ALKALOIDS OF THE GENUS RAUWOLFIA

ALKALOIDS OF THE AFRICAN **RAUWOLFIA** **SPECIES** OF **THE SUB-SECTION** **OPHIOXYLANTHUS**

Thesis submitted by

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to the University of Bradford for the degree

of Doctor of Philosophy

Postgraduate School of Studies in Pharmacy

University of Bradford

November, 1978

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. W. E. Court for his invaluable help, advice, guidance and supervision throughout this work. My thanks are also extended to Professor J. M. Rowson for his encouragement and interest in the work.

I am grateful to Professor P. W. Le Quesne, Professor R. C. Elderfield, Professor J. L. Pousset, Dr. A. Bonati and Dr. C. Thal for valuable alkaloid samples. My thanks are extended to Mr. T. Mettrick, Mrs. D. Lee and the entire laboratory staff of the Pharmacognosy and Pharmaceutical Chemistry departments for technical assistance and to Mr. R. Nettleton for mass spectrum measurements and to Mr. A. Nasser for the spectra of ajmalidine and geissoschizol.

I am grateful to my colleagues and friends in the Pharmacognosy and Pharmaceutical Chemistry departments for their valuable discussions.

My thanks also go to Mrs. Wynne Smith for typing this thesis.

I am indebted to my wife without whose forbearance and encouragement at all times this work could never have been completed.

Finally, I would like to thank Ahmadu Bello University, Zaria, Nigeria for the study fellowship award and the Federal Government of Nigeria for the financial assistance which has enabled me to pursue this investigation.

ABSTRACT

The alkaloids of the Rauwolfia species have been reviewed and spectroscopic methods of identification discussed.

Detailed phytochemical investigations have "been carried out on the two species of the section Ophioxylanthus of Pichon's classification of the genus Rauwolfia. In all, ninety alkaloids were isolated and their structures elucidated.

R. volkensii stapf root yielded twenty-six alkaloids including two new compounds, tetraphyllicine-17-O-acetyl and rauvolcinine. The stem bark gave seventeen alkaloids including the new alkaloid pre-perakine. Thirteen alkaloids were isolated from the leaves, four of which are new natural products i.e. methoxy-pleioearpamine, methoxy-pleiocarpamine 2 : 7 dihydro, methoxy-pleioearpamine N-oxide and volkensine. The alkaloid pleiocarpamine and its derivatives have not previously been isolated from any Rauwolfia species.

l\- oreogiton Mgf. root yielded thirteen alkaloids of which three have not been previously reported in the plant. The leaves yielded twenty-one alkaloids including five new ones, akuammiline-5-formyl, oreog U, akuammiline *2* -dihydro, deacetyl akuammiline 2/4 -dihydro and an important biogenetic intermediate alkaloid adirubine acetate 19 : 20 dehydro.

The inter-relationship of the isolated alkaloids has been discussed and biosynthetic pathways proposed. The evidence obtained concerning the alkaloids of the section Ophioxylanthus species has been used to relate the section chemotaxonomically to the other African Rauwolfia sections and the closely related genus Cabucala.

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INTRODUCTION

Historical Introduction

It has long been known that certain plants and groups of plants contain 'principles' which can exert marked physiological effects on the central nervous systems of animals. According to the nature and advancement of the particular human society, these plants have been variously regarded as a source of medicinal preparations, magical syrups or essences, or potent poisons.

The use of plants of the genus Rauwolfia in native medicine dates back many centuries. The Indian species, R. serpentina Benth. was mentioned in ancient Sanskrit writings and, in particular, in the 'Charaksamhita', a medica-pharmaceutical treatise, the date of which has been variously estimated as 100-800 B.C. (Srivastava, 1954) or 100 A.D. (Hora, 1951). Rauwolfia serpentina was then known under the names 'Sarpagandha' meaning serpent repellent and 'Chundrika' meaning moon and alluding to its use in lunacy (Dymock, 1891; Watt, 1932; Younkman, 1954).

From early times its main use appeared to be as an antidote for snake bite although mention was also made of its use in Indian native medicine for the relief of various nervous derangements (Younkman, 1954; Lesser, 1954).

In Africa Rauwolfia plants known by various native names have been used for the treatment of afflictions ranging from rashes to nervous breakdowns and abdominal disorders.

The ancient remedy R. serpentina became known in Europe in the 16th century as 'insanity root' and was reputed to control epilepsy, insomnia, fever, cholera, dizziness and headaches. It was not until about 1930 that the plant was seriously considered as a rational medicine.

The work of the Indian chemists Siddiqui and Siddiqui (1931) initiated a vast amount of interest in the genus Rauwolfia all over the world. They isolated five crystalline alkaloids from the root of R. serpentina but the pharmacological activities demonstrated by these alkaloids was found not to fully reflect the medical value of the crude plant extract (Chopra et al, 1941). Further research revealed that it was the weakly basic 'oleo-resin' fraction that was largely responsible for the hypotensive properties of the crude plant extract (Gupta et al, 1944).

The work of Vakil (1949) highlighted the efficiency of both plant extracts and sieved root powder in lowering elevated blood pressure. By 1950 the crude drug was available for clinical trials in the treatment of hypertension and was used extensively in Europe for this purpose.

In 1952 the Swiss chemists Müller, Schlittler and Bein isolated a feebly basic alkaloid from the roots of R. serpentina and called it reserpine. Reserpine was found to possess not only an antihypertensive activity but also a tranquilising effect. This discovery led to the investigation of many other Rauwolfia species as alternative sources of reserpine.

Consequently, one of the outstanding developments in the field of natural products in recent times has been the discovery and investigation of the therapeutic properties of Rauwolfia alkaloids. In addition to providing useful agents for the treatment of hypertension and mental diseases, this research stimulated interest in medicinal plant products the world over.

This led to a rapid expansion in knowledge of not only the field of natural product chemistry but also of the understanding of the pharmacology of the central nervous system. As newer and more sophisticated techniques of structural elucidation were developed, the structures and chemistry of the large number of newly isolated indole alkaloids began to unfold. This resulted in hypotheses concerning biogenetic transformations and the relationships of the diverse skeletal structures encountered.

The African Rauwolfia species figures prominently in this resurgence of interest in Rauwolfia and in plants of medicinal value in general. As more became known of the commercial importance of the Indian species R. serpentina, the Indian Government placed intermittent embargoes on its export. This stimulated a widespread survey of other Rauwolfia species which might be of commercial value and the African species became of interest especially to workers in Europe.

Paris (1943) demonstrated that the tree R. vomitoria Afz. was more commercially workable than the small Indian shrub. Schlittler (1961) also reported that R. vomitoria roots contained a higher reserpine content than the Indian species.

Court and his co-workers in a series of publications (1957 - 1978) have made systematic studies of, in particular, the African Rauwolfia species. Their work ranged from microscopy to quantitative chromatographic assay and general phytochemistry of the various African species of the genus.

Current research emphasis on African Rauwolfia species centres on the isolation and characterisation of the alkaloids from the plants using the more sophisticated modern techniques with a view to elucidating the chemical and biosynthetic relationships of the alkaloids within the plant species, to investigating the possible inter-relationships of the alkaloids in different parts of the individual species and to relating the individual species within the genus.

THE GENUS RAUWOLFIA

Systematics and Chemotaxonomy

Occurrence and habit

The section Ophioxylanthus

Indigenous usage

Systematics and Chemotaxonomy

The genus Rauwolfia was established in 1703 by Charles Plumier and the name Rauwolfia honoured the sixteenth century German physician and explorer Leonhart Rauvolf. Rauvolf had travelled extensively in the Middle East searching for plants of medicinal value although he never saw a Rauwolfia plant. The correct form of the name of the genus, in agreement with the international convention on Botanical Nomenclature, is Rauvolfia but, as the form Rauwolfia is so well established in medical and chemical literatures, it is used in this work.

Rauwolfia belongs to the family Apocynaceae, one of the largest families of the sympetalous order Gentianales (Engler, 1964). Other families placed in this order by Engler include Rubiaceae, Loganiaceae, Asclepidaceae, Menyantheceae and Gentianaceae.

Common morphological and chemical features exist amongst these families and all except the Asclepidaceae species have been found to produce precursors of indole alkaloids if not the alkaloids themselves (Timmins, 1975; Siegler, 1977). Plants of the families Gentianaceae and Menyantheceae produce iridoid units such as loganin or the more appropriate seco-loganin, which are the currently accepted precursors of most indole alkaloids.

The family Asclepidaceae, although very closely related morphologically to the Apocynaceae, is not known to yield indole alkaloids (Siegler, 1977).

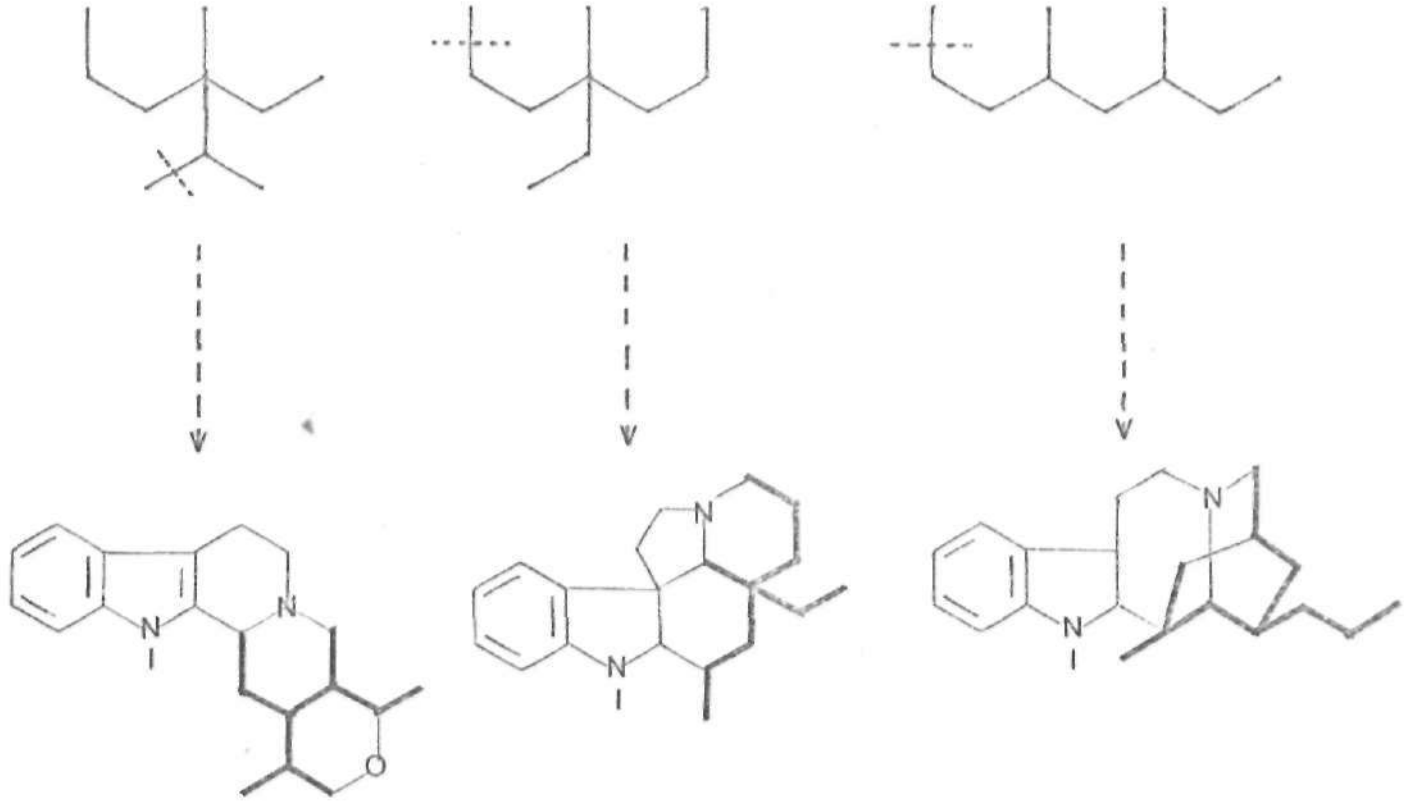
The family Apocynaceae consists of about 155 genera and can be distinguished by the universal occurrence of laticiferous canals and intraxylary phloem (Metcalfe and Chalk, 1957). The family has been divided into three sub-families, Cerberioideae, Echitoideae and Plumerioideae (Pichon, 1948).

Chemical evidence supports this division as members of the Cerberioideae elaborate monoterpenoid bases such as the alkaloidal amine skytanthines from Skytanthus species (Naffauf, 1970). The Echitoideae produce steroidal glycosides such as the cardiac glycoside k-strophanthoside from Strophanthus kombe Oliv. (Trease and Evans, 1978). The Plumerioideae group form the true indole alkaloid producing sub-family and Rauwolfia belongs to this group.

Pichon (1948) divided the Plumerioideae sub-family into seven tribes viz. Alstonieae, Allamandeae, Ambelanieae, Carisseae, Chilocarpeae, Rauvolfiieae and Tabernaemontaneae. The genus Rauwolfia is classified in the sub-tribe Rauvolfineae of the tribe Rauvolfiieae.

Plants from four of the seven tribes of the sub-family Plumerioideae have been well investigated chemically for alkaloids because of their medicinal importance. Whilst it may be necessary to await further work on the other tribes before a conclusive claim can be made either in support of, or against, the chemotaxonomic division into tribes, evidence from the four tribes so far investigated indicates that they all produce monoterpenoid indole alkaloids of three main classes (Snieckus, 1968).

It is now established, as a result of numerous feeding experiments, that the C-9, C-10 components of these indole alkaloids are derived via the acetate-mevalonate pathway and an iridoid skeleton is incorporated into the indole alkaloids through condensation with tryptamine. Since the incorporation of the non-tryptamine C-9, C-10 unit can be effected in three ways, incorporation results in three classes of indole alkaloids, A, B and C (Fig. 1).



Class A

Corynanthe-Akuammine type

Class B

Aspidosperma type

Class C

Iboga type

Fig.1. The three main classes of skeleta

All three skeletal classes have been found to occur in the sub-family Plumerioideae. The occurrence of an individual skeletal class amongst the four tribes examined has been found to vary considerably.

In a review article Snieckus (1968) has shown the distribution of the different skeletal classes of indole alkaloids in plants. Table 1 summarises the occurrence of the three classes in the four tribes of the sub-family Plumerioideae investigated.

The tribe Rauvolfiieae produces predominantly skeletal type class A alkaloids. The alkaloids of classes A and B occur simultaneously in the tribe Alstonieae but Class A type predominates. The skeletal type Class C appears to be restricted to the tribe Tabernaemontaneae. Its occurrence therefore could be used as a possible fingerprint for this tribe. The tribe Cerisseae also produces alkaloids of both classes A and B but, as in the tribe Alstonieae, Class A alkaloids predominate. In general, it is still not possible at this stage to produce chemical evidence in support of the morphological division into tribes.

Within the tribe Rauvolfiieae, although the plants elaborate mainly the Class A type alkaloids, several of the individual alkaloids occur in more than one genus. For example, reserpine has been found in Rauwolfia, Cabucala Pichon., Ochrosia Juss. and Vallesia Ruiz et Pav. (Bisset, 1958; Douzoua, 1974); isoreserpiline occurs in species of Rauwolfia, Ochrosia and Bleckaria Hassk. (Bisset, 1961; Kilminster, Sainsbury and Webb, 1972).

TABLE I

Tribes	Number of species examined	Class occurrence			Class occurrence ratio within tribe		
		Class A	Class B	Class C	Class A	Class B	Class C
Alstonieae	75	66	39	0	2	1	0
Rauvolfiieae	75	69	9	0	8	1	0
Cerisseae	8	7	2	0	3	1	0
Tabernaemontaneae	42	19	12	31	2	1	3

Distribution of alkaloids within the genus Rauwolfia is apparently not systematic; no occurrence of a particular alkaloid seems to be diagnostically peculiar to the genus. Also the unrestricted occurrence of the alkaloids of Rauwolfia in various other genera may preclude chemotaxonomic significance at the genus level.

It was emphasised, however, (Hegnauer, 1963) that it is not so much the mere occurrence of an alkaloid or alkaloidal type that represents a chemical character of taxonomic importance but the accumulation of this compound in the plant. Tetenyi (1970) also noted that a trace alkaloid or by-product can only be of taxonomic importance if its occurrence is significant in a homologous biogenetic route. Therefore, until further and more thorough investigations are undertaken, features upon which the chemotaxonomy of Rauwolfia will be based may remain obscure.

Occurrence and Habit

Plants of the genus Rauwolfia are found mainly in rain forests or well-watered grassland areas in tropical and sub-tropical zones, occurring as shrubs or trees of variable size.

Typically, the leaves are oblong, shiny and evergreen and usually occur in whorls. The genus is characterized by the occurrence of phloem sclereids, non-articulate laticiferous tubes, non-septate fibres and heterogeneous rays (Metcalfe and Chalk, 1957).

The stem can be differentiated from the root by the presence of a well-defined central pith and unlignified fibres in the pericyclic region. The plant usually bears inconspicuous white or greenish flowers or drupes.

Members of the genus vary considerably in size and habit. The small Rhodesian shrub R. nana E. A. Bruce attains a height of about 15 cm. when fully grown (White, 1962) and the large Peruvian tree, R. praecox K. Schum., can be as tall as 30 m (Woodson, 1957).

The geographical distribution of Rauwolfia species includes Central and South America, Africa, India and Burma, Philippine Islands, Hawaii, New Guinea, New Caledonia and some other Pacific Islands.

Three species in particular are of wide geographical distribution: R. serpentina occurs in India and Malaysia, R. vomitoria throughout tropical Africa and R. tetraphylla L., a species indigenous to Central America, has been introduced to India and Australia (Court, 1961).

The Index Kewensis and its supplements list more than 175 species of Rauwolfia but considerable synonymy has been observed between the entries. The works of Pichon (1947, 1948), Rao (1956), Woodson (1957) and Bisset (1958, 1961) have reduced this number to about 76 species. A recent addition to this list is R. capuronii Mgf. found in Malagasy Republic (Miet et al, 1977). The species R. discolor (Combes, et al, 1966) reputedly collected in Malagasy Republic, does not in fact exist. Boiteau (personal communication, 1975) has pointed out that the only

Rauwolfia species growing in the region of Malagasy where R. discolor was collected is R. confertiflora Pichon. (Timmins, 1975).

Pichon (1948) divided the genus Rauwolfia into fourteen sections. The division, which is largely geographical, comprises four African sections, Afrovolfia, Endolobus, Ophioxylanthus and Rhopalanthus. Rao (1957) modified this classification and produced three sections, the section Rhopalanthus being merged with Endolobus.

TABLE II

Distribution, habit and synonymy of African Rauwolfia species

Section	Species	Synonyms	Distribution	Habit
Afrovolfia	R. caffra Sond.	R. goetzi Stapf		
		R. inebrians K. Schum.		
		R. natalensis Sond.		
		R. obliquinervis Stapf	Widespread	Tree, 9-25 m.
		R. ochrosioides Stapf		
		R. oxyphylla Stapf		
		R. welwitschii Stapf		
	R. concolor Pichon	—	Comoro Islands	Shrub
	R. confertiflora Pichon	—	Malagasy Republic	Shrub
	R. macrophylla Stapf	R. gonioclada K. Schum.	Guinea, Cameroon	Tree, 12-18 m.
		R. leucopoda K. Schum.		

Section	Species	Synonyms	Distribution	Habit
Afrovolfia contd.	R. mannii Stapf	R. cardiocarpa K. Schum.	Guinea, Zaire	Shrub, 2-3 m.
		R. preussii K. Schum.	Mozambique	
	R. mayombensis Pellegrin	—	Zaire	Tree, 10-12 m.
	R. media Pichon	—	Malagasy Republic	Shrub
	R. nana E. A. Bruce	—	Rhodesia and Zambia	Sub-shrub, 15 cm.
	R. obscura K. Schum.	R. longeacuminata De Wild.	Zaire	Shrub, 0.5-1.5 m.
	R. rosea K. Schum.	—	Tanzania, Mozambique	Shrub, 0.5-2 m.
	R. tchibangensis Pellegrin	—	Zaire	Shrub

Section	Species	Synonyms	Distribution	Habit
Endolobus	R. cumminsii Stapf	R. liberensis Stapf R. ivorensis Cheval	West Africa	Shrub, 2-3 m.
	R. mombasiana Stapf	R. monopyrena K. Schum.	East Africa	Shrub, 2 m.
	R. vomitoria Afz.	R. senegambiae A.DC. R. stuhlmannii K. Schum.	West and Central Africa	Tree, 2 m.
	R. obtusiflora A.DC.	—	Malagasy Republic	Shrub
Ophioxylanthus	R. oreogiton Mgf.	—	Tanzania	Shrub
	R. volkensii Stapf	—	Tanzania, Mozambique	Shrub
Unclassified	R. capuronii Mgf.	—	Malagasy Republic	Shrub

The section Ophioxylanthus

The African divisions in Pichon's classification of the genus Rauwolfia include the section Ophioxylanthus. It consists of two species only, R. volkensii Stapf. and R. oreogiton Mgf. The section is characterised by short, thick, stamen filaments. It can be differentiated from the section Afrovolfia, the main characteristic features of which are stamens with longer filaments and filiform towards the base. Members of the section Endolobus possess corolla lobes inflexed in the buds (Court, 1968).

R. volkensii was first described by Schumann in 1895 as Tabernaemontana volkensii and recorded as such in the Supplement I of the Index Kewensis (1886-95). Later, in 1904, Stapf described the shrub as R. volkensii and the Index Kewensis was amended accordingly in Supplement III (1901-1905).

In 1923 Markgraf described R. oreogiton but this species is regarded as doubtfully distinct from R. volkensii. Court (1958) noted some similarities between them and African silviculturists claimed that plants of the two species were difficult to distinguish and were regarded as synonymous. According to Greenway (1955) and Willan (1960), Pichon (1947), in his classification of the genus Rauwolfia, grouped the species together in the section Ophioxylanthus but without examining specimens of R. oreogiton as no authentic specimens were available. However, botanists in East Africa where the plants grow still regard them as distinct species although closely related.

R. volkensii is a shrub with slender branches growing to a height of about 2 m. It is found in Tanzania in the West Usambara mountains and the Kilimanjaro mountains at an elevation in excess of about 1300 m. It may also be found in Mozambique.

R. oreogiton is also a shrub growing to a height of about 2 m. (Timmins and Court, 1974). The plant is indigenous to Tanzania and can be found in the West Usambara mountains, and the Kilimanjaro mountains at an elevation in excess of 1200 m. (Feuell, 1955; Timmins and Court, 1974).

Indigenous Usage

Plants of Rauwolfia species are very popular in the traditional medicine of the indigenous African people. The Chagga tribe, people of the Kilimanjaro region of Tanzania, are reported to frequently add the bark of R. caffra to traditionally brewed beer to give the beer a kick, and to conveniently intoxicate a drinker after imbibing only a moderate amount (Madati et al, 1977).

Several other African communities incorporate plants of Rauwolfia in different forms in traditional medicine (Feuell, 1955). Those indicated in the literature are given in Table III.

TABLE III

Vernacular names and Indigenous usage of some African Rauwolfia species

Species	Vernacular names	Indigenous Usage
R. caffra	Um Hlambamanzi (Zulu) Mulimbalimba (Katanga) Msesswe (Chigga, Tanzania) (Madati, 1977) Quinine tree (Europeans) (Court, 1958)	Among the South Africans the plant is regarded as insecticidal and used against maggots in wounds; the leaves are used in various skin diseases; the root bark is strongly purgative whereas the stem bark is used in making native beer; it has occasionally been used as an arrow poison supplement (Feuell, 1955).
R. cumminsii	—	In admixture with other species, the roots are used in the treatment of high blood pressure, piles, stomach ailments and veneral diseases (Hakim, 1965).

Species	Vernacular names	Indigenous Usage
R. macrophylla	Ompege, Esoma, Munenjii Mokande (Feuell, 1955)	The bark is used as a purgative; in admixture with other plants, it is used against syphilis (Feuell, 1955).
R. mannii	Mbara	Used as arrow poison supplement; powdered bark is applied to wounds (Feuell, 1955)
R. mombasiana	Umboza makili, Mbozumakii Mumasia	Roots are ground with coconut oil to treat pimples; included in a treatment for gonorrhoea (Feuell, 1955); has also been used to poison dogs (Watt et al, 1962).
R. obscura	Dimadini, Dusangulatati, Lopundu	Used in the treatment of pimples; the roots are employed in arrow poison mixtures (Feuell, 1955).
R. volkensii / R. oreogiton	—	Employed for the treatment of gonorrhoea and snakebite and to increase lactation in nursing mothers (Wood, 1964).

Species	Vernacular names	Indigenous Usage
R. vomitoria	Swizzle Stick tree (Europeans) Ira-Igbo, Asofeiyeje, Akata, Akanta (Yoruba, S. Nigeria) Mongemba-Utoenyn (Ibo, S. Nigeria) (Feuell, 1955)	Used by Sierra Leone people to allay the spasmodic action of tetanus; in Nigeria powdered root is used in the treatment of snakebite; leaves and roots used against gonorrhoea; powdered bark is applied as a dressing for sores and a skin application for nettle-rash, measles, herpes etc. (Ainslie,1937; Feuell, 1955). Leaves and berries are strongly purgative and emetic; the leaves have been used to poison animals; a decoction of the root has been employed as a sedative for maniacal symptoms (Feuell, 1955). Insecticidal properties have also been claimed; Hausa traders (Nigeria) use bark extract mixed with pawpaw juice to preserve kola nuts from weevils; also the latex and a decoction of the leaves have been employed against head lice and other parasitic skin diseases (Feuell, 1955).

ALKALOIDS OF *RAUWOLFIA*

Introduction

Classification and Structure

Introduction

Plants of the genus Rauwolfia produce a wide range of indole alkaloids, the composition of which varies considerably from one species to another and with geographical location. The presence of alkaloids in the genus was first observed by the Dutch chemist Greshoff in 1890. It was his belief that the physiologically active principles of Rauwolfia were alkaloids that prompted the investigations of the Indian chemists Siddiqui and Siddiqui in 1931.

More than 50 Rauwolfia alkaloids are now known (Schlittler, 1977). Chemical investigations showed that alkaloids could be found in the leaves, stem and roots. Greatest amounts were found in the roots and particularly in the root bark (Court, Trease and Evans, 1958; Court, Khalil and Stewart, 1967; Court, Hakim and Stewart, 1962; Court and Timmins, 1975; Court and Iwu, 1977).

Rauwolfia alkaloids are of diverse skeletal structures and variable basicity. Tertiary, quaternary, phenolic and N-oxide bases have all been isolated from various species of the genus.

In medical practice, alkaloids such as reserpine, deserpidine and rescinnamine are used as antihypertensive and tranquilising agents. Some Rauwolfia alkaloids possess other activities while many others have, as yet, no reported pharmacological effect.

Classification and Structure

Previous classification schemes of indole alkaloids including those of Rauwolfia were based on colour, pKa values or the presence of chromophoric systems (Chatterjee et al, 1956; Chatterjee and Ray, 1962; Schlittler, 1957; Pakrashi and Achari, 1968) and these were not entirely satisfactory. Classifications based on chemical structures (Schlittler and Bein, 1967; Snieckus, 1968) or on biogenetic pathways (Poisson, 1965; Kompis et al, 1971) are more informative.

All known Rauwolfia alkaloids are monoterpenoid indole bases of Class A (Corymanthe) skeletal type (Fig. 1). The C-9, C-10 iridoid unit can be recognised in their gross skeletal structures. A great number of these alkaloids exhibit minor structural variations in the basic iridoid moiety as shown in Fig. 2. Such variations can be used to classify the alkaloids of Rauwolfia into four main groups, viz.

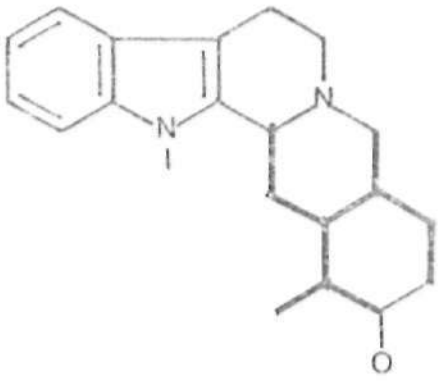
I Yohimbane

II Heteroyohimbane

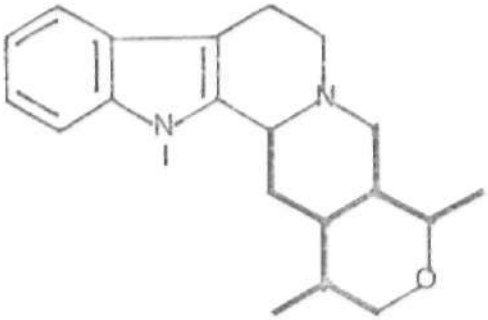
III Ajmaline - sarpagine

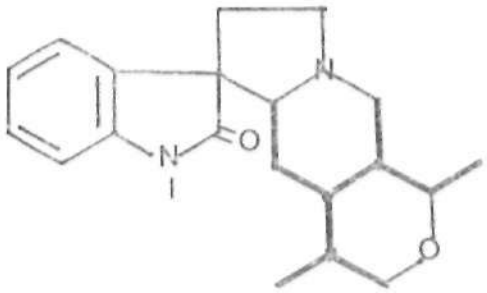
IV Miscellaneous

Fig. 2.

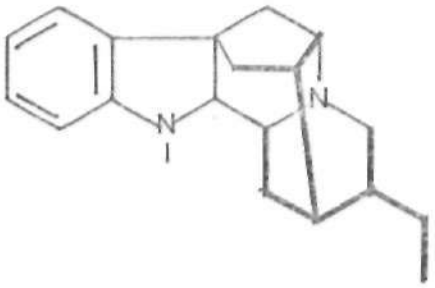


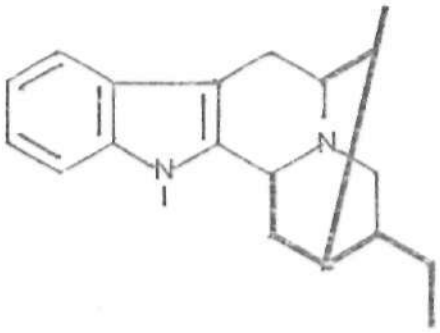
Yohimbane type

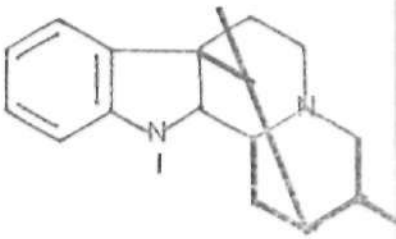




Heteroyohimbane type







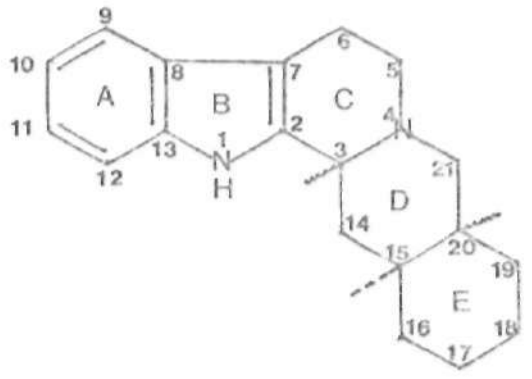
Ajmalan - sarpagan - akuammiline type

I Yohimbane Group

This group comprises alkaloids based on the yohimbane nucleus (X). Such alkaloids are characterised by a pentacyclic ring structure with a homocyclic 'E' ring and fall into two categories:-

(i) the yohimbines

and (ii) the 18-hydroxy yohimbines.



(X) Yohimbane nucleus

The absolute configuration of the nucleus has been established and confirmed by extensive studies on the synthesis and degradation processes of yohimbine and its isomers.

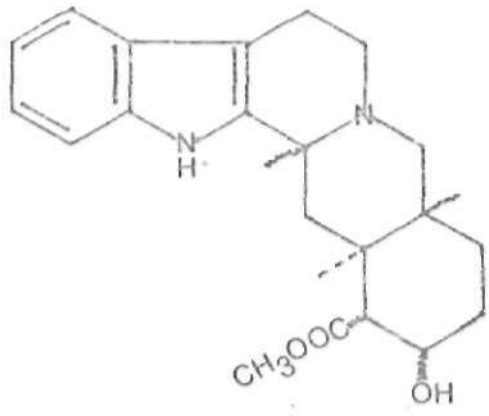
Associated with the pentacyclic ring structure are three assymmetric centres at the C-3, C-15 and C-20 positions. Therefore 8 theoretical isomers are possible. However, the configuration at C-15 in all known naturally occurring compounds is α and is suggested to remain constant on biogenetic grounds (Wenkert and Bringi, 1959). As a result of the configurational variation of the hydrogen atoms at C-3 and C-20, four stereochemical arrangements or configurations of the yohimbine nucleus occur and form compounds of four stereochemical series, viz. normal, allo, epiallo and pseudo.

C - 3H	C - 20H		
α	β	normal	series
α	α	allo	"
β	α	epiallo	"
β	β	pseudo	"

(i) Yohimbine Group

Yohimbine, the alkaloid from which the group takes its name was first isolated from Corynanthe yohimbe K. Schum. (Rubiaceae) and several isomers of yohimbine have subsequently been isolated from many members of the Apocynaceae family.

Fig. 3. Yohimbine Group



	C-16, $COOCH_3$	C-17, OH	
Normal series			
Yohimbine	α	α	
Corynantheine	β	α	
β -yohimbine	β	β	
11- methoxy-yohimbine	α	α	C-11, OCH_3
Allo series			
α-yohimbine	β	α	
Allo-yohimbine	α	α	
Serpine	α	β	
Seredine	β	α	C-10, C-11, OCH
Epiallo series			
3-epi- α -yohimbine	β	α	
Pseudo series			
Pseudo-yohimbine	α	α	
11-methoxy-ψ-yohimbine	α	α	C-11, OCH_3

The structure of yohimbine embodies the yohimbane nucleus (X) with a carbomethoxy substituent at the C-16 position and an alcoholic hydroxy function at the C-17 position. The presence of substituents at the C-16 and C-17 positions creates two more assymmetric centres on the nucleus thereby permitting theoretically 16 possible yohimbine isomers. Nine of these have been isolated from Rauwolfia species (Fig. 3).

Methoxy substituted analogues are also known to occur, the substitution usually occurring on the aromatic ring A. Recent examples are the two methoxy yohimbines isolated from R. caputronii (Miet et al, 1977).

(ii) 18-hydroxy yohimbine group

Members of this group differ from the yohimbines by possessing an additional hydroxy function at the C-18 position. This alcoholic hydroxy group is usually esterified although the free 18-hydroxy-yohimbines have been found naturally occurring (Majumdar et al, 1973; Inu, 1977).

The medicinally important Rauwolfia alkaloid reserpine belongs to this group. Similar pharmacologically important analogues such as rescinnamine and deserpidine also belong to the group. Although reserpine has been found to occur in several of the species of Rauwolfia so far investigated, the other analogues are of mere limited occurrence quantitatively and qualitatively.

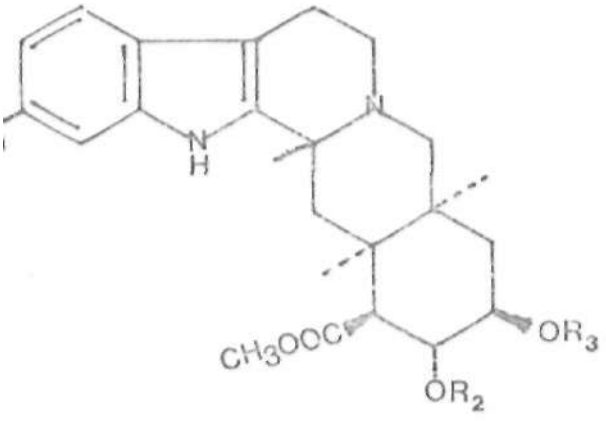
Degradation and synthetic studies provided independent proof of the structure and stereochemistry of reserpine and consequently also of the analogues.

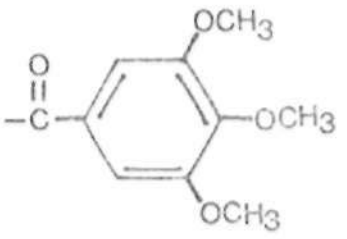
The 18-hydroxy yohimbines (Fig. 4) possess six assymmetric centres permitting theoretically sixty four isomers. Fortunately very few of these have been found to occur in nature. Reserpine and its other naturally occurring analogues belong to the epiallo series. Isoreserpine (3-epi-reserpine), the only reserpine type alkaloid that belongs to the allo series, may be an acid induced rearrangement product of reserpine as reserpine epimerises very easily at C-3 to give isoreserpine under acidic conditions (Taylor, 1966). No trans D/E ring fused analogue has so far been recognised.

Beta configuration at positions C-3, C-16 and C-18 has been shown to be essential for the pharmacological activity of reserpine and its analogues. Synthetic analogues, differing from reserpine at these crucial positions showed no significant pharmacological effect (Comer, 1970). Deserpidine, an alkaloid which differs from reserpine by the absence of the 11-methoxy substituent, is generally considered to produce the same pharmacological effect as reserpine both quantitatively and qualitatively. Substitution on the aromatic ring is therefore apparently of no consequence (Comer, 1970; Schlittler, 1977).

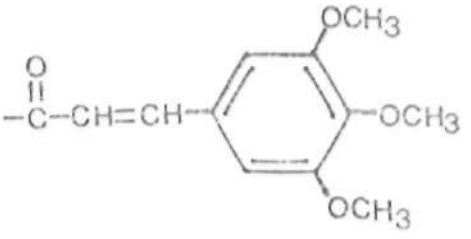
A methoxy group rather than a hydroxy group in position C-17 is, however, essential for pharmacological activity since raunescine, pseudo-reserpine and rescidine which lack the 17-methoxy group have been shown to be much less active pharmacologically than reserpine or rescinnamine (Schlittler, 1977).

Fig. 4. 18-Hydroxy-yohimbines





Trimethoxy-benzoyl (TMB)



Trimethoxy-cinnamoyl (TMC)

	R_1	R_2	R_3	
Epiallo series				
Deserpidine	H	CH_3	TMB	
Raunescine	H	H	TMB	
Isoraunescine	H	TMB	H	
Methylreserpate	OCH_3	CH_3	H	
Pseudoreserpine	OCH_3	H	TMB	
Reserpine	OCH_3	CH_3	TMB	
Raugustine	OCH_3	TMB	H	
Rescidine	OCH_3	H	TMC	
Rescinnamine	OCH_3	CH_3	TMC	
Renoxidine	OCH_3	CH_3	TMB	N_b-oxide
Allo series				
Isoreserpine	OCH_3	CH_3	TMB	

19-20, Dehydro - 18-hydroxy-yohimbines

Raujemidine = 19-20, Dehydro reserpine

Deserpidéine = 19-20, Dehydro deserpidine

Raujemidine N_b-oxide

II Heteroyohimbine Group

This group includes the following alkaloid types:-

(i) the heteroyohimbines,

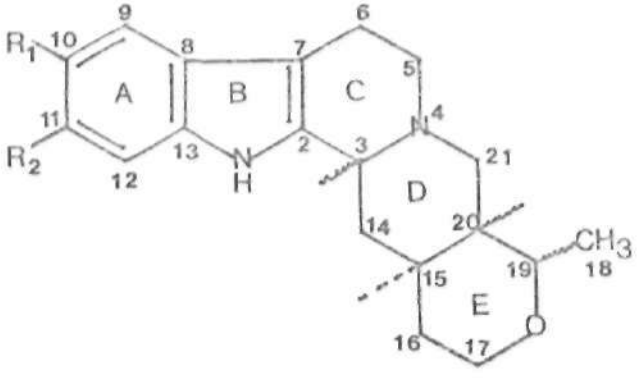
(ii) the anhydronium bases,

(iii) the oxindoles and pseudoindoxyls,

(iv) the E-seco indoles.

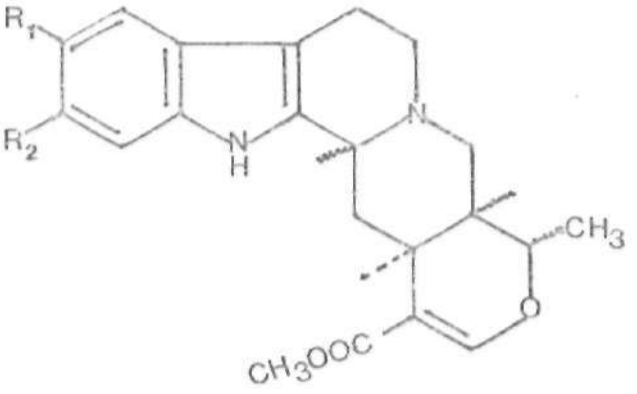
(i) Heteroyohimbines

The heteroyohimbine alkaloids possess the skeleton (X1) where R_1 and R_2 can either be hydrogen atoms or methoxy substituents. They are weakly basic alkaloids and form by far the largest group of alkaloids occurring in Rauwolfia species.



(X1) Heteroyohimbine skeleton

Fig. 5. Heteroyohimbines



	R_1	R_2	C-19, CH_3	C/D ring fusion
Normal series				
Ajmalicine	H	H	α	Trans
Tetraphylline	H	OCH_3	α	Trans
Raumitorine	OCH_3	H	β	Trans
Rauvanine	OCH_3	OCH_3	β	Trans
10, 11-Dimethoxy-ajmalicine	OCH_3	OCH_3	α	Trans
Tetraphyllinine (16, 17-dihydro-17-hydroxy tetraphylline)	H	OCH_3	α	Trans

	R_1	R_2	C-19, CH_3	C/D ring fusion
Allo series				
Tetrahydroalstonine	H	H	α	Trans
Reserpinine	H	OCH_3	α	Trans
Aricine	OCH_3	H	α	Trans
Isoreserpiline	OCH_3	OCH_3	α	Trans
Rauniticine	H	H	β	Trans
Raunitidine	OCH_3	H	β	Trans
Epiallo series				
Reserpiline	OCH_3	OCH_3	α	Cis
Isoreserpinine	H	OCH_3	α	Cis
N_b-methyl reserpiline	OCH_3	OCH_3	α	Cis
Pseudo series				
Raufloridine	H	OCH_3	α	Trans
Unknown configuration				
Neoreserpiline	OCH_3	OCH_3	α	Trans

The four basic stereochemical configurations, namely allo, epiallo, normal and pseudo, occur as in the yohimbines. For each stereochemical series the C-19 methyl substituent may assume an α - or β - configuration thus permitting theoretically 8 stereoisomers. Derivatives of many of these have been isolated from Rauwolfia species.

A number of different conformations are again possible for each basic configuration. Although the stereochemical-relationship of these alkaloids is extremely interesting, the group is not considered important pharmacologically.

(ii) Anhydronium bases

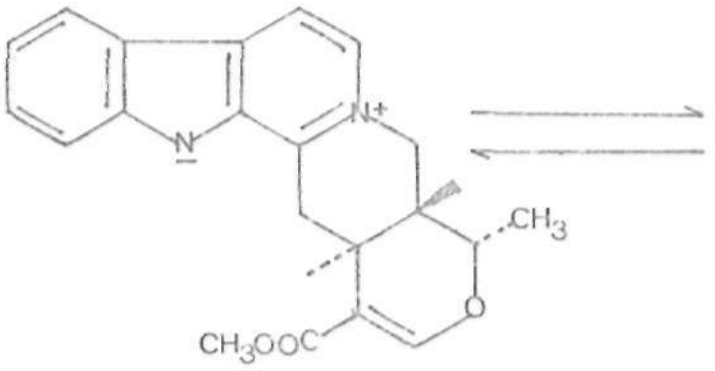
These are strongly basic alkaloids. The two most commonly occurring in Rauwolfia species are serpentine and alstonine. Serpentine was first isolated from R. serpentina by Siddiqui and Siddiqui (1931) and alstonine was isolated later from R. vomitoria by Schlittler et al (1952). The dimeric alkaloid serpentine has also been isolated from a few Rauwolfia species (Siddiqui and Siddiqui, 1931; Schlittler et al, 1954; Djerassi and Fishman, 1955).

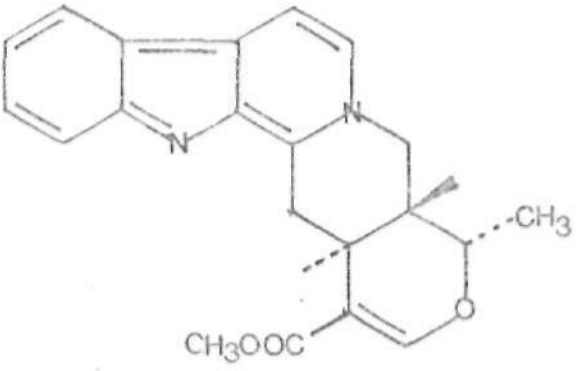
The anhydronium bases can occur as a completely aromatic ionic form (I) or as an O-quinoid form (II)(Fig. 6). They show a close structural relationship to the heteroyohimbines and are probably biogenetically related to them. The following interconversions are possible:

$$\text{Ajmalicine} \underset{NaBH_4}{\overset{Pb(OAc)_4}{\rightleftharpoons}} \text{Serpentine}$$

$$\text{Tetrahydroalstonine} \underset{NaBH_4}{\overset{Pb(OAc)_4}{\rightleftharpoons}} \text{Alstonine}$$

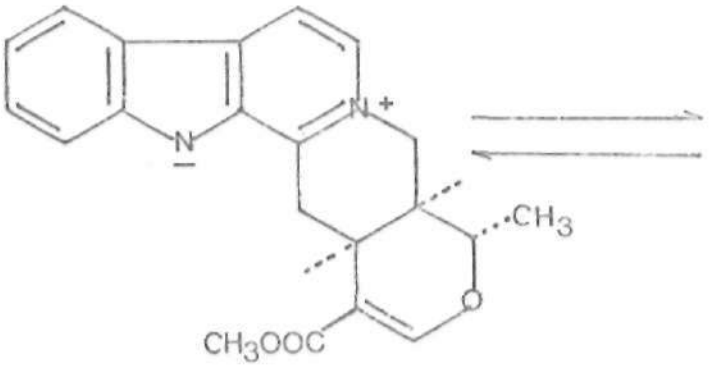
Fig. 6. Anhydronium bases

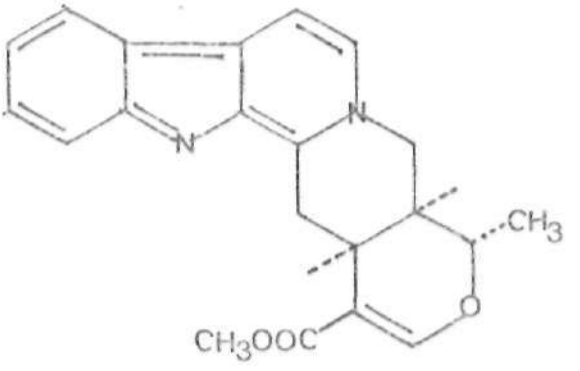




(I) (II)

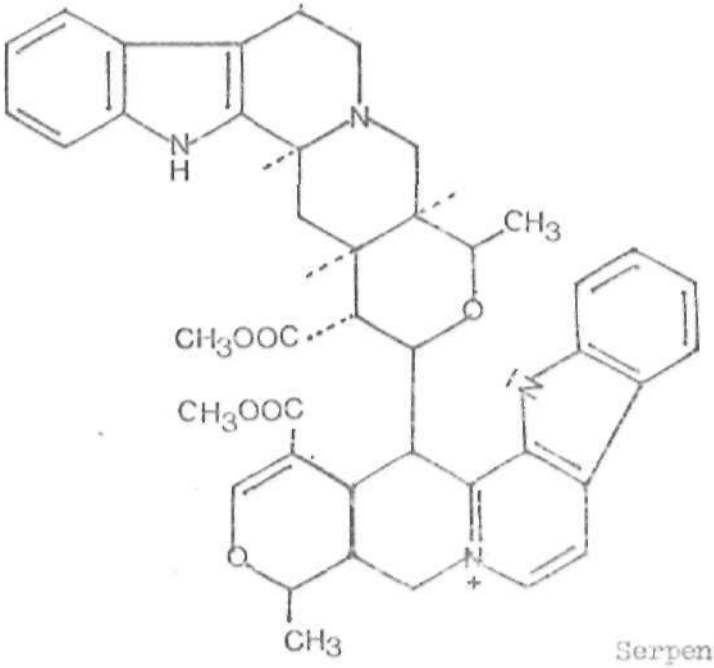
Serpentine





(I) (II)

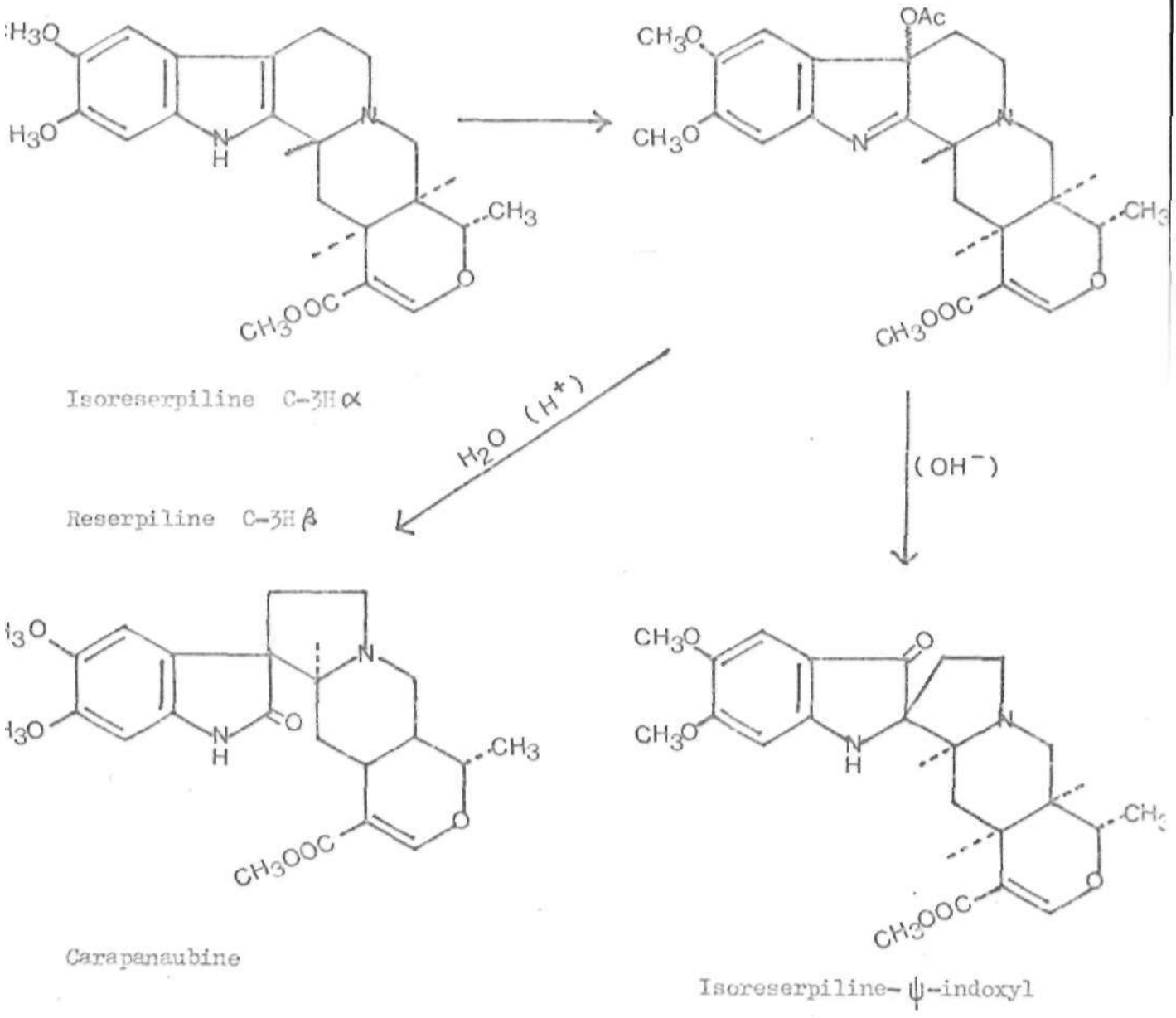
Alstonine



Serpentinine

(iii) Oxindoles and Pseudoindoxyls

Only oxindoles and pseudoindoxyls related to reserpiline and isoreserpiline are known to occur in Rauwolfia species. Chemically they can be obtained from the parent two alkaloids by oxidative methods employing lead tetra-acetate or tert-butyl hypochlorite (Finch and Taylor, 1962). Acid rearrangement of the resultant indolenines produces the oxindoles and alkaline rearrangement will yield the corresponding pseudo-indoxyls (Scheme I)



SCHEME I

Generally with oxindoles, for each stereochemical series i.e. normal, allo, etc., two series of oxindoles, 'A' and 'B', can arise because a further chiral centre is created in the formation of a spiro carbon atom at C-5. The oxindoles with the lactam carbonyl oriented below the plane of the C/D ring are designated 'A' series and those in which the lactam carbonyl is situated above the plane of C/D ring are designated 'B' series.

As only oxindoles related to reserpiline and isoreserpiline occur in Rauwolfia species, Rauwolfia oxindoles are therefore only of the allo and epiallo stereochemical series and are Allo 'A' and 'B' and Epiallo 'A' and 'B' (Fig. 7a).

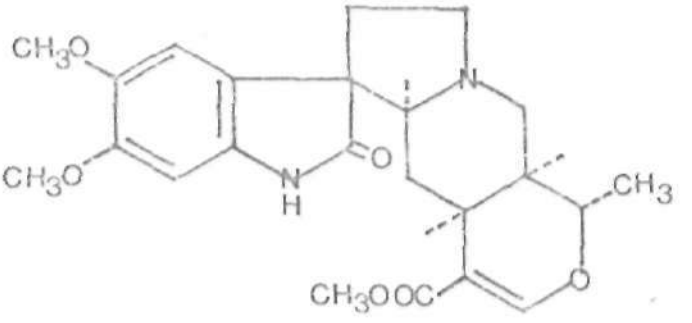
The pseudo series of oxindoles are considered to be too unstable to exist normally because of a serious steric interference between the oxindole ring and the underside of ring D (Shamma et al, 1967).

The only pseudoindoxyl alkaloid so far isolated from Rauwolfia species is isoreserpiline- ψ-indoxyl (Finch et al, 1963; Mueller, 1963).

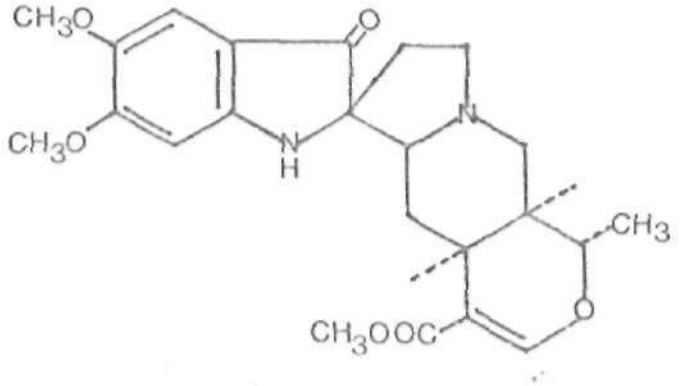
(iv) E-seco indoles

The E-seco indole alkaloids possess tetracyclic ring structures with an open E-ring. Those known to occur in Rauwolfia species have in common the gross skeleton (XIII) (Fig. 8) with variable substituents at R_1, R_2, R_3 and R_4. The C-20 side chain may be vinyl or ethylidene.

