



CHAPTER IV

RESULT AND DISCUSSION

The 0.5 Kg of dried stems of *Pycnarrhena lucida* (Teijsm. & Binn.) Miq. was macerated with ethanol. The ethanol extract was separated by chromatographic technique to afford five pure compounds. The structure elucidations of these compounds were based on data from UV, IR, NMR and Mass spectra, and further confirmed by comparison with the previously data reported in literature, as discussed below.

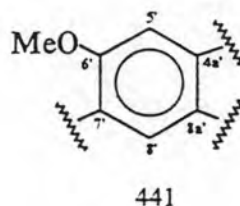
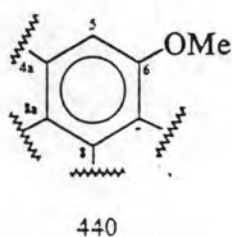
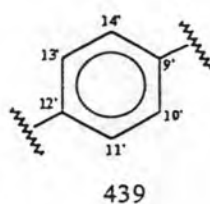
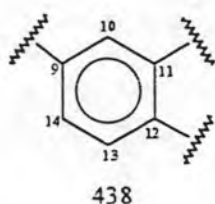
The Structure Elucidation of The Isolated Compounds

Compound CH01

Compound CH01 was crystallized as colorless crystals from benzene. The eims spectrum of compound CH01 (Figure 2) shows a molecular ion peak at m/z 594 (12%) and establishes the tentative molecular formula of $C_{36}H_{38}O_6N_2$. The uv absorption band (Figure 3) at λ_{max} 284 nm (ϵ 6357), indicates the bisbenzylisoquinoline structure (Shamma, 1972 and Brossi, 1987). The ir spectrum (Figure 4) exhibits the band at ν 3400 (broad), 1650, and 1230 cm^{-1} indicating the presences of hydroxyl, aromatic ring and ether functional groups, respectively.

As evident from the resonances at δ 2.29, 2.51, 3.78 and 3.91 ppm in the 1H -nmr spectrum (Figure 5), the structure of CH01 includes two *N*-Me and two aromatic OMe groups.

The aromatic region of the spectrum provides four structural parts (438-441) as followed:



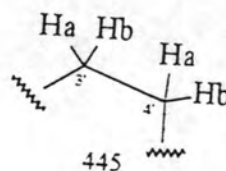
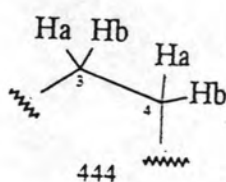
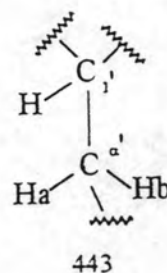
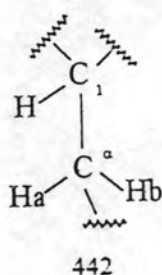
Fragment 438 is established from H-14 at δ 6.63 ppm (dd, $J = 7.93, 1.83$ Hz) coupling to the signals of H-10 at δ 6.24 ppm (d, $J = 1.83$ Hz) and H-13 at δ 6.76 ppm (d, $J = 7.93$ Hz). By the C-H correlations observed in the HETCOR spectrum (Figure 11), the correlated carbons are assigned accordingly as C-10 (δ 115 ppm), C-13 (δ 114.5 ppm) and C-14 (δ 123 ppm).

Fragment 439 exhibits the signals of H-10' (δ 6.45 ppm, dd, $J = 8.24, 2.14$ Hz), H-11' (δ 6.81 ppm, dd, $J = 8.24, 2.14$ Hz), H-13' (δ 7.05 ppm, dd, $J = 8.24, 2.14$ Hz) and H-14' (δ 7.32 ppm, dd, $J = 8.24, 2.14$ Hz). The signal of H-10' couples to H-11' with the coupling constant of 8.24 Hz, and to H-14' with the coupling constant of 2.14 Hz. The signal of H-11' couples to H-10' and H-13' or the signal of H-13' couples to H-14' and H-11', and the signal of H-14' couples to H-13' and H-10' are as same as the signal of H-10'. All of these protonated carbons are achieved by the analysis of the HETCOR spectrum. The chemical shift of these carbons are as followed : C-10' (δ 132 ppm), C-11' (δ 123 ppm), C-13' (δ 123 ppm) and C-14' (δ 130.2 ppm).

Fragment 440 consists of the signal of H-5 (δ 6.35 ppm, s) which correlates to the other methoxyl proton at δ 3.78 ppm. The HETCOR spectrum clearly shows the C-5 resonance at δ 107 ppm.

Fragment 441 contains the connectivity of H-5' ($\delta 6.74$ ppm,s), H-8' ($\delta 6.06$ ppm,s). In H-H COSY spectrum (Figure 9) the signal of H-5' shows the correlation to the signal of H-8', although those signals are not splitted. It is assigned to locate in para-position of each other in the same aromatic ring. The H-H COSY spectrum also shows H-5' couples with methoxy proton at $\delta 3.91$ ppm. It is proposed in ortho position with H-5'. From HETCOR spectrum, the carbon signal at $\delta 113$ and 121.3 ppm are assigned to C-5' and C-8', respectively.

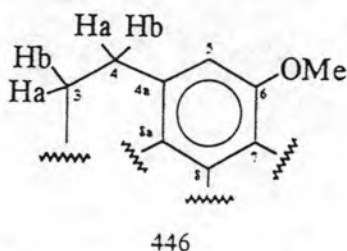
The aliphatic region of the spectrum contains four structural parts (442-445) as followed:



Fragment 442 consists of the signal of H-1 ($\delta 4.01$ ppm, dd, $J = 10.98, 2.44$ Hz) and the methylene proton, H- α_a ($\delta 2.64$ ppm, dd, $J = 15.56, 10.98$ Hz) and H- α_b ($\delta 2.92$ ppm). Based on the C-H COSY spectrum, the chemical shifts of C-1 and C- α are $\delta 61$ and 39.1 ppm, respectively.

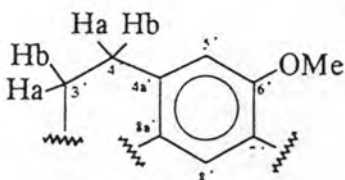
As same as fragment 442, fragment 443 is established from H-1' at δ 3.62 ppm (dd, $J = 10.68, 4.88$ Hz) coupling to the signals of methylene protons, H- α' a (δ 2.8 ppm, m) and H- α' b (δ 3.26 ppm, dd, $J = 14.34, 10.68$ Hz). the chemical shifts of C-1' and C- α' are δ 65 and 38.2 ppm, respectively.

