrated benzene extract
    - methanol-ether (1 : 2)

- Crystals (Alkaloid I)
  - m.p. 286°
- Mother liquor and washings concentrated
  - Crystals
    - ether-methanol (1 : 2)
  - Crystals (Alkaloid II)
    - m.p. 212°
Alkaloid I crystallizes out from methanol-ether mixture as slightly yellowish crystals melting at 286° (vac. cap., dec.). Analysis gave the following results: 69.66% C, 7.00% H, 7.29% N. Molecular weight: 754 (osmotic pressure method). Rf value = 0.79 in the system n-BuOH : CH₂COOH. H₂O (4 : 1 : 4) using Dragenthorff as developing agent. [α]D⁰−₀₂ = −322° (c = 1, CHCl₃). IR spectrum: 3335 cm⁻¹, (v.s.) (bonded NH), 3000–2900 cm⁻¹, (CH₃, CH₂), 1610 cm⁻¹ (v.s.) (CO–NH). The infra-red spectrum of the first alkaloid isolated and that of authentic vohtusine are shown to be super-imposable. NMR spectroscopic analysis of the base further substantiates that Alkaloid I is vohtusine.

Alkaloid II crystallizes out from the mother liquor as needle-like prisms melting at 212° (vac. cap., dec.). Analysis: 71.80% C, 7.82% H, 7.61% N. Molecular weight: 716 (Rast.). Rf value = 0.86. [α]D⁰ = −49° (c = 1, CHCl₃). IR Spectrum: 3450 cm⁻¹ (sec. a mine, str.), 2925 cm⁻¹ (CH₂: str.), 1730 cm⁻¹ (v.s.) (acetate), 1467 cm⁻¹ (v.s.) (–C = C–). Comparison of the IR spectrum of Alkaloid II with that of authentic voacamine reveals that both compounds are identical.

In an earlier report (Santos and co-workers 1961) made on the alkaloidal content of Voacanga globosa (Blanco) Merr. no definite conclusions were drawn as to the identity of the two alkaloids isolated. However, the authors reported that the analysis of the ether-insoluble alkaloid indicated that it may be vohtusine, and that the ether-soluble base gave very close analytical data to two alkaloids of the Voacanga species, voacamine or voacaraine.

In view of the above definite identification of the alkaloids isolated from Voacanga megacarpa Merr. as vohtusine and voacamine, which thus became available as authentic materials for comparison purposes, we thought of following up our previous work on the alkaloids of Voacanga globosa (Blanco) Merr. which gave analytical data agreeing with those already reported before. Mixed melting point determinations of the respective alkaloids gave no depression thus definitely establishing now that the two alkaloids previously isolated from Voacanga globosa (Blanco) Merr. were really voacamine and vohtusine.

Recently, Buchi and collaborators (1963) established the structure of voacamine, a dimeric indole alkaloid which was first isolated from Voacanga africana Stapf. That deshydroxy-vobasionol (IIc), a base with an unusual cyclo-octoisindole structure, and voacangine (Ia), a typical ibogaine type base, are the moieties which comprise the dimer, was established after a series of chemical investigations. Djerassi, Percheron and co-workers (1963) confirmed this structure with the application of mass spectrometry.

Vohtusine, one of the alkaloids obtained, was first isolated from Voacanga africana Stapf. and V. thousii Reem and Schultzes (var. obusa K. Schum) by Janet and Goutarel (1955 a). It exhibits a characteristic blue color with nitric acid. The UV spectra of the base showing maxima at 220, 257, 296 and 328 μ. indicates the presence of a chromophore which is not found in voacamine. Selenium dehydrogenation of vohtusine yields quinoline (Schuler, Verbeck and Warren 1958). The chemical structure of the alkaloid is still under investigation.
Acknowledgements

This work has been aided by a financial grant from the National Science Development Board and the National Research Council of the Philippines.

The authors are indebted to Dr. Marvin Gorman of the Lilly Research Laboratories, Eli Lilly and Company, U. S. A., for the IR and NMR spectroscopic determinations of the two alkaloids isolated from Voacanga megacarpa Merr.

Miss Glory Lleander former research assistant of the National Science Development Board participated in the early part of this work.

REFERENCES

ISOLATION OF NIMBIN
AND THE STUDY
OF ITS CHEMICAL
CONSTITUTION

BY

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NEEM (Melia azadirachta Linn.) also called the Indian Lilac, is a large ever-green tree, growing to about 40 to 50 ft. in height and found in many parts of South East Asia. The bark, leaves and fruits of this tree have been used in Ayurvedic medicine from antiquity, and are mentioned in the earliest Sanskrit medical writings as the 'Susruta'. The bark is bitter, and astringent and is used in fever nausea and skin diseases Chopra (1958). The principal crystalline constituent nimbin, obtained from the bark and other parts of the tree has been the subject of recent chemical studies (Mitra, 1956, 1957; Narasimhan, 1959; Sen Gupta et al., 1960). The original molecular formula (C₁₈H₃₆O₉±CH₂) suggested by Mitra (1956) for nimbin was later disputed as C₃₀H₃₈O₉ Narasimhan (1959) and as C₂₉H₃₈O₉ (Sen Gupta et al., 1960). By careful elemental analysis of a number of nimbin derivatives, and by mass and NMR spectra, it was found (Narayanan et al., 1962) that the correct molecular formula of nimbin is C₃₀H₃₈O₉. The functional groups of nimbin were also confirmed by NMR spectroscopy and further chemical evidence. It showed the two carbomethoxy groups at δ 6.27 and 6.36, the acetate at δ 7.97 and the β-substituted furan protons at δ 2.66, 2.73 and 3.65. Two partial structures for nimbin (I) with a trisubstituted double bond and (II) with a cis disubstituted double bond has been suggested before (Sen Gupta et al., 1960). Of these, (II) has now been found to be correct and the ring is confirmed to be six-membered, since in the NMR spectra, nimbin shows a typical AB quartet with doublets centred at δ 3.63 and 4.2 (J 10 cps) which disappears in the spectra of dihydronimbin wherein the conjugated double bond is saturated. On catalytic hydrogenation nimbin absorbs 3 moles of hydrogen and gives hexahydronimbin whose ultraviolet spectrum λ₂₀₈ (ε8500)
indicates the presence of a tetrasubstituted double bond and which titrates with permanganate periodate or iodine monobromide for one double bond. The NMR spectrum of nimbin also shows that it contains a cyclic ether. Nimbin is therefore tetracarboxylic and contains a basic skeleton of 26 carbon atoms.

Recent elucidation of the structure and stereochemistry of bitter principles having a basic skeleton of 26 carbon atoms and a β-substituted furan ring have shown that they are invariably derivable from a triterpene precursor of the apoeuphol type (Gopinath et al., 1961; Hodges et al., 1963). Structure (IX) is now proposed (Narayanan et al., 1964) for nimbin which is readily derivable from apoeuphol (VII) by oxidative cleavage of ring C between C₁₂ and C₁₃ and appropriate oxidations at other sites as indicated in (VIII). This is in complete agreement with all the physical and chemical evidence.

Dihydrornimbin (IV) forms a monooxime whose ultra-violet spectra shows the absence of the R-band for a ketone. This proves that nimbin contains one and only one ketone and hence the 9th oxygen function in nimbin has to be incorporated in an ether linkage. Part structure (II) can be readily accommodated only in ring A of the triterpenoid skeleton. The ready decarboxylation of nimbin on hot hydrolysis which does not take place when the conjugated double bond is hydrogenated, requires the placing of the carboxyl group at C₄ vinylogously β to the C₁-ketone. The alkyl substituent at C₄ has now been shown to be a methyl group by comparing the NMR spectra of 'pyronimbic' acid (III) with that of desacetyl nimbin (XI) since a methyl group on a quaternary carbon atom in desacetyl nimbin (XI) is shifted down to τ 7.9 in ‘pyronimbic’ acid (III).

Dihydrornimbinic acid (V) on treatment with acetic anhydride and pyridine gives a neutral product (VI), C₃₆H₅₈O₈ whose IR and UV spectra show the absence of hydroxyl and ketonic groups but reveal the presence of an enol o-lactone (1754, 1645 cm⁻¹) and a γ-lactone (1773 cm⁻¹) formed by the lactonization of the two carboxyl groups with the enol of the C₁-ketone and the C₈-hydroxyl group. Hexahydrornimbinic acid (XIII) obtained by the hydrolysis of hexahydrornimbin(XII) also gives a similar product (VI) on the same treatment. The C₁-carboxyl group cannot form the enol -δ- lactone with the C₁-ketone as that will involve a double bond at the bridge head. This fixes the position of the hydroxyl group γ- to the C₈-carboxyl group. As the hydroxyl is shown to be secondary by its oxidation to a ketone, the acetate in nimbin is attached to the C₈-carbon atom.
The proton appearing at $\tau 4.77$ (J 3 cps and 12.5 cps) in the NMR spectra of nimbin which moves upfield (about 1 to 2 $\tau$ units) in desacetyl nimbin has to be assigned to the $C_8$-H. One of the protons that couples with $C_8$-H is found at $\tau 5.94$ (J 3 cps). This is assigned to $C_7$ since the chemical shift indicates a proton on a carbon having an oxygen function on it. The coupling pattern of $C_8$-H can then fit in only for $C_5$-$C_6$ diaxial, $C_6$-$C_7$ axial equatorial disposition of the protons in a rigid six-membered ring. The acetic acid side chain from $C_9$ needed for the formation of the enol $\delta$-lactone with the $C_7$-ketone in these cases can readily be accommodated only with the cleavage of ring C between $C_{12}$ and $C_{13}$, in the triterpenoid skeleton. The dilactones could thus be assigned structure (IV) and pyronimibic acid, structure (XIV). The $C_8$ proton appears as a doublet at $\tau 6.34$ (J 12.5 cps) in nimbin, but in pyronimibic acid acetate this doublet shows evidence of further coupling whereas the doublet assigned to $C_1$-H is still sharp. This would mean that the proton which is axially coupled to $C_8$ in allylic and axial to the $\Delta^3$, double bond and couples with $H_3$ (Collins, Hobbs and Sternhell, 1963). This confirms the configurations assigned to the protons at $C_8$, $C_9$, and $C_7$, and shows besides that A/B rings are transfused.

The sharpness of the doublet of the $C_5$ and $C_7$ protons in nimbin indicates that the $C_4$, $C_{10}$ and $C_9$ carbon atoms are quaternary. The methyl groups at these positions appear as singlets at $\tau 8.64$ (6 protons) and 8.71 (3 protons) consistent with their environment. One of these shifts considerably down to $\tau 8.4$ in desacetyl nimbin. In 'pyronimibic' acid (XIV) this shift is to $\tau 7.9$ from 8.1 in its acetate (XV) showing thereby that it is the $C_4$-Me that is involved in these shifts. Such a deshielding of a methyl group by a hydroxyl group requires a 1,3-diaxial or equivalent disposition of the two groups and hence in the case of the $C_4$-equatorial hydroxyl group, it would require the $C_4$ methyl group also to be equatorial as shown in (IX). The $\alpha$-axial orientations of the $C_2$-H-bond (in the trans A/B rings) and the $C_7$-O-bond (in a five-membered ring) would require the $C_{11}$-and $C_9$-methyl groups to be oriented $\beta$ and axial.

Nimbin on lithium aluminium hydride reduction and further dehydrogenation gives (i) 1, 2, 5-trimethylnaphthalene and (ii) a compound regenerated from its crystalline TNB adduct showing UV spectra markedly resembling that of naphtho (2, 3-b) furan. This fully supports the placing of the methyl groups at $C_8$ and $C_{10}$ and the ether oxygen at $C_7$.

The $\beta$-substituted furan ring and the remaining six carbon atoms in nimbin are assigned to ring D to accommodate the following facts. Hexahydonimbin is still unsaturated and shows chemical evidence for the presence of one double bond. Its NMR spectrums shows a methyl group on a double bond ($\tau 8.3$). The splitting of this methyl group into a doublet (J 1.5 cps) clearly visible in nimbin and many of its derivatives is due to long range coupling with $C_{15}$-H. (Pinhey and Sternhell, 1963; Narayanan and Venkatasubramanian 1964). The $C_{15}$-H besides being coupled to $C_{19}$-methyl is also coupled to the adjacent methylene at $C_{18}$ and shows up as a broad triplet at $\tau 4.42$ (J 6.5 cps) in nimbin. On acid catalysed isomerization, hexahydonimbin gives isohexahydonimbin which shows no strong absorption in the ultraviolet above 210 m$\mu$. Nimbin and dihydronimbin on the other hand
on similar isomerization gives iso-
niminbin and iso-dihydroniminbin respectively both having a new
chromophore $\lambda_{\text{max}} = 233$ $\text{m} \mu\epsilon = 9000$
which persists on lithium aluminium
hydride reduction. The NMR spectra
of both the compounds show that the
furan is now conjugated with the
double bond (Govindachari and co-
workers, 1961). On acid treatment, the
double bond in (IX) could move into
conjugation with the furan ring to give
(i) a 13, 17-double bond with the C$_{13}$
methyl on it, or (ii) a 16, 17-double
bond with a proton on C$_{13}$ causing the C$_{13}$
methyl to appear as a doublet
at higher fields or (iii) a 16, 17-double bond with an allylic migration of the ether
from C$_{16}$ to C$_{13}$ as in (XVI). Both (ii) and (iii) but not (i) will have an additional
vinyl proton (C$_{16}$). The NMR spectra of isoniminbin and isodihydroniminbin show
the four methyl groups as sharp singlets at $\tau 8.35$, $8.7$, $8.73$ and $9.05$, and a new
vinyl proton as a narrow triplet at $\tau 4.2$. Hence isoniminbin and isodihydroniminbin
should be regarded as (XVI). As expected of this assignment the coupling constants
and chemical shifts of the protons in the AB rings are not very different from those
of nimbin. This is in complete agreement with structure (XVI) for the D ring of
the iso-compounds. The ORD curve of hexahydroniminbin is very similar to that of
l-ketocholestane and hence structure (IX) would represent the absolute configuration
of nimbin.

Nimbin is thus the first of the naturally occurring C$_{30}$-triterpenoids in which ring
C is oxidised and broken.

REFERENCES

Gopinath, K. W., Govindacheri, T. R., Ruth Parthasarathi, P. C., Viswanathan, N., Arigoni, D.
Ibid., (1956) *ibid.*, 15b, 477.
Narayanan, C. R., Pradhan, S. K., Panchpumar, R. V. and Narasimhan, N. S. (1962). *Chem. and
Ind. (Rev.)*, 1283.
VARIATION OF
THE RESERPINE CONTENT
IN Rauwolfia serpentina
WITH RESPECT TO
LOCALITY AND TIME OF
YEAR

BY

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AND
M. CHIT SEIN

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Chemistry Research Department,
Union of Burma Applied Research
Institute, Rangoon, Burma.

The roots of Rauwolfia serpentina have been used for centuries in Indian medicine as
a sedative and as a cure for insanity. The genus Rauwolfia is widespread in the
tropics and nearly 131 species are known to exist (Mukerji, 1955) of which six species,
namely, R. serpentina, R. densiflora, R. microcarpa, R. ophiorrhizoides, R. rivularis
and R. verticillata are reported to occur in Burma (Hundley, 1956). Rauwolfia
serpentina is distributed widely over Burma, as far north as Myitkyina, east in the
Shan States, south to the Amherst district and west as far as Kalemyo at the foot of
the Chin Hills. The yield of the root drug has been estimated at 118 tons per year.
Prior to the use of this drug on a large scale it is desirable to know the variation of
reserpine content of indigenous R. serpentina roots with respect to locality and time
of year. The project was undertaken as a joint venture between the Government
Silviculturist Department and the Union of Burma Applied Research Institute.
The R. serpentina roots were analysed by the method of Dhar and Bhattacharji (1955)
with the modification that ammonium salt of T. M. B.*., after paper chromatography,
was eluted with 1% ammonia (8 c.c.) and T. M. B. concentration was read from a
standard T. M. B. curve (see Fig.). Reserpine percentage was then calculated using
the expression:

\[
\frac{\text{wt. of weak bases (in mg.)} \times \text{T. M. B. concn} \times 2.87 \times 8}{100}
\]

The roots were also analysed for the total alkaloids content (Indian Pharmacopoeia,
1955) and the analytical results are presented in Table 1.

*3, 4, 6—trimethoxybenzoic acid.
Fig. Calibration curve of 3,6-dihydropyrrolo-triazine in 0.1N hydrochloric acid.
<table>
<thead>
<tr>
<th>Dated of collection (1)</th>
<th>Forest Range (2)</th>
<th>Total alkaloids % (4)</th>
<th>Reserpine % (4)</th>
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131
Table I—Variation of Reserpine content of *R. serpentina* with respect to locality and time of year—contd.

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<tr>
<th>Dated of collection (1)</th>
<th>Forest Range (2)</th>
<th>Total alkaloids % (3)</th>
<th>Reserpine % (4)</th>
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Discussion

The analysis of *R. serpentina* roots has shown that the total alkaloid content varies from 1.2 to 3.1 per cent and the reserpine content from 0.003 to 0.18 per cent. It has also been shown that, on the whole, locality and time of year has little effect on the total alkaloid content and that reserpine content varies with variation in the time of collection. The results indicate that optimum time for collection of *Rauwolfia* is November to December the period by which the plant has flowered and dropped the major portion of its crop of fruit. Apparently total alkaloid content and reserpine content in the indigenous *R. serpentina* could not be correlated.

Acknowledgements

Our thanks are due to Mr. H. G. Hundley, Silviculturist, Forest Department without whose help this investigation would not have been possible. We also thank Chas Pfizer and Co., U. S. A. for a sample of reserpine.

REFERENCES

A REVIEW OF WORK CARRIED OUT ON INDIGENOUS PLANTS

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AND
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INDIGENOUS plant material is the basis of Ayurvedic therapeutics. There is a host of indigenous plants which are reputed to possess therapeutic action and their use in Indigenous Medicine dates back to early beginnings of Ceylon history. There has not been a proper scientific evaluation of these claims and it is quite possible that some at least of the indigenous drugs do possess valuable pharmacological properties. It has been our view that if any system of medicine had held its own for centuries there was something worthwhile at the back of it which warrants systematic scientific investigation.

It is with this view that we undertook our investigations in the field of indigenous plants. Our aim was to isolate the active principles of those indigenous medicinal plants which had first been clinically and pharmacologically screened and found to be therapeutically active. The present review, however, will deal with some of the chemical constituents isolated from indigenous plant material and their structural investigations.

Terminalia chebula (Sinhalese, Aralu) is used in Ayurvedic medicine for practically all diseases which human flesh is heir to and enters into combinations of many of the stock preparations prescribed by the Ayurvedic practitioners. Terminalia chebula is claimed to be a tonic, and laxative. In addition to its therapeutic usage Terminalia chebula finds considerable use in the tannin industry. In the absence of any controlled clinical trials which confirmed any therapeutic actions attributed to it, our attention was directed towards the elucidation of the structure of chebulinic acid, the major tannin, present in Terminalia chebula.

Chebulinic acid was first isolated by Fridolin in 1884. Freudenberg (1919–1927) and his collaborators found that the tannin had a molecular formula \( \text{C}_4\text{H}_3\text{O}_{37} \). It was a monobasic acid and on partial hydrolysis yielded equimolecular proportions of gallic acid, 3 : 6 digalloyl glucose and chebulic acid \( \text{C}_{14}\text{H}_{14}\text{O}_{11} \).

Chebulic acid (I, \( R = \text{H} \)) titrated in the cold as a tribasic acid and on hot titration consumed 4 equivalents of alkali (Schmidt et al., 1947). Schmidt and Mayer (1951) on the basis of the analysis of the crystalline triamide modified Freudenberg's

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formula to C_{14}H_{12}O_{11}. Chebulic acid was methylated by diazomethane to methyl tri-O-methyl chebulate C_{20}H_{24}O_{17}. The ester on hydrolysis afforded tri-O-methyl chebulic acid C_{17}H_{18}O_{11}. Tri-O-methyl chebulic acid (I, R = CH\textsubscript{3}) titrated in the cold as a tribasic acid and on hot titration consumed 4 equivalents of alkali indicating the presence of a lactone ring.

Schmidt et al., (1952) failed to isolate tri-O-methyl phthalic acid (II) by all the oxidative methods employed. However by acid permanganate oxidation tri-O-methyl chebulic acid gave a crystalline acid C_{8}H_{6}O_{8}. It contained 3 COOH groups and one lactone group. The trimethyl ester C_{11}H_{14}O_{8} gave a tetramide C_{8}H_{14}O_{6}N_{4} and a triamide C_{8}H_{11}O_{6}N_{3}. Schmidt and Mayer (1953) proposed the structure n-1 butanol 1 : 2 : 3 : 4 tetracarboxylic acid γ lactone (III) for the acid C_{8}H_{8}O_{11} and considered the 3 : 4 dihydroisocoumarin structure for chebulic acid (I, R = H). Thus during the acid permanganate oxidation the entire side chain was preserved and the aromatic nucleus was oxidized to a single-COOH group. Further the proposed formula was attractive because chebulic acid could be derived from ellagic acid (IV) by the oxidative degradation of one of the phenolic rings.

It is evident that no chemical evidence for the phthalic acid grouping has been advanced in support of Schmidt’s formula for chebulic acid. By the use of alkaline ferricyanide Haworth and de Silva (1951) obtained 3 : 4 : 5 tri-O-methyl phthalic acid in yields approaching 40 per cent thus confirming the aromatic nucleus.
Further evidence for the aromatic nucleus was obtained by the alkali fusion of tri-O-methyl chebulic acid to yield oxalic acid and tri-O-methyl gallic acid. (Haworth and de Silva, 1954).

The production of 3 : 4 : 5 tri-O-methyl phthalic acid together with Schmidt's isolation of n-l butanol 1 : 2 : 3 : 4 tetracarboxylic acid γ-lactone lend support to Schmidt's formula (I, R = H) for chebulic acid.

Schmidt had not considered the formula in with a δ-lactone (V, R = H) which could also fit the experimental facts. The I R spectrum of the crystalline triamide had a sharp band at 1727 cm⁻¹ and a broad intense band at (1666-1695) cm⁻¹. The band at 1727 cm⁻¹ indicated the presence of a conjugated 6-membered lactone ring and the broad intense band at (1666-1695) cm⁻¹ was due to the amide carbonyl group. This clearly eliminated (V) and supported 3 : 4 dihydroisoumarin structure for chebulic acid (I) (Haworth and de Silva, 1954).

Further confirmatory evidence for the structure was obtained by the pyrolysis of tri-O-methyl chebulic acid with copper powder under reduced pressure (Haworth and de Silva, 1954) when 5 : 6 : 7 tri-O-methyl isocoumarin 3-carboxylic acid (VI) and succinic acid were obtained. This could be readily explained on the basis of Schmidt's formula as a type of reversed Michael Reaction.
Terminalia belerica (Sinhalese, Bulu) is the other ingredient which enters into decoctions prescribed by the ayurvedic physicians and its uses are nearly the same as those of Terminalia chebula.