Fig. 7a Oxindoles and Pseudoindoxyl

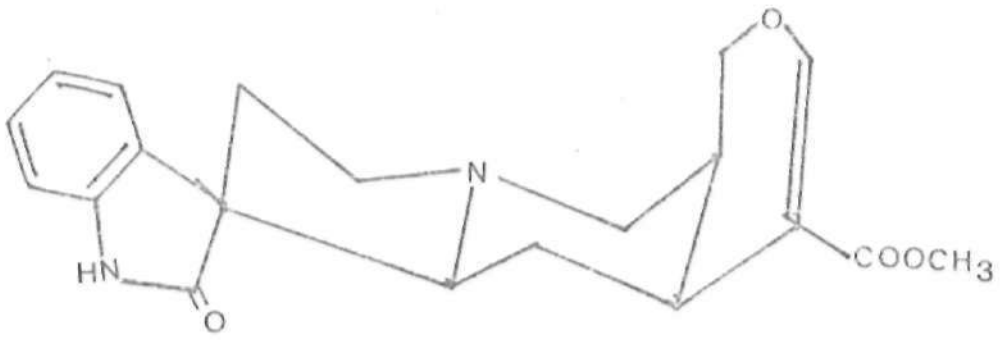


		C-3H
Isocarapaunabine	Allo 'A'	α
Carapaunabine	Allo 'B'	α
Rauvoxinine	Epiallo 'A'	β
Rauvoxine	Epiallo 'B'	β

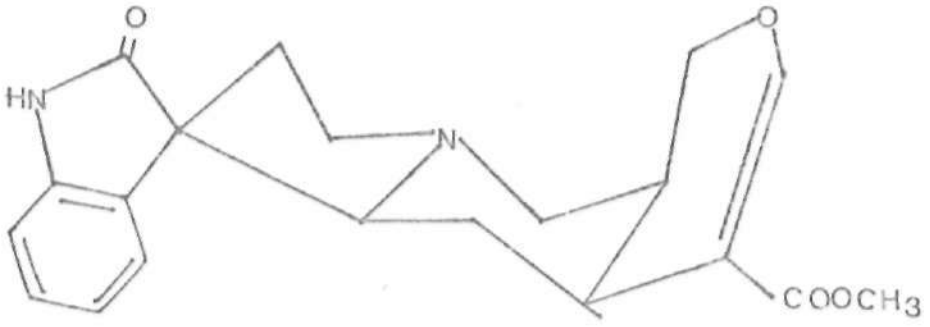


Isoreserpiline- ψ -indoxyl

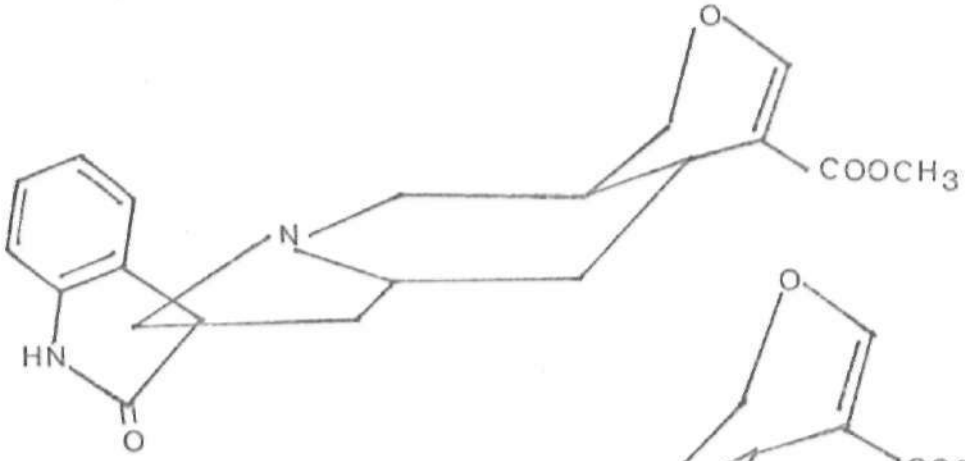
Fig. 7b. Stereochemical configuration of Oxindole alkaloids



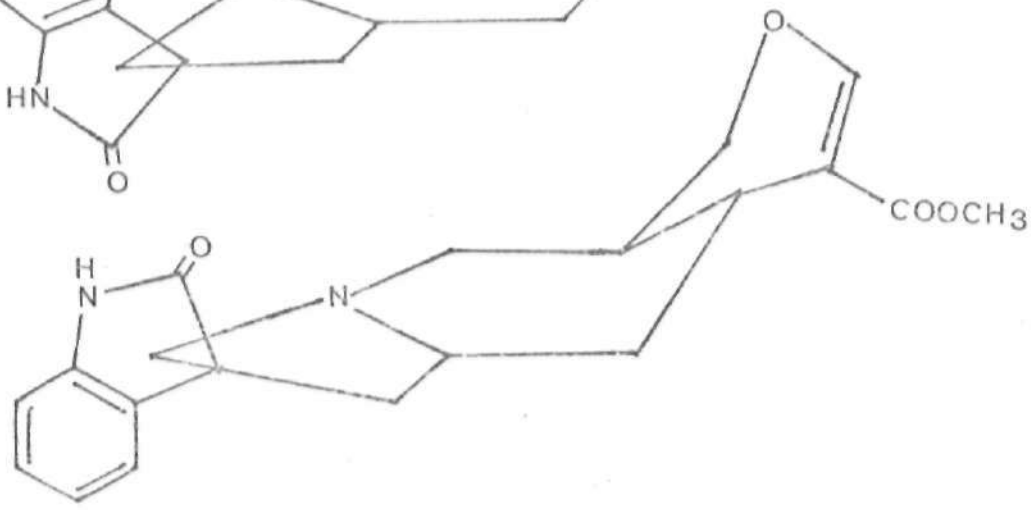
Allo A



Allo B



Epiallo A

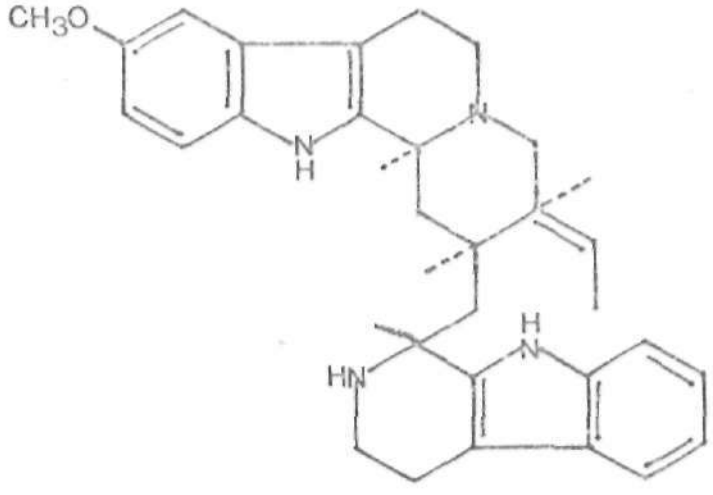


Epiallo B

The alkaloids of this group are believed to be early products in the biosynthesis of indole alkaloids.

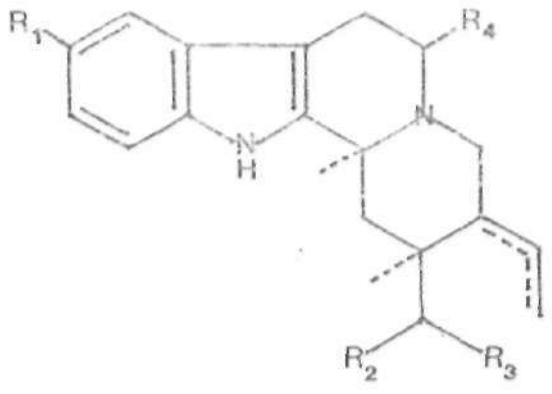
Geissoschizol, the first reported member of this group in Rauwolfia species, was obtained from the leaves of R. vomitoria by Pousset et al, (1965). Subsequently other workers (Timmins, 1975; Iwu, 1977; Sabri and Court, 1978) have demonstrated the presence of several of the E-seco indole alkaloids in other Rauwolfia species.

The sesquimeric E-seco indole alkaloid obsurifoline (XII) was obtained from R. obscura (Timmins and Court, 1975).



(XII) Obscurifoline

Fig. 8. E-seco indole alkaloids



(XIII) E-seco indole skeleton.

	R_1	R_2	R_3	R_4
Normal series				
Coryantheal	H	H	-CHO	H
Corynantheol	H	H	$-CH_2OH$	H
Demethylcorynantheine	H	$-COCH_3$	=CHOH	H

	R_1	R_2	R_3	R_4
Allo series				
Geissoschizal	H	H	-CHO	H
Geissoschizol	H	H	$-CH_2OH$	H
10-Hydroxy-geissoschizol	OH	H	$-CH_2OH$	H
10-Methoxy-geissoschizol	OCH_3	H	$-CH_2OH$	H
Geissoschizine	H	$-COOCH_3$	=CHOH	H
Isositsirikine	H	$-COOCH_3$	$-CH_2OH$	H
Geissoschizol acetyl	H	H	$-CH_2OAC$	H
Demethyl-corymantheidine	H	$-COOCH_3$	=CHOH	H
5-Carboxy-corynantheidine	H	$-COOCH_3$	=CHOH	-COOH

III Ajmaline-Sarpagine Group

This group comprises two main alkaloidal types; the ajmalan type and the sarpagan type.

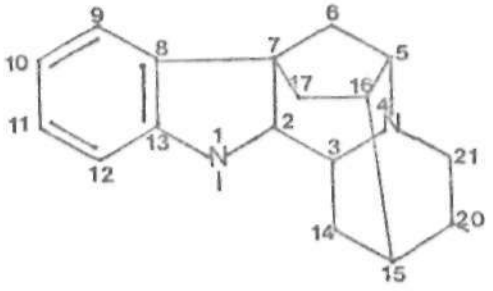
The ajmalan type comprises a group of dihydroindole alkaloids possessing the basic skeleton (XIV). Ajmaline, the best known alkaloid of the group, has been isolated from several species of Rauwolfia and is frequently the principal alkaloid in the roots of the plant.

Alkaloids of the ajmalan type fall into three series:-

(a) the ajmaline series, a group characterised by an ethyl substituent at C-20 and an alcoholic hydroxy group at C-21,

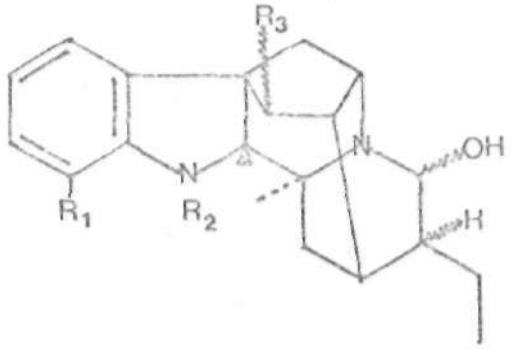
(b) the tetraphyllicine series, possessing an ethylidene group at C-20 and no hydroxy group at C-21 and,

(c) the perakine series, indolenines having other substituents at C-20.



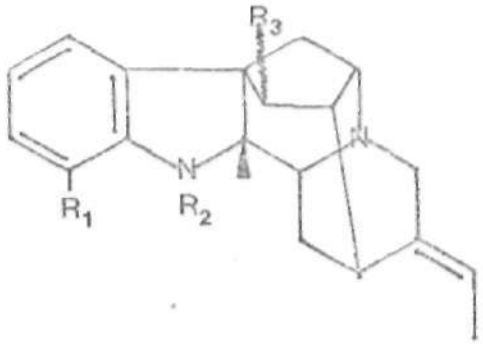
(XIV) Ajmalan type skeleton.

Fig. 9. Ajmaline Series



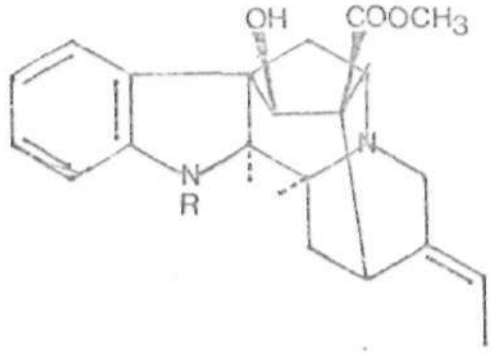
	R_1	R_2	R_3	C-20(H)	C-21(OH)
Ajmaline	H	CH_3	OH(β)	α	α
Norajmaline	H	H	OH(β)	α	α
Isoajmaline	H	CH_3	OH(β)	β	β
Sandwicine	H	CH_3	OH(α)	α	α
Isosandwicine	H	CH_3	OH(β)	β	β
17-acetyl-ajmaline	H	CH_3	$OCOCH_3$(β)	α	α
12-methoxy-ajmaline	OCH_3	CH_3	OH(β)	α	α
Diacetyl-ajmaline	H	CH_3	$OCOCH_3$(β)	α	$OCOCH_3$(α)
Ajmalidine	H	CH_3	=O	α	α
Vomalidine	OCH_3	CH_3	=O	α	α

Fig. 10. Tetraphyllicine Series



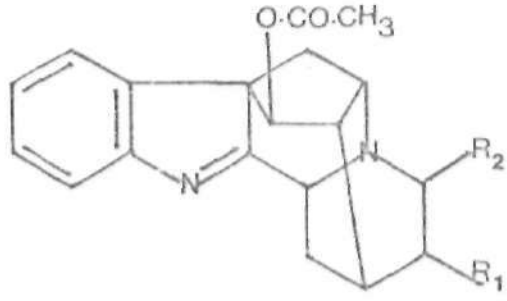
	R_1	R_2	R_3
Tetraphyllicine	H	CH_3	OH(β)
Mauiensine	H	CH_3	OH(α)
Rauvomitine	H	CH_3	OTMB
Mitoridine	OH	CH_3	OTMB
Purpeline	OCH_3	CH_3	=O
Seredamine	OCH_3	CH_3	OH(β)
Reflexine	OCH_3	CH_3	OH(α)
Norseredamine	OCH_3	H	OH(β)
Rauflorine	H	H	=O
Nortetraphyllicine	H	H	OH(β)
Norpurpeline	OCH_3	H	=O
Endolobine	H	H	=O, C-10, OCH_3

Vincamajine and norvincamajine (quebrachidine) belong to this series but the C-2 (H) is α (Fig. 11).



Vincamajine R = CH_3

Quebrachidine R = H

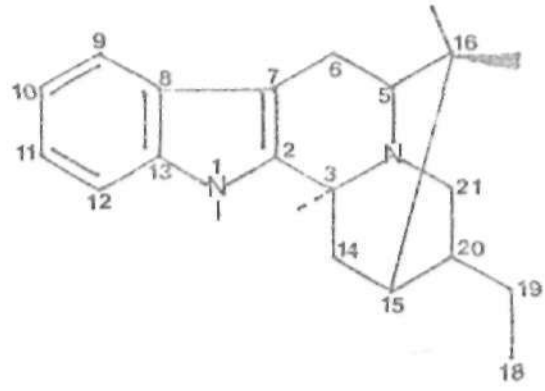


	R_1	R_2
Vomilenine	= $CH.CH_3$	OH
Raucaffricine	= $CH.CH_3$	O-galactose
Perakine	- CHO	CH_3
Raucaffrinoline	- CH_2OH	CH_3
RP 7	= $CH.CH_3$	H . (Kiang et al, 1964)

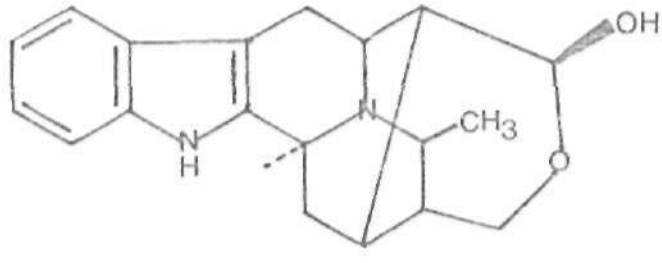
Fig. 11.

Alkaloids of the sarpagan group are believed to be formed from E-seco indoles by C-16→C-5 ring closure and possess indole UV chromophores (Fig. 12).

Vomifoline, an alkaloid structurally related to the sarpagan group and isolated from R. vomitoria (Pousset et al, 1967), was thought to be an epimer of peraksine (XV), but it has now been shown that the two alkaloids are identical (Pousset et al, 1977).

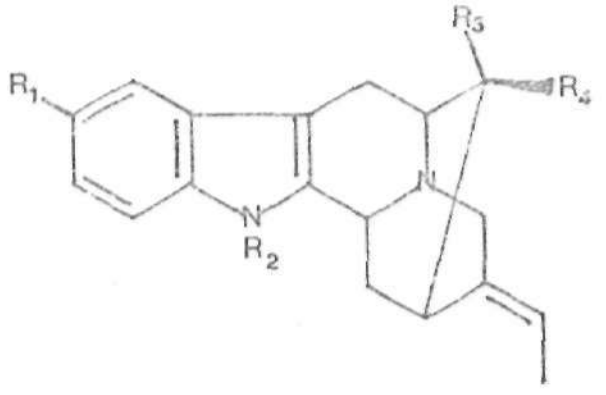


Sarpagan skeleton



(XV) Peraksine = Vomifoline

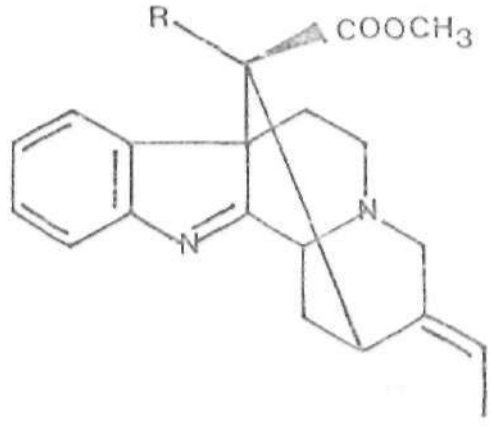
Fig. 12. Sarpagan Group



	R_1	R_2	R_3	R_4
Sarpagine	OH	H	H	$-CH_2OH$
Normacusine B	H	H	H	$-CH_2OH$
Polyneuridine	H	H	$-CH_2OH$	$-COOCH_3$
Pericyclivine	H	H	H	$-COOCH_3$
Spegatrine	OH	H	H	CH_2OH, $N_b(CH_3)$
Lochnerine	OCH_3	H	H	CH_2OH
Neosarpagine	OH	H	H	CH_2OH
Tombozine	H	H	CH_2OH	H
Vellosimine	H	H	H	CHO
N_a, N_b-dimethyl-sarpagine	OH	CH_3	H	CH_2OH, $N_b(CH_3)$

Akuammiline Type

Alkaloids of this group possess a structure resembling sarpagan but with a C-16 ⟶ C-7 bridge. All of the reported members of this group occurring in Rauwolfia species are indolenines.

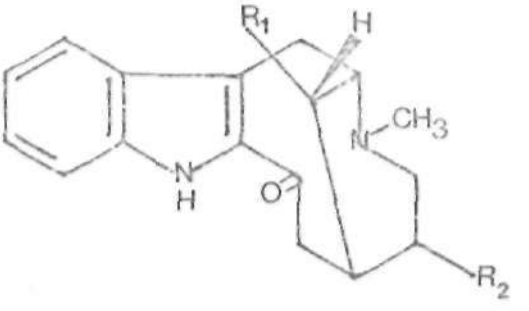


	R_1	R_2
Deacetyl-desformo-akuammiline	H	H
Raufloricine	OCH_3	CH_2OA_c

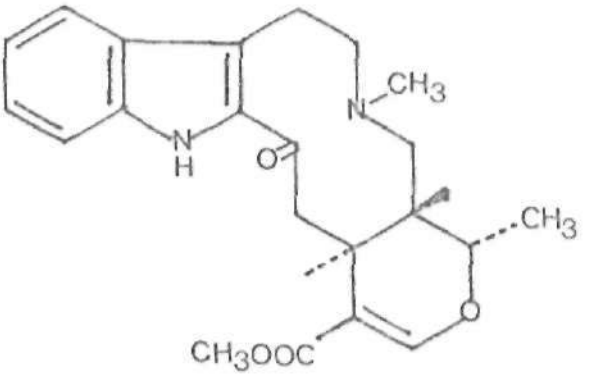
Miscellaneous Group

This group includes the 2-acyl indoles, the picrinine type alkaloids, other ajmaline related compounds not discussed above and the macroline type alkaloid, sauveoline.

Fig. 13. 2-Acyl indole



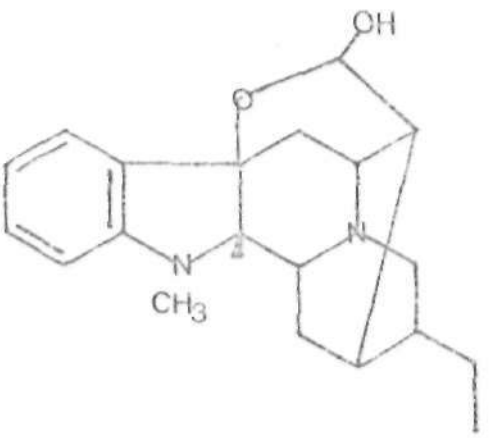
	R_1	R_2
Tabernaemontanine	$-COOCH_3$	CH_2CH_3
16 Decarbomethoxy-dihydro-vobasine	H	CH_2CH_3
Vobasine	$COOCH_3$	$=CHCH_3$
*Pelirine	CH	$=CH.CH_3$



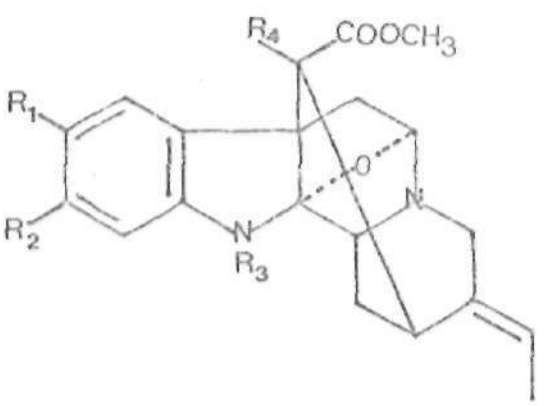
20-epi-picraphylline

* The structure of pelirine has not been fully established. It is known to have an aromatic methoxy substituent but the exact position has not been determined. The stereochemistry at C-16 is also not established.

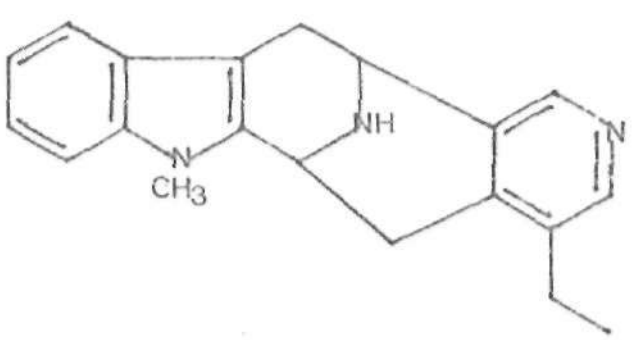
Fig. 14.



Rauwolfinine



	R_1	R_2	R_3	R_4
Picrinine	H	H	H	H
Deacetyl-picraline	H	H	H	CH_2OH
Quaternine	OCH_3	OCH_3	CH_3	H



Suaveoline

SPECTROSCOPIC METHODS OF IDENTIFICATION

RAUWOLFIA ALKALOIDS

I Ultraviolet spectroscopy

II Infrared spectroscopy

III Nuclear magnetic resonance spectroscopy

IV Mass spectroscopy

Ultraviolet Spectroscopy (UV)

Ultraviolet spectra can provide valuable information aiding the identification of Rauwolfia alkaloids. Measurements can be obtained using very small samples and the basic chromophoric systems in the molecules can usually be established by inspection of the UV spectra.

Usually the indole moiety in the molecule is responsible for the chromophore. The terpenoid part, except when it possesses a conjugated double bond, exerts little or no effect on ultraviolet absorption.

Correlation between ultraviolet spectra and details of molecular structure has been used in the classification of indole alkaloids (Pakrashi and Achari, 1968). Rauwolfia alkaloids were classified by four basic chromophoric systems:-

(i) Indole

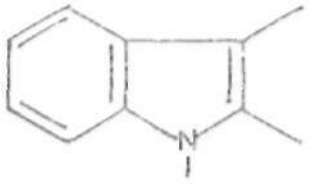
(ii) Dihydroindole or indoline

(iii) Indolenine

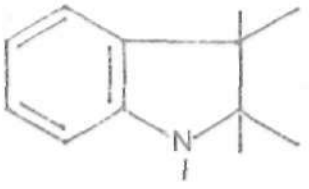
(iv) Oxindole and pseudoindoxyl

(The original classification of Pakrashi et al did not include the oxindoles and ψ-indoxyls)

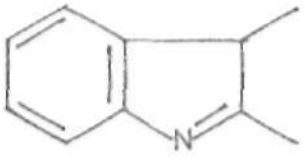
Fig. 15.



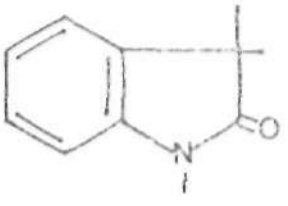
(I) Indole



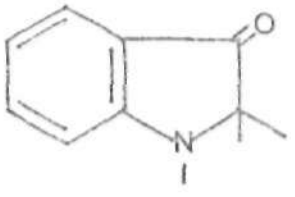
(II) Dihydroindole



(III) Indolenine



(IV) Oxindole



Pseudoindoxyl

(I) Indole alkaloids

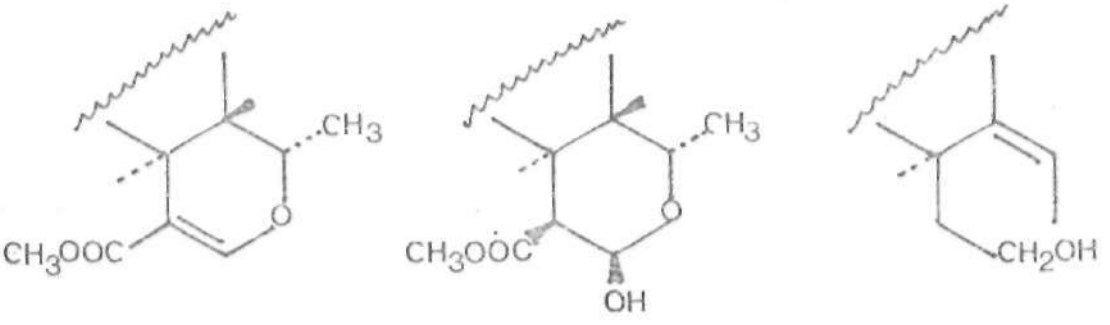
Common Rauwolfia alkaloids demonstrating indole UV absorption are the yohimbines, the heteroyohimbines, the E-seco indoles and the sarpagans. Sangster and Stuart (1965) reviewed the ultraviolet absorption spectra of the then known indole alkaloids including those of Rauwolfia and observed that unsubstituted indole alkaloids show two major absorption bands, one at 225-227 nm and the other at 280-290 nm.

The latter maximum usually displays fine structures and this is especially true of the spectra of yohimbine and normacusine B. The spectra

of these two alkaloids are similar and are almost superimposable on the spectrum of 2, 3, - dimethyl-indole. Both show maxima at 226, 283 and 291 nm with a deep minimum at around 240 nm.

Heteroyohimbines and some E-seco indole alkaloids exhibit similar absorption maxima to the yohimbines but can be differentiated by the presence of an inflexion at around 250 nm. This inflexion is attributed to the presence in the molecule of the ß-alkoxy-acrylic ester chromophore. Compounds in which one of the conjugated double bond systems in this moiety is saturated e.g. ajmalicinine, or the carbomethoxy group is absent e.g. geissoschizol, produce a normal indole absorption as in the yohimbines.

Fig. 16.



Ajmalicine Ajmalicinine Geissoschizol

Substitution on the aromatic ring affects the UV spectra of indole alkaloids, usually causing a bathochromic shift of the longer wavelength maximum. The magnitude of the shift depends on the number of substituents.

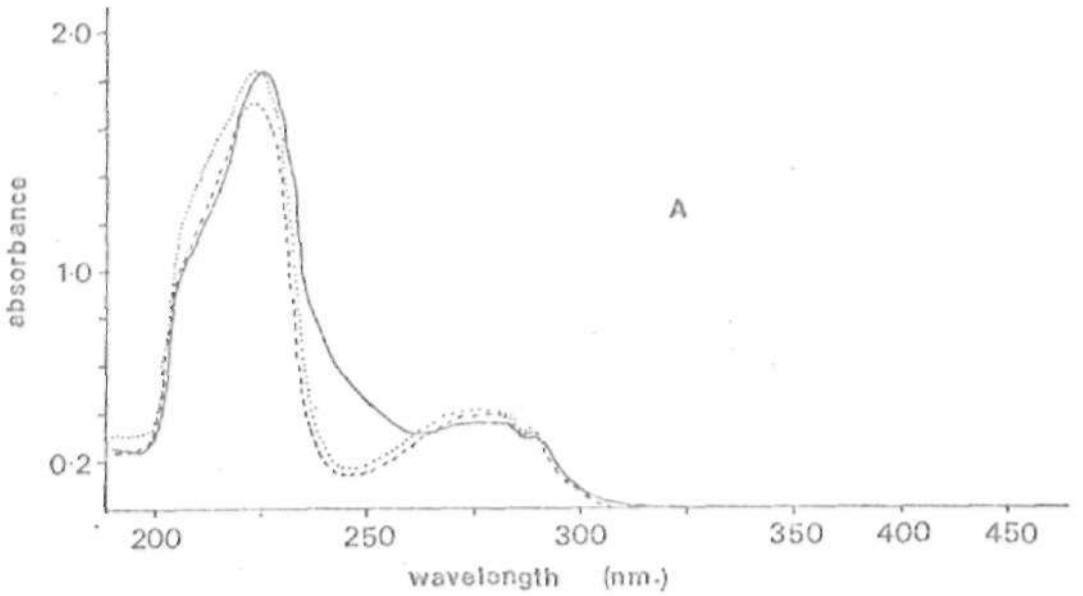
Thus tetrahydroalstonine show maxima at 226, 280 and 291 nm; aricine, the 10-methoxy analogue, shows maxima at 227, 282 and 295 nm and reserpiline, the 10, 11-dimethoxy analogue shows maxima at 229, 300 and and 304 nm.

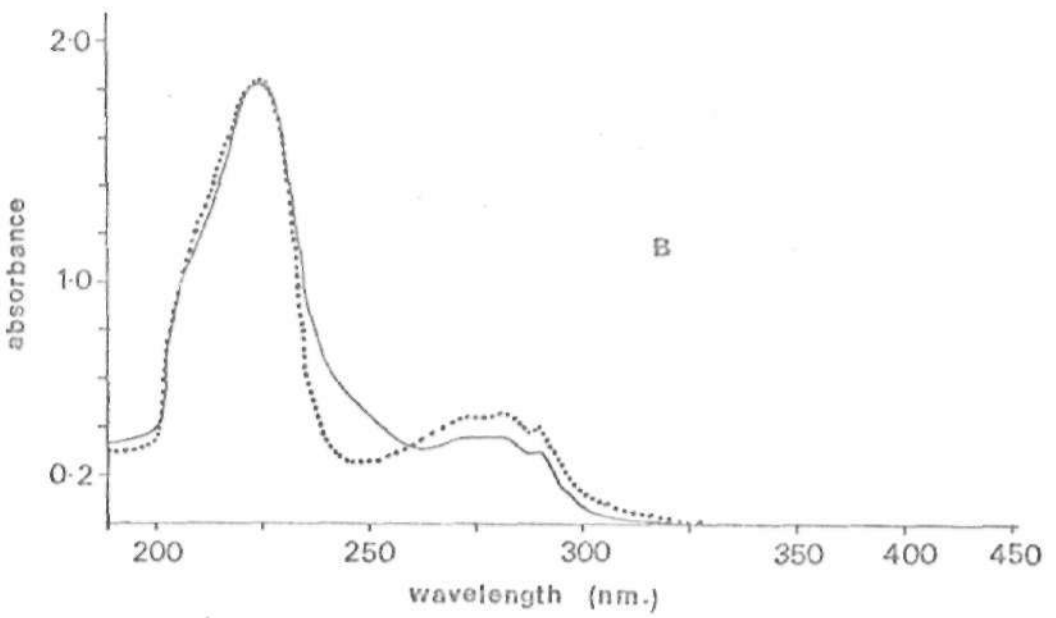
The salts of anhydronium bases exhibit three distinct peaks at about 252, 308 and 370 nm whilst in the corresponding bases there is a large bathochromic shift (Kump et al, 1963). Serpentine base yields absorption maxima at 284, 329 and 424 nm but the salt serpentine hydrochloride produces absorption maxima at 252, 308 and 370 nm. In serpentinine where there is a combination of β-carbolinium and indole chromophores, the composite spectrum exhibits the characteristic maxima of both systems. Serpentinine shows maxima at 227, 257, 281, 294, 304 and 373 nm.

The reaction of alkalis with phenolic indoles is accompanied by a bathochromic shift in the UV absorption spectrum e.g. sarpagine base absorbs at 230, 278, 293 sh, 310 nm but in alkaline medium a bathochromic shift of 10 nm occurred (Inu, 1977).

The 18-hydroxy yohimbine esters show composite spectra of indole and the esterifying acids, 3,4, 5-trimethoxy-benzoic acid in the case of reserpine and deserpidine and 3, 4, 5-trimethoxy-cinnamic acid in the case of rescinnamine. Reserpine shows maxima at 216, 267 and 292 nm and rescinnamine absorbs at 228 and 302 nm.

Fig. 17 Ultra-violet spectra





(A) ——— Ajmalicine

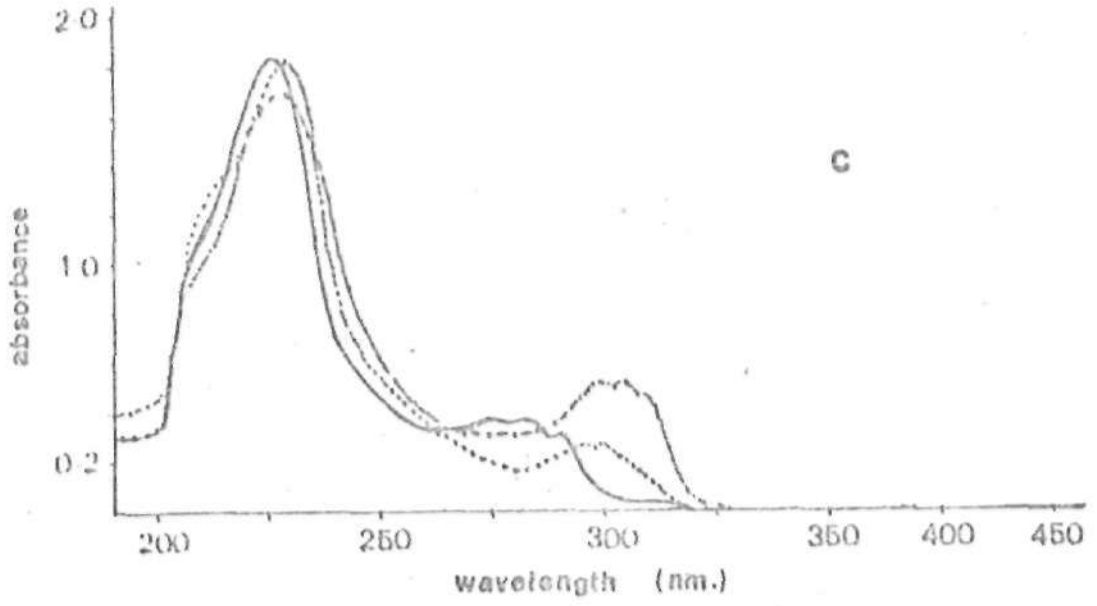
..... Normacusine B

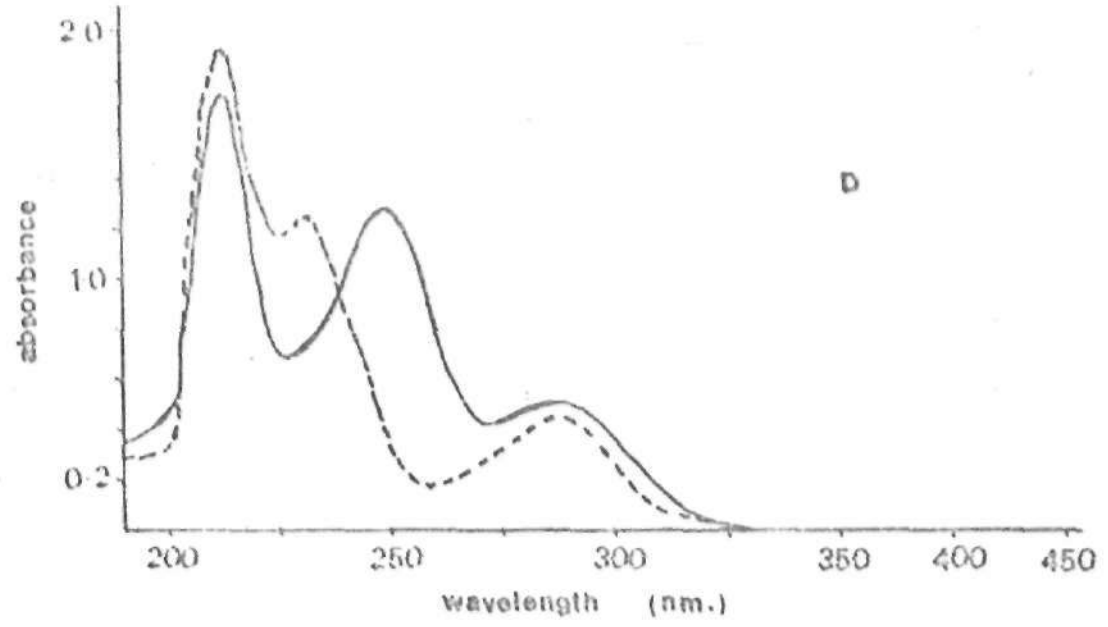
- - - - - Yohimbine

(B) ——— Ajmalicine

..... Ajmalicinine

. Fig.17 Contd.





(C) ——— Tetrahydroalstonine

..... Ajmaline

-.-.-.- Isoreserpiline

(D) ——— Ajmaline

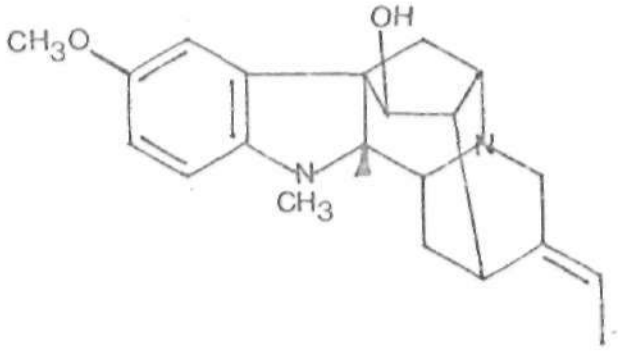
– – – – Picrinine

(II) Dihydroindole or Indoline alkaloids

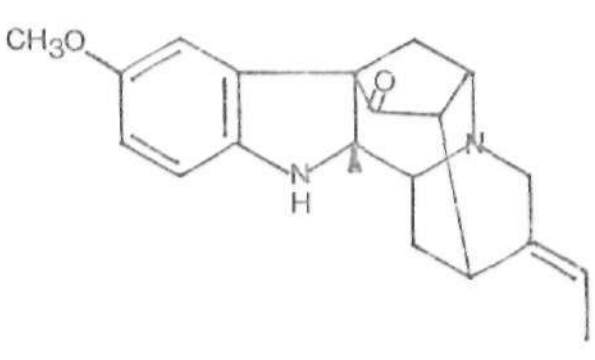
Rauwolfia alkaloids exhibiting indoline chromophores are of two types, the normal dihydroindole alkaloids such as ajmaline or tetraphyllicine and the modified indoline alkaloids such as the picrinine type.

Indoline alkaloids exhibit two distinct and characteristic peaks at about 245 and 295 nm while unsubstituted modified indolines absorb some 8-10 nm downfield. For example, ajmaline shows absorption maxima at 247 and 295 nm and picrinine absorbs at 237 and 287 nm (Gabetta and Mustich, 1975).

Substitution on the aromatic ring has been reported to affect the UV spectra of indoline alkaloids (Robinson and Spitteler, 1964; Sangster and Stuart, 1965; Farnsworth and Taylor, 1973) but vomalidine, purpeline (Gabetta and Mustich, 1975) and endolobine (Iwu and Court, 1978a), the methoxy-substituted indoline alkaloids of the Rauwolfia species, do not show any appreciable shift, absorption maxima occurring at about 250 and 290 nm. Nevertheless, vincamajoreine, a C-10 methoxy-tetraphyllicine isolated from Vinca species, exhibits a shift of the longer wavelength maximum to about 310 nm (Farnsworth, 1973).



Vincamajoreine



Endolobine

Methyl group substitution on the N_a-nitrogen produces no significant effect on UV absorption as ajmaline, norajmaline, tetraphyllicine and nortetraphyllicine all exhibit absorption maxima at about 245 and 292 nm (Gabetta and Mustich, 1975; Sabri and Court, 1978).

The UV spectra of picrinine and its analogues are not affected by the addition of dilute acids or alkalis; in concentrated perchloric acid (70%) however, a reversible change occurs, with maxima at about 241, 246 and 310 nm (Saxton, 1965).

Indolenine alkaloids

Rauwolfia indolenine alkaloids such as vomilenine, raucaffricine and perakine exhibit maxima at about 218 and 257 nm. In the presence of strong acids indolenines yield an indolic spectrum. Akuammiline type indolenine alkaloids show maxima at 216 and 264 nm (Gabetta and Mustich, 1975).

Oxindoles and Pseudoindoxyls

Unsubstituted oxindole alkaloids yield maxima at about 210 and 250 nm with a shoulder at 280 nm (Sangster and Stuart, 1965). The dimethoxy analogues encountered in Rauwolfia species show a series of shoulders at 218, 240, 280 and 300 nm rather than distinct maxima.

The pseudo-indoxyls exhibit spectra consistent with the spiro-cyclopentane - 1, 2 - pseudo-indoxyl which absorbs at 235 nm and 400 nm. Pseudo-indoxyl alkaloids are not common in Rauwolfia species, the only example so far isolated being isoreserpiline- ψ -indoxyl and it exhibits absorption maxima at 251, 283 and 405 nm.

II Infrared Spectroscopy

Infrared spectroscopy can be used for the detection of functional groups and is also of value in deciding stereochemical assignments of some Rauwolfia alkaloids.

Wenkert et al (1956) suggested that the infrared bands in the region 2800 cm^{-1} could be used in the case of yohimbinoid alkaloids to identify the stereochemistry of the C-3 hydrogen relative to the N_b lone pair of electrons. It was observed that, in spectra obtained using chloroform as the solvent, all compounds possessing C-3Hα (i.e. normal and allo series) exhibit two or more distinct bands of medium intensity at around 2900 - 2700 cm^{-1} while those compounds possessing C-3Hβ (pseudo and epiallo series) show indistinct shoulders in this region.

Bohlmann (1958), however, indicated that the relative simplicity or complexity of the observed bands in this region is due to the equatorial or axial orientation of the hydrogen atom α to the N_b nitrogen lone pair in a quinolizidine system. Compounds in which there are two or more hydrogen atoms in a trans diaxial relationship to the lone pair electrons on the nitrogen produce a complex absorption in the 2900 - 2700 cm^{-1} region. It follows therefore that it was conformation at C-3H rather than the configuration that could be determined. It was stressed that this rule can only be applied if the compound exists substantially in one conformation and the relationship between conformation and configuration is known (Rosen, 1961).

Neuss and Boaz (1957) were able to use infrared spectra to relate the stereochemistry of methoxy-heteroyohimbines to either the ajmalicine (normal) or the tetrahydroalstonine (allo) series. For example, reserpinine (11-methoxy-tetrahydroalstonine) showed infrared bands that corresponded exactly in their position and intensity with those of the summation spectrum of an equimolecular solution of 2, 3 - dimethyl-6-methoxy - indole and tetrahydroalstonine. They also claimed to be able to use the appearance of the ester bands at 1200 cm^{-1} region to identify the stereochemical groups of heteroyohimbine alkaloids. Evidence based on spectra measured in carbon disulphide showed that the normal series alkaloids such as ajmalicine and tetraphylline exhibited a simple ester band at 1190 cm^{-1}; allo series e.g. tetrahydroalstonine and reserpinine exhibited three distinct peaks at 1240, 1200 and 1190 cm^{-1} while the epiallo series e.g. reserpiline and isoreserpinine showed broad centred ester bands at 1210 cm^{-1}.

The spectrum region between 3500 - 1600 cm^{-1} is particularly helpful in determination of functional groups. The ß-alkoxy-acrylic ester moiety of heteroyohimbines shows characteristic twin absorption maxima at about 1695 and 1600 cm^{-1}.

The substitution pattern in the aromatic ring can be determined from the infrared spectrum. For example, unsubstituted indole alkaloids show absorption in the 750 cm^{-1} region corresponding to the 1, 2 - disubstituted benzene ring of the indole system.

Compounds with substituents at the C-9 or C-12 position exhibit bands at about 760 cm^{-1} while those with substituents at C-10 or C-11 show bands at about 810, 780 and 760 cm^{-1}.

III Nuclear Magnetic Resonance (NMR)

The techniques of nuclear magnetic resonance spectroscopy is of wide application in the deduction of stereochemistry of Rauwolfia alkaloids. It may also provide valuable help in establishing the presence or absence of certain structural features in the molecule such as ethyl or ethylidene side-chains or the substitution pattern on the aromatic ring.

Uskokovic and co-workers (1964) noted that in all quinolizidine systems the angular proton of trans conformers resonates at a higher field than the cis conformers. Thus, by locating the NMR signal for the angular proton C-3H in Rauwolfia alkaloids, the stereochemistry of the C/D ring fusion can be established.

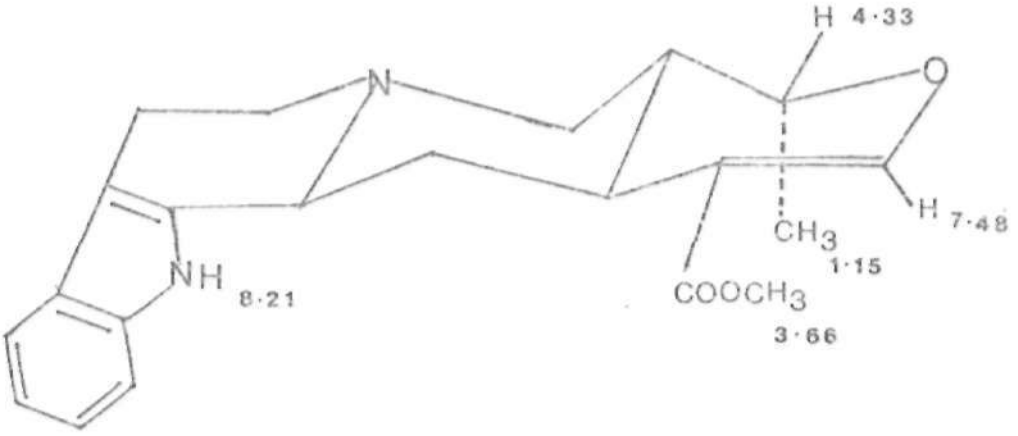
In methyl reserpate (C/D ring cis fused) this proton resonates at δ 4.45 and in methyl-3-isoreserpate (C/D ring trans fused) it absorbed at a higher field of δ 3.30 (Rosen and Shoolery, 1961). Similarly C-3 epi-ajmalicine (C/D ring cis fused) gives a signal for the C-3 proton at δ 4.5 but ajmalicine, the C/D ring trans fused analogue, resonates at a higher field (Wenkert et al, 1961).

It has also been established that in a series of N-methyl quinolizidinium cations the N-methyl protons of methiodides with cis fused rings give signals at lower fields than their trans analogues (Moynehan et al, 1962). Thus in a series of yohimbine alkaloids Shamma et al (1963) observed that the N-methyl peak in C/D ring cis fused analogues appeared near δ 3.5 while the corresponding peak in the trans fused series appeared at δ 3.3. The same relationship was also found for the heteroyohimbine alkaloid methiodides.

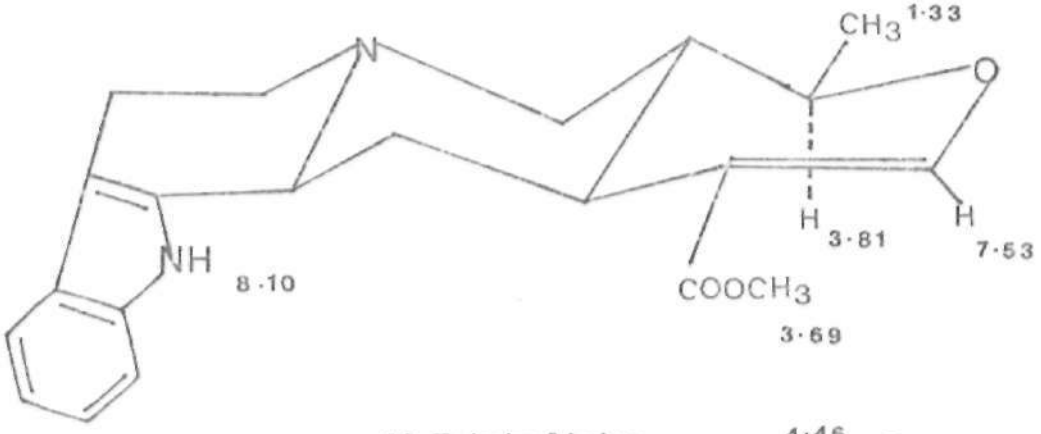
The C-19 methyl proton doublet absorption signal at about δ 1.35 in heteroyohimbine alkaloids has been found to be characteristic of the stereochemical group and not dependent on the methoxy substitution on the aromatic ring (Shamma et al, 1963). The influence of stereochemistry on the NMR signals of the C-19 proton and the C-19 methyl protons in ajmalicine and related structures is shown in Fig. 18 and Table IV.

Results from both Fig. 18 and Table IV indicate that the absorption position of the C-19 methyl protons is not only characteristic of the stereochemical group but also indicative of the marked shielding effect on α -oriented groups in the series. Thus an α -oriented C-19 methyl proton or C-19 proton will absorb at a higher field than the corresponding ß-analogues in the same stereochemical series. The C-19 methyl protons of allo series compounds absorb at a very low field compared with the other stereochemical groups indicating their proximity to the N_b nitrogen electron lone pairs.

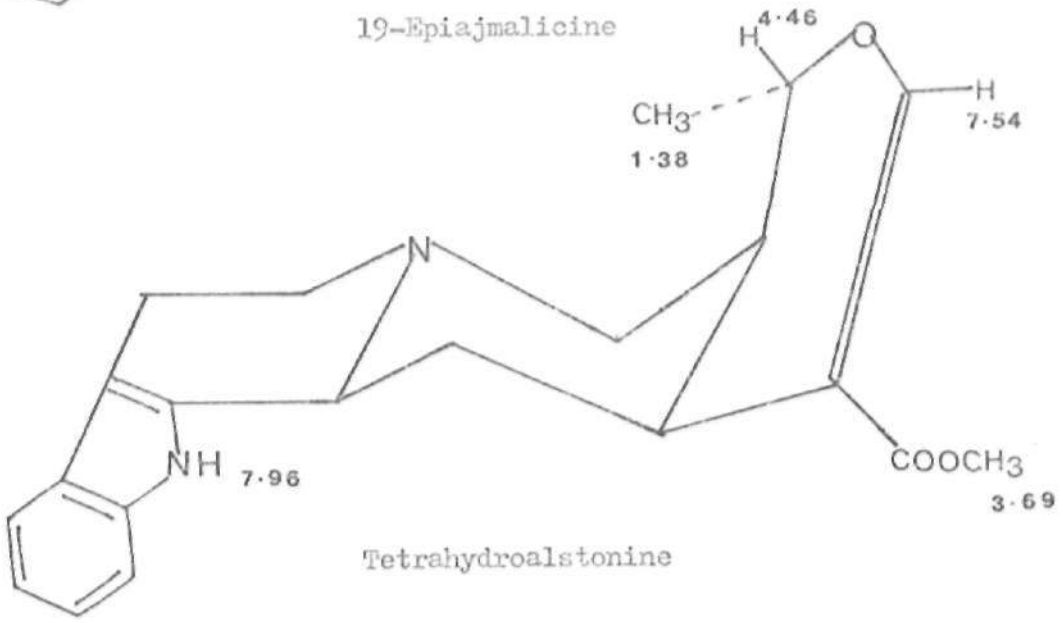
Fig. 18.



Ajmalicine (in $CDCl_3$)



19-Epiajmalicine



Tetrahydroalstonine

TABLE IV

Stereochemical group and alkaloids	C-19 CH_3 configuration	NMR Absorption Signal -values (j in c.p.s)		C/D Ring fusion
Normal series				
Ajmalicine	α	1.15	(6.7)	Trans
Tetraphylline	α	1.16	(6.5)	"
Tetraphyllinine	α	1.05		"
Raumitorine	β	1.29		"
Rauvanine	β	1.34		"
Allo series				
Tetrahydroalstonine	α	1.38	(6.1)	"
Reserpinine	α	1.38	(6.1)	"
Aricine	α	1.37	(6.3)	"
Isoreserpiline	α	1.37	(6.2)	"
Raunitidine	β	1.42	(6.7)	"
Raunitidine	β	1.42	(7.1)	"
Epiallo series				
Isoreserpinine	α	1.32	(6.5)	Cis
Reserpiline	α	1.35	(6.3)	"
Pseudo series				
Raufloridine	α	0.91		Trans
Isoajmalicine	α	0.80		Cis

The relationship between NMR spectra and the stereochemistry of the yohimbine alkaloids was briefly studied by Albright et al (1963) and the configuration of the C-17 substituents can also be determined from the NMR spectra.

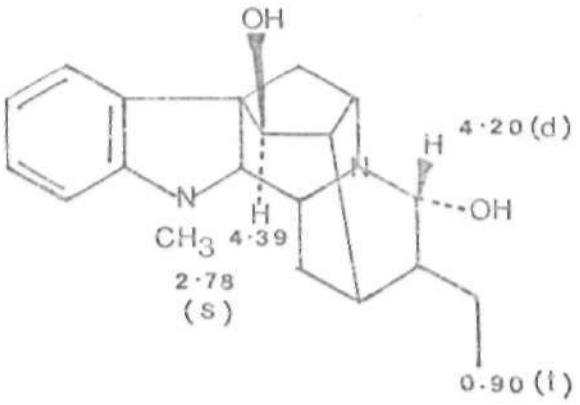
A common feature in the structure of sarpagan-ajmalan group alkaloids is the presence of either an ethyl or an ethylidene side-chain and this can be determined from the NMR spectra.

The ethylidene side-chain shows two absorption signals, one, a doublet at about δ 1.55, corresponding to the C-18 methyl protons and the other, a quartet at about δ 5.4, corresponding to the C-19 proton. The ethyl group however shows a triplet absorption signal at about δ 0.8.

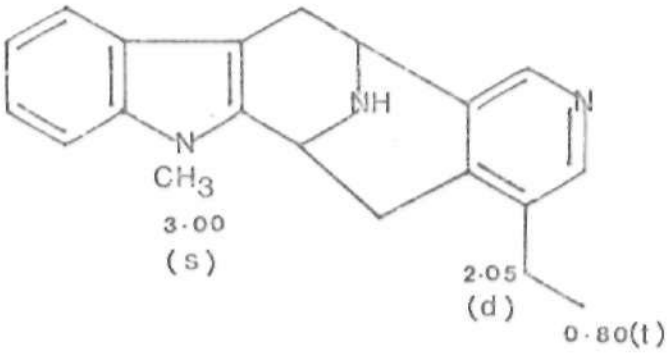
The correlation of structure and NMR spectra establishing the axial/equatorial orientation of the ethyl side-chain in alkaloids related to corynantheidine has been described (Lee et al, 1967). It was observed that isomers with $-CH_2CH_3$ group axial orientation gave a better resolved triplet absorption signal than the equatorially oriented analogues. This was presumably because when the ethyl group is axial (i.e. cis to the N_b nitrogen electron lone pair) the $-CH_2CH_3$ protons are deshielded relative to the equatorial $-CH_2CH_3$ protons.

The NMR spectrum is also useful in confirming the presence of functional groups, and other substituents in Rauwolfia alkaloids. Fig. 19 shows the structure of some Rauwolfia alkaloids with some relevant NMR data.

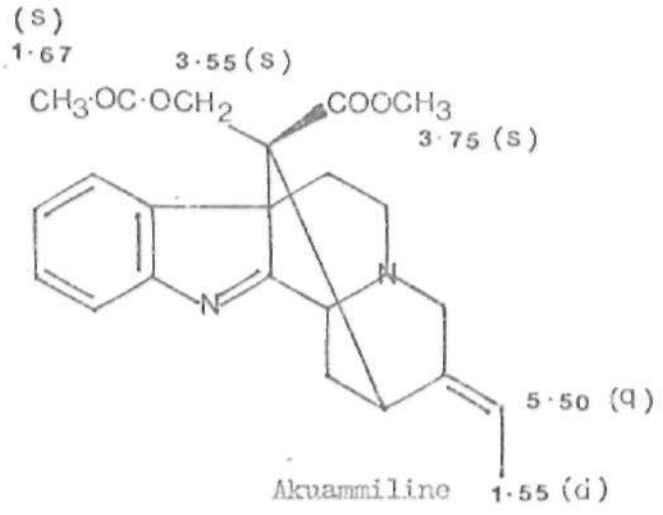
Fig. 19.



Ajmaline (in $CDCl_3$)



Suaveoline



Akuammiline

In the sarpagan type alkaloids such as polyneuridine or akuammidine or the akuammiline type compounds, the stereochemistry at C-16 can be determined by the absorption signals of C-16 substituents (Crabb, 1975). Substituents with orientation nearer the aromatic ring are more shielded and therefore absorb further upfield than their C-16 epi analogues. This is because of their cis relationship with the aromatic ring.

IV Mass Spectrometry (MS)

Mass spectrometry is a valuable technique in the structural elucidation and differentiation of indole alkaloids. The pioneering studies in this field were reported by Biemann and his collaborators in 1960. Since then characterization of the structures of several indole alkaloids has been achieved primarily by MS interpretation.

Budzikiewicz et al (1964) suggested two reasons why indole alkaloids lend themselves so readily to MS interpretation. Firstly, they possess two centres which have a great capability for stabilising a positive charge, (i) the indole nucleus i.e. the aromatic π electrons and/or the indole nitrogen atom and (ii) the basic N_b nitrogen atom. These centres sustain characteristic peaks on electron impact which can be used to recognise the alkaloids embodying them. Secondly, indole alkaloids possess a carbocyclic framework in which certain bonds are particularly prone to rupture thus giving rise to a few very intense but characteristic fragment ions e.g. as in the 2-acyl indole alkaloids.

Information concerning functional groups and other substituents can be obtained at the high mass range. Frequently, though not always, the M-R peaks in the high mass region for indole alkaloids are important indicators of the presence of certain substituents. Thus the existence of M-17 or M-18 points towards the elimination of water or OH groups although elimination of water has been observed in the

mass spectra of some ketones and certain carboxylic acids (Budzikiewicz et al, 1964).

The loss of 15 mass units refers to the elimination of a methyl radical. Aromatic methoxyl groups may occasionally give rise to M-30 peaks and aliphatic methoxyl groups may be indicated by an M-31 peak. Other diagnostically important M-R fragments are M-28 (C = O or $CH_2 = CH_2$), M-29 (CHO or $-CH_2CH_3$), M-42 (CH_2CO^+), M-43 (CH_3CO^+), M-59 ($-COOCH_3$), M-60 (H. $COOCH_3$) and M-73 ($-CH_2OCOCH_3$).

In cases where an M-R corresponds to an unlikely or impossible combination of atoms, it is attributed to impurities. Thus M-2, although possible owing to loss of 2 hydrogen atoms, is very rare; M-3, M-4, M-5 and M-13 are virtually impossible if ordinary organic compounds are considered. Similarly M-19, M-22 and M-35 are extremely unlikely.