Fragment 444, exhibits the connectivity of H-4a (δ 2.38 ppm, dd, $J = 20.14, 4.88$ Hz), H-4b (δ 2.8 ppm, m), H-3a (δ 2.8 ppm, m), and H-3b (δ 3.25 ppm, dd, $J = 23.19, 4.88$ Hz). From HETCOR spectrum, the protons at δ 2.38 ppm and 2.8 ppm are placed on C-4 (δ 23 ppm) and the other methylene protons in this fragment are on C-3 (δ 43.9 ppm). And in H-H COSY spectrum, the signal at δ 2.38 ppm shows long range correlation with aromatic proton at δ 6.35 ppm. It is proposed to be fragment 446 which is shown below.



But the additional data from various experiments must be continuously studied

Like the fragment 444, fragment 445 is proposed by the analyses of 1-D ^1H nmr, H-H COSY and HETCOR spectrum. Two protons at δ 2.95 ppm are placed on carbon at δ 26 ppm of which is assigned to be C-4'. The last methylene protons which place on C-3' (δ 46 ppm) are analysed as H-3a' (δ 2.8 ppm, m) and H-3b' (δ 3.38 ppm, m). The H-H COSY spectrum also shows the connectivity from the methylene protons at δ 2.95 ppm to aromatic proton at δ 6.74 ppm and support the presence of the fragment 447.



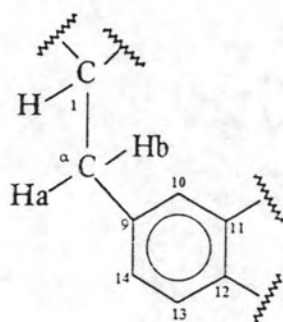
447

Certainly, like the fragment 446, the additional data is necessary.

All of the fragments are linked together based on the analyses of C-H long range correlations, and some fragments will be confirmed.

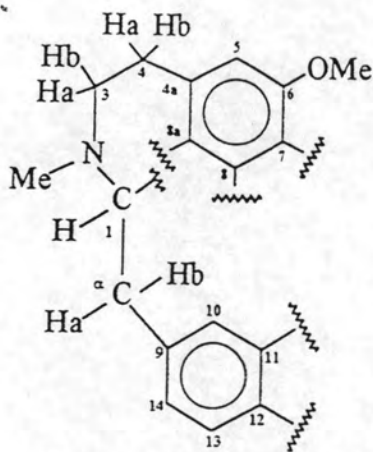
Fragment 446 is confirmed by the correlation between 23 ppm) and H-5' (δ 6.35, s) as same as that between C-4' (δ 26 ppm) to H-5' (δ 6.74 ppm, s) in fragment 447.

The long-range coupling exhibits the correlation of C- α (δ 39.1 ppm) and H-1 (δ 4.01, dd) and H-10 (δ 6.24 ppm, d) supports the proposed fragment 442 of being placed on ortho position to C-10 (δ 115 ppm). The COLOC spectrum is also displayed the long-range coupling at δ 132.8 ppm and both C- α protons so the ortho position to C-10 of δ 132.8 ppm of which fragment 442 is placed on. When the aromatic oxygenated carbons region is focused, the long-range correlations of one carbon at δ 143.4 ppm and H-10 (δ 6.24 ppm, d), H-13 (δ 6.76 ppm,d), the other carbon at δ 148 ppm and H-10 (δ 6.24, d), H-13 (δ 6.76, d) are also observed. The propose that carbon at δ 143.4 ppm and δ 148 ppm are in ortho position to each other, is more reasonable. So the fragment 438 contains the signals of C-9 (δ 132.8 ppm), C-10 (δ 115 ppm), C-11 (δ 143.4 ppm), C-12 (δ 148 ppm), C-13 (δ 114.5 ppm) and C-14 (δ 123 ppm). The fragment 438 and fragment 442 are linked together to form fragment 448, as below:



448

From HETCOR spectrum, the carbon in *N*-CH₃ δ 2.29 ppm(s) shows attach at δ 42.4 ppm. The C-H long-range correlations of C-1 (δ 61 ppm) and the *N*-CH₃ protons (δ 2.29 ppm, s) are shown in COLOC spectrum. The COLOC spectrum also exhibits the C-H long-range correlations between C-3 (δ 43.9 ppm) and the *N*-CH₃ protons (δ 2.29ppm, s). The fragment 449 is proposed, as below:



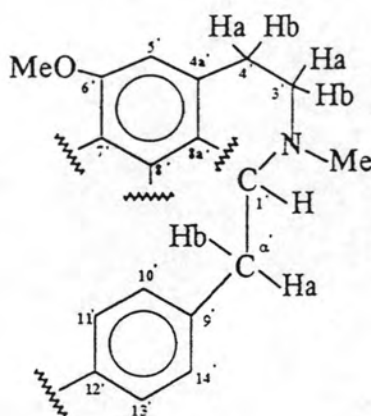
449

One of the carbon groups at δ 123 ppm displays long-range coupling with H-1 (δ 4.01 ppm, dd), H- α (δ 2.92 ppm, dd) and H-5 (δ 6.35 ppm, s), but the carbon at δ 124.3 ppm has only long-range correlation with H-4 (δ 2.38 ppm, dd). So the

resonances at δ 123 ppm and δ 124.3 ppm are therefore assigned to C-8a and C-4a, respectively

Carbons signals of C-6 and C-7 are assigned to resonances at δ 147 and δ 136.3 ppm as a result of the C-H long-range correlation of C-6 (δ 147 ppm) and the methoxy protons (δ 3.78 ppm, s) are observed and the correlations of both C-6 and C-7 and H-5 (δ 6.35 ppm, s) are also exhibited.

Examination of the HETCOR spectrum allows the assignments of signal of carbon at δ 43 ppm of *N*-CH₃ of which protons at δ 2.51 ppm (s) is attached and these protons show the connectivity to C-1' (δ 65 ppm) by long-range coupling. The C-3' (δ 46 ppm) shows the correlation with the *N*-CH₃ protons (δ 2.51 ppm, s). And the C-9' is linked to fragment 443 at C- α' since it also shows the correlation to H- α' (δ 2.8 ppm, m). From these data, the fragment 450 is proposed, as below:



450

The fragment 450 can be confirmed by the correlation of C-10' (δ 132 ppm) and H- α' (δ 2.8 ppm, m and 3.26, dd).

The oxygenated aromatic carbon at $\delta 135.6$ ppm is assigned to C-9' because COLOC spectrum exhibits C-H long-range correlations of carbon at $\delta 135.6$ ppm and H- α' ($\delta 2.8$ ppm, m and 3.26 ppm, dd).