The only isolable compound from Terminalia belerica was gallic acid which was obtained in high yield.

Terminalia arjuna (Sinhalese, Kumbuk)—The bark of the tree is used in the indigenous medicine as an astringent. It is also claimed that the extracts of kumbuk bark possess cardiotonic action.

Continuous extraction of the powdered bark with ligroin yielded a pale yellow solution with suspended solid. The solid was recrystallised from methanol to give a crystalline solid, of molecular formula C_{30}H_{49}O_{3} yielding a monoacetate C_{32}H_{60}O_{4}, and a methyl ester C_{31}H_{59}O_{3}. The crystalline solid was found to be oleanolic acid (VII) by comparison with an authentic specimen.

The orange yellow oil obtained by evaporation of the ligroin extract after careful chromatography in benzene over alumina and elution with benzene methanol yielded "β-Sitosterol" characterised by its bezoate C_{35}H_{54}O_{2} and the acetate C_{32}H_{59}O_{2}.

Continuous ether extraction of the bark afforded the main triterpene C_{29}H_{49}O_{3}, giving a methyl ester C_{31}H_{59}O_{3}. The triterpene was found to be arjunolic acid (VIII) by comparison with an authentic specimen.

![Chemical structures of VII and VIII](image)

Oleanolic acid and arjunolic acid were isolated from the heartwood of Terminalia arjuna by T. J. King (1954).

Dipterocarpus sp.—There are five species of the Dipterocarpus family indigenous to Ceylon and in the present work we have examined two of them namely Dipterocarpus zeylanicus (Sinhalese Hora) and D. glandulosus (Sinhalese Dorana).

D. zeylanicus is a tree about 170 ft. high found in the S-W Wet Zone and the Central and Uva Provinces. The timber is of economic value and the heartwood is claimed to be used in indigenous medicine as an ingredient in decoctions for fever. A light petroleum extract of the powdered bark was evaporated to a small bulk. By careful chromatography through an alumina column followed by extensive
crystallisations from light petroleum a crystalline product was isolated. Elemental analysis indicated a formula $C_{30}H_{20}O_2$; the compound gave a positive response to Liebermann-Burchard reaction and gave a yellow colouration with tetranitro methane. It yielded an oxime $C_{30}H_{18}O_2N$ and a semicarbazone $C_{31}H_{36}N_2O_2$. This compound was found to be dipterocarpol (IX) by comparison of the mps and mixed m.p. as well as the infra red spectra with an authentic specimen made available to us by Dr. Mac Lean (de Silva, Rodrigo and Wijesekera, 1962).

After the removal of dipterocarpol, the bark was then exhaustively extracted with alcohol and the alcoholic extract was run through a column of activated charcoal and then evaporated to a thick syrup. The concentrated alcoholic extract was then treated with acetone and allowed to stand for a few days when a crystalline solid separated out. Recrystallisations from water yielded a crystalline compound melting ca. 133° which analysed for $C_{14}H_{16}O_9H_2O$. Repeated recrystallisation from alcohol yielded the anhydrous product $C_{14}H_{16}O_9$, m.p. 238° (decomp.).

This compound on paper partition chromatography using BuOH/ACOH/H$_2$O gave a single spot with a blue-green fluorescence which turned orange when sprayed with diazotised sulphanilic acid. It contained a lactone ring, one methoxy group, two free phenolic groups and no free carboxyl group. Treatment with ethereal diazomethane gave a di-O-methyl derivative $C_{15}H_{20}O_6$ m.p. 200–201°. Acetic anhydride/NaA afforded the penta-acetate $C_{14}H_{11}O_4$ (OCOCH$_3$)$_5$ m.p. 205–206°. The U. V. spectrum of the di-O-methyl derivative had $\lambda$ max 275 log $\varepsilon$ 3.85, $\lambda$ max 220, log $\varepsilon$ 4.42 and was identical with that of methyl tri-o-methyl gallate. A comparison of the physical and chemical properties of this compound with those of bergenin (X) a colourless crystalline polyphenol isolated from the roots of Bergenia crassifolia the bark of the Corylopsis opicata and the heartwood of Shorea leprosula indicated a possible identity. This was confirmed by a comparison of the mps of the two compounds and their derivatives. This was further confirmed by their I. R. Spectra which were also found to be identical.

There has been no instance reported previously where bergenin has been isolated from any of the Dipterocarpaceae.

\[ \text{D. glandulosus is also found in the S.-W. Zone and yields a balsam known as 'Dorana thel'. This is light brown in colour, resembles honey and is used as a solvent for paints.} \]
Light petroleum extraction of the commercially available bark and hardwood failed to yield any dipterocarpol. However chromatography of the commercially available balsam through alumina followed by several recrystallisations from light petroleum gave dipterocarpol (IX) (de Silva, Rodrigo and Wijesekera 1962.)

It is possible that the bark and heartwood used by us in this case had previously been exploited for its balsam.

*Catharanthus roseus* syn. *Vinca rosea* (Sinhalese, *Minimal*) is a perennial herb about 1½ ft. high and having leaves about 2½ ins. long. The leaves have been used as a household remedy for diabetes.

The defatted leaves were extracted with the boiling acetone and after concentration of the resulting solution to a small bulk, a dark green solid separated which was purified by extensive crystallisation from alcohol. The colourless product mp. 288–290°, analysed for C_{30}H_{49}O_{3} and gave a positive response to the Liebermann Burchardt-reaction.

\[ \text{Diagram of the molecule} \]

With aqueous sodium hydroxide a sparingly soluble salt was obtained which reacted with methyl sulphate to give the methyl ester C_{31}H_{50}O_{3}. The compound was thus identified as a triterpene carboxylic acid and the hydroxyl function of the third oxygen was established by the preparation of the monoacetate with acetic anhydride and basic catalysts and the monobenzoate. With acetic anhydride alone a crystalline compound is obtained with two acetyl groups; this probably is the monoacetate mixed anhydride as it is converted to the monoacetate with aqueous alcohol. A comparison of the physical properties of this acid and its derivatives with those of the relevant known triterpenes indicated a possible identity with ursolic acid (XI). This was confirmed by comparison of the physical properties of the two acids and their derivatives. After the conclusion of these experiments King and co-workers (1955) have reported the isolation of ursolic acid from *Vinca rosea*.

*Knoxia zeylanica* (Sinhalese, *Visadhuliya*) is a small shrub which belongs to the family of Rubiaceae. It is found in most parts of Ceylon particularly in the West and its leaves are used in the traditional medicine as an antidote for snake-bite.
The dried powdered leaves were first extracted with petroleum ether and then with methanol. The petroleum ether extract, after chromatography through alumina yielded a golden brown oil. The oil on hydrolysis with alcoholic KOH gave a sterol C_{29}H_{44}O giving an acetate C_{28}H_{48}O_{2} and a methyl derivate C_{27}H_{46}O.

We propose to give the name Knoxiol for this sterol (de Silva and Wijesekera, 1961). It gave a positive Liebermann-Burchardt reaction and Solkowski reaction and a negative Halpen-Hichs and Noller reactions for triterpenes. The structure of Knoxiol has not been elucidated yet.

The methanolic extract yielded a product which after fractional chromatography through alumina and several crystallisations from alcohol afforded a triterpene Ursolic acid (XI) (de Silva and Wijesekera, 1961).

*Samadera indica* (Simarubaceae)—The investigation of this plant was begun by Mme. J. Polonsky of the Institute de Chimie des Substances Naturelles, Paris. in collaboration with us.

*S. indica* is a tall tree found in certain regions of Ceylon and Java. Its bark furnishes a viscous oil used in indigenous medicine in the treatment of rheumatic arthritis. Previous studies carried out on this plant have referred to the presence of an uncharacterised bitter principle "samaderine" (Vander Marck 1900, Tonningen 1858) in the bark.

In the recent investigations carried out (Polonsky *et al.* 1962) three compounds viz. taraxerone (XII), stigmastanone (XIII) and stigmasterol (XIV) were identified in the petroleum ether percolate. Taraxerone was the major constituent.
Three "samaderines" designated A, B and C were separated from a boiling water extract of the defatted bark together with 2:6-dimethoxybenzoquinone.

The physical properties of the three samaderines are given below:

<table>
<thead>
<tr>
<th>Samaderine</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Yellow</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C_{19}H_{15}O_{6}</td>
<td>C_{19}H_{20}O_{7}</td>
<td>C_{19}H_{24}O_{7}</td>
</tr>
<tr>
<td>(α)_{D} pyridine</td>
<td>−18-6°</td>
<td>+67-5°</td>
<td>+58-5°</td>
</tr>
<tr>
<td>Rf</td>
<td>--</td>
<td>0-7</td>
<td>0-1</td>
</tr>
<tr>
<td>UV Max</td>
<td>290 mpe</td>
<td>290 mpe</td>
<td>295 mpe</td>
</tr>
<tr>
<td>Percent Yield</td>
<td>Log 4-26</td>
<td>Log 3-98</td>
<td>Log 3-63</td>
</tr>
<tr>
<td></td>
<td>0-012</td>
<td>0-03</td>
<td>0-041</td>
</tr>
</tbody>
</table>

The bitter principle of the bark was the samaderine C.

REFERENCES

PHYTOCHEMICAL
STUDIES ON
Holarrhena antidysenterica
GROWN IN BURMA

BY

M. PE THAN, TIN TIN NU,
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AND

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A literature survey shows that a large volume of work has been done on the barks and seeds of Holarrhena both in Europe as well as in India. The European workers have mainly worked on Holarrhena congoensis (Pyman, 1919) while the Indian workers have chiefly investigated Holarrhena antidysenterica, and Holarrhena febrifuga. (Siddiqui and Pillay, 1932; Siddiqui, Misra and Sharma, 1945). The chemical investigation of H. antidysenterica was made by Peacock and Chowdhury (1935) and the authors reported the isolation of a new alkaloid, lettcocine, from the Burmese H. antidysenterica. In recent years there has been a revival of interest in this drug and the isolation of two new alkaloids, trimethylkonkurchine and holarrhesimine, has been reported by Tschesche and co-workers. (Tschesche and Peterson, 1954; Tschesche and Roy, 1956). The isolation, constitution and biological activity of kurchi alkaloids have been critically reviewed by Roy and Mukerji (1958).

Holarrhena antidysenterica Wall, a member of the family Apocynaceae, grows wild all over Burma and the yield of stem-barks of the drug has been estimated at 500 tons per year. Confusion could be encountered about the identity of this plant as it could be often mistaken for or adulterated with another different species, Wrightia tomentosa Roem. The bark which is official in India is required to contain not less than 2 per cent of total alkaloids and kurchi bismuth iodide is a preparation much used for chronic amoebic dysentery. A preparation of the alkaloid, conessine, in the form of dihydrochloride, has also been used (Mukerji, 1961). The bark of Holarrhena is also used in Burma as a remedy for dysentery and as a febrifuge. As Holarrhena species possess curative properties in the treatment of amoebic dysentery, it was thought desirable to undertake a research project to study the suitability of indigenous Holarrhena for the preparation of Holarrhena alkaloidal salts, the clinical tests of which would be studied later. The present paper describes the experimental results carried out in this connection.
The *H. antidysenterica* samples collected from the Pegu Forest Division, were analysed by the method given in the Indian Pharmaceutical Codex (1961) with a view to study the suitability of the raw material and the analytical results are represented in Table I.

<table>
<thead>
<tr>
<th>Locality and Date of collection</th>
<th>Approximate age of plant</th>
<th>Part of plant material used</th>
<th>per cent of total alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonshwegan 10.7.61</td>
<td>10 years</td>
<td>Stem bark</td>
<td>1.88</td>
</tr>
<tr>
<td>Tsemiingon 18.4.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>3.77</td>
</tr>
<tr>
<td>Pwesiagiyon 19.4.62</td>
<td>5-6 years</td>
<td>Stem bark</td>
<td>3.16</td>
</tr>
<tr>
<td>Pwesiagiyon 19.4.62</td>
<td>5-6 years</td>
<td>Stem wood</td>
<td>0.52</td>
</tr>
<tr>
<td>Thabyegon 24.4.62</td>
<td>1-2 years</td>
<td>Leaves</td>
<td>0.83</td>
</tr>
<tr>
<td>Thabyegon 24.4.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>3.47</td>
</tr>
<tr>
<td>Kyatchaung 27.4.62</td>
<td>1-2 years</td>
<td>Leaves</td>
<td>0.48</td>
</tr>
<tr>
<td>Kyatchaung 27.4.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>3.06</td>
</tr>
<tr>
<td>Sitpinseik 3.5.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>2.96</td>
</tr>
<tr>
<td>Kadok 3.5.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>3.05</td>
</tr>
<tr>
<td>Hmaw-in 8.5.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>3.04</td>
</tr>
<tr>
<td>Egayitpin 10.5.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>2.83</td>
</tr>
<tr>
<td>Nyaungbintha 14.5.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>2.07</td>
</tr>
<tr>
<td>Kyungon 23.5.62</td>
<td>1-2 years</td>
<td>Stem bark</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Dilute mineral acids, ether-alcohol mixture (Schroff and Dhir, 1939; Siddiqui and Pillay, 1932; Siddiqui *et al.*, 1945; Peacock and Chowdhury, 1935) and ammoniacal alcohol (U Sein Gwan, 1959) have been employed in the extraction of *H. antidysenterica* bark. Benzene, 95 per cent ethyl alcohol and dilute sulphuric acid have been tried out in the extraction of Holarrhena bark in order to develop optimum process conditions for the preparation of Holarrhena alkaloidal salts and the small scale extraction processes are given below.
(a) Extraction of Holarrhena with benzene as solvent

The air-dry coarsely powdered stem barks of *H. antidysenterica* (100 g) were moistened with water (75 ml) and thoroughly triturated with calcium hydroxide (20 g) and the mixture was extracted with warm benzene (100 ml) for 6 hr. The extract was drained off and the crude drug was extracted four times more with the same volume of the solvent. The benzene extracts were then combined, and concentrated under reduced pressure, to a small volume (150 ml). The benzene concentrate was shaken with dilute hydrochloric acid solution (3 × 50 ml); the acid extracts were combined, basified with dilute ammonia solution and the alkaloidal bases were collected in chloroform (4 × 250 ml). Removal of chloroform afforded a brown gummy residue which was dried to constant weight and the weight of total alkaloids was determined.

(b) Extraction of Holarrhena with 95 per cent ethyl alcohol as solvent

The same sample (100 g) was moistened with dilute ammonia solution (75 ml) and the basified drug was extracted with warm 95 per cent ethanol (1 L) for 5 hr. The extract was drained off and the marc was extracted three times more with the same volume of alcohol. The alcoholic extracts were combined and distilled off under reduced pressure to a small volume (50 ml). The alcoholic concentrate was acidified with dilute hydrochloric acid solution (30 ml) with continuous stirring and the resulting solution was kept in the ice box overnight. The insoluble matter that separated out on standing was removed by filtration and the alkaloids were collected in chloroform after basification of the filtrate with dilute sodium carbonate solution. The chloroform solution was distilled off under vacuum and the dark brown gummy residue thus obtained was dried to constant weight and the weight of total alkaloids was recorded.

(c) The extraction of Holarrhena with dilute sulphuric acid as solvent

The powdered Holarrhena sample (100 g) was moistened with dilute ammonia solution (40 ml) and allowed to stand for ½ hr. The basified drug was macerated with distilled water (1 L) for 2 hr. The supernatant water extract was drained off and the water-soluble impurities were extracted by percolation with more distilled water (1.6 L). The marc was then macerated with 10 per cent sulphuric acid (1 L) for 2 hr, the acid extract was drained off and the remaining alkaloids were extracted by percolation with more dilute acid until complete extraction of alkaloids was effected. The total acid percolate (2.5 L) pale red in colour was basified with dilute ammonia solution and the alkaloids were collected in chloroform. (4.5 L) Removal of chloroform under vacuum gave a pale yellowish brown residue which was dried to constant weight and the weight of alkaloidal bases was recorded. The aforementioned experiments were repeated and the experimental results are presented in Table 2.
TABLE 2—COMPARISON OF YIELD OF TOTAL ALKALOIDS OF Holarrhena BY USING BENZENE, 95 PER CENT ALCOHOL AND 10 PER CENT SULPHURIC ACID AS SOLVENTS

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Yield of total alkaloids g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzene</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
</tr>
<tr>
<td>95% alcohol</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
</tr>
<tr>
<td>10% sulphuric acid</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>1.62</td>
</tr>
</tbody>
</table>

After the small scale extraction experiments, larger scale extraction of Holarrhena using 95 per cent ethanol and 10 per cent sulphuric acid as solvents was attempted and the Holarrhena bismuth iodide salts were prepared. Benzene could not be employed for a larger scale extraction of Holarrhena as the solvent is not available locally. And for the preparation of Holarhena total alkaloids hydrochlorides, ethanol was used as solvent for the extraction of Holarhena. The larger scale extraction processes are given below:

Process No. 1—Semi-Pilot Plant scale extraction of Holarrhena antidysenterica using 95% alcohol as solvent and preparation of Holarrhena bismuth iodide

(a) Extraction of Holarrhena

The air-dry coarsely powdered Holarrhena antidysenterica stem barks (9 Kg) were moistened with 10 per cent ammonia solution (4.5 L) and kept aside for 1 hr. The basified drug was charged into the extractor wherein it was extracted with warm 95 per cent ethanol (52 L) with continuous stirring, for 4-5 hr. The extract was drained off and the marc was extracted with the same volume of the solvent four times more. A sample of the fifth extraction usually shows only a slight opalescence when it was evaporated to dryness, acidified and treated with Mayer’s reagent. The alcoholic extracts were combined, transferred to the still and concentrated to a small volume under vacuum of 27–27.5 inches/Hg. The concentrated alcoholic solution was then acidified with dilute hydrochloric acid solution with continuous stirring and the resulting solution was kept in the ice-box overnight. The insoluble matter which principally consisted of resins, separated out and this was removed by filtration. The filtrate was then basified with strong ammonia solution (2 L) and the precipitated total alkaloids, light yellowish brown in colour, were collected in chloroform (5L). The chloroform solution was washed with water, dried over anhydrous sodium sulphate and the solvent was distilled off under reduced pressure. The alkaloid residue was dissolved in a little absolute ethanol and the solution was evaporated to dryness under vacuum until the residue was void of chloroform. The experiment was repeated twice and the Holarrhena bismuth iodide preparation was attempted (Chopra, 1958).
(b) Preparation of Holarrhena bismuth iodide

The residue from above was dissolved in 95 per cent ethanol (500 ml) and titrated to neutrality with N hydrochloric acid solution and freshly prepared Dragendorff's reagent (1·9 L) was slowly added to the solution of the hydrochlorides, with gently stirring, yielding bright orange-coloured Holarrhena bismuth iodide precipitate. The precipitate was filtered and washed with distilled water several times until the washings were almost neutral to litmus. The Holarrhena bismuth iodide was then dried in the vacuum oven, and was analysed for total alkaloids, iodine and bismuth content determinations. (Indian Pharmacopoeia, 1955; Schroff and Dhir, 1939).

Process No. 2—A larger scale extraction of Holarrhena antidysenterica using dilute sulphuric acid as solvent and preparation of Holarrhena Bismuth Iodide.

The coarsely powdered Holarrhena stem barks (3 Kg) were thoroughly mixed with 10 per cent ammonia solution (1·5 L) and kept for about 1 hr. The basified drug was then macerated with distilled water for 2 hr and the supernatant water extract was drained off and discarded. The water-soluble impurities were exhaustively extracted by continuous percolation with more distilled water (20 L). The marc was then macerated with 10 per cent sulphuric acid for 2 hr, the acid extract was drained off and the remaining alkaloids were extracted by percolation with more acid solvent until complete extraction of alkaloidal bases was effected. (44 L) Freshly prepared Dragendorff's reagent (870 ml) was slowly added to the solution of the alkaloids sulphates with gentle stirring, yielding pale red-coloured Holarrhena bismuth iodide precipitate. The precipitate was allowed to settle and the supernatant liquid was removed by decantation. The precipitate was collected in a Buchner funnel, washed thoroughly with distilled water and dried in vacuum oven at 40° C. The dried precipitate was then analysed for total alkaloids, iodine and bismuth content determinations. The experiment was repeated with 5 Kg quantity of Holarrhena and the results are represented in Table 3.

**Table 3—Yield of T. A. and Analysis of Holarrhena B. I. obtained from Process No. 1 and 2**

<table>
<thead>
<tr>
<th>Quantity of Holarrhena</th>
<th>Solvent</th>
<th>Yield of T. A. g.</th>
<th>Yield of Holarrhena B. I. g.</th>
<th>Percent of T.A.I.P. method</th>
<th>Percent of T. A. I., Bi. Schroff and Dhir's method</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 Kg.</td>
<td>ethanol</td>
<td>126·0</td>
<td>232·7</td>
<td>24·4</td>
<td>28·3 47·8 20·6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24·6</td>
<td>28·1 47·6 20·5</td>
</tr>
<tr>
<td>9 Kg.</td>
<td>ethanol</td>
<td>143·6</td>
<td>277·0</td>
<td>26·4</td>
<td>30·2 45·6 19·8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26·3</td>
<td>30·3 45·4 19·7</td>
</tr>
<tr>
<td>9 Kg.</td>
<td>ethanol</td>
<td>187·3</td>
<td>282·3</td>
<td>23·2</td>
<td>31·6 45·8 21·5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23·4</td>
<td>31·3 45·4 21·2</td>
</tr>
<tr>
<td>3 Kg.</td>
<td>10% H₂SO₄</td>
<td>121</td>
<td></td>
<td>14·6</td>
<td>17·4 42·1 37·0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14·4</td>
<td>17·7 42·4 36·7</td>
</tr>
<tr>
<td>5 Kg.</td>
<td>10% H₂SO₄</td>
<td>234·6</td>
<td></td>
<td>11·86</td>
<td>15·52 44·8 29·63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11·88</td>
<td>15·43 44·7 29·65</td>
</tr>
</tbody>
</table>

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Process No. 3—*Extraction of H. antidysenterica and preparation of Holarrhena total alkaloids hydrochlorides*

The air-dry, coarsely powdered stem barks of *H. antidysenterica* (10 Kg) were exhaustively extracted with warm 95 per cent ethanol in the usual manner and the alcoholic concentrate (5·5 L) which was mixed with 10 per cent hydrochloric acid solution (3·5 L) with continuous stirring and filtered. The filtrate was then raised to pH 9·6 with strong ammonia solution and the precipitated alkaloids were collected in chloroform (6 L). Removal of chloroform afforded a dark brown resinous residue (227 g). To effect purification of the crude mixture, it was redissolved in dilute hydrochloric acid solution, filtered and the filtrate was basified with strong ammonia and the alkaloids were taken up in chloroform. Removal of the solvent under reduced pressure afforded a brown resinous residue (230 g). The residue was then dissolved in dry acetone (2 L) and the solution was cooled with ice to 10° C. A stream of dry hydrochloric acid gas was passed with continuous stirring until the liquid showed reaction to Congo test paper. (Hammerslag, 1950). As the suspension of the material became thicker more dry acetone was added. (1 L) Stirring was continued for ½ hr and the material was filtered, washed with dry acetone and dried in vacuum oven at 45° C (140 g). The experiment was repeated with 10 and 15 Kg quantities of *Holarrhena* and the T. A. hydrochlorides were assayed according to the Indian Pharmacopoeia method of assay for K. B. I. The results are presented in Table 4.

