

CHAPTER IV

RESULT AND DISCUSSION

The extraction of the root of *Clausena guillauminii* Tanaka by refluxing with hexane and separation by column chromatography, resulted to isolate at least six compounds. Each compound was identified on the basis of spectroscopic method, physical constant and some chemical reactions, and related to known data on authentic samples. The authentic samples were obtained from the isolated of *Clausena harmandiana* Pierre by Jinda Wangboonskul. Only compound VI, the more additional spectroscopic methods were needed in order to determine the correct structure.

The identification of six isolated compounds were demonstrated of the basis of the following data.

1) Compound I

A Yellow needle crystal (29.2 mg, 0.292% yield) was obtained from fractions 16-18 (a).

TLC : The compound was spotted on TLC plate and developed in solvent system 1, 2, 4, 6, 7 and 8. After the plate was dried in open air, the detection was sprayed with ferric chloride reagent. A blue color was developed, indicated that the compound contains phenolic group. The R_f value in different solvent system are the follows :-

system 1 : 0.47
 system 2 : 0.70
 system 4 : 0.85
 system 5 : 0.92
 system 6 : 0.43
 system 7 : 0.77
 system 8 : 0.69

Melting point : 171-172°C (uncorrected)

Infrared Absorption Spectrum (Fig. 24)

$\nu_{\text{max}}^{\text{KBr}}$ = 3300 cm^{-1} (-OH or -NH stretching)
 2740-3125 cm^{-1} (-CH stretching)
 1612 cm^{-1} (aldehyde, -C=C stretching)
 1450-1600 cm^{-1} (aromatic)

Nuclear magnetic resonance spectrum

^{13}C NMR (62.89 MHz, CDCl_3 , off resonance and APT technique)
 (Fig. 25, 26, 27)

Chemical shift (δ , ppm)	Carbon position	Off resonance	APT
195.38	CHO	doublet	CH
157.19	C_2	singlet	C
145.11	C_{15}	singlet	C
140.13	C_9	singlet	C
134.2	C_{10}	singlet	C
125.9	} $\text{C}_7, \text{C}_{14}$	singlet	C
125.3			

Chemical shift (δ , ppm)	Carbon position	Off resonance	APT
123.7	C ₁₁	singlet	C
121.29	C ₄	doublet	CH
120.9	C ₅	doublet	CH
119.82	C ₆	doublet	CH
117.4	C ₁₂	singlet	C
115.57	C ₃	singlet	C
110.8	C ₈	doublet	CH
109.11	C ₁	singlet	C
22.9	C ₁₃	triplet	CH ₂
18.12	} C ₁₆ , C ₁₇	quartet	CH ₃
25.7			

¹H NMR (250 MHz/CDCl₃/TMS)

(Fig. 28)

Chemical shift (δ , ppm)	Proton position	multiplicity	Coupling constant (J, Hz)
11.65	- OH	singlet	
9.899	- CHO	singlet	
8.214	- NH	singlet	
8.039	4 (H)	singlet	
7.99	6 (H)	double doublet	J = 11.5, 7.7
7.37	} 5 (H) 8 (H)	doublet	} J = 7.7 J = 4.2
7.41			

Chemical shift (δ , ppm)	Proton position	multiplicity	Coupling constant (J, Hz)
7.27	7 (H)	double doublet	J = 11.5, 4.2
5.317	14 (H)	triplet	J = 6.85
3.635	13 (2H)	doublet	J = 6.85
1.9	} 16 (3H) } 17 (3H)	} singlet	
1.77			

The comparison of IR spectrum between compound I and Heptaphylline are showed Fig. 24.

All these informations suggested that compound I is Heptaphylline. They are identical in R_f value, melting point (Heptaphylline, 171-172°C) color and form of crystal (Yellow needle) and also IR spectrum.^(9,17)

2) Compound II

A colorless rod crystal (230 mg, 2.3% yield) was obtained from fraction 30-38 (b).

TLC : The compound was spotted on TLC plate and developed in solvent system 1, 2, 3, 4, 5, 6, 7, and 8 the detection was performed by spraying with benzidine and ferric chloride.

