PHYTOCHEMICAL STUDIES ON SOME SPECIES OF THE LEGUMINOSAE

A thesis submitted for the degree of Doctor of Philosophy of the University of Strathclyde

by

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SUMMARY

Five species from four genera of the sub-families Caesalpinioideae and Papilionoideae of the Leguminosae have been investigated.

From the root bark of Cassia sieberiana (-)-epiafzelechin lupeol and β-sitosterol were isolated. (-)-Epiafzelechin was identified by spectral analysis with particular use of ^{1}H NMR in resolving stereochemistry of the C-ring. Lupeol and β-sitosterol were identified by direct comparison with authentic samples.

The stem bark of Cynometra hankei yielded four imidazole alkaloids, two of which were novel. The major alkaloids were identified as cynometrine and N_1-demethylcynometrine (novel) by interpretation of ^{1}H NMR, ^{13}C NMR and mass spectral data and by the conversion of N_1-demethylcynometrine to cynometrine. One of the minor alkaloids was identified as cynodine (cynometrine-11-benzoate); this being confirmed by its saponification to cynometrine. The fourth alkaloid, isolated in very small amounts, was also novel. Its identification as N_1-demethylcynodine was confirmed by its conversion to cynodine. The seeds of this species gave the first three mentioned alkaloids. A similar examination of the stem bark of Cynometra manii did not reveal any alkaloids.

The seeds of Camoensia brevicalyx yielded five quinolizidine/indolizidine alkaloids including the known compounds camoensine and camoensidine. ^{13}C NMR spectra of these alkaloids were interpreted and proved valuable in assigning H-9-H-11 stereochemistry. The remaining three alkaloids were novel. The major compound was identified as 12-α-hydroxycamoensine on the basis of detailed interpretation of ^{1}H NMR, ^{13}C NMR and mass spectral data.

^{13}C NMR was of particular value in assigning the position of the hydroxyl substituent and ^{1}H NMR in deciding its stereochemistry. A very minor component was tentatively identified as 12-hydroxycamoensidine. The fifth alkaloid appeared to be 12-hydroxy-16-methoxy-11:12,13:14-tetradehydrocamoensine, a hitherto unrecorded type of quinolizidine/indolizidine alkaloid. The biological significance of these alkaloids was briefly discussed.

The seeds of Sesbania macrocarpa yielded a single flavonoid glycoside. Hydrolysis of the glycoside gave a flavone and a sugar, identified as kaempferol and rhamnose respectively. On the basis of the UV spectra of the glycoside and the aglycone with a range of different reagents the glycoside was identified as kaempferol-3,7-dirhamnoside.

DEDICATION

This thesis is dedicated to my wife Frances and children Selwyn, Folasade and especially Temitayo.

INTRODUCTION

1:1 TAXONOMY OF THE LEGUMINOSAE

The Leguminosae Juss. is a plant family of world wide distribution [1] consisting of trees, shrubs and herbs. They constitute one of the largest of plant families, exceeded in number of species only by the Compositae and Orchidaceae [2]. Whether the Leguminosae is regarded as an order or a family has been a controversial point among taxonomists since early plant classification. According to Bentham [3] the Leguminosae is a family consisting of three sub-families:-

(i) Caesalpiniaceae R.Br.; Flinders, Voy. Terra Austr. 2:551 (1814)

(ii) Mimosaceae R.Br.; Flinders, Voy. Terra Austr. 2:551 (1814)

(iii) Papilionaceae Ciseke, Praelect Ord. Nat. Pl. 415 (1792)

This classification is supported by Taubert in the Pflazenfamilien[4] (Table 1) but with a different sequence for the families and tribes, with the latter also showing a variation in content.

The Bentham and Taubert approach has been accepted by most authors. Melchoir's version of Taubert's system (in A. Engler's Syllabus der Pflazenfamilien[5]) shows some differences in the definition of tribes (Table 1). According to Melchoir [5] the Leguminosae is a family with three sub-families, a view that is shared by Cronquist [6] who places the Leguminosae in the order Rosales.

Recent classifications have not resolved the question of

whether the Caesalpiniaceae, Mimosaceae and Papilionaceae should be regarded as families or as sub-families of the Leguminosae. Both Takhtajan[7] and Dahlgren[8] regard the three as separate families belonging to the order Fabales. Thorne[9], on the other hand, regards them as sub-families of the Leguminosae which is, in turn, part of the order Rosales. Some of the various suggestions that have been made for the classification of the Leguminosae are shown in Table 2.

According to Heywood[10] the three sub-families share few morphological diagnostic characters in common. The principal ones are :

(i) monocarpellary of the gynoecium with parietal placentation which develops into a pod or legume dehiscing by dorsal suture

(ii) the androecium of diplostemonous origin but showing variations

(iii) the more or less cup shaped receptacle.

All three sub-families are world-wide in distribution. The Caesalpiniaceae and Mimosaceae are mainly tropical and sub-tropical while the Papilionaceae, regarded as the most advanced of the three, occurs in temperate regions in larger numbers[10]. The main morphological characteristics of the three families are:-

CAESALPINIACEAE:-

(i) Trees, shrubs or rarely herbs and scramblers

(ii) leaves pinnate or bipinnate rarely simple or 1-foliolate

(iii) stipules paired, mostly caducous

(iv) stipels mostly absent

(v) flowers showy, racemose, spicate or rarely cymose; zygomorphic, rarely subactinomorphic

(vi) sepals 5 or 4 by the union of 2, free or partly united, imbricate or rarely valvate, often much reduced when the bracteoles are large and calyx-like covering the bud

(vii) petals 5 or fewer or absent, the adaxial (upper) one inside, the others variously imbricate

(viii) stamens 10 or fewer often free or variously connate

(ix) anthers various sometimes opening by terminal pores

(x) ovary superior 1-locular

(xi) fruit a legume but mostly indehiscent, often winged

(xii) seeds with copious, thin, or no endosperm and large embryo.

MIMOSACEAE :-

(i) trees or shrubs and rarely herbs

(ii) leaves bipinnate or rarely simply pinnate

(iii) calyx is tubular valvate or rarely, as in the Parkieae, imbricate 5-lobed or toothed

(iv) petals valvate free or connate into a short tube, mostly hypogynous

(v) the stamens same in number as the sepals, or more numerous or indefinite, free or monadelphous

(vi) anthers small 2-locular, opening lengthwise, often with a deciduous gland at the apex

(vii) ovary superior

(viii) fruit a legume or indehiscent with scanty or no endosperm.

PAPILIONACEAE :-

(i) trees or shrubs or often herbs

(ii) flowers zygomorphic, mostly hermaphrodite

(iii) sepals usually 5, more or less connate into a tube

(iv) petals 5, imbricate, free, the upper exterior and forming the standard, the two lateral (wings) more or less parallel with each other; the lower two interior and connate by the lower margins into a keel

(v) stamens inserted with the petals, often 10, monadelphous or diadelphous, mostly all perfect

(vi) anthers mostly opening, all perfect

(vii) fruit usually a legume or indehiscent, sometimes jointed and breaking up into 1-seeded segments, seed with or without very scanty endosperm.

TABLE 1 EARLY CLASSIFICATIONS OF THE LEGUMINOSAE; BENTHAM [3](A), TAUBERT [4](B), AND MELCHOIR [5](C)

	A		B		C
	PAPILIONACEAE		MIMOSOIDEAE		MIMOSOIDEAE
1	Podalyrieae	1	Igneae		Igneae
2	Genisteae	2	Acacieae		Mimoseae
3	Trifolieae	3	Eumimoseae		Adenanthereae
4	Loteae	4	Adenanthereae		Mimozygantheae
5	Galegeae	5	Piptandenieae		Parkieae
6	Hedysareae	6	Parkieae		
7	Vicieae				FABOIDEAE
8	Phaseoleae		CAESALPINIOIDEAE		Sophoreae
9	Dalbergieae	1	Dimorphandreae		Podalyrieae
10	Sophoreae	2	Cynometreae		Dalbergieae
11	Swartzieae	3	Amherstieae		Genisteae
		4	Bauhinieae		Astragaleae
	CAESALPINIACEAE	5	Cassieae		Loteae
12	Sclerobieae	6	Kramperieae		Coronilleae
13	Eucaesalpinieae	7	Eucaesalpinieae		Ononideae
14	Cassieae	8	Sclerobieae		Trifolieae
15	Bauhinieae	9	Swartzieae		Fabeae
16	Amherstieae				Phaseoleae
17	Cynometreae		PAPILIONATE		
18	Dimorphandreae	1	Sophoreae		CAESALPINIOIDEAE
		2	Podalyrieae		Dimorphandreae
	MIMOSACEAE	3	Genisteae		Caesalpinieae
19	Parkieae	4	Trifolieae		*Amherstieae
20	Adenanthereae	5	Loteae		Cassieae
21	Eumimoseae	6	Galegeae		Swartzieae
22	Acacieae	7	Hedysareae		
23	Igneae	8	Dalbergieae		
		9	Vicieae		*(Including Cynometreae
		10	Phaseoleae		and Sclerobieae)

TABLE 2 COMPARATIVE CLASSIFICATIONS OF THE LEGUMINOSAE

AUTHOR	ORDER	CLOSEST ORDERS OR FAMILIES
Engler [5]	Rosales	Chrysobalanaceae
		Connaraceae
		Krameriaceae
		Hystachyaceae
Bentham [3]	Rosales	Myratales
		Passiflorales
		Sapindales
		Celastrales
Hutchinson [2]	Leguminales	Corrariales
		Rosales
		Cunoniales
		Styracales
Takhtajan [7]	Fabales	Rosaceae
		Chrysobalanaceae
		Neuradaceae
		Droseraceae
		Nepenthaceae
Cronquist [6]	Rosales	Neuradaceae
		Chrysobalanaceae
		Podostemaceae
		Haloragaceae
		Gunneraceae
Dahlgren [8]	Fabales	Sapindales
		Rosales
		Proteales
Thorne [9]	Rosales	Rosaceae
		Crossosmataceae
		Connaraceae
		Crassulaceae
		Cephalotaceae

1:2 A BRIEF REVIEW OF THE SECONDARY METABOLITES OF THE LEGUMINOSAE

Many types of secondary metabolites have been isolated from the Leguminosae, reflecting not only the size of the family but also the diversity in chemical structures it is able to biosynthesise. Examples of the various classes of compounds discussed below are shown in Table 3.

ALKALOIDS

The most widespread alkaloid types are the quinolizidines[11,12] (I, II), with which are included variants of the matrine type (III), found in *Sophora* species [13], and the alkaloids of *Ormosia* [14] (IV). The similarly derived pyrr olizidine alkaloids (V) seem to be restricted almost exclusively to the genus *Crotolaria* L.[15] Other types are the tyrosine-derived *Erythrina* L. alkaloids [16] (VI), the *Phytostigma* Bal. alkaloids [17] (VII), which are simple derivatives of tryptophan, and histidine-derived imidazoles [18](VIII). The pseudo-alkaloids (IX) of species of *Erythropleum* Afzel [19] are ethanolamine esters of diterpenes.

FLAVONOIDS

These are plant secondary metabolites based on an aromatic heterocyclic phenylbenzopyrone nucleus. In the Leguminosae such compounds are common, the most abundant types being simple flavones[20] (X), chalcones [21] (XI), flavanones [22] (XII), rotenoids [23] (XIII), neo-flavonoids [24] (XIV) and isoflavonoids [25] (XV). The latter three are unique to the subfamily Papilionaceae [10A]. Also predominantly derived from the isoflavonoid precursors are the phytoalexins [26], antimicrobial compounds synthesised in large amounts

in response to invasion of healthy tissue by fungi. These are generally found in highest concentrations around the damaged cells. Two of the best known phytoalexins are pisatin [27] (XVI) and phaseollin [28] (XVII). Another group of cinnamic derived compounds are the furanocoumarins (XVIII) which occur widely among species of Psorela [29].

POLYKETIDES

These are mainly anthracene derivatives, notably anthraquinones [30,31] (XIX,XX), anthrones [32] (XXI), and bianthraquinones [33] (XXII). They are mainly found in species of the genus Cassia L.[10] From the genus Cassia is obtained the economically important senna [34], which specifically refers to drug preparations from dried leaves of Cassia acutifolia Del. and C. angustifolia Vahl. The active ingredients in senna are anthracene derivatives, in particular emodin (XXIII), aloe-emodin (XXIV), rhein (XXV), physcion (XXVI), chrysophanol (XXVII); and sennosides A, B, C and D (XXVIII), which are bianthrone diglycosides [35]. Sennosides B and D are the mesoforms of A and C, respectively.

AMINO ACIDS

Many amino acids have been identified from the Leguminosae. As well as the common protein amino acids [36], a large number of unusual non-protein amino acids have been isolated. The latter are often the most dominant secondary compounds in some parts of a species, particularly the seeds [37]. In extreme cases they can make up in excess of 5% of the dry weight of the seeds. Two of the most well known non-protein amino acids are canavanine [38](XXIX) and lathyrine [39] (XXX).

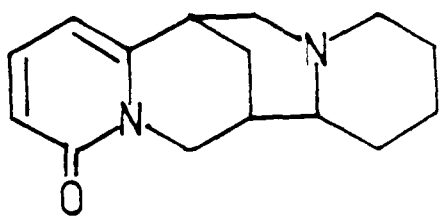
TRITERPENOIDS

All the main categories of triterpenes are found e.g. plant sterols [40] (XXXI), pentacyclic triterpenes [41] (XXXII) and saponins and sapogenins [42] (XXXIII). They include the pharmacologically important triterpenes present in Glycyrrhzia glabra Reg . and Henl. Liquorice [34] refers to the sweet wood of varieties of this species; its activity being due to glycyrrhizinic acid [34] (XXXIV) which has a triterpenoid structure. Its use results from the deoxycorticosterone effects it exhibits and it appears that it may give symptomatic relief from peptic ulcer.[34]

PHYTOHAEMAGLUTTININS

These protein substances are widespread in the seeds of the Leguminosae [106]. They are known to coagulate erythrocytes[43,44] and are used to aid identification of certain human blood groups and in studies on serological distinction between various animal species. Phytohaemagluttinins that act as precipitins of soluble blood group substances are referred to as lectins [45]. Those that are specific and of practical value are Anti-A [46], obtained from Dolichos biflorus L.; Anti-B [47], from Caragana frutex C. Koch.; Anti A+B [48], from Calpurnia aurea E. Mey; Anti-H;[49] from Cytisus sessilifolius L; and Anti-N [50], from Vicia species.

TABLE 3 SOME EXAMPLES OF SECONDARY METABOLITES FOUND IN THE LEGUMINOSAE.



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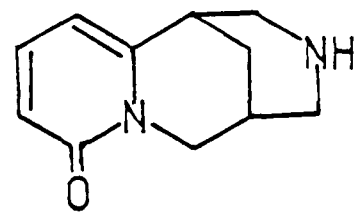

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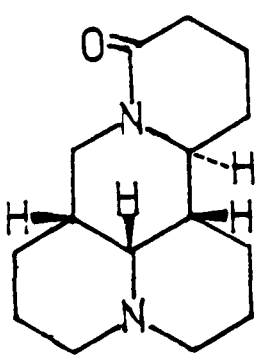

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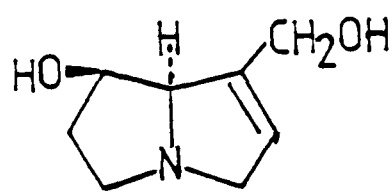

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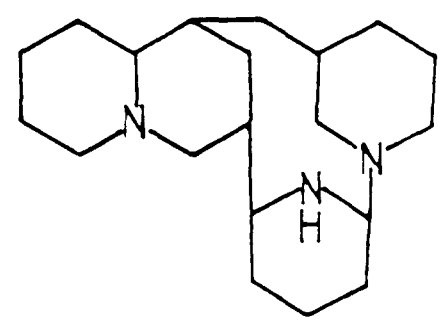

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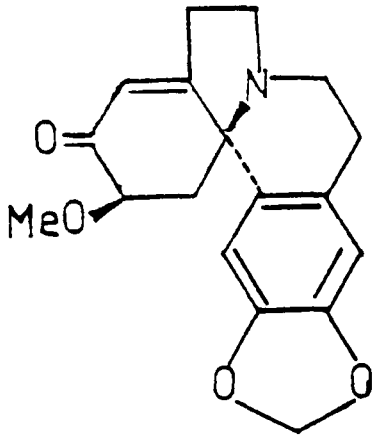

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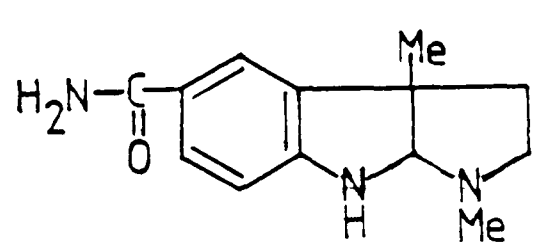

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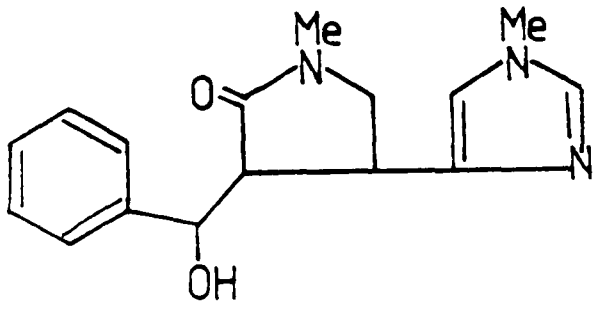

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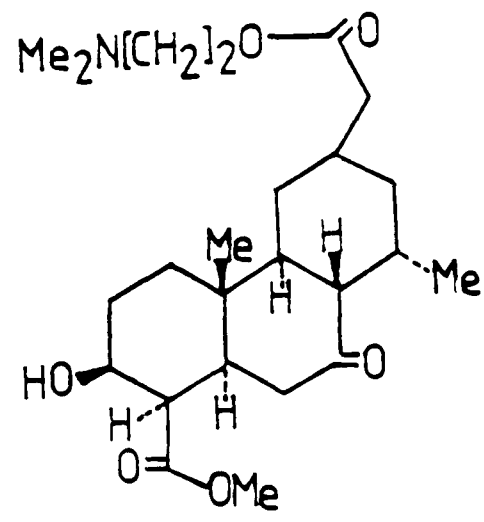

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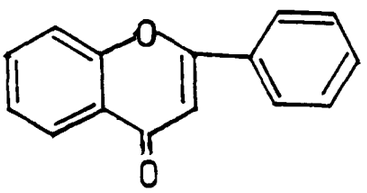

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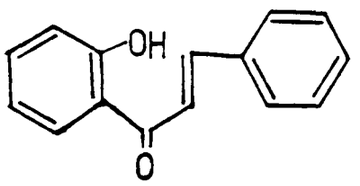

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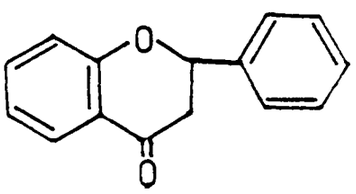

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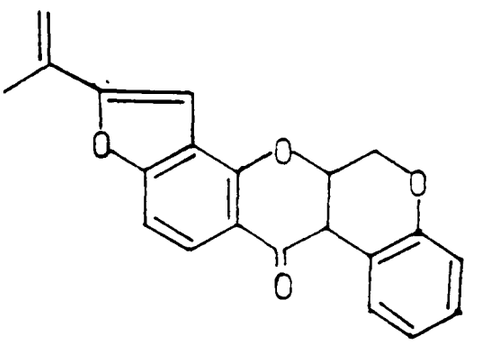

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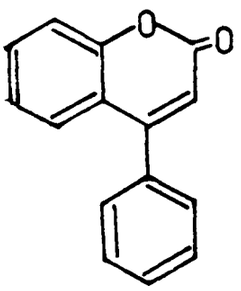

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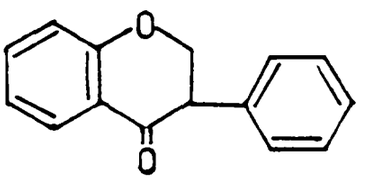

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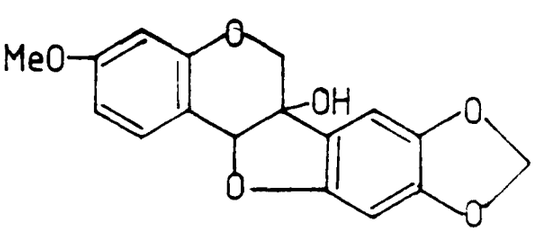

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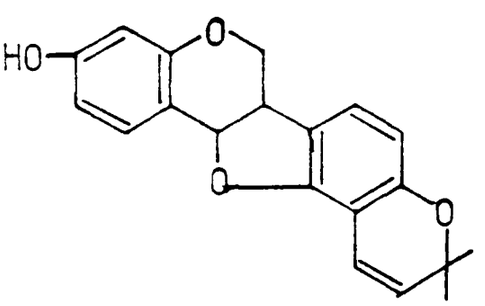

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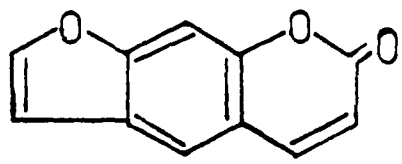

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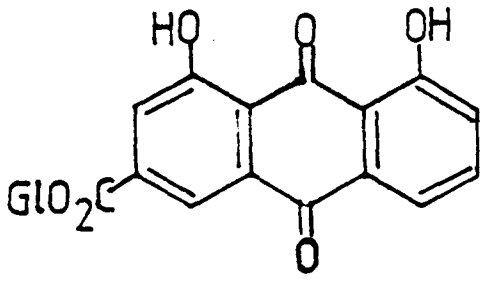

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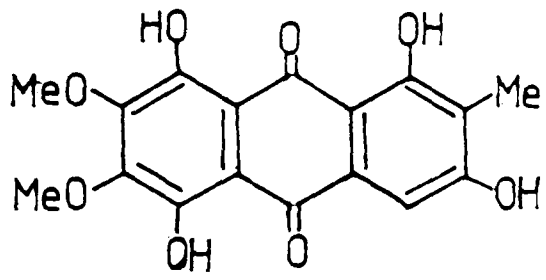

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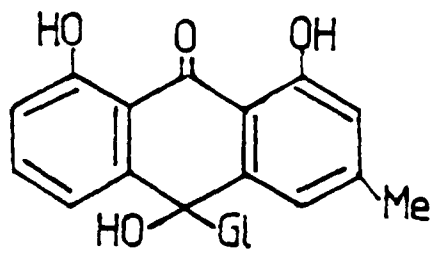

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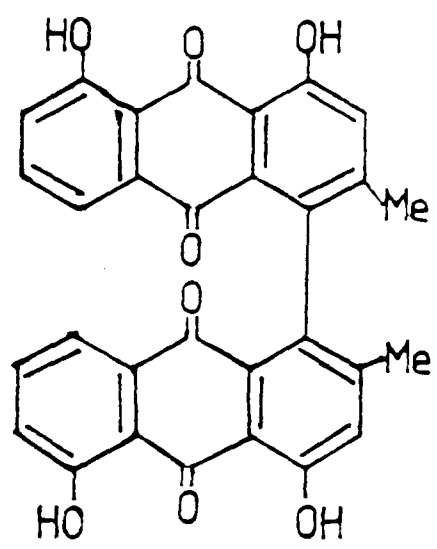

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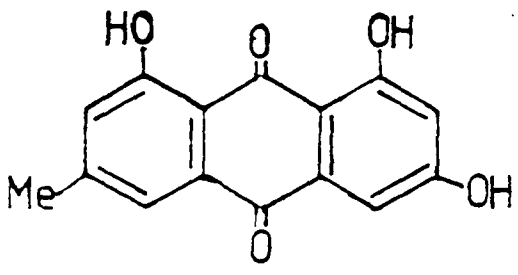

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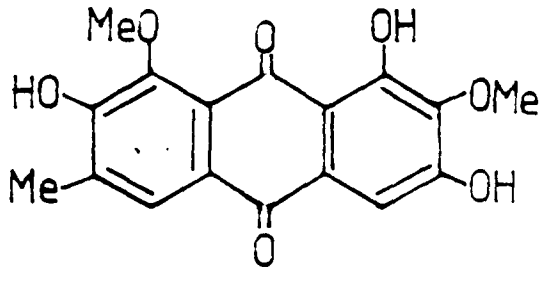

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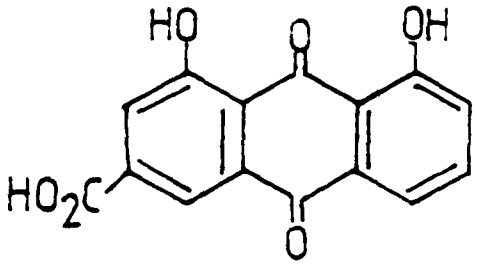

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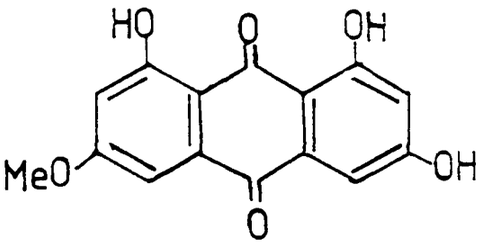

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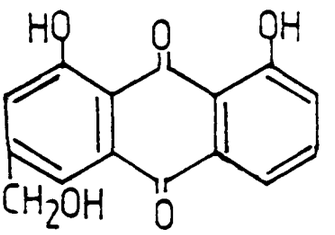

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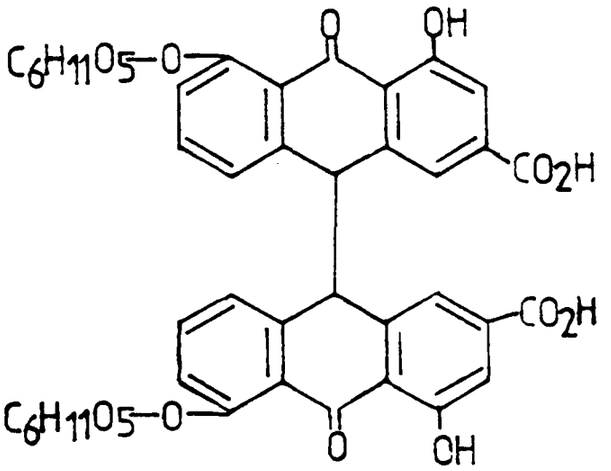
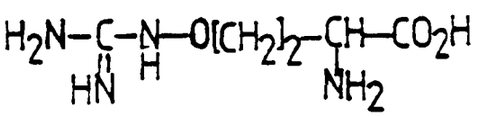
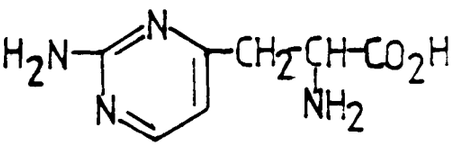


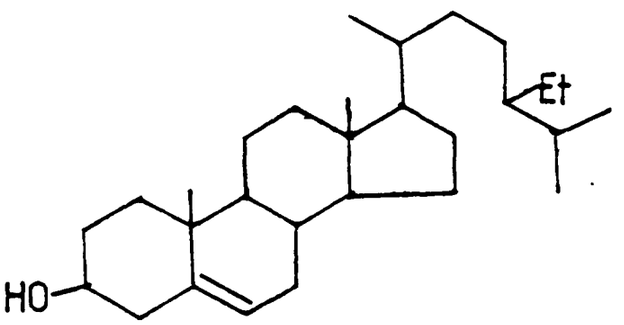
XXVIII



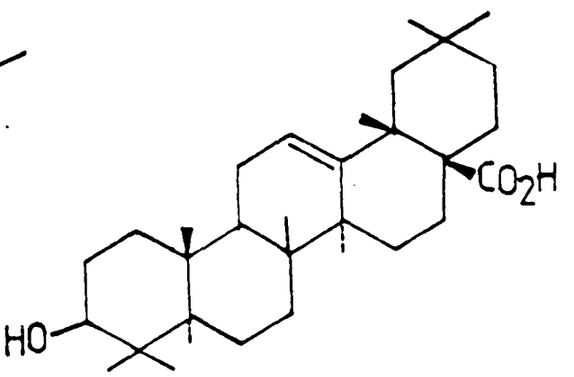
XXIX



XXX



XXXI



XXXII

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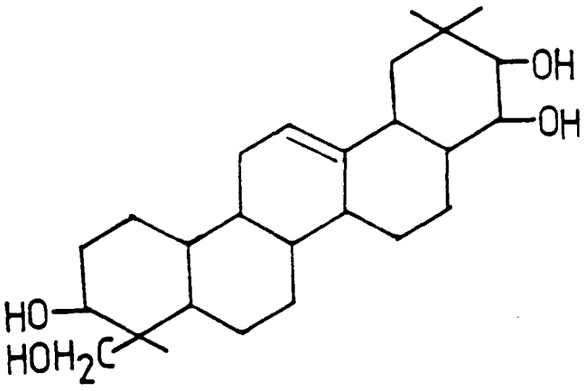
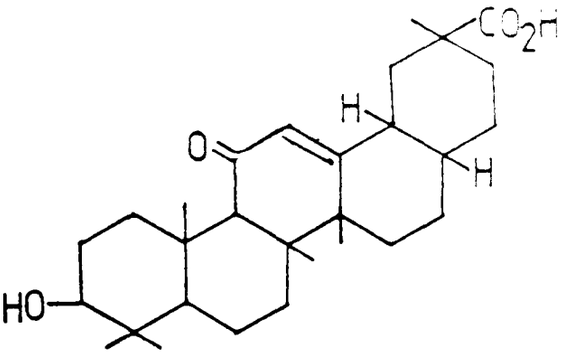
/Table 3 contd.



XXXIII



XXXIV

1:3: TAXONOMY AND CHEMISTRY OF THE GENERA AND SPECIES INVESTIGATED IN THIS STUDY

1:3:1 CASSIA L. F.T.A. 268 Benth. in Trans. Linn. Soc. 27 503 - 591 (1871)

The genus Cassia is normally placed in the tribe Cassiaeae of the sub-family Caesalpiniaceae[1]. It contains about 600 species which occur in tropical and sub-tropical areas of the world[1]. The distinguishing morphological features of the genus are :-

(i) Flowers bisexual, yellow or rarely white or rosy in axillary or terminal racemes

(ii) Petals 5, imbricate, spreading, subequal or lowermost larger and uppermost inside the other

(iii) Stamens 10, fertile or partly abortive

(iv) Anthers uniform or those of lower stamens larger

(v) Ovary sessile or stipitate, free within calyx

(vi) Ovules numerous

(vii) Fruit various in shape and side often 2-valved. Sometimes indehiscent with endosperm.

Several species of Cassia have medicinal uses, notably C. augustifolia (see page 9). The seeds of C. absus are used in India as a cathartic while in South Africa they are used in the treatment of ringworm and eye diseases[1]. The genus has been the subject of numerous chemical studies, and the secondary metabolites isolated are shown in Table 4. The major types of secondary metabolites isolated are flavonoids, sometimes as glycosides, and polyketides. The polyketides include anthraquinones, anthracene derivatives, bi-anthroquinones and sennosides. The latter

bi-anthrone glycosides are believed to be the active ingredients responsible for the laxative effects of decoctions of Cassia species. Also reported from Cassia are a number of alkaloids (LXXIX-LXXXVII), predominantly of the piperidine type. Several common triterpenes have been isolated.

1:3:1:1 CASSIA SIEBERIANA D.C.Prod. ii 489 (1864 - 68)

A tree up to about 15m in height. In Sierra Leone it is found in savannah areas [51]. This species, first recorded by De Candolle [52], is found over a wide area in tropical Africa [1].

The main morphological features are, according to Hutchinson [53]:-

(i) Fruits without a longitudinal septum, the seeds in 1-series, fruits seldom straight, terete, 5 -80 cm long and about 1.5 cm diameter

(ii) Bracts linear-lanceolate, 1-2 cm long pubescent

(iii) Pedicel present

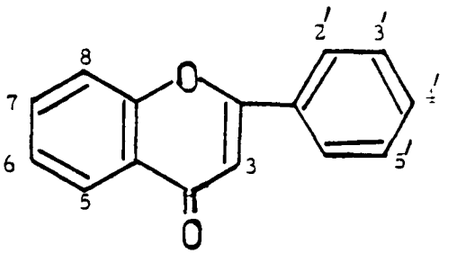
(iv) Leaflets 5-8 pairs, elliptic or oblong,shortly subacute or emarginate at apex, 5-10 cm long, 2.5-5 cm broad

(v) Petiole and rhachis up to 20 cm long.

There have been three reports on secondary compounds of C. sieberiana. Duquenois and Anton [54] examined the leaves and recorded the presence of sitosterol, (XXXI) (also reported in another publication [55]), quercetin (XLIII), rhein (XXV), and glycosides of all three. Paris and Etchpare [56] have isolated leucopelargonidin (LIV) and epicatechin (LIII) from the roots. Decoctions of this plant are known to have diuretic and purgative properties, which are attributed to the phenolics they contain [57].

In one of two reported cases [58] of use in Sierra Leone two adults drank decoctions of the root bark and had acute stomach pains which needed urgent medical attention. In the second case a similar decoction was given to an infant who showed the symptoms of severe stomach pains, became unconscious, and died within 24 hours. The use of the root bark of C. sieberiana resulted from confusion with senna which is widely used in Sierra Leone for its purgative properties.

TABLE 4 SECONDARY METABOLITES OF SPECIES OF THE GENUS CASSIA.



3	5	6	7	8	3'	4'	5'			
	OH		OH			OH		Apigenin	(XXXV)	C. absus Linn.[59] C. siamea Lam.[60]
	OH	Me	ORh	Me		OMe		Matteucinol-7-Rhamnoside	(XXXVI)	C. occidentalis Linn.[61]
OH	OH		OH			OH		Kaempferol	(XXXVII)	C. alata Linn.[83] C. augustifolia Linn.[63] C. fistula Linn.[64] C. marginata Roxb.[30] C. rogeonii Ghesq.[65] C. siamea[60]
OGl	OH		OH			OH		Kaempferol-3-Glucoside	(XXXVIII)	C. fistula[66] C. marginata[30,67]
ONh	OH		OH			OH		Kaempferol-3-neohesperido-side	(XXXIX)	C. fistula[66]
OH	OH		OMe			OH		Rhamnocitrin	(XL)	C. garrettiana Craib[68]
	OH		OH		OH	OH		Luteolin	(XLI)	C. absus[59] C. rogeonii[65]
	OH		OH		OMe	OH		Cassiglucin	(XLII)	C. multijuga Rich.[69]

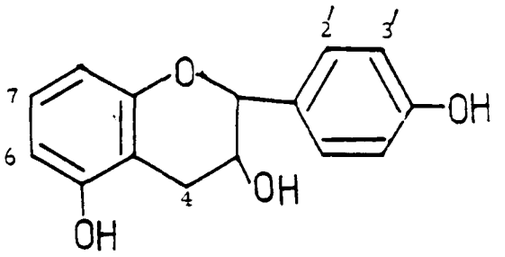
/contd...

