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STUDIES ON *FAGARA RHOIFOLIA* Lam.

AND

DIPLORRHYNCHUS CONDYLOCARPON Pichon

C O N T E N T S

C O N T E N T S

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

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P R E F A C E

Recently considerable interest has been shown in compounds isolated from members of the plant family Rutaceae, most especially in the alkaloids of Ruta graveolens which have been shown to have a spasmolytic action. Also in the quaternary bases of South American species of the genus Fagara, and in the chemotaxonomy of the Rutaceae, in particular of the genera Fagara and Zanthoxylum. The distribution of alkaloidal types has been used in an attempt to classify species within these genera.

Research has been directed towards finding suitable methods for the extraction, separation and purification of the tertiary and quaternary bases and to their subsequent pharmacological examination.

Part I of the present work deals with the isolation and characterisation of the tertiary and quaternary bases of Fagara rhoifolia Lam., a South American species not previously examined but related to another South American species, F. coco Engl., on which extensive work had been reported: also with a preliminary examination for quaternary bases in the four African species Fagara leprieurii Engl., F. macrophylla Engl., F. viridis A. Chev., and F. xanthoxyloides Lam., which had previously been shown to contain tertiary alkaloids. Suitable extraction and chromatographic techniques, particularly thin-layer methods, were devised for the separation and identification of these bases. A chemotaxonomic comparison was made of the tertiary and quaternary alkaloids of these four species.

Part II of this work is concerned with an alkaloidal survey of a number of species of the family Apocynaceae, the alkaloids of which have aroused considerable pharmacological and chemo-taxonomic interest. The presence of alkaloid was demonstrated in *Ambelania acida* Aubl., *Diplorrhynchus condylocarpon* Pichon, *Mandevilla hirsuta* Malme, *Ochrosia elliptica* Labill, *Odontadenia nitida* Muell., *Prestonia quinquangularis* Spreng, *Tabernaemontana angolensis* Stapf, and *T. pachysiphon* Stapf. One of these, *Diplorrhynchus condylocarpon* Pichon, was further investigated, with the isolation of three known tertiary indole bases.

P A R T I

THE QUATERNARY AND TERTIARY ALKALOIDS OF *FAGARA RHOIFOLIA* LAM.

THE EXAMINATION OF FOUR AFRICAN *FAGARA* SPECIES FOR TERTIARY AND QUATERNARY ALKALOIDS

I N T R O D U C T I O N

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THE PLANT FAMILY RUTACEAE

The family Rutaceae has the seven subfamilies Rutoideae, Dictyolomatoideae, Flindersioideae, Spaethelioideae, Toddalioideae, Aurantioideae and Rhabdodendroideae comprising 146 genera and more than a thousand species[1,2]. Geographically the family Rutaceae is widely distributed in tropical and subtropical habitats, from North and South America across Africa and Australia to Asia. The members of the Rutaceae are mostly trees and shrubs but with some herbs, for example Ruta and Dictamnus species, and some heath-like plants such as Diosma species[2,3]. In most species the leaves are gland-dotted and a large proportion of the family is relatively rich in essential oils which are present in members of the four subfamilies Aurantioideae, Flindersioideae, Rutoideae and Toddalioideae. Coumarins[2] are found in 18 genera and alkaloids in 42 genera of these same four subfamilies, with alkaloids also reported in the genus Dictyoloma which, according to Willis[3], is the only genus of the subfamily Dictyolomatoideae. This subfamily is placed, by Metcalfe and Chalk[4], in the closely related family Simarubaceae. Medicinally, the species most important at the present time are Pilocarpus heterophyllus Griseb., P. jaborandi Holmes, P. microphyllus Stapf, P. pennatifolius Lem., P. racemosus Vahl and P. spicatus A. St. Hil., grown in South America, chiefly in Brazil, and known collectively as "Jaborandi"[5]. The

leaves contain the imidazole alkaloid pilocarpine which is used in ophthalmic surgery to contract the pupil of the eye.

Economically, the most important genus is Citrus, fruit of which are sources of Vitamin C and essential oils, the latter being used as flavouring agents. The plants yielding essential oils with reputed medicinal use are mostly species of the genera Barosma, Citrus, Galipea, Ruta and Toddalia[3]. Barosma betulina B. & W., known by its Hottentot name "Buchu", yields a volatile oil, having antiseptic and diuretic properties[6].

The wood of many genera is used as timber. The citron tree of Ceylon, Chloroxylon swietenia DC.[7], and two West African Fagara species, F. heitzii known locally as "Olon", and F. macrophylla, "Olonvogo", are valuable sources of yellow-coloured timber[7]. These three species are alkaloid yielding plants.

THE GENUS *FAGARA*

Taxonomy.

This genus, of the subtribe Evodiinae, tribe Xanthoxyleae and subfamily Rutoideae[1], cannot be considered without reference to the closely allied genus *Zanthoxylum*. Since the early nineteenth century confusion has existed as to the position of certain species within these two genera.

The genus *Fagara* was instituted by Linnaeus in the 10th edition of the Systema Naturae, in 1759, in conjunction with the genus *Zanthoxylum*. Two major revisions of the genera were by Engler in 1874[8] and Engler and Prantl in 1931[1], while more recently Escalente[9] has carefully defined the differences between the two genera.

Fagara and *Zanthoxylum* species[1] are shrubs or small trees, having imparipinnate evergreen leaves, often with large or small thorns on the leaves and twigs. The leaves of the genus *Fagara* may also be simple or tripinnate. Both genera have small dioecious flowers, sessile or with short pedicels, mostly in clusters in the genus *Fagara* and in associated panicles or, less frequently, in clusters in the genus *Zanthoxylum*[1]. The two genera differ in the arrangement of the floral parts, more precisely in the number of whorls. The perianth of *Fagara* species[1,9] is constituted of a calyx of 4-5 sepals and a corolla of 4-5 petals, the sepals alternating with the dome-shaped imbricate petals. In the male flowers the stamens are positioned in front of the sepals, alternating with the petals. The perianth of the *Zanthoxylum*[1,9] is

constituted of a single whorl which, in the male flowers, has its parts alternating with the stamens.

The androecium and gynaecium are very similar in shape and arrangement in both genera.

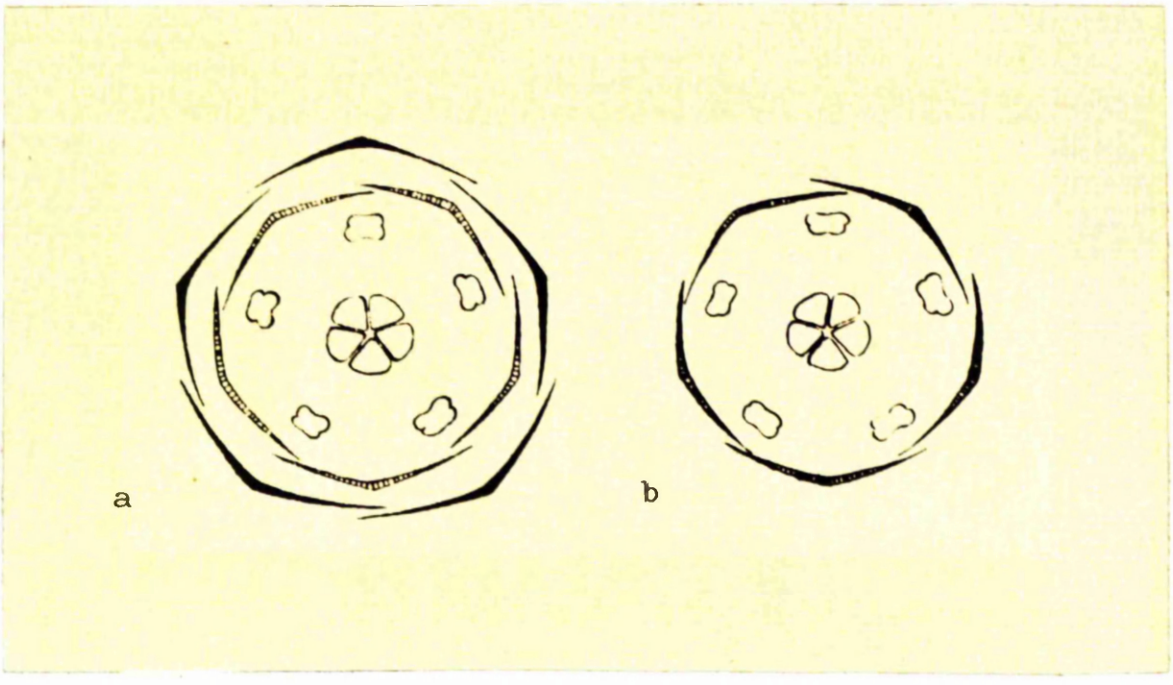


Figure I. Floral Diagrams of Male Flowers[9],

(a) Fagara species, (b) Zanthoxylum species.

According to the botanical classifications of Engler and Prantl[1] and the more recent volumes of the Index Kewensis[10], the following species correctly belong to the genus Fagara, although in the chemical literature, reporting the presence of alkaloids, they have been ascribed to the genus Zanthoxylum:-

F. ailanthoides Engl., F. avicennae Lam., F. brachyacantha Engl., F. budrunga Roxb., F. hamiltoniana Engl., F. nitida Roxb., F. oxyphylla Reeder and S.Y. Cheo, F. peckoltiana Engl., F. rhetsa Roxb., F. scandens Engl., F. schinifolia Engl. and F. venenefica Engl.

Ethnobotany.

Throughout the continents of Asia, Australia, Africa and tropical America, the local inhabitants have used Fagara species for the treatment of many diseases. Of the Asiatic species F. scandens Engl. (Z. scandens Bl.), found in Java, has been used by the natives as a fish poison and is reported to contain alkaloids[11]. The Australian F. venenefica Engl. (Z. veneneficum F.M. Bailey) is a violent convulsive poison[11].

In Africa Artar root, the root of F. xanthoxyloides Lam. (Z. senegalense DC.), has alleged antiseptic properties[12], produces salivation, and is a strong fish poison[13]: the fruits have an antiparasitic action[13]. In Nigeria a decoction of the bark of F. macrophylla Engl. is used as a genito-urinary antiseptic and is applied topically as a rubefacient and also in toothaches[13], while F. viridis A. Cheval. is used as an aphrodisiac[13]. In central Africa the bark of F. macrophylla is reputed to have a narcotic action, the bark also being used as a fish poison and for poisoning the tips of hunting weapons[14]. Similarly the barks of F. angolensis Engl. and F. lemairei De Wild are used as fish poisons[14]. The bark of F. leprieurii Engl. is reported[15] to contain a vermifuge of rapid action and the root is used for infections.

In the Argentine F. naranjillo Engl. has been used as a sudorific, diuretic and depressant in the same way as the leaves of "Jaborandi"[16]. Fagara coco Engl., F. hyemalis Engl.[16], F. rhoifolia Lam. and F. tingoassuiba Hoehne[17] have also been utilised for various

medicinal purposes, the latter being included in the Brazilian Pharmacoepia of 1929. These five species are closely related taxonomically.

The species F. rhoifolia, the main subject of the present work, is found principally along the banks of the River Plate in the Argentine, where it is known as "mamica de cadel", but it is also found in Bolivia, Paraguay and Brazil, and in Formosa[9].

THE PHARMACOLOGY OF THE ALKALOIDS AND EXTRACTS OF *FAGARA* SPECIES

At present a complete report on the pharmacology of the alkaloids of the genus *Fagara* does not exist. Though both Palmer[18] and Price[2] reviewed the known alkaloids from a chemical aspect, neither commented on the pharmacology. Price[2] was primarily concerned with the chemo-taxonomic significance of the alkaloids.

One of the first reviews of importance was made in 1889 by Giacosa and Soave[16], who prefaced a study on the chemistry and pharmacology of *F. xanthoxyloides* Lam. (*Z. senegalense* DC.), with a review on the pharmacology of a number of *Zanthoxylum* species, many of which are now included in the genus *Fagara*.

Since that time a number of alkaloids found in the genus *Fagara* have been investigated pharmacologically. The effects will be considered for the alkaloids classified into groups as follows:- amides, protoberberines, protopines, aporphines, benzylisoquinolines and furoquinolines.

Amides.

Probably the first report on the pharmacological action of a pure compound from the genus *Fagara* was that on fagaramide by Thoms and Thümen[19] who isolated the base from *F. xanthoxyloides* Lam.; later Goodson[20] isolated the same amide from *F. macrophylla* Engl. Fagaramide was shown to have a narcotic action on cold-blooded animals[19] and, more recently,

it has been demonstrated as a synergist for pyrethrin insecticides[21]. It is of interest to note here that the insecticide N-isobutyl-2,8-dodecadienamide, with approximately the same order of paralytic action and toxicity to house flies as the pyrethrins[22], was found in the bark of Zanthoxylum americanum Engl. (Z. clava-herculis L.), whose exact position within the two genera Fagara and Zanthoxylum still causes confusion[23]. From the light petroleum fraction of F. xanthoxyloides, Bowden and Ross[24] isolated the related substance N-isobutyl-trans-2-trans-4-decenamide, an amide having a formication action on mucous membranes followed by local anaesthesia. The leaves of Afraegle paniculata Engl., Rutaceae[25], were reported as having a piquant taste followed by local anaesthesia of the tongue; possibly this species also contains a similar amide.

Protoberberines.

In 1913 Laidlaw[26] gave a complete report on the pharmacology of the quaternary alkaloid, methylcanadine, the α-(-) form of which had recently been isolated from Zanthoxylum brachyacanthum Muell.[27], (considered by Engler and Prantl[1] to be F. brachyacantha Engl.). The alkaloid produced gradual paralysis of all voluntary muscle of the frog, the action being similar to that of curare and quaternary ammonium bases. The β form was ten times more active than the α form. In mammals the base caused a large fall in the blood pressure, paralysed the ganglion cells of the vagus nerve and toxic doses paralysed the respiratory centre. The isolated uterus of the guinea-pig was mildly stimulated. Relative

activities of the four stereochemical forms of the base were as follows:-

(-)- α : (+)- α : (-)- β : (+)- β :: 1 : 9 : 12 : 28[26].

Small doses of the tertiary base, canadine, produced drowsiness and depression; larger doses produced excitement, succeeded by depression and paralysis of the central nervous system. When given parenterally, canadine produced violent peristalsis with diarrhoea. No effect was observed on the blood pressure unlike the effect of the quaternary base methylcanadine[28].

Protopines.

α-Allocryptopine (β -homochelidonine) was isolated from F. brachyacantha Engl. by Jowett and Pyman[27] in 1913, and later as α-fagarine from F. coco by Stuckert[29]. α-Allocryptopine exerted a depressant action on cardiac function[30]. Later work showed that the threshold was raised for both auricular and ventricular fibrillation arising from faradic stimulation[31a,b,c]. In 1945 Deulofeu et al.[32], reported its use as a substitute for quinidine for controlling cardiac arrythmias. Benthe[33] showed that α-allocryptopine, acting as an antifibrillatory agent on the ventricle, increased the refactory period causing a decrease in the conducting velocity more effectively than quinine or procaine.

Clinical trials[34] revealed that in four patients with coronary heart disease and in two with mitral stenosis the sinus rhythm was

restored within thirty minutes, normal doses of quinidine being of no value in five of the patients. However Scherf et al.[35] later found that fatal ventricular fibrillation appeared in three patients of a group of thirteen while five others developed dangerous multifocal ventricular extrasystoles. They concluded that α-allocryptopine was not adequately safe or efficient.

Aporphines.

The tertiary aporphines produced depression of the central nervous system with a raising of thermal pain threshold and hypothermia[36]. A number of synthetic aporphines, substituted in ring D but not in ring A (see p. 26), were produced by Weisbach et al.[36] and though they produced the above effects none was of therapeutic value.

The pharmacology of a number of quaternary aporphines has been investigated. Chang et al.[37] found that the dihydroxy-quaternary aporphine, magnoflorine, found in a number of Zanthoxylum species, when injected intravenously in cats, rats and dogs produced a prompt and significant fall in blood pressure of 1 - 2 hours duration. Some curare-like action was also noticed, the conclusion being drawn that the hypotensive action of magnoflorine was mainly associated with ganglionic block.

The monohydroxy quaternary aporphine, N-methyl-isocorydine isolated from F. coco[38], F. brachyacantha[39] and F. venenefica[39], presents toxicological characteristics resembling those of tertiary aporphine alkaloids[40]. Respiratory failure was produced before heart failure.

Hyperexcitability was produced in the rabbit and rat but hypo-excitability occurred in the toad where administration by way of the lymphatics produced intense and persistent miosis, an effect modified neither by ether anaesthesia nor destruction of the C.N.S. Mesantoin and coramine increased the tolerance for N-methyl-isocorydine while lobeline and the barbiturates did not modify it[40].

The action of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethyl-aporphinium chloride, isolated first from F. tingoassuiba[42] and now from F. rhoifolia[43], has not been elucidated. It is an isomer of N-methyl-isocorydine.

Benzylisoquinolines

The quaternary alkaloid (+)-tembetarine has been reported in a number of Fagara species including F. rhoifolia[44] but the pharmacological action has not been reported.

Phellodendrine, a related quaternary base found in Phellodendron amurense Rupr. Rutaceae, had activity similar to that of its isomer, cyclanoline. Phellodendrine depressed the action of acetylcholine, producing a fall in blood pressure, which was not affected by atropine[45]. It is of interest to note here that an aqueous extract of P. amurense inhibited the in vitro hydrolysis of acetylcholine catalysed by human plasma cholinesterase[46]. This phenomenon was also exhibited by aqueous extracts of two other Rutaceous plants, Ptelea trifoliata Linn., being nine times and Zanthoxylum americanum Engl. eleven times more active than P. amurense[46].

Furoquinolines.

The pharmacological study of the furoquinolines is made difficult by the insolubility of the free bases and the acidity of their salts.

Early work, on rather impure samples of skimmianine (isolated as chloroxylonine from Chloroxylon swietenia DC.[47]) resulted in a description of its irritant effect on skin[47] and of its toxic properties[48]. Recently skimmianine was shown to have a weaker spasmolytic action than the total alkaloids of Ruta graveolens[49]. When the dose was increased skimmianine increased the spontaneous contraction of guinea-pig uterus[49]. γ-Fagarine also possessed a weak spasmolytic action[49].

For Dictamnine, Kovalenko[50] recommended further clinical trials after showing that, amongst other effects, the base had a potent action on smooth muscle, strongly contracting guinea-pig and rabbit uterine muscle.

Lahey et al.[51] investigated the properties of acronycidine, showing that in its most marked properties, the effect on voluntary muscle, the base resembled skimmianine.

Alkaloids of unknown structure.

Xanthofagarine, isolated from F. parvifolia[52] A. Cheval, and F. macrophylla[53], when injected intravenously in the dog produced an abrupt fall in arterial pressure with brachycardia, followed by a short phase of hypertension; the action was similar to that produced

by other alkaloids of the African Fagara species, in particular fagaridine, also from F. macrophylla[53].

Artarine, isolated from F. xanthoxyloides[54], was reported to act on voluntary muscle, the rate of contraction of the heart being slowed and the amplitude increased.

Paris and Moyse-Mignon [52] found in the light petroleum extract of F. viridis an irritant substance, producing salivation and tingling of the tongue: also a crystalline non-nitrogenous compound, m.p. 162°, with ichthyotoxic properties. Aqueous infusions of both the stem and root bark of F. viridis were toxic to fish causing loss of balance, whereas similar extracts of F. parvifolia exhibited no such toxicity[52].

A butanol-soluble fraction of the stem bark of Fagara rhoifolia (fraction I of the extract used in this work, see Figure XII p. 109) was reported as having ganglionic blocking activity[55].

The pharmacological actions of the remaining alkaloids of unknown structure, listed in Table I, p. 25 , have not been reported.

THE ALKALOIDS OF RUTACEOUS SPECIES

General.

Species of the genus Fagara have been chemically investigated since the early part of the nineteenth century, possibly because of their wide use in folk-medicine in Africa and America. Recently greater interest has centred on the chemo-taxonomy of the alkaloids as a possible means of differentiating between the closely related genera Fagara and Zanthoxylum[2].

The Rutaceae, as a family, is of tremendous interest from the chemo-taxonomical aspect: it is probably unique in the diversity of the chemical types of alkaloid present. Biogenetically the alkaloids are noteworthy since they fall into two main groups. The first group, derived from benzylisoquinoline, includes the aporphines, protoberberines, protopines, benzophenanthridines, phthalide isoquinolines and benzyl-isoquinolines The second biogenetically homogeneous group is based on the "anthranilic acid unit" and includes the acridines, the quinazolino-carbolines and the quinolines, the latter including the isopropylfuro-quinolines, the furoquinolines and the dihydrofuroquinolines. Two additional small groups of alkaloids occur in the family, the imidazoles, including pilocarpine, found in the genera Pilocarpus and Casimiroa and the amide derivatives of the Fagara and Zanthoxylum genera, such as fagaramide.

The furoquinoline group is of particular taxonomic significance since it is found extensively in the Rutaceae and has not been reported in any other plant family. The acridines, chemically related to the

furoquinolines, have also been found exclusively in the subfamily Rutoideae[2].

Tertiary alkaloids of the Rutaceae.

As stated earlier, 42 genera of the Rutaceae contain alkaloids, 30 containing furoquinolines all of which occur as tertiary bases with the exception of the two isopropylfuroquinoline quaternary bases, lunasine (I, R = H, p.20) and O-methylbalfourodine (II, R = OH, p.20). Tertiary bases of the Rutaceae have been reviewed to some extent by Price[2], Ritchie[56], and Stambouli[57], and all such bases known prior to 1960 have been dealt with by Boit[58]. Since the present work is concerned mainly with quaternary compounds, a complete review of all tertiary bases in the Rutaceae would be inappropriate but it is of interest to note that in the past six years typical furoquinoline, acridine and quinazoline types of alkaloid have been isolated from the genera Balfourodendron[59], Evodia[61], Flindersia[62], Halfordia[63], Haplophyllum[64], Murraya[65], Platydesma[66], Ptelea[67], Ruta[49], Teclea[68], and Vepris[69]. Some of these alkaloids were known compounds, others were new compounds.

A number of new types of base have also been discovered: the dihydrofuroquinolines, dubinine (III, p.20), in Haplophyllum dubium Korovin[64], and ifflaiamine (IV, p. 20), in Flindersia ifflaiana F. Muell[62]., and the oxazole bases halfordinol, (V, R = H, p. 20), halfordine, (VI, R = $CH_2CHOHCOHMe_2$, p.20), and halfordinone, (VII, R = $CH_2COCHCHMe_2$, p. 20) in Halfordia scleroxyla F. Muell.[63]. More

unusual was the isolation of the carbazole derivative murrayine (VIII, p. 20) from Murraya koenigii Spreng.[65].

Quaternary alkaloids of the Rutaceae.

Quaternary bases have so far been reported in only seven genera, four of which, Balfourodendron, Halfordia, Phellodendron and Toddalia, occur in the subfamily Toddalioideae and three, Fagara, Lunasia and Zanthoxylum occur in the subfamily Rutoideae. This may not be a true picture of the distribution of quaternary bases in the Rutaceae. Quaternary bases may not have been extracted by the particular methods used for investigating some species or may have been rejected in aqueous extracts. Palmer[18] did not observe the presence of quaternary bases in Fagara leprieurii, F. macrophylla, F. viridis and F. xanthoxyloides, though the present work shows their occurrence in all four species.

Quaternary bases of the isopropylfuroquinoline type occur exclusively in Lunasia and Balfourodendron species, representing both subfamilies named above. The genus Halfordia, represented by H. scleroxyla, contains N-methylhalfordine, a quaternary oxazole base[70]. The genus Phellodendron contains quaternary bases of the aporphine, benzylisoquinoline and protoberberine types. These three types also occur in the genus Fagara along with quaternary bases of the benzophenanthridine type. Accepting the Engler and Prantl[1] classification of the genera Fagara and Zanthoxylum, then the alkaloidal-bearing

species remaining in the genus Zanthoxylum contain only quaternary bases of the aporphine type.

At present ten known quaternary bases are reported amongst fifteen Fagara species and three such bases between two Zanthoxylum species: several uncharacterised quaternary bases occur in a further six Fagara species. In the genus Phellodendron five known quaternary bases are reported amongst three species and three varieties of one of these species.

The first quaternary base to be isolated from the Rutaceae was lunasine, from Lunasia costulata Miq. in 1900[71], but the structure was not elucidated until 1959[72], using infra-red and N.M.R. analyses. In 1913, α-(-)-methylcanadine* was isolated from F. brachyacantha Engl.[27] and this was the first example of a natural compound with an asymmetric nitrogen atom. The protoberberines palmatine (1926) and jateorrhizine (1958) were isolated from Phellodendron amurense Rupr.[73] and P. amurense var. sachalinense Sargent[74] respectively: chelerythrine* (toddaline) was found in Toddalia aculeata Pers.[75] in 1933: N-methylisocorydine* was reported in Fagara brachyacantha Engl. in 1953[39]. The benzylisoquinoline phellodendrine and the aporphine magnoflorine* were found together in Phellodendron amurense (1957)[76]. Laurifoline* was isolated from Fagara ailanthoides Engl. (1958)[77].

Since 1958 seven new quaternary bases have been isolated,

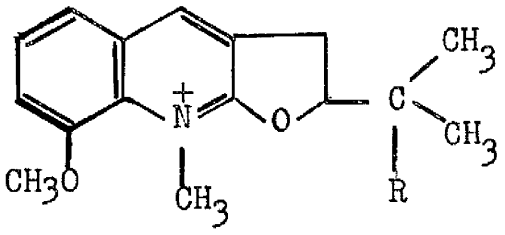
(* All compounds marked thus have been isolated from Fagara species and are included in Table I, pp.25 - 36 , where the structures, physical data and sources are quoted)

some of these being exclusive to the family Rutaceae. The benzophenanthridine nitidine* was isolated from Fagara nitida Roxb. in 1958[78] and the related base avicine* from Fagara avicennae Lam. in 1959[79]. Also in 1959, Rapoport and Holden[80] isolated the isopropylfuroquinoline O-methylbalfourodine from Balfourodendron riedelianum Engl. The aporphine 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphine* was isolated from Fagara tingoassuiba Hoehne in 1961[42]. The oxazole N-methylhalfordine was isolated from Halfordia scleroxyla[70] in 1963. Finally, in 1964, the benzylisoquinoline (+)-tembetarine* was isolated from Fagara naranjillo Engl. and F. rhoifolia Lam.[44], and the new quaternary alkaloid (+)-N-methylcorydine* was discovered in the bark of F. nigrescens Fries[81].

The presence of quaternary bases in the genus Fagara has become of increasing importance since 1953, when N-methylisocorydine and α-(-)-methylcanadine were shown to be the major alkaloids of F. brachyacantha Engl.[39]. During the subsequent years quaternary bases have been reported as the major alkaloids in 19 Fagara species. The present work shows that the quaternary bases α-(-)-methylcanadine and 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphine are the principal bases of F. rhoifolia[43]. Without doubt a number of Fagara species known to contain tertiary bases will require re-investigation for the presence of quaternary bases. The present work shows that quaternary bases are present in F. leprieurii, F. macrophylla, F. viridis and F. xanthoxyloides, all previously known to contain tertiary bases[18,52,12].

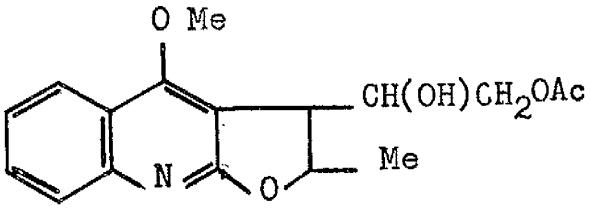
In the closely related genus Zanthoxylum, quaternary bases

were reported as the major alkaloids of *Z. planispinum*[82]. The roots contained magnoflorine (0.17%) and xanthoplanine (0.0075%) in greater concentration than the tertiary bases skimmianine (0.0015%), dictamnine (0.001%) and γ-fagarine (0.00015%). The wood and bark of the stem contained magnoflorine (0.02%) and xanthoplanine (0.01%)

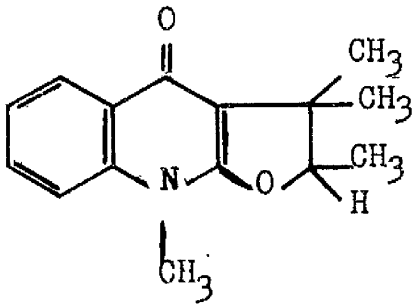


I, R = H

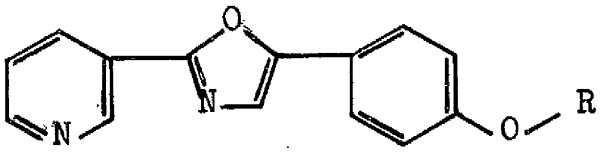
II, R = OH



III



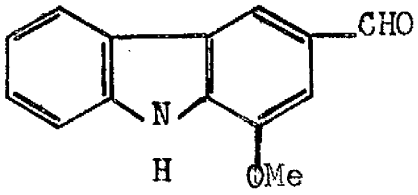
IV



V, R = H

VI, R = $CH_2CHOHCOHMe_2$

VII, R = $CH_2COCHCHMe_2$



VIII

Classification of the Fagara alkaloids.

The Fagara alkaloids of known structure follow the general pattern of the Rutaceous alkaloids and are divided into three groups. These three groups can be subdivided chemically as follows:-

Group I. Amides:- comprising two compounds, both derivatives of isobutylamide. One is an aryl compound, fagaramide[19], the other an alkyl compound, N-isobutyl-trans-2-trans-4-decenamide[24].

Group II. Benzylisoquinoline derivatives:- comprising 14 compounds, which can be further divided into five groups according to the type of nucleus and ultraviolet spectra.

A. Benzylisoquinolines:- This group contains one quaternary alkaloid, (+)-tembetarine chloride.

B. Aporphines:- This group comprises five quaternary alkaloids which can be further divided into two sub-groups according to their substitution pattern and ultraviolet spectra. Subgroup (i) contains three quaternary bases magnoflorine, N-methyl-isocorydine and (+)-N-methylcorydine which are substituted at positions 3, 4, 5 and 6 and have an ultraviolet spectrum with low absorption in the region of 300 mμ [83]. Subgroup (ii) contains two quaternary alkaloids (+)-laurifoline and 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride, both of which are substituted at positions 2, 3, 5 and 6 and have intense absorption in the 300 mμ region[83].

C. Protopines:- This group consists of the two tertiary isomers, α-allocryptopine and fagarine II, which have similar ultraviolet spectra[83] and differ only in the position of substituents at positions 9, 10 and 11.

D. Tetrahydroprotoberberines:- This group has one quaternary alkaloid, α-(-)-methylcanadine chloride.

E. Benzophenanthridines:- This group has five bases which can be divided into three subgroups on their ultraviolet spectra. Subgroup (i) contains the three quaternary bases nitidine, avicine and chlerythrine: subgroup (ii) has the tertiary alkaloid oxynitidine: subgroup (iii) contains dihydrochelerythrine.

Group III. Anthranilic acid group:- comprising five tertiary alkaloids which are in two subgroups, the furoquinolines and the quinazolinocarbolines.

A. Furoquinolines:- This group has three tertiary alkaloids dictamnine, γ-fagarine and skimmianine, the latter being the most common alkaloid in the genus Fagara occurring in ten species. These three bases differ only in the type of substitution at positions 7 and 8.

B. Quinazolinocarbolines (Indolo-quinazolines):- This group contains three tertiary bases evodiamine and rutaecarpine, which have a five-ringed nucleus, and rhetsinine which has only four rings in the nucleus, Catalytic reduction of rhetsinine gave evodiamine (rhetsine)[127].

Alkaloids of Fagara rhoifolia Lam.

F. rhoifolia Lam. was first investigated by Albonico et al.[44]

who, in 1964, reported the presence of (+)-tembetarine. The presence of two additional quaternary bases, α-(-)-methylcanadine and 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphine, and the tertiary base α-allocryptopine has recently been reported by the author[43] and their isolation is described in the present work.

Alkaloids of unknown structure.

The literature on Fagara species contains references to twenty-five crystalline alkaloids of unknown structure. Palmer[18] has shown, by paper chromatography, that several of these were in fact mixtures: alkaloids A and B from F. macrophylla[53], parvifagarine from F. parvifolia[52] and alkaloids A_2 and A_3 (fagaridine) from F. xanthoxyloides[12] have all been shown to be mixtures of two or three components. Palmer[18] has also reported tertiary bases in F. pubescens A. Chev. and both tertiary and quaternary bases in F. melanacantha Engl. Ill-defined alkaloidal material is also reported[84] in F. peckoltiana Engl. F. schinifolia Engl. is reported[96] to contain a large amount of thermo-labile quaternary base which decomposed during extraction. The remaining fifteen bases are included in Table I (page 25). Palmer[18] isolated two new alkaloids, angoline and angolinine, from F. angolensis: the present work shows that angolinine is a mixture of two bases.

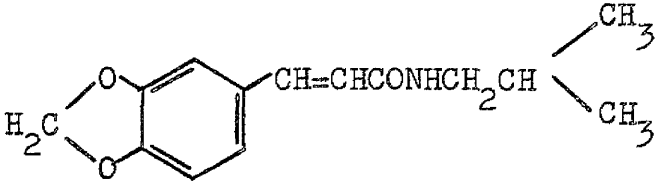
Alkaloid A of F. tingoassuiba[17] would appear, from the infrared spectra of the base and its hydrochloride salt, to be a protopine derivative. The ultraviolet spectrum also suggests a protopine base. The

ultraviolet spectrum of alkaloid B from this species is very similar to that obtained in the present work for base X in F. rhoifolia; base X is possibly a mixture of two alkaloids.

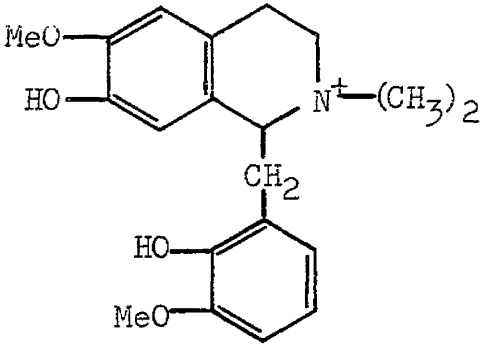
Table I

Alkaloids of the Fagara

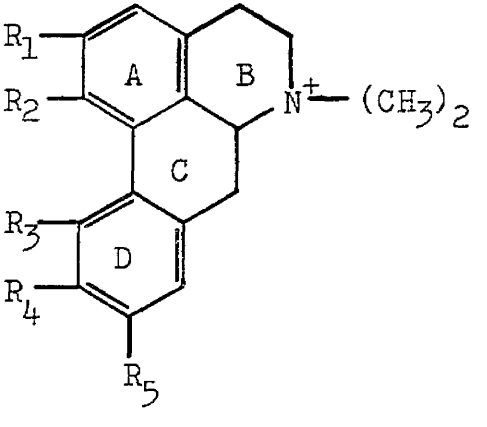
Amides

Alkaloid m.p. °C	Structure	Source	Refs.
Fagaramide $C_{14}H_{17}O_3N$ 119-120		Fagara macrophylla Engl. F. xanthoxyloides Lam. (Zanthoxylum senegalense DC.)	20 19
N-Isobutyldecenamide $C_{14}H_{25}ON$ 90-90.5	$CH_3(CH_2)_4(CH{=}CH)_2CONHCH_2CH(CH_3)_2$	F. xanthoxyloides Lam. (Z. senegalense DC.)	24

Benzylisoquinolines.