Studies of the MS of several groups of indole alkaloids revealed that characteristic fragmentation features exist in their spectra which served readily to permit assignment of some of the alkaloids into specific groups. Most of the Rauwolfia alkaloids belong to the tetrahydro- β -carboline group. The mass spectra of alkaloids of this group have been studied extensively by Antonaccio et al (1962), Biemann (1961), (1964) and Beckett et al (1969). The spectra fall into three main categories:-

(I) Yohimbine and heteroyohimbine alkaloids,

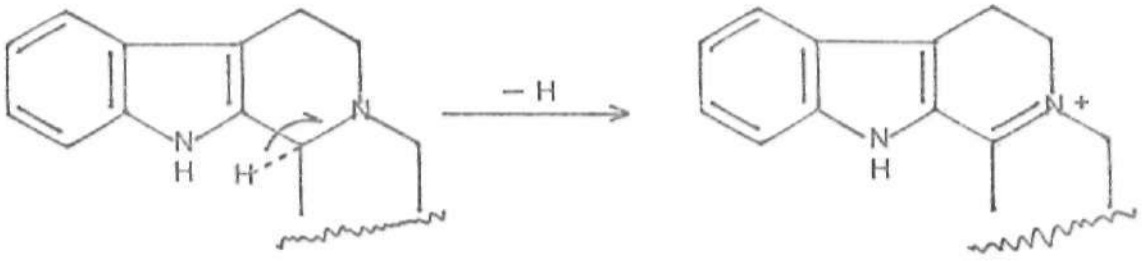
(II) Sarpagine and related compounds,

(III) Ajmaline and related compounds.

I Yohimbine and heteroyohimbine alkaloids

A characteristic feature in the mass spectra of this group is the prominent M-1 peak, which in the case yohimbine and its isomers is usually more intense than the molecular peak. The genesis of this peak has been shown by deuterium labelling to be predominantly due to the loss of C-3H with the formation of species a (Fig. 20). The resultant positive charge is stabilised by conjugation with the indole system as well as by participation of the electron pair on the N_b nitrogen (Antonaccio et al, 1962).

Fig. 20.



a

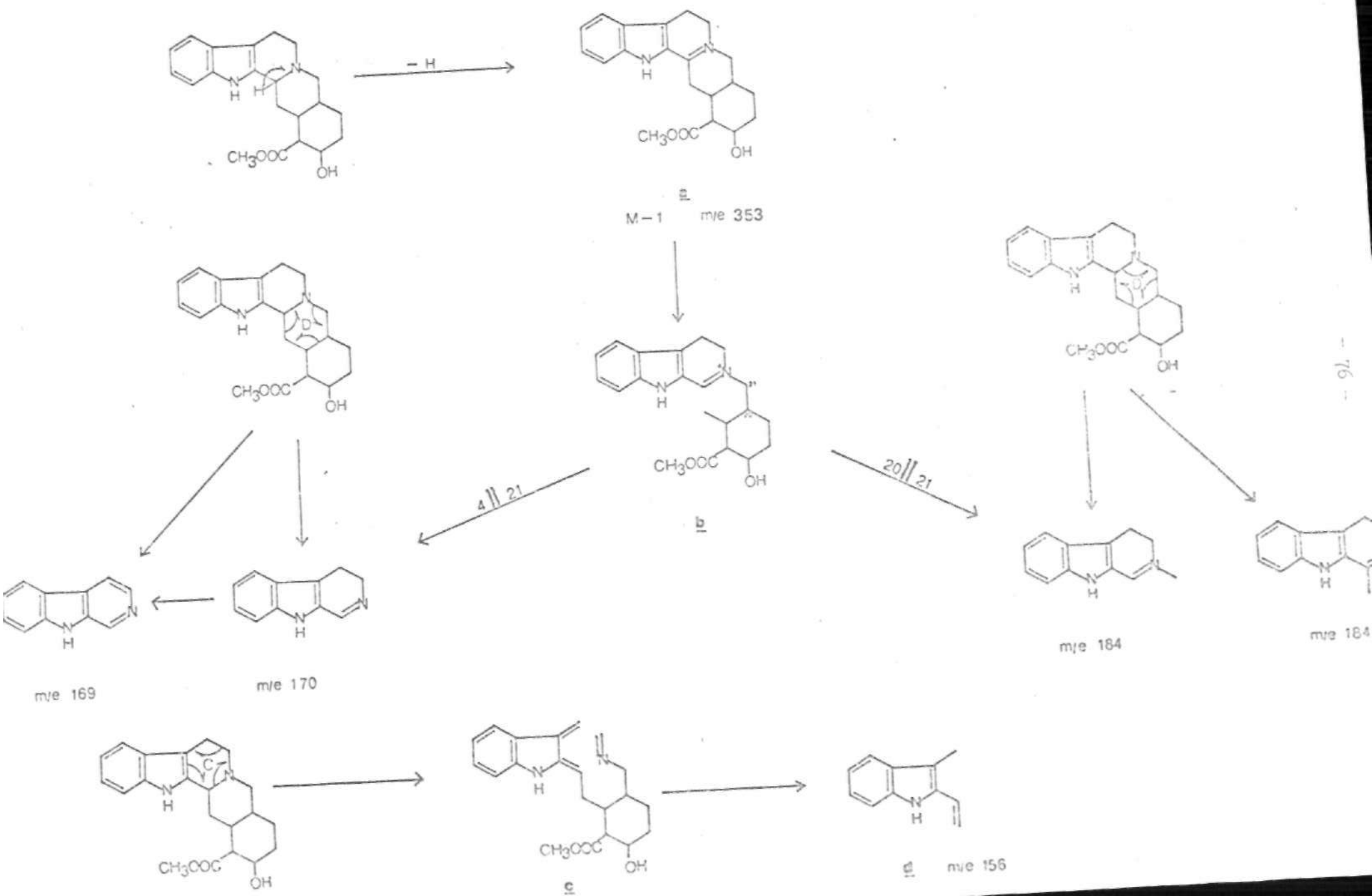
Both yohimbine and heteroyohimbine alkaloids are believed to follow similar fragmentation paths showing characteristic peaks at m/e 184, 170, 169 and 156; the principal difference is the appearance of the peaks at m/e 225 and 223 in the heteroyohimbine alkaloids (Djerassi, 1963). The genesis of these two peaks is believed to involve a retro-Diels-Alder fragmentation of the heterocyclic ring E as shown in Scheme III.

The production of the ions at m/e 156, 169, 170 and 184 is also associated with a simultaneous retro-Diels-Alder fragmentation of rings C and D. The fragmentation of ring C, followed by a homolytic fission of the allylically activated C-14, C-15 bond in species c results in the generation of the conjugated ion d, m/e 156 (Scheme II). The m/e 156 peak is usually more intense in the mass spectra of heteroyohimbine compounds than in the mass spectra of the yohimbines. This is presumably because cleavage of the C-14, C-15 bond in the former is more favoured by the presence of the C-16, C-17 double bond in the heterocyclic ring E.

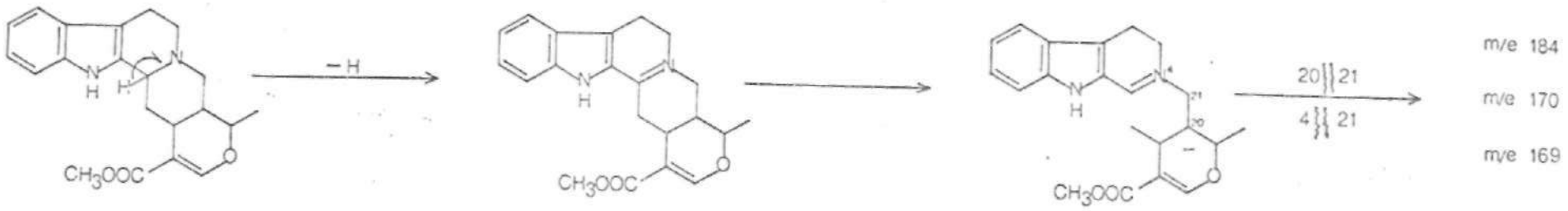
Similarly, retro-Diels-Alder fragmentation of ring D produces the peaks at m/e 184, 170 and 169 as shown in Scheme II.

It has been shown (Antonaccio et al, 1962) that for the yohimbine group stereochemical assignment by mass spectrometry is of no significance as characteristic fragments of yohimbine and its isomers vary only slightly in abundance. In the heteroyohimbine group, however, the relative intensities of the peaks at m/e 184, 170, 169

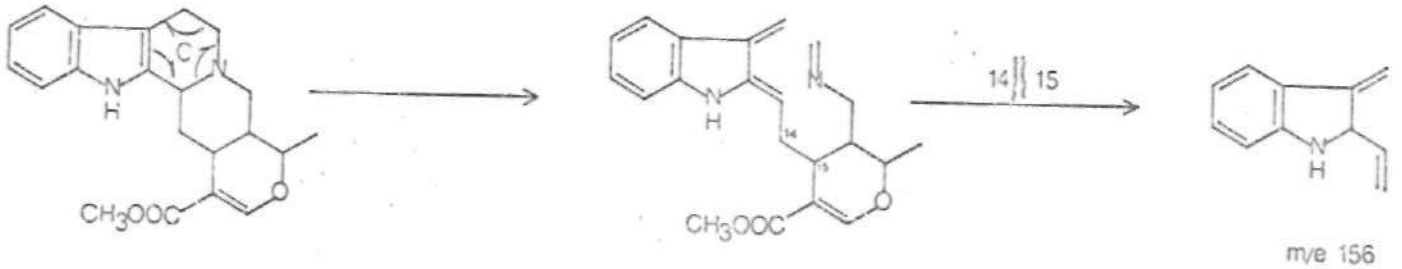
Scheme II MS Fragmentation pattern of Yohimbine



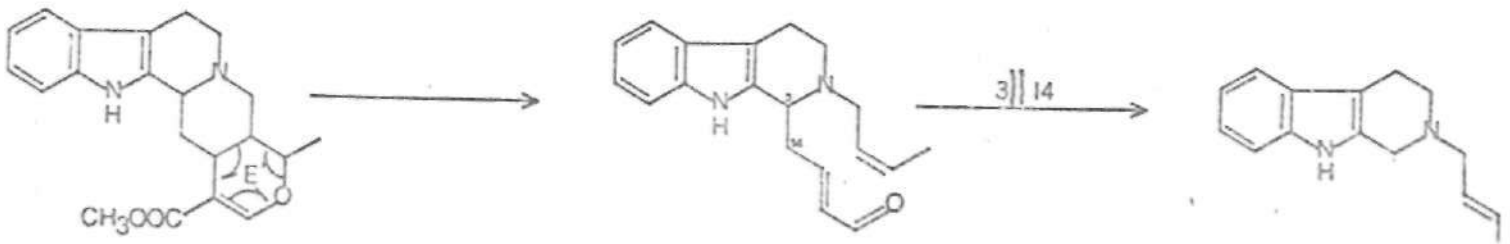
Scheme III. MS Fragmentation Pattern of Ajmalicine



M−1 m/e 351



m/e 156



m/e 225

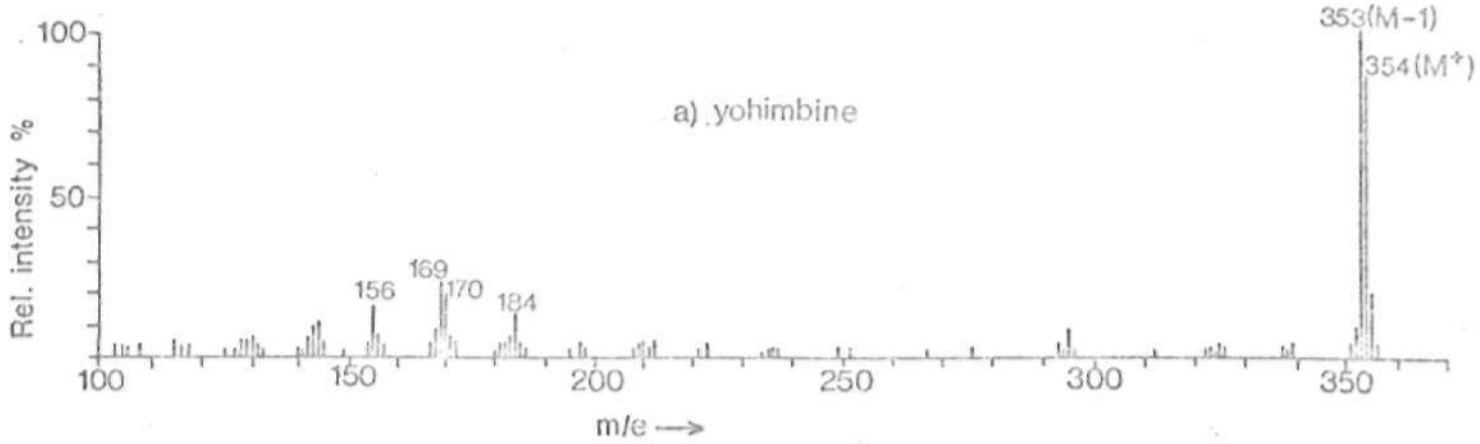
and 156 vary significantly to allow stereochemical assignment of the D/E ring junction.

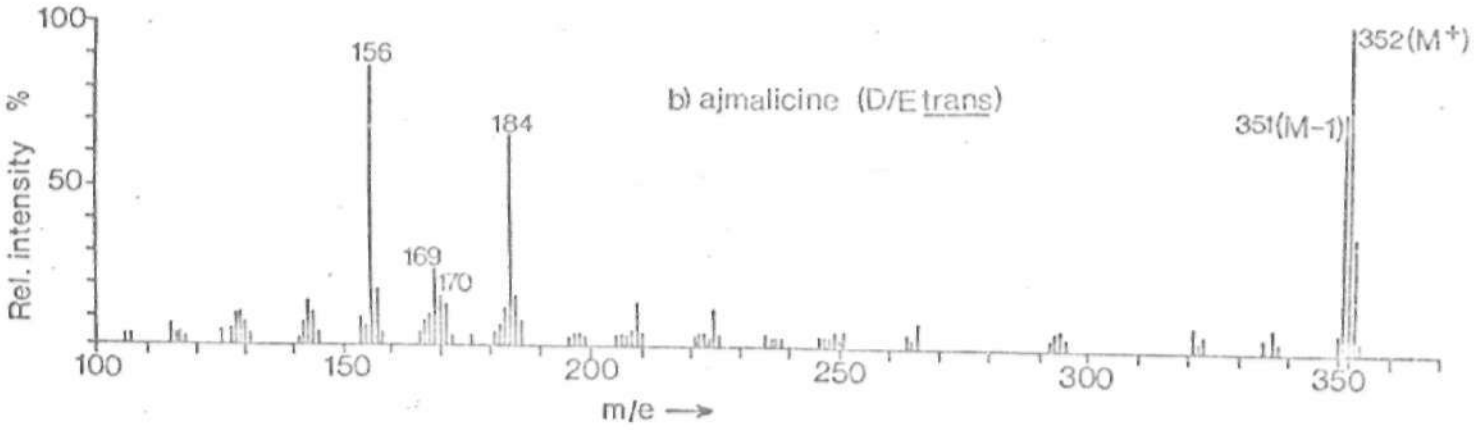
Ajmalicine, which is D/E ring trans fused, yields a fragment ion at m/e 184 which is relatively more abundant than those at m/e 170 and 169, but for tetrahydroalstonine, which is D/E ring cis fused, the fragment ions at m/e 184 is of equal or lower abundance than the ions at m/e 170 and 169 (Fig. 21).

The intensities of peaks at m/e 251, 225, 223 and 209 can also be used to differentiate stereochemical groups among the heteroyohimbine alkaloids (Hesse, 1974; Iwu, 1977). In compounds of normal series, peaks at m/e 251 and 223 are usually of relatively low intensity whilst those at m/e 225 and 209 are of moderate intensity; the m/e 223 peak is usually more prominent than the m/e 225 peak in the same spectrum (Hesse, 1974). The reverse obtains for compounds of the allo series i.e. the peaks at m/e 251 and 223 are of moderate intensity whilst those at 225 and 209 are of low intensity. The intensity of the m/e 225 peak is greater than that of the peak at m/e 223.

Substitution on the indole nucleus causes peaks associated with the ß-carboline moiety to shift correspondingly e.g. an OH group causes an appropriate shift of 16 mass units, OCH_3 group a shift of 30 mass units and two OCH_3 groups a shift of 60 mass units. This is in agreement with Biemann's observation (1960) that substitution in the aromatic portion of an indole or dihydroindole alkaloid does not affect

Fig.21. Mass spectra of yohimbine and heteroyohimbine alkaloids.





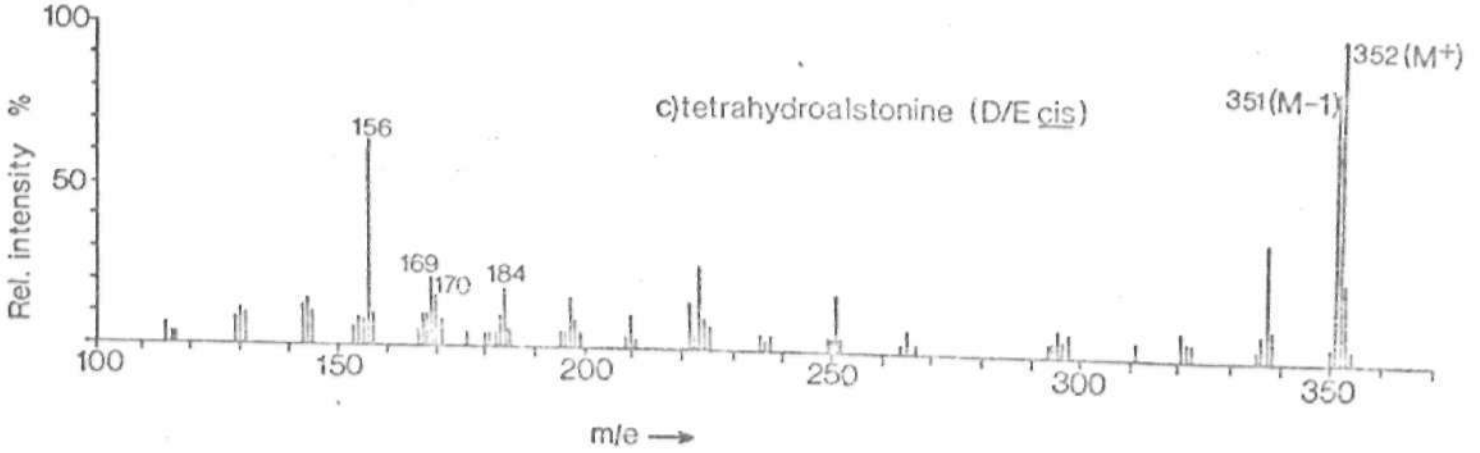
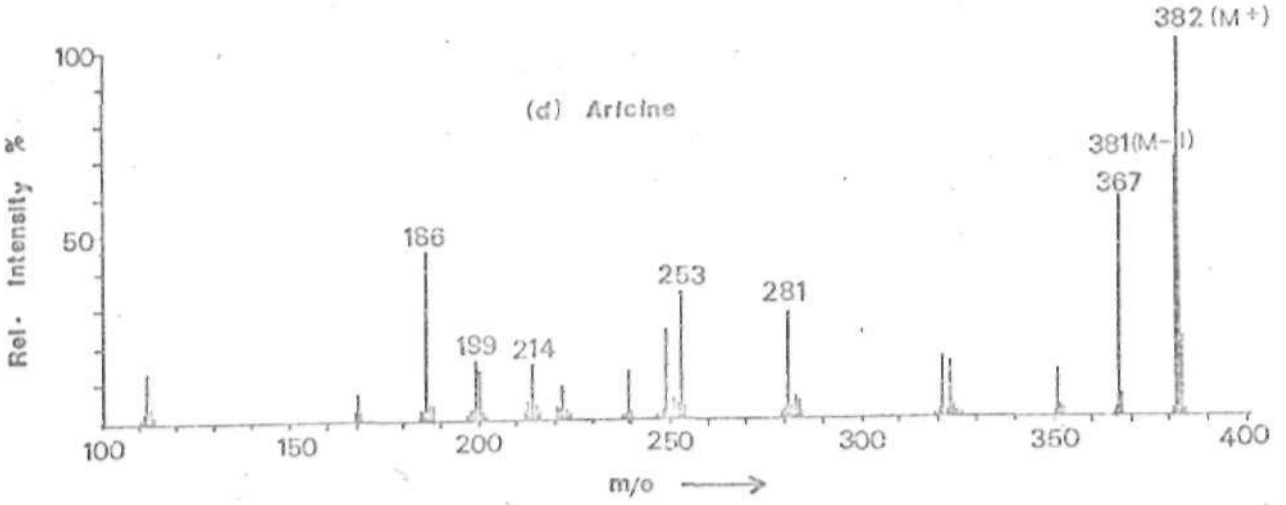
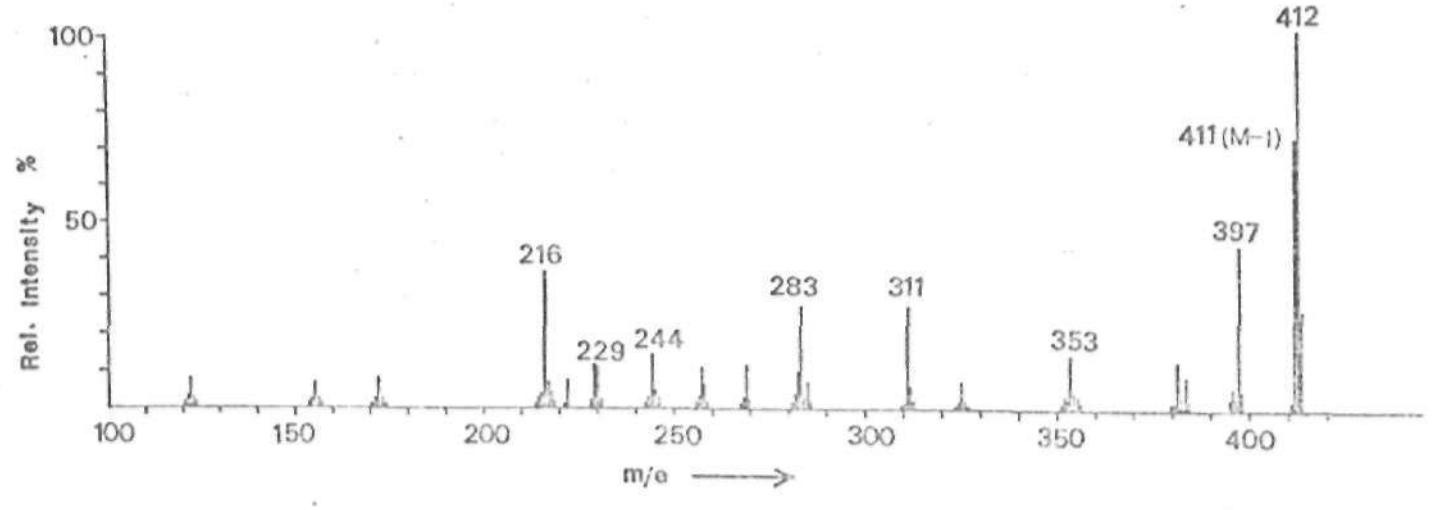


Fig. 22. MS of Aricine and Reserpiline



(e) Reserpiline

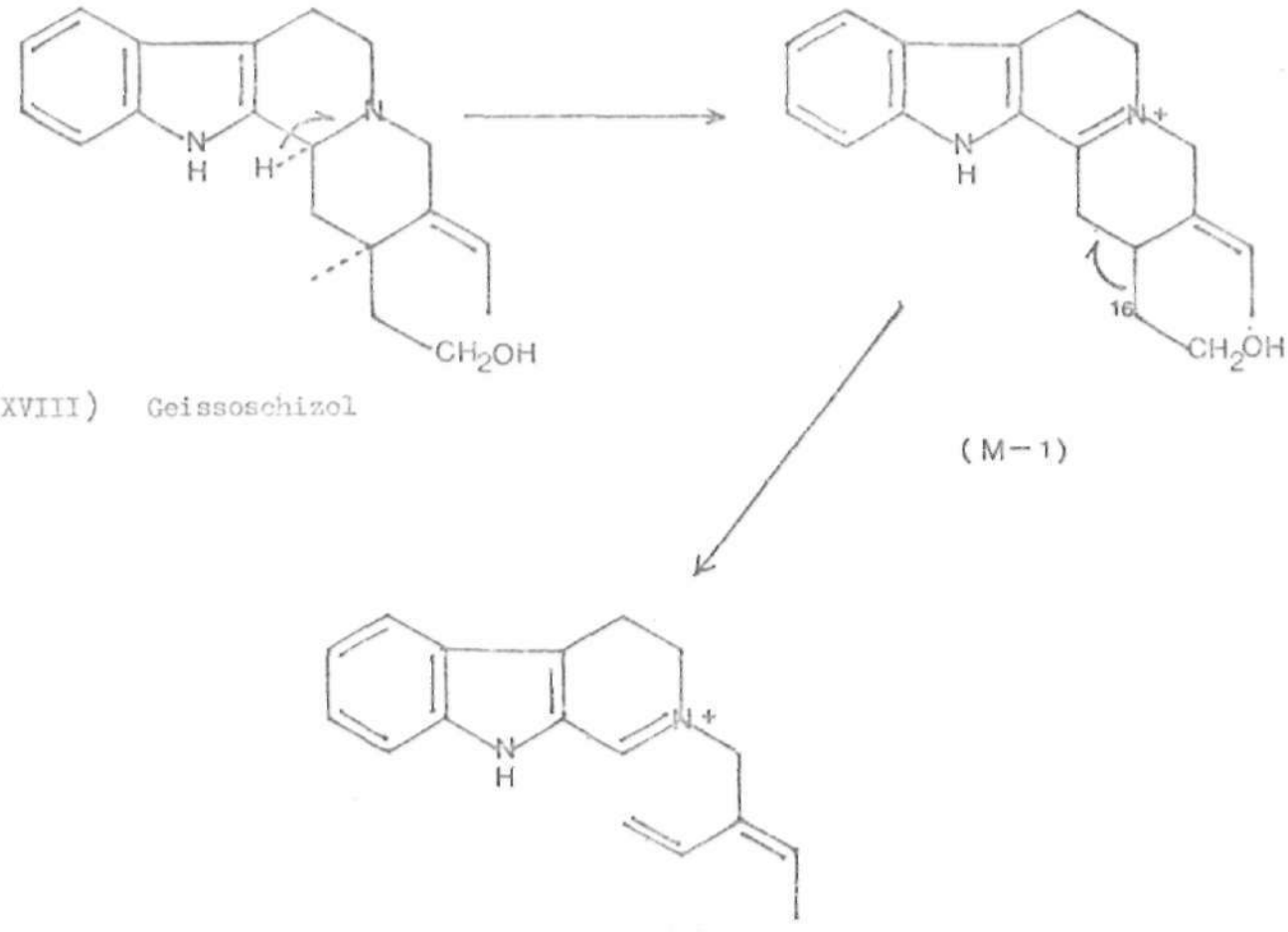


the overall mass spectral pattern of the molecule, fragments of compounds with an additional group on the aromatic ring will be shifted by the appropriate increment (Biemann, 1960).

Thus aricine, the C-10 methoxy analogue of tetrahydroalstonine, shows the β-carboline peaks at m/e 214, 200, 199 and 186 and reserpiline, the C-10, C-11-dimethoxy analogue, shows the β-carboline peaks at m/e 244, 240, 239 and 216 (Fig. 22).

The D-seco indole alkaloids show fragmentation patterns similar to those of the heteroyohimbines. They exhibit the characteristic pronounced M-1 peak and the β-carboline peaks occur at m/e 184, 170, 169 and 156 as in the heteroyohimbines. Particularly valuable diagnostically in their mass spectra is the loss of the C-16 carbon atom and its substituents to give a peak at m/e 251 in analogues possessing an ethylidene side-chain e.g. geissoschizol (XVIII) or a peak at m/e 253 in analogues having an ethyl side-chain e.g. dihydrocorynantheol (XIX) (Scheme IV) (Budzikiewicz et al, 1964; Hesse, 1974).

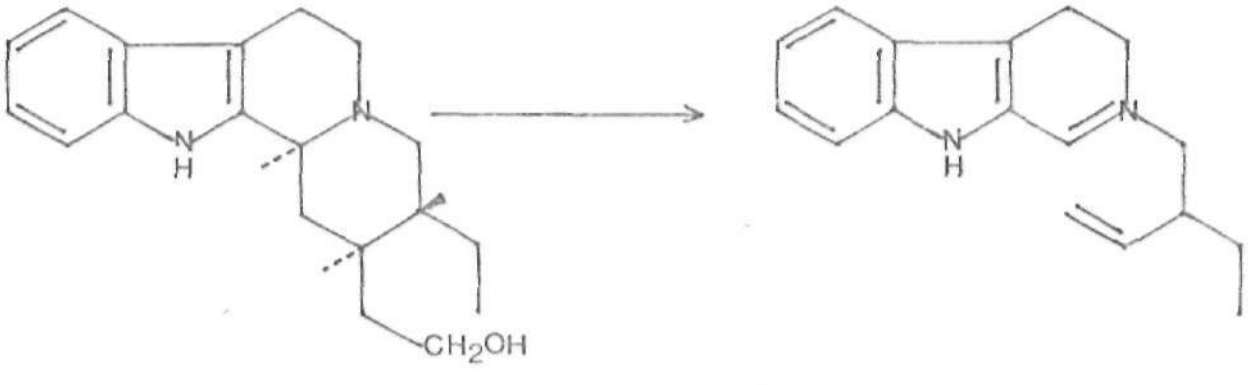
Compounds having an ethyl side-chain fragment further to give a peak at m/e 225 in normal and pseudo series or a peak at m/e 223 in allo and epiallo series. The m/e 225/223 peak is usually more intense than the m/e 253 peak.



(XVIII) Geissoschizol

(M−1)

m/e 251

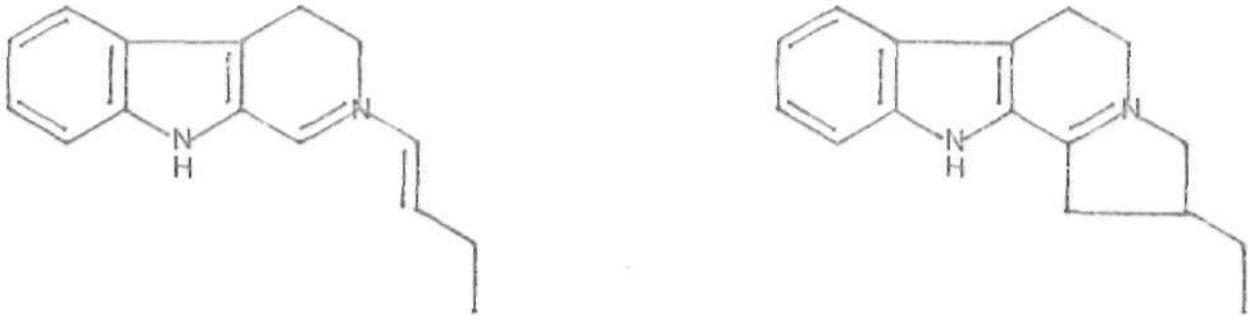


m/e 253

(XIX) Dihydrocorynantheol

SCHEME IV

E-seco indole compounds possessing a double bond at the C-16, C-17 position, as in corymantheidine, fragment to give an additional peak at m/e 239 which is attributed to species f as in Fig. 23 (Beckett et al, 1969; Hesse, 1974).

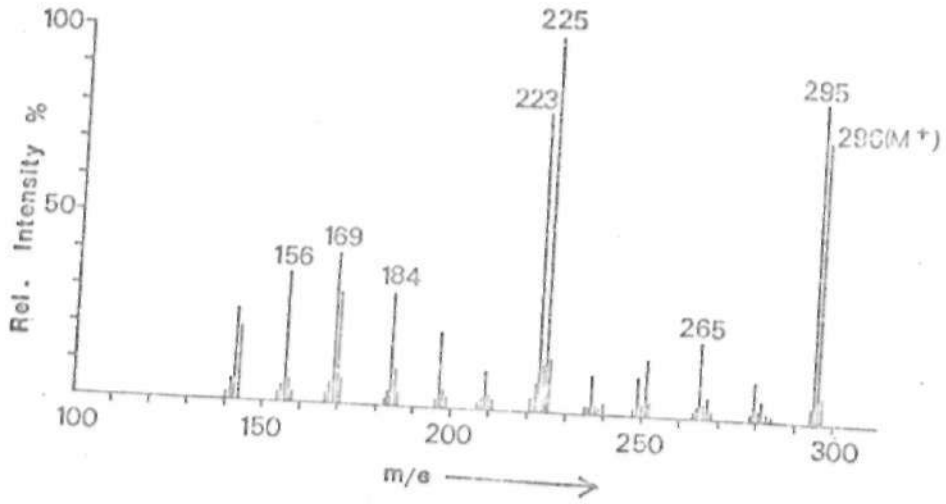


m/e 225 (f) m/e 239

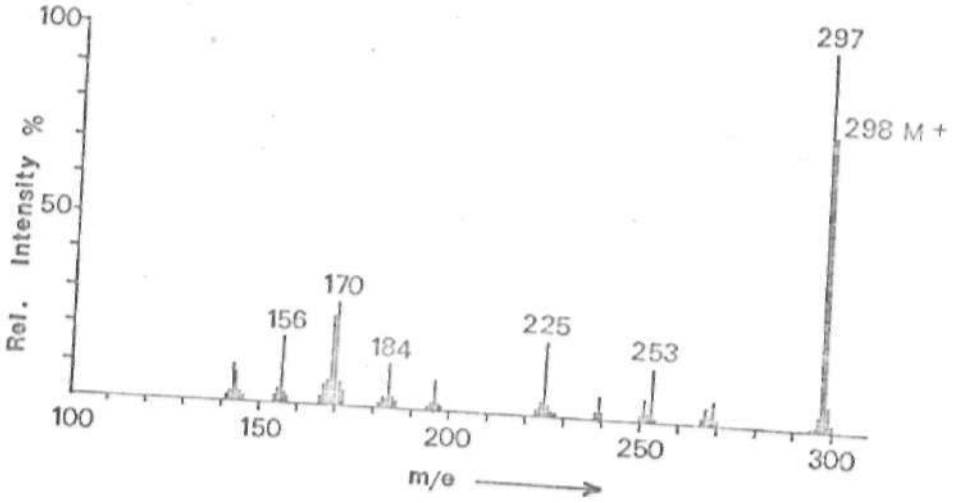
Fig. 23.

18-Hydroxy-yohimbine esters fragment to yield peaks corresponding to the esterifying 3, 4, 5 - trimethoxy-benzoyl (reserpine or deserpidine) or cinnamoyl (rescinnamine) moieties and the yohimbane nucleus. The fragmentation patterns are, in general, similar to those of the yohimbines except that the species k (Scheme V; m/e 221 in deserpidine or m/e 251 in reserpine or rescinnamine) seems more prominent in this group. The mechanism of fragmentation leading to the formation of species k is shown in Scheme V (Hesse, 1974).

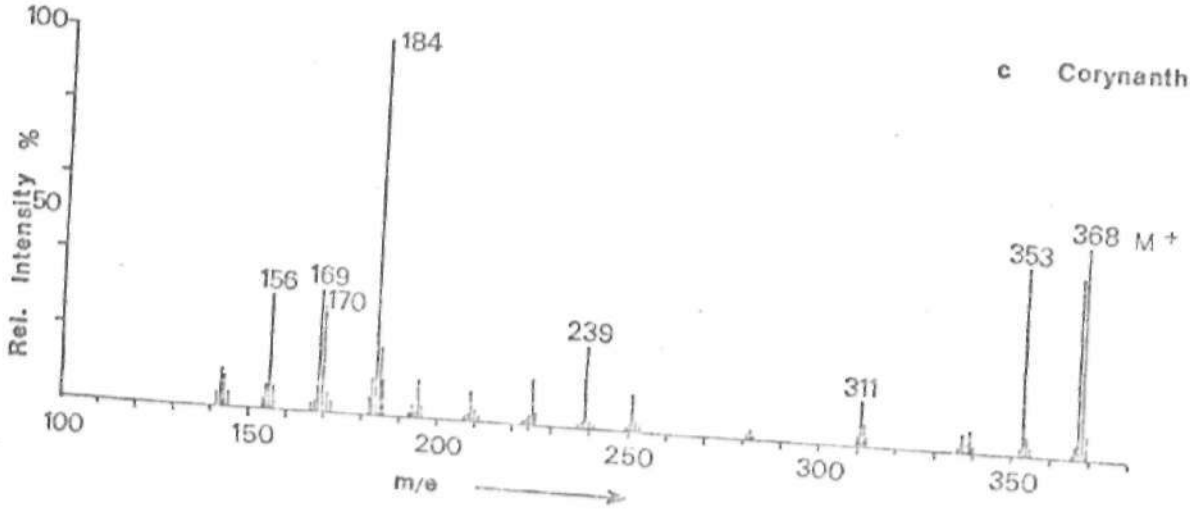
Fig. 24 : MS of E-Seco Indole Alkaloids



a Geissocchizol

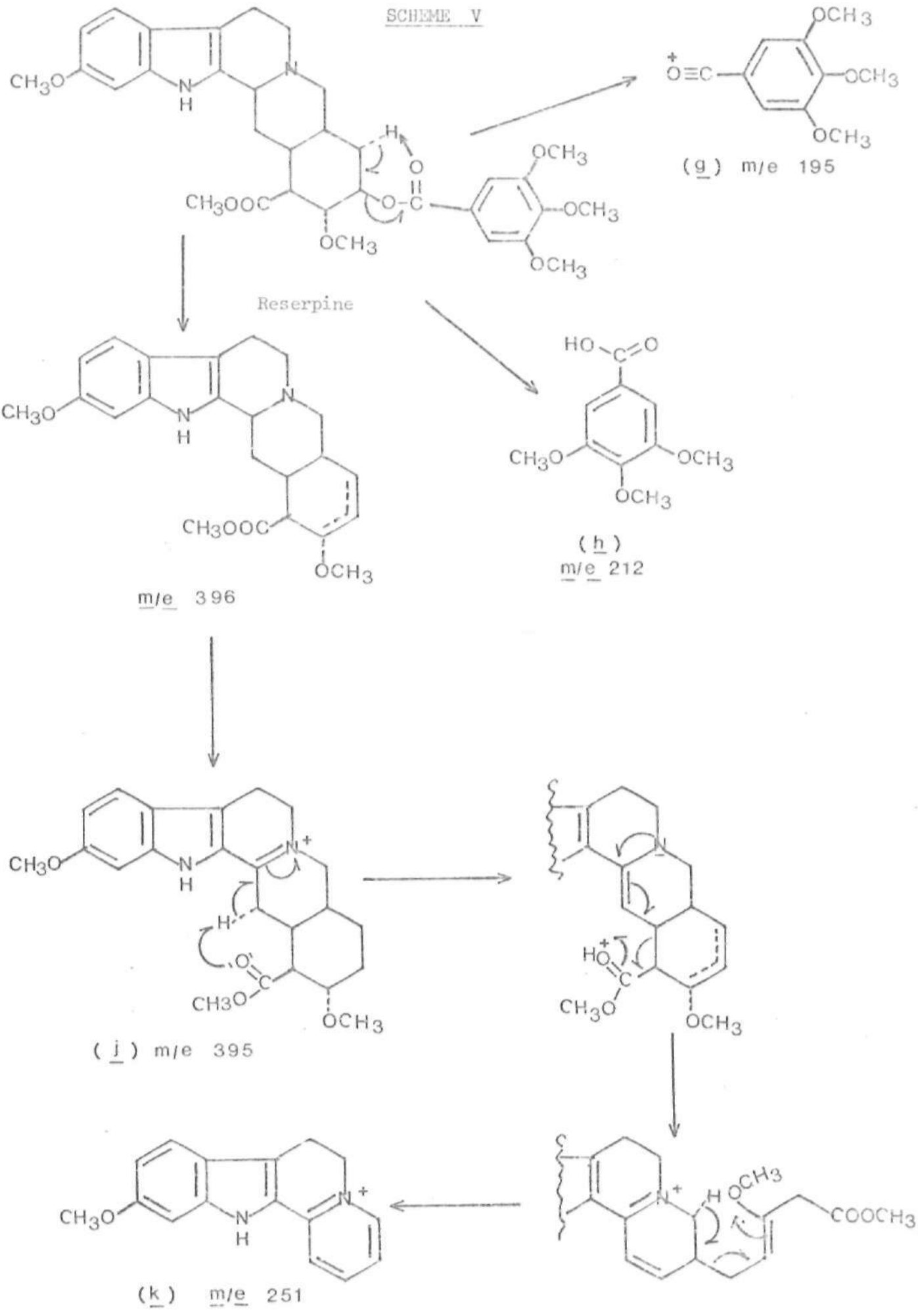


b Dihydrocorynantheol



c Corynantheidine

SCHEME V



The mass spectrum of reserpine reveals a prominent peak at M-1 and the β-carboline peaks occur at m/e 214, 200, 199 and 186. The intense peaks at m/e 212 and 195 are due to the 3, 4, 5 - trimethoxybenzoyl fragment i.e. species g and h respectively (Scheme V).

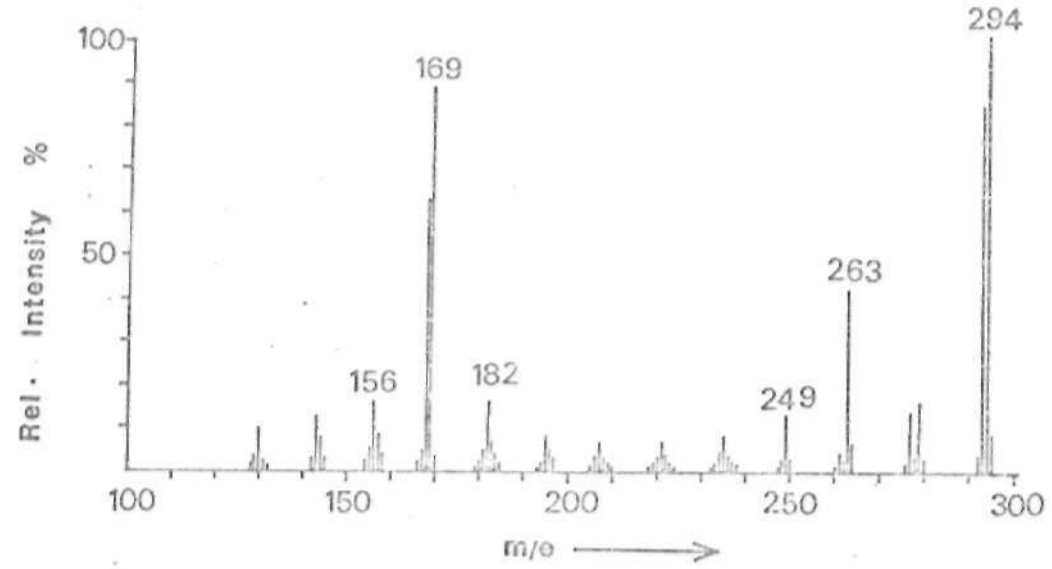
II Sarpagine and related alkaloids

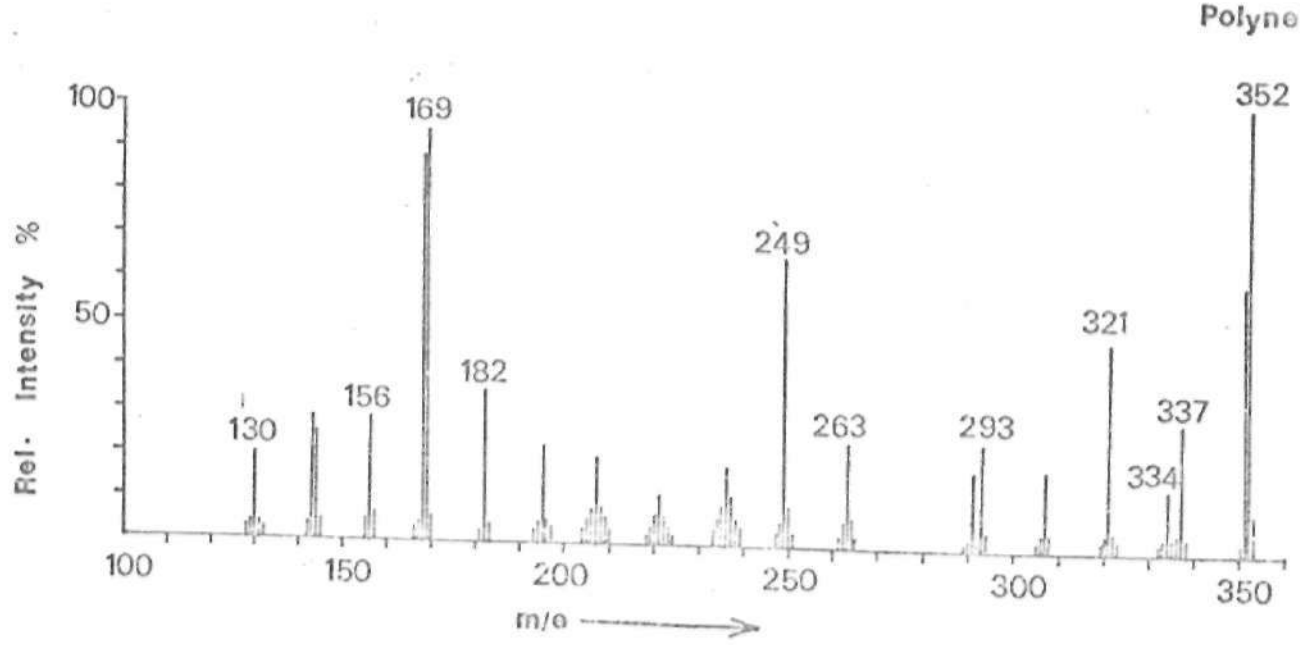
A characteristic feature in the fragmentation pattern of alkaloids possessing a sarpagan type skeleton is the loss, on electron impact, of the C-16 carbon bridge with its attached substituents and an additional hydrogen atom (Antonaccio et al, 1962). The appropriate peak occurs at m/e 249 and is particularly pronounced when C-16 is disubstituted. Its intensity is considerably reduced when only one functional group is attached to C-16 (Fig. 25).

The proposed genesis of this peak involves homolysis of the C-5, C-16 bond to produce an intermediate i followed by transfer of the C-18 hydrogen atom to C-16 and rupture of the C-15, C-16 bond (Scheme VI.)

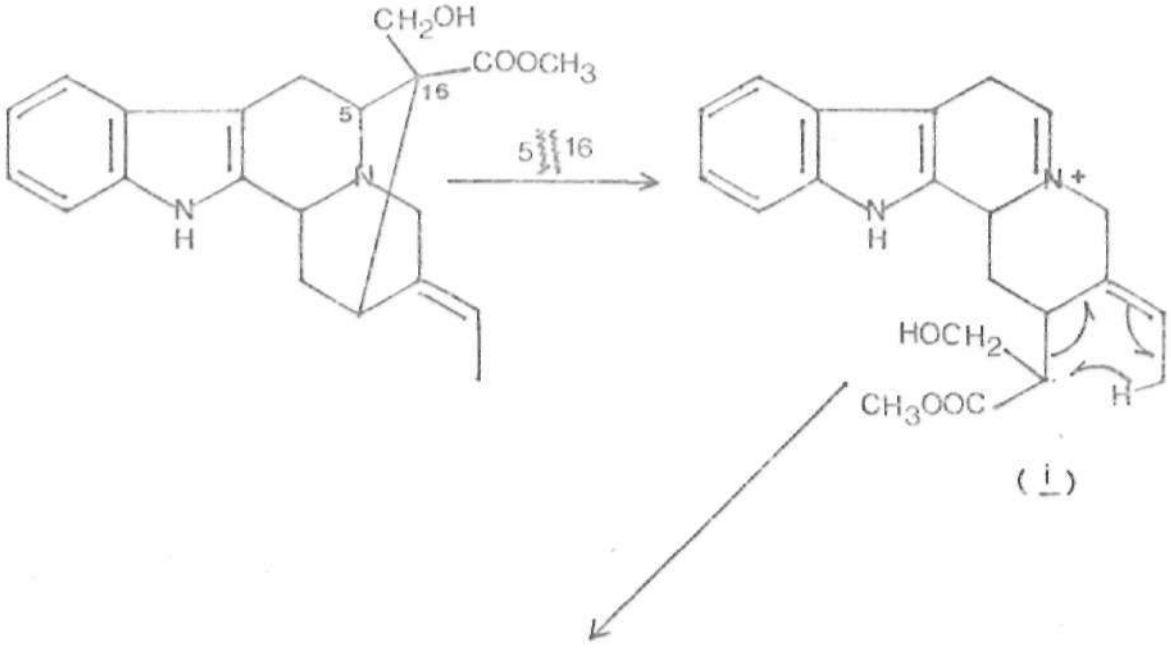
In analogues having an ethyl side-chain, the appropriate m/e 251 peak is virtually absent but a substantial m/e 223 peak occurs and this peak is associated with species j (Biemann et al, 1961).

Fig. 25. MS of Sarpagan Type Alkaloids

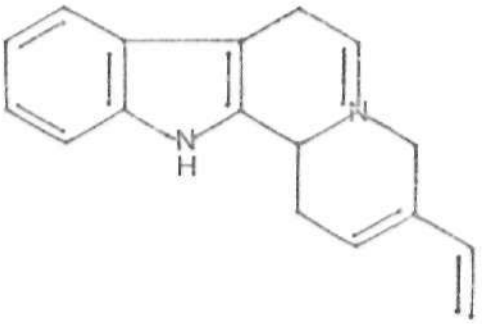




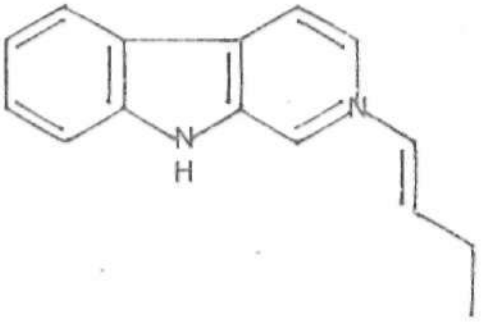
Scheme VI



(i)



m/e 249

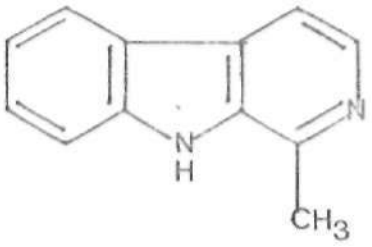


m/e 223

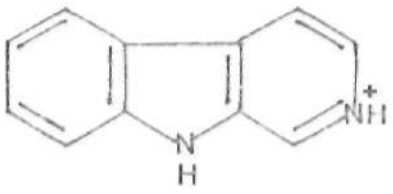
(i)

Sarpagan type alkaloids also exhibit prominent M-1 peaks, the origin of which is still not clearly understood. The participation of the C-3H, as proposed for the yohimbine group, is considered unlikely because the additional ring terminating at C-5 would make a C-3, N_b double bond sterically very unfavourable (Antonaccio et al, 1962).

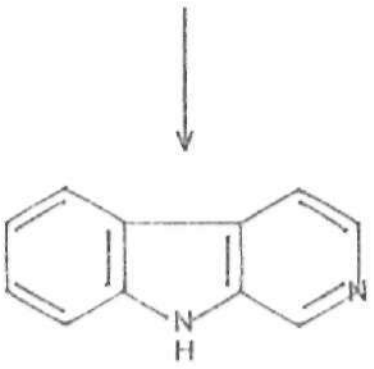
A distinguishing feature in the mass spectra of the sarpagan type compounds is the appearance of the β-carboline peaks at m/e 182, 169, 168 and 156 instead of at m/e 184, 170, 169 and 156 as in the yohimbine and heteroyohimbine alkaloids. This difference is considered to be due to the additional bond at C-5, C-16 that has to be cleaved in ring C before the β-carboline ions can be formed.



m/e 182

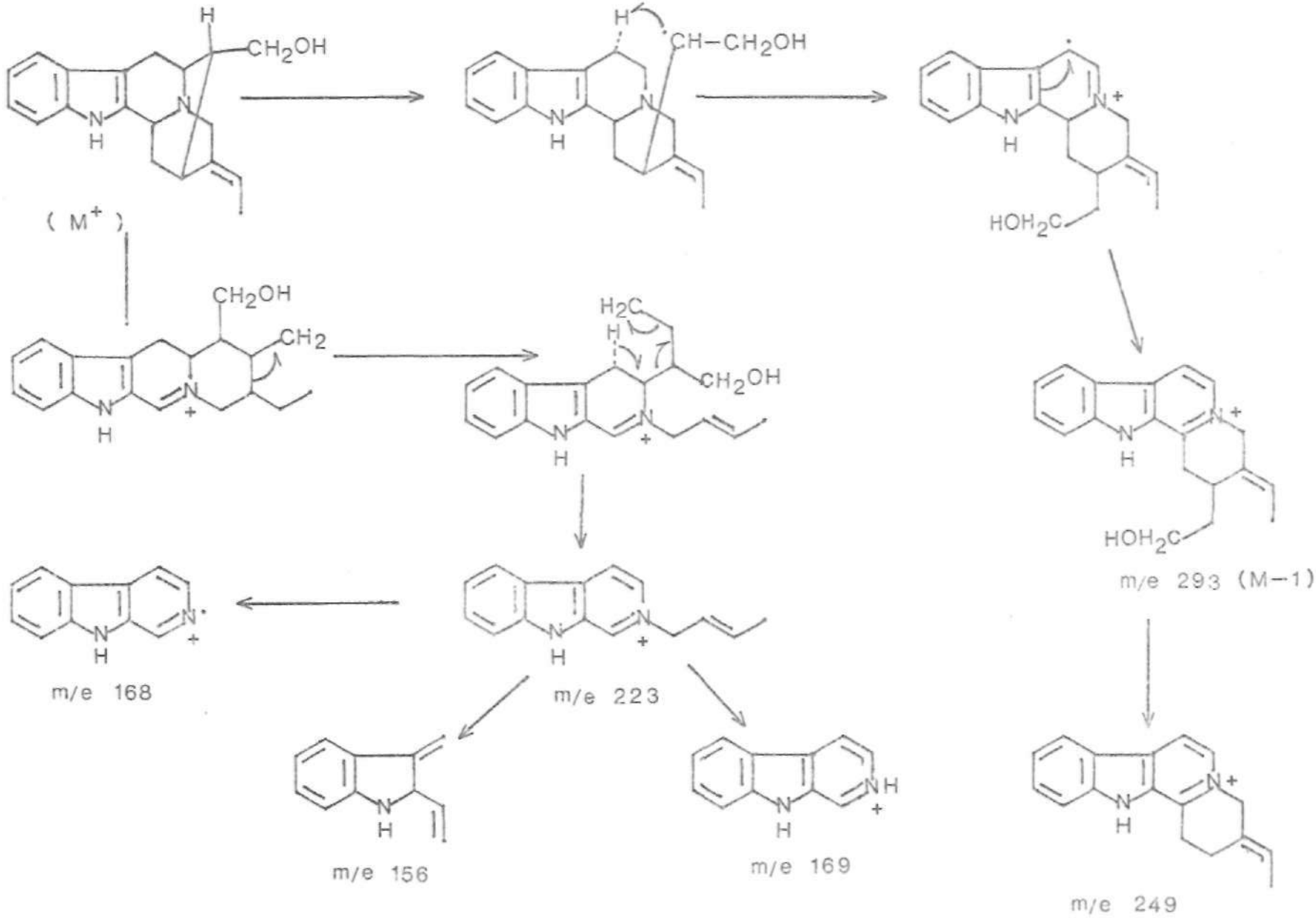


m/e 169



m/e 169

SCHEME VII MS Fragmentation Pattern of Normacusine B



Scheme VII shows the fragmentation pattern leading to significant peaks in the mass spectrum of normacusine B.

Ajmaline and related alkaloids

It has been shown that some alkaloids belonging to this group, although possessing the same carbon skeleton as ajmaline, do vary in their mass spectral fragmentation pattern because of subtle differences in structure and substitution (Biemann et al, 1964). There are three general sub-divisions of the group:-

a) those with spectra resembling ajmaline,

b) those resembling ajmalidine,

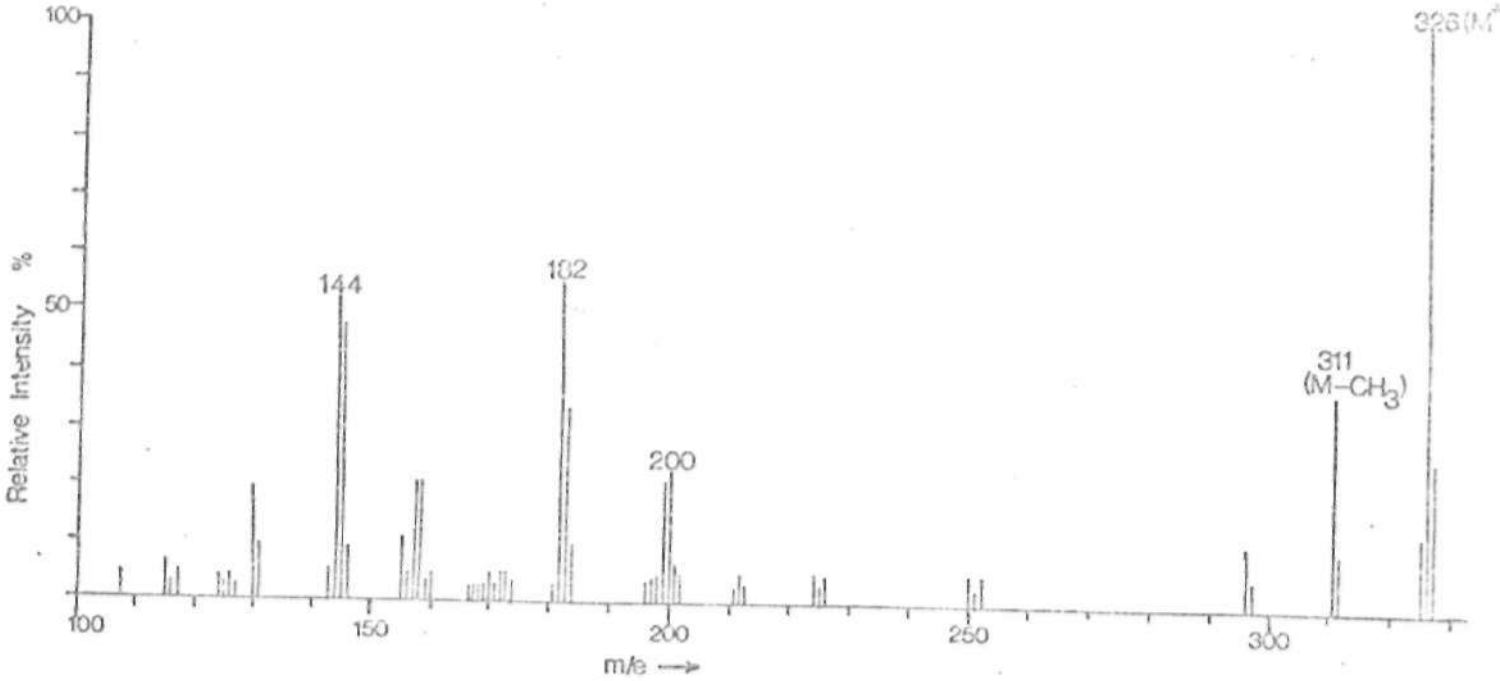
c) those resembling 2-epi-ajmaline.