Table 4 contd.

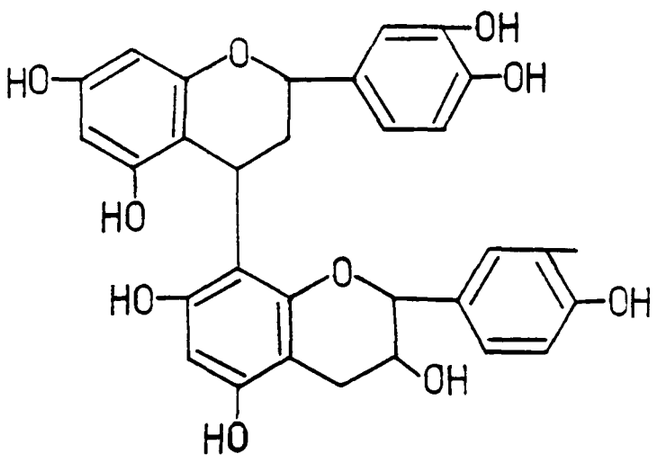
3	5	6	7	8	3'	4'	5'			
OH	OH		OH		OH	OH		Quercetin	(XLIII)	C. absus[70] C. garrettiana[68] C. laevigata Willd[72] C. marginata[30]
OGl	OH		OH		OH	OH		Quercetin - 3-Glucoside	(XLIV)	C. marginata[30]
OGal	OH		OH		OH	OH		Quercetin - 3-Galactoside	(XLV)	C. marginata[30,67]
OH	OH		OMe		OH	OH		Rhamnetin	(XLVI)	C. garrettiana[68]
OGal	OH		OMe		OH	OH		5,3',4'-trihydroxy 7-methoxy-3-Galactoside	(XLVII)	C. sophora Linn.[73]
OGal	OH		OMe		OH	OMe		5,3'-dihydroxy-7, 4'-dimethoxy-3-0-Galactoside	(XLVIII)	C. laevigata[72]
$(OGal)_2$	OH		OMe		OH	OMe		5,3'-dihydroxy-7, 4'-dimethoxy-3-0-digalactoside	(XLIX)	C. laevigata[72]
OMe	OH	OMe	ORh		OMe	OH		Jaceiden-7-Rhamnoside	(L)	C. occidentalis
OMe	OH	OMe	OH		CH	OH		Quercetagetin-3,6-dimethyl ether	(LI)	C. renigera Wall[74]
	ORh	OMe	OMe		OMe	OMe	OMe	5,7,3',4',5'-pentamethoxy-7-Rhamnoside	(LII)	C. renigera[74]

/contd...

/Table 4 contd.

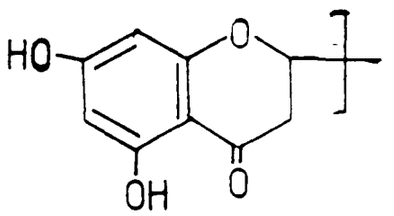


4	7	2′	3′			
	OH		OH	epicatechin	(LIII)	C. sieberiana D.C.[56]
OH	OH		OH	leucopelargonidin	(LIV)	C. sieberiana[56]
OH	OH			goratensidine	(LV)	C. auriculata Linn.
OH		OH		Auriculacacidin	(LVI)	C. auriculata[76]



Auricassidin (LVII) C. auriculata[77]

R =



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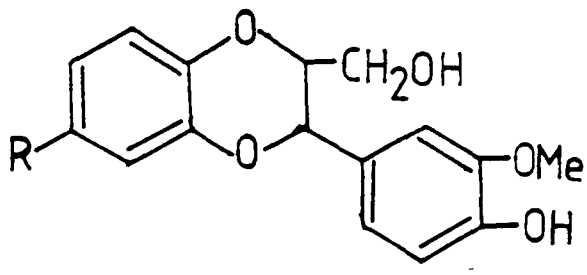
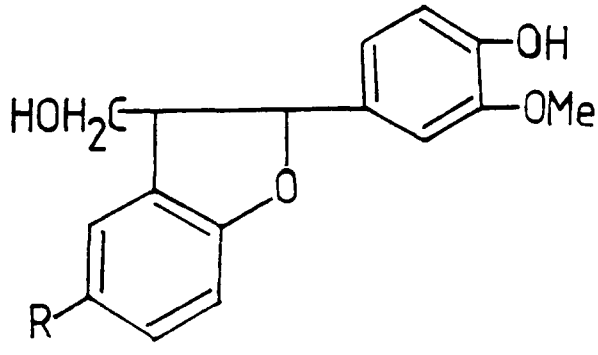
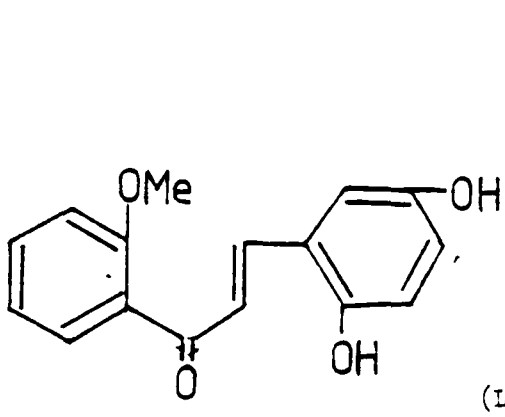


hydnocarpin (LVIIA) C.absus [59]

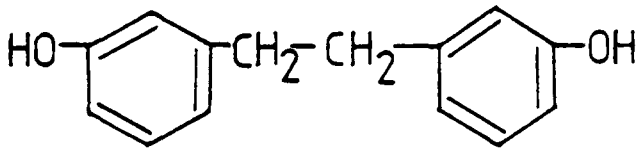


isohydnocarpin (LVIIIB) C.absus [59]



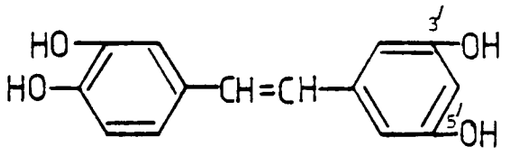
(LIX)

3' 6' dihydroxy 6 methoxy chalcone C.javanica Linn.[98]

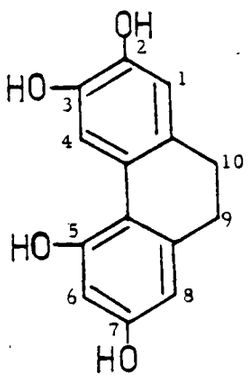


4	5'			
H	H	3 3' dihydroxybibenzyl	(LX)	C. garrettiana [68]
OH	H	3 3' 4 trihydroxybibenzyl	(LXI)	C. garrettiana [68]
OH	OH	procatechuic aldehyde	(LXII)	C. garrettiana [68]

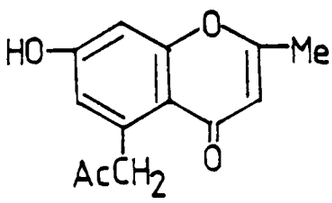
/Table 4 contd..



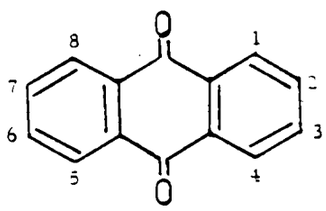
3 4 3′, 5′-tetrahydroxy-Stilbene (LXIII) C. marginata[30]



2,3,5,7-tetrahydroxy-9,10-dihydrophenanthrene (LXIV) C. marginata[30]



2-methyl-5 acetonyl-7-hydroxychromone (LXV) C. siamea[71]



1	2	3	5	6	7	8				
OH	Me					OH	Chrysophanol	(XXVII)	C. absus[70], C. alata[79]	
									C. angolensis Welw.[80]	
									C. marginata[30]	
									C. siamea[81]	
									C. sophera[73]	
OH	COOH					OH	Rhein	(XXV)	C. acutifolia Del.[35]	
									C. alata[32,33]	
									C. podisa Buch.[84]	
									C. siamea[81]	

/contd..

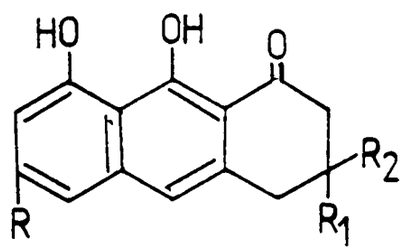
/Table 4 contd.

1	2	3	5	6	7	8			
OH	COOH					OAcGl	Rhein-O-acyl-Glucoside	(LXVI)	C. marginata[67]
OH	COOH					OGl	Rhein-O-Glucoside	(LXVII)	C. acutifolia[35]
OH	COOH					$(OGl)_2$	Rhein-(O-Glucoside)$_2$	(LXVIII)	C. acutifolia[35]
OH	Me	OH				OH	1,3,8-trihydroxy-2-methyl-anthraquinone	(LXIX)	C. spectabilis D.C.[86]
OH	Me		OH			OH	Emodin	(XXIII)	C. alata[79,82,83] C. angolensis[80] C. javanica Linn.[87] C. torosa Cav.[88,93]
OH	Me		OH			OGl	Emodin-8-Rhamnoside	(LXX)	C. javanica[87]
OH		COOGl				OH	1,8-dihydroxy-3-carbo-glucopyranosyloxy-anthraquinone	(XIX)	C. marginata[30]
OH		Me		OMe		OH	Physcion	(XXVI)	C. alata[79,89] C. angolensis[80] C. siamea[81] C. torosa[90]
OH		Me		OMe		OGl	Physcion-8-O-Glucoside	(LXXI)	C. alata[89]
OH	Me	OMe				OMe	1,-hydroxy,-3, 8-dimethoxy-2-methyl-anthraquinone	(LXXII)	C. occidentalis[91]

/contd...

/Table 4 contd.

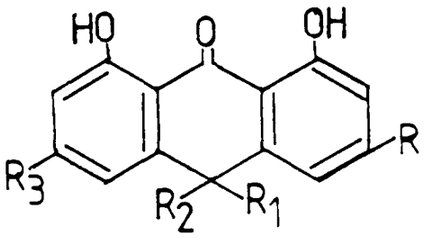
1	2	3	5	6	7	8			
OH		Me	OH			OH	Xanthorin	(LXXIII)	C. torosa[38,93]
OH		Me	OH			OH	5-hydroxy emodin	(LXXIV)	C. javanica[87]
OH		Me	OH			OGl	5-hydroxy-emodin 8-O-Glucoside	(LXXV)	C. javanica[87]
OMe	OH	Me		OH	Me	OH	Aloe-emodin	(XXIV)	C. absus[70] C. acutifolia[35]
OMe	OH	Me		OH	Me	OGl	Aloe-emodin-8-O-Glucoside	(LXXVI)	C. alata[35,82,83,89]
OH	Me	OH	OH	OMe	OMe		1,3 5,-trihydroxy-6, 7-dimethoxy-2-methyl-anthraquinone	(LXXVII)	C. torosa[31]



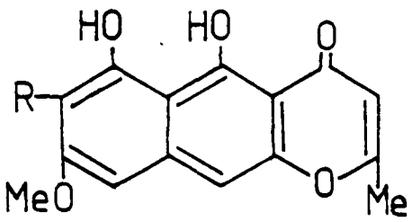
R	R_1	R_2			
Me	OH	H	Germichrysone	(LXXVIII)	C. torosa[90]
OMe	OH	Me	Torachrysone	(LXXIX)	C. torosa[90,93]

/contd...

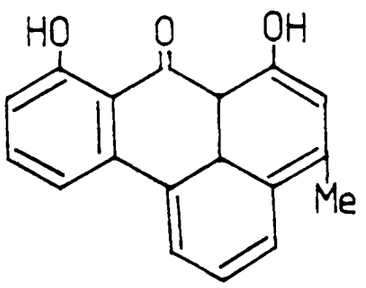
Table 4 contd.



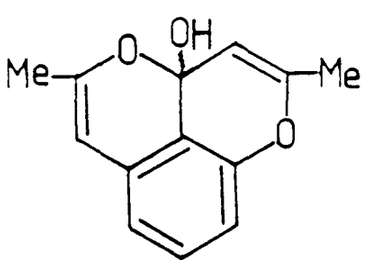
R	R_1	R_2	R_3			
H	H	H	H	Chrysophanic acid 9 anthrone	(LXXX)	C. tora Linn.[94]
H	OGl	H	H	(-)10-Deoxyaloin	(LXXXI)	C. garrettiana[32]
H	OGl	OH	H	Cassialoin	(LXXXII)	C. garrettiana[32]



R = H	Robrofursarin	(LXXXIII)	C. quinquangulata Rich.[95]
R = Me	Quinquangulin	(LXXXIV)	C. quinquangulata[95]

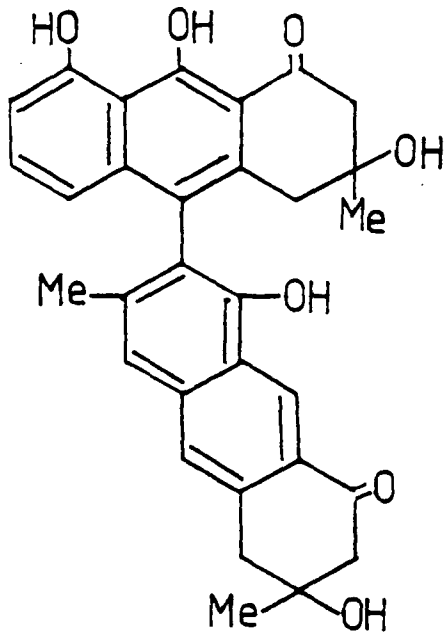


Chrysophanol benzanthrone (LXXXV) C. garrettiana[68]

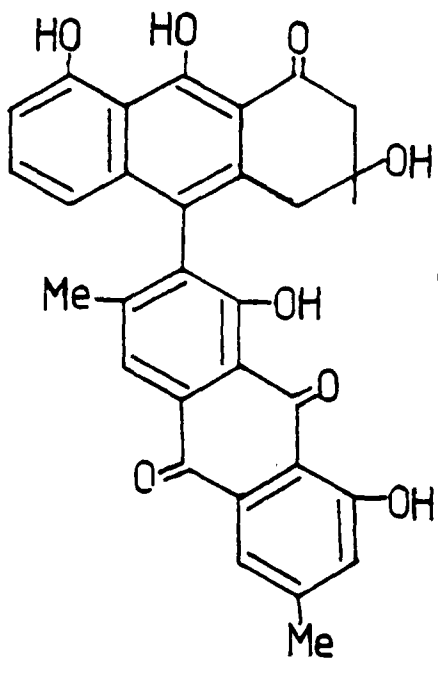


Barakol (LXXXVI) C. siamea[60,96]

/Table 4 contd.

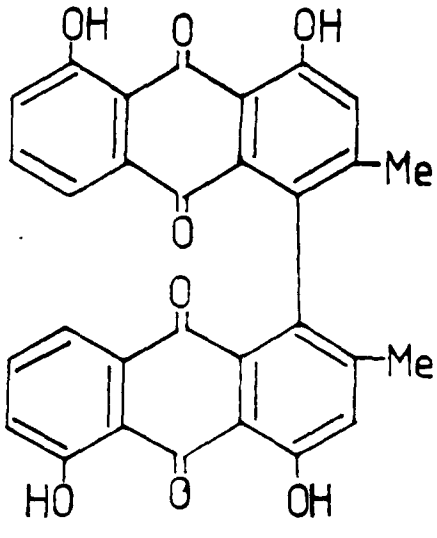


Phlegamacin (LXXXVII) C. torosa[84]



Anhydrophlegamacin

9, 10-quinone (LXXXVIII) C. torosa[84]

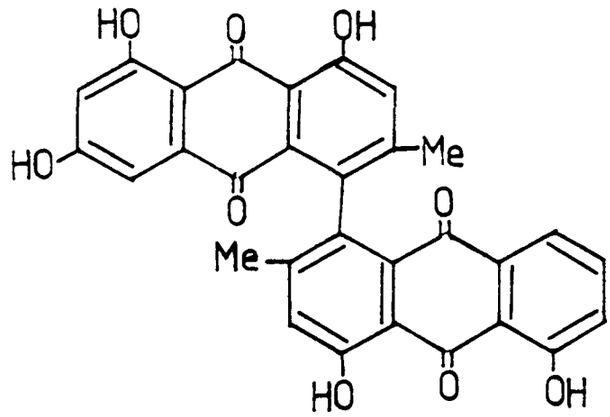


Siameanin (XXII) C. occidentalis[33]

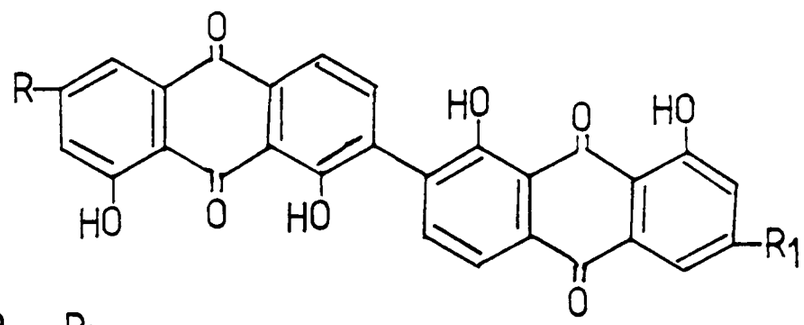
C. siamea[97]

/contd..

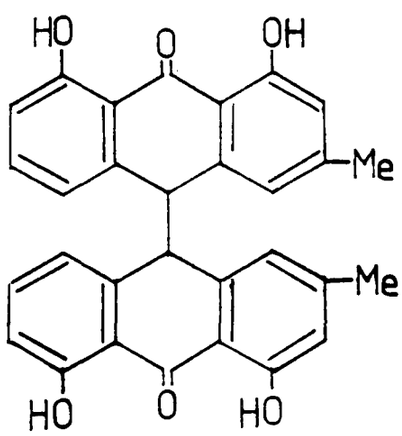
/Table 4 contd.



Cassiamin (LXXXIX) C. siamea[97]



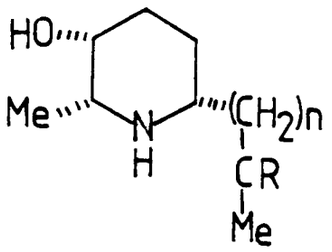
R	R_1			
H	OH	Cassiamin A	(XC)	C. siamea[98]
OH	OH	Cassiamin B	(XCI)	C. siamea[98]
H	H	Cassiamin C	(XCII)	C. siamea[98]



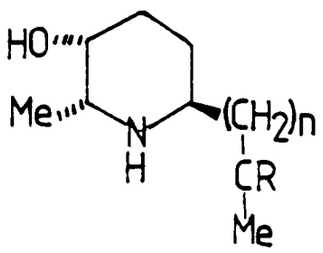
Chrysophanol-dianthrone (XCIII) C. occidentalis[33]

/contd...

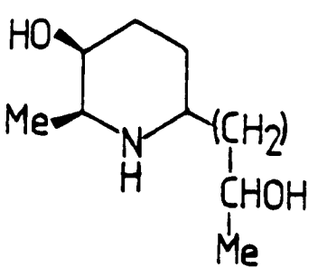
/Table 4 contd.



n	R			
10	=O	Cassine	(XCIV)	C. spectabilis[86] C. excelsa Schrad.[99]
12	=O	Cassinicine	(XCV)	C. spectabilis[86]
10	HOH	Carnavaline	(XCVI)	C. carnaval Linn.[100,101]



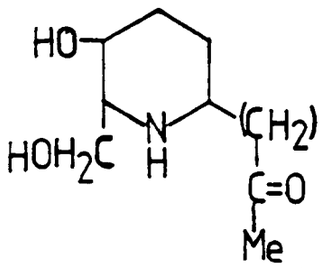
n	R			
10	=O	Isocassine	(XCVII)	C. spectabilis[102]
10	HOH	Isocarnavaline	(XCVIII)	C. spectabilis[103]



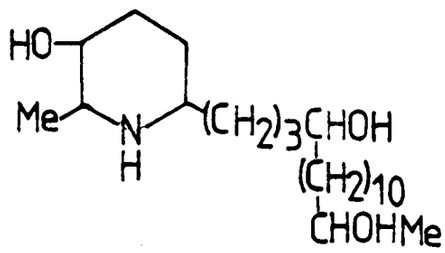
Spectalinine (XCIX) C. spectabilis[102.103]

/contd...

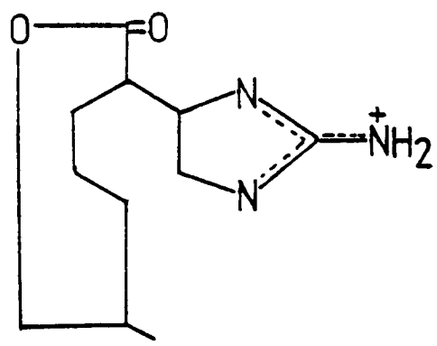
/Table 4 contd.



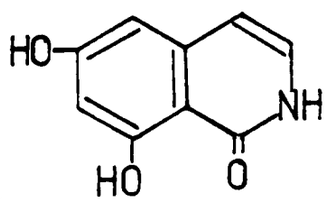
Prosopinone (C) C. carnaval Linn.[104]



Alkaloid D (CI) C. carnaval[104]



Chaksine (CII) C. absus[105]

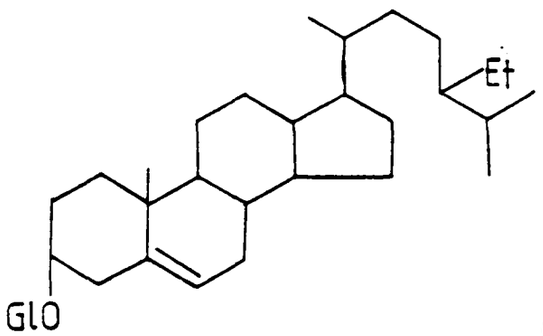


Siamine (CIII) C. siamea[106]

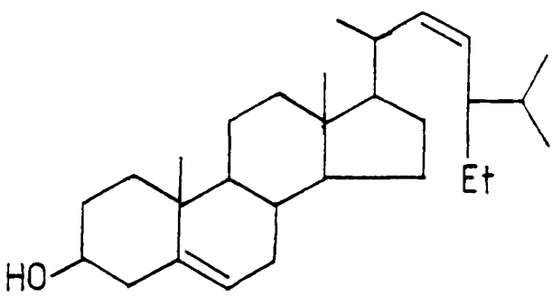
/contd...

Table 4 contd.

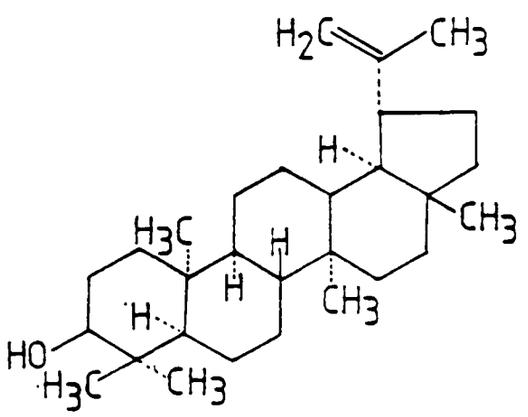
β- Sitosterol (CXCVI) C.alata [83]
C.javanica [42]
C.modesa Buch & Ham [107]
C.occidentalis [107]
C.siamea [108]
C.sieberiana [54.55]
C.spetabilis [86]



β- Sitosterol Glucoside (CIV) C.marginata [30]
C.sieberiana [54]



Stigmasterol (CV) C.spectabilis [86]



Lupeol (CVI) C.siamea [97,108]

1:3:2 CYNOMETRA L. Sp. Pl. 382 (1753)

The genus Cynometra is a member of the tribe Cynometraea of the sub-family Caesalpiniaceae. This genus, which includes at least 60 species, is found predominantly in Africa, but has a pantropical distribution.[112] According to Hutchinson[53] the genus has the following morphological features :-

(i) Trees and shrubs

(ii) Leaves paripinnate, leaflets 1-few pairs,coriaceous, oblique, sometimes with a gland at the base

(iii) Petals 5, subequal or the lower minute, imbricate

(iv) Stamens 10 rarely numerous

(v) Ovary sessile or shortly stipitate, free or nearly so, 2 ovulate

(vi) Flowers small, racemose, racemes short, often fasiculate, axillary, lateral on the branches or on the trunk

(vii) Calyx tube short

(viii) Style filiform; stigma terminal truncate or capitate

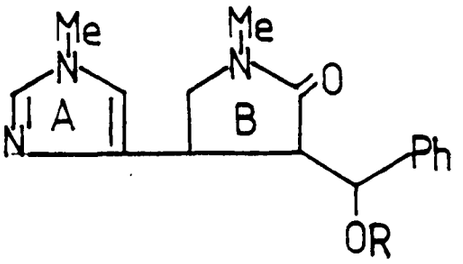
(ix) Fruit arcuate-ovoid or subreniform, rarely straight, thick turgid or subcompressed, varrucose or rarely smooth, 2-valved

(x) Seed filling the cavity thick and compressed

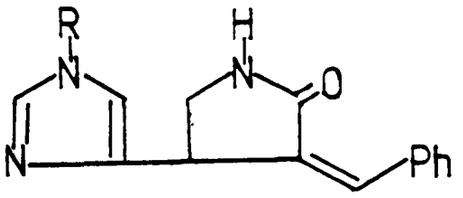
(xi) Cotyledons thick and fleshy

Phytochemical work reported on this genus has been restricted to two African species, C.ananta Hutchinson and Dalz.,[18] from Ivory Coast, and C.lujae De Willd[109] from Congo-Brazzaville. Both have yielded a novel type of imidazole alkaloids. These compounds, seven in all (Table 5), are similar in structure to pilocarpine (CVII), but differ in that .

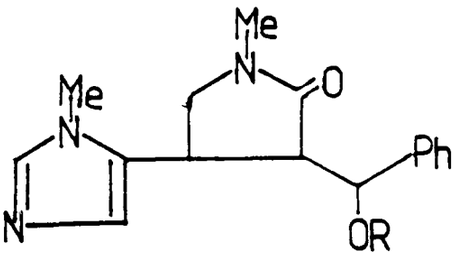
TABLE 5 SECONDARY METABOLITES FROM SPECIES OF *CYNOMETRA*



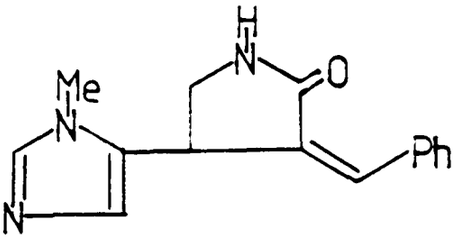
R = H Cynometrine	(VIII)	*C. ananta* Hutchinson & Dalz.[18] *C. lujae* De Wild[109]
R = COPh Cynodine	(CIX)	*C. ananta*[18]



R = Me Anantine	(CX)	*C. ananta*[18]
R = H Nor-anantine	(CXI)	*C. lujae*[109]

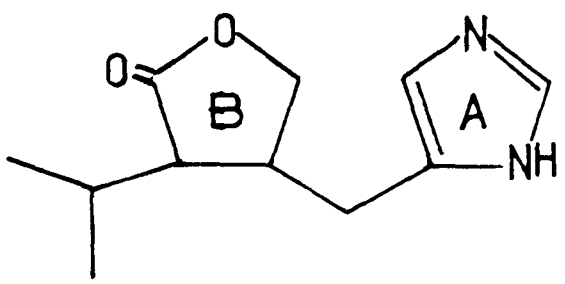


R = H Isocynometrine	(CXII)	*C. lujae*[109]
R = COPh Isocynodine	(CXIII)	*C. lujae*[109]



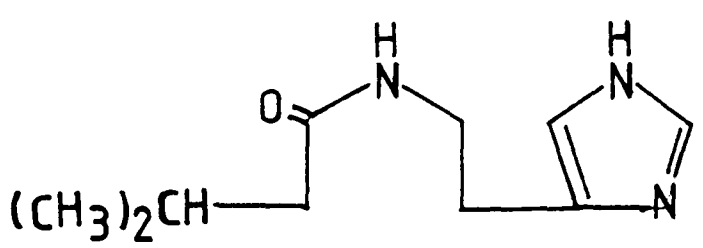
Isoanantine	(CXIV)	*C. lujae*[109]

they possess a lactam B-ring instead of a lactone B-ring [109].



CVII

The biogentic pathway by which the Cynometra imidazoles are formed appears more likely to be allied to that leading to dilocotheline (CVIII), isolated



CVIII

from Dilocothele sphaerica Britton and Rose (family Cactaceae)[110]. Whereas in pilocarpine the precursor amino acid, histidine (CXV), undergoes deamination; in dilocotheline [111] and the Cynometra alkaloids the side chain nitrogen is retained and decarboxylation occurs to give histamine (CXVI). It seems plausible that the resulting amine then interacts with cinnamic acid (CXVII) or a similar compound to give the amide (CXVIII). Cyclisation could then proceed to yield nor-anantine (CX) which by N-methylation,

hydroxylation and subsequent esterification could give rise to all the known Cynometra alkaloids. The speculative pathway proposed is outlined in Scheme 1.

Pharmacological studies on cynometrine (VIII), anantine (CIX) and cynodine (CIX) have revealed spasmolytic but weak central depressive activity; marked analgesic action with no secondary effect. The LD_{50} was 140-200 mg/kg.[92]

1:3:2:1 CYNOMETRA HANKEI Harms. Notizbl. Bot. Gart. Berl. Appl. 21:39 (1911)

This species is a forest tree which, according to Hutchinson [53], is found in Southern Nigeria, Cameroun and Zaire. According to the same author it grows to about 40 m high and shows the following morphological features:-

(i) Leaflets about 10 pairs, shortly pubescent on the mid-rib beneath

(ii) Flowers very small in panicles shorter than the leaves

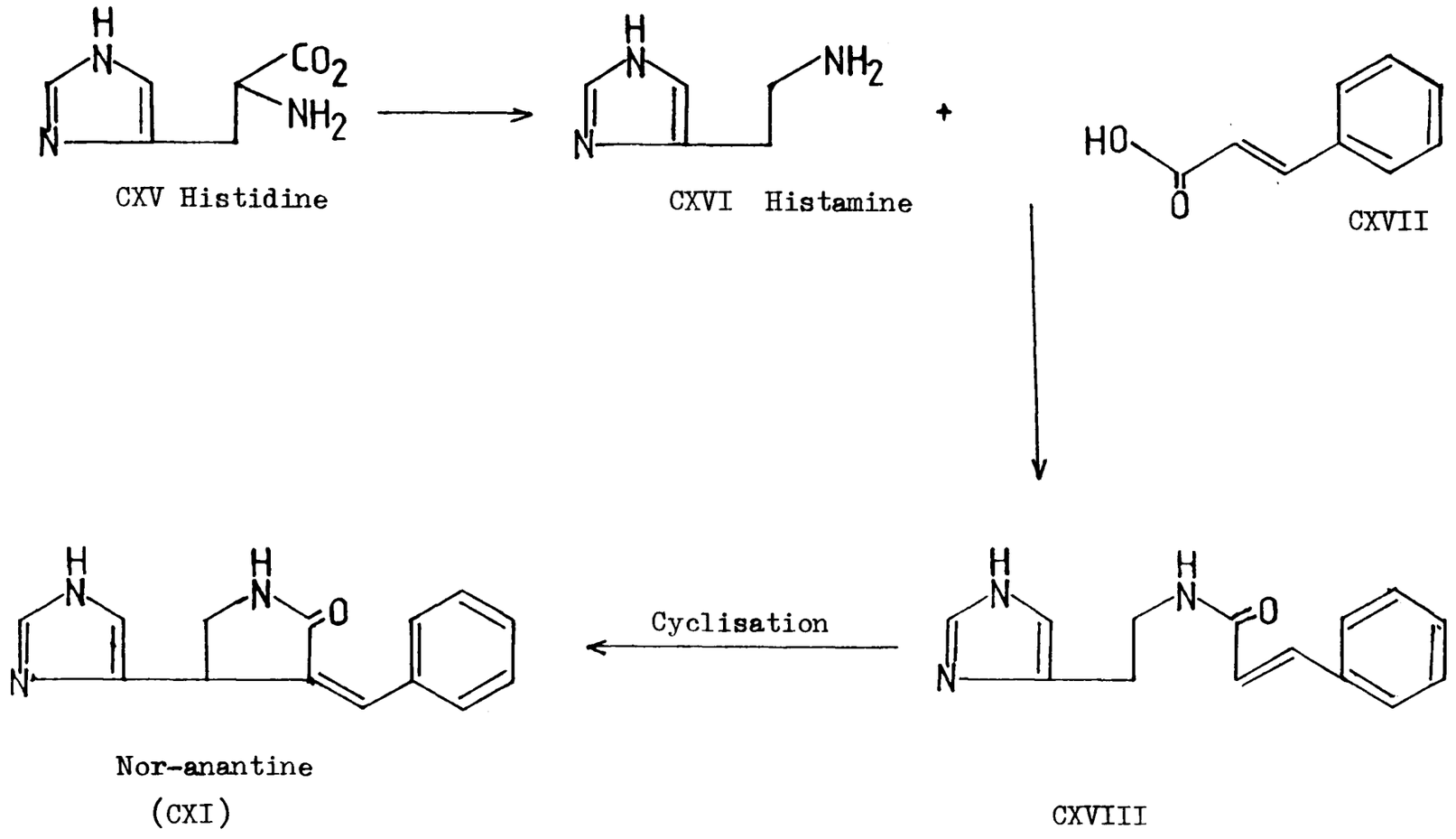
(iii) Pedicels 5-8 mm long shortly pubescent

(iv) Bracts ovate, long acuminate, about 6 mm long

The material studied was collected in the Douala-Edea Forest Reserve, Cameroun, where C. hankei is one of the dominant canopy species [113].

1:3:2:2 CYNOMETRA MANNII Oliv. F.T.A. 2: 317

This species has approximately the same distribution as C.hankei. It grows to about the same height but appears to prefer wetter areas than C. hankei. According to Hutchinson [53] it has



SCHEME 1. SPECULATIVE BIOSYNTHETIC PATHWAY OF CYNOMETRA ALKALOIDS

the following morphological features:-

(i) Acumen rather long and gradual, deeply emarginate

(ii) Bracts glabrous and closely striate

(iii) Leaflets obliquely obovate-oblanceolate, the uppermost pair the largest up to 6 cm long and 2.5 cm broad, glabrous

(iv) Inflorescence short and densely bracteate

(v) Pedicels rather densely pubescent

(vi) Fruits elliptic, about 4 cm long and very rugose

The material studied was collected in the Douala-Edea Forest Reserve, Cameroun, where it is a rare tree of swamp areas.

No phytochemical data on either C. hankei or C. mannii has been previously reported.