**Table 4—Yield of T. A., T. A. Hydrochlorides and per cent of T. A. in T. A. Hydrochlorides of *H. antidysenterica***

<table>
<thead>
<tr>
<th>Quantity of Holarrhena</th>
<th>Yield of T. A.</th>
<th>Yield of T. A. hydrochlorides</th>
<th>Per cent of T. A. in T. A. salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Kg.</td>
<td>195 g.</td>
<td>132 g.</td>
<td>58·05</td>
</tr>
<tr>
<td>10 Kg.</td>
<td>230 g.</td>
<td>140 g.</td>
<td>58·2</td>
</tr>
<tr>
<td>15 Kg.</td>
<td>262 g.</td>
<td>220 g.</td>
<td>63·5</td>
</tr>
</tbody>
</table>

*Analysis of Holarrhena Bismuth Iodide from Process No. 1, Process No. 2 and ‘Anabin’ tablets by paper chromatography.*

Holarrhena bismuth iodide powder from Process No. 1 (1 g) was triturated well with 2N sodium hydroxide solution (10 ml) and transferred to a separator and extracted with chloroform (5 × 20 ml). The total chloroform solution was then washed with water and the solution was evaporated to dryness under vacuum. The residue was dissolved in absolute ethanol (5 ml) and the solution was evaporated to dryness again. The residue thus obtained was designated T. A. 1. Holarrhena bismuth iodide powders from Process No. 2 and the ‘Anabin’ tablets (Bengal Chemical and Pharmaceutical works, Ltd., Calcutta, India) were treated in a similar
manner and the residues obtained were designated T. A.-2 and T. A.-3 respectively. The residues were then dissolved in suitable volumes of chloroform and with the help of micro-pipette aliquot portions of the solutions (0.03-0.06 ml) were applied to Whatman filter paper No. 1, buffered with M/10 phosphate buffer of pH 5.4 and the chromatogram was developed overnight at room temperature, with water saturated n-butanol, in an atmosphere saturated with water and n-butanol. The alkaloidal spots were then located by spraying the chromatogram with modified Dragendorff’s reagent. (Munier and Machebouef, 1953). The alkaloidal distribution pattern of the paper chromatogram is shown in the figure.

![Chromatogram Image]

**Isolation of conessine from H. antidysenterica**

Coarsely powdered *H. antidysenterica* stem barks were basified with dilute ammonia solution (3 L) and extracted with warm 95 per cent ethanol as usual until complete extraction of the alkaloids was effected. The total alcoholic extracts were concentrated under reduced pressure to a small volume (1.7 L). The alcohol concentrate was treated with 10 per cent hydrochloric acid solution with stirring and the resulting solution was kept in the ice-box overnight. The insoluble matter that separated out was removed by filtration and the filtrate was extracted with chloroform to remove resins and colouring matter. The resulting acid solution was filtered again and the filtrate was basified with saturated sodium carbonate solution. The precipitated alkaloids were repeatedly extracted with petroleum ether b.p. 60-80° C. (ca 3 L) and the solvent was distilled off under reduced pressure. After crystallisation from acetone the residue yielded 9.8 g of crude conessine.

The crude conessine had m.p. 108°, which was raised to 122-124° on repeated recrystallisation from acetone. The m.p. was not depressed on admixture with authentic conessine. It also afforded a picrate, shining needles from aqueous ethanol, and had m.p. 116-120°, which on repeated recrystallisation from dilute ethanol, was raised to 224-226°. The m.p. of the picrate was undepressed on admixture with conessine picrate m.p. 222° prepared from the authentic conessine. A chloroplatinate and an auric chloride prepared from the base had m.p. 269° and 184-185° respectively and the derivatives on admixture with conessine chloroplatinate
and conessine aurichloride prepared from the authentic conessine sample, showed no melting point depression. The homogeneous nature of the base was also checked by paper chromatography.

Discussion

Young Holarrhena antidysenterica stem barks were shown to contain a fairly high percentage of total alkaloids. Paper chromatography has shown that both the indigenous H. antidysenterica and the foreign Holarrhena preparation (Bengal Chemical and Pharmaceutical Works Ltd., Calcutta, India) have almost identical alkaloidal distribution patterns. For the analysis of Holarrhena bismuth iodide the titrimetric method of the Indian Pharmacopoeia gives reliable and concordant results while the gravimetric method of Schroff and Dhir (1939) affords rather high percentage of total alkaloids. Schroff and Dhir’s method, however, gives concordant results in the estimation of iodine and bismuth content. The Holarrhena Bismuth Iodide from process No. 1, was found to conform to the Indian Pharmacopoeia standard requirements.

The red-coloured Holarrhena bismuth iodide from Process No. 2 was not up to the I. P. requirements as the material contained a low percentage of total alkaloids. The use of dilute acids, although inexpensive, might not be suitable for the preparation of Holarrhena bismuth iodide, from the indigenous young Holarrhena plants. The total alkaloids hydrochlorides from process No. 3 were found to be rather deliquescent and contained 58–64 per cent of total alkaloids. The Holarrhena stem barks were shown to contain 0.2 per cent of conessine, the identity of which was proved by the preparation of derivatives and paper chromatographic behaviour.

Acknowledgements

We thank Dr. M. M. Gale for his help, interest and advice in the early stages of this work and Professor Dr. R. Tschesche for a sample of chromatographically pure conessine. For the collection and identification of plant material our thanks are due to Systematic Botanist, U Hla Maw of U. B. A. R. I. without whose help this phytochemical work would not have been possible.

REFERENCES

Mukerji, B. (1951). The Indian Pharmaceutical Codex, 1, 135.
U Sein Gwan (1961). Private communication. (Medical College I, Faculty of Medicine, University of Rangoon.

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CARBON-14 BIOSYNTHESIS
CHAMBERS FOR
STUDIES WITH
MEDICINAL PLANTS

BY

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APPLICATIONS of tracer methodology in biology and medicine have resulted in unprecedented advances in our knowledge of the structural and functional aspects of living organisms and man in normality and disease. Processes such as photosynthesis are today understood in intimate detail only as a result of application of radiotracer techniques (Bassham, 1962; Calvin and Bassham, 1962). The biosynthesis of steroids and terpenoids is yet another beautiful example where the application of these elegant techniques has led to an almost complete understanding of the biosynthetic pathways in a brief span of time (Wright, 1961). The present day knowledge of the in vivo transformations of steroidal hormones is largely a product of experimentation involving tracer methods (Engel and Langer, 1961). It would not be possible here to attempt a summary of all the areas of investigation in biology and medicine, where tracer techniques have been successfully employed. Instead, we will confine ourselves to the field of medicinal plants and outline briefly the application of tracer methods in the study of plant biosynthesis, formation of uniformly C\(^{14}\)-labelled essential principles of plants and their metabolic fate in vivo.

A study of plant biosynthesis and metabolism of drugs can be carried out by using specifically or randomly H\(^{3}\)labelled or specifically or uniformly C\(^{14}\)labelled compounds as precursors. Alternatively plant biosynthesis can be studied by growing plants in an atmosphere of C\(^{14}O_2\). Both approaches are complementary to each other. The basic question in the study of plant biosynthesis is to know how CO\(_2\), the one carbon unit, is converted into alkaloids, steroids and other materials. Also it is important to find out the intermediates involved in the various transformations leading to the final products. Real progress in this field began when organic compounds labelled with carbon-14 became available and as a result, during the last decade the biosynthetic pathways leading to the formation of alkaloids belonging to Datura, Narcissus, Nicotiana, Papaver, Rauwolfia and a number of other plant genera have been investigated and understood in great detail. The relationships
between the possible precursors, e.g., ornithine, lysine, putrescine, tyrosine, norlaudanosine, tryptophan, etc., on the one hand and a variety of alkaloids on the other have been presented in some excellent reviews (Dawson, 1960; Battersby, 1961, 1963; Mothes and Schutte, 1963; Barton, 1963; Leete, 1963; Ramstad and Agurell, 1964); Similarly, the relationship between acetate and diosgenin in *Dioscorea floribunda* (Heftmann, Bennett and Bonner, 1961) and mevalonic acid and sapogenins and sterols in *Dioscorea spiculiflora* (Bennet et al., 1963) has been studied by using C\textsubscript{14} labelled precursors.

Important contributions to the problem of plant biosynthesis have been made by growing plants in C\textsubscript{14}O\textsubscript{2} atmosphere and by studying the rate of incorporation in different constituents and in different parts of the same constituent. Rapoport and co-workers have carried out painstaking and systematic work on the biosynthesis of morphine alkaloids in *Papaver somniferum* (Rapoport, Sternitz and Baker, 1961) and of nicotine in *Nicotiana glutinosa* (Alworth, De Selms and Rapoport, 1964) from C\textsubscript{14}O\textsubscript{2}. They have demonstrated that in case of *Papaver*, the formation of alkaloids follows the sequence thebaine-codeine-morphine. In case of *Nicotiana*, the formation of nicotine from C\textsubscript{14}O\textsubscript{2} has been shown independently both in root and shoot regions. Further, Rapoport and his collaborators have shown that pyridine ring acquires an early and high specific activity and these studies strengthen the earlier findings that the pyridine ring should arise from the simple precursors such as acetate, propionate, succinate, aspartate, glycerol, etc. The relatively slow incorporation of carbon into pyrroline has been interpreted as consistent with the earlier reports that amino acids, e.g., glutamic acid, ornithine, proline, etc., are the precursors of this ring.

Important advances in our knowledge of drug metabolism have been attained by using specifically and uniformly labelled drugs. Metabolic fate of specifically labelled morphine (Ellison *et al.*, 1963; Elliot, Tolbert, Adler and Anderson, 1954) codeine (Adler *et al.*, 1955; Way and Baker, 1962; Adler, 1963) nicotine (Mckennis *et al.*, 1962) and reserpine (Numore *et al.*, 1960) etc., has been studied in considerable detail. Using specifically labelled precursor, it is quite convenient to follow the changes at the site of the label, e.g., trans or demethylation using N or O-methyl labelled materials. But for a thorough study of all the metabolites that may result from a drug, the use of a uniformly labelled material offers the obvious advantage that it is possible to follow all the degradation products in minute quantities. By combination of chromatographic and tracer techniques it is possible to isolate and follow the metabolic products and evolve a pattern of metabolic fate of various drugs. Using uniformly labelled nicotine (Ganz *et al.*, 1951; Bennet *et al.*, 1954; Owen and Larson, 1958) formation of a number of metabolites was indicated in animal experiments. Using uniformly labelled morphine Rapoport and co-workers (Rapoport *et al.*, 1963) confirmed their earlier finding (using N-C\textsubscript{14}H\textsubscript{3} morphine) that the patterns of excretion in addicts and non-addicts are almost similar. Okita *et al.* (1955) carried out blood level studies and rate of disappearance of biosynthesized C\textsubscript{14} digitoxin in cardiac patients.
From the foregoing it is clear that any programme of work dealing with growing plant material in C¹⁴O₂ atmosphere serves a two-fold purpose: (i) to obtain an insight into the problem of biosynthesis as a whole and (ii) to produce uniformly labelled essential principles of plants which would greatly facilitate metabolic studies. In fact, a facility like this can also be used for investigating some important physiological problems, relating to plant growth, flowering, mineral nutrition and evaluation of short term photosynthetic efficiency. With this in view the fabrication of a C¹⁴O₂ biosynthesis facility has been undertaken in Trombay. Several such facilities have been fabricated in different parts of the world, e.g., Argonne National Laboratory, Argonne III U. S. A. (Scully et al., 1956); Soil and Water Conservation Research Division of the U. S. Agriculture Research Service, Washington D. C., U. S. A. (Smith et al., 1962); Rothamsted Experimental Station, Harpenden, England (Jenkinson, 1960); Bio-organic group, University of California, Berkeley, California. U. S. A. (7); Radiological Nutritiology Laboratory, Medical College of Virginia. Richmond, Va., U. S. A.; Danish Atomic Energy Commission Research Establishment, Ris, Denmark (Anderson et al., 1961); and the University of Bonn, Germany (von Sauerbeck, 1960).

The biosynthesis facility under fabrication in Trombay, which will be the first of its kind in India consists of the following essential parts:

1. System for generating C¹⁴O₂
2. Biosynthesis chambers
3. Ionization chamber—vibrating reed electrometer
4. Infrared gas analyser
5. Time relay circuit for activating a panel of Solenoid valves.
Three chambers $36'' \times 36'' \times 36''$ connected in parallel are being fabricated from $\frac{3}{8}''$ thick perspex and are based on the design described by Rapoport (7) suitably modified to meet our requirements. The hermetically sealed chambers are connected to the ionization chamber and the CO$_2$ analyser by means of $\frac{1}{4}''$ stainless steel tubing.

Air pumps located in the system continuously supply air samples at a desired rate to both the ionization chamber and infra-red gas analyser which assay periodically each chamber atmosphere for C$^{14}$ activity and CO$_2$ concentration respectively. While the gas after analysis is returned to the respective chamber, any mixing of gases is avoided by flushing the sample cell with N$_2$ after each assay. All these changeovers are regulated by a programme controller activating a panel of solenoid valves.

The chambers are provided with an arrangement for the supply of nutrient solutions. A provision for breather expansion bag which will expand or collapse in response to change in pressure inside the chamber also exists.

The relative humidity in the chamber is to be regulated with the help of a humidistat which will activate the flow of cold water through coils on which the extra water vapour will condense.

Illumination of desired spectral range, photoperiod and intensity is to be provided by suitable combination of fluorescent tubes and incandescent bulbs.

In order to avoid fluctuations of environmental temperature, the chambers are to be installed in a temperature controlled room.

The present facility will be a forerunner of a larger and more diversified system which as a national facility will be useful, among others, to:

(a) Foresters for labelling young trees and wood.
(b) Agronomists in the study of the decomposition of labelled humus as a source of plant food, and
(c) Animal physiologists in carrying out short time animal metabolism studies by feeding labelled gases.

In addition the growing of plants in a gaseous atmosphere containing a known radioactivity offers opportunities to radiation botanists for studies on soft beta radiation effects of such plants as well as the collection of radioactive seeds eventually.

REFERENCES


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PRELIMINARY SCREENING TEST FOR ANTIMICROBIAL ACTIVITY ON TAIWAN FRESH PLANTS

BY

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In folk medicines certain plants ground into slush are applied on the surface of the wounds to prevent inflammation. It is said that the alcoholic extract of certain plants might be administered orally to treat snake-bite; and ointment prepared from some other plants might cure trauma or some surgical illness.

We have collected 100-200 gm of different species of fresh Taiwan plants for antimicrobial activity tests. In this series of experiments, 69 families of plants were investigated.

Material

The fresh plants with their antimicrobial activity tested are as follows:

Acanthaceae: Lepidagathis formosensis Clarke
Hypoeostes purpurea, R. Brown

Aceraceae: Acer rubescens, Hayata
Acer serrulatum Hayata

Alangiaceae: Alangium platanifolium Harms

Amaranthaceae: Achyranthes opalata, Yamamoto
Amaranthus spinosus, Linn.
Alternanthera sessilis R. Br.

Anacardiaceae: Rhus javanica, L. var. roxburghii, Merr.
Semecarpus vernicifera, Hayata et Kawakami

Annonaceae: Annona reticulata, Linn.

Apocynaceae: Strophanthus dichotomus DC.

Araceae: Epipremnum mirabile, Schott.
Alocasia macrorrhiza, Schott.
Colocasia antiquorum, Schott. var. esculentum, Schott.
Araliaceae: Heptaleurum arboricolum, Hayata
Aralia bipinnata Blanco
Dendropanax pellucido punctatus Kanch.
Fatsia japonica Decne. et Planch.
Hedera formosana Nakai

Aristolochiaceae: Asarum leptophyllum, Hayata

Aquifoliaceae: Ilex lantecelolia, Hayata

Boraginaceae: Cynoglossum formosum, Nakai

Camelliaceae: Adinandra formosana, Hayata
Eurya acuminata DC.
Ternstroemla gymnana, Sprague

Caprifoliaceae: Viburnum awabucki, K. Koch

Caryophyllaceae: Drymaria cordata, Linn Willd.

Cephalotaxaceae: Cephalotaxus wilsoniana, Hayata

Chenopodiaceae: Chenopodium ambrosioides, Linn.

Combretaceae: Terminalia arjuna, Wight et Arn.

Commelinaceae: Zebrina pendula, Schniz.

Compositae: Wedelia biflora, Linn DC.
Cirsium brevicaule, A. Gray
Aster indicus L.
Myriactis longiperunculata, Hayata
Ageratum conyzoides, Linn
Solidago virga-aurea, Linn
Cnyza japonica, Less
Crepis japonica, Benth
Aster baccharoides, Steetz.
Bidens pilosa, Linn
Sonchus arvensis, Linn
Artemisia vulgaris, Linn. var. indica, Maxim.
Senecio caianensis, Hayata
Eupatorium formosanum, Hayata
Erigon linifolius, Willd.

Convolvulaceae: Pharbitis nil, Chois.
Ipomoea obscura, (Linn) Ker.

Crassulaceae: Bryophyllum pinnatum (Lam) H. urz.

Cupressaceae: Chamaecyparis formosanis, Matsum.

Dilleniaceae: Actinidia chinensis, Planch

Ebenaceae: Diospyros discolor, Willd.
Diospyros erlantla, champ.
Diospyros maritima, Blume.
Maha buxifolia, Pers.

Elaeocarpaceae: Elaeocarpus japonicus, Sieb et Zucc.

Ericaceae: Vaccinium emarginatum, Hayata
Pieris taiwanensis, Hayata
Vaccinium randalense, Hayata
Euphorbiaceae:  
  *Brevina officinalis*, Hemsl.  
  *Homonia riparia*, Lour.  
  *Phyllanthus acdus*, Skeels.  
  *Euphorbia pulcherrima*, Willd.

Fagaceae:  
  *Castanopsis carlesii*, Hayata  
  *Lithocarpus koris*, Hayata  
  *Quercus paucia* a, French.

Gesneriaceae:  
  *Lysionotus warleyensis*, Willmott  
  *Jitatorichum toldami*, Solereds.

Ginkgoaceae:  
  *Ginkgo biloba*, Linn.

Gramineae:  
  *Cox lachryma-jobi*, Linn.

Guttiferae:  
  *Garcinia mangosthana* L.

Hamamelidaceae:  
  *Liquidambar formosana*, Hance

Icacinaceae:  
  *Gonocaryum diospyrosifolium*, Hayata

Labiatae:  
  *Hyptis caprata*, Taq.

Lardizabalaceae:  
  *Stauntonia hexaphylla*, DC.  
  *Akebia longiracemosa*, Matsum.

Lauraceae:  
  *Actinodaphne musaensis*, Hayata  
  *Actinodaphne nantoonensis*, Hayata  
  *Cinnamomum randaiense*, Hayata.  
  *Machilus kusanoii*, Hayata  
  *Machilus pseudolongifolia*, Hayata.  
  *Notaphoebe konishii*, Hayata.  
  *Sassafras randaiense*, Rehder.

Leguminosae:  
  *Cassia torosa* Gaven  
  *Tamarindus indica*, Linn.  
  *Bauhinia championii*, Benth.  
  *Caesalpinia pulcherrima*, (Linn) SW  
  *Desmodium microphyllum* DC.  
  *Phaseolus mungo*, Linn.  
  *Acacia confusa*, Merrill.  
  *Erythrina coralloidendron* L.  
  *Lespedeza cuneata*, G. Don  
  *Aeschynomene indica*, Linn.  
  *Clitoria ternatea*, Linn.

Liliaceae:  
  *Aletris foliata* Franch.

Liliaceae:  
  *Polygonatum japonicum*, Morren et Decaisne. var. *formosanum*, Hayata  
  *Pleomele augustifolia* N.E. Brown.  
  *Sansevieria zeylanica*, Willd. var. *caurentii* Net R. C.

Lycopodiaceae:  
  *Lycopodium carinatum* Desv  
  *Lycopodium clavatum* L.

Magnoliaceae:  
  *Illicium arborescens*, Hayata  
  *Kadsura japonica*, Linn.

Malvaceae:  
  *Abutilon indicum*, Sweet

Melastomataceae:  
  *Barthea formosana*, Hayata  
  *Sarcepyramis delicata*, C. B. Robinson

Moraceae:  
  *Ficus swinhoei*, King  
  *Broussonetia papyrifera* Vent.
Myricaceae : Myrica nagi, Thunb
Oenotheraceae : Jussiaea erecta, Linn.
Orchidaceae : Liparis caespitosa Lindl.
Phaius gracilis, Hayata
Pandanaceae : Pandanus graminifolius, Hurz.
Pinaceae : Tsuga chinensis Pritzel var. formosana Hayata
Piperaceae : Peperomia nakaharai, Hayata
Peperomia dindylguleensis, Miq.
Polygonaceae : Rumex maritimus, Linn.
Rumex crispus, Linn.
Polygonum alatum Ham. var. nepalense Hookf.
Polygonum multiflorum Thunb
Plantaginaceae : Plantago major L. var. asiatica, Decne
Polypodiaceae : Nephrolepis cordifolia, Presl.
Drymoglossum carnosum Hook
Davallia bullata, Wall.
Plagiogyria formosana, Nakai
Plagiogyria stenoptera, Diels
Ranunculaceae : Clematis floribunda Yama
Clematis gouriana, Roxburgh
Rosaceae : Stranvaesia nittakayamensis Hayata
Rubiaceae : Damnacanthus angustifolius, Hayata
Psychotria elliptica Ker
Gardenia florída L. var. grandiflora fr Et Sav.
Psederia uralensis, Hayata
Psederia chinensis, Hance
Neonauclea reticulata, Mctr.
Rutaceae : Glycosmis pentaphylla, (Retz) Correa
Fagada nitidz, Roxb.
Murraya puniculata, (Linn) Jack
Atalanta bilocularis, Wall.
Saururaceae : Saururus chinensis, Baild.
Saxifragaceae : Hydrangea integrifolia, Hayata
Hydrangea longifolia, Hayata
Mitella japonica, Miq. var. formosana, Hayata
Salicaceae : Salix babylonica, Linn.
Selaginellaceae : Selaginella canaliculata Baker
Solanaceae : Lycianthes biflora Bitter
Solanum nigrum Linn.
Solanum verbascifolium Linn.
Styracaceae : Styrax fo. mosanum Matsum
Thymelaeaceae : Daphne arisanensis, Hayata
Umbelliferae : Hydrocotyle javanica, Thunb
Sanicula satsumana, Maxim.
Urticaceae:  *Memorialis pentandra*, (Benn) Wedd. var. *hypericifolia*, (Bl) Wedd
*Pilea kankaensis*, Hayata
*Elatostema lineolatum* Forst. var. *major*, Thunb
*Pilea nokozarenisis*, Yamamoto
*Boehmeria frutescens*, Thunb. var *concolor*, Nakai

Verbenaceae:  *Callicarpa fomosana*, Rolfe
*Clerodendron trichotomum*, Thunb
*Vitex negundo*, Linn.