Some other changes in the molecule such as epimerisation at C-20, removal of C-21 OH-group, epimerisation of the C-17 OH-group and unsaturation of the C-19, C-20 bond have no effect on the basic fragmentation pattern.

The mass spectrum of ajmaline and the fragmentation pathways leading to significant peaks in the spectrum are shown in Fig. 26.

Conversion of the C-17 alcoholic function to a keto group gives rise to an ajmalidine-like spectrum where the carbonyl is eliminated as C = O (Fig. 27).

Fig. 26. Mass spectrum of ajmaline.

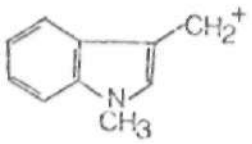






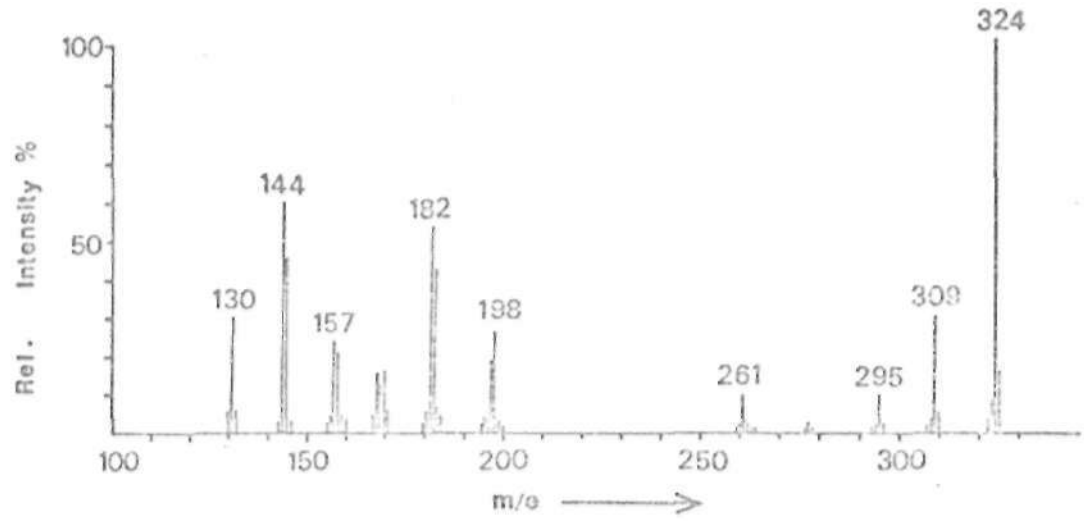
m/e 200.

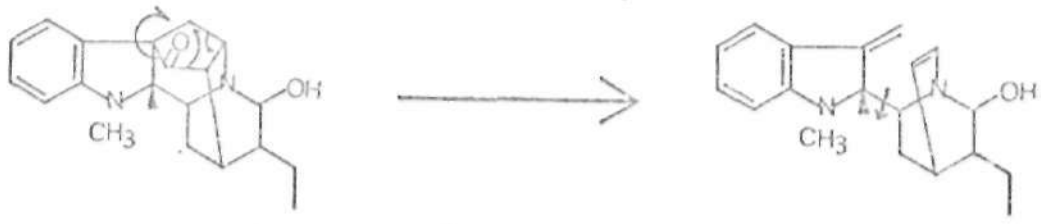
m/e 182
(m/e 183 if protonated).



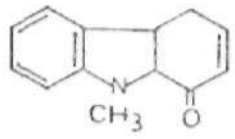
m/e 144.

Fig. 27. MS of Ajmalidine

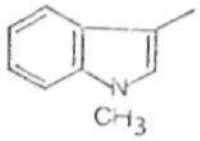




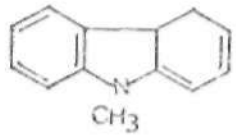
m/e 295



m/e 198

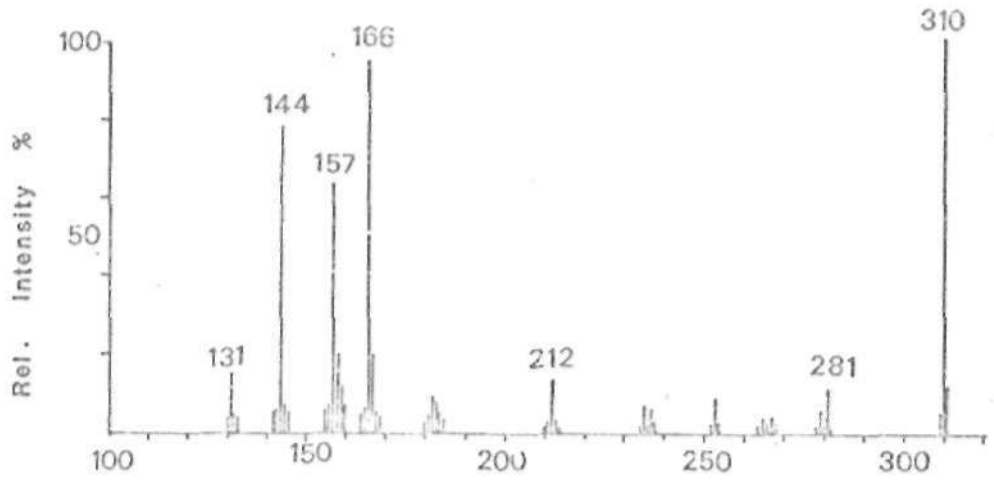


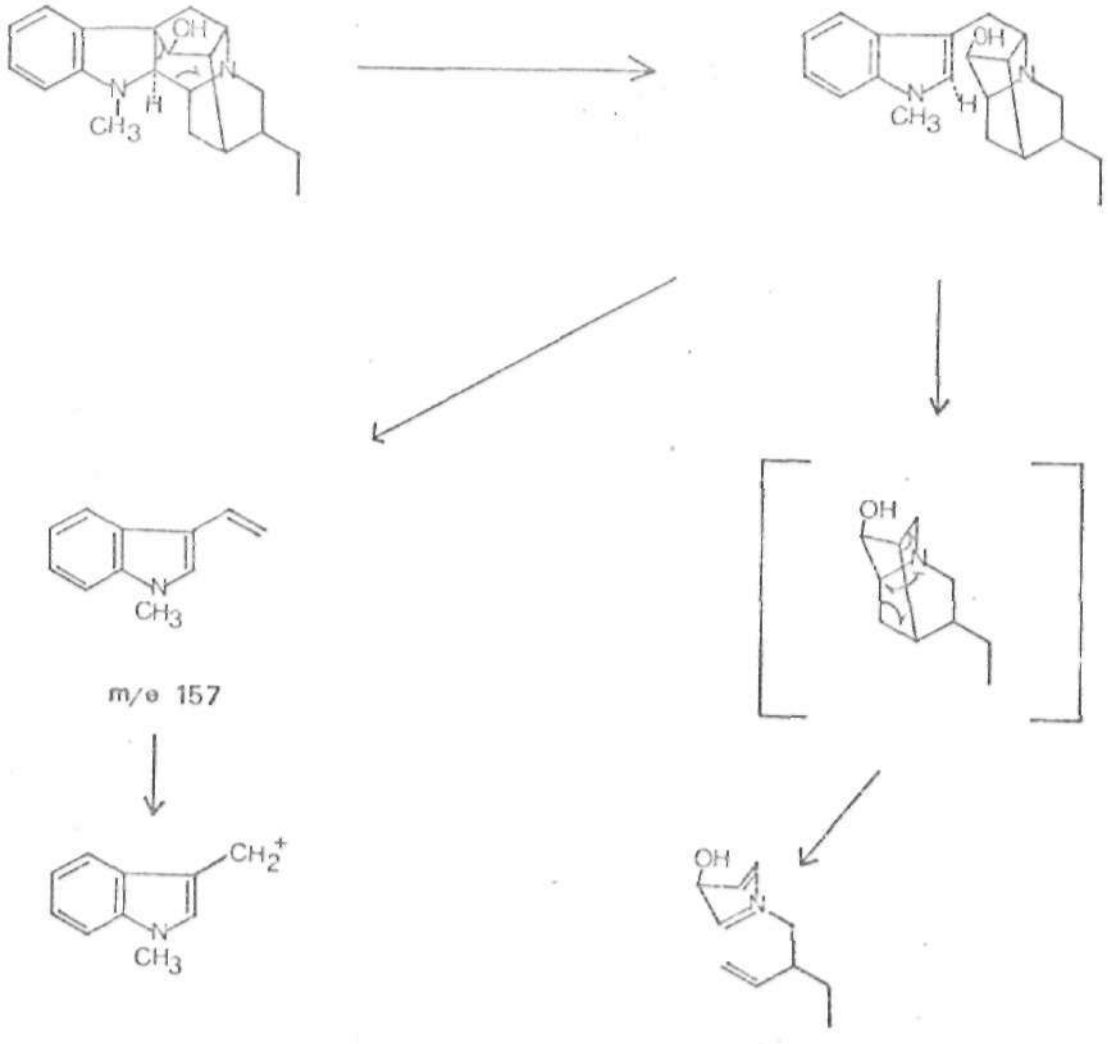
m/e 144



m/e 182

Fig. 28. MS of 2-Epi-ajmaline-21-deoxy





The spectrum exhibited by 21-deoxy-2-epi-ajmaline is different from those of the unepimerised analogues discussed above. The peaks at m/e 183 and 182 are virtually absent and a new peak appears at m/e 166. This peak is attributed to species S (Fig. 28) and is the most intense peak in the spectrum. The fragmentation pathways leading to the genesis of the m/e 166 peak and other significant peaks in 21-deoxy-2-epi-ajmaline is shown in Fig. 28 (Biemann et al, 1964).

Picrinine group

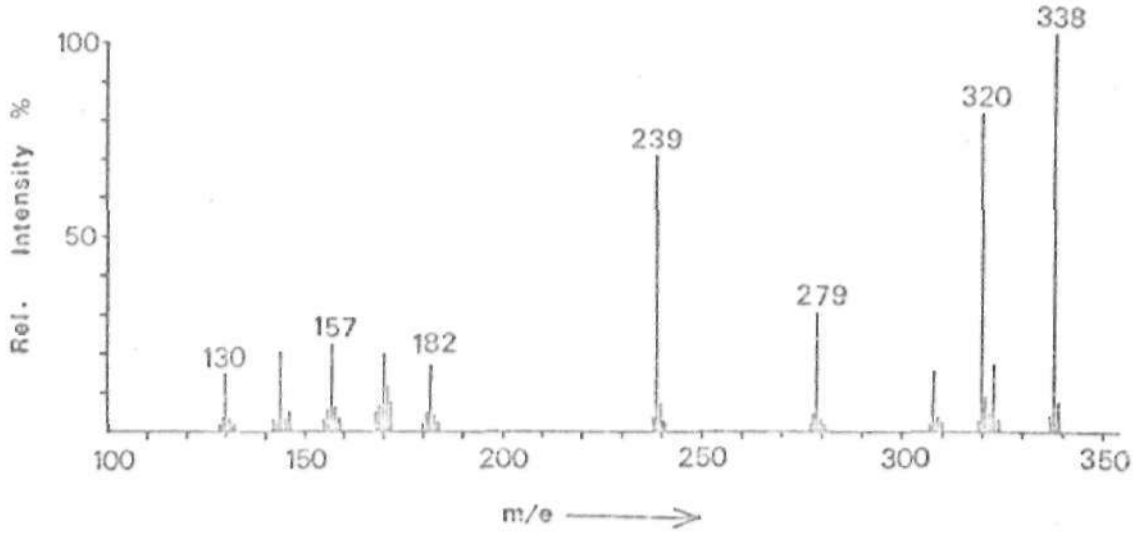
Compounds of this group show spectra consistent with both the C-16, C-7 bridge structure (sarpagan-like) and the modified indoline structure. Particularly diagnostic in their spectrum is the prominent m/e 239 peak which has been attributed to expulsion of the C-16 carbon atom and its attached substituents as well as the C-5 carbon atom together with the oxygen bridge as a C = O group (Britten, Smith and Spiteller, 1963), (Scheme VIII).

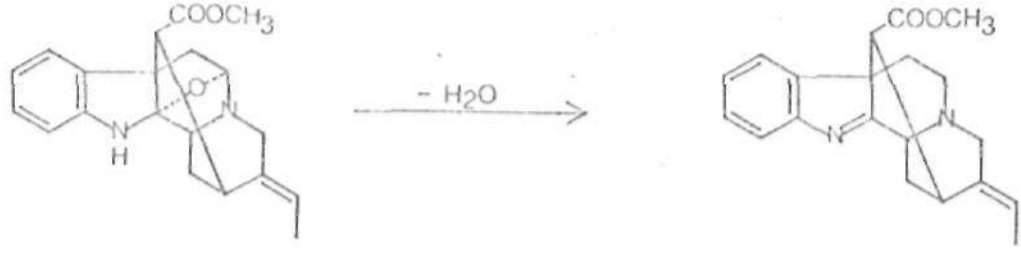
Oxindole and-ψ-indoxyl compounds

The most intense peak in the spectrum of carapanaubine and its isomers occurs at m/e 223 (Gilbert et al, 1963). The production of this peak and other important peaks in the spectrum is shown in Scheme IX.

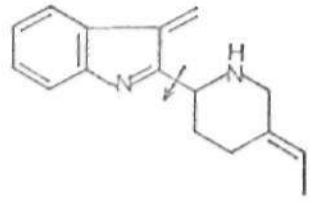
The peak at m/e 223 has been shown to be derived from the molecular ion by the homolytic rupture of the C-5, C-6 and C-3, C-7 bonds. The molecular ion could also decompose to yield the peak at m/e 180 (Scheme IX).

Scheme VIII. MS of Picrinine

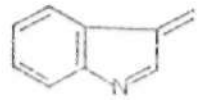


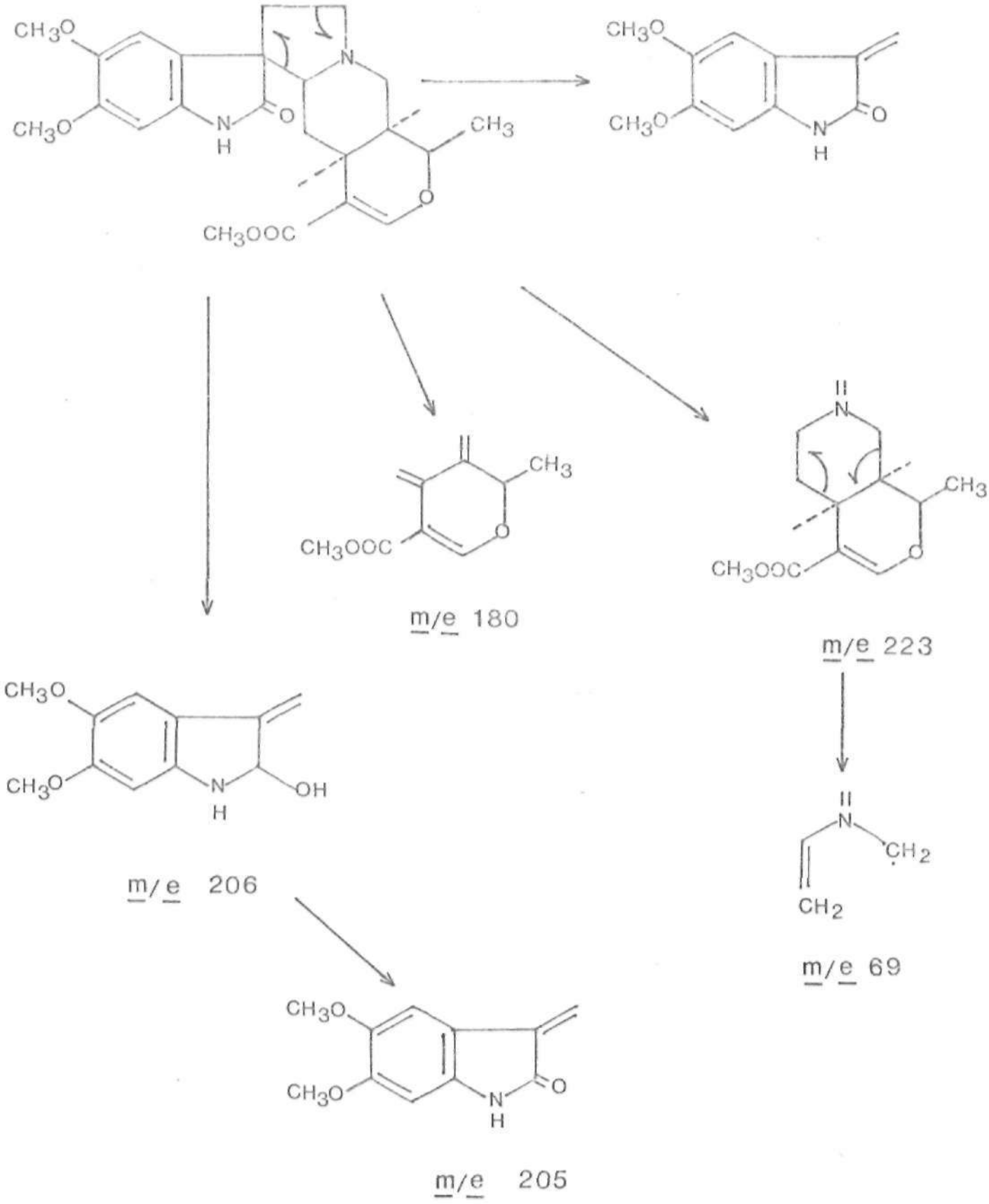


m/e 320

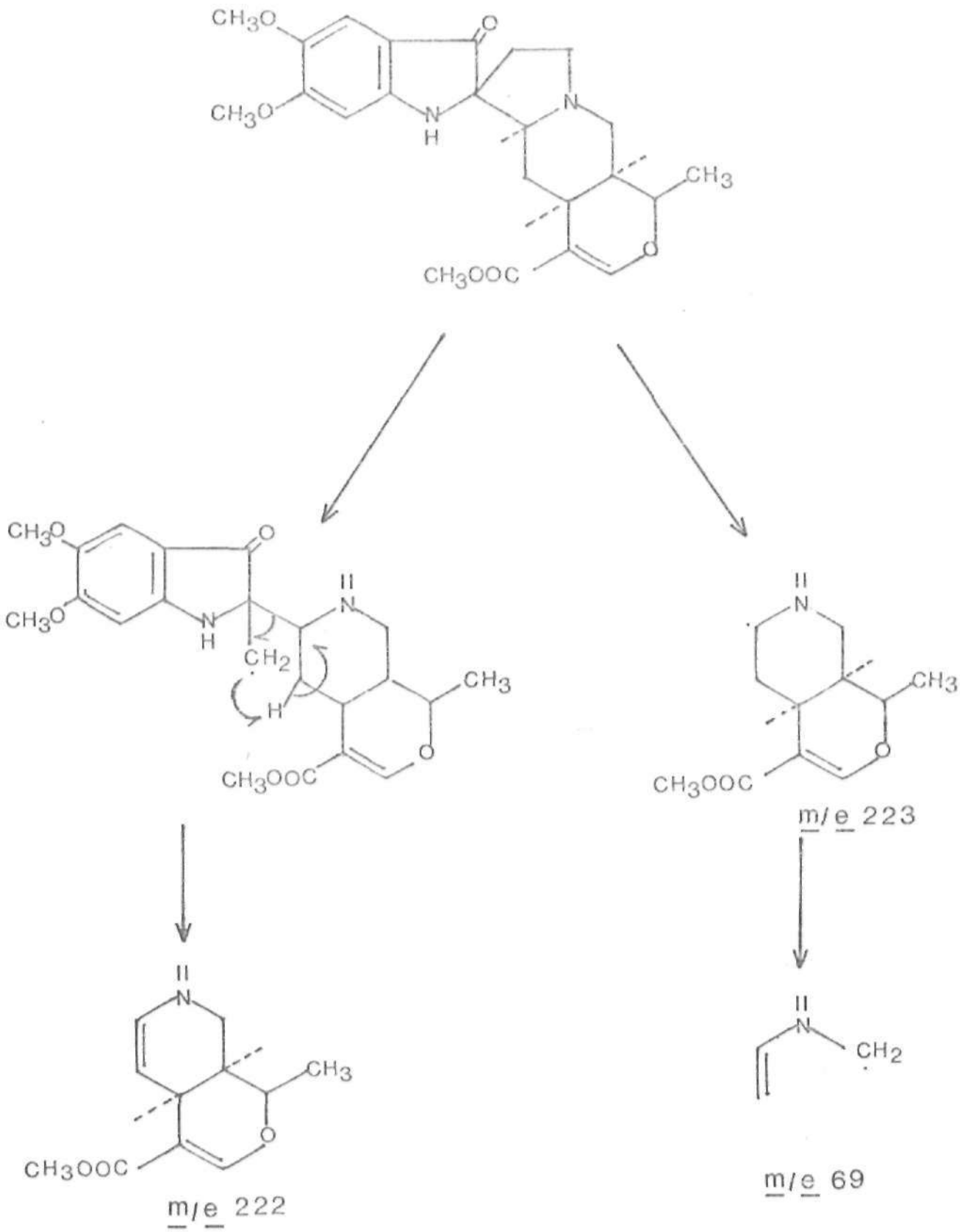


m/e 239





Scheme IX. MS Fragmentation pattern of carapaunabine



Scheme X. MS Fragmentation of Isoreserpiline-ψ-indoxyl (Hesse, 1974)

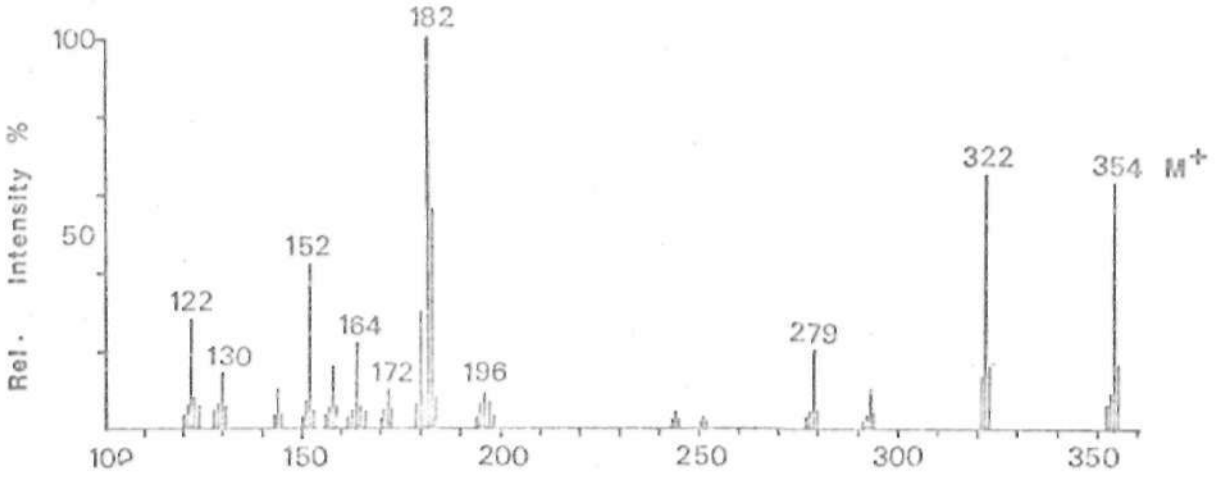
The pseudoindoxyls show fragmentation patterns similar to those of the oxindoles, the most striking difference being in the relative intensities of the peaks at m/e 222 and 223. The latter is the base peak in spectra of oxindoles and the m/e 222 peak is usually of low intensity. The reverse obtains for pseudoindoxyls. The fragmentation pathways of isoreserpiline-ψ-indoxyl is shown in Scheme X.

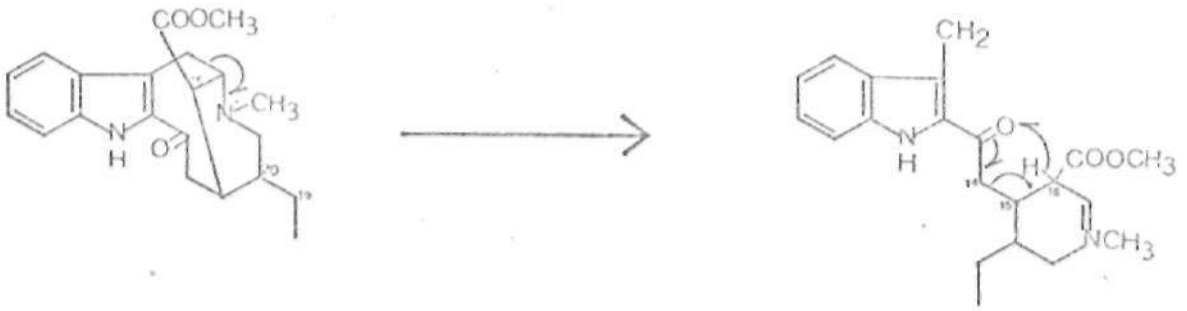
2-Acyl-indole alkaloids

The typical fragmentation pattern of this group of alkaloids is exhibited by tabernaemontanine (Fig. 29) (Combes et al, 1966). The 2-acyl-indoles undergo an initial rupture of the bond connecting the indole moiety with the N_b nitrogen atom. The resulting species q then undergoes β-cleavage with transfer of the γ-hydrogen atom to give the ion r as shown in Fig. 29. The most intense peak in the spectrum occurs at m/e 182 and is attributed to species r. In 19-20-dehydro analogues this peak occurs at m/e 180 which corresponds to structure (a) (Fig. 30). Substituents other than the $-COOCH_3$ group at C-16 show a corresponding shift of this intense peak. Thus in vobasinic acid (C-16, COOH) the peak occurs at m/e 166 (structure (b); Fig. 30) and in affinine (C-16, CH_2OH), it occurs at m/e 152 (structure (c); Fig. 30) (Noranjo et al, 1972).

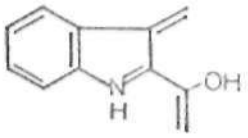
Another significant peak in the spectrum of tabernaemontanine occurs at m/e 196. It has been rationalised in terms of structure p,

Fig. 29. MS of Tabernaemontanine

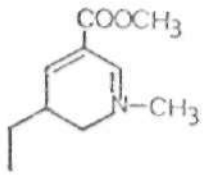




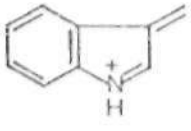
(9)



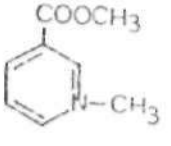
m/e 172



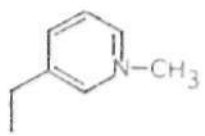
(7) m/e 182



m/e 130

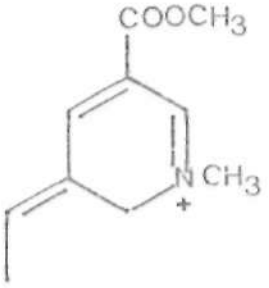


m/e 152



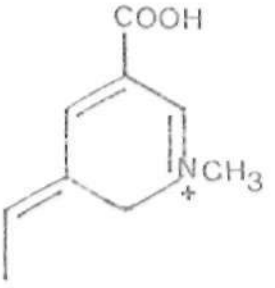
m/e 122

Fig. 30.



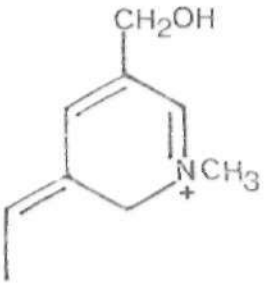
(a)

m/e 180



(b)

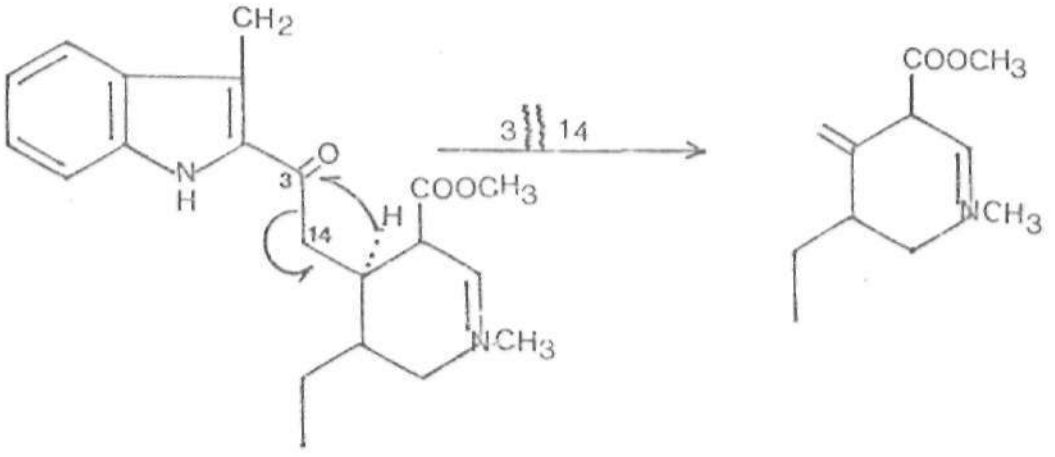
m/e 166



(c)

m/e 152

the formation of which can be visualised as cleavage of the C-3, C-14 bond in species q (Fig. 29) with the transfer of one hydrogen atom as shown below (Combes et al, 1966)



q

m/e 196

2-acyl indole alkaloids of the picraphylline type show similar spectra to the tabernaemontanine/vobasine type but the indolic peaks are more pronounced (Levy et al, 1964). The fragmentation pattern leading to significant peaks in picraphylline is shown in Scheme XI (Hesse, 1974).

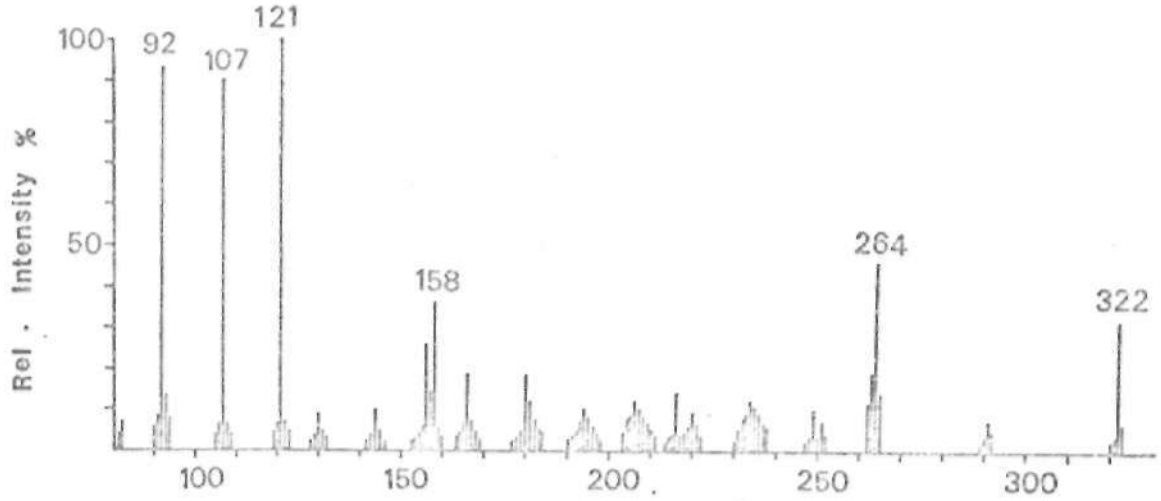
Akuammicine and related compounds

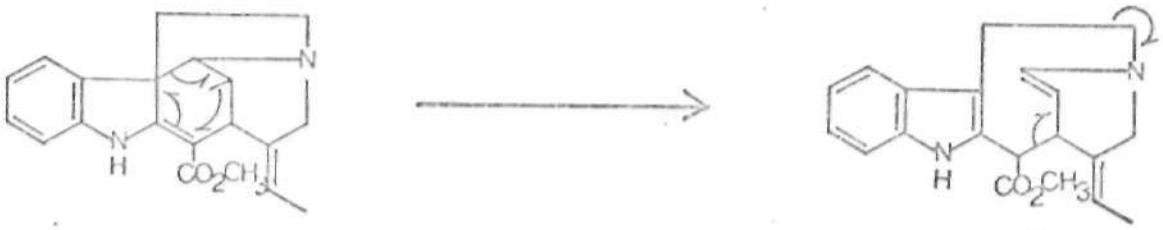
The mass spectrum of akuammicine is considerably more complicated and more difficult to interpret than the mass spectra of the β-carboline compounds which indicates that several fragmentation processes occur. This in turn presumably indicates that no bonds are especially liable to cleavage or that none of the possible modes of cleavage on electron impact are energetically favourable to any marked extent. The general pattern is defined by the appearance of strong peaks in the lower mass range; particularly diagnostic in the spectrum of akuammicine are the peaks at m/e 121, 107 and 92 (Fig. 31). These peaks have been shown by deuterium labelling experiments not to encompass the indole system (Budzikiewicz et al, 1964). The indole peaks in the akuammicine spectrum are usually of low intensity. Another clearly recognisable peak, apart from the molecular peak, is the M-59 peak which corresponds to loss of the $-COOCH_3$ functional group.

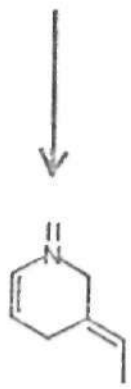
Mass Spectrometry - Direct Inlet Silylation (MS-DIS)

MS-DIS is a useful technique for the qualitative detection of active hydrogen atoms such as OH, NH and COOH by mass spectrometry in

Fig. 31. MS of Akuammicine







m/e 121

m/e 107

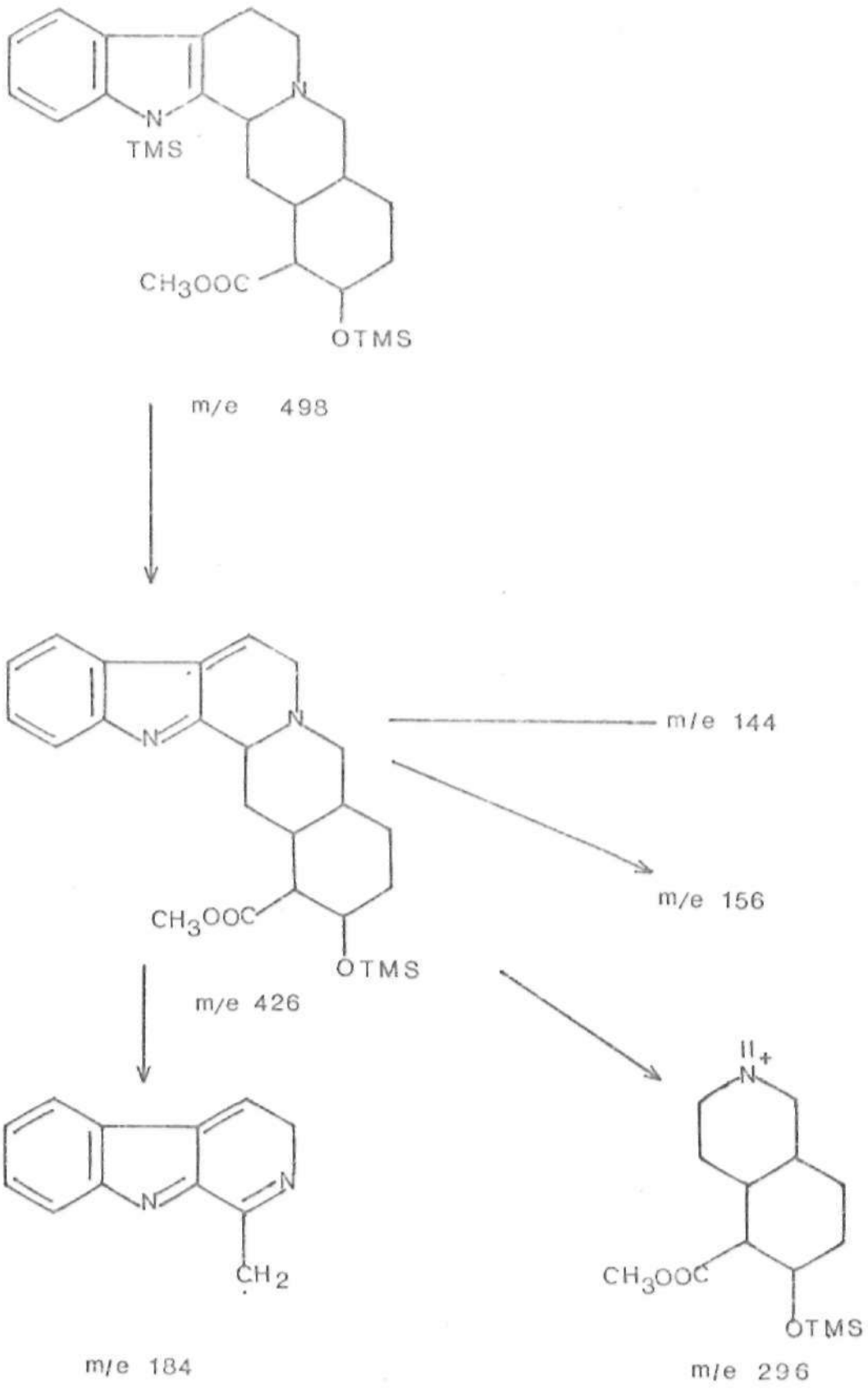
m/e 92

Rauwolfia alkaloids (Iwu, 1977). The alkaloids are treated with N, O-bis-trimethylsilyl (TMS) - trifluoro-cetamide (without prior purification) and the active hydrogen atoms in the molecule are replaced by TMS units.

In each case where a TMS unit is accepted, there is an increase of 72 mass units on the molecular ion and peaks associated with the active hydrogen atom show a corresponding shift of 72 mass units. For example, ajmaline (Fig. 26) possesses two active hydrogen atoms, the OH groups at C-17 and C-21 positions. The spectrum of the silylation product showed a molecular peak ion at m/e 470 (326 + 144) and the peak at m/e 200 (Fig. 26) occurred at m/e 272.

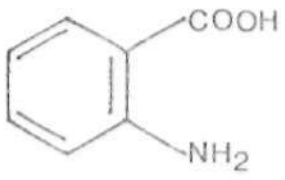
Studies by the same author on yohimbine, α -yohimbine and ψ -yohimbine revealed that stereochemical assignment by mass spectrometry is possible for the yohimbine group of alkaloids if the silylated spectrum is used. The peaks at m/e 184, 170, 169 and 156 varied according to stereochemical groups and agreed with the observations on the heteroyohimbines (Djerassi, 1963). Scheme XII shows the probable fragmentation pattern of yohimbine - TMS (Iwu, 1977).

SCHEME XII MS Fragmentation pattern of Yohimbine TMS

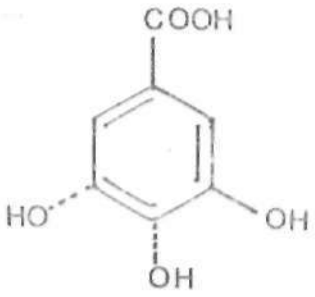


BIOSYNTHESIS OF INDOLE ALKALOIDS WITH PARTICULAR REFERENCE TO THE *RAUWOLFIA* ALKALOIDS

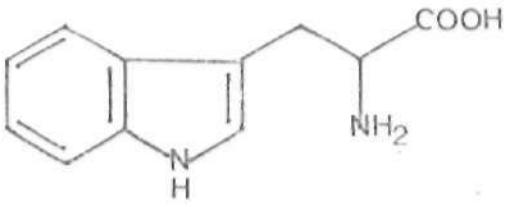
Robinson (1919) originally suggested that the two nitrogen atoms and the aromatic portion of all the then known indole alkaloids originated from tryptophan via its decarboxylation product tryptamine. More recent experiments with labelled tryptophan performed on a number of indole alkaloid-producing plants, including *R. serpentina*, have confirmed this fact (Leete *et al*, 1960-65; Groger *et al*, 1964; Kutney *et al*, 1968). Leete (1960, 1961) demonstrated the incorporation of radioactively labelled tryptophan into ajmalicine, serpentine and reserpine in *R. serpentina* plant.



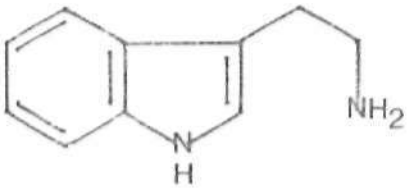
(XX) Anthranilic Acid



(XXI) Shikimic Acid

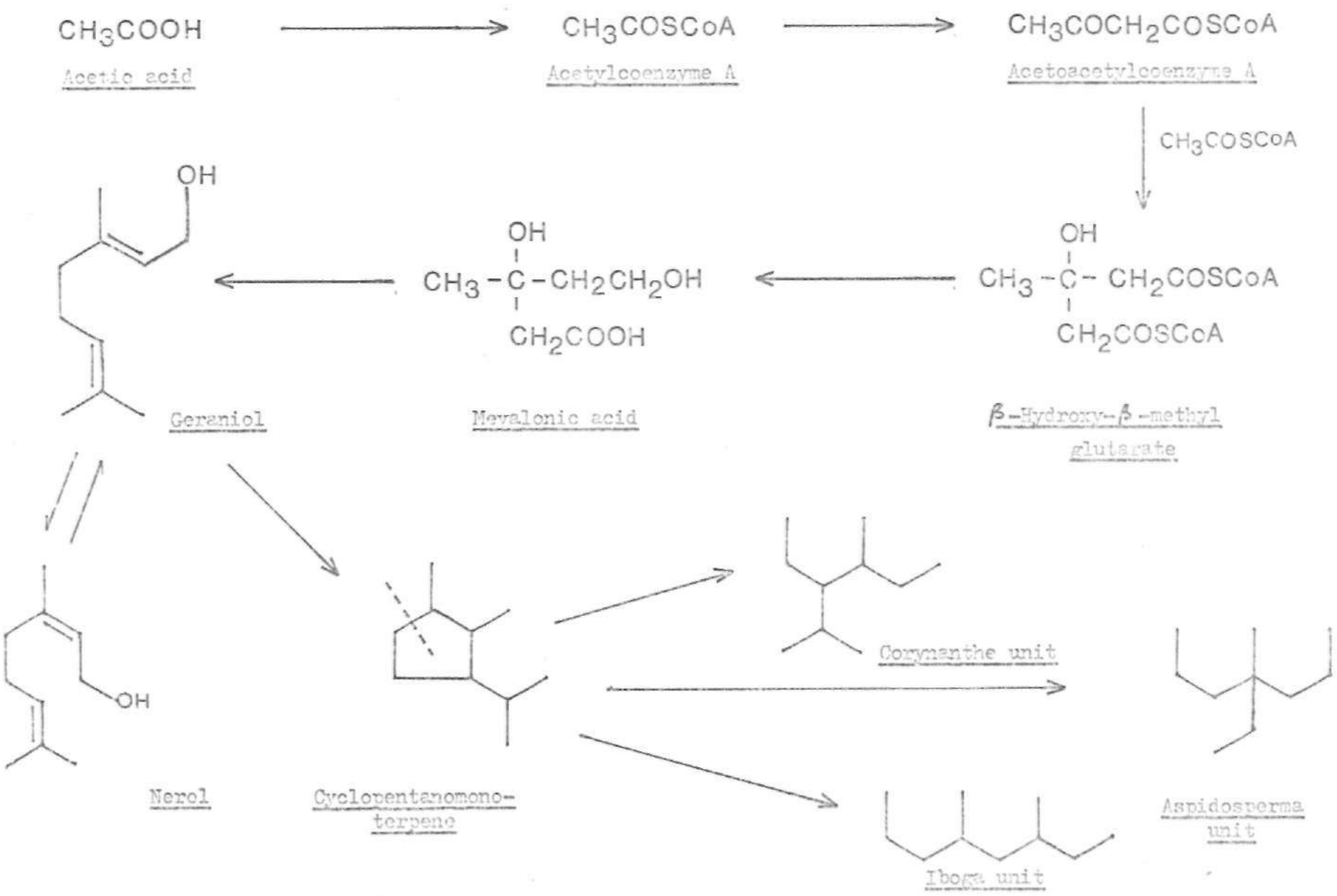


(XXII) Tryptophan



(XXIII) Tryptamine

SCHEME XIII Schematic derivation of various 10-carbon units from a monoterpene



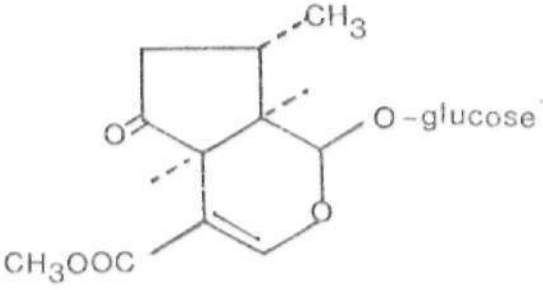
Other biogenetic precursors of tryptophan, such as anthranilic acid (XX) and shikimic acid (XXI), have also been shown to be incorporated into indole alkaloids in Vinca and Catharanthus species (Stolle et al, 1965; Groger et al, 1966; Bjothe Harvath et al, 1969).

The non-tryptamine derived part of the indole alkaloids contains a 9 or 10 carbon unit chain and it was suggested independently by Thomas (1961) and Wenkert (1962) that this chain was of monoterpenoid origin. The hypothesis suggested a route from acetate via mevalonate and geraniol to a cyclopentanomonoterpene as shown in Scheme XIII.

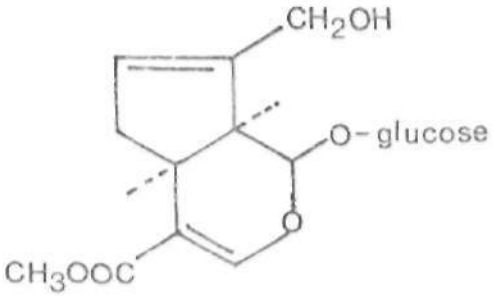
Experimental evidence has established the sequence from the mevalonate stage to the cyclopentanomonoterpene stage. Labelled acetate, however, gave rise to uniformly labelled alkaloids : thus ajmaline in R. serpentina was uniformly labelled (Leete et al, 1965; Battersby et al, 1963; Goeggel et al, 1965). This suggested that an alternative route probably produced the mevalonate.

Feeding experiments with labelled mevalonate and geraniol produced the expected pattern of specific labelling in several indole alkaloids. For example, ajmalicine, serpentine, strychnine, catharanthine, vindoline and pervine were specifically labelled (Cordell, 1974).

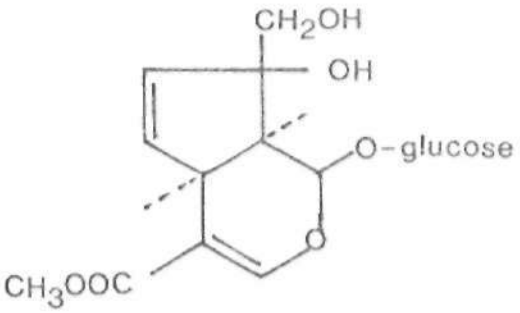
Considerable attention was therefore focussed on the role of some natural cyclopentanomonoterpenes. Labelled verbenalin, genepin, monotropeine methyl ester and loganin were fed into plants. Only loganin was found to be incorporated in significant quantity into several alkaloids including ajmaline in R. serpentina (Battersby et al, 1968).



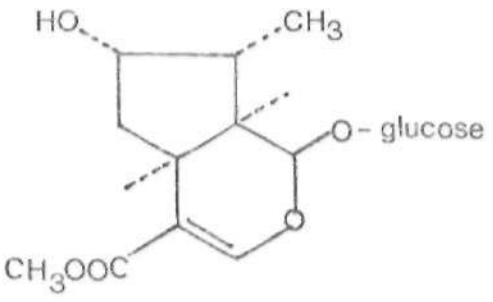
Verbenalin



Genepin



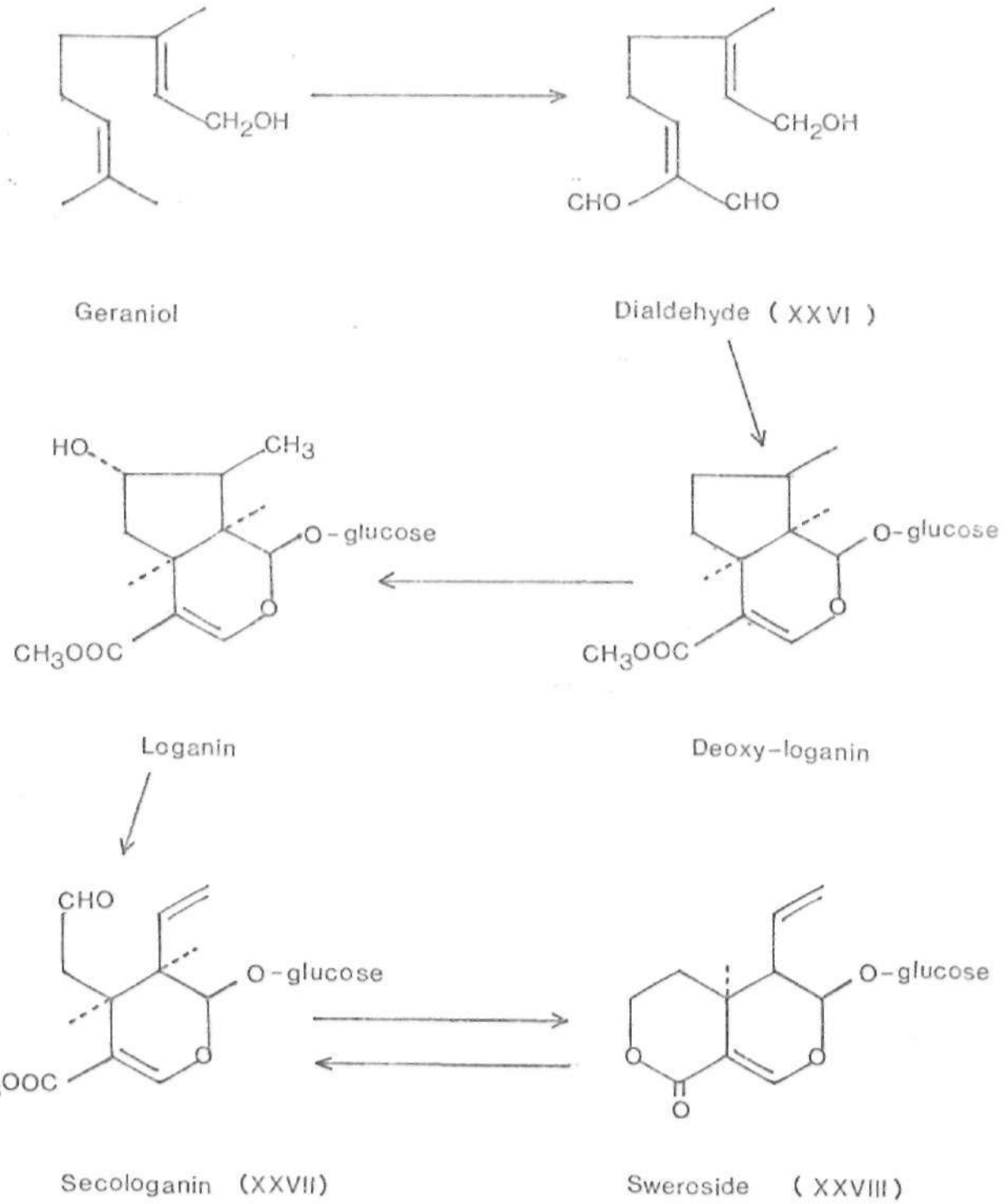
Monotropeine methyl ester



Loganin

Loganin was therefore considered as the probable cyclopentanomonoterpene precursor. The terpenoid origin of loganin was established by biosynthetic studies. Labelled mevalonate and geraniol were found to incorporate into loganin and loganic acid in Catharanthus and Swertia species (Cordell, 1974).

More significantly, loganin has been found to be a good precursor for all three skeletal classes of indole alkaloids. Radio-actively labelled loganin was incorporated into ajmalicine, serpentine, ajmaline, catharanthine and vindoline (Cordell, 1974). Loganin has also been isolated from a few indole alkaloid-producing plants (Battersby, 1971a).


CH2OH
CH2OH
CHO
CHO
Geraniol
Dialdehyde (XXVI)
HO
CH3
O-glucose
CH3OOC
O-glucose
CH3OOC
Loganin
Deoxy-loganin
CHO
O-glucose
CH3OOC
O-glucose
O
O
Secologanin (XXVII)
Sweroside (XXVIII)

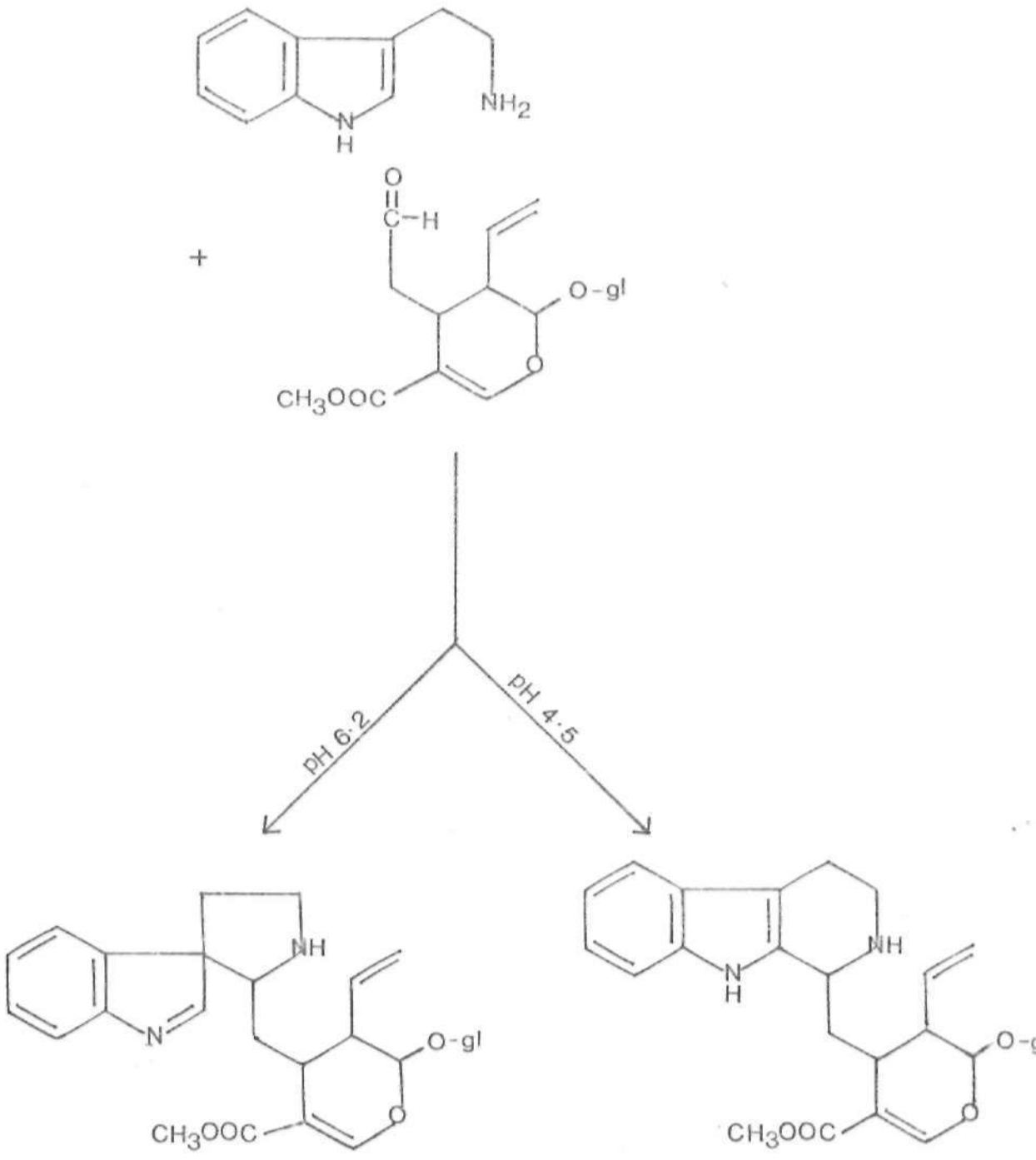
Scheme XIV

Concerning the biosynthetic stages between geraniol and loganin, a dialdehyde of the structure (XXVI) (Scheme XIV) is considered to be an important intermediate. The theory that hydroxylation at C-7 of the loganin molecule was a late stage in the biosynthesis received experimental confirmation (Inouye et al, 1969; 1972).

