CHAPTER 2

RESULTS AND DISCUSSION

2.1 DISTRIBUTION OF *UNCARIA* SPECIES IN PENINSULAR MALAYSIA

One of the primary objectives of this study is to collect, identify and screen all the *Uncaria* species in Peninsular Malaysia for their alkaloidal content. An extensive program to collect *Uncaria* species was carried out throughout the country, and each sample was documented, identified (whenever possible) and screened for its alkaloids. The sampling exercise is summarized in Table 3.1. Areas selected are representative of the regions under study, i.e. Northern, Central, Western, Southern and Eastern regions of the Peninsula (Figure 2.1).

Although many samples were collected, not all could be identified. This is in part due to the fact that for this taxonomically problematic genus, a firm identification requires the presence of not only leaf samples but of flowers and fruit samples as well, which were more often than not absent during the time of collection.

The results are presented in Table 2.1, where it can be seen that *Uncaria* is fairly widely distributed over the lowland regions of the country. Of the 14 species reported to be present in Malaysia, 8 species were collected. In addition, several 'new' species were also collected (see Table 3.2).

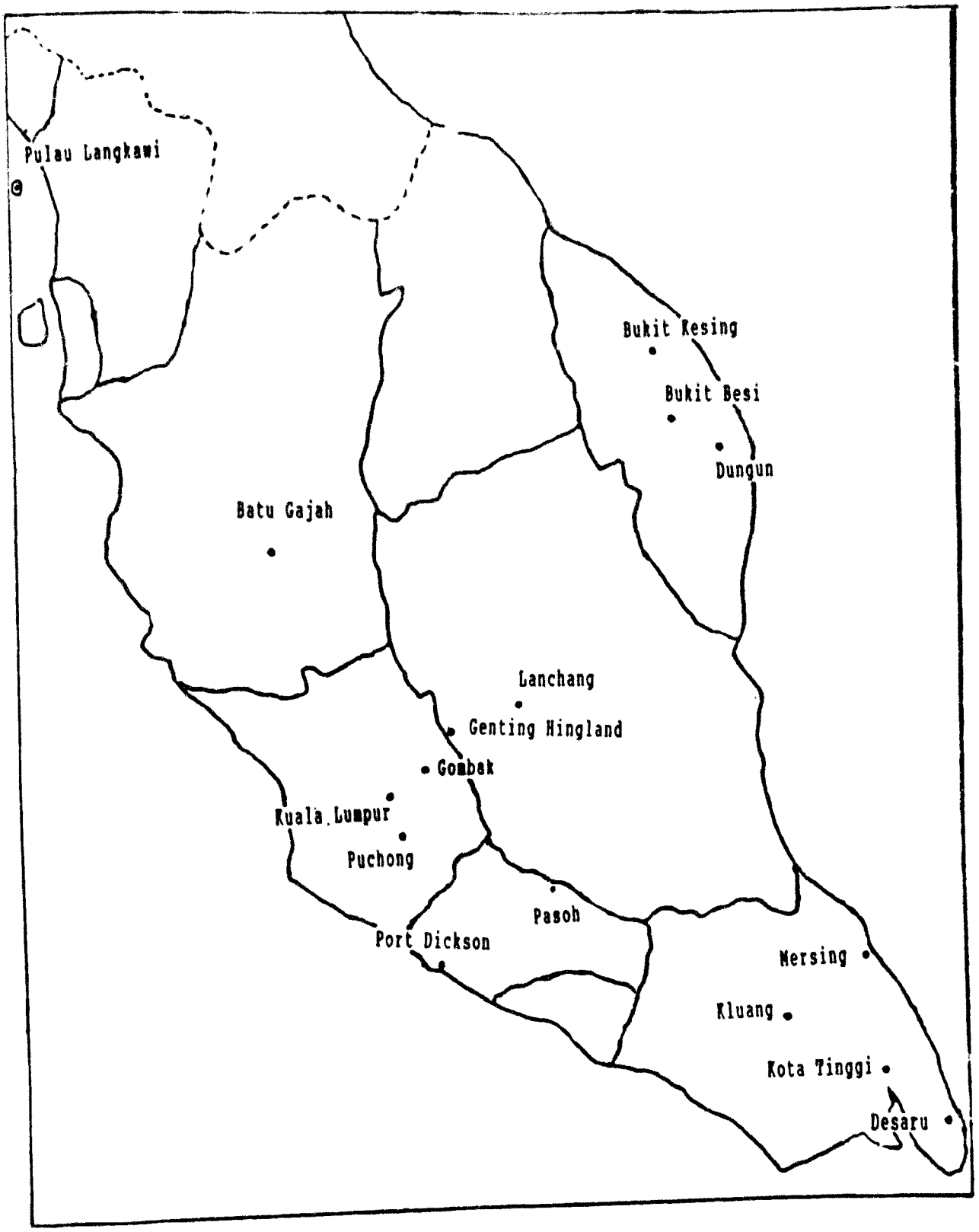


Figure 2.1 : Distribution of *Uncaria* species in Peninsular Malaysia

Table 2.1 : Distribution of *Uncaria* species in Peninsular Malaysia

	NORTHERN REGION			WEST COAST				SOUTHERN REGION				CENTRAL REGION			EAST COAST	
	Pulau Langkawi	Batu Gajah	Puchong	Kuala Lumpur	Pasoh	Port Dickson	Kota Tinggi	Kluang	Mersing	Desaru	Lanchang	Genting Highland	Gombak	Bukit Kesing	Bukit Besi	Dungun
U. callophylla	-	+	+	-	+	-	+	+	+	+	-	-	-	+	+	+
U. acida[1]	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-	-
U. lanosa v. glabrata	-	+	+	-	-	-	-	-	+	-	+	+	+	+	-	+
U. lanosa v. ferrea	-	+	+	-	-	-	+	-	+	+	+	+	-	+	+	-
U. gambir	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
U. cordata[1]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
U. cordata v. cordata f. cordata	-	-	+	-	-	-	-	-	-	-	+	+	-	+	-	-
f. sundaica	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-
U. cordata v. ferruginea f. leiantha	-	-	+	-	-	-	-	-	-	-	+	+	-	+	-	+
f. ferruginea	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
U. elliptica	-	+	+	-	-	-	+	-	+	+	-	-	-	+	+	-
U. borneensis	-	+	+	-	-	-	+	-	+	-	-	-	-	+	+	-
U. longiflora v. pteropoda	-	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+
U. longiflora v. longiflora	-	+	+	-	-	-	-	-	+	+	-	-	-	+	+	-

[1] partial identification only.

The more commonly encountered species are *U. cordata*, *U. callophylla* and *U. longiflora var. pteropoda*. Surprisingly, it was difficult to locate *U. gambir*, which in the past was of considerable economic importance as a source of gambier used for dyeing and tanning.[3] Another significant result arising from this extensive screening exercise was the recognition of a distinct alkaloidal pattern as shown by t.l.c. for *U. callophylla* and *U. elliptica* (Fig. 2.2); which provided a quick and convenient chemotaxonomic-based aid to the identification of these troublesome species. Identification of these species in particular have posed problems in the past for the group of taxonomically related species which include *U. callophylla*, *U. acida*, *U. elliptica* and *U. gambir*.[1,2] The present results provide a useful phytochemical-based method as an aid for species verification.

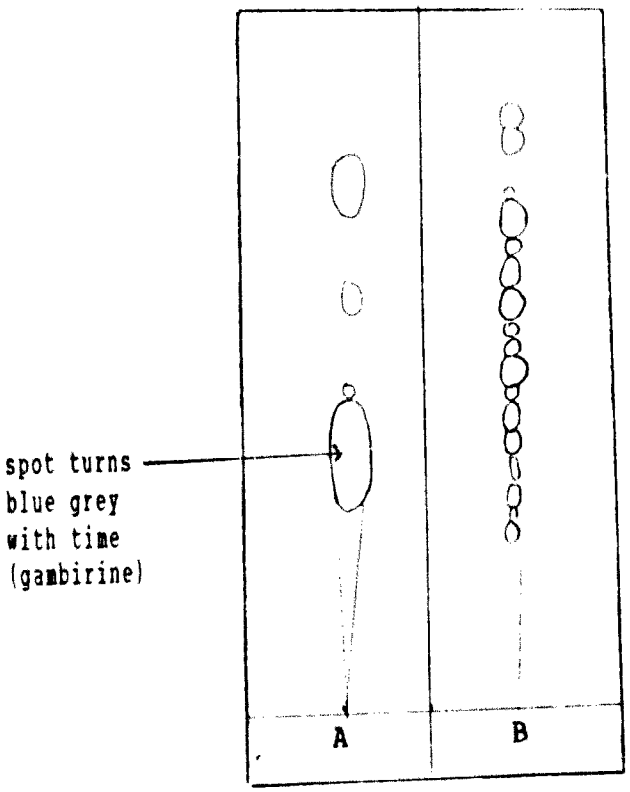
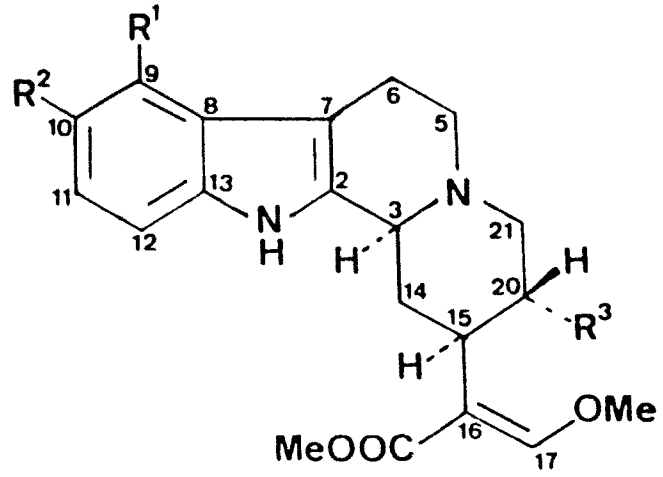


Figure 2.2 : Thin layer chromatogram of crude alkaloid extract from A) *U. callophylla* ($CHCl_3$: MeOH, 10:1) and B) *U. elliptica* ($CHCl_3$: MeOH, 15:1).

2.2 STRUCTURE ELUCIDATION

2.2.1 Tetracyclic Heteroyohimbine Alkaloids

The structures of the tetracyclic heteroyohimbine alkaloids isolated are shown below. Isogambirine [86] and Gambireine [87] are new alkaloids.



	R^1	R^2	R^3
[20] DIHYDROCORYNANTHEINE	H	H	CH_2CH_3
[22] GAMBIRINE (9-hydroxydihydrocorynantheine)	OH	H	CH_2CH_3
[86] ISOGAMBIRINE (10-hydroxydihydrocorynantheine)	H	OH	CH_2CH_3
[87] GAMBIREINE (9-hydroxycorynantheine)	OH	H	$CH{=}CH_2$

2.2.1.1 Dihydrocorynantheine [20]

Dihydrocorynantheine has been found in a number of *Uncaria* species[2] and is also common in other plant families.[44] The absolute configuration of dihydrocorynantheine has been established as having the normal configuration.[45]

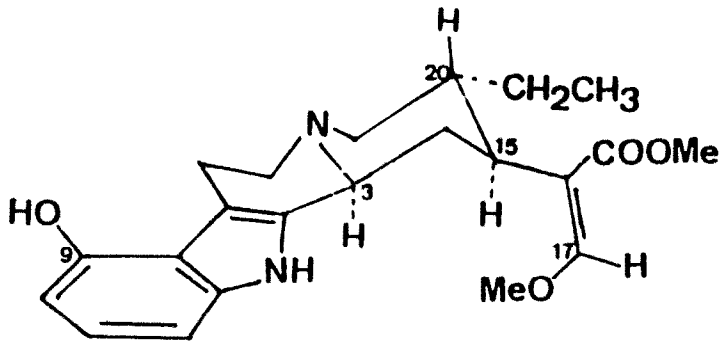
The ^{1}H NMR spectrum of dihydrocorynantheine isolated in the present study shows the presence of an ethyl group, indicated by an unsymmetrical triplet at 0.85 ppm, an unsubstituted indole nucleus (multiplet, 4H, 7.01-7.48 ppm), an olefinic hydrogen (singlet, 1H, 7.27 ppm), two methoxy singlets at 3.69 and 3.73 ppm due to carbomethoxy and enol ether methoxy groups respectively, and a broad NH singlet at 8.16 ppm. The remaining methylene and methine hydrogens appear as overlapping multiplets from 1.04 to 3.50 ppm. The ^{1}H NMR assignments are summarized in Table 2.2. The ^{13}C NMR spectral data of dihydrocorynantheine is presented in Table 2.3 and correlates well with published data.[46,47]

2.2.1.2 Gambirine [22]

Gambirine was first reported from the leaves of '*U. gambir*' by Merlini *et. al.* and was identified as 9-hydroxy-dihydrocorynantheine.[48] It has subsequently been established that only one *Uncaria* species namely, *U. callophylla*, provides the only source of this alkaloid, the name of which has thus remained a misnomer.[2,12,16]

The mass spectrum of gambirine shows a molecular ion at m/z 384, a strong M^+-1 fragment, and other ion fragments which are consistent with a hydroxy-substituted heteroyohimbine.[49] The ^1H NMR spectrum of gambirine is shown in Figure 2.3 and the assignments are summarized in Table 2.2. The high field region from 1.0 to 3.8 ppm resembles that of dihydrocorynantheine, accounting for the carbomethoxy, enol ether methoxy and ethyl groups. A broad NH singlet and olefinic hydrogen singlet are observed at 7.67 and 7.36 ppm respectively. The aromatic region however shows substantial differences. The observed pattern is suggestive of aromatic substitution. Thus a one proton doublet of doublet with J = 7.5, 2.0 Hz is observed at 6.40 ppm being characteristic of 9- or 12-substituted heteroyohimbine. Examination of the aromatic ^{13}C NMR carbon resonances allows placement of the hydroxy substitution at C9. The doublet of doublet centered at 6.40 ppm is thus assigned to H-12. The H-10 and H-11 signals are not resolved at 100 MHz and are observed as multiplets from 6.79 to 7.02 ppm. The hydroxy proton is consistently not detected in $CDCl_3$ solution.

The ^{13}C NMR spectral data of gambirine is presented in Table 2.3, where some of the previous assignments[12] have been revised in view of larger amounts of material available for more definitive spectra.



[22]

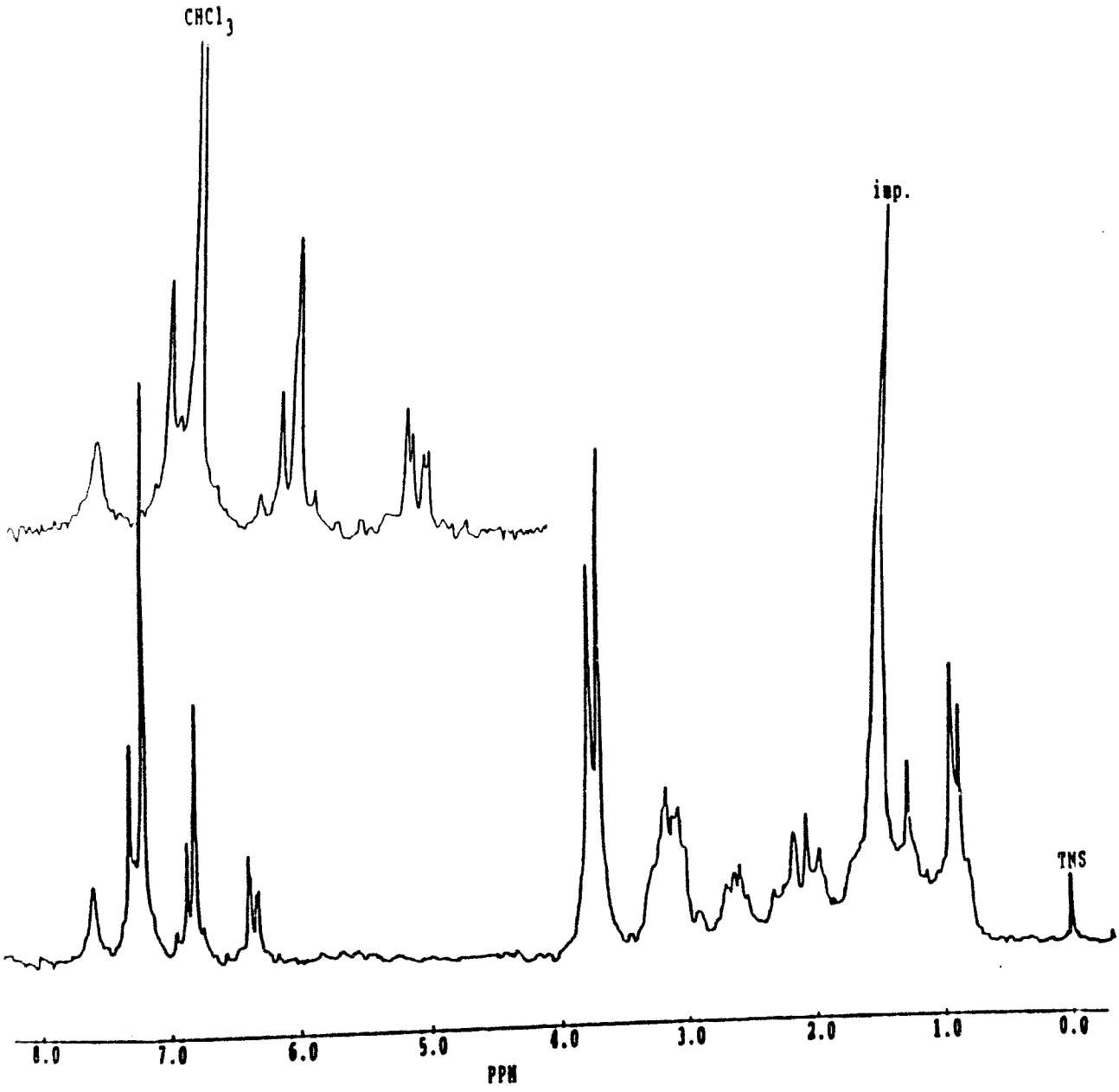


Figure 2.3 : ^{1}H NMR Spectrum ($CDCl_3$, 100MHz) of Gambirine.