1:3:3 CAMOENSIA Welw. ex. Benth. - F.T.A. 2: 251

The genus Camoensia belongs to the tribe Sophoraea of the sub-family Papilionaceae and consists of two species, C. maxima Welw. and C. brevicalyx Benth., both of which occur in West Africa. According to Bentham and Hooker [115] the genus is unique in the Leguminosae in that, "it combines the lofty climbing stem and habits of many Dalbergeae with the digitately trifoliolate leaves of the Podalyrieae and Genisteae, whilst the flowers place it amongst the Sophoreae". It has the following morphological characters:-

(i) Petals papilionaceous with long claws, the standard orbicular, the others narrower, all free

(ii) Calyx turbinate with imbricated lobes

(iii) Anthers uniform linear, versatile

(iv) Ovary stipitate, many ovuled

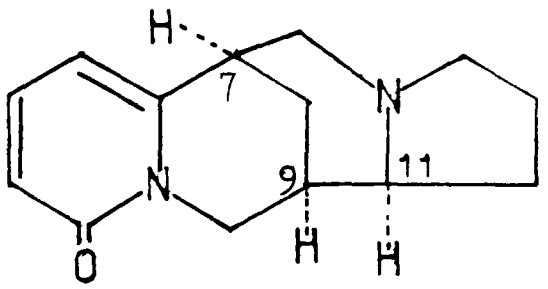
(v) Style filiform involute in the unexpanded flower

(vi) Stigma terminal small capitate

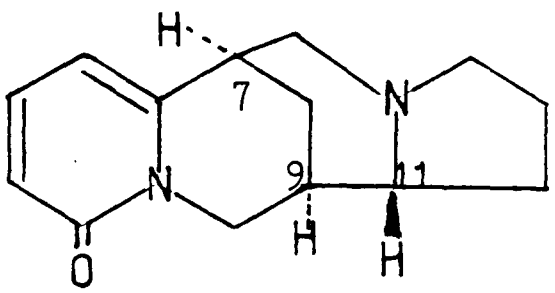
(vii) Pod broad linear flattened, coriaceous two valved

(viii)Wide climbing shrubs with digitately trifoliolate leaves.

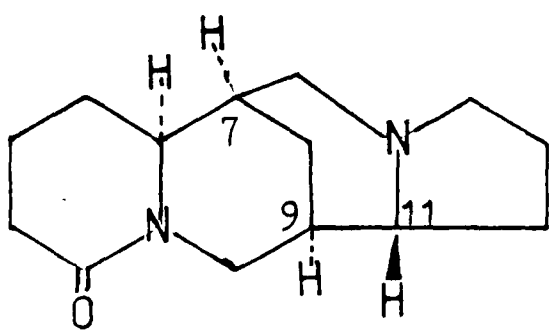
Phytochemical studies on the powdered roots of C.maxima [116] have resulted in the isolation of three alkaloids, leontidine (CXIX) camoensine (CXX) and camoensidine (CXXI).



CXIX



CXX



CXXI

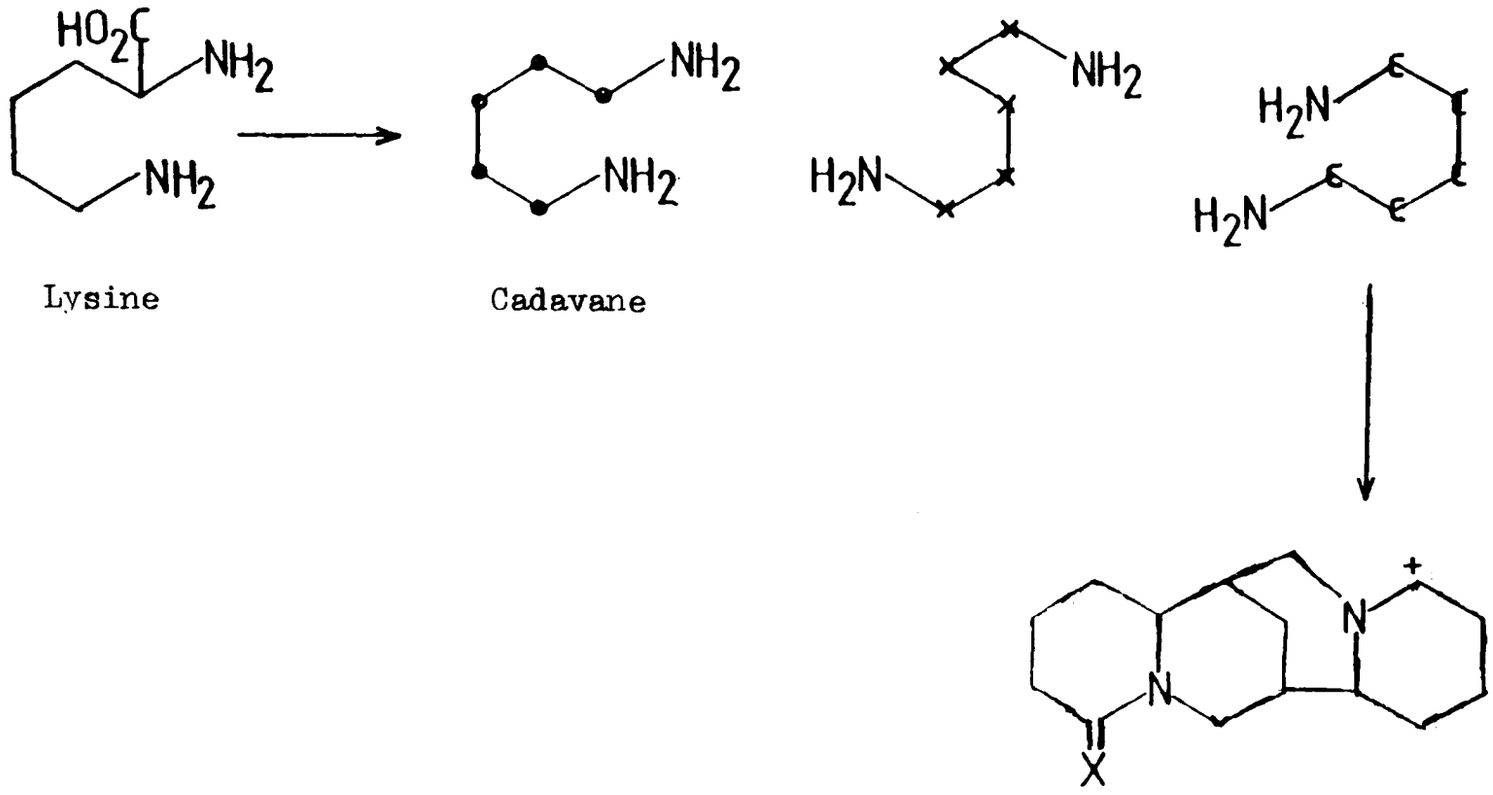
These alkaloids, of the quinolizidine type, are similar to anagyrine (I) except that the D ring is 5-membered instead of 6-membered. Leontidine has been previously reported from the epigeal parts of Leontice alberti Regel [117] and L. ewersmanii Bunge [118] of the family Berberidaceae. Leontidine and cameonsine are diastereomers, leontidine (CXIX) being cis 9 cis 11 whilst camoensine (CXX) is cis 9 trans 11. Camoensidine is the reduced tetrahydro quinolizidine of camoensine with cis stereochemistry at C-6, C-7.

The lupin alkaloids, to which these alkaloids are similar, are believed to be biosynthesised from C-5 units derived from lysine via its decarboxylation product cadaverine [119] (CXXII), with

the retention of two of the nitrogen atoms present in two cadaverine dimers (Scheme 2, p.40).

No biosynthetic work has been reported on the tetracyclic alkaloids with a terminal pyrolidine ring. It seems plausible that formation of these compounds involves loss of the terminal (+) carbon before cyclisation (Scheme 2).

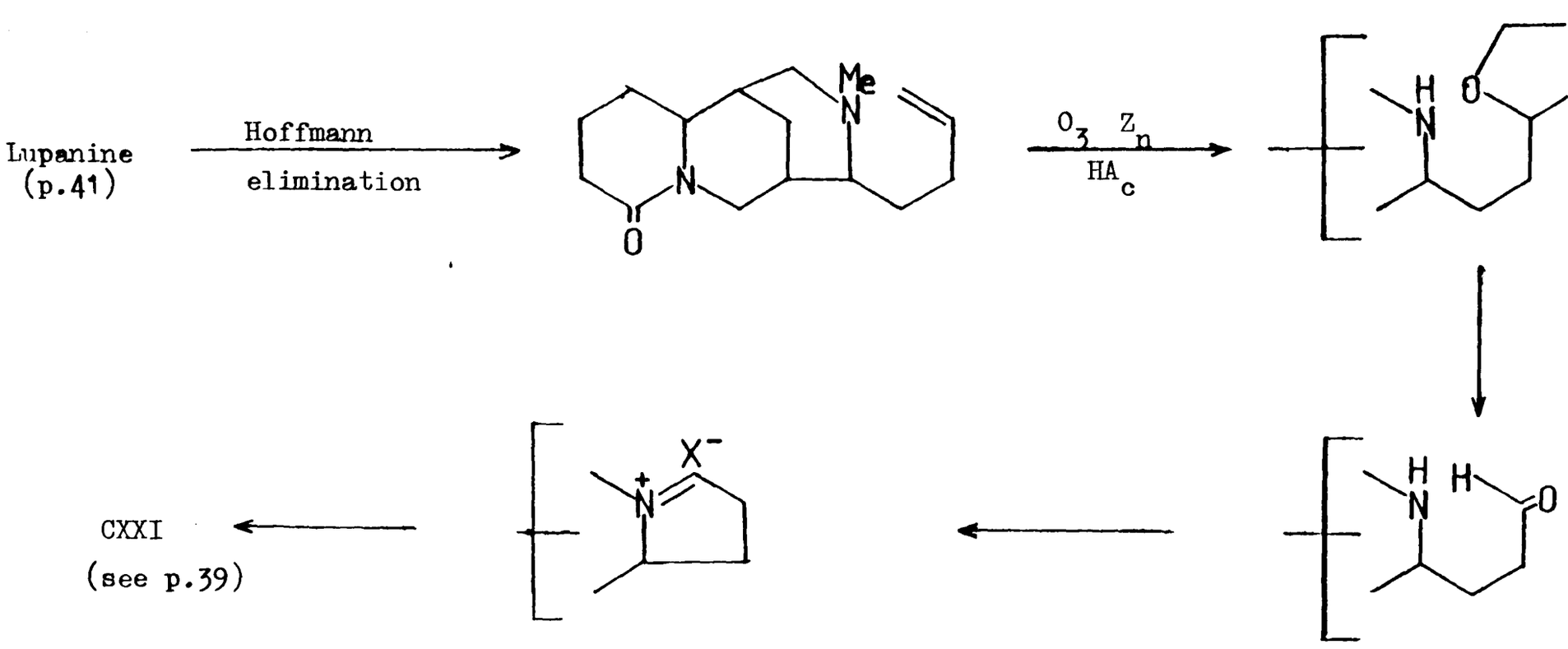
(±)-Camoensidine can be synthesised from (±) lupanine, the tetrahydro-analogue of anagyrine (see Scheme 3). This has been reported by Santa Maria and Khuong Huu, [116] using Hoffman degradation and reaction of one of the products (CXXIV) with ozone followed by treatment with zinc and acetic acid. The authors favour a mechanism in which ozone effects the oxidative demethylation of the N-methyl amino group (Scheme 3). The conversion of lupanine into camoensidine in this way may mirror the biosynthetic pathway in which initial oxidation of the terminal double bond of the Hoffman elimination product would be via the epoxide.



Lysine Cadavane

X = O Lupanine
X = H_2 Sparteine

SCHEME: 2 BIOSYNTHETIC PATHWAY FOR TETRACYCLIC QUINOLIZIDINES



SCHEME: 3 SYNTHETIC ROUTE FOR THE FORMATION OF QUINOLIZIDINE/INDOLIZIDINE ALKALOIDS OF CAMOENSIA MAXIMA [116]

1:3:3:1 CAMOENSIA BREVICALYX Benth. F.T.A. 2: 251

Linn. Trans. XXV 302

Morphological features distinguishing it from C. maxima are, according to Hutchinson [53]:-

(i) A woody forest climber at least 20m high

(ii) Petals white or mauve with a yellow mark in the centre of the standard

The present work deals with compounds isolated from seeds of C. brevicalyx collected from plants growing in the Douala-Edea Forest Reserve, Cameroun.

1:3:4 SESBANIA Scop. Intrd. 308 (1777)

The genus Sesbania is a member of the tribe Galegaea, sub-family Papilionaceae. It consists of about seventy species[1] of herbs and shrubs found mainly in tropical and sub-tropical areas of the world[1]. According to Abrams [120] the genus shows the following morphological features :-

(i) Leaves abruptly pinnate, the rhachis ending in a setaceous point

(ii) Stipules small, scarious caducous

(iii) Leaflets numerous

(iv) Flowers linear oblong in axillary racemes bracteate

(v) Calyx campanulate, the lobe shorter than the tube

(vi) Corolla yellow of standard purplish

(vii) Keel petals obtuse or rounded

(viii) Stamens diadelphous

(ix) Ovary short stipitate many ovuled

(x) Pod linear serete, or highly compressed with septa between the seeds, 2-valved; seeds many, narrowly oblong.

There has been little phytochemical work reported on Sesbania. A survey of the seed of several species (Table 6) revealed the presence of saponins which, on hydrolysis, gave the triterpenes β- sitostanol(CXXVI), oleanolic acid (XXVII), stigmasterol (CV) and queretoroic acid (CXXVI). Simple amino acids, such as canavanine (XXIX) are also present.[121] Some species of Sesbania have been shown to have antitumor activity[122] and a cytotoxic alkaloid, sesbanine (CXXVIII), has been isolated from S. drummondii.[123]

Glottidium Desv., a related genus, contains only one species, G. vesicarium Jacq., a shrub with long pods which taper at each end, almost 2cm wide in the middle with one or two seeds.[124] According to Rickett[124] this species is sometimes mistaken for Sesbania exaltata. The relationship between Glottidium and Sesbania is far from settled, many botanists preferring to merge them under Sesbania.

Phytochemical studies on Glottidium vesicarium seeds have shown them to contain three unidentified phenolic compounds.[125] In one of two recent reports in which Glottidium vesicarium has been investigated under the name Sesbania vesicarium[126] hydrolysis of the saponins revealed the presence of two triterpenes; glottidigenins A, (oleanolic acid) (XXXII) and D (CXXVIII). In the second report[122] only glottidigenin A was isolated together with queretaroic acid (CXXVII).

1:3:4:1 SESBANIA MACROCARPA Muhl. Cat. 65 (1813)

This species of Sesbania is restricted to mid southern and Atlantic coast areas of the United States. According to Abrams[12] the species has the following morphological features :-

(i) Annual, glabrous stems 30 - 100cm high

(ii) Striated leaves 5 - 10cm long

(iii) Leaflets 20 - 70 linear-oblong, 10 - 25mm long rounded or oblong at each end, somewhat glaucous beneath

(iv) Racemes 2-6 flowered, bracts and bractlets narrowly linear

(v) Pedicels 5 - 10mm long

(vi) Calyx about 5mm long and as broad

(vii)Lobes triangular-subulate, scarcely 2mm long

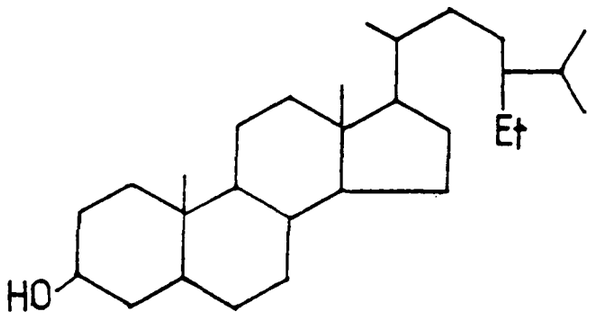
(viii) Corolla about 15mm long, yellowish, the standard streaked and dotted with purple

(ix) Pod 10 - 15cm long, 3mm wide, tipped a slender beak 15 - 30 seeded; seeds 4mm long.

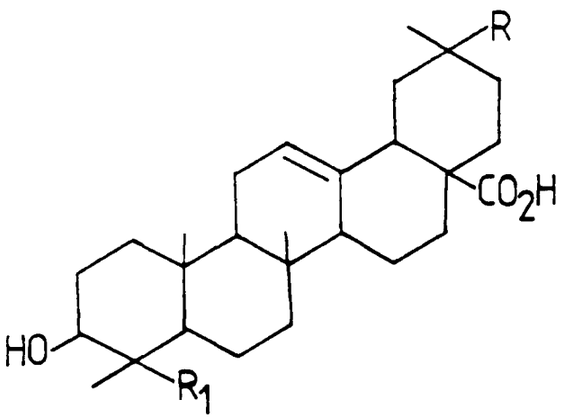
There is no previous record of phytochemical work on Sesbania marcocarpa.

TABLE 6.

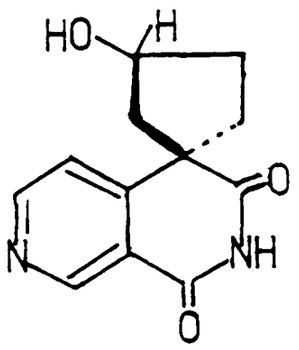
SECONDARY METABOLITES OF THE GENUS SESBANIA



Sitostanol	(CXXVI)	S. speciosa Soland[127]
Oleanolic acid	(XXXII)	Glottidium vesicarium Jacq.[126]
		S. aegyptica Buch-Ham.[128]
		S. bispinosa Jacq.[129]
		S. drummondii Ryd.[123]
		S. punicea Benth.[123]
		S. vesicaria Jacq.[123]
Stigmasterol	(CV)	S. drummondii[123]
		S. punicea[123]
		S. vesicaria[123]



R	R_1			
CH_2OH	CH_3	Queretaroic acid	(CXXVII)	S. vesicaria[123]
CH_3	CO_2H	Glottidigenin B	(CXXVIII)	Glottidium vesicarium[126]



Sesbanine (CXXIX) S. drummondii[124]

RESULTS AND DISCUSSION

2. 1 PRELIMINARY EXTRACTION AND SEPARATION

2:1:1 Plant Materials

The plant materials examined in this work are shown in Table 7.

2:1:2 Collection and Drying of Specimens

The Cassia sieberiana specimen was collected in Sierra Leone (see acknowledgement p. 144) and dried in the herbarium at Fourah Bay College in Freetown. The Cynometra and Camoensia specimens were collected from the Douala-Edea Reserve in West Cameroun and sun dried. The Sesbania macrocarpa seeds were collected near Lubbock, Texas, U.S.A. and sun dried. (see voucher details p. 124).

2:1:3 Extraction

The extractions were carried out in a Soxhlet apparatus using, separately and successively, solvents of increasing polarity, usually light petroleum (b.p. 40 - 60°), followed by chloroform, and finally methanol. The extracts were then separately concentrated using a rotary film evaporator.

2:1:4 Screening of Extracts

Thin layer chromatography was used to screen the concentrates of the various plant extracts. The concentrates were spotted on TLC plates of either silica gel or alumina (see Experimental pp. 125 - 127) and the plates developed in various solvent systems. These solvent systems were mixtures of pure solvents freshly made up and left to equilibrate in a chromatography tank before the chromatoplates were developed. Development was over

TABLE 7. PLANT MATERIALS EXAMINED

PLANT SPECIES	PLANT MATERIAL		
	Stem bark	Root bark	Seeds
CASSIA SIEBERIANA		*	
CYNOMETRA HANKEI	*		*
CYNOMETRA MANII	*		
SESBANIA MACROCARPA			*
CAMOENSIA BREVICALYX			*

* Plant materials studied.

10 - 15cm at room temperature.

2:1:5 Selection of Chromatographic Systems

Alumina plates were used for the examination of the alkaloids of Camoensia brevicalyx and Cynometra hankei because they gave the best resolution. The solvent systems used to develop the plates, mixtures of chloroform and methanol,were solvent systems 2 and 3 (See Table 8). Silica gel G. chromatoplates were used in the initial detection of the compounds of Cassia sieberiana and Sesbania macrocarpa using solvent systems 1, 3, 4, 5 and 6 (Table 8). Cellulose plates were used to detect the sugars obtained from the hydrolysis of the glycoside from Sesbania macrocarpa. Cellulose chromatoplates were developed in solvent system 7 (Table 8).

2:1:6 Detection of the Isolated Compounds

2:1:6 :1 Alkaloids

Each of the chromatoplates was examined under UV light (366nm). Both the imidazole and quinolizidine alkaloids showed up as pale blue fluorescences, only cynodine showed up as a yellowish fluorescence. The compounds were detected by spraying the chromatoplate with either modified Dragendorff's reagent. which gave an orange brown spot against a pale yellow background, or with Iodoplatinate spray reagent, which normally gave a deep purple colour against a pink background.

2:1:6 :2 Triterpenes and Flavonoids

Vanillin/sulphuric acid spray reagent; made up of 2% vanillin (w/v) in concentrated sulphuric acid, was used to detect the triterpenes. The chromatoplate was sprayed with the above

reagent, and then heated in an oven at 100° for about 5mins. β- sitosterol gave a pink colour and lupeol a bluish colour.

When flavonoids were suspected from initial viewing under UV light, the plate was exposed to NH_3 and revisualised under UV light to observe any colour change. The plate was then sprayed with alcoholic ferric chloride, (3% ferric chloride in methanol) to determine whether the compounds were phenolic. Over spraying with vanillin/sulphuric acid spray reagent and heating in an oven, as above, gave a black-brown spot.

2:1:6:3 Sugars

Aniline phthalate spray reagent for reducing sugars, made up of 0.93g Aniline and 1.66g of o-phthalic acid in 100ml H_2O, saturated with n-butanol, was used to spray the chromatoplates. The plates were then heated at 100° for 10mins to observe the sugar from the hydrolysis of SM-1 (p. 138) which showed up as a brown spot.

TABLE 8. SOLVENT SYSTEMS FOR TLC AND PREPARATIVE TLC

SOLVENT SYSTEM	SOLVENTS	RATIO
No. 1	Light petroleum (b.p. 60-80°)/ ethyl acetate	3:1
2	Chloroform/Methanol	49:1
3	Chloroform/Methanol	19:1
4	Chloroform/Methanol	9:1
5	Chloroform/Methanol	4:1
6	Chloroform/Methanol	1:2
7	n-Butanol: Acetic acid (Glacial): Water (BAW)	4:1:5

2:2 THE ISOLATION OF COMPOUNDS FROM CASSIA SIEBERIANA ROOT BARK.

2:2:1 Preliminary Analysis

The powdered root bark was extracted separately and successively with light petroleum (b.p. 40 - 60°), chloroform and methanol. The concentrated light petroleum extract of *C. sieberiana* was examined by TLC in solvent system 1, (Table 8 p.54) which revealed two major spots on spraying with vanillin/sulphuric acid reagent and heating to 100°C. These two compounds, termed CS-1 and CS-2, were similarly identified in the chloroform extract. Other compounds were only found in trace amounts in these two extracts.

TLC screening of the concentrated methanolic extracts (systems 1, 2, 4 and 5 p.54) indicated that it contained only one compound in significant amounts. This compound, termed CS-3, was visualised by its strong colour reaction with ferric chloride spray reagent.

2:2:2 Isolation of compounds from the light petroleum extract of C.sieberiana root bark

The concentrated light petroleum extract was column chromatographed over silica gel eluting with light petroleum (b.p. 60 - 80°)/ethyl acetate (19:1) to give, firstly, compound CS-1 (recrystallised from acetone as white needles) and then CS-2, which precipitated as a white crystalline material.

2:2:2:1 Characterization of CS-1 as β-sitosterol (CXXVI p.31)

The white crystalline substance gave Rf values and

colour reactions that were similar to those of an authentic sample of β-sitosterol. The melting point (138°) was in close agreement with literature data.[85] Accurate mass measurement showed an empirical formula $C_{29}H_{50}O$. The IR 3300cm^{-1} (OH) and 2760-2980 (CH_2) and the ^{1}H NMR spectra δ 5.40 = C<u>H</u>-CH_2 and δ 3.40<u>m</u> (C<u>H</u> OH) were in agreement with published data.[130,131] The mass spectrum gave a fragmentation pattern that was in agreement with that anticipated for β-sitosterol. The co-identity of CS-1 and β-sitosterol was confirmed by direct comparison with an authentic sample (co-chromatography -3 systems, IR).

2:2:2:2 <u>Characterization of CS-2 as lupeol (CV1 p.31)</u>

Thin layer chromatography of CS-2 gave Rf values and a colour reaction on spraying with vanillin/sulphuric acid identical to those of an authentic sample of lupeol. The melting point of the crystalline material (213°) was in close agreement with published data.[131a] High resolution mass spectroscopy gave an exact mass of 426.386 indicating an empirical formula of $C_{30}H_{50}O$. The IR spectrum (3150(CH=CH_2); 3300 (OH) was identical to that of the known compound. The mass fragmentation pattern was identical to that obtained for an authentic sample of lupeol. The co-identity of CS-2 was confirmed by direct comparison with an authentic sample (co-chromatography -3 systems, IR).

2:2:2:3 <u>Isolation of the compounds from the methanol extract of C. sieberiana root bark</u>

The concentrated methanol extract was precipitated with diethyl ether to give a cream coloured powder. This was then

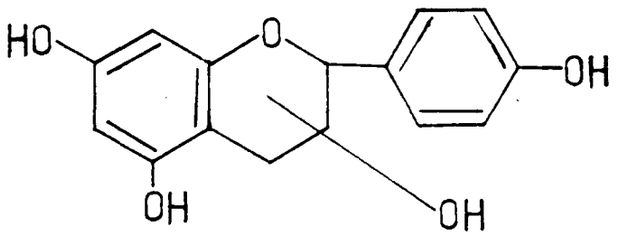
chromatographed over silica gel. Elution of the column with ethyl acetate/ chloroform 2:3, followed by concentration of the eluate and recrystallisation of the solid from hot acetone gave the compound CS-3.

2:2: 4 Characterization of CS-3 as (-)epiafzelechin

The cream coloured crystalline material CS-3, melting point 312°, reacted on the chromatoplate with alcoholic ferric chloride to give a black spot suggesting it to be phenolic. This was confirmed by a bathochromic shift in the UV to 296nm (original 275nm) on the addition of sodium hydroxide. The IR spectrum showed a large band between 3600 and 3200 cm^{-1} for hydroxyl substituents but lacked any carbonyl band. Accurate mass measurement of the parent ion indicated an empirical formula $C_{15}H_{14}O_5$.

The ^1H NMR spectrum, run in $(CD_3)_2CO$, revealed signals for all 14 protons. A broad singlet at δ 8.10, for 3H, replaceable by D_2O, could be assigned to three phenolic hydroxy substituents. A further replaceable signal at δ 3.60 (1H) could be assigned to an alcoholic hydroxyl. Of the remaining protons six occurred in the aromatic region in the form of two AB quartets (4H and 2H respectively). The four proton system, centred at δ 7.41 and 6.89 (J=8Hz) was typical of the 2'3'5' and 6' protons of a 4' substituted B- ring of a flavonoid.[132] The second AB quartet system, at δ 6.06 and 6.01 (J= 2Hz), was typical of the meta coupled 6 and 8 protons of the 5,7 disubstituted A ring of a flavonoid.[133] These data, together with the absence of a carbonyl (from the IR spectrum), indicate that CS-3 is a 5,7 4'

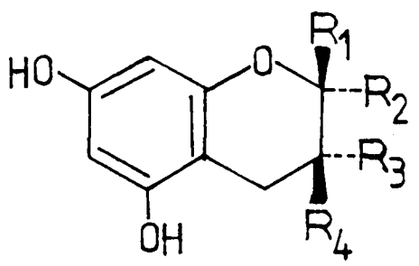
trihydroxy flavonoid of the flavan type with a further hydroxyl in the C-ring (partial structure CXXIX).

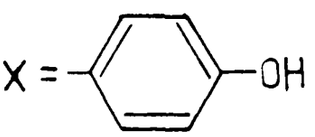


CXXIX

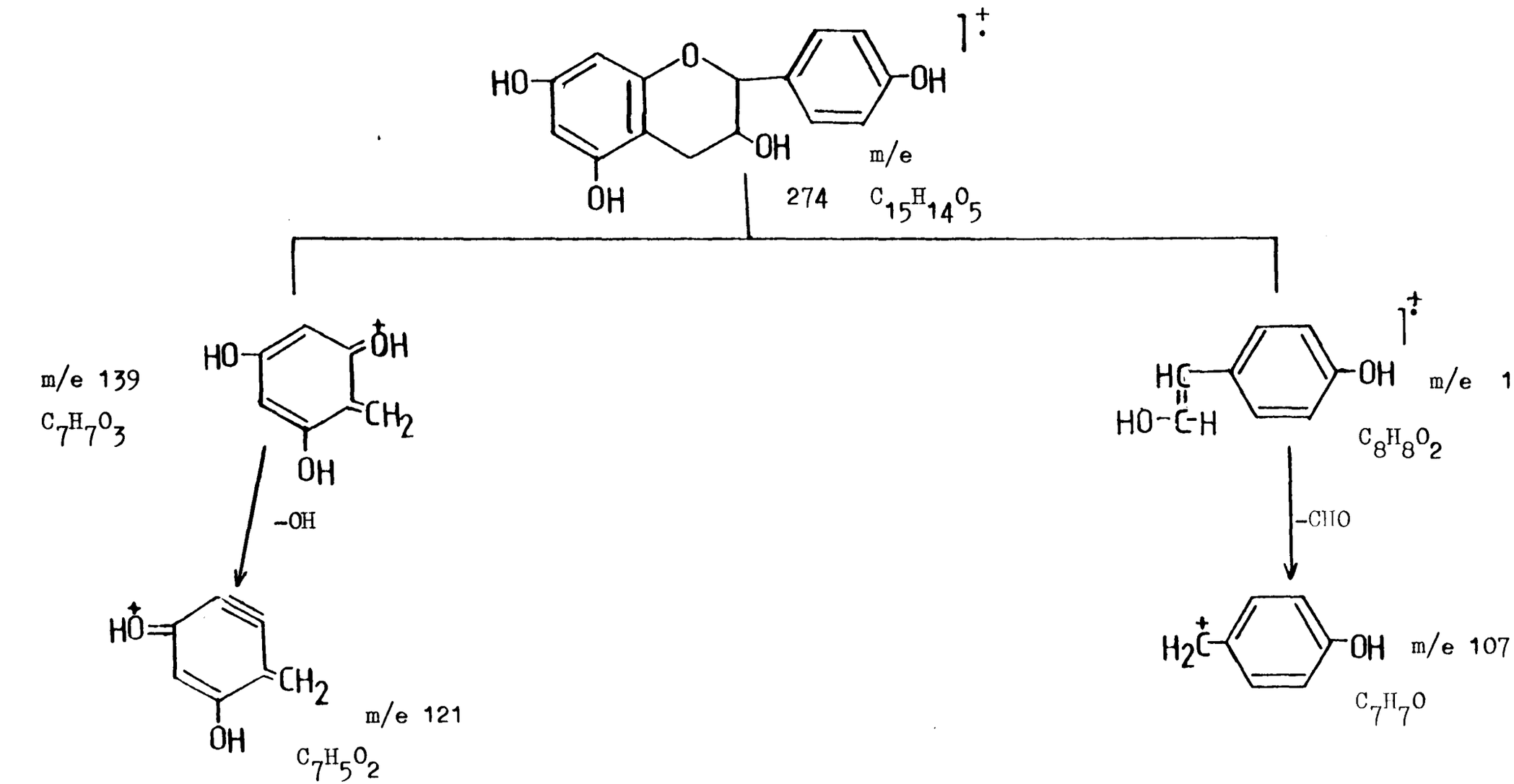
This hypothesis receives further support from the mass spectral fragmentation pattern (Scheme 4) which yields the major fragments A (m/e 139) and B (m/e 137) typical of a flavan. Further fragmentation, also in agreement with the hypothesis and permits the assignment of the C-ring hydroxyl to C-3.[134]

On the basis of the above data CS-3 must be one of the four possible isomers of 5,7 4'trihydroxy flavan-3-ol, (+) afzelechin (a); (-)-epiafzelechin (b); (+)-epiafzelechin (c); and (-)-afzelechin (d).





	R_1	R_2	R_3	R_4	
(a)	H	X	H	OH	(+)-afzelechin
(b)	H	X	OH	H	(-)-epiafzelechin
(c)	X	H	H	OH	(+)-epiafzelechin
(d)	X	H	OH	H	(-)-afzelechin



SCHEME 4. MASS FRAGMENTATION PATTERN OF (-)- EPIAFZELECHIN (CS-3)

The laevorotatory nature of CS-3 (-47°) immediately restricts possible structures to b or d. Differentiation between b and d can be made on the basis if the ^{1}H NMR spectra of the protons at C-2, C-3 and C-4. In a and d the relationship between the substituents at C-2 and C-3 is transoid whereas in b and c they are cisoid. A comparison of the ^{1}H spectra of (+)-catechin (sterochemistry a) and (-)-epicatechin (sterochemistry b) shows that there is a marked difference in the resulting ^{1}H spectra (Figure 1). In (+)-catechin the C-2 proton is observed as a doublet ($\underline{J}$ = 8Hz), C-3 as a multiplet and the C-4 protons in the form of a double-doublet ($\underline{J}_1$= 6Hz, $\underline{J}_2$ = 9Hz). By contrast in (-)epicatechin the C-2 proton is observed as a singlet the C-3 proton as a triplet and the C-4 proton as a doublet. Thus in the cisoid configuration no coupling occurs between C-2 and C-3 protons.

In CS-3 the signals for C-2, C-3 and C-4 protons (Figure 1) were observed as a singlet at δ 4.99 (1H) for H-2, a triplet (J = 5Hz) at δ 4.29 (1H) for H-3 and a doublet (J = 5Hz) for the methylene protons at δ 2.82. The occurrence of H-2 as a singlet rather than a double-doublet is indicative of either a 2R: 3R or 2S :3S configuration (b or c) as in epicatechin rather than the 2R: 3S or 2S: 3R configuration (a or d), of catechin.[135] The relevant portions of the ^{1}H spectra of (+)-catechin, (-)-epicatechin and CS-3 are shown in Figure 1.