It is now believed that loganin is converted to seco-loganin (XXVII) and that the initial stage of biosynthesis of indole alkaloids involves the condensation of tryptamine or tryptophan with seco-loganin. The actual mode of condensation, although always assumed, has now been validated experimentally by Battersby et al (1969a). The reaction as demonstrated by in vitro experiments resulted, depending on the pH condition of the reaction mixture, in the formation of two epimeric β-carboline alkaloids, vincoside and isovincoside, and the appropriate spiro-indolenine derivative (Scheme XV).

Independently, and almost simultaneously, strictosidine was isolated from a natural source (Rhazya stricta Decaisne, family Apocynaceae), and, by direct comparison, it was identified as isovincoside (Smith, 1968; Battersby, 1971a).

Vincoside was originally assumed to possess a C-3H α configuration but subsequently this was reversed to C-3H β whilst the epimer, strictosidine or isovincoside, was shown to possess the C-3H α configuration (Battersby et al, 1969a; 1971b; De Silva et al, 1971; Blackstock et al, 1971). This revision has been confirmed by X-ray diffraction studies (Mattes et al, 1975).



Spiroindolenine

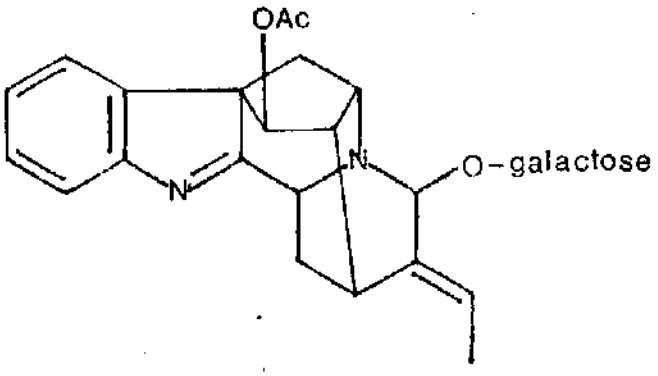
C−3Hα Strictosidine
or Isovincoside

C−3Hβ Vincoside

Scheme XV

As a result of feeding experiments which demonstrated the exclusive incorporation of labelled vincoside into all three classes of indole alkaloids (Corynanthe, Aspidosperma and Iboga) in Vinca rosea L., but not isovincoside, a precursor role was adduced to vincoside (Battersby, 1969). The recent works of Zenk (1977) and Brown et al (1978) have shown that it is strictosidine (isovincoside) which correctly possesses the C-3H α configuration that is incorporated into the indole alkaloids of Vinca rosea, and not vincoside with C-3H β as earlier indicated by Battersby (1969).

The incorporation studies on vincoside and isovincoside by Battersby (1969), Zenk (1977) and Brown et al (1978) were undertaken using Vinca species. No such work has yet been performed on Rauwolfia species but the incorporation of loganin into ajmaline in R. serpentina (Battersby, 1968) and the isolation of glycosidal alkaloids such as raucaffricine from R. caffra (Khan, 1970) indicates that a similar biogenetic process probably obtains in Rauwolfia species. Nevertheless, neither vincoside nor isovincoside has been isolated as yet from any Rauwolfia species. Also whether loganin is an obligatory precursor in all indole alkaloid-producing plant still needs to be confirmed.



Raucaffricine

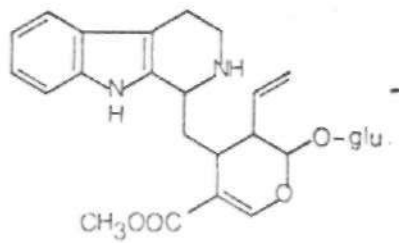
The process of biosynthetic transformation from strictosidine or vincoside to the various indole alkaloid types is still speculative in spite of the numerous experimental works on indole alkaloid biosynthesis.

Some of the experimental techniques employed included isolation of minor biosynthetic intermediates, tissue culture and isotope labelling, tracer work and dilution analysis. Based on these researches many biogenetic routes have been proposed to account for the origin of indole alkaloids (Battersby et al, 1966; Kompis et al, 1971; Cordell, 1974; Timmins and Court, 1975).

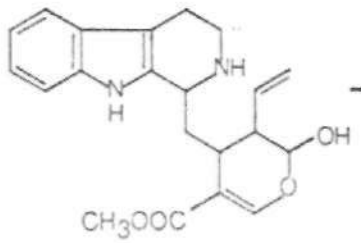
More recently Zenk and his co-workers (1977; 1978), performing tissue culture experiments, have been able to implicate two compounds, 4, 21-dehydrocorynantheine aldehyde (XXIII) and cathenamine (XXIV) (Scheme XVI) as key intermediates in the biosynthetic transformation of strictosidine to ajmalicine and some E-seco indole alkaloids. The transformation involved an initial enzymatic cleavage of glucose from strictosidine (XXIX) to yield an unstable aglycone (XXXI) which in turn produced the dialdehyde (XXXII) and subsequently formed the intermediate 4, 21-dehydrocorynantheine aldehyde (XXXIII).

The authors also demonstrated that, under certain enzymatic conditions, compound (XXXIII) would yield ajmaline, 19-epi-ajmaline and tetrahydroalstonine via the intermediate cathenamine (XXXIV). Under different enzymatic conditions compound (XXXIII) produced the E-seco indole alkaloids, sitsirikine and 16-epi-sitsirikine, as shown in Scheme XVI.

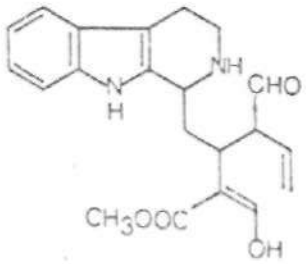
Scheme XVI



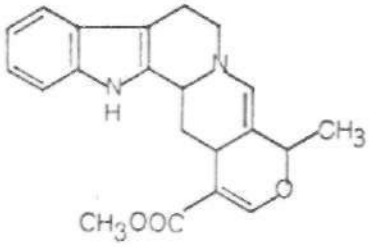
XXIX Strictosidine



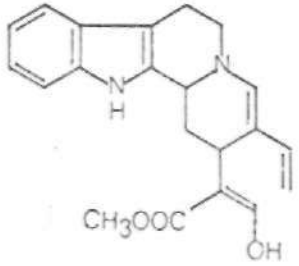
XXXI Aglycone



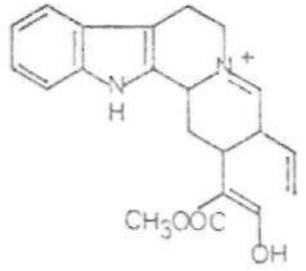
XXXII Aldehyde



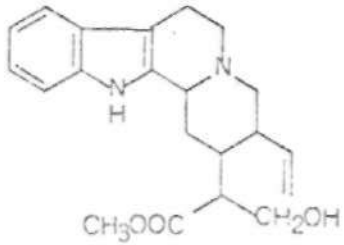
XXXV Cathenamine



XXXIV



XXXIII



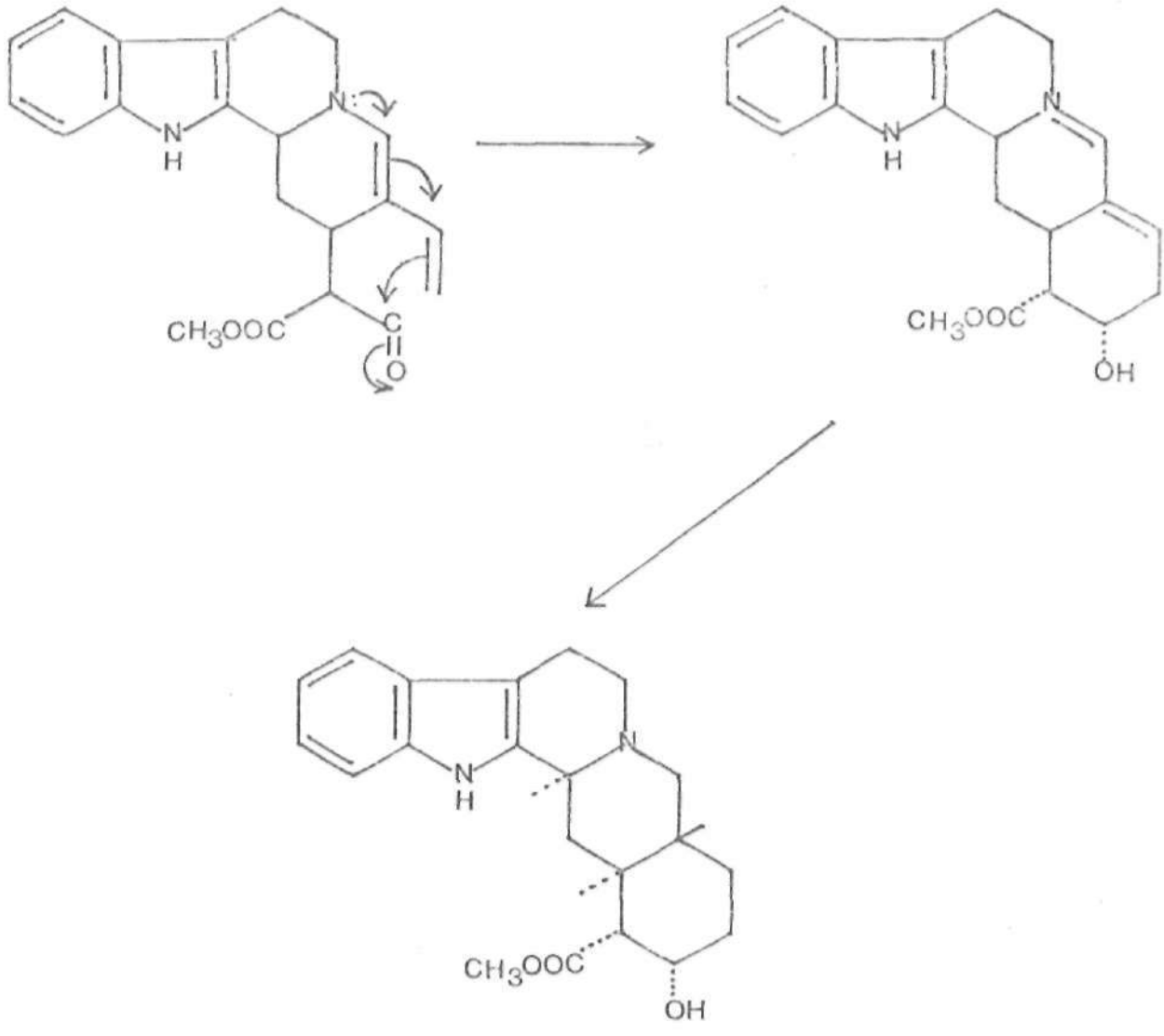
Sitsirikine
&

Ajmalicine

19-Epi-ajmalicine

Tetrahydroalstonine

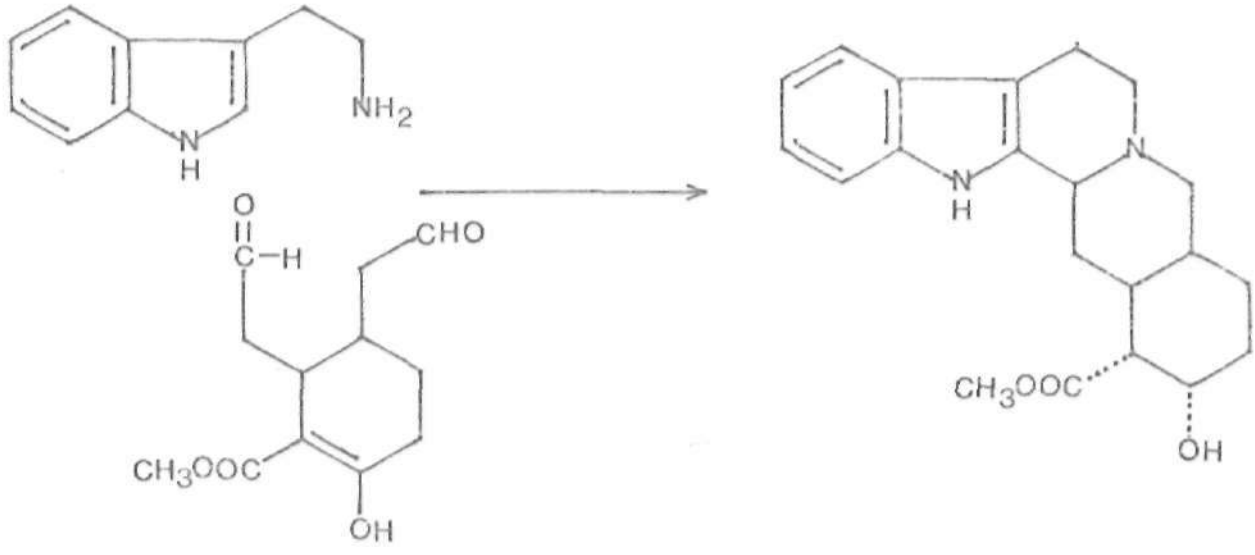
No biosynthetic studies have so far been made on the yohimbine group of alkaloids but it was suggested that their biosynthesis probably involves a C-C cyclization of an intermediate E-seco indole alkaloid as shown in Scheme XVII (Cordell, 1974).



Yohimbine

Scheme XVII

Earlier Inouye (1968) had suggested an alternative route involving the condensation of tryptamine with a seco-iridoid of the type compound (XXXVI) (Scheme XVIII).

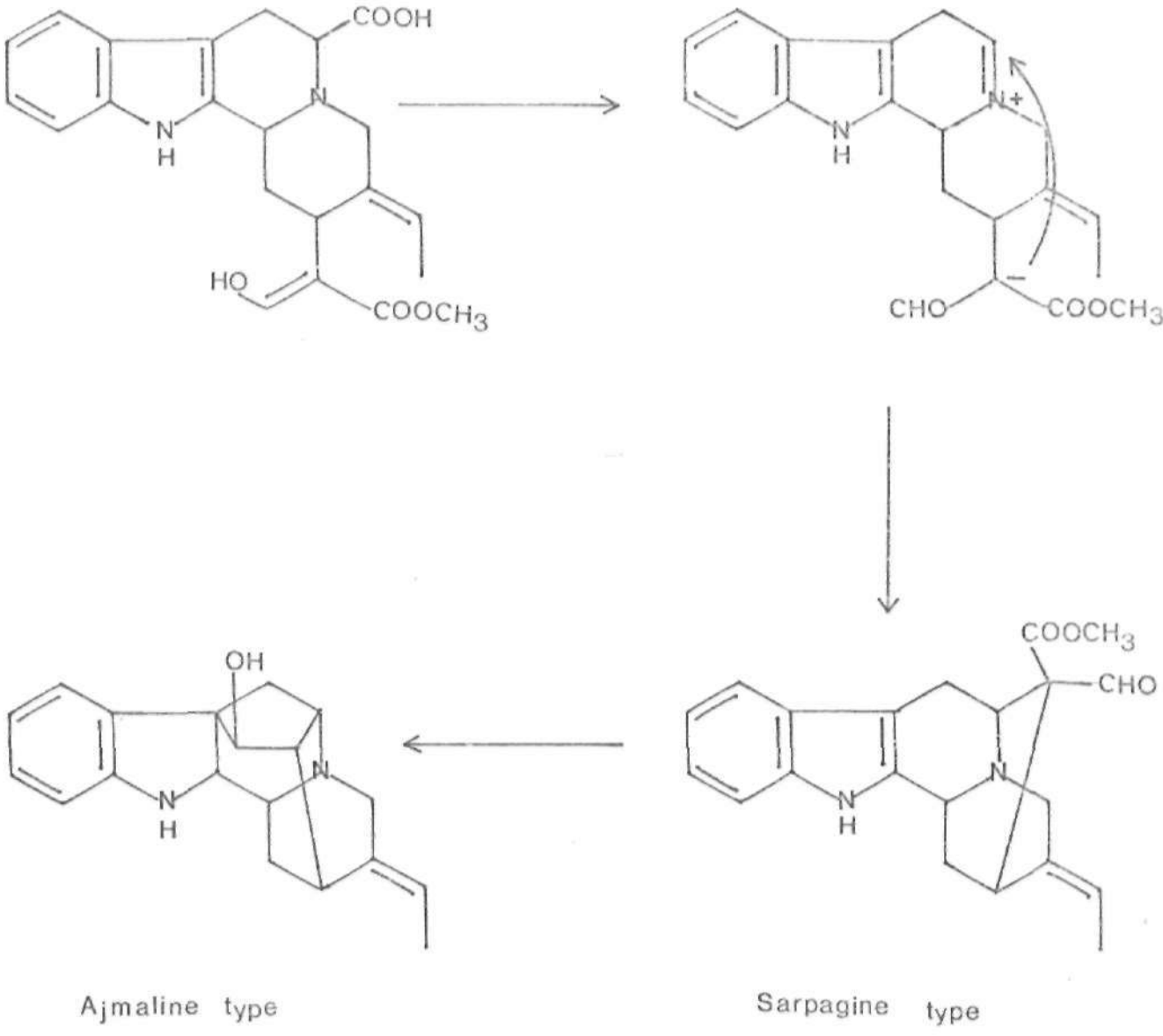


(XXXVI)

Scheme XVIII

Van Tamelen (1968) proposed that alkaloids of the sarpagan-ajmaline group are formed by the reaction of the C-16 carbon atom of the E-seco indole alkaloids on an electrophilic imine at C-5, which was formed by oxidative decarboxylation of 5-carboxy-geissoschizine type compounds. Thus alkaloids of the adirubine type, could be possible precursors of the sarpagan-ajmaline group (Scheme XIX).

There seems to be little doubt about the biosynthetic steps leading to the ajmaline skeleton since isolated alkaloids such as normacusine B, vellosimine, polyneuridine and vomilenine can be regarded as trapped intermediates (Taylor, 1965).



Ajmaline type

Sarpagine type

Scheme XIX

The co-occurrence of ajmaline, nor-ajmaline, tetraphyllicine and nor-tetraphyllicine in the same plant may be related to the biogenesis of ajmaline. The N_a-methyl group in ajmaline was shown to be derivable from methionine and the C-21 hydroxylation may be a late stage in the biosynthesis of ajmaline as 21-deoxy-ajmaline has been shown to be a good precursor of ajmaline in R. serpentina (Barton et al, 1965).

The more recent works of Timmins and Court (1975), Iwu and Court (1977) and Sabri and Court (1978) which demonstrated that the nor-bases such as nor-ajmaline, nor-tetraphyllicine and endolobine occurred mainly in the aerial parts of the plants whilst the N_a-methyl analogues occurred in the lower parts show that the nor-bases are the probable precursors of the N_a-methyl analogues.

It is, however, not certain at what stage of biosynthesis the methoxylation of heteroyohimbine and sarpagine-ajmaline compounds takes place, but the isolation of hydroxy-geissoschizol and methoxy-geissoschizol from Rauwolfia species (Timmins and Court, 1975; Sabri and Court, 1978) suggests that this may be at the E-seco indole alkaloid stage.

There is therefore a need for thorough and careful analysis of the individual plant members in order to demonstrate the alkaloidal pattern in the plant and to trap the more important biogenetic intermediates. With such data it may be possible to piece together the biogenetic routes and to explain the occurrence of the wide range of indole alkaloids in the Rauwolfia species.

SCOPE OF THE PRESENT WORK

Scope of the present work

The section Ophioxylanthus of Pichon's classification of the genus Rauwolfia comprises two species viz. R. volkensii and R. oreogiton. Plants of the two species have been investigated in order to compare the distribution of the alkaloids in the species. The information obtained has also been used to relate the section Ophioxylanthus to the other African sections of Pichon's classification.

As no work had previously been reported concerning R. volkensii, the alkaloid distribution in the root, stem and leaves of this plant was investigated and the inter-relationship of the alkaloids within the plant is discussed.

The root alkaloids of R. oreogiton were studied previously by Court (1964; 1968) under the name R. volkensii and by Timmins and Court (1974). Earlier investigations indicated the presence of ajmalicine, reserpine, rescinnamine, renoxydine, reserpiline, α-yohimbine, an unidentified yohimbine isomer, ajmaline and serpentine. The root alkaloids were re-investigated and the total root extract compared chromatographically with that of R. volkensii roots.

The leaf alkaloids were investigated and the results are compared with those for R. volkensii leaves.

E X P E R I M E N T A L

Plant Material

R. volkensii Stapf.

The root, stem and leaf samples used were collected at the same time and from the same plants at the Kindoroko Forest Reserve, Same District, Kilimanjaro Region, Tanzania in January 1974.

Voucher specimens of the materials are lodged with Pharmaceutical Society of Great Britain Collection of Material Medica and Harbaria, University of Bradford under the reference numbers RAU 111-741, 111-742 and 111-743.

R. oreogiton Markgraf.

The root samples were collected by the Forestry Department, Lushoto, Tanzania in 1959 and 1960 under collectors' numbers Semsei 2827 and Semsei 2948. The identity of the samples was confirmed at the East African Herbarium, Nairobi, Kenya. Voucher specimens are lodged with Pharmaceutical Society Collection of Material Medica and Harbaria, University of Bradford under the reference numbers RAU 106-591 and RAU 102-601.

The leaf samples were collected in April 1970 in the Mkussu Forest Reserve, West Usambaras, Tanzania. Voucher specimens, reference number RAU 106-701, are also lodged in the University of Bradford.

Extraction and Fractionation

The general extraction procedure entailed defatting the powdered material in sufficient quantities of petroleum spirit (b.p. 40-60°C) and extracting the dried marc consecutively with methanol, methanol-ammonia (2% concentrated ammonia in methanol) and 1% sodium hydroxide solution in methanol. Overnight maceration was employed in each extraction procedure.

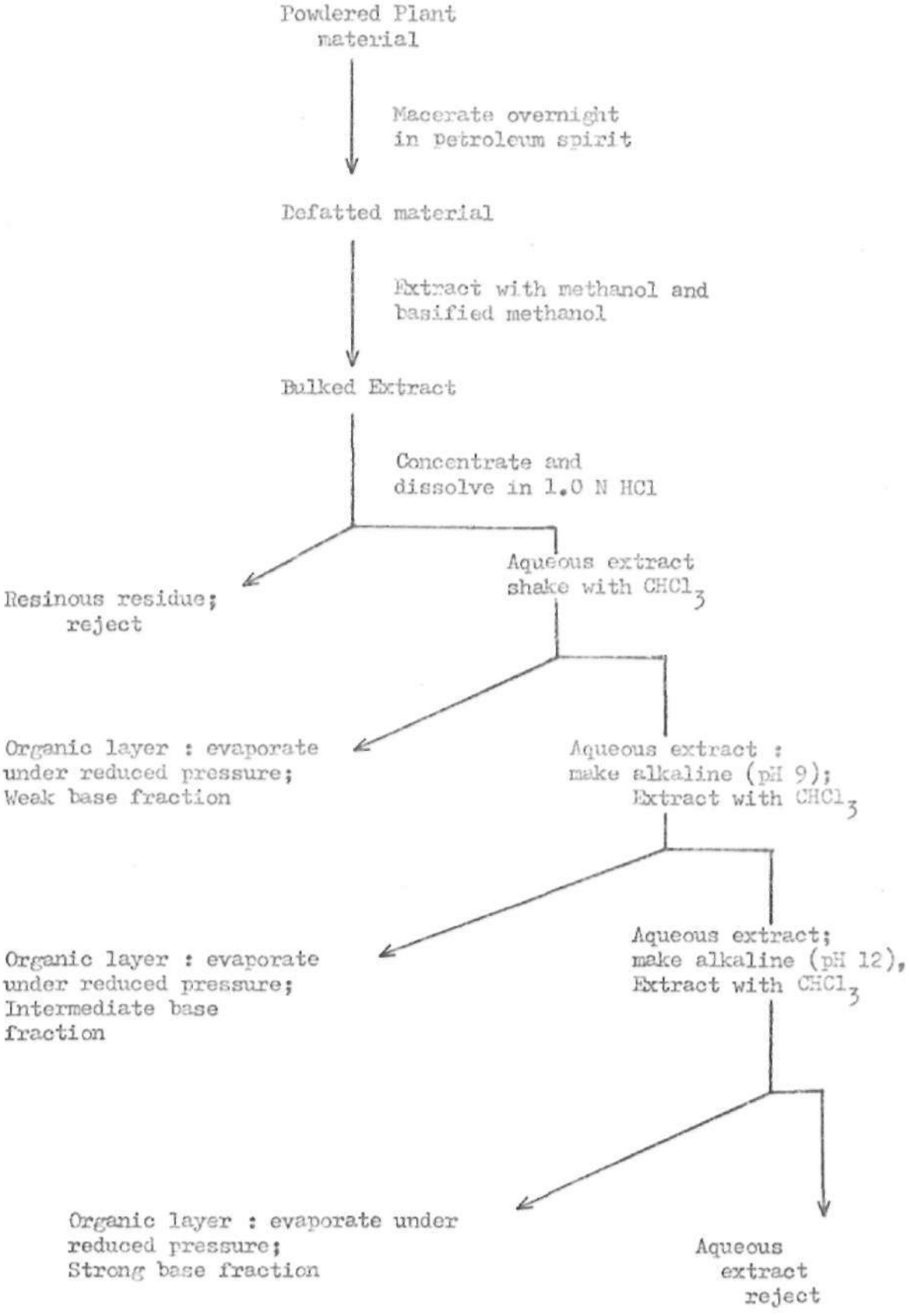
Adequate precautions were taken throughout the extraction procedure to avoid contact of the alkaloids with strong acids or alkali or excessive heat. The alcoholic extracts were freed of the solvent in vacuo and the residue obtained dissolved in 1.0 N hydrochloric acid. The insoluble resinous matter was removed by filtration and the aqueous layer fractionated into weak, intermediate and strong base fractions as described in the flow diagram based on Court (1958).

The weak, intermediate and strong base fractions were then separately passed through a preparative chromatographic column and the freed alkaloids separated and purified by thin layer chromatography.

Analytical Methods

1. Column Chromatography

Technique	:	Gradient elution.
Column	:	Glass column, 50 cm length by 3.5 cm internal diameter.
Adsorbent	:	Aluminium Oxide Active (neutral Brockmann grade 1) for chromatographic analysis (BDH Chemical Ltd., England). The column was packed by the slurry method and eluates were collected manually.
Solvents	:	For details see text.


Powdered Plant material
Macerate overnight in petroleum spirit
Defatted material
Extract with methanol and basified methanol
Bulked Extract
Concentrate and dissolve in 1.0 N HCl
Resinous residue; reject
Aqueous extract shake with $CHCl_3$
Organic layer : evaporate under reduced pressure; Weak base fraction
Aqueous extract : make alkaline (pH 9); Extract with $CHCl_3$
Organic layer : evaporate under reduced pressure; Intermediate base fraction
Aqueous extract; make alkaline (pH 12), Extract with $CHCl_3$
Organic layer : evaporate under reduced pressure; Strong base fraction
Aqueous extract reject

2. Thin-layer Chromatography (TLC)

Technique : Ascending; development distance 15 cm.

Adsorbents : Silica gel GF_{254} (Merck, Darmstadt, West Germany) or Silica gel Type 60 with added fluorescent indicator F_{254}. Thin-layer plates (20 x 20 cm) with layers 250 μm thick were prepared using a BTL motorised TLC coater. The plates were activated at 110°C for 2 hrs. and stored in a desiccator. Activated plates were used within 72 hrs. of preparation.

Development : Spots or streaks were applied to the baselines manually using a fine capillary tube and the chromatograms were developed at room temperature (20 - 22°C) using Shandon Chromato-tanks (22 x 24 x 9 cm) (Shandon, London).

For qualitative work the tanks were lined with Whatman No. 1 filter paper and the tank atmospheres were allowed to saturate for 30 minutes. Where an ammonia atmosphere was required a small beaker containing 5 mls. ammonia (0.880) was placed centrally in the tank.

Visualisation : Developed plates were usually air dried but, if relatively non-volatile solvent systems were used, plates were dried in an oven at 100°C for 10 minutes before visualisation. Methods of visualisation were:-

(a) Ultraviolet light at wavelength 254 nm was employed to detect fluorescent quenching spots or bands and at wavelength 366 nm to detect fluorescent spots.

(b) Detecting reagents:-

(i) Ferric chloride - perchloric acid reagent:-

5% ferric chloride in 35% v/v aqueous perchloric acid.

(ii) Ceric sulphate reagent:-

1% ceric sulphate in 10% aqueous sulphuric acid solution

(iii) Dragendorff's reagent:-

Solution I

Bismuth submitrate	0.85 G.
Glacial acetic acid	10 mls.
Water	40 mls.

Solution II

Potassium iodide	8.0 G.
Distilled water to	20 mls.

Solutions I and II were mixed to make the stock solution ;

10 mls. of stock solution was diluted with 20 mls. of glacial acetic acid and the volume adjusted to 100 mls. with water to prepare the spray reagent.

3. Elution from PLC

The presence of alkaloid spots on the chromatograms was detected by fluorescent quenching in ultraviolet light or by spraying the edges of the plates with a suitable alkaloid detecting reagent. The appropriate bands were removed using a small spatula and eluted by standing in methanol

with intermittent agitation for three hours. The resultant solutions were filtered through sintered glass and the filtrates on evaporation to dryness under reduced pressure provided the respective alkaloids as amorphous residues. Wherever quantities permitted the alkaloids were further purified by crystallisation from suitable solvents.

4. Ultraviolet spectroscopy

The UV spectra of the isolated alkaloids were recorded using methanol (Analar grade) as solvent and a Unicam S.P. 800 spectrophotometer. If the alkaloids were eluted directly from the adsorbent, blank solutions were prepared from equivalent weights of silica gel obtained from chromatoplates which had been similarly treated but without the test alkaloids (Harris et al, 1968).

5. Infrared spectroscopy

The infrared spectra were recorded using a Unicam S.P. 200 spectrophotometer or a Perkin-Elmer infrared spectrophotometer 297 with a beam condensing unit and attenuator to compensate the reference beam. Samples were prepared in potassium bromide microdiscs or in some cases where quantities permitted in chloroform solution.

6. Nuclear Magnetic Resonance Spectrometry

The NMR spectra were recorded using a JEOL NMR instrument, model JNM-MH-100 (100 mHz). Alkaloid samples were prepared in deuterochloroform solutions and tetramethyl-silane (TMS) was employed as the internal standard.

7. Mass spectrometry

Mass spectra were obtained using an A.E.I., MS 902 spectrometer. Samples were introduced on a direct insertion probe and spectra were recorded under the following conditions:-

Acceleration voltage	8 kv
Electron beam	70 eV
Emission	100 uA
Source temperature	250°C

8. Melting point apparatus

Melting points were determined using a Kofler hot stage microscope. For mixed melting points a Buchi melting point apparatus was employed.

9. Reference Alkaloids

Ajmalicine, ajmaline, reserpine, rescinnamine and serpentine hydrogen tartrate were obtained commercially from Koch-Light Ltd. (Colnbrook, England).

Yohimbine hydrochloride, alpha-yohimbine, pseudo-yohimbine, tetraphyllicine and reserpiline oxalate were obtained commercially from K and K Laboratories (Plainview, New York, U.S.A.).

Aricine was kindly donated by S. B. Penick and Co. (New York and Chicago, U.S.A.).

Tetrahydroalstonine samples were kindly donated by Dr. P. W. Le Quesne and Professor R. C. Elderfield (University of Michigan, Michigan, U.S.A.). Sarpagine was obtained as a gift from Dr. A. Bonati (Inverni-Della, Baffa, Milano, Italy). Quaternine was kindly supplied by Dr. Claude Thal (Institut de Chimie des Substances Naturelles, C.N.R.S. 91190 Gif-sur-Yvette, France) and peraksine was kindly given by Dr. J. L. Pousset, Paris.

10. Developing solvents

(i) Xylene : iso-octane : ethyl acetate : diethyl ether (3 : 9 : 1 : 2);

(ii) n - Butanol : ethyl acetate : ethylene dichloride (1 : 3 : 6);

(iii) Acetone : petroleum ether (b.p. 40-60°C) : carbon tetrachloride : iso-octane (7 : 6 : 4 : 3);

(iv) Methanol : methylethylketone : n-heptane (8 : 4.33 : 6.58);

(v) Acetone : petroleum ether (b.p. 40-60°C) : glacial acetic acid (9 : 9 : 2);

(vi) Acetone : methanol : glacial acetic acid (14 : 5 : 1);

(vii) Acetone : n-butanol : iso-octane (33.6 : 8.4 : 5.8);

(viii) Ether : methanol (9 : 1);

(ix) Acetone : petroleum ether : diethylamine (7 : 2 : 1);

(x) Chloroform : petroleum ether : methanol (8 : 1 : 1);

(xi) Acetone : methanol : diethylamine (7 : 2 : 1);

(xii) Chloroform : methanol (3 : 1);

(xiii) Ethyl acetate : hexane : carbon tetrachloride : butanol (4 : 3 : 2 : 1);

(xiv) Tert-butanol : acetic acid : water (4 : 1 : 5);

(xv) Ethanol : ethyl acetate : dimethylformamide : diethylamine (12 : 6 : 1 : 1);

(xvi) Chloroform : methanol (saturated with ammonia) (99 : 1);

(xvii) Heptane : methyl ethylketone (5 : 4);

(xviii) Chloroform : acetone (5 : 4);

(xix) Ether : ethanol (19 : 1);

(xx) Ethyl acetate : iso-propanol : conc. ammonia (16 : 3 : 1);

(xxi) Ether : ethyl acetate (1 : 1);

(xxii) Ether : methanol (1 : 1);

(xxiii) Ethyl acetate : methanol : acetone (7 : 2 : 1).

EXTRACTION AND SEPARATION

Rauwolfia volkensii

1. Root bark and wood
2. Stem bark
3. Leaves

Rauwolfia oreogiton

4. Root bark and wood and comparison with *R. volkensii* root
5. Leaves

1. R. volkensii root

5 kg powdered root wood and bark was macerated overnight in 15 litres of petroleum spirit (b.p. 40-60°C). After removal of solvent, the marc was air dried at room temperature.

The dried powder was extracted with 15 litres of methanol employing overnight maceration with occasional manual agitation and stirring. The solvent was removed by filtration and the marc re-extracted in the same manner using 15 litres of methanol-ammonia (2% concentrated ammonia in methanol). Two further successive extractions were carried out with fresh solvent and the extracts were recovered by filtration.

The filtered extracts were bulked and evaporated to dryness under reduced pressure. The resultant dry extract was then shaken with 500 ml 1.0 N hydrochloric acid and residual insoluble matter removed by filtration.

Extraction of the aqueous filtrate with chloroform (6 x 150 ml) and evaporation of the separated and bulked chloroform extracts under reduced pressure yielded the weak base fraction (8.5 g).

The aqueous phase was made alkaline with dilute ammonia (pH about 9) and extracted with chloroform (6 x 150 ml). Removal of the separated and bulked chloroform layers under reduced pressure produced the intermediate base fraction (2.2 g).

The aqueous phase was rendered more alkaline with 5% sodium hydroxide solution (pH 12) and similarly extracted with chloroform (6 x 150 ml) to yield the strong base fraction (0.8 g).

The alkaloidal extract of the weak base fraction was further fractionated by column chromatography. The extract was introduced to the top of the well-packed column as a slurry, with filter paper and anti-bumping granules separating the adsorbent and the alkaloidal extract. The column was then eluted with the following solvents in successive 500 ml quantities:-

Hexane : ethyl acetate	1 : 1
Ethyl acetate	
Ethyl acetate : methanol	19 : 1
Ethyl acetate : methanol	9 : 1
Ethyl acetate : methanol	17 : 3
Ethyl acetate : methanol	4 : 1
Ethyl acetate : methanol	7 : 3
Ethyl acetate : methanol	3 : 2
Ethyl acetate : methanol	1 : 1
Methanol	

Forty fractions of 75 ml volumes were collected and representative concentrates were monitored by TLC using the solvent systems:-

(i) Acetone : petroleum ether (b.p. 40-60°C) : carbon tetrachloride : iso-octane (7 : 6 : 4 : 3);

(ii) Chloroform : petroleum ether (b.p. 40-60°C) : methanol (8 : 1 : 1);

(iii) Ethyl acetate : iso-propanol : ammonia (16 : 3 : 1).

By the mixing of similar fractions the collected fractions were reduced to seven and designated fractions A-G. The alkaloids in each fraction were separated and purified by TLC.

Fraction A was separated using the solvent system acetone : petroleum ether (b.p. 40-60°C) : carbon tetrachloride : iso-octane (7 : 6 : 4 : 3) and three alkaloids were obtained, RVOK 1 (5 mg), RVOK 2 (50 mg) and RVOK 3 (10 mg).

Fraction B, on separation using the solvent system diethyl ether : methanol (19 : 1) provided two alkaloids, RVOK 4 (30 mg) and RVOK 5 (1500 mg). Fraction C was similarly separated and yielded RVOK 6 (50 mg) and RVOK 7 (5 mg). Fraction D treated in the same manner provided RVOK 8 (7 mg) and RVOK 9 (5 mg).

Fraction E alkaloids were separated by the solvent system acetone : chloroform : ethanol (1 : 8 : 1) and the alkaloids RVOK 10 (15 mg) and RVOK 11 (10 mg) were obtained. Fraction F yielded two amorphous alkaloids RVOK 12 (8 mg) and RVOK 13 (8 mg) when separated as for Fraction E.

Alkaloids of Fraction F were separated using the solvent system ethyl acetate : iso-propanol : ammonia (16 : 3 : 1) and two alkaloids RVOK 14 (50 mg) and RVOK 15 (800 mg) were obtained.

The intermediate and strong base fractions were combined and fractionated by column chromatography into five fractions designated H-L in the same manner as for the weak base fraction. The alkaloids of each fraction were also separated and purified by TLC.

Fraction H provided two alkaloids RVOK 16 (50 mg) and RVOK 17 (8 mg) on separation using the solvent system n-butanol : ethyl acetate : ethylene dichloride (1 : 3 : 6). Fraction I gave one alkaloid, RVOK 18 (10 mg), using the same solvent system.

Three alkaloids, RVOK 19 (8 mg), RVOK 20 (15 mg) and RVOK 21 (12 mg), were separated from Fraction J using the solvent system chloroform : methanol (3 : 1).

Fraction K yielded three alkaloids, RVOK 22 (10 mg), RVOK 23 (8 mg), RVOK 24 (30 mg), when separated using the solvent system acetone : methanol : diethylamine (7 : 2 : 1).

Fraction L on separation using acetone : methanol : acetic acid (7 : 2 : 1) provided two alkaloids, RVOK 25 (20 mg) and RVOK 26 (15 mg).

2. *R. volkensii* stem bark

2 kg powdered stem bark was defatted with petroleum ether, dried and rendered alkaline with 20 g of calcium hydroxide and a small quantity of water. The plant material was extracted with 10 litres of methanol by overnight maceration.

The solvent was removed and two more successive extractions were carried out using 1% sodium hydroxide solution in methanol.

The filtered extracts were bulked and evaporated to dryness under reduced pressure. The concentrated extract was dissolved in 200 ml 1.0 N hydrochloric acid and the insoluble resinous matter removed by filtration. The filtrate was shaken with chloroform (6 x 100 ml portions) and processed as before for the root to yield the weak base (5.2 g), intermediate base (3.1 g) and the strong base (0.9 g) fractions.

The weak base fraction was fractionated by preparative layer chromatography using the solvent system chloroform : acetone : methanol : *iso*-octane (13 : 3 : 2 : 2) into four bands designated bands A-D.

Band A on separation and purification by TLC using the solvent system acetone : petroleum ether (b.p. 40-60°C) : carbon tetrachloride : *iso*-octane (7 : 6 : 4 : 3) yielded three alkaloids RVOSB 1 (2 mg), RVOSB 2 (50 mg) and RVOSB 3 (50 mg).

Band B was separated using the solvent system diethylether : methanol (19 : 1) to yield two alkaloids RVOSB 4 (30 mg) and RVOSB 5 (800 mg).

Band C produced three alkaloids RVOSB 6 (25 mg), RVOSB 7 (10 mg) and RVOSB 8 (10 mg) by separation in the solvent system ethyl acetate : iso-propanol : ammonia (16 : 3 : 1). Alkaloids of Band D were separated in the same manner and two compounds, RVOSB 9 (80 mg) and RVOSB 10 (200 mg), were obtained.

The alkaloids of the strong and intermediate base fractions were separated using the solvent system chloroform : acetone : methanol (8 : 1 : 1) and the seven compounds, RVOSB 11 (15 mg), RVOSB 12 (10 mg), RVOSB 13 (20 mg), RVOSB 14 (20 mg), RVOSB 15 (20 mg), RVOSB 16 (10 mg) and RVOSB 17 (10 mg) were obtained.

3. R. volkensii leaves

3 kg of powdered leaves was extracted and processed as before to yield the weak base (10.5 g), intermediate base (0.8 g) and strong base (0.2 g) fractions.

The weak base fraction was further fractionated by column chromatography and, by monitoring of the concentrated eluates by TLC using the solvent systems chloroform : methanol : acetone : iso-octane (6 : 2 : 1 : 1) and ethyl acetate : iso-propanol : ammonia (17 : 3 : 1) the three fractions A-C were obtained.

Fraction A on separation by TLC using the solvent system acetone : chloroform : (4 : 5) yielded five alkaloids designated in order of

decreasing hRf values RVOLF 1 (8 mg), RVOLF 2 (10 mg), RVOLF 3 (8 mg), RVOLF 4 (60 mg) and RVOLF 5 (5 mg).

Alkaloids of Fraction B were separated using the solvent system chloroform : petroleum ether (b.p. 40-60°C) : methanol (8 : 1 : 1) and three alkaloids, RVOLF 6 (60 mg), RVOLF 7 (600 mg) and RVOLF 8 (15 mg), were obtained.

Fraction C alkaloids remained near the base line with most of the polar solvent systems used. The alkaloids were successfully separated using the solvent system chloroform : methanol (3 : 1) and RVOLF 9 (10 mg) and RVOLF 10 (15 mg) were obtained.

The intermediate and strong base fractions yielded three major alkaloids on separation by TLC using the solvent system chloroform : methanol : acetone : iso-octane (6 : 2 : 1 : 1). RVOLF 11 (8 mg), RVOLF 12 (8 mg) and RVOLF 13 (5 mg) were obtained.

4. R. oreogiton root

35 g of powdered root was extracted and fractionated into weak base and strong base fractions by the method described for the extraction of R. volkensii root. The yields obtained were 300 mg weak base fraction and 100 mg strong and intermediate base fraction.

Comparison with R. volkensii root

35 g of powdered R. volkensii root was similarly extracted and yielded 280 mg weak base fraction and 120 mg strong and intermediate base fractions.

The weak base fraction from each plant was dissolved in 10 ml chloroform : methanol (1 : 1) and the extracts co-chromatographed in six solvent systems. The alkaloids of each plant extract were examined and chromatographic characters compared.

The methods employed involved visualisation of the chromatograms under UV light, spraying with alkaloid detecting reagents such as Dragendorff's reagent, ferric chloride-perchloric acid reagent and ceric sulphate reagent, and comparison of hRf values. The strong base fractions of each plant were similarly compared.

Confirmation of identity of detected alkaloids

The extracts from R. volkensii root and R. oreogiton root were each applied as streaks to the baselines of freshly prepared and activated plates and the plates were developed in the solvent system acetone : petroleum ether : carbon tetrachloride : iso-octane (7 : 6 : 4 : 3). After location under UV light, the appropriate and corresponding bands in each extract respectively were scraped off and eluted in methanol.

The recovered alkaloids were co-chromatographed on TLC plates and with 0.01% solution of reference alkaloids using six solvent systems.

5. R. oreogiton leaves

6 kg of defatted, leaf powder was macerated overnight in 20 litres of methanol and further extracted with 3 x 20 litre quantities of methanol - ammonia. The extracts were bulked and concentrated under reduced pressure to yield 500 ml of a dark viscous liquid.

The concentrated extract was dissolved in 1 litre of 1.0 N hydrochloric acid and the resinous matter removed by filtration. The filtrate was extracted with 10 x 500 ml portions of chloroform and the combined chloroform extracts on evaporation to dryness under reduced pressure yielded the weak base fraction (15.0 g).

The aqueous layer was made alkaline to pH 9 with ammonia solution and extracted with 10 x 500 ml volumes of chloroform. Removal of the chloroform provided the intermediate base fraction (8.5 g).

The remaining aqueous phase was made further alkaline with 5% sodium hydroxide solution to pH 12 and extracted with chloroform (5 x 500 ml quantities). Evaporation of the chloroform extract provided the strong base fraction (5.2 g).

The weak base fraction was further fractionated by column chromatography in the same manner as reported earlier for the root of R. volkensii. Representative concentrated eluates were monitored by TLC in the solvent systems:-

Acetone : petroleum ether : carbon tetrachloride : iso-octane (7 : 6 : 4 : 3);

Chloroform : ethyl acetate : methanol : iso-octane (7 : 1 : 1 : 1) ;

Ethyl acetate : iso-propanol : ammonia (16 : 3 : 1).

By mixing of similar eluates six main fractions were obtained and designated fractions I-VI.

Fraction I yielded three alkaloids, Oreog 1 (4 mg), Oreog 2 (8 mg) and Oreog 3 (10 mg), when separated by TLC using the solvent system acetone : petroleum ether : carbon tetrachloride : iso-octane (7 : 6 : 4 : 3).

Oreog 4 (50 mg) crystallised from the methanolic solution of Fraction II and the supernatant solution on separation using the solvent system ether : methanol (9 : 1) provided Oreog 5 (100 mg) and Oreog 6 (50 mg).

From the methanolic solution of Fraction III Oreog 10 (100 mg) crystallised out and separation of the supernatant solution by TLC using the solvent system ethyl acetate : iso-propanol : ammonia (16 : 3 : 1) provided three more alkaloids, Oreog 7 (5 mg), Oreog 8 (15 mg) and Oreog 9 (100 mg).

Fraction IV treated in the same manner yielded four alkaloids, Oreog 11 (150 mg), Oreog 12 (120 mg), Oreog 13 (5 mg) and Oreog 14 (100 mg).

Fraction V provided three alkaloids Oreog 15 (5 mg), Oreog 16 (10 mg) and Oreog 17 (10 mg) when separated using the solvent system ether : methanol (1 : 1).

Fraction VI yielded Oreog 18 (30 mg) when separated using the solvent system chloroform : methanol : petroleum ether (8 : 1 : 1).

The intermediate and strong base fractions were combined and on separation using the solvent system ethyl acetate : iso-propanol : ammonia (17 : 3 : 1) yielded three identifiable alkaloids Oreog 19 (10 mg), Oreog 20 (10 mg) and Oreog 21 (20 mg).

RESULTS AND DISCUSSION

ALKALOIDS of *R. VOLKENSII* ROOT

ALKALOIDS of *R. VOLKENSII* STEM BARK

ALKALOIDS of *R. VOLKENSII* LEAVES

ALKALOIDS of *R. OREOGITON* ROOT

ALKALOIDS of *R. OREOGITON* LEAVES

Alkaloids of R. volkensii root

RVUK 1 : Light yellow crystals; m.p. 228 - 230°C.

UV : λ_{max}^{MeOH} nm 226, 282, 290 nm; inflexion at 250.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2900 m, 2850 w, 2750 w, 1695 s, 1600 s, 1460 w, 1450 s, 1360 m, 1310 w, 1300 s, 1260 s, 1200 s, 1180 s, 1150 m, 1110 s, 1060 w, 740 s.

MS : m/e (relative intensity per cent) 352 M^+ (100), 351 (M - 1) (76), 337 (M - 15) (29), 321 (M - 31) (7), 309 (4), 293 (M - 59) (9), 281 (4), 267 (4), 251 (4), 239 (5), 223 (18), 211 (7), 209 (9), 197 (15), 184 (13), 170 (13), 169 (20), 168 (11), 156 (51).

Chromogenic reactions: A greyish colour was obtained with ferric chloride-perchloric acid reagent and a faint yellow colour obtained with ceric sulphate reagent.

The UV spectrum suggested a heteroyohimbine chromophore. This was confirmed by the twin absorption IR maxima at 1695 and 1600 cm^{-1} indicating the presence of the β-alkoxy-acrylic ester moiety in the molecule. The IR spectrum also showed a strong absorption maximum at 740 cm^{-1} due to a 1, 2 - disubstituted benzene ring and indicating absence of substitution on the aromatic ring.

The MS showed the typical fragmentation pattern of an unsubstituted heteroyohimbine compound; the β-carboline peaks were indicated at m/e 184, 170, 169 and 156. The relative intensity of the peaks at m/e 184, 170 and 169 and the peaks at m/e 225 and 223 indicated that the compound belongs to the allo or epiallo series (Djerassi, 1963; Hesse, 1974).

The above data suggested the structure of tetrahydroalstonine. Co-chromatography with an authentic sample supplied by Professor R. C. Elderfield and Dr. Le Quesne in six solvent systems and an undepressed mixed melting point confirmed RVOK 1 as tetrahydroalstonine.

RVOK 2 : Light yellow crystals; m.p. 216 - 218°C.

UV : λ_{max}^{MeOH} nm 228, 299, 305; inflexion at 250.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2900 m, 2850 w, 2800 w, 2750 w, 1700 s, 1630 s, 1570 w, 1480 s, 1440 m, 1340 m, 1300 s, 1210 s, 1190 m, 1150 s, 1110 m, 1080 m, 1030 w, 830 w, 770 w, 740 w.

MS : m/e (relative intensity per cent) 412 M^+ (100), 411 (M - 1) (82), 397 (M - 15) (23), 381 (M - 31) (9), 353 (M - 59) (9), 311 (14), 283 (18), 269 (9), 257 (9), 244 (11), 230 (11), 229 (11), 222 (9), 216 (41).

Chromogenic reactions: An indigo blue colour was produced with ferric chloride - perchloric acid reagent and a yellow colour obtained with ceric sulphate reagent.

The UV spectrum indicated a substituted heteroyohimbine chromophore. MS showed a typical heteroyohimbine fragmentation pattern but the β - carboline peaks were indicated at m/e 244, 230, 229 and 216 thus suggesting two methoxy substituents on the indole nucleus. MS relative peak intensities at m/e 244, 230 and 229 indicated cis D/E ring fusion (Djerassi, 1963). Chromogenic reactions and spectral data suggested that the alkaloid resembled reserpiline but differed chromatographically. A sample of isoreserpiline was prepared by acid epimerisation of an authentic sample of reserpiline and subsequent purification by PLC (MacPhillamy et al, 1955).

Direct comparison of RVOK 2 and the synthetic sample (UV, IR, MS, co-TLC) and the undepressed mixed melting point confirmed that RVOK 2 was isoreserpiline.

RVOK 3 : Yellow crystals; m.p. 260°C.

UV : λ_{max}^{MeOH} nm 226, 282, 290; inflexion at 250.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2900 m, 1700 s, 1610 s, 1465 w, 1450 s, 1360 m, 1310 w, 1300 s, 1200 s, 1180 s, 1150 m, 1110 s, 1060 w, 1020 w, 1000 w, 960 w, 920 w, 740 s.

MS : m/e (relative intensity per cent) 352 M^+ (100), 351 (M - 1) (75), 337 (M - 15) (13), 321 (M - 31) (13), 293 (M - 59) (17), 225 (21), 223 (17), 209 (25), 196 (21), 184 (58), 170 (25), 169 (33), 156 (83).

Chromogenic reactions: A greyish colour was obtained with ferric chloride - perchloric acid reagent and a faint yellow colour produced with ceric sulphate reagent.

The data obtained agrees with published data for ajmalicine (Timmins and Court, 1974). Mixed melting points were undepressed and identity was confirmed by co-TLC and comparison of the IR spectrum with that of an authentic sample of ajmalicine.

RVOK 4 : Yellow amorphous powder.

UV : λ_{max}^{MeOH} nm 227, 282, 292; inflexion at 250.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2900 s, 2850 w, 1700 m, 1620 m, 1570 s, 1430 m, 1280 m, 1240 w, 1200 m, 1160 w, 1130 w, 1080 m, 830 w, 800 w, 770 w.

MS : m/e (relative intensity per cent) 382 M^+ (100), 381 (M - 1) (69), 367 (M - 15) (58), 351 (M - 31) (13), 323 (M - 59) (15), 321 (16), 281 (29), 251 (33), 249 (24), 239 (13), 222 (9), 214 (15), 200 (13), 199 (16), 186 (43).

NMR : ($CDCl_3$) δ 8.12 (dd 1H, NH), 7.72 - 6.92 (m, 3H, aromatic), 3.88 (S, 3H, OCH_3), 3.76 (S, 3H, OCH_3), 3.00 (m, 1H), 2.64 (m, 1H), 2.16 (S, 2H), 1.76 (S, 1H), 1.32 (d, 3H, C-19, CH_3).

Chromogenic reactions: A greyish brown colour was obtained with ferric chloride - perchloric acid reagent and a dirty brown colour produced with ceric sulphate reagent.

Spectral data (UV, IR, MS, NMR) agrees with published data for aricine (Boonchuay and Court, 1976). Co-TLC with an authentic sample of aricine in six solvent systems confirmed the identity of RVOK 4 as aricine.

RVOK 5 : Light yellow crystals; m.p. 104 - 106°C.

UV : λ_{max}^{MeOH} nm 228, 299, 304; inflexion at 250.

IR : ν_{max}^{KBr} cm^{-1} 3350 s, 2925 s, 2825 m, 1700 s, 1620 s, 1560 w, 1480 s, 1440 m, 1380 w, 1340 m, 1300 s, 1240 m, 1200 s, 1140 m, 1080 s, 1020 w, 830 m, 790 m, 760 m, 740 w.

MS : m/e (relative intensity per cent) 412 M^+ (100), 411 (M - 1) (71), 397 (M - 15) (43), 381 (M - 31) (11), 353 (M - 59) (14), 325 (7), 311 (25), 283 (29), 263 (11), 257 (11), 244 (14), 230 (11), 229 (11), 222 (7), 216 (36).

Chromogenic reactions: An indigo blue colour was produced with ferric chloride - perchloric acid reagent and a yellow colour obtained with ceric sulphate reagent.

The UV spectrum indicated a substituted heteroychimbine chromophore. MS indicated the presence of two methoxy substituents on the indole nucleus. The β-carboline peaks occurred at m/e 244, 230, 229 and 216. The relative peak intensities at m/e 244, 230 and 229 indicated trans D/E ring fusion (Djerassi, 1963).

Spectral data (UV, IR, MS) and colour reactions demonstrated that this alkaloid was identical with reserpiline. Mixed melting points were undepressed and co-TLC with reference reserpiline confirmed the identity of the compound as reserpiline.

RVOK 6 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm 218, 246 sh, 278, 302 sh.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2950 s, 2820 w, 2800 w, 1700 s, 1620 s, 1500 s, 1460 m, 1380 w, 1330 m, 1260 w, 1210 m, 1190 m, 1080 m, 1060 w, 1000 w, 850 w, 820 w, 770 m.

MS : m/e (relative intensity per cent) 428 M^+ (100), 413 (M - 15) (7), 411 (M - 17) (9), 397 (M - 31) (9), 367 (M - 61) (7), 353 (7), 223 (73), 222 (40), 219 (29), 208 (38), 204 (22), 198 (11), 190 (16), 180 (20), 164 (24), 124 (29), 109 (47), 69 (91).

Chromogenic reactions: A pale pink colour was obtained with ferric chloride - perchloric acid reagent and a buff colour produced with ceric sulphate reagent.

The UV spectrum indicated an oxindole chromophore and the MS showed a typical oxindole fragmentation pattern. The indole peaks appeared at

m/e 204 and 190, suggesting dimethoxy substitution on the aromatic ring. Other spectral data agreed with the structure of carapanaubine (Finch et al, 1963; Pousset et al, 1967).

A sample of carapanaubine was prepared by reacting 15 mg of isoreserpiline dissolved in 30 mls methylene dichloride with lead tetra-acetate solution for 20 minutes (Finch et al, 1963). The reaction mixture was poured into ice-cold potassium bicarbonate solution and the separated organic layer concentrated under reduced pressure. The concentrate was refluxed in 30 mls methanol : acetic acid mixture for 2 hours and the reaction product on dilution with cold potassium bicarbonate solution was extracted with chloroform. The chloroform extract on concentration and purification yielded 8 mg crystalline carapanaubine, m.p. 225°C.

Direct comparison (UV, IR, MS and co-TLC) of the synthetic product with RVOK 6 confirmed the identity as carapanaubine.

RVOK 7 : Light yellow amorphous powder.

UV : λ_{max}^{MeOH} nm 225, 251 sh, 281, 400 sh.

IR : ν_{max}^{KBr} cm^{-1} 3750 s, 2950 s, 2850 m, 1700 s, 1620 s, 1500 s, 1460 m, 1330 m, 1280 w, 1210 m, 1190 m, 1140 m, 1080 m, 1060 w, 870 w, 820 w, 770 s.

MS : m/e (relative intensity per cent) 428 M^+ (100), 413 (M - 15) (20), 411 (M - 17) (40), 397 (30), 381 (20), 253 (40), 223 (60), 222 (100), 219 (30), 208 (30), 204 (20), 190 (30), 180 (30), 120 (20), 69 (40).

Chromogenic reactions: A faint brown colour was produced with ferric chloride - perchloric acid reagent and a buff colour obtained with ceric sulphate reagent.

The UV spectrum suggested an oxindole or pseudo-indoxyl chromophore. The MS showed the base peak at m/e 222 whilst the m/e 223 peak was small. This suggested a pseudo-indoxyl fragmentation pattern (Budzikiewicz et al, 1964).

UV, IR and MS data agrees with published data for isoreserpiline-ψ-indoxyl (Finch et al, 1963). Co-TLC with a reference sample isolated from R. vomitoria (Iwu and Court, 1977) confirmed the identity of the compound as isoreserpiline-ψ-indoxyl.