Alkaloid m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
(+)-Tembetarine chloride $C_{20}H_{26}O_4N^+Cl^-$ 236-237	+123.3° (EtOH)		F. naranjillo Engl. F. hyemalis Engl. F. nigrescens Fries F. pterota Engl. F. rhoifolia Lam.	44, 85 44, 85 44, 85 44, 85 44, 85

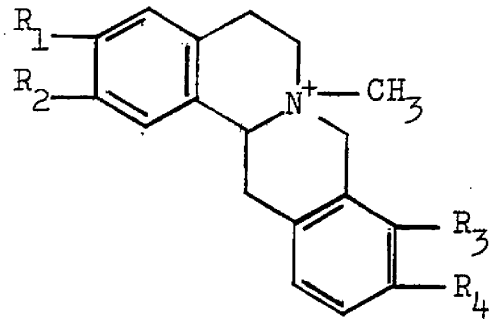
Aporphines.

Laurifoline chloride $C_{20}H_{24}O_4N^+Cl^-$ 253	+26° (H_2O)	 R_1 OMe; R_2 OH; R_3 H; R_4 OMe; R_5 OH	F. ailanthoides Engl. (Z. ailanthoides Sieb & Zucc.) F. pterota Engl.	77 44, 85

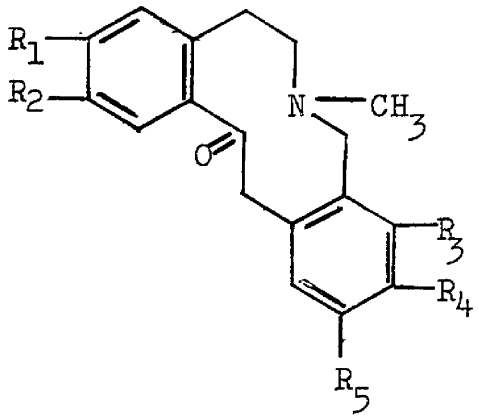
Aporphines, (contd.)

Alkaloid M.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Magnoflorine Iodide $C_{20}H_{24}O_4{}^+I^-$ 248-249	-214° (MeOH)	R_1 OMe; R_2 OH; R_3 OH; R_4 OMe; R_5 H	F. ailanthoides Engl. (Z. ailanthoides Sieb & Zucc.)	77
5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride $C_{21}H_{26}O_4N^+Cl^-$ 215-219	+30.2° (H_2O)	R_1 OMe; R_2 OH; R_3 H; R_4 OMe; R_5 OMe	F. tingoassuiba Hoehne F. rhoifolia Lam.	42 43
N-Methylisocorydine chloride $C_{21}H_{26}O_4N^+Cl^-$ 217-218	-168.6° (H_2O)	R_1 OMe; R_2 OMe; R_3 OH; R_4 OMe; R_5 H	F. coco Engl. F. brachyacantha Engl. (Z. brachyacanthum F. Muell.) F. venenefica Engl. (Z. veneneficum F.M. Bailey)	38 39 39
(+)-N-Methylcorydine styphnate $C_{21}H_{26}O_4N^+$ 206-207	+28.7° (Me_2CO)	R_1 OMe; R_2 OH; R_3 OMe; R_4 OMe; R_5 H	F. nigrescens Fries	81

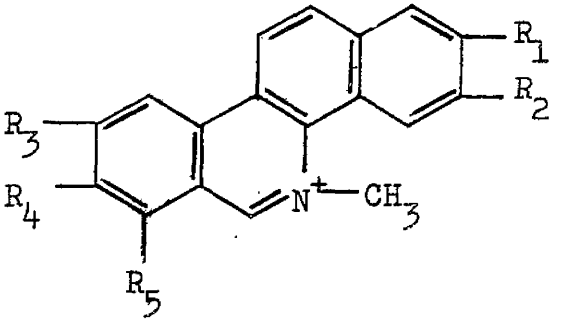
Protoberberines

Alkaloid m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
α-(-)-Methylcanadine chloride $C_{21}H_{24}O_4N^+Cl^-$ 262	-137° (H_2O)	 R1 = R2 = -O-Me-O-; R3 = OMe; R4 = OMe	F. brachyacantha Engl. (Z. brachyacanthum F. Muell.) F. venenefica Engl. (Z. veneneficum F.M. Bailey) F. rhoifolia Lam.	27 39 43

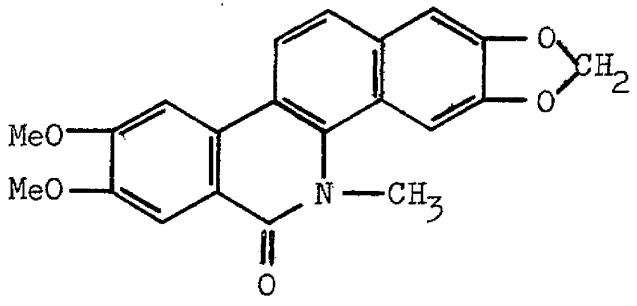
Protopines

α-Allocryptopine $C_{21}H_{23}O_5N$ 160	0°	 R1 = R2 = -O-Me-O-; R3 = OMe; R4 = OMe; R5 = H	F. brachyacantha Engl. (Z. brachyacanthum F. Muell.) F. coco Engl. F. rhoifolia Lam. F. venenefica Engl. (Z. veneneficum F.M. Bailey)	27 86 43 39

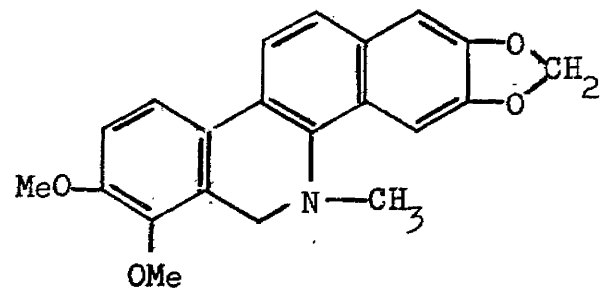
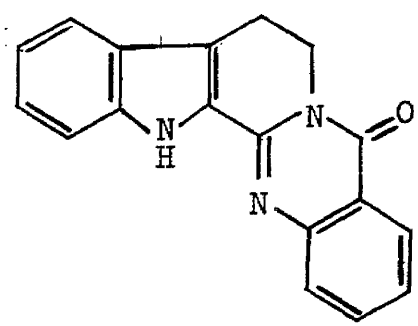
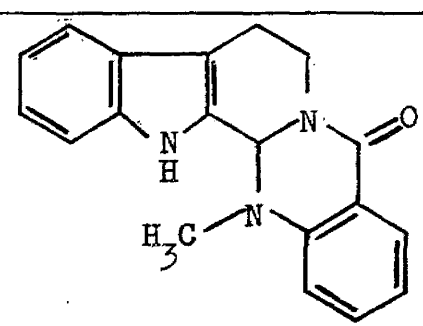
Protopines (contd.).

Alkaloids m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Fagarine II $C_{21}H_{23}O_5N$ 198-199	0°	R_1 R_2 R_3 R_4 R_5 -O-Me-O- H OMe OMe	F. coco Engl.	86
Benzophenanthridines.				
Avicine $C_{20}H_{14}O_4N^+OH^-$	0°	 R_1 R_2 R_3 R_4 R_5 -O-Me-O- -O-Me-O- H	F. avicennae Lam.	79

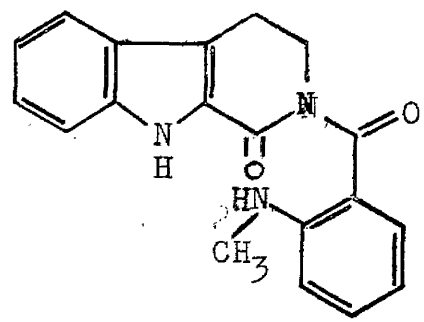
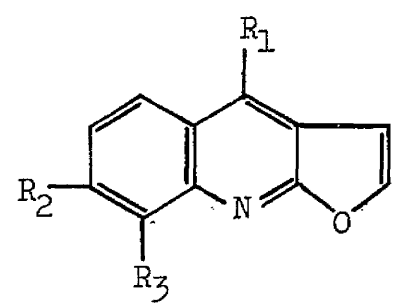
Benzophenanthridines (contd.).

Alkaloids m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Nitidine $C_{21}H_{18}O_4N^+OH^-$ 285-286	0°	R_1 R_2 R_3 R_4 R_5 -O-Me-O- OMe OMe H	F. nitida Roxb. (Z. nitidum DC.) F. hamiltonia Engl. (Z. hamiltonianum Wall.)	78 87
Chelerythrine (Toddaline) $C_{21}H_{18}O_4N^+Cl^-$ 213-214 yellow	0°	R_1 R_2 R_3 R_4 R_5 -O-Me-O- H OMe OMe	F. brachyacantha Engl. (Z. brachyacanthum F. Muell.) F. rhetsa Roxb. (Z. rhetsa DC.) F. semiarticulata St. John & Hosaka F. venenefica Engl. (Z. veneneficum F.M. Bailey)	39 88 89 39
Oxynitidine $C_{21}H_{17}O_5N$ 284-285 yellow	0°		F. nitida Roxb. (Z. nitidum DC.)	78

Benzophenanthridines, (contd.).

Alkaloid m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Dihydrochelerythrine $C_{21}H_{19}O_4N$ 166-167	0°		F. semiarticulata St. John & Hosaka	89
Quinazolinocarbolines.				
Rutaecarpine (Rhetine) $C_{18}H_{13}ON_3$ 258	0°		F. rhetsa Roxb. (Z. rhetsa DC.)	88, 91
Evodiamine (Rhetsine) $C_{19}H_{17}ON_3$ 277-278	0°		F. rhetsa Roxb. (Z. rhetsa DC.)	88, 91

Quinazolinocarbolines, (contd.).

Alkaloid m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Rhetsinine $C_{19}H_{17}O_2N_3$ 196	0°		F. rhetsa Roxb. (Z. rhetsa DC.) F. oxyphylla R. & C. (Z. oxyphyllum)	88,127 90
Furoquinolines.				
Dictamnine $C_{12}H_9O_2N$ 132-133	0°	 R_1 OMe, R_2 H, R_3 H	F. ailanthoides Engl. (Z. ailanthoides Sieb & Zucc.)	77

Furoquinolines, (Contd.).

Alkaloids m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
γ-Fagarine $C_{13}H_{11}O_3N$ 142-143	0°	 R_1 R_2 R_3 OMe H OMe	F. coco Engl.	92
Skimmianine (β-Fagarine) $C_{14}H_{13}O_4N$ 176-177	0°	 R_1 R_2 R_3 OMe OMe OMe	F. ailanthoides Engl. (Z. ailanthoides Sieb. & Zucc.) F. angolensis Engl. F. coco Engl. F. leprieurii Engl. F. macrophylla Engl. F. mantchurica Honda F. rhetsa Roxb. (Z. rhetsa DC.) F. viridis A. Cheval F. xanthoxyloides Lam. (Z. senegalense DC.) F. schinifolia Engl. (Z. schinifolium Sieb. & Zucc.)	77 93 92 18 18 94 95 52 12 96

Alkaloids of unknown structure.

Alkaloid	m.p. °C.	$[\alpha]_D$	Source	Refs.
Alkaloid B	$C_9H_{17}ON$.HCl 186 .picrate 187		F. tingoassuiba Hoehne	17
Xanthofagarine	$C_{18}H_{21}O_8N$ 278-280 yellow -23 .HCl 245	0°	F. macrophylla Engl. F. parvifolia A. Chev.	53 52
Un-named Alkaloid from F. macrophylla	$C_{20}H_{21}O_7N$.HCl 276 (dec.) yellow		F. macrophylla Engl.	97
Fagaridine	$C_{19}H_{23}O_7N$ 238-240 red -25		F. macrophylla Engl.	53
Alkaloid A	$C_{20}H_{19}O_4N$.HCl 185-186 .picrate 202	0°	F. tingoassuiba Hoehne	17
Artarine	$C_{21}H_{23}O_4N$.HCl 194 yellow .nitrate 212		F. xanthoxyloides Lam.	54
Angoline (Base A) (Base A_2)	$C_{22}H_{21}O_4N$ 209 .HCl 267-268 yellow .picrate 238-239		F. angolensis Engl. F. parvifolia A. Chev. F. xanthoxyloides Lam.	93 18 18

Alkaloids of unknown structure. (contd.).

Alkaloid	m.p. °C	$[\alpha]_D$	Source	Refs.
Fagarine III	$C_{22}H_{25}O_4N$. 181-183 -27 .HCl 232	-300° ($CHCl_3$)	F. coco Engl.	86
Angolinine	$C_{24}H_{23}O_6N$. 272-273 .HCl 299 .picrate 278-280		F. angolensis Engl.	93
Melanacanthine	$C_{23}H_{23}O_5N$. 208 .HCl 280-281 .picrate 247		F. melanacantha Engl.	18
Melanacanthinine	$C_{44}H_{46}O_9N$. 202-203 .HCl 287 .picrate 269-270		F. melanacantha Engl.	18
α-Heitzine	$C_{19}H_{17}O_4N$. 280		F. heitzii Aubreville & Pellegrin	18
β-Heitzine	$C_{19}H_{17}O_2N_3$ 261-263 .HCl 304-305 .picrate 273		F. heitzii Aubreville & Pellegrin	18

Alkaloids of unknown structure, (contd.).

Alkaloid	m.p. °C	$[\alpha]_D$	Source	Refs.
γ-Heitzine	$C_{25}H_{25}O_5N$. 199-200 .HCl 278-280 .picrate 272		F. heitzii Aubreville & Pellegrin	18
δ-Heitzine	$C_{21}H_{19}O_5N$. 254-255 .HCl 254-255 .picrate 272		F. heitzii Aubreville & Pellegrin	18

METHODS FOR EXTRACTION AND SEPARATION OF TERTIARY AND QUATERNARY BASES

One of the conventional methods for the isolation of tertiary alkaloids is the extraction of the suitably basified plant material by a non-polar solvent, such as benzene. The alkaloids, as salts, are then removed from the extract, by extracting with dilute aqueous acid: the acid solution is basified and the alkaloids, as free bases, extracted with an organic solvent, commonly ether or chloroform. This method almost certainly does not extract the quaternary bases.

An alternative method is the extraction of the dried non-basified material with ethanol[38], or other polar solvent, under either acid or neutral conditions. Under neutral conditions, after removal of most of the solvent, the residual extract is treated with dilute aqueous acid. Both tertiary and quaternary alkaloids are removed by this method and the tertiary bases can be obtained by basifying the acid solution and extracting with an organic solvent. Because of their water-solubility, the quaternary bases remain in the aqueous phase and can be recovered by precipitation as complexes with mercuric chloride[38], potassium mercuric iodide[98] or, more commonly, as insoluble salts such as picrates[42] and reineckates[99] or, more recently, by partition with a water-immiscible solvent such as n-butanol[55].

Comin and Deulofeu[38] precipitated N-methylisocorydine from aqueous solution as a mercuric chloride complex, while Riggs et al.[42] used the picrate method for the precipitation of 5-hydroxy-2,3,6-trimethoxy-N,N,dimethylaporphine. Occasionally mixtures of such salts

may be fractionated by crystallisation, but they may be converted, by double decomposition or by ion-exchange resins[98,100] to chlorides which are then separated. A serious disadvantage of the precipitation of quaternary alkaloids as insoluble complexes is the risk of decomposition of the base which may occur concurrently with the breakdown of the complex. Petaline reineckate, isolated from Leontice leontopetalum L., Berberidaceae, undergoes Hofmann degradation on passage through a column of anion exchange resin, producing the corresponding methine base which was previously described as leonticine[101]. Where decomposition may be expected, partition with butanol is a safer method, the disadvantage of this method being the formation of stable liquid-liquid emulsions due to the emulsifying properties of quaternary salts. This difficulty can be avoided by the use of liquid-liquid extractors, though the high boiling point of n-butanol is a disadvantage.

Isolation of individual quaternary compounds can sometimes be achieved by fractional crystallisation or by micro-sublimation but these conventional methods often fail to yield pure quaternary compounds. In such cases various chromatographic methods or countercurrent distribution, or a combination of such techniques are used to achieve separation. (+)-N-Methylcorydine was separated from the mixture of quaternary bases, isolated from F. nigrescens Fries[81], by countercurrent distribution and column chromatography on cellulose.

Adsorption chromatography, on alumina, has been used successfully in the separation of tertiary bases. Iriarte et al.[102] separated

the three closely related tertiary furoquinoline alkaloids, dictamnine, γ-fagarine and skimmianine from an alkaline benzene extract of the bark of Casimiroa edulis La Lave, Rutaceae, by chromatography on an alumina column. The use of this method, however, for the separation of quaternary salts, both reineckate[99,103] and chlorides[104], has been criticised, since well-separated fractions from alumina columns contain the same alkaloids. Adsorption chromatography has, however, been useful in the removal of non-alkaloidal impurities, giving fractions from which crystalline material can be obtained.

The most efficient and satisfactory method for resolution of the quaternary alkaloids is that developed by Schmid and Karrer[105], and Wieland[106], utilising cellulose columns and a variety of solvent systems for the separation of calabash curare alkaloids. Bartlett et al.[107] have satisfactorily separated the quaternary alkaloids of Hunteria eburnea Pichon, Apocynaceae, on cellulose, using very simple monophasic solvent systems such as acetone saturated with water. Recently Battersby and Yeowell[108] separated the indole alkaloids macusine B and macusine C, on cellulose, using methyl ethyl ketone saturated with water.

This separation of quaternary alkaloids by partition chromatography was a major advance, but pure compounds are obtained only by working under strictly controlled conditions, constant temperature being of special importance. Large amounts of starting material are required, with the subsequent reworking or rejection of mixed fractions, before appreciable quantities of pure alkaloids can be obtained. With smaller

amounts of starting material, preparative paper chromatography has been successful in the isolation of pure alkaloids, for example, in the separation of quaternary bases from certain Strychnos species of the family Loganiaceae[109] and Aspidosperma subincanum Mart., Apocynaceae[110].

Another useful method for the separation of complex mixtures of natural products is based on countercurrent distribution. The application of this technique to the alkaloid field has been reviewed by Casinovi[111], who succeeded in resolving a total extract from Strychnos amazonica Krukoff by repeated distribution between methyl ethyl ketone and water.

One advantage of countercurrent distribution lies in the possibility of following the course of fractionation at any stage. As the process is continuous, there is no danger of diffusion which, as a result of interruptions in flow or the long duration of the process, may occur in and reduce the efficiency of chromatographic methods. Also absent are irreversible adsorption phenomena and wall effects which in column chromatography may bring about a loss of substances with consequent reduction of efficiency. Further improvements in this type of separation have been made possible by the introduction of steady-state distribution.

The principal disadvantage of this method is the formation of emulsions formed under experimental conditions. Control of emulsion formation is very difficult when dealing with crude natural extracts, especially with the presence of quaternary compounds. Qaisuddin[99] was unable to separate indole type quaternary alkaloids in a mixture from Aspidosperma peroba F. Allem, Apocynaceae.

Electrophoresis has been widely used for the separation of

amino acids. Only recently has the technique been applied to the isolation and separation of quaternary alkaloids. Winek *et al.*[112] have used a gel electrophoretic procedure for the separation of the quaternary aporphine magnoflorine from plant extracts of *Aquilegia caerulea* var. Scott-Elliot, Ranunculaceae.

ALKALOIDAL SCREENING OF PLANT MATERIAL.

In surveys for alkaloids in plants of N. Borneo[113], Hongkong[114], Malaya[115,116], and Hawaii[117,118,119], various workers have commented on the difficulties of extraction and detection of these compounds and, in 1960, Raffauf[120] stated that none of the usual methods was completely acceptable. His review highlighted, amongst other points, the difficulty of purifying extracts without loss of certain types of alkaloids and the failure of most procedures to detect quaternary alkaloids. As recently as 1963 a method was advocated for the extraction of alkaloids from fresh plant material, which would certainly fail to detect quaternary alkaloids[121].

The more recent screening methods have attempted with some success to avoid these losses. The method of Farnsworth and Euler[122] has overcome the failure to extract quaternary alkaloids by following the extraction of tertiary alkaloids with a second extraction using acidified ethanol. The weight of plant material used was only 2g though Raffauf[120] had recommended a minimum of 20g. Farnsworth and Euler[122], in addition, screened the tertiary and quaternary alkaloid fractions on thin-layer silica gel chromatoplates. The pigments present in the crude extracts interfered with the interpretation of the chromatograms, a difficulty which was overcome by partial purification of the extracts. Their purification of the tertiary base extract can be criticised because the chloroform extract of the acidic alkaloidal aqueous phase was rejected without further examination. The present work shows that, under these conditions, alkaloids may be extracted in the chloroform phase. Gilbert *et al.*[123]

have also shown, in the examination of certain Aspidosperma species, that such a chloroform solution contained alkaloids.

Farnsworth and Euler[122] used Dragendorff's reagent for the detection of alkaloids, this reagent having been recommended as one of the most useful for this purpose[124].

In a chemo-taxonomic study Sandberg and Michel[125] utilised two dimensional thin-layer chromatograms of the alkaloidal fractions of Pancratium maritimum L., Amaryllidaceae, to show the existence of qualitative and quantitative variation in plants from various habitats. In the genus Ptelea, Rutaceae, a survey[126] of the chromatographic patterns of leaf extracts has been used as an aid to phylogenetic studies on the genus. The chromatograms gave distinctive patterns when observed in ultra-violet light.

The screening method used in the present work has taken account of the above considerations.

D I S C U S S I O N

INTRODUCTION TO THE PRESENT WORK

The main object of this investigation was the isolation and identification of the quaternary and tertiary alkaloids from Fagara rhoifolia Lam. Additionally, since the presence of quaternary alkaloids had recently been shown in a number of Fagara species, the stem and root barks of Fagara leprieurii Engl., F. macrophylla Engl., F. viridis A. Cheval. and F. xanthoxyloides Lam., known to contain tertiary alkaloids, were examined for the presence of quaternary alkaloids.

PRELIMINARY EXTRACTIONS AND SEPARATIONS

Materials.

The starting materials for this work were three crude extracts obtained from the bark of authenticated[55] Fagara rhoifolia Lam., according to the extraction scheme shown in Figure XII, pp.109 - 111. Due to different solubility characteristics the quaternary and tertiary alkaloids were present in different fractions. Fractions I and II were subsequently shown to contain only quaternary compounds while the tertiary alkaloids were present in fraction III together with a chloroform-soluble quaternary base. The residues, as received, were in the form of pale brown amorphous powders. Each fraction was partially soluble in water, the three solutions being strongly positive to Meyer's and Dragendorff's reagents. The water-soluble alkaloids were present as the chlorides, the aqueous solutions giving a heavy white precipitate with solution of silver nitrate.

PAPER CHROMATOGRAPHY

Since most other Fagara species so far investigated had shown the presence of complex mixtures of alkaloids, both tertiary and quaternary, it was felt that a suitable paper chromatographic method would be required to determine the number of alkaloids present in fractions of the material obtained from F. rhoifolia bark.

As the literature showed a wide range both of possible solvent systems, monophasic and biphasic, and of actual chromatographic technique, several solvent systems were tried in order to determine the most suitable for this particular case. The paper used for all chromatograms was Whatman No. 1, and the alkaloids were detected using either modified Dragendorff's[128] or iodoplatinic acid reagent[129]. The former reagent gave orange to orange-brown areas which contrasted well with the pale yellow background. Iodoplatinic acid reagent[129], alleged to give differently coloured areas, usually blue to green and blue-black, in this case gave little colour difference and, since the areas did not contrast well with the red-purple background, this reagent was abandoned in favour of Dragendorff's which has been reported as one of the most sensitive reagents for the detection of alkaloids[124].

Development of chromatograms.

Ascending development was first used and of the solvents tested No. 1 (Table III, p.115), gave good definition but did not separate all the quaternary bases of fraction I, although good separation of the tertiary bases in fraction III was achieved. The similar solvent No. 2, used

successfully for the separation of bases in F. semiarticulata[89], did not separate all the bases of fraction I and gave only one large unseparated area for fraction III. Since the R_f values were high, the organic phase of solvents Nos. 1 and 2 was obviously too polar and therefore, part of the n-butanol portion was replaced by ether to give solvent system No. 4. This system did reduce the R_f values but without improved separation and it had the disadvantage of producing diffuse areas. Solvent No. 8[18] was particularly good for the tertiary bases of fraction III but No. 9[55] gave the best overall picture, showing the presence of five bases in fraction I and three in fraction III, although the areas tended to be elongated and to merge.

Of the three solvents used for descending development No. 7[107] gave diffuse elongated areas, while No. 8[18], though excellent for the tertiary bases of fraction III, gave only two large compact areas for the quaternary bases of fraction I. As in the case of ascending development, solvent No. 9[55] was the most efficient, giving very good definition and showing the presence of six, possibly eight, bases in fraction I and three in fraction III.

Of the four solvents used for radial development none gave good definition although No. 3 was useful for separating the tertiary bases of fraction III. Even solvent No. 9[55], so efficient in the ascending and descending methods, gave poorly defined separation with this more rapid method. This may have been due to the short run or, as was subsequently seen with descending technique, to the relatively low temperature (20°).

It is interesting to note that neither solvent No. 2 nor

No. 8, both used successfully for other Fagara species[18,89], was completely successful in this instance, although No. 2 was of some use for the separation of quaternary bases of fraction I and No. 8 for the tertiary bases of fraction III. Generally, solvents which were of use for quaternary bases gave poor separation with tertiary bases and vice versa, an exception being No. 9, although even this solvent gave better separation of quaternary bases. Another interesting point was the small amount of movement of the bases with solvent No. 5 which had been very successful in separating tertiary indole bases of Voacanga[130] species.

Solvent No. 9, with descending development, was chosen as the most efficient for identifying the bases and henceforth in this work it is referred to as the "standard solvent".

Effect of temperature variation.

The standard solvent was used to run a series of chromatograms over the temperature range 21-32°. The constant temperature tank used was similar to that described by Fairbairn and Wassel[131]; improved temperature control ($\pm$ 0.2°) was achieved by using contact thermometer thermostats.

The results (Figure II, p. 49) indicated that 25° was the optimum temperature for this system. At this temperature the alkaloidal areas were well separated and compact. As the temperature increased from 21 to 32° all the R_f values increased, although not proportionately. An increase or decrease in temperature from 25° resulted in considerable "tailing" which produced merging of the alkaloidal spots, especially at the higher temperatures.

A development time of 16 hours was necessary to achieve adequate movement and separation at 25°.

Figure II

Effect of temperature variation on R_f values of compounds A-E in fraction I

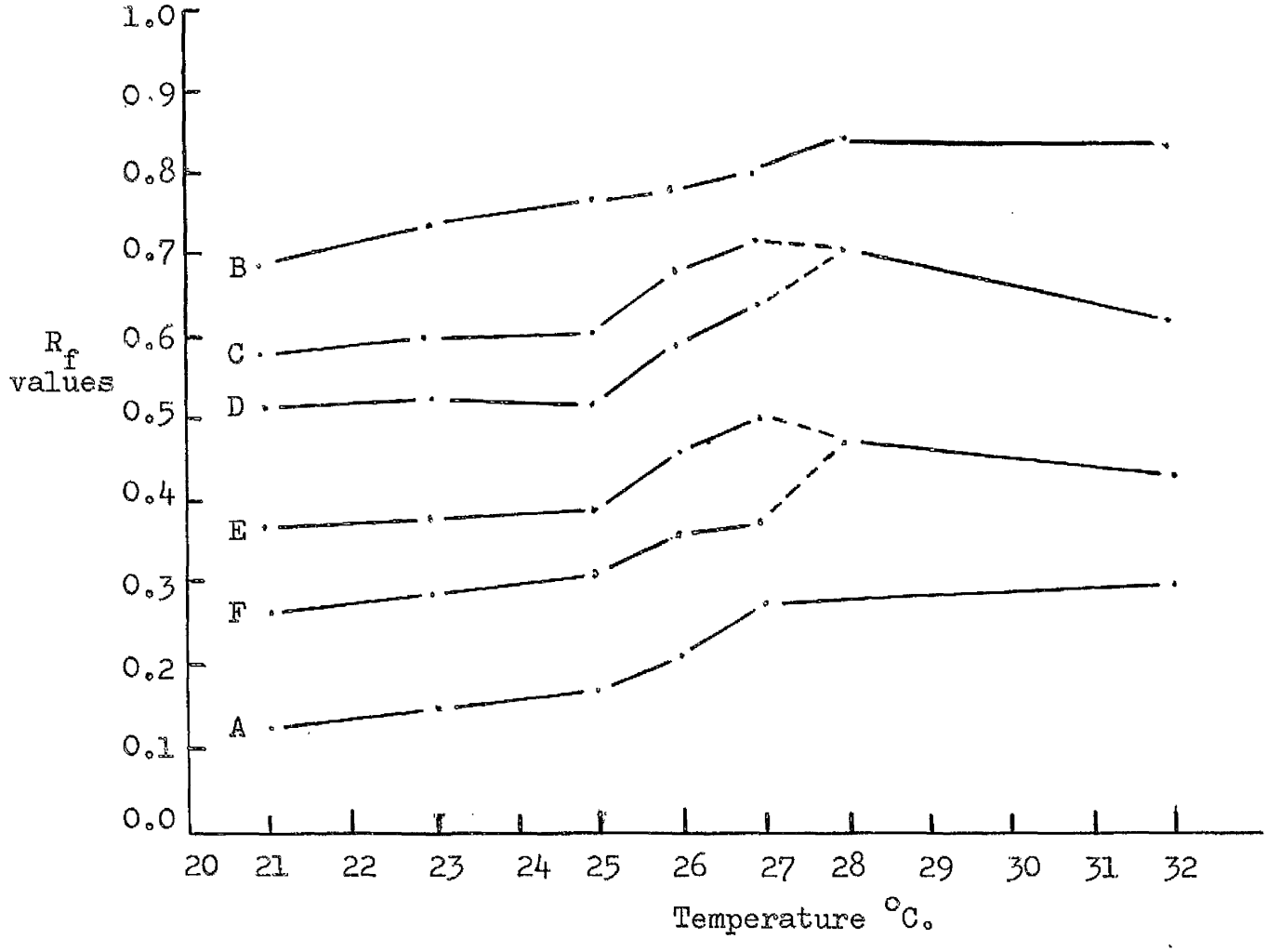
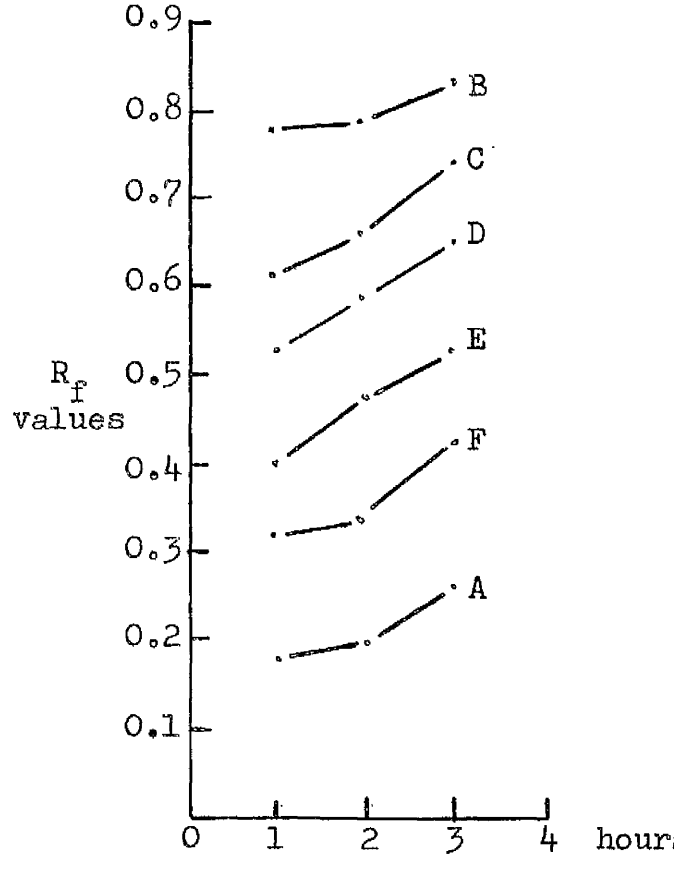


Figure III

Effect of variation in saturation time of papers prior to development



Effect of variation in saturation time.

Having determined the best solvent and optimal development temperature on papers equilibrated for an arbitrary period of one hour, a series of experiments was carried out to determine the optimal saturation time for the papers prior to development; one hour was found to give the most satisfactory result (Figure III, p. 49). Increase in saturation time over 1 hour produced an increase in R_f values accompanied by considerable "tailing". Prior saturation of 0 and 0.5 hours duration was inadequate; in both cases the solvent front travelled a shorter distance than with 1 hour saturation. Fraction I showed the presence of only three bases whereas six bases were observed with 1 hour prior saturation. Similarly Bush and Crowshaw[132] observed poor separation of steroids due to inadequate prior saturation, this being accompanied, as in the present case, by diffuse elongated spots.

Results.

Chromatograms, obtained by downward development using the standard solvent at 25°, showed the presence, in fraction I, of six bases which were designated as compounds A,(R_f 0.18), B,(0.78), C,(0.62), D,(0.53), E,(0.40), F,(0.32). Bases A and B were most abundant, C and D moderately abundant and E and F present in low concentration. Occasionally two additional alkaloidal areas (R_f 0.57 and 0.69) appeared in chromatograms. Fraction II contained compounds A, B, C, E and F but only B was present in significant amount; compound D was absent. The composition of fraction

III was markedly different: base B again appeared but gave a slightly lower R_f value, 0.73, (subsequent work showed this to be a mixture of two bases, see p. 83): two other bases were present in trace amounts one, H, of R_f 0.57 but different from C, the other, G, of R_f 0.96.

THIN-LAYER CHROMATOGRAPHY

Having determined, by paper chromatography, the possible number of alkaloids present in the fractions, use was made of thin-layer chromatography in an attempt to obtain a quicker method of separation and also to differentiate between tertiary and quaternary alkaloids in the crude base fractions.

Thin-layer chromatograms were developed at 25°, in a constant temperature room, on Alumina G, Silica Gel G and powdered cellulose using various organic solvents. It was anticipated that the results on powdered cellulose might correspond to the results on paper chromatograms.

Cellulose.

The standard solvent, No. 3^{55}, and solvents 1(i) and 1(ii), p.117 , produced continuous streaks with fractions I and II but solvent 1(iii) separated the quaternary bases into three groups. Solvent 2^{107}, methyl ethyl ketone/water (saturated), gave the best results, showing four alkaloidal areas, Table V, p.119.

Silica Gel.