2.2.1.3 UC7 (Isogambirine [86])

UC7 is a minor alkaloid isolated from the leaves of *U. callophylla*. The mass spectrum shows a molecular ion at m/z 384 which is also the base peak. HRMS (M^+ 384.2050) gave the molecular formula $C_{22}H_{28}N_2O_4$ (calcd. 384.2049). The presence of a strong M^+-1 ion suggests that UC7 is a heteroyohimbine since in oxindole alkaloids the M^+-1 fragments are absent.[49] The proposed pathway leading to the principal fragment ions is shown in Figure 2.4. The main fragments at m/z 172 (a), 185 (b), 186 (c), 200 (d), 241 (e) and 255 (f) are characteristic of tetrahydro β-carboline compounds.[50,51] These peaks are in fact also seen in the mass spectrum of gambirine and dihydrocorynantheine (shifted by 16 mass units). The mass spectral data of UC7 thus suggests that it is an isomer of gambirine.

The ^1H NMR spectrum of UC7 (Figure 2.5 and Table 2.2) shows the presence of an imino group at 7.54 ppm (1H, s, disappears upon deuteration), a carbomethoxy group at 3.69 ppm (3H, s), an enol ether methoxy group at 3.75 ppm (3H, s), an olefinic proton singlet at 7.36 ppm which is typical of an alkaloid possesing the conjugated chromophore MeOOC-C=CHOMe and a poorly resolved triplet at 0.85 ppm (3H) attributed to the equatorial C18 hydrogens. The aromatic region shows an ABX pattern for the three aromatic protons with resonances at 7.09 ppm (1H, d, J = 8.5 Hz), 6.77 ppm (1H, dd, J = 8.5, 2.4 Hz) and 6.84 ppm (1H, d, J = 2.4 Hz). This allows placement of the hydroxyl group

at C10 which is also corroborated by the ^{13}C NMR spectral data. The ^{13}C NMR spectral data is also in accord with a normal configuration for UC7 by correlation with gambirine and dihydrocorynantheine (see Table 2.3). UC7 is a new alkaloid for which the name isogambirine [86] (10-hydroxydihydrocorynantheine) is proposed.

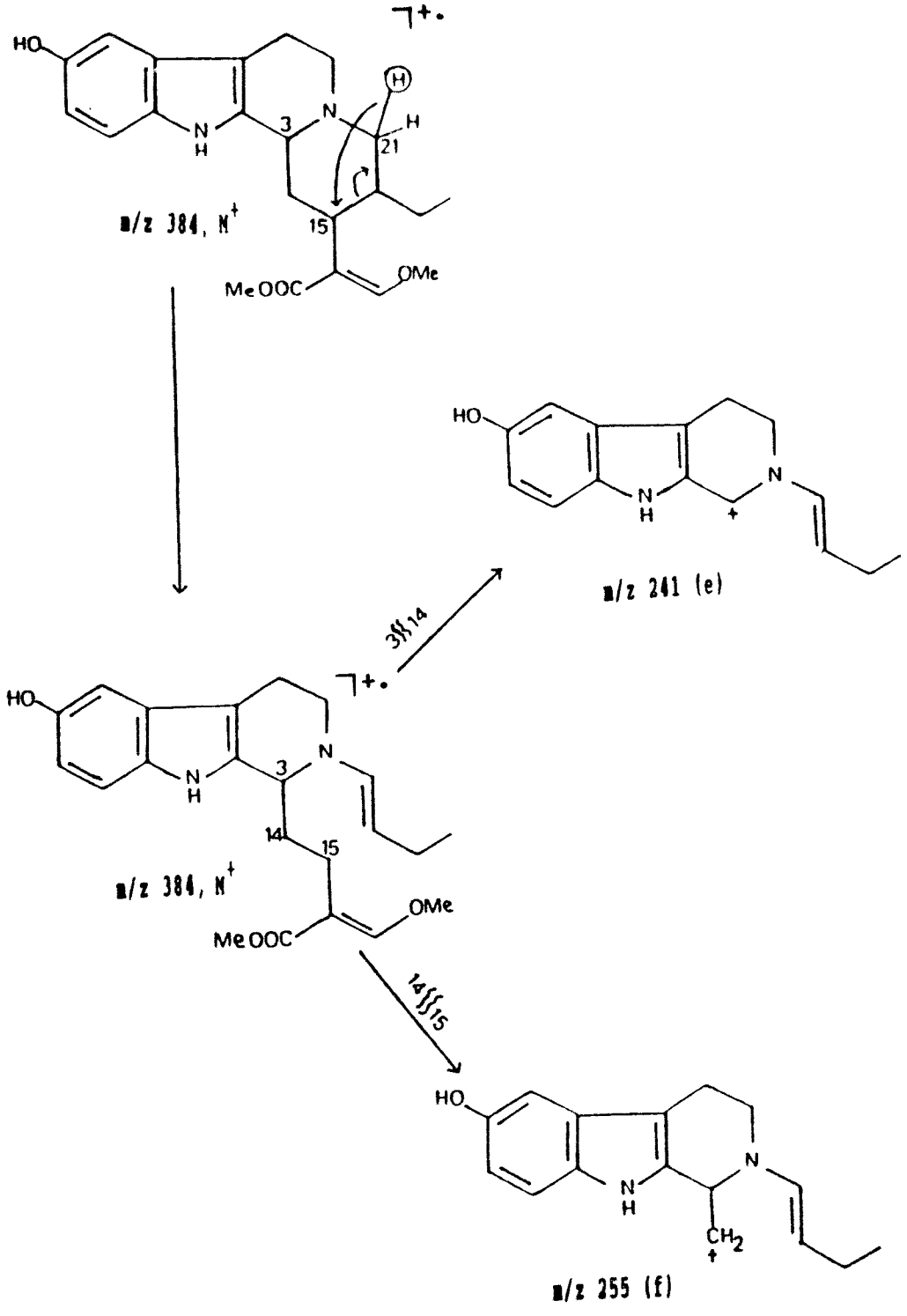


Figure 2.4a : MS Fragmentation of Isogambirine.

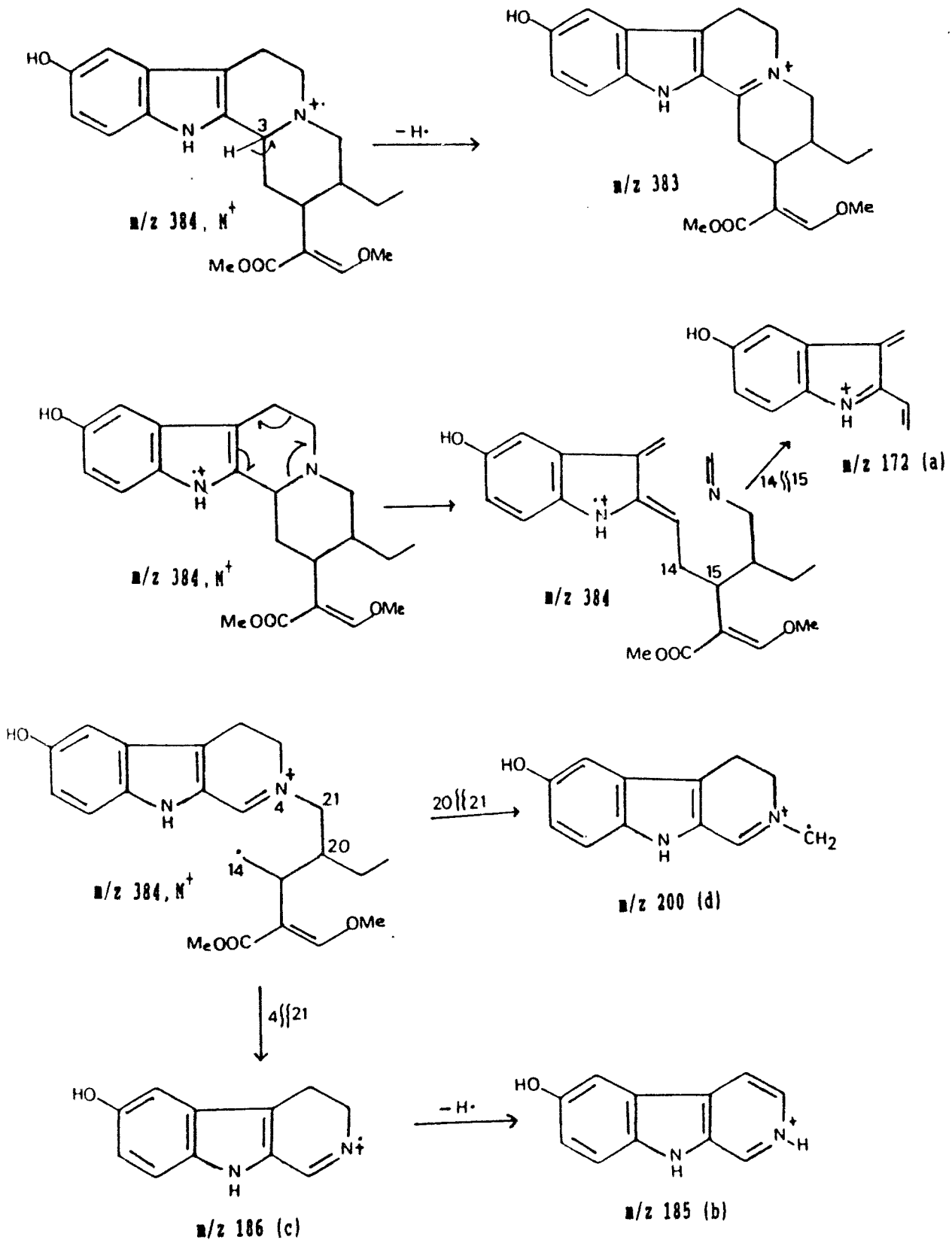
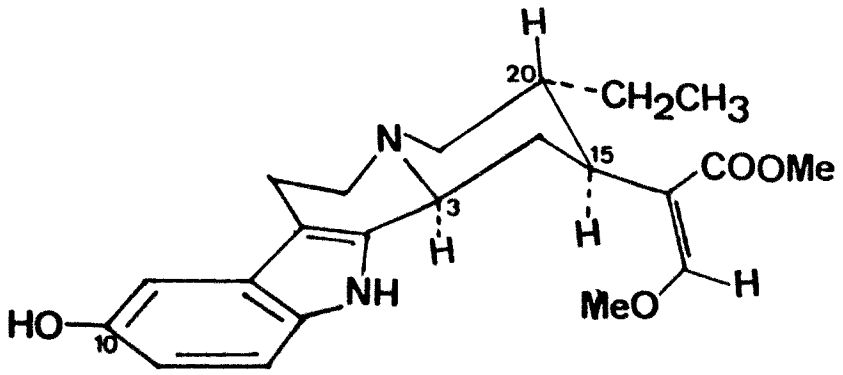


Figure 2.4b : MS Fragmentation of Isogambirine.



[86] ISOGAMBIRINE

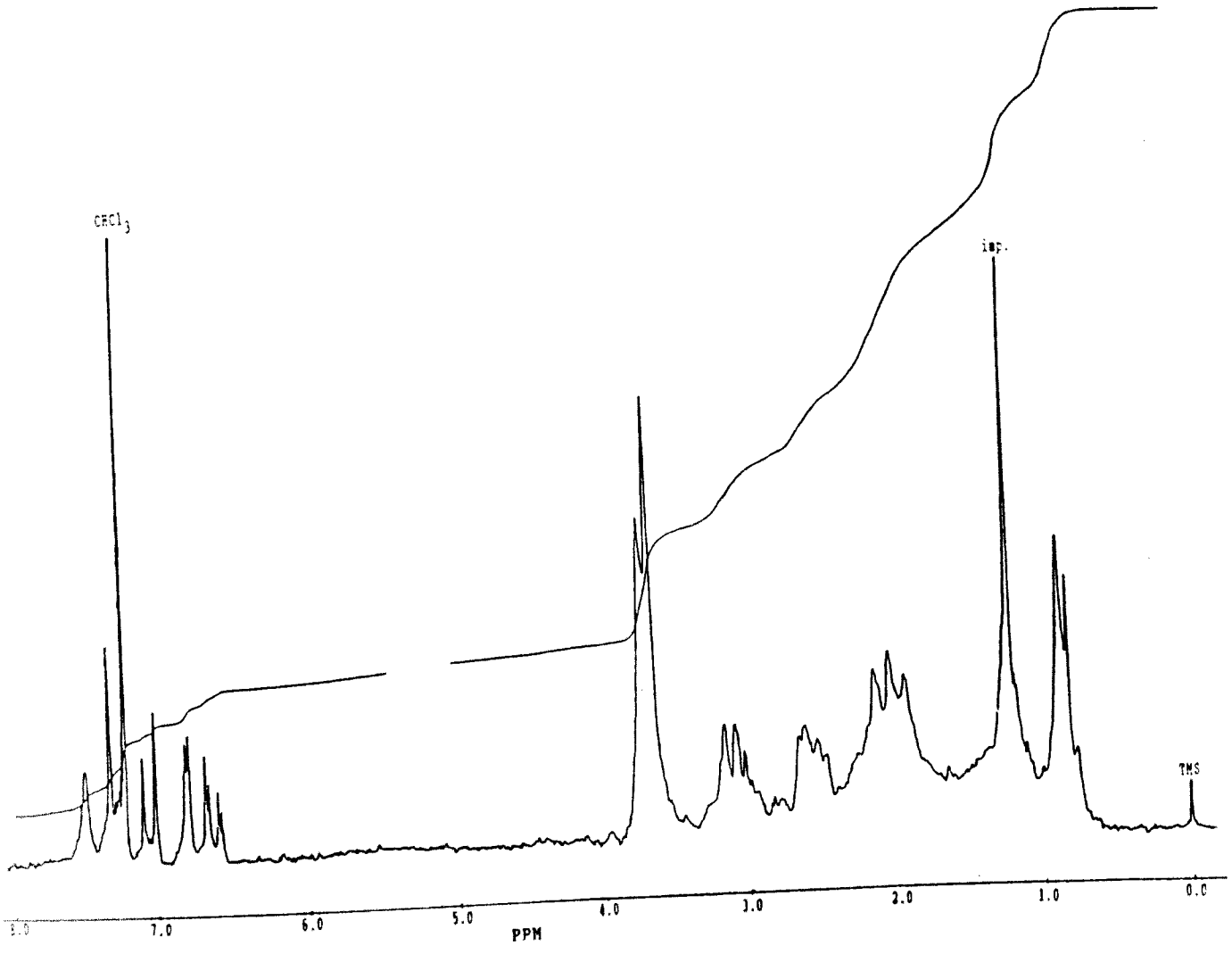
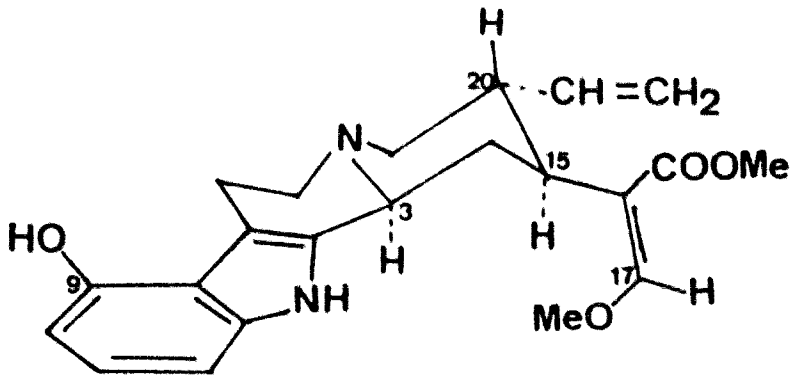


Figure 2.5 : ^{1}H NMR Spectrum ($CDCl_3$, 100MHz) of Isogambirine.

2.2.1.4 UC5 (Gambireine) [87]

UC5 is a minor alkaloid isolated from the leaves of *U. callophylla*. The mass spectrum shows molecular ion at m/z 382 (HRMS 382.1868) corresponding to the molecular formula $C_{22}H_{26}N_2O_4$ (calcd. 382.1892). (The main fragments at m/z 172, 185, 186, 200, 239 and 253 are characteristic of a tetrahydro β-carboline compound and also suggest that UC7 is an vinyl analogue of gambirine[49,51]). The ^1H NMR spectrum accounts for the presence of indole NH (7.74 ppm, 1H, s, disappears upon deuteration), carbomethoxy group (3.68 ppm, 3H, s), methoxy group (3.75 ppm, 3H, s) and an olefinic hydrogen (7.32 ppm, 1H, s). In addition, the presence of a vinyl group is indicated by signals at 4.8-5.0 ppm. (m, 2H, -CH=$\underline{CH_2}$) and 5.3-5.7 ppm. (1H, m, -$\underline{CH}$=CH_2). The aromatic region shows an ABX pattern for the three aromatic protons with resonances at 6.78-6.91 ppm (2H, m) and 6.38 ppm (1H, dd, J = 7,2 Hz) consistent with a C9 or C12 substituted heteroyohimbine. The hydroxy substituent is placed on C9 on the basis of the ^{13}C NMR spectral data in which the aromatic carbon chemical shifts are very similar to those of gambirine. The presence of the vinyl substituent at C20 is indicated by characteristic carbon shifts at 115.6 and 139.2 ppm for C18 and C19 respectively. The other carbon resonances are readily assigned based on their multiplicities and by correlation with gambirine and corynantheine [21] (see Table 2.3), thus, establishing UC5 as the vinyl analogue of gambirine.

UC5 is a new alkaloid, for which the name gambireine [87] (9-hydroxycorynantheine) is proposed.