*Vitis shigunensis*, Hayata.

**Experiment**

1. The procedure for this experiment was outlined as follows:

   Fresh plant (50 g.)

   ![Diagram]

   Extract with H₂O (100 ml.)  Extract with 50% alcohol (100 ml.)

   Concentrate  Concentrate

   Add H₂O (to 5 ml.)  Add 50% alcohol to make 5 ml.

   1000% T.S.  1000% T.S.

   | 1 ml.  | 0.1 ml.  | 1 ml.  | 0.1 ml.  |

   - cultured medium (9 ml.)
   - add cultured medium (9.9 ml.)
   - add cultured medium (9 ml.)
   - add cultured medium (9.9 ml.)

   100% Extract  10% Extract  100% Extract  10% Extract

   (I)  (II)  (I)  (II)  (I)  (II)  (I)  (II)

(I) Crude extract in nutrient agar-medium.

(II) Crude extract in nutrient agar-medium.
The antimicrobial activity of the plant extracts by the agar streaking method has been shown in the above figures.

\[ W = \text{Petri dish contains: } 1 \text{ ml. } H_2O + 9 \text{ ml. nutrient agar medium (Control)} \]
\[ A = \text{Petri dish contains: } 1 \text{ ml. 50\% Alc. + nutrient agar medium (Control)} \]
\[ a = \text{Petri dish contains: } 1 \text{ ml. plant aq. extract + 9 ml. nutrient agar medium} \]
\[ b = \text{Petri dish contains: } 0.1 \text{ ml. plant aq. extract + 9.9 ml. nutrient agar medium} \]
\[ c = \text{Petri dish contains: } 1 \text{ ml. plant 50\% Alc. extract + 9 ml. nutrient agar medium} \]
\[ d = \text{Petri dish contains: } 0.1 \text{ ml. plant 50\% Alc. extract + 9.9 ml. nutrient agar med.} \]

2. Preparation and sterilization of culture media:

(a) Nutrient agar-medium:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypeptone</td>
<td>1 g</td>
</tr>
<tr>
<td>Meat Extract</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Distilled water to make</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

This was used for cultivation of bacteria. Nutrient broth had also been used. The ingredients were the same as above but without agar. After preparation, all nutrient media were sterilized in the autoclave at 15 lb for 15 minutes. The pH of the solution was adjusted to 7.2-7.4 before sterilization.

(b) Sabouraud's medium:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypeptone</td>
<td>1 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>4 g</td>
</tr>
<tr>
<td>Agar</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Distilled water to make</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

This was used for cultivation of fungi. Sabouraud's broth has also been used.

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3. Preparation of the microbial strains and bacteriological tests by agar streaking method (see fig.)

The following species of fungi and bacteria were used:—

Fungi:  
C: *Candida albicans* NO.YU 1200  
A: *Aspergillus niger*  
S: *Saccharomyces cerevisiae*  
T: *Trichophyton mentagrophytes*  

Bacteria:  
S: *Staphylococcus aureus* FDA 209 P.  
B: *Bacillus subtilis* PCI 219  
E: *Escherichia coli* NIHJ  
P: *Pseudomonas aeruginosa*  
C: *Corynebacterium xerose*  
M: *Mycobacterium tuberculosis* (*Mycobacterium smegmatis*) ATCC 607

4. The experimental procedure was tabulated as follows:

(1) For fungi:

<table>
<thead>
<tr>
<th>Fungi Stock Cultures C. &amp; S.</th>
<th>Fungi Stock Culture A subculture on Sabouraud's agar slant</th>
<th>Fungi Stock Culture T subculture on Sabouraud's agar slant</th>
</tr>
</thead>
<tbody>
<tr>
<td>subculture on Sabouraud's agar slant</td>
<td>incubate at 25°C for 3 days</td>
<td>incubate at 25°C for 7 days</td>
</tr>
<tr>
<td>incubate at 25°C for 3 days</td>
<td>add 3 ml. of sterile normal saline</td>
<td>add 3 ml. of sterile normal saline</td>
</tr>
<tr>
<td>subculture on Sabouraud's broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>incubate at 25°C for 3 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>streak a loopful susp. on specific section of prepared agar plate</td>
<td>incubate at 25°C for 3 days</td>
<td>incubate at 25°C for 7 days</td>
</tr>
<tr>
<td>observe inhibition of microbial growth</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(2) For bacteria:

<table>
<thead>
<tr>
<th>Bacteria Stock Cultures S.B.E.P.C.</th>
<th>Bacteria Stock Culture M</th>
</tr>
</thead>
<tbody>
<tr>
<td>subculture on nutrient slant</td>
<td>subculture on nutrient slant</td>
</tr>
<tr>
<td>incubate at 37°C for 24 hrs.</td>
<td>incubate at 37°C for 48 hrs.</td>
</tr>
<tr>
<td>inoculate in nutrient broth</td>
<td>inoculate in nutrient broth</td>
</tr>
<tr>
<td>incubate at 37°C for 48 hrs.</td>
<td>incubate at 37°C for 48 hrs.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seed Cultures S.B.E.P.C.</th>
<th>Seed Culture M</th>
</tr>
</thead>
<tbody>
<tr>
<td>streak a loopful susp. on specific section of prepared agar plate</td>
<td>streak a loopful susp. on specific section of prepared agar plate</td>
</tr>
<tr>
<td>incubate at 37°C for 24 hrs.</td>
<td>incubate at 37°C for 48 hrs.</td>
</tr>
<tr>
<td>observe inhibition of microbial growth</td>
<td></td>
</tr>
</tbody>
</table>
Summary

We carried out the preliminary antimicrobial activity test for 69 families of fresh Taiwan plants in the form of (1) 100 per cent. and 10 per cent. aqueous extracts and (2) 100 per cent. and 10 per cent. alcoholic extracts against six species of bacteria and four species of fungi. We obtained positive reactions to the fungi tested in the case of the following four species of plants:

(1) Aq. extracts:
   (a) Pandanus graminifolius Kurz of Pandanaceae.
   (b) Lithocarpus kodaishoensis Hayata of Fagaceae.

(2) Alcoholic extracts:
   (a) Bidens pilosa L. of Compositae.
   (b) Ardisia chinensis Benth of Myrsinaceae.

From the results of this experiment the extracts of only 11 species of plants extract appeared to have antimicrobial effect against all of the six bacteria tested.

They were—

(1) Aq. extracts:
   (a) Euphorbia thymifolia Linn. of Euphorbiaceae.
   (b) Aster indicus L. of Compositae.
   (c) Rhus javanica var. Roxburghi of Anacardiaceae.
   (d) Fasineta japonica Deca., of Araliaceae.
   (e) Barthea formosana Hayata of Melastomataceae.
   (f) Lycopodium carinatum of Lycopodiaceae.
   (g) Ipomoea congesta R. Br. of Convolvulaceae.

(2) Alcoholic extracts:
   (a) Kadsura japonica L. of Magnoliaceae.
   (b) Homonia riparia Lour of Euphorbiaceae.
   (c) Tamarindus indica L. of Leguminosae.
   (d) Annona reticulata L. of Annonaceae.
PHARMACOLOGY
HYPOTENSIVE ACTION
OF
JATAMANSONE (Valerenone)--
A sesquiterpene from
Nardostachys jatamansi DC

BY

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AND
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This paper presents a study on the hypotensive action of jatamansone with an attempt to elucidate the mechanism of its hypotensive action. Jatamansone is a sesquiterpene ketone isolated from the rhizomes of Nardostachys jatamansi, a plant indigenous to India and has been reported in Ayurvedic and Unani system of medicine to be useful in the treatment of a variety of ailments such as spasmodic hysteric fits, epilepsy and other nervous disorders (Chopra, 1958). From the rhizomes of this herb Govindachari (1958) isolated a sesquiterpene ketone, jatamansone. It has been shown to be the same as valerenone, a ketonic principle of the roots of Valeriana officinalis (Krepinski et al., 1960 a). Govindachari et al., (1959 and 1961 b) Krepinski et al. (1960 a, b and 1962) established the chemical structure of Jatamansone as shown in Fig. 1. The absolute stereo-chemistry of valerenone has been studied by a number of workers and particularly by Klyne, Bhattacharya, Sorm and their coworkers (1964). Jatamansone is a cis-decalone having two angular methyl groups and has the absolute stereo-chemistry shown in Fig. 2.

Fig. 1
Earlier studies report its antiepileptic activity in rats (Arora et al., 1962 a), antiarrhythmic activity in rats, (Arora et al., 1958), tranquilizing activity in rats and monkeys (Arora et al., 1962 a) and hypotensive activity in various animal species (Arora and Arora, 1963). Subacute toxicity studies conducted on rats and teratogenic effect studied on mice did not reveal any contraindication to the use of drug in man. Carefully controlled clinical investigations have shown it be a promising agent in patients of hypertension where it causes significant reduction in systolic and diastolic blood pressure in standing as well as supine position without intolerable side effects (Arora and Arora, 1963).

In a previous paper (Arora et al., 1962 b) biochemical attribute of this drug was studied presuming that its hypotensive property might be mediated through a change in amine levels or related enzymes. The effect on brain serotonin (5HT), catecholamine content of brain, dopa-decarboxylase inhibitory activity and monoamine oxidase activity was studied after administration of jatamansone. This drug produced depletion of serotonin (5HT) and catecholamine content from the rabbit’s brain. The drug inhibited decarboxylase enzyme whereas MAO remained unaltered. Though these actions are significant, the depletion of brain amines and decarboxylase inhibition is not as pronounced so as to account for the marked hypotensive activity of this drug.

In view of the fact that preliminary clinical trials with this drug have shown it to be a promising agent it was thought worth while to study the mechanism of hypotensive activity of this drug. Also, the prolonged chronic toxicity study was deemed essential. The present paper gives an insight into the mechanism of hypotensive action of jatamansone and the results of chronic (52 weeks) studies conducted on rats.

Methods

Cat blood pressure—Cats of either sex weighing between 2–5 kg were used. Anaesthesia was induced with ether and maintained with chloralose 80 mgm/kg intravenously. Carotid arterial blood pressure was recorded on a Sanborn polyviso through a pressure transducer. To prevent clotting of blood heparin 400 I.U./kg of body weight was given intravenously Drugs were injected through a canula in femoral vein. In some experiments (pressor response to bilateral carotid artery
occlusion) femoral arterial blood pressure was recorded. An emulsion of the drug was prepared in sodium alginate (one fourth of the weight of jatamansone). Control experiments with sodium alginate were performed.

**Injection of Jatamansone into the lateral cerebral ventricles of cats**

To study the central vasomotor effects, Jatamansone was injected into cerebral ventricles of the cat. The method of Feldberg and Sherwood (1953) was used to insert the cannula inside the lateral cerebral ventricles.

Jatamansone 10 to 30 mg/kg was injected into the lateral cerebral ventricle and the effect on blood pressure noted.

**Blood pressure of spinal cats**—Spinal cat preparations were made following the method of Burn (1952) Cats of either sex with body weight ranging from 3 to 5 kg were used. Jatamansone was administered in 15 and 20 mg/kg dose and the effect observed for 6 hours after drug administration.

**Study of effect on the peripheral sympathetic nervous system**

**Contractions of nictitating membrane**—Cats weighing between 2.5 kg were anaesthetized with chloralose 80 mg/kg intravenously. Recordings of the isometric contractions of the smooth muscles of nictitating membrane were obtained with a force displacement transducer, coupled to one channel of Sanborn polyviso. The resting tension of nictitating membrane was 12 gm. Equilibration of the membrane at this tension was indicated by the establishment of a stable base-line. This generally required 20 to 30 minutes after initial application of 12 gm tension. The pre- and post-ganglionic fibres of the cervical sympathetic trunk were dissected free and immersed in a layer of warm oxygenated liquid petroleum. The pre-ganglionic fibres were severed in all experiments. Shielded bipolar platinum electrodes were placed on either the pre- or post-ganglionic nerve segment. Cervical sympathetic trunk was dissected clear from the vagus. Pre-ganglionic fibres of the sympathetic chain were given electrical stimulation with a current of 3V, 5 m. sec. in duration and 30 per second delivered by a Grass stimulator. Contractions of the nictitating membrane were recorded in response to electrical stimulation of the sympathetic trunk and to intravenous administration of adrenaline (2 mcg/kg). Jatamansone emulsified in sodium alginate was injected intravenously in dosages ranging from 10 to 15 mgm/kg through a cannulated femoral vein. Contractions of the nictitating membrane were again recorded in response to electrical stimulation of the pre-ganglionic fibres of sympathetic chain and intravenous injection of adrenaline.

**Effect on cardiovascular responses in anaesthetized cats**

**Bilateral carotid occlusion.**—Carotid arteries of both sides were isolated. Gentle pressure by the clamps for 10 sec. brings about occlusion of the arteries and rise in blood pressure occurs. Pressure on arteries is applied at a point just below the bifurcation of carotid arteries into internal and external carotid arteries. Pressor response to bilateral carotid artery occlusion was seen before and after administration of Jatamansone 15 mg/kg I. V.
Stimulation of vagus nerve

Vagus nerves on both sides were isolated, made free from the sympathetic chain and cut. Central and peripheral cut ends were separately stimulated by electrical stimulus (5 V, 5 mill. sec. duration, 30 per sec. for 10 sec.) before and after administration of Jatamansone.

Effect on pressor/depressor effect of some drugs

Effect of administration of adrenaline 0.5 to 1 mcg/kg I. V., noradrenaline 1 mcg/kg I. V., response to injecting metacholine 2 mcg/kg I. V., amphetamine sulphate 500 mg/kg I. V. and histamine 2 mcg/kg subcutaneously was studied before and after administration of jatamansone 15 mg/kg I. V.

Effect on functioning of adrenal medulla

Dimethyl-phenyl-piperazinium (DMPP) when injected intravenously in doses of 50 to 100 mcg causes liberation of catecholamines from the adrenal medulla in the cat and stimulation of sympathetic ganglion thus bringing about a rise in blood pressure. Pressor response to DMPP (injection of 50 to 100 mcg) was studied before and after administration of jatamansone.

Stimulation of splanchnic nerve

Cats were anaesthetized as before. The abdominal splanchnic nerves were exposed according to Sherrington's (1929) method. The right splanchnic nerve was stimulated by supramaximal stimulus of 15/sec. for 40 sec. delivered through a Grass stimulator. Pressor response to splanchnic nerve stimulation was seen before and after intravenous injection of 15 mg/kg of jatamansone.

Hypotensive effect in dibenamine pretreated animals

Dibenamine 15–20 mg/kg given intravenously causes a reversal of the pressor response to adrenaline (0.5 mcg/kg I. V.) to 1.0 mcg/kg I. V.) and blocks the pressor effect of noradrenaline (1 mcg/kg I. V.). This dose of dibenamine was given to cats thus producing blockade of alpha receptors. Effect of jatamansone 15 mg/kg I. V. was studied in such dibenamine pretreated animals.

Effect of Jatamansone on cardiac output

Healthy adult mongrel dogs weighing between 18–22 kg were used in this study. The technique employed was essentially the same as described by Krayer and Mendez (1942). Jatamansone 50 or 100 mg. was put into the venous reservoir directly. The blood volume was 1,400–1,500 ml. at 39°C. Cardiac output was measured with Wease stromflhr. Blood pressure, cardiac output, venous pressure and electrocardiogram (Lead II) were recorded on a four channel Sanborn polyviso.
Chronic toxicity study on rats

Adult albino rats of either sex weighing between 100-200 gms. were used for oral chronic toxicity studies. Food and water were available at all times. Body weights were obtained before treatment and at the end of each week and the dose of drug for the following week was recalculated on the basis of the new body weight. Haematologic studies included haemoglobin percentage and total and differential leukocyte count. Haematologic studies were conducted before, in the middle and at the termination of the treatment. Every day record of food consumption by each animal was maintained. At the termination of the toxicity studies surviving rats were killed with ethereal for post-mortem studies. Some rats that died during the course of the study were examined at autopsy and tissues removed for histomorphologic studies if autolytic changes were not too advanced. 60 rats divided in three groups of 20 each were given jatamansone in oral doses of 30 and 100 mg/kg by a stomach tube seven days a week for 52 weeks. Third group of rats was kept as control group and was given the solvent.

Results

Cat blood pressure:

Jatamansone 15 to 20 mg/kg I. V. produced a prolonged and pronounced fall in blood pressure. Maximum fall was 50 to 60 per cent. There was an immediate fall after injection, followed after one to two minutes by rise in blood pressure which lasted for 2 to 5 minutes. This was again followed by a prolonged fall. Maximum fall in blood pressure occurred 1 to 1½ hrs. after drug administration. There was no further fall after 3 hrs., after which there was tendency for recovery but it did not reach normal level up to 5-6 hrs. after drug administration (period up to which it was observed). Fig. 3 shows the results. At lower doses (10 mg/kg I. V.) fall in blood pressure was 20-40 per cent. and lasted for 2 hrs., after which blood pressure started rising and touched predrug level within 3 hrs. In control experiments where only sodium alginate was given, there was no fall in blood pressure.

Effect of intracerebro-ventricular injection

There was only 10 to 20 mm Hg fall in blood pressure when these high doses were given intraventricularly. Blood pressure came back to control level within one hour of the injection of the drug. Then, after 20-30 minutes when the blood pressure was at control level, the same dose of Jatamansone was injected intravenously and again the effect on blood pressure studied. Thus in the same animal the central as well as peripheral actions could be studied. Jatamansone 15 to 20 mg/kg in the femoral vein caused a 35 to 50 mm Hg fall in blood pressure in contrast with the effect of same dose given intraventricularly (Fig. 4). These experiments make it clear that Jatamansone did not produce significant hypotensive action when the drug was injected into the cerebral ventricles and had a direct access to the vasomotor area, thus suggesting that central site of action does not play important role in the mechanism of hypotensive action of jatamansone.
(a) Immediately after injection of jatamansone 15 mg/kg I.V.
(b) 3 hours after injection.

Fig. 3—Shows the effect of jatamansone on blood pressure of cat.
Spinal cat

At doses of 15 mg/kg i. v. Jatamansone produced fall in blood pressure. Action started immediately after drug injection and was prolonged. There was 40-50 per cent fall in blood pressure at this dose. Maximum effect and the duration of hypotensive action in spinal animals was same as in cats with the central nervous system intact.

Effect on nictitating membrane contraction in cats

Jatamansone in doses of 10 to 15 mg/kg caused a marked reduction in the amplitude of contraction in response to electrical stimulation of sympathetic chain and to intravenous administration of adrenaline (Fig. 5).

Stimulation of vagus nerve.—Jatamansone did not show any effect upon the pressor action elicited by central vagal stimulation. It caused a significant reduction in the response following peripheral vagal stimulation.
Fig. 5—Record of nictitating membrane contraction in cats in response to electrical stimulation of preganglionic fibres of sympathetic chain

(a) before jatamansone.
(b) after 15 minutes of jatamansone administration.
(c) after one hour of jatamansone administration.
Bilateral carotid occlusion

There was marked reduction in the pressor response to bilateral occlusion of carotid arteries after administration of Jatamansone (Fig. 6).

Effect on pressor/depressor effect of some drugs

Jatamansone in doses of 15 mgm/kg I. V. caused reduction of pressor response to adrenaline and noradrenaline administration. However, the effect of metacholine and histamine on blood pressure remained unaltered. At this dose Jatamansone blocked the pressor effect elicited by amphetamine but did not show any effect upon the pressor action elicited by central vagal stimulation.

Effect on functioning of adrenal medulla

Jatamansone in 15 mgm/kg intravenous dose reduced and in some animals completely blocked the hypertensive effect of 50 mcg DMPP. I.V. (Fig. 7).

Stimulation of splanchnic nerve

Jatamansone reduced the intensity and duration of pressor effect of splanchnic nerve stimulation.

Hypotensive effect in dibenamine pretreated animals

In animals pretreated with dibenamine, Jatamansone failed to produce any hypotensive effect. This is shown in Fig. 8.

Heart lung preparation of dog

Our studies on isolated denervated heart lung preparation reveal that jatamansone in 50 and 70 mg total dose per heart lung preparation of the dog does not have any significant and consistent action. There is no effect on heart action; neither the frequency nor the rhythm nor the cardiac output are affected. However, it was observed that within 5 minutes of administration of 100 mgm of jatamansone in venous reservoir, there was decrease in cardiac output, which tended to recover within 10-15 minutes but was always below the normal. At 50 mg dose there was no effect on the cardiac output but the fall in blood pressure remained throughout the course of experiment. It appears that fall in blood pressure is not related to the effect of drug if any, on the cardiac output as at 50 mg dose hypotensive action of the drug was manifested, though there was no decrease in cardiac output at that dose.

Chronic toxicity studies on rats

Slight sedation was noted at all dosage groups. Weight of the animal and food consumption were not affected by drug. No other signs of drug effect were noticed. No significant change was observed in the haemoglobin concentration and total and differential leucocyte count. Differential changes were minor and infrequent. Autopsy examination of the surviving rats killed after receiving the drug in oral doses of 30 and 100 mg/kg for 52 weeks, revealed no gross lesions attributable to treatment.
Fig. 6—Record of blood pressure of cat, pressor response to bilateral carotid occlusion.

(a) before drug.

(b) 30 minutes after administration of jatamansone 15 mg/kg.
Fig. 7—Records of blood pressure of cat showing pressor response to dimethyl-phenylpiperazinium 50 mcg. intravenously

(a) before jatamansone.
(b) after 30 minutes of jatamansone administration—15 mg/kg I. V.
Fig. 8—Record of blood pressure of cat

(a) shows effect of dibenamine 15 mg/kg intravenously.

(b) shows reversal of pressor response to epinephrine confirming that alpha receptors have been blocked.

(c) shows effect of jatamansone 15 mg/kg intravenously after dibenamine.
A detailed microscopic examination was made of the formaline fixed haematoxylin-eosin stained paraffin sections of heart, liver, spleen, kidney and brain. In brain, cardiac muscle and spleen no microscopic lesion attributable to treatment were observed. In rats treated with 100 mg/kg dose liver showed slight cloudy swelling and fatty degeneration near the centrilobular region and no evidence of damage at the peripheral regions. No haemorrhages were seen. At 30 mg/kg dose there was no evidence of damage.

Kidney showed healthy glomeruli but slight cloudy swelling was present in some areas in the urinary tubules. At 30 mg/kg dose there was no evidence of damage.