With the solvents used, p.117 , the quaternary bases of fractions I and II did not move from the base line. Using the ethanol/chloroform mixtures, the tertiary bases of fraction III were not separated as efficiently as on Alumina G (Table V, p.119). Unexpectedly, solvent 2^{133}, p.117 , gave no movement of compounds. Solvent 3^{134} gave excellent separation and definition but this system was rejected for general use in

favour of the equally successful and simpler ethanol/chloroform mixtures on alumina.

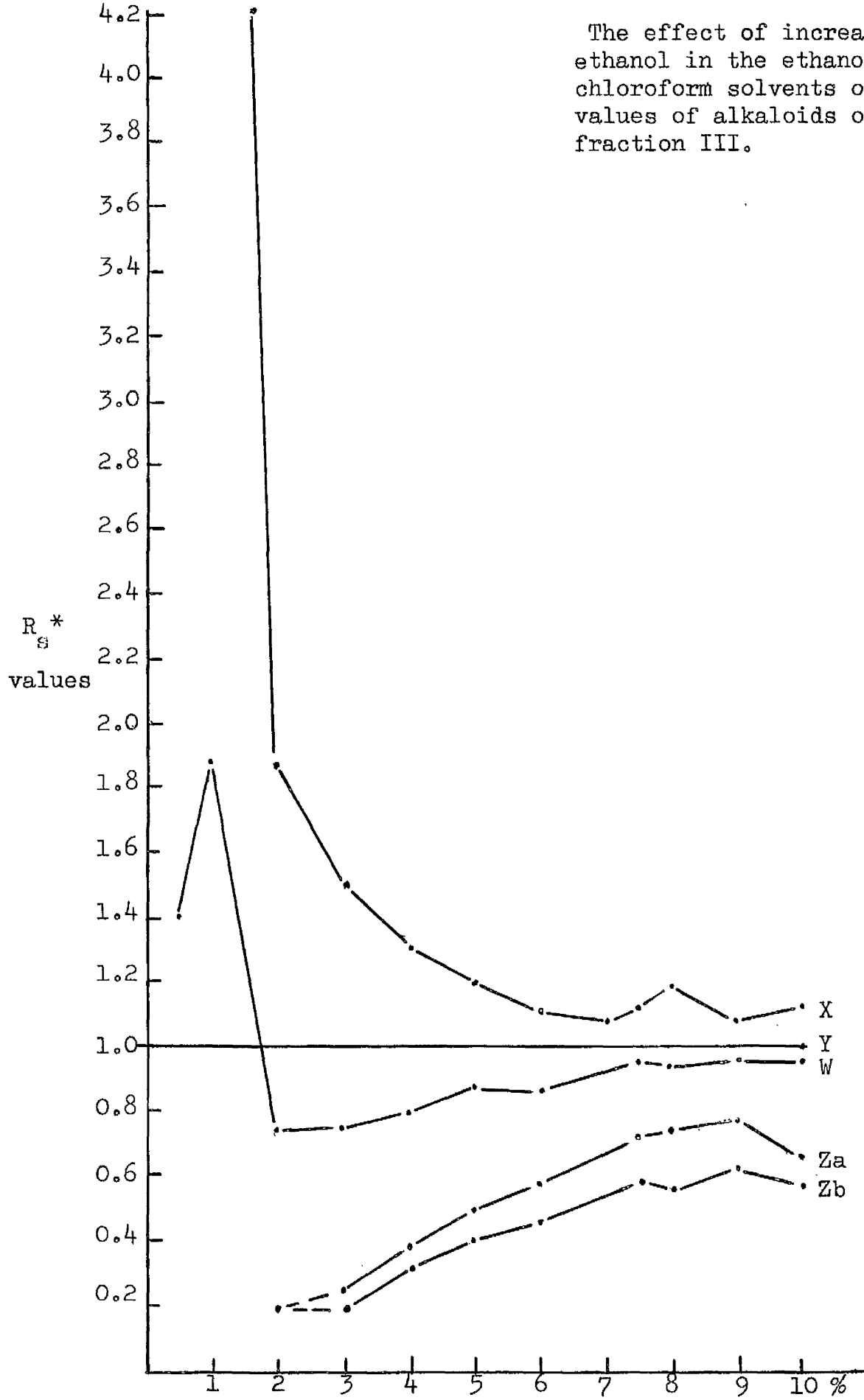
Alumina.

The results (Table IV, p. 118) showed that, using ethanol/chloroform mixtures, the quaternary bases remained on the starting line while the tertiary bases moved. In fractions I and II all the bases remained on the starting line, confirming the deduction that they contained only quaternary bases. Initially fraction III showed the presence of three tertiary bases but subsequent purification and concentration of the material revealed the presence of three additional tertiary bases (see p. 85). Four bases gave the usual orange colours with Dragendorff's reagent; exceptions being the blue-grey colour given by base W and the red-violet colour by base Za. Palmer[18] reported that the furoquinoline base, skimmianine, gave a rose-violet colour with Dragendorff's reagent.

The result of increasing the concentration of ethanol in chloroform (Table IV, p. 118) was to produce an increase in R_f values, the optimal concentration for separation of tertiary bases X, Y and Z being 2%. However, by increasing the concentration to 8%, base Z was most clearly resolved into two fractions which were designated Za and Zb while base Y because of its increased R_f value was also separated more clearly from a minor base which was called compound W. The results (Figure IV, p. 54) were expressed as R_s values (using compound Y = α-allocryptopine as standard) since these, determined from a number of chromatograms, were found to be more constant than R_f values.

Figure IV

The effect of increasing % ethanol in the ethanol/chloroform solvents on the R_s values of alkaloids of fraction III.



(* reference standard = (Y) = α-allocryptopine)

Neither ether nor ethyl acetate gave good separation of the bases in fractions I, II and III.

Conclusions.

Separation of the quaternary bases of fractions I and II on cellulose chromatoplates was inefficient compared with that obtained on paper chromatograms, using the standard solvent. Separation of the tertiary bases of fraction III was most efficient and rapid on alumina chromatoplates, using the ethanol/chloroform solvents.

ISOLATION OF COMPOUNDS FROM FRACTION I

Attempted removal of non-alkaloidal material by solvent extraction.

Fraction I contained six quaternary bases which required separation. The material was dark brown in colour and very hygroscopic, a property at first thought to belong to the non-alkaloidal material but later shown to be due largely to the presence of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride. Attempts to crystallise the bases failed. Obviously further purification was necessary, since the bases were present as water-soluble quaternary chlorides, extraction with a non-polar solvent was considered a possible means of removing the non-alkaloidal material. Methylene chloride was chosen as a suitable non-polar solvent of low boiling point, which might extract some of the non-alkaloidal material yet leave the quaternary bases in the residue.

Fraction I was mixed with acid washed sand to give a larger surface area and prevent the material from compacting. The material was extracted with hot methylene chloride in Soxhlet apparatus. The solvent preferentially extracted the quaternary bases instead of the non-alkaloidal material, probably due to the presence of a small percentage of ethanol in the methylene chloride. Three extracts were obtained and on concentration all yielded several fractions of crystalline material. Fractions shown to be similar by paper chromatography (Table VI, p. 122) were bulked as shown on page 121. Fractions I(1), p. 121, was mainly base A (subsequently shown to be 5-hydroxy-2,3,6-trimethoxy-N,N-dimethoxylaporphinium chloride) and fraction I(2) was mainly base B (subsequently shown to be

α-(-)-methylcanadine chloride). Bases C, D, E and F were present in both bulked fractions but no separation of these had been achieved.

5-HYDROXY-2,3,6-TRIMETHOXY-N,N-DIMETHYLAPORPHINE

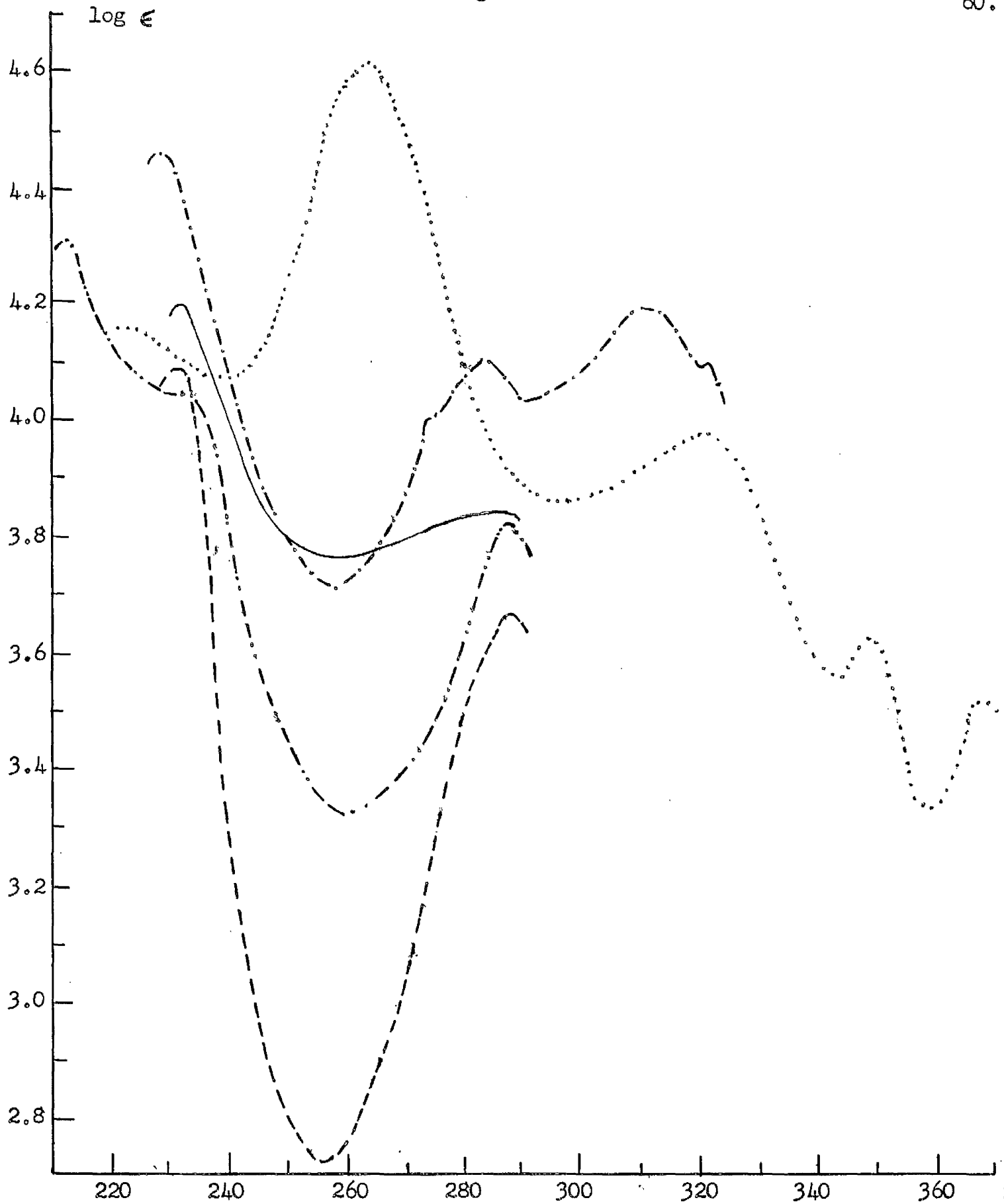
5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride.

The pale brown crystals of fraction I(1) gave, after recrystallisation from methanol/ethyl acetate, colourless needles m.p. 216-218° (decomp.). The ultraviolet spectrum (Figure V, p. 60) was similar to that of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride[42] being characteristic of a 2,3,5,6-oxygenated aporphine, since oxygenation in positions 3, 4, 5 and 6 leads to light absorption of much lower intensity in the vicinity of 300 mμ [42,83]. The low magnitude of the specific rotation also indicated that position 4 was not substituted[135]. The infrared spectrum of this compound (Figure VI, p. 61) showed the presence of methoxyl and hydroxyl groups.. The presence of a phenolic hydroxyl group was confirmed by a positive test with ferric chloride and by the large bathochromic shift in the spectrum on addition of alkali[42]. A negative Gibb's reaction indicated that the phenol was substituted in the para-position[136]. The presence of chloride ion was confirmed by a positive test with silver nitrate. It is interesting to note that the colour reaction with Froehde's reagent was identical with that given by the isomeric alkaloid N-methylisocorydine chloride[38] but the colour with nitric acid differed[38]. The empirical formula $C_{21}H_{26}O_4NCl$, based on elementary analysis of the compound, fitted exactly with that of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride, isolated from Fagara tingoassuiba by Riggs et al[42]. 5-Hydroxy-2,3,6-trimethoxy--N,N-dimethylaporphinium chloride was originally described

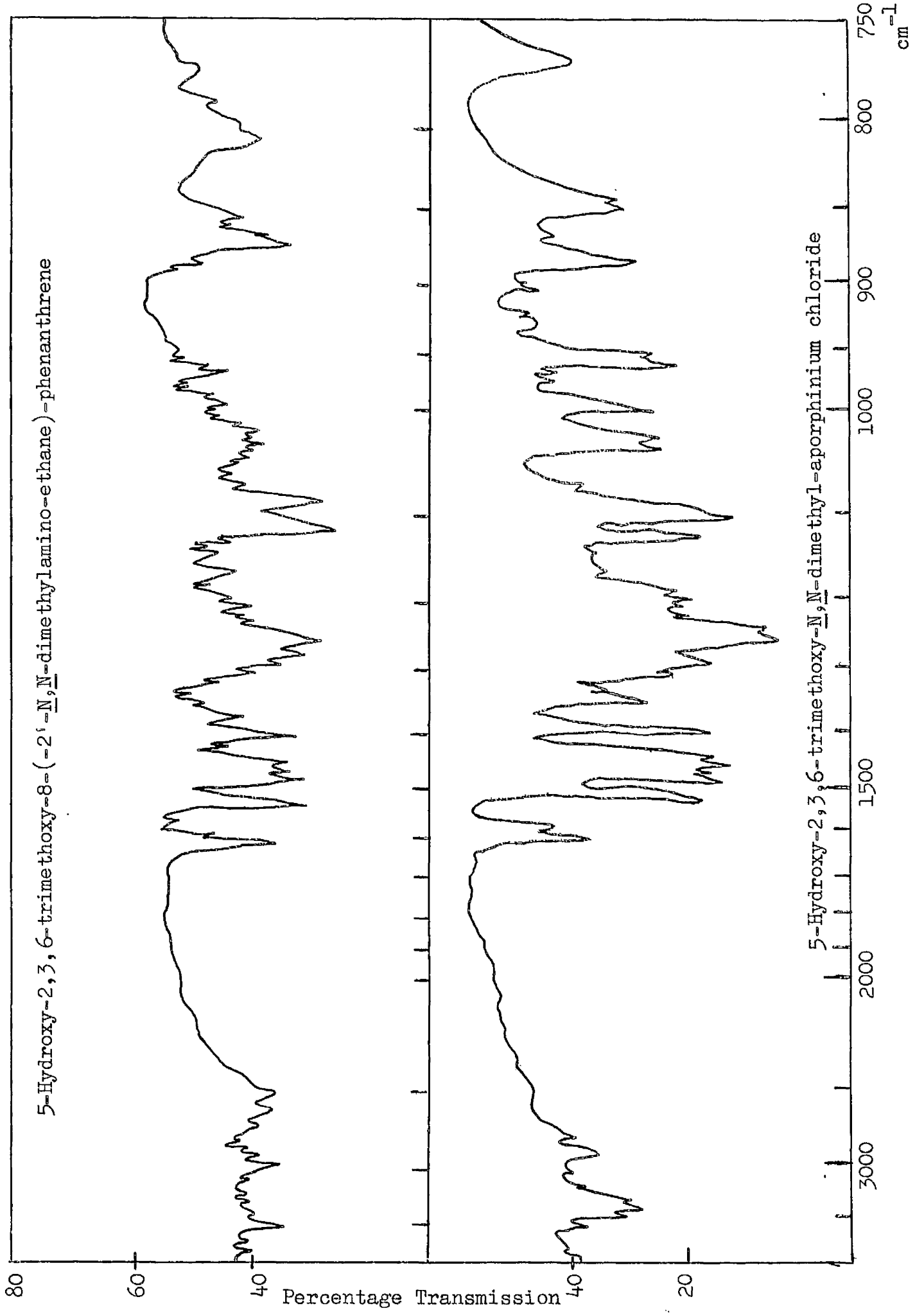
as being the 6-hydroxy derivative[42]. Subsequently the compound was shown to have its hydroxyl group at position 5[137].

The tentative identification of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride was confirmed by the preparation of iodide, picrate, the tetramethoxy iodide and the acetoxy chloride. The melting points and analyses of all these compounds were in good agreement with the values reported in the literature[42]. The infrared spectrum of the acetoxy derivative confirmed the presence of a phenolic acetate[138]. The picrate salt was identical with an authentic sample of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium picrate by virtue of mixed melting point and infrared spectra[42].

Figure V



a —·—·—·— 5-Hydroxy-2,3,6-trimethoxy-N,N-dimethyl-aporphine
b ····· 5-Hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino-ethane)-phenanthre
c ———— α -Allocryptopine
d —··—·· α -(-)-Methyl-canadine
e ———— (-)-Canadine


5-Hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino-ethane)-phenanthrene
5-Hydroxy-2,3,6-trimethoxy-N,N-dimethyl-aporphinium chloride
Percentage Transmission
80
60
40
40
20
750
800
900
1000
1500
2000
3000
cm-1

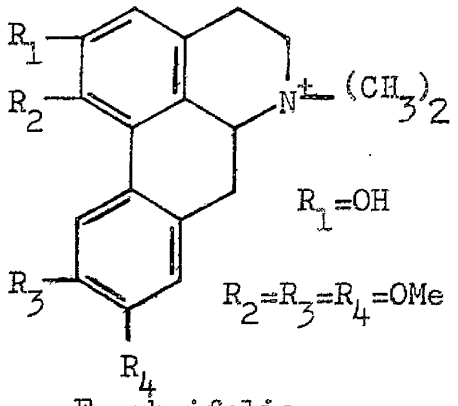
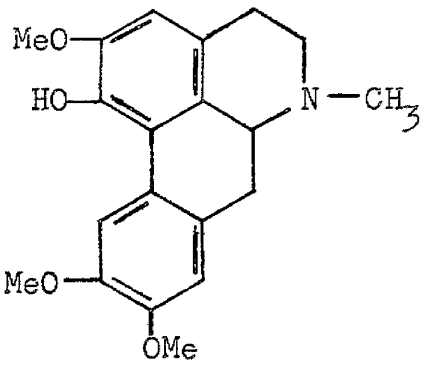
5-HYDROXY-2,3,6-TRIMETHOXY-8-(-2'-N,N-DIMETHYLAMINO-ETHANE-)-PHENANTHRENE

Attempted conversion of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride to 5-hydroxy-2,3,6-trimethoxyaporphine.

Prior to 1965[137] the structure of the quaternary base isolated from F. tinggassuiba[42] was considered to be 6-hydroxy-2,3,5-trimethoxy-N,N-dimethylaporphine. Theoretically N-demethylation of 6-hydroxy-2,3,5-trimethoxy-N,N-dimethylaporphinium chloride (Table II, p. 63) should have yielded 6-hydroxy-2,3,5-trimethoxyaporphine, which was the alleged structure for thalicmidine isolated from Thalictrum minus, Ranunculaceae, by Yunusov and Progressov[139]. Both Shamma[135] and Tomita and Furukawa[140] doubted the correctness of the proposed structure of thalicmidine. Shamma observed that the reported laevo-rotation did not agree with his suggested relationship between ring substituents and absolute configuration, and the sign of the specific rotation at 589 mμ , in the aporphine series. He proposed that thalicmidine might be 3-hydroxy-2,5,6-trimethoxyaporphine but the physical data of the quaternary base cocsarmine (Table II, p. 63), isolated from Cocculus sarmentosus, Menispermaceae, by Tomita and Furukawa[140], showed this proposal to be incorrect since cocsarmine iodide was not identical with thalicmidine methyl iodide. Though Yunusov and Progressov[139] had degraded O-methyl-thalicmidine methiodide to 2,3,5,6-tetramethoxy-8-phenanthrene-carboxylic acid, the physical characteristics of thalicmidine did not agree with those of any one of the four then known mono-hydroxy-trimethoxy isomers of 2,3,5,6-tetra-

Table II

Physical data, as known prior to 1965, on four mono-hydroxy-trimethoxyaporphine isomers

Alkaloid	6-Hydroxy-2,3,5-trimethoxy-N,N-dimethylaporphine*	Glaucentrine+	Cocsarmine	Xanthoplanine
			R_3 = OH $R_1=R_2=R_4$=OMe	R_4 = OH $R_1=R_2=R_3$=OMe
Source	F. rhoifolia F. tingoasuiba[42]	Dicentra exima[58]	Cocculus sarmentosus[140]	Zanthoxylum planispinum[82]
m.p. chloride	216-218(dec.) 215-219(dec.)	238		218-220
Iodide	228-229 226-229		205-207	148-149
Picrate	147-149 146-151		226-227	228

Cont. on p. 64.

* = now known to be 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride[137].

+ = now known to be identical with corydine, 5-hydroxy-3,4,6-trimethoxyaporphine[137].

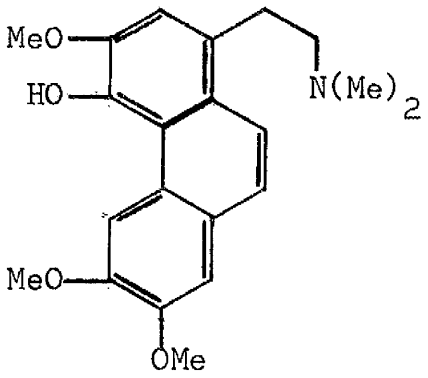
Table II contd.

Physical data, as known prior to 1965, on four mono-hydroxy-trimethoxyaporphine isomers.

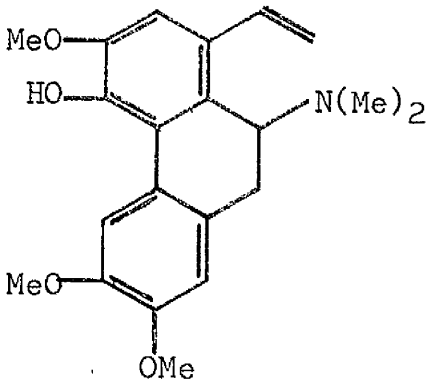
Alkaloid	6-Hydroxy-2,3,5-trimethoxy-N,N-dimethylaporphine*	Glaucentrine+	Cocsarmine	Xanthoplanine
Methyl iodide		204-206		
$[\alpha]_D$	+30.2°	+	+27.9°	+71°
Base		148		

substituted-aporphines (see Table II, p. 63). However, Shamma and Slusarchyk[137] subsequently (at a later date than the attempted N-demethylation described in the present work) reported the synthesis of 5-hydroxy-2,3,6-trimethoxy-aporphine and showed it to be identical with thalicmidine, concurrently proving that the new structure proposed by Tschesche et al.[141] for the quaternary base, from F. tinguassuiba[42] and F. rhoifolia (here reported) was 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride. Thalicmidine methiodide may thus be regarded as a laevo form of the iodide of the Fagara alkaloid[141]. Glaucentrine is identical with corydine[137] (Table I, p. 25) and all 2,3,5,6-tetra-oxygenated aporphines with structures elucidated by comparison with the assigned structure of glaucentrine require reconsideration.

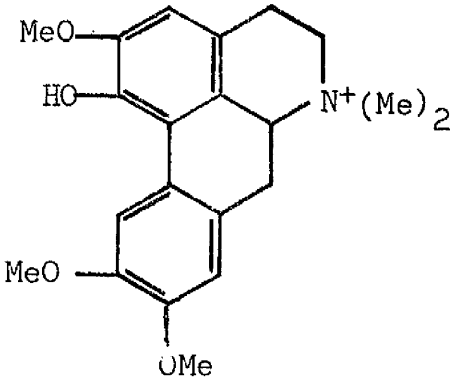
The N-demethylation of the quaternary base chloride was attempted by the method reported by Battersby and Yeowell[108] for macusine B, an indole type base, even though Schittler and Müller[142] had reported that dry distillation of the aporphine, isothebaine methyl ether methohydroxide, yielded a mixture of the optically inactive methine and the optically active isomethine, both of which gave the same trimethoxyvinylphenanthrene on further degradation. Further, N-demethylation of N-methyl-isocorydinium iodide[143], (V, p.66) by heating with ethanolamine, at 160-170°, did not produce the corresponding tertiary base. The compound underwent Hofmann degradation accompanied by demethylation of the methoxyl group at position 5 with the formation of the methine IV, p. 66. Normal Hofmann degradation of N-methylisocorydinium chloride[144] gave VI, p. 66.



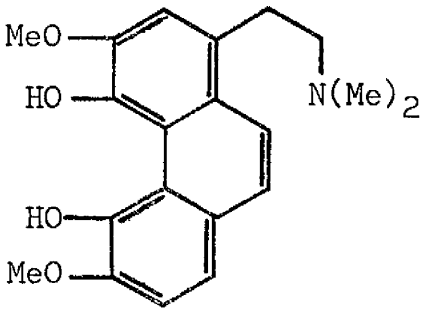
I



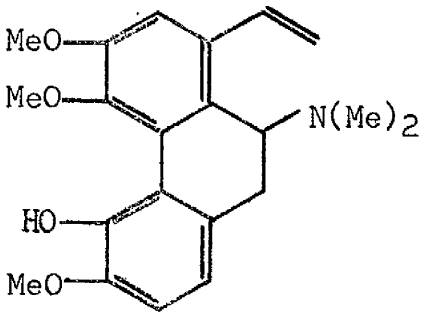
II



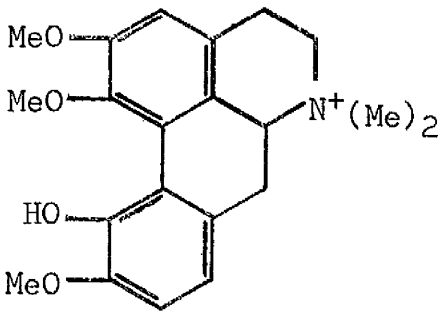
III



IV



VI



V

Pyrolysis of the quaternary chloride III, p. 66 , here yielded as the major constituent (60%) the methine 5-hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino ethane-)-phenanthrene, I, p. 66 .. Paper and thin-layer chromatography showed that a single tertiary compound, different from the parent quaternary base, had been produced. Absence of chloride was shown by a negative silver nitrate test. It is of interest to note that with concentrated nitric acid and Froehde's reagent the colours obtained differed from those with the quaternary base. With the former reagent the colour was cherry-red compared with a deep green; with the latter the colour sequence was green, blue then purple instead of blue turning green. The compound recrystallised from chloroform as needles, m.p. 225-227° (decomp.), the ultraviolet spectrum (Figure V, p. 60) indicated a substituted phenanthrene[145]. On the addition of alkali a bathochromic shift was observed, indicating that the phenolic hydroxyl group was intact and the phenolic phenanthrene was confirmed by the infrared spectrum[146] (Figure VI, p. 61). The unusual purple colour, with ferric chloride, was similar to that of the phenolic petaline methine[101]. The infra-red spectrum also showed the presence of methoxyl and the absence of an end vinyl group (-CH=CH_2)[147] which would have been present in the isomethine II, p. 66 . The absence of specific rotation indicated the absence of an asymmetric atom and confirmed that the compound had structure I, p. 66 . The empirical formula $C_{21}H_{25}O_4N$, based on elementary analysis of the compound, fitted that required for 5-hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino-ethane-)-phenanthrene, I, p. 66.

The tentative identification of the compound as 5-hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino-ethane-)-phenanthrene was confirmed by the preparation of the picrate, the analysis for which agreed with the required formula $C_{27}H_{27}O_{11}N_4$.

Adsorption chromatography of fraction I(2).

Evidence in the literature suggests that alkaloidal salts, especially those of quaternary bases, cannot be separated by adsorption chromatography on alumina columns. Both Battersby[103] and Qaisuddin[99] have shown that well separated bands from an alumina column contain the same compounds when alkaloidal reineckates are chromatographed. Although separation was not accomplished, Qaisuddin[99] did, however, find that some degree of purification was achieved.

Fraction I(2) was adsorbed on an alumina column and eluted with ethanol/methylene chloride (1-10% ethanol) and finally pure ethanol. The fractions were examined by paper chromatography. The results were similar to those obtained by Qaisuddin[99], the initial fractions being relatively free from non-alkaloidal colouring material but subsequent fractions were highly coloured due to the effect of increasing concentration of ethanol. Most of the fractions (Table VII, p. 131) contained the same bases; fractions I(2)b and c being notable exceptions. Fraction I(2)b contained only base B and fraction I(2)c contained principally B with smaller amounts of bases C and D. Crystallisation of fraction I(2)b yielded colourless plates, m.p. 242-244°, which gave a positive Labat test[148]. The colour reactions and the infrared spectrum of the compound were identical with those of α-(-)-methylcanadine chloride, subsequently isolated as the principal base of Fraction III (pp. 75 and 76).

Fractions I(2)c, I(2)d, and I(2)e did not yield crystalline compounds.

ATTEMPTED SEPARATION OF MINOR QUATERNARY BASES

Partition chromatography of residue of fraction I.

Little separation of bases C, D, E and F was achieved by adsorption chromatography and yet separation was excellent on paper chromatography. Partition chromatography was therefore attempted on cellulose columns.

Bartlett *et al.*[107] were of the opinion that solvent systems giving poor resolution on paper chromatograms often gave good separation on cellulose columns. The system ethyl acetate/glacial acetic acid/water (63:27:10) was used first since it had given some separation (p. 115), even though it was inferior to the standard solvent on paper chromatograms. It was cheaper and, more important, had a lower boiling point which facilitated concentration of eluates and reduced the risk of decomposition of the bases. Before use the column was washed with 8-hydroxyquinoline to remove possible traces of metal ions[105] and then tested with a solution of methyl red to ensure even flow through the column. The column chromatogram was developed at constant temperature to minimise production of mixed fractions due to temperature fluctuations.

The results were fairly satisfactory. Fractions 111-270 (Table VIII, p. 134) contained the single base B in a residue pure enough for crystallisation. The compound, crystals m.p. 242-244°, was shown by infrared spectrum to be identical with α-(-)-methylcanadine chloride. Attempts to obtain crystalline material from the remaining residues failed.

The bulked residues were again partitioned on a cellulose column using one of the solvents, acetone/water (8%), recommended by Bartlett *et al.*[107]. The results are given in Table IX, p. 136. Attempts to crystallise compounds from the four alkaloid-positive residues failed.

ISOLATION OF COMPOUNDS FROM FRACTION II

Fraction II was treated in the same way as Fraction I with the isolation of α-(-)-methylcanadine chloride as the major quaternary alkaloid; a significantly smaller amount of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride was also obtained. The identity of the compounds was achieved by comparisons of melting points, ultraviolet and infrared spectra.

The minor quaternary alkaloids were present in even smaller concentration than in Fraction I. None of these was obtained in pure crystalline form. Tertiary alkaloids were absent from Fraction II.

Examination of this fraction was carried out in order to show that the principal quaternary alkaloids were identical with those of Fraction I.

ISOLATION OF COMPOUNDS FROM FRACTION III

Adsorption chromatography of fraction III on alumina.

According to the extraction flow sheet (p.109) and consideration of solubility characteristics, fraction III should have contained all the tertiary bases and been devoid of quaternary alkaloids. The fraction was examined by thin-layer chromatography (p.116) and showed the presence of three tertiary bases X, Y and Z together with quaternary bases. Base Y was the major tertiary component. Paper chromatography, using the standard solvent (p. 48), showed the presence of a major base, R_f 0.73, which was suspected to be base B running with a closely related base to give an Rf value slightly lower than that observed for base B in fraction I. Two minor bases of R_f 0.57 and 0.96, corresponding perhaps to the minor tertiary bases X and Z, were also observed.

Fraction III was chromatographed on a grade III alumina (Woelm). On elution with ethanol/chloroform (1:99), the column did not retain the quaternary base B, which was present in high concentration in eluate fractions III(a) and III(b) and in low concentration in fractions III(c) and III(d) (Table X, p. 138). Tertiary base Y was present in all fractions, base X in fractions III(b), III(c) and III(d). The fact that base Y preceded base X from the column and that there was no separation of tertiary bases may have been due to the presence of the quaternary base B. An advantage of the column chromatography was that much of the non-alkaloidal, brown colouring matter was fairly strongly adsorbed and was not eluted until after much of the alkaloid had been collected. Thus,

fraction III(d), which represented about 1/30 of the original weight of fraction III applied to the column, contained most of the colouring matter, while fractions III(a), III(b) and III(c) were pale brown in colour and crystallised on concentration.

α-(-)-METHYLCANADINE

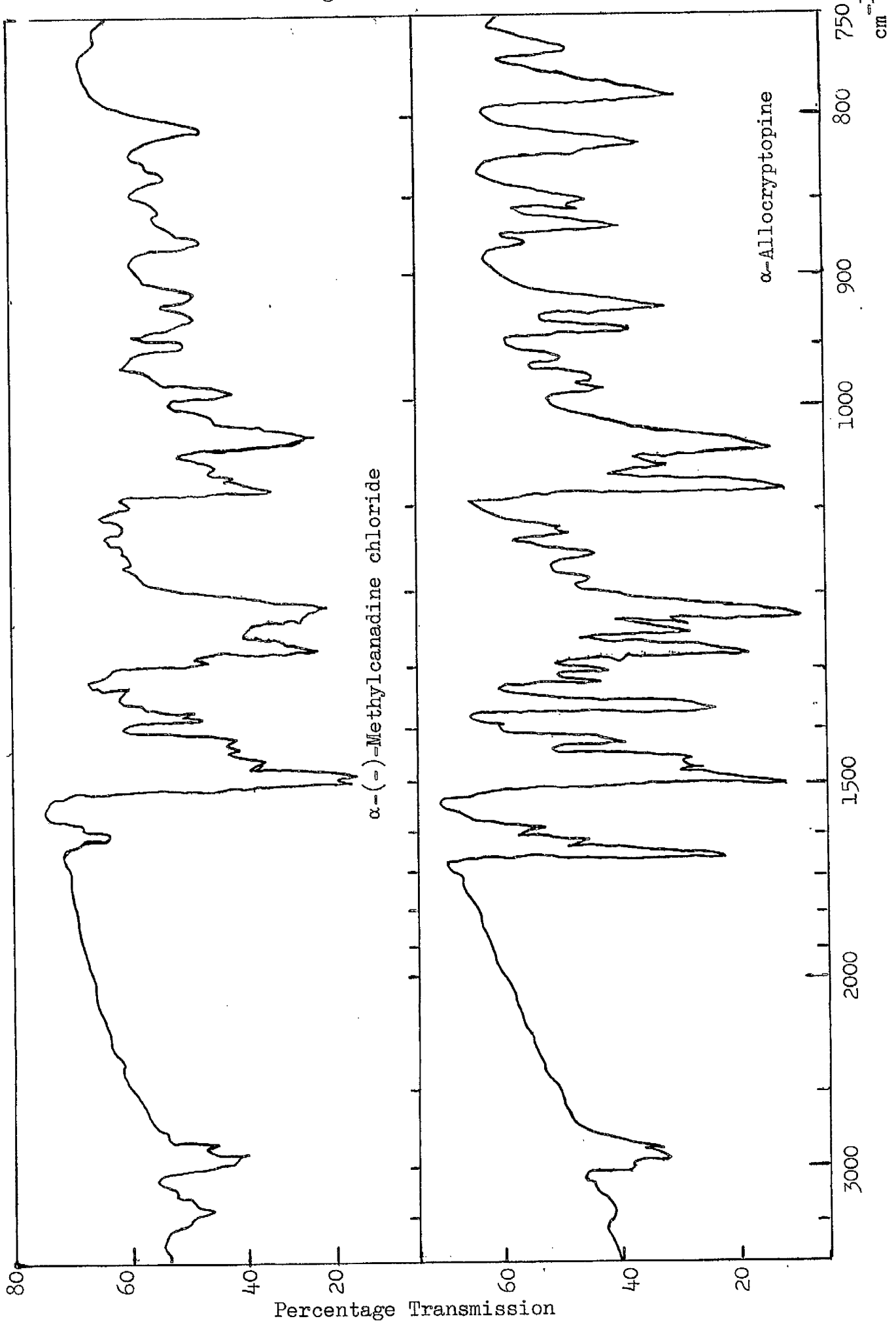
Isolation of α-(-)-methylcanadine chloride from fraction III(b).