CS-3 must therefore be (-)-epiafzelechin. Further support for the identity of CS-3 was obtained from the ^{13}C-NMR. The values

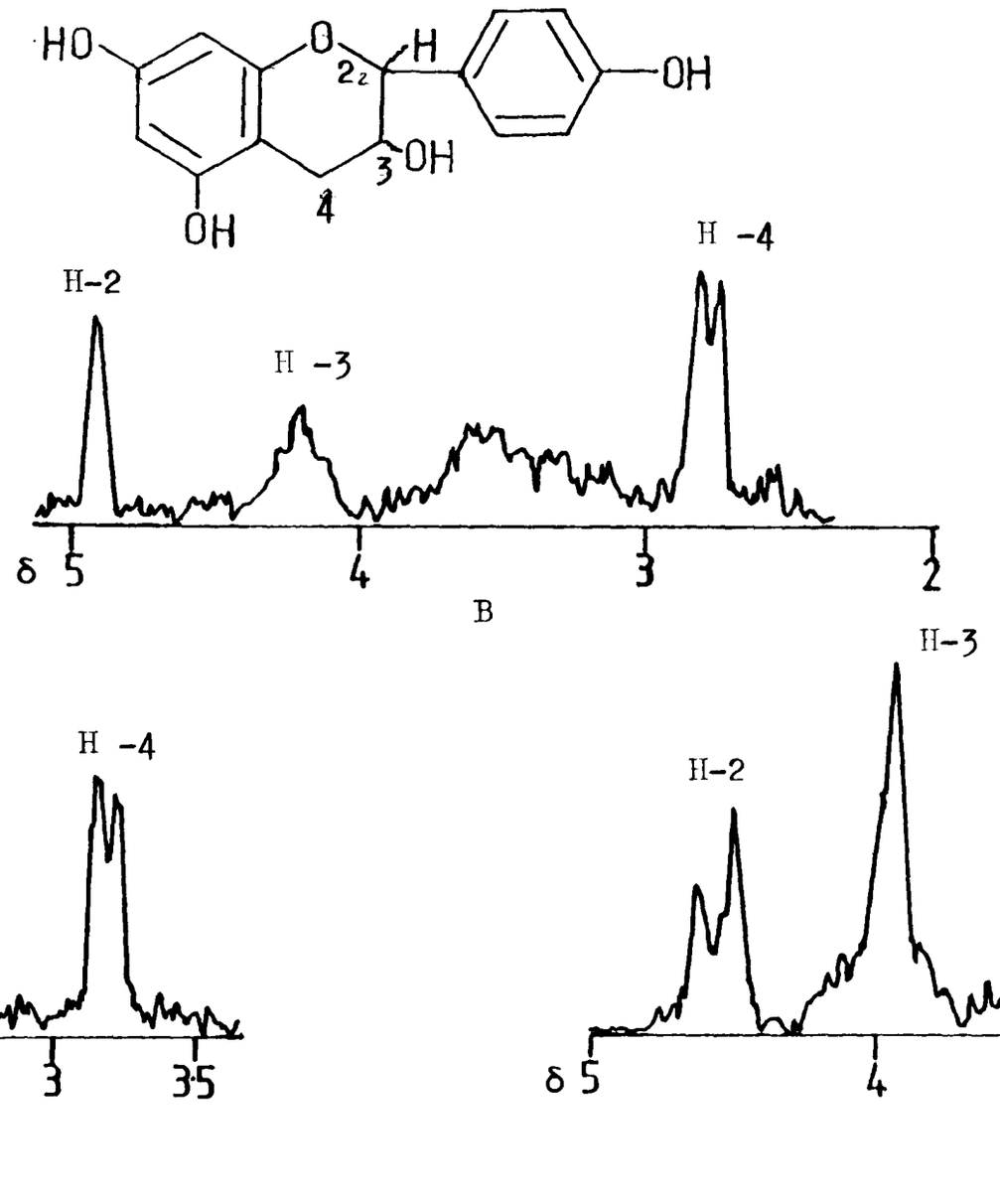
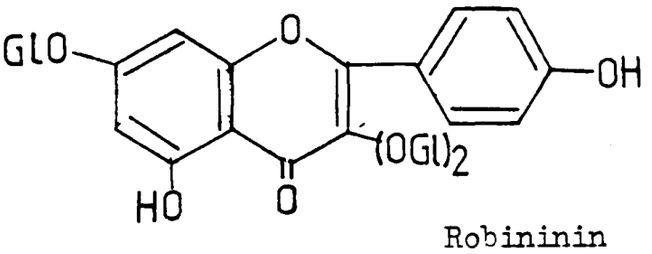


FIGURE 1. COMPARATIVE ^{1}H NMR OF THE C-2, C-3 AND C-4 PROTONS OF (-) EPICATECHIN (A), (-) EPIAFZELECHIN (B) AND (+) CATECHIN (C)

obtained (Table 8) were in close agreement to those published [136] for catechin except in the C-3', C-4' and C-5' regions. The values for these carbons were however similar to those of robininin (below) which, like CS-3, has a 4' hydroxy B ring.

(-)-Epiafzelechin has been previously reported in Afzelia,[137] a genus of the Leguminosae, and more recently from Cassia marginata,[35] while (+)-epiafzelechin is known only from the Palmae.[138]

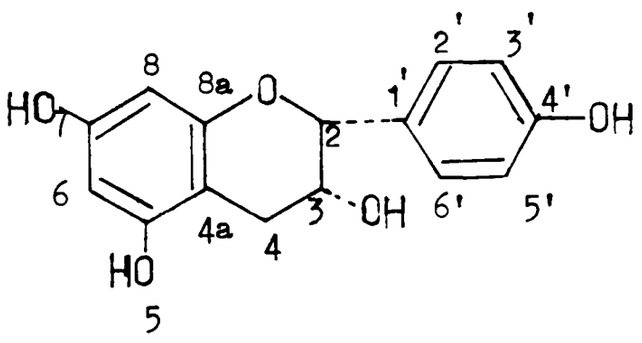
The identified compound is unlikely to have been the cause of the toxicity of a decoction of C. sieberiana observed by the author.[58]



Robininin

TABLE 9. ^{13}C NMR SHIFT VALUES FOR (-)-EPIAFZELECHIN (+) CATECHIN AND THE B-RING CARBONS OF ROBININ

CARBON NO.	(-)-EPIAFZELECHIN	(+)-CATECHIN [136]	ROBININ [136]
2	79.4 d	81.2	
3	66.3 d	66.6	
4	29.0 t	28.1	
4a	99.7 s	99.4	
5	157.0 s	156.3	
6	96.4 d	95.5	
7	157.0 s	156.6	
8	95.8 d	94.3	
8a	157.0 s	155.8	
1'	131.3 s	130.8	
2'	128.9 d	114.7	131.1
3'	115.5 d	145.0	115.2
4'	157.0 s	145.0	157.0
5'	115.5 d	115.4	115.2
6'	128.9 d	118.8	131.1



(-)-Epiafzelechin

2:3 THE ISOLATION OF COMPOUNDS FROM CYNOMETRA HANKEI STEM BARK

2:3:1 Preliminary Analysis

The powdered stem bark was extracted separately and successively with light petroleum (b.p. 40-60°), chloroform and methanol. The concentrated light petroleum and chloroform extracts were screened by TLC (systems 1,2,3 and 5 p.54) but did not show any identifiable compounds when sprayed with various spray reagents. The methanol extract, on initial screening, gave similar results. When the dried methanol extract was treated with 0.88NH_3 and then subjected to liq-liq extraction with chloroform, the concentrated chloroform extract gave positive reactions for alkaloids with Dragendorff's and Iodoplatinate reagents. The extract was then screened on alumina chromatoplates (systems 2 and 3 p.54) and revealed four spots when sprayed with Dragendorff's reagent.

2:3:2 Isolation of compounds from the methanol extract of C. hankei stem bark

The chloroform fraction of the NH_4OH treated methanol extract was chromatographed on alumina column to give, on elution with chloroform containing increasing amounts of methanol, four compounds; CHM 1-4. Elution with chloroform alone gave CHM-1 as a gummy solid. CHM-2 was obtained by elution with chloroform-methanol 49:1 and was recrystallised from chloroform as off-white needle-like crystals. CHM-3 was obtained subsequently by continued elution with the same solvent as CHM-2 and gave white needle crystals. Between the pure bands of CHM-2 and CHM-3 fractions was a mixture which was subjected to preparative thin layer chromatography (PTLC) on alumina (solvent system 2 p.54) to give a

further compound CHM-4 as a brown amorphous solid.

2:3:2:1 Characterization of CHM-2 as Cynometrine (VIII p.33)

The off-white crystals had a sharp melting point (211^{o}) and gave a rubic colour typical of alkaloids when sprayed with Dragendorff's reagent. The compound gave positive analysis for nitrogen with the sodium fusion test. The high resolution mass spectrum gave an exact mass of 285.1477 indicating an empirical formula of $C_{16}H_{19}N_3O_2$.

The UV spectrum showed weak absorption maxima at 252, 258 and 264 nm in close agreement with published data for cynometrine.[18,92] The IR spectra exhibited a carbonyl absorption at 1690 cm^{-1} typical of a lactam amide.[18] Further maxima circa 1600 cm^{-1} supported the presence of a phenyl moiety. There was also a broad band at 3300 cm^{-1} indicative of an OH substituent.

The PMR spectrum(Figure 2) run in $CDCl_3$ showed signals for all 19 protons. Two singlets(3H each) at δ 3.48 and 2.88 were typical of N-methyl substituents on imidazole and α - lactam ring systems respectively.[18] A complex series of signals centred at δ7.25 integrated for 6 protons. Five of these could be assigned to a monosubstituted phenyl ring. The sixth can be attributed to the (Me)N CH=N - proton of an N-methyl imidazole.[18] A doublet (J = 7Hz) at δ 5.06 was typical of the Ph CH OH-proton (cf. nor - ephedrine δ 5.20 [130A]). These data agree with those already published for cynometrine. The fact that the Ph(CH)OH-proton appears as a doublet rather than as a singlet confirms it must be attached to the lactam ring rather than to the imidazole ring.

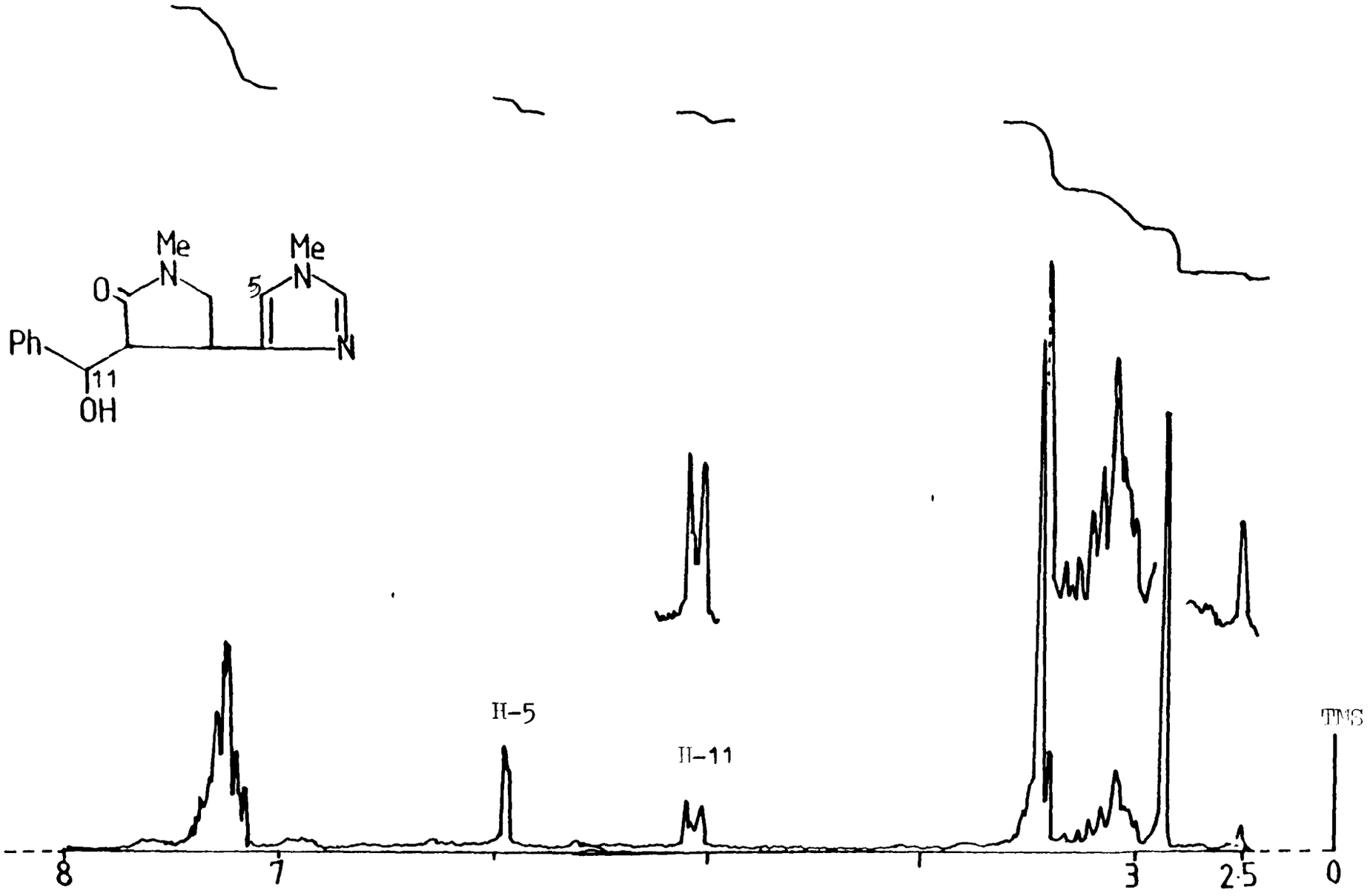


FIGURE 2. ^{1}H NMR SPECTRUM OF CYNOMETRINE (CHM-2)

The four lactam ring protons occurred as an ill-defined multiplet between 3.0 and 3.5.

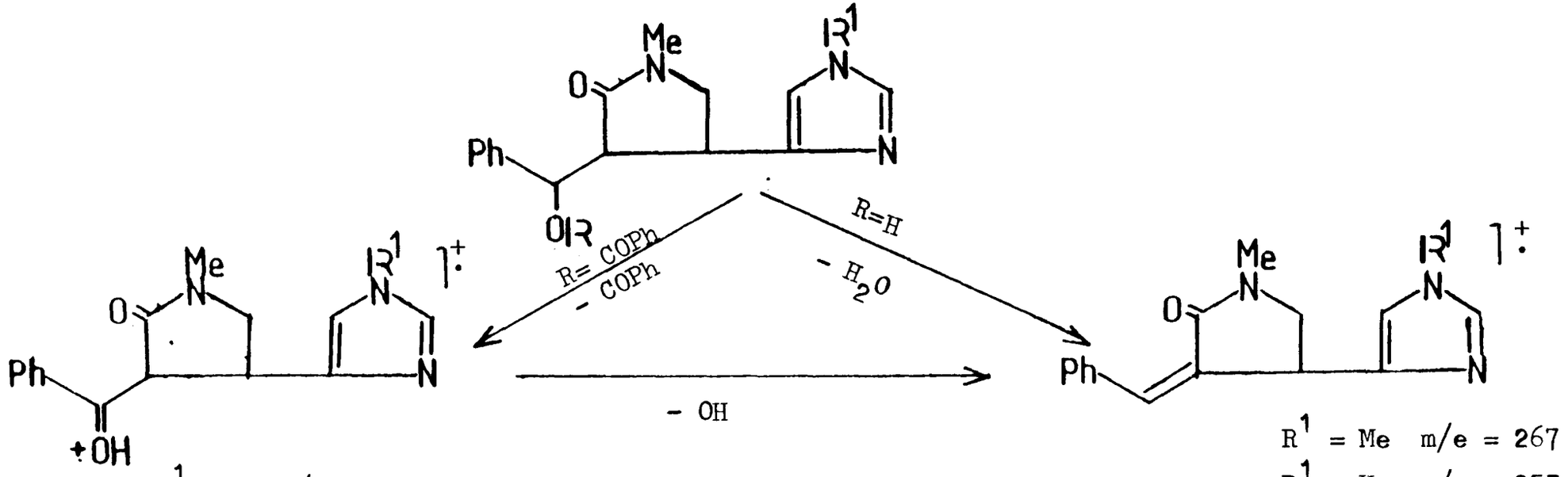
Support for the cynometrine structure is obtained from the mass spectral fragmentation pattern of CHM-2 (Scheme 5). The occurrence of a significant fragment at m/e 267 ($-H_2O$) could only occur if the hydroxyl was in the position adjacent to the lactam ring. Further support for the proposed structure is seen from the remainder of the fragmentation pattern which yields characteristic ions [18] at m/e 179 and 108. Further fragmentation of m/e 253 and m/e 179 give rise to a series of further ions that appear diagnostic of this type of compound. A tentative hypothesis of the formation of these ions is given in Scheme 6. The ^{13}C-NMR spectrum showed shifts that were consistent with those observed for cynometrine (Table 10).[18] The N-methyl carbons were observed at 33.0ppm and 29.56 ppm. and can be assigned to the imidazole and lactam nitrogens respectively.

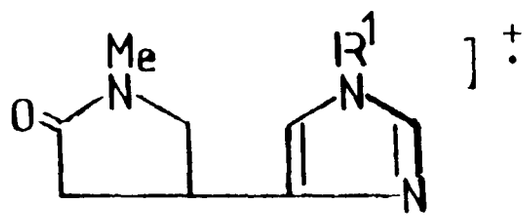
2:3:2:2 Characterization of CHM-3 as N_1-demethylcynometrine

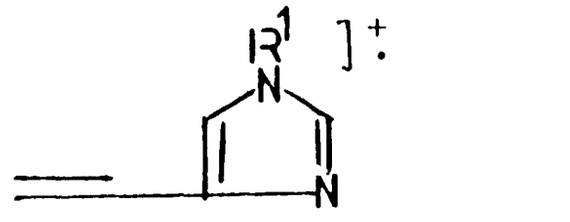
(CXL p.74)

The white crystalline compound, melting point 204°, gave identical reactions for nitrogen and with Dragendorff's reagent to CHM-2. The UV spectrum with peaks at 252, 257 and 267 (Figure 3), was very similar to that of the previous compound. The IR spectrum (Figure 4) was also basically similar to CHM-2 but showed a sharp additional band at 3200 cm^{-1} typical of an N-H group. The mass spectrum gave an empirical formula $C_{15}H_{17}N_3O_2$ which is 14 mass units (CH_2) less than for cynometrine.

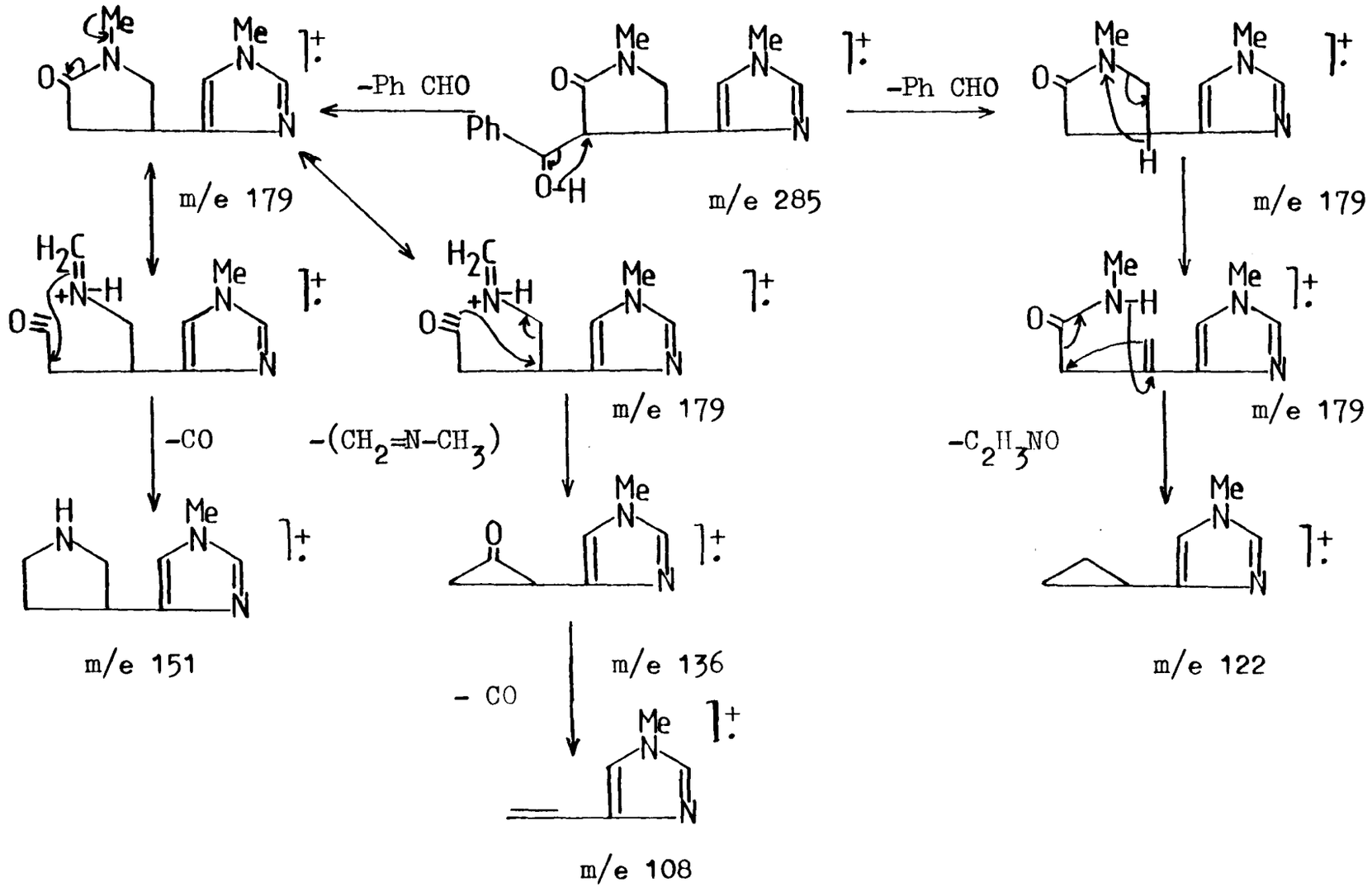
The ^1H NMR spectrum (Figure 5) run in $CDCl_3$ showed one







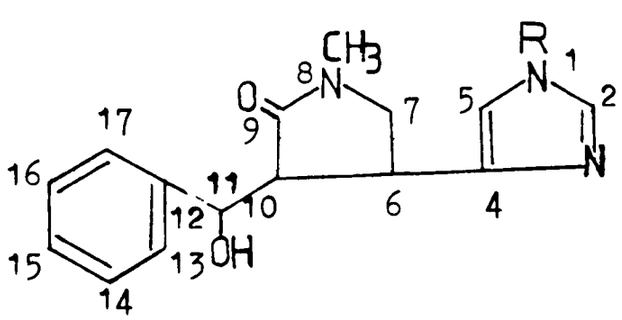
SCHEME 5. MASS FRAGMENTATION FOR CYNOMETRA ALKALOIDS



SCHEME 6. MASS FRAGMENTATION PATTERN OF CYNOMETRINE CHM-2

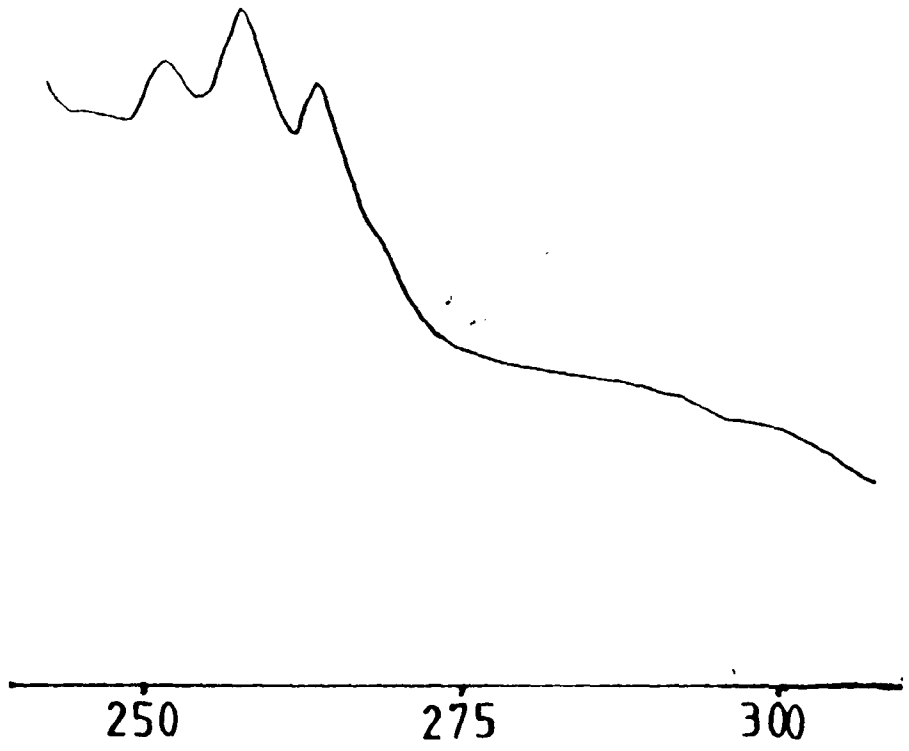
TABLE 10. ^{13}C NMR SHIFTS OF CYNOMETRA ALKALOIDS

Carbons		CHM-2		CHM-3		CYNOMETRINE [18]
1	(NCH_3)	33.1 q			(NCH_3)	32.5
2		137.0 d		136.8 d		136.9
4		141.7 s		142.6 s		141.7
5		116.5 d		115.6 d		115.8
6		33.9 d		33.6 d		32.8
7		52.9 t		55.1 t		51.8
8	(NCH_3)	29.6 q	(NCH_3)	29.8 q	(NCH_3)	28.8
9		174.9 s		176.5 s		173.8
10		54.3 d		56.6 d		53.5
11		75.0 d		75.1 d		73.8
12		141.7 s		142.6 s		146.8
13		128.05d		129.4 d		126.8
to				129.0 d		127.3
17		127.5 d		128.1 d		

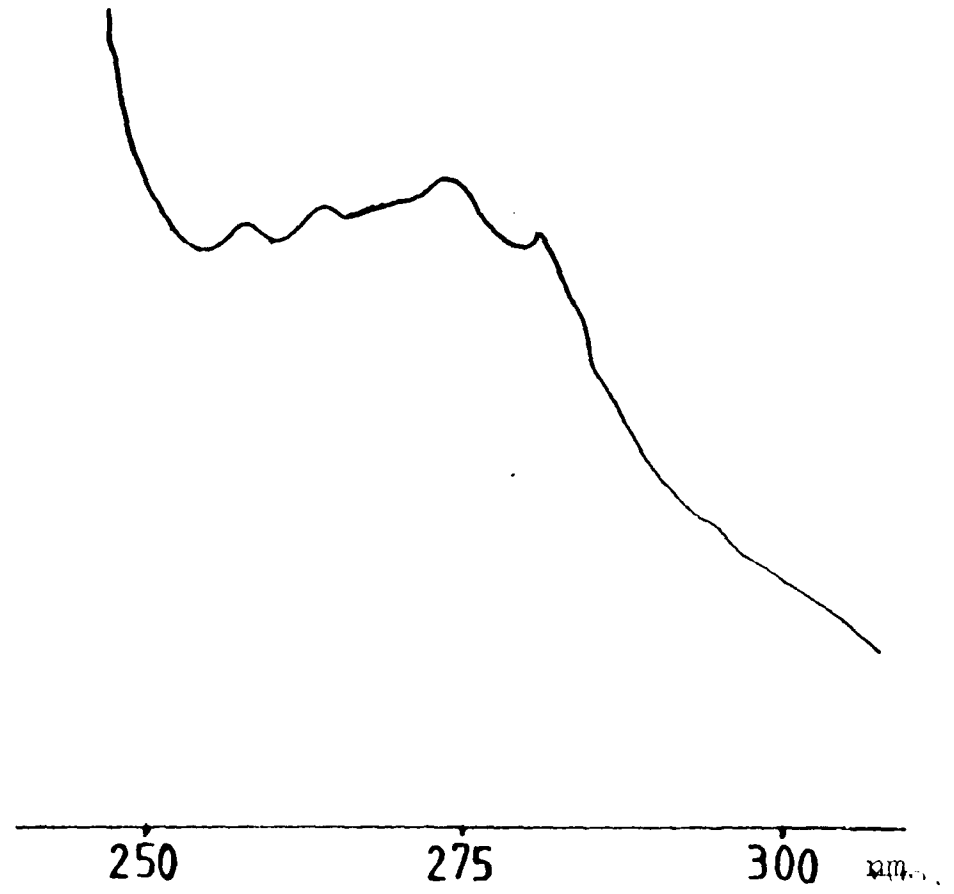


R = CH_3 CHM-2 and Cynometrine

R = H CHM-3



A



B

FIGURE 3. UV SPECTRA OF CHM-2 AND CHM-3 (A) CHM-1 AND CHM-4 (B)

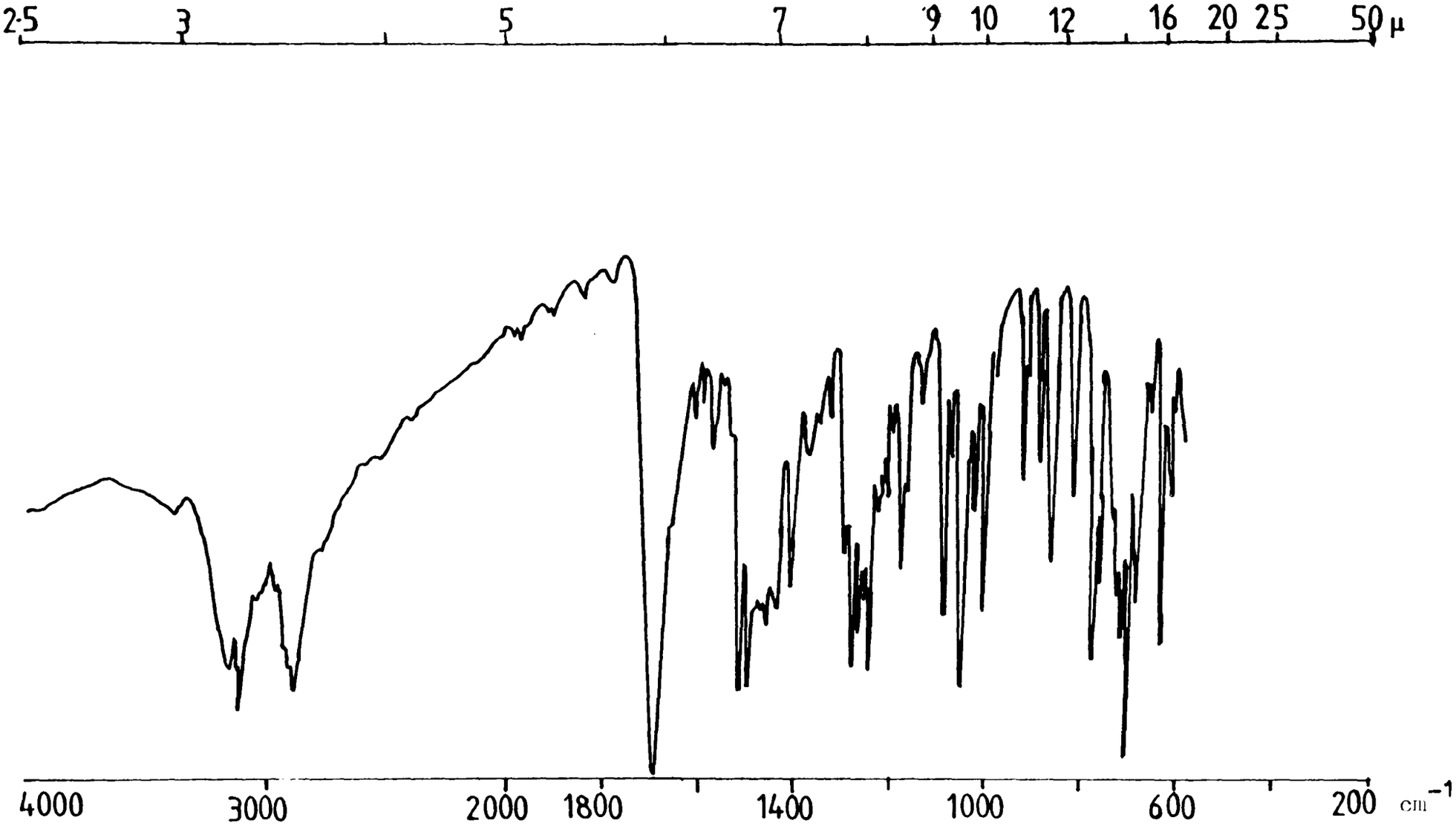


FIGURE 4. INFRA RED SPECTRUM OF CHM-3; N_1-DEMETHYLCYNOMETRINE

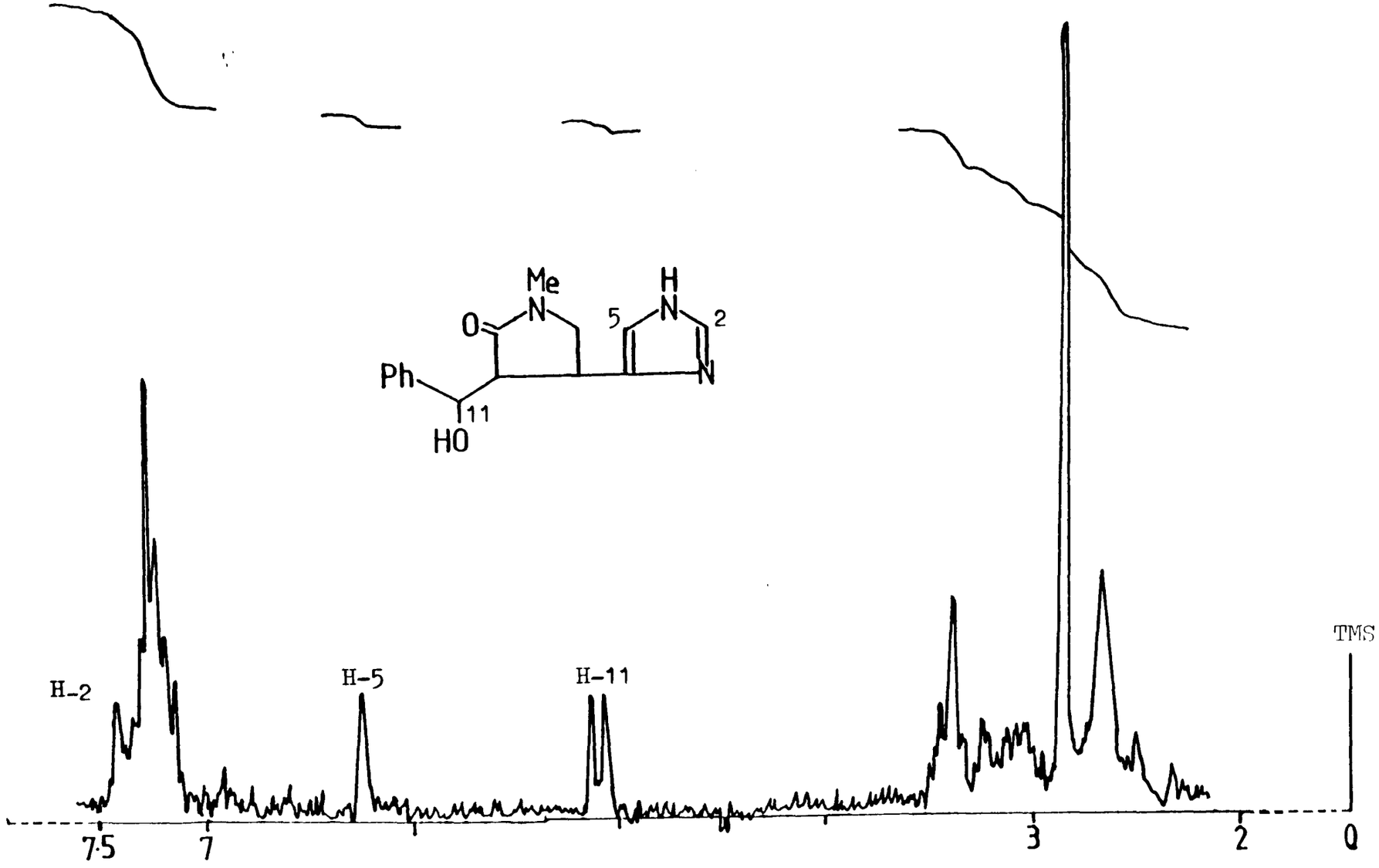
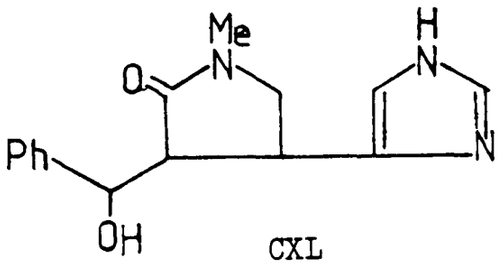


FIGURE 5. ^{1}H NMR SPECTRUM OF N_1-DEMETHYLCYNOMETRINE (CHM-3)

3-H singlet at 2.87 typical of the lactam N-methyl. A doublet at δ 5.09 (J= 7Hz) compared closely with that observed for the C-11 proton of cynometrine. The aromatic region showed a singlet at δ7.44 (1H) and a multiplet centred at δ 7.26 (5H). The 5-H multiplet can be assigned to the phenyl ring system. The 7.44ppm signal must be assigned to the C-2 proton of the imidazole ring because the change from $N-CH_3$ to N-H would result in the proton on this position being further deshielded. Similarly the C-5 proton is seen at δ6.26 showing a deshielding, relative to cynometrine, of 0.34ppm. The remainder of the spectrum was in close agreement with that of cynometrine.