The COLOC spectrum shows the C-H long-range correlations among the quaternary carbon at $\delta 130.5$ ppm and H- α' ($\delta 3.26$ ppm, dd), H-5' ($\delta 6.74$ ppm, s) and the quaternary carbon at $\delta 130.8$ ppm has long-range connectivity to H-8' ($\delta 6.06$ ppm, s). The C-4'a and C-8'a are assigned to resonance at $\delta 130.8$ and 130.5 ppm, respectively.

The last two oxygenated carbons that can be assigned, are at $\delta 143.2$ ppm and 149 ppm. The signal at $\delta 149$ ppm is assigned to C-6', based on the long-range correlation to the OCH₃ protons at $\delta 3.91$ ppm (s), leaving the oxygenated carbon at $\delta 143.2$ ppm which show connectivity to H-5' ($\delta 6.74$ ppm, s) and H-8' ($\delta 6.06$ ppm, s) to be assigned to C-7'.

When the C-1 and C-8a, C-1' and C-8'a are connected together to form isoquinoline skeleton, fragment 449 and 450 are formed to become bisbenzylisoquinoline parts.

The peak at m/z 191 in the ms spectrum, together with the two *N*-methyl groups signals at $\delta 2.29$ ppm (s) and 2.51 ppm (s) in the ¹H-nmr spectrum, strongly indicate that CH01 belongs to the subgroup C (8-7', 11-12')(Guinaudeau et al., 1986), with two methoxy groups and one hydroxyl group on the upper part of the molecule. In addition, ¹H-nmr spectral features suggested that CH01 is a tail-to-tail bisbenzylisoquinoline containing two diaryl ether bridges from C-8 to C-7' and C-11 to C-12'. So leaving the last 34 mass units, which equal to two hydroxyl group, are assigned to locate on C-7 and C-12.

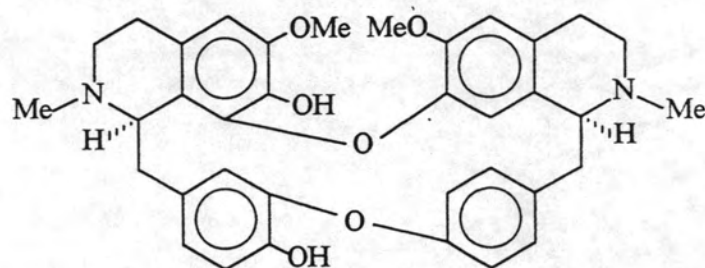
The relative stereochemistry between H-1 and H-1' is deduced as a *syn* configuration from the following observations: (1) H-10 (δ 6.24 ppm, d) is visible between δ 6.20-6.60 ppm. (2) H-10' (δ 6.45 ppm, dd) appears upfield, in the vicinity of δ 6.40 ppm. The positive sign of the specific optical rotation of CH01 ($+270^{\circ}$ in chloroform) indicates that the absolute configuration of CH01 is 1*R*,1'*S* (Cassels and Shamma, 1980). The assignments of protons of compound CH01 are confirmed by comparison with the data of obamegine (193) previously reported by Guinaudeau et al (1986). The assignments are summarized in table 6.

Table 6 ^1H - and ^{13}C -nmr assignments of CH01*

Position	^1H	^{13}C	Position	^1H	^{13}C
1	4.01(dd,10.98,2.44)	61	1'	3.62 (dd, 10.68, 4.88)	65
3	2.80 (m)	43.9	3'	2.80 (m)	46
	3.25 (dd,23.19, 4.88)			3.38 (m)	
4	2.38 (dd, 20.14, 4.88)	23	4'	2.95 (m, 2H)	26
	2.8 (m)				
4a	-	124.3	4a'	-	130.8
5	6.35 (s)	107	5'	6.74 (s)	113
6	-	147	6'	-	149
7	-	136.3	7'	-	143.2
8	-	144.1	8'	6.06 (s)	121.3
8a	-	123	8a'	-	130.5
α	2.64 (dd,15.56,10.98)	39.1	α'	2.80 (m)	38.2
	2.92 (m)			3.26 (dd,14.34,10.68)	
9	-	132.8	9'	-	135.6
10	6.24 (d, 1.83)	115	10'	6.45 (dd, 8.24, 2.14)	132
11	-	143.4	11'	6.81 (dd, 8.24, 2.14)	123
12	-	148	12'	-	154
13	6.76 (d, 7.93)	114.5	13'	7.05 (dd, 8.24, 2.14)	123
14	6.63 (dd, 7.93, 1.83)	123	14'	7.32 (dd, 8.24, 2.14)	130.2
2-NMe	2.29 (s)	42.4	2'-NMe	2.51(s)	43
6-OMe	3.78(s)	56	6'-OMe	3.91	56