The pale brown crystals obtained from fraction III(b) gave, after recrystallisation from methanol/ethyl acetate, colourless needles, m.p. 235-237° (decomp.). The ultraviolet spectrum (Figure V, p. 60) was similar to that of tetrahydroprotoberberine[83,149]. The infrared spectrum of this compound (Figure VII, p. 77) showed bands indicative of a methylenedioxy group (-O-CH_2-O-), subsequently confirmed by a positive Labat test[148]. Two strong bands at 1222 and 1276 cm^{-1} suggested the presence of methoxyl groups (OCH_3), the presence of two methoxyl groups being confirmed by analysis. The colours given with concentrated nitric acid and Froehde's reagent differed from those obtained with the quaternary alkaloid 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride described earlier in this work. The compound gave a single spot of R_f 0.79 on paper chromatograms and did not move from the base line of thin-layer alumina plates, using the solvent ethanol/chloroform (2:98). The empirical formula $C_{21}H_{24}O_4NCl.H_2O$, based on elementary analysis of the compound, fitted exactly with that of α-(-)-methylcanadine chloride first isolated from Fagara brachyacantha Engl. (Zanthoxylum brachyacanthum F. Muell.) by Jowett and Pyman[27]. The optical rotation was in good agreement with the value reported in the literature[27] but the melting point of the chloride was not identical with that reported[27,39]. The crystals were dimorphic, the crystal form depending on the solvent used for crystallisation; from methanol, the compound gave needles

m.p. 235-237°, while methanol/ethyl acetate gave plates m.p. 242-244°. Jowett and Pyman[27] found the m.p. of α-(-)-methylcanadine chloride, with one molecule of water of crystallisation, to be 262° and that of the β-form also 262°, while Pyman[150], later in the same year[150], found the m.p. of synthetic α-(±)-tetrahydroprotoberberine methochloride (α-(±)-methylcanadine chloride) to be 150° and that of the β-form to be 288°. Cannon et al.[39] reported a m.p. of 262° for α-(-)-methylcanadine chloride isolated from Fagara venenefica Engl. (Zanthoxylum veneneficum F. Muell.). Jowett and Pyman[27] reported that the action of heat converted the α-form into the β-form and this conversion may account for the discrepancies in melting point figures.

The tentative identification of α-(-)-methylcanadine chloride was confirmed by the preparation of the iodide and by N-demethylation to the corresponding tertiary base (-)-canadine. The melting point and optical rotation of the prepared iodide were in good agreement with the values reported in the literature[27,39]. α-(-)-Methylcanadine iodide had m.p. 216-218°; when heated to 230° the α-form was converted to the β-form which crystallised and remelted at 246°. Analytical figures for the iodide agreed well with calculated data. The equivalent weight (527.0, by Volhard titration[151]) also fitted very closely with the calculated value (527.4) for α-(-)-methylcanadine iodide. The picrate was prepared but could not be crystallised; the picrate is not reported in the literature.

Figure VII


α-(-)-Methylcanadine chloride
α-Allocryptopine
Percentage Transmission
80
60
40
20
750
800
900
1000
1500
2000
3000
cm⁻¹

(-)-CANADINE

Pyman[150], in 1913, pyrolised α-(-)-methylcanadine hydroxide, using the normal Hofmann degradation technique, to yield a mixture of two methines, (I) (p. 81), the major constituent (53%) (optically inactive) and (II) (optically active), together with the racemate of the original salt and the racemate of the methine base (II). The methine (I) had a m.p. 135-136°, very similar to that of (-)-canadine (IV) m.p. 133°. The conditions used did not produce the tetrahydroprotoberberine (-)-canadine.

More recently Watanbe[152] pyrolised the tetrahydroprotoberberine base O,O-dimethyl-steponine iodide, at 270° and 0.09 mm Hg, to yield O,O-dimethyl-N-demethylsteponine. Similarly[152] the quaternary compound O,O-diethyl-cyclanoline iodide was converted by heating at 260-270° at 2×10^{-4} mm Hg, to the tertiary base O,O-diethyl-sculerine.

In the present case α-(-)-methylcanadine chloride (III) was pyrolised at 325°, 10^{-4} mm Hg for 20 seconds, according to the method of Battersby[108], the heating time being reduced from forty to twenty seconds to minimise decomposition of the product. The product was chromatographed on an alumina column to yield two fractions A (69%) and B (17%).

Fraction A was crystallised from methanol to yield needles, m.p. 132-133°, the ultraviolet spectrum (Figure V, p. 60) indicating a tetrahydroprotoberberine nucleus and not the stilbene[149] type nucleus of the optically inactive methine (I) nor the styrene type of optically active methine (II). The infrared spectrum was similar to that of the parent substance and did not show strong absorption at 900 and 1,000 cm^{-1},

indicating the absence of an end vinyl group ($-CH=CH_2$) and therefore of the optically active methine (II). The behaviour of the compound on thin-layer alumina plates indicated that a tertiary base had been produced. The specific rotation confirmed that (-)-canadine (IV) had been produced. Further confirmation was given by positive colour tests with concentrated nitric acid and Froehde's reagent[28], the colours differing from those obtained with the starting material. In addition the compound reduced a mixture of potassium ferricyanide and ferric chloride[153]. The infrared spectrum also indicated the presence of methylene-dioxy and methoxyl groups. The empirical formula $C_{20}H_{21}NO_4$, based on elementary analysis, agreed with that calculated for (-)-canadine.

The tentative identification of (-)-canadine was confirmed by the preparation of the nitrate, the melting point for which had not been reported in the literature. The analysis fitted the formulae required for canadine nitrate ($C_{20}H_{22}N_2O_7$).

Berberine

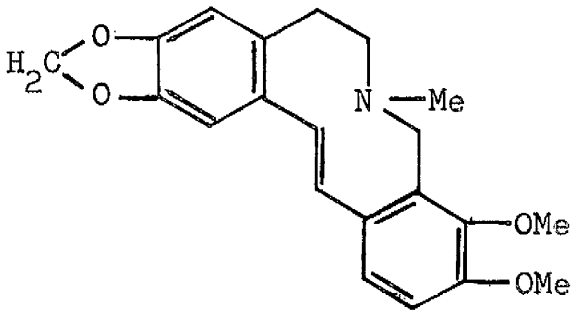
A crystalline compound was not obtained from fraction B. The ultraviolet spectrum of the yellow residue from this fraction was similar to that of berberine hydrochloride and the observed bathochromic shift, in 0.25N potassium hydroxide, was also similar to that published for berberine[154] (V). The absence of specific rotation indicated the presence of berberine. The material gave positive colour tests with Mandelin's and Froehde's reagents[153]. The bright red colour which developed with bromine water is alleged to be specific for berberine[153]. A positive Labat test

showed the presence of a methylenedioxy group[148].

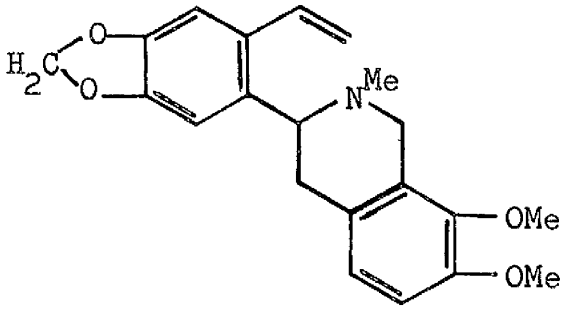
On paper chromatographic examination the material had the same R_f value as an authentic sample of berberine hydrochloride.

The infrared spectrum of the prepared berberine aurichloride was identical with that of an authentic sample of berberine aurichloride.

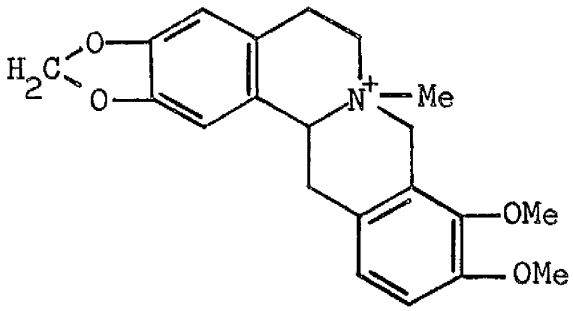
These results confirm the usefulness of the Battersby method for N-demethylation of quaternary bases. The slightly modified method here gave a 70% yield of the required product, (-)-canadine, together with a smaller yield of the related base berberine.



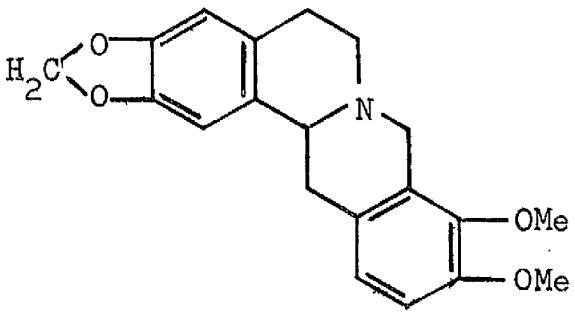
I



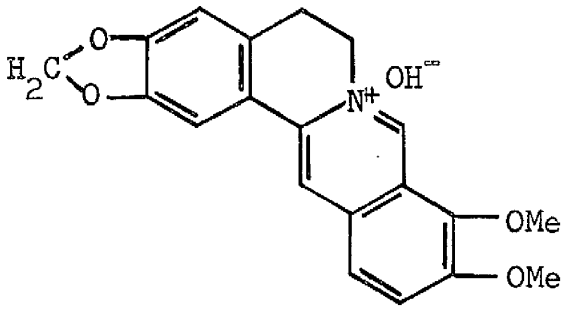
II



III



IV



V

α-ALLOCRYPTOPINE

The mother liquors left after the crystallisation of α-(-)methylcanadine chloride contained at least three tertiary bases X, Y and Z, whose presence was shown by thin-layer chromatography on alumina (Table X, p. 138). The bulked, dried residue (4.99g) was chromatographed on an alumina column, eluting first with ethanol-free chloroform, then ethanol (10%) in chloroform and finally with ethanol. When the fractions were tested on thin-layer alumina plates the principal tertiary base Y was present in greatest concentration in fractions III(1b) and III(1c) (Table XI, p. 145) accompanied in fraction III(1b) by base X. Base X was absent from fractions III(1d - 1f) which contained principally Z with small amounts of Y. A small amount of α-(-)-methylcanadine chloride was also present in the final fraction III(1f) which was dark brown in colour and contained most of the non-alkaloidal colouring material. Whilst complete separation of the tertiary bases had not been achieved, there was some fractionation of the compounds present and the bulk of the tertiary bases had been separated from the remaining trace of the quaternary α-(-)-methylcanadine chloride.

Isolation of α-allocryptopine from fractions III(1b) and III(1c).

These fractions were combined and base Y was crystallised from ethanol as colourless prisms, m.p. 161-162°. The ultraviolet spectrum (Figure V, p. 60) showed maxima characteristic of both tetrahydroprotoberberine and protopine bases[83]; the minimum absorption showed that the base belonged to the protopine series[83]. This conclusion was supported by the infrared spectrum (Figure VII, p. 77) which showed a very strong

peak at 1660 cm^{-1}, characteristic of a carbonyl group in the ten-membered ring of a protopine base[155]. This rather low value for carbonyl frequency is the result of the close proximity of the nitrogen atom with a lone pair of electrons, resulting in a direct effect upon the carbonyl group across the intramolecular space. The proximity of the nitrogen atom and carbonyl groups in this system is supported by the production of a bridged-ring derivative with hydriodic acid. The infrared spectrum of α-allocryptopine hydriodide lacked a strong band at 1660 cm^{-1} (=CO): this phenomenon was first observed by Mottus et al.[156] with the alkaloid protopine. In addition the infrared spectrum showed the presence of methylenedioxy and methoxyl groups. The presence of the former group was confirmed by a positive Labat test[148]. The infrared and ultraviolet spectra corresponded to those obtained with an authentic sample of α-allocryptopine. The compound gave a positive colour test for a protopine[157]. The compound also gave positive colour tests with concentrated nitric acid[28] and Froehde's reagent[28].

The compound when chromatographed on paper, using the standard method, had an R_f value of 0.71 showing that α-allocryptopine had been the base, in fraction III, which had been suspected of moving with base B, α-(-)-methylcanadine chloride, to give base B a lower R_f value (0.73) than it had (0.79) in fraction I.

The empirical formula $C_{21}H_{23}NO_5$, based on elementary analysis, fitted exactly with that of α-allocryptopine[86,158]. The melting point of the base confirmed that the α-form of the base was present, the melting point of the β-form being 170° [86].

The identification of α-allocryptopine was confirmed by the preparation of the iodide and picrate salts. The melting points and analyses of both salts were in good agreement with the values reported in the literature[86,158].

It is interesting to note the close structural relationship between α-allocryptopine (Table I, p. 25) and α-(-)-methylcanadine chloride. The latter occurs with its cryptopine analogue, α-allocryptopine, in Fagara brachyacantha (Z. brachyacanthum), F. venenefica (Z. venenefica)[27,39], and their co-occurrence is now established in F. rhoifolia[43].

ATTEMPTED SEPARATION OF THE MINOR TERTIARY ALKALOIDS OF FRACTION III

Preparative thin-layer chromatography of fraction III residue.

Adsorption column chromatography of fraction III had yielded the quaternary alkaloid α-(-)-methylcanadine and the major tertiary base α-allocryptopine. Four minor bases, previously designated X, W, Za and Zb, remained in the mother liquors, little separation of these having been achieved by adsorption column chromatography.

The material for preparative thick-layer chromatography was not available, therefore preparative thin-layer chromatography was attempted using Alumina G and ethanol/chloroform as solvent. From the bulked starting material (fraction III(1)), three alkaloidal fractions were obtained, III(2a), III(2b) and III(2c).

Thin-layer chromatography of fraction III(2a), Table XII, p. 149, showed the presence of one base, X; fraction III(2b) was a mixture of α-allocryptopine and a trace of X; fraction III(2c) was a mixture of α-allocryptopine and bases W, X, Za and Zb. Base Za gave a rose-violet colour with Dragendorff's reagent. Palmer[18] reported that the furoquinoline base skimmianine gave a similar colour reaction, but in the author's experience this colour did not always develop although it could be produced by subsequent treatment of the chromatogram with ammonia. Further, comparison on chromatoplates with an authentic sample, showed that base Za was not skimmianine.

Fraction III(2a).

This was the only fraction, shown by uni-dimensional thin-layer chromatography, to contain a single compound but this could not be obtained in a crystalline form. The ultraviolet spectrum (p.150) of the residue was very similar to that of the unknown tertiary base, A, isolated from F. tingoassuiba[17], but the infrared spectra were different. Subsequently two-dimensional chromatography (p.150) indicated the possibility that "X" was a mixture of two bases.

These fractions containing minor tertiary alkaloids were not further investigated.

EXAMINATION OF FOUR AFRICAN *FAGARA* SPECIES FOR THE PRESENCE OF TERTIARY AND QUATERNARY ALKALOIDS

The presence of both tertiary and quaternary alkaloids had been demonstrated in South American species of *Fagara*[38,44] and Australian species of this genus[27,39] and although tertiary alkaloids had been reported in a number of African *Fagara* species[18,52,53] there had been mention of uncharacterised quaternary bases only in the African species *F. melanacantha*[18].

Stem and root barks of the following African species have now been examined for both tertiary and quaternary bases:- *F. leprieurii* Engl., *F. macrophylla* Engl., *F. viridis* A. Cheval. and *F. xanthoxyloides* Lam.

DEMONSTRATION OF POOR AFRICAN *FAGARA* SPECIES FOR THE PRESENCE OF TERTIARY AND QUATERNARY ALKALOIDS

The presence of both tertiary and quaternary alkaloids had been demonstrated in South American species of *Fagara*[73,44] and Australian species of this genus[27,39] and although tertiary alkaloids had been reported in a number of African *Fagara* species[19,72,73] there had been mention of unobtrusive quaternary bases only in the African species *F. melanacantha*[19].

Stem and root barks of the following African species have now been examined for both tertiary and quaternary bases:- *F. leprieurii* Engl., *F. macrophylla* Engl., *F. viridis* A. Cheval. and *F. xanthoxyloides* Lam.

FAGARA LEPRIEURII Engl.

Preliminary extractions and separations.

The powdered stem and root barks of Fagara leprieurii Engl., extracted with petroleum ether, chloroform and ethanol, gave alkaloid-positive fractions.

The dried ethanolic extracts were fractionated to give alkaloid-positive, chloroform-soluble and butanol-soluble fractions and this chloroform-soluble fraction, from the larger sample of bark, was then further separated into acidic and alkaline chloroform fractions, the latter being alkaloid negative. The unusual phenomenon of alkaloids being extracted by chloroform from an acidic aqueous phase was also observed with F. macrophylla and has been reported[123] in the extraction of alkaloids from Aspidosperma species. Such extraction can be attributable either to slight solubility of the base hydrochlorides in chloroform, in which case the effect would be accentuated by continuous liquid/liquid extraction, or perhaps to the fact that hydrochlorides were not formed under the experimental conditions used. It has been shown, for example with the quinazolinocarbolines, that some hydrochlorides are formed with difficulty and only in the presence of concentrated acid[88].

Chromatography.

The results of paper and thin-layer chromatography (Table XIV, p. 157) showed conclusively that tertiary and quaternary alkaloids were present in both stem and root barks. Thin-layer chromatography on alumina, using the ethanol/chloroform solvent, was again an effective means of

differentiating between tertiary and quaternary bases and of indicating the number of tertiary bases present. Subsequent thin-layer chromatography on micro-crystalline cellulose gave more rapid and more efficient separation of the quaternary bases than conventional paper chromatography (Table XX, p. 102 and Figures X and XI, pp. 104 and 105).

Qualitative and quantitative differences existed in the alkaloidal content of stem and root bark. The former contained at least four tertiary and six quaternary bases, the latter three and seven respectively but the root bark is quantitatively richer in total alkaloids. This work, on sample 2 of the stem bark, considerably extended the preliminary observations which had been made on the small sample (sample 1) in which there were indications of at least three tertiary and two quaternary bases (Table XIII, p. 154).

Tertiary alkaloids of sample 2.

Tertiary bases were largely extracted by petroleum ether and to a lesser extent by chloroform, while the ethanolic extract contained mainly quaternary bases with smaller amounts of tertiary bases. The extraction of tertiary bases by petroleum ether from a vegetable drug, without prior basification, is unusual but not unknown: the presence of a yellow alkaloid in a petroleum ether extract of F. macrophylla[97] has been reported. At least one yellow tertiary alkaloid was present in the petroleum ether and chloroform extracts of the stem and root barks of F. leprieurii. Palmer[18] reported the presence in F. angolensis and F. leprieurii of two bases, angoline and angolinine, of unknown

structure, both forming yellow hydrochlorides. The yellow alkaloid was shown to have the same R_s value (1.11, relative to skimmianine) as an authentic sample of angoline. Palmer[18] also showed that base A from F. parvifolia[52] and base A_2 from F. xanthoxyloides[12] were identical, by mixed melting point, with angoline. The yellow base had an orange fluorescence. A colourless base with a blue fluorescence (R_s value 0.93) was identified as skimmianine even though the R_s value was slightly reduced, probably due to the presence of other bases in the mixture. Skimmianine had been previously reported in F. leprieurii[18] also in F. macrophylla[18], F. viridis[52] and F. xanthoxyloides[12]. In the present work skimmianine was subsequently also observed in the petroleum ether and chloroform extracts of both stem and root barks of F. macrophylla, F. viridis and F. xanthoxyloides. When the chromatograms which had been sprayed with Dragendorff's reagent were resprayed with dilute solution of ammonia, the orange colour with skimmianine changed to a rose-violet, a colour previously reported[18] for skimmianine with Dragendorff's reagent, without any subsequent treatment. The base gave a purplish-brown colour changing to grey when sprayed with potassium permanganate solution following Dragendorff's reagent; such colour changes have been reported[159] for quinoline alkaloids.

The third base, R_s value 0.17, with a yellow fluorescence, was present in both the petroleum ether and chloroform extracts of the root barks and in the chloroform extract of the stem bark of F. leprieurii. A sample supplied as authentic angolinine was shown, by thin-layer chromatography, to be a mixture of two bases the major one being identical in R_s value (0.17) and in fluorescence with this third tertiary base. This base was

subsequently also shown to be present in F. macrophylla but not in F. viridis nor F. xanthoxyloides. The minor tertiary base of the angolinine mixture had an R_s value of 1.15; it gave a violet colour with Dragendorff's reagent, a purple fluorescence when examined in ultraviolet light and did not appear to be identical with any base of F. leprieurii, F. macrophylla, F. viridis or F. xanthoxyloides.

The fourth tertiary base of F. leprieurii, R_S value 0.58, was observed only in the chloroform and ethanolic extracts of the root bark. The same base appeared to be present also in the root bark of F. xanthoxyloides.

Quaternary alkaloids of sample 2.

As noted previously quaternary alkaloids did move from the base line when chromatographed with ethanol/chloroform on thin-layer alumina plates. Four quaternary alkaloids were observed, by paper chromatography using the standard solvent, in the butanol-soluble fraction of the dried ethanolic extract of the root bark (Table XIV, p. 157). When, however, the same fraction was chromatographed two-dimensionally on microcrystalline cellulose thin-layer chromatoplates (Figures X and XI, pp. 104 and 105), using both the standard solvent and butanol/glacial acetic acid/water (10:1:3), two additional bases were observed. A total of seven bases was observed in the stem bark, a considerable similarity existing between these and the bases found in the root bark (Table XX, p. 102). One of the bases, R_f value 0.22, appeared to be identical with the phenolic base, 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride, giving

the same R_f values with both solvents and an identical blue-green colour with ferric chloride reagent. The same base was observed in the root and stem barks of F. macrophylla, F. viridis and F. xanthoxyloides.

None of the bases was identical with α-(-)-methylcanadine chloride. Two bases, R_f values 0.22 and 0.28, in both the stem and root barks, gave a blue-green colour with ferric chloride, indicating that these were phenolic alkaloids.

Palmer[18] did not report the presence of quaternary bases in F. leprieurii, though by 1956 he was obviously aware of their existence in Fagara species. Indeed he reported their presence in F. melanacantha Engl., stating that they were not extracted by chloroform from an alkaline aqueous phase. His initial extraction procedure was capable of removing both tertiary and quaternary bases from the powdered drug, but he only column chromatographed the chloroform-soluble fraction and neglected the aqueous phase. Moreover, he dissolved the residue in a small volume of chloroform before partition chromatography, thereby losing any quaternary bases which had transferred from the aqueous phase via water dissolved in the chloroform.

FAGARA MACROPHYLLA Engl.

Preliminary extractions and separations.

The powdered stem and root barks of Fagara macrophylla Engl. were extracted with petroleum ether and ethanol, all four extracts giving positive alkaloidal tests.

The dried ethanolic extract from the stem bark was fractionated to yield alkaline chloroform and butanol-soluble fractions, both giving positive alkaloidal tests. The dried ethanolic extract from the root bark was fractionated to give acidic chloroform, alkaline chloroform and butanol-soluble fractions, all giving positive alkaloidal tests.

Chromatography.

The results of paper and thin-layer chromatography (Table XVI, pp. 162 , 163) showed the presence of tertiary and quaternary alkaloids in both stem and root bark. As with F. leprieurii the root bark was, qualitatively and quantitatively, richer in alkaloids than the stem bark. It contained four tertiary and seven quaternary bases compared with three and five, respectively, in the stem bark.

Tertiary alkaloids.

Tertiary bases were again largely extracted by petroleum ether and chloroform, the ethanolic extract of the root bark containing mainly quaternary bases with small amounts of the tertiary bases. The root bark had four tertiary bases, three of these being present in the stem bark. One of the tertiary bases had a yellow colour before spraying with Dragendorff's reagent. The existence of two yellow bases has been reported

in F. macrophylla[53,97]. Xanthofagarine hydrochloride, $C_{18}H_{23}O_8N.HCl$, m.p. 278-280°, was isolated by Paris and Moyse-Mignon[53]. King et al.[97], while investigating the coumarins of F. macrophylla, found, in the petroleum ether extract, a yellow alkaloid $C_{20}H_{21}O_7N.HCl$, m.p. 276° (dec.). It is possible that these are identical with angoline hydrochloride m.p. 267-268°, the presence of which has been established in the present sample. The presence of skimmianine and of the major base present in the sample supplied as angolinine has also been established. A tertiary alkaloid, R_s value 1.15 on thin-layer chromatograms, was present in the ethanolic extract of the root bark but not in the stem bark. This alkaloid appeared to be absent from the stem and root barks of F. leprieurii and the stem bark of F. xanthoxyloides.

Quaternary alkaloids

Quaternary bases have not previously been reported in F. macrophylla. Two dimensional thin-layer chromatography on micro-crystalline cellulose (Figure X, p. 104) showed the presence of five bases in the stem bark, and seven in the root bark. In both cases there was one phenolic alkaloid which had the same R_f values as that of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride. Certain, but not all, of the alkaloids appeared to be similar to those found in F. leprieurii, F. viridis and F. xanthoxyloides. None was identical with α-(-)-methylcanadine chloride.

FAGARA VIRIDIS A. Cheval.

Preliminary extractions and separations.

The powdered stem and root barks of Fagara viridis A. Cheval., were extracted with petroleum ether and ethanol to give alkaloid-positive extracts. The dried ethanolic extracts were further fractionated to yield alkaline chloroform and butanol-soluble fractions, all four fractions giving positive alkaloidal tests.

Chromatography.

The results of paper and thin-layer chromatography, Tables XVIII and XX pp. 167 and 102 , showed conclusively that tertiary and quaternary alkaloids were present in both stem and root barks.

Qualitative and quantitative differences existed in the alkaloidal content of stem and root bark: the root bark is richer in alkaloids, containing at least four tertiary and eight quaternary bases compared with five and seven respectively in the stem bark. As with F. leprieurii and F. macrophylla, marked differences were observed in the distribution of alkaloids in the chloroform and petroleum-soluble fractions, particularly as shown on paper chromatograms. This work on sample 2 of the stem and root barks considerably extended the preliminary observations which had been made on the small sample (sample 1) in which there were indications of at least three tertiary and two quaternary bases (Table XVII, p. 165).

Tertiary alkaloids.

Of the tertiary alkaloids (Table XVIII, p. 167), a yellow base

with orange fluorescence and a colourless base with blue fluorescence appeared to be identical with angoline and skimmianine respectively (p.90). A third alkaloid, red in colour, with purple fluorescence, and R_s value 0.08 on thin-layer chromatograms, was present in both stem and root barks but was absent from the other three African species. A red base, fagaridine, (Table I, p. 25), of unknown structure, has previously been reported in F. macrophylla[53] and F. xanthoxyloides[12]. A fourth base, R_s value 0.56, appeared to be similar to a base found in the root bark of F. leprieurii. The fifth base, R_s value 1.15, present in both stem and root barks was similar to that observed in the root barks of F. macrophylla.

Quaternary alkaloids.

Quaternary bases have not previously been reported in F. viridis. Six and five quaternary alkaloids were observed in the butanol-soluble extracts of the stem and root barks respectively, using the standard method of paper chromatography. When, however, the same fractions were chromatographed two-dimensionally on micro-crystalline cellulose chromatograms (Figures X and XI, pp. 104 and 105) one and three additional bases were observed in the stem and root barks respectively. Two phenolic bases were common to both stem and root bark, one having the same R_s value (0.22, Table XVIII, p. 167) as 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride. None of the bases appeared to be identical with α-(-)-methylcanadine chloride. Six of the quaternary bases appeared to be common to both stem and root barks (Table XX, p. 102).

FAGARA XANTHOXYLOIDES Lam.

Preliminary extractions and separations.

The powdered stem and root barks of Fagara xanthoxyloides Lam. were extracted and processed as for F. viridis, (pp. 95 and 96) yielding petroleum ether, alkaline chloroform and butanol-soluble fractions, all six giving positive alkaloidal tests with Dragendorff's and Meyer's reagents.

Chromatography.

The results of paper and thin-layer chromatography (Tables XIX and XX pp. 172 and 102) showed that tertiary and quaternary alkaloids were present in both stem and root barks. The comparative richness of alkaloids in the root bark was even more marked than with F. leprieurii, F. viridis and F. macrophylla.

Tertiary alkaloids.

Six tertiary bases were present in the root bark, only one of these, skimmianine, R_s value 0.93, p.172, being present in the stem bark. Skimmianine was previously reported in this species by Paris and Moyse-Mignon[12]. The presence of artarine[16], a base with a yellow hydrochloride, was reported in 1889 and Paris and Moyse-Mignon also reported the presence of a yellow base. Palmer[18], however, found this to be a mixture of two bases, one being colourless, the other yellow. The latter was identical, by mixed melting point, with angoline, whose presence has now been shown in the present sample (cf. p. 90). Generally the tertiary alkaloids of F. xanthoxyloides were similar to those of F. leprieurii, F. macrophylla and F. viridis, an

exception being the bases of R_s values 0.26, 0.36 and 0.43 (Table XXI, p. 107.

Quaternary alkaloids.

The presence of quaternary alkaloids has not previously been reported in F. xanthoxyloides. Three and four quaternary alkaloids were observed in the butanol-soluble extracts of the stem and root barks respectively, using the standard method of paper chromatography. When, however, the same fractions were chromatographed two-dimensionally on micro-crystalline cellulose chromatograms (Figures X and XI, pp. 104 and 105) four and three additional bases were observed in the stem and root barks respectively, though the root bark appeared to contain the larger amount of alkaloid. Five bases appeared to be common to the butanol-soluble extracts of both stem and root barks; one of the common bases had an R_f value identical with that of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride, and gave the same blue-green colour with ferric chloride reagent. None of the bases was identical with α-(-)-methylcanadine chloride.

Thin-layer chromatography, on micro-crystalline cellulose, of the butanol-soluble fractions of Fagara leprieurii, F. macrophylla, F. viridis and F. xanthoxyloides.

Previously, p.52, thin-layer chromatography of fraction I of F. rhoifolia, on cellulose, gave poor separation of quaternary alkaloids. The type of cellulose powder used originally contained a binding agent which possibly interfered with the separation of the bases. Recently Giacopello[160] described the use of a new type of micro-crystalline cellulose powder, without binder, for thin-layer chromatography of alkaloids. The new Whatman cellulose powder CC41 was of this type. Plates of this material were developed using the standard solvent (p.48 , i.e. solvent No. 2, p.174) and solvent No. 1 (p.174), recommended by Giacopello[160].

Thin-layer chromatography of the butanol-soluble fractions, of stem and root barks, from the above four Fagara species was attempted on the micro-crystalline cellulose plates. The standard solvent, No. 2, (p.174) gave better separation than solvent No. 1. With both solvents, resolution was improved by prior saturation of the plates, for one hour, in the presence of the aqueous phase before development of the chromatograms with the organic phase (Figures VIII and IX, pp. 100 and 101).

In addition to the advantage of faster development, this method required a smaller quantity of starting material and gave, even with the standard solvent, better separation than paper chromatography. Although the thin-layer chromatograms showed the same principal bases in the butanol-soluble fractions as paper chromatograms, certain additional minor bases were also observed (Table XX, p. 102).

Figure VIII

Thin-layer Chromatography, on cellulose, of butanol-soluble fractions; effect of prior saturation (standard solvent).

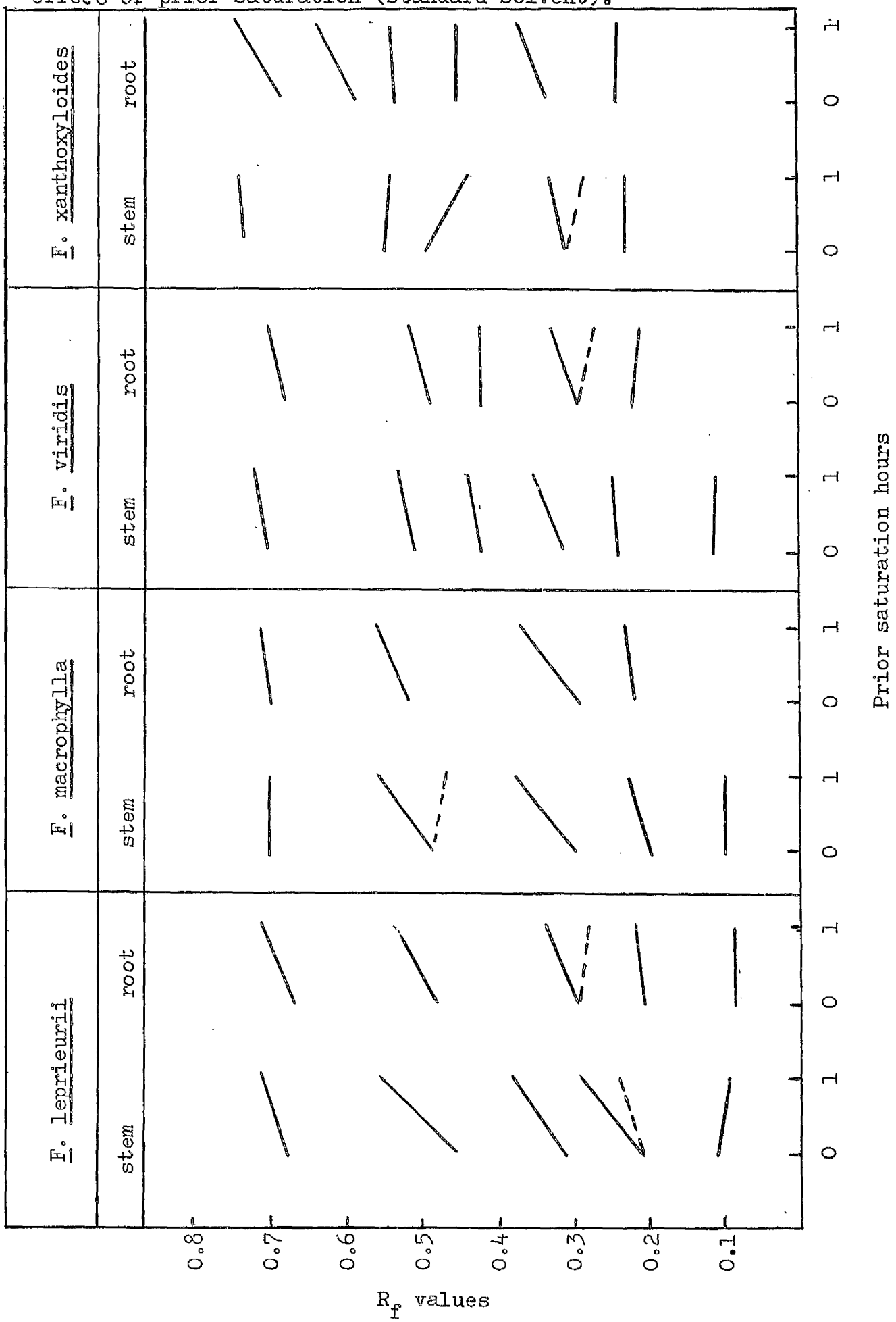


Figure IX

Thin-layer chromatography, on cellulose, of butanol-soluble fractions: effect of prior saturation (butanol solvent).