Discussion

Drugs currently available for the therapy of hypertension act by diverse mechanisms and sites. A drug which produces lowering of blood pressure may act either by its central vasomotor effects or its effect on the peripheral sympathetic system and both effects may contribute in the mechanism of hypotension.

Central vasomotor effect of jatamansone was studied by injection into the cerebra ventricles so that a direct access to the vasomotor area was obtained. Experiments on spinal cats also show that the central site of action does not play a significant role in the mechanism of hypotensive action of jatamansone. Jatamansone shows evidence for blockade of adrenergic mechanism as indicated by the fact that the pressor response to carotid occlusion, is depressed, pressor effect of dimethyl-phenyl-piperazinium iodide (a ganglion stimulant resembling nicotine) is depressed, pressor effect of intravenous administration of adrenaline and noradrenaline is blocked or depressed, pressor effect of splanchnic nerve stimulation in cats is depressed and in dibenamine (an alpha adrenergic blocking agent) pretreated animals, jatamansone fails to produce any marked hypotensive action. Also, there is marked reduction in the amplitude of contraction of nictitating membrane after administration of jatamansone.

The activity of various homeostatic reflexes is closely related to every change in arterial pressure and so the state of these reflexes in man and animals and the alterations produced by various hypotensive drugs is important. These reflexes are designed for the reflex control of arterial pressure. Reserpine, bretylium tosylate, guanethidine, adrenergic and ganglion blocking agents, all produce typical pattern of events on these vascular responses which helps in their categorization. When these effects of jatamansone are compared with the other known anti-hypertensive agents (Table I), it helps in understanding the mechanism underlying its hypotensive action. Reduction of pressor response to amphetamine and lack of effect on central vagal response elicited by jatamansone are identical with alpha methyl dopa. All these agents reserpine, guanethidine and alpha methyl dopa are known to lower catecholamines from certain peripheral structures and jatamansone also has been earlier reported to deplete catecholamines from certain peripheral areas (Arora, et al., 1963). The reduction in response to amphetamine can be explained by the possession of such actions as catecholamine depletion, a property in common with reserpine, guanethidine and alpha methyl dopa.
TABLE I

<table>
<thead>
<tr>
<th>Response</th>
<th>Jatamansone</th>
<th>Alpha-methyl dopa</th>
<th>Reserpine</th>
<th>Guanethidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pressor response to amphetamine</td>
<td>Blocked</td>
<td>Blocked</td>
<td>Blocked</td>
<td>Blocked</td>
</tr>
<tr>
<td>2. Pressor response to electrical stimulation of central end of cut vagus nerve</td>
<td>No effect</td>
<td>No effect</td>
<td>Blocked</td>
<td>Blocked</td>
</tr>
<tr>
<td>3. Pressor response to electrical stimulation of peripheral end of cut vagus nerve</td>
<td>Significant consistent reduction</td>
<td>Significant consistent reduction</td>
<td>No significant effect</td>
<td>No significant effect</td>
</tr>
<tr>
<td>4. Pressor response to bilateral carotid occlusion</td>
<td>Reduced</td>
<td>No effect</td>
<td>Blocked</td>
<td>Blocked</td>
</tr>
<tr>
<td>5. Pressor response to intravenous injection of norepinephrine</td>
<td>Reduced</td>
<td>No effect</td>
<td>Potentiated</td>
<td>Potentiated</td>
</tr>
</tbody>
</table>

Lack of effect upon central vagal response, a property shared only by alpha methyl dopa, cannot be explained properly. The reason for this may be found in the explanation put forward for alpha methyl dopa (personal communication data prepared by the staff of the Merck Institute). The hypothesis states that “Separate stores of catecholamines may exist in the tissues subserving the responses which are differently affected by the agents studied. Hence, reserpine and guanethidine may influence a store of catecholamines in adrenergic neurones which presumably is involved in response to the nerve impulse. These two agents would also deplete a catecholamine store located more diffusely in cardiovascular structures which is the store presumably influenced by amphetamine. It is perhaps, this latter store which is lowered by alpha methyl dopa. Its action therefore, would extend only to amphetamine. The existence of separate stores of physiologically active catecholamines, however, is speculative and requires more direct evidence for acceptance.” As earlier suggested by Trendelenburg (1961), noradrenaline store seems to consist of two compartments, a small compartment A of easily releasable (available) and a large compartment B of more tightly bound noradrenaline.

The reduction in pressor response to bilateral carotid occlusion was not related to a decrease in mean arterial pressure, an effect which is known to decrease carotid occlusion pressor response. Jatamansone caused a significant reduction in the response following peripheral vagal stimulation. This action suggests that this agent may be possessing weak ganglionic blocking and/or atropine like activity. This assumption for ganglion blocking activity is further confirmed by the findings of our experiments on nictitating membrane contraction in cats where at the same
dose (15 mg/kg) jatamansone produces reduction in contraction of nictitating membrane in response to electrical stimulation of preganglionic fibres of sympathetic chain. Thus as shown in these experiments jatamansone has close similarity with a alpha methyl dopa and they both produce similar pattern of events on the various cardiovascular responses viz., both agents reduce pressor response to amphetamine, both agents lack any effect on central vagal stimulation response and both produce significant and consistent reduction in blood pressure response to peripheral vagal stimulation. These findings differ from the results with other known anti-hypertensive agents like reserpine, guanethidine and bretylium tosylate.

Bretlyium tosylate and guanethedine both are known to potentiate the pressor response to noradrenaline. Alpha methyl dopa has no effect on this response while after Jatamansone administration there is reduction in this response.

Impairment of pressor response to stimulating the adrenal medulla either by splanchnic nerve stimulation or by the intravenous injection of dimethyl phenyl piperazinium shows effect on functioning of the adrenal medulla. Further evidence of adrenergic blocking action is provided by the experiments on dibenamine pretreated animals where jatamansone fails to produce any hypotensive action. No untoward toxic actions with the drug have been revealed in chronic toxicity studies.

Summary

In cats, jatamansone produces the following effects:—

Pressor response to bilateral carotid occlusion, to adrenaline and noradrenaline administration and to amphetamine, is depressed. There is no effect on pressor response to central vagal stimulation. Pressor effect of splanchnic nerve stimulation and injection of dimethylphenyl piperzinium is depressed. There is marked reduction in the amplitude of contraction of nictitating membrane following preganglionic sympathetic nerve stimulation. In debenamine pretreated animals the drug does not produce its hypotensive action. In experiments on heart-lung preparation of the dog cardiac output is not effected by drug administration.

Chronic toxicity studies in rats do not reveal any untoward effect of the drug.

Acknowledgements

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REFERENCES


Feldberg, W. and Sherwood, S. L. (1953). J. Physiol. (Lond.), 120, 3 P.


ANTHELMINTIC EFFECTS
OF
Diospyros mollis
EXTRACT
IN MAN AND DOG

BY

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*Diospyros mollis, Griff. is one of the indigenous plants of Thailand which has been
made use of for several hundreds of years and is known in Thailand as Ma-gluea or
Ma-guea. Its fruit and leaves have been used as a fast black dyestuff. Before the
recognition of synthetic dyes among the natives of the Far East and of the South-East
Asia regions, people of different countries, especially of China, used to ship their
fabrics into Thailand to have them dyed with the freshly prepared juice of Diospyros
mollis, after which the fabrics were sent back to the producing countries. Neither
the fruit nor the leaves of ma-gluea could be shipped for dyeing of the fabrics outside
Thailand, because the fruit and leaves would turn black within a few days and thus
became useless for dyeing purposes. No satisfactory method has ever been
discovered which could prevent these changes.

Another useful property of ma-gluea is its anthelmintic activity, which has been
widely recognized in Thailand for hundreds of years. The native herb doctors
gave their patients a number of units of ma-gluea fruit equal to the number of years
in their age. The fresh fruit is crushed with a small amount of water, strained
through a cloth, and mixed with coconut water in order to mask its very disagreeable
and nauseating taste. This mixture is taken either in the morning or at bed time.
The maximum dosage for an adult is 25 units. Used in this manner, except for the
rather frequent nausea and vomiting observed, no other toxic symptoms have been
reported. However, the fruit becomes inactive after becoming black in color.

Ma-gluea, or Diospyros mollis, Griff. (Family, Ebenaceae) is a tree of moderate
size about 2 or 3 metres high, which grows wild all over the central and northern
parts of the country. It is also cultivated in some places. It bears fruit only during
Fig. 1—The ripe fruits of Ma-gluea (*Diospyros mollis*, Griff)

Fig. 2—The young and the ripe fruits of Ma-gluea

Fig. 3—The inactive vs. the active fruits of Ma-gluea
the months of June through October, which is the rainy season. Its beneficial application is thus limited and dependent upon the seasonal change. The fruit is more or less round in shape and varies in size. The mature fruit is about 2 to 3 cms. in diameter. The immature fruit is usually green and becomes greenish yellow as it matures. It will turn black within 5 to 7 days after dropping from the tree, and darkens in a few hours if crushed. The juice has a characteristic smell with a very disagreeable and nauseating taste. The leaves and the flowers also turn black several days after separation from the branch. (Figs. 1, 2 and 3).

Because of the seasonal limitations and color changes referred to above, attempts have been made in Thailand to extract the active principles of this fruit. The crushed fruit is blended with 95% alcohol and the mixture is suction filtered through a Buchner funnel. The filtrate is treated with 10–20% acetic acid solution until precipitation is complete, and then suction filtered and dried in a vacuum oven at 55–60°C. A dry powder is obtained which has a yellowish color and is practically devoid of smell. However, this powder will rapidly become grey and then black within a day and the inherent anthelmintic property of the yellowish powder is also lost. In fact, the color of this powder is a good indication of its anthelmintic effectiveness. The only successful method of storage of this extract is to keep the powder under vacuum in hermetically sealed ampoules. The anthelmintic dose of this extract in an adult man is about 2 to 4 grams.

Attempts have been made to elucidate the chemical constituents of this extract. In her investigation of the coloring matter in this extract, Benchakarnchan (1948) concluded that it is a rhamnoside. The phenolic compound obtained from hydrolysis of the rhamnoside was studied and certain derivatives were prepared and characterized. The structure of the active principle of this extract was considered to be a dihydrodihydroxy derivative of acenaphthene. Loder et al. (1957) on the other hand, found it to be a readily oxidizable tetrahydric phenol of the formula C_{22}H_{18}O_{4} which is devoid of methoxy groups but contains a 2, 2′—dinaphthyl chromophore and which was named by them "diospyrol". This disagreement was brought up to the attention of the scientists at the Ninth Pacific Science Congress held in Bangkok, Thailand in 1957 by Nilanidhi and Prachankadee, but no resolution was established in this discussion.

Several clinical trials have been carried out by many physicians in Thailand with excellent results, but there has never been any publication of the recorded data in English. It is therefore the aim of this paper to bring to the attention of scientists the beneficial anthelmintic effects of this extract both in man and in dog.

**Methods**

In the following clinical trials and experimentation in dogs, the extracts prepared in the Department of Medical Sciences and stored in hermetically sealed ampoules of 2 grams each were used. No demonstrable difference in activity was observed between the various batches of extract. One batch which is now about four years old still retains its yellowish color.

Clinical trials were done in fifty cases both in clinics and hospitals. Stool examination was performed prior to the treatment in every case, but egg counts
were not carried out. Positive parasites or eggs of the parasites were indicative of the initiation of the treatment. In some of the cases, the drug was administered at bedtime after a light meal in the evening. The first purgation was usually observed early in the following morning, and this might be repeated several times until about noon depending upon the dose. In others, the patients took the drug early in the morning followed by no or only a light breakfast. Purgation usually followed within a few hours and was repeated several times until afternoon. In some of these cases the purged stools were collected and examined for the parasites. Co-operation of the patient in this matter was however rather poor in some cases. Re-examination of the stools was done about one week after the treatment. In most of the cases no more ova were demonstrable in the stools after the treatment, but in certain instances repeated treatments were required to eradicate the parasites.

The dosage of the drug used in each treatment was 2 to 4 grams for an adult, while children received 0.1 gram per year of age. The powder was given mixed with cow’s milk or coconut water.

Similar treatments were also given to fifteen dogs who showed parasitic ova in their stools; the drug was administered usually in the morning, through stomach tubes, mixed with water. The dosage was also 2 to 4 grams for each animal. Many of the animals had nausea and vomiting after the administration. No animal was killed after the treatment.

**Results**

*In man.*—Excellent anthelmintic results were obtained in almost all cases although many of the patients required more than one treatment. Parasites were found in the purged stools in several cases. Patient co-operation in the collection of the stools was, however, rather difficult to obtain.

**Table I—Anthelmintic effects of Diospyros mollis extract in man**

<table>
<thead>
<tr>
<th>Types of Parasites</th>
<th>Cases of Positive Ova in Stools Before B</th>
<th>Cases of Demonstrated Expelled Worms</th>
<th>No. of B</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ancylostoma duodenale and Necator americana</td>
<td>18</td>
<td>18</td>
<td>1–3</td>
<td>Many of these cases required more than one treatment to eradicate the parasites</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>The stools of one of the patients were not available after the treatment</td>
</tr>
<tr>
<td>Enterobius vermicularis</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Strongyloides stercoralis</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Scolex demonstrable in 2 cases</td>
</tr>
<tr>
<td>Taenia saginata</td>
<td>12</td>
<td>10</td>
<td>1</td>
<td>Scolex demonstrable in 1 case</td>
</tr>
<tr>
<td>Taenia solium</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fasciolopsis buski</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>1–3</td>
</tr>
</tbody>
</table>
Ancylostoma duodenale and Necator americana.—In all of the eighteen cases who showed parasitic ova in their stools, the worms were expelled after the administration of the drug and were demonstrable in the stools. Many of these cases, however, required more than one treatment to eradicate the parasites. Three treatments were needed in a few of them. These patients showed very rapid improvement of the symptoms of anaemia after the treatment, and this might be accelerated by the later administration of iron.

Ascaris lumbricoides.—All the five patients who revealed the presence of ascaris ova in their stools received each only one treatment. No parasites or ova were found after the treatment.

Trichuris trichiura.—Only one treatment was given to each of three patients, one of whom passed parasites. The stools of one patient still showed the presence of the ova after the treatment, but no attempts had been made to repeat it.

Enterobius vermicularis.—The six patients harbouring pin worms received only one treatment. All of them showed no more ova after one week, and the parasites were passed in five of them. The stools of the sixth patient were not available after the treatment.

Strongyloides stercoralis.—A satisfactory result was obtained in one patient after only one treatment.

Taenia saginata.—Excellent results were also obtained in the twelve beef tapeworm cases, ten of whom passed the long segments of the parasites. Scolex was demonstrable in two cases. No ova were found in the stools of any case one week after the treatment. Only one dose of the drug was given to each patient.

Taenia solium.—In both cases of pork tapeworm the parasites were expelled after one treatment of the drug, and scolex was found in one of them. No ova were seen after the treatment.

Fasciolopsis buski.—Strangely enough, after the administration of the drug the worms were seen in the stools of all the three patients harboring the parasites. One patient required three treatments before the stools showed the absence of ova.

In dog.—Similarly, excellent results were obtained in all the fifteen dogs harbouring either Ancylostoma caninum, Diphyllobothrium latum, Dipyldium caninum or Trichuris vulpis. No more ova were found after the treatment. But some of these animals required two or even three treatments. Many of the animals showed nausea and vomiting very shortly after the administration.
TABLE II—ANTHELMINTIC EFFECTS OF_Diospyros mollis_ EXTRACT IN DOG

<table>
<thead>
<tr>
<th>Types of Parasites</th>
<th>Cases of Positive Ova in Stools</th>
<th>Cases of Demonstrated Expelled Worms</th>
<th>No. of B</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before B</td>
<td>After B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ancylostoma caninum</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>1-3</td>
</tr>
<tr>
<td>Diphyllobothrium latum</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dipylidium caninum</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Trichuris vulpis</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1-2</td>
</tr>
</tbody>
</table>

Side effects

The most frequent side effects encountered in the use of this extract were nausea and vomiting, and these were seen in many of the dogs. The symptoms were moderately severe in three of the patients, one of whom was pregnant. But all of them recovered in a day without any other complications or any treatment.

Discussion

From the results obtained both in man and in dogs it is apparent that the extract of _Diospyros mollis_ is effective as a broad-spectrum anthelmintic. It is a safe product although nausea and vomiting are quite frequent. However, in some cases more than one treatment is required to eradicate all the parasites. The major disadvantage of the extract is its rapid discoloration with concurrent loss of the activity. But if it is stored in a hermetically sealed ampoules under vacuum it can retain its effectiveness for years.

Summary

The extract of _Diospyros mollis_ was tried in fifty humans and fifteen dogs in this series with excellent anthelmintic results. It was found to be safe and broad-spectrum in nature and required no stringent preparation of the patients prior to the administration.

Acknowledgments

The authors wish to express their gratitude to Professor Luang Lipidharm Sributta, Professor Chamlong Suvagondha, Dr. Prakorb Tuchinda, Mr. Porn Tamprateep, and Miss Thanomwong Amatayakul of the Department of Medical Sciences, Dr. Sombodhi Bukkavesa of Siriraj Hospital, Rear Admiral Kamol Champoondhopong and Captain Piriya Hotrapavanond, R. T. N., of the Navy Hospital, Colonel Supot Khwanmit and Lieutenant Anothai Jamjim of the Army Hospital, Dr. Mukda Piyakesin of Lerdson Hospital, Professor Luang Chai-Asvaraks, Dr. Prasidhi Podhipaks and Mr. Prakai Jitrakorn of the College of Veterinary Medicine, for their assistance and use of their facilities.

REFERENCES

PHARMACOLOGICAL SCREENING OF MEDICINAL PLANTS

BY

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Royal Pharmaceutical Institute
Stockholm, Sweden

In this department a research program on new medicinal plants is going on for some years. Potential drugs have been collected in tropical and semitropical zones of Asia, Africa and South America. Regardless of the reason for collection of the plants (on taxonomic grounds, being a folklore remedy, a plant avoided by grazing cattle) they have been screened for pharmacological effects. For this purpose the procedure of Malone and Robichaud (1962) has been applied and modified.

This paper deals with a detailed description of this method in our modification and our experiences of the method, the aim being to give the participants of this symposium a manual for direct practical use. The author has several times in Asian countries been asked for a screening procedure that could be used in the evaluation of plant extracts. It is my hope that this paper may serve that purpose.

Equipment

The following special equipment is required:

**Observation rink.**—This is made of wood, the size being 54 × 58 cm, with a height of 7.5 cm. There is no bottom in the rink which is placed on a sheet of paper, which is reasonably absorbant. The paper is changed after all observations made each day in order to notify the amount and nature of excreta during that period.

**Rectal Thermometer.**—A glass thermometer with the scale 28-42° C. The part to be inserted is 3 mm in diameter and 24 mm long.

**Test screen.**—This is a reinforced wire mesh screen used to check skeletal muscle strength and coordination. The size is 30 × 36 cm and constructed with 13 threads per 10 cm.

**Stop watch.**—A timer divided into seconds and a 30-minute capacity.

**Horse-hair Probe.**—A 5 cm long horsehair is attached to the end of a 15 cm long wooden dowel having a 2 mm diameter. Attachment is effected with thread and gum leaving 4 cm of the horsehair extending beyond the dowel. The hair end is used to check corneal and pinna reflexes, the wood end for the head tap response.
Filter paper.—5.5 cm in diameter. Whatman no. 1 or similar quality.

Cardboard sheet.—Along one side of a cardboard sheet, 2 x 15 cm, with interval of about 1 cm, black dots are made with the following diameters: 1/16, 1/8, 1/4, 1/2, 1, 2, 3, 4 mm. It is used for the measurement of pupil size.

Standardised work sheet.—The sheet is shown in Fig. 1. The size of the mimeographed work sheet used in our laboratory is 21 x 30 cm.

Experimental procedure

Animals.—Non-fasted female rats, in the weight range of 160–180 gm are used. Food and water are withheld during the 6-hour observation period the first day; thereafter, ad libitum again.

Route.—As a rule injections are made intraperitoneally preferably in the same place. However, if the suspension is too viscous or too coarse, it is administered orally.

Test solution.—All raw plant extracts and purified extracts are suspended in an aqueous vehicle of 0.25 or sometimes 0.50 percent agar. The extracts are freeze-dried and reduced to a powder which facilitates the preparation of a suspension (made by trituration). If there are difficulties in preparing a suspension of an alkaloid containing extract hydrochloric acid is added to pH 6. Some raw extracts may be resinous in nature and difficult to make suspensions from. For those the higher percentage of agar is used and they are given orally. The highest concentration used of an extract is 100 mg/ml and the maximum volume of the suspension used is 10 ml/kg. This means that highest dose given is 1000 mg/kg. For a pure chemical (reference substance or new drug), 250 mg is required, for a raw or purified extract 2–5 gm to perform the screening.

Dosage and number of animals.—The primary aim is to determine a lethal dose, one complete ineffective and at least three doses with some effects in between these two levels.

For raw extracts of plants we start with a dose of 500 mg/kg (in a suspension of 10 mg/ml—if possible—otherwise 50 mg/ml). If this dose is ineffective, 1,000 mg/kg is given. If the last mentioned dose is effective or lethal, 750 mg/kg is given.

In the case that 500 mg/kg produces some effects (but not lethal effect) both higher (750, 1,000 mg/kg) and lower doses are given—for instance 250, 100, 50, 10, 1 mg/kg depending on the effects obtained.

In the initial dose of 500 mg/kg, is lethal, 50 mg/kg. is given as a second dose. Depending on the results thus obtained, further doses are given.