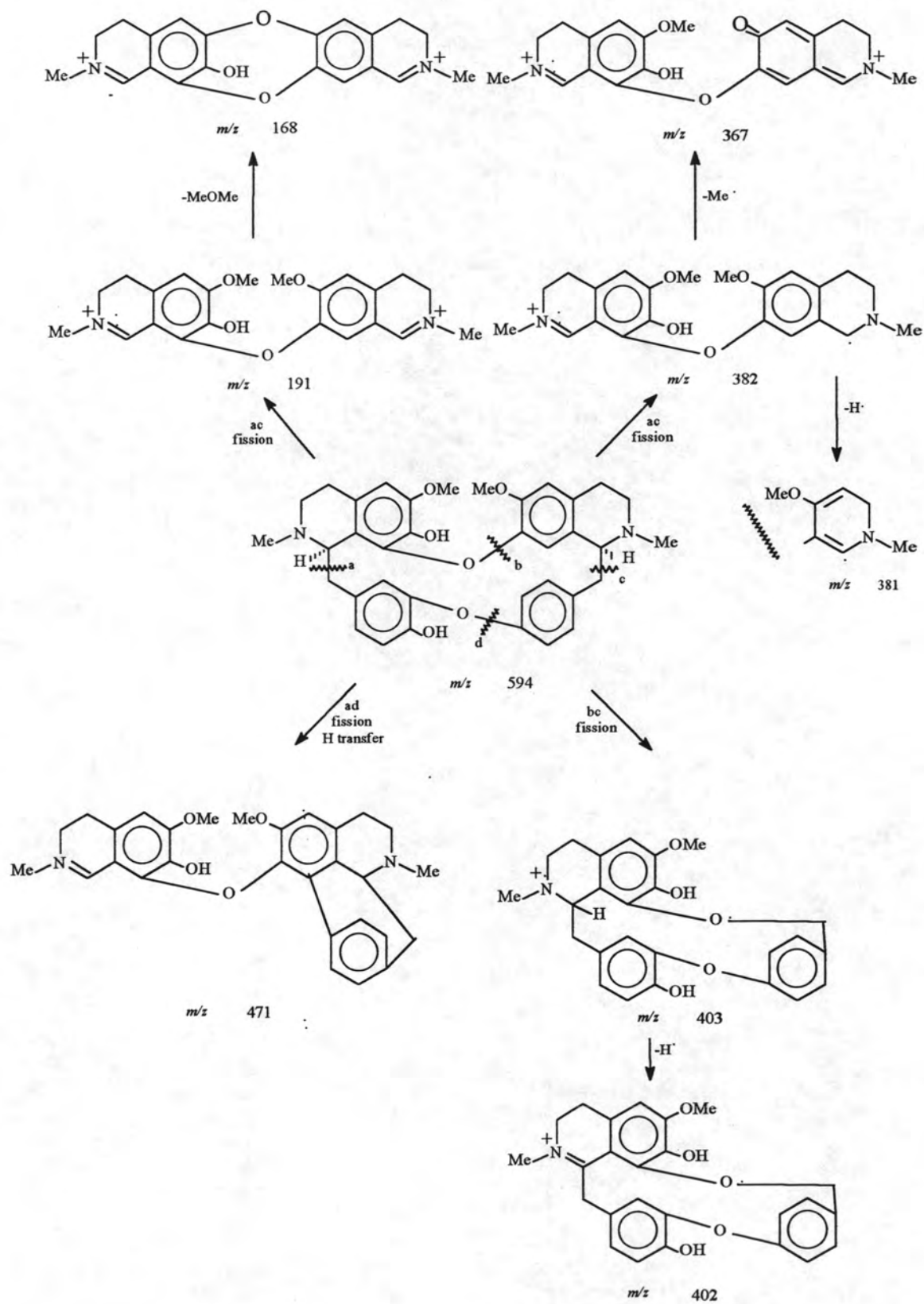
*Chemical shifts are reported in ppm from TMS in CDCl_3 ; multiplicity and coupling constants in Hz are in parentheses.

The structure of obamegine (193) is shown below.



193 Obamegine

This structure is confirmed by the analysis of the mass fragmentation (Scheme 6).

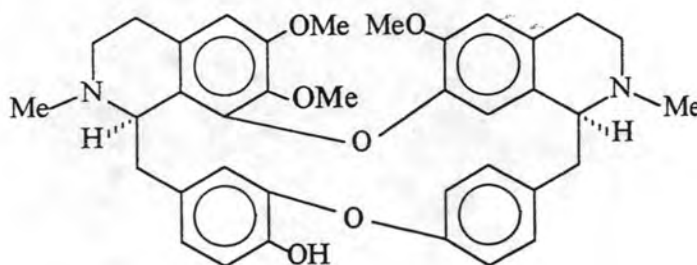


Scheme 6 Mass fragmentation of CH01

COMPOUND CH02

Compound CH02 was obtained as colorless needles. The eims spectrum of CH02 (Fig 15) exhibits the molecular ion peak at m/z 608 (11%), and establishes the tentative molecular formula of $C_{37}H_{40}O_6N_2$. The uv absorptions (Figure 16) at λ_{max} 283 nm(ϵ 7877), indicates the bisbenzylisoquinoline structure (Shamma, 1972 and Brossi, 1987). The ir spectrum (Figure 17) exhibits the band at ν 3450 (broad), 1650 and 1225 cm^{-1} indicating the presences of hydroxyl, aromatic ring and ether functional groups, respectively.

Compound CH02 is assigned as a known bisbenzylisoquinoline, berbamine (152), by the 1H -nmr (Figure 18), ^{13}C -nmr (Figure 20), H-H COSY (Figure 22), HETCOR (Figure 24) and COLOC (Figure 26) spectra as same as compound CH01. The chemical shifts of protons and carbons are summarized in table 7. Only one position at C-7 is different from CH01, therefore the methoxy group is assigned to C-7 in CH02. The COLOC spectrum(Figure 26) exhibits the C-H long-range correlation of C-7 (δ 136.9 ppm) and OCH_3 (H δ 3.12(s), C δ 60.4 ppm), that in CH05 is not observed. The structure of berbamine is supported by the eims fragmentation (Scheme 7). Its absolute configuration is assigned to be $1R,1'S$ (Specific optical rotation of CH02 is $+100^\circ$ in chloroform) as same as CH01. The assignments of protons and carbons of compound CH02 are confirmed by comparison with the data of berbamine (152) previously reported by Guinaudeau et al (1986) and Koike et al (1982).