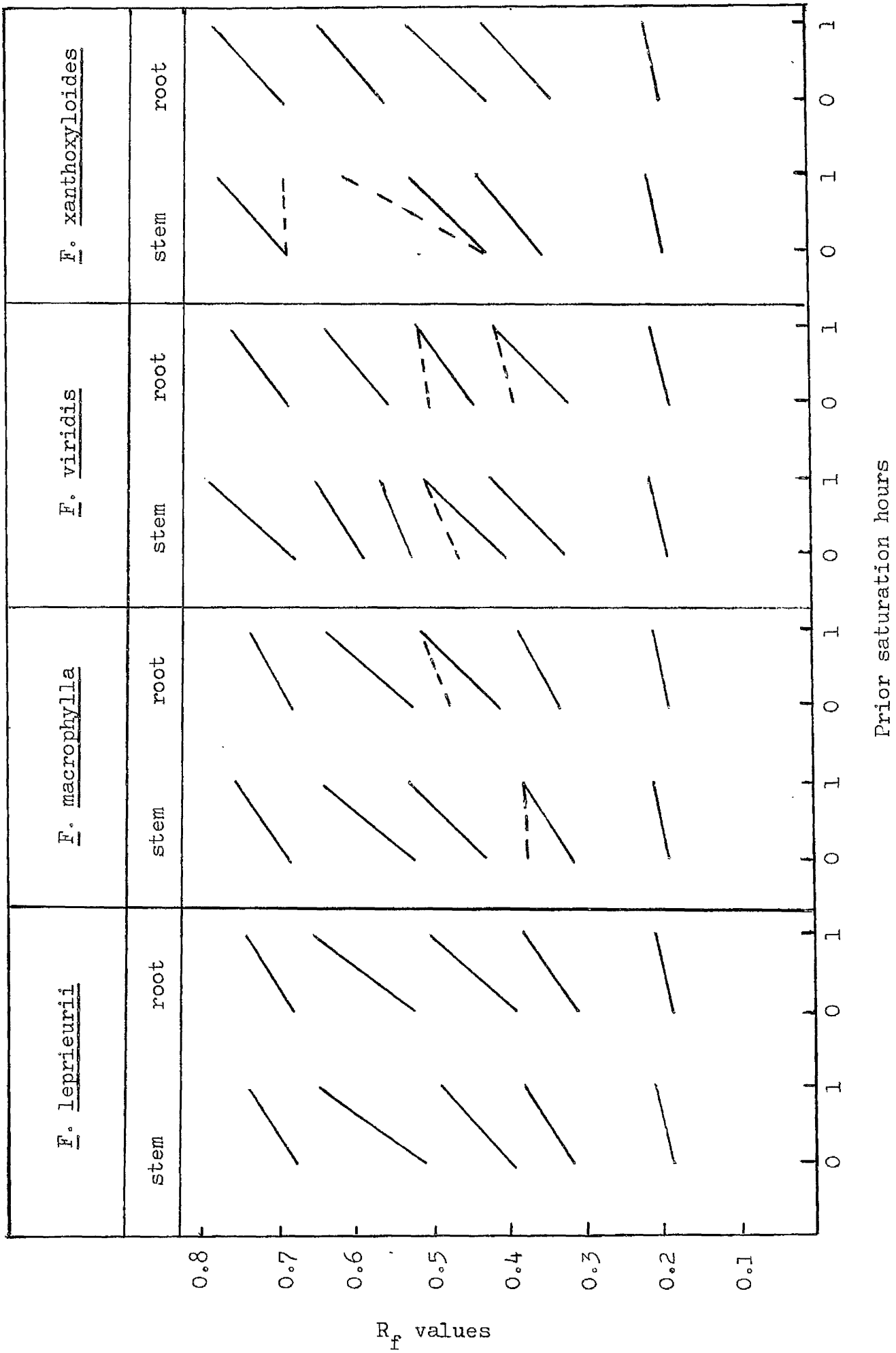


Table XX

Thin-layer chromatography, on cellulose, of butanol-soluble fractions of ethanolic extracts of four African Fagara species using the standard solvent.

Average R_f value	Fagara leprieurii		Fagara macrophylla		Fagara viridis		Fagara xanthoxyloides	
	stem	root	stem	root	stem	root	stem	root
0.10	+	+	+	+	+	+	+	+
0.22* phenolic	++++	+++++	+	++	++	+++++	+++	+
0.28 phenolic	+++	+				++++	+	
0.34	++++	+++			+++	+++	++	++++
0.38			+++	+++				
0.44					++++	+++	+	+
0.56	+	++	++++	++++	+++	++	+	++
0.63								++
0.70	++	++	+	++	+	+	+	+

* 5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride had the same R_f value.

A comparison of the quaternary bases of *F. leprieurii*, *F. macrophylla*, *F. viridis* and *F. xanthoxyloides*.

Two-dimensional chromatography, on thin-layer cellulose chromatoplates, (Figures X and XI, pp. 104 and 105), showed that the quaternary bases of *F. leprieurii*, *F. macrophylla* and *F. xanthoxyloides* were very similar though sufficient differences existed for their separation into individual species. Two of the quaternary bases of *F. viridis* appeared to be present in the other three African *Fagara* species, but the other bases were dissimilar.

It is of interest to note that one of the major quaternary alkaloids present in the two South American species, *F. rhoifolia* and *F. tinguassoiba*[42], was also present in the four African species: however, the second major quaternary compound of *F. rhoifolia*, α-(-)-methylcanadine, is absent from the above African species.

Figure X

Two dimensional thin-layer chromatography of butanol-soluble fractions of F. leprieurii and F. macrophylla.

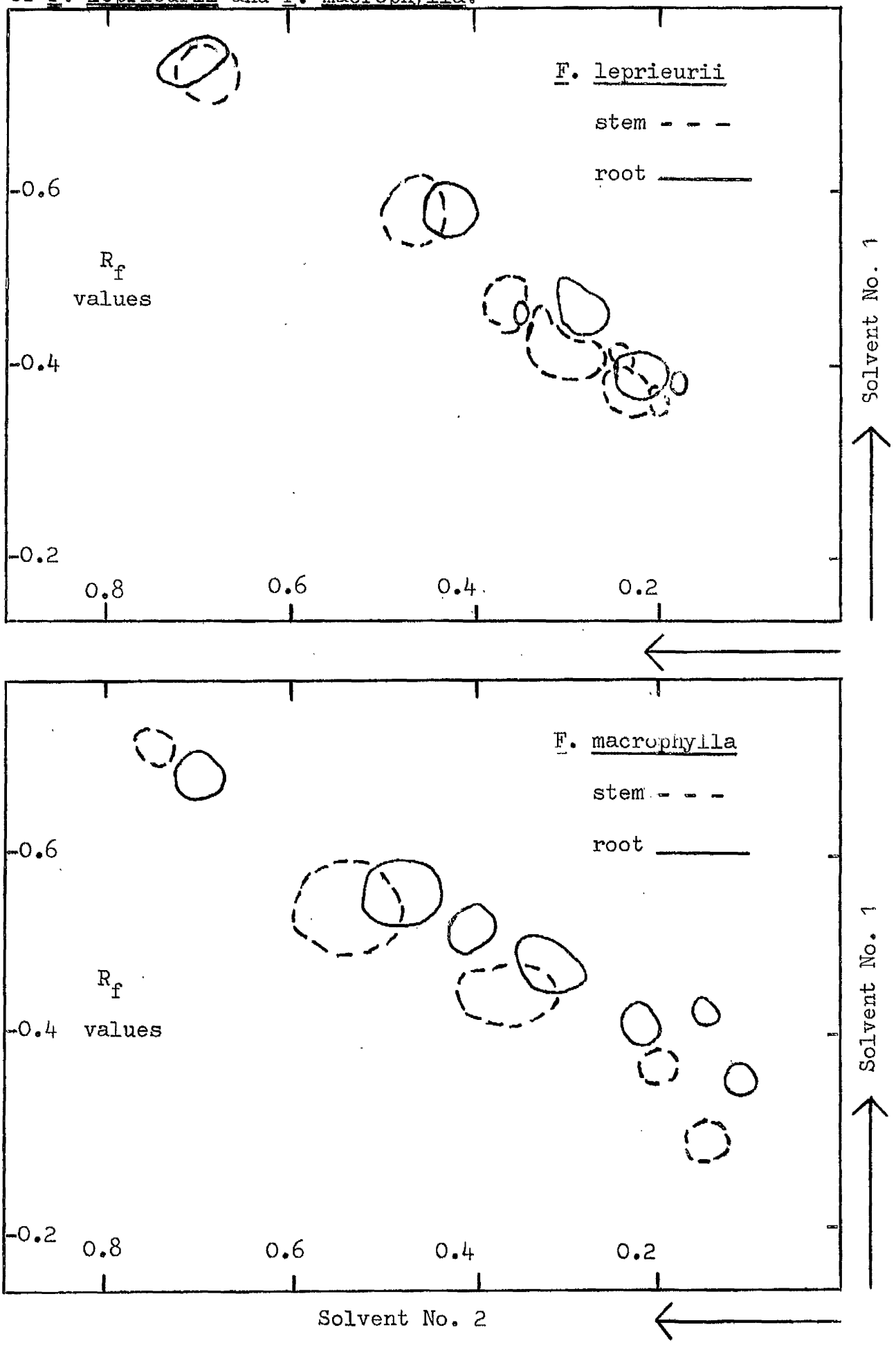
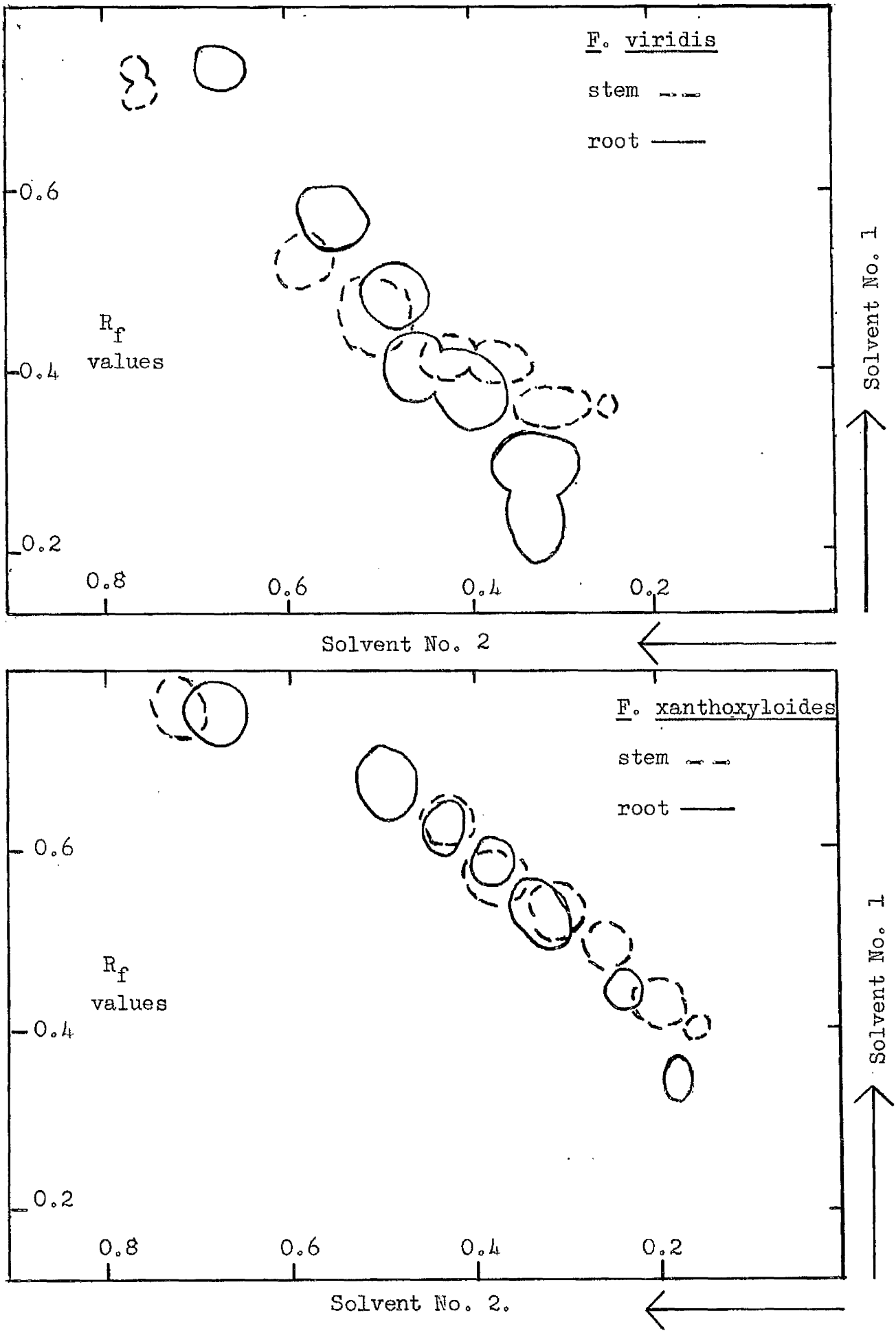


Figure XI

Two dimensional thin-layer chromatography of butanol-soluble fractions of F. viridis and F. xanthoxyloides.



A comparison of the tertiary bases of F. leprieurii, F. macrophylla, F. viridis and F. xanthoxyloides.

The major tertiary bases skimmianine and angoline were present in all four African Fagara species (Table XXI, p.107). The major base of "angolinine" was present as a minor tertiary base of the stem and root barks of F. leprieurii and F. macrophylla. Seven minor tertiary bases were present, but not all in any one species: the distribution of these seven minor bases was sufficiently different to warrant the existence of four distinct species. Though F. leprieurii and F. macrophylla differed only by the presence of one of the two bases with R_s values 0.58 and 1.15, a greater variation existed in the minor tertiary bases of F. viridis and F. xanthoxyloides.

Table XXI

The relative distribution of tertiary bases in the stem and root barks of F. leprieurii, F. macrophylla, F. viridis and F. xanthoxyloides (from alumina thin-layer chromatoplates).

Average R_s* values	Fagara leprieurii		Fagara macrophylla		Fagara viridis		Fagara xanthoxyloides	
	stem	root	stem	root	stem	root	stem	root
0.08					++	+++		
0.17 angolinine	++	+++	++	+				
0.26								+
0.42								+
0.58		+						
0.61								+
0.95 skimmianine	+++	++++	++	++	+++	++++	++	++++
1.12 angoline	+++	++++	+	++	++	+++		+++
1.15				+	+	++		+

(*reference standard = skimmianine)

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

PRELIMINARY EXTRACTIONS AND SEPARATIONS

Materials.

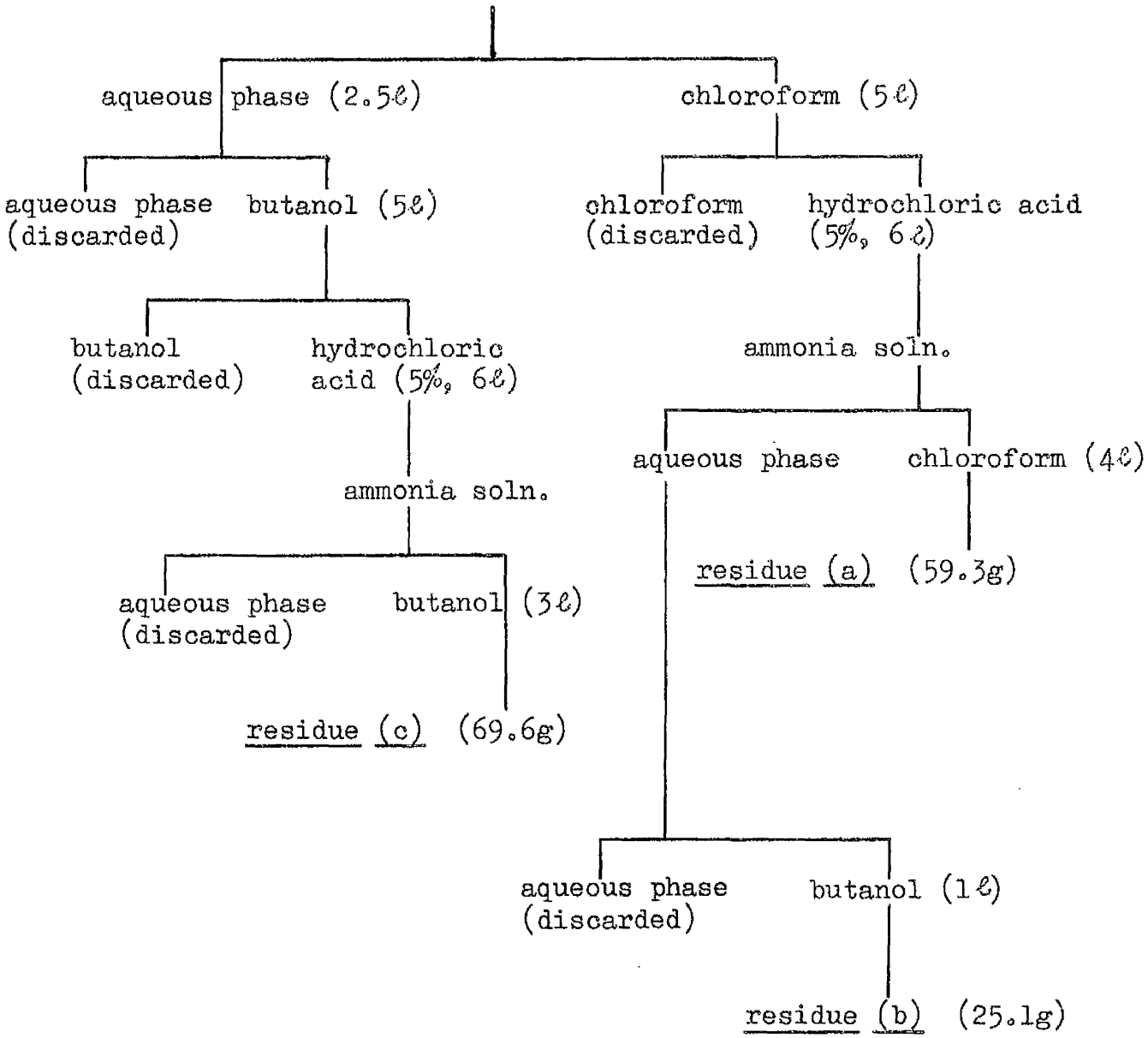
The dried stem bark of authenticated[55] Fagara rhoifolia Lam., collected in the Argentine, was extracted by Smith, Kline and French Laboratories, Philadelphia, according to the scheme given in Figure XII, p.109. The three crude, dry extracts designated fractions I, II and III constituted the starting materials for this part of the work.

Each fraction was examined for water solubility and the solutions obtained were tested for alkaloids, using Dragendorff's and Meyer's reagents, and for the presence of chloride using silver nitrate solution (1%).

Figure XII

Method of extraction of alkaloids from the dried stem bark of Fagara rhoifolia Lam.

2.9kg. bark extracted to exhaustion with warm ethanol (95%). The extract concentrated in vacuo to a thick syrup which was partitioned between chloroform and dilute solution of ammonia.



continued/

Figure XII contd.

These resinous materials were then treated as follows to effect some degree of purification.

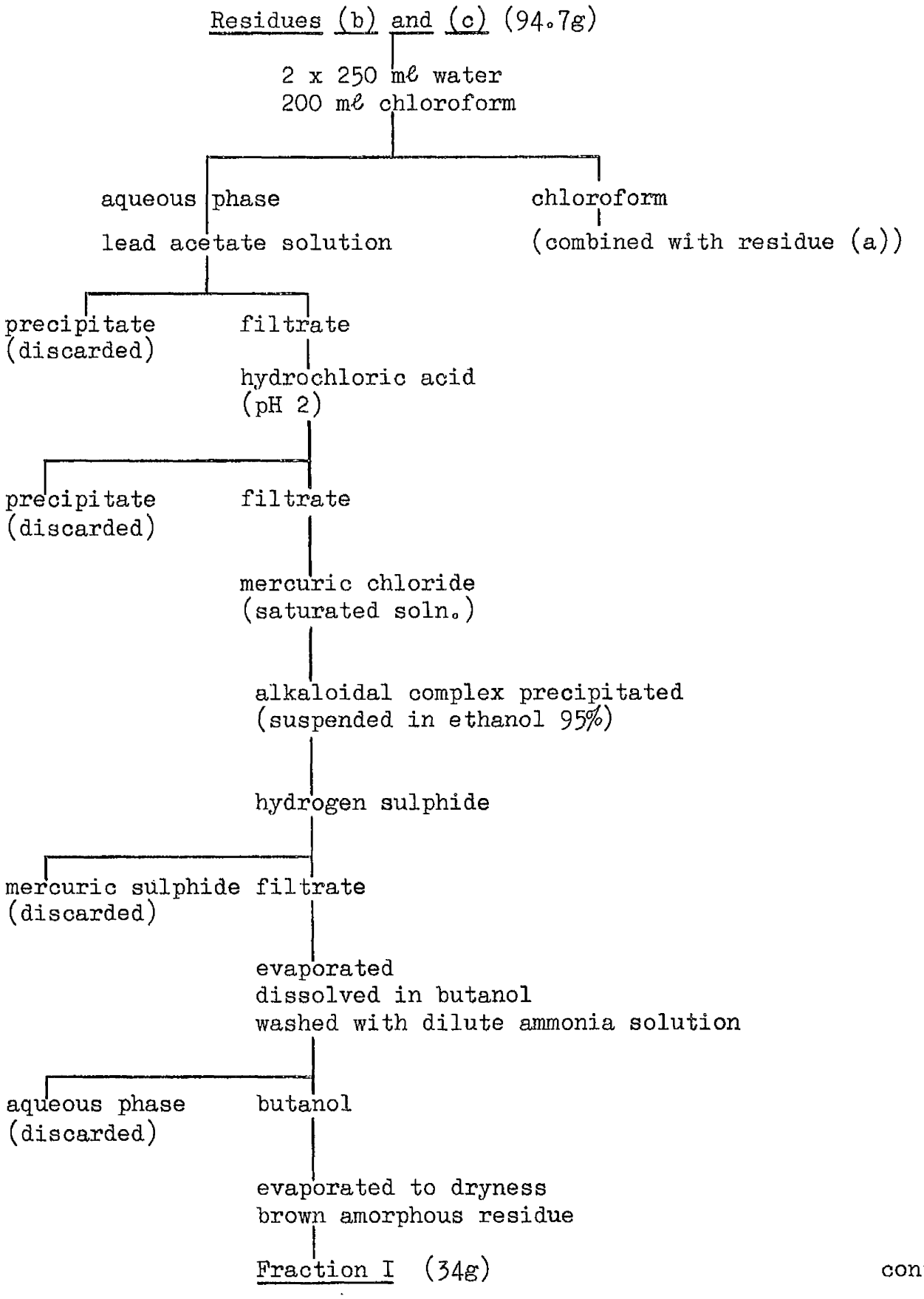


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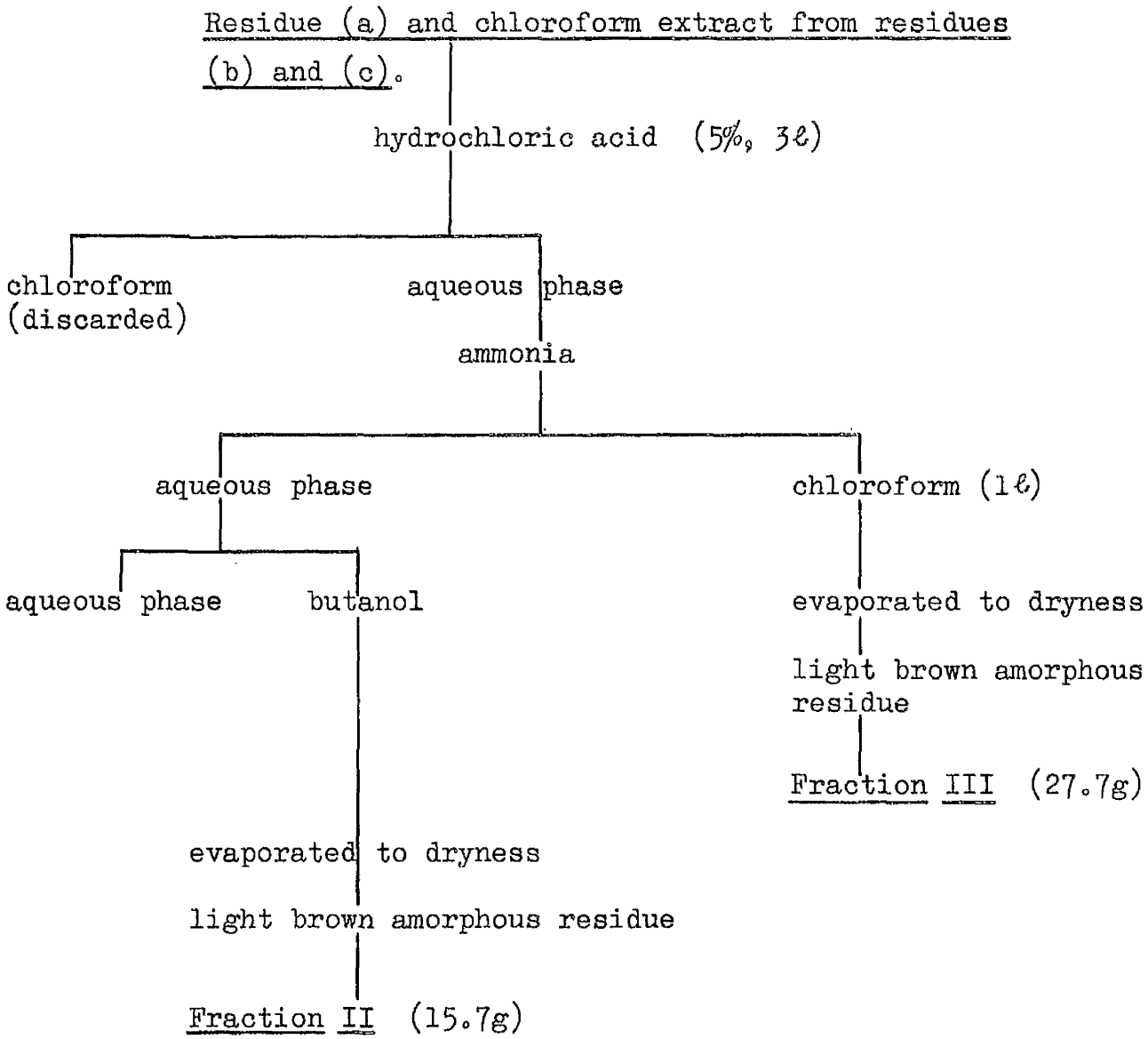
Figure XII contd.



The alkaloidal material of all three fractions was very soluble in water so that it was necessary to extract in a liquid-liquid extractor.

PAPER CHROMATOGRAPHY

Paper chromatography of alkaloidal fractions I, II and III.

Chromatographic separation of alkaloidal fractions I, II and III was attempted using the undernoted solvent systems:-

1. n-butanol/glacial acetic acid/water (5:1:4),
2. n-butanol/glacial acetic acid/water (63:10:27)[89],
3. n-butanol/ether/glacial acetic acid/water (32:31:10:27),
4. n-butanol/ether/glacial acetic acid/water (28:35:10:27),
5. ether/phthalate buffer pH 4.0, (saturated)[130],
6. ethyl acetate/glacial acetic acid/water (63:10:27),
7. methyl ethyl ketone/water (saturated)[107],
8. iso-amyl alcohol/petroleum ether, B.P. 60-80°/glacial acetic acid/water (12:4:12:8.5)[18],
9. iso-amyl alcohol/tertiary-amyl alcohol/formic acid/water (1:1:1:5)[55].

In all cases Whatman No. 1 paper was used, being equilibrated with the vapour of the stationary phase of the solvent for one hour.

(a) Ascending development.

Solvents 1, 2, 4, 8 and 9 were used on 51 cm lengths at 20°.

The papers were dried in a current of air at room temperature and then sprayed with either modified Dragendorff's reagent[128] or iodo-platinic acid reagent[129] to detect the alkaloids. The results are shown in Table III, p. 115.

(b) Descending development.

Solvents 7, 8 and 9 were used on 50 cm lengths of paper at 25°. The alkaloids were detected as above and the results are shown in Table III, page 115.

(c) Radial development.

Using circular chromatography on paper discs, 24 cm diameter, solvents 3, 5, 6 and 9 were used at 20°. The alkaloids were detected as above and the results are shown in Table III, p. 115.

Effect of temperature variation.

The standard solvent No. 9 was used to run a series of chromatograms of fraction I over the temperature range 21-32°. Descending development was carried out in a glass chromatography tank immersed in a constant temperature bath[131]. The insulated bath was heated by an immersion coil and cooled by a refrigeration coil, both controlled independently by means of contact thermometers. The water was circulated, by means of a suitable pump, through perforated pipes to minimise local temperature variation. This bath was capable of maintaining a constant temperature ± 0.2°. The papers were sprayed with modified Dragendorff's reagent and the results are shown in Figure II, p. 49.

Effect of variation in saturation time.

A series of chromatograms was run utilising the standard solvent No. 9 at 25°, the saturation time prior to running the chromato-

grams being varied from 0 to 3 hours. The papers were sprayed as above and the results are shown in Figure III, p. 49.

Table III

Paper chromatography of fractions I and III

Solvent No.*	Temperature °C	R_f values of alkaloids		Remarks
		Fraction I	Fraction III	
Ascending development				
1	20	0.39 0.61 0.82	0.59 0.76 0.83	Compact areas
2	25	0.38 0.48 0.63 0.77	0.59 (large area)	compact areas
4	20	0.18 0.30 0.46 0.57	0.58 (large area)	diffuse areas
8	22	0.56 0.69	0.67 0.70 0.95	compact areas
9	25	0.19 0.29 0.49 0.56 0.63	0.58 0.63 0.93	elongated areas
Descending development				
7	25	0.11 0.38 0.54 0.63 0.70	no experiment	diffuse areas
8	25	0.64 0.75	0.57 0.73 0.93	compact areas
9	25	0.18 0.32 0.40 0.53 0.62 0.78	0.57 0.73 0.96	compact areas
		0.57 0.69		two additional areas occasionally observed
Radial development				
3	20	0.47 0.72 0.79	0.40 0.76 0.98	diffuse areas
5	20	0.05 0.14	0.09	little movement
6	20	0.16 0.27 0.37 0.76 0.88	0.22 0.82	diffuse areas
9	20	0.27 0.35 0.43 0.65 0.72 0.77	0.77	diffuse areas

(* Solvent composition p. 112.).

THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography of alkaloid fractions I, II and III.

(a) On Alumina G.

Using a Camag applicator, glass plates (20 x 10 cm) were spread with a suspension of Alumina G for thin-layer chromatography (Merck, according to Stahl, 40g in 95 ml water) to give a layer 0.25mm thick. The plates were dried at 120° for three hours and used immediately after cooling or, within five days (stored in a dessicated atmosphere). The mixtures to be tested were applied in methanol or chloroform solution and the chromatograms were developed, for a distance of 10 cm at a temperature of 25 $\pm$ 0.5°, using the following solvent systems:-

1. ethanol/chloroform (0-10% ethanol),
2. ether,
3. ethyl acetate.

After drying the plates were sprayed with modified Dragendorff's reagent to detect the alkaloids. One base (W) gave a blue-grey colour; bases X, Y, Za and Zb gave orange to orange-brown colours. The results are given in Tables IV and V, pp. 118 and 119, and Figure IV, p. 54.

(b) On Silica Gel G.

The above method was used to prepare plates of Silica Gel G (Merck). The plates were dried at 105° for 1 hour. The plates were stored and used as above. The chromatograms were developed, at 25°,

using the following solvent systems:-

1. ethanol/chloroform (0-10% ethanol),

2. chloroform/benzene/formamide/methanol (40:50:0.5:10)[133],

3. chloroform/methanol/diethylamine (13.5:1:0.5)[134].

The results obtained after spraying are given in Table V, p. 119.

(c) On cellulose.

Plates similar to the above were prepared using Cellulose (Merck Cellulose Powder for thin-layer chromatography), the plates being dried, in a current of air, at 40° for two hours. The chromatograms were developed, at 25°, using the following solvent systems:-

1(i). ethyl acetate/water/glacial acetic acid (68:27:5),

1(ii). ethyl acetate/water/glacial acetic acid (63:27:10),

1(iii). ethyl acetate/water/glacial acetic acid (58:27:15),

2. methyl ethyl ketone/water (saturated)[107],

3. iso-amyl alcohol/tertiary-amyl alcohol/formic acid/water (1:1:1:5)[55].

The results obtained after spraying are given in Table V, p. 119.

Table IV

Thin-layer chromatography of fractions I, II, III, on Alumina G using ethanol/chloroform mixtures as developing solvents

Solvents	R_s^* values/Remarks						
	Fraction III alkaloids						Fractions I and II
	Za	Z	Zb	W	Y	X	
1. chloroform							
+0% ethanol	-	-	-	-	-	-	
1% ethanol		-		1.90	1.00	6.80	
2% ethanol		0.25		0.75	1.00	1.88	
3% ethanol		0.25		0.76	1.00	1.50	No movement from starting line
4% ethanol		0.39		0.80	1.00	1.30	
5% ethanol		0.44		0.90	1.00	1.20	
6% ethanol		0.58		0.88	1.00	1.10	
7% ethanol		0.60		-	1.00	1.08	
7.5% ethanol	0.58		0.72	0.95	1.00	1.12	
8% ethanol	0.56		0.74	0.93	1.00	1.18	0.1
9% ethanol	0.62		0.77	0.95	1.00	1.08	0.1
10% ethanol	0.57		0.66	0.95	1.00	1.12	0.1
Relative amounts	+		+	+	++ ++	+	++ ++

(* reference standard = (Y) = α-allocryptopine)

Table V

Thin-layer chromatography of fractions I, II and III.