RVOK 8 : White crystals; m.p. 260 - 262°C.

UV : λ_{max}^{MeOH} nm 220, 269, 296; λ_{min}^{MeOH} 247, 287.

IR : ν_{max}^{KBr} cm^{-1} 3380 m, 2915 s, 1700 s, 1670 m, 1530 s, 1480 s, 1290 m, 1160 s, 810 m.

MS : m/e (relative intensity per cent) 608 M^+ (100), 607 (M - 1) (55), 595 (11), 477 (8), 450 (8), 414 (8), 397 (41), 381 (19), 365 (11), 363 (8), 337 (8), 321 (8), 267 (11), 252 (11), 251 (36), 214 (19), 213 (8), 212 (22), 200 (22), 199 (19), 195 (44), 186 (19).

Chromogenic reactions: A greenish brown colour was produced with both ferric chloride - perchloric acid reagent and ceric sulphate reagent.

Spectral data (UV, IR, MS) and chromogenic reactions agreed with published work on reserpine (Gaskell and Joule, 1967; Timmins and Court, 1974). Mixed melting points were undepressed and co-TLC with an authentic sample of reserpine in six solvent systems confirmed RVOK 8 as reserpine.

RVOK 9 : Light yellow crystals; m.p. 224 - 226°C.

UV : λ_{max}^{MeOH} nm 228, 303; λ_{min}^{MeOH} 262.

IR : ν_{max}^{KBr} cm^{-1} 3400 m, 2850 s, 1700 s, 1620 m, 1542 s, 1520 m, 1280 s, 1160 s, 840 s.

MS : m/e (relative intensity per cent) 634 M^+ (89), 633 (M - 1) (52), 414 (15), 398 (40), 397 (100), 396 (13), 382 (33), 367 (23), 265 (17), 252 (19), 251 (50), 238 (26), 223 (17), 221 (39), 214 (19), 212 (17), 200 (28), 199 (27), 195 (33), 186 (27).

Chromogenic reactions: A greenish brown colour was obtained with both ferric chloride - perchloric acid reagent and ceric sulphate reagent.

The spectral data agreed with published data for rescinnamine (Klohs et al, 1955; Gabetta and Mustich, 1975). Mixed melting points were undepressed and co-TLC with an authentic sample of rescinnamine confirmed the identity of the compound as rescinnamine.

RVOK 10 : White amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 210 (4.65), 249 (3.92), 293 (3.35).

λ_{min}^{MeOH} nm 252, 271.

IR : ν_{max}^{KBr} cm^{-1} 1720 s, 1600 s, 1460 s, 1300 m, 1260 m, 1200 m, 1120 m, 1040 m, 980 m, 850 w, 760 s, 740 s.

MS : m/e (relative intensity per cent) 350 M^+ (100), 335 (M - 15) (15), 307 (M - 43) (18), 291 (M - 59) (36), 227 (M - 73) (9), 275 (5), 263 (9), 249 (5), 225 (9), 222 (5), 198 (14), 183 (73), 169 (32), 168 (36), 157 (45), 144 (32), 130 (32).

Chromogenic reactions: An instant red colour reaction was produced with ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

The UV spectrum and chromogenic reaction suggested a dihydroindole chromophore. The IR spectrum indicated a strong carbonyl absorption maximum at 1720 cm^{-1}. The MS fragmentation pattern resembled that of tetraphyllicine (Biemann et al, 1964) but the molecular peak occurred at m/e 350 which is 42 mass units more than that of tetraphyllicine. The MS in the high mass region showed peaks at m/e 307 (M - 43) and 291 (M - 59) which were attributable to loss of CH_3CO^+ and $COOCH_3$ fragments respectively. The IR spectrum indicated no hydroxyl absorption maximum at 3400 cm^{-1} region.

The above evidence suggested that the structure was tetraphyllicine-17-O-acetyl. A semi-synthetic tetraphyllicine-17-O-acetyl was prepared by reacting 10 mg of tetraphyllicine with acetic anhydride-pyridine mixture (2:1) at room temperature for 24 hr (Antonaccio et al, 1962). The solvent was evaporated under reduced pressure and on purification by TLC tetraphyllicine-17-O-acetyl (6 mg) was obtained.

Direct comparison of UV, IR, MS and the chromatographic behaviour of the synthetic product and RVOK 10 confirmed the identity of RVOK 10 as tetraphyllicine-17-O-acetyl.

RVOK 11 : White crystals; m.p. 320 - 322°C.

UV : λ_{max}^{MeOH} nm 213, 248, 293; λ_{min}^{MeOH} 226, 270.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2900 s, 2850 m, 1600 s, 1460 s, 1420 w, 1300 w, 1260 w, 1200 w, 1120 m, 1050 m, 960 m, 850 w, 760 s, 740 s.

MS : m/e (relative intensity per cent) 308 M^+ (100), 293 (M - 15) (5), 291 (M - 17) (8), 277 (11), 263 (9), 249 (5), 225 (6), 200 (5), 199 (8), 183 (55), 169 (11), 168 (14), 157 (23), 144 (35), 131 (17), 130 (12).

Chromogenic reactions: A red colour reaction was produced with ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

The UV spectrum and chromogenic reactions indicated a dihydroindole chromophore. The IR spectrum showed a broad absorption band at 3400 cm^{-1} indicating the presence of a free OH-group in the molecule. The MS fragmentation pattern agreed with the spectrum of tetraphyllicine (Biemann et al, 1964). Mixed melting points were undepressed. CO-TLC with an authentic sample of tetraphyllicine and direct comparison of spectral data, (UV, IR and MS) with data for the authentic sample confirmed the compound as tetraphyllicine.

RVOK 12 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 225 (4.29), 284 sh, 291 (4.00), 327 (4.25);

λ_{min}^{MeOH} nm 265, 315.

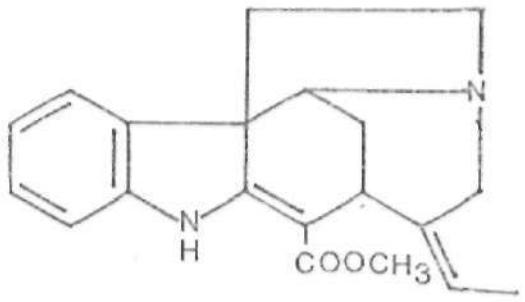
IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2925 s, 2850 w, 1670 s, 1600 s, 1580 m, 1460 s, 1440 m, 1400 m, 1240 m, 1200 m, 1130 w, 1100 w, 740 m.

MS : m/e (relative intensity per cent) 322 M^+ (60), 307 (M - 15) (16), 292 (19), 291 (M - 31) (16), 279 (12), 263 (M - 59) (32), 252 (26), 234 (19), 216 (16), 206 (29), 183 (24), 182 (26), 180 (29), 168 (32), 167 (44), 144 (21), 130 (19), 121 (100), 107 (55), 92 (55).

Chromogenic reactions: A bluish green colour was produced with ferric chloride - perchloric acid reagent and a greyish colour obtained with ceric sulphate reagent.

The UV spectrum indicated a 2-methylene-indoline chromophore. Spectral data agreed with published data to akuammicine (Budzikiewicz et al, 1964; Boonchuay and Court, 1976b).

Co-TLC with an authentic sample of akuammicine confirmed the identity of compound RVOK 12 as akuammicine.



Akuammicine

RVOK 13 : Off-white amorphous powder.

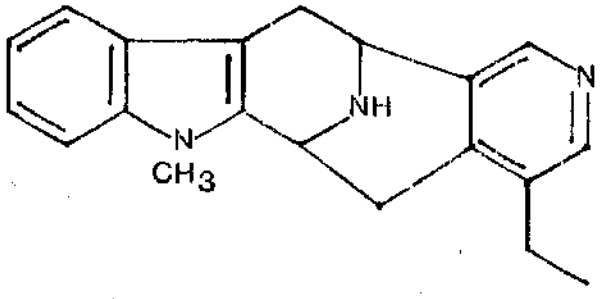
UV : λ_{max}^{MeOH} nm 225, 273, 284.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2920 s, 1600 m, 1580 m, 1460 s, 1420 s, 1200 w, 1080 w, 1010 w, 980 w, 820 m, 740 s.

MS : m/e (relative intensity per cent) 303 M^+ (100), 286 (M - 17) (33), 260 (17), 257 (17), 245 (17), 211 (17), 183 (67), 168 (42), 158 (25), 144 (25).

Chromogenic reactions: A pink colour reaction was produced with ferric chloride - perchloric acid reagent but no colour was observed with ceric sulphate reagent.

The UV spectrum indicated an indole chromophore. The MS demonstrated the β-carboline peaks at m/e 183, 168, 158 and 144 which is indicative of a methyl group attached to the indole nitrogen atom. Other spectral data (UV, IR, MS) agreed with published data on suaveoline (Majumdar et al, 1972; Iwu and Court, 1977). Co-TLC with a sample of suaveoline isolated from R. vomitoria roots confirmed the identity of the compound as suaveoline.



Suaveoline

RVOK 14 : White crystals; m.p. 216°C.

UV : λ_{max}^{MeOH} nm 212, 248, 289; λ_{min}^{MeOH} nm 226, 270.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2950 s, 2850 w, 1730 s, 1610 s, 1460 s, 1420 m, 1350 m, 1300 m, 1100 s, 1040 s, 980 s, 910 m, 850 m, 790 s, 760 s, 740 s.

MS : m/e (relative intensity per cent) 368 M^+ (80), 353 (M - 15) (27), 339 (7), 325 (M - 43) (13), 309 (M - 59) (13), 286 (13), 226 (27), 200 (20), 196 (40), 195 (33), 183 (53), 182 (87), 168 (100), 167 (60), 157 (40), 144 (93), 131 (40).

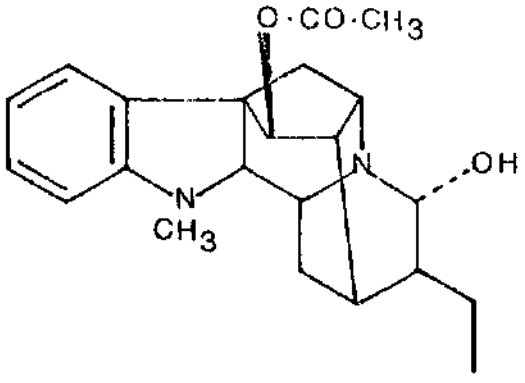
Chromogenic reactions: A red colour reaction was produced with both ferric chloride - perchloric acid reagent and ceric sulphate reagent.

The UV spectrum and chromogenic reactions indicated a dihydroindole structure. The IR spectrum resembled that of ajmaline except for the carbonyl band at 1730 cm^{-1}. The MS spectrum showed a fragmentation pattern consistent with the structure of ajmaline-17-O-acetyl (Bombardelli et al, 1967).

Acetylation of RVOK 14 by reacting it with acetic anhydride : pyridine mixture (2:1) at room temperature for 12 hr yielded an acetyl derivative. UV : λ_{max}^{MeOH} nm 249, 294; IR : ν_{max}^{KBr} cm^{-1} 1735 s, 1715 m, 1610 m, 1410 m, 1100 m, 760 s. MS : m/e (relative intensity per cent) 410 (70), 395 (45), 368 (20), 367 (40), 325 (80), 200 (60), 199 (50), 183 (100), 182 (40), 158 (30), 144 (18), 130 (30).

An ajmaline sample similarly treated formed a di-acetyl derivative which showed identical spectral data and chromatographic behaviour with that of the acetyl derivative of RVOK 14.

The above evidence therefore confirmed that RVOK 14 was ajmaline-17-O-acetyl.



Ajmaline-17-O-acetyl

RVOK 15 : White crystals: m.p. 207°C.

UV : λ_{max}^{MeOH} nm 223, 249, 292; λ_{min}^{MeOH} nm 225, 270.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2950 s, 2875 w, 1610 s, 1460 s, 1420 m, 1350 m, 1300 m, 1200 w, 1140 w, 1110 m, 1040 s, 1020 w, 860 s, 790 s, 760 s, 740 s.

MS : m/e (relative intensity per cent) 326 M^+ (96), 311 (M - 15) (38), 297 (8), 279 (4), 253 (4), 226 (6), 224 (8), 216 (8), 200 (29), 199 (29), 183 (44), 182 (65), 170 (19), 169 (10), 168 (15), 158 (27), 157 (35), 145 (67), 144 (100), 131 (42).

Chromogenic reactions: A red colour was obtained with ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

Spectral data (UV, IR, MS) agreed with published data for ajmaline (Timmins and Court, 1974). Mixed melting points were undepressed. Co-TLC with an authentic sample of ajmaline confirmed the identity of the compound as ajmaline.

RVCK 16 : Yellow crystals; m.p. 238 - 340°C.

UV : λ_{max}^{MeOH} nm 227, 282, 291; λ_{min}^{MeOH} nm 250.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2920 s, 2800 w, 2750 w, 1720 m, 1650 w, 1580 s, 1430 s, 920 w, 740 s.

MS : m/e (relative intensity per cent) 354 M^+ (98), 353 (M - 1) (100), 339 (M - 15) (9), 337 (M - 17) (4), 323 (9), 295 (M - 59) (11), 223 (11), 221 (9), 211 (6), 209 (6), 197 (9), 184 (17), 170 (23), 169 (23), 156 (17), 144 (15).

Chromogenic reactions: A greyish colour was produced with ferric chloride - perchloric acid reagent and a yellow colour obtained with ceric sulphate reagent.

The UV spectrum indicated an indole chromophore with a well-defined minimum at 250 nm. The IR showed absorption maxima at 2800 and 2750 cm^{-1}

characteristic of C-3H α compounds (Wenkert, et al, 1956). The IR absorption maximum at 740 cm^{-1} suggested non-substitution on the aromatic ring. The MS showed a typical yohimbine type fragmentation pattern (Djerassi, 1963). The chromatographic behaviour of the compound differed from that of yohimbine. Co-TLC with an authentic sample of α-yohimbine in six solvent systems established the identity of the alkaloid as α-yohimbine. The mixed melting points were undepressed.

RVOK 17 : Light yellow amorphous powder.

UV : λ_{max}^{MeOH} nm 227, 282, 290; λ_{min}^{MeOH} nm 248.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2910 s, 2850 w, 2800 w, 2750 w, 1710 s, 1620 m, 1570 m, 1430 s, 1290 m, 1200 s, 1150 m, 740 s.

MS : m/e (relative intensity per cent) 354 M^+ (86), 353 (M - 1) (100), 339 (M - 15) (2), 337 (M - 17) (2), 325 (3), 321 (33), 312 (2), 295 (M - 59) (10), 249 (4), 224 (14), 184 (16), 170 (32), 169 (26), 156 (18).

Chromogenic reactions: A greyish colour was obtained with ferric chloride - perchloric acid reagent and a light yellow colour was produced with ceric sulphate reagent.

Spectral data agreed with published data for yohimbine (Gabetta and Mutstich, 1975). Co-TLC with an authentic sample of yohimbine confirmed the identity of the compound and the mixed melting points were undepressed.

RVOK 18 : White amorphous powder.

UV : λ_{max}^{MeOH} nm 227, 280, 300 sh, 310 sh; λ_{min}^{MeOH} nm 252.

The UV spectrum exhibited a bathochromic shift in an alkaline medium ($\lambda_{max}^{MeOH : NaOH}$ 235, 278, 300 sh, 312 sh.)

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2900 s, 1630 s, 1590 m, 1450 s, 1300 w, 1200 w, 1150 m, 1130 w, 1020 w, 800 m, 740 m.

MS : m/e (relative intensity per cent) 310 M^+ (100), 309 (M - 1) (90), 295 (M - 15) (20), 293 (M - 17) (20), 279 (M - 31) (50), 265 (20), 251 (10), 226 (20), 223 (20), 211 (20), 198 (10), 185 (70), 184 (60), 172 (15), 169 (30), 168 (25), 159 (20).

Chromogenic reactions: A steel grey colour reaction was obtained with ferric chloride - perchloric acid reagent. A yellow colour was produced with ceric sulphate reagent.

The UV spectrum indicated an indolic chromophore but in an alkaline medium (N/10 NaOH) a bathochromic shift of 8 nm occurred in the lower wavelength maximum, thus suggesting a phenolic indole chromophore. The IR absorption maxima at 800 and 740 cm^{-1} confirmed the presence of a substituent on the aromatic ring. The MS fragmentation pattern agreed with published spectrum of sarpagine (Hesse, 1974).

Co-TLC with an authentic sample of sarpagine confirmed the identity of the compound as sarpagine.

RVOK 19 : White amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 226 (4.5), 282 (4.0), 290 (3.2) sh;

λ_{min}^{MeOH} nm 250.

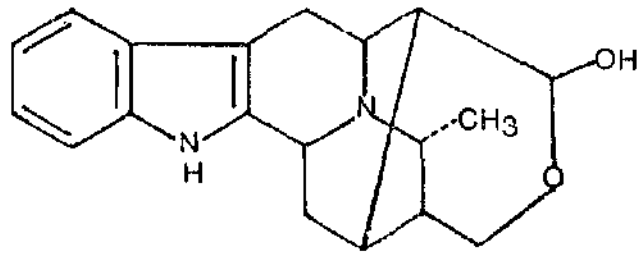
IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2900 s, 2850 w, 1650 s, 1570 m, 1440 s, 1300 s, 1070 w, 1020 w, 980 w, 820 w, 740 s.

MS : m/e (relative intensity per cent) 310 M^+ (73), 309 (M - 1) (100), 292 (M - 18) (14), 282 (M - 29) (18), 279 (M - 31) (23), 263 (9), 249 (9), 239 (9), 226 (36), 211 (41), 209 (27), 195 (18), 182 (27), 169 (55), 168 (45), 156 (27).

Chromogenic reactions: A greyish colour was obtained with ferric chloride - perchloric acid reagent and a faint yellow colour produced with ceric sulphate reagent.

The UV spectrum exhibited an indole chromophore with a well-defined minimum at 250 nm. The spectrum recorded was unchanged when run in an alkaline or acidic medium. The IR and MS spectra suggested a non-substitution of the aromatic ring.

Other spectral data (UV, IR, MS) agreed with published data for peraksine (Pousset, 1977). Co-TLC with an authentic sample of peraksine confirmed the identity of the compound.



Peraksine

RVOK 20 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm 220, 260; λ_{min}^{MeOH} nm 248.

IR : ν_{max}^{KBr} cm^{-1} 1740 s, 1710 m, 1660 m, 1590 s, 1450 m, 1230 s, 1120 s, 1030 m, 740 s.

MS : m/e (relative intensity per cent) 350 M^+ (86), 335 (M - 15) (7), 321 (M - 29) (21), 307 (M - 43) (21), 291 (M - 59) (21), 279 (9), 263 (14), 251 (21), 237 (32), 226 (21), 223 (21), 211 (71), 196 (50), 183 (21), 182 (29), 169 (64), 168 (100), 156 (21), 144 (36), 130 (36), 115 (15).

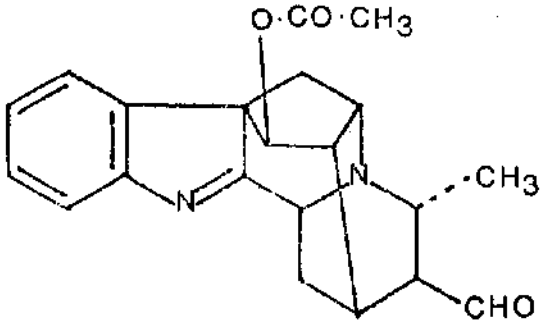
NMR ($CDCl_3$) : δ 9.92 (s, 1H, aldehyde), 7.80 - 6.88 (m, 4H, aromatic), 4.92 (s, 1H), 4.16 (dd, 1H), 3.84 (s, 1H), 2.12 (s, 3H, $OCOCH_3$), 1.56 (dd, 1H), 1.24 (d, 3H, C-21 CH_3).

Chromogenic reactions: No colour was obtained with ceric sulphate reagent but a faint pinkish colour was produced with ferric chloride - perchloric acid reagent.

The UV spectrum revealed an indolenine chromophore; a bathochromic shift to an indolic chromophore occurred in concentrated acid medium ($\lambda_{max}^{MeOH : HCl}$ 229, 283 nm). The IR spectrum indicated two carbonyl absorption bands at 1740 and 1710 cm^{-1}. The upper mass region of the mass spectrum exhibited peaks at m/e 335 (M - 15), 321 (M - 29), 307 (M - 43) and 291 (M - 59) which were attributable to loss of CH_3, CHO, CH_3CO and $COOCH_3$ fragments respectively. Evidence of the presence of these functional groups in the molecule was confirmed by the NMR signals at δ 9.92 (CHO), δ 2.12 (s, 3H, $OCOCH_3$) and δ 1.24 (d, 3H, C-21, CH_3).

Other spectral data (UV and IR) agreed with published data for perakine (Taylor et al, 1962). NMR data similarly agreed with that for perakine recorded by Khan and Siddiqui (1972). The MS fragmentation pattern is consistent with the structure of perakine.

The compound was therefore identified as perakine.



Perakine

RVOK 21 : Light yellow amorphous powder.

UV : λ_{max}^{MeOH} nm 220, 225, 265.

IR : ν_{max}^{KBr} cm^{-1} 3380 s, 2920 s, 1720 s, 1610 m, 1580 s, 1450 s, 1300 w, 1230 w, 1080 m, 1030 w, 820 m, 770 w, 740 s.

MS : m/e (relative intensity per cent) 352 M^+ (100), 335 (M - 17) (47), 321 (M - 31) (35), 309 (M - 42) (41), 293 (M - 59) (35), 279 (M - 73) (35), 263 (41), 251 (12), 249 (12), 226 (41), 211 (41), 196 (53), 183 (55), 169 (94), 168 (94), 156 (25), 144 (68), 143 (49), 130 (47), 115 (20).

NMR ($CHCl_3$) : δ 7.80 - 7.00 (m, 4H, aromatic), 5.12 (s, 1H), 4.18 (dd, 1H, C-3H), 3.40 (s, 1H, OH), 2.00 (s, 3H, $OCOCH_3$), 1.26 (d, 3H, C-21, CH_3).

MS-DIS : m/e (relative intensity per cent) 424 M^+ (100), 409 (M - 15) (21) 281 (M - 43) (24), 365 (M - 59) (18), 335 (29), 321 (59), 303 (26), 291 (44), 277 (35), 259 (29), 221 (24), 196 (79), 187 (53), 183 (50), 169 (91), 168 (97), 156 (32), 144 (44), 143 (56), 130 (59).

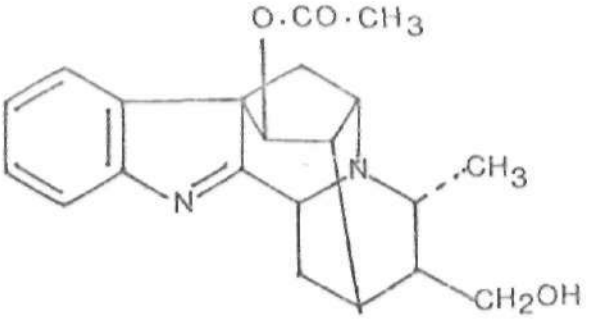
Chromogenic reactions: A faint pinkish colour was obtained with ferric chloride - perchloric acid reagent but no colour was produced with ceric sulphate reagent.

The UV spectrum indicated an indolenine chromophore. The IR spectrum showed a strong OH-absorption maximum at 3400 cm^{-1} and a carbonyl absorption band at 1720 cm^{-1}. MS-DIS indicated the presence of one active hydrogen atom which is most likely to be an OH group since the compound is an indolenine.

The MS showed in the upper mass region peaks at m/e 335 (M - 17), 321 (M - 31), 309 (M - 43) and 293 (M - 59) which probably corresponded to loss of OH, CH_2OH, CH_3CO and $OCOCH_3$ fragments respectively.

A close examination of the spectral data of the compound and that of perakine (RVCK 20) revealed that the two alkaloids are closely related. The aldehydic signal which appeared in the NMR spectrum of perakine at δ 9.92 was absent in the spectrum of RVCK 21 but instead a signal occurred at δ 3.40 which corresponded to an OH-proton resonance. The MS fragmentation patterns of both alkaloids were similar. It was therefore concluded that RVCK 21 differed from perakine (RVCK 20) by the presence of a CH_2OH group at C-20 instead of a CHO group as in perakine.

The above evidence suggested the structure of raucaffrinoline. Spectral data (UV, IR, MS and NMR) agreed with published data for raucaffrinoline (Khan and Siddiqui, 1972).



Raucaffrinoline

RVCK 22 : White amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 226 (4.5), 284 (3.9), 291 (3.7);

λ_{min}^{MeOH} nm 252.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, (broad), 2950 s, 2850 w, 2800 w, 2740 w, 1710 s, 1620 m, 1500 w, 1460 s, 1420 s, 1360 w, 1280 w, 1200 m, 1150 m, 740 s.

MS : m/e (relative intensity per cent) 370 M^+ (97), 369 (M - 1) (100), 355 (M - 15) (11), 353 (M - 17) (9), 339 (13), 222 (13), 221 (14), 197 (14), 184 (29), 170 (30), 169 (37), 156 (26).

HRMS : Accurate mass weight 370.19093 (calculated for $C_{21}H_{25}N_2O_4$ requires 370.189245; error 0.00159).

Chromogenic reactions: A greyish colour was produced with ferric chloride - perchloric acid reagent and a yellow colour obtained with ceric sulphate reagent.

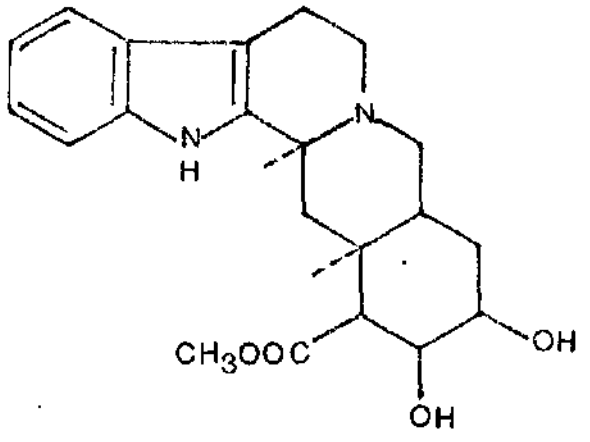
The UV spectrum indicated an indole chromophore with a well-defined λ min at 252 nm. The IR spectrum revealed a broad OH absorption band at 3400 cm^{-1} and a carbonyl band at 1710 cm^{-1}. The IR absorption bands at 2850, 2800 and 2750 cm^{-1} are characteristic of compounds possessing C-3H α (Wenkert, 1956).

The MS exhibited a typical yohimbine-type fragmentation pattern, the β-carboline peaks occurring at m/e 184, 170, 169 and 156 thus suggesting non-substitution on the indole nucleus. The molecular peak appeared at m/e 370 which is 16 mass units more than for yohimbine.

The acetyl derivative prepared by reacting acetic anhydride : pyridine mixture (2:1) with 5 mg of the compound was a diacetate. MS : m/e (relative intensity per cent) 454 (70), 453 (53), 412 (25), 411 (30), 395 (28), 370 (70), 369 (100), 353 (8), 330 (18), 311 (8), 293 (10), 197 (14), 184 (30), 170 (45), 169 (50), 156 (28), 144 (40). This indicated the presence of two hydroxyl groups in the molecule.

Comparison of the above data with that of 18-hydroxy yohimbine (Iwu, 1977) suggested that the two compounds were probably identical.

Co-TLC with a sample of 18-hydroxy-yohimbine isolated from R. mombasiana root (Iwu, 1977) confirmed the identity of the alkaloid as 18-hydroxy-yohimbine.



18-hydroxy-yohimbine

RVOK 23 : Off-white crystals; m.p. 218 - 220°C.

UV : λ_{max}^{MeOH} nm 227, 284, 291; λ_{min}^{MeOH} nm 250.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2920 s, 2850 w, 2800 w, 1720 s, 1580 s, 1400 s, 1200 m, 1160 m, 1120 m, 1010 w, 860 m, 840 m, 740 s.

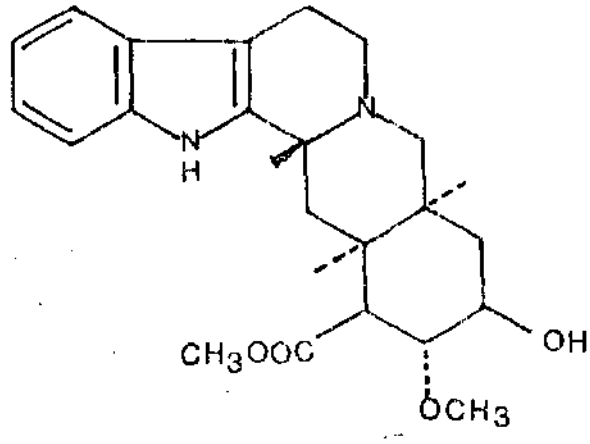
MS : m/e (relative intensity per cent) 384 M^+ (93), 383 (M - 1) (100), 353 (M - 31) (23), 323 (M - 61) (15), 295 (15), 221 (23), 184 (23), 170 (31), 169 (31), 156 (23), 144 (15).

Chromogenic reactions: A greyish green colour was produced with ferric chloride - perchloric acid reagent and a greyish colour was obtained with ceric sulphate reagent.

Spectral data suggested a yohimbine-type structure. The MS showed the molecular ion at m/e 384 which is 30 mass units greater than that of

yohimbine. The β-carboline peaks occurred at m/e 184, 170, 169 and 156 indicating non-substitution on the aromatic ring.

Chromatographic behaviour was similar to that of methyl deserpidate as reported by Timmins (1975). Mixed melting points with an authentic sample of methyl deserpidate were undepressed. Co-TLC confirmed the identity of the compound as methyl-deserpidate.



methyl-deserpidate

RVOK 24 : White amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 226 (4.40), 282 (3.64), 291 (3.70);

λ_{min}^{MeOH} nm 252.

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2950 s, 2850 w, 2800 w, 1710 s, 1620 m, 1500 w, 1460 s, 1420 s, 1360 w, 1330 w, 1280 m, 1200 m, 1150 m, 1130 w, 980 m, 950 w, 940 w, 800 s, 740 w.

MS : m/e (relative intensity per cent) 400 M^+ (97), 399 (M - 1) (100), 385 (M - 15) (11), 383 (M - 17) (15), 369 (M - 31) (30), 341 (M - 59) (15), 339 (10), 311 (10), 297 (30), 281 (15), 253 (10), 251 (15), 239 (10), 222 (14), 214 (10), 200 (16), 199 (17), 186 (29).

HRMS : Accurate mass weight 400.20175 (calculated for $C_{22}H_{28}N_2O_5$, requires 400.199809; error 0.00195).

NMR ($CDCl_3$) : δ 8.00 (s, 1H, NH), 7.22 - 6.50 (m, 3H, aromatic), 4.40 (s, 1H), 3.99 (s, 1H), 3.84 (s, 3H, $COOCH_3$), 3.78 (s, 3H, OCH_3), 3.10 (s, 1H), 2.88 (m, 1H), 2.48 (dd, 2H), 2.13 (s, H), 1.76 (dd, 2H), 1.24 (d, 3H, C-19, CH_3).

Chromogenic reactions: A greyish-green colour was produced with ferric chloride - perchloric acid reagent.

The UV spectrum exhibited a yohimbine-type indole chromophore. The IR spectrum demonstrated a strong OH-absorption band at 3400 cm^{-1} and the maxima at 1710, 1280 and 1150 cm^{-1} indicated the presence of a carbomethoxy group and an ether linkage. The IR absorption maxima at 2850, 2800 and 2750 cm^{-1} are characteristic of compounds with C-3H α. (Wenkert, 1956).

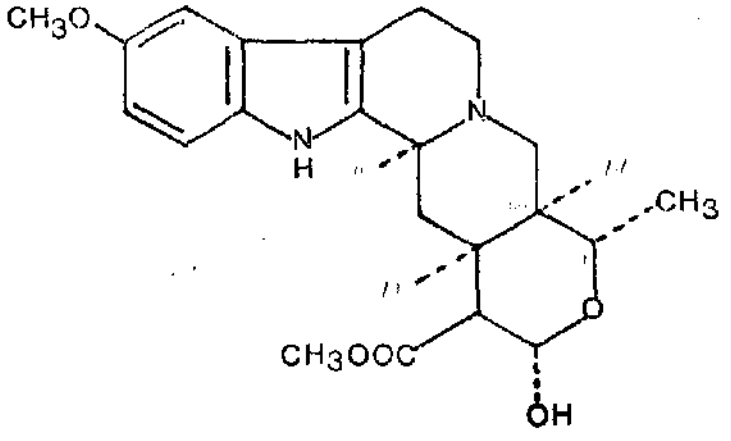
The MS indicated the presence of a methoxy substituent on the indole nucleus, β -carboline peaks occurring at m/e 214, 200, 199 and 186. The NMR aromatic multiplet signals at δ 7.22 - 6.50 and the OCH_3 singlet signal at δ 3.78 supported by the IR peaks at 740 and 800 cm^{-1} indicated a 1,2,4-pattern of substitution; therefore the methoxy group was at the C-10 position. The NMR spectrum also indicated the presence of $COOCH_3$ protons by the signals at δ 3.84 and of aliphatic $-CH_3$ protons at δ 1.24.

The IR spectrum showed twin absorption maxima at 1710 and 1620 cm^{-1} characteristic of heteroyohimbine alkaloids but the UV spectrum did not exhibit an inflexion at 250 nm thus suggesting a structure resembling cabucinine (Bombadelli et al, 1974).

The large coupling constant (10.0 c.p.s.) between the C-19 and C-20 protons of this compound implied a trans-diaxial relationship; from this it followed that the 19-methyl group had an α-configuration and the C-20 proton had an α-configuration (Wenkert et al, 1961). Also the intensities of the MS peaks at m/e 214, 200 and 199 indicated cis D/E ring fusion (Djerassi, 1963).

The NMR spectrum showed that the C-17, OH proton absorbed further upfield than reported for cabucinine, thus suggesting an axial orientation (Albright et al, 1963).

Considering the earlier isolation of the allo compounds tetrahydroalstonine, aricine and isoreserpiline from the weak base fraction of this plant, RVOK 24 is probably an allo epimer of cabucinine with additional epimerisation at the C-17 OH. The structure below is therefore proposed and the name rauvolcinine assigned to the compound which is 17-epi,20-epi cabucinine.



Rauvolcinine (17, 20-epi-cabucinine)

RVOK 25 : Yellow crystals; m.p. 286°C.

UV : λ_{max}^{MeOH} nm 225, 253, 306, 360; λ_{min}^{MeOH} nm 242, 280.

IR : ν_{max}^{KBr} cm^{-1} 3420 w, 2900 s, 1695 s, 1620 m, 1540 m, 1420 s, 1125 s, 740 s.

MS : m/e (relative intensity per cent) 348 M^+ (100), 333 (M - 15) (15), 319 (18), 317 (M - 31) (20), 305 (15), 289 (M - 59) (18), 261 (15), 247 (13), 221 (19), 206 (16), 205 (17).

The spectral data and chromatographic behaviour suggested an anhydronium base structure. The compound chromatographed differently from a reference sample of serpentine. The chromatographic behaviour in the solvent system ethanol : ethyl acetate :diethylamine : diethylformamide (12 : 6 : 1 : 1) agreed with the results reported by Bonati and Pesce (1966) and the compound was identified as alstonine.

Sodium borohydride reduction in cold yielded a compound which was identified as tetrahydroalstonine (RVOK 1). The UV, IR, MS and chromatographic behaviour were identical to that of an authentic sample of tetrahydroalstonine.

RVOK 25 was therefore confirmed as alstonine.

RVOK 26 : White crystals; m.p. 260°C (HCl).

UV : λ_{max}^{MeOH} nm (log E) 221 (4.80), 279 (4.00), 290 (3.80), inflexion at 250 nm.

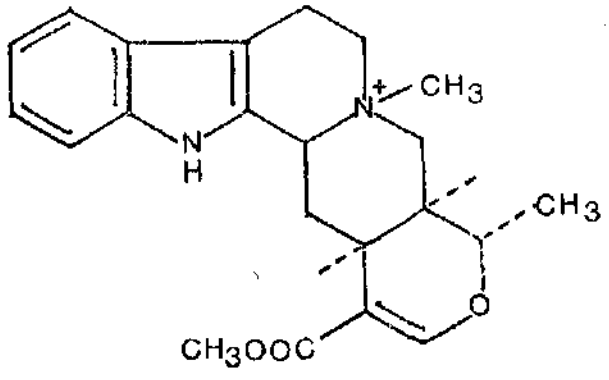
IR : ν_{max}^{KBr} cm^{-1} 3450 s, 2900 m, 1695 w, 1610 s, 1580 s, 1450 s, 1410 s, 1340 m, 1300 m, 1280 w, 1195 m, 1100 m, 1050 m, 1020 s, 750 s.

MS : m/e (relative intensity per cent) 366 M^+ (30), 365 (M - 1) (18), 352 (M - 15) (100), 351 (75), 337 (14), 321 (14), 307 (9), 293 (11), 279 (11), 265 (11), 249 (9), 239 (11), 225 (14), 223 (16), 209 (18), 198 (29), 184 (23), 169 (57), 156 (75), 144 (14), 130 (11).

Chromogenic reactions: A greyish colour was produced with ferric chloride - perchloric acid reagent and a faint yellow colour was obtained with ceric sulphate reagent.

The UV and IR spectra revealed a typical heteroyohimbine chromophore. On chromatographic analysis the compound remained at the base line when most neutral and basic solvent systems were used.

The MS fragmentation pattern resembled that of tetrahydroalstonine N_b-methyl (Hesse, 1965). A synthetic compound was prepared by reacting 5 mg of authentic tetrahydroalstonine with 2 mls of methyl iodide in cold for 18 hr. The reaction product was separated and purified by TLC to give a mixture of tetrahydroalstonine N_b-methyl and tetrahydroalstonine, N_a, N_b-methyl. The spectral data (UV, IR and MS) of RVCK 26 agreed with that of synthetic tetrahydroalstonine N_b-methyl and Co-TLC in 6 solvent systems confirmed the alkaloid as tetrahydroalstonine N_b-methyl.



Tetrahydroalstonine N_b-methyl

Alkaloids of R. volkensii stem bark

RVOSB 1 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with tetrahydroalstonine isolated from the root (RVOK 1).

RVOSB 2 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with isoreserpiline isolated from the root (RVOK 2).

RVOSB 3 : Showed identical spectral data (UV, IR, MS), chromogenic reactions and hRf values with carapanaubine isolated from the root (RVOK 6).

RVOSB 4 : Showed identical spectral data (UV, IR, MS, NMR), chromogenic reactions and hRf values with aricine isolated from the root (RVOK 4).

RVOSB 5 : Showed identical spectral data (UV, IR, MS), chromogenic reactions and hRf values with reserpiline isolated from the root (RVOK 5).

RVOSB 6 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 228 (4.55), 283 (3.90), 290 (3.82);

λ_{min}^{MeOH} nm 257.

IR : ν_{max}^{KBr} cm^{-1} 3450 s, 2950 s, 2850 w, 1720 m, 1650 m, 1590 w, 1520 w, 1470 s, 1430 w, 1380 w, 1280 m, 1220 m, 1160 w, 1120 s, 1040 w, 740 s.

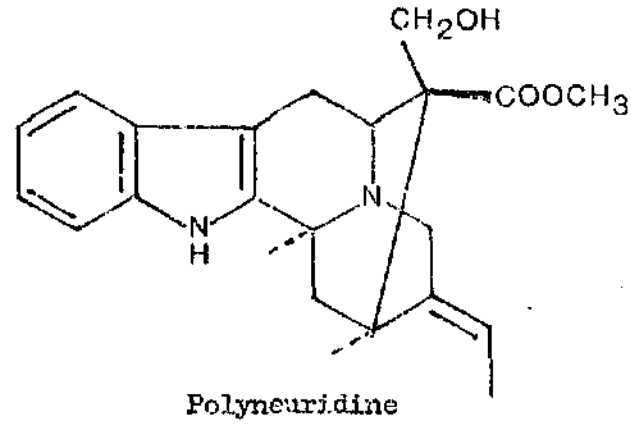
MS : m/e (relative intensity per cent) 352 M^+ (100), 351 (M - 1) (53), 337 (M - 15) (29), 334 (M - 18) (12), 321 (M - 31) (47), 307 (M - 42) (18), 293 (M - 59) (24), 291 (18), 263 (24), 249 (65), 236 (18), 221 (11), 207 (18), 198 (21), 183 (29), 182 (35), 169 (94), 168 (93), 156 (29).

HRMS : Accurate mass weight 352.17992 (calculated for $C_{21}H_{24}N_2O_3$, requires 352.178628; error 0.00124).

Chromogenic reaction: A greyish colour was obtained with ferric chloride - perchloric acid reagent and a yellow colour obtained with ceric sulphate reagent.

The UV spectrum indicated an unsubstituted indole chromophore. The MS fragmentation pattern suggested a sarpagan structure as MS peaks occurred at m/e 249, 182, 169, 168 and 156. The intensity of the m/e 249 peak suggested disubstitution at the C-16 position (Antonaccio et al, 1962). The MS in the upper mass region revealed peaks at m/e 321 and 293 which were attributable to the loss of CH_2OH and $COOCH_3$ fragments respectively. The IR spectrum exhibited absorption bands at 3450 cm^{-1} and 1720 cm^{-1}, thus confirming the presence of OH and C=O groups respectively in the molecule.

The above evidence suggested the structure of polyneuridine or akuammidine. The presence of the diagnostic M-18 peak at m/e 334 established the stereochemistry at C-16 as in polyneuridine (Antonaccio et al, 1962). RVOSB 6 was therefore identified as polyneuridine.



Polyneuridine

RVOSB 7 : White amorphous powder.

UV : λ_{max}^{MeOH} nm 228, 283, 290; inflexion at 250.

IR : ν_{max}^{KBr} cm^{-1} 3450 s, 2950 s, 2850 w, 1720 s, 1580 s, 1440 w, 1410 w, 1400 w, 740 s.

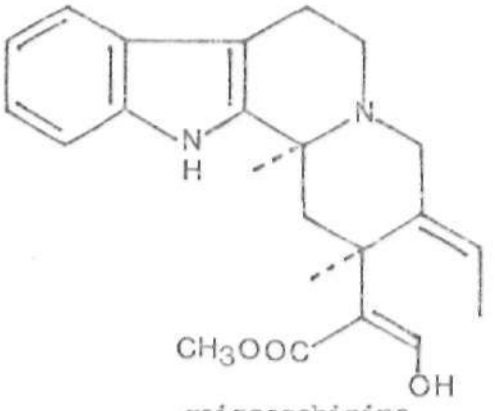
MS : m/e (relative intensity per cent) 352 M^+ (100), 351 (M - 1) (92), 337 (M - 15) (15), 321 (M - 31) (16), 293 (M - 59) (19), 277 (8), 253 (15), 251 (23), 239 (30), 237 (15), 223 (18), 221 (15), 211 (15), 209 (15), 184 (22), 170 (38), 169 (34), 156 (31).

Chromogenic reaction: A greenish colour was obtained with ferric chloride - perchloric acid reagent and a green-brown colour obtained with ceric sulphate reagent.

The UV spectrum indicated an indole chromophore of the heteroyohimbine type. The chromogenic reactions however suggested a possible E-seco indole structure. The MS fragmentation pattern indicated non-substitution of the indole nucleus, as β-carboline peaks occurred at m/e 184, 170, 169 and 156. The prominent MS peaks at m/e 251, 239 and 237 confirmed an E-seco indole structure with a C-16, C-17 double bond (Beckett et al, 1969; Hesse, 1974).

The IR spectrum revealed the presence of OH, NH and CH_3OOC groups (absorption bands at 3450 cm^{-1}, 2950 cm and 1720 cm^{-1} respectively). The UV spectrum also showed an inflexion at 250 nm which is characteristic of indole chromophores with a ($CH_3OOC - C = C - O -$) grouping in the molecule.

The above data agreed with the published data for *geissoschizine* (Yamada *et al*, 1974). Co-TLC with an authentic sample confirmed the alkaloid as *geissoschizine*.



geissoschizine

RVOSB 8 : Off-white crystals; m.p. 280°C (dec).

UV : λ_{max}^{MeOH} nm (log E) 243 (4.07), 292 (3.60);

λ_{min}^{MeOH} nm 222, 264.

IR : ν_{max}^{KBr} cm^{-1} 3350 m, 2910 s, 2810 w, 2700 w, 1600 m, 1460 s, 1440 w, 1390 w, 1320 s, 1300 s, 1220 m, 1160 w, 1100 m, 1080 w, 1070 w, 760 s, 730 s.

MS : m/e (relative intensity per cent) 294 M^+ (100), 279 (M - 15) (8), 277 (M - 17) (12), 263 (20), 249 (11), 235 (8), 223 (6), 211 (9), 184 (11), 169 (15), 168 (49), 164 (23), 156 (14), 144 (20), 143 (29), 130 (34), 117 (20).

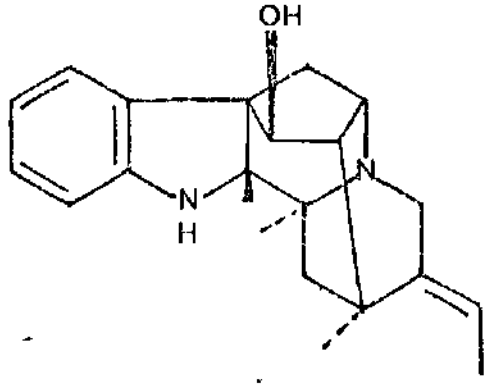
NMR ($CDCl_3$) : δ 7.30 - 6.60 (m, 4H, aromatic), 5.30 (q, 1H, C-19, H), 3.80 (s, 1H), 3.55 (m, 2H), 3.10 (d, 2H), 2.00 (s, 1H), 1.87 (s, 1H), 1.65 (d, 3H, C-19, CH_3).

Chromogenic reactions: An orange colour was produced with ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

The UV spectrum and chromogenic reactions indicated a dihydroindole chromophore. The MS fragmentation pattern and chromogenic reaction suggested a nor-dihydroindole structure (the β -carboline peaks occurred at m/e 169, 168, 144, 143, 130 and 117). The MS is consistent with the structure of N_a-demethyl-tetraphyllicine; the molecular ion peak occurred at m/e 294 which is 14 mass units less than that of tetraphyllicine.

By the absorption signals at δ 5.30 and 1.65 the NMR revealed the presence of an exocyclic ethylidene side chain.

The above data agreed with the data for nor-tetraphyllicine (Sabri and Court, 1978). Co-TLC with an authentic sample isolated from R. vomitoria stem bark confirmed the identity of the alkaloid as nor-tetraphyllicine. Mixed melting points were undepressed.



Nor-tetraphyllicine

RVOSB 9 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with tetraphyllicine isolated from the roots (RVOK 11).

RVOSB 10 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with ajmaline (RVOK 15).

RVOSB 11 : Showed identical spectral data (UV, IR, MS), chromogenic reaction and hRf values with α-yohimbine (RVOK 16).

RVOSB 12 : Showed identical hRf (UV, IR, MS), spectral data and chromogenic reactions with yohimbine (RVOK 17).

RVOSB 13 : White amorphous powder.

UV : λ_{max}^{MeOH} nm 227, 282, 290; λ_{min}^{MeOH} nm 249

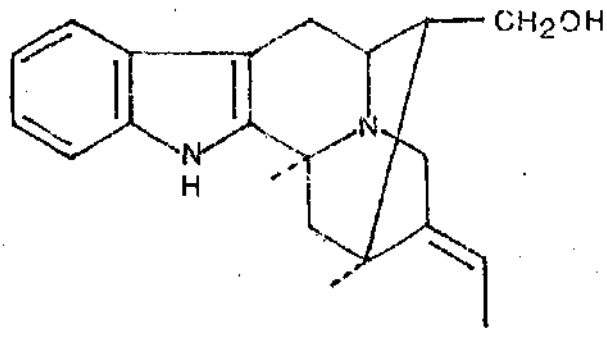
IR : ν_{max}^{KBr} cm^{-1} 3450 s, 2950 s, 2850 w, 1340 w, 1280 m, 1220 m, 1160 w, 1120 s, 1040 w, 830 w, 740 s.

MS : m/e (relative intensity per cent) 294 M^+ (100), 293 (M - 1) (93), 279 (M - 15) (27), 263 (M - 31) (30), 249 (27), 211 (19), 198 (20), 182 (21), 169 (60), 168 (47), 156 (20), 144 (13), 130 (20).

Chromogenic reactions: A greyish colour was obtained with ferric chloride - perchloric acid reagent and a faint yellow colour produced with ceric sulphate reagent.

The UV spectrum indicated an indole chromophore with a well-defined absorption minimum at 249 nm. The MS fragmentation pattern indicated a sarpagan-type structure and agreed with published data for normacusine B (deoxy-sarpagine) (Antonaccio et al, 1962).

Co-TLC with an authentic sample of normacusine B confirmed the identity of the alkalcid as normacusine B.



Normacusine B

RVOSB 14 : Off-white amorphous powder; $[\alpha]_D^{20^\circ}$ + 45° ($CHCl_3$) .

UV : λ_{max}^{MeOH} nm (log E) 226 (4.24), 283 (3.74), 290 (3.68);

λ_{min}^{MeOH} nm 250 (3.66).

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2925 m, 1700 m, 1660 w, 1580 s, 1440 m, 1230 s, 1120 s, 1030 m, 780 w, 740 s.

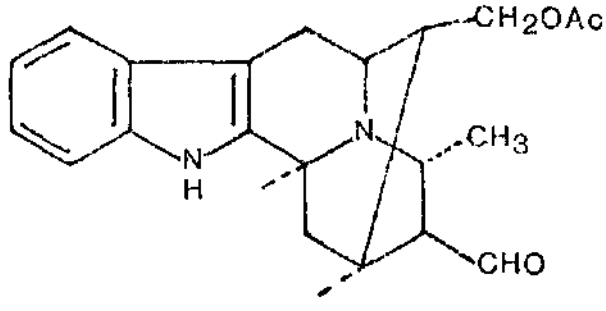
MS : m/e (relative intensity per cent) 352 M^+ (42), 351 (M - 1) (40), 323 (17), 309 (M - 43) (25), 279 (M - 73) (58), 265 (15), 251 (42), 237 (25), 223 (25), 209 (46), 196 (25), 183 (20), 182 (25), 169 (100), 168 (88), 156 (25).

NMR ($CDCl_3$) : δ 9.92 (d, 1H, CHO), 7.8 - 7.0 (m, 4H, aromatic), 4.92 (s, 1H), 4.16 (dd, 1H), 3.88 (s, 2H, CH_2-OAc), 2.74 (dd, 1H), 2.44 (q, 1H), 2.14 (s, 3H, $-OCOCH_3$), 1.56 (dd, 1H), 1.24 (d, 3H, CH_3).

Chromogenic reactions: A greyish colour reaction was obtained with ferric chloride - perchloric acid reagent and a yellow colour obtained with ceric sulphate reagent.

The UV spectrum exhibited an indole chromophore. The IR spectrum indicated two carbonyl absorption bands at 1700 cm^{-1} and 1660 cm^{-1}. The MS in the upper mass region indicated peaks at m/e 323 (M - 29), 309 (M - 43) and 279 (M - 73) which were attributable to loss of CHO, CH_3CO^+ and $-CH_2OCOCH_3$ fragments respectively. The general MS fragmentation pattern resembled that of perakine (Taylor et al, 1962; Khan et al, 1972) but RVOSB 14 spectrum exhibited a prominent M-1 peak as in sarpagan structure and the molecular ion peak was indicated at m/e 352 which is two mass units more than that of perakine.

The NMR spectrum confirmed the presence of the CHO, $OCOCH_3$ and the C-21, CH_3 protons (absorption signals at δ 9.92, 2.13 and 1.24 respectively). The structure of the compound was therefore concluded as the sarpagan-equivalent of perakine and the structure below is proposed.



Pre-perakine

RVOSB 15 : Small colourless prisms; m.p. 210°C; $[\alpha]_D^{20°}$ + 15° ($CHCl_3$).

UV : λ_{max}^{MeOH} nm (log E) 225 (4.50), 280 (3.85), 289 (3.70);

λ_{min}^{MeOH} nm 245 (3.00).

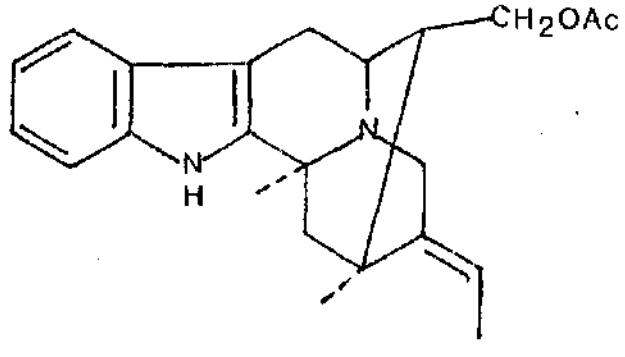
IR : ν_{max}^{KBr} cm^{-1} 2950 s, 1720 s, 1610 m, 1580 s, 1470 s, 1430 w, 1380 w, 1360 w, 1300 m, 1240 s, 1120 w, 1060 m, 1040 m, 1030 w, 860 m, 740 s.

MS : m/e (relative intensity per cent) 336 M^+ (100), 321 (M - 15) (10), 293 (M - 43) (20), 277 (M - 59) (35), 263 (M - 73) (20), 249 (15), 183 (20), 182 (20), 169 (50), 168 (45), 156 (20).

NMR ($CDCl_3$) : δ 7.4 - 6.4 (m, 4H, aromatic), 5.24 (q, 1H), 4.36 (dd, 1H), 4.23 (bd, 1H), 3.80 (s, 2H), 3.07 (dd, 1H), 2.96 (dd, 1H), 2.71 (dd, 1H), 1.86 (dd, 1H), 1.72 (dd, 1H), 1.54 (d, 3H, CH_3), 1.24 (s, 3H, $OCOCH_3$).

Chromogenic reactions: A greyish colour reaction was obtained with ferric chloride - perchloric acid reagent and a faint yellow colour produced with ceric sulphate reagent.

The spectral data agreed with published data for normacusine B acetate (Antonaccio et al, 1962). The synthetic normacusine B acetate prepared by reacting normacusine B with acetic anhydride : pyridine mixture at room temperature for 12 hr yielded identical spectral data, UV, IR, MS and chromatographic behaviour as RVOSB 15. This confirmed the identity of the alkaloid as normacusine B acetate.



Normacusine B acetate

RVOSB 16 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with peraksine isolated from the root (RVOK 19).

RVOSB 17 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with alstonine isolated from the root (RVOK 25).

Alkaloids of R. volkensii leaves

RVOLF 1 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with tetrahydroalstonine (RVOK 1).

RVOLF 2 : Showed identical spectral data (UV, IR, MS) and hRf values, and chromogenic reactions with geissoschizine (RVOSB 7).

RVOLF 3 : Off-white crystals; m.p. 158 - 160°C.

UV : λ_{max}^{MeOH} nm 227, 285; λ_{min}^{MeOH} nm 265.

IR : ν_{max}^{KBr} cm^{-1} 1730 s, 1670 m, 1580 s, 1500 w, 1460 m, 1420 s, 1200 w, 1120 s, 1040 w, 840 w, 740 s.

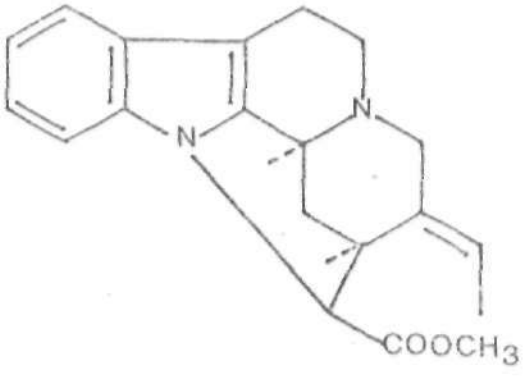
MS : m/e (relative intensity per cent) 322 M^+ (100), 307 (M - 15) (11), 291 (M - 31) (71), 263 (M - 59) (95), 249 (7), 246 (11), 234 (33), 232 (22), 223 (30), 210 (15), 205 (26), 194 (22), 182 (22), 181 (26), 180 (82), 167 (33), 155 (27), 140 (56), 131 (34), 121 (41), 108 (56).

NMR ($CDCl_3$) : δ 7.76 - 7.04 (m, 4H, aromatic), 5.48 (q, 1H), 4.72 (bd, 1H), 4.16 (s, 1H), 3.88 (s, 1H), 3.68 (s, 3H, OCH_3), 2.52 (s, 2H), 1.56 (d, 3H, C-19, CH_3), 1.28 (s, 1H).

Chromogenic reactions: A greyish colour was produced with ferric chloride - perchloric acid reagent.

The UV spectrum indicated a normal indole chromophore. The IR spectrum indicated the presence of a carbonyl group (1730 cm^{-1}) but showed no evidence of OH, NH and aromatic substitution. Attempted acetylation and MS-DIS indicated the absence of an active hydrogen atom (no change was observed in the MS of either product).

The MS fragmentation pattern was not typical of the commonly encountered Rauwolfia alkaloids. The spectrum indicated a prominent peak at m/e 263 (M - 59) which is attributable to the loss of a $COOCH_3$ fragment and three other prominent peaks occurred at m/e 234, 232 and 180. The fragmentation pattern agreed with published spectrum of pleiocarpamine (Hesse, et al, 1964; Le Quesne et al, 1973). Other spectral data (UV, IR, MS and NMR) and the melting point of this alkaloid agreed with the identification as pleiocarpamine.



Pleiocarpamine

RVOLF 4 : Light yellow amorphous powder; $[\alpha]_D^{20^\circ}$ - 75.3 (C = 0.1 in $CHCl_3$).

UV : λ_{max}^{MeOH} nm (log E) 230 (4.4), 283 (3.8); λ_{min}^{MeOH} 263 (3.6).