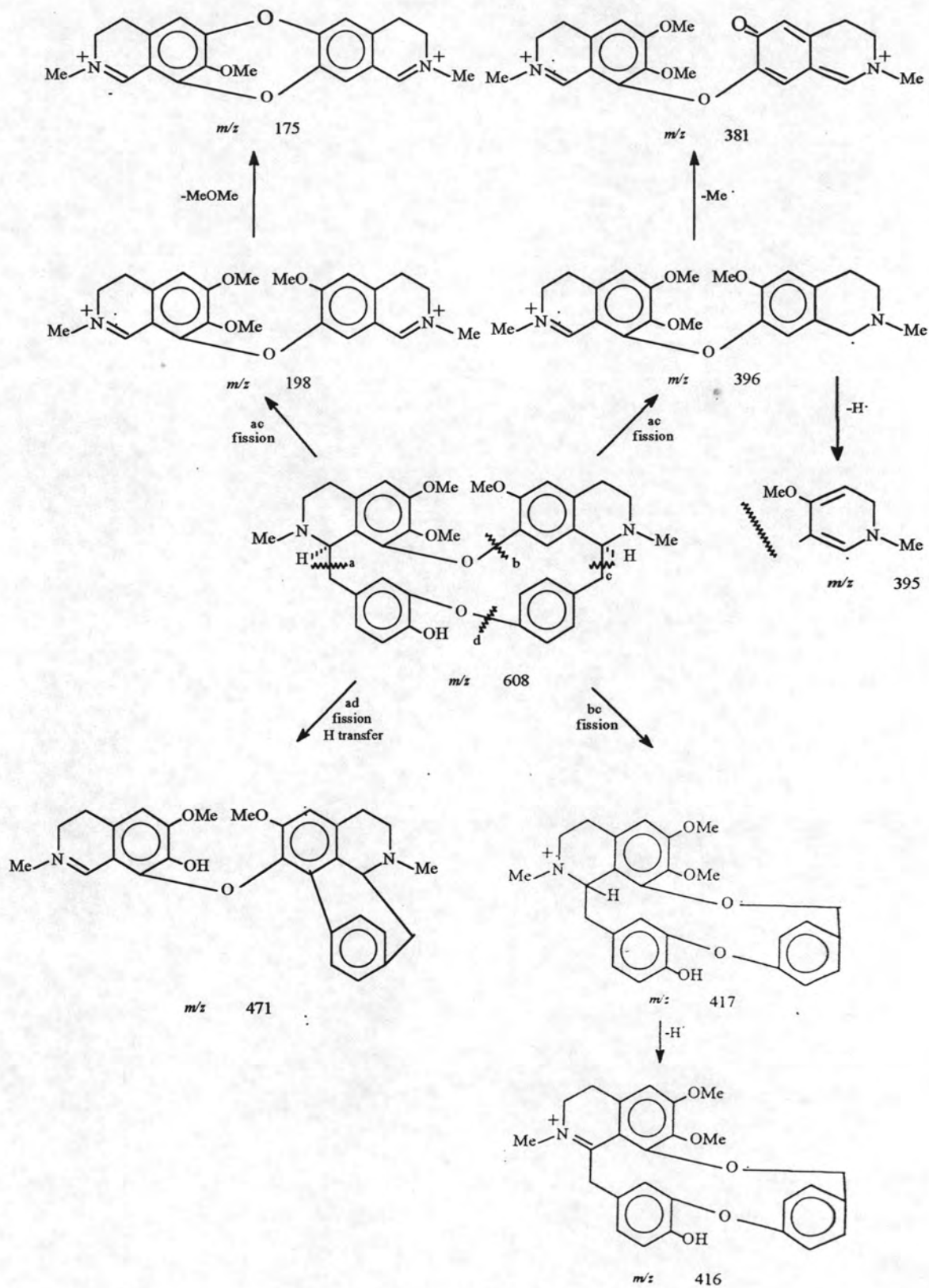


152 Berbamine

Table 7 ^1H - and ^{13}C -nmr assignments of CH02*

Position	^1H	^{13}C	Position	^1H	^{13}C
1	3.85 (br)	62.1	1'	3.85 (br)	63.4
3	2.80(m)	45.9	3'	2.80 (m)	45.9
	3.25 (dd, 12.81, 6.41)			3.40 (m)	
4	2.40(brd)	25.5	4'	2.9 (m, 2H)	25.5
	2.80 (m)				
4a	-	128.3	4a'	-	128.7
5	6.27 (s)	105.4	5'	6.52 (s)	111.2
6	-	151.7	6'	-	149.8
7	-	136.9	7'	-	143.5
8	-	147.4	8'	5.98 (s)	119.8
8a	-	120.7	8a'	-	127.5
α	2.59 (m)	38.5	α'	2.80 (m)	37.7
	3.0 (d, 14.04)			3.25 (dd, 12.81, 6.41)	
9	-	135.6	9'	-	135.6
10	6.43 (br)	115.2	10'	6.43 (br)	132.2
11	-	143.6	11'	6.62 (dd, 8.24, 2.44)	121.6
12	-	148.1	12'	-	153.7
13	6.81 (d, 8.24)	114.4	13'	7.09 (dd, 8.24, 2.44)	121.3
14	6.74 (dd, 8.24, 1.53)	123.4	14'	7.25 (dd, 8.24, 2.44)	130.2
2-NMe	2.24 (s)	42.7	2'-NMe	2.56 (s)	42.7
6-OMe	3.74 (s)	55.6	6'-OMe	3.57 (s)	55.6
7-OMe	3.12 (s)	60.4			

*Chemical shifts are reported in ppm from TMS in CDCl_3 ; multiplicity and coupling constants in Hz are in parentheses.



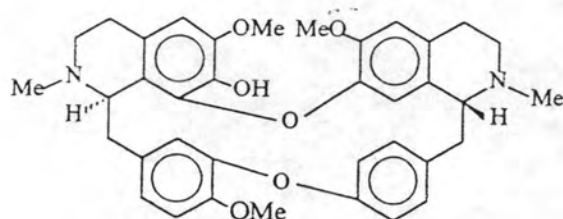
Scheme 7 Mass fragmentation of CH02

COMPOUND CH03

Compound CH03 was obtained as colorless needles. The eims spectrum of CH03 (Figure 28) exhibits the molecular ion peak at m/z 608 (6%), and establishes the proposed molecular formula of $C_{37}H_{40}O_6N_2$. The uv absorptions (Figure 29) at λ_{max} 284 (ϵ 8765), indicates the bisbenzylisoquinoline structure (Shamma, 1972 and Brossi, 1987). The ir spectrum (Figure 30) exhibits the band at ν 3450 (broad), 1645 and 1240 cm^{-1} indicating the presences of hydroxyl, aromatic ring and ether functional groups, respectively.

Compound CH03 is assigned as a known bisbenzylisoquinoline, limacine (177), by the ^1H -nmr (Figure 31), ^{13}C -nmr (Figure 33), H-H COSY (Figure 34), HMQC (Figure 36) and HMBC (Figure 38) spectra as same as compound CH01. The chemical shifts of protons and carbons are summarized in table 8. Only one position at C-12 is different from CH01, therefore the methoxy group is assigned to attach to C-12 in CH03. The HMBC spectrum (Figure 38) exhibits the C-H long range correlation of C-12 (δ 146.7 ppm) and OCH_3 (H δ 3.93 ppm (s), C δ 56.1 ppm), that in CH01 is not observed.

The relative stereochemistry between H-1 and H-1' is deduced as an *anti* configuration from the following observations: (1) H-10 (δ 6.56 ppm, d) lies between δ 6.45-6.60 ppm (2) the protons of 6'-OMe (δ 3.35 ppm, s) are observed between δ 3.20-3.40 ppm. The negative sign of the specific optical rotation of CH03 (-210° in chloroform) indicates that the absolute configuration of CH03 is 1*R*,1'*R* (Cassels and Shamma, 1980). The assignments of protons and carbons of compound CH03 are confirmed by comparison with the data of limacine (177) previously reported by Guinaudeau et al (1986) and Schiff (1987). The structure of limacine (177) is supported by the eims fragmentation (Scheme 8).