R_f values/Remarks		
Solvent No.*	Fraction III	Fractions I and II
	Alumina G	
2. ether	0.05	No movement from starting line
3. ethyl acetate	0.14 0.78	
	Silica G	
1. chloroform +0-10% ethanol	Separation less efficient than with alumina G	No movement from starting line
2.	no movement	
3.	0.10 0.33 0.68 0.92	Slight movement (0.1)
	Cellulose	
1(i).	not tested	continuous streak
1(ii)	0.78 0.95	
1(iii)	not tested	0.41 0.63 0.80
2.	0.33 0.95	0.25 0.35 0.55 0.88
3.	continuous streak	continuous streak

(* Solvent composition see pp. 116 and 117.).

ISOLATION OF COMPOUNDS FROM FRACTION I

Removal of non-alkaloidal material of fraction I by solvent extraction.

Crude, dry, finely powdered fraction I (6.2g) (dried over phosphorous pentoxide) was mixed with acid washed sand (80g) and extracted in a Soxhlet apparatus with methylene chloride in successive periods of 3, 4 and 14 hours. The resultant brown solutions were concentrated separately, under reduced pressure at 50°, and all the concentrates deposited crystals on standing. The crystals, mainly rosettes of fine needles, were removed and the filtrates concentrated to yield more crystalline material. This process was repeated for each concentrate until crystallisation ceased. Five crystalline fractions were deposited from extract 1, two from extract 2 and three from extract 3. The weights of the crystalline fractions and the melting points are recorded in Table VI, p. 122. The mother liquors from the three extracts were combined and taken to dryness (2.4g)

The residue, from the Soxhlet apparatus, was dried at 40°, in a current of air, and re-extracted with ethanol (95%) for 12 hours. The ethanolic extract, on concentration, deposited a small amount of white granular powder (0.052g): this material dissolved in dilute solution of hydrochloric acid but gave a negative test with Meyer's reagent and was not examined further. The remainder of the ethanolic extract was evaporated to dryness, at 60°, to yield an amorphous brown alkaloidal residue (0.74g).

Paper chromatography of crystalline fractions.

Paper chromatographic analysis of the crystalline fractions and other residues dissolved in methanol, was carried out on 50 cm Whatman No. 1 paper using descending technique at 25° with the standard solvent. These results are also recorded in Table VI, p. 122.

As a result of the paper chromatographic examination of the crystalline materials of fraction I the following fractions, containing principally, or solely, alkaloid A, were combined:-

Extract 1: fractions 1, 4 and 5

Extract 2: fractions 1 and 2,

Extract 3: fractions 1, 2 and 3, Total weight 1.85g.

This crystalline material was designated fraction I(1).

The following fractions were combined and designated fraction I(2):-

Extract 1: fractions 2 and 3, Total weight 1.37g.

Table VI

Distribution of alkaloids in extracts of fraction I.

Fractions	Weight g	m.p. °C	Alkaloids R_f values	Relative intensities on chromatograms
Extract 1 fraction 1	0.86	172-178	A 0.19 B 0.79	++++ +
fraction 2	1.09	204-214	A 0.18 B 0.78 C 0.63 D 0.53 F 0.33	+ ++++ + + +
fraction 3	0.28	197-222	A 0.20 B 0.74 C 0.65 D 0.54	++ +++++ + +
fraction 4	0.14	197-207	A 0.20	++++
fraction 5	0.07	192-202	A 0.21	++
Extract 2 fraction 1	0.35	178-208	A 0.19 B 0.79 C 0.63 D 0.52 F 0.32	+++++ + + + +
fraction 2	0.02		A 0.20	+++
Extract 3 fraction 1	0.20	189-216	A. 0.19	++
fraction 2	0.04	202-216	A 0.18 F 0.33	+ +
fraction 3	0.07	194-202	A 0.19	++
Residue from extracts 1, 2 and 3	2.40		A 0.17 B 0.70 C 0.60 D 0.52 E 0.41 F 0.27	+++ +++++ ++ ++ + +
Ethanol (95%) extract	0.74		A 0.19 CD 0.67 E 0.41	++ +++ ++

5-HYDROXY-2,3,6-TRIMETHOXY-N,N-DIMETHYLAPORPHINE

Isolation of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride from fraction I(1).

The crystalline material (1.85g) was recrystallised twice from warm methanol/ethyl acetate to give long colourless needles (0.78g), m.p. 216-218° (decomp.) (Lit.[42], m.p. 215-219° (decomp.)), $[\alpha]_D^{22}$ + 29.2° (c, 0.50 in water) (Lit.[42], + 30.2 (in water)); $\lambda_{max.}^{EtOH}$ 229mμ (log ε 4.55), 273(sh.)(3.98), 283(4.10), 310(4.19), 320(sh.)(4.09); $\lambda_{min.}^{EtOH}$ 259(3.71), 290(4.03). In 0.3N sodium hydroxide in ethanol (95%) a bathochromic shift occurred, $\lambda_{max.}^{EtOH}$ very strong below 240mμ, 265-271(sh.)(4.21), 349(4.09); $\lambda_{min.}^{EtOH}$ 316(3.88).

The infrared spectrum (Figure VI, p.61) showed a strong band at 3350-3450 cm^{-1}, indicative of -OH vibration (phenolic -OH)[138] and one at 1255 cm^{-1} ($-OCH_3$). The compound was treated with aqueous solution of ferric chloride and gave a green colour. The compound gave no colour with 2,6-dichlorobenzoquinone chlorimide[136] (Gibb's reaction). The compound was treated with concentrated nitric acid and gave a deep green colour rapidly turning brown and with Froehde's reagent a deep blue turning green. The compound dissolved easily in water; the solution giving a white precipitate with silver nitrate solution.

Analysis, found: C,63.60; H,6.85; N,3.53; Cl,9.35.

Calculated for $C_{21}H_{26}NO_4Cl$: C,64.34; H,6.68; N,3.60; Cl,9.04%

5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium iodide.

5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride (0.055g) in water (1 mℓ) was treated with excess saturated aqueous solution of potassium iodide. The resultant white precipitate was collected, washed with water and dried (P_2O_5) to give a colourless solid (0.067g). This was recrystallised twice from ethanol/ether to give 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium iodide (0.058g) as colourless prisms m.p. 228-229° (decomp.)(Lit.[42], 226-229° (decomp.)).

Analysis, found: C,52.11; H,5.34; N,2.88; I,26.12.

Calculated for $C_{21}H_{26}NO_4I$: C,52.16; H,5.42; N,2.90; I,26.25%.

5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium picrate.

5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride (0.035g) in water (0.5 mℓ) was treated with excess of saturated aqueous solution of sodium picrate; the precipitate was washed with water, giving a yellow powder (0.063g). This, after two recrystallisations from aqueous acetone, gave 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium picrate (0.037g) as small prisms, m.p. 147-149° (dried at 80° at 0.1 mm Hg. for 18 hours)(Lit.[42], 146-151°).

Analysis, found: C,55.5; H,4.83; N,9.15; OCH_3,15.0.

Calculated for $C_{27}H_{28}N_4O_{11}$: C,55.7; H,4.83; N,9.6; $3OCH_3$,15.92%.

The infrared spectrum of the compound was identical with that of an authentic sample of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium picrate. The compound was mixed with an authentic sample of 5-hydroxy-

2,3,6-trimethoxy-N,N-dimethylaporphinium picrate and had a m.p. of 147-150°.

2,3,5,6-Tetramethoxy-N,N-dimethylaporphinium iodide.

5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride (0.062g), dissolved in dry methanol (1 mℓ), cooled below 5°, was treated with diazomethane. Nitrosomethylurea (0.12g) was added to a distillation flask, containing aqueous solution of potassium hydroxide (50%, 0.3 mℓ) and ether (2 mℓ), cooled below 5°. The diazomethane, with ether, was distilled over into the methanolic solution which turned yellow. The solution was evaporated to dryness, under reduced pressure at 50°, and the residue (0.059g) dissolved in methanol; on the addition of an excess of saturated aqueous solution of potassium iodide a white precipitate was produced. This was collected, washed with water and recrystallised twice from methanol/ether to give 2,3,5,6-tetramethoxy-N,N-dimethylaporphinium iodide as small needles m.p. 216-218° (decomp.) (after softening 195-200°) (Lit.[42], m.p. 216-217°, softening 200-210°).

Analysis, found: C,52.9; H5.64; N,2.79; I,25.46; (OCH_3),24.0.

Calculated for $C_{22}H_{28}NO_4I$: C,53.1; H,5.67; N,2.79; I,25.51; (OCH_3)4, 24.94%

5-Acetoxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride.

5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride (0.116g) was heated with acetic anhydride (2 mℓ) and pyridine (0.25 mℓ) at 50° for 1 hour. The solution was evaporated to dryness under reduced

pressure giving a colourless solid (0.126g). This was recrystallised twice from methanol/acetone/ether to give 5-acetoxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride (0.094g) as small prisms, m.p. 234-236° (decomp.) (Lit.[42], 234-238°).

Analysis, found: C,63.09; H,6.63; N,2.83.

Calculated for $C_{23}H_{28}NO_5Cl.C_3H_6O$: C,63.48; H,6.96; N,2.85%.

The infrared spectrum showed a strong band at 1750 cm^{-1} (C = O, aryl ester) and one at 1205 cm^{-1} (C-O- stretching vibration, phenolic acetate)[138].

5-HYDROXY-2,3,6-TRIMETHOXY-8-(-2'-N,N-DIMETHYLAMINO-ETHANE)-PHENANTHRENE

Attempted conversion of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride to 5-hydroxy-2,3,6-trimethoxyaporphine.

5-Hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride (0.096g) was dissolved in ethanol (10 mℓ), distributed around the lower halves of four round-bottom flasks (100 mℓ) and dried (P_2O_5). One flask at a time was evacuated to 0.0001 mm Hg. and the lower half of the flask dipped into a bath (Wood's metal) at 325-330° for 15-20 secs. Immediately the flask was filled with white fumes which condensed, on the cooler upper half of the flask, as a white sublimate. The white sublimate (the material darkened on exposure to air), dissolved in pure chloroform, was chromatographed on an alumina column (Woelm III, 10g, 1.2 x 7.5 cm). The material was eluted with ethanol/chloroform (2:98, 100 mℓ). The combined eluate was evaporated, at 60°, to give a yellowish-brown amorphous powder (0.058g). The material was chromatographed, along with the original quaternary chloride, on paper by the standard method (p.48) and on alumina plates, using ethanol/chloroform (2:98) as the developing solvent. In both systems the substance gave a single spot, R_f value 0.33 on paper and 0.42 on alumina as compared with R_f value 0.18 and 0.00, respectively, for the parent substance. The compound was recrystallised from warm chloroform to give brown needles (0.029g), m.p. 225-227° (decomp.), $[\alpha]_D^{22}$ 0° (c, 0.31 in chloroform); $\lambda_{max.}^{EtOH}$ 221mμ (log ε 4.15), 263(4.61), 320(3.98), 349(3.58), 367(3.52); $\lambda_{min.}^{EtOH}$ 240(4.07), 296(3.86), 344(3.56), 358(3.33). In 0.3N sodium

hydroxide in ethanol (95%) a bathochromic shift occurred, $\lambda_{max.}^{EtOH}$ 233mμ (log ε 4.38), 250(4.36), 283(sh.)(3.98), 293(sh.)(3.88), 366(3.71); $\lambda_{min.}^{EtOH}$ 243(4.35), 316(3.13). The infrared spectrum was determined (Figure VI, p. 61) and showed the presence of a sharp strong band at 3610 cm^{-1} indicative of -OH stretching vibration[146] and at 1255 cm^{-1} ($-OCH_3$). The spectrum, when compared with that of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride, showed the appearance of two bands at 2500 and 2625 cm^{-1} and the shifting of two bands to 800 and 867 cm^{-1}. The compound gave a purple colour turning red with aqueous ferric chloride solution[101]. The compound was treated with concentrated nitric acid and gave a cherry red colour and with Froehde's reagent a deep green turning, first to a blue, then a purple colour. The compound was insoluble in water and the aqueous solution gave no precipitate with silver nitrate.

Analysis, found: C,62.92; H,7.39; N,3.44.

$C_{21}H_{25}NO_4.3H_2O$ requires: C,61.59; H,7.63; N,3.42%.

5-Hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino-ethane-)-phenanthrene picrate.

5-Hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino-ethane-)-phenanthrene (0.015g) in ethanol was treated with excess of saturated ethanolic solution of picric acid; the precipitate was washed with water giving a yellow-brown powder (0.019g). This, after two recrystallisations from aqueous acetone, gave 5-hydroxy-2,3,6-trimethoxy-8-(-2'-N,N-dimethylamino-ethane-)-phenanthrene picrate (0.016g) as small brown hygroscopic needles, m.p. 166-168°, softening at 110° (dried at 80° at

0.1mm Hg for 24 hours).

Analysis, found:	C,54.63;	H,4.90;	N,9.36.
$C_{27}H_{27}N_4O_{11}.H_2O$ requires:	C,53.92;	H,4.86;	N,9.31%.

Adsorption chromatography of fraction I(2).

The crystalline fraction I(2) (1.37g) referred to on page 121, was dissolved in ethanol/methylene chloride (2:98, 10 mℓ) and chromatographed on an alumina column (Woelm IV, 1.2 x 25 cm). The column was eluted successively with ethanol/methylene chloride (1:99, 400 mℓ), (2:98, 200 mℓ), (4:96, 400 mℓ), (6:94, 200 mℓ), (10:90, 200 mℓ), and finally ethanol (200 mℓ). Fractions of eluate (25 mℓ) were collected and examined by paper chromatography using the standard solvent with downward development at 25°. The fractions were combined according to the bases present. The results are given in Table VII, p. 131.

Crystallisation of α-(-)-methylcanadine chloride from eluate fraction I(2)b.

Fraction I(2)b (250 mℓ) was evaporated to dryness, at 60°C, to give pale brown crystals (0.38g), plates, m.p. 238-241° (decomp.). The material was recrystallised twice from methanol/ethyl acetate to give colourless plates m.p. 242-244° (decomp.). The compound was treated with concentrated nitric acid and gave a yellow colour turning deep orange-red. The compound, with Froehde's reagent, gave a deep green colour turning brown. The compound, dissolved in ethanol, gave an intense blue colour when heated with concentrated sulphuric acid and ethanolic solution of gallic acid (5%)[148]. The compound dissolved readily in water; the solution gave a white precipitate with aqueous solution of silver nitrate. The infrared spectrum was identical with that of α-(-)-methylcanadine chloride isolated from fraction III (see p.139).

Table VII

Distribution of quaternary alkaloids in eluate fractions from an alumina column (see p.130).

Fractions volume mℓ	Alkaloids	R_f values	Relative intensities on chromatograms
I(2)a 200 mℓ	negative		
I(2)b 250 mℓ	B	0.79	+++++
I(2)c 350 mℓ	B	0.79	++++
	C	0.63	++
	D	0.52	++
I(2)d 350 mℓ	B	0.79	+++
	C	0.61	++
	D	0.51	++
	F	0.31	+
I(2)e 450 mℓ	A	0.17	++
	B	0.79	+++
	C	0.60	+
	D	0.52	+
	E	0.39	+
	F	0.31	+

Attempted crystallisation of residues from fractions I(2)c, I(2)d, and I(2)e.

These fractions were evaporated to dryness, at 50°, and yielded residues I(2)c (0.25g), I(2)d (0.32g) and I(2)e (0.44g). Crystallisation of these residues was attempted from methanol/ethyl acetate. Crystalline material was not obtained from any of the three fractions which were then reserved for further investigation by partition chromatography.

ATTEMPTED SEPARATION OF MINOR QUATERNARY BASES

Partition chromatography on cellulose columns.

Cellulose columns.

Columns of cellulose powder (Whatman standard grade for chromatography) were prepared by the method recommended by Bartlett *et al.*[107]. The cellulose powder was covered with the aqueous phase of the appropriate solvent system and placed in a vacuum desiccator which was evacuated until all the air was expelled from the cellulose powder. The column was packed tightly, as quickly as possible, with successive portions of the cellulose suspension. Each portion was compressed using a tamping rod with a perforated disc, of diameter almost equal to the internal diameter of the column. The column was washed with a solution of 8-hydroxy-quinoline (0.5g) dissolved in the organic phase, then with pure organic phase until the eluate was free of 8-hydroxy-quinoline[105]. The column was then tested, for uniformity of packing, with methyl red (5mg) dissolved in the organic phase of the solvent system. The dye travelled down a correctly prepared column as a horizontal, discrete band. The column was washed with organic phase until the eluate was free of methyl red.

Partition chromatography of the residue from fraction I(2).

The combined residues from fractions I(2)c, I(2)d and I(2)e were examined by paper chromatography, using the standard method at 25°, and showed the presence of bases A, B, C, D, E and F.

The material (0.79g) was dissolved in ethanol (5 mℓ), the solution mixed with powdered cellulose (5g) and the mixture dried in a vacuum desiccator, then equilibrated with the vapour of the aqueous phase of the solvent system below. The impregnated powder was added to a cellulose column (350g, 64 x 4.5cm) which had been prepared, as above, using the solvent system ethyl acetate/glacial acetic acid/water (63:10:27). The chromatogram was developed with the organic phase, the eluate being collected at a flow rate of 5 mℓ per minute, at a temperature of 25 ± 0.5° in a constant temperature room.

Fractions (400 x 14 mℓ) were collected, concentrated individually and chromatographed on paper using the standard method. The results are shown in Table VIII, p. 134, fractions containing the same bases having been combined.

Table VIII

Distribution of quaternary alkaloids in fractions from a cellulose column (see p. 133).

Fractions Nos	Weight g	Alkaloids R_f values	Relative intensities on chromatograms
0-111	-	negative	
111-270	0.39	B 0.78	+++++
271-280	0.09	B 0.79 C 0.61	+++ +++
301-310	0.06	B 0.79 C 0.70 D 0.61 E 0.42 F 0.29	+ ++ +++ + ++
321-330	0.02	C 0.66 D 0.56 E 0.42 F 0.29	++ +++ + ++
331-340	0.02	D 0.56 E 0.42 F 0.29	++ + ++
341-350	0.04	D 0.56 E 0.42 F 0.29	++ + +

Crystallisation of residue from fractions 111-270

The combined fractions 111-270 were evaporated to dryness, at 50°, and the residue (0.39g) recrystallised twice from methanol/ethyl acetate to give colourless plates m.p. 242-244° (decomp.)(0.12g). The compound had an infrared spectrum identical with that of α-(-)-methylcanadine chloride.

Attempted crystallisation of residues from fractions 271-280, 301-310, 321-330, 331-340, and 341-350.

Attempts were made to crystallise the residues from the above fractions from methanol/ethyl acetate. No crystalline compounds were obtained. The residues were bulked (0.21g).

Partition chromatography of the residues from fractions 271-350.

A column of cellulose powder (180g, 48 x 3.5 cm) was prepared, as before, using the solvent system acetone/water (8%). The residue (0.21g) was dissolved in ethanol (2 mℓ), mixed with cellulose powder (2g), dried and placed on top of the column. The column was eluted with acetone (water saturated), at 25°, at a flow rate of 5 mℓ per minute. Fractions (411 x 15 mℓ) were collected until all the bases had been eluted.

The fractions were combined in groups of ten, concentrated to small volume (5 mℓ), and examined by paper chromatography using the standard solvent with downward development at 25°. Fractions containing the same bases were combined, the results are recorded in Table IX, p. 136.

Table IX

Distribution of quaternary alkaloids in fractions from a cellulose column, solvent acetone/water (8%).

Fractions Nos.	Weight g	Alkaloids R_f values	Relative intensities on chromatograms
1- 30	0.07	negative	
31- 50	0.02	B 0.79 C 0.68 D 0.58	+ ++++ ++
51-130	0.05	C 0.69 D 0.57	++ +++++
131-150	0.03	C 0.65 D 0.47	++ +++++
151-200	0.01	E) F) 0.37	+++++
201-330	0.01	negative	
331-410	0.01	A 0.18	+ trace

Attempted crystallisation of residues from fractions 31-50, 51-130, 131-150, and 151-200.

Attempts were made to crystallise the above residues using ethanol/ethyl acetate and acetone. No crystalline compounds were obtained.

ISOLATION OF COMPOUNDS FROM FRACTION II

Fraction II was treated in exactly the same way as fraction I. A major crystalline compound was identical, by melting point, and ultraviolet and infrared spectra, with an authentic sample of α-(-)-methylcanadine chloride. A second crystalline base, in much smaller amount, was identical, by melting point and infrared and ultraviolet spectra with an authentic sample of 5-hydroxy-2,3,6-trimethoxy-N,N-dimethylaporphinium chloride. Both compounds gave the same colour reactions with concentrated nitric acid and Froehde's reagent as the respective authentic compounds.

ISOLATION OF COMPOUNDS FROM FRACTION III

Adsorption chromatography of fraction III on alumina.

The crude dry fraction III (15.8g), dissolved in ethanol/chloroform (2:98, 20mℓ), was chromatographed on an alumina column (Woelm III, 100g, 2 x 36 cm). The column was eluted with ethanol/chloroform (1:99, 150 mℓ); (5:95, 300 mℓ); (10:90, 200 mℓ); and ethanol (150 mℓ). Fractions (25 mℓ) were collected and chromatographed on alumina plates using ethanol/chloroform (2:98) as solvent. Fractions containing the same alkaloids were combined, the combined fractions were evaporated to dryness at 60°. The results are recorded in Table X.

Table X

Distribution of alkaloids in fractions from an alumina column.

Fractions	Eluant volume mℓ	Residues				
		Weight g	Alkaloids, relative intensities on chromatograms. B	Y	X	Z
III(a)	75	3.89	+++	++		
III(b)	50	9.39	+++++	+++	+	
III(c)	175	1.38	++	++++	+	++
III(d)	450	0.45	+	+	+	+

α-(-)-METHYLCANADINE

Isolation of α-(-)-methylcanadine chloride from fraction III(b).

The pale brown dried fraction III(b) (9.39g), Table X, p. 138, was crystallised from methanol to give buff coloured needles, m.p. 235-237° (decomp.) (6.87g). The crystals were recrystallised twice from methanol/ethyl acetate to yield colourless plates, m.p. 242-244° (decomp.) (Lit.[27,39], 262°), $[\alpha]_D^{22}$ -134.2 (c, 0.23 in water) (Lit.[27,39], $[\alpha]_D^{22}$ -136.4 (water)), $\lambda_{max.}^{EtOH}$ 212mμ (log ε 4.31), 233(sh.)(4.04), 287(3.82), $\lambda_{min.}^{EtOH}$ 259(3.32). The infrared spectrum (Figure VII, p. 77) showed two strong bands at 1222 and 1276 cm^{-1} indicative of $-OCH_3$ and the presence of a methylene-dioxy group ($-O-CH_2-O-$) was evident from the combination of a small peak at 1620 and two strong peaks at 934 and 1040 cm^{-1}. The compound gave a yellow solution turning deep orange-red when moistened with concentrated nitric acid and a deep green colour turning brown with Froehde's reagent. The compound, in ethanolic solution, gave an intense blue colour when heated with concentrated sulphuric acid and ethanolic solution of gallic acid (5%)[148]. The compound dissolved readily in water, the solution giving a white precipitate with solution of silver nitrate. The compound when examined by paper chromatography using downward development at 25° with the standard solvent, gave a single spot of R_f 0 79. When examined by thin-layer chromatography on alumina plates using the solvent ethanol/chloroform (2:98) the base did not move from the starting line.

Analysis, found: C,61.8; H,6.65; N,3.3; Cl,8.6; OCH_3,15.9.

Calculated for $C_{21}H_{24}NO_4Cl.H_2O$: C,61.8; H,6.4; N,3.4; Cl,8.7; $(OCH_3)_2$,16.9%.

α-(-)-Methylcanadine iodide.

α-(-)-Methylcanadine chloride (0.07g) in water (1 mℓ) was treated with excess saturated solution of potassium iodide. The resultant white precipitate (0.108g) was collected, dried (P_2O_5) and recrystallised twice from ethanol to give colourless prisms (0.066g), m.p. 216-218° (decomp.) resolidifying when heated to 230° and remelting at 246° (decomp.) (Lit.[39], 218° then 245° decomp.) (Lit.[27], 220° then 250°(decomp.)).

Analysis, found: C,51.9; H,5.7; N,2.8; I,24.1.

Calculated for $C_{21}H_{24}NO_4I.C_2H_5OH$: C,52.4; H,5.7; N,2.7; I,24.1%.

The equivalent weight was determined[151] by dissolving the iodide (10.6 mg) in methanol (1 mℓ) and adding dilute nitric acid (2 mℓ); a known excess (2 mℓ) of N/50 silver nitrate solution was then added and the residual silver nitrate back titrated with N/50 ammonium thiocyanate solution using ferric alum as indicator. The equivalent weight was found to be 527.0 (calculated molecular weight for $C_{21}H_{24}NO_4I.C_2H_5OH$ = 527.4).

α-(-)-Methylcanadine picrate.

α-(-)-Methylcanadine chloride (0.06g) in water (1 mℓ) was treated with excess of saturated solution of sodium picrate and the precipitate washed with water and then dried to give a yellow amorphous solid (0.06g) m.p., softened at 98°. The solid did not crystallise from ethanol, acetone or aqueous mixtures of these solvents.

(-)-CANADINE

Conversion of α-(-)-methylcanadine chloride to (-)-canadine.

α-(-)-Methylcanadine chloride (0.13g) was pyrolised by the Battersby[108] method (see p.127). The yellowish-white sublimate was dissolved in chloroform (5 mℓ) and chromatographed on an alumina column (Woelm III, 10g, 1.2 x 13 cm); elution was continued with chloroform until the eluate was free from alkaloid (Meyer's reagent): the total eluate was designated fraction A. A band of yellow material remained near the top of the column and this was eluted with ethanol/chloroform (5:95, 100 mℓ); the eluate gave a white precipitate with Meyer's reagent and was designated fraction B.

Fraction A (40 mℓ) was evaporated to give an almost white solid (0.069g) which crystallised readily from methanol as fine, colourless needles (0.05g), m.p. 132-133° (Lit.[28], m.p. 133°), $[\alpha]_D^{22}$ -297.7° (c, 0.17 in chloroform) (Lit.[28], $[\alpha]_D$ -299° (in chloroform); $\lambda_{max.}^{EtOH}$ 230mμ (sh.) (log ε 4.09), 287(3.67), $\lambda_{min.}^{EtOH}$ 256(2.72). The infrared spectrum was determined. The compound was paper chromatographed using the standard method and gave an R_f value 0.79 compared with R_f 0.79 for the parent substance α-(-)-methylcanadine chloride. When chromatographed on alumina plates using ethanol/chloroform (2:98)as solvent the compound gave a single spot of average R_f value 0.69, while α-(-)-methylcanadine chloride, used as a control, gave a R_f value of 0.00.

The compound was treated with concentrated nitric acid and gave an orange colour; with Froehde's reagent it gave an olive-green

colour turning brownish-violet[28]. The compound tested with a mixture of solutions of potassium ferricyanide and ferric chloride gave a prussian blue colour[153]. The compound, in ethanol, was heated with concentrated sulphuric acid and ethanolic solution of gallic acid (5%) and gave an intense blue colour[148].

Analysis, found: C,69.5; H,6.9; N,3.78.

Calculated for $C_{20}H_{21}NO_4.CH_3OH$: C,67.9; H,6.8; N,3.8%.

(-)-Canadine nitrate.

(-)-Canadine (0.025g) was dissolved in ethanol (1 mℓ) and dilute nitric acid (0.5 mℓ) added. The white precipitate (0.026g) was collected and dried (P_2O_5). The compound was crystallised from ethanol to give colourless leaflets, m.p. 222-224°.

Analysis, found: C,60.4; H,5.60; N,6.92.

Calculated for $C_{20}H_{22}N_2O_7$: C,59.7; H,5.51; N,6.97%.

Berberine.

Fraction B (100 mℓ) was evaporated to dryness at 60° and gave an orange-yellow solid (0.017g). The material was dissolved in water, giving a yellow solution neutral to litmus; the addition of ammonia solution, until alkaline to litmus, did not produce a precipitate. The compound was dissolved in dilute hydrochloric acid and gave a yellow precipitate with Meyer's reagent. The compound did not crystallise from ethanol, ether or acetone. The material was dissolved in concentrated sulphuric acid and gave a yellow solution; on the addition of a crystal

of potassium dichromate a violet colour developed[153]. The material was treated with Mandelin's reagent and gave a violet colour[153]; a yellow solution turning green was produced with Froehde's reagent[153]. An aqueous solution of the compound was treated with a drop of bromine water and gave a bright red colour[153]. The material was dissolved in sulphuric acid (10%) and treated with a mixture of concentrated sulphuric acid and ethanolic solution of gallic acid (5%): it gave a green colour in the cold turning blue on heating[148].

The ultraviolet spectrum had $\lambda_{max.}^{EtOH}$ 229, 344; $\lambda_{min.}^{EtOH}$ 255, 310, in 0.25N potassium hydroxide in 95% ethanol a bathochromic shift[154] occurred, $\lambda_{max.}$ 289mμ, 264; $\lambda_{min.}$ 314; $[\alpha]_D^{22}$ 0° (c, 0.14 in chloroform) (Lit.[154], 0°).

Paper chromatography

The compound was paper chromatographed using the standard method and gave an R_f value (0.45) which was identical with that of an authentic sample of berberine.

Berberine aurichloride

The compound, dissolved in water (1 mℓ), was treated with solution of gold chloride to give a yellow-orange precipitate. The dried precipitate (0.015g) would not crystallise from ethanol, ether or acetone. The infra-red spectrum was identical with that of an authentic sample of berberine aurichloride.

α-ALLOCRYPTOPINE

Adsorption chromatography on alumina of the mother liquors of crystallisation of α-(-)-methylcanadine chloride.

The mother liquors remaining after crystallisation of α-(-)-methylcanadine chloride from fractions III(a), III(b) and III(c) (Table X, p. 138 were combined with fraction III(d) and evaporated to dryness at 60°. The dried (P_2O_5) residue (4.99g) was dissolved in pure chloroform (15 mℓ) and chromatographed on an alumina column (Woelm III, 100g, 2 x 35 cm). The column was eluted with pure chloroform (500 mℓ) (ethanol free) then with ethanol/chloroform (10:90, 100 mℓ) and finally with ethanol (250 mℓ). Fractions (25 mℓ) were collected and chromatographed on alumina plates at 25° using ethanol/chloroform (2:98) as solvent; fractions containing the same bases were combined and evaporated to dryness at 60°. The fraction weights and the R_f values and the distribution of the bases are given in Table XI, p. 145.

Isolation of α-allocryptopine from fractions III(1b) and III(1c).

Fractions III(1b) and III(1c) were combined (1.52g) and extracted with cold ether (3 x 10 mℓ); a brownish-yellow crystalline residue (0.71g) m.p. 154-156° was separated by centrifuging; the ether solution, on concentration, yielded pale yellow prisms (0.41g), m.p. 158-159°. The two crystalline fractions were chromatographed on alumina plates using ethanol/chloroform (2:98), both fractions contained alkaloid Y with a

Table XI

Distribution of tertiary alkaloids in fractions from an alumina column (see p.144).

Fractions	Eluant volume	Residues				
		Weight	Alkaloids, relative intensities on chromatograms			
	mℓ	g	B	Y	X	Z
III(1a)	75	0.05	negative			
III(1b)	75	1.30		+++++	+++	
III(1c)	50	0.22		+++		
III(1d)	75	0.11		++		++
III(1e)	250	0.16		+		++
III(1f)	275	2.22	+	+		++

trace of X. The fractions were combined (1.12g) and twice recrystallised from ethanol to give colourless prisms (0.5g) m.p. 161-162°, (Lit.[86], 160°), $[\alpha]_D^{22}$ 0° (c, 0.5 in chloroform), (Lit.[86], 0°); $\lambda_{max.}^{EtOH}$ 231mμ (sh.) (log ε 4.19), 286(3.83), $\lambda_{min.}^{EtOH}$ 256(3.76). The crystals were chromatographed on alumina using both ethanol/chloroform (2:98 and 8:92) and showed the presence of one base Y with R_f values, in both solvent systems, identical with those obtained with authentic α-allocryptopine. The compound was paper-chromatographed using the standard solvent and had an R_f value of 0.71.

The compound was dissolved in concentrated sulphuric acid and gave an intense reddish-violet colour[157]. In ethanol, it gave an intense green colour when heated with concentrated sulphuric acid and solution of gallic acid (5% in ethanol[148]). The compound was treated with concentrated nitric acid and gave an orange-red colour turning yellow. When moistened with Froehde's reagent it gave a violet colour rapidly turning green[28]. The infrared spectrum (Figure VII, p. 77) showed a strong band at 1660 cm^{-1} indicative of =CO vibration[155], a strong band at 1225 (OCH_3) and a combination of a weak band at 1620 and two strong bands at 934 and 1036 cm^{-1} indicative of a methylenedioxy group ($-O-CH_2-O-$). The infrared spectrum was identical with that of an authentic sample of α-allocryptopine.

Analysis, found: C,69.0; H,6.4; N,3.8; (OCH_3), 16.0.

Calculated for $C_{21}H_{23}NO_5$: C,68.3; H,6.3; N,3.8; $(OCH_3)_2$,16.8%.

α-Allocryptopine hydriodide.

α-Allocryptopine (0.032g) was treated with an ethanolic solution (0.6 mℓ) of potassium iodide (0.024g) and glacial acetic acid (8mg). Crystals (0.034g) formed immediately and were recrystallised from ethanol to give α-allocryptopine hydriodide as prisms m.p. 192-193° (decomp.) (Lit.[86], m.p. 194°).

Analysis, found: C,51.4; H,4.9; N,2.8; I,25.1.

Calculated for $C_{21}H_{24}NO_5I$: C,50.7; H,4.8; N,2.8; I,25.5%.

The infrared spectrum showed the absence of a strong band at 1660 cm^{-1} (=CO)[155].

α-Allocryptopine picrate.

α-Allocryptopine (0.027g) in ethanol (2 mℓ) was treated with picric acid (0.018g) in ethanol (0.4 mℓ); crystallisation occurred on warming the solution. The crystals (0.032g) were washed with ethanol and dried (P_2O_5). The yellow crystals were recrystallised from ethanol to yield α-allocryptopine picrate (0.026g) as slender yellow prisms, m.p. 207-209° (decomp.) (Lit.[86], m.p. 207-208°).

Analysis, found: C,54.2; H,4.4; N,9.2.

Calculated for $C_{27}H_{25}N_4O_{12}$: C,54.2; H,4.2; N,9.4%.

ATTEMPTED SEPARATION OF THE MINOR TERTIARY ALKALOIDS OF FRACTION III

Preparative thin-layer chromatography of fraction III residue.