[87] GAMBIREINE

Table 2.2 : ^{1}H NMR Spectral Data ($CDCl_3$, 100 MHz) of Dihydrocorynantheine [20], Gambirine [22], Isogambirine [86] and Gambireine [87].

Alkaloid	$-CH_2\underline{CH}_3$	OMe	COOMe	H-9	H-10	H-11	H-12	H-17	NH
Dihydrocorynantheine	0.85t	3.73s	3.69s	(---------7.01-7.48m-------)				7.27s	8.16s
Gambirine	0.88t	3.77s	3.69s	-	6.79-7.02 m		6.40dd	7.36s	7.67s
Isogambirine	0.85t	3.75s	3.69s	6.84d	-	6.77dd	7.09d	7.36s	7.54s
Gambireine	*	3.75s	3.68s	-	6.78-6.91 m		6.38dd	7.32s	7.74s

* $-\underline{CH}=CH_2$ 5.3-5.7 m; $-CH=\underline{CH}_2$ 4.8-5.0 m

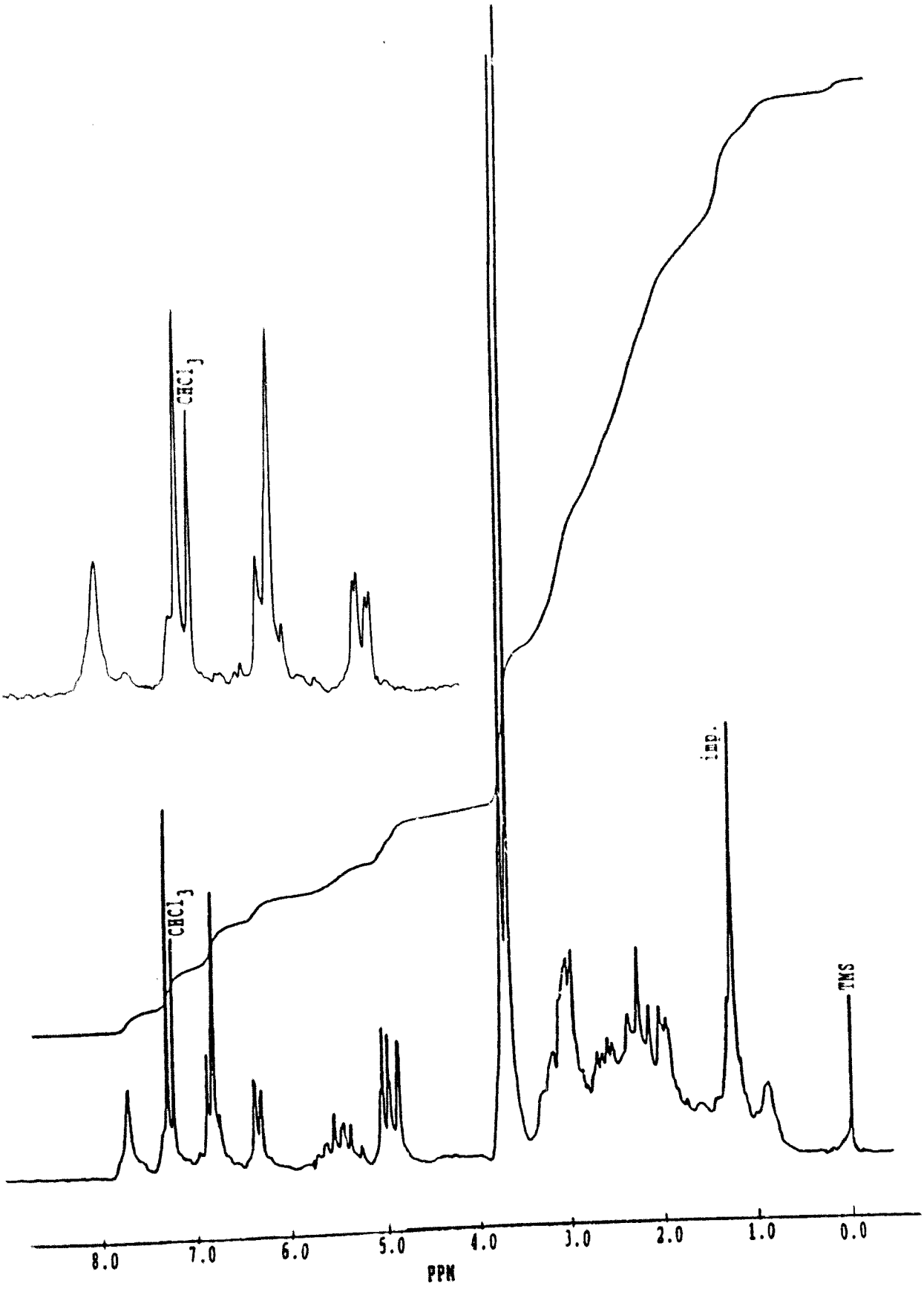


Figure 2.6 : ^{1}H NMR Spectrum ($CDCl_3$, 100 MHz) of Gambireine

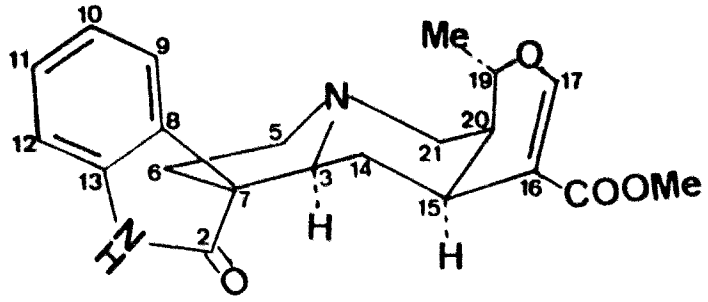
Table 2.3 : ^{13}C NMR Spectral Data ($CDCl_3$, 25 MHz) of Dihydrocory-nantheine [20], Gambirine [22], Isogambirine [86], Gambireine [87] and Corynantheine [21].

Carbon	[20]	[22]	[86]	[87]	[21]*
C2	135.1	132.4	131.1	133.2	135.2
C3	60.3	60.3	60.3	59.9	59.9
C5	53.1	53.2	52.9	52.9	52.6
C6	21.8	23.0	21.4	23.5	21.8
C7	107.8	106.7	107.4	106.9	107.5
C8	127.4	116.7	127.9	116.5	127.4
C9	118.0	150.8	103.1	150.1	117.9
C10	121.0	103.5[a]	149.8	104.4[b]	120.9
C11	119.2	121.7	111.3	122.1	119.0
C12	110.7	103.0[a]	110.9	103.8[b]	110.8
C13	136.0	138.1	135.7	138.0	136.2
C14	33.9	33.2	33.5	33.3	33.1
C15	38.7	38.1	38.1	38.6	38.8
C16	111.7	111.5	111.6	111.5	111.7
C17	159.9	160.2	160.1	159.9	159.8
C18	11.3	10.9	11.2	115.6	115.4
C19	24.4	24.2	24.4	139.2	139.2
C20	39.5	39.2	39.2	42.7	42.4
C21	60.9	60.6	61.6	61.3	61.3
C=O	168.9	169.4	169.2	168.8	168.9
COOMe	51.1	51.2	51.5	51.3	51.1
CHOMe	61.5	61.5	60.7	61.1	61.3

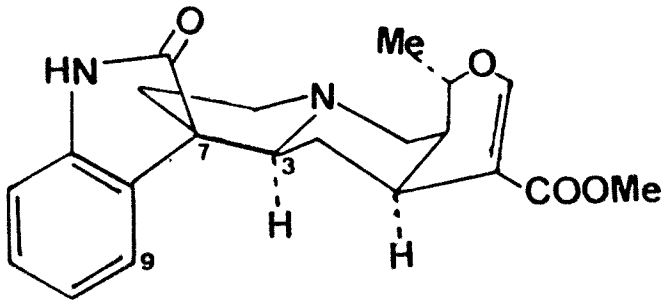
*From reference 46
[a,b]assignments may be interchanged

2.2.2 Pentacyclic Oxindole Alkaloids

The structures of the pentacyclic oxindole alkaloids isolated are shown below.



[34] ISOPTEROPODINE



[35] PTEROPODINE

2.2.2.1 Isopteropodine [34] and Pteropodine [35]

Isopteropodine and pteropodine were first isolated from *U. pteropoda* (now referred to as *U. longiflora var. pteropoda*[1]) by Chan and co-workers[27] and its stereochemistry was later established by ^{1}H NMR spectroscopy.[52]

In this study, isopteropodine and pteropodine were also isolated from other *Uncaria* species. Their physical and spectral

properties are in good agreement with published data.[20,52] The ^1H NMR spectrum of isopteropodine shows the presence of C19-CH_3 as indicated by a doublet at 1.41 ppm, one methoxy group (singlet, 3.60 ppm), C19-H (multiplet, 4.2-4.5 ppm), four aromatic protons (multiplet, 6.80-7.30 ppm), olefinic H (singlet, 7.41 ppm), and indole NH (broad singlet, 8.31 ppm). The remaining hydrogens are overlapped from 0.68-3.45 ppm. The ^1H NMR spectrum of pteropodine is very similar to that of isopteropodine with some minor differences in their δ values. These data are summarized in Table 2.4. The ^{13}C NMR spectrum of isopteropodine and pteropodine show the presence of a total of 21 carbon atoms. The oxindole carbonyl resonance at *ca.* 182 ppm. as well as the characteristic aromatic carbon resonances can readily be assigned as are the remaining carbon resonances based on their multiplicities and using isorhynchophylline and rhynchophylline as reference compounds.[46] The carbon assignments are summarized in Table 2.5.

Table 2.4 : ^1H NMR Spectral Data ($CDCl_3$, 100 MHz) of Isopteropodine [34] and Pteropodine [35].

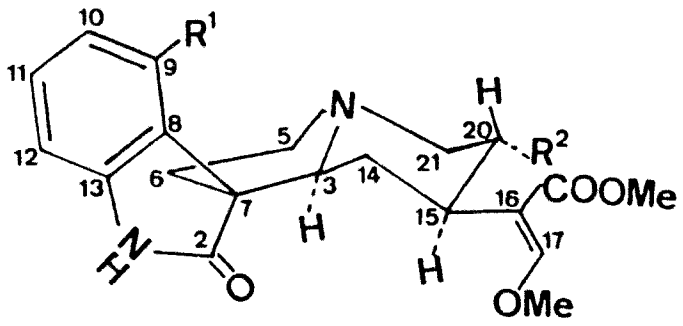
Hydrogen	δ / ppm Isopteropodine	Pteropodine
C19-CH_3	1.41, d, J = 7 Hz	1.33, d, J = 7 Hz
COOMe	3.60, s	3.53, s
H-19	4.2-4.5, m	4.3-4.7, m
4 Aromatic H	6.80-7.30, m	6.80-7.20, m
H-17	7.41, s	7.41, s
NH	8.31, br s	8.40, br s

Table 2.5 : ^{13}C NMR Spectral Data ($CDCl_3$, 25 MHz) of Isopteropodine [34] and Pteropodine [35].

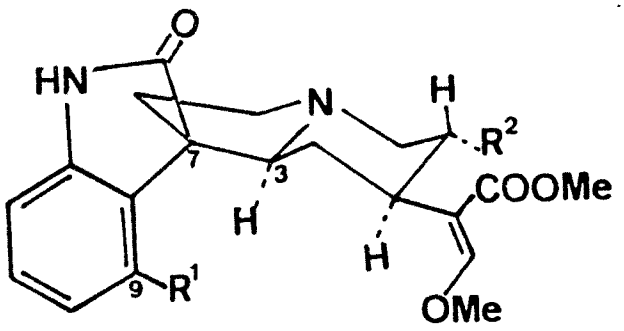
Carbon	[34]	[35]
2	181.3	181.5
3	71.3	74.4
5	54.1	55.2
6	30.2	31.1
7	57.0	56.2
8	133.8	133.5
9	124.5	123.0
10	122.5	122.6
11	127.7	127.9
12	109.7	109.2
13	140.3	140.9
14	34.9	34.7
15	30.5	29.6
16	109.9	109.7
17	155.0	155.3
19	72.2	72.2
20	37.9	37.9
21	53.5	53.7
C19-$\underline{CH}_3$	18.6	18.6
C=O	167.6	167.7
OCH_3	50.9	50.9

2.2.3 Tetracyclic Oxindole Alkaloids

The structures of the tetracyclic oxindole alkaloids isolated are shown below.



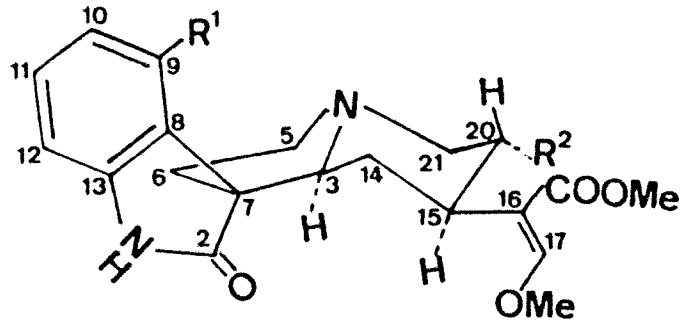
	R^1	R^2
[38] ISORHYNCHOPHYLLINE	H	CH_2CH_3
[40] ISOCORYNOXEINE	H	$CH{=}CH_2$
[42] ROTUNDIFOLINE	OH	CH_2CH_3



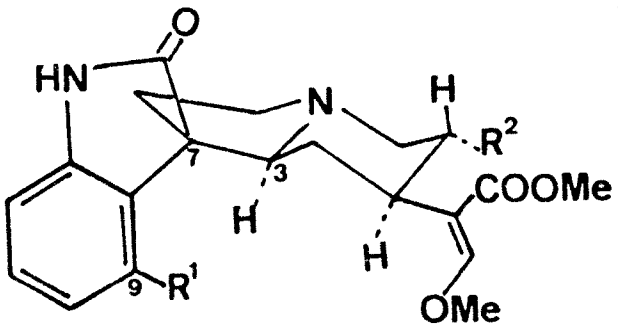
	R^1	R^2
[39] RHYNCHOPHYLLINE	H	CH_2CH_3
[41] CORYNOXEINE	H	$CH{=}CH_2$

2.2.3 Tetracyclic Oxindole Alkaloids

The structures of the tetracyclic oxindole alkaloids isolated are shown below.



	$\underline{R}^1$	$\underline{R}^2$
[38] ISORHYNCHOPHYLLINE	H	CH_2CH_3
[40] ISOCORYNOXEINE	H	$CH{=}CH_2$
[42] ROTUNDIFOLINE	OH	CH_2CH_3



	$\underline{R}^1$	$\underline{R}^2$
[39] RHYNCHOPHYLLINE	H	CH_2CH_3
[41] CORYNOXEINE	H	$CH{=}CH_2$

2.2.3.1 Isorhynchophylline [38] and Rhynchophylline [39]

Isorhynchophylline and rhynchophylline are known tetracyclic oxindole alkaloids which have been previously isolated from *Uncaria*[2] as well as other plant species.[53] The ^{1}H NMR spectral data of rhynchophylline and isorhynchophylline isolated in this work are summarized in Table 2.6 and are consistent with published data[54] accounting for the presence of ester and enol ether methoxy functions, aromatic protons, H-17 olefinic and imino hydrogens and the C20 ethyl substituent. The upfield aliphatic hydrogens (methylene and methine) are seen as overlapping multiplets from 0.4 - 3.5 ppm. The ^{13}C NMR spectral data of isorhynchophylline and rhynchophylline obtained in this work are given in Table 2.9. The data correlates well with published values.[46] A noteworthy feature of the ^{13}C NMR data is the chemical shifts of C3 and C9 which are diagnostic of the configuration of the spiro carbon, C7.

Table 2.6 : ^{1}H NMR Spectral Data ($CDCl_3$, 100 MHz) of Isorhynchophylline [38] and Rhynchophylline [39]

Hydrogen	δ / ppm	
	Isorhynchophylline	Rhynchophylline
$CH_2\underline{CH_3}$	0.79, t	0.77, t
OMe	3.60, s	3.62, s
COO<u>Me</u>	3.52, s	3.55, s
4 Aromatic H	6.80-7.20, m	6.80-7.20, m
H-17	7.14, s	7.18, s
NH	8.98, br, s	8.92, br, s

2.2.3.2 Isocorynoxeine [40] and Corynoxeine [41]

Isocorynoxeine and corynoxeine are C20 vinyl analogues of isorhynchophylline and rhynchophylline respectively. All four alkaloids are usually found to occur together in various species of *Uncaria*[22] and *Mitragyna*.[55]

The same pattern is also observed in the present study where the four alkaloids are found to occur together in *U. longiflora var. longiflora* and in *U. borneensis*.

The ^{1}H NMR spectrum of corynoxeine and isocorynoxeine are generally very similar to that of rhynchophylline and isorhynchophylline (see Table 2.6) except that the signal due to the C18 methyl group is now replaced by signals due to the vinyl substituent at C20. These data are summarized in Table 2.7. The same is true in the ^{13}C NMR spectrum where it can also be seen that except for C18, C19 and to a lesser extent C20, the other carbon shifts are very similar to those of rhynchophylline and isorhynchophylline. The carbon assignments are summarized in Table 2.9.

Table 2.7 : ^{1}H NMR Spectral Data ($CDCl_3$, 100 MHz) of Isocorynoxeine [40] and Corynoxeine [41].

Hydrogen	δ /ppm Isocorynoxeine	Corynoxeine
-CH=CH_2	5.30 - 5.70, m	5.35 - 5.69, m
-CH=CH_2	4.85 - 5.02, m	4.86 - 5.02, m
OMe	3.68, s	3.70, s
COOMe	3.57, s	3.60, s
4 Aromatic H	6.84 - 7.48, m	6.88 - 7.27, m
H-17	7.23, s	7.22, s
NH	8.89, br s	8.83, br s

2.2.3.3 Rotundifoline [42]

Rotundifoline has been previously isolated from several *Uncaria* species.[2] It was isolated as a minor alkaloid from *U. callophylla* in the present study. The ^{1}H NMR spectrum shows the presence of ethyl and two methoxy groups, olefinic, aromatic, and imino hydrogens as summmarized in Table 2.8. The coupling pattern of the aromatic hydrogens (6.36, d; 6.57, d; 7.04, t) is characteristic of a 9-substituted tetracyclic oxindole and is in fact in good agreement with the reported spectrum of rotundifoline.[56] This is further supported by the ^{13}C NMR spectrum (Table 2.9) where the observed aromatic carbon shifts also confirm hydroxy substitution at C9.