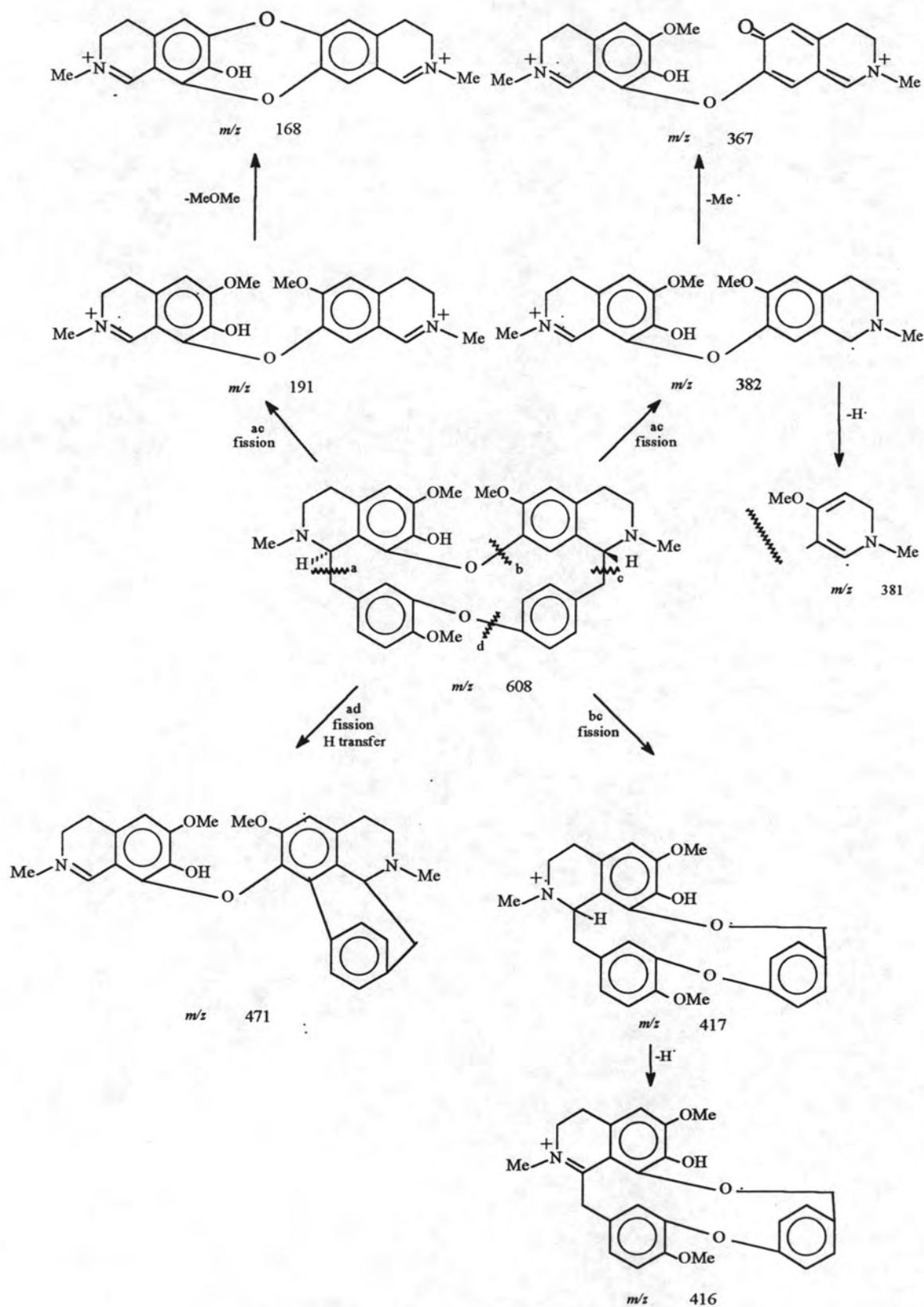


177 Limacine

Table 8 ^1H - and ^{13}C -nmr assignments of CH₃*

Position	^1H	^{13}C	Position	^1H	^{13}C
1	3.78 (overlapping)	61.2	1'	3.93 (overlapping)	63.8
3	2.9 (m)	44.9	3'	2.80 (m)	45.1
	3.53 (m)			3.43 (m)	
4	2.46 (dd, 15.96, 4.28)	21.8	4'	2.9 (m, 2H)	25
	2.9 (m)				
4a	-	123	4a'	-	127.6
5	6.30 (s)	105	5'	6.52 (s)	112.5
6	-	145.7	6'	-	148.4
7	-	135	7'	-	143
8	-	142.5	8'	6.05 (s)	120.8
8a	-	123	8a'	-	128
α	2.57 (d, 13.74)	42	α'	2.8 (m)	38
	2.8 (m)			3.30 (dd, 12.51, 5.49)	
9	-	135	9'	-	135
10	6.56 (d, 1.84)	116	10'	6.32 (dd, 8.24, 2.13)	132.3
11	-	149	11'	6.81 (dd, 8.24, 2.13)	121
12	-	146.7	12'	-	154
13	6.84 (d, 8.24)	111.8	13'	7.13 (dd, 8.24, 2.13)	121
14	6.90 (dd, 8.24, 1.84)	123	14'	7.33 (dd, 8.24, 2.13)	130
2-NMe	2.35 (s)	42.5	2'-NMe	2.65 (s)	42.5
6-OMe	3.78 (s)	56.1	6'-OMe	3.35 (s)	56.1
12-OMe	3.93 (s)	56.1			

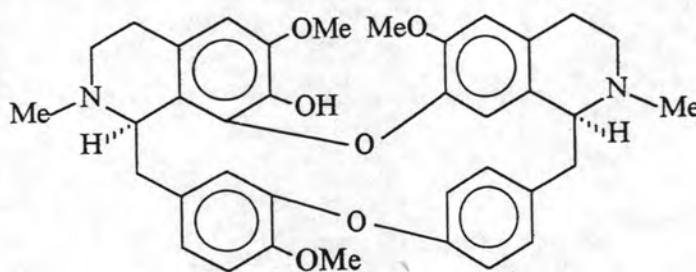
*Chemical shifts are reported in ppm from TMS in CDCl₃; multiplicity and coupling constants in Hz are in parentheses.



Scheme 8 Mass fragmentation of CH03

COMPOUND CH04

Compound CH04 was obtained as pale yellow needles. The eims spectrum of CH04 (Figure 40) exhibits the molecular ion peak at m/z 608 (10%), and establishes the tentative molecular formula of $C_{37}H_{40}O_6N_2$. Compound CH04 is assigned as a known bisbenzylisoquinoline, thalrugosine (206), by the 1H -nmr (Figure 43), ^{13}C -nmr (Figure 45), H-H COSY (Figure 46), HMQC (Figure 48) and HMBC (Figure 50) spectra as same as compound CH01. The chemical shifts of protons and carbons are summarized in the table 9. Only one position at C-12 is different from CH01, therefore the methoxy group is assigned to attach to C-12 in CH04. The HMBC spectrum (Figure 50) exhibits the C-H long rang correlation of C-12 (δ 146.8 ppm) and OCH_3 (H δ 3.94 ppm (s), C δ 56 ppm), that in CH01 is not observed. Its absolute configuration is assigned to be $1R,1'S$. (Specific optical rotation of CH04 is $+85^\circ$ in MeOH), likes CH01. The assignments of protons of compound CH04 are confirmed by comparison with the data of thalrugosine (206) previously reported by Guinaudeau et al (1986). The structure of thalrugosine (206) is supported by the eims fragmentation (Scheme 9).