A portion (0.05g) of the fraction III(1) residue, left after the removal of α-(-)-methylcanadine chloride and α-allocryptopine, was dissolved in chloroform (2 mℓ) and applied to an alumina thin-layer plate (20 x 20cm) as an even, continuous streak by means of a mechanical applicator[161]. The chromatogram was developed for a distance of 15 cm with the solvent ethanol/chloroform (2:98); a narrow strip (width 1cm) down one edge was sprayed with Dragendorff's reagent to indicate the position of alkaloidal fractions. Three well separated fractions, designated III(2a), III(2b) and III(2c) of R_f values, 0.65, 0.30 and 0.06 respectively, were shown, and the adsorbent from the corresponding unsprayed zones was scraped from the plate.

The alkaloidal material of fractions III(2a), III(2b) and III(2c) was eluted from the alumina by ethanol, using Soxhlet extraction. The dried weights (P_2O_5) of the three fractions are given in Table XII p. 149.

Chromatography of fractions III(2a), III(2b) and III(2c).

The residues were chromatographed, together with pure skimmianine, on thin-layer alumina plates, using as solvents ethanol/chloroform (2:98 and 4:96). The chromatograms were sprayed with Dragendorff's reagent. The results are given in Table XII p. 149. With Dragendorff's reagent, base W gave a grey colour and base Za a rose-violet. This latter colour was also sometimes given by skimmianine: on those occasions when only the normal

orange colour was given, the rose-violet colour could be produced by spraying with dilute solution of ammonia.

Table XII

Distribution of tertiary alkaloids in fractions from a preparative thin-layer chromatogram (see p.148).

Solvents	R_f values of Alkaloids					Weight of fractions
	Za	Z	Zb	W	Y	X	
	Fraction III(2a)						0.012
Chloroform +2% ethanol						0.65	
+4% ethanol						0.73	
Relative intensities						++	
	Fraction III(2b)						0.027
Chloroform +2% ethanol					0.30	0.63	
+4% ethanol					0.56	0.73	
Relative intensities					++++	+	
	Fraction III(2c)						0.011
Chloroform +2% ethanol		0.06		0.23	0.31	0.66	
+4% ethanol	0.19		0.22	0.49	0.62	0.76	
Relative intensities	+		+	++	+	±	

Fraction III(2a)

The crystallisation of residue III(2a) was attempted using ethanol, acetone, and chloroform; crystalline material was not obtained. The ultraviolet spectrum of fraction III(2a) had $\lambda_{max.}^{EtOH}$ 231mμ , 283, 323(sh.); $\lambda_{min.}^{EtOH}$ 257. The infrared spectrum showed strong absorption peaks at 805, 1027, 1091, 1262, 1466 and 2945 cm^{-1}.

Two-dimensional, thin-layer chromatography of fraction III(2a) on alumina G using ethanol:chloroform (0.5:99.5) along the first axis and ethanol:chloroform (1:99) along the second, gave two alkaloidal spots X_1 and X_2.

Fractions III(2b) and III(2c).

These fractions were not further examined.

EXAMINATION OF FOUR AFRICAN *FAGARA* SPECIES FOR THE PRESENCE OF TERTIARY AND QUATERNARY ALKALOIDS

Stem and root barks of the following African species were examined for both tertiary and quaternary bases:- *Fagara leprieurii* Engl., *F. macrophylla* Engl., *F. viridis* A. Chev. and *F. xanthoxyloides* Lam.

FAGARA LEPRIEURII ENGL.

(Sample 1)

Preliminary extractions and separations.

Material

The starting material was the stem bark of authenticated Fagara leprieurii Engl. collected in the Ilesha district of Western Nigeria. The dried bark (1.53g) was finely ground and extracted in a Soxhlet apparatus with ethanol (95%). The extract was evaporated to dryness under reduced pressure at 60°, giving a yellowish-brown amorphous powder (0.13g) with a bitter taste and acrid odour. The dried ethanolic residue was extracted with chloroform (ethanol free) (2 x 5 mℓ) and the residue re-extracted with dilute acetic acid (3 x 5 mℓ). The acidic extract was basified (pH 8.0) with dilute solution of ammonia and extracted with chloroform, in a liquid-liquid extractor. The chloroform extracts were combined and reduced to dryness at 60° (0.042g). The basified aqueous phase was then extracted with n-butanol and the butanol extract reduced to dryness at 60° (0.037g). The original dried ethanolic extract and the chloroform and the butanol extracts from this, dissolved in dilute hydrochloric acid, gave white precipitates with Meyer's reagent.

Chromatography of ethanol, chloroform and butanol extracts

Paper chromatography

Chromatographic separation of the dried extracts was attempted

using the standard solvent system on Whatman No. 1 paper with downward development at 25°, for 16 hours. The solvent iso-amyl alcohol/petroleum ether (b.p. 60-80°)/ glacial acetic acid (12:4:12:8.5), described by Palmer[18] for chromatographic examination of a number of African Fagara species, was also used. After development the papers were examined in screened ultraviolet light followed by spraying with modified Dragendorff's reagent. The results are given in Table XIII, p. 154.

Thin-layer chromatography.

The three dried extracts were chromatographed on alumina plates, prepared as on p. 116, using ethanol/chloroform (2:98) as solvent. After drying, the developed plates were examined in screened ultraviolet light then sprayed with modified Dragendorff's reagent, followed by spraying with an aqueous solution of potassium permanganate (1%)[159]. The results are given in Table XIII, p. 154.

Table XIII

Distribution of alkaloids in extracts from the stem bark of *Fagara leprieurii* Engl. (Sample 1).

Extracts	R_f values			Fluorescence	Relative intensities on chromatograms
	Paper		T.L.C.		
	Standard solvent	Palmer solvent	2% Ethanol		
Ethanol	0.20				++
			0.00		+++
	0.36	0.72			+++++
			0.75	Blue	++
	0.58	0.91			+
			0.86	Violet	++
	0.75	0.98			++++
Butanol	0.20				++
	0.36	0.72	0.00		+++++
	0.58				+
Chloroform	0.70	0.91	0.80	Blue	++
	0.75	0.98	0.95	Violet	+

FAGARA LEPRIEURII ENGL.

(Sample 2)

Preliminary extractions and separations.

Material

The starting materials were the stem and root barks of authenticated Fagara leprieurii Engl. The finely powdered dried barks (25g) were separately extracted in a Soxhlet apparatus with petroleum ether (b.p. 40-60°), chloroform, and ethanol. The extracts were reduced to dryness at 60° under reduced pressure. The weights of the extracts are recorded in Table XV, p. 159. The extracts, dissolved in the appropriate solvent, were applied to filter paper which was sprayed with Dragendorff's reagent. All three extracts gave a positive brown-orange colour.

The bright yellow petroleum ether extracts, of both stem and root barks, on concentration, gave colourless non-alkaloidal crystals and a bright yellow alkaloidal viscous oil.

The dark yellow chloroform extracts, of both stem and root barks, on concentration, gave brownish-yellow resins, which dissolved partially in dilute hydrochloric acid, the solutions giving white precipitates with Meyer's reagent.

The dried, brown, resinous ethanolic extracts of both stem and root barks were extracted with hydrochloric acid (2 x 10 mℓ 5%, 2 x 10 mℓ 2.5%). The non-alkaloidal resinous precipitate was removed by filtration. The acidic alkaloidal filtrate was extracted in a liquid-

liquid extractor with chloroform, until the extracting solvent was negative to Meyer's extract; the acidic chloroform-soluble extract gave a white precipitate with Meyer's reagent. The aqueous phase was then basified (pH 8.0) with strong solution of ammonia and re-extracted with chloroform, to give an alkaline chloroform-soluble extract which was non-alkaloidal. The ammoniacal aqueous phase, which still gave a positive test with Meyer's reagent, was finally extracted with n-butanol until the aqueous phase was negative to Meyer's reagent. The acidic chloroform, the alkaline chloroform and the butanol-soluble extracts were reduced to dryness, at 60° under reduced pressure. The weights of the dried extracts are given in Table XV, p. 159.

Chromatography of the extracts.

Paper chromatography

Chromatographic separation of the six extracts, dissolved in 1 mℓ of chloroform or ethanol, was attempted, using the standard solvent on No. 1 Whatman paper with downward development at 25° for 16 hours. After development the dried papers were examined in screened ultraviolet light, then sprayed with modified Dragendorff's reagent. The results are given in Table XIV, p. 157 and p. 158.

Thin-layer chromatography

The six extracts were chromatographed on alumina plates, using ethanol/chloroform (2:98) as solvent. After drying, the plates were treated as before (p. 153). The results are given in Table XIV, p. 157 and 158.

Table XIV

Paper and thin-layer chromatography of the extracts of F. leprieurii.

Stem bark

Extract	R_f values Paper	R_s values* T.L.C.	Colour unsprayed chromatograms	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.70 0.76		orange	orange	++++ +++
		0.93 1.11	yellow	blue orange	++ ++++
Chloroform	0.23 0.38 0.69		yellow yellow	green yellow-orange	++ +++ +++
		0.17 0.93 1.12	yellow	yellow blue orange	++ ++ ++
Ethanol	0.11 0.25 0.44 0.49				+ +++ ++ ++
		0.00			++++
Acidic chloroform (Ex ethanol)	0.77			blue	++++
		0.94 1.13	yellow	blue orange	++++ ++
Alkaline chloroform (ex ethanol)	no bases				
Butanol (ex ethanol)	0.17 0.30 0.51				+++ +++++ +
		0.00			+++

(* reference standard = skimmianine)

Table XIV contd.

Paper and thin-layer chromatography of the extracts of F. leprieurii.

Root bark

Extract	R_f values	R_s values	Colour unsprayed chromato-grams	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.24 0.43 0.72 0.77		 yellow	green blue orange	+ + +++ +
		0.17 0.92 1.11	 yellow	yellow blue orange	+++ +++ ++++
Chloroform	0.15 0.34 0.77		 yellow yellow	green yellow	++ ++++ ++
		0.17 0.58 0.93 1.13	 yellow	yellow blue orange	++ + ++ +++
Ethanol	0.13 0.25 0.44				+++ +++ +
		0.00			+++
Acidic chloroform (Ex ethanol)	0.32 0.44 0.53 0.77		yellow		+++ + + +++
		0.17 1.01 1.14	 yellow	 blue orange	+ +++ ++++
Alkaline chloroform (Ex ethanol)	no bases				
Butanol (Ex ethanol)	0.07 0.16 0.29 0.50				+ ++++ ++++ ++
		0.60			+++

Table XV

Weights of extracts of the four African Fagara species.

Extract	Petroleum ether g.	Chloroform g.	Ethanol g.	Dried ethanolic extract		
				Chloroform soluble		Butanol soluble g.
				Acidic g.	Alkaline g.	
F. leprieurii						
stem bark	0.98	0.32	1.32	0.07	0.08	0.85
root bark	1.38	0.33	1.81	0.16	0.21	0.83
F. macrophylla						
stem bark	0.27		0.99		0.19	0.69
root bark	0.61		0.86	0.09	0.05	0.59
F. viridis						
stem bark	0.84		2.11		0.25	0.59
root bark	1.78		3.82		0.84	1.68
F. xanthoxyloides						
stem bark	0.40		2.27		0.21	0.90
root bark	0.52		0.97		0.33	0.53

FAGARA MACROPHYLLA Engl.

Preliminary extractions and separations.

Material

The starting materials were samples of the stem and root barks of authenticated Fagara macrophylla Engl. The finely powdered dried barks (25g) were separately extracted, in a Soxhlet apparatus, with petroleum ether (b.p. 40-60°) and ethanol 95%. The extracts were reduced to dryness at 60° under reduced pressure. The weights of the extracts are recorded in Table XV, p. 159. The four extracts gave a reddish-brown precipitate with Dragendorff's reagent and a yellowish-white precipitate with Meyer's reagent.

The bright yellow petroleum ether extract of the root bark, on concentration, gave white non-alkaloidal crystals, insoluble in dilute hydrochloric acid, and a yellow viscous oil. The similar bright yellow extract of the stem bark, on concentration, gave a pale yellow waxy solid. The oil from the root bark and the waxy solid from the stem bark both dissolved partially in dilute hydrochloric acid to give solutions which yielded yellowish-white precipitates with Meyer's reagent.

The brown resinous dried residue from the ethanolic extract of the stem bark was extracted with hydrochloric acid (5%, 2 x 10 mℓ ; 2.5%, 2 x 10 mℓ). The non-alkaloidal resinous precipitate was removed by filtration and discarded. The acidic alkaloidal filtrate was basified (pH 8.0) with strong solution of ammonia and extracted in a liquid-liquid extractor with

chloroform. A red precipitate remained in the alkaline aqueous phase which gave a white precipitate with Meyer's reagent. The aqueous phase was further extracted in a liquid-liquid extractor with n-butanol, until the aqueous phase was negative to Meyer's reagent. The alkaline chloroform- and the butanol-soluble extracts were reduced to dryness at 60° under reduced pressure. The dried weights are given in Table XV, p. 159.

The dried, brown resinous residue from the ethanolic extract of the root bark was extracted with hydrochloric acid as for the stem bark. The acidic aqueous phase was extracted in a liquid-liquid extractor with chloroform, until the chloroform ceased to extract alkaloid. The acidic chloroform extract gave a white precipitate with Meyer's reagent. The aqueous phase was basified (pH 8.0) with ammonia solution and further extracted with chloroform, until the chloroform ceased to extract alkaloid. Finally the alkaloidal aqueous phase was extracted with n-butanol until the aqueous phase was negative to Meyer's reagent. The two chloroform extracts and the butanol-soluble extract were separately reduced to dryness at 60° under reduced pressure. The weights are given in Table XV, p. 159.

Chromatography of the extracts.

The extracts of both the stem and root barks were examined by paper chromatography and thin-layer chromatography as described for F. leprieurii p. 156. The results are given in Table XVI, pp. 162-163.

Table XVI

Paper and thin-layer chromatography of the extracts of F. macrophylla.

Stem bark

Extract	R_f values Paper	R_s values* T.L.C.	Colour	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.79		yellow		+
		0.98		blue	+
Alkaline chloroform (Ex ethanol)	0.37 0.56 0.78		yellow yellow	yellow yellow blue/orange	+++ +++ +++++
		0.19 0.92 1.10	yellow	yellow blue orange	++ ++ +
Butanol (Ex ethanol	0.28 0.52 0.68				++++ +++++ +

(* reference standard = skimmianine)

Table XVI contd.

Paper and thin-layer chromatography of the extracts of F. macrophylla.

Root bark

Extract	R_f values Paper	R_s values T.L.C.	Colour	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.81			blue/orange	++
		0.19		yellow	+
		0.94		blue	+
		1.13	yellow	orange	++
Ethanol	0.22				+++
	0.37				+++
	0.51				+++
	0.59				++++
	0.73			blue	+
		0.00			+++
		0.17		yellow	+
		0.93		blue	+
		1.13	yellow	orange	+
		1.15			+
Acidic chloroform (Ex ethanol)	0.36		yellow		+++
	0.45				++++
	0.52				+++
	0.82			blue/orange	+
		0.17		yellow	+
		0.93		blue	+
		1.13	yellow	orange	++++
Alkaline chloroform (Ex ethanol)	0.46			yellow	+++
		0.17		yellow	+++
Butanol (ex ethanol)	0.16				+++
	0.30				++++
	0.48				++++
	0.66	0.00			++++

FAGARA VIRIDIS A. CHEVAL.

(Sample 1.).

Preliminary extractions and separations.

Material

The starting materials were the stem bark and whole root of authenticated Fagara viridis A. Cheval collected in the Igbajo district of Western Nigeria. The dried bark (0.77g) and the dried whole root (0.3g) were separately finely powdered and extracted by the method used for F. leprieurii, (p.152) and the extracts evaporated, at 60°, to give dried extracts, the weights of which are given in Table XVII, p. 165. All the extracts gave a white precipitate with Meyer's reagent.

Chromatography of the ethanol, chloroform and butanol extracts of the bark and root.

The ethanol, butanol and chloroform extracts were examined by paper and thin-layer chromatography using the methods for F.leprieurii given on p.152. The results are given in Table XVII, p. 165.

Distribution of alkaloids in extracts from the stem and root barks of Fagara viridis A. Cheval. (sample 1.).

Extracts Weights	Chromatography		Fluorescence	Relative intensities on chromatograms
	Paper Standard solvent R_f values	Thin-layer 2% ethanol in chloroform R_s values*		
Stem bark				
Ethanol 0.1g	0.22 0.38 0.42 0.66 0.76			+++ +++ ++++ +++ +++++
		0.00 0.57 0.96 0.13	blue orange	++ + ++ ++
Butanol 0.042g	0.23 0.39			+++ +++
		0.00		++
Chloroform 0.058g	0.42 0.67 0.76			+++ +++ +++
		0.57 0.96 1.13	blue orange	+ ++ ++
Root bark				
Butanol 0.045g	0.23 0.42			++ ++
		0.00		++
Chloroform 0.025g	0.42 0.59 0.66 0.76			++++ + ++++ +++++
		0.55 0.96 1.13		+ ++ ++

(* reference standard = skimmianine)

FAGARA VIRIDIS A. CHEVAL.

(Sample 2.).

Preliminary extractions and separations.

Material

The starting materials were the stem and root barks of authenticated Fagara viridis A. Cheval. The dried finely powdered barks (25g) were separately extracted in a Soxhlet apparatus with petroleum ether (bp. 40-60°) and ethanol. The extracts were reduced to dryness at 60° under reduced pressure. The weights of the extracts are recorded in Table XV, p. 159.

On concentration, the yellow petroleum ether extract of the stem bark gave a greenish-yellow waxy residue which partially dissolved in dilute hydrochloric acid, the solution giving a white precipitate with Meyer's reagent.

The petroleum ether extract of the root bark was concentrated; colourless crystals separated from the orange-yellow viscous residue. The crystals were insoluble in dilute hydrochloric acid and were non-alkaloidal. The orange-yellow residue partially dissolved in dilute hydrochloric acid and the solution gave a white precipitate with Meyer's reagent.

The dried, brown, resinous ethanolic extracts of both the stem and root barks were treated as for the stem bark of F. macrophylla p. 160, to yield both chloroform-soluble and butanol-soluble dried extracts. All four extracts, dissolved in dilute hydrochloric acid, gave a white precipitate with Meyer's reagent.

Table XVIII

Paper and thin-layer chromatography of the extracts of F. viridis.

Stem bark

Extract	R_f values Paper	R_s values* T.L.C.	Colour	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.74 0.89		yellow		+++ +
		0.08 0.93 1.12	red yellow	purple blue orange	+ +++ +
Chloroform (Ex ethanol)	0.28 0.50 0.60 0.74		yellow	violet	+++ +++ ++ +++++
		0.08 0.94 1.13 1.15	red yellow	purple blue orange	+++ ++++ ++++ +
Butanol (Ex ethanol)	0.06 0.17 0.29 0.41 0.51 0.67				+ +++ +++ ++++ +++ ++
		0.00 0.56			++++ ++

(* reference standard = skimmianine)

Table XVIII contd.

Paper and thin-layer chromatography of the extracts of F. viridis.

Root bark

Extract	R_f values Paper	R_s values T.L.C.	Colour	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.74		yellow	blue/orange	+++++
		0.92		blue	++++
		1.13	yellow	orange	++
		1.15			++
Chloroform (Ex ethanol)	0.21		yellow	yellow	+++
	0.44				++++
	0.54				+++
	0.77			violet	+++++
		0.08	red	purple	++
		0.93		blue	+++
		1.12	yellow	orange	+++
		1.15			++
Butanol (Ex ethanol)	0.06				+
	0.19				++++
	0.31				++++
	0.42				++
	0.51				++
		0.00			++++

FAGARA XANTHOXYLOIDES LAM.

(Sample 1.).

Preliminary extractions and separations.

Material

The starting material was the stem bark of authenticated Fagara xanthoxyloides Lam. collected at Lagos, Nigeria. The dried bark (0.65g) was extracted by the method used for F. leprieurii (p. 152). The extracts did not give precipitates with Meyer's or Dragendorff's reagents.

FAGARA XANTHOXYLOIDES LAM.

(Sample 2.).

Preliminary extractions and separations.

Material

The starting materials were the stem and root barks of authenticated Fagara xanthoxyloides Lam. The dried finely powdered barks (25g) were separately extracted in a Soxhlet apparatus with petroleum ether (bp 40-60°) and ethanol. The extracts were reduced to dryness at 60° under reduced pressure. The weights of the extracts are recorded in Table XV, p. 159.

On concentration the yellow petroleum ether extract of the stem bark gave a yellowish-white waxy solid. Some of the residue dissolved in dilute hydrochloric acid and gave a white precipitate with Meyer's reagent.

On concentration the yellow petroleum ether extract of the root bark gave colourless crystals and a deep yellow viscous oil. The crystals were insoluble in dilute hydrochloric acid and were non-alkaloidal. The yellow oil, dissolved partially in dilute hydrochloric acid, gave a white precipitate with Meyer's reagent.

The dried residue from the ethanolic extract of the stem bark was treated as for the ethanolic residue of F. macrophylla stem bark p.160 , to yield both alkaline chloroform-soluble and butanol-soluble extracts. On treatment with strong solution of ammonia, the yellowish-brown acid extract gave a red precipitate, which dissolved in butanol.

The dried, resinous brown residue from the ethanolic extract of the root bark was extracted with hydrochloric acid (5%, 2 x 10 mℓ ; 2.5% 2 x

10 mℓ). The non-alkaloidal resinous precipitate was removed by filtration and discarded. The acidic alkaloidal filtrate was basified (pH 8.0) with strong solution of ammonia giving a copious red precipitate. The alkaline aqueous phase was extracted in a liquid-liquid extractor with chloroform until the extracting chloroform gave a negative test with Meyer's reagent. The red precipitate was still present in the alkaline aqueous phase and this was re-extracted with n-butanol which dissolved a little of the red precipitate. The remainder of the precipitate was removed by filtration, and dissolved in ethanol, giving a bright yellow solution which on evaporation gave an amorphous yellow solid. The yellow solid was insoluble in dilute hydrochloric acid. The alkaloidal alkaline aqueous phase was further extracted with n-butanol until the aqueous phase gave a negative test with Meyer's reagent.

The alkaline chloroform-soluble and butanol-soluble extracts of both the stem and root barks were reduced to dryness at 60° under reduced pressure and the weights are given in Table XV, p. 159.

Chromatography of the extracts.

The petroleum ether, alkaline chloroform-and butanol-soluble extracts of both the stem and root barks were examined by paper and thin-layer chromatography, as described for F. leprieurii p.156 . The results are given in Table XIX, pp. 172-173.

Table XIX

Paper and thin-layer chromatography of the extracts of F. xanthoxyloides.

Stem bark

Extract	R_f values Paper	R_s* values T.L.C.	Colour	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.79			purple	++
		0.93		purple	++
Chloroform (Ex ethanol)	0.54 0.63 0.75			 pale blue blue	+ ++ +++
		0.00 0.93		 blue	++ +++
Butanol (Ex ethanol)	0.15 0.38 0.54				++ ++++ ++
		0.00			++++

(* reference standard = skimmianine)

Table XIX contd.

Paper and thin-layer chromatography of the extracts of F. xanthoxyloides.

Root bark

Extract	R_f values Paper	R_s values T.L.C.	Colour	Fluorescence	Relative intensities on chromatograms
Petroleum ether	0.56 0.80		yellow	yellow	++ +++
		0.26 0.42 0.93 1.13 1.16	yellow	yellow blue blue orange	+ + ++++ ++ +
Chloroform (Ex ethanol)	0.38 0.66 0.80		yellow	yellow pale blue purple	+++ ++ +++
		0.25 0.44 0.61 0.93 1.12	yellow	yellow blue blue blue orange	+ + + +++ ++++
Butanol (Ex ethanol)	0.16 0.30 0.56 0.67				++ ++++ +++ +++
		0.36			+++

Thin-layer chromatography, on micro-crystalline cellulose, of the butanol-soluble fractions of Fagara leprieurii, F. macrophylla, F. viridis and F. xanthoxyloides.

Chromatoplates, similar to those described on p.117, were prepared using cellulose powder (Whatman Chromedia CC41) (30g) suspended in water (75 mℓ), by mixing in a blender for 20 secs.[160]. The chromatoplates were dried in a current of air at 60°, for 30 mins. and stored in the absence of a dessicant. The butanol-soluble fractions were dissolved in ethanol. The chromatograms were equilibrated, before development, for either 0 or 1 hour, in the presence of the appropriate solvent. The chromatograms were developed for 15 cm, using the following systems:-

1. n-butanol/glacial acetic acid/water (10:1:3)[160], at 22°
2. iso-amyl alcohol/tertiary-amyl alcohol/formic acid/water (1:1:1:5), at 25°.

For an effective movement of 15 cm the development time was approximately 2.5 hours for solvent No. 2, at 25°, and 2 hours for solvent No. 1 at 22°. After development the chromatograms were dried in a current of air at 40°, before spraying with an ethanolic solution of ferric chloride, followed by Dragendorff's reagent. The results are given in Table XX, p. 102, and Figures VIII and IX, pp. 100 and 101.

Two-dimensional chromatography

The above butanol-soluble fractions were chromatographed on cellulose chromatoplates (20 x 20 cm) for a distance of 15 cm. The

chromatoplates were developed along the first axis, using solvent No. 1. The solvent was removed by drying, in a current of air at 30°. Then solvent No. 2 was used along the second axis, the chromatoplates being dried and sprayed with the above reagents. The results are given in Figures X and XI, pp. 104 and 105.

R E F E R E N C E S

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P A R T II

EXAMINATION OF CERTAIN APOCYNACEOUS DRUGS FOR ALKALOIDS

THE ALKALOIDS OF *DIPLORRHYNCHUS CONDYLOCARPON* PICHON

I N T R O D U C T I O N

THE FAMILY APOCYNACEAE

The family Apocynaceae, containing 180 genera and about 1,400 species[1], is known for its richness in alkaloid- and glycoside-bearing plants.

Of the three subfamilies[2,3], the Plumerioideae, one of the two major subfamilies, consists almost exclusively of alkaloid-bearing plants, including species of the genera Aspidosperma, Tabernaemontana (including Conopharyngia), Diplorrhynchus and Ochrosia[2,3]: the genus Carissa is glycoside-bearing. Within the subfamily Plumerioideae, Bisset (after Pichon)[2,3] placed the genus Carissa in the tribe Carisseae, the genus Tabernaemontana in the tribe Tabernaemontaneae, Diplorrhynchus and Aspidosperma in the tribe Alstonieae and Ochrosia in the tribe Rauwolfieae.

In the second major subfamily Echitoideae, the genus Prestonia was included in the tribe Parsonsieae, the genus Odontadenia in the tribe Ecdysanthereae and Mandevilla in the closely related tribe Ichnocarpeae[2,3]: some species within these three genera are alkaloid-bearing. Steroidal amines have been isolated from species of the genera Chonemorpha, Funtumia, Kibatalia and Malouetia, four of the twenty genera reported to contain alkaloids in this subfamily of approximately 56 genera[4].

The chemistry and pharmacology of the numerous indole alkaloids of the Apocynaceae have recently been reviewed[5]. The physical data and sources of the alkaloids which have been isolated from the species of the genera Diplorrhynchus, Ochrosia and Tabernaemontana, involved in this survey, are listed in Table XXII, p. 186. Of the genera involved in this

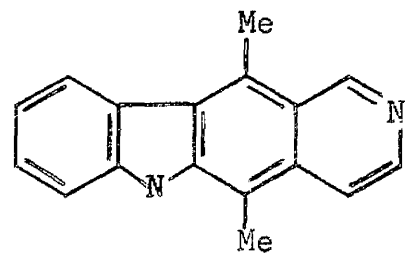
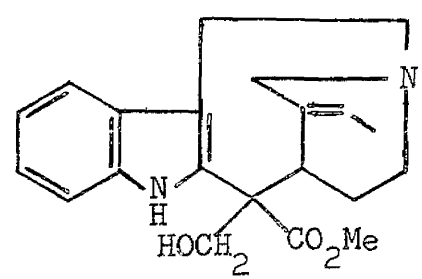
alkaloidal survey only these three genera have yielded alkaloids of known structure.

Table XXII

Alkaloids isolated from Apocynaceous drugs

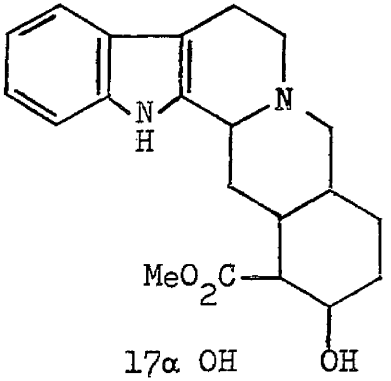
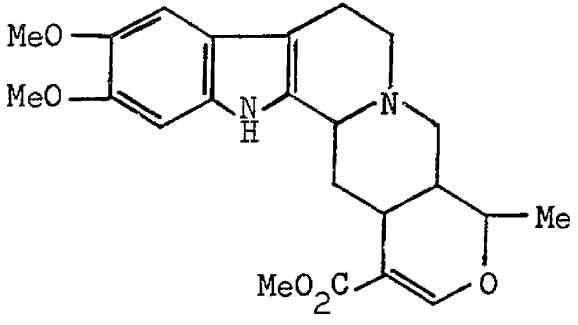
Tetracyclic indoles.

Pyridocarbazoles.

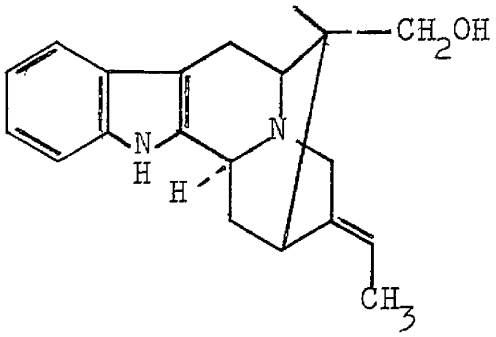
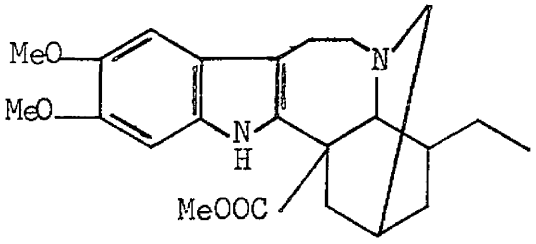
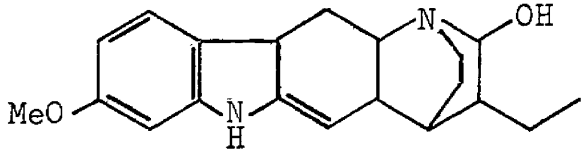
Alkaloid	m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Ellipticine $C_{17}H_{14}N_2$	311-315	0°		Ochrosia elliptica Labill.	6
Methoxy-ellipticine $C_{18}H_{16}ON_2$	280-285	0°		Ochrosia elliptica Labill.	6
Stemmadenine $C_{21}H_{26}O_3N_2$ Stemmadenine type	189-191	+329		Diplorrhynchus condylocarpon Pichon var. mossambicensis Duvign.	7, 10

Pentacyclic indoles.

Yohimbine type.

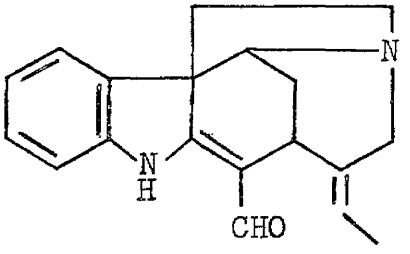
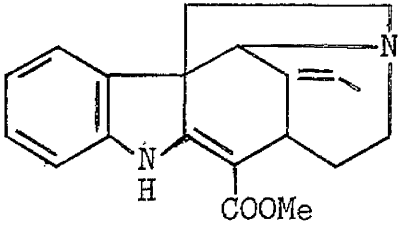
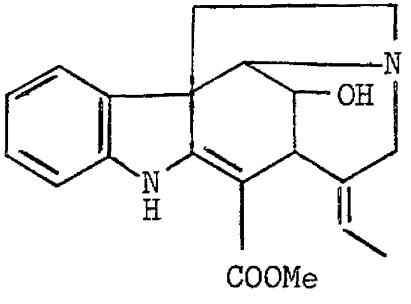
Alkaloid	m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Yohimbine $C_{21}H_{26}O_3N_2$	233-234	+110° (pyridine)	 17α OH OH	Diplorrhynchus condylocarpon Pichon var. mossambicensis Duvign.	7
β-Yohimbine $C_{21}H_{26}O_3N_2$	226-230	-47° (pyridine)	17β OH	D. condylocarpon var. mossambicensis	7
Reserpiline type.					
Isoreserpiline $C_{23}H_{28}O_5N_2$	211-212	-82° (pyridine)		O. elliptica Labill.	6

Isoquinuclidine type.

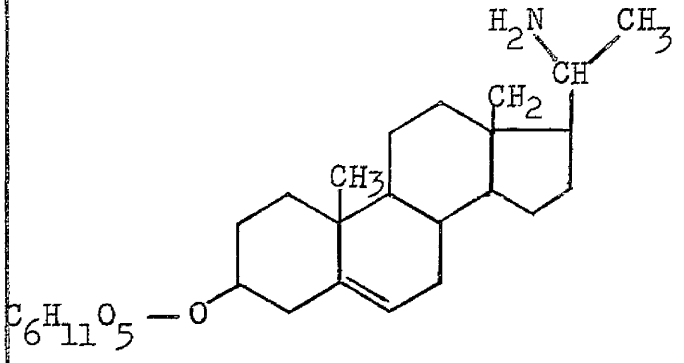
Alkaloid	m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Normacusine B (Tombozine) $C_{19}H_{22}ON_2$	270-272	+38° (EtOH)		D. condylocarpon var. mossambicensis Duvign.	7
Conopharyngine $C_{23}H_{30}O_4N_2$	144-145	-40.5° ($CHCl_3$)		Tabernaemontana pachysiphon var. cumminsi Huber	9
Pentacyclic dihydroindoles					
Elliptinine $C_{20}H_{24}O_2N_2$	231-233	-255° (EtOH)		O. elliptica Labill.	6

Pentacyclic dihydroindoles (contd.)

α-Methylene indoline type.

Alkaloid	m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Norfluorocurarine $C_{19}H_{20}ON_2$	184-186	-1230°		D. condylocarpon var. mossambicensis	7
Condylocarpine $C_{20}H_{22}O_2N_2$	159-162	+900° ($CHCl_3$)		D. condylocarpon var. mossambicensis	7, 11
Mossambine $C_{20}H_{22}O_3N_2$	238-242	-470° ($CHCl_3$)		D. condylocarpon var. mossambicensis	7, 12

Steroidal Alkaloids

Alkaloid	m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
3β-hydroxy-20α-aminopregnene-(5)-glucoside	285-288	-		Conopharyngia pachysiphon Stapf.	13

D I S C U S S I O N

APOCYNACEOUS DRUGS

Extraction of alkaloids.

The samples of Apocynaceous drugs listed on page 200 were extracted with petroleum ether (bp 60-80°), chloroform, ethanol 95%, and ethanol 75%. Each concentrated percolate was tested with Dragendorff's and Meyer's reagents for the presence of alkaloids. The following samples were found to be devoid of alkaloid:-

2a and 2b, stem bark and wood of Cameraria belizensis Standley,

3a and 3b, stem bark and wood of Carissa edulis Vahl,

4a, root wood of Diplorrhynchus condylocarpon Pichon,

5a, stem of Mandevilla hirsuta Malme.