IR : ν_{max}^{KBr} cm^{-1} 1730 s, 1650 w, 1600 s, 1485 s, 1440 s, 1360 m, 1330 w, 1305 w, 1200 m, 1120 m, 1100 m, 1060 m, 1040 s, 1000 w, 910 m, 880 w, 850 w, 830 m, 790 m, 780 m, 740 w.

MS : m/e (relative intensity per cent) 352 M^+ (100), 351 (M - 1) (66), 321 (11), 293 (M - 59) (47), 271 (11), 264 (14), 262 (12), 253 (5), 244 (8), 238 (6), 212 (10), 210 (15), 198 (8), 186 (18), 168 (8), 154 (8), 121 (18).

NMR ($CDCl_3$) : δ 7.36 - 6.60 (m, 3H, aromatic), 5.45 (q, 1H), 4.64 (d, 1H) 4.12 (s, 1H), 3.92 (s, 1H), 3.80 (s, 3H, OCH_3), 3.68 (s, 3H, $COOCH_3$), 3.48 (bd, 1H), 3.16 (s, 1H), 3.00 (s, 1H), 2.68 (m, 3H) 2.16 (s, 1H), 2.08 (d, 1H), 1.56 (d, 3H, C-19, CH_3), 1.26 (s, 1H)

HRMS : Accurate mass weight 352.17735, calculated for $C_{21}H_{24}N_2O_3$ requires 352.178682; error 0.00133; m/e 293.16494, calculated for $C_{19}H_{21}N_2O$ requires 293.165370; error 0.00043.

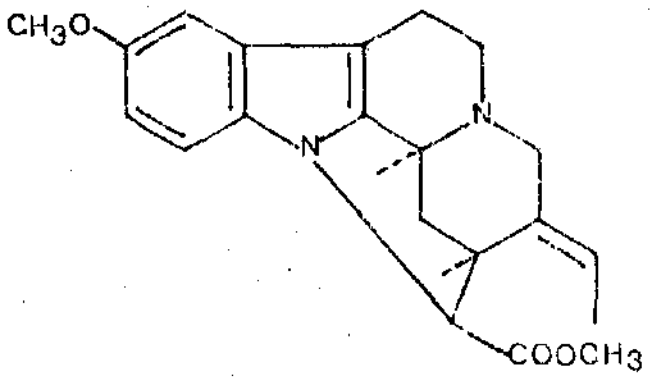
Chromogenic reactions: A greyish colour reaction was obtained with ferric chloride - perchloric acid reagent.

The UV spectrum indicated an indole chromophore. The IR spectrum revealed the presence of a carbonyl absorption maximum at 1730 cm^{-1} but showed no evidence of OH or NH groups. The absorption bands at 830 cm^{-1} and 790 cm^{-1} indicated a 1, 2, 4-pattern of aromatic substitution thus suggesting a substituent at the C-10 position. The MS fragmentation pattern resembled that of pleiocarpamine but the molecular ion peak occurred at m/e 352, which is 30 mass units greater than that of pleiocarpamine.

The NMR spectrum revealed the presence of two methoxy proton groups (δ 3.80 and δ 3.68). The aromatic multiplet signals at δ 7.36 - 6.60 and the OCH_3 singlet signal at δ 3.80 confirmed the 1, 2, 4-pattern of aromatic trisubstitution. The presence of an ethylidene side chain was indicated by the signals at δ 5.45 and 1.56.

Attempted acetylation and MS-DIS indicated the lack of an active hydrogen atom.

The above evidence indicated that the identity of RVOLF 4 was 10 - methoxy-pleiocarpamine and supported the conclusion that RVOLF 3 was pleiocarpamine.



10-methoxy-pleiocarpamine

RVOLF 5 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 210 (4.47), 249 (4.06), 297 (3.48).

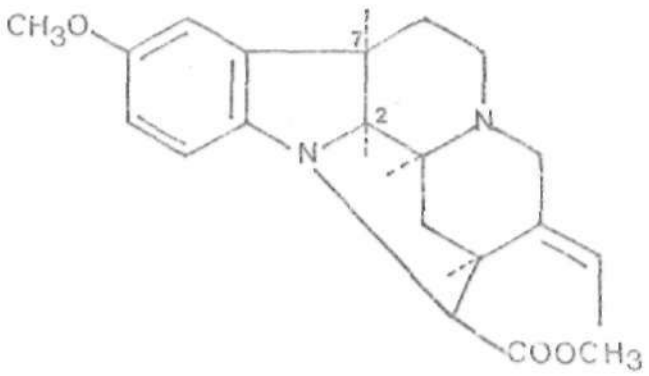
IR : ν_{max}^{KBr} cm^{-1} 1730 s, 1660 m, 1610 s, 1500 w, 1460 s, 1400 w, 1340 w, 1260 w, 1200 m, 1160 s, 1130 m, 1030 s, 820 m.

MS : m/e (relative intensity per cent) 354 M^+ (100), 339 (M - 15) (31), 323 (13), 295 (M - 59) (22), 281 (13), 263 (13), 232 (13), 224 (13), 200 (14), 180 (13), 174 (13), 173 (13), 161 (19), 160 (13).

Chromogenic reactions: A reddish-brown colour was produced with ferric chloride - perchloric acid reagent and a distinct red colour obtained with ceric sulphate reagent.

The UV spectrum and chromogenic reactions indicated a dihydroindole chromophore. The MS fragmentation pattern resembled that of 2:7 dihydro-pleiocarpamine (Hesse et al, 1964). The indole peaks however, appeared at m/e 174, 161 and 160 which suggested the presence of a methoxy substituent on the aromatic ring. The IR spectrum indicated a carbomethoxyl ester band (1730 cm^{-1} and 1200 cm^{-1}). There was also a medium absorption band at 820 cm^{-1} which confirmed the 1, 2, 4 substitution pattern of the aromatic ring of the indole moiety.

By reference to RVOLF 4 the compound was therefore identified as 10-methoxy-pleiocarpamine 2:7 dihydro.



10-methoxy-pleiocarpamine - 2:7 dihydro

RVOLF 6 : Off-white amorphous powder; $[\alpha]_D^{20°}$ - 56 ± 2 (C = 0.1 in $CHCl_3$)

UV : λ_{max}^{MeOH} nm (log E) 210 (4.70), 243 (4.1), 305 (3.90);

λ_{min}^{MeOH} 232 (3.9), 272 (3.3).

IR : υ_{max}^{KBr} cm^{-1} 3450 w, 2950 s, 1730 s, 1610 m, 1500 s, 1460 m, 1410 w, 1390 w, 1360 w, 1320 w, 1270 w, 1240 m, 1200 m, 1160 m, 1070 s, 1020 s, 990 m, 820 s, 750 w.

MS : m/e (relative intensity per cent) 412 M^+ (100), 397 (M - 15) (20), 383 (9), 381 (5), 353 (M - 59) (24), 325 (8), 313 (77), 276 (38), 268 (6), 254 (6), 231 (16), 218 (7), 204 (9), 169 (9), 136 (14).

HRMS : Accurate mass weight 412.19681, calculated for $C_{23}H_{28}N_2O_5$ requires 412.199809; error 0.00299; m/e 313.15591, calculated for $C_{18}H_{21}N_2O_3$ requires 313.155208, error 0.00071; m/e 276.12285 calculated for $C_{15}H_{18}N\,O_4$ requires 276.123574, error 0.00072.

NMR ($CDCl_3$) : δ 7.24 (s, 1H, aromatic), 6.20 (s, 1H, aromatic), 5.32 (q, 1H, C-19; H), 4.72 (d, 1H), 3.80 (s, 3H, $COOCH_3$), 3.72 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 3.36 (s, 1H), 3.24 (s, 1H), 2.88 (s, 3H, N_a-CH_3), 1.44 (d, 3H, C-19, CH_3).

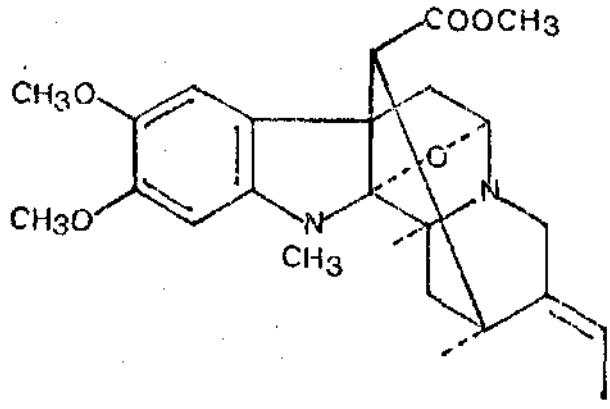
Chromogenic reactions: A brownish-red colour was produced with ferric chloride - perchloric acid reagent and an orange-yellow colour was produced with ceric sulphate reagent.

The UV spectrum and chromogenic reactions suggested a modified indoline chromophore. The UV absorption maximum at 305 nm indicated aromatic substitution. The NMR spectrum showed two separate methoxy absorption signals at δ 3.72 and 3.60 in addition to the carbomethoxy protons signal at δ 3.80. That these were aromatic methoxy groups was confirmed by the characteristic, 1, 4-pattern of the two aromatic protons at δ 7.42 and 6.20. The presence of an exocyclic ethylidene side chain was demonstrated by the signals at δ 5.32 and 1.44.

The MS fragmentation pattern was similar to that given by members of the picrinine group (Britten et al, 1963). The characteristic M-99 peak appeared at m/e 313 and, by applying the Biemann (1960) MS shift principle, the spectrum agreed with that of quaternine (10, 11, dimethoxy, N_a-methyl picrinine). The indole peaks (i.e. m/e 204 and 218) were 74 mass units greater than in the peaks of picrinine confirming the two aromatic methoxy groups and the N_a-CH_3 substituent.

The above data agreed with published data for quaternine isolated from Alstonia quaternata Heucket Muell (Apocynaceae) (Mamatas-kalamaras et al, 1975). Co-TLC with an authentic sample supplied by Dr. Claude Thal confirmed the identity of the compound.

It should be noted that quaternine as reported by the above authors is 10, 11, dimethoxy N_a-methyl-picrinine and not the nor base as stated in the abstract.



Quaternine

RVOLF 7 : White needles; m.p. 280; $[\alpha]_D^{20^\circ}$ - 65° (C = 0.1 in $CHCl_3$).

UV : λ_{max}^{MeOH} nm (log E) 213 (4.3), 244 (4.0), 303 (3.9);

λ_{min}^{MeOH} 231 (4.0), 267 (3.2).

IR : ν_{max}^{KBr} cm^{-1} 3400 m, 2900 m, 1730 s, 1610 m, 1500 m, 1460 s, 1380 w, 1330 m, 1250 w, 1230 w, 1195 m, 1160 s, 1120 s, 1070 m, 1030 m, 1020 m, 1000 m, 870 m, 820 m.

MS : m/e (relative intensity per cent) 398 (M^+) (100), 383 (M - 15) (13), 380 (M - 18) (79), 365 (M - 33) (29), 339 (M - 59) (25), 321 (13), 307 (8), 299 (M - 99) (50), 292 (13), 269 (13), 262 (28), 254 (10), 232 (13), 217 (13), 213 (13), 204 (10), 190 (17), 136 (25), 108 (21).

HRMS : Accurate mass weight 398.19063, (calculated for $C_{22}H_{26}N_2O_5$ requires 398.184159, error 0.00064); m/e 299.14391, (calculated for $C_{17}H_{19}N_2O_3$ requires 299.139558, error 0.00435); m/e 262.10674 (calculated for $C_{14}H_{16}N\,O_4$ requires 262.107925, error 0.00118).

NMR ($CDCl_3$) : δ 7.24 (s, 1H, aromatic), 6.36 (s, 1H, aromatic), 5.36 (q, 1H, C-19, H), 4.80 (s, 1H), 3.78 (s, 3H, $COOCH_3$), 3.74 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.40 (s, 1H), 3.26 (s, 1H), 3.12 (s, 1H), 2.96 (s, 1H), 2.40 - 2.02 (m, 3H), 1.88 (s, 1H), 1.48 (d, 3H, C-19, CH_3).

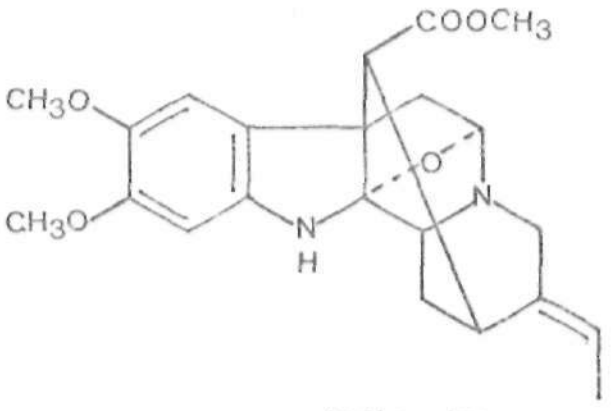
MS-DIS : 470 M^+ (25), 455 (10), 398 (100), 383 (13), 380 (79), 365 (29), 339 (25), 321 (13), 307 (8), 299 (50), 292 (13), 269 (13), 262 (28), 254 (10), 232 (13), 217 (13), 213 (13), 204 (10), 190 (17).

Chromogenic reactions: A brownish-red colour reaction was obtained with ferric chloride - perchloric acid reagent and an orange-yellow colour was obtained with ceric sulphate reagent.

The UV spectrum and chromogenic reactions indicated a modified indoline chromophore. The MS fragmentation pattern resembled that of quaternine (RVOLF 6) but the molecular ion peak appeared at m/e 398 which is 14 mass units less than that of quaternine. The indole peaks occurred at m/e 204 and 190 thus indicating two methoxy substituents on the aromatic ring. The MS-DIS indicated the presence of an active hydrogen atom. The MS exhibited a prominent M-18 peak at m/e 380. Similar peaks were observed in the MS of picrinine and picralstonine, both of which possess active N_a-hydrogen atoms (Chatterjee et al, 1965; Banerji et al, 1972).

The NMR spectrum indicated the presence of two methoxy proton signals at δ 3.74 and 3.64 and a $COOCH_3$ methoxy signal at δ 3.78. The characteristic 1,4-pattern of the two aromatic protons at δ 7.24 and 6.36 confirmed the two methoxy groups at 10 and 11 positions. The absorption signals at δ 5.36 and 1.48 indicated the presence of an exocyclic ethylidene side chain.

The above evidence identified RVOLF 7 as the N_a-demethyl analogue of quaternine (RVOLF 6). All spectral data (UV, IR, MS, NMR, MS-DIS) were consistent with this structure and as this alkaloid was the major alkaloid of the leaves of R. volkensii the name volkensine is proposed.



Volkensine

RVOLF 8 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm 223, 292, 335; λ_{min}^{MeOH} 263, 302.

$\lambda_{max}^{MeOH + N/10\ NaOH}$ nm 248, 299, 354; λ min nm 278, 312.

IR : ν_{max}^{KBr} cm^{-1} 3450 s, 2950 s, 1730 m, 1660 m, 1600 s, 1470 w, 1440 m, 1380 w, 1300 m, 1260 m, 1240 m, 1200 w, 1150 w, 1100 m, 1070 w, 780 m, 750 w, 730 w.

MS : m/e (relative intensity per cent) 338 M^+ (64), 324 (M - 15) (9), 322 (9), 309 (9), 307 (9), 295 (9), 279 (M - 59) (19), 268 (9), 265 (9), 263 (9), 250 (9), 232 (17), 222 (9), 212 (9), 199 (9), 196 (9), 182 (14), 180 (9), 172 (18), 170 (18), 169 (12), 121 (100), 107 (55), 92 (55).

HRMS : Accurate mass weight 338.16505 (calculated for $C_{20}H_{22}N_2O_3$ requires 338.163032; error 0.0020).

Chromogenic reactions: A bluish green colour reaction was produced with ferric chloride - perchloric acid reagent and a steel grey colour obtained with ceric sulphate reagent.

The UV spectrum demonstrated a 2-methyleno indole chromophore. A bathochromic shift of up to 10 nm occurred in N/10 sodium hydroxide solution thus indicating a phenolic methylene indole.

The MS fragmentation pattern resembled that of akuammicine except for the displacement of 16 m/e units of the upper end of the spectrum. The principle fragment ions appeared at m/e 121, 107 and 92 as in akuammicine (Budzikiewicz et al, 1964).

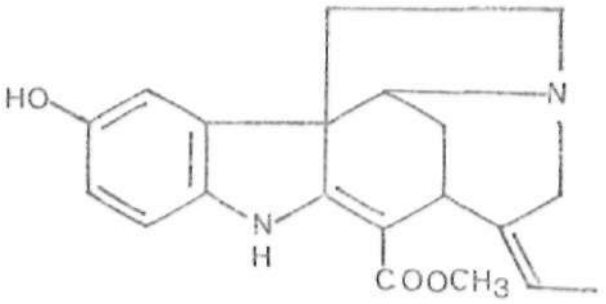
The acetyl derivative prepared by reacting 5 mg of RVOLF 8 with acetic anhydride : pyridine mixture at room temperature produced a mono - acetate

UV : λ_{max}^{MeOH} nm 230, 310, 340 .

MS : m/e (relative intensity per cent) 380 M^+ (56), 365 (M - 15) (7), 363 (3), 351 (14), 349 (7), 338 (7), 337 (7), 321 (14), 310 (7) 274 (17), 263 (7), 250 (3), 241 (7), 232 (3), 222 (7), 172 (7), 121 (100), 107 (55), 92 (50).

The MS of the acetyl derivative confirmed the acyl group on the indole moiety (m/e 232 in RVOLF 8 shifted to m/e 274 in the acetyl product).

The above data agreed with published data for sewerine (10-hydroxy akuammicine) (Ahmad and Le Quesne, 1970). The position of the phenolic OH-group was found to be similar to sewerine by comparing the magnitude of the bathochromic shift of the UV spectrum in alkaline medium with that of p-hydroxy aniline and m-hydroxy-aniline as described by the same authors.



10—Hydroxy-akuammicine (sewerine)

RVOLF 9 : Yellow amorphous powder.

UV : λ_{max}^{MeOH} nm 233, 279; λmin 258.

IR : ν_{max}^{KBr} cm^{-1} 1730 s, 1660 w, 1600 s, 1490 s, 1440 s, 1360 w, 1340 w, 1300 w, 1280 s, 1200 s, 1180 w, 1160 w, 1140 w, 1120 w, 1090 w, 1030 s, 910 w, 820 m, 780 w, 760 w.

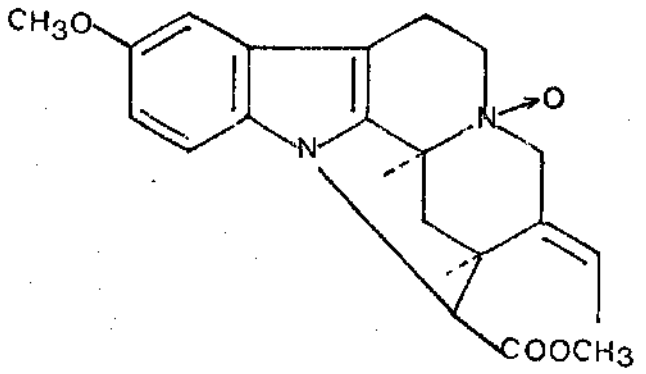
MS : m/e (relative intensity per cent) 368 M^+ (71), 352 (M - 16) (100), 351 (M - 17) (82), 337 (11), 321 (11), 310 (12), 293 (39), 291 (21), 277 (9), 271 (9), 263 (18), 250 (22), 224 (14), 210 (18), 198 (12), 197 (12), 186 (21).

Chromogenic reactions: A purplish colour turning grey was observed with ferric chloride - perchloric acid reagent and a yellow colour turning greenish was obtained with ceric sulphate reagent.

The UV and IR spectra were similar to those of methoxy-pleiocarpamine (RVOLF 4). In neutral solvent systems the compound stayed mainly on the base line. The MS fragmentation pattern indicated an N-oxide type of compound (prominent M-16 peak occurred at m/e 352). The remainder of the fragmentation was identical with the spectrum of methoxy-pleiocarpamine. The compound was therefore probably methoxy-pleiocarpamine N-oxide.

A synthetic methoxy-pleiocarpamine N-oxide was prepared by reacting 20 mg of m-chloroperbenzoic acid in 5 mls chloroform with 35 mg of methoxy-pleiocarpamine in dichloromethane (Cymerman et al, 1970). The reaction was continuously stirred and cooled in an ice-bath at about 0.5°C for three hours. The solvent was evaporated under reduced pressure and the residue made alkaline with 2 mls dilute ammonia solution. Extraction of the aqueous solution with chloroform (2 x 3 mls) provided the methoxy-pleiocarpamine N-oxide which was further purified by TLC (yield 15 mg).

Spectral data (UV, IR, MS) and co-TLC of this compound agreed with the data for RVOLF 9.



Methoxy-pleiocarpamine N-oxide

RVOLF 10 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with tetrahydroalstonine N_b-methyl (RVOK 26).

RVOLF 11 : Showed identical hRf values, spectral data and chromogenic reactions with nor-tetraphyllicine (RVOSB 8).

RVOLF 12 : Showed identical spectral data, hRf values and chromogenic reactions with peraksine (RVOK 19).

RVOLF 13 : Spectral data, hRf values and chromogenic reactions agreed with that of normacusine B (Deoxy-sarpagine) (RVOSB 13).

R. Oreogiton root alkaloids

ROG 1 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with ajmalicine (RVOK 3).

ROG 2 : Showed identical hRf values, spectral data (UV, IR and MS) and chromogenic reactions with isoreserpiline (RVOK 2).

ROG 3 : Showed identical hRf values, spectral data (UV, IR, MS and NMR) and chromogenic reactions with aricine (RVOK 4).

ROG 4 : Showed identical hRf values, spectral data (UV, IR and MS) and chromogenic reactions with carapaunabine (RVOK 6).

ROG 5 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with reserpiline (RVOK 5).

ROG 6 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with reserpine (RVOK 8).

ROG 7 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with rescinnamine (RVOK 9).

ROG 8 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with α -yohimbine (RVOK 16).

ROG 9 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with yohimbine (RVOK 17).

ROG 10 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with tetraphyllicine (RVOK 11).

ROG 11 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with ajmaline (RVOK 15).

R. Oreogiton leaves alkaloids

Oreog 1 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with geissoschizine (RVOSB 7).

Oreog 2 : Showed identical hRf values, spectral data (UV, IR, MS, NMR) and chromogenic reactions with aricine (RVOK 4).

Oreog 3 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 223 (4.20), 265 (3.75);

λ_{min}^{MeOH} nm 248 (3.72).

IR : ν_{max}^{KBr} cm^{-1} 3400 m, 2925 w, 1740 s, 1620 w, 1600 w, 1440 m, 1360 w, 1240 s, 1120 m, 1080 m, 750 m.

MS : m/e (relative intensity per cent) 424 M^+ (80), 409 (M - 15) (50), 392 (M - 32) (20), 382 (20), 367 (25), 365 (M - 59) (30), 351 (M - 73) (100), 333 (M - 91) (60), 292 (20), 279 (20), 260 (20), 247 (35), 232 (25), 218 (20), 206 (20), 194 (25), 182 (30), 168 (30), 156 (25).

NMR ($CDCl_3$) : δ 7.80 - 7.12 (m, 4H, aromatic), 5.52 (q, 1H, C-19 H), 4.48 (s, 1H), 4.08 (s, 1H), 3.80 (s, 3H, $COOCH_3$), 3.48 (s, 2H, CH_2OA_c), 3.20 (s, 2H), 3.00 (s, 1H), 2.36 (s, 1H), 1.96 (d, 1H), 1.64 (d, 3H, C-19, CH_3), 1.56 (s, 3H, $OCOCH_3$), 1.24 (s, 1H).

Chromogenic reactions: No colour reaction was obtained with ferric chloride - perchloric acid reagent or with ceric sulphate reagent. A positive reaction was however obtained with Dragendorff's reagent.

The UV spectrum and chromogenic reaction indicated an indolenine chromophore. A bathochromic shift occurred in concentrated acid medium; $\lambda_{max}^{MeOH + HCl}$ nm 229, 285; λmin 255.

The MS and NMR spectra indicated no evidence of aromatic substitution (MS showed β-carboline peaks at m/e 182, 168 and 156 and the NMR indicated four aromatic proton multiplet signals at δ 7.80 - 7.12).

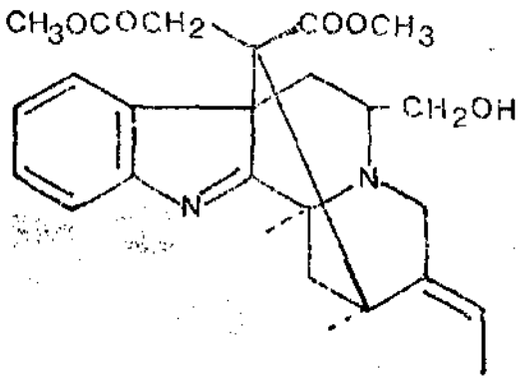
The MS showed in the upper mass region prominent peaks at m/e 409 (M - 15), 392 (M - 32), 382 (M - 42), 365 (M - 59), 351 (M - 73), and 333 (M - 91) which can be attributed to loss of the following fragment ions:- CH_3, CH_3OH, CH_2CO, $COOCH_3$, CH_2OCOCH_3 and $CH_3OH + COOCH_3$ respectively. The NMR spectrum confirmed the presence of $COOCH_3$ and $OCOCH_3$ protons by the absorption signals at δ 3.80 and 1.56 respectively. Evidence of an ethylidene side chain was indicated by the absorption signals at δ 5.52 and 1.64.

The MS peak at m/e 279 is attributable to loss of C-16 carbon atom and attached substituents plus one hydrogen atom ($CH_3OCOCH_2 - CH - COOCH_3 + H$) while the peak at m/e 247 could be m/e 279 - MeOH.

The IR spectrum indicated the presence of an unconjugated ester band at 1740 cm^{-1} and a hydroxyl band at 3400 cm^{-1}. The alkaloid on acetylation produced an amorphous mono-acetyl derivative (M.wt.466).

The MS general fragmentation pattern resembled that of alkaramiline (Hesse, 1974). However the loss of a CH_3OH fragment i.e. (M - 32) and the occurrence of the β-carboline peaks at m/e 182, 168 and 156 are characteristic features of tetrahydro β-carboline compounds with substituents at the C-5 position. (Budzikiewicz et al, 1964; Brown et al, 1972).

The above evidence is consistent with the structure of akuammiline 5-formyl. الدهلول



Akuammiline 5-formyl الدهلول

Oreog 4 : White crystals; m.p. 180°C.

UV : λ_{max}^{MeOH} nm (log E) 214 (4.23), 249 (4.00), 284 (3.67);

λ_{max}^{MeOH} nm 234 (3.91), 273 (3.54).

IR : ν_{max}^{KBr} cm^{-1} 2950 s, 2850 s, 1740 s, 1610 m, 1480 s, 1330 w, 1120 m, 1070 m, 1020 w, 940 m, 790 w, 740 s.

MS : m/e (relative intensity per cent) 340 M^+ (10), 312 (M - 28) (16), 295 (1), 282 (3), 267 (7), 255 (22), 239 (9), 224 (40), 210 (4), 199 (16), 196 (18), 182 (21), 168 (100), 158 (54), 144 (78), 138 (57), 131 (13), 122 (13), 108 (15), 96 (33), 94 (89).

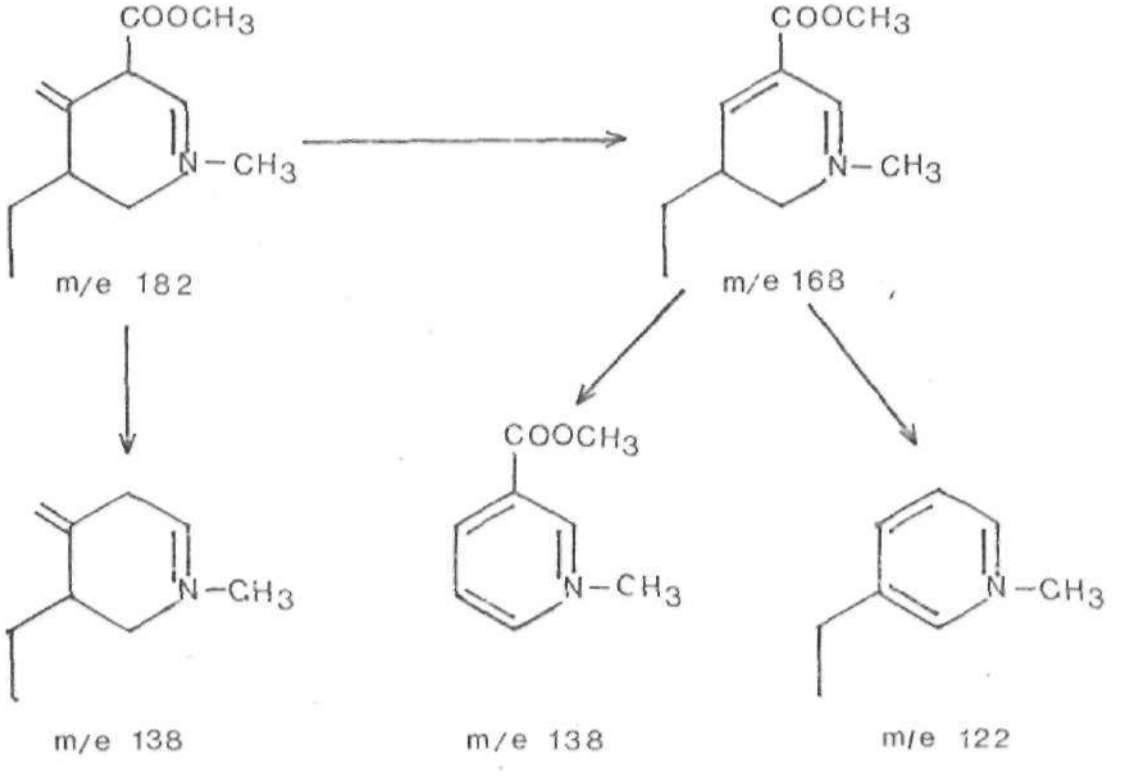
NMR ($CDCl_3$) : δ 7.40 - 6.72 (m, 4H, aromatic), 3.92 (s, 1H), 3.72 (bd, 3H), 3.44 (s, 1H), 3.36 (s, 1H), 2.76 (s, 3H, N-CH_3), 2.72 (s, 3H, N-CH_3), 2.64 (s, 1H), 2.52 (s, 1H), 1.84 (s, 1H), 1.72 (s, 1H), 1.56 (dd, 3H), 1.28 (d, 2H, C-19 H), 1.00 (t, 3H, C-19 CH_3).

Chromogenic reactions: An instant red colour was obtained with both ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

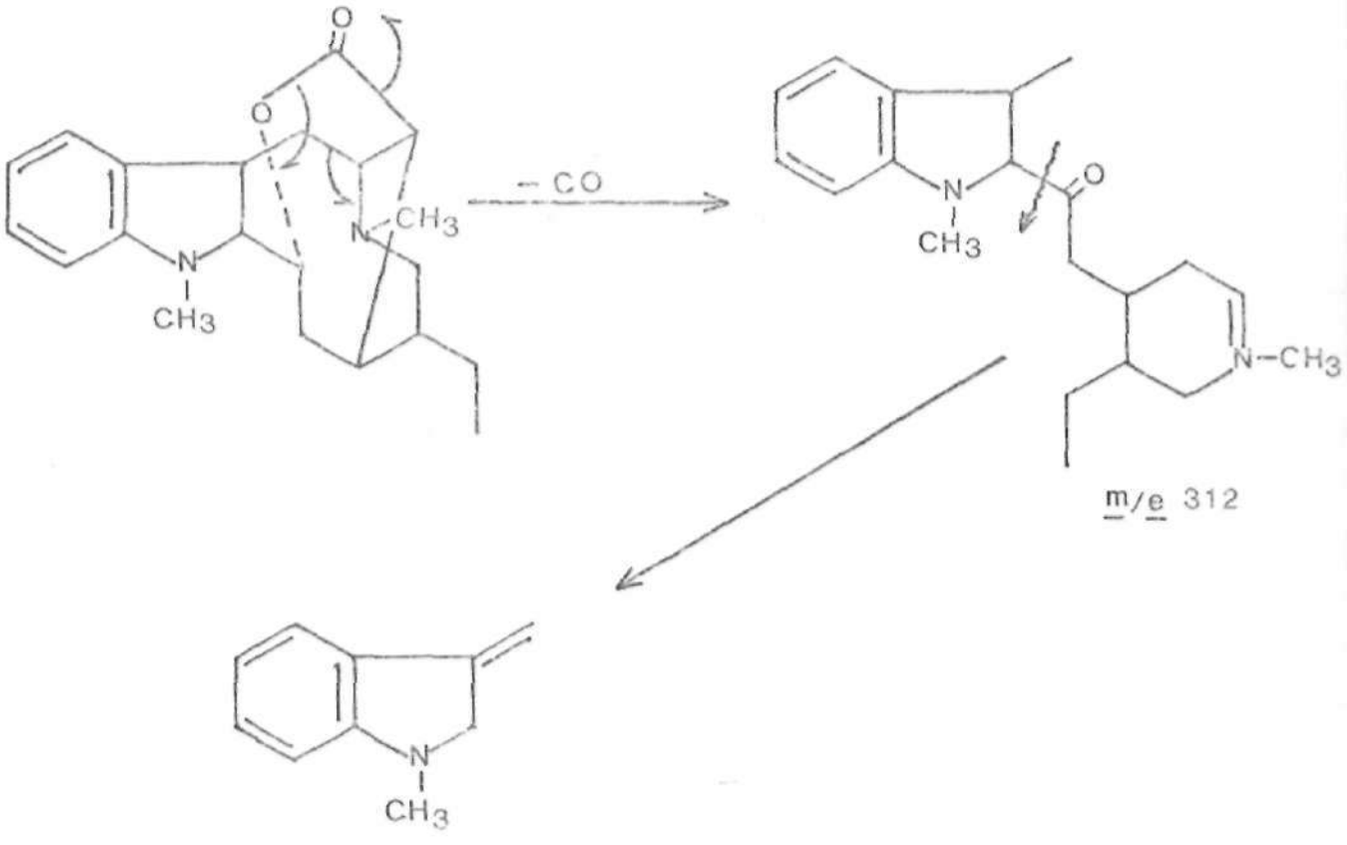
The UV spectrum and chromogenic reactions indicated a dihydroindole chromophore. The IR spectrum revealed the presence of a carbonyl absorption band at 1740 cm^{-1} but no evidence of a free hydroxyl group. Attempted acetylation produced no acetyl derivative.

The NMR spectrum showed evidence of an ethyl side chain (doublet signal at δ 1.28 corresponding to C-19 CH_2 protons and the triplet signal at δ 1.00 corresponding to C-19 CH_3 protons). The spectrum also indicate evidence of two aliphatic N-methyl proton signals at δ 2.76 and 2.72. Th MS indicated the indole peaks at m/e 158 and 144 thus confirming evidence of an N_a-methyl substituent.

The MS showed in the upper mass region a sharp M-28 peak at m/e 312 which is characteristic of a ketone group, in a ring system (Biemann et a 1964). The overall fragmentation pattern resembled that of acyl indole alkaloids. By analogy to tabernaemontanine (Combes et al, 1966) the peaks at m/e 182, 168, 138 and 122 corresponded both in intensity and position t the peaks at m/e 196, 182, 152 and 122 respectively in tabernaemontanine and could be represented as shown below:-

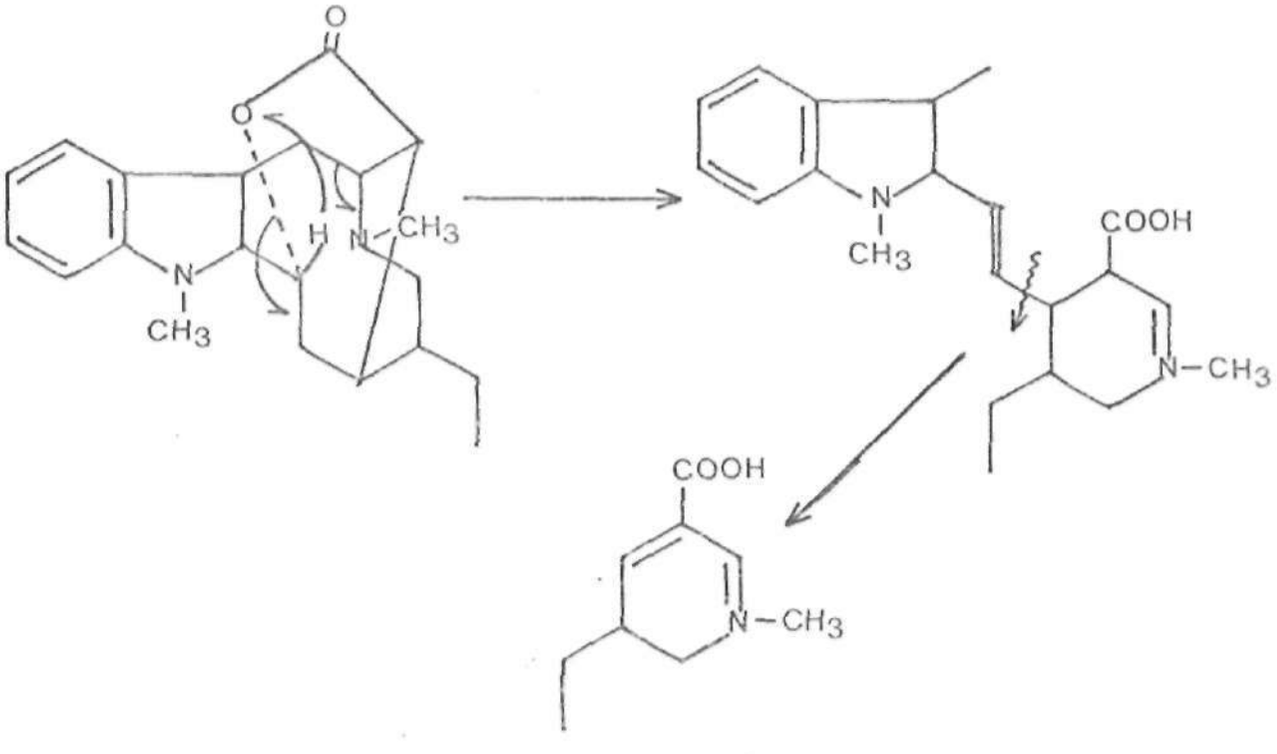


Proposed Fragmentation pattern by analogy to tabernaemontamine (Combes et al. 1966).



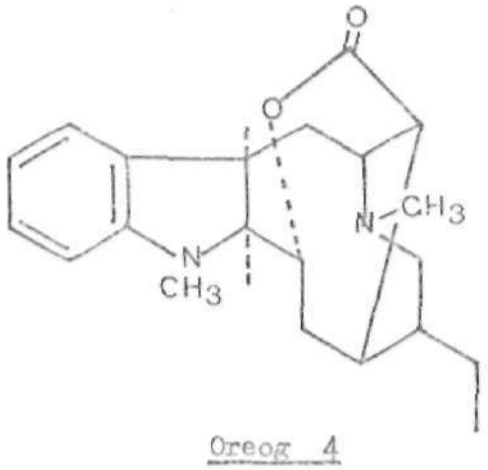
m/e 312

m/e 144



m/e 168

The sharp M-28 peak in the MS and the inability of the alkaloid to form an acetyl derivative indicated that the COOH group at C-16 position is in a bonded form. On the above evidence the structure below is proposed for the alkaloid



Oreog 4

Oreog 5 : White crystals; m.p. 158°C.

UV : λ_{max}^{MeOH} nm (log E) 217 (4.08), 222 (4.08), 267 (3.54);

λ_{min}^{MeOH} nm 243 (3.39).

IR : ν_{max}^{KBr} cm^{-1} 3400 m, 2980 w, 1730 s, 1620 m, 1600 m, 1440 s, 1280 m, 1230 s, 1100 m, 1080 w, 1060 m, 1030 m, 960 s, 840 m, 780 m, 750 s.

MS : m/e (relative intensity per cent) 394 M^+ (100), 379 (M - 15) (4), 363 (M - 31) (6), 352 (M - 42) (6), 335 (M - 59) (40), 321 (M - 73) (68), 306 (8), 292 (8), 275 (10), 261 (8), 249 (14), 232 (12), 223 (8), 218 (8), 206 (8), 194 (10), 180 (16), 170 (14), 168 (16), 156 (12).

NMR ($CDCl_3$) : δ 7.40 - 7.04 (m, 4H, aromatic), 5.52 (q, 1H), 4.60 (d, 1H), 4.16 (s, 1H), 4.00 (s, 1H), 3.76 (s, 3H, $COOCH_3$), 3.56 (s, 2H, CH_2OAc), 3.20 (s, 1H), 3.04 (s, 1H), 2.64 (d, 1H), 2.52 (d, 1H), 2.36 (t, 1H), 2.16 (s, 1H), 1.96 (dd, 1H), 1.64 (d, 3H, C-19 CH_3), 1.56 (s, 3H, $OCOCH_3$), 1.24 (s, 1H).

Chromogenic reactions: No colour reaction was obtained with ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

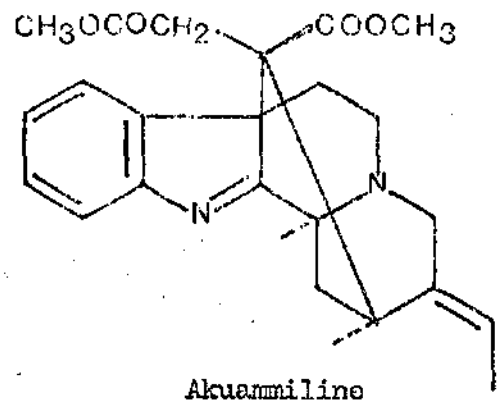
The UV spectrum demonstrated an indolenine chromophore : a bathochromic shift was observed in concentrated acid medium.

$\lambda_{max}^{MeOH + HCl}$ nm 228, 283; λ_{min} nm 225.

The IR spectrum disclosed an unconjugated carbonyl ester band at 1730 cm^{-1} and an ortho-disubstituted benzene nucleus at 750 cm^{-1} thus indicating non-substitution on the indole aromatic ring. The MS and NMR spectra confirmed non-substitution on the aromatic ring (β -carboline peaks occurred at m/e 180, 170, 168 and 156) : the NMR spectrum showed the typical four aromatic proton multiplet at δ 7.40 - 7.04.

The MS in the upper mass region indicated prominent peaks at m/e 379 (M - 15), 363 (M - 31), 352 (M - 42), 335 (M - 59) and 321 (M - 77) which were attributable to the loss of CH_3, CH_3O, CH_3CO, $COOCH_3$ and CH_2OCOCH_3 fragments respectively. The NMR signals at δ 3.76 confirmed the presence of $COOCH_3$ protons and the signals at δ 3.56 and 1.56 were indicative of the CH_2OAc and the $OCOCH_3$ protons respectively. The absorption signals at δ 5.52 and 1.64 demonstrated evidence of an exocyclic ethylidene side chain.

The MS peak at m/e 249 is attributable to the loss of the C-16 carbon atom and its attached substituents plus one hydrogen atom. The above data agreed with published data for akuammiline (Robinson and Thomas, 1954; Oliver et al, 1964; Hesse, 1974).



Akuammiline

Oreog 6 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 222 (4.25), 267 (3.76); λ max nm 247 (3.70).

IR : ν_{max}^{KBr} cm^{-1} 3350 s, 2950 s, 1730 s, 1660 m, 1620 w, 1600 m, 1440 s, 1230 s, 1100 m, 1070 m, 960 w, 920 w, 840 m, 780 m, 750 s.

MS : m/e (relative intensity per cent) 352 M^+ (100), 335 (M - 15) (11), 321 (M - 31) (95), 307 (5), 293 (M - 59) (23), 261 (14), 249 (18), 232 (18), 206 (14), 194 (14), 180 (23), 170 (18), 168 (23), 156 (16).

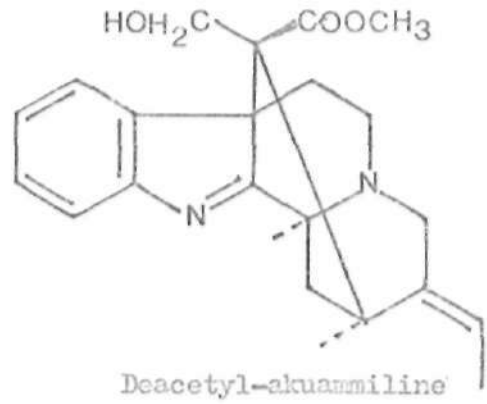
NMR ($CDCl_3$) : δ 7.84 - 7.24 (m, 4H, aromatic), 5.56 (q, 1H), 4.24 (s, 1H), 4.12 (s, 1H), 3.90 (s, 3H, $COOCH_3$), 3.76 (dd, 1H), 3.28 (s, 1H), 3.08 (s, 1H), 2.96 (d, 2H), 2.68 (s, 1H), 2.56 (s, 1H), 2.36 (m, 1H), 2.00 (s, 1H), 1.64 (d, 3H, C-19, CH_3), 1.28 (s, 1H).

Chromogenic reactions: No colour reaction was obtained with ferric chloride - perchloric acid reagent or with ceric sulphate reagent.

The UV spectrum indicated an indolenine chromophore. Other spectral data (IR, MS and NMR) revealed characters resembling akuammiline (Oreog 5). The IR spectrum however demonstrated a broad hydroxyl absorption band at 3350 cm^{-1} and the MS showed the molecular ion peak at m/e 352 which is 42

mass units less than that of akuammiline. The methyl protons signal at δ 1.50 of the acetate group in akuammiline was absent from the NMR spectrum of the compound. Oreog 6 which is therefore deacetyl-akuammiline. (Oliver et al, 1965).

An acetyl derivative of the compound was prepared and the spectral data (UV, IR, MS and NMR) compared with that of akuammiline (Oreog 5). Such data were found to be identical and mixed melting points of the compounds showed no depression. The chromatographic behaviours were identical (Co-TLC in six different solvent systems). The alkaloid was therefore identified as deacetyl-akuammiline.



Deacetyl-akuammiline

Oreog 7 : Colourless crystals; m.p. 226°C.

UV : λ_{max}^{MeOH} nm (log E) 210 (4.18), 232 (3.90), 287 (3.50);

λ_{min}^{MeOH} nm 225 (3.85), 258 (3.08).

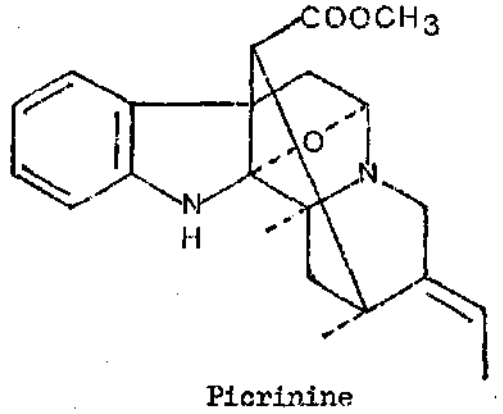
IR : υ_{max}^{KBr} cm^{-1} 3400 s, 2900 s, 1720 s, 1610 m, 1490 w, 860 w, 810 w, 750 m.

MS : m/e (relative intensity per cent) 338 M^+ (100), 323 (M - 15) (17), 320 (M - 18) (80), 308 (15), 279 (M - 59) (30), 239 (70), 182 (17), 170 (20), 168 (17), 157 (22), 144 (20), 130 (15).

Chromogenic reactions: An orange colour turning brown on heating was produced with ferric chloride -- perchloric acid reagent.

The UV spectrum indicated a dihydroindole chromophore which changed to a 3H-indolium type in the presence of concentrated perchloric acid (70%); $\lambda_{max}^{MeOH + 70\% HClO_4}$ nm 234, 245, 305.

Other spectral data (UV, IR, MS) agreed with published data for picrinine (deacetyl-desformo-picraline) (Chatterjee et al, 1965; Iwu and Court, 1978). Co-TLC with an authentic sample confirmed the identity of the alkaloid as picrinine. Mixed melting points were undepressed.



Picrinine

Oreog 8 : Showed identical hRf values, spectral data (UV, IR, MS, NMR) and chromogenic reaction with quaternine (RVOLF 6).

Oreog 9 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 213 (4.19), 244 (3.89), 303 (3.52);

λ_{min}^{MeOH} nm 227 (3.68), 268 (3.00).

IR : ν_{max}^{KBr} cm^{-1} 3400 m, 2950 s, 1740 s, 1600 m, 1580 m, 1470 m, 1380 m, 1200 m, 1120 m, 1040 m, 990 m, 920 w, 890 w, 800 w, 780 m, 740 s.

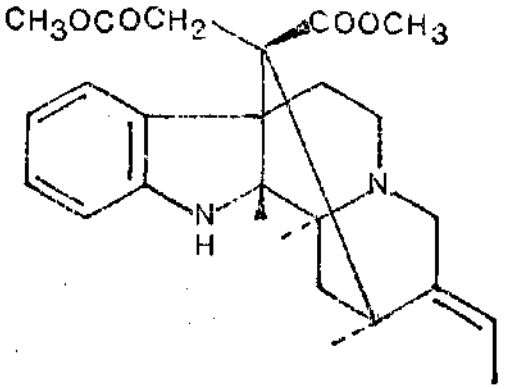
MS : m/e (relative intensity per cent) 396 M^+ (100), 381 (M - 15) (35), 369 (4), 354 (4), 337 (M - 59) (35), 323 (4), 321 (4), 277 (4), 263 (4), 251 (4), 235 (4), 221 (4), 194 (4), 182 (9), 168 (17), 156 (13), 144 (13), 130 (13).

NMR ($CDCl_3$) : δ 7.88 (d, 1H, NH), 7.24 - 6.52 (m, 4H, aromatic), 5.58 (s, 1H, C-19 H), 4.92 (d, 1H), 4.12 (s, 1H), 4.00 (s, 1H), 3.84 (s, 3H, $COOCH_3$), 3.80 (s, 1H), 3.58 (s, 1H), 3.36 (s, 1H), 3.12 (s, 1H), 2.96 (s, 1H), 2.08 (s, 3H, $OCOCH_3$), 1.80 (d, 3H, C-19, CH_3), 1.28 (s, 1H).

Chromogenic reactions: A red colour reaction was obtained with ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

The UV spectrum and chromogenic reactions indicated a dihydroindole chromophore. The MS fragmentation pattern resembled that of akuammiline (Oreog 5) but the molecular ion peak occurred at m/e 396 which is two mass units more than for akuammiline. The NMR spectrum showed an NH proton signal at δ 7.88 and the MS showed a distinct indole peak at m/e 144 and 130. The compound could therefore be a dihydroindole derivative of akuammiline.

Catalytic hydrogenation of akuammiline (at pH 10) using 10% palladium on alumina for 12 hrs yielded on purification a relatively small quantity of the dihydro derivative (Biemann et al, 1963). Direct comparison of the spectral data (UV, IR, MS) and chromogenic reactions of this product with that of Oreog 9 showed that the two compounds were identical. The intensity of the indole peaks at m/e 144 and 130 indicated a C-2 H β- dihydroindole compound (Biemann et al, 1964). The alkaloid was therefore identified as 2β-dihydro - akuammiline.



2 - β-dihydro-akuammiline

Oreog 10 : Colourless crystals; m.p. 242°C.

UV : λ_{max}^{MeOH} nm (log E) 213 (4.23), 245 (4.10), 310 (3.70);

λ_{min}^{MeOH} nm 227 (3.83), 273 (3.08).

IR : ν_{max}^{KBr} cm^{-1} 3400 w, 2950 s, 2890 m, 1730 s, 1600 m, 1480 s, 1440 s, 1280 s, 1220 w, 1100 m, 1070 w, 1030 w, 1000 m, 940 m, 900 m, 860 w, 800 m, 720 m.

MS : m/e (relative intensity per cent) 396 M^+ (100), 381 (M - 15) (4), 379 (M - 17) (4), 366 (M - 30) (30), 350 (M - 45) (11), 335 (M - 61) (14), 323 (14), 307 (46), 296 (21), 293 (25), 279 (14), 269 (18), 264 (21), 260 (14), 245 (11), 228 (7), 222 (13), 214 (21), 200 (18), 198 (14), 188 (18), 187 (14), 174 (18), 121 (25).

Chromogenic reactions: An orange-red colour reaction was obtained with ferric chloride - perchloric acid reagent and a red colour produced with ceric sulphate reagent.

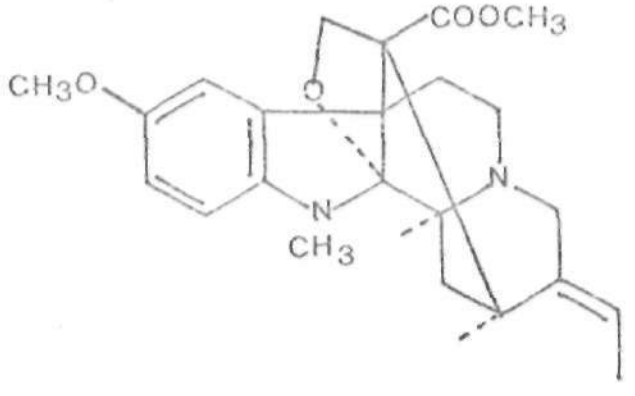
The UV spectrum and the chromogenic reactions were typical of indoline bases. The UV spectrum showed a marked bathochromic shift in

70% perchloric acid medium, which is characteristic of 3H-indolinium ion ($\lambda_{max}^{MeOH:HClO_4}$ nm 212, 257, 248; λ min 236, 278).

The IR spectrum showed absorption at 800 cm^{-1} which is indicative of a 1, 2, 4-trisubstituted benzene ring and thus suggested aromatic substitution at the C-10 position of the indole nucleus. The IR spectrum also indicated a carbonyl ester band at 1730 cm^{-1}.

The MS revealed β-carboline peaks at m/e 214, 200, 188 and 174 which corresponded by application of the MS shift technique to a shift of 44 m/e units and thus indicated the presence of a methoxy group on the aromatic ring and a methyl group on the N_a-nitrogen atom.

The above data agreed with published data for akuammine, O-methyl (Rakhimov et al, 1971). Identical melting points and chromatographic behaviour confirmed the identity of the alkaloid as akuammine O-methyl.



Akuammine O-methyl

Oreog 11 : White crystals; m.p. 182°C.

UV : λ_{max}^{MeOH} nm (log E) 210 (4.16), 232 (3.85), 287 (3.45);

λ_{min}^{MeOH} nm 225 (3.85), 258 (3.08).

IR : υ_{max}^{KBr} cm^{-1} 3400 s, 2950 s, 1730 s, 1610 w, 1450 m, 1380 w, 1360 w, 1280 w, 1230 s, 1100 m, 1020 m, 920 w, 850 w, 810 w, 750 m.

MS : m/e (relative intensity per cent) 410 M^+ (64), 392 (M - 18) (25), 382 (M - 28) (9), 367 (M - 43) (9), 351 (M - 59) (28), 337 (M - 73) (100), 333 (50), 305 (9), 291 (13), 260 (9), 247 (22), 239 (88), 232 (16), 206 (9), 197 (31), 194 (22), 182 (22), 170 (31), 157 (33), 144 (16), 130 (22).

NMR ($CDCl_3$) : δ 7.60 - 6.80 (m, 4H, aromatic), 5.48 (q, 1H), 5.00 (m, 1H), 4.84 (d, 1H), 4.68 (s, 1H), 4.56 (s, 1H), 4.00 (s, 1H), 3.92 (s, 1H), 3.76 (s, 3H, $COOCH_3$), 3.36 (s, 1H), 3.24 (d, 1H), 2.04 (dd, 2H), 1.64 (d, 3H, C-19 CH_3), 1.60 (s, 3H, $OCOCH_3$), 1.32 (s, 1H).

Chromogenic reactions: An orange colour turning brown on heating was produced with ferric chloride - perchloric acid reagent and a red colour reaction obtained with ceric sulphate reagent.

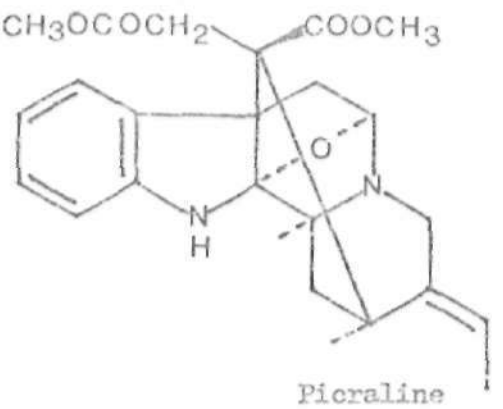
The UV spectrum and chromogenic reactions indicated an indoline chromophore. The IR spectrum contained bands due to NH (2950 cm^{-1}), carbonyl ester (1730 cm^{-1}) and an ortho - disubstituted benzene nucleus (750 cm^{-1}), thus suggesting non-substitution on the indole moiety.

The NMR confirmed the presence of the carbomethoxy protons (signals at δ 3.76) and also showed evidence of acetoxy protons (δ 1.60) and an ethylidene side chain (δ 5.48 and 1.64). The absorption signal at δ 4.84 is characteristic of a carbinolamine proton (Chatterjee et al. 1965).

The MS in the upper mass region showed prominent peaks at m/e 392 (M-18) 351 (M - 59), 337 (M - 73) and 333 (M - 77) which were attributable to the loss of H_2O, $COOCH_3$ and CH_2OAc and to m/e 392 - $COOCH_3$ fragments respectively. The spectrum also showed a prominent m/e 239 peak which is attributable to loss of the C-16 carbon bridge and its attached substituents

and C = O from the C-5 carbon atom plus one hydrogen unit (Britten et al, 1963). The overall fragmentation pattern agreed with that of picraline (Britten et al, 1963; Oliver et al, 1963).

Spectral data (UV, IR, MS, NMR) and the melting point agreed with published data for picraline (Gabetta and Mustich, 1975).



Picraline

Oreog 12 : White crystals; m.p. 228 - 230°C.