Table 2.8 : ^{1}H NMR Spectral Data ($CDCl_3$, 100 Mz) of Rotundifoline

Hydrogen	δ/ppm	J/Hz
$CH_2\underline{CH}_3$	0.80, t	7
CHOMe	3.69, s	-
COOMe	3.58, s	-
H-10	6.36, d	8
H-11	7.04, t	8
H-12	6.57, d	8
H-17	7.22, s	-
NH	8.30, br s	-

Table 2.9 : ^{13}C NMR Spectral Data ($CDCl_3$, 25 MHz) of Isorhynchophylline [38], Rhynchophylline [39], Isocorynoxeine [40], Corynoxeine [41], and Rotundifoline [42].

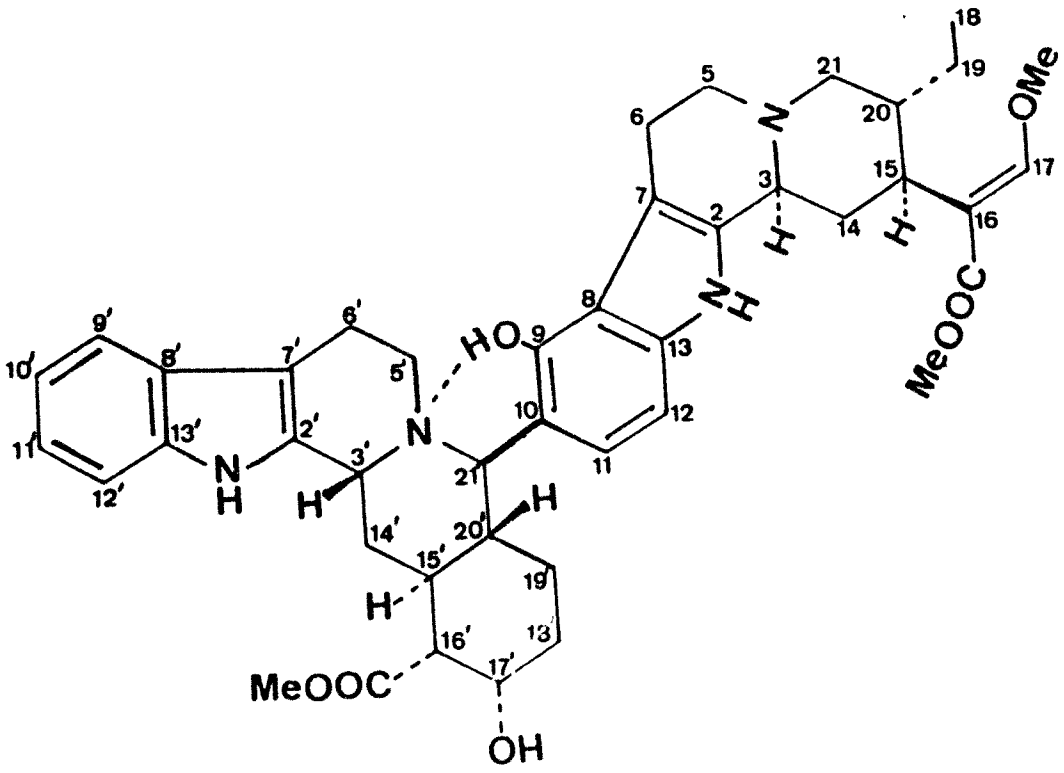
Carbon	[38]	[39]	[40]	[41]	[42]
2	182.4	182.0	182.2	181.9	179.5
3	72.3	74.8	72.0	75.0	68.6
5	54.2	54.6	53.9	54.8	53.1
6	35.4	34.5	35.2	34.8	33.9
7	56.9	55.9	56.9	56.1	57.0
8	133.9	133.5	133.9	133.7	116.7
9	125.1	122.7	125.1	123.0	154.3
10	122.2	122.0	122.2	122.3	111.6
11	127.4	127.5	127.4	127.8	129.2
12	109.5	109.5	109.5	109.8	101.1
13	140.3	141.2	140.3	141.2	140.8
14	29.6	28.8	29.4	28.8	29.3
15	38.2	37.6	37.7	38.5	37.6
16	112.0	111.4	112.4	111.6	111.6
17	159.4	159.5	159.4	159.6	159.7
18	11.2	11.0	115.2	115.3	11.0
19	24.3	23.9	139.6	139.4	24.1
20	39.0	39.4	42.5	42.0	37.6
21	58.2	57.8	58.7	58.7	56.8
C=O	168.3	168.3	168.3	168.6	168.3
COOMe	50.9	50.8	50.9	51.1	51.0
CHOMe	61.2	61.2	61.2	61.4	61.3

2.2.4 Dimeric Indole Alkaloids

An unidentified dimeric alkaloid was first reported by Nasini *et al*, purportedly from "*U. gambir*".[57] Based on spectral data it was proposed that this dimer was constituted from substituted dihydrocorynantheine-type and yohimbine-type moieties. Subsequently Goh *et al*[12] reported the isolation of a dimeric alkaloid callophylline from a verified sample of *U. callophylla* which had ^{1}H NMR spectrum identical to that of the dimer reported by Nasini *et al*.[57] Additional spectral data *viz.* ^{13}C NMR allowed the assignment of the structure of callophylline [89] as being constituted from the monomeric moieties gambirine and pseudoyohimbine, both of which were also present in the leaves and stems of *U. callophylla*.[31] In view of the well known taxonomic difficulties associated with the genus *Uncaria* and in particular the difficulties in differentiating between *U. callophylla*, *U. gambir*, *U. acida* and *U. elliptica*[1,2,16] and the fact that gambirine has been consistently detected by us[12,31,41] and others[2] only in *U. callophylla*,[2] it is likely that the unknown dimer, as well as gambirine and dihydrocorynantheine which Nasini *et al* attributed to "*U. gambir*", actually originated from *U. callophylla*. Additional data have been accumulated on this dimeric alkaloid, callophylline, which is discussed below (2.3.4.1). The occurrence of gambirine (9-hydroxydihydrocorynantheine) and a dimeric alkaloid constituted from gambirine and pseudoyohimbine moieties, as well as the detection of other minor, hitherto

elusive alkaloidal spots on t.l.c. chromatograms during isolation of alkaloids, led us to speculate that other hydroxylated derivatives including dimeric alkaloids might be produced by *U. callophylla*. These expectations were fulfilled when we undertook to isolate minor alkaloids from a large scale extraction (10kg). In this manner we were able to isolate and characterize additional new hydroxy-substituted heteroyohimbines (named isogambirine [86] and gambireine [87], discussed earlier in Section 2.2.1) and new dimeric alkaloids (callophyllines A [89] and B [90]).

2.2.4.1 UC4, Dimer 1 (Callophylline [88])



[88] CALLOPHYLLINE

Callophylline is obtained in relatively small amounts (compared to gambirine and dihydrocorynantheine) as an amorphous powder, m.p. 330 ^{o}C (dec.). The spectral data (UV, IR, MS ^{1}H & ^{13}C NMR) of callophylline indicate that it is a dimeric alkaloid constituted from gambirine and pseudoyohimbine moieties. The mass spectrum (FAB) shows a MH^{+} ion at m/z 737.4 corresponding to the molecular formula $C_{43}H_{52}N_4O_7$. The presence of gambirine as one of the two units of the dimer is indicated by strong IR bands at νmax 1705 and 1690 cm^{-1} and an olefinic hydrogen singlet at 7.37 ppm characteristic of alkaloids possesing the conjugated chromophore MeOOC-$\overset{|}{C}$=CHOMe. This is confirmed by the presence of an unsymmetrical triplet at 0.87 ppm in the ^{1}H NMR spectrum (300 MHz) due to the C18 hydrogens. Other absorptions due to the gambirine unit include the indole NH (7.69 ppm) and the carbomethoxy and enol ether methoxy groups (3.80 and 3.70 ppm). The aromatic hydrogens of the gambirine unit are observed as a pair of AB doublets centered at 6.58 and 6.69 ppm with J_{AB} = 8 Hz, indicating adjacent hydrogens. This suggests that the dimeric alkaloid is bonded at the 10- or 12- position of gambirine. Examination of the ^{13}C NMR data (*vide infra*) as well as some partial 2-D NMR data allows the assignment of the dimer bond at C10 of the gambirine unit. The recognition of the other unit of the dimeric alkaloid as pseudoyohimbine is facilitated by the observation of two broad one proton singlets at 4.70 and 4.17 ppm characteristic of the *cis* H-3 and *cis* H-17 pseudoyohimbine as well as the characteristic four hydrogen aromatic multiplet at

7.1 to 7.5 ppm. The imino hydrogen of the pseudoyohimbine unit is observed at 8.29 ppm and exchanges with D_2O or CD_3OD at a faster rate compared to the gambirine NH at 7.69 ppm. The C17'-OH of the pseudoyohimbine unit is not observed and is possibly overlapped in the complex high field part of the spectrum. Likewise, the phenolic hydrogen of the gambirine unit is not observed in $CDCl_3$ solution but is more likely to be seen in DMSO solution. The ^1H NMR spectrum is shown in Figure 2.7 and the assignments are shown in Table 2.10. The assignment of the monomer units as gambirine and pseudoyohimbine is corroborated by the ^{13}C NMR spectral data of the dimeric alkaloid (Table 2.11) where it can also be seen that the carbon shifts support the attachment of the monomer units at C10 and C21' of gambirine and pseudoyohimbine respectively. The assignment of the multiplicities of the carbon resonances is now greatly facilitated by the 75 MHz DEPT spectrum which has been obtained (Fig. 2.8). Examination of the carbon shifts (Table 2.11) shows that the chemical shifts at or near the positions of coupling are shifted accordingly while the chemical shifts of the other carbons remain essentially the same as in the monomer alkaloids. Thus the chemical shift of C21' (52.0 ppm) of pseudoyohimbine is shifted to 63.8 ppm in the dimer and the chemical shift of C10 of gambirine to 113.1 from 103.5 ppm. Furthermore, the multiplicity of C21' of the pseudoyohimbine unit has changed from triplet to doublet (CH_2 to CH) and that of the aromatic C10 of the gambirine unit from doublet to singlet (CH to $C^{\#}$) providing additional support for the C10-C21'

attachment. These assignments are also supported by some 2-D NMR data (^1H-^1H COSY, Fig. 2.9 and ^1H-^{13}C correlation, Fig. 2.10). The ^1H-^1H, ^1H-^{13}C correlation spectrum confirms the assignment of the AB doublets at 6.58 and 6.69 ppm to H-11 and H-12 of gambirine respectively, thus leaving C10 on gambirine for dimer formation. Similarly, the doublet at 3.56 ppm assigned to H-21' of the pseudoyohimbine unit corelates with δ_C 63.8 ppm attributed to C21' of the pseudoyohimbine unit of the dimer which is shifted from 52.0 ppm in pseudoyohimbine.

It can be envisaged that the dimer is probably formed *via* electrophilic ortho attack of an imminium ion of pseudoyohimbine on gambirine. In view of the steric requirement, the C21' attachment is likely to be beta. Inspection of Dreiding models shows that a conformational arrangement is possible which allows for intramolecular H-bonding between the phenolic hydroxy proton of the gambirine unit and the 4'-nitrogen of the pseudoyohimbine unit. This would qualitatively account for the relative R_f values observed for dihydrocorynantheine, callophylline and gambirine (0.65, 0.58 and 0.43 respectively in 10% MeOH/$CHCl_3$) in t.l.c. chromatograms of the leaf alkaloid extracts where the dimeric alkaloid shows a higher R_f value compared to gambirine, a feature which is rationalized by the occurrence of intramolecular H-bonding in the dimeric alkaloid.

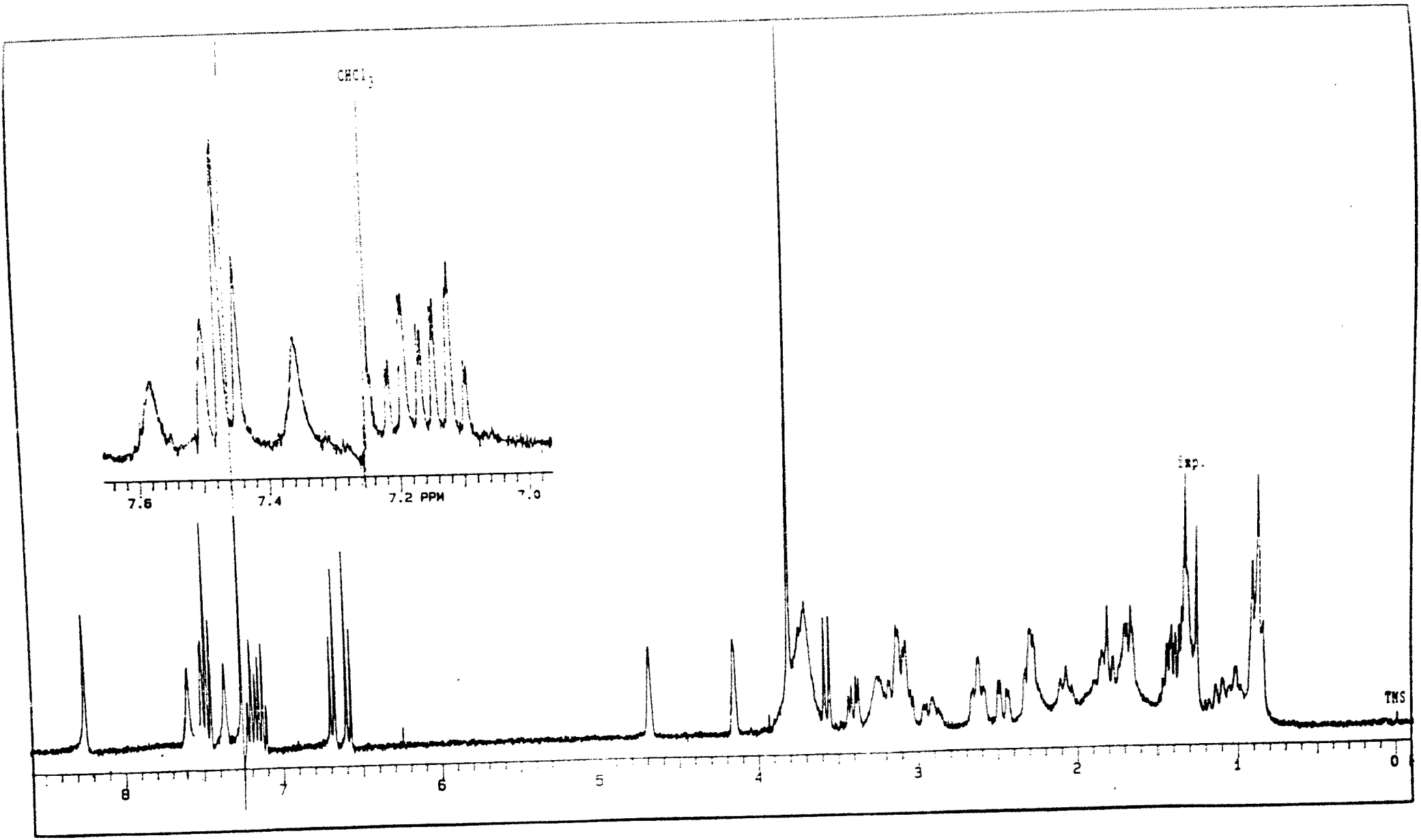


Figure 2.7 : ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of Callophylline.

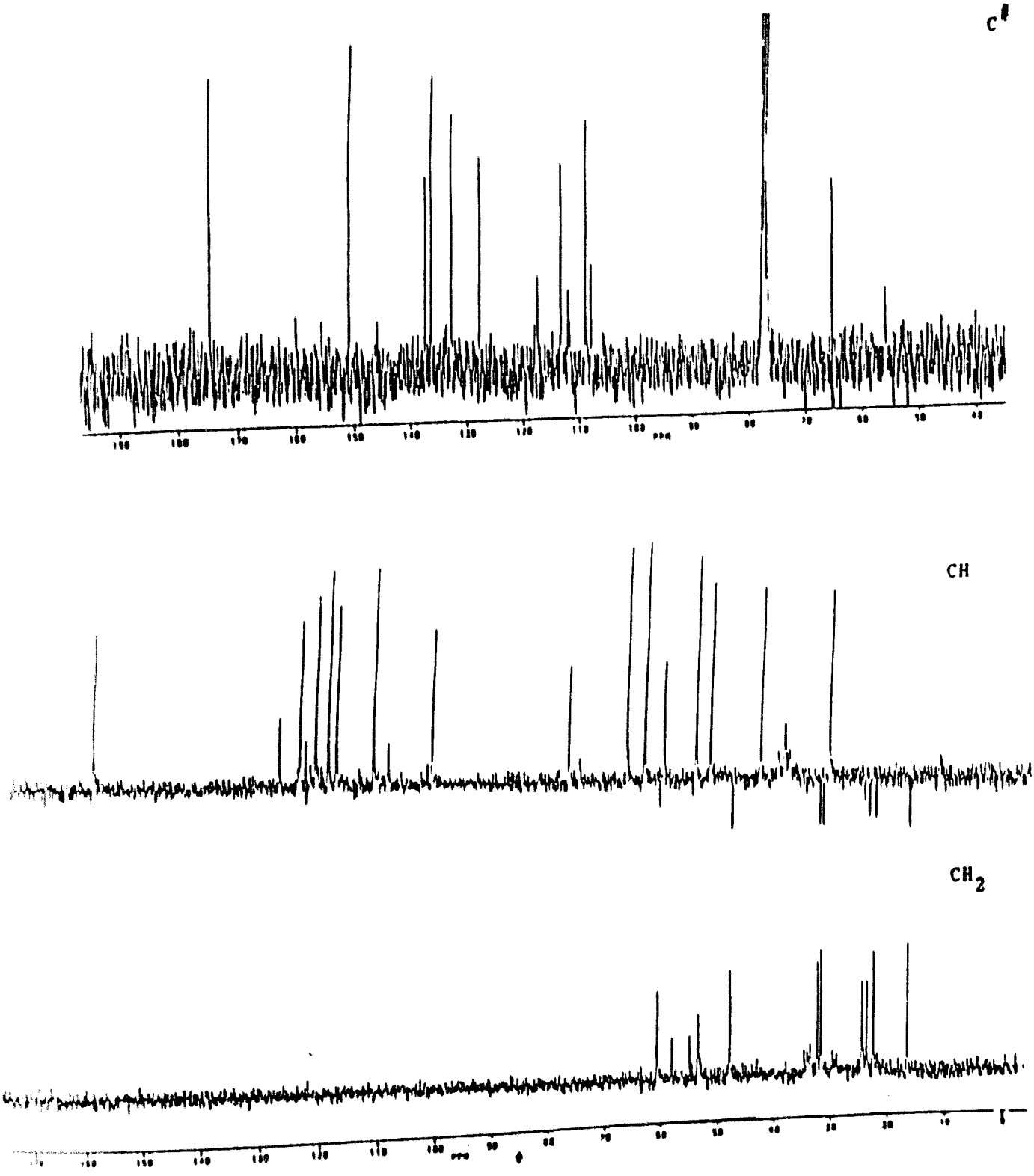


Figure 2.8 : ^{13}C NMR DEPT Spectrum ($CDCl_3$, 75 MHz) of Callophylline.