The net result of the ^1H NMR assignments was to suggest a structure identical to cynometrine except that the imidazole ring was demethylated. Support for this is found in the fragmentation pattern of the mass spectrum of CHM-3 (Scheme 5). This again showed ions at m/e 254 and 253 associated with the loss of OH and H_2O respectively. These ions both have mass values at 14 mass units (CH_2) less than the corresponding values for CHM-2. Similarly the m/e ion at 179 of cynometrine is replaced by a base peak at m/e 165. The occurrence of a major ion at m/e 94 instead of m/e 108 (c.f. cynometrine) is further proof that the loss of N-methyl has occurred in the imidazole ring. The compound CHM-3 was therefore identified as N_1-demethyl cynometrine. (structure below).



CXL

The ^{13}C-NMR spectrum was also in complete agreement with the proposed structure. With the exception of the absence of the signal at δ 33.1, previously assigned to the imidazole N-methyl carbon, the shift values compared favourably to those of CHM-2 (Table 8).

(i) Conversion of N_1-demethylcynometrine to cynometrine

N_1-demethylcynometrine was refluxed under alkaline conditions in dry acetone with excess methyl iodide for 24hrs. From the reaction mixture a brown gummy material was obtained which gave positive reaction with Dragendorff's reagent, but gave Rf of 0.0 with all normal systems.

The UV and IR spectra were very similar to those of cynometrine. High resolution mass spectroscopy failed to give a satisfactory parent ion. The ^1H NMR spectrum was characterized by the occurrence of three N-methyl signals at δ 2.8 for the lactam ring and at δ 3.80 and δ 3.84. The latter two must be assigned to the imidazole ring presumably due to an $\overset{+}{N}(Me_2)$ quaternary group. Further support for the PMR assignments is observed in the mass fragmentation pattern which shows major fragments at m/e 193 and m/e 122 (Scheme 5).

In a second methylation experiment equimolar amount of CHM-3 and methyl iodide were used. This reaction yielded crystalline compound melting point 211° which, besides the positive result with Dragendorff's, had an Rf value identical to CHM-2.

The spectral characteristics of this compound were all identical to those of cynometrine thus confirming the relationship between cynometrine and N_1-demethylcynometrine.

2:3:2:3 Characterization of CHM-1 as Cynodine (CIX) p.33

The brown gummy substance (Rf 0.74 in solvent system 3 p. 54) gave similar reactions to cynometrine with Dragendorff's spray reagent and the sodium fusion test. The UV spectrum however, Figure 3 showed an extra maximum at 281nm. The IR spectrum showed an extra carbonyl at 1730 cm^{-1} typical of a benzoic acid ester but lacked the hydroxyl absorption band of cynometrine. Accurate mass measurement indicated an empirical formula $C_{23}H_{25}N_3O_3$ in agreement with that required for the 11-benzoate of cynometrine. This compound has been previously reported, under the name cynodine, from the leaves of C. ananta [18] but little spectral data has been reported.

The PMR spectrum (Figure 6) accounted for all 23 protons. Two sharp singlets (3H each) at chemical shifts identical to those recorded in cynometrine suggested N-methyl-substituents on the lactam and the imidazole rings. Eleven protons were found in the aromatic region, eight between δ7.25 and 7.35 and three, deshielded to δ 7.79 - 7.96. The latter must include the two protons ortho to the carbonyl moiety of the benzoic acid substituent. The third must be the deshielded 2 -proton of the N-Me imidazole system. In accord with the proposed structure, the doublet for the C-11 proton (J =5Hz) was shifted downfield to δ 6.6 (cf. 5.05 in cynometrine) due to the deshielding effect of the esterifying benzoic acid group. The C-5-proton of the N-methyl imidazole overlapped the C-11 signal at δ 6.6 (J = 1Hz). The ill-defined multiplet between δ 3.50 and 3.00 represented the remaining protons of the lactam ring, as in cynometrine and N_1-demethylcynometrine (pp. 66, 77).

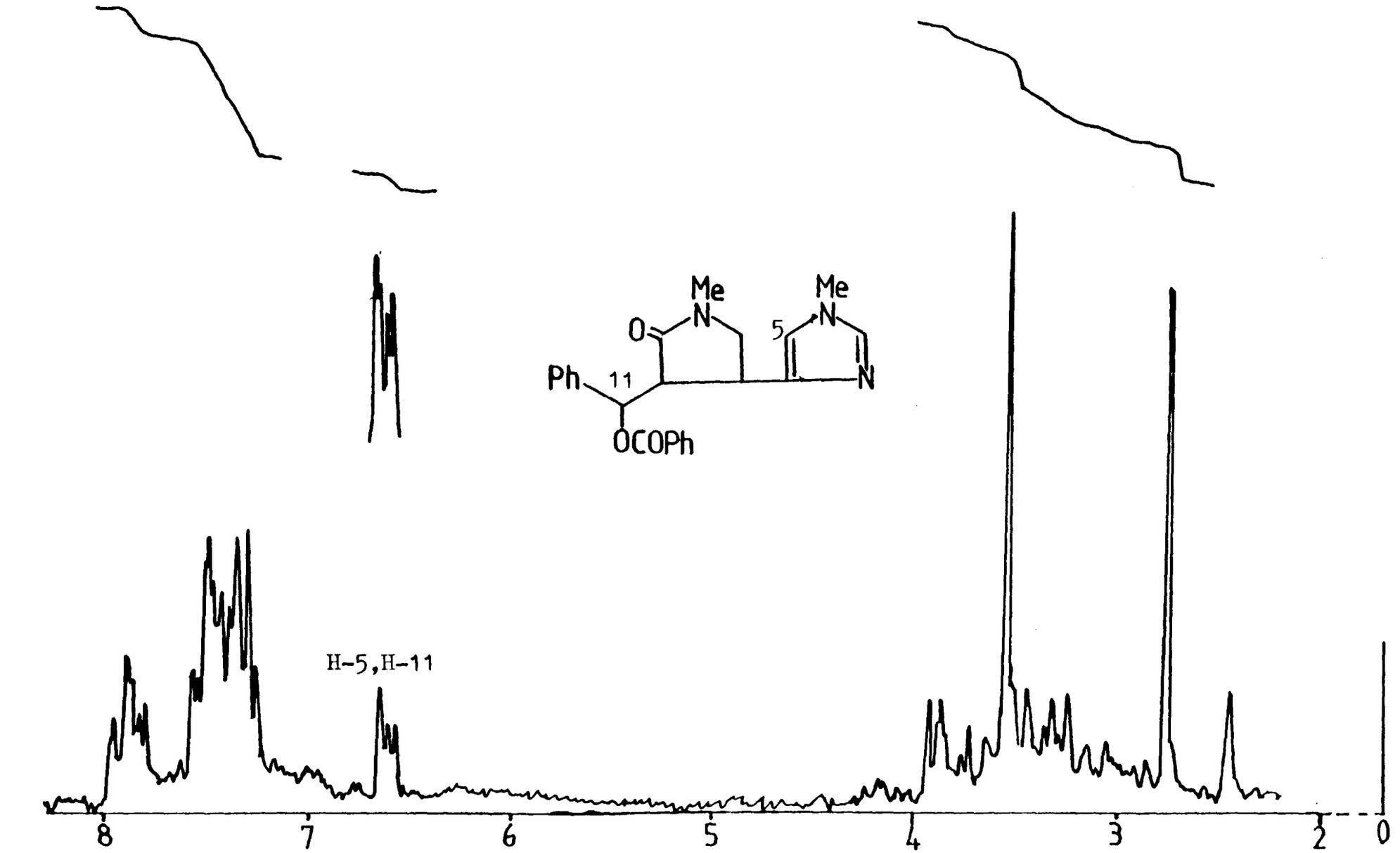
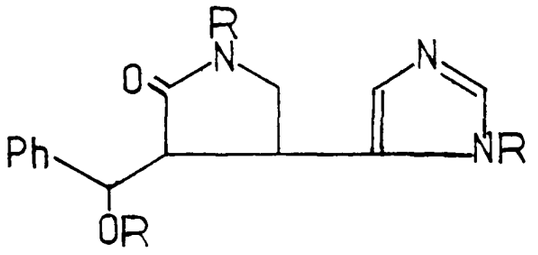


FIGURE 6. ^{1}H NMR SPECTRUM OF CYNODINE (CHM-1)

Further support for compound CHM-1 being the benzoic acid derivative of cynometrine is obtained from its fragmentation pattern which showed major ions at m/e 284 ($M^+ - C_7H_5O$) and m/e 105 characteristic of compounds with a benzoate substituent. It further loses OH^- to give m/e 267 ($M^+ - C_7H_5O$, OH) identical to that recorded for loss of water from cynometrine (Scheme 5). The remaining significant fragments are also identical to those of cynometrine (Scheme 5).

The deshielding of the C-2 and C-5 protons of the imidazole moiety relative to cynometrine need to be explained. It seems possible to rationalise this phenomenon in terms of spatial effects of the benzoic acid substituent but a possible alternative would be the occurrence of the iso-form of the imidazole ring, below



Iso-alkaloids of this class have been recorded from C.lujae[109] and show a strong deshielding of the C-4 proton because of the loss of the electron donating effect on the adjacent N-methyl substituent. However unpublished data for isocynodine (CXII)[140] shows C-11 and the C-4 proton signals to be separated by about 0.3ppm. whereas in CHM-1 they overlap. CHM-1 must therefore be cynodine and the deshielding of the C-2 proton must be due to the spatial effects of the C = O of the benzoic acid ester group.

The interrelationship of CHM-1 and cynometrine was confirmed by saponification of the former which gave a product identical, in

all respects, with the latter.

2:3:2:4 Characterization of CHM-4 as N_1-demethylcynodine (CXLi) p.82

The brown amorphous compound showed up as a pale blue spot under UV light and gave a colour reaction with Dragendorff's reagent and the nitrogen sodium fusion test identical to the other alkaloids characterized. The UV spectrum (Figure 3) was identical to that of cynodine. The IR spectrum (Figure 7) was similar to that of cynodine in the carbonyl region and lacked a hydroxyl band. However a sharp absorption band circa 3200 cm^{-1}, reminiscent of nor-cynometrine, indicated an NH group. The high resolution mass spectrum gave an exact mass of 375.1551 indicating an empirical formula of $C_{22}H_{21}N_3O_3$, 14 mass units (CH_2) less than for cynodine.

The ^1H NMR spectrum (Figure 8) in $CDCl_3$ showed only one 3H singlet, at δ 2.77, for the lactam N-methyl. The aromatic region showed 12 protons, nine associated with the signal centred at δ 7.22-7.68 and three at δ 7.75 - 7.96. Two of the latter must be assigned to the protons ortho to the carbonyl of the benzoic acid substituent and the third to the C-2 proton of the imidazole ring. There is again a doublet at δ 6.60 (1H, J = 2Hz) assosiated with the C-11 proton as in cynodine. Another 1-H doublet, at δ 6.82 (J = 2Hz), can be assigned to the C-5 proton of the imidazole nucleus. Since only 8 or the 9 protons in the δ 7.22 - 7.68 multiplet can be assigned to the phenyl rings five due to C-3' - C-5' the ninth must be associated with the N-H proton of the imidazole.

The mass fragmentation gave an M^+ - 105 ion at m/e 270 (Scheme 5) which then lost OH^- to give an ion at m/e 253 (p.68) identical to the fragmentation exhibited by nor-cynometrine.

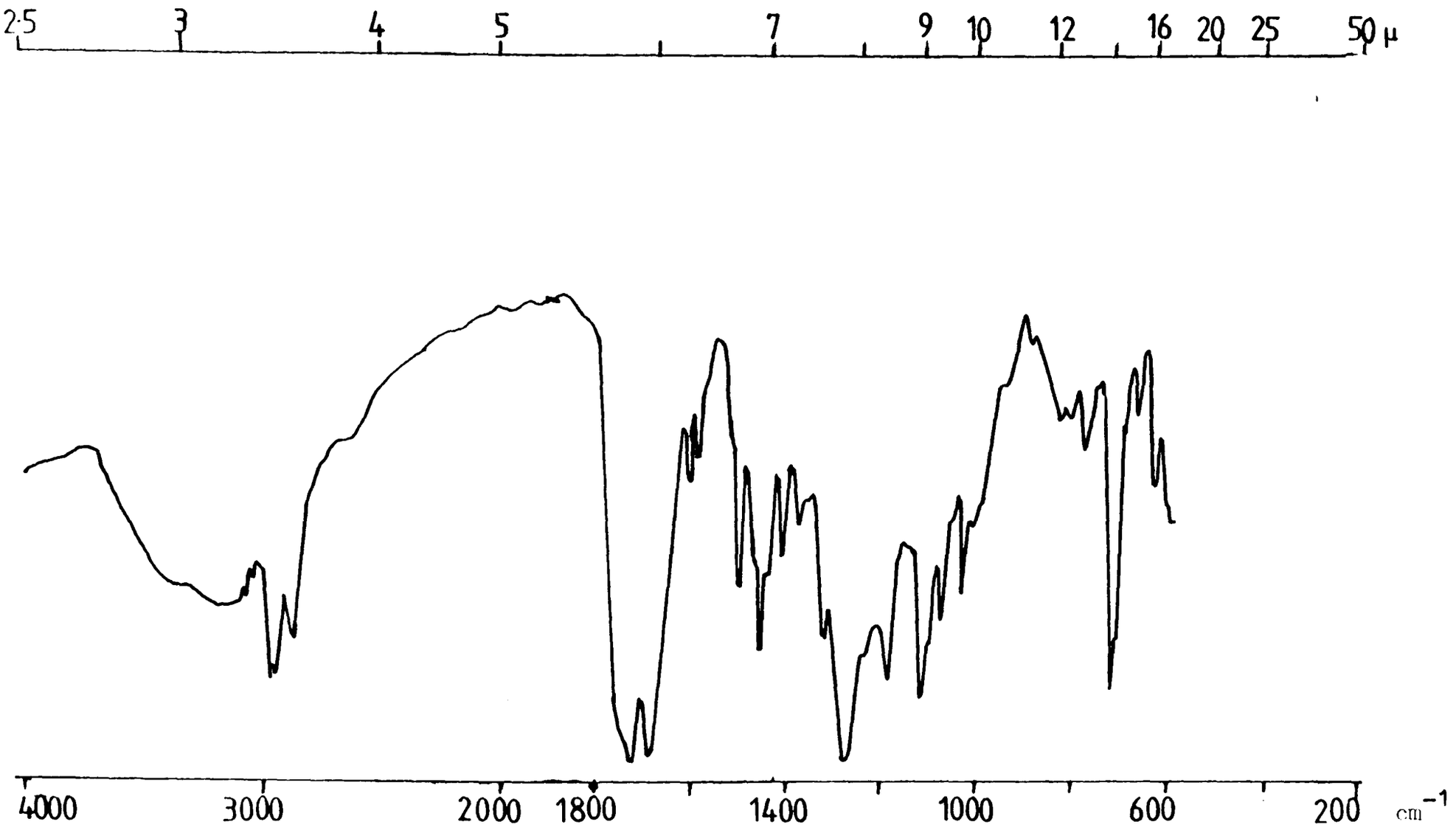


FIGURE 7. INFRA RED SPECTRUM OF N_1-DEMETHYLCYNODINE (CHM-4)

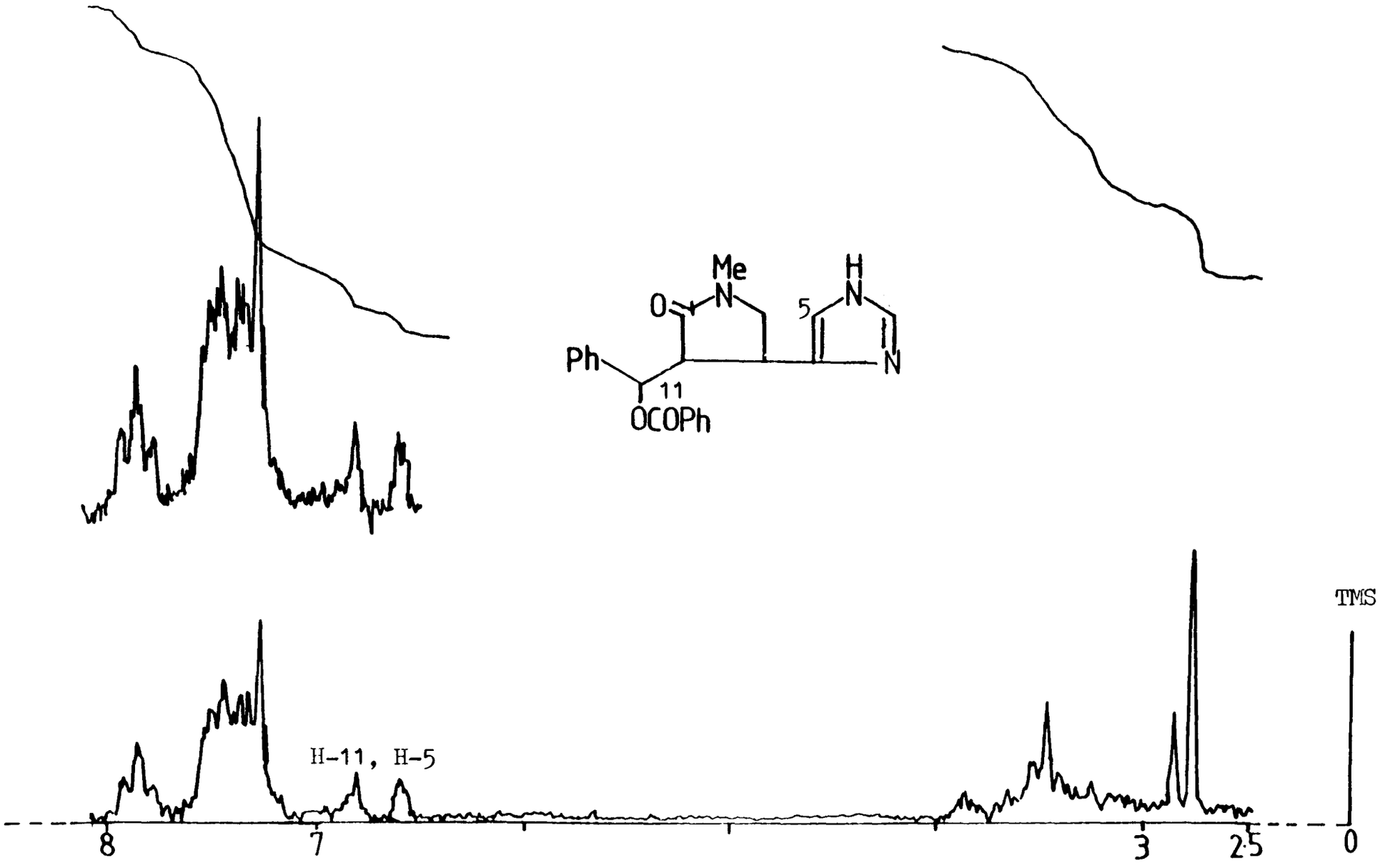
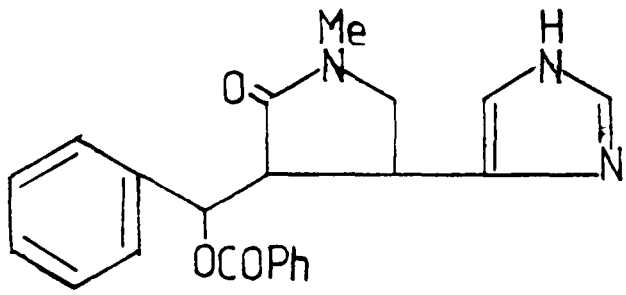


FIGURE 8. ^{1}H NMR SPECTRUM OF N_1-DEMETHYLCYNODINE (CHM-4)

Further major mass fragments were observed at m/e 165 and 94 which are 14 mass units ($-CH_2$) less than corresponding values for cynodine but identical to those of N_1-demethylcynometrine (Scheme 5). These data therefore identify CHM-4 as the benzoate of N_1-demethylcynometrine and the compound was accordingly named N_1-demethylcynometrine.

An attempted methylation of CHM-4 using an equimolar amount of MeI, in dry acetone under alkaline conditions gave, on TLC of the resulting brown gummy residue, a compound with an Rf value and reaction with Dragendorff's reagent that were comparable to those of cynodine. The ^1H NMR of the residue showed an additional 3-H singlet at δ 3.53 for the imidazole methyl substituent. This confirms the relationship between cynodine and N_1-demethylcynodine which must therefore have the structure below.



CXL1

2:3:3 THE ISOLATION OF COMPOUNDS FROM CYNOMETRA HANKEI SEEDS

2:3:3:1 Preliminary Analysis

The ground seeds were extracted separately and successively with light petroleum (b.p.40 - 60°) chloroform and methanol. The concentrated light petroleum and chloroform extracts did not reveal any compound when screened in various solvent systems on both alumina and silica gel. The concentrated methanol extract however revealed three spots, typical of alkaloids, when sprayed with Dragendorff's reagent.

2:3:3:2 Isolation of the compounds from the methanol extract of C. hankei seeds

The methanol extract was chromatographed over an alumina column to give, on elution with chloroform containing increasing amounts of methanol, three compounds; CHS-1 obtained from chloroform alone as the eluent; CHS-2 obtained with a solvent mixture of chloroform/methanol 49:1; CHS-3 obtained on further elution with the same solvent.

2:3:3:3 Characterization of CHS-1 as Cynodine (CIX) CHS-2 as Cynometrine (VIII) and CHS-3 as N_1-demethylcynometrine (CXL1)

The physical and spectral characteristics of CHS-1, CHS-2 and CHS-3, were identical to those previously recorded for cynodine (p.76) cynometrine (p.65) and N_1-demethylcynometrine (p.67), respectively.

2:3:4 THE ATTEMPTED ISOLATION OF COMPOUNDS FROM CYNOMETRA MANNII STEM BARK

The powdered stem bark was extracted separately and successively with light petroleum (b.p. 40 - 60°), chloroform and methanol. The concentrated extracts when screened by TLC in various solvent systems on both alumina and silica gel did not reveal any identifiable compounds.

The dried methanol extract when treated with NH_3 and partitioned with $CHCl_3$, did not give positive tests for the presence of alkaloids, unlike *C. hankei* extracts after identical treatment.

2:3:5 BIOLOGICAL SIGNIFICANCE OF THE ALKALOIDS OF C. HANKEI

These alkaloids were not initially observed in the methanol extract of the stem bark on screening, and could have been missed altogether. It seems likely that this initial resistance to visualisation occurred because they were bound in some way and treatment with alkali was necessary to release them. The stem bark of C. hankei is known to contain very large amounts of condensed tannins (about 20% w/w)[114] and the presence of the alkaloids as tannates could account for their original non-appearance. This assumption is supported by the fact that in seeds, which contain a very small amount of tannins relative to the stem bark, the alkaloids were observed immediately on screening of the methanol extract. Hennart[92] did comparable alkali extraction from the stem bark of C. ananta to isolate the alkaloids listed in Table 11. The iso-analogues were similarly isolated by Tchissambou.[109, 139]

The alkaloids cynodine, cynometrine and anantine have been shown to have pharmacological activities including spasmolytic, weak central depressant and analgesic effects but are not toxic.[92] C. hankei, a common species in the Douala-Edea Reserve, appears to be a potential alternative source of the first two of the alkaloids.

The alkaloids, including their iso-analogues appear, to date, to occur exclusively in the genus Cynometra (Table 11). The alkaloids have been found in more than one location in the plants studied. Whilst, in two species, only the normal alkaloids occur, in C. lujae the iso-analogues are also found. The widespread occurrence of the alkaloids in various parts of the plants seems, therefore, to be of chemotaxonomic significance. The absence, however, of any of the alkaloids from the stem bark of C. mannii indicates that

not all species of Cynometra need necessarily be expected to produce these alkaloids,

TABLE 11. THE DISTRIBUTION OF ALKALOIDS IN CYNOMETRA SPECIES

	C.lujae	C.ananta	C.hankei
Anantine	Sb.,rb.,1	Sb. 1	
Isoanantine	Sb.,rb.		
Nor-anantine	Sb.,rb.		
Cynodine		Sb. 1	Sb. sds.
Isocynodine	Sb.,rb.,1		
N_1-demethylcynodine			Sb.
Cynometrine	Sb., 1	Sb. 1	Sb., sds.
Isocynometrine	Sb.		
N_1-demethylcynometrine			Sb., sds.
Meta-hydroxy-anantine	Sb.,rb.		
Cynolujine	1		

Sb. = Stem bark; rb. = Root bark; 1 = leaves; sds. = seeds

2:4 THE ISOLATION OF COMPOUNDS FROM SESBANIA MACROCARPA SEEDS

2:4:1 Preliminary Analysis

The powdered seeds were extracted with methanol. The concentrated methanol extract was examined by TLC. Visualisation with ferric chloride showed a black spot (Rf 0.18) after development on silica gel chromatoplates in solvent system 6, (p.54). The same compound reacted with vanillin/sulphuric acid at 100° to give a black brown spot.

2:4:2 Isolation of compounds from the methanol extract of S. macrocarpa

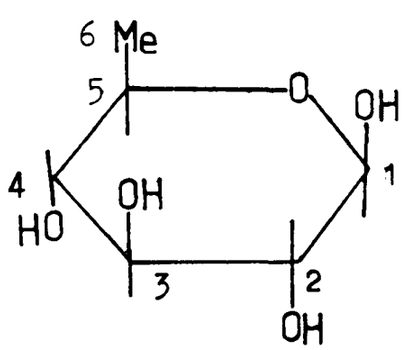
The concentrated methanol extract was initially partitioned with light petroleum (b.p. 60 - 80°). The petrol insoluble material was dried to give a brownish mass. Retreatment of the residue with methanol gave a soluble fraction which was filtered to remove the insoluble remainder. The methanol soluble fraction was then column chromatographed over silica gel to give, on elution with chloroform/methanol 4:1, a brownish amorphous mass designated SM-1.

2:4:2:1 Characterization of SM-1 as Kaempferol-3,7 di-rhamnoside (CXLIII p. 93)

The brownish deliquescent amorphous substance gave no definite melting point. When examined by TLC in solvent system it showed a pale yellow spot when viewed under UV light. Exposure to NH_3 vapour made the spot intensely yellow when re-examined under UV light, a reaction typical of a flavone glycoside.[140] On spraying with an alcoholic ferric chloride SM-1 gave, almost immediately, a black spot, suggesting the compound was phenolic.

The effect of the addition of different reagents on the UV spectra of flavonoids has proved to be a valuable technique in the structure elucidation of these compounds.[141] Table 12 gives data for the maxima observed when the UV spectrum of SM-1 was run in a number of these reagents. A bathochromic shift to 396 nm (original 348nm) on the addition of NaOH is typical of a 4' hydroxy flavonoid. The addition of $AlCl_3$ also gave a bathochromic shift to 400nm. This shift is associated with flavonoids with a free 5 hydroxy group. The addition of NaOAc and subsequently of H_3BO_3 to the same solution did not produce any change in the UV. The absence of a bathochromic shift on addition of NaOAc indicates that there is no free 7-hydroxy substituent. Absence of a shift on further addition of H_3BO_3 indicates there is not 3' and 4' substitution.

Support for the hypothesis that the compound was a glycoside was obtained from the ^1H NMR spectrum which showed signals at δ5.25 (1H, d, J = 2Hz, H-1) δ4.12 - 3.53 (m H-2, H-3, H-4, H-5); δ1.27 - 1.16 (3H , d, 6-CH_3); which are typical of a rhamnosyl moiety (CXL11). Integration of the identified resonances against those of the aromatic region of the flavonoid indicated the presence of two rhamnosyl units.



(CXL11)

Further supporting evidence for the compound being a glycoside was obtained from its hydrolysis using 0.5N HCl. This yielded an

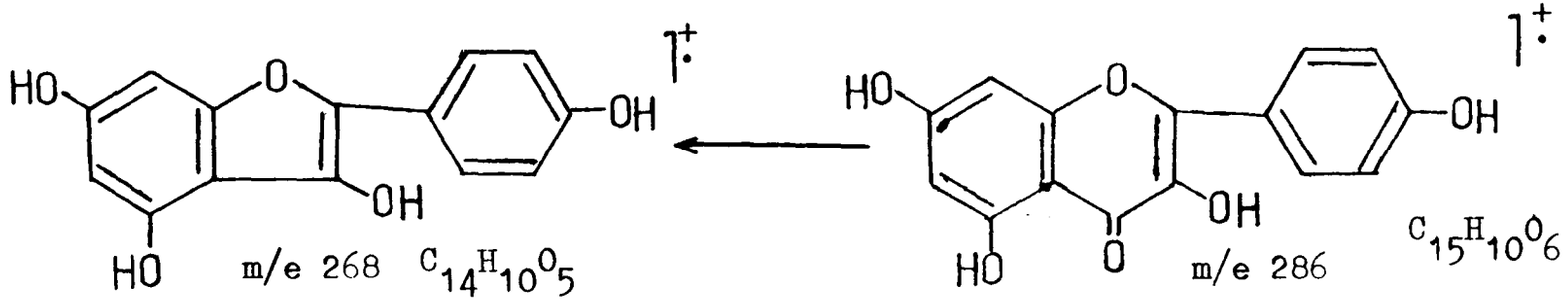
	BAND I	BAND II			SHIFT	INFERENCE
SM-1	268		348			
+ NaOH	270		396		+ 48nm	phenolic
+ $AlCl_3$	268	354		400	+ 52nm	5-OH
+ $AlCl_3$+HCl	268	348		400		
+ NaOAc	268	348			no free	7-OH
+ NaOAc+H_3BO_3	268	348				
SM-2	268	364			11nm	hypsochromic shift rel.to quercetin Band I
+ NaOH	278			410	+ 46nm	3-OH phenolic
+ $AlCl_3$	272(sh)	354		425	+ 61nm	5-OH
+ $AlCl_3$+HCl	272(sh)	354		425		
+ NaOAc	272	380			+ 16nm	7-OH
NaOAc+H_3BO_3	268	364				

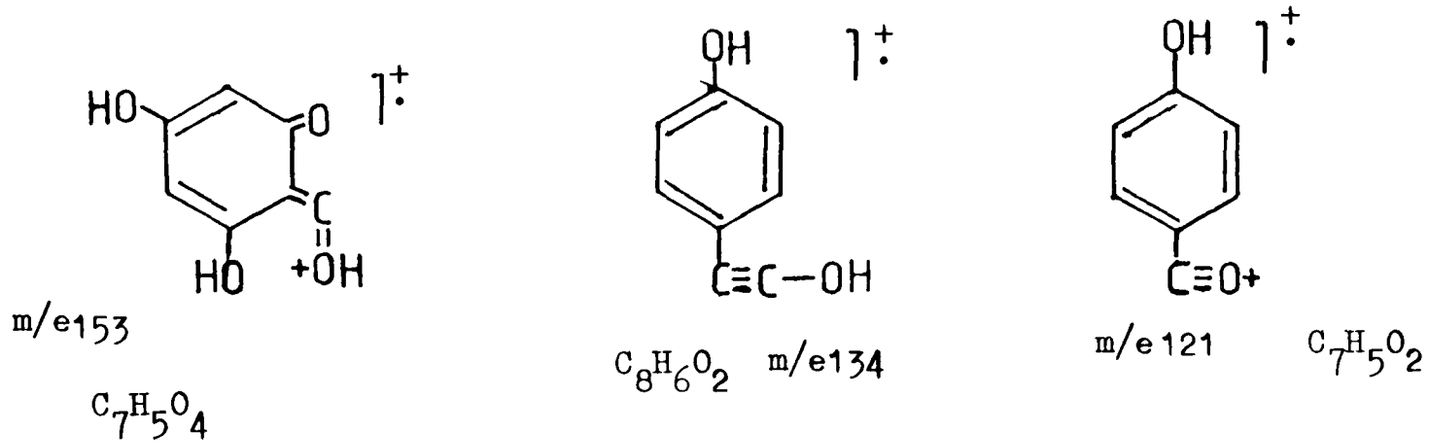
TABLE 12. UV SPECTRAL SHIFTS FOR SM-1 AND SM-2 ON ADDITION OF VARIOUS REAGENTS

ethyl acetate soluble compound SM-2 which had an Rf value of 0.46 in solvent system 6. Rhamnose was identified by TLC in the aqueous mother liquor (p.138) (cf. SM-1, Rf. 0.18).