In the original description of this method the authors have claimed that the effective doses should be given in logarithmic scale. We have found that for practical reasons this has to be somewhat modified as indicated above.
**FIGURE 1**

**Qualitative and Semi-Quantitative Screening and Toxicity Report of Dicodiphosphate**

**Test Animal:** Rat  
**Fasted:** Yes  
**Sex:** Male  
**Mark:** 1  
**Color Mark:**  
**Weight (g):** 197  
**Cage:** Halley

**Vehicle for Sample:**  
**Conc. (mg/ml):** 30  
**Sample Dosage:** 30 mg/kg  
**Notebook no.:** pp.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time (min. post dosage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor Activity</td>
<td></td>
</tr>
<tr>
<td>Ataxia</td>
<td></td>
</tr>
<tr>
<td>Loss Righting Reflex</td>
<td></td>
</tr>
<tr>
<td>Analgesia</td>
<td></td>
</tr>
<tr>
<td>Anesthesia</td>
<td></td>
</tr>
<tr>
<td>Resp. Rate (Bp)</td>
<td></td>
</tr>
<tr>
<td>Loss Corneal Reflex</td>
<td></td>
</tr>
<tr>
<td>Loss Pinna Reflex</td>
<td></td>
</tr>
<tr>
<td>Paralysis: Forelegs</td>
<td></td>
</tr>
<tr>
<td>Paralysis: Hind legs</td>
<td></td>
</tr>
<tr>
<td>Paralysis: Head</td>
<td></td>
</tr>
<tr>
<td>Screen Crisp: H.L. loss</td>
<td></td>
</tr>
<tr>
<td>Screen Crisp: F.L. loss</td>
<td></td>
</tr>
<tr>
<td>Sterile Reaction</td>
<td></td>
</tr>
<tr>
<td>Motor Activity</td>
<td></td>
</tr>
<tr>
<td>Fine Body Tremors</td>
<td></td>
</tr>
<tr>
<td>Coarse Body Tremors</td>
<td></td>
</tr>
<tr>
<td>Fatculations</td>
<td></td>
</tr>
<tr>
<td>Tonic Convulsions</td>
<td></td>
</tr>
<tr>
<td>Mixed Types Convulsations</td>
<td></td>
</tr>
<tr>
<td>Resp. Rate (Bp)</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td></td>
</tr>
</tbody>
</table>

| EYES                        |                         |
| Enophthalmus                |                         |
| Exophthalmus                |                         |
| Palpebral Plots             |                         |
| Pupil Size (mm)             |                         |
| Pupil Size (mm, (lit)       |                         |
| Nystagmus                   |                         |
| Lacrimation                 |                         |
| "Bloody" Tears              |                         |

**OTHER NOTES:** Associate each symptom or observation with a specific time post-dosage. Note stereotypy.

**SUBJECTIVE**

- Head Tap: Aggressive
- Body:
  - Grasp: Passive
  - Status Positions: Passive
  - Excess Curiosity: Passive

**DEATH AND AUTOPSY NOTES** (note: Resp. or Cardiac Arrest, systole or diastole, color: intent. Wall and lungs, etc.)
In the case of an effective extract one or two rats are injected at each dose level resulting in a minimum of five rats per complete evaluation and an optimum of ten rats. However, for inactive extracts two rats for 1,000 and 500 mg/kg, respectively, might be enough.

**Observation.**--The observations must be performed at 5, 15, 30, 60 minutes, 2, 4, 6 hours after administration during the first day. Each day from the second to the seventh day the rats are observed once daily. Observations at intermediate times may also be selected.

Each blank in the work sheet must be filled in at each time interval given above, either with a rating [0, (+), +, ++, ++++, +++++] or with an actual measurement. For the evaluation of a work sheet the absence of a symptom is as important as the presence of a symptom. Filling in even the absence of a symptom at a certain time means that there actually is a check of that item.

The completely filled out work sheets are shown in Fig. 1-3.

**Evaluation of parameters.**--The parameters are put into groups on the work sheet to facilitate the evaluation of the results, but the order of tests is different when carrying out the screening (see below).

First group shows central nervous system depression.

*Decrease in motor activity* is rated as follows: (+), animal is quiet, occasionally moves spontaneously; +, does not move spontaneously, but when handled will move slowly; ++, when handled will move very sluggishly; ++++, when handled will not move at all.

*Ataxia rating*: (+), when moving shows occasionally detectable incoordination; +, when moving constant incoordination but the course of progress is true; ++, the rat cannot walk straight, the course is erratic; ++++, cannot walk on any course.

*Loss of righting reflex* is rated: (+) the hind part of the body including the hind legs can be placed one side; +, the rat can be placed only on one side; ++, the rat can be placed on either side equally well; ++++, the rat can be placed on back as well as either side: ++++, the rat can not be aroused from back position by a hind leg toe pinch.

*Analgesia* is rated as follows: (+), when the investigator's fingernail is firmly pressed down across one of the toes of one of the rat's hind feet and there is a sluggish response with vocalization and/or attempts to escape or bite; +, when fingernail is pressed down there is neither vocalization nor attempts to escape and bite, but the rat attempts to calmly withdraw the foot from pressure; ++, no response.

*Anaesthesia+:*, the rat is placed on its side and remains so and cannot be aroused from this position by handling.

*Respiratory rate and depth.* The time for 50 breaths is measured with the stopwatch, and the number of seconds is noted in the work sheet. Thus, a decrease in respiratory rate is reflected by a higher figure than for the control period, and increase
### FIGURE 3

#### Qualitative and Semi-Quantitative Screening and Toxicity Report of 2,4-D "Bougainvillea" Extract of Root

**Vehicle:** control

**Sample Dosage:** 250 mg/kg, Note no. 1607-03

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CNS Motor Activity</th>
<th>CNS Somatic Reaction</th>
<th>EYES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>Response ( )</td>
<td>Response ( )</td>
<td>Response ( )</td>
</tr>
</tbody>
</table>

#### CNS Motor Activity
- **Ataxia**: 0
- **Loss Righting Reflex**: 0
- **Analogues**: 0
- **Anesthesia**: 0
- **Respiration Rate (x)**: 30
- **Loss Corneal Reflex**: 0
- **Loss moist reflex**: 0
- **Paralysis: Frontal**: 0
- **Paralysis: Hind Legs**: 0
- **Paralysis: Head**: 0
- **Screen Crush: Hind Legs**: 0
- **Y-Loss**: 0

#### CNS Somatic Reaction
- **Motor Activity**: 0
- **Joint Body Tension**: 0
- **Skeletal Body Tension**: 0
- **Fasciculations**: 0
- **Clonic Convulsions**: 0
- **Tonic Convulsions**: 0
- **Mixed Types Convulsions**: 0
- **Respiration Rate (x)**: 30
- **Depth of Breathing**: 0

#### EYES
- **Exophthalmus**: 0
- **Enophthalmus**: 0
- **Pupillary Size (mm)**: 2.0
- **Pupillary Size (mm) (Light)**: 2.0
- **Mydriasis**: 0
- **Lacrimation**: 0
- **"Bloody" Tears**: 0

#### Subjective
- **Head Tap**: 0
- **Passive**: 0
- **Active**: 0

#### Systolic (mmHg): 0

#### Other Notes:
- Associate each symptom or observation with a specific time post-dosage. Note stereotypy.
in respiratory rate is shown by a lower figure, decrease in respiratory depth is rated:
(+), moderate decrease; +, definite decrease; ++, pronounced decrease. Cheyne-
Stoke respiration is either absent (0) or present (+).

Corneal and pinna reflex (+), sluggish response only, when the horsehair end of the probe is touched to the cornea and ear canal; +, no response at all.

Paralysis of forelegs, hindlegs, head. This is checked by manipulation of the respective parts and is rated: (+), the head or legs fall sluggishly back, after lifting and dropping; +, the head or legs fall rapidly back after lifting and dropping, the rat cannot perform any muscular movement.

Loss of screen grip. In this test for checking muscular strength and coordination, the rat is placed on the centre of the horizontal screen and the screen is then inverted and shaken gently back and forth in a horizontal plane. The test is rated as follows: ++++, the rat falls off as the screen is tilted to a 45° angle; +++, the rat falls off as the screen is at a 90° angle; ++, the rat falls off, when the screen has been inverted; +, the rat falls at the first shake of the screen; (+) an equivocal loss. In the sections for notes at the bottom of the work sheet, the investigator should note whether grip fails first in the hind legs or whether grip strength appears to fail simultaneously in both the front and hind paws.

In the second group of parameters symptoms of central nervous system stimulation are included.

Startle reaction is checked by sharply slapping the outside of the observation rink with a folded metal sheet. It is rated as follows: (+), the rat gives a mild start; +, the rat visibly jerks; ++, the rat jerks, jumps and makes a sudden frantic escape attempt; ++++, the rat instantly goes into clonic convulsions.

Increase in motor activity is rated as follows: (+), the rat looks around, moves the body but does not run around the rink; +, the rat moves constantly, but at a normal rate about the rink and wipes its nose; +++, moves constantly and rapidly, often attempts to escape; +++++, runs constantly, makes frequent escape attempts.

Fine body tremors are not seen by the eye on gross observation; they are best detected by lightly placing the fingers on the rat’s back over the spine. Fine body tremors are rated: (+), equivocal presence; +, definite presence but only sporadic outbursts are noted; ++, continuous tremor; ++++, occasionally tremors are detectable by observation alone, without recourse to touching the back.

Coarse body tremors Ratings are made by eye alone: (+), equivocal presence; +, definite, but only sporadic presence; ++, continuous tremors; ++++, pronounced tremors, nearly clonic convulsions.

Fasciculations are wave-like movement of the skin towards the hind part and are rated in this section of the work sheet because of the importance of differentiating it from coarse body tremors and mild clonic convulsions (next parameter). Fasciculations are seen by the eye on gross observation and rated as follows: (+), equivocal response; +, definite fasciculations in one area, but movement is not continuous;
**Figure 3**

Qualitative and Semi-Quantitative Screening and Toxicity Report of *Physostigma venenosum*, root extract of seeds

**Test Animal:** Rat  
**Fasted:** No  
**Sex:** Female  
**Mark:**  
**Color Mark:**  
**Red Weight (g):**  
**Cage:**  
**Vehicle for sample:** Conc. 10  
**mg/ml:**  
**ml. injected:**  
**Route Inj.:** Oral  
**Time Inj.:** 6:00  
**Evaluated by:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time:</th>
<th>5, 15, 30, 60, 120, 240 min. past dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>Motor Activity</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Ataxia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss Righting Reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Analgesia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aniridia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resp. Rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I.o.: Corneal Reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss Pinea Reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paralysis: Forelegs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paralysis: Hind legs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raphaeal: Head</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Screen Grip: H., L., P.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tonic Reflex</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time:</th>
<th>5, 15, 30, 60, 120, 240 min. past dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>EARS: ORAL MUCOSA</td>
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</tr>
<tr>
<td></td>
<td>Blushing</td>
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<tr>
<td></td>
<td>Hyperemia</td>
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</tr>
<tr>
<td></td>
<td>Synesthesia</td>
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</tr>
<tr>
<td>GEMEAL</td>
<td>Salivation</td>
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</tr>
<tr>
<td></td>
<td>Tail Erection</td>
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</tr>
<tr>
<td></td>
<td>Pilemoter Erection</td>
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</tr>
<tr>
<td></td>
<td>Micturition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mastismo</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Pupiloid Test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Circulating Motions</td>
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</tr>
<tr>
<td></td>
<td>Tail Lashing</td>
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</tr>
<tr>
<td></td>
<td>Abdominal Grappling</td>
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<tr>
<td></td>
<td>Rectal Temp. (°C)</td>
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</tr>
<tr>
<td></td>
<td>Body Weight, Gm.</td>
<td>188 10</td>
</tr>
<tr>
<td>ZONES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUBJUNCTIVE</td>
<td>Head Tap: Aggressive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Passive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fearful</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Body</td>
<td>Agressive</td>
</tr>
<tr>
<td></td>
<td>Passive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fearful</td>
<td>0</td>
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<tr>
<td></td>
<td>Status: Positive</td>
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</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Excess Curiosity</td>
<td>0</td>
</tr>
<tr>
<td>DEATH AND AUTOPSY NOTES</td>
<td>Note: Resp. or Cardiac Arrest, systole, crepitations, color, intact, Wall and Lungs, etc.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red spots on the lungs</td>
<td></td>
</tr>
</tbody>
</table>

**Other Notes:** Associate each symptom or observation with a specific time post-dosage. Note stereotypy.
+, fasciculations in more than one area, but movement is not continuous; ++, fasciculation is seen in several areas (hind legs, back, neck) simultaneously with response persisting in at least one area continuously; ++++, continuous total involvement.

**Clonic convulsions** are rated depending upon the severity and the duration of the seizure: +, sporadic convulsions; ++, almost continuous convulsions; ++++, the convulsions are very severe and the rat dies.

**Tonic convulsions** are rated similarly: +, the rat stretches the hind legs and the body is rigid and reacts when touched at; ++, repeated convulsions, in between the rat lies on its side; ++++, the convulsions are very severe and the rat dies.

**Mixed type of convulsions** with both clonic and tonic convulsions are rated analogously.

**Respiratory rate.** Is the same recordings as described before.

**Increase in respiratory depth** is rated as follows: (+), equivocal increase; +, definite increase; ++, pronounced increase.

The third group of symptoms derives from the eyes.

**Enophthalmos and exophthalmos** are rated subjectively on a scale from (+) to ++ after checking one's evaluation with a look at the natural, untreated rat in observation rink: (+), equivocal response; +, definite response; ++, pronounced response. Agents affecting hypotension produces enophthalmos while hypertensive agents will produce exophthalmos.

**Palpebral ptosis** is ascertained after manually alerting the rat to make sure that the ptosis is a permanent displacement. The ptotic response is scored according to Rubin et al., (1957): 0, normal opening of the eyelids; 1, noticeable closure of the eyelids; 2, half closure; 3, almost complete closure; 4, complete closure. The test response, which ranged from 0-8 for each rat, is the sum of the scores for both eyes. Whenever complete closure is seen, the eyes should be checked to make sure that the phenomenon is not due to encrustation from eye secretions.

**Pupil size** is measured accurately using a matching process holding in close proximity to the pupil the cardboard sheet, described above. The pupil should be measured under a constant but diffuse source of illumination; care should be taken not to excite the animal. After the regular reading the pupil is measured for its responsiveness to light: a flashlight is held approximately 4 cm away and directed towards the pupil. Normally the rat shows a contraction of the pupil, which is marked 0, lack of concentration is marked + in the work sheet. The responsiveness to light can also be tested by keeping the hand in front of the eye and then rapidly withdraw it, so the light reaches the eye.

**Nystagmus** is rated by looking at both eyes simultaneously from a head-on position.
Lachrymation, chromodacryorrhea are measured according to Malone et al. (1961) by folding in half a single piece of 5, 5 cm diameter filter-paper (Whatman no. 1 or similar quality) and placing the fold corner of this gently but firmly into the inner canthus of the rat eye. The absorbant paper quickly drains the fluids from the conjunctival sae within 20 seconds and provides a permanent document of the dual lachrymation-chromodacryorrhea response. By repeated refolding a single piece of filter-paper will contain the control reading and the five test readings. After draining the eye, the paper is unfolded (Fig. 4) and the length of \( W_2 \) and \( L_2 \) is measured in mm across the noncolored moistened section. The distances \( W \) and \( L_1 \) are measured in mm across the red-brown colored section. In the worksheet the figures \( L_2/W_2 \) are given for lachrymation and the figures \( L_1/W_1 \) are given for chromodacryorrhea ("bloody" tears).

![Fig. 4](image)

The fourth group of symptoms are obtained from the ears. In order to provide a sound basis for rating blanching and hyperaemia of the skin, the investigator counts the number of small vessels that are visibly detectable in either the right or the left ear during the control period. The ear should be placed between the light and the eye of the investigator in order to facilitate the observations. A small drawing of the tree-shaped vascular should be drawn on the little square of the work sheet so that one can keep the relative positions of the vessels in mind. When blanching occurs, the number of visible vessels decreases, while in hyperaemia the visible number increases. Rating of blanching and hyperemia is done subjectively based on the number of blood vessels apparent and the general color of the ear: (+), equivocal effect; +, definite response; ++, pronounced response.

Cyanosis is rated subjectively by viewing the color of the ears, feet and oral mucosa [ (+) to ++ ]. The degree of cyanosis is determined by comparing the test animal with control rat.

The fifth group of parameters are headed General.
Salivation is scored according to Malone et al. (1961) using the following numerical scale: +2 = maximal response with saliva actively dripping down from the jaws; +1 = jaws and chin fur wet, no drooling noted; +0, 5 = jaws and fur not noticeably wet, but moistening of the filter-paper (the same as for lachrymation test) noted when the paper is wiped beneath the jaw; 0 = absence of response, normal control-like response, filter-paper wiped beneath jaw remains dry.

Tail erection or the Straub response is rated as follows: (+), the tail is erected 10–40° or only intermittently to this level; +, the tail is erected 40–60°; ++, the tail is erected 60°–90°; ++++, the tail is erected more than 90°, a tense tail that arches closely over the back of the rat.

Pilomotor erection is rated subjectively from (+), equivocal response, the pilomotor erection disappears when touched upon; +, definite pilomotor erection in the neck region; ++, the hair of the entire rat is erect and rough-appearing.

Micturition is noticed from the spots of urine on the paper and if the rat is wet on the upper parts on the hindlegs.

Diarrhoea is observed when the rectal temperature is measured and by observing the degree of faeces staining on the paper in the observation rink. Diarrhoea is rated as follows: 0, the thermometer is clean, when taken out or only firm pellets follow the thermometer; (+), pellet when flicked off on the paper leaves a small but detectable stain; +, semi-firm pellet leaves a stain roughly the size of the pellet; ++, soft pellet and the stain exceeds the boundary of the pellet; ++++, a shapeless faeces mass with pronounced staining of the paper.

Brown or red colouring of the urine and black or red colouring of the faeces should be checked using Hematest Reagent Tablets or Paper (Ames Co).

Colpectasia in the female rat is instead of priapism rated by noting the presence (+) or absence (0) of discharge from the vaginal canal, whether discharge is clear or milky, and whether the mucosa around the vaginal is engorged and red in colour. In the male rat priapism (erection of penis) is rated as (+) equivocal; −, present.

Robichaud-Test consists of picking up the loose skin of the rat’s back between the thumb and forefinger until the animal has been just raised off its feet and then suddenly releasing the skinfold. In a normal animal the skin will promptly readjust to the contours of the body. If a perpendicular fold persists for 3–5 seconds after release, the response is rated as +; and persisting for longer than 5 seconds as ++. Agents promoting skeletal muscle relaxation, excessive diureses, and/or acidosis produce this phenomenon.

Circling motions is a common form of stereotypy seen with rats on test. However, this symptom may also be produced by infections of the inner ear which are fairly common in most rat colonies. An infected rat when picked up by the tail begins to spin around, even though it may not show circling motions when placed in the observation rink. A rat suspected of an ear infection should not be used as a test animal. Circling motions are rated as being either present (+) or absent (0).
Tail lashing from side to side is a spontaneous phenomenon which can be precipitated in certain treated rats by stroking the animal’s back downward towards the tail. Presence or response is rated as +, absence as 0.

Abdominal griping or writhing is rated in the following manner: (+), equivocal present; +, present but, not seen continuously; ++, continuously seen.

Rectal temperature is usually not determined until the 30 minute reading after injection. It is always important to insert the thermometer to the same point. Recordings are followed and noted at the maximal value.

Body weight is not determined until the 1-hour reading.

The sixth group of parameters is called Subjective

Head tap test.—The wood end of the horsehair probe is used to make three brisk, successive vertical taps on the skull between the rat’s eyes in order to check the animal’s reaction to “head tap”. Rats have a normal reaction pattern that falls into three distinguishable types: aggression (biting or attacking the wood probe), passivity (ignoring the probe or tolerating it) and fear (turning and fleeing). It is desirable to perform the test in two three-tap sessions about 15 seconds apart. One should not test a drug on only passive or only aggression rats, but should attempt to disperse animals having all three control reaction patterns randomly, within the test series, if possible.

Body grasp test is performed as follows: When the investigator’s hand closes about the rat’s body with the fingertips meeting in the abdominal region, and when gentle but firm grasping pressure is applied, the test animal again displays one of the three basic reaction patterns. The bodygrasp is much more psychologically challenging to the rat than the head tap test.

Stature positions or catalepsy is based upon the length of time that a rat will stay placed in an unnatural position. One of the rat’s foreleg is placed on a piece of wood, 7 cm high. Normally the rat immediately leaves this position. When catalepsy is present, it is rated as follows: (+), 30–60 seconds; +, 1–2 minutes; ++, 2–3 minutes; ++++, 3–4 minutes; +++++, more than 4 minutes.

Where catalepsy is present it is important to note whether the animal can be aroused by a sharp noise, such as that used to determine the startle reaction.

Excess curiosity is rated subjectively as follows:

(+) equivocal;
+ present;
++ continuously present.

Autopsy.—If the rat dies acutely from drug effects, it is important to autopsy immediately and determine whether death is due to cardiac or respiratory arrest. If the heart has stop; it is noticed whether in systole or diastole (only on quite recently dead animals) and whether the auricles are beating in normal rhythm.
Routinely is also noted colour of the inside of the abdominal wall, around the place of injection to see local irritation, degree of motility of intestines, relative size of mesenteric vessels, colour of lungs, whether blood clots normally, and anything unusual that may be seen at gross observation.

After observations have been made on the seventh day the rat is killed and autopsied. The investigator must note the items above as well as the colour and size of liver, kidneys, uterus and testes.

**The performance of the screening**

On a bench in a quiet laboratory where nothing else than the screening is performed, four observation rinks are placed. In each rink two rats, each with a identification mark, are placed and are left for 15 minutes to become acclimatised to the rink. One rat serves as a control (on the following day it will be injected with the drug material) and on the rat to be injected the screening program is carried out [to be filled in the column C (control)] in the following sequence:

1. Motor activity
2. Respiratory rate and depth. The presence of (+-) of Cheyne-Stokes respiration and dyspnoea is also noted, if so this is noted at the bottom of the worksheet together with an onset time and a time of disappearance.
3. Tremors, fasciculations
4. Symptoms of the eye
5. Symptoms headed Subjective
6. Robichaud-test
7. Cornea and pinna reflex
8. Analgesia
9. Ataxia, loss righting reflex
10. Anaesthesia
11. Paralysis
12. Screen grip
13. Convulsions
14. Symptoms headed Ears, Oral mucosa
15. Symptoms headed General with the exception of the Robichaud-test in the order they are given in the work sheet.
The injections and observations are performed according to the following timetable, which may serve as a model:

<table>
<thead>
<tr>
<th>Rat in Rink No.</th>
<th>Time of Injection</th>
<th>Time of observations after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>I</td>
<td>8:30</td>
<td>8:35*</td>
</tr>
<tr>
<td>II</td>
<td>8:35</td>
<td>8:40</td>
</tr>
<tr>
<td>IV</td>
<td>9:45</td>
<td>9:50</td>
</tr>
</tbody>
</table>

*Performed immediately after injection of rat No. II and IV respectively.

Lunch break could be taken between 10.50 and 11.40 or from 11.50 to 12.30. In the free intervals autopsy on the seventh day's animals is performed, planning the screening for next day, preparing suitable dilutions of suspensions to be used next day.

The screening is performed as a double-blind test. Thus, the suspensions of plant extracts and solutions of reference substances to be injected is prepared by one of my chemical assistants under a serial code number, and given to the pharmacological assistant, who performs the screening. After the evaluation of the results is made by the author, the identity of the test solution is revealed.

Once again should be pointed out the importance that all blanks of the worksheet are filled in. Any observed symptom that is not listed on the worksheet is also noted at the bottom of the page and associated with an onset time and a time of disappearance. The investigator must never ignore a phenomenon either because it is unexpected or difficult to clearly describe. When the investigator feels that the phenomenon seen is equivocal it is still noted; then, when the higher dosage is administrated, the phenomenon should reappear to a greater degree, if it is truly part of the effects of the drug material. If any symptom does not follow a dose-response pattern, then it usually must be classified as an idiosyncratic response. Evaluation of the effects of the drug is based on the response profile of the entire animal population receiving the five dosages rather than on one isolated animal.