UV : λ_{max}^{MeOH} nm (log E) 210 (4.04), 244 (3.58), 302 (3.23);

λ_{min}^{MeOH} nm 227 (3.56), 267 (2.90).

IR : ν_{max}^{KBr} cm^{-1} 3450 s, 2950 s, 2850 w, 1720 s, 1600 m, 1460 s, 1440 s, 1210 s, 1110 m, 1040 m, 920 w, 800 w, 740 s.

MS : m/e (relative intensity per cent) 354 (100) M^{+}, 339 (M - 15) (34), 323 (M - 31) (8), 295 (M - 59) (9), 277 (3), 263 (6), 251 (9), 235 (8), 221 (5), 206 (5), 194 (5), 182 (5), 168 (13), 167 (9), 156 (9), 144 (6), 143 (6), 130 (9).

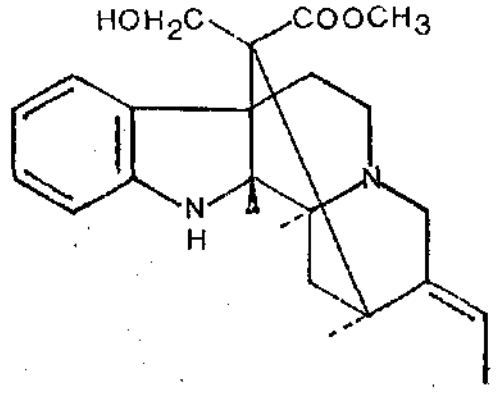
NMR ($CDCl_3$) : δ 7.60 - 6.60 (m, 4H, aromatic), 5.56 (q, 1H), 4.25 (s, 1H), 4.12 (s, 1H), 3.80 (s, 3H, $COOCH_3$), 3.76 (dd, 1H), 3.28 (s, 1H), 3.08 (s, 1H), 2.96 (d, 2H), 2.68 (s, 1H), 2.56 (s, 1H), 2.36 (m, 1H), 2.00 (s, 1H), 1.64 (d, 3H, C-19, CH_3), 1.28 (s, 1H).

Chromogenic reactions: A red colour was obtained with ferric chloride - perchloric acid reagent and with ceric sulphate reagent.

The UV spectrum and chromogenic reactions indicated a dihydroindole chromophore. The IR spectrum showed evidence of OH (3450 cm^{-1}), NH (2950 cm^{-1}) and carbonyl ester (1720 cm^{-1}) groups. The absorption band at 740 cm^{-1} indicated a 1, 2, disubstituted benzene ring thus confirming non-substitution of the indole nucleus.

The NMR spectrum and MS fragmentation pattern closely resembled those of deacetyl-akuammiline (Oreog 6). The MS, however, indicated the molecular ion peak at m/e 354 which is two mass units more than that of deacetyl akuammiline. The MS also exhibited distinct indole peaks at m/e 144, 143 and 130. The compound could therefore be a dihydroindole derivate of deacetyl-akuammiline.

Catalytic hydrogenation of deacetyl-akuammiline at pH 10 using palladium 10% on alumina for 12 hrs yielded a compound which, on purification, gave identical spectral data (UV, IR, MS and NMR), chromogenic reactions and hRf values with Oreog 12. Mixed melting points were undepressed. The alkaloid was therefore confirmed as 2-ß-dihydro-deacetyl-akuammiline.



2-ß-dihydro-deacetyl-akuammiline

Oreog 13 : Showed identical hRf values, spectral data (UV, IR, MS and NMR) and chromogenic reactions with pleiocarpamine (RVOLF 3).

Oreog 14 : Showed identical hRf values, spectral data (UV, IR, MS and NMR) and chromogenic reactions with volkensine (RVOLF 7).

Oreog 15 : Off-white amorphous powder.

UV : λ_{max}^{MeOH} nm (log E) 227 (4.40), 285 (3.80), 291 (3.82);
λ_{min}^{MeOH} nm 252 (3.00).

IR : ν_{max}^{KBr} cm^{-1} 3400 s, 2950 m, 2800 w, 2760 w, 1740 s, 1620 w, 1600 w, 1440 m, 1360 w, 1240 s, 1120 m, 1080 m, 740 s.

MS : m/e (relative intensity per cent) 440 M^+ (67), 426 (M - 14) (44), 409 (M - 31) (44), 394 (M - 46) (56), 383 (M - 57) (78), 381 (M - 59) (33), 367 (M - 73) (33), 352 (56), 337 (56), 325 (44), 305 (44), 292 (33), 277 (44), 262 (44), 250 (67), 234 (100), 222 (56), 206 (56), 204 (44), 194 (67), 183 (67), 182 (56), 168 (67), 167 (65), 156 (78), 144 (56).

Chromogenic reactions: A greyish brown colour turning green on heating was obtained with ferric chloride - perchloric acid reagent.

The UV spectrum demonstrated an indole chromophore with a well-defined minimum at 252 nm. The chromogenic reaction and MS fragmentation pattern suggested a compound structurally related to the E-seco indoles. The β-carboline peaks occurred at m/e 182, 168 and 156 as opposed to m/e 184, 170 and 156 in the E-seco indoles. This was analogous to tetrahydro-β-carboline alkaloids possessing a C-5 carbon bridge (Budzikiewicz et al, 1964). The MS in the upper mass region indicated prominent peaks at m/e 409 (M - 31), 394 (M - 46), 381 (M - 59) and 337 (M - 73) which

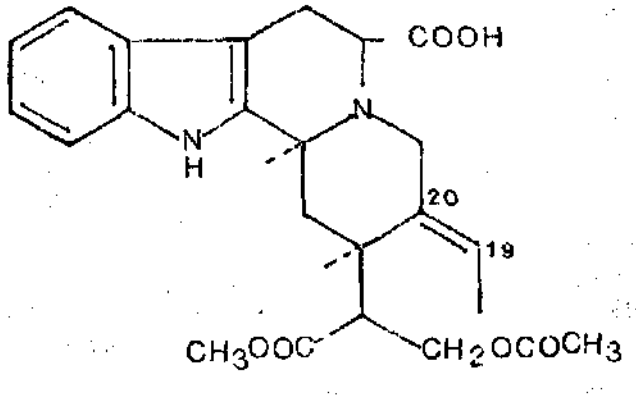
were attributable to loss of CH_3O, COOH + H, $COOCH_3$ and CH_2OCOCH_2 fragments respectively. That a COOH group is attached to the C-5 position is evident in the expulsion, on electron impact, of 46 mass units from the molecular ion and this is attributable to loss of COOH + H (species a). Analogous peaks were observed in the mass spectra of adirubine, and 5-carboxytetrahydroalstonine, both of which possess a similar C-5 functional group (Brown et al, 1972; Brown and Charalambides, 1974).

Loss of the C-16 carbon atom and its attached substituents and expulsion of the C-5, COOH + H substituents produced the peak at m/e 250.

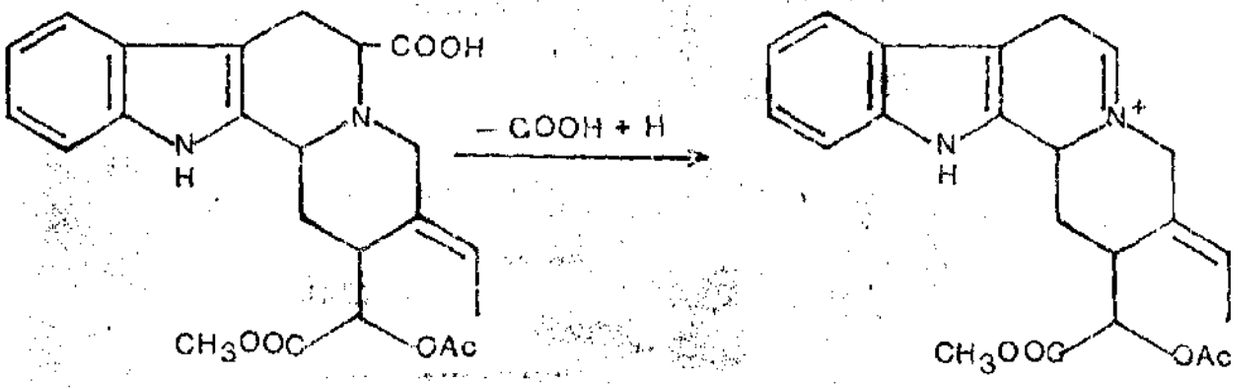
The IR spectrum revealed evidence of OH (3400 cm^{-1}), NH (2950 cm^{-1}) and carbonyl ester (1740 cm^{-1}) groups. Bohlmann bands appeared at 2800 cm^{-1} and 2760 cm^{-1} and indicated the presence of C-3H α.

Insufficient material remained for NMR examination but the above data showed a close resemblance to data for adirubine acetate (Brown et al, 1972) although the molecular ion peak occurred at m/e 440 which is two units less than that reported for adirubine acetate. The occurrence of the m/e 394 peak (Species a) as opposed to the m/e 396 peak in adirubine acetate indicated that Oreog 15 possesses an ethylidene side chain while adirubine acetate possessed an ethyl side chain.

Oreog 15 could therefore be adirubine acetate 19 : 20 dehydro.



Adirubine acetate 19 : 20 Dehydro



M^+ m/e 440 (a) m/e 394

Oreog 16 : Showed identical spectral data (UV, IR, MS), chromogenic reactions and hRf values with ekuammicine (RVOK 12).

Oreog 17 : Showed identical hRf values, spectral data (UV, IR, MS) and chromogenic reactions with sewerine (RVOLF 8).

Oreog 18 : Showed identical hRf values, spectral data (UV, IR, MS, NMR) and chromogenic reactions with methoxy-pleiocarpamine (RVOLF 4).

Oreog 19 : Showed identical spectral data, (UV, IR, MS and NMR), hRf values and chromogenic reactions with nortetraphyllicine (RVOSB 8).

Oreog 20 : Showed identical spectral data (UV, IR, MS), hRf values and chromogenic reactions with peraksine (RVOX 19).

Oreog 21 : Showed identical spectral data (UV, IR, MS), hRf values and chromogenic reactions with normacusine B (RVOSB 13).

TABLE V Co-TLC of *R. volkensii* and *R. oreogiton* root bark extracts

Detected Alkaloids	*R. volkensii* root extract	*R. oreogiton* root extract	Solvent systems I	II	III	IV	V	VI
			hRf values					
Ajmalicine	+	+	83	97	96	83	97	96
Isoreserpiline	++	++	73	83	80	92	90	94
Aricine	+	+	67	82	96	92	63	94
Carapanaubine	+	+	57	83	80	92	67	94
Reserpine	+	+	50	97	96	90	71	96
Rescinnamine	+	+	47	97	96	90	67	96
∝ -Yohimbine	+	+	20	50	80	40	52	90
Yohimbine	+	+	18	47	80	35	50	88
Reserpiline	+++	+++	10	60	60	92	47	80
Tetraphyllicine	+	+	7	40	73	80	36	80
Ajmaline	+++	+++	5	37	63	78	31	78

Solvent Systems relevant to Table V

I Acetone : petroleum spirit (b.p. 40 - 60°C) : carbon tetrachloride : iso-octane (7 : 6 : 4 : 3).

II Acetone : methanol : diethylamine (7 : 2 : 1).

III Ethyl acetate : iso-propanol : ammonia (16 : 3 : 1).

IV Acetone : methanol : glacial acetic acid (7 : 2 : 1).

V Diethylether : methanol (9 : 1).

VI Chloroform : methanoli acetone : iso-octane (5 : 2 : 1 : 1).

hRf values stated are the mean values from five determinations.

key to alkaloid abundance

\+ = small amount

++ = moderate amount

+++ = abundant

Table VI

Distribution of Alkaloids detected in R. volkensii

Skeletal type	Alkaloid	Leaf	Stem	Root
E-seco indole	Geissoschizine	+	+	-
Sarpagan	Normacusine B	+	+	-
	Normacusine B-O-acetyl	-	+	-
	Polyneuridine	-	+	-
	Pre-peraksine	-	+	-
	Sarpagine	-	-	+
Dihydroindole	Nortetraphyllicine	+	+	-
	Tetraphyllicine	-	+	+
	Tetraphyllicine-O-acetyl	-	-	+
	Ajmaline	-	+	+
	Ajmaline-O-acetyl	-	-	+
Indolenine	Perakine	-	-	+
	Raucaffrinoline	-	-	+
Yohimbine	α-Yohimbine	-	+	+
	Yohimbine	-	+	+
18-Hydroxy-yohimbine esters	Reserpine	-	-	+
	Rescinnamine	-	-	+
	18-Hydroxy-yohimbine	-	-	+
	Methyl deserpidate	-	-	+

Skeletal type	Alkaloid	Leaf	Stem	Root
Heteroyohimbine	Tetrahydroalstonine	+	-	+
	Ajmalicine	-	-	+
	Aricine	-	+	+
	Isoreserpiline	-	+	+
	Reserpiline	-	+	+
	Rauvolcinine	-	-	+
	Tetrahydroalstonine N_b-methyl	+	-	+
Oxindole and -indoxyl	Carapanaubine	-	+	+
	Isoreserpiline-ψ-indoxyl	-	-	+
Picraline	Volkensine	+	-	-
	Quaternine	+	-	-
Pleiocarpamine	Pleiocarpamine	+	-	-
	Methoxy-pleiocarpamine	+	-	-
	Methoxy-pleiocarpamine-2 : 7-dihydro	+	-	-
	Methoxy-pleiocarpamine - 2:7-dihydro N_b-oxide	+	-	-
Akuammicine	Akuammicine	+	-	+
	Sewerine	+	-	-
Anhydronium-base	Alstonine	-	+	+
Miscellaneous	Peraksine	+	+	+

Table VII

Distribution of alkaloids detected in R. oreomiton

Skeletal type	Alkaloid	Leaf	Root
E-seco-indole	Geissoschizine	+	-
	Oreog 15	+	-
Sarpagan	Normacusine B	+	-
Dihydroindole	Nortetraphyllicine	+	-
	Tetraphyllicine	-	+
	Ajmaline	-	+
	Oreog 4	+	-
	Akuammiline-2β -dihydro	+	-
	Deacetyl-akuammiline-2β -dihydro	+	-
	Akuammine-O-methyl	+	-
Indolenine	Akuammiline	+	-
	Akuammiline-5-formyl	+	-
	Deacetyl-akuammiline	+	-
Yohimbine	α -Yohimbine	-	+
	Yohimbine	-	+
	Yohimbine isomer	-	+
18-Hydroxy-yohimbine esters	Reserpine	-	+
	Rescinnamine	-	+
	Renoxidine	-	+

Skeletal type	Alkaloid	Leaf	Root
Heteroyohimbine	Ajmalicine	-	+
	Aricine	+	+
	Isoreserpiline	-	+
	Reserpiline	-	+
Oxindole	Carapaunabine	-	+
Picraline	Picrinine	+	-
	Picraline	+	-
	Volkensine	+	-
	Quaternine	+	-
Pleiocarpamine	Pleiocarpamine	+	-
	Methoxy-pleiocarpamine	+	-
Akuammicine	Akuammicine	+	-
	Sewerine	+	-
Anhydronium base	Serpentine	-	+
Miscellaneous	Peraksine	+	-

Occurrence and distribution of alkaloids in R. volkensii

Employing previously developed methods of chromatographic analysis (Court and Habib, 1973; Court and Timmins, 1975; Iwu and Court, 1977), chromogenic and fluorogenic reactions (Court, 1966; Court and Timmins, 1975) and a combination of spectroscopic and other physical methods, the presence in R. volkensii of thirty eight alkaloids was demonstrated during the course of this investigation. The distribution of the alkaloids in the plant is shown in Table V|

Isolation and characterisation of the alkaloids indicated that the following structural types were recognisable:- E. seco indole, sarpagan, ajmalan dihydroindole, yohimbine, heteroyohimbine, oxindole and ψ -indoxyl, picraline, pleiocarpamine, akuammicine and anhydronium bases.

The major alkaloids of the roots and stem bark were reserpiline and ajmaline. Isoreserpiline and α-yohimbine also occurred in appreciable quantities in the roots and tetraphyllicine and nortetraphyllicine were obtained in good yield from the stem bark. In the leaves, however, alkaloids of the picraline and pleiocarpamine type (volkensine and methoxy-pleiocarpamine) were the major alkaloids. The inability to demonstrate the presence of reserpiline and isoreserpiline or their corresponding oxindoles in the leaves of this plant is noteworthy in view of the occurrence of high yields in the roots and stem bark.

That a marked difference exists between the alkaloids occurring at differing levels of the plant axis is a phenomenon observed in several other species of Rauwolfia e.g. R. mannii (Patel et al, 1965), R. vomitoria (Pousset et al, 1965; Iwu and Court, 1977; Sabri and Court, 1978),

R. obscura (Timmins, 1975) and R. oreogiton (Timmins, 1975; this investigation). R. volkensii presents a similar pattern. Thirteen identifiable alkaloids were obtained from the leaves, seventeen from the stem bark and twenty six from the roots.

As in other species of the genus, the E-seco indole alkaloids and the sarpagan group occurred more abundantly in the aerial parts; the sarpagan group being more conspicuous in the stem bark.

Alkaloids of the yohimbine group and their derivatives occurred chiefly in the roots, being less abundant in the stem bark and not detected in the leaves. The pharmacologically important alkaloids reserpine and rescinnamine, were isolated from the plant but in minute quantities only from the roots.

The heteroyohimbines occurred throughout the plant, the 10, 11-dimethoxy analogues constituting more than 50% of the total alkaloid content of the plant. Judging by the isolation of a large number of compounds of the allo stereochemical series from this plant, it would seem that the dominant stereochemical form is allo (C-3Hα, C-20H α). Although the major alkaloid of the plant is reserpiline, which belongs to the epiallo series (C-3Hβ, C-20Hα), it is possible that isoreserpiline, an allo compound also a constituent of the plant, is converted to the epiallo form as the latter is considered to possess the more stable configuration. It has been shown that the stability relationship between compounds of the allo and epiallo series possessing substituents on the D and E, rings depends upon the nature of the substituents and their configurations (Wenkert et al, 1955).

The oxindole and Ψ-indoxyl alkaloids isolated from R. volkensii were related to reserpiline and isoreserpiline. This is in agreement with the

hypothesis proposed by Shellard and Houghton (1973) that the presence of oxindole alkaloids may be related to the corresponding heteroyohimbines. R. vomitoria is another Rauwolfia plant from which oxindole and ψ-indoxyl alkaloids have been reported (Pousset et al, 1967; Sabri and Court, 1978) and only oxindoles corresponding to reserpiline and isoreserpiline were detected. Isoreserpiline-ψ-indoxyl occurs in R. ligustrina Roem et Schutt, a species also known to produce reserpiline and isoreserpiline (Finch et al, 1963). Oxindoles corresponding to other heteroyohimbine alkaloids have been reported in other Apocynaceous genera but in R. volkensii and R. vomitoria oxindoles derived from compounds such as tetrahydroalstonine, aricine etc. were not found. This may be due to the relatively low yield of these alkaloids in Rauwolfia plants.

The dihydroindole alkaloids were distributed throughout the plant but the N_a-methyl analogues occurred mainly in the lower axis. Dihydroindole alkaloids with hydroxy or methoxy-substituents on the aromatic ring such as purpeline, seredamine, vomalidine and endolobine were not found although such compounds have been reported in several other Rauwolfia species and particularly from species of the section Endolobus. For example mitoridine, seredamine, purpeline, vomalidine and endolobine (Fig. 10) occurred in R. vomitoria (Poisson et al, 1964; Vale, 1963 ; Iwu, 1977), R. cumminsii and R. mombasiana (Iwu, 1977). 12-Methoxy-ajmaline and vomalidine were isolated from R. obscura, a section Afrovolfia species (Timmins and Court, 1974b) and norseredamine occurred in the stem bark of the Pacific Island species R. sumatrana Jack., a section Crytosiphonia species (Hanaoka et al, 1970).

That the trimethoxybenzoyl esters of dihydroindole alkaloids e.g. rauvomitine or mitoridine (Fig. 10) did not occur in this plant is scarcely surprising as the 18-hydroxy-yohimbine ester alkaloids (reserpine and rescinnamine) also occurred only in trace quantities.

The ajmaline related indolenine alkaloids perakine and raucaffrinoline were obtained from the roots. Perakine is probably not an artefact as suggested by Taylor (1966) because it has been obtained not only from the mother liquors of reserpine manufacture from R. vomitoria (Ulshafer et al, 1961) but also from fresh plant extracts of R. perakensis (Kiang and Wan, 1960) and R. caffra (Habib and Court, 1974).

The picraline bases volkensine and quaternine occurred in significantly high yields in the leaves of R. volkensii and were apparently confined to this part of the plant. Although picrinine and deacetyl-desformo-akuammiline were obtained from R. vomitoria leaves (Pousset et al, 1965) and picrinine and deacetyl picraline were found in R. cumminsii (Iwu and Court, 1978a; 1978b), the occurrence of typical Picralima bases in Rauwolfia plants is very uncommon. Such compounds have been regarded as by-products of the biosynthetic route but their occurrence in large quantities in R. volkensii leaves suggests a greater significance.

Picralima bases do occur in several other genera of the family Apocynaceae. For example, picrinine, picralinal and quaternine have been reported in some species of Alstonia (Banerji et al, 1972; Rastogi et al, 1970; Mamates-Kalamaras et al, 1975). Deacetyl-picraline was found in some Hunteria species (Bartlett et al, 1963) and akuammicine and akuammine occurred in Cabucala and Vinca species (Douzoua et al, 1974; Farnsworth et al, 1964; Svoboda et al, 1963).

The isolation of pleiocarpamine, methoxy-pleiocarpamine and its N-oxide from the leaves of R. volkensii is the first reported occurrence of this skeletal type in a Rauwolfia species. Pleiocarpamine was first isolated from Pleiocarpa mutica Benth (Hesse et al, 1964) and has since proved to be widespread in other Apocynaceous genera where it occurs either free or as part of a complex alkaloid. Pleiocarpamine is known to occur in Hunteria (Bartlett et al, 1963), Vallessia (Walser and Djerassi, 1964) and Alstonia species (Burke et al, 1973). Pleiocarpamine also occurred as part of the complex alkaloid villastonine in Alstonia muelleriana Domin. (Burke et al, 1973).

The inter-relationship of the alkaloids

The currently accepted theory of the biogenesis of indole alkaloids proposes that D-seco compounds such as strictosidine or vincoside or their 5-carboxy-derivatives undergo enzymatic removal of the glucoside side chain and subsequent cyclization to yield the E-seco indole alkaloids. Such alkaloids then undergo various forms of ring closure e.g. C-16 -> C-5 (sarpagan), C-16 -> C-7 (picrinine), C-16 -> C-5, C-20 -> C-16 (peraksine), C-16 -> C-5, C-17 -> C-7 (ajmalan), to produce a wide range of alkaloidal types.

The highly reactive D-seco alkaloids have not as yet been isolated from Rauwolfia species but the E-seco compounds have been obtained from several species of the genus. The occurrence of a wide variety of indole alkaloids structural types in R. volkensii prompts interest in their biogenetic relationship.

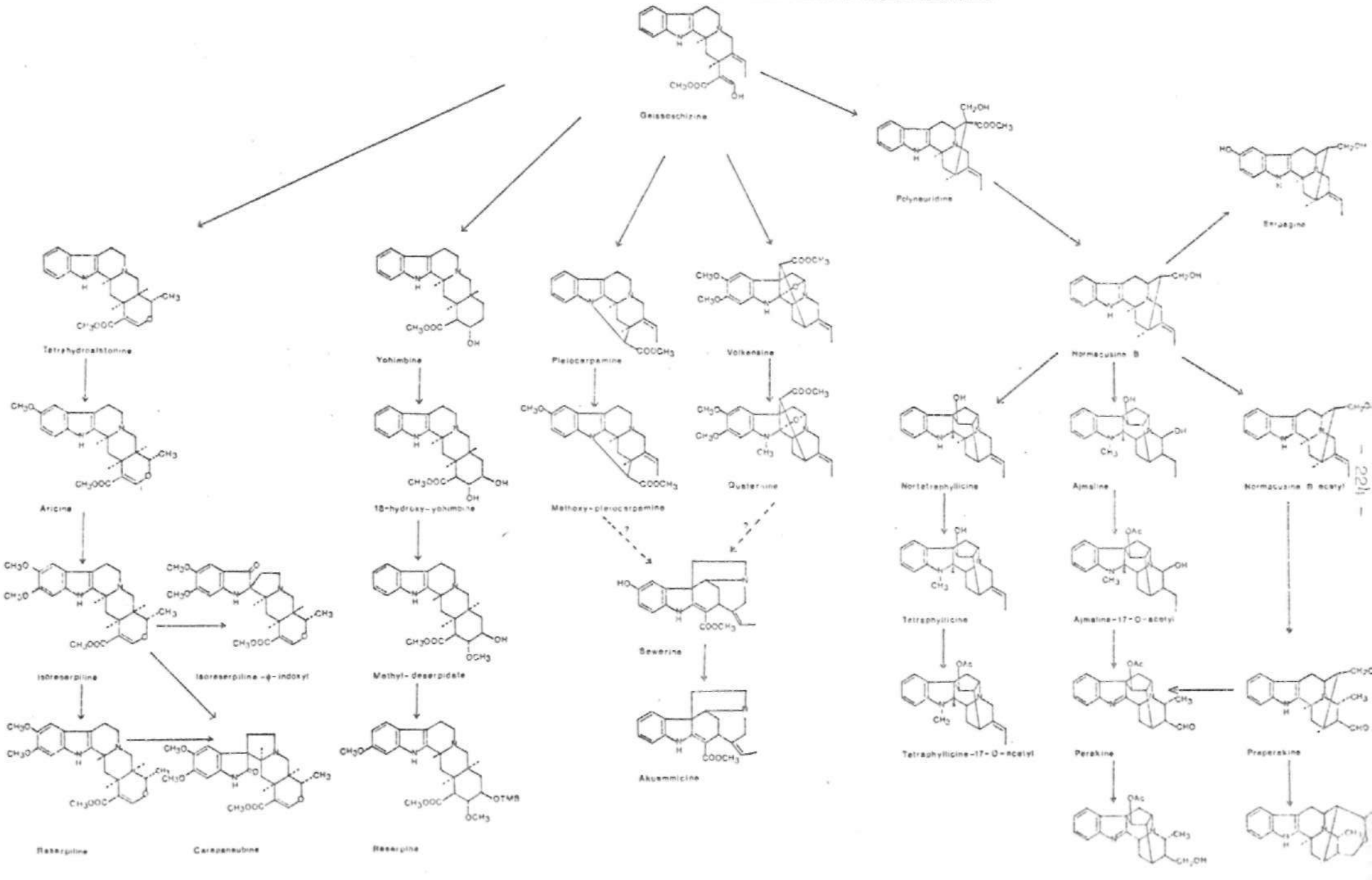
The E-seco indole alkaloid geissoschizine found in the leaves and stem bark occupies a unique position since all the cogeneric alkaloids can be envisaged as arising from it. The postulate that geissoschizine is a probable precursor is substantiated because this alkaloid has been implicated in the sequential metabolism of vincoside or isovincoside in Vinca species and was one of the first detected alkaloids (Scott, 1970). Also, results from direct feeding experiments have demonstrated incorporation of labelled geissoschizine in ajmaline, tetrahydroalstonine, serpentine, akuammicine and vindoline (Battersby, 1969). Many of these compounds are well-known Rauwolfia alkaloids.

Geissoschizine could thus be considered as a key intermediate in the biosynthesis of the alkaloids of R. volkensii. The C-16 decarboxy analogues such as geissoschizol and geissoschizal isolated from R. vomitoria (Pousset et al, 1965; Sabri and Court, 1978) and R. obscura (Timmins and Court, 1976) are probably derived from geissoschizine itself.

Battersby (1969) has demonstrated the incorporation of geissoschizine into ajmaline in Vinca species and Djakoure et al (1975) have shown in a series of interconversions, that E-seco indole alkaloids can be converted to heteroyohimbinoid and yohimbinoid alkaloids. The exact manner of bioconversion is not known but geissoschizine may undergo an oxidative ring closure to produce the pentacyclic heteroyohimbine alkaloids as shown in Scheme XX. An alternative ring closure involving a C-17 → C-19 linkage could produce the yohimbine group of alkaloids.

It is not known whether a similar route produces the aromatic methoxy analogues such as aricine and reserpiline. Nevertheless, it has been shown

Scheme XX Inter-relationship of *R. volkensii* alkaloids



that bacteria can methoxylate the aromatic ring in the yohimbine type alkaloids (Iizuka et al, 1976). A similar enzymatic methoxylation process in the plant could produce the methoxy heteroyohimbine and yohimbine alkaloids. The stage of biosynthesis at which such methoxylation takes place is not clear. If it is after closure of the E-ring, then alkaloids such as tetrahydroalstonine could act as precursors of aricine which in turn could be the precursor of isoreserpiline and reserpiline.

18-Hydroxy-yohimbine and methyl deserpidate may arise from a yohimbine precursor and may by esterification and methoxylation produce reserpine and rescinnamine.

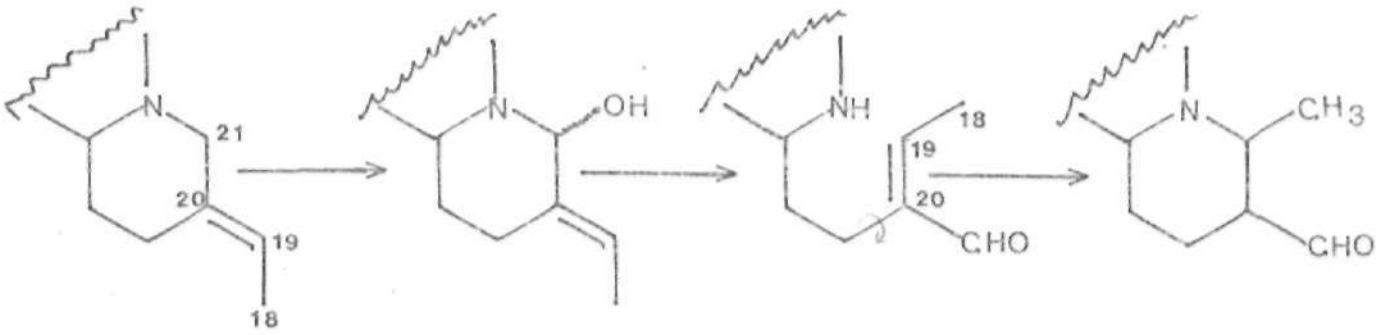
The occurrence of such a high yield of dimethoxy-picrinines (volkensine and quaternine) and methoxy-pleiocarpamine in the leaves of R. volkensii as opposed to reserpiline and isoreserpiline which were the major alkaloids of the stem bark and root may suggest that the methoxy-heteroyohimbines are probably not synthesised directly from E-seco indoles. Further work on the leaves of other reserpiline producing species is necessary to establish any connection between these two skeletal types.

Cordell (1974) referred to the biosynthesis of ajmaline type dihydro-indole alkaloids from E-seco precursors via sarpagan type intermediates. The co-occurrence of geissoschizine and a number of sarpagan and ajmalan type alkaloids in R. volkensii supports this contention. Thus geissoschizine, or its 5-carboxyl-derivative as proposed by Van Tamelen (1968), could be converted to a reactive imino form which would cyclise to yield the sarpagan skeleton by linkage of the C-16 carbon to the electrophilic imine on C-5 so producing polyneuridine which could then undergo oxidative decarboxylation to produce normacusine B. (Scheme XX).

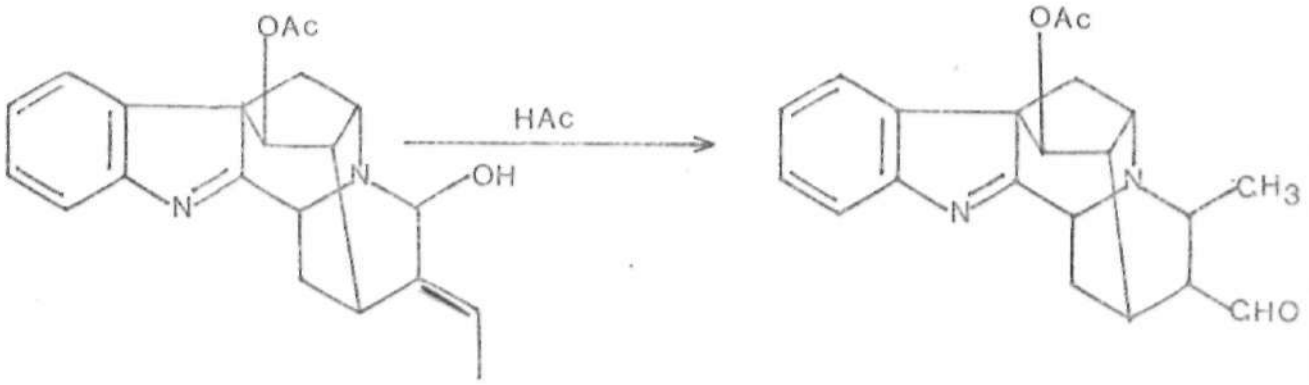
Normacusine B could further ring close by C-17 → C-7 linkage to produce the ajmalan skeletal type as shown in Scheme XX. The occurrence of N_a-demethyl ajmalan type dihydroindole alkaloids in the leaves and the stem bark only indicates that the demethyl compounds are probably formed first and subsequently methylated further down the plant axis. Barton and co-workers (1965) have shown that norajmaline is a good precursor of ajmaline in R. serpentina, the N_a-methyl radical being derived from methionine. The inability to isolate norajmaline from R. volkensii may be attributed to its rapid conversion to ajmaline. Norajmaline has been obtained from several other ajmaline producing plants e.g. R. suaveolens (Majumdar et al, 1973), R. obscura (Timmins, 1975), R. vomitoria (Iwu, 1977) and R. macrophylla (Timmins and Court, 1974c). Nortetraphyllicine, a closely related dihydroindole alkaloid, was isolated from the leaves and the stem bark of R. volkensii.

The co-occurrence of several ajmaline related alkaloids viz. perakine, preperakine, peraksine and raucaffrinoline in R. volkensii presents an interesting biogenetic sequence. A common feature in the structures of these alkaloids is the linkage of the C-19 to the N_b-nitrogen of the tryptamine moiety. The relationship of these alkaloids to ajmaline is evident, not only in the closeness of their structure and chemistry but also in their frequent co-occurrence in plants. Perakine, peraksine and ajmaline have been isolated together in R. vomitoria (Ulshafer et al, 1961) in R. perakensis (Kiang, et al, 1966) and in R. caffra (Habib and Court, 1973).

Kompis et al (1971) have suggested that a macroline type precursor may be involved in their biosynthesis or, alternatively, they may arise from a sarpagan type compound which is hydroxylated at C-21 and the molecule rearranged such that the C-19 becomes attached to the N_b-position.

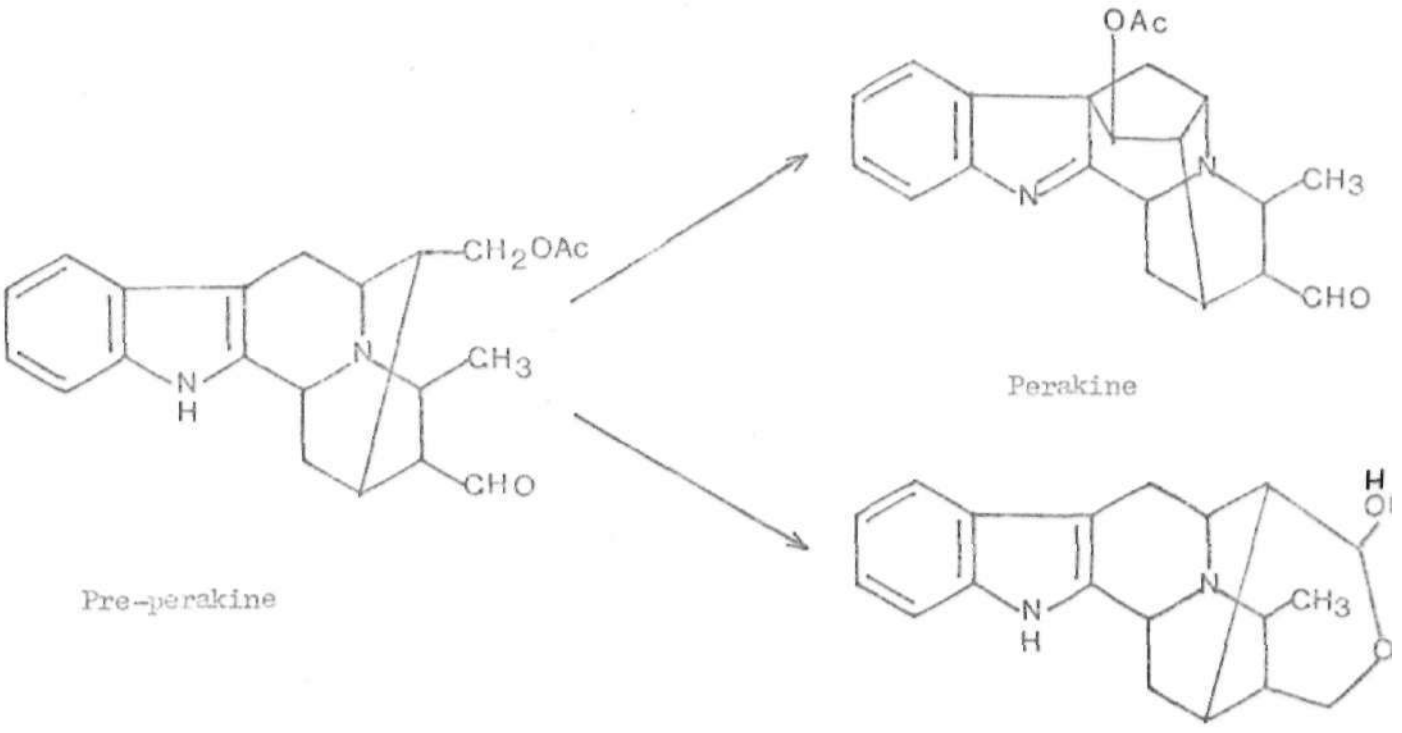


The ease of conversion of vomilenine to perakine by brief treatment with boiling acetic acid (Taylor, 1965) suggests that such a rearrangement is possible in the plant.



Vomilenine Perakine

The isolation of pre-perakine from R. volkensii further supports the sarpagan route hypothesis as preperakine constitutes a link between the sarpagan skeleton and the perakine structure. The C-17 -> C-7 ring closure of preperakine will yield perakine whilst an alternative ring closure involving C-17 -> C-21 will elaborate the peraksine type of skeleton.



Pre-perakine

Perakine

Peraksine

The picraline bases may also be derived from an E-seco precursor such as geissoschizine. Their production would involve a C-16 → C-7 linkage as shown in Scheme XX. Possible intermediates in this pathway could be the indolenine alkaloids of the akuammiline type (Scheme XXI). Although such alkaloids were not isolated from R. volkensii they were obtained from R. oreogiton leaves and deacetyl-desformo-akuammiline occurred in the leaves of R. vomitoria (Pousset et al, 1965).

Very little is known about the biosynthesis of pleiocarpamine. Cordell (1974) suggested three routes by which it could be formed from an E-seco indole alkaloid such as geissoschizine. It may be formed by direct attachment of the C-16 carbon atom to the indole nitrogen or by a 1:3 shift of the C-16 carbon bridge in akuammiline types or via an intermediate with C-16 → C-2 linkage. It is significant therefore that both pleiocarpamine and picraline bases occurred in R. volkensii.

Alkaloids of R. oreogiton

Thirteen identifiable alkaloids were obtained from the roots of R. oreogiton. The results are in general agreement with earlier investigations concerning the alkaloids of this root (Court, 1966; Timmins and Court, 1974). The major alkaloids are reserpiline and ajmaline. However, the presence of three other minor alkaloids, ambicine, isoreserpiline and carapanaubine, not reported in the previous works, was demonstrated.

It was not possible to differentiate the roots of R. volkensii from the roots of R. oreogiton by chromatographic analysis. The migration patterns, fluorescent spots and chromogenic reactions of the alkaloids in both extracts were identical in the six solvent systems used.

It is significant that silviculturists in East Africa are unable to differentiate the two species and regard them as doubtfully distinct (East African Harbarium, private communication).

The alkaloids obtained from the leaves of R. oreogiton included a 5-carboxy-E-seco indole compound. Van Tamelen et al (1970) proposed a biosynthetically modelled synthesis of ajmaline that required a 5-carboxy-E-seco indole alkaloid. It is significant therefore that this alkaloid was isolated from a Rauwolfia plant. Its occurrence also suggested that tryptophan as well as tryptamine is involved in the biosynthesis of some Rauwolfia alkaloids.

The distribution pattern of the alkaloids of R. oreogiton leaves is very similar to that obtained from the leaves of R. volkensii. As in the latter, the dominant alkaloidal group comprised the picraline bases, but many other closely related alkaloids were also obtained.

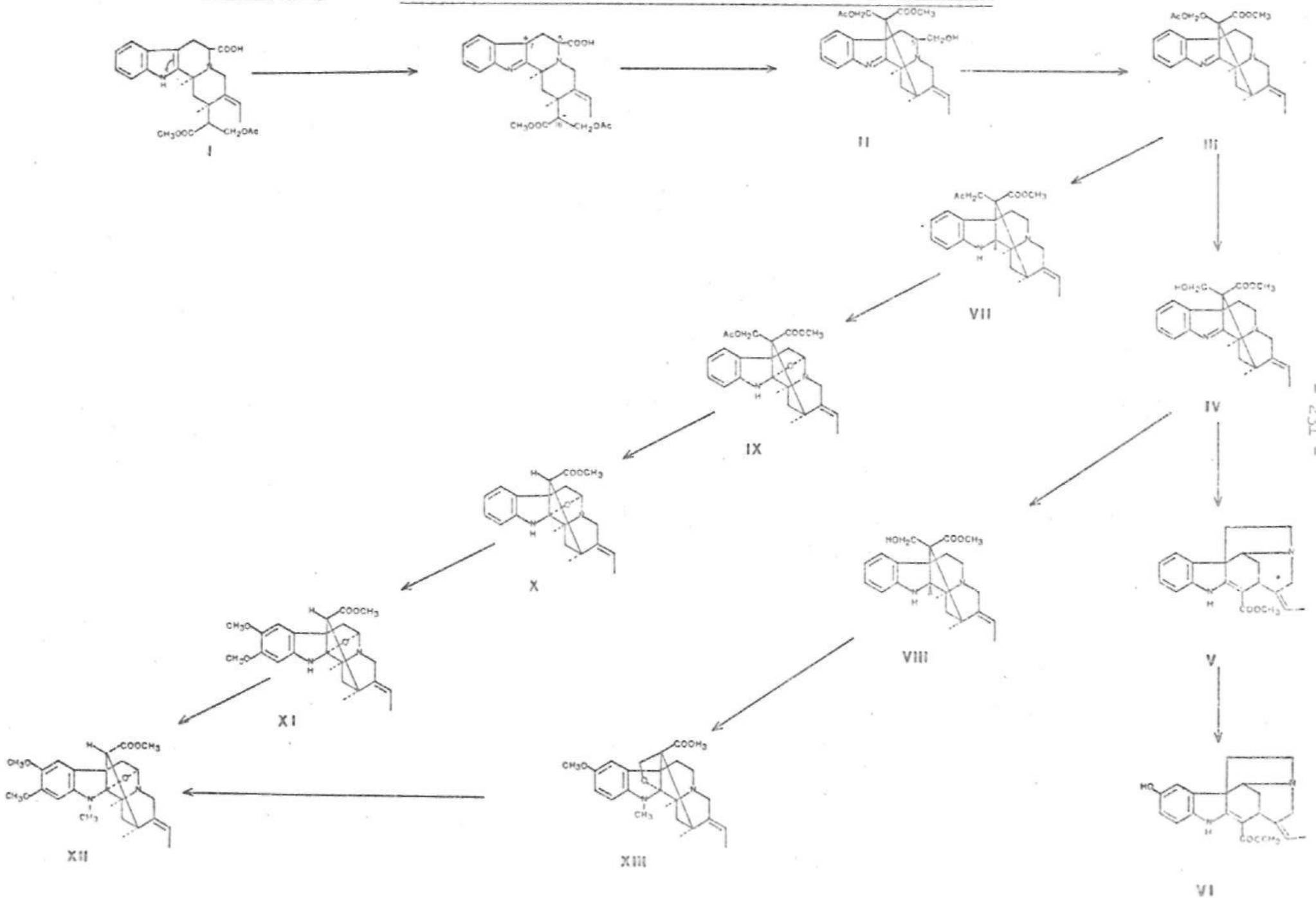
The isolation from a single batch of material of such a large number of alkaloids belonging to the same structural group suggested a close biogenetic relationship.

Saxton (1966) proposed that picraline bases were derived from a 2-acyl indole precursor of the picraphylline type. This ingenious proposal was based mainly on the fact that burnamicine co-occurred with deacetyl-picraline in Hunteria eburnea Pichon. Although a large number of the picraline type alkaloids were isolated from R. oreogiton, no such acyl indole alkaloid was found. On the basis of this experimental evidence the 5-carboxy-E-seco indole alkaloid seems a more likely precursor than an undetected acyl indole. Smith (1961), commenting on the biogenesis of echitamine, had suggested that burnamicine (E-seco 2-acyl indole) could itself be derived from geissoschizine. It thus follows that an E-seco indole could be a common precursor of the acyl indole and the picraline bases obtained in Hunteria eburnea.

As shown in Scheme XXI re-arrangement of the indole ring in structure I to an indolenine form will make the C-7 position highly electrophilic and thus susceptible to attack from the C-16 carbon atom. Thus a C-16 -> C-7 ring closure could take place to produce species II in which the 5-carboxy group has been reduced to a formyl group. Precedent for such re-arrangement has been demonstrated (Wenkert and Wickberg, 1965).

Deformylation of the 5-formyl group in compound II could yield akuammiline (III) which could then produce akuammicine (V) and sewerine (VI) via the intermediate deacetylakuammiline (IV).

Scheme XXI Inter-relationship of the picraline bases of R. oreogiton



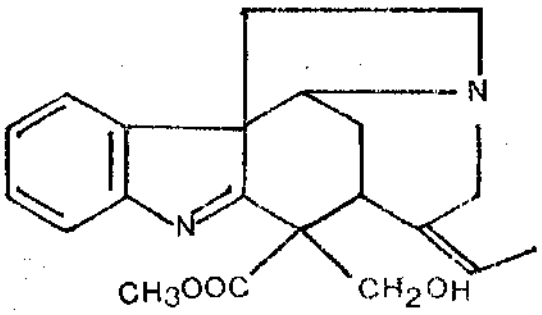
Enzymatic oxidation of the akuammiline skeleton could produce picraline (IX), volkensine (XI) and quaternine (XII). Thus a route from akuammiline may yield the picraline bases in a manner reminiscent of that postulated by Saxton (1968).

Evidence that the indolenine compounds of the akuammiline type are possible precursors of the akuammicine type was demonstrated by the French workers. Janot et al (1965) observed that the reaction of potassium - t -butoxide with deacetylakuammiline resulted in a retro-aldol loss of formaldehyde followed, presumably, by a re-arrangement with the sole production of (-)akuammicine. When deacetyl-desformo-akuammiline (a constituent alkaloid of R. vomitoria) was similarly treated, akuammicine was produced (Pousset et al, 1965). It follows therefore that a similar enzyme controlled reaction could take place within the plant to produce akuammicine.

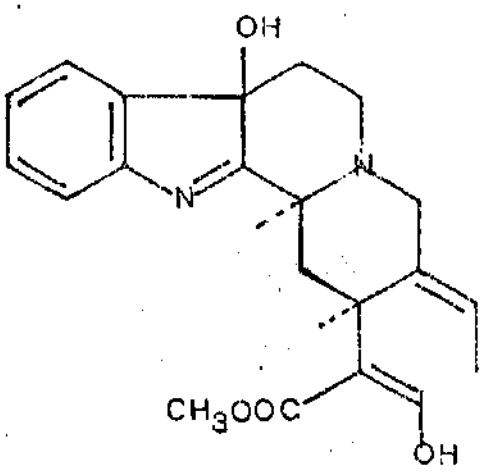
No rigid order of biogenetic reaction is implied by the arrows in Scheme XXI. For example, it is not certain whether the 1:2-dihydro-akuammiline types (compounds VII and VIII) are by-products in the biosynthetic route or are directly in the pathway. The extremely resistant nature of the indolenine bond of akuammiline to lithium aluminium hydride reduction (Oliver et al, 1964; Saxton, 1968) rules out their occurrence as artefacts.

It is also possible that many more intermediates are involved which have not been isolated because they occur in trace amounts only or are very highly reactive. For example preakuammicine (although not isolated from this plant) appears to be a more immediate precursor of akuammicine. A transformation can also be achieved by treatment with a base (Scott and Qureshi, 1969). It could be an intermediate between deacetyl-akuammiline (IV) and akuammicine (V) as indicated by Scott (1970). In sequential isolation of alkaloids in Vinca rosea young seedling, Scott (1970) reported

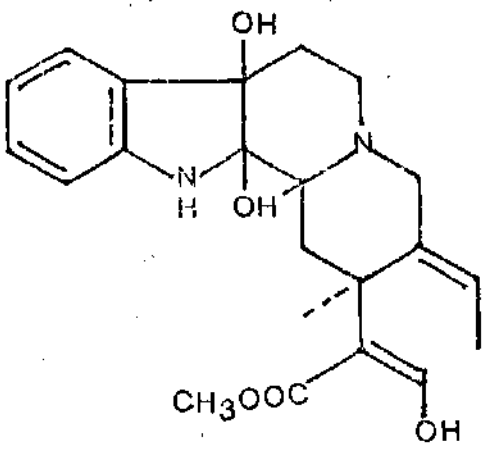
the isolation of 'β -hydroxyindolenine' and 'diol'. These compounds are possible intermediates between the akuammiline skeleton and picraline type.



Pre-akuammicine



ß - hydroxyindolenine



Diol

Considering the quantity of sewerine and akuammicine isolated from R. oreogiton, it would seem that the production of these alkaloids is secondary and the major biosynthetic route yields the picraline bases.

An interesting feature of the two plants, R. volkensii and R. oreogiton, is the production of large quantities of picraline bases and pleiocarpamine type alkaloids in the leaves, but further down the axis the typical Rauwolfia alkaloids are elaborated. The precise function of these alkaloids in the leaves is not clear but their occurrence in such a high yield suggests biogenetic significance.

A possible explanation is that the leaves, being the most active metabolic sites in the plant, synthesise the precursors to the alkaloids. Such precursors are probably strictosidine, vincoside and/or the E-seco indoles and these bases are quickly transformed to either the picraline or pleiocarpamine types which are simpler to form in order to:-

(a) Continuously remove the precursor compounds from the reaction medium and thus permit further syntheses;

(b) Protect the precursor alkaloids from rapid conversion to other metabolites in the plant;

(c) Act as a transport compound facilitating the translocation of the E-seco compounds in a less reactive form to the stem and root where they can be re-converted to the E-seco compounds and used for further syntheses.

A similar role was suggested for obscurifoline, an alkaloid of R. obscura leaves (Timmins and Court, 1975). It is significant that in many other Apocynaceous genera where such bases have been reported they have occurred mainly in the aerial parts or in the young seedlings (Saxton, 1968; 1970).

Kompis et al (1971) suggested a possible way by which pleiocarpamine and picraline type alkaloids could be derived directly from vincoside or strictosidine which implied that these alkaloids may be produced very early in the biogenetic process.

Formylstrictamine (picraline type) and pleiocarpamine were isolated from Rhazya stricta and Cordell (1974) suggested that these alkaloids are probably involved in the biosynthesis of akuammicine and the strychnos alkaloids. The N_a --> C-16 bond in pleiocarpamine would need to unlink at a certain stage of the biosynthesis in order that such conversions could take place.

We therefore support Cordell's (1974) call for more work on the biosynthesis of pleiocarpamine and the picraline (strictamine) bases in order to establish their relationship with the corynanthe bases and other indole alkaloids. It is envisaged that the difficult techniques of feeding experiments and sequential isolation of labelled alkaloids will shed more light on this problem.

Chemotaxomonic implication of the isolated alkaloids

Only alkaloids of the skeletal type Class A (Fig. 1) occurred in the species investigated. Both R. volkensii and R. oreogiton justified their identities as Rauwolfia plants by their ability to elaborate the five characteristic skeletal types found in typical Rauwolfia species i.e. the E-seco indoles, sarpagans, ajmalans, yohimbans and heteroyohimbans. Although many other closely related Apocynaceous genera e.g. Alstonia (Saxton, 1965; 1973), Vinca (Aynilian et al, 1973) and Aspidosperma

Gilbert et al, 1965) do elaborate these skeletal types, Rauwolfia species appear to be different by their apparent inability to produce alkaloids of the skeletal types Class B and C (Fig. 1) which are generally common in Alstonia, Vinca and Aspidosperma species.

Pichon (1948) classified the genus Rauwolfia morphologically into 14 sections. It is not possible at the moment to provide any chemical support for this division from the alkaloidal distribution patterns found in the Ophioxylanthus species since thorough chemical investigations in the other sections have been confined to a few species only. The chemical characters of R. oreogiton and R. volkensii are closely related and may justify their grouping together in the section Ophioxylanthus. Nevertheless, the principal alkaloids of these species occurred in various species of other sections and frequently as principal alkaloids. For example ajmaline, reserpiline and isoreserpiline occur in R. vomitoria (Endolobus) (Iwu and Court, 1977) and R. confertiflora (Afrovolfia) (Danielli et al, 1971). The production of the pleiocarpamine skeletal type and the picraline bases would at first sight appear to be peculiar to the section Ophioxylanthus but pleiocarpamine is now known to occur in R. caffra (Afrovolfia) (Nasser and Court, unpublished work). Picraline bases have also been isolated from R. vomitoria (Endolobus) (Pousset et al, 1965), R. cumminsii (Endolobus) (Iwu and Court, 1978) and R. confertiflora (Afrovolfia) (Danielli et al, 1971).

Cabucala is a close relative of Rauwolfia, both genera being members of the sub-tribe Rauvolfinae of the tribe Rauvolfiieae (Pichon, 1948). The Ophioxylanthus species have shown by the isolations reported here to share several common chemical features with Cabucala. For example, R. oreogiton elaborates akuammicine and C-methyl-akuammine which are typical Cabucala

alkaloids (Douzoua et al, 1974; Titeux et al, 1974). Also the isolation of the 17-oxy-heteroyohimbine alkaloid rauvolcinine, carapanaubine and akuammicine from R. volkensii further supports the close relationship of these species with Cabucala. However, Cabucala is not known to be able to produce anhydronium bases.

By the elaboration of tetrahydroalstonine, alstonine, reserpiline, isoreserpiline and oxindole and ψ-indoxyl alkaloids, R. volkensii shows a close chemical relationship to R. vomitoria but can apparently be differentiated by its inability to produce 12-oxy-indoline bases such as purpeline, seredamine, and vomalidine (Fig. 10). It is significant that R. vomitoria itself has been found to show a different alkaloidal pattern from the other members of the Endolobus section (Iwu, 1977).

Iwu (1977) proposed that Rauwolfia plants could be divided into two main groups; the reserpilinate species and the non-reserpilinate species. This may well prove true particularly of the African sections. The Ophioxylanthus species would be reserpilinate species as reserpiline was the principal alkaloid of both species. However, reserpiline and isoreserpiline are known to occur in several other non Rauwolfia Apocynaceous genera e.g. Bleekaria (Ochrosia) (Kilminster et al, 1972; Kanji and Sainsbury, 1974), Aspidosperma (Dastoor and Schmid, 1963) and Vinca (Aynilian et al, 1973).

On the basis of current knowledge, the dimeric alkaloid serpentinine would appear to take a more significant role as a chemical marker for the genus Rauwolfia. So far it has not been isolated from the African Rauwolfia species although it is known to be present in many Asian, Pacific and American species. If its absence in the African Rauwolfia species holds true, a division of the genus could be attempted on the basis of ability to elaborate serpentinine.

The classical belief that many alkaloids were confined to certain genera is questioned as a result of this investigation. Examination of minor constituents has revealed the presence of several different alkaloidal types in a single species, many of which are not considered typical of the genus. This would probably prove true of many other members of the genus if scrutinised more carefully. Similar results were observed with Aspidosperma (Gilbert et al, 1965) and Alstonia (Burke et al, 1973) species.

The frequent occurrence of similar indole alkaloids in closely related Apocynaceous genera suggests that the general biosynthetic routes in the indole alkaloid producing plants are similar. Although there are a large number of different structural types, they are all formed from the same building units.

The alkaloidal distribution within the different plants does differ as the accumulative capabilities and the enzyme systems of the individual plants vary environmentally and genetically.

A careful analysis of the alkaloidal pattern of the entire plant rather than of an organ is essential in establishing the true chemical character of the plant. Isolation of minor constituents may provide the solutions to the problems concerning the biosynthetic routes to the alkaloids. However, a meaningful chemical classification would have to be made on the basis of the occurrence of a significant quantity of a characteristic type.

The fact that two taxa may use the same starting material to elaborate different alkaloids or pool the same product by different pathways indicates that the mere presence of a compound does not necessarily indicate a biosynthetic relationship. There is therefore need for radio-active tracer work to follow up the isolation work with the aim of proving the proposed pathways.

Significantly nothing could be inferred from the above investigation concerning the function of the alkaloids in the plant. The classical view that these compounds are waste or excretory products is untenable. The complexity of the reactions involved in their formation and the body of information currently accumulating (Whittaker and Feeny, 1971; Freeland and Janzen, 1974; Van Emden, 1973), suggest that they may have co-evolutionary defensive and attractive roles as well as primary metabolic significance.

The detailed investigation of the occurrence of alkaloids in the plants has provided considerable information on the structural detail and inter-relationship of these compounds. Further detailed investigation using large quantities of starting material is essential before more detailed biosynthetic experiments involving labelled compounds can be usefully devised.

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