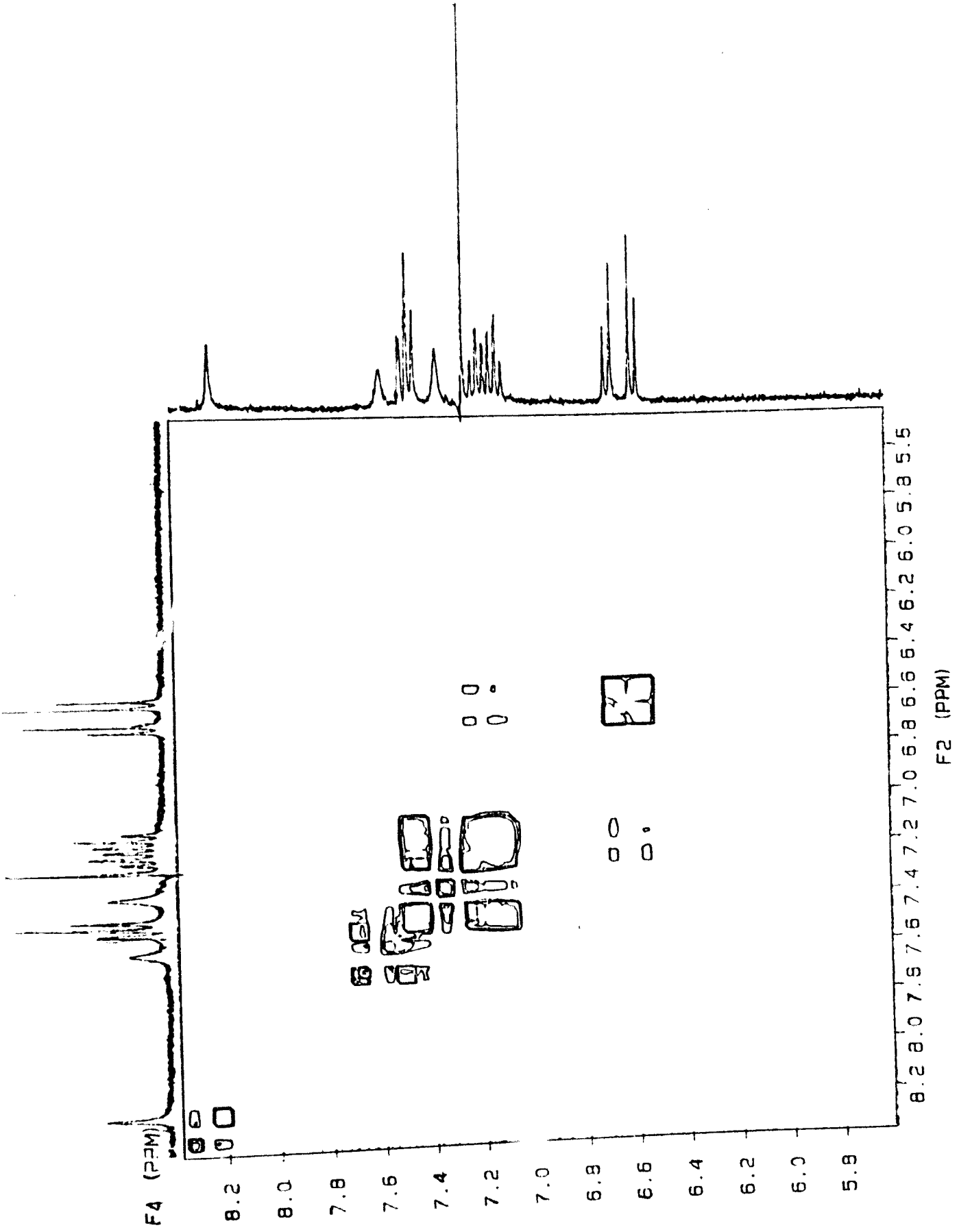


Fig. 2.9 : ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 300 MHz) of Callophylline.

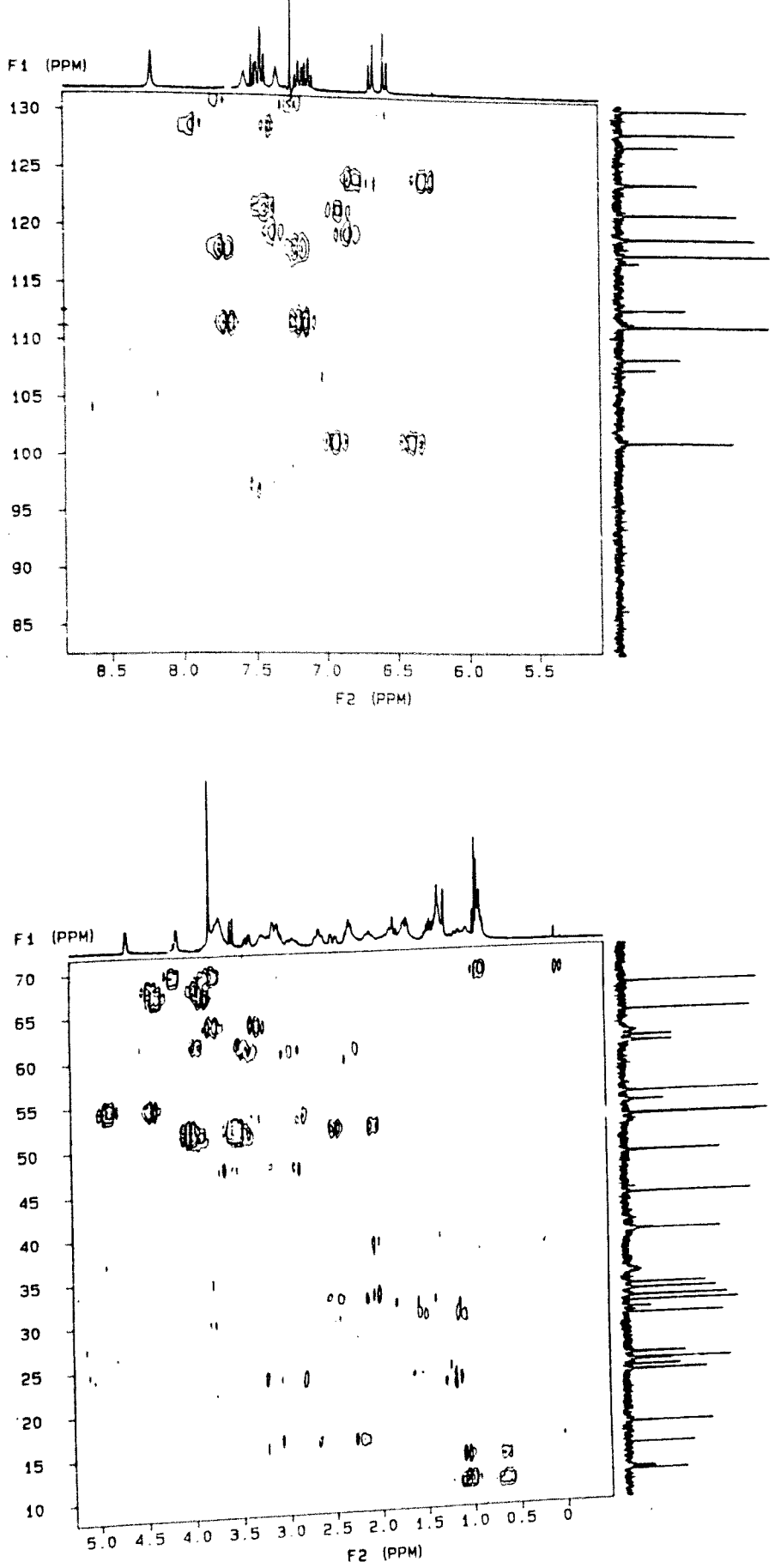


Figure 2.10 : ^{1}H-^{13}C Correlation Spectrum ($CDCl_3$, 300 MHz) of Callophylline.

Table 2.10 : ^{1}H NMR Spectral Data ($CDCl_3$, 300 MHz) of Callophylline [88].

Hydrogen	δ / ppm	J/Hz
H-11	6.58, d	8
H-12	6.69, d	8
H-17	7.37, s	-
H-9'	7.48, d	7
H-10'	7.12, ddd	7, 7, 1
H-11'	7.19, ddd	7, 7, 1
H-12'	7.45, d	7
H-21'	3.56, d	9
H-3'	4.70, br s	-
H-17'	4.17, br s	-
NH	7.69, br s	-
NH	8.29, br s	-
OMe	3.70, s	-
COOMe	3.70, s	-
COOMe'	3.82, s	-
$CH_2\underline{CH}_3$	0.87, t	7

Table 2.11 : ^{13}C NMR Spectral Data ($CDCl_3$, 25 MHz) of Callophylline [88], Gambirine [22] and Pseudoyohimbine [50].

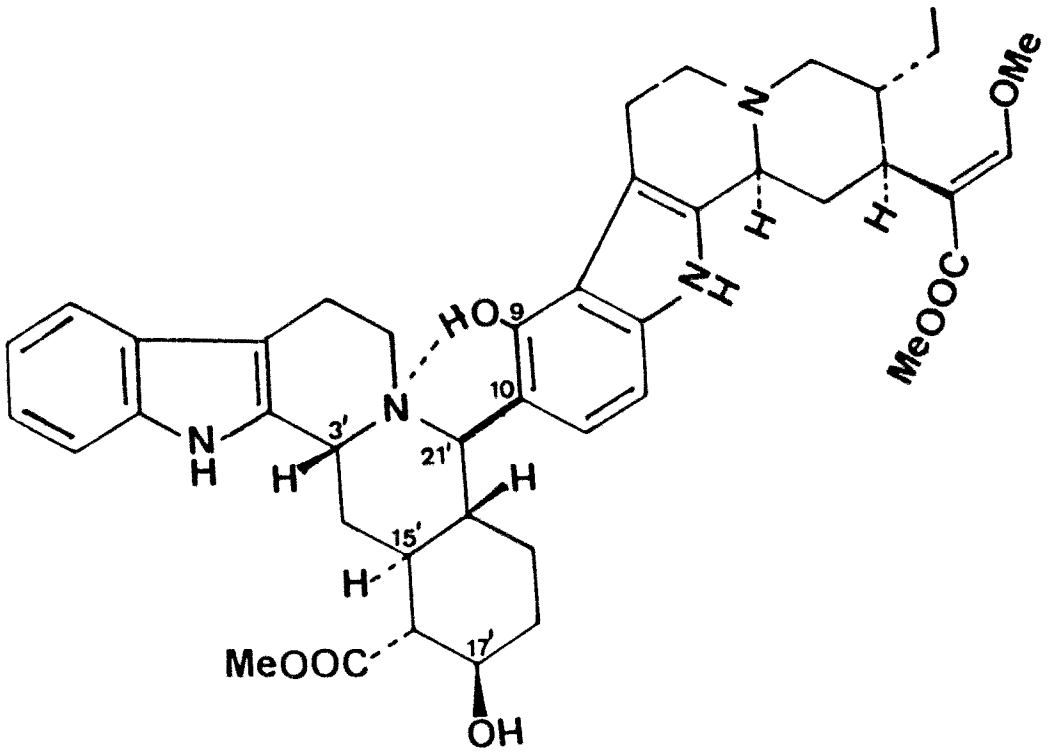
Carbon	[88]	[22]	Carbon	[88]	[50]
2	133.4[a] *s*	132.4	2'	132.7[a] *s*	132.0
3	60.2 *d*	60.3	3'	54.2 *s*	54.6
5	53.5 *t*	53.2	5'	47.5 *t*	51.0
6	23.5 *t*	23.0	6'	16.4 *t*	16.9
7	107.6[b] *s*	106.7	7'	108.8[b] *s*	107.8
8	117.2 *s*	116.7	8'	127.5 *s*	127.0
9	150.8 *s*	150.8	9'	117.8 *d*	117.0
10	113.1 *s*	103.5[c]	10'	119.2 *d*	119.0
11	124.2 *d*	121.7	11'	121.4 *d*	121.2
12	101.3 *d*	103.0[c]	12'	111.6 *d*	111.3
13	137.3 *s*	138.1	13'	136.3 *s*	135.9
14	33.7 *t*	33.2	14'	32.3 *t*	31.6
15	38.5 *d*	38.1	15'	30.9 *d*	32.5
16	111.8 *s*	111.5	16'	52.0 *d*	52.1
17	159.9 *d*	160.2	17'	67.1 *d*	67.0
18	11.2 *q*	10.9	18'	31.9 *t*	31.4
19	24.4 *t*	24.2	19'	22.0 *t*	23.0
20	39.5 *d*	39.2	20'	42.9 *d*	40.2
21	60.8 *t*	61.5	21'	63.8 *d*	52.0
CO	169.2 *s*	169.4	CO	174.8 *s*	174.0
CHOMe	61.4 *q*	60.6	COOMe	51.8 *q*	50.9
COOMe	51.9 *q*	51.2			

a,b,c assignments may be interchanged

2.2.4.2 UC 9, Dimer 2 (Callophylline A [89])

Dimer 2, isolated in small amounts from *U. callophylla* is an isomer of callophylline as indicated by its FAB mass spectrum (MH^+ 737.4562, calcd. $C_{43}H_{52}N_4O_7$ + H 737.3914). The UV spectrum of Dimer 2 also shows similar absorption maxima as callophylline suggesting the presence of similar chromophores in both compounds (Fig. 2.11). The ^1H NMR spectrum (Fig. 2.12) of Dimer 2 with the exception of a few signals, is strikingly similar to that of callophylline accounting for two indole imino hydrogens, olefinic H-17 and the C20 ethyl group of gambirine, the characteristic aromatic multiplet of an unsubstituted yohimbine-type unit, a pair of AB doublets due to adjacent H-11 and H-12 of gambirine, 3 methoxy groups and a broad singlet (4.69 ppm) due to the *cis* H-3' of a yohimbine-type moiety. The assignments are summarized in Table 2.12. The major difference in the ^1H NMR spectrum of Dimer 2 compared to callophylline is the absence of the *cis* H-17 signal at *ca.* 4.2 ppm indicating that pseudoyohimbine has now been replaced by another yohimbine isomer as the other unit linked to gambirine. The identity of the yohimbine unit was established by the ^{13}C NMR spectrum of the dimer which showed that it was 3-epi-β-yohimbine. The monomer alkaloid 3-epi-β-yohimbine which was in hand, having isolated it from *U. borneensis* (see Section 3.5.2) was used as reference compound. As in the previous dimer, attachment of the monomer units is from C21' of 3-epi-β-yohimbine to C10 of gambirine as deduced from the ^1H and ^{13}C NMR spectral data (Table 2.12 and 2.13) as described previously (see section 2.2.4.1).

The structure of Dimer 2 for which the name callophylline A is proposed is as shown in [89].