Results and discussion

In order to train a person to perform the screening it is necessary first to study the effects of various doses of certain reference substances. For the evaluation of the effects of a new drug comparison must be made with the profile of effects of reference substances.
The following reference substances (and their dosage) have been used:

Adrenaline bitartrate: 4, 2, 1, 0.5, 0.25 mg/kg (calcd. as base).
Amylobarbital: 20, 50, 25, 10 mg/kg.
Amphetamine sulphate: 80, 40, 20, 10, 5, 2, 1 mg/kg.
Atropine sulphate: 10, 5, 2.5, 1.25, 0.63 mg/kg.
Carbacholine HCl: 4, 2, 1, 0.5, 0.25, 0.125 mg/kg.
Chlorpromazine HCl: 50, 40, 20, 10, 5 mg/kg.
Codeine phosphate: 120, 60, 30, 15, 7.5, 3.8 mg/kg.
Ephedrine HCl: 80, 40, 20, 10, 5, 2.5 mg/kg.
g-Strophanthine: 50, 30, 20, 10, 5, 3 mg/kg.
Hexobarbital: 20, 100, 50, 25, 12.5, 6.2 mg/kg.
Lidocaine HCl: 80, 40, 20, 10 mg/kg.
Mephenesin: 600, 400, 300, 200, 100, 50 mg/kg.
Meprobamate: 1600, 800, 400, 200, 100, 50 mg/kg.
Methadone HCl: 25, 12.5, 6.2, 3.1 mg/kg.
Morphine HCl: 320, 160, 80, 40, 20, 10 mg/kg.
Noradrenaline bitartrate: 10, 5, 2.5, 1.25, 0.5 mg/kg.
Papaverine HCl: 50, 30, 20, 10, 5 mg/kg.
Pentazol: 80, 50, 40, 30, 20, 10 mg/kg.
Reserpine: 20, 10, 5, 2.5, 1.25 mg/kg.
Scopolamine HBr: 10, 2, 1, 0.5, 0.12, 0.06 mg/kg.
Strychnine nitrate: 2, 1.6, 1.4, 1.2, 1 mg/kg.
Suxamethonium iodide: 5, 3.8, 2.5, 1.9, 1.25 mg/kg.
Physostigmine methylsulphate: 1, 0.5, 0.25, 0.12, 0.06, 0.03 mg/kg.
Transergran: 200, 100, 50, 10, 5 mg/kg.
Tubocurarine chloride: 0.3, 0.2, 0.15, 0.075, 0.05 mg/kg.

Each of this reference substances shows one or more positive responses in the parameters included in the worksheet. Such reference substances like reserpine, strychnine and physostigmine have a very characteristic profile of effects. Other substances such as amphetamine, atropine, codeine (Fig. 1), hexobarbital and tubocurarine show a profile characteristic of a whole group of drugs, where as, for instance, adrenaline, g-strophanthine and mephenesin is less characteristic.

The battery of reference substances may vary, the larger the better; it shows what kind of effects and kind substances that can be detected with this screening.

At present we have tested 238 raw plants extracts (alcoholic, freeze-dried extracts) and purified extracts made from the raw-extracts. As mentioned before the screening is performed as a double blind test and in order to test the validity of the method some plants known phytochemically and pharmacologically have been included.

In roots of Rauwolfia vomitoria (Fig. 2) and seeds of Physostigma venenosum (Fig. 3) the effects of reserpine and physostigmine (synstigmine) were easily recognized, respectively.

On the other hand the cardioactive alkaloids in the bark of Erythrophleum guineense and Erythrophleum africanaum show only lethal effect, preceded by CNS-depressive symptoms.

The hallucinogenic effects of the alkaloids in Tabernanthe iboga are only reflected by increased motor activity and coarse body tremor.
Thus, it is easy to overlook important effects and it shows the difficulty involved in evaluating the results obtained. It means that this method is only a broad screening, and that for instance also lethal effect, preceded by CNS-depressive symptoms must be considered.

From the 238 raw extracts 35 have been considered worth further purification. During the chemical purification of the raw extracts it has been possible to follow the increasing potency of an effect originally found in the raw extract. New combinations of actions have been found and also quite unexpected effects. Thus, for instance muscle relaxing action of tertiary alkaloids from African Loganiaceae plants has been established.

In conclusion it may be stated that the method described is well suited for a general broad screening of plant extracts.

On the basis of the results obtained a fairly large number of raw extract must be chosen for further purification. At this later stage other screening procedures for testing (for instance isolated intestine, and Langendorff heart preparation, blood-pressure on cats and so on) and discrimination of effects must be used.

Acknowledgements

I want to thank Dr. M. H. Malone for good advice, when we met in the U. S. A. and by correspondence. Astra Ltd., Södertälje is thanked for financial support of this investigation; my sincere thanks also go to my perfect assistant and coworker, Mrs. Kerstin Fungdal, who performed most of the screening work.

REFERENCES

PHARMACOLOGY
AND
CLINICAL TRIAL OF
HAYATIN METHIODIDE

BY

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Several derivatives of hayatin, an alkaloid from the roots of an Indian plant, *Cissampelos pareira* Linn. (Bhattacharji, Sharma and Dhar, 1952 and 1956; Bhattacharji, Roy and Dhar, 1962), were tested for their pharmacological activities. In these tests hayatin methiodide (HMI) was found to be a potent neuro-muscular blocking agent, which was also confirmed in subsequent clinical trial in a series of 100 patients undergoing surgery. This report summarises the available pharmacological and clinical information relating to the compound.

Neuro-muscular blocking effect

The muscle relaxing effect of HMI was tested on several species of animals, either on the intact animal or on isolated tissues by different methods such as the inclined screen test in mice, head-drop method in rabbits, sciatic nerve-gastrocnemius preparation in cats and dogs and phrenic nerve-diaphragam preparation of rats (Pradhan, Way and Varadan, 1952, 1958; Pradhan and De, 1953).

Table I—Comparative data for Hayatin Methiodide (HMI) and Tubocurarine Chloride (TC)

<table>
<thead>
<tr>
<th></th>
<th>HMI (mg/kg)</th>
<th>TC (mg/kg)</th>
<th>Potency Ratio HMI/TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;, Mice, s. c.</td>
<td>0.20</td>
<td>0.40</td>
<td>2</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;, Mice, s. c.</td>
<td>0.36</td>
<td>0.60</td>
<td>1.6</td>
</tr>
<tr>
<td>HD&lt;sub&gt;50&lt;/sub&gt;, Rabbit, i. v.</td>
<td>0.05</td>
<td>0.11</td>
<td>2.1</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;, Rabbit, i. v.</td>
<td>0.07</td>
<td>0.15</td>
<td>2.2</td>
</tr>
<tr>
<td>HD, Rabbit, i. v.</td>
<td>0.08</td>
<td>0.17</td>
<td>2.0</td>
</tr>
<tr>
<td>(mean, cross over test)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. M. Block, Dog, i. v.</td>
<td></td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>
The subcutaneous ED$_{50}$ in mice was found to be $0.195 \pm 0.0187$ mg/kg and the intravenous HD$_{50}$ (50% head-drop dose) by the quantal assay procedure in rabbits was $0.0526 \pm 0.0009$ mg/kg. The mean head-drop dose for HMI by the cross-over test in rabbits was found to be $0.0840 \pm 0.0176$ mg/kg. The corresponding dose for (+)-tubocurarine chloride (TC) was $0.1713 \pm 0.0364$ mg/kg. In mice and rabbits HMI appeared to be approximately twice as active as TC. These tests, however, did not provide information regarding the site of relaxant effect of HMI, for which purpose experiments on isolated nerve-muscle preparations were helpful.

The activity of HMI was 70 to 75% of that of TC on the rat phrenic nerve-diaphragm preparation (Kreb's bicarbonate Ringer; 5% CO$_2$/95% O$_2$). The block due to HMI was dependent on the frequency of stimulation in the same way as the block due to TC, suggesting that the block was post-synaptic in nature.

In the sciatic nerve-gastrocnemius preparation of cats and dogs HMI blocked the effect of electrical stimulation of the nerve, though the muscle could still be stimulated directly. The effect of this drug has not been studied on nerve conduction; however, it can be presumed from its many similarities to TC that HMI acts mainly by blocking the neuro-muscular junction. HMI depressed the indirectly elicited muscular contraction by 66–90% at a dose of 0.20 to 0.33 mg/kg. It appeared to be about 1.14 times as potent as TC in dogs.

HMI showed resemblance to TC in many aspects, such as the onset, development and degree of paralysis in different muscles; the limb muscles were more depressed than the respiratory muscles, the diaphragm being least affected. This was further investigated by comparing the dose of HMI causing complete respiratory paralysis with that causing total limb paralysis; the ratio of these two doses was approximately two, the corresponding ratio for TC being 1.25.

HMI also resembled TC in producing a flaccid type of paralysis in pigeons after intravenous injection, in antagonizing acetylcholine-induced contracture of the frog's abdominis and in being readily antagonised by the anti-cholinesterases, e.g., neostigmine and physostigmine, in all species of animals tested upon.

Effect on ganglion transmission

No significant effect on the autonomic ganglia has been observed at low doses (0.2–0.4 mg/kg) either in experiments on pressor response to acetylcholine in atropinized cats and dogs or on contraction of nictitating membrane of cats following pre-ganglionic stimulation (Pradhan, Ray and Varadan, 1958). However, at doses of 1 and 2 mg/kg, it produced blockade of contraction of nictitating membrane upto 20–30% and 30–70% respectively. Further, it showed differential block at the autonomic ganglia, the parasympathetic ganglia being more susceptible than the sympathetic ganglia (Patnaik, Pradhan and Vohra, to be published, 1965.)
Cardiovascular effects

At doses of 0.1–0.2 mg/kg which depress neuro-muscular transmission considerably, HMI lowered blood pressure very slightly or none at all. At doses of 0.3 to 0.4 mg/kg a fall of 30 to 40% in blood pressure was observed. The fall in blood pressure decreased on repeated administration of doses and appeared to be less than that caused by a corresponding dose of TC. This effect was also observed in spinal preparations, though to a lesser extent. It did not appear to be due to a central effect, nor was it cardiac in origin, since no depression of heart was seen on the isolated heart, on the heart in situ and in the electrocardiogram (loc. cit.) Vohra, M.M., unpublished data.) Leg volume and spleen volume in cats and dogs, however, increased showing vasodilatation as the main cause of hypotension. The fall could be prevented almost completely by anti-histaminics such as promethazine. Thus, in the causation of hypotension by HMI, a release of histamine plays an important role, along with its ganglion blocking effect discussed before. Release of histamine by HMI can be further corroborated from its stimulating effect on gastric and bile sections (loc. cit.) and from the formation of a weal on intradermal injection.

Respiratory effect

After small doses of HMI there was an initial depression of respiration followed by stimulation and subsequent depression. With the increase of dose a partial or complete paralysis of respiration would occur. These changes reflect the drug effect on inter-costal muscles and diaphragm described earlier.

Gastro-intestinal effects

HMI increased the volume and acidity of gastric juice and also bile secretion in dogs. Though no significant effect was seen on isolated intestinal loops of rabbits or on the intestine in situ in cats, marked contraction was seen on the isolated guinea pig gut.

Effect on central nervous system

Following the intravenous administration of HMI in cats, dogs or rabbits no central effect was manifested. However, after its local injection (intra-cerebroventricular, intracisternal, interthecal) in a dose of 0.4 mg. in cats and 0.5 mg in dogs, HMI caused a rise of blood pressure, stimulation of respiration, increase of somatic reflexes (e.g. knee-jerk, crossed extensor, flexor and linguo-lingual) and sometimes convulsions (Sur and Pradhan, 1964).

Absorption and excretion

HMI did not appear to be absorbed from the gastro-intestinal tract, since rabbits fed orally with 125 times HD_{100} failed to show paralysis of either limb or neck muscles within 3 to 4 hours (Pradhan, Ray and Varadan, 1958). Following intravenous administration in dogs as well in patients the peak concentration of HMI (estimated spectrophotometrically) in blood was reached within 2 min., after which
it gradually decreased and could not be detected after 15 min. About 4 to 8% of the total dose injected in dogs was excreted in urine within 3 hr; the amount excreted thereafter was very slight or nil (Basu and Pradhan, 1964).

Toxicity

Animals injected with lethal doses of HMI gradually showed ataxia of hind limbs, respiratory embarrassment, twitching or convulsions (anoxic), urination and finally death due to respiratory paralysis. LD₅₀ in mice and rabbits were 0.355 and 0.696 mg/kg respectively. At sub-lethal doses the manifestations disappeared with the re-establishment of respiratory movement. Salivation was noticed in some of the dogs and rabbits. In rabbits injected up to 0.055 mg/kg (HD₁₀₀) daily for 5 consecutive days it did not produce any untoward effect or cause significant loss of body weight (Pradhan and De, 1953). With sub-paralytic doses given daily over a period of six weeks in rats and with doses causing paralysis for 4 to 6 hours and given daily for 7 days in a dog, no significant change attributable to the drug was observed on body weight, haemoglobin content, clotting time, total count of red and white blood cells and also on gross and histological picture of several vital organs, e.g., liver, kidneys, spleen, adrenals, heart, lungs and stomach (Lahiri and Pradhan, 1964).

Comparative pharmacological effects of other hayatin derivatives

Comparative pharmacological studies of several derivatives of hayatin showed that methylation of the two OH groups of the hayatin methiodide enhanced the curariform activity; activity decreased on substituting methyl by ethyl or butyl groups. The ganglion blocking action appeared to be more with ethyl and butyl derivatives (Pradhan and De, 1959). The central action of the methiodide and methochloride of hayatin was less than that of the dimethyl-ether methiodide (Sur and Pradhan, 1964).

Clinical trial of HMI

HMI has been subjected to clinical trial in a series of 100 patients undergoing surgery (Badola, Pande and Pradhan, 1964; Pradhan, Pande, and Badola, 1964). The nature and number of operations, 87 of which were abdominal are given in Table II; the duration of these operations ranged from 0.5 to 3 hr.

HMI provided adequate relaxation for endotracheal intubation and surgery; at an intravenous dose of 1.5 mg/kg maximum relaxation was seen within 1 to 2.5 minutes after injection of the drug. In some cases a small supplementary dose (5–15 mg) was needed for establishment of complete control of respiration and surgical relaxation. It appeared to be about one-third as potent as tubocurarine (and 1.5 times as potent as gallamine). The duration of action of hayatin methiodide and (+)——tubocurarine chloride appeared to be equal for equipotent doses both from the clinical observations as well as from the recordings of finger movements following electrical stimulation over the appropriate nerve at the wrist in several patients. The neuro-muscular block produced by this drug could be completely reversed by neostigmine.
### Table II—Nature and Number of Operations

#### Abdominal (87 cases)
- Gastrectomy and gastro-jejunostomy: 10
- Ileocolostomy: 2
- Hemicolecotomy: 1
- Appendectomy: 4
- Cholecystectomy: 3
- Hysterectomy: 20
- Myomectomy: 1
- Caesarean Section: 8
- Ovariectomy: 11
- Operations on Fallopian tube: 4
- Prostatectomy: 4
- Lumbar sympathectomy: 4
- Laparotomy: 10
- Herniae: 5

#### Others (13 cases)
- Vaginal operations: 6
- Amputation leg: 1
- Mastectomy: 1
- Orchidectomy: 1
- Urethroplasty: 3
- Ureteric catheterisation: 1

**Total** 100

**Side effects**

HMI caused transient hypotension of varying degrees in about 70 per cent of the patients. The maximum fall of blood pressure occurred within 5 minutes reaching the normal level within 10–15 minutes. This hypotension, which was partly due to histamine release and partly to ganglion blocking action, rarely needed any supportive treatment.

In 8 cases of Caesarean section the relaxant did not produce any untoward effect on the foetus or the uterine tone.

In three cases of this series a generalised urticarial rash was observed after injection of HMI, but disappeared spontaneously in about half an hour. The drug appears to be relatively free from serious side-effects.
In conclusion then HMI shows itself as a potent neuro-muscular blocking drug in experimental animals as well as in man. Its potency is about one-third that of tubocurarine. Its effects can be antagonised by an anti-cholinesterase like neostigmine and it is free from serious side-effects. It appears to be promising as a substitute for tubocurarine.

Acknowledgements

The material incorporated in this paper represents the collaborative work of Drs. N. N. De, C. Ray, K. S. Varadan, R. N. Sur, D. K. Basu and P. K. Lahiri of this Institute and Drs. K. Pande and R. P. Badola of the Department of Anaesthesiology, King George's Medical College, Lucknow, who have been associated with different aspects of the problem.

REFERENCES


BIOLOGICAL ACTIVITY OF PLANT PHENOLICS

by

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St. Coombs, Talawakele

Plant tissues contain a wide variety of compounds possessing phenolic hydroxy groups, and the importance of these compounds in the life cycle of the plant is becoming increasingly evident. In addition some of them have been shown to exert different types of pharmacological activity in animals and to also be of use in the food industry.

First, with regard to plants, interest in phenolics as the "antiseptics" of the Plant Kingdom is growing, and many studies have been made on disease resistance and the interaction of micro-organisms with phenols. For example, studies of the so-called "brown rot" disease of apples, caused by the fungus, Sclerotinia fructigena, have shown that whilst no variety of apple is immune to attack, there are differences in varietal susceptibility. In a series of studies (Byrde et al., 1960) at the Long Ashton Research Station, Bristol, it was shown that the resistant varieties of apple, (e.g., Yarlington Mill), had a significantly higher polyphenol content than the susceptible varieties. The work of Byrde and his co-workers established that the resistance to disease was due to inactivation by oxidised polyphenols of the extra cellular pectolytic enzymes of the fungus—which enzymes are necessary for pathogenicity. This finding may be considered together with that of Vincent and Segonzac that tannins in low concentration inhibit the enzyme, hyaluronidase, which is secreted by certain organisms pathogenic to animals. The function of hyaluronidase in animal pathogens is similar to that of pectolytic enzymes in plant pathogens in that these enzymes depolymerise mucopolysaccharides and pectin respectively, both of which function as cement substances between cells. In addition to the action of oxidised polyphenols in combating infections in this way, unoxidised polyphenols have also been shown to have a direct fungistatic effect on infection of, for example, onions and apples, by other fungi pathogenic to these hosts.

Coming now to the inactivation of plant viruses by polyphenols, the inhibitory activity was found to be a function of molecular size—simple phenols such as flavanoids and leucoanthocyanins which have no tanning action did not protect against infection. Furthermore, tannic acid or raspberry leaf extract (both of which contain polymeric polyphenols), decrease infectivity when mixed with the virus inoculum prior to infecting the plant, although they have no effect when sprayed on leaves which have been already infected. Also the union between virus and tannin is loose and easily dissociable by dilution or increase in pH. Hence
although viruses are inactivated by polyphenolic compounds in vitro, there is no unequivocal evidence that they confer protection in the field against viruses that are transmitted through the soil or by insect vectors.

In addition to their action on fungi and viruses, polyphenols have been found to inhibit the germination of certain seeds and fungal spores. Seeds of sugar beet were found to contain a potent germination inhibitor, which was identified as ferulic acid, and this acid, as well as the closely related compound, vanillic acid, has been shown to be present in different species of Chenopodiaceae, Gramineae and Leguminoseae. In the case of spores, studies were made to find out the cause of the erratic germination of uredospores of Puccinia graminis var. triticum (wheat rust). It was found that here too ferulic acid, as well as p-hydroxybenzoic acid and certain coumarins, strongly inhibited the germination of these spores. Moreover, it was found that there was a correlation between rust resistance and phenolic content. Apart from this well studied problem, there is some evidence that the resistance of onions and potatoes to infections is related to the amounts of protocatechuic, caffeic and chlorogenic acids present in these crops.

What has been said so far suggest the use of polyphenolic compounds as antimicrobials, but the greater part of research effort has been directed to their pharmacological properties. This field of investigation was initiated in 1936 by Szent-Gyorgi and co-workers when they discovered that 'citrin', a mixture of flavonoid compounds, which included hesperidin, had the property of reducing capillary fragility and permeability. They gave the term "Permeabilitas-vitamin " and Vitamin P to this complex of factors, and it has since been claimed that many flavonoids (rutin, dihydroquercetin, catechin, epicatchin, myricetin) have a similar activity. There is some evidence in support of these claims, but there is doubt as to whether Vitamin P meets all the classical requirements of a vitamin and the term has been dropped. Instead of the word "bioflavonoid" has been suggested to designate flavonoid compounds having biological activity. Such compounds have been used for the treatment of many widely different conditions, e.g., diabetes, hypertension, rheumatic fever, arthritis, the common cold, radiation disease, frostbite and others (Pearson, 1957) too numerous to mention, but their use in these has not, for the larger part, been justified by clinical trials. However, the oestrogenic activity of isoflavones (Biggers, 1959) has been well demonstrated in studies with animals. The most important of those investigated so far is genistein, which has shown to be the probable cause of infertility in sheep feeding on subterranean clover in Australia. In this connection it is interesting to note that genistein occurs in soya beans. Recently a coumarin-like compound, coumestrol, has been isolated from clover and leciner and shown to have considerable oestrogenic activity.

The well known story of cattle bleeding to death after feeding on spoiled sweet clover hay led to the isolation of dicoumarol -3 -' methylene-bis (4-hydroxy coumarin). The haemorrhagic condition caused by this compound was found to be characterised by prothrombin deficiency, and use has been made of dicoumarol for the prevention of the clotting of blood and in the treatment of coronary thrombosis. Another polyphenolic compound known to have a definite action in man is the
dihydrochalcone glucoside, phloridzin, which produces glucosuria. This was isolated in 1835 from the root bark of the apple tree and later shown to have an inhibitory effect on phosphorylase and dehydrogenase systems, but the mechanism of its action in causing glucosuria in man is not known with certainty. Besides being present in apple, phloridzin also occurs in other members of the Rosaceae, e.g., *Pyrus* and *Prunus*.

In the food investigation field, it has been found, that polyphenolic compounds occur in nearly half of the food-yielding plants. They are responsible for the colour and astringency of unripe fruits, and also sometimes detract from the aesthetic value of foods—as when they cause "browning" of potatoes and apples, although this same "browning" reaction is indispensable to the manufacture of tea and cocoa, and also in the production of tobacco. In tea and cocoa manufacture, the oxidation of polyphenols, besides imparting a desirable colour to the "liquor", also play a part in regulating the extent of enzymic reactions that occur during "fermentation". This regulating action is due to the inhibitory action of oxidised polyphenols on enzyme systems, including the polyphenolase which is responsible for their formation. Hence the enzymic reactions which occur during "fermentation", cease when the level of oxidised polyphenols reaches the inhibitory concentration (Wickremasinghe and Swain, 1964). Finally the ease of oxidation of polyphenols has led to their use as anti-oxidant additives to foods, e.g., dihydroquercetin, quercetin and myricetin have been added to lard, peanut and other oils, and also been used to prevent the oxidation of ascorbic acid.