The compound gave only one spot on TLC and gave a positive test with benzidine reagent (red color) and ferric chloride (blue color), indicated that the phenolic presented in the molecule. The R_f value in different solvent system are the following :-

System 1	:	0.16
System 2	:	0.55
System 3	:	0.63
System 4	:	0.28
System 5	:	0.75
System 6	:	0.25
System 7	:	0.51
System 8	:	0.36

Melting point : 200-202°C (uncorrected)

Infrared Absorption Spectrum (Fig. 29)

$\nu_{\text{max}}^{\text{KBr}}$	=	3170 cm^{-1}	(-OH stretching)
		2880-3010 cm^{-1}	(-CH stretching)
		1672 cm^{-1}	(coumarin carbonyl)
		1645 cm^{-1}	(conjugated double bond at C-3, C-4)
		1610 cm^{-1}	(CH=C- stretching)
		1450-1600 cm^{-1}	(aromatic)

All these informations suggested that compound II is Clausarin.

The comparison of IR spectrum between compound II and Clausarin are showed Fig. 29. They are identical in Rf value, melting point (Clausarin 198-200°C,⁽⁹⁾ 208-210°C⁽⁷⁾), color and form of crystal (colorless rod) and IR spectrum^(9,25) with Clausarin.

3) Compound III

A colorless elongated prism crystal (373 mg, 3.73% yield) was obtained from the rechromatography of fraction 40-47 (c).



TLC : The compound was spotted on TLC plate and developed in solvent system 1, 2, 3, 4, 5, 6, 7 and 8 the detection was performed under UV and was sprayed with spraying reagent. The compound gave only one spot on TLC plate and fluoresced under UV light (long wavelength). It also gave a negative test with benzidine and ferric chloride reagent, but gave a yellow spot with iodine vapor. The R_f value were shown below :-

System 1	:	0.19
System 2	:	0.51
System 3	:	0.69
System 4	:	0.37
System 5	:	0.83
System 6	:	0.41
System 7	:	0.55
System 8	:	0.65

Melting point : 95°C (uncorrected)

Infrared Absorption Spectrum (Fig. 30)

KBr max	=	2870-3080 cm ⁻¹	(-CH stretching)
		1725 cm ⁻¹	(coumarin carbonyl)
		1630 cm ⁻¹	(conjugated double bond at C-3, C-4)
		1610 cm ⁻¹	(-CH=C stretching)
		1450-1600 cm ⁻¹	(aromatic)

All these informations suggested that compound III is Dentatin. The comparison of IR spectra between compound III and Dentatin are

showed Fig. 30. They are identical in Rf value, melting point (Dentatin 95°C⁽⁹⁾, 95-96°C⁽¹³⁾), color and form of crystal (colorless prism) and IR spectrum^(9,13) with Dentatin.

4) Compound IV

A Colorless prism crystal (93.5 mg, 0.935% yield) was obtained from fraction 67-73 (d).

TLC : The compound was spotted on TLC plate and developed in solvent system 1, 2, 3, 4, 5, 6, 7 and 8 the detection was performed under UV and was sprayed with spraying reagent. The compound gave only one spot on TLC and fluoresced under long-wavelength UV light. It also gave negative with benzidine and ferric chloride reagent, but gave a yellow spot in iodine vapor. The Rf value were the following;-

System 1	:	0.19
System 2	:	0.47
System 3	:	0.71
System 4	:	0.27
System 5	:	0.71
System 6	:	0.33
System 7	:	0.66
System 8	:	0.64

Melting point : 81-82°C (uncorrected)

Infrared Absorption Spectrum (Fig. 31)

$\nu_{\text{max}}^{\text{KBr}}$	=	2840-3010 cm^{-1}	(-CH stretching)
		1728 cm^{-1}	(coumarin carbonyl)
		1635 cm^{-1}	(conjugated double bond at C-3, C-4)
		1610 cm^{-1}	(-CH=C stretching)
		1450-1600 cm^{-1}	(aromatic)

All these informations suggested that compound IV is Osthol. The comparison of IR spectra between compound IV and Osthol are showed Fig. 31. They are identical in Rf value, melting point (osthol 78-81°C⁽⁹⁾), color and form of crystal (colorless prism) and IR spectrum.^(9,29)

5) Compound V

A pale yellow prism crystal (60.5 mg, 0.605% yield) from fraction 81-82 (e).