The UV spectrum (Table 12 p.90) of SM-2 gave a maximum (Band 1) at 364nm, a bathochromic shift of 18nm by comparison with SM-1. The methylation or glycosylation of 3,5 and 4' hydroxyl groups on the flavonoid structure results in a hypsochromic shift particularly of Band 1 (range 300 - 400nm) of the spectrum.[142] The glycoside SM-1 has already been shown to have 5 and 4' hydroxyl groups; the bathochromic shift in the spectrum of SM-2 must therefore be associated with a 3 hydroxyl group on the aglycone. The addition of NaOH and ACl_3 again led to bathochromic shifts. In addition, however, there was now also a bathochromic shift with NaOAc (-16cm) unlike the situation observed in the glycoside. This shift is typical of flavonoids with a free 7 hydroxy group. There was no shift in the spectrum, on the addition of H_3BO_3 to the NaOAc/SM-2 solution, confirming the absence of a 3' hydroxy group. The UV spectrum therefore suggested the aglycone to be a 3,5,7,4' oxygenated flavone.

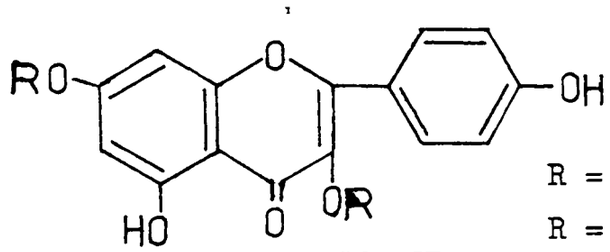
A high resolution mass spectrum of SM-2 gave an exact mass of 286.0478 indicating an empirical formula of $C_{15}H_{10}O_6$, which is in agreement with that required for a 3,5,7,4' tetrahydroxy flavone. Support for the proposed substitution was obtained from the fragmentation pattern of SM-2 (Scheme 7) which gave an M^+ - CO fragment at m/e 268, an ion at m/e 153 (A_1+H) unique to flavonols with 3-OH or 3-OCH_3 groups, and the m/e 134 (B_1) and m/e 121 (B_2) fragments which are also typical of flavonols.[142a] This pattern is similar to that noted for kaempferol (CXLIII, p.95) by Khalid.[143]





SCHEME 7. MASS FRAGMENTATION PATTERN FOR KAEMPFEROL (SM-2)

The proposed structure for SM-2 was confirmed by a ^{1}H NMR spectrum. Signals centred at δ 8.10 (2H) and δ 6.89 (2H) in the form of an AB quartet (J = 8Hz) are typical of the 2'3'5'6' protons of the 4' substituted B-ring of a flavonoid.[132] Signals at δ 6.22 and 6.42, again in the form of an AB quartet (J= 1Hz), are typical of the meta coupled 6 and 8 protons of the 5, 7—disubstituted hydroxy A-ring of a flavonoid.[133]



CXLIII

R = H Kaempferol

R = Rh Kaempferol-3,7,-di-rhamnoside

These data indicate that SM-1 was kaempferol 3, 7 diglycoside. Since the aqueous layer from the hydrolysis reaction showed only the presence of rhamnose and ^{1}H NMR of SM-1 showed only two rhamnosyl units to be present SM-1 can only be assigned the structure of kaempferol-3-7-di-rhamnoside (CXLIII). This is the first recorded characterization of kaempferol-3,7-di-rhamnoside in a Sesbania species. It has been previously reported from the leaves of Indigofera arrecta Hochst ex Rich [144,145] which belongs to the tribe Indigofereae of the sub-family Papilionaceae of the Leguminosae.

2:5 THE ISOLATION OF COMPOUNDS FROM THE SEEDS OF CAMOENSIA BREVICALYX

2:5 :1 Preliminary Analysis

The ground seeds of Camoensia brevicalyx were extracted separately and successively with light petroleum (b.p. 40-60°), chloroform and methanol. The concentrated light petroleum extract did not show any significant compounds, when developed in various solvent systems, on alumina or silica gel plates, and sprayed with different spray reagents. The concentrated chloroform extract revealed three compounds which, when developed on alumina plates in solvent system 3,(p.54) and sprayed with either Dragendorff's or Iodoplatinate spray reagent, gave reactions typical of alkaloids . The methanol fraction when similarly developed on alumina plates in solvent system 3(p.54) and sprayed with similar spray reagents to the chloroform extract, showed four alkaloidal spots three of which were identical to those of the chloroform extract. When the methanol extract was developed on a silica gel plate in solvent system 7,(p. 54) and the chromatoplate sprayed with Dragendorff's reagent another spot, in addition to those already observed, also gave an alkaloid positive reaction.

2:5:2 Isolation of compounds from the chloroform extract of C. brevicalyx

The concentrated chloroform extract was chromatographed over an alumina column and eluted with chloroform methanol 99:1 to give a mixture of compounds CC-M1 and CC-M2. Further elution with chloroform/methanol 49:1 gave a pure band which was concentrated to give a brown gummy compound CC-1. The mixture of CC-M1 and

CC-M2 gave, on standing, crystalline CC-M2. The remaining supernatant was separated by preparative TLC on alumina (solvent system 2, p.54) to give CC-M1 as a brown gummy mass.

2:5:2:1 Characterization of CC-1 as camoensine (CXX p.39)

The brown gummy solid has a UV spectrum (Figure 9) which showed a hypsochromic shift on the addition of 0.1N HCl to 297nm (original 308nm). This type of shift is associated with the formation of the salt of an alkaloid base having an α-pyridone ring.[146] The IR showed an absorption band at 1650cm^{-1} typical of an pyridone carbonyl and others at 1550cm^{-1} and 1570cm^{-1} typical of C = C bands. The mass spectrum gave an exact mass of 230.1419 indicating an empirical formula $C_{14}H_{18}N_2O$ identical to that of the two known quinolizidine alkaloids camoenside (CXX) and leontidine (CXIX) (see page 39).

The ^{1}H NMR spectrum (Figure 10) showed three pairs of double doublets centred at δ 7.31 (J_1 = 9Hz, J_2 = 7Hz, 1H); 6.43 (J_1 = 9Hz, J_3 = 1Hz, 1H) and 6.03 (J_2 = 7Hz, J_3 = 1Hz, 1H), identical to those of the H-4, H-5 and H-3 protons of camoensine, respectively.[116] In addition the AB part of an ABX system was centred at δ 4.2 and 3.96 (J_{AB} = 14Hz, J_{AX} = 7Hz). These signals can be assigned to the protons at C-10 which, being to the nitrogen of the pyridone nucleus and to the carbonyl of that nucleus are deshielded. They form the AB protons of an ABX system by coupling with the C-9 methine proton. The J-values obtained were similar to those of the known compound camoenside (CXX p.37).[116]

Further support for the co-identity of CC-1 and camoensine was obtained from the mass spectrum fragmentation pattern of camoensine.

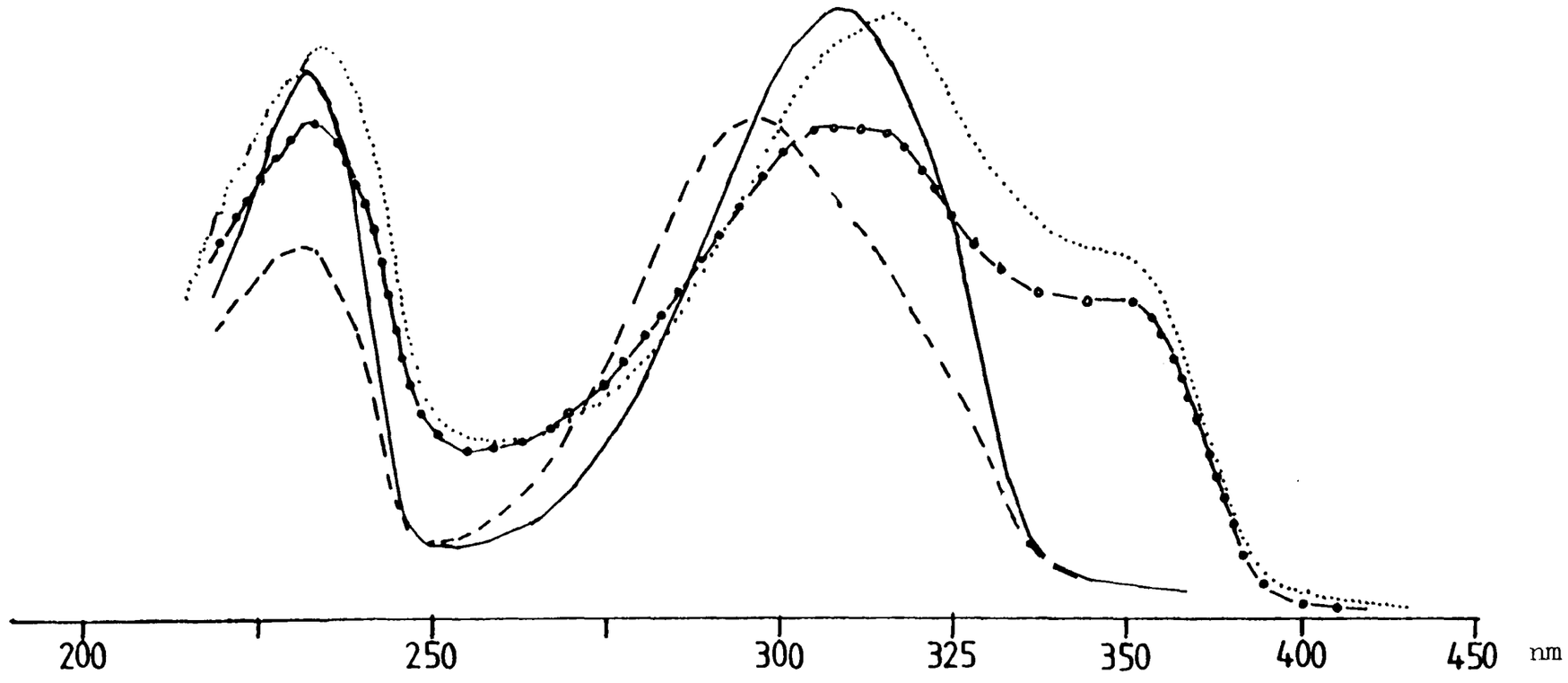


FIGURE 9 . UV SPECTRUM OF CAMOENSIA ALKALOIDS

CC-1/CC-2:(-) (+HCl)---; CC-3:(.....), (+HCl) (o-o-o)

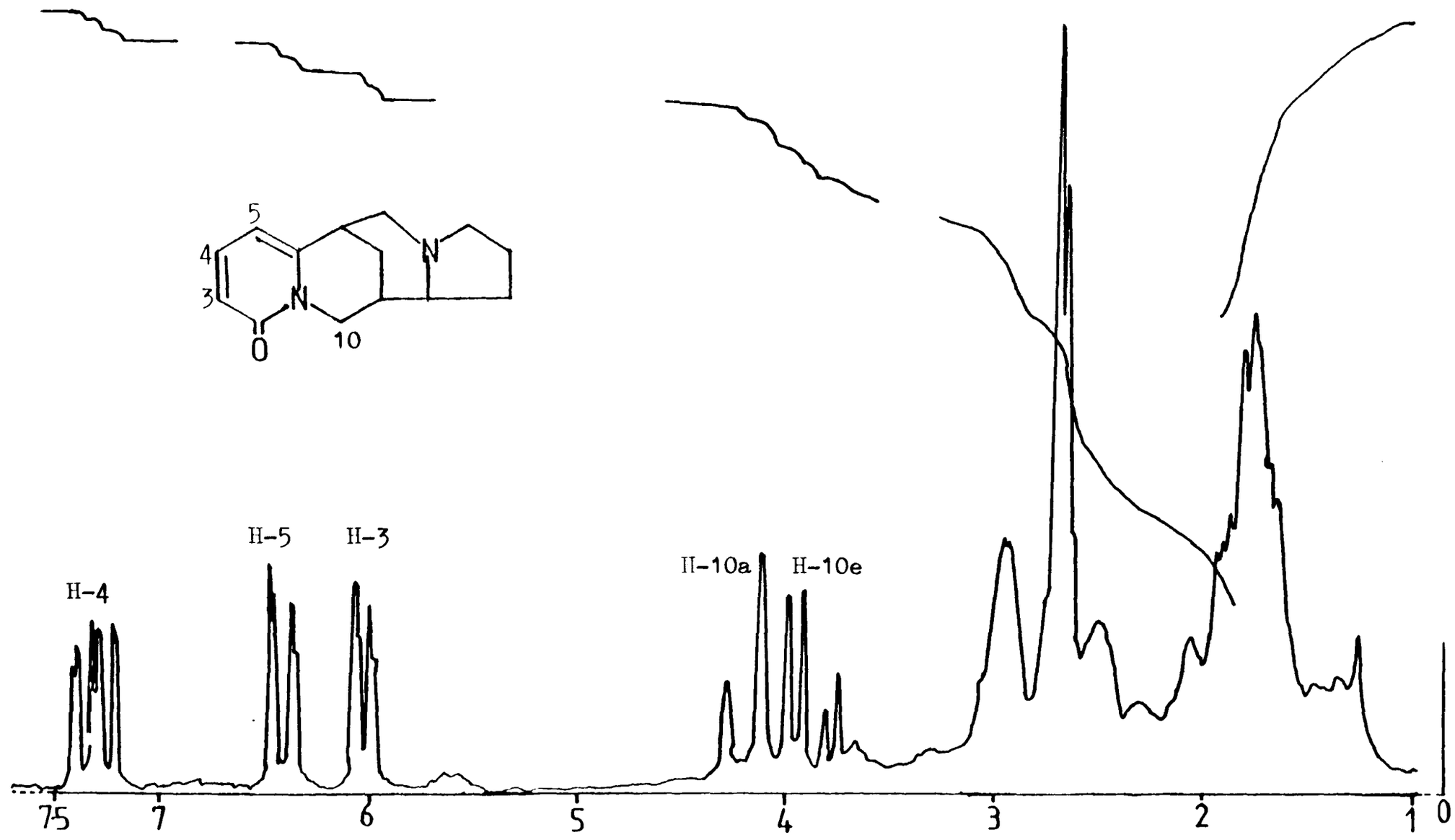
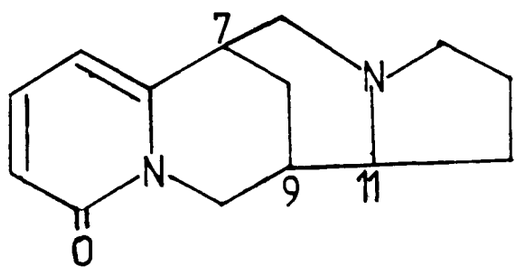


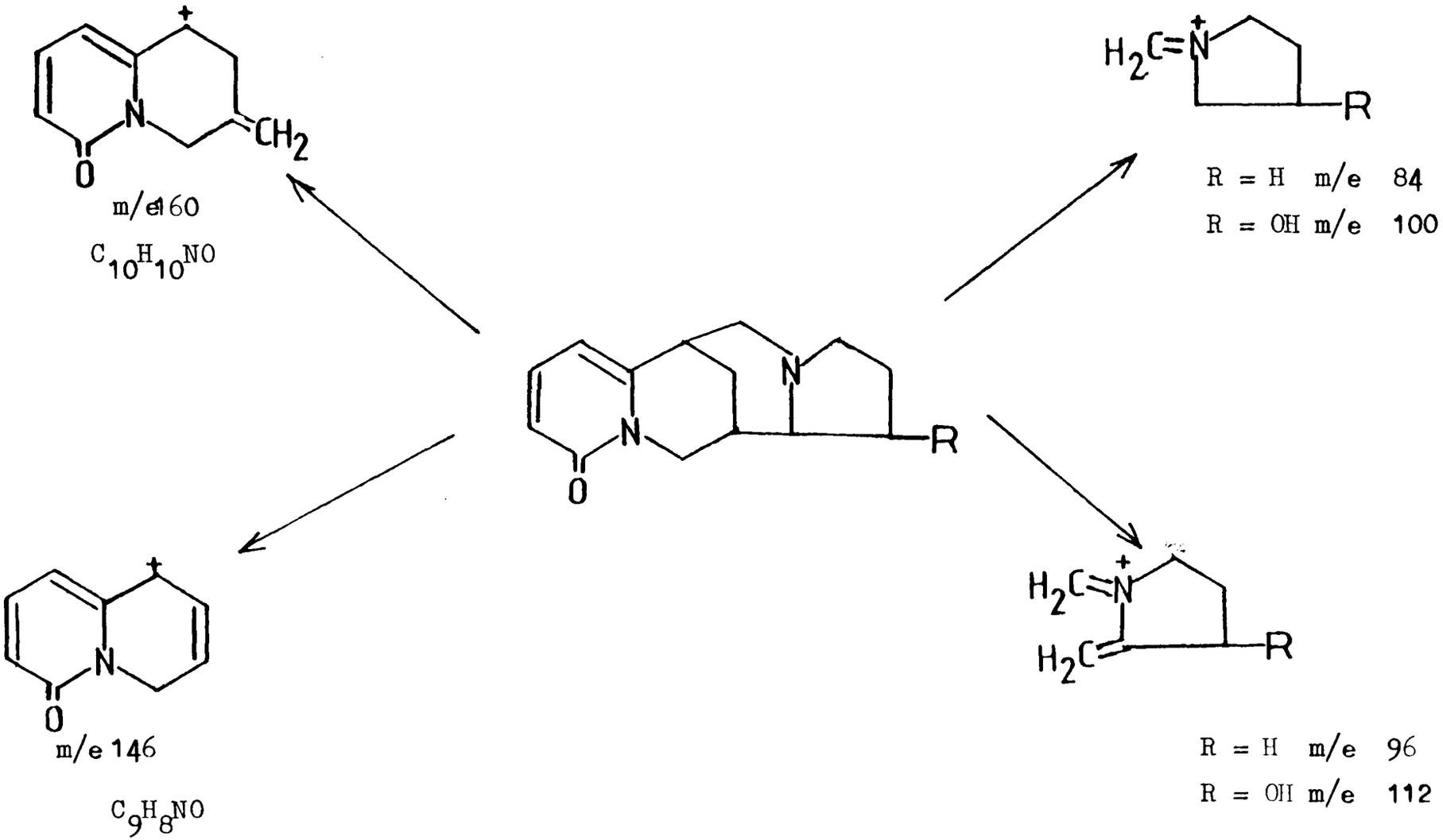
FIGURE 10. ^{1}H NMR SPECTRUM OF CAMOENSINE (CC-1)

(Scheme 8). Major fragments at m/e 160 ($C_{10}H_{10}NO$), m/e 146 (C_9H_8NO) and m/e 84 ($C_5H_{10}N$) were typical of the fracture of the C-ring of a tetracyclic quinolizidine.[147] The m/e 84 ion is of particular significance as it confirms the five membered D-ring (cf. m/e 98) in typical tetracyclic quinolizidines.[147]

These data indicate that CC-1 has a structure identical to that of leontidine (CXIX) and camoensine (CXX). No data have been previously reported on the ^{13}C-NMR spectra of this type of quinolizidine alkaloid. The ^{13}C-NMR spectrum gave chemical shift values (Table 12) for the carbon atoms of the A and B rings that were comparable to those of C-1 to C-10 of anagyrine (I, p. 11)[148] except at C-9 where CC-1 showed a shielding of about 3.5ppm. A plausible explanation of the difference of 3.5ppm between C-9 of CC-1 and C-9 of anagyrine would be the change in stereochemistry that would result from the D-ring being pentacyclic instead of hexacyclic. The rest of the spectrum of CC-1 gave values that were similarly comparable to anagyrine if the C-13 of anagyrine is excluded and the C-13, C-14 and C-16 of CC-1 were compared with C-14, C-15 and C-17 of anagyrine respectively.

^{13}C-NMR can be employed to elucidate the stereochemistry of the C-9/C-11 bond and can therefore be used to distinguish between the isomers camoensine and leontidine. Anagyrine-type quinolizidines have three asymmetric carbon atoms (C-7, C-9 and C-11) but steric requirements necessitate the methylene

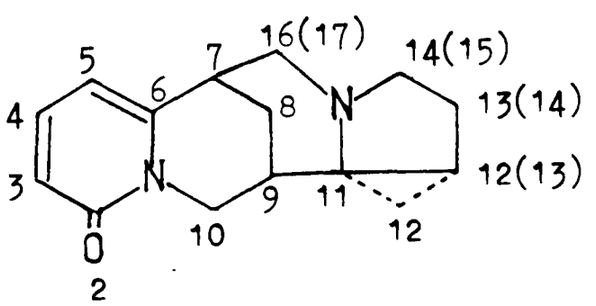




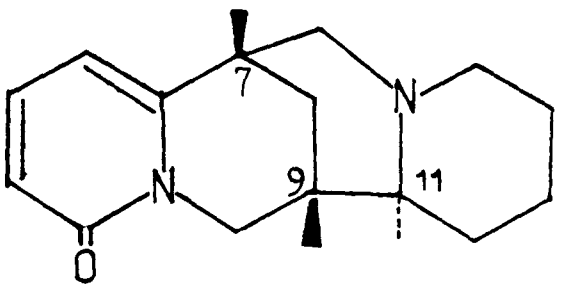
SCHEME 8. MASS FRAGMENTATION PATTERN OF CAMOENSINE (R=H) AND 12 HYDROXY CAMOENSINE (R=OH)

TABLE 13., ^{13}C NMR SHIFT VALUES FOR ANAGYRINE, CAMOENSINE, AND 12 HYDROXY CAMOENSINE.

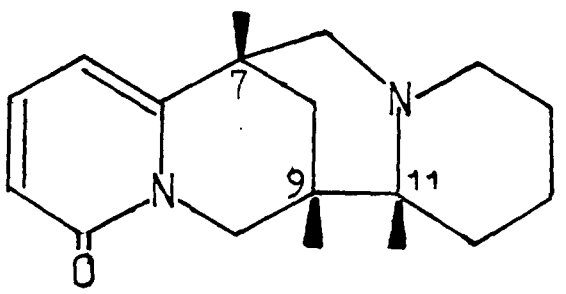
CARBON NO.	CC-1	CC-2	ANAGYRINE[148]
1			
2	164.1 s	164. 7 s	163.5
3	117.2 d	117.0 d	116.6
4	139.2 d	139.5 d	138.6
5	105.2 d	106.0 d	104.3
6	151.4 s	152.6 s	151.1
7	35.2 d	34.4 d	35.6
8	21.4 t	22.9 t	22.6
9	29.2 d	27.2 d	32.7
10	55.1 t	52.10 t	51.6
11	66.3 d	74.7 d	63.3
12	25.4 t	68.7 d	25.7
13	20.9 t	34.00 t	19.2
14	51.9 t	52.1 t	20.8
15			53.0
16	55.3 t	57.9 t	
17			54.4



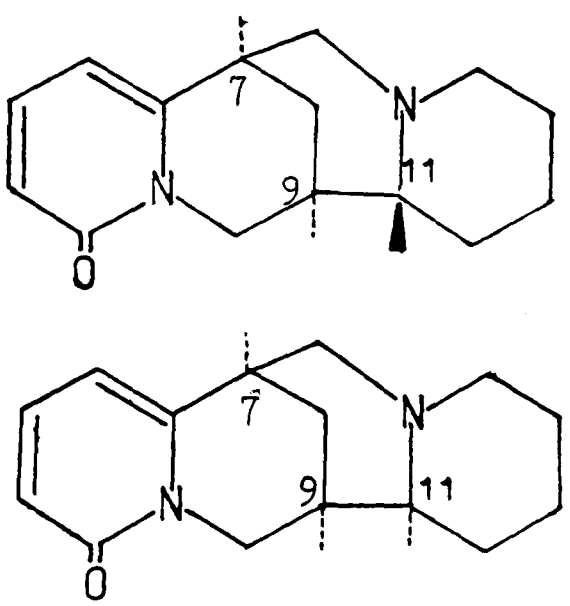
bridge between C-7 and C-9 being cis and the asymmetries at those positions are consequently interdependent.[149] This makes possible the existence of 4 stereoisomers (two racemic pairs of each structure below.)



CXLIV
(+)-Anagyrine



CLXV
(+)-Thermopsine



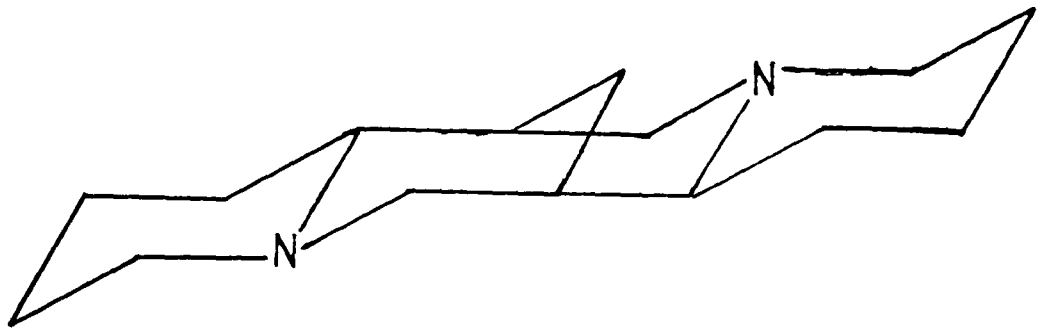
CLXVI
(-)-Anagyrine

CLXVII
(-)-Thermopsine

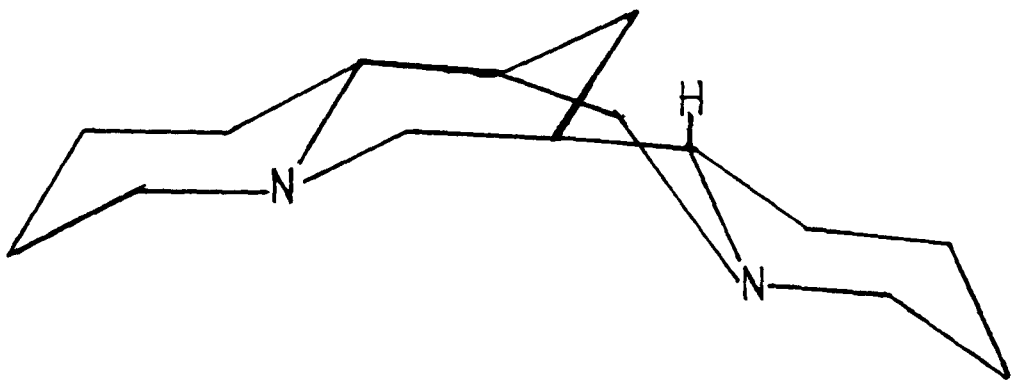
The laverotatory nature of CC-1 eliminates the configuration 7S: 9S:11S of (+)-anagyrine and 7S: 9S: 11R of (+)-thermopsine. This would restrict possible configurations to 7R: 9R: 11R and 7R: 9R: 11S of (-)-anagyrine and (-)-thermopsine respectively.

The ^{13}C-NMR spectra of quinolizidines [148] shows a correlation between the configuration of the C-11 proton and the C-8 chemical shift because of the so-called bowsprite/flagpole interaction [150] between C-8 and the C/D ring nitrogen.

This is illustrated by a comparison of the ^{13}C NMR spectra of sparteine CXLVIII and isosparteine CXLIX.



CXLVIII



CXLIX

The chemical shift of C-8 is always above 30ppm. when the C-9 and C-11 protons are in a cis configuration to one another e.g. as in isosparteine (CXLIX)[151] and thermopsine (e.g. 9R: 11S or 9S: 11R). On the other hand, when the C-9 and C-11 protons are in trans configuration (9R: 11R or 9S: 11S), as in sparteine (CXLVIII)[151] and anagyrine then the chemical shift for C-8 is below 30ppm.

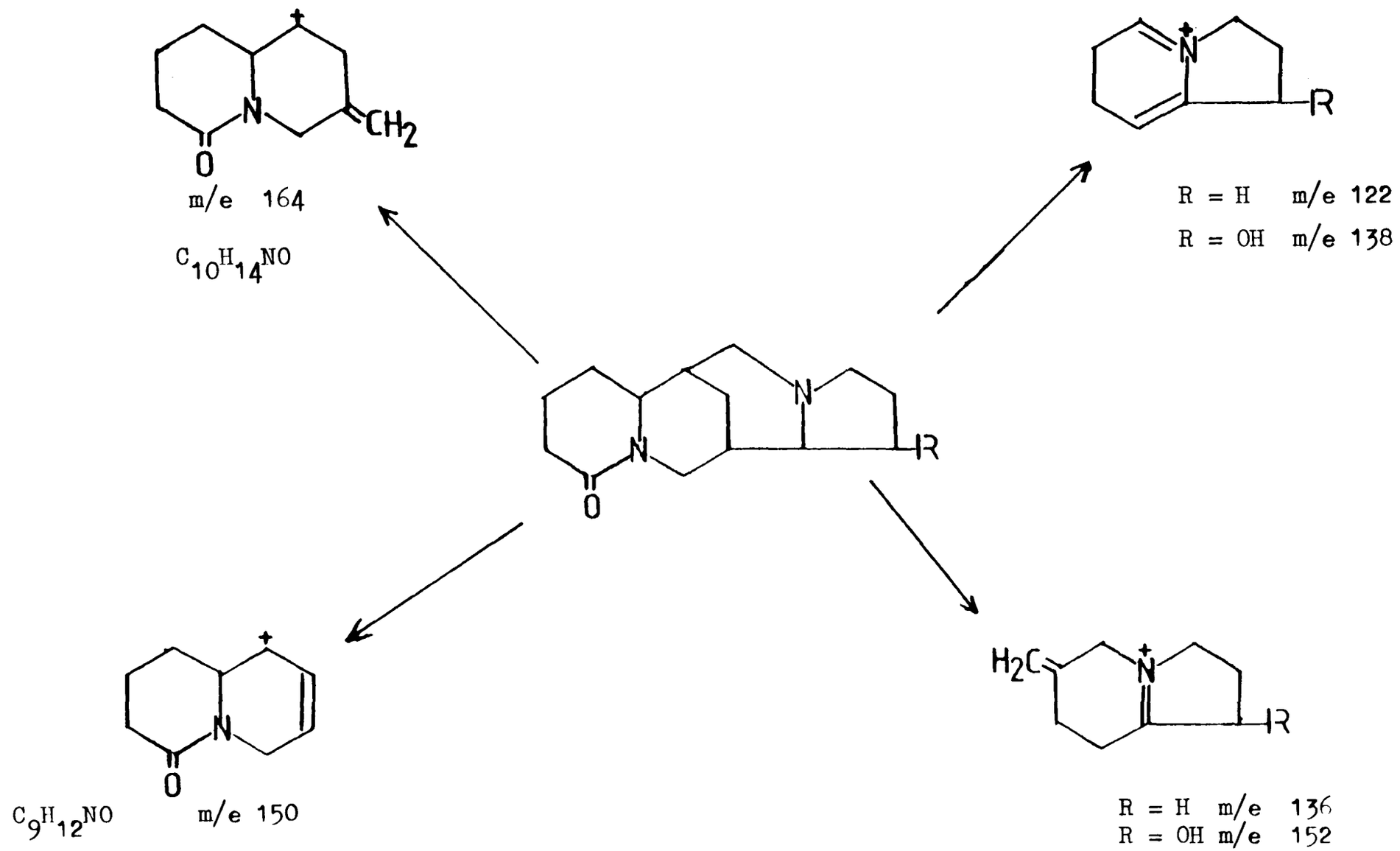
As the value obtained for the chemical chift of C-8 was 21.4ppm it must be in the 9R: 11R configuration. This therefore confirms the identity of CC-1 as camoensine (7R:9R:11R) rather than leontidine (7R:9R:11S).

2:5:2:2 Characterization of CC-M1 as Camoensidine (CXX1) p.39

The brown gummy compound gave no m.p. and no UV spectrum. The IR spectrum showed a carbonyl band at 1670cm^{-1} consistent with a six-membered lactam; and also CH_2 stretch bands between 2850 - 3000cm^{-1}. The IR lacked the C=C bands of CC-1 at 1550cm^{-1} and 1570cm^{-1}. Accurate mass measurement gave an empirical formula of $C_{14}H_{22}N_2O$. This formula and the above UV and IR data are consistent with a tetrahydrogenated derivative of a camoensine - type quinolizidine. Only one such compound has been reported: camoensidine (CXX1, p. 39),from the roots of Camoensia maxima.[116]

The ^1H NMR spectrum showed one part of an ABX system at δ3.8 - 4.10 (J_{AB} = 11Hz, J_{AX} = 9Hz), this resulting from the interaction of the methylene proton at C-10 and the methine C-9 proton. These data conflict with the reported resonance positions for the C -10 protons of camoensidine (δ 4.6, 3.40).[116] The absence of signals in the aromatic region further supports the hypothesis that CC-M1 is a fully saturated quinolizidine.

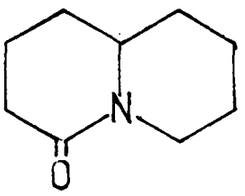
The mass spectrum fragmentation pattern of CC-M1 (Scheme 9) showed a major ion at m/e 164 ($C_{10}H_{14}NO$) due to the A and B rings of a saturated quinolizidine (cf. m/e 160 Scheme 9). The fragment at m/e 150 is similarly related to the m/e 146 ion



SCHEME 9. MASS FRAGMENTATION PATTERNS FOR CAMOENSIDINE AND 12 HYDROXYCAMOENSIDINE

which again confirmed the five-membered D-ring structure. Other ions at m/e 136, and m/e 122 do not occur in the spectrum of camoensine (Scheme 8 p.99). These ions occur due to cleavage of the quinolizidine through the B-ring, a pathway inhibited in camoenside because of the presence of the conjugated system of double bands that favour localization of the positive change in the A/B rings.[117] These data all suggest that CC-M1 is a tetrahydro derivative of camoensine or leontidine.

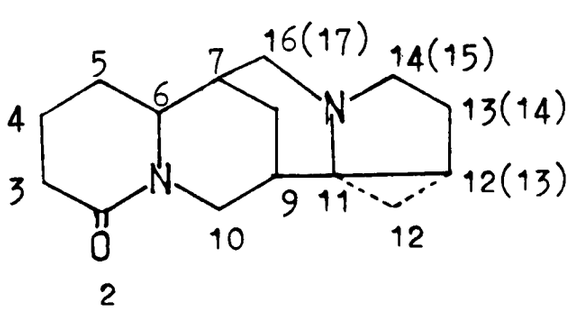
The ^{13}C-NMR of CC-M1 ($CDCl_3$) (Table 13) showed shifts for the A-ring that were identical to those of 4-oxoquinolizidine[148] (<u>vide infra</u>). The B, C and D rings had values that were similar

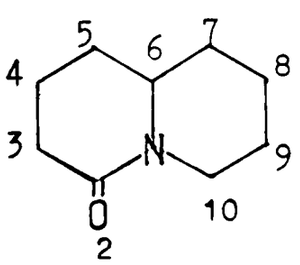


to those of lupanine.[148] The values of the C-8 chemical shift, as in camoensine, permits resolution of a <u>trans</u> configuration of H-9 and H-11 and indicates that CC-M1 is camoensidine.