CALLOPHYLLINE A [89]

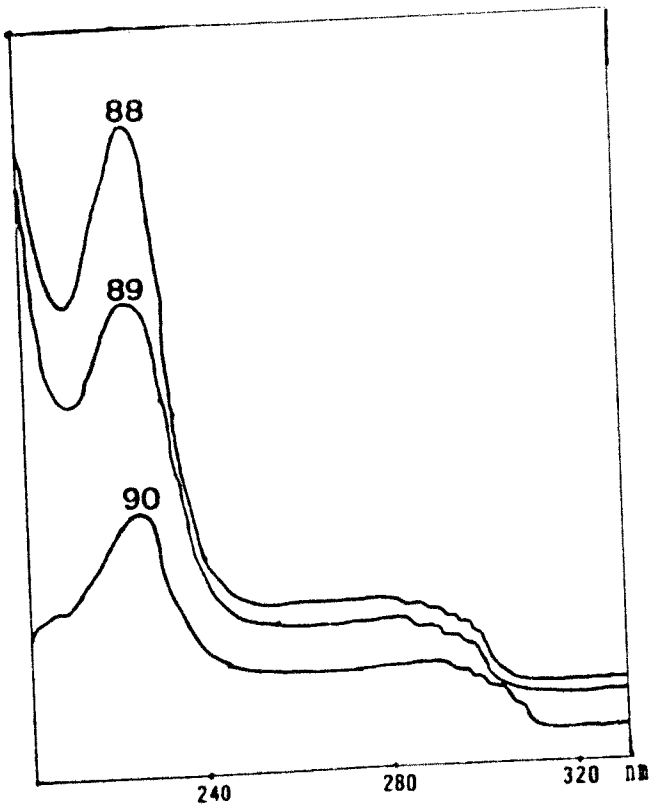


Figure 2.11 : UV spectrum of Callophylline [88], Callophylline A [89] and Callophylline B [90]

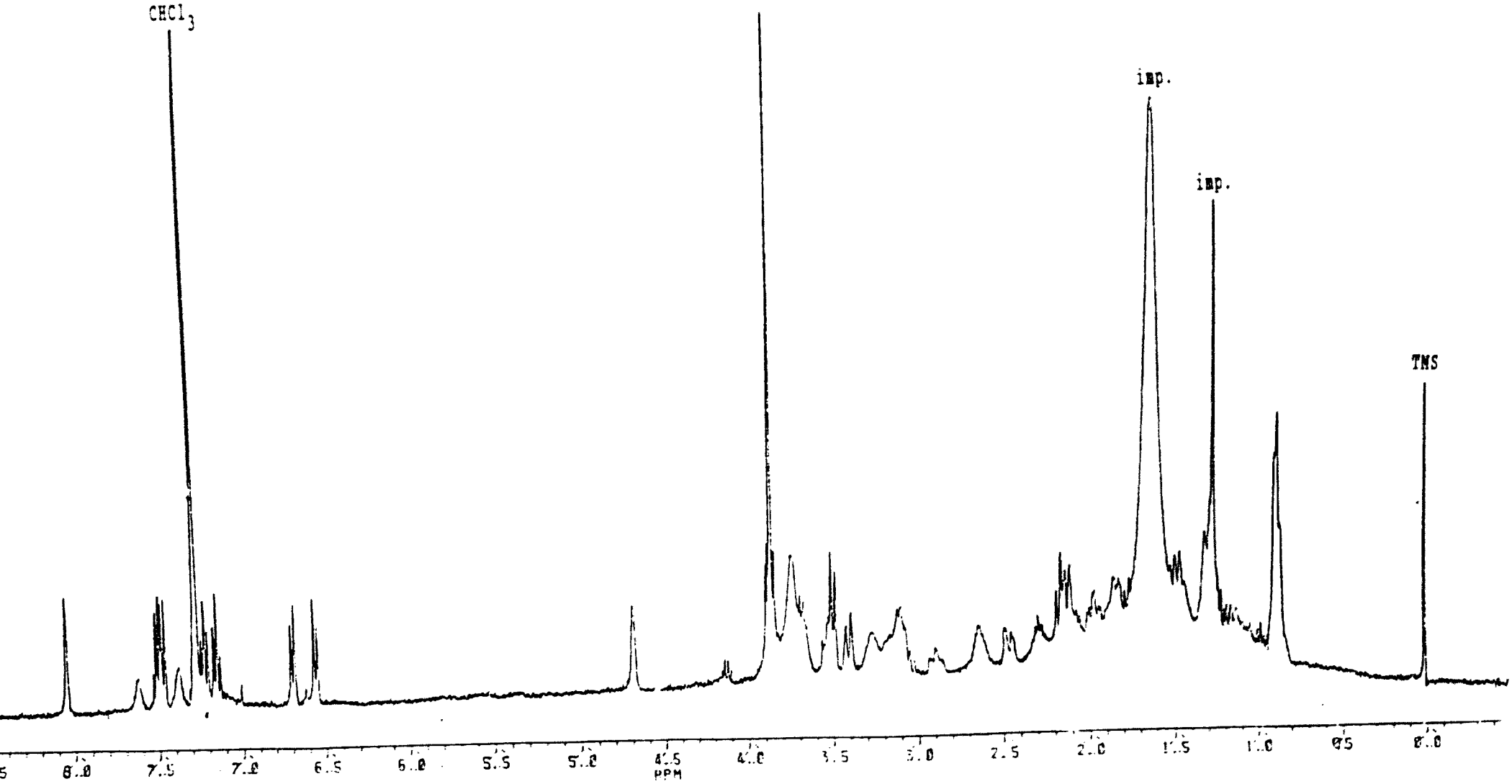


Fig. 2.12 : ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of Callophylline A

Table 2.12 : ^{1}H NMR Spectral Data ($CDCl_3$, 300 MHz) of Callophylline A [89]

Hydrogen	δ/ppm	J/Hz
H-11	6.54, d	8
H-12	6.69, d	8
H-17	7.37, s	-
H-9'	7.51, d	7
H-10'	7.12, dd	7, 7
H-11'	7.19, dd	7, 7
H-12'	7.46, d	7
H-21'	3.57, d	9
H-3'	4.69, br s	-
NH	7.62, br s	-
NH'	8.02, br s	-
OMe	3.75, s	-
COOMe	3.75, s	-
COOMe'	3.86, s	-
$CH_2\underline{CH}_3$	0.87, t	7

Table 2.13 : ^{13}C NMR Spectral Data ($CDCl_3$, 25 MHz) of Callophylline A [89], Gambirine [22] and 3-epi-β -yohimbine [52].

Carbon	[89]	[22]	Carbon	[89]	[52]
2	133.5[a]	132.4	2'	132.2[a]	131.6
3	60.3	60.3	3'	54.4	53.6
5	53.4	53.2	5'	47.7	50.8
6	23.7	23.0	6'	16.4	16.7
7	107.8[b]	106.7	7'	108.9[b]	107.1
8	117.4	116.7	8'	127.5	127.0
9	150.7	150.8	9'	118.1	117.8
10	113.0	103.5[c]	10'	119.6	119.2
11	124.1	121.7	11'	121.7	121.4
12	101.4	103.0[c]	12'	111.5	111.3
13	137.4	138.1	13'	136.1	136.1
14	33.8	33.2	14'	32.1	31.5
15	38.7	38.1	15'	36.3	36.6
16	111.5	111.8	16'	56.6	56.9
17	159.9	160.2	17'	71.8	71.7
18	11.3	10.9	18'	34.2	33.8
19	24.4	24.2	19'	27.9	27.8
20	39.4	39.2	20'	42.4	38.9
21	60.9	61.5	21'	63.8	50.3
CO	169.1	169.4	CO	174.2	172.7
CHOMe	61.6	60.6	COOMe	51.3	51.8
COOMe	52.1	51.2			

a,b,c assignments may be interchanged.

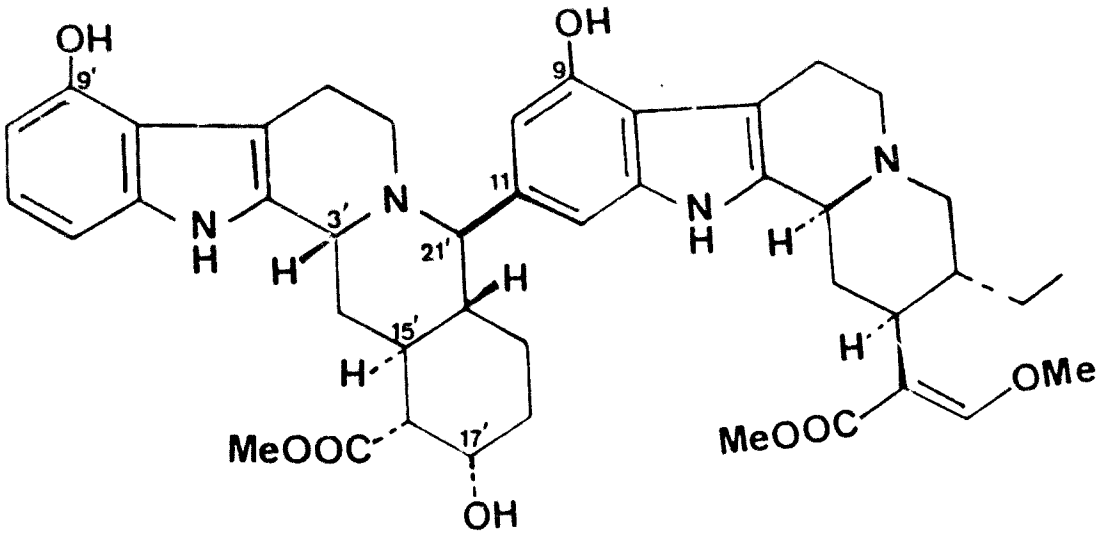
2.2.4.3 UC8, Dimer 3 (Callophylline B [90])

Dimer 3 was isolated in very small amounts from *U. callophylla*. The mass spectrum (FAB) shows a MH^+ ion at m/z 752.4763 corresponding to the formula $C_{43}H_{52}N_4O_8$. The formula obtained indicates the presence of an additional hydroxyl group compared to callophylline ($C_{43}H_{52}N_4O_7$). The ^1H NMR spectrum of Dimer 3 is shown in Fig. 2.13a and 2.13b. The lower field portion of the spectrum (downfield from the methoxy absorption at *ca.* 3.8 ppm) is more informative compared to the highly overlapped high field region which resembles that of callophylline [88]. Thus, the presence of a pseudoyohimbine unit is immediately apparent from the presence of the two characteristic broad singlets at 4.66 and 4.16 ppm due to the *cis* H-3' and *cis* H-17' hydrogens respectively. The aromatic region however does not show the characteristic multiplets associated with an unsubstituted pseudoyohimbine, thus suggesting aromatic substitution on the pseudoyohimbine portion. Two of the low field signals at 8.24 and 7.62 ppm exchange with D_2O or CD_3OD and are assigned to the indole imino hydrogens. The singlet at 7.37 ppm and the unsymmetrical triplet at 0.86 ppm are due to the olefinic hydrogen (MeOOC-$\underset{|}{C}$=CHOMe) and the C18 hydrogens respectively of a tetracyclic heteroyohimbine. The heteroyohimbine unit is deduced to be gambirine from the molecular formula and the ^{13}C NMR data (*vide infra*).

As in the case of callophylline, a total of three methoxy groups are discerned, two due to the gambirine unit and one due to the substituted pseudoyohimbine unit. The spectrum shows a total of five aromatic hydrogens, a one proton doublet of doublet at 6.48 ppm (J = 8, 2 Hz), a two proton multiplet at 6.6-6.7 ppm and another two proton multiplet at 6.9-7.1 ppm. Decoupling experiments show that the doublet of doublet at 6.48 ppm is coupled to the multiplet at 6.9-7.1 ppm, thus indicating that these hydrogens are from the same indole ring. The coupling pattern (ABX) and the chemical shift values are in fact characteristic of a 9-substituted indole, which in this case is attributed to the pseudoyohimbine unit. The doublet of doublet at 6.48 ppm is thus assigned to H-12' and the multiplet at 6.9-7.1 ppm are ascribed to H-10' and H-11'. The other unit of the dimer is deduced to be gambirine from the MS and ^{1}H and ^{13}C NMR data. Assuming the mode of Dimer 3 formation is similar to that of the Dimer 1, callophylline, i.e *via* electrophilic attack of an imminium ion of pseudoyohimbine on gambirine, the dimer bond attachment is likely to be on the beta face of C21' of the substituted pseudoyohimbine moiety. The attachment on gambirine has to be on C11 since attachment on either C10 or C12 would give a pair of AB doublets which is not observed. The ^{1}H NMR assignments are summarized in Table 2.14.

The ^{1}H NMR assignments of the third dimer are again corroborated by the ^{13}C NMR spectral data (Table 2.15) which supports the assignment of the monomer units as 9-hydroxypseudoyohimbine and

gambirine as well as the attachment of the monomer units at C11 and C21' of gambirine and 9-hydroxypseudoyohimbine respectively. As in the case of callophylline, it is also noted that the chemical shifts at or near the position of coupling are shifted while those of more remote carbons remain essentially unchanged as in the monomer alkaloids. Based on the foregoing arguments, the proposed structure of Dimer 5, named callophylline B, is as shown in [90].



CALLOPHYLLINE B [90]

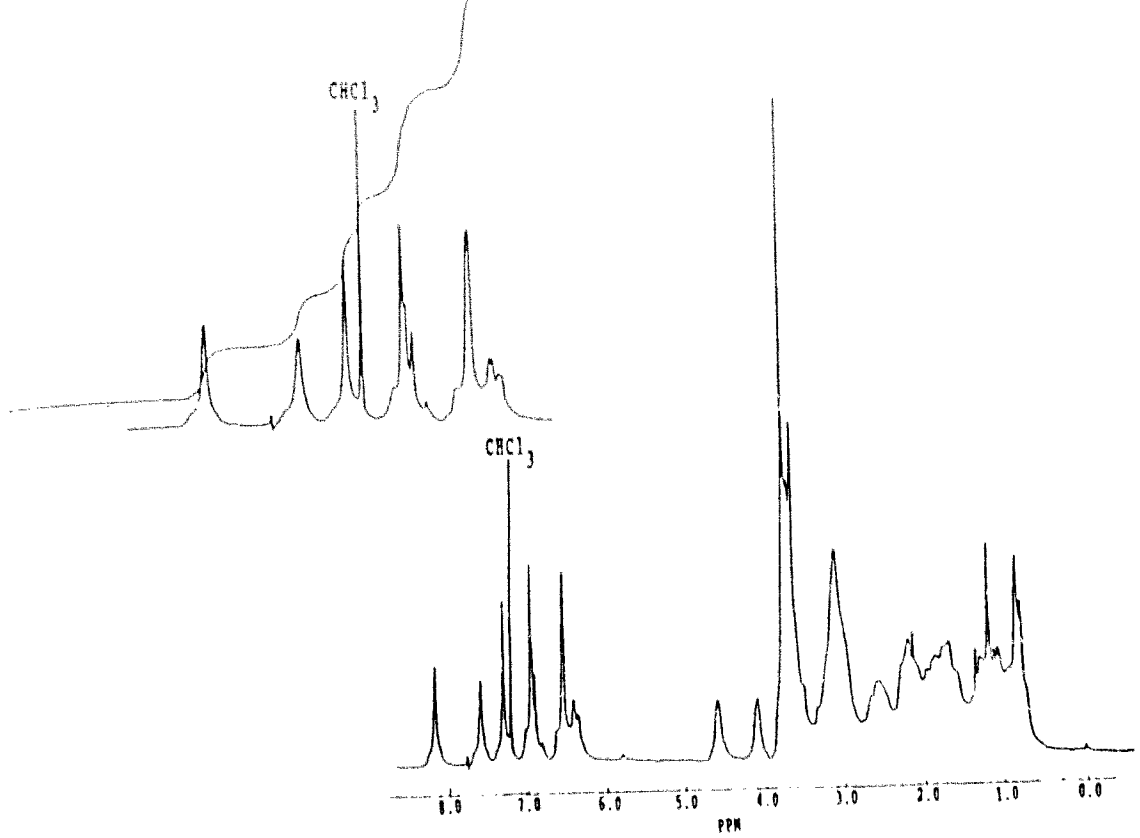


Figure 2.13a : ^{1}H NMR Spectrum ($CDCl_3$, 100 MHz) of Callophylline B

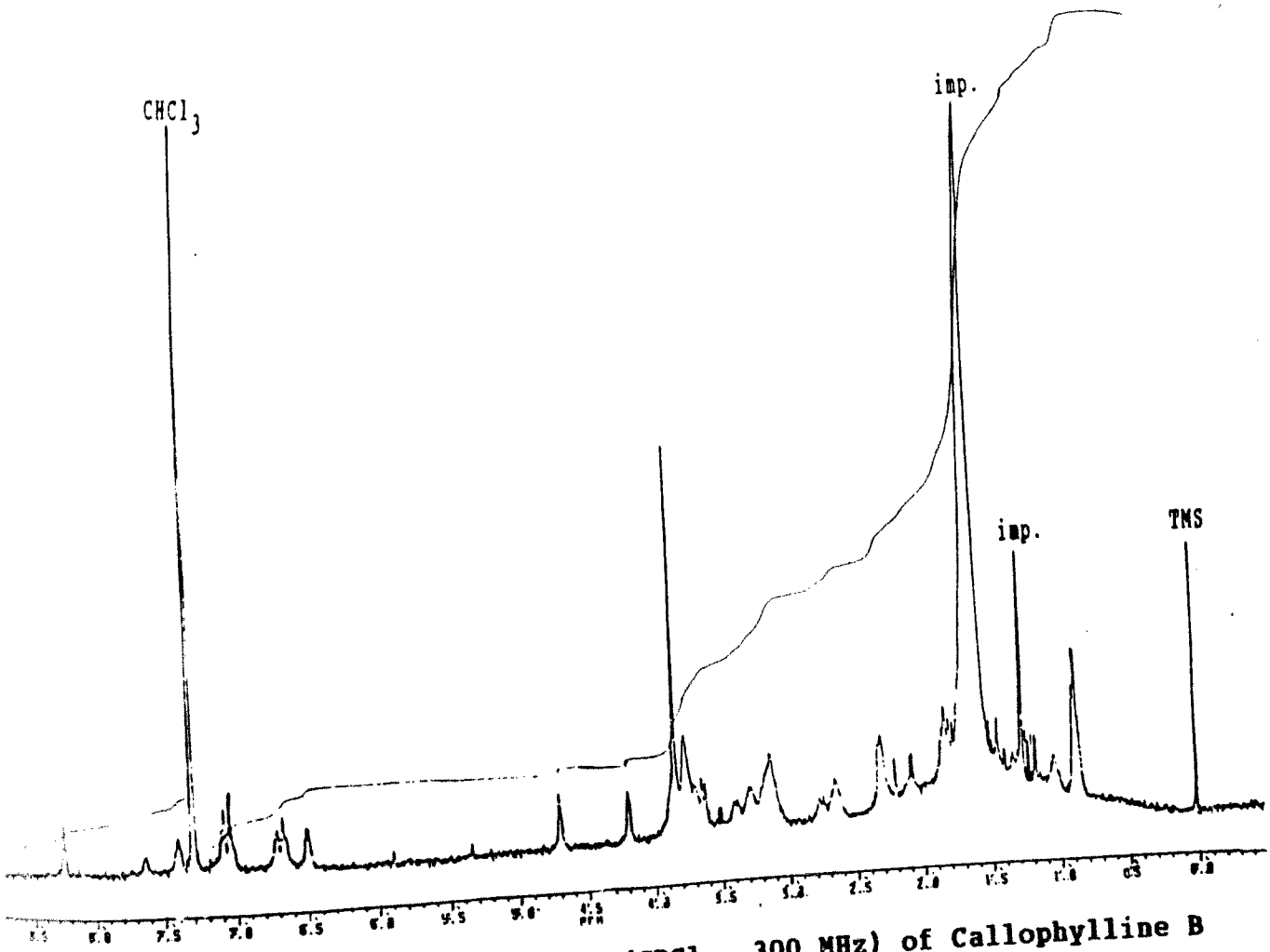


Figure 2.13b : ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of Callophylline B

Table 2.14 : ^{1}H NMR Spectral Data ($CDCl_3$) of Callophylline B [90]

Hydrogen	100 Mz δ /ppm	J/Hz	300 Mz δ/ppm	J/Hz
H-10 & H-12	6.6-6.7, m	-	6.6-6.7, m	-
H-17	7.36, s	-	7.37, s	-
H-10' & H-11'	6.9-7.1, m	-	6.9-7.1, m	-
H-12'	6.45, dd,	8,2	6.48, dd	8,2
H-21'	not resolved	-	3.62, d	8
H-3'	4.65, br s	-	4.66, br s	-
H-17'	4.15, br s	-	4.16, br s	-
NH	7.57, br s	-	7.62, br s	-
NH	8.19, br s	-	8.24, br s	-
OMe	3.73, s	-	3.72, s	-
COOMe	3.73, s	-	3.72, s	-
COOMe'	3.81, s	-	3.81, s	-
$CH_2\underline{CH_3}$	0.89, t	7	0.86, t	7

Table 2.15 : ^{13}C NMR Spectral Data ($CDCl_3$, 25 MHz) of Callophylline B [90], Gambirine [22] and Pseudoyohimbine [50].