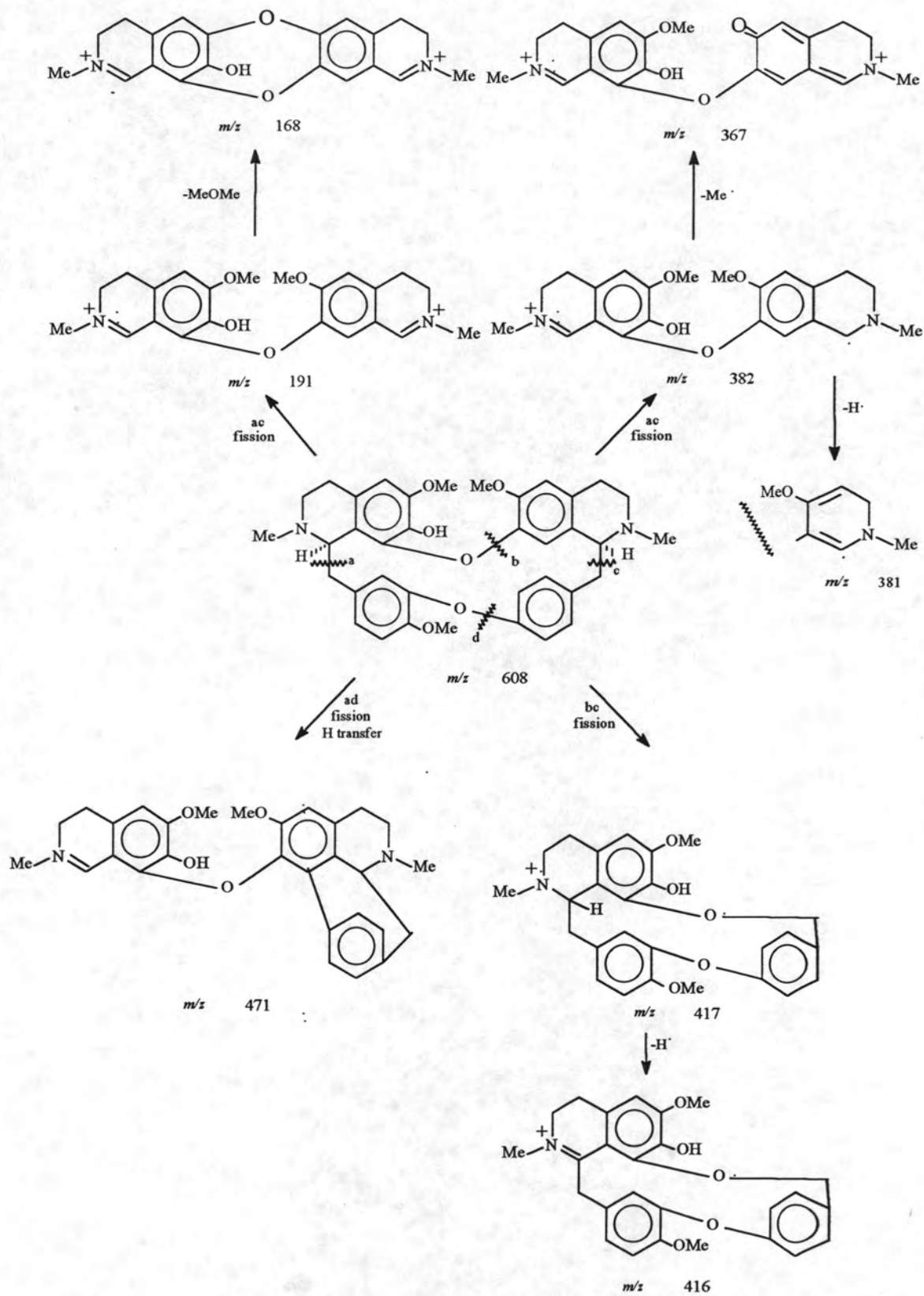


206 Thalrugosine

Table 9 ^1H - and ^{13}C -nmr assignments of CH04*

Position	^1H	^{13}C	Position	^1H	^{13}C
1	4.01 (dd, 10.68, 2.14)	60.2	1'	3.61 (overlapping)	65.2
3	2.8 (m)	44	3'	2.80 (m)	46
	3.23 (m)			3.38 (m)	
4	2.35 (dd, 16.48, 6.80)	21.3	4'	2.9 (m, 2H)	25.9
	2.8 (m)				
4a	-	124.4	4a'	-	131
5	6.35 (s)	107.9	5'	6.75 (s)	112.5
6	-	147	6'	-	149.2
7	-	136.5	7'	-	143.6
8	-	144.3	8'	6.06 (s)	121.5
8a	-	122.3	8a'	-	130.8
α	2.64 (dd, 14.65, 10.68)	39.3	α'	2.8 (m)	38.1
	2.9 (m)			3.23 (m)	
9	-	133.6	9'	-	135.6
10	6.29 (d, 1.83)	115	10'	6.47 (dd, 8.24, 2.14)	132
11	-	150.3	11'	6.86 (dd, 8.24, 2.14)	123
12	-	146.8	12'	-	154.5
13	6.80 (d, 8.24)	111.5	13'	7.06 (dd, 8.24, 2.14)	122.8
14	6.69 (dd, 8.24, 1.83)	122	14'	7.31 (dd, 8.24, 2.14)	130
2-NMe	2.32 (s)	42.1	2'-NMe	2.51 (s)	43.3
6-OMe	3.78 (s)	56	6'-OMe	3.94 (s)	56
12-OMe	3.94 (s)	56			

*Chemical shifts are reported in ppm from TMS in CDCl_3 ; multiplicity and coupling constants in Hz are in parentheses.



Scheme 9 Mass fragmentation of CH04

COMPOUND CH05

CH05 was obtained as colorless needles and recrystallized from hexane. It gave green color to Liebermann-Burchard's test. Thus, it appeared to be the steroidal compound.