I have by no means exhausted all the types of biological activity of plant phenolics, but hope that I have indicated their wide range and the possible use of this class of compounds.

REFERENCES


CARDIOVASCULAR
PHARMACOTHERAPEUTICS
OF SOME EASTERN
MEDICINAL PLANTS

BY
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Life destroying animals and life saving plants are co-existing in the Indian forests from time immemorial. Ever since the dawn of Indian civilization, some of these plants have been worshipped and celebrated in the folk-lore medicine which is developed from century-old human experience. Fortunately, but sometimes most unfortunately, some of these natural treasures are gradually disappearing from the encyclopedia of human knowledge by the advent of synthetic medicines before proper evaluation and clear understanding of the natural sources could be made out. Even a poisonous plant like *Thevetia neriifolia* which is notoriously used by vagrers for suicidal and homicidal purposes, can be of utmost significance in saving the life from congestive heart-failure, if properly studied in the animals and carefully applied in humans.

That India has a rich source of indigenous medicinal plants can be supported by a comment of Prof. Greenish of the London school of Pharmacy, “India, owing to the remarkable variations she possesses of climate, altitude and soil, is in a position to produce successfully every variety of medicinal herb required by Europe” (Chopra and Chopra, 1955).

Ever since the advent of modern knowledge of botany, chemistry, pharmacology and biochemistry, a systemic and integrated study of various indigenous plants of medicinal importance are being carried in India. Utilization of the local resources and substitution of the indigenous products for the more expensive imported foreign drugs is the only way to bring the treatment of human ailments within the means of common masses. With these objectives, the study on the Indian indigenous plants was carried out in this department and the work conducted till today has brought the following 6 medicinal plants upto the stage of clinical trials in human patients with successful results.

I. Development of drugs for the treatment of congestive heart failure and cardiac oedema

A. *A cardioactive glycoside—peruvoside from Thevetia neriifolia* Juss

*Thevetia neriifolia* has been used clinically by ancient physicians of India. It is a wild plant growing throughout India. The recent interest in this plant was aroused by Kohli and Vohra's (1960) preliminary publications wherein they have reported...
it to possess cardiotonic properties. Rangaswamy and Rao (1959) have isolated a new digitaloid glycoside—peruvoside from the kernels of *Thevetia neriifolia* Juss. The process of extraction and isolation of this is inexpensive.

Detailed pharmacological studies of peruvoside, conducted by Arora *et al.* (1965) revealed that this orally administrable drug is as potent as ouabain. In the heart-lung preparation it showed a marked positive inotropic effect on cat—papillary muscle and restored the force of contraction diminished by pretreatment with sodium pentobarbitone. It exhibited a marked cardiotonic activity in the isolated, denervated heart-lung preparation (HLP) of the dog. When administered by continuous infusion method into HLP of the dog, it exerts its dominant effects on the cardiac output, atrial pressure and heart rate as depicted in Fig. 1. The therapeutic, irregularity and lethal doses of peruvoside as determined on the heart-lung preparation are similar to those of ouabain.

![Graph showing the effects of peruvoside on cardiac output and arterial pressure.](image)

*Fig. 1.—The effect of a constant infusion of peruvoside on pentobarbital heart failure in the H. L. P. of the dog*

Subacute toxicity experiments in the dogs showed that electrocardiographic changes are typical of cardiac glycosides. This quick acting drug is well absorbed from gastrointestinal tract and has low cumulative toxicity. Mild degree of vomiting is observed in the animals receiving high doses of peruvoside.
It appears to be superior over ouabain in following aspects:

(i) It can be administered orally unlike ouabain, and is completely absorbed from the gastrointestinal tract;

(ii) It has a wide margin of safety;

(iii) The compound is less cumulative and has no major side effects.

Preliminary clinical trials conducted on the patients of congestive heart failure due to mitral stenosis have shown very promising results with this drug. The patients could be "digitalized" easily at a daily dose of 2 to 3 microgram per kilogram and maintained on the maintenance dosages for a period of more than four to six weeks without any sign of toxicity. Further results are awaited.

Some of the early clinical trials were conducted at the Institute of History of Medicine and Medical Research, New Delhi, by us under the guidance of Hakim Abdul Hamid Saheb and Dr. Siddiqui.

B. New Indigenous diuretic agent—a glucoside and its aglucone from Caccina gauaca

*Caccina gauaca* Savi, commonly known as Gaozaban, is frequently used in irritation of the bladder, strangury, and in several other conditions. Various uses of this plant have been described by ancient writers. Recently, a glucoside, caccinin, and its aglucone, caccinetin, which is the dimethylallyl ester of caffeic acid, have been isolated from it in our department in collaboration with Delhi University. In view of the reputation and extensive use of the drug by practitioners of the indigenous system of medicine an investigation was undertaken by Arora and Arora (1962) to elucidate the pharmacological properties of the pure compounds.

The most prominent property of the compounds isolated from *Caccina gauaca* is their diuretic activity. This property is exhibited after oral as well as parenteral administration in the conscious and anaesthetized rats and anaesthetized dogs. Activity is manifested in doses which do not have other pharmacological actions.

It appears that these compounds act by increasing glomerular filtration. This very much resembles the action of xanthines, which have their main value in cases resistant to mercurial diuretics due to a low glomerular filtration and are the only agents available at present which effectively increase glomerular filtration. The xanthines, however, possess undesirable side effects especially in large doses on the cardiovascular, gastrointestinal and central nervous system. On the contrary, the isolated glucoside
and the aglucone, even in large doses, do not exhibit any such undesirable actions as shown by this study. They may, therefore, be considered to have some promise for further studies. Clinical trials are in progress.

II. Development of newer compounds from indigenous sources for the therapy of cardiac arrhythmias

A. Ajmaline—esters of ajmaline and serpajmaline

*Rauwolfia serpentina* has been used in the Indian indigenous system of medicine for many centuries but its pharmacological actions were not known till the thirties of the present century. Almost twenty years were to elapse before an active interest was aroused in the scientific field for the detailed investigation of this plant. From the present knowledge of 35 to 40 alkaloids of *Rauwolfia serpentina*, it is considered that possibly reserpine is by far the only promising product of this plant. But we feel that reserpine is not what is all in rauwolfia. There is plenty of hope in the other alkaloids and principles which have shown better results in our hands and we are waiting for the results of the clinical trials.

We have reported earlier that ajmaline and serpentine, derived from Rauwolfia, possess antiveratrinic (Arora, 1956) and antiarrhythmic properties (Arora and Madan, 1956). These publications evoked clinical interests and in 1959, Klinsorge of the University Polyclinic, Jena, Europe, tried this drug in cases of ventricular arrhythmia, and extra systoles, thus confirming our experimental findings. The special advantage of ajmaline appears that it can be used in acute heart attacks since it does not produce cardiac depressant action like that of quinidine. Encouraged by the favourable findings we tried esters and other preparations of ajmaline series of compounds in experimental cardiac arrhythmias.

Since no single antiarrhythmic method can predict adequately the overall activity of a compound, following techniques were used to screen the antiarrhythmic properties of ajmaline, serpajmaline and certain esters of ajmaline.

(1) Ectopic ventricular tachycardia produced according to the technique of Harris (1950).

(2) Acetylcholine induced ventricular tachycardia produced according to the technique of Schallek (1952), a modification of the method of Scherf and Chick (1951) as shown in Fig. 2.

(3) Hydrocarbon-epinephrine induced ventricular tachycardia produced according to the method of Riker et al. (1955).
Fig. 2—It illustrates the different stages in the initiation and spontaneous reversion of acetylcholine induced auricular fibrillation.

A. Direct electrocardiogram from the right atrium.
B. Bipolar Lead II—Paper speed 25 m.m./sec.
1. Normal.
2. At the arrow the pledget of acetylcholine has been applied. Note the marked bradycardia.
3. After the atrium has been pinched, note the irregular contours and spacing of auricular complexes. The ventricular rate is irregularly irregular. No presence of P waves and F waves can be seen.
4. The auricular rate has showed down and the ventricular rate is increased.
5. The fibrillation has disappeared and sinus rhythm has been restored. Note the marked bradycardia that is still present.
(4) Auricular arrhythmias:

(a) Auricular flutter as described by Rosenblueth and Garcia Ramos (1947).
(b) Auricular fibrillation as described by Scherf (1947) (Fig. 3).

The results of these drugs as observed in ectopic ventricular tachycardia due to two stage coronary artery ligation of dogs are given in the Table I. It is evident that serpajmaline and certain esters of ajmaline such as monopropionyl ajmaline hydrochloride, monobenzoyl ajmaline hydrochloride and monobutyryl ajmaline hydrochloride may prove still better and promising drugs for various complicated disorders of the cardiac rhythm.

### Table I—Antiarrhythmic Action of Ajmaline, Serpajmaline and Esters of Ajmaline

<table>
<thead>
<tr>
<th>Drugs Used</th>
<th>Equimolar Route of Doses Administra-</th>
<th>Duration of reversion</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajmaline</td>
<td>5</td>
<td>80–100</td>
<td>In some experiments ectopiventricular tachycardia reverted to nodal rhythm</td>
</tr>
<tr>
<td>Serpajmaline</td>
<td>10</td>
<td>100</td>
<td>Reversion to normal sinus rhythm</td>
</tr>
<tr>
<td>Monopropionyl Ajmaline Hydrochloride</td>
<td>6.5</td>
<td>100 70–100</td>
<td>do.</td>
</tr>
<tr>
<td>Monobutyryl Ajmaline Hydrochloride</td>
<td>6.4</td>
<td>100</td>
<td>do.</td>
</tr>
<tr>
<td>Monobenzoyl Ajmaline Hydrochloride</td>
<td>6.4</td>
<td>90</td>
<td>do.</td>
</tr>
<tr>
<td>Diproionyl Ajmaline Hydrochloride</td>
<td>7.5</td>
<td>—</td>
<td>Variable results some reversion to nodal rhythm</td>
</tr>
<tr>
<td>Dibutyryl Ajmaline Hydrochloride</td>
<td>6.5</td>
<td>—</td>
<td>Variable results</td>
</tr>
</tbody>
</table>

Our preliminary experiments have also shown that the combination of ajmaline and serpajmaline potentiate their antiarrhythmic action, thereby provide an opportunity for using them at minimal dosages with a wide margin of safety (Arora, Bagchi & Sharma, 1965).

These results are compatible with the clinical observations of Brauch of Germany (1964) who has reported that ajmaline is effective in the treatment of paroxysmal tachycardia, ventricular extrasystoles and early stages of auricular fibrillation. We hope that ajmaline series of compounds will be useful addition to the physician’s armamentarium in therapy of cardiac arrhythmias.*

**B. Reserpine**

Reserpine is an alkaloid obtained from the roots of *Rauvolfia serpentina*, a plant indigenous to India. The extracts of the roots of this plant have been employed for centuries in the folk lore of ancient Indian medicine. Its cardiovascular actions

*The first sample of serpajmaline was kindly supplied to us by Dr. Sahmuzaman Siddiqui, Director General, Council of Scientific and Industrial Research, Pakistan.
Fig. 3—It illustrates the development of auricular fibrillation after application of aconitine. Direct auriculogram was recorded along with bipolar standard limb lead.

Fig. 4
of bradycardia and hypotension were well established but its actions on arrhythmias were not known. Arora (1958) brought first evidence regarding its antiarrhythmic actions which were demonstrated in experimental animal employing the classical methods for production of arrhythmias as depicted in Fig. 4. Subsequent to this report Marangoni and Cavusoghi (1959) have observed the beneficial effects of reserpine in the patients of cardiac arrhythmias. They advocate the administration of reserpine is such patients at a dose of 1 to 3 mg per day for the reversion of abnormal rhythm to normality and for subsequent maintenance.

Reserpine may possess superiority over other antiarrhythmic agents in those cases where psychological factors precipitate the ventricular extrasystoles, since it is known to produce ataraxia as well, unfortunately little work has been done in this direction and therefore, this claim needs further support by more extensive experimental work which is already being undertaken in our department.

C. Acorus calamus

The ancient medical text books of Charak and Sushruta record that many beneficial cures with indigenous medicines in diseases pertaining to the psyche. But these authors seldom recommend the use of a single drug. Combination of many drugs such as uragandha (Acorus calamus), Brahmi, (Herpestis monniera) and Sarpan-gandha (Rauwolfia) and many others have been reported to be useful in various disorders. In an effort to shift the grain from the chaff, one single drug from amongst the many was selected for its pharmacological studies.

Madan, Arora and Kapila (1960) investigated the essential oil of Acorus calamus for its antiarrhythmic, antiveratric and anticonvulsant activities. Its antiarrhythmic actions are illustrated graphically in Fig. 5. The concept that pathophysiology of

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**Fig. 5**—Hypoprothrombinenic activity of Culophyllolide, DicumaroI, Tromexan and Sintrom after single identical doses of 50 mg/kg p. o. in male albino rabbits.
convulsions and arrhythmia due to myocardial infraction has a number of similarities led us to this investigation of finding out the effectiveness of the essential oil in experimental epilepsy as well as in experimental ventricular arrhythmia and it was found to be effective in both these conditions.

This particular drug being more effective in psychosomatic disorders rather than cardiac diseases, the emphasis was shifted to clinical trials in patients of anxiety, neurosis and schizophrenia, where its efficacy has been confirmed.

III. Development of new complex coumarin for coronary thrombosis, related thromboembolic episodes and spasmodic condition of blood vessels

A. Anticoagulant activity of calophyllolide, an indigenous complex coumarin

Calophyllolide is a complex 4-phenyl coumarin, isolated from an Indian plant Calophyllum inophyllum, Linn. (N. O. Guttiferae). Calophyllolide was extracted from defatted kernels of nuts by Ormaneay (Potier et al., 1951) and the chemical structure was established by Polonsky (1956). Since its chemical nature is of coumarin derivative, its anticoagulant activity was tested and at the same time its cardiovascular actions were also studied.

Calophyllolide, possesses a significant anticoagulant activity in rabbits. The hypoprothrombinaemic response was achieved earlier with calophyllolide than with dicoumarol and acenocoumarol although it is slower than ethyl biscoumacetate in this respect. The anticoagulant activity of calophyllolide was found related to blood plasma levels and the peak plasma levels of calophyllolide were reached during the lag period before the hypoprothrombinaemic response was obtained.

With calophyllolide, therefore, a rapid onset and gradual recovery of hypoprothrombinaemic response and less likelihood of fluctuations of prothrombin time can be obtained. Calophyllolide thus behaves like an intermediate acting compound in comparison to dicoumarol which is slow and long acting and ethyl biscoumacetate which is rapid and short acting. The antithrombotic activity of calophyllolide is represented by the marked increase in platelet clumping time. The whole blood clotting and bleeding times were also increased moderately.

Calophyllolide possesses antiarrhythmic activity as well and is as effective as quinidine in suppressing ventricular ectopic tachycardia resulting from acute myocardial infarction in unanaesthetised dog. It also possesses bradycardiac and coronary dilator actions; in addition it raises the blood pressure. Hence it may be useful in the treatment of myocardial infarction, complicated with shock and tachycardia (Arora et al., 1962).

Preliminary single dose clinical trials with this compound has given encouraging results.

B. Relationship between structure and anticoagulant activity of coumarin derivatives

Since calophyllolide was found to have a puissant anticoagulant activity this investigation was extended to study in more exact terms the structural requirement for anticoagulant activity.

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With this view 35 coumarins, with or without a 4-hydroxyl group, have been examined, for their anticoagulant activity in rabbits by determining prothrombin time by Quick's one-stage modified method (Quick et al., 1935; Montigel and Pulver, 1952). The compounds include methoxylated dico.,marols substituted 4-hydroxycoumarins and coumarins devoid of a 4-hydroxyl group such as 3-and 4-phenyl coumarins, 4-methyl coumarins and some complex coumarin derivatives having additional rings. The results show the complexity of the problem and the involvement of various factors. Among these the importance of molecular geometry is emphasized by the high activity of calophyllolide and a new synthetic compound, 4-methyl 2-2, 5-dioxi-3-phenyl-2H, 5H-pyrano (3, 2-C) (V)-benzopyran. In terms of molar activity, calophyllolide ranks second among the compounds studied and is more active than dicoumarol.

The importance for the anticoagulant activity of a substitute in position 8 of the coumarin moiety and the role of ability to ionize with regards to the Vitamin-K-like property of some hydroxylated phenyl coumarins, are also indicated (Aurora and Mathur, 1963). The study of indigenous coumarin anticoagulant calophyllolide has given us an idea of the intimate nature of the structural requirement for anticoagulant activity and also a clue to the development of new complex coumarins as anticoagulants.

IV. New vistas in the search for anti-hypertensive agents

Treatment of severe form of hypertension is still a difficult problem, despite dynamic research work in the subject. In this meeting, the other day I presented our work on hypotensive activity of Jatamansone—a sesquiterpene ketone isolated from an Indian indigenous plant material—Nardostachys jatamansi. Results of double-blind clinical trial on 28 patients with different grades of hypertension have revealed this drug to be effective in mild, moderate and in few cases of severe hypertension. It reduces the systolic and diastolic blood pressure in standing as well as in supine position. It is relatively free from side effects, effects associated with depression of parasympathetic nervous system and with central depression. Within the framework of this study it appears to be promising agent.

A point of further interest is that this drug is a terpenoid compound. Lately, great emphasis has been laid on the potentialities of terpenoid group of compounds. Extensive investigations by various workers have revealed a broad spectrum of pharmacological activities in this group and some of these have gained clinical application. This wide field of naturally occurring compounds certainly hides many areas which need thorough exploration to assess the application of terpenoid chemistry to medicinal chemistry. On the basis of our work and studies performed by many other workers I can safely presume that research in this field will prove fruitful. One compound mecamylamine—a nitrogenous monoterpene—has already proved useful in the drug treatment of hypertension.
V. Indigenous Indian plants and drugs in ischaemic heart diseases

Before I close I would like to draw your kind attention to the fact that while it has been possible for us in practically most of the countries to conquer infectious diseases and a number of other ailments and to increase the longevity, in its wake it has revealed a greater importance of treating cardiovascular diseases and cancer. For example, on June 10th, 1964, the W.H.O. experts at a meeting in Geneva opined that the deaths due to cardiovascular diseases, especially heart attacks, called by the lay people as coronary, amounts to about 48 per cent of the total deaths in the civilised countries of the world.

We are at present envisaging at the All-India Institute a programme of work of finding out the drugs from indigenous sources—Unani and as well as Ayurveda for improving the histopathology of myocardial infarction.

For the past four years we have been developing the condition simulating human coronary thrombosis and atherosclerosis in the Indian domestic pig. The pig has a large number of similarities with the human as far as the cardiovascular system is concerned. This has been clearly elaborated by Nobel Laureate Sir Howard Florey in his Jophcott lecture in 1960. For example, it has an end artery system like the humans, is omnivorous, develops coronary thrombosis, etc. after coronary occlusion and the development of spontaneous atherosclerosis is common in aged pigs, like the humans. There are also mythological, i.e. Varaha Avtar and anatomical similarities of the pig's heart with humans. We have developed two techniques of producing myocardial infarction, one acute and the other chronic, resulting from atherosclerosis. We are utilizing both these techniques in our study of the effectiveness of drugs in myocardial infarction.

We have obtained very interesting results with two of the Unani medicines used in coronary thrombosis in the Unani system of medicine. One of them is Badranj-boy. This plant grows in temperate Himalaya from Garhwal to Sikkim. An alcoholic extract of this herb, when given at a dose of 20 mg/kg per day to the pigs over a period of four days, has shown marked hypocholesteremic effect in artificially induced hypercholesteremia. In addition, it also produces some beneficial effects in the histopathology of myocardial infarction. These drugs were brought to our notice by Hakim Abdul Hamied Saheb of Hamdard Waqf, a renowned Unani physician of South-East Asia, who is the Director of the Institute of the History of Medicine and Medical Research, Delhi. The results of clinical trials with this drug in patients of coronary thrombosis though encouraging, are still in a preliminary stage and unpublished.

I would end my talk here with an appeal to the audience to explore the possibility of developing drugs for the treatment of ischaemic heart diseases and it may not be impossible to find one from the Eastern medicinal plants.

Acknowledgements

This work was made possible by funds and facilities from Indian Council of Medical Research, Council of Scientific and Industrial Research and Unichem Laboratories for which the author is deeply obliged. The utilization of some to
the author's published data from the Archives International Pharmacodynamie et de Therapie, Journal of Pharmacology and Experimental Therapeutics and American Journal of Pharmaceutical Sciences, to make the lecture more complete and comprehensive is gratefully acknowledged.

Finally confirmatory clinical trials conducted by Hakim Abdul Hamied Saheb, Delhi, Col. M.S. Rao, M.D., F.R.C.P., Indian High Commission, U.K., Dr. M. J. Shah, M.R.C.P., K.E.M. Hospital, Bombay, are gratefully acknowledged.

REFERENCES

New Delhi : Indian Council of Medical Research.


CONCLUDING SESSION
OF SYMPOSIUM

BY

R. O. B. WIJESEKERA,
Secretary,
Symposium Organizing Committee

The following resolutions were unanimously adopted at the concluding session:—

(1) Regular symposia in the region

While appreciating the success of the present Symposium and the useful contacts between scientists of our region that this event has made possible, it is recommended that UNESCO continue to sponsor similar Symposia on Medicinal Plants at regular 3-year intervals in countries of the region.

(2) International co-operation in research on medicinal plants

In view of the great interest shown by scientists of this region on medicinal plants from the point of view of research and economic potential, this Symposium recommends to UNESCO that it takes steps to approach the Governments of this region with a view to formulating a concrete programme of co-operative research on plants of this region.

(3) Conservation of naturally occurring drug plants

In view of the fact that in many countries valuable drug plants are now being collected on a large scale for commercial purposes and that such a collection may lead to the ultimate extermination of these plants in their natural environments, this Symposium recommends to the countries of the region that when drug plants are collected from natural environments adequate provision should be made to see that sufficient seed and plants are left to ensure the continued existence of the various species in their natural homes.

(4) Strict national reserves

In view of the possibility that a large number of plants which are at present not used in medicine, are potential sources of drugs and recognizing the fact that with the increasing populations in most countries at least some of the forested areas will have to be brought under cultivation, this Symposium recommends that when new areas are opened up for rehabilitation schemes or cultivation purposes, adequate sample areas of national vegetation types should be set aside and protected as Strict National Reserves to serve as reservoirs of indigenous plants for future studies.
Dr. A. G. Evstafiev on behalf of UNESCO, thanked the delegates and the host country and expressed pleasure at the success of the Symposium. He added that UNESCO would be pleased to take action on the resolutions passed.

Prof. Finn Sandberg on behalf of the delegates, thanked UNESCO and the Government of Ceylon for the arrangements for the Symposium.

The Chairman, Dr. Gunasekara, responding expressed the hope on behalf of the host country that the delegates had enjoyed themselves whilst making useful contacts and gathering useful information. He thanked the delegates for their courtesy and co-operation, and declared the Symposium closed.