Carbon	[90]	[22]	Carbon	[90]	[50]
2	133.5[a]	132.4	2'	133.6[a]	132.0
3	60.3	60.3	3'	54.5	54.6
5	53.4	53.2	5'	48.0	51.0
6	23.7	23.0	6'	18.4	16.9
7	107.6[b]	106.7	7'	107.9[b]	107.8
8	113.1[c]	116.7	8'	113.2[c]	127.0
9	150.1	150.8	9'	150.6	117.0
10	124.4	103.5[d]	10'	103.9	119.0
11	130.8	121.7	11'	122.3	121.2
12	100.9	103.0[d]	12'	103.9	111.3
13	138.2[e]	138.1	13'	137.4[e]	135.9
14	33.9	33.2	14'	32.4	31.6
15	38.7	38.1	15'	30.9	32.5
16	111.9	111.5	16'	52.0	52.1
17	160.0	160.2	17'	67.1	67.0
18	11.3	10.9	18'	31.7	31.4
19	24.5	24.2	19'	22.6	23.0
20	39.2	39.2	20'	43.0	40.2
21	60.9	61.5	21'	63.8	52.0
CO	168.0	169.4	CO	174.9	174.0
CHOMe	61.5	60.6	COOMe	51.4[f]	50.9
COOMe	51.9[f]	51.2			

a,b,c,d,e,f assignments may be interchanged.

2.2.5 Yohimbine Alkaloids

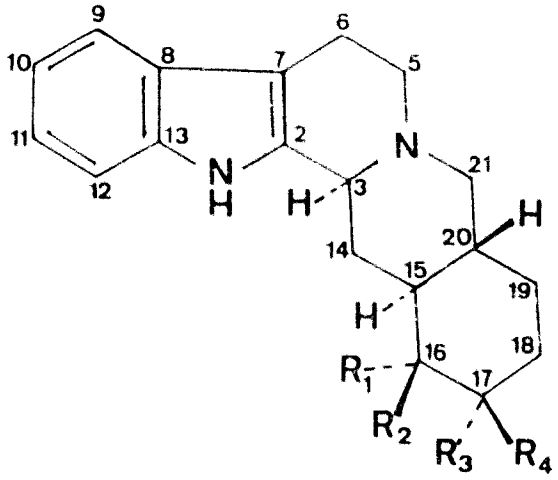
The yohimbines are divided into four groups, *viz.*, normal, pseudo, allo and epiallo according to the relative configurations of the stereo centres at C3, C15 and C20. Only one yohimbine alkaloid, namely, pseudoyohimbine has been previously isolated from *Uncaria* and only from *U. callophylla*[12] and *U. attenuatta*.[13]

In this study, 5 more yohimbine alkaloids have been isolated from *U. callophylla* and *U. borneensis*. The distribution of the yohimbine alkaloids in the *Uncaria* species investigated are given in Table 2.16, where it can be seen that while the normal, allo, and pseudo isomers have been isolated, the epiallo compounds are conspicuously absent.

Table 2.16 : Yohimbine Alkaloids Isolated From *Uncaria* Species

Yohimbines	Configuration	*U. callophylla*	*U. borneensis*
Yohimbine [47]	normal	+	-
β-Yohimbine [49]	normal	+	-
Pseudoyohimbine [50]	pseudo	+	+
3-epi-β-Yohimbine [52]	pseudo	-	+
Alloyohimbine [53]	allo	-	+
α-Yohimbine [54]	allo	+	-
Isomers	epiallo	-	-

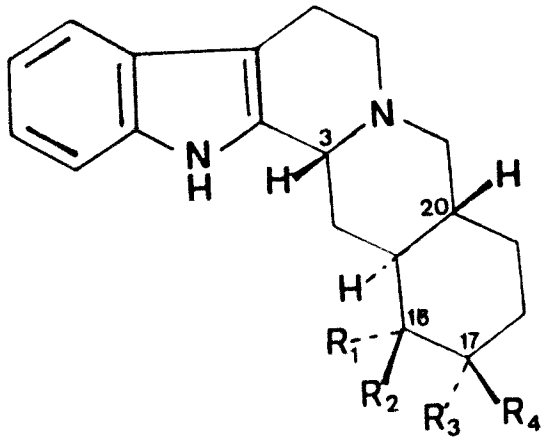
+ indicates alkaloid present
- indicates alkaloid absent



Normal Series

[47] Yohimbine $R_1=CO_2CH_3$; $R_3=OH$; $R_2=R_4=H$

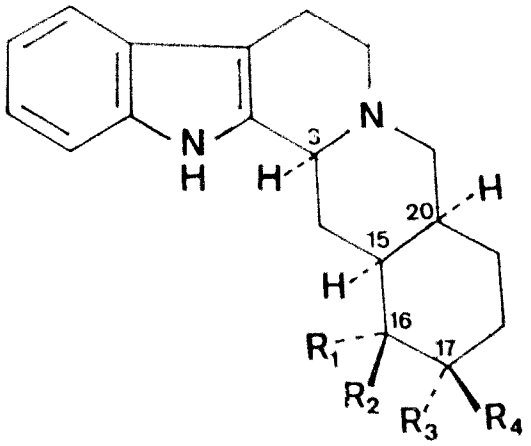
[49] β-Yohimbine $R_1=CO_2CH_3$; $R_4=OH$; $R_2=R_3=H$



Pseudo Series

[50] Pseudoyohimbine $R_1=CO_2CH_3$; $R_3=OH$; $R_2=R_4=H$

[52] 3-epi-β-yohimbine $R_1=CO_2CH_3$; $R_4=OH$; $R_2=R_3=H$



Allo Series

[53] Alloyohimbine $R_1=CO_2CH_3$; $R_3=OH$; $R_2=R_4=H$

[54] α-Yohimbine $R_2=CO_2CH_3$; $R_3=OH$; $R_1=R_4=H$

The yohimbine isomers can be distinguished by their characteristic molecular ion and MS fragmentation pattern as well as their ^1H NMR and ^{13}C NMR spectral data. In ^1H NMR, the signal of the H-3 *cis* to the nitrogen lone pair electrons (whether axial or equatorial) will appear downfield relative to that of the H-3 with *trans* orientation. Thus, the preferred conformation of the pseudo and epiallo configurations should show a downfield (i.e.› 3.8 ppm) one proton multiplet (seen as a broad singlet at 100 MHz) with a band width of about 8 Hz since both have the equatorial H-3 *cis* to the nitrogen lone pair electrons.[58] This low field signal is usually observable as it is not hidden within the methoxy and upfield aliphatic multiplet signals whereas in the preferred conformation of the normal and allo configurations, this signal will be hidden. Other characteristic absorptions in the ^1H NMR spectrum of the

yohimbines are the signals due to the methoxy group (3H, s, 3.7-3.8 ppm), the aromatic protons (4H, multiplet, 7.0-7.5 ppm) and NH proton (broad singlet, 7.7-8.0 ppm).

The C17-OH resonance is not distinct and is probably hidden under the aliphatic proton multiplets (a feature which is common in spectra obtained in $CDCl_3$ solution). The C17-Hβ absorption is more distinct compared to the C17-Hα signal as shown in the spectrum of pseudoyohimbine (C17-Hβ at 4.25 ppm). These data are summarized in Table 2.17.

Table 2.17 : ^{1}H NMR Spectral Data ($CDCl_3$, 100 MHz) of Yohimbine Alkaloids

Alkaloid	H-17	H-3	COOMe	NH
Yohimbine [47]	4.22, β	- , α	3.81	7.80
β-Yohimbine [49]	- , α	- , α	3.83	7.81
Pseudoyohimbine [50]	4.25, β	4.45, β	3.78	7.95
3-epi- β -Yohimbine [52]	- , α	4.44, β	3.82	8.01
Alloyohimbine [53]	3.90, β	- , α	3.77	7.82
α-Yohimbine [54]	3.99, β	- , α	3.84	7.75

^{13}C NMR spectroscopy proved the most useful spectroscopic tool for the characterization of the yohimbines in view of the large amount of data accumulated due to the fundamental work of Wenkert.[7,9] The ^{13}C NMR spectral data for the yohimbine

alkaloids isolated in this work is presented in Table 2.18. The data obtained is in good agreement with previously published data.[6,7,9] A yohimbine alkaloid which has not been previously characterized is 3-epi-β-yohimbine (isolated from *U. borneensis*) for which ^{1}H and ^{13}C NMR data is presented for the first time. In the ^{1}H NMR spectrum only the *cis* H-3 resonance is observed at 4.44 ppm ruling out the normal and allo configurations. The structure is assigned as 3-epi-β-yohimbine [52] (pseudo configuration, C16-Hβ, C17-Hα) from the ^{13}C NMR spectrum which is assigned by correlation with those of the other isomers using in particular pseudoyohimbine [50] and β-yohimbine [49] as reference compounds.

Table 2.18 : ^{13}C NMR Spectral Data ($CDCl_3$, 25MHz) of Yohimbine [47], β-Yohimbine [49], Pseudoyohimbine [50], 3-Epi-β-yohimbine [52], Alloyohimbine [53] and α-Yohimbine [54].

Carbon	[47]	[49]	[50]	[52]	[53]	[54]
2	134.5	134.3	132.0	131.6	134.0	134.5
3	59.9	59.6	54.6	53.6	60.4	60.3
5	52.4	53.0	51.0	50.8	53.3	53.6
6	21.8	21.7	16.9	16.7	21.7	21.8
7	108.3	108.3	107.8	107.1	108.3	108.1
8	127.4	127.4	127.0	127.0	127.4	127.1
9	118.2	118.2	117.0	117.8	118.1	118.1
10	119.4	119.5	119.0	119.2	119.4	119.4
11	121.4	121.5	121.2	121.4	121.3	121.4
12	110.8	110.8	111.3	111.3	110.9	110.8
13	136.0	136.1	135.9	136.1	135.8	136.0
14	34.3	34.2	31.6	31.5	31.7	27.7
15	36.7	41.9	32.5	36.6	38.1	37.9
16	52.9	57.4	52.1	56.9	50.9	54.7
17	67.0	72.3	67.0	71.7	67.3	66.1
18	31.5	34.2	31.4	33.8	31.1	33.2
19	23.3	27.9	23.0	27.8	25.2	24.6
20	40.8	39.9	40.2	38.9	32.8	36.6
21	61.4	61.1	52.0	50.3	60.2	60.6
COOMe	51.9	51.9	50.9	51.8	51.9	52.0
C=O	175.6	174.9	174.0	172.7	174.5	174.7

2.3 DISTRIBUTION OF ALKALOIDS IN MALAYSIAN *UNCARIA*

Eight species of Malaysian *Uncaria* were investigated for their alkaloid content in this study and the results are summarized in Table 2.19. All the alkaloids reported represent isolated and thoroughly characterized compounds from verified plant samples. Detailed chemical studies were carried out on two species, *viz.* *U. callophylla* for which previous studies reported only the major alkaloids,[12,31] and *U. borneensis* for which there has been no previous detailed chemical study.[2] Other species which were collected and investigated include *U. longiflora*, *U. pteropoda*, *U. lanosa*, *U. cordata*, *U. gambir*, *U. acida* and *U. elliptica*.

Table 2.19 : Distribution of Alkaloids in Malaysian Uncaria

Species	Alkaloids[#]
U. callophylla	**dihydrocorynantheine [20], gambirine [22],** isogambirine [86], gambireine [87], rotundifoline [42], **callophylline [88],** callophylline A [89], callophylline B [90], yohimbine [47], pseudoyohimbine [50], β-yohimbine [49], α-yohimbine [54],
U. borneensis	**isorhynchophylline [38], rhynchophylline [39], isocorynoxeine [40], corynoxeine [41],** alloyohimbine [53], pseudoyohimbine [50], 3-epi-β-yohimbine [52].
U. longiflora var. longiflora	**isorhynchophylline [38],** rhynchophylline [39], **isocorynoxeine [40],** corynoxeine [41].
U. longiflora var. pteropoda	isopteropodine [34], pteropodine [35].
U. lanosa var. glabrata	**isopteropodine [34],** pteropodine [35].

Table 2.19 continued.....

Species	Alkaloids#
U. lanosa *var. ferrea*	isopteropodine [34], pteropodine [35].
U. cordata *var. cordata* *f. sundaica*	dihydrocorynantheine [20]
U. cordata *var. ferruginea* *f. ferruginea*	dihydrocorynantheine [20]
U. cordata *var. cordata* *f. cordata*	-ve
U. elliptica	mixture of alkaloids but no roxburghines [5]
U. acida	-ve
U. gambir	-ve

#major alkaloids in **bold** type; -ve = not detectable

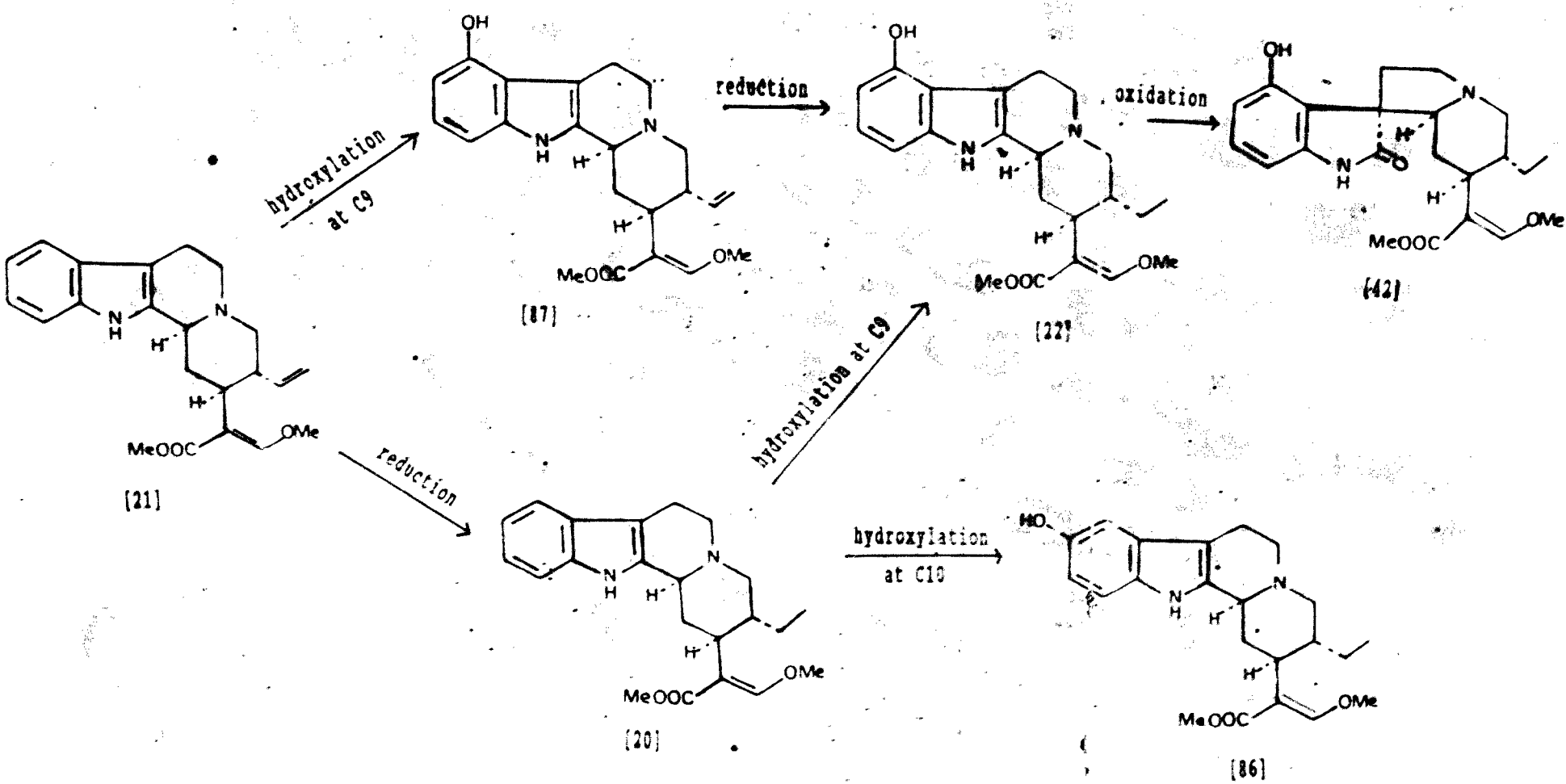
The nature of the alkaloids in the leaves and stems of *U. callophylla* was found to be different. Whereas the stem furnishes mainly dihydrocorynantheine, pseudoyohimbine and yohimbine, the leaves provide mainly dihydrocorynantheine, the dimeric alkaloid, callophylline and gambirine. There is also a seasonal dependance of the alkaloidal content (Section 2.4) not recognized before in the genus.