The ir spectrum of CH05 (Figure 53) suggested that functional groups of CH05 were hydroxyl group, methyl group, methylene group and alkyl group. (Table 10)

Table 10 IR Spectrum Assignment of CH05

Range of Absorption (cm ⁻¹)	Assignment
3500-3200	O-H stretching of R-OH
3000-2800	C-H stretching of CH ₃ , CH ₂
1640	C=C stretching of alkene
1470	C-H bending of CH ₃ (asymmetric), CH ₂
1390	C-H bending of CH ₃ (symmetric),
1090	C-O stretching

CH05 can be assigned as a known sterol, β -sitosterol through analysis of its ¹H- and ¹³C-nmr spectra. The ¹H-nmr spectrum of CH05 (Figure 54) shows the signals at δ 0.68-1.01 ppm which are the signals of methyl protons that substituted at C-18, C-19 and at side chain of the steroidal compounds. The signals at δ 1.1-2.3 ppm are the signals of methylene and methine proton of steroid. The signal at δ 3.52 ppm (m) is the signal of proton at C-3. The olefinic signal at δ 5.35 ppm (m) can be assigned as H-6 which was trisubstituted vinyl proton. The ¹³C-nmr spectrum (Figure 56) shows the signals of 3 quarternary, 9 methine, 11 methylene and 6 methyl carbons. The carbon assignments of CH05 are shown in table 11. Both the ¹H- and ¹³C-nmr spectra (Figure 54 and 56) are in accordance with those published previously of β -sitosterol (Ogura, Cordell and Farnsworth, 1977; Robinstein et al, 1976; Wright et al,

1976). It is concluded that CH05 is β -sitosterol, the structure of which is shown below.

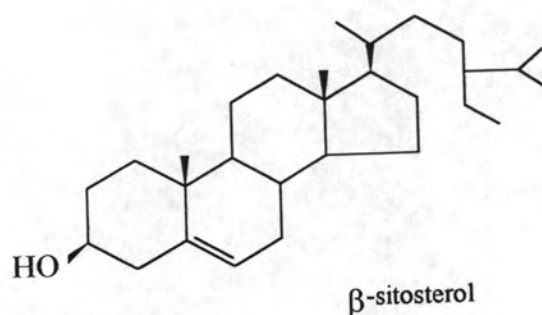
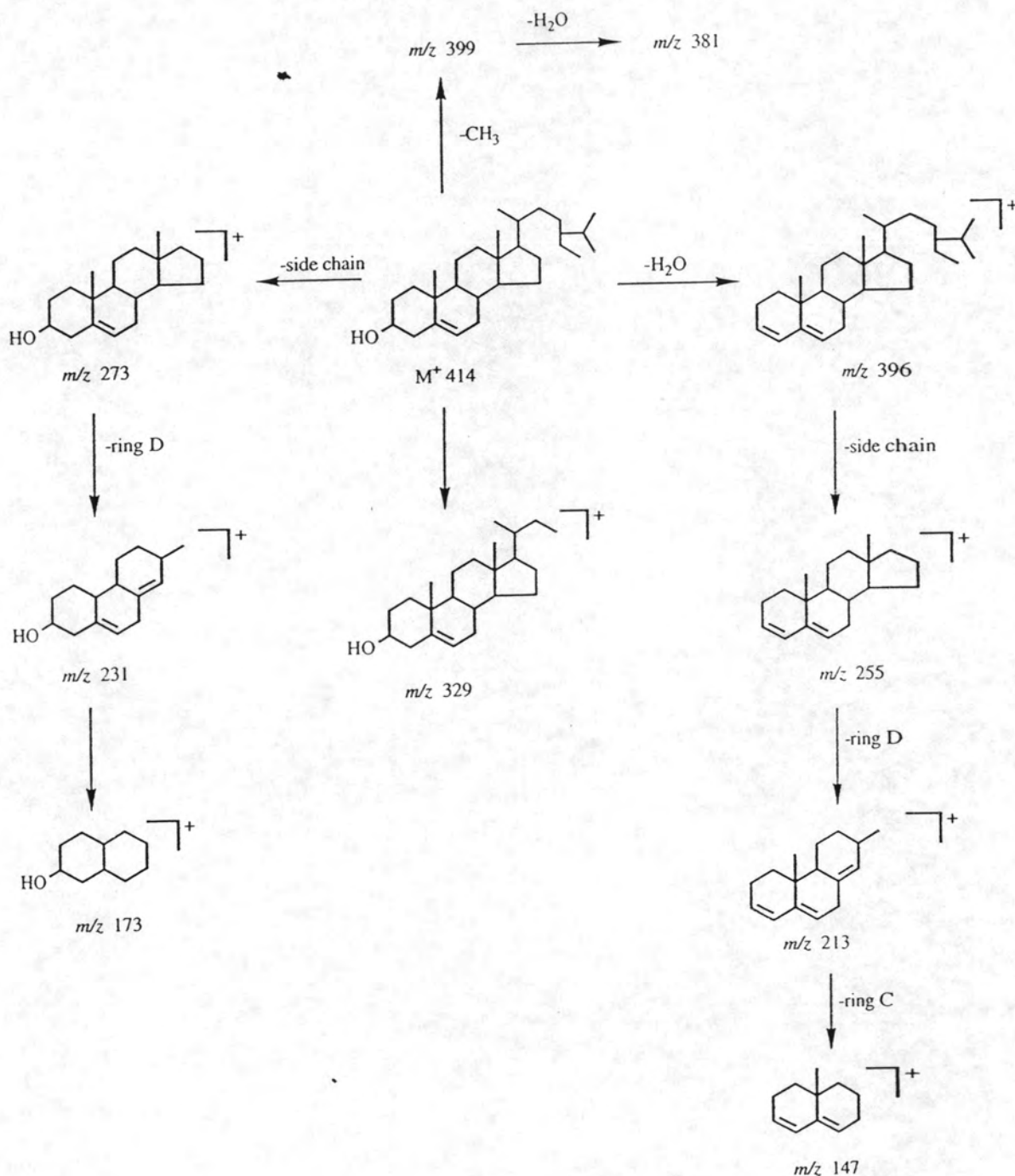


Table 11 ^{13}C -nmr Assignments of CH05

C position	δC (ppm)	C position	δC (ppm)
1	37.2	16	28.3
2	31.7	17	56
3	72	18	12
4	42.3	19	19.4
5	140.5	20	36.2
6	122	21	18.8
7	32	22	33.9
8	32	23	26.1
9	50.1	24	45.9
10	36.4	25	29.1
11	21	26	19.8
12	39.8	27	19
13	42.3	28	23
14	56.8	29	11.9
15	24.3		

This structure is confirmed by the analyses of mass fragmentations. The eims

spectrum (Figure 52) exhibits a weak molecular ion peak at m/z 414 (9%) corresponding to molecular formula $C_{29}H_{50}O$. The proposed fragmentation patterns are shown below.



Scheme 10 Mass fragmentation of CH05