TABLE 14. ^{13}C-NMR OF CC-M1, LUPANINE AND 4-OXOQUINOLIZIDINE

CARBON NO.	CC-M1	LUPANINE [148]	4-OXOQUINOLIZIDINE [148]
1			
2	175.2 s		
3	33.4 t	33.0 t	33.1
4	20.6 t	19.6 t	19.5
5	30.1 t	26.7 t	30.7
6	62.0 d	61.7 d	56.8
7	33.8 d	34.9 d	34.1
8	23.8 t	27.3 t	24.7
9	29.7 d	32.4 d	25.6
10	43.3 t	46.6 t	42.0
11	64.8 d	63.8 d	
12	30.5 t	33.5 t	
13	25.8 t	24.5 t	
14	55.7 t	25.3 t	
15		55.3 t	
16	60.5 t		
17		57.2 t	



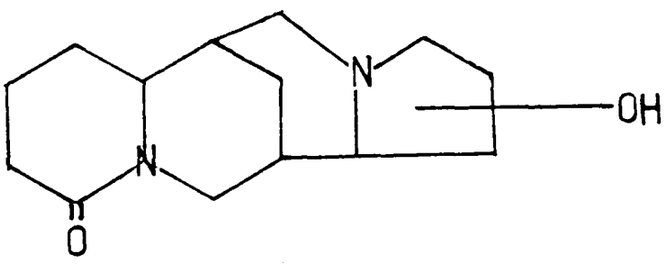


4-Oxoquinolizidine

2: 5:2:3 Tentative characterization of CC-M2 as 12-hydroxycamoensidine (CLI p.108)

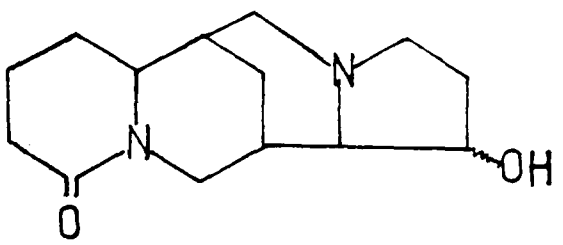
The crystalline compound melting point 200° did not give a UV spectrum. The IR spectrum was similar to that of CC-M1 but had, in addition, a sharp hydroxy band at 3250cm^{-1}. The mass spectrum gave an exact mass measurement indicating an empirical formula of $C_{14}H_{22}N_2O_2$. In the absence of sufficient material for a ^{1}H NMR spectrum attempts at structure elucidation are made on the basis of the other data available, particularly the mass spectrum.

The mass fragmentation pattern gave identical ions at m/e 164 and m/e 150 to those of CC-M1 (Scheme 9) suggesting that the A/B ring system was identical to that of camoensidine. The fragments associated with the C/D and D-rings of camoensidine were displaced by an additional 16 mass units and were found at m/e152, m/e 138, m/e 112 and m/e 100 respectively. These data further support the occurrence of an OH group and fix it in the D ring, giving the partial structure below.



From the data available it is not possible to identify the position at which the hydroxyl substituent occurs. Circumstantial evidence from the isolation of 12 - hydroxycamoensine (see page 109),

suggest that CC-M2 is 12-hydroxycamoensidine (CL1).



CL1

2:5:3 The Isolation of compounds from the methanol extract of the seeds of C. brevicalyx

An aliquot of the methanol extract was column chromatographed over alumina, eluting with chloroform containing increasing amounts of methanol. Four compounds were obtained, a mixture of CC-M1 and CC-M2 (in trace amounts), an additional quantity of CC-1; and a new pure compound CC-2. CC-2 gave a brown gummy non crystallisable solid. When another aliquot of the methanol extract was column chromatographed over silica gel, eluting with the same solvents, another alkaloidal compound, CC-3, was obtained.

2:5:3:1 Characterization of CC-M1 as camoensidine, (CXX1) CC-M2 as 12-hydroxycamoensidine (CL1) and camoensine (CX1X)

The physical and spectral data for CC-M1, CC-M2 and CC-1 were in agreement with that recorded previously for camoensidine, (p. 103), 12- hydroxycamoensidine (p. 107) and camoensine(p. 95).

2:5:3:2 Characterization of CC-2 as 12-α-hydroxy-camoensine (CL111)

The compound was non-crystalline and had a UV spectrum that was similar to that of CC-1 (Figure 9) with a band at 314nm showing an identical hypsochromic shift on the addition of 0.1 N HC1. The IR spectrum (Figure 11) was similar to that of camoensine with bands at 1657 (C = O, α - pyridone), 1570 and 1550cm^{-1} (C = C). An additional band for hydroxyl absorption was observed at circa 3400cm^{-1}. The high resolution mass spectrum gave an exact mass determination of 246.1368 which indicated an empirical formula of $C_{14}H_{18}N_2O_2$, differing from that of camoensine by the presence of an additional oxygen.

The ^{1}H NMR spectrum (Figure 12) was similar to that of camoensine and identical in the aromatic region where three double doublets, for H-3, H-4 and H-5, were observed. The H-10 protons were again observed as part of an ABX system. Coupling between H-10a and H-10e was 14Hz. Coupling between H-10e, centred at δ 4.20, and H-9 was in the order of 1Hz; and between H-10a, centred at δ 3.70, and H-9 was 6Hz. In 13-hydroxylupanine coupling between H-10e and H-9 is quoted as 2Hz.[151] A broad multiplet

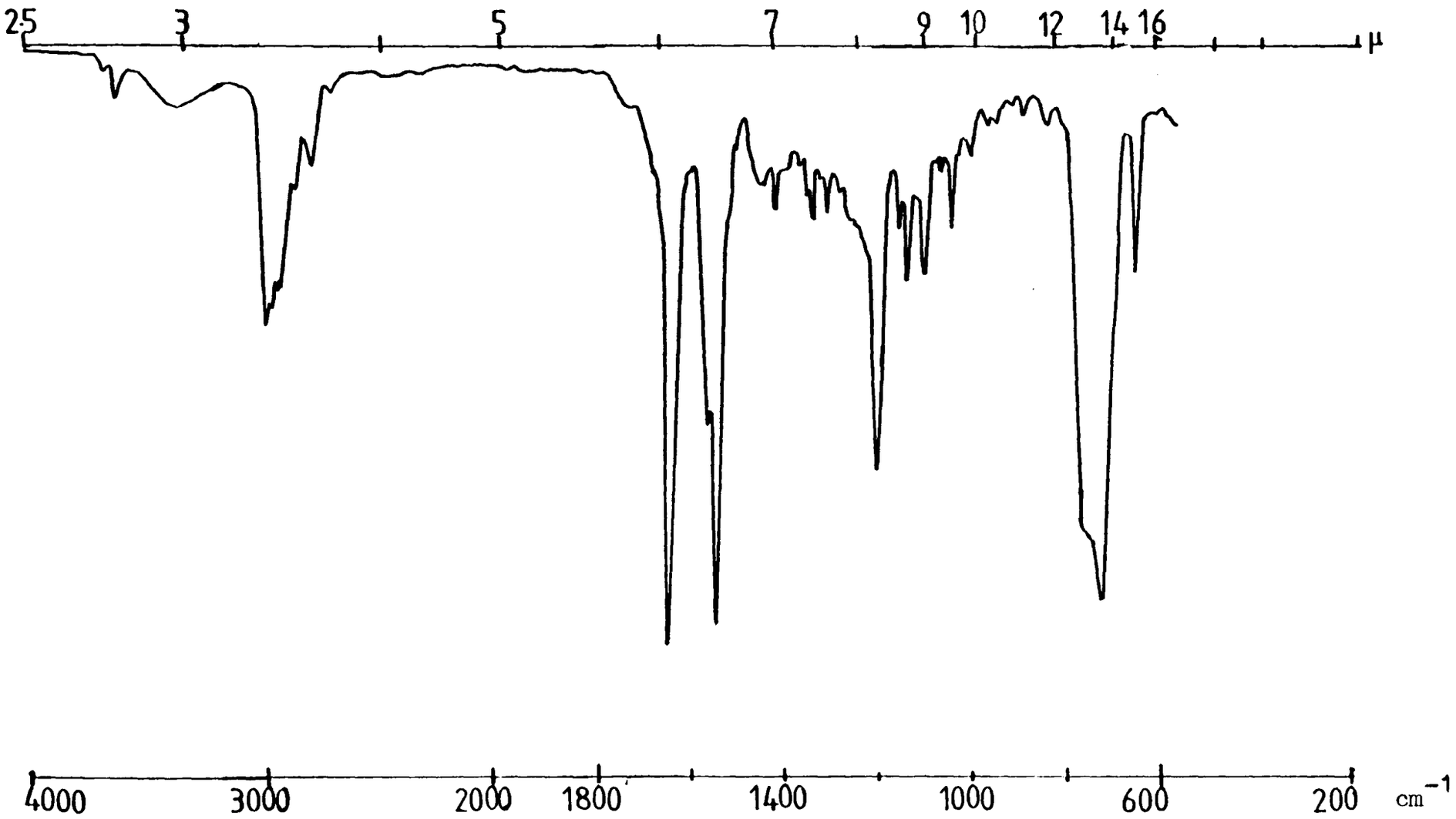


FIGURE 11. INFRA RED SPECTRUM OF 12- -HYDROXYCAMOENSINE

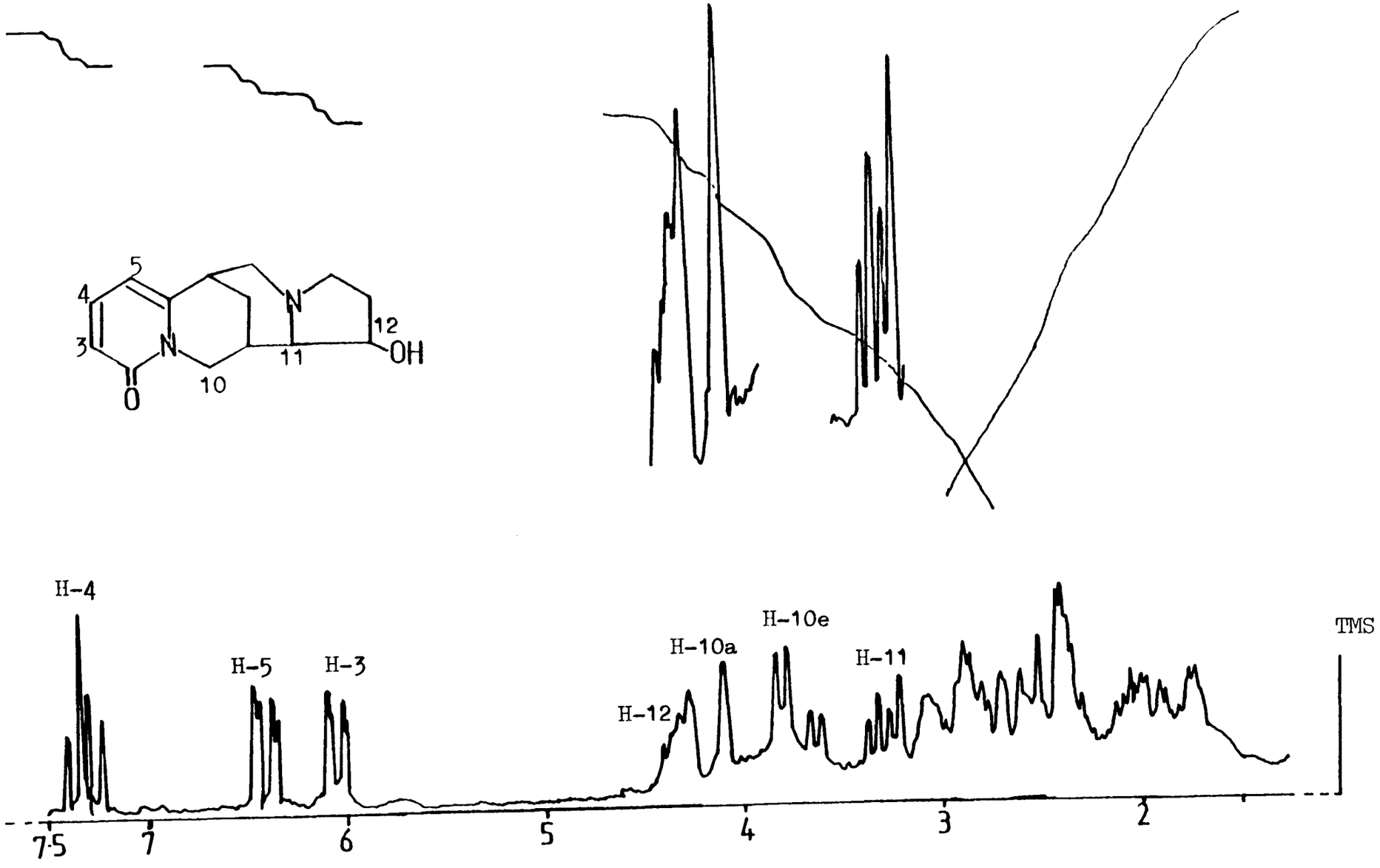
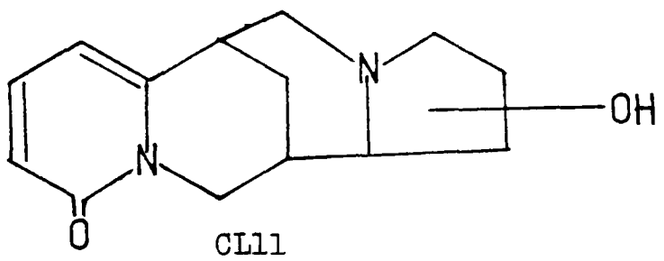


FIGURE 12. ^{1}H NMR SPECTRUM OF 12 HYDROXY CAMOENSINE (CC-2)

at δ 4.35 was typical of the CH-OH proton; recorded at δ 4.0 in 13 -hydroxylupanine [151] A well defined double-doublet at δ 3.3 (J_1 = 10Hz, J_2 = 5Hz) can be assigned to H-11.

The tentative conclusion that CC-2 must be a hydroxy-derivative of camoensine was supported by the mass spectral fragmentation pattern which showed ions at m/e 160 ($C_{10}H_{10}NO$) and m/e 146 (C_9H_8NO), typical of the AB rings of the α-pyridone quinolizidines (Scheme 8). The base peak at m/e 100 ($C_5H_{10}NO$) differed from the base peak of camoensine (m/e 84, $C_5H_{10}N$) by an oxygen which indicates that the hydroxyl substituent must be associated with the D-ring. This would therefore give CC-2 the partial structure:



CL11

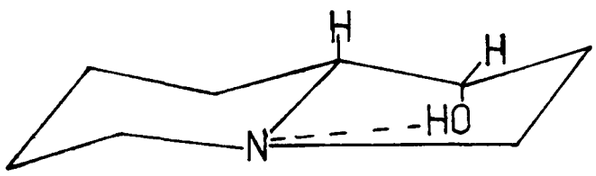
As the H-11 signal is clearly visible in the ^1H NMR spectrum then the hydroxyl substituent must be located at C-12, C-13 or C-14. Furthermore, the occurrence of H-11 as a well defined double-doublet produced by interaction with H-9 and the methine proton at C-12 strongly suggest that the hydroxyl substituent must be at C-12. If this were not the case then it is anticipated that H-11 would be coupled to two protons at C-12 as well as to H-9, and this would result in its appearance as a more complex multiplet.

Support for the proposed structure was obtained for the ^{13}C NMR spectrum (Table 13). Shift values obtained for the A

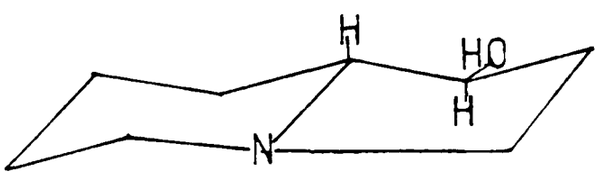
and B rings compared with those obtained for camoensine (Table 13), the C-9 signal undergoing a shielding of 2ppm, consistent with its placement relative to the carbon with an hydroxyl substituent.[148] Of the remaining chemical shifts those at δ 58.0 and 52.1 ppm (both triplets) can be assigned to C-16 and C-14, respectively. Carbons α to an hydroxyl show a deshielding in the order of 6-10ppm.[148] On this basis the signals at 34.0ppm (triplet) and 74.8ppm (doublet) can be assigned to C-13 and C-11, respectively. The remaining signal at 68.7ppm (doublet) is in agreement with that recorded for CHOH of D-ring hydroxyquinolizidines.[148]

The occurrence of the triplet for C-8 at 22.9ppm permits the assignment of 7R:9R; 11R stereochemistry, thus relating the alkaloid to camoensine rather than to leontidine (7R: 9R: 11S).

The remaining problem concerned the stereochemistry of the hydroxyl substituent at C-12. Valuable information relating to assignment of the stereochemistry of indolizidines can be gained from the ^{1}H NMR spectrum. Aaron *et al*[152] have shown that in 1-hydroxyindolizidines the broadening of the H-1 carbinol proton is much greater where H-1 and H-9 are in a *trans* configuration than when they are in a *cis* configuration structures below.

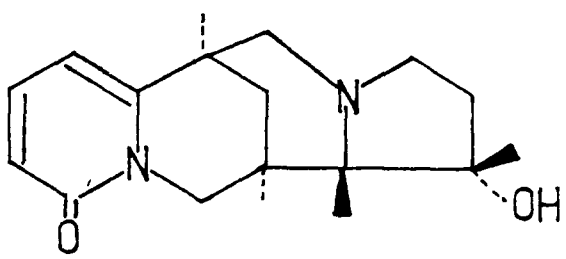


cis 1,9-H



trans 1,9-H

In the ^{1}H spectrum of CC-2 the W value [152] observed for H-12 was 11 Hz, in close agreement with that anticipated for a cis -1,9-H configuration. [153] These data would suggest the following structure, (CL111) with the 12-hydroxy substituent in the pseudo-axial position.



CL111

The stereochemistry is supported by published data on the thermodynamic stability of 9-hydroxy-indolizidine which show a 1,9-cis configuration to be much preferred. [154]

2:5:3:3 Characterization of CC-3 as 12-hydroxy-16-methoxy-11:12, 13:14-tetradehydrocamoensine (CLV)

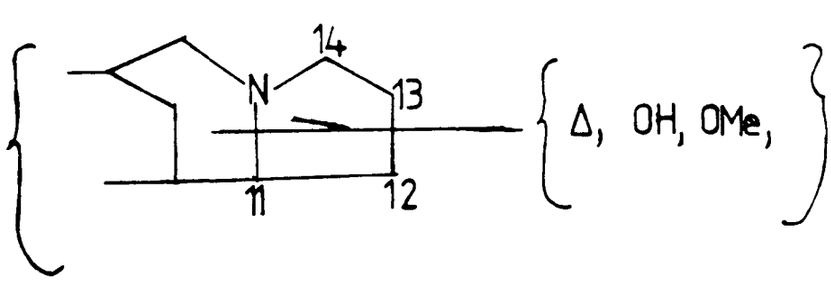
The compound, an amorphous solid, had a UV spectrum (Figure 10 p.96) which showed a maximum at 315nm and a shoulder at 350nm. There was only a slight hypsochromic shift (to 309nm) [146] on the addition of 0.1N HC1. The IR spectrum showed a carbonyl band ca 1660cm^{-1} (C=O, α - pyridone), with bands at 1550cm^{-1}, 1570cm^{-1} (C=C) and an hydroxyl absorption band at 3400cm^{-1}. The high resolution mass spectrum gave an exact mass of 272.1161 indicating an empirical formula of $C_{15}H_{16}N_2O_3$. This formula would suggest

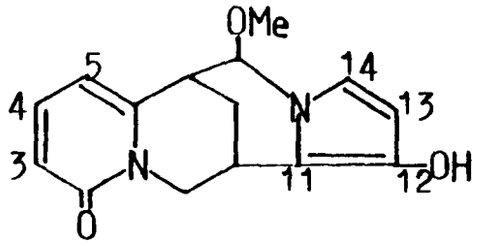
that, relative to camoensine, compound CC-3 contained two further double bonds in its structure.

The ^{1}H NMR spectrum (Figure 13) showed the AB of an ABX system in the region of δ4.00, as in camoensine and 12-hydroxy-camoensine. The aromatic region of the spectrum showed signals at δ7.30 (1H) and δ6.28 (2H), associated with H-4, H-3 and H-5 of the pyridone A-ring of a quinolizidine respectively. In addition there was a doublet centred at δ 7.90(1H $\underline{J}$ = 6Hz) which on decoupling, reduced a doublet at δ 4.90(1H, $\underline{J}$ = 6Hz) to a singlet. These signals represent an AB system; the shifts being due to protons α and β to the nitrogen of an aromatic system.[155] There was also a singlet at δ 3.15 (3H) indicating the presence of a methoxy substituent.

The mass spectral fragmentation showed ions at m/e 160 ($C_{10}H_{10}NO$) and at m/e 146 (C_9H_8NO) typical of the AB rings of α- pyridone quinolizidines. (Scheme 10 p.117). The presence of a methoxy substituent is supported by fragments M^+ - 15, M^+ -31 and M^+- 43, which are consistent with the loss of $CH_3^{\cdot}$, $OCH_3^{\cdot}$ and $CH_3C{=}O^{\cdot}$ from the parent ion. These spectral data suggest a quinolizidine alkaloid in which the A and B rings are normal but in which the C and D rings are both unsaturated and substituted with an OMe and an OH.

On the assumption that CC-3 retains the nucleus of other alkaloids isolated from Camoensia brevicalyx, the following part structure can be drawn for the C/D ring systems.





Solvent - DMSO-d_6

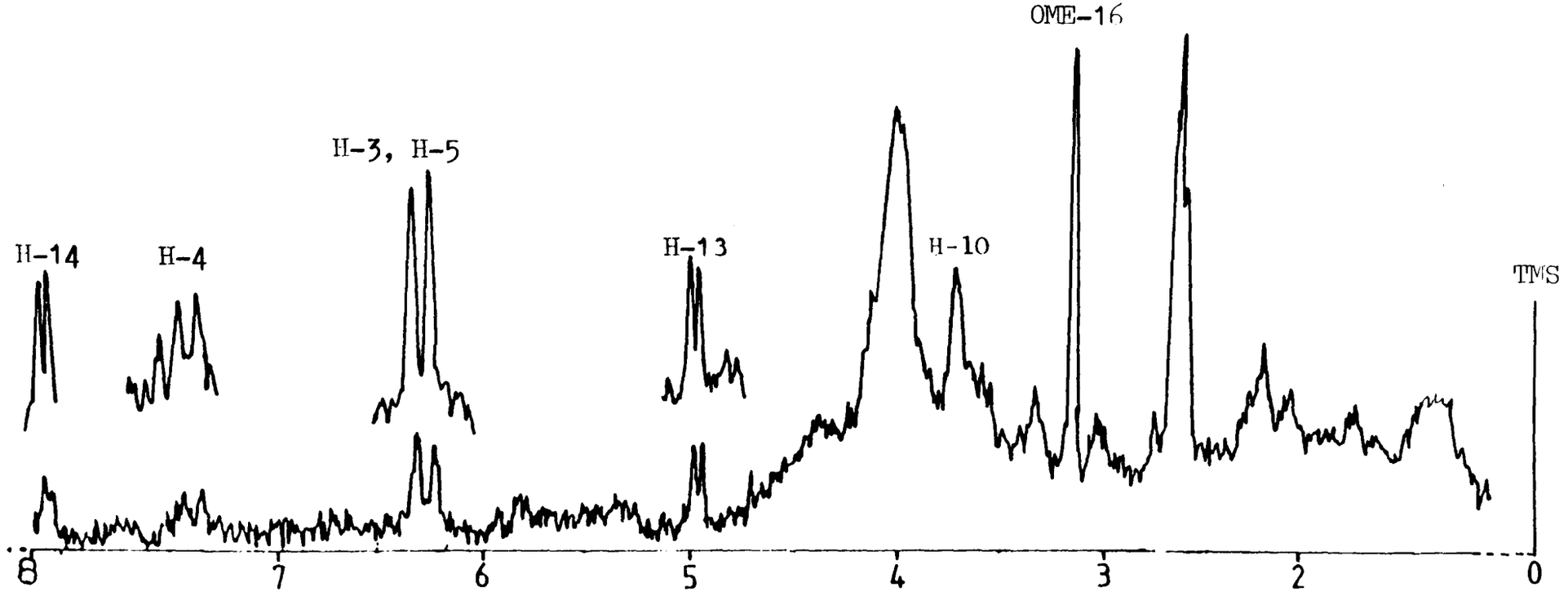
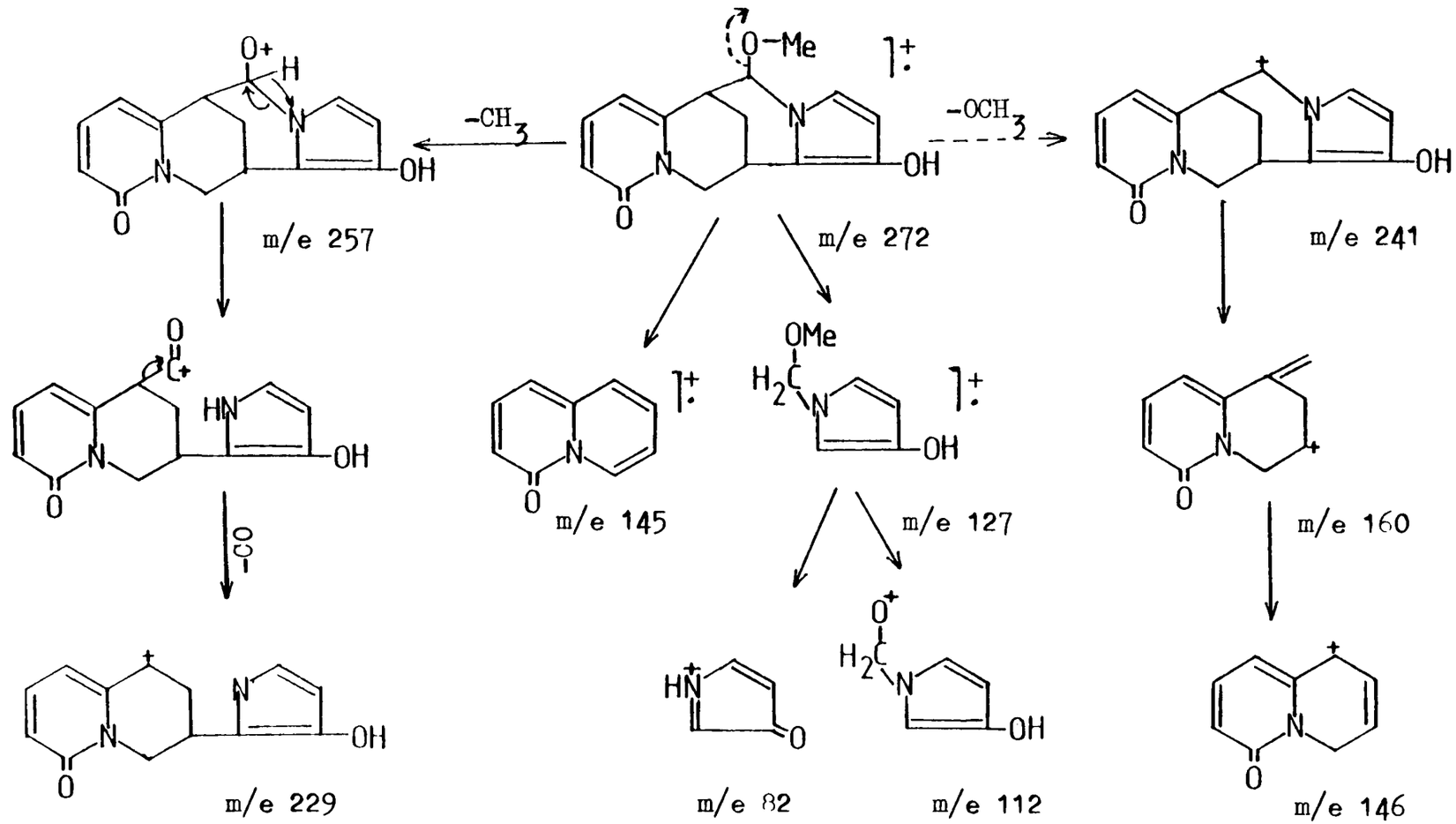
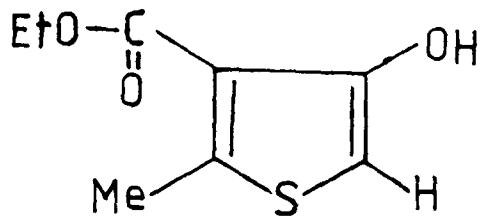


FIGURE 13. ^{1}H NMR SPECTRUM OF 12-HYDROXY-16-METHOXY,11:12,13:14-TETRADEHYDROCAMOENSINE



SCHEME 10. MASS FRAGMENTATION PATTERN FOR 12HYDROXY-16-METHOXY-11:12,13:14TETRADEHYDROCAMOFENSINE (CC-3)

As the upfield part of the AB quartet, which is due to H-13, is not further split it follows that C-12 must be fully substituted. This requires that the additional double-bond be placed between C-11 and C-12. The absence in the mass fragmentation pattern of M^+ -17 ($-OH^\cdot$) and M^+-18 ($-H_2O$) ions is indicative of the OH being on a C =C bond as in 3-hydroxy-4-carbethoxyl-5-methyl-thiophene. (CL1V).[156]



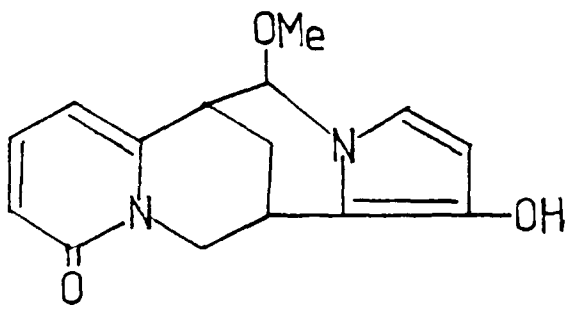
CL1V

It therefore appears probable that CC-3 has a 12-hydroxy substituent and that the - OMe must therefore be placed at C-16.

The observed fragmentation pattern of CC-3 supports the proposed structure (Scheme 10). In addition to the pattern of usual major A/B ring fragments at m/e 160 and 146 there is a further fragment from this system at m/e 145 (C_9H_7NO). The latter occurs together with the complimentary fragment at m/e 127 ($C_6H_9NO_2$, 47.1%), suggesting fragmentation through the C-ring. The m/e 127 ion (fragment K) gives rise to ions consistent with further loss of ($-CH_3$), ($-OCH_3$) and ($-CH_2OCH_3$). These further confirm the presence of the OMe and OH groups in the C/D rings.

These data permit the tentative identification of CC-3

as 12-hydroxy-16-methoxy-11:12,13:14-tetradehydrocamoensine (CLV).



CLV

The unsubstituted isomer of this compound, tetradehydro leontidine, has been produced by Iskandarov *et al* [117] but no spectral details were given.

2:6 CHEMOTAXONOMIC SIGNIFICANCE OF CAMOENSIA ALKALOIDS

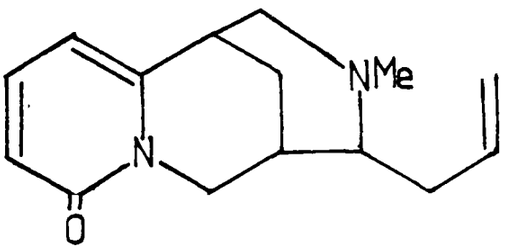
The alkaloids isolated from C. brevicalyx are the same as those previously reported from C. maxima [116] (camoensine and camoensidine p. 39) or oxidation products of these two alkaloids. Since there are only two species in the genus this alkaloid type can therefore be considered as characteristic of the genus. To date no similar quinolizidine/indolizidine alakloids have been reported other than those discussed before (p. 37).

These Camoensia alkaloids are structurally and biogenetically similar to the typical quinolizidines of the Papilionaceae, in particular the 'anagyrine' type and its saturated analogues. The main difference between the 'anagyrine' type and the Camoensia alkaloids is the reduction of the D-ring by one carbon unit.

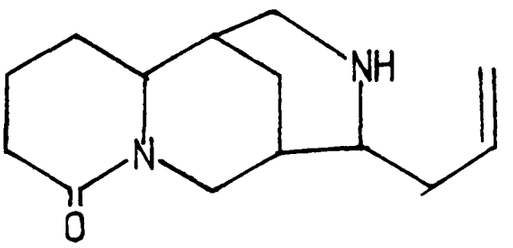
The Leguminosae are the major source of quinolizidine alkaloids.[157] Within the family their distribution is restricted to genera of the Papilionaceae.[158] Thus their presence in Camoensia spp. can be taken as very strong evidence in favour of the placement of Camoensia in the Papilionaceae and contradicts the opinion of Yakovlev [159] who placed this genus in the Caesalpiniaceae.

Salatino and Gottlieb [160] have recently discussed the use of quinolizidine alkaloids as chemotaxonomic markers in the Papilionaceae. Their studies suggest that the source of quinolizidine alkaloids in the sub-family is of African origin and that a number of different alkaloid-producing groups have radiated from this central core. Unfortunately Salatino and Gottlieb [160] omitted Camoensia from their study.

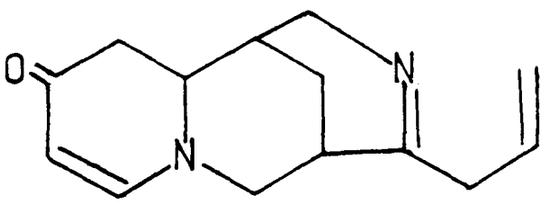
The closest alkaloids to these of Camoensia, in terms of final isolated structures, are tinctorine(CLV1) from Genista tinctoria L.[161] and Thermopsis alteniflora Regel and Schmalh ex Regel,[162] and augustifoline (CLV11)from Sarothamnus catalaunious Webb.[163] Both of these alkaloids have a 3-carbon moiety at C-11 but it is uncertain how this unit is derived.



CLV1



CLV11



CLV111

A third alkaloid with this 3C group at C-11 is dehydroalbine (CLV111)from Lupinus albus Linn.,[164] but in this case there is a different A-ring structure.

If the Camoensia alkaloids are closely related to the above then they would be classified as belonging to the cytisine/paracytisine types.[160] The former are widespread in all alkaloid

containing sub-families of the Papilionaceae, the latter occur predominantly in Ormosia in the Sophorea and to a much smaller extent in the Genisteae; in the genera Lupinus and Sarothamus which are considered to be most closely akin to the Sophoreae.[160]

The chemotaxonomic interpretation of the alkaloids of Camoensia thus seems to favour those authorities who place it in the Sophoreae. However without clear evidence of the mode of formation of the Camoensia alkaloids, in relation to the typical quinolizidines, considerable caution should be used in applying alkaloid chemistry to systematic problems concerning the relationship of this genus.