The following samples were shown (Table XXIII, p. 201) to contain alkaloids and extracts of each were chromatographed on alumina plates in order to estimate the possible number of alkaloids present (Table XXIV, p. 203):-

1, stem bark of Ambelania acida Aubl.,

4b, stem bark of Diplorrhynchus condylocarpon Pichon,

5b, root of Mandevilla hirsuta Malme,

6, root of Ochrosia elliptica Labill,

7, root of Odontadenia nitida Muell.,

8, root of Prestonia quinquangularis Spreng,

9, stem bark of Tabernaemontana angolensis Stapf,

10, stem bark of Tabernaemontana pachysiphon, Stapf.

THE ALKALOIDS OF CERTAIN APOCYNACEOUS SPECIES

Ambelania acida Aubl.

The presence of at least five alkaloids (Table XXIV, p. 203) was demonstrated, two of these occurring in the chloroform extract. Alkaloids have not previously been reported in this South American genus, closely allied to the genus Macoubea[2,3] in which it was included by Pichon[4]. A potent alkaloid, macoubeine, of unknown structure, was reported in M. guianensis Aubl.[14].

Cameraria belizensis Standley.

In British Honduras, this species is known as "Savvanah white poisonwood". Alkaloidal material was absent from the stem wood and stem bark. Chemical investigations of the genus have not been reported in the literature.

Carissa edulis Vahl.

The stem wood and bark of this species were devoid of alkaloids. Similarly Abisch and Reichstein[15] subsequently did not find alkaloids in the roots while Pernet[2] found unimportant traces of alkaloid in the branches and leaves of Madagascan plants of this species. From the bark of C. carandas L., Greshoff[2] isolated a trace of alkaloid but he did not find any in the bark of C. diffusa Roxb. Dymock et al.[2] found alkaloid in the ether extract of the root of C. carandas. In contrast, Joshi and Boyce[2] in an investigation of the roots of the closely related,

but distinct, C. congesta Wight did not find alkaloid. Webb[16] found alkaloid in the bark of C. ovata R. Br. but not in the leaves nor stems; the bark of the variety stolonifera also gave negative tests. Mohr et al.[17] found a trace of alkaloid in the roots of C. ovata var. stolonifera but none in the leaves, nor in the leaves of C. lanceolata R. Br. Abisch and Reichstein[15] reported that C. congesta, roots and seeds, C. grandiflora A.DC., branches, branch thorns and leaves, and C. oppositifolia Pichon, roots, were devoid of alkaloid.

The presence of cardiac glycosides has been reported in Carissa carandas L.[18], C. lanceolata[17], C. ovata var. stolonifera[17] and C. spinarum[18].

Diplorrhynchus condylocarpon (Muell. Arg.) Pichon.

The presence of at least six alkaloids was demonstrated in the stem bark of this species (Table XXIV, p. 203). In earlier work (unpublished) the author had isolated three crystalline, tertiary, indole alkaloids (see p. 205) which were subsequently shown to be normacusine B, condylocarpine and mossambine, three of the seven alkaloids isolated at a later date, by Stauffacher[7], from D. condylocarpon Pichon var. mossambicensis Duvign. Melting points, ultraviolet (Figure XIII, p. 199) and infrared spectra, elementary analyses and the results of chemical tests, obtained on the three crystalline compounds, were in agreement with those published by Stauffacher[7]. He isolated four known alkaloids yohimbine, β-yohimbine, tombozine (later shown to be identical with normacusine B) and stemmadenine, the latter of unknown structure and previously isolated from Stemmadenia

donnellsmithii R.E. Woodson[19] together with three alkaloids, then of unknown structure, condylocarpine, mossambine and norfluorocurarine. The structure of the latter was subsequently proved by conversion to C-fluorocurarine chloride[20], and the structures of condylocarpine[11], mossambine[12] and stemmadenine[10] were also subsequently elucidated.

The examination of this species was the initial major aim of the present work but it was curtailed after the publication of Stauffacher[7]: the report now forms only a minor part of the thesis, being included with the survey of other Apocynaceous plants. The extraction methods used were similar to those used previously[21] for the separation of some tertiary indole alkaloids of the Apocynaceae.

Mandevilla hirsuta Malme.

The presence of at least four alkaloids was demonstrated in the root (Table XXIV, p. 203), two occurring in the chloroform extract. The stem was devoid of alkaloids.

Mandevilla hirsuta Malme has not previously been investigated. Abisch and Reichstein[15] found no trace of alkaloid in the seeds of M. laxa (Ruiz et Pav.) Woods (synonym: M. suaveolens Lindl.). Peckolt[2] found an amorphous alkaloid dipladenine in the bulbous roots of M. illustris (Vell.) Woods (formerly Dipladenia illustris (Vell.) M. Arg.), in the leaves and roots of M. fragrans (Stadelm.) Woods (D. fragrans DC.), and in the underground parts of M. atroviolacea Woods (D. atroviolaceae A. DC.). M. velame Pichon (syn. Echites velame A. St. Hil. = Macrosiphonia velame M. Arg.) also contained amorphous alkaloidal material[2]. Raffauf[4] has also reported the presence of alkaloid in the genus Mandevilla.

Ochrosia elliptica Labill.

The presence of at least three alkaloids was demonstrated (Table XXIV, p. 203), quaternary bases (R_f value 0.00 being particularly abundant.

Goodwin et al.[6] reported the presence of three tertiary alkaloids of known structure, isoreserpiline, ellipticine and methoxy-ellipticine and one elliptinine, of unknown structure, in this species. The occurrence of alkaloidal material in the genus Ochrosia and the chemistry of the known alkaloids of O. coccinea Miq., O. elliptica Labill., O. glomerata Valeton, O. moorei F. Muell., O. oppositifolia K. Schum., O. poweri F.M. Bailey and O. tuberculata (Vall.) Pichon (O. sandwicensis A. DC.) have been reviewed by Bisset[2,3] and by Gilbert[5]. N_b-methylisoreserpilinium chloride (holeinine), from O. tuberculata[8], was the first known quaternary base of the ajmalicine type of indole alkaloid to be isolated from a member of the family Apocynaceae. The presence of quaternary alkaloids has also been reported in O. sandwicensis A. Gray[22].

Odontadenia nitida Muell.

The root of this species gave strongly positive alkaloidal tests. The presence of at least six bases was demonstrated (Table XXIV, p. 203), four being present in the petroleum ether extract. The possible presence of quaternary base (R_f value 0.00) was also indicated in addition to five tertiary bases.

Speir, through Bisset[2], reported that alkaloid tests on the bark of O. hoffmannseggiana (Steud.) Woods (syn. O. grandiflora Miq., O. macrantha Markg.) gave strongly positive results.

Prestonia quinquangularis (Jacq.) Spreng.

The presence of at least two alkaloids was demonstrated in the ethanolic extract of the root (Table XXIV, p. 203).

Speir, through Bisset[2], also reported the presence of alkaloid in the bark of P. quinquangularis. Peckolt[2] did not find alkaloid in the leaves of P. tomentosa R. Br. Bisset[2,3] has reviewed the alleged isolation of N,N-dimethyltryptamine from a reputed sample of P. amazonica (Benth.) Macbr. However, Schultes and Raffauf[3] concluded that the plant sample investigated was not a Prestonia species.

Tabernaemontana angolensis Stapf.

The presence of at least five alkaloids was demonstrated in the ethanolic extract (Table XXIV, p. 203) and these were separated into two groups by solvent extraction in a liquid/liquid extractor. The first group consisted of four chloroform-soluble tertiary alkaloids, the second group being of n-butanol-soluble quaternary bases.

This sample of bark was supplied as Conopharyngia angolensis Stapf. Pichon[23] incorporated Conopharyngia in the genus Tabernaemontana, together with the genera Anacampta, Gabunia, Pescheria and Stenosolen. Bisset[2,3] and Taylor[5] have reviewed the occurrence of alkaloidal

material and of alkaloids of known structure in the genus Tabernaemontana. Since the latter review, the isoquinuclidine base, coronaridine, has been isolated from Tabernaemontana jollyana[24] and T. pandacaqui[27].

Tabernaemontana pachysiphon Stapf.

This sample of bark was supplied as Conopharyngia pachysiphon Stapf. The presence of four alkaloids was demonstrated in the ethanolic extract (Table XXIV, p.203); none of these appeared to correspond to the bases of T. angolensis.

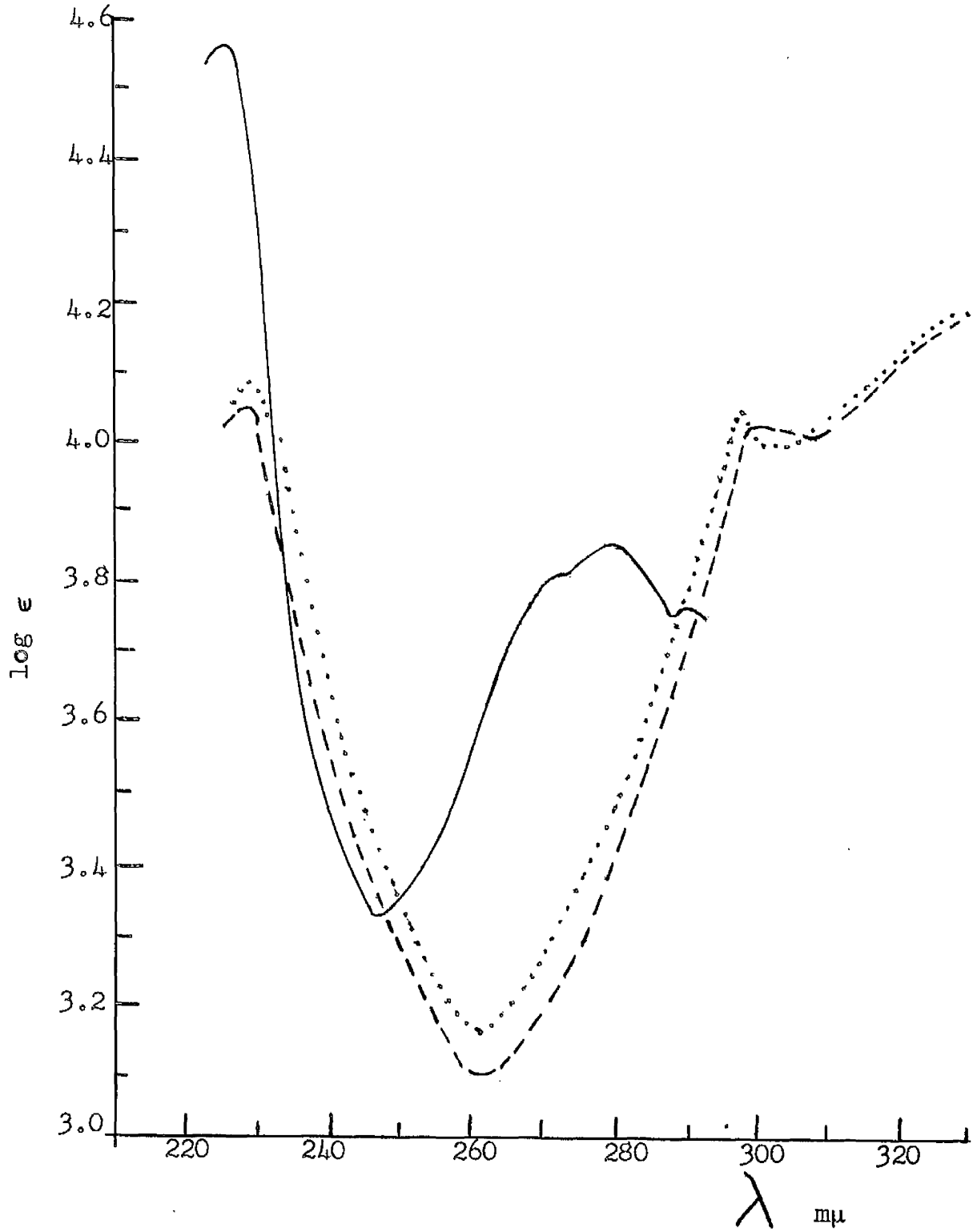
Dickel et al.[13] and Lucas et al.[25] isolated two alkaloids from the roots of this species. A pure, hypotensive principle was shown to be 3β-hydroxy-20α-amino-pregn-5-ene-β-D-glucoside hydrochloride and a compound with respiratory depressant activity was 3β-hydroxy-20α-amino-pregn-5-ene hydrochloride. Raffauf and Flagler[26] thought that the presence of a steroidal alkaloid suggested that the plant material did not belong to the genus Tabernaemontana which usually contained isoquinuclidine, and related, alkaloids. In 1963, Thomas et al.[9] investigated the leaves of T. pachysiphon var. cumminsi (Stapf) H. Huber, isolating conopharyngine as the major base and showing the presence of other, minor alkaloids. Conopharyngine, an isoquinuclidine alkaloid, was previously isolated from T. durissima Stapf (C. durissima Stapf)[28], along with isovoacangine, and three alkaloids of unknown structure, conodurine, conoduramine and Alkaloid E. The following species of the genus Tabernaemontana[5] have related isoquinuclidine alkaloids:- T. australis, T. coronaria, T. oppositifolia and T. psychotrifolia.

The bark of T. durissima has been used in W. Africa to relieve

fatigue[29]. Recently, the neutral fraction of a methanolic extract of the stem and root barks of T. durissima was examined with the subsequent isolation of α-amyrin acetate, β-amyrin acetate, lupenyl acetate and clion asterol[29].

The presence of alkaloids has also been reported[2] in T. ventricosa Hochst ex A. DC. and in T. holstii K. Schum.

Figure XIII



——— Tombozine

– – – – Mossambine

.... Condylocarpine

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

APOCYNACEOUS DRUGS

Preliminary extractions

Materials

The following samples of drugs were used:-

1. *Ambelania acida* Aubl., stem bark,
2a. *Cameraria belizensis* Standley, stem bark,
2b. *C. belizensis* Standley, stem wood,
3a. *Carissa edulis* Vahl, stem bark,
3b. *C. edulis* Vahl, stem wood,
4a. *Diplorrhynchus condylocarpon* Pichon, root wood,
4b. *D. condylocarpon* Pichon, stem bark,
5a. *Mandevilla hirsuta* Malme, stem,
5b. *M. hirsuta* Malme, root,
6. *Ochrosia elliptica* Labill, root,
7. *Odontadenia nitida* Muell., root,
8. *Prestonia quinquangularis* Spreng, root,
9. *Tabernaemontana angolensis* Stapf, stem bark,
10. *Tabernaemontana pachysiphon* Stapf, stem bark.

Each sample (25g) was finely powdered and extracted, by the Soxhlet method, with petroleum ether (bp 40-60°), chloroform and ethanol and by cold percolation with ethanol (70%). Extraction with each solvent was continued until a sample (5ml) gave no residue when evaporated to dryness. The extracts were concentrated to small volume, under reduced pressure, tested with Dragendorff's and Meyer's reagents and finally

concentrated to a dry residue. The weights of the extracts and the results of alkaloid tests are given in Table XXIII, p. 201.

Table XXIII

Weights of dried extracts from, and the occurrence of alkaloids in, certain Apocynaceous drugs.

Drug sample No.*	Extracts							
	Petroleum ether		Chloroform		Ethanol		Ethanol 70%	
	Weight g	Alk-aloid	Weight g	Alk-aloid	Weight g	Alk-aloid	Weight g	Alk-aloid
1	0.74	-	0.21	+	0.35	+	-	-
2a	0.12	-	0.46	-	2.11	-	0.95	-
2b	0.23	-	0.56	-	1.56	-	1.24	-
3a	0.43	-	0.77	-	2.43	-	1.36	-
4a	0.37	-	0.85	-	1.89	-	1.43	-
4b	0.48	-	0.67	-	2.45	+	1.38	+
5a	0.16	-	0.49	-	1.17	-	1.66	-
5b	0.27	+	0.41	+	1.54	+	-	-
6	0.35	-	0.98	+	1.87	+	0.88	+
7	0.61	+	1.39	+	3.22	+	-	-
8	0.46	-	0.84	-	1.67	+	-	-
9	0.82	-	0.41	-	1.85	+	-	-
10	0.76	-	0.61	-	1.98	+	-	-

(*see page 200 for drug sources)

Tabernaemontana angolensis.

The dried ethanolic extract of T. angolensis was extracted with hydrochloric acid (1%, 4 x 25ml), the acidic aqueous phase being filtered free of undissolved non-alkaloidal material. The aqueous extract was basified (pH 8.0) with strong solution of ammonia and extracted with chloroform. The alkaloid which remained unextracted in the aqueous phase was extracted with n-butanol. The chloroform and butanol extracts were chromatographed on alumina plates using the solvent ethanol/chloroform (2:98) (Table XXIV, p. 203).

Thin-layer chromatography.

Extracts from samples 1, 4b, 5b, 6, 7, 8, 9 and 10, showing positive alkaloidal tests, were selected for thin-layer chromatographic examination using Alumina G plates (prepared by the method on p.116) with solvents ethanol/chloroform (2:98 and 8:92). The plates were sprayed with modified Dragendorff's reagent. The results are given in Table XXIV, p.203 .

Table XXIV

Thin-layer chromatography of extracts from samples 1, 4b, 5b, 6, 7, 8, 9 and 10.

Drug Sample No.*	Extract	R_f values with solvent ethanol in chloroform		Relative intensities on chromatograms
		2%	8%	
1	chloroform	0.00 0.10	0.00 0.09	+ ++
	ethanol	0.00 0.05 0.33 0.66 0.77	 0.00 0.07 0.72 0.78	++++ ++ ++ + ++ ++
4b	ethanol	0.00	0.00 0.33 0.47 0.64 0.73 0.80	++ + + +++ +++ ++
5b	petroleum ether	0.00 0.04		+ ++
	chloroform	0.04	0.60	+++
	ethanol	0.00	 0.04 0.76	+++ + +
6	ethanol		0.00 0.70 0.79	++++ +++ ++
7	petroleum ether	0.00 0.25 0.59 0.76	0.00 0.32 0.56	+++ + ++++ + + +

(*see page 200 for drug sources)

Table XXIV (Contd.)

Drug Sample No.*	Extract	R_f values with solvent ethanol in chloroform		Relative intensities on chromatograms
		2%	8%	
7	chloroform	0.00 0.10 0.25	0.00 0.10 0.60 0.68	+++ +++++ +++ +++ +++
8	ethanol	0.00	0.00 0.10	+++ +
9	ethanol	0.00 0.27 0.36 0.51 0.63		++ + +++ ++++ +++
	chloroform (ex.ethanol)	0.00 0.38 0.59		+ +++ ++++
	butanol (ex.ethanol)	0.00		+++
10	ethanol	0.05 0.20 0.32 0.47		+++++ +++ + ++++

DIPLORRHYNCHUS CONDYLOCARPON Pichon

Isolation of alkaloids.

The finely powdered stem bark of Diplorrhynchus condylocarpon Pichon, (3kg), moistened with dilute solution of ammonia (10%, 350mℓ), was macerated in benzene (7ℓ) for 24 hours. The drug was percolated with benzene until the percolate (20ℓ) was negative to Meyer's reagent. The percolate was reduced to small volume (250mℓ), at 50°, ethanol (100mℓ) was added and the mixture extracted with hydrochloric acid solution (1%, 12 x 200mℓ). The acidic solutions were washed with chloroform (50mℓ), bulked, basified (pH 8.0) with strong solution of ammonia and extracted with chloroform (8 x 150mℓ). The chloroform extracts, washed with water (100mℓ), bulked and dried (Na_2SO_4), were evaporated to give a dark-brown resinous material (35g). To this residue, dissolved in ethanol (120mℓ), was added ether (80mℓ) until precipitation of a brown amorphous material was complete. The precipitate (9.5g) was collected, washed with ether and dried; the material, dissolved in hydrochloric acid (5%), gave a negative test with Meyer's reagent and was discarded. The filtrate was evaporated, at 50°, to yield a pale-brown powder (24g), designated fraction I.

Adsorption chromatography of fraction I

Fraction I (10g), dissolved in benzene (20mℓ), was chromatographed on an alumina column (B.D.H., 55 x 2cm). The column was eluted with benzene (450mℓ), ether (200mℓ), ether/ethanol (99:1, 400mℓ) and finally

ethanol (300mℓ). Fractions (35 x 10mℓ) were collected and examined by paper chromatography using the solvent n-butanol/glacial acetic acid/water (63:10:27), with upward development, for 14 hours at 22°C, and the sequential fractions, showing the presence of the same bases, were combined as shown in Table XXV below.

Table XXV

Distribution of alkaloids in eluate fractions from an alumina column.

Fractions	Combined eluate fractions	Weight g	R_f values of bases	Relative intensities on chromatograms
	0 - 10	0.28	-	
A	11 - 35	0.45	0.90	++
B	51 - 65	0.32	-	
C	75 - 105	1.57	0.95 0.90 0.85	++++ ++ +
D	112- 135	4.33	0.90 0.86	++ ++

NORMACUSINE B (TOMBOZINE)

Isolation from fraction C.

Fraction C (1.57g), a pale brown amorphous powder, was recrystallised twice from warm methanol to yield colourless needles (0.26g), m.p. 273-274° (Lit.[7], m.p. 270-272°), $[\alpha]_D^{25}$ + 35.4° (c, 1.059 in methanol); $\lambda_{max.}^{EtOH}$ 225mμ (log ε 4.56), 272(sh.)(3.81), 280 (3.85), 290(3.76), $\lambda_{min.}^{EtOH}$ 247(3.33), 288(3.75)(Figure XIII, p. 199). The infrared spectrum was identical with that published[7]. The compound gave a yellow colour with concentrated nitric acid[7] and an olive-green colour turning purple with Froehde's reagent. The compound, when examined by paper chromatography using radial development at 22° with ether/phthalate buffer (pH 4.0) as solvent on buffer-saturated Whatman No.1 paper, gave a single spot of R_f value 0.43.

Analysis, found: C,73.8, H,7.79, N,8.55.

Calculated for $C_{19}H_{22}ON_2.CH_3OH$: C,73.6, H,8.03, N,8.59%.

Normacusine B hydrochloride.

Normacusine B (0.032g) in ethanol (1mℓ) was treated with ethanolic hydrochloric acid (0.1mℓ, 1.23N) and the hydrochloride precipitated with ether. The colourless precipitate was washed twice with ether to give normacusine B hydrochloride (0.024g) which could not be crystallised.

Normacusine B picrate.

Amorphous normacusine B hydrochloride (0.024g) was dissolved

in water (1mℓ) and a saturated solution of picric acid was added until precipitation was complete. The yellow precipitate was collected, washed with water, and recrystallised twice from warm methanol to yield normacusine B picrate, yellow prisms (0.02g), m.p. 238-240° (decomp.), (Lit.[30], m.p. 239-245° (decomp.)).

Analysis, found: C,58.10, H,4.7, N,13.1.

$C_{25}H_{25}O_8N_5$ requires: C,57.35, H,4.8, N,13.4%.

CONDYLOCARPINE

Isolation from fraction A.

Fraction A (0.45g), a brown amorphous powder, was recrystallised, three times, from ether to give colourless prisms (0.065g), m.p. 158-160° (decomp.) (Lit.[7], m.p. 159-162°), $[\alpha]_D^{26}$ + 875° (c, 0.21 in chloroform); $\lambda_{max.}^{EtOH}$ 228mμ (log ε 4.08), 298(4.03), 330(4.18), $\lambda_{min.}^{EtOH}$ 260(3.16), 299(3.99), (Figure XIII, p. 199). The compound, when treated with concentrated nitric acid, gave a greenish-yellow colour turning yellow[7].

Analysis, found: C,74.3, H,6.6, N,8.6.

Calculated for $C_{20}H_{22}O_2N_2$: C,74.5, H,6.9, N,8.7%.

Ethanolic extract II.

A further batch of finely powdered bark (2.5Kg), macerated with ethanol 70% for 24 hours, was percolated with ethanol (70%) to exhaustion (negative to Meyer's reagent). The percolate (50ℓ) was concentrated, at 50°, to a dark brown aqueous extract (2ℓ). The aqueous extract (2ℓ) was extracted with ethyl acetate (4 x 250mℓ) and each extract washed with water (200mℓ). The combined ethyl acetate solution was extracted with acetic acid (5%, 5 x 250mℓ); the acidic extracts were washed with ethyl acetate (100mℓ), combined, and strong solution of ammonia (50mℓ) added to pH 7.0 and the precipitated bases extracted with ethyl acetate (3 x 250mℓ), each extract being washed with water (100mℓ). The combined ethyl acetate extract, dried (Na_2SO_4), was evaporated to dryness, at 50°, to give a dark brown residue IIA (1.06g) (Figure XIV, p. 212) containing strong bases. On crystallisation, from warm methanol, fraction IIA yielded colourless prisms m.p. 242-243°. When chromatographed by radial development, on Whatman No.1 paper (24cm) saturated with phthalate buffer (pH 4.0) using ether/phthalate buffer as solvent, the compound showed the presence of one spot R_f 0.42. The infrared spectrum was identical with that of mossambine isolated from fraction IIC, p. 213.

The ethyl acetate solution, after the acetic acid extraction, still contained alkaloidal material (positive with Meyer's reagent). The ethyl acetate solution was extracted with hydrochloric acid (5%, 4 x 200mℓ); the acidic extract basified with strong solution of ammonia (160mℓ) was extracted with ether (3 x 150mℓ). The combined, dried

(Na_2SO_4) ether extract was evaporated, at 50°, to a brown resinous residue (0.3g), fraction IIB.

The original aqueous solution (pH 7.0) still contained alkaloids, positive to Meyer's reagent, which were extracted by adjusting to pH 9.0 with sodium carbonate and treating with ethyl acetate (5 x 250mℓ), the ethyl acetate extracts being washed with water (50mℓ). The combined ethyl acetate solution was extracted with acetic acid (5%, 4 x 200mℓ). The combined acidic extract, basified (pH 9.0) with strong solution of ammonia (40mℓ) was extracted with ether (3 x 250mℓ), the ether extracts being washed with water (50mℓ). The dried (Na_2SO_4) ether solution was evaporated to dryness, at 50°, giving brownish-yellow powder (2.03g), fraction IIC.

Paper chromatography of fractions IIA, IIB and IIC.

These fractions were examined by paper chromatography, using the method described on p. 210. The papers were dried and sprayed with modified Dragendorff's reagent; the results are given in Table XXVI, p. 212.

Figure XIV

Fractionation of ethanolic extract II.

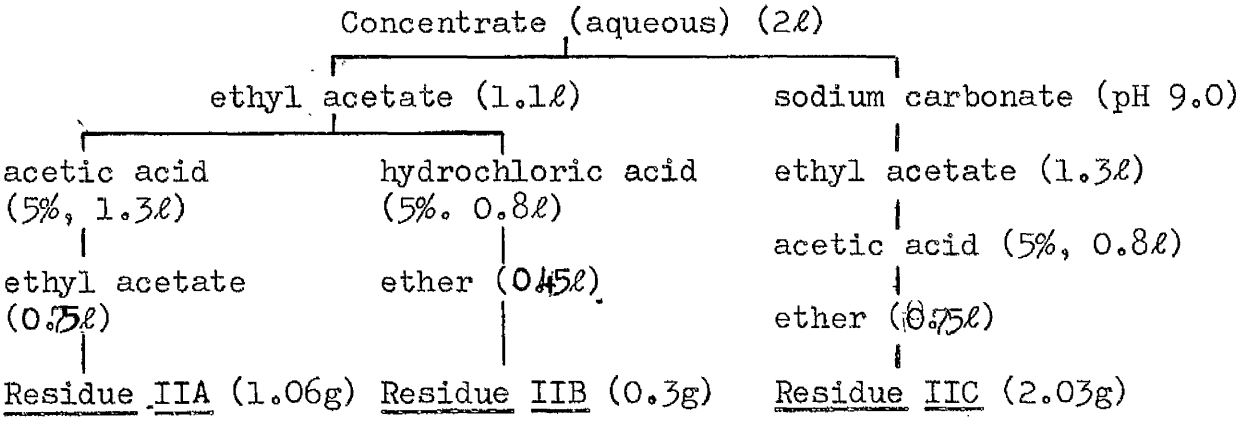


Table XXVI

Distribution of alkaloids in fractions IIA, IIB and IIC.

Fraction	R_f values of bases	Relative intensities on chromatograms
IIA	0.43 0.59 0.62	++ +++ ++++
IIB	0.17 0.59	+ ++
IIC	0.19 0.41 0.68	+ ++++ ++

MOSSAMBINE

Isolation from fraction IIC

Fraction IIC, a pale brown amorphous powder (2.03g), was recrystallised, three times from methanol, to yield colourless prisms (0.095g), m.p. 242° (Lit.[7], 238-242°), $[\alpha]_D^{22}$ -467° (c, 0.26 in chloroform); $\lambda_{max.}^{EtOH}$ 229mμ (log ε 4.05), 301(4.02), 330(4.18), $\lambda_{min.}^{EtOH}$ 261 (3.09), 307(4.01)(Figure XIII, p. 199). The infrared spectrum showed bands at 3416 (OH), 2937 (NH) and 1671 cm^{-1} (carbonyl function of $COOCH_3$). With concentrated nitric acid the compound gave an intense blue colour rapidly turning a deep green[7]. To the compound, dissolved in sulphuric acid (80%), was added a crystal of potassium dichromate; a green colour developed turning to pale purple. The compound gave no reaction with Froehde's reagent.

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S U M M A R Y

S U M M A R Y

Part I of this work presents a review of the tertiary and quaternary alkaloids of the genus Fagara, family Rutaceae, comprising a detailed account of the physical data of the alkaloids and a survey of the pharmacological actions of these and related alkaloids. Methods are discussed for the extraction, separation and purification of tertiary and quaternary alkaloids, with particular reference to the screening for alkaloids in plant material.

The alkaloidal compositions of three fractions obtained from the stem bark of Fagara rhoifolia Lam. were studied; fractions I and II were shown to contain similar quaternary alkaloids whilst fraction III was shown to consist of a mixture of one quaternary and five tertiary alkaloids. The two major quaternary alkaloids of fractions I and II were isolated and shown to be 5-hydroxy-2,3,6-trimethoxy-N,N-dimethyl-aporphinium chloride and α-(-)-methylcanadine chloride. The remaining minor quaternary alkaloids were not isolated as pure compounds. α-(-)-Methylcanadine chloride was identified as the major alkaloid, and the only quaternary base, of fraction III which also contained α-allocryptopine, as the principal tertiary base, together with other minor uncharacterised, tertiary alkaloids.

The stem and root barks of four African Fagara species, Fagara leprieurii Engl., F. macrophylla Engl., F. viridis A. Chev. and F. xanthoxyloides Lam., were examined for the presence of tertiary and

quaternary bases. A suitable method was devised for the separation of these two groups of bases. The mixtures of tertiary and quaternary alkaloids of the four African Fagara species were compared by means of suitable thin-layer chromatographic systems. The results showed sufficient variation in the constituent alkaloids to differentiate the four species, but the similarities showed that the four had a close chemical relationship. Although several authors have reported tertiary alkaloids in these African Fagara species, the present work is the first to report the presence of quaternary alkaloids.

Part II comprises the results of an alkaloidal screening of some Apocynaceous plant materials and a review of the alkaloidal constituents previously reported in other species of the genera involved in this survey. The presence of alkaloids was demonstrated in extracts from plant material of Ambelania acida Aubl., Diplorrhynchus condylocarpon Pichon, Mandevilla hirsuta Malme, Ochrosia elliptica Labill, Odontadenia nitida Muell., Prestonia quinquangularis Spreng, Tabernaemontana angolensis Stapf and T. pachysiphon Stapf. This is the first report of alkaloidal material in Ambelania acida, Mandevilla hirsuta, Odontadenia nitida and Tabernaemontana angolensis, though alkaloids have previously been reported in the genera Macoubea (in which Pichon includes the genus Ambelania), Mandevilla, Odontadenia and Tabernaemontana. Extracts from Cameraria belizensis Standley and Carissa edulis Vahl were devoid of alkaloid.

The alkaloids of the stem bark of Diplorrhynchus condylocarpon Pichon were isolated and three pure alkaloids were identified as the known bases normacusine B, condylocarpine and mossambine.

S U M M A R Y

Part I of this work presents a review of the tertiary and quaternary alkaloids of the genus Fagara, family Rutaceae, comprising a detailed account of the physical data of the alkaloids and a survey of the pharmacological actions of these and related alkaloids. Methods are discussed for the extraction, separation and purification of tertiary and quaternary alkaloids, with particular reference to the screening for alkaloids in plant material.

The alkaloidal compositions of three fractions obtained from the bark of Fagara rhoifolia Lam. were studied; fractions I and II were shown to contain similar quaternary alkaloids whilst fraction III was shown to consist of a mixture of one quaternary and five tertiary alkaloids. The two major quaternary alkaloids of fractions I and II were isolated and shown to be 5-hydroxy-2,3,6-trimethoxy-N,N-dimethyl-aporphinium chloride and α-(-)-methylcanadine chloride. The remaining minor quaternary alkaloids were not isolated as pure compounds. α-(-)-Methylcanadine chloride was identified as the major alkaloid, and the only quaternary base, of fraction III which also contained α-allocryptopine, as the principal tertiary base, together with other minor, uncharacterised, tertiary alkaloids.

The stem and root barks of four African Fagara species, Fagara leprieurii Engl., F. macrophylla Engl., F. viridis A. Chev. and

F. xanthoxyloides Lam., were examined for the presence of tertiary and quaternary bases. A suitable method was devised for the separation of these two groups of bases. The mixtures of tertiary and quaternary alkaloids of the four African Fagara species were compared by means of suitable thin-layer chromatographic systems. The results showed sufficient variation in the constituent alkaloids to differentiate the four species, but the similarities showed that the four had a close chemical relationship. Although several authors have reported tertiary alkaloids in these African Fagara species, the present work is the first to report the presence of quaternary alkaloids.

Part II comprises the results of an alkaloidal screening of some Apocynaceous plant materials and a review of the alkaloidal constituents previously reported in other species of the genera involved in this survey. The presence of alkaloids was demonstrated in extracts from plant material of Ambelania acida Aubl., Diplorrhynchus condylocarpon Pichon, Mandevilla hirsuta Malme, Ochrosia elliptica Labill, Odontadenia nitida Muell., Prestonia quinquangularis Spreng, Tabernaemontana angolensis Stapf and T. pachysiphon Stapf. This is the first report of alkaloidal material in Ambelania acida, Mandevilla hirsuta, Odontadenia nitida and Tabernaemontana angolensis, though alkaloids have previously been reported in the genera Macoubea (in which Pichon includes the genus Ambelania), Mandevilla, Odontadenia and Tabernaemontana. Extracts from Cameraria belizensis Standley and Carissa edulis Vahl were devoid of alkaloid.

The alkaloids of the stem bark of Diplorrhynchus condylocarpon Pichon were isolated and three pure alkaloids were identified as the known bases normacusine B, condylocarpine and mossambine.