A number of minor alkaloids including novel heteroyohimbines (isogambirine and gambireine) and dimeric alkaloids (callophyllines A and B) were isolated from a large scale extraction of leaf samples (5-10kg). Other additional alkaloids

which were not previously reported include the yohimbines (yohimbine, β-yohimbine and α-yohimbine). There are additional minor polar alkaloids present but were not further investigated.

Four of the heteroyohimbine alkaloids isolated from *U. callophylla* (gambirine [22], gambireine [87], isogambirine [86] and dihydrocorynantheine [20]) are closely related in structure, differing mainly in their substitution at C9, C10 and C20. In addition, the sole oxindole isolated, *viz.* rotundifoline [42], is the oxindole derived from gambirine [22]. It has been previously suggested that a possible origin for gambirine in *U. callophylla* derives from hydroxylation of dihydrocorynantheine which occurs in the leaf.[12] In the light of the present results where additional hydroxy-substituted heteroychimbines have been found, this hypothesis can be extended to rationalize the relationship between the five alkaloids as shown in Scheme 4. However the precursor alkaloid, corynantheine [21], was not isolated in this study. From the relative amounts obtained which is in the order gambirine > dihydrocorynantheine > rotundifoline > isogambirine ~ gambireine, it can be postulated that most of the dihydrocorynantheine is converted to gambirine with only a minor diversion to yield isogambirine.

It is interesting to note that the stability of these compounds do not parallel the relative amounts present. Instead, the observed stability order is dihydrocorynantheine ~ rotundifoline > isogambirine > gambirine ~ gambireine.



Scheme 4 : Proposed relationship between the heteroyohimbine alkaloids in *U. callophylla*.

The relative amounts of the dimeric alkaloids isolated were in the order callophylline > callophylline B > callophylline A. Of these, callophylline B appears to be the most unstable and prone to decomposition when exposed to air at room temperature. It is interesting to note that gambirine is the heteroyohimbine unit common to all three dimers and also that the other units in all the three dimers are constituted from yohimbine alkaloids, all of which possess the pseudo configuration. Two of these yohimbines 9-hydroxypseudoyohimbine and 3-epi-β-yohimbine were not isolated but were probably present in the more polar fractions which were not investigated due to lack of time.

U. borneensis and *U. longiflora* were found to have the same major alkaloids which are the oxindoles, isorhynchophylline, rhynchophylline, isocorynoxeine and corynoxeine. *U. borneensis* however furnished in addition to these, 3-epi-β-yohimbine, alloyohimbine and pseudoyohimbine as minor alkaloids.

U. pteropoda and *U. lanosa* both furnished the pentacyclic oxindoles *viz.* isopteropodine and pteropodine while *U. cordata* gave dihydrocorynantheine, which are in accordance with previous reports. Only one sample of flowering *U. gambir* (wild species) was encountered despite a concerted search over three years, but was found to contain virtually no alkaloids.

Various samples of *U. elliptica* were screened and were shown to contain numerous alkaloids. However the yields of alkaloids were low and isolation of the alkaloids proved particularly difficult. Nevertheless, roxburghines were consistently not detected.

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2.4 SEASONAL VARIATION OF THE ALKALOIDAL CONTENT IN *U. CALLOPHYLLA*

In the early stages of this study it was observed that the alkaloidal content of *U. callophylla* was not always constant. For example, in some samples gambirine which is a diagnostic alkaloid unique to *U. callophylla*,[2,12,16] was found to be absent. The relative amounts of the dimer, callophylline, as well as pseudoyohimbine in the stem were also observed to show some variation as seen in t.l.c. In view of this, a systematic study of the seasonal variation of the alkaloids appears to be in order. Leaf samples from specifically tagged bushes were collected from the Puchong (see Fig. 2.1) area at monthly intervals over a period of ten months spanning the flowering season. The alkaloids were extracted and screened by t.l.c. which distinguished the three major alkaloids present *viz.* dihydrocorynantheine, callophylline and gambirine. The results are shown in Fig. 2.14 and 2.15 where it can be seen that there is a sharp drop in the gambirine content from November to January which also coincides with the flowering period. This result then establishes the basis of the previously observed erratic pattern, namely, gambirine production in the leaves drops drastically during the flowering stage, a phenomenon which is not unexpected although little investigated. Another noteworthy feature of the alkaloidal content of *U. callophylla* is the difference shown in the leaf and stem. The leaf produces mainly dihydrocorynantheine, callophylline and gambirine (in addition to the other minor alkaloids isolated) while the stem produces

dihydrocorynantheine, yohimbine and pseudoyohimbines as the major alkaloids. It was also established that pseudoyohimbine is present in significant quantities in the stem only during the flowering stage.

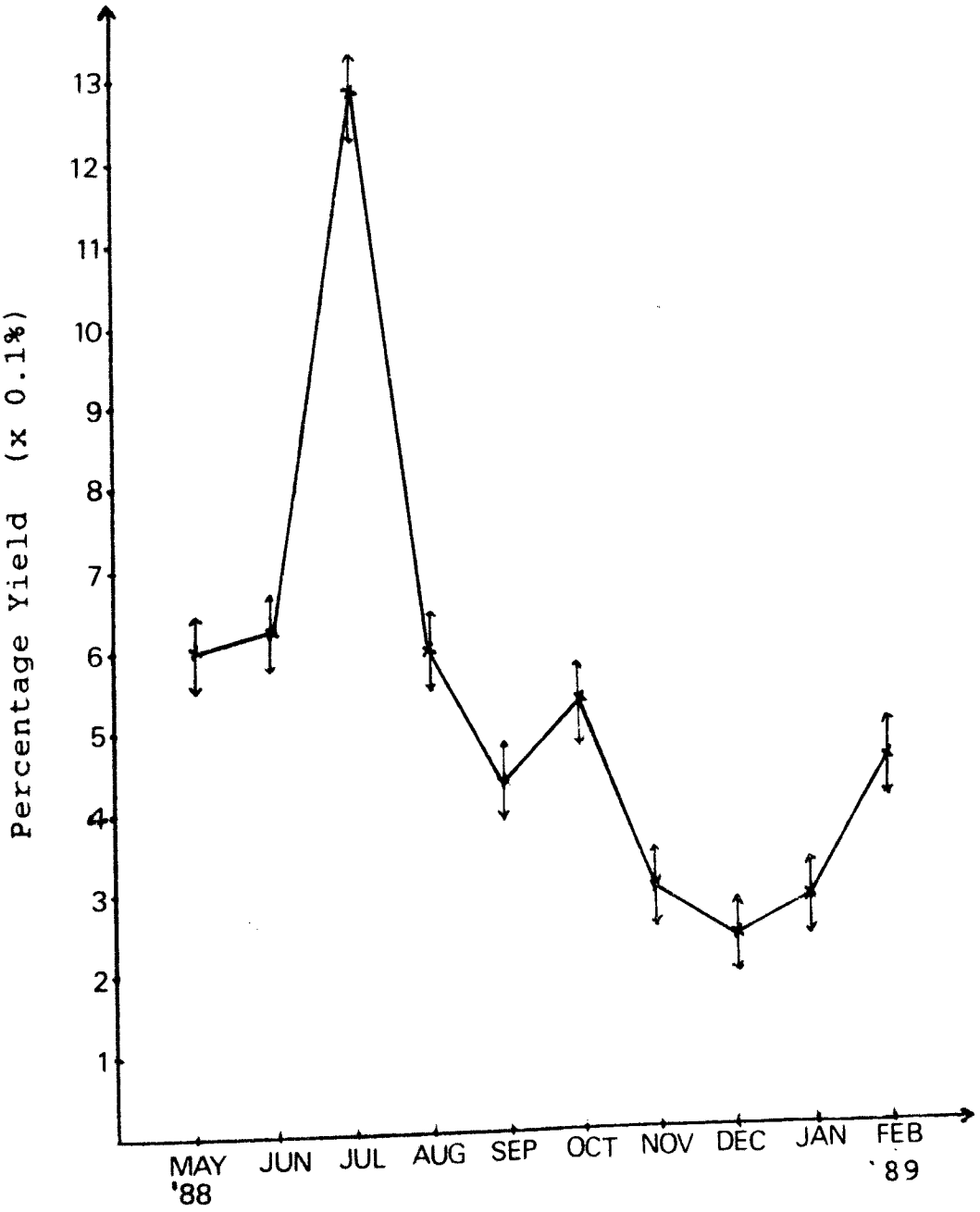


Figure 2.14 : Monthly Variation of Total Alkaloids in *U. callophylla* (data from 5 bushes).

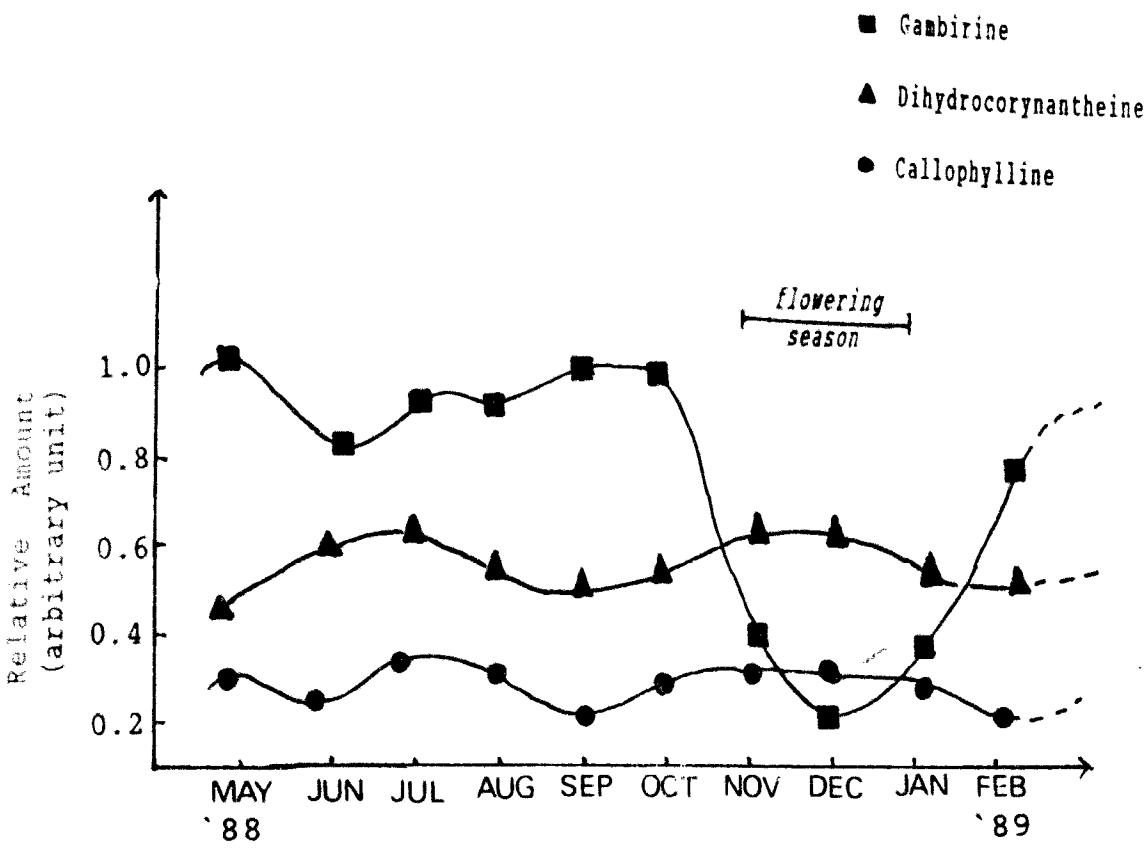
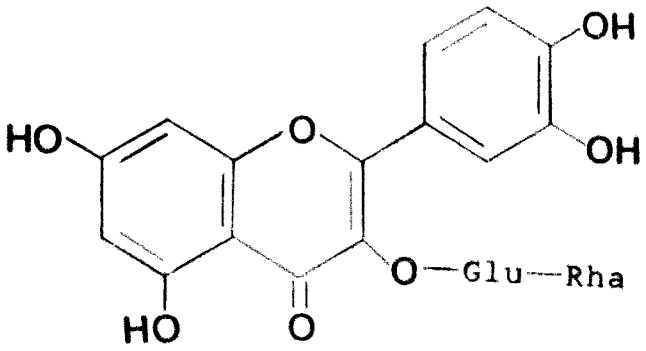


Figure 2.15 : Monthly Variation of Major Alkaloids (gambirine, dihydrocorynantheine and callophylline) in leaves of *U. callophylla*.

2.5 RUTIN FROM MALAYSIAN *UNCARIA* SPECIES

In view of the use of *Uncaria* species in local traditional medicine and the known therapeutic use of rutin [91] in decreasing capillary fragility,[59] a survey of the rutin content in *Uncaria* was carried out. 12 samples representing 9 species of *Uncaria* were investigated for their rutin content. The rutin was isolated by extraction with ethanol, followed by preparative t.l.c. and then determined by UV spectroscopy.



[91] RUTIN

From the 9 species investigated, the single major source of rutin appears to be *U. callophylla* (2.6%). Other *Uncaria* species in which rutin is found include *U. lanosa var. ferrea* (0.8%), *U. acida* (0.6%), *U. gambir* (0.4%), *U. cordata var. ferruginea f. ferruginea* (0.10%), *U. cordata var. cordata f. cordata* (0.08%) and *U.elliptica* (0.08%). The others contain little or no rutin.

It is noteworthy that *U. callophylla* is presently found to provide much larger amounts of rutin than *U. elliptica* which was previously considered to be a good source of rutin.[60]

2.6 PHARMACOLOGICAL ACTIVITY OF SOME ISOLATED ALKALOIDS

One of the aims of this work is to isolate biologically active alkaloids from the *Uncaria* species investigated since they are known to have a wide spectrum of uses in traditional medicine.[2,3] Some preliminary pharmacological investigations have been initiated where alkaloidal extracts of a number of *Uncaria* species collected were tested for cardiovascular effects in Sprague-Dawley normotensive rats. The test were carried out on conscious or anaesthetized rats (200-250g). Blood pressure was monitored from a common carotid artery *via* Statham pressure transducer (P23 AC) coupled to a Grass Polygraph (Model 7D). Alkaloidal extracts and pure alkaloids were solubilized in dilute aqueous hydrochloric acid and partially neutralized by dilute sodium hydroxide (pH *ca.* 5). Dilutions were with normal saline. All injections were done through a cannulated femoral vein. (The pharmacological studies were carried out by our collaborators at the Department of Pharmacology, University of Malaya, Kuala Lumpur.) The results show that virtually all the extracts of *Uncaria* species investigated (including *U. elliptica*, *U. callophylla*, *U. longiflora*, *U. pteropoda*, *U. cordata* and *U. lanosa*) exhibit antihypertensive activity in varying degrees (Table 2.20). The pure alkaloids isolated in the present study were also evaluated and these compounds when tested displayed moderate antihypertensive activity (Table 2.21). The results are particularly promising in the case of gambirine where further studies are presently in progress.

Table 2.20 : **Antihypertensive Activity of Alkaloidal Extracts of Some Malaysian *Uncaria* Species.**

Plant	Part	Dosage mg/kg	Activity* (Duration)# Anaesthetized	Conscious
U. callophylla	leaf	5	5+ (l)	5+ (l)
	stem	5	4+ (m)	4+ (m)
U. elliptica	leaf	5	2+ (m)	2+ (s)
	stem	5	3+ (m)	3+ (s)
U. longiflora var. longiflora	leaf	10	2+ (m)	3+ (m)
	stem	10	4+ (m)	3+ (m)
U. longiflora var. pteropoda	leaf	10	2+ (s)	2+ (s)
U. lanosa var. ferrea	leaf	10	2+ (s)	3+ (s)
U. cordata var. cordata f. sundaica	leaf	10	2+ (m)	2+ (m)

*5+, 4+, 3+, 2+ and 1+ refer to bp depression of 81-100, 61-80, 41-60, 21-40 and ‹20 mm Hg respectively.

#l, m and s refer to duration of ›5, 5-2 and ‹2 minutes respectively

Table 2.21 : Antihypertensive Activity Of Alkaloids From Malaysian *Uncaria*

Alkaloid	Dosage mg/kg	Activity* (Duration)# (Anaesthetized rats)
Dihydrocorynantheine [20]	5	3+ (m)
Isopteropodine [34]	5	3+ (s)
Pteropodine [35]	5	1+ (s)
Yohimbine [47]	5	5+ (m)
Gambirine [22]	5	3+ (l)
Pseudoyohimbine [50]	10	2+ (l)
Isocorynoxeine [38]	10	2+ (s)
Corynoxeine [41]	10	2+ (s)
Isorhynchophylline [38]	10	3+ (m)
Rhynchophylline [39]	10	1+ (s)
Callophylline [88]	5	1+ (s)

*5+, 4+, 3+, 2+ and 1+ refer to bp depression of 81-100, 61-80, 41-60, 21-40 and <20 mm Hg respectively.

#l, m and s refer to duration of >5, 5-2, and <2 minutes respectively.