EXPERIMENTAL

3 EXPERIMENTAL

3: 1 PREAMBLE

Melting points were determined on a Kofler Hot Stage and are uncorrected. Ultra-Violet (UV) Spectra were run on a Pye Unicam SP 800 Spectrophotometer, in methanol unless otherwise stated. The Infra-Red (IR) spectra were run, as KCl discs, on a Perkin Elmer 197 spectrophotometer. The ^{1}H - NMR spectra were run, in appropriate deuterated solvents, using either a Perkin Elmer R32 B 90 MHz, or Perkin Elmer R12 B 60 MHz Nuclear Magnetic Resonance Spectrometer. TMS was used as the internal standard. ^{13}C - NMR were run at 25.15 MHz on a JOEL PFT, 100 MHz Spectrometer in the Fourier transform mode. Solvents used in ^{13}C - NMR are given in the text. Mass spectra were run on an AEI M.S. 9 02 Mass Spectrometer at 70e.V. using probe insertion and elevated temperatures. The optical rotations were determined on a Perkin Elmer Polarimeter, Model 241.

3: 2 Vouchers for Plant Materials Studied

The voucher details of the plant materials collected are listed below:-

Cassia sieberiana L. voucher: N.H.A. Cole F/1 at the Herbarium of the Royal Botanic Garden, Edinburgh.

Cynometra hankei Harms. voucher: Waterman and McKey 852 at the Herbarium of the Royal Botanic Gardens, Kew.

Cynometra mannii Oliv. voucher: McKey and Gartlan 206 at the Royal Botanic Gardens, Kew.

Camoensia brevicalyx Benth. voucher: McKey and Gartlan 208 at the Royal Botanic Gardens, Kew.

Sesbania macrocarpa Muhl. No voucher: Collected by McKey, near Lubbock, Texas.

3:3 Preparation of Plant Materials

The various plant materials were ground separately in a Christy Norris Hammer Mill fitted with a 3 mm aperture screen. The ground powdered plant materials were extracted separately and successively in a Soxhlet with solvents of increasing polarity; usually light petroleum (b.p. 40 - 60°), followed by chloroform and finally methanol. The extracts were then filtered hot and concentrated under reduced pressure using a 'Buchi Rotavapor'.

3:4 Chromatographic Techniques

3:4:1 Thin Layer Chromatography (TLC)

Neutral alumina (Aluminium Oxide G, Merck No.1090), Silica gel G (Type 60; Merck No. 7731) and cellulose (Microcrystalline, Merck No. 2330) were used to prepare the TLC plates. The plates were spread to a thickness of 0.25 mm and were initially allowed to dry in the atmosphere before being dried in an oven at 100° for at least 2hr.

3:4:2 Preparative Thin Layer Chromatography

The plates (20 x 20 cm) were spread with either silica gel G or alumina (make as above) to a thickness of 0.50 mm. The plates were dried in the same way as the normal TLC plates.

3:4:3 Application of the Samples in Preparative TLC

The mixtures to be separated were streaked onto the preparative TLC plates by means of a Burkard TLC applicator (Type SA 40) using an Agla syringe. The developed plates were

observed under UV light to identify the band(s) required or were sprayed to a width of 1 cm from the edge of the plate with the appropriate detection reagent. The required bands were then scraped from the plates and the adsorbed substance(s) eluted with a polar solvent.

3:4:4 Column Chromatography

The column was wet packed with either silica gel 60 (70 - 230 mesh, ASTM, Merck No. 7734), or alumina (Aluminium Oxide 90, Merck No. 1097) mixed with 2.5% (v/v) of water. The extract to be chromatographed was adsorbed onto a small amount of packing material by evaporating a mixture of the extract and the packing material under reduced pressure. This material was then applied to the top of the column.

3:4:5 Visualisation of the Chromatoplates

The TLC plates, spotted with the various extracts or with the column eluate, were initially viewed under UV light (366 nm) before being developed in the appropriate solvent system to a height of 10 - 15 cm. The developed chromatoplates were then revisualised under UV light and the fluorescent spots, if any, were marked. The plate was then sprayed with a visualising reagent as determined from the initial screening of the extracts (see p. 50) When flavonoids were suspected from the visualisation under UV light the plate was first exposed to NH_3 vapours and then re-examined under UV light to observe any colour change. The plate was then sprayed with an alcoholic solution of $FeCl_3$ (3%) to determine whether the compounds present were phenolic. Dragendorf's [165] and Iodoplatinate [166] spray reagents were used to identify alkaloids.

Vanillin/sulphuric acid spray reagent (see p. 52) followed by heating to 100° was used as a general spray reagent to visualise all compounds present in an extract.

3:6 ANALYSIS OF CASSIA SIEBERIANA L.

3:6:1 Extraction and Isolation

The ground root bark (730g) was extracted separately and successively with light petroleum (b.p. 40 - 60°), chloroform and methanol. The concentrated light petroleum extract, on screening on silica gel in solvent system 1 (p. 54), gave two dominant spots with the vanillin/sulphuric acid spray reagent.

A concentrate of the petroleum extract (2g) was column chromatographed over silica gel to give, on elution with light petroleum (b.p. 60 - 80°)/ethyl acetate (19:1), a compound designated as CS-1 (20mg). On continued elution with the same solvent another pure band was obtained which gave a crystalline compound CS-2, (15mg).

The chloroform extract, on screening, showed traces of compounds which were identical to CS-1 and CS-2. In addition, traces of some unidentifiable compounds were also present.

The methanol extract, on screening, showed one dominant spot. Viewed initially under UV light (366nm) this appeared as a pale yellow spot which intensified on exposure to NH_3 vapour and revisualisation under UV light. The concentrated methanol extract was precipitated with diethyl ether to give a creamy coloured solid (5g) which was column chromatographed over silica gel and gave, on elution with chloroform/ethyl acetate (2:3), a single compound CS-3 (200mg, Rf. 0.42 on silica gel in solvent system 5 p. 54). The compound had the TLC characteristics of the major compound described above.

3:6:2 Characterization of the compounds isolated from Cassia sieberiana root bark

3:6:2:1 Characterization of CS-1 as β- sitosterol (XXXl)p. 14

Recrystallised as white needles from acetone, melting point 138° (Lit.[85] 140°). Found : M^+ 414.3867; $C_{29}H_{50}O$ requires 414.3862. Optical rotation $[\alpha]_D^{20}$ - 35° (C = 1.00 in $CHCl_3$) (Lit.[85] - 37°). UV: no λmax above 225nm. IR ν max. cm^{-1} : 3300 (OH), 2980 - 2760 (CH_2). ^1H NMR ($CDCl_3$): δ5.40 (1H, d, J = 4Hz , =CH - CH_2), δ3.40 (1H, m , CHOH). Mass spectrum m/e (%) : 414 (M^+, 100), 400 ($C_{28}H_{48}O$, 29.9), 303 ($C_{22}H_{39}$, 14.4), 255 ($C_{19}H_{27}$, 28), 159 ($C_{12}H_{15}$, 14.4).

3:6:2:2 Characterization of CS-2 as lupeol (CVl p.31)

Recrystallised as white needles from acetone, melting point 213°, (Lit. [131] 215 - 216°) Found: M^+ 426.3866 ; $C_{30}H_{50}O$ requires 426.3861. UV: no λ max above 225nm. IR ν max cm^{-1} : 3300 (OH^-), 3150 (CH = CH_2), Mass spectrum m/e (%): 426 (M^+, 70.0), 383 (6.4), 220 (17), 218 (98.4), 207 ($C_{14}H_{23}O$, 100), 189 (24.2).

3: 6:2:3 Characterization of CS-3 as (-)-epiafzelechin (p. 58)

Cream amorphous compound from acetone, melting point 312° (Lit.[136] 240 - 243° decomp.) Found: M^+ 274.8836; $C_{15}H_{14}O_5$ requires 274.8841. Optical rotation $[\alpha]_D^{20}$ - 47° (C = 1.00 in acetone) (Lit.[136] - 59°). UV. λ max nm: 275 max (+NaOH) 296. IR ν max.cm^{-1} : 3300 (OH), 2900 (CH_2).

^{1}H NMR (60 MHz, $(CD_3)_2$ CO): δ 8.10 (3H, broad s , replaceable by D_2O, H-5, H-7, H-4'), 7.41 (2H, ABq, J = 9Hz, H-2', H-6'); 6.89 (2H, ABq, J = 9Hz, H-3', H-5'), 6.06 (1H, ABq, J = 2Hz, H-6); 6.01 (1H, ABq, J = 2Hz, H-8), 4.99 (1H, s H-2), 4.29 (1H, t, H-3), 3.60 (1H, broad s replaceable by D_2O, H-O-3), 2.82 (2H, d, J = 5Hz, H-4). ^{13}C - NMR ($(CD_3)_2$ CO): δ157.0 (4 x s C-5,C-7, C-8a, C-4'); 131.3 (s, C-1'), 128.9 (2 x d, C-2', C-6'), 115.5 (2 x d, C-3', C-5'); 99.7 (s, C-4a), 96.4 (d, C-6), 95.8 (d, C-8), 79.4 (d, C-2), 66.3 (d, C-3), 29.0 (t, C-4). Mass spectrum, m/e (%): 274 (M^+, 23.0), 139 ($C_7H_7O_3$, 100), 137 ($C_8H_8O_2$, 37), 121 ($C_7H_5O_2$, 22), 107 (C_7H_7O, 33).

3:7 ANALYSIS OF CYNOMETRA HANKEI STEM BARK

3:7:1 Extraction and Isolation

The ground stem bark of C. hankei (340g) was extracted in a Soxhlet separately and successively with light petroleum (b.p. 40 - 60°), chloroform, and methanol. The methanol extract gave a reddish-brown residue (100g). Screening of the concentrated light petroleum and chloroform extracts did not reveal any identifiable compound.

Initial screening of the methanol extract was similarly negative. However, when the dried extract was treated with NH_4OH and extracted with chloroform, four alkaloids were detected in the chloroform extract when it was screened on alumina TLC plates (systems 2, and 3, p. 54). Column chromatography of the chloroform concentrate over alumina (containing 2.5% water v/v) gave, on elution with chloroform, CHM -1 (15mg. Rf.0.74 on alumina in solvent system 2, p 54). Elution with chloroform/methanol (99:1) gave CHM - 2 (117 mg, Rf. 0.65 on alumina chromatoplate in solvent system 2, p 54). Further elution with the same solvent gave a band which contained a mixture. This was separated by preparative TLC on alumina chromatoplates (solvent system 2 p. 54) to give another compound, CHM -4, as a brown amorphons solid (20mg, Rf 0.39 on alumina chromatoplate in solvent system 2). Continued elution with the same solvent mixture then gave another pure band designated CHM -3 (185mg. Rf. 0.38 on alumina in solvent system 3, p.54 ; Rf. 0.27 on alumina chromatoplate in solvent system 2, p. 54).

3:7:2 Characterization of the compounds isolated from Cynometra hankei stem bark.

3:7:2:1 Characterization of CHM-1 as Cynodine (CIX) p.33

A non crystallisable brown powder with no sharp melting point. Found: M^+ 389.1710; $C_{23}H_{23}N_3O_3$ requires 389.1737. Optical rotation $[\alpha]_D^{20}$ + 11.5° (c = 0.10, $CHCl_3$) (Lit.[18] + 15°). UV λ max nm: 259, 264, 272, 281. IR ν max cm^{-1} : 3250 (N = CH), 1720 (ArC = O), 1685 (lactam C= O). ^1H NMR ($CDCl_3$): δ 7.90 (3H, m, H-2, H-2', H-6'), 7.60 - 7.25 (8H, m, H-13 - H-17, H-3' - H-5'), 6.65 (1H, d, J = 1Hz, H-5), 6.58 (1H, d, J = 6Hz,H-11), 3.56 (3H, s, N_1- CH_3), 4.00 - 3.00 (4H, m, H-6,H-7, H-10), 2.77 (3H, s , N_8 - CH_3). Mass spectrum m/e (%) : 389 (M^+, 71.2), 284 (M^+ C_6H_5CO, 30.2), 268 (M^+ O-COPh, 557), 267 (M^+ PhCO, OH, 46.0), 179 ($C_9H_{13}N_3O$, 40.2), 137 ($C_7H_{11}N_3$, 100).

(i) Saponification of CHM-1

CHM-1 (10mg) was heated in a water bath for 2hr with 2ml. of 20% alcoholic KOH. The reaction mixture was diluted with water and extracted with chloroform to give a compound with physical properties identical to those of cynometrine (see below).

3:7:2:2 Characterization of CHM-2 as Cynometrine (VIII)p. 33

Recrystallised as needles from chloroform, melting point 210° (Lit.[18] 213°). Found: M^+ 285.1477; $C_{16}H_{19}N_3O_2$ requires 285.1470. Optical rotation $[\alpha]_D^{20}$ - 27.1° (c 0.1, $CHCl_3$) (Lit.[18] - 30.0°). UV λ max nm: 251, 257, 264, IR ν max. cm^{-1}: 3300 (OH), 3250 (N=CH), 1695 (lactam C=O). ^1H NMR ($CDCl_3$): δ 7.40 - 7.17 (6H, m, H-2, H-13 -H-17); 5.96 (1H, d, J = 1Hz, H-5); 5.06 (1H, d, J = 5Hz, H-11), 3.47 (3H, s, N_1-CH_3); 4.00 - 3.00 (4H, m , H-6, H-7, H-10), 2.87 (3H, s,N_8 - CH_3).

^{13}C-NMR ($CDCl_3$); δ 174.9 (s, C-9), 141.7 (2 x s, C-4, C-12); 137.0 (d, C-2); 128.0 (3 x d, C-13, C-15, C-17); 127.5 (2 x d, C-14, C-16); 116.5 (d, C-5); 75.0 (d, C-11); 54.3 (d, C-10); 52.9 (t, C-7); 33.1 (q, N_1 -CH_3), 29.8 (d, C-6), 29.6 (q, N_8-CH_3). Mass spectrum m/e (%): 285 (M^+ 39.4), 268 (M^+ - OH, 15.7), 267 (M^+ - H_2O, 5.7), 179 ($C_9H_{13}N_3O$, 64.6), 151 ($C_8H_{13}N_3$, 4.7), 136 ($C_7H_8N_2O$, 66.4), 122 ($C_6H_6N_2O$, 37.7), 108 ($C_6H_8N_2$, 100).

3:7:2:3 Characterization of CHM-3 as N_1-demethyl-cynometrine (CXL)p.74

Recrystallised as needles from methanol, melting point 204°. Found: M^+ 271.1335; $C_{15}H_{17}N_3O_2$, requires 271.1321. Optical rotation $[\alpha]_D^{20}$ - 31.0° (C 0.1, $CHCl_3$). UV λ max nm: 252, 257, 267. IR ν max cm^{-1}: 3300 (OH), 3250 (N - H), 1690 (lactam C=O), ^{1}H NMR ($CDCl_3$): δ 7.44 - 7.18 (6H, m, H-2, H-13 - H -17); 6.27 (1H, d, J = 1Hz, H-5); 5.11 (1H, d, J = 6Hz, H-11); 3.51 - 3.05 (4H, m, H-6, H-7, H-10), 2.87 (3H, s, N_8-CH_3) ^{13}C-NMR $CDCl_3$: δ 176.5 (s, C-9), 142.6 (2 x s, C-4, C-12), 136.8 (d, C-2); 129.4 (2 x d, C-13, C-17), 129.0 (d, C-15), 128.1 (2 x d, C-14, C-16), 115.6 (d, C-5), 75.1 (d, C-11), 56.6 (d, C-10), 55.1 (t, C-7), 33.6 (d, C-6); 29.8 (q, N_8-CH_3). Mass spectrum m/e (%) : 271 (M^+, 64.8); 253 (M^+-H_2O, 21.0); 165 ($C_8H_{11}N_3O$, 100); 94 ($C_5H_6N_2$, 88.9).

(i) Conversion of CHM-3 to cynometrine

CHM-3 (40mg) was dissolved in dry acetone (40ml) and refluxed for 24hr with methyl iodide (20mg) and fused K_2CO_3 (0.5g). The reaction mixture was filtered and concentrated.

Recrystallisation from $CHCl_3$ gave cynometrine(15mg), identical in all respects (TLC, m.p., UV, IR, ^{1}HNMR, MS) with CHM -2 (p.132).

(ii) CHM-3 (40mg) was methylated as above but using 40mg of methyl iodide. The reaction mixture was filtered and gave 15mg of a methanol soluble gummy solid (Rf 0.0 on alumina chromatoplates in solvent systems 2, 3, 4 and 5, p. 54). ^{1}H NMR (CD_3OD), δ 3.84 (3H, s, N_1-CH_3), 3.80 (3H, s, N_1-CH_3), 2.90 (3H, s, N_8-CH_3). Mass spectrum m/e (%) : 283 (M^+ - OH, $C_{17}H_{21}N_3O$, 0.9), 282 (M^+ -H_2O, $C_{17}H_{20}N_3O$, 1.5). 1.94 ($C_{10}H_{16}N_3O$, 2.8) 123 ($C_7H_{11}N_2$, 8.1).

3:7:2:4 Characterization of CHM-4 as N_1-demethylcynodine (CXL)p.82

A brown non-crystallisable solid. Found : M^+ 375.1590; $C_{22}H_{21}N_3O_3$ requires 375.1583. Optical rotation $[\alpha]_D^{20}$ -31° (c = 0.1, MEOH). UV λ max nm: 258, 264, 274, 281. IR ν max cm^{-1} : 3250 (N-H), 1720 (Ar C=O) 1685 (lactam C = O). ^{1}H NMR ($CDCl_3$): δ 7.95 - 7.86 (3H, m, H-2, H-2^1, H-6^1): 7.49 -7.23 (8H, m, H-13 - H-17, H-3^1 - H-5^1), 6.82 (1H, d, J = 2Hz, H-5), 6.62 (1H, d, J = 4Hz, H-11), 3.72 - 3.00 (4H, m, H-6, 1H-7, H-10): 2.78 (3H, s, N_8-CH_3). Mass spectrum m/e (%): 375 (M^+, 45.2), 270(M^+- C_6H_5CO, 55.8), 254 ($C_{15}H_{16}N_3O$, 47.3), 253 ($C_{15}H_{15}N_3O$, 100), 165 ($C_8H_{11}N_3O$, 41.4), 94 ($C_5H_6N_2$, 30).

(i) Conversion of CHM-4 to cynodine

CHM-4 (15mg) was methylated using the method previously described (p. 133) and gave a product identical by TLC (systems 2 and 3 on alumina chromatoplates) with cynodine (p. 132).

3:7:3 ANALYSIS OF CYNOMETRA HANKEI SEEDS

The ground seeds of C. hankei (27g) were extracted separately and successively with light petroleum (b.p. 40 - 60°), chloroform and methanol. The concentrated light petroleum and chloroform extracts did not yield any identifiable compound. The methanol extract on screening on alumina chromatoplates in solvent system 2 (p. 54) gave three spots, Rf. 0.74, 0.65 and 0.27 when visualised with Dragendorff's reagent. These were identical to cynodine, cynometrine and N_1-demethylcynometrine respectively. Column chromatography of the methanol extract on alumina chromatoplates gave all three compounds: CHS-1 (10mg), on elution with chloroform; CHS-2 (20mg) on elution with chloroform/methanol (99:1); and CHS-3 (25mg) on further elution with the same solvent.

3:7:4 Characterization of CHS-1 as Cynodine (CIX) CHS-2 as Cynometrine (VIII) and CHS-3 as N_1-demethylcynometrine (CXL).

The spectral characteristics of CHS-1, CHS-2 and CHS-3 were identical to those of cynodine (p. 132), cynometrine (p. 132) and N_1-demethylcynometrine (p. 133) respectively.

3:7:5 ANALYSIS OF CYNOMETRA MANII STEM BARK

3:7:5:1 Extraction and Isolation

Ground C. manii stem bark (5g) was extracted with solvents of increasing polarity, initially light petroleum (b.p. 40 - 60°), then chloroform, and finally methanol. The screened concentrates did not show any identifiable compound. The methanol extract was concentrated and treated with NH_4OH into chloroform. This chloroform extract did not give positive results when screened for alkaloids.

3:8 ANALYSIS OF SESBANIA MACROCARPA SEEDS

3:8:1 Extraction and Isolation

The ground seeds of S. macrocarpa (235g) were extracted with methanol. The concentrated extract was examined by TLC (systems 5 and 6 on silica gel) and revealed a pale yellow fluorescent spot under UV light which intensified when exposed to NH_3 vapours and re-examined under UV light. Spraying the TLC plate with ferric chloride reagent (p.53) gave a black spot (Rf. 0.46).

The concentrated methanol extract was partitioned with light petroleum (b.p. 60 - 80°) and the petrol insoluble fraction evaporated to dryness. The methanol fraction of the residue was then chromatographed over silica gel. Elution with chloroform/methanol (4:1) gave a single comound SM-1 (100mg). Visualisation under UV light (366nm) and exposure to ammonia vapour showed TLC characteristics identical to those noted previously.

3: 8:2 Characterization of SM-1 as Kaempferol-3, 7-dirhamnoside (CXL111 p.93)

Brown amorphous solid from methanol. No mass spectrum. UV (Cecil CE505 Double Beam UV spectrophotometer) λ max, nm: 268, 348 (MeOH + NaOH) 270, 396; (MeOH + $AlCl_3$) 268, 354 400; (MeOH + NaOAc) 268, 348; (MeOH + NaOAc + H_3BO_3) 268, 348.

^1H NMR (CD_3OD) : δ 8.10 (2H, ABq J = 7Hz, H-3', H-5'); 6.89 (2H, ABq, J = 7Hz, H-2', H-6'); 6.42 (1H, ABq, J = 2Hz, H-6); 6.22 (1H, ABq, J = 2Hz, H-8); 5.25 (1H, d, J = 2Hz, H_1- 7Rh.); 4.12 - 3.53 (11H, broad m, protons of two rhamnosyl units). 1.27 - 1.16 (6H, broad d, methyl groups of two rhamnosyl units)

Hydrolysis of SM-1 - SM-1 (80mg) was hydrolysed in 0.5 N HCl on a boiling water bath for 3hr. The reaction mixture was partitioned with EtOAC to give an amorphous solid, SM-2 (20mg). The aqueous layer was neutralised and spotted on a cellulose chromatoplate (0.25m, solvent system 7, p.54) and run against known sugars to determine the sugar residue. The cellulose chromatoplate was then sprayed with aniline phthalate (see p. 53) and heated for 10min, at 100°, to give a red-brown spot (Rf. 0.45), that was identical with a reference sample of rhamnose.

3:8:2:1 Characterization of SM-2 as Kaempferol (CXLIII) p.93

Brown amorphous solid from methanol. Found: M^+ 286.0471; $C_{15}H_{10}O_6$ requires 286.0477. UV (Cecil CE505 Double Beam UV spectrophotometer) λ max nm : 268, 364, (MeOH + NaOH) 278, 320, 410; (MeOH + $AlCl_3$) 272, 354, 425; (MeOH + NaOAc) 274, 380; (MeOH + NaOAc + H_3BO_3) 268, 364. IR ν max cm^{-1} 3300 (OH), 1740 (benzopyrone C = O). ^1H NMR (CD_3OD) δ 8.10 (2H, ABq, J = 8Hz, H-3', H-5'); 6.89 (2H, ABq, J = 8Hz, H-2', H-6'), 6.42 (1H, ABq, J = 2Hz, H-6), 6.22 (1H, ABq, J = 2Hz, H-8). Mass spectrum m/e (%): 286 (M^+, 100), 258 (M^+ -CO, 6.8); 153 ($C_7H_5O_4$, 5.4); 134 ($C_8H_6O_2$, 1.4); 121 ($C_7H_5O_2$, 31.0);

3:9 ANALYSIS OF THE SEEDS OF CAMOENSIA BREVICALYX SEEDS

3: 9:1 Extraction and Isolation

The dried ground seeds (130g) were extracted separately and successively with light petroleum (b.p. 40 - 60°), chloroform and methanol. The concentrated light petroleum extract, on screening in solvent systems 1, 2, 4 and 6 on both alumina and silica gel plates (p. 54), did not show any significant compounds. The concentrated chloroform extract, when examined in system 2 (p.54) on alumina, was found to contain three alkaloid spots when sprayed with Dragendorff's and Iodoplatinate spray reagents; Rf values 0.65, 0.57 and 0.39 respectively.

The methanol extract, on similar analysis to the chloroform extract ,showed the same three compounds previously noted in the chloroform extract and, in addition, a fourth compound (Rf. 0.18). When the methanol concentrate was examined on silica gel plates (system 2 p. 54) a fifth compound giving a positive reaction with the alkaloid spray reagents was observed (Rf. 0.59).

The chloroform extract was chromatographed over alumina (containing 2.5% v/v water) to give, on elution with chloroform, a mixture of compounds. This mixture, on standing, gave a crystalline solid, CC-M 2 (4mg, Rf. 0.57 in solvent system 2, p. on alumina chromatoplates). The supernatant solution from which CC - M2 has been obtained was subjected to preparative TLC on alumina chromatoplates (solvent system 2) and gave a second compound CC - M1 (10mg, Rf. 0.65 in solvent system 2,p.54 on alumina chromatoplates). Continued elution of the column with chloroform /methanol (99:1) gave a pure band which yielded a third compound CC-1 (150mg, Rf. 0.39 in solvent system 2, p. 54 on alumina chromatoplates).

The methanol extract was similarly chromatographed over alumina and gave, on elution with chloroform, a mixture of CC-M1 and CC-M2. On preparative TLC, the mixture gave additional amounts of CC-M1 (2mg) and CC-M2 (2mg). Continued elution of the column with chloroform/methanol (99:1) gave further CC-1 (200mg), followed by another pure compound CC-2 (150mg, Rf. 0.18 on alumina plate in solvent 2, 0.47 in solvent 3 p.54)

Column chromatography of an aliquot of the methanol concentrate on silica gel gave, on elution with chloroform/methanol (99:1), a pure band which on concentration gave CC-3 (20mg, Rf. 0.59 in system 2). Continued elution with chloroform/methanol (49:1) gave trace amounts of CC-M1 and CC-M2 and further quantities of CC-1 (100mg) and CC-2 (40mg).

3: 9:2 CHARACTERIZATION OF COMPOUNDS ISOLATED FROM CAMOENSIA BREVICALYX

3:9:2:1 Characterization of CC-1 as Camoensine (CXX) p. 39

A brown non crystallisable solid. Found : M^+ 230.1400; $C_{14}H_{18}N_2O$ requires 230.1455. Optical rotation $[\alpha]_D^{20}$ - 108° (c, 1.0 $CHCl_3$) (Lit.[116] - 186°). UV λ max nm: 230, 308 (MeOH + HCl) 230, 297. IR ν max cm^{-1} : 1650 (α -pyridone) 1570, 1560 (C = C). ^1H NMR ($CDCl_3$) : δ 7.31 (1H dd J_1 = 9Hz, J_2 = 7Hz, H-4), 6.43 (1H, dd, J_1 = 9Hz J_2= 1Hz, H-5), 6.03 (1H, dd, J_1 = 7Hz , J_2 = 1Hz, H- 3). 4.20 (1H, ABX, J_{AB} = 14Hz, J_{AX} = 1Hz H - 10_a) 3.88 (1H ABX, J_{AB} = 14Hz, J_{BX} = 7Hz, H - 10_e) 13C - NMR ($CDCl_3$) : 164.1 (s, C-2), 151.4 (s, C- 6), 139.2 (d, C-4), 117.2 (d, C-3), 105.2 (d, C-5), 66.3 (d, C-11), 55.3 (t, C-16), 55.1 (t, C-10), 51.9 (t, C-14), 35.2 (d, C-7), 29.2 (d, C-9), 25.4 (t, C-12), 21.4 (t,C-8), 26.9 (t, C-13). Mass spectrum m/e (%) : 230 (M^+, 40.6), 160 ($C_{10}H_{10}NO$, 5.5), 146 (C_9H_8NO, 13.9), 84 ($C_5H_{10}N$, 100).

3: 9:2:2 Characterization of CC -M1 as Camoensidine (CXXi) p. 39

A brown amorphous solid. Found: M^+ 234.1721: $C_{14}H_{22}N_2O$ requires 234.1732. UV: no λ max above 225 nm. IR ν max cm^{-1} : 1670 (piperidone C = 0). ^1H NMR ($CDCl_3$) : δ 4.40 - 3.50 (2H, m, H-10). ^{13}C - NMR ($CDCl_3$) : δ 175.2 (s, C-2), 64.8 (d, C-11), 62.0 (d, C-6), 60.4 (t, C-16), 55.7 (t, C-14), 43.3 (t, C-10), 33.8 (d, C-7), 33.4 (t, C-3), 30.5 (t, C-12), 30.1 (t, C-5), 29.7 (d, C-9), 25.8 (t,C-13), 23.8 (t,C-8), 20.6 (t, C-4). Mass spectrum m/e (%) :

234 (M^+, 71.6), 164 ($C_{10}H_{14}NO$, 7.2), 150 ($C_9H_{12}NO$, 100), 136 ($C_9H_{14}N_1$ 91.9), 122 ($C_8H_{12}N$, 26.7), 84 ($C_5H_{10}N$, 90.6).

3:9:2:3 Tentative Characterization of CC-M2 as 12- hydroxycamoensidine (CL1) p. 108

Recrystallised as needles from methanol, melting point, 200°. Found: M^+ 250.1692; $C_{14}H_{22}N_2O_2$, requires 250.1681. Optical rotation $[\alpha]_D^{20}$ - 50.0° (c, 0.1 MeOH) UV : no λ max above 225nm. IR ν max cm^{-1}: 3250 (sharp) (OH) 1660 (piperidine C = O). Mass spectrum m/e (%) : 250 (M^+, 100), 164 ($C_{10}H_{14}NO$, 0.8), 150 ($C_9H_{12}NO$, 11.5), 138 ($C_8H_{12}NO$, 44.9), 136 ($C_9H_{14}N$, 25.1), 122 ($C_8H_{12}N$, 18.8), 100 ($C_5H_{10}NO$, 19.7).

3: 9:2:4 Characterization of CC-2 as 12-α-hydroxy-camoensine (CL111) p. 114

A brown amorphous solid. Found: M^+ 246.1362 ; $C_{14}H_{18}N_2O_2$ requires 246.1368. Optical rotation $[\alpha]_D^{20}$ - 115° (c 1.00, MeOH). UV λ max nm : 230, 309 (MeOH + HCl): 230, 297. IR ν max. cm^{-1} : 3300 (OH), 1645 (C = O α-pyridine). 1563, 1550 (C = C) ^1H NMR ($CDCl_3$) : δ 7.3 (1H, dd, J_1 = 9Hz, J_2 = 7Hz, H-4), 6.43 (1H, dd, J_1 = 9Hz, J_2 = 1Hz, H-5), 6.03 (1H, dd, J_1 = 7Hz, J_2 = 1Hz, H-3), 4.35 (1H, broad m, H-12), 4.16 (1H, ABX, J_{AB}=16Hz, J_{AX}= 1Hz, H-10a), 3.73 (1H, ABX, J_{AB} = 16Hz, J_{BX} = 6Hz, H-10e), 3.30 (1H, dd, J_1 = 10Hz, J_2 = 5Hz, H-11), 13C NMR ($CDCl_3$) : δ 164.6 (s, C-2), 152.6 (s, C-6), 139.5 (d, C-4), 117.0 (d, C-3), 106.1 (d, C-5), 74.7 (d, C-11), 68.7 (d, C-12), 57.9 (t, C-16), 52.1 (2 x t, C-10, C-14), 34.4 (d, C-7), 34.0 (t, C-13), 27.2 (d, C-9), 22.9 (t, C-8). Mass spectrum m/e (%):

246 (M^+, 64.3), 229 ($M^+ - OH^-$, 4.7), 228 ($M^+ - H_2O$, 7.4), 160 ($C_{10}H_{10}NO$, 27.0), 146 (C_9H_8NO, 33.1), 100 ($C_5H_{10}NO$, 100).

3:9:2:5 Characterization of CC-3 as 12-hydroxy-16-methoxy, 11:12, 13:14-tetradehydrocamoensine (CLV)p. 119

A brown amorphous compound from methanol. Found: M^+ 272.1153; $C_{15}H_{16}N_2O_3$ requires 272.1161. UV λ max nm: 235, 315, 350 (sh) (+ HCl) 235, 369, 350 (sh). IR ν max cm^{-1} : 3400 (OH), 1650 (C=O, α-pyridone). ^1H NMR (DMSO $-d_6$) δ 7.90 (1H, d, J = 5Hz, H-14), 7.30 (1H, dd, J_1 = 5Hz, J_2 = H-4) 6.28 (2H, dd, J_1 = Hz, J_2 = Hz, H-3, H-5), 4.90 (1H, d, J = 5Hz, H-13), 3.15 (3H, s, OCH_3 - 16). Mass spectrum m/e (%) : 272 (M^+, 100), 257 ($M^+ - CH_3$, 39.0), 241 ($M^+ - OCH_3$, 8.2) 229 ($M^+ - CH_3$, C=O, 30.2), 160 ($C_{10}H_{10}NO$, 16.7), 146 (C_9H_8NO, 65.4), 145 (C_9H_7NO, 11.5), 127 ($C_6H_9NO_2$, 47.4), 112 ($C_5H_6NO_2$, 1.4), 96 (C_5H_6NO, 3.1), 82 (C_4H_4NO, 10.3).

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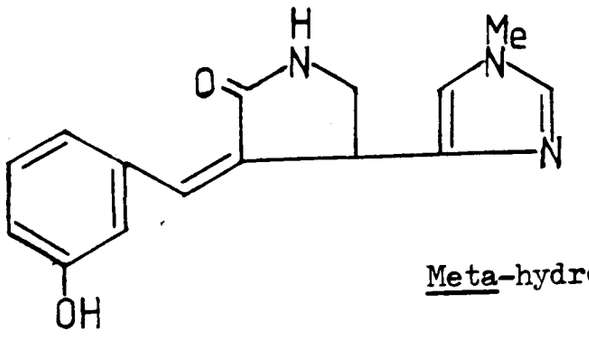
ADDENDUM

Since the commencement of the typing of the thesis correspondence has been received from Md. F. Khuong Huu, Universite de Paris, Sud. on the thesis of L. Tchissambou.[139] In it is reported, in addition to those alkaloids listed in their publication,[109] two more alkaloids from Cynometra lujae. These are meta-hydroxy anantine and cynolujine. The spectral details of these compounds were not made available, although one can safely predict the structure of meta-hydroxy anantine as that given below.



Meta-hydroxy anantine

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Dr. R.M. Polhill, Royal Botanic Gardens, Kew, kindly confirmed the identity of plant species studied.

Mrs. J. Struthers is particularly thanked for her patience in typing the thesis, as are my colleagues in the laboratory and members of staff who at one time or another were helpful.

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