TLC : The compound was spotted on TLC plate and developed in solvent system 1, 2, 3, 4, 5, 6, 7 and 8. The compound gave only one spot on TLC and gave positive test (red color) with benzidine reagent but gave a negative test with ferric chloride. It gave positive test (yellow spot) in iodine vapor. The Rf were the following :-

System 1	:	0.013
System 2	:	0.145
System 3	:	0.25
System 4	:	0.054
System 5	:	0.63

System 6 : 0.053

System 7 : 0.074

System 8 : 0.15

Melting point : 177-180°C (uncorrected)

Infrared Absorption Spectrum (Fig. 32)

$\nu_{\text{max}}^{\text{KBr}}$	=	3240 cm^{-1}	(-OH stretching)
		2860-2980 cm^{-1}	(-CH stretching)
		1685 cm^{-1}	(coumarin carbonyl)
		1640 cm^{-1}	(conjugated double bond at C-3, C-4)
		1605 cm^{-1}	(-CH=C- stretching)
		1450-1600 cm^{-1}	(aromatic)

All these informations suggested that compound V is Nordentatin. The comparison of IR spectra between compound V and Nordentatin are showed Fig. 32. They are identical in R_f value, melting point (Nordentatin, 183-186°C⁽⁹⁾, 178-180°C⁽⁷⁾, 182°C⁽¹³⁾), color and form of crystal (pale yellow prism) and IR spectrum.^(9,13)

6) Compound VI

A bright yellow needle crystal (27 mg, 0.27% yield) from the rechromatography of fraction 40-47 (e).

TLC : The compound showed only one spot on TLC in different solvent system. It gave a positive test with ferric chloride, indicate that the molecule contained phenolic. The R_f value were showed below :-

System 1	:	0.28
System 2	:	0.61
System 4	:	0.38
System 5	:	0.86
System 6	:	0.30
System 7	:	0.66
System 8	:	0.68

Molecular weight : 309 (eims)

Melting point : 159-160°C (uncorrected)

Ultraviolet absorprion spectrum (Fig. 33)

$\lambda_{\text{max}}^{\text{MeOH}}$	=	207 nm
		235 nm
		253 nm
		299 nm
		338 nm

Infrared absorption spectrum (Fig. 34)

$\nu_{\text{max}}^{\text{KBr}}$	=	3320 cm^{-1} (-OH or -NH stretching)
		2850-2980 cm^{-1} (-CH stretching)
		1618 cm^{-1} (-C=C stretching)
		1630, 2860 cm^{-1} (-CHO aromatic)
		1450-1600 cm^{-1} (aromatic)
		1160-1300, 1000-1060 cm^{-1} (-OCH ₃ aromatic)

Nuclear magnetic resonance spectrum

^{13}C NMR (62.89 MHz, CDCl_3 , off resonance and APT technique)

(Fig. 35, 36, 37)

Chemical shift (δ , ppm)	Carbon position	Off resonance	APT
195.39	CHO	doublet	CH
159.1	C_7	singlet	C
157.34	C_2	singlet	C
145.21	C_{15}	singlet	C
141.46	C_{10}	singlet	C
134.13	C_9	singlet	C
124.0	C_5	doublet	CH
121.34	} $\text{C}_4, \text{C}_{14}$	doublet	CH
120.52			
117.52	$\text{C}_{11}, \text{C}_{12}$	singlet	C
117.29			
115.42	C_3	singlet	C
108.96	C_6	doublet	CH
108.96	C_1	singlet	C
95.69	C_8	doublet	CH
55.7	OCH_3	quartet	CH_3
25.7	} $\text{C}_{16}, \text{C}_{17}$	quartet	CH_3
18.2			
22.8	C_{13}	triplet	CH_2

$^1\text{H NMR}$ (250 MHz/ CDCl_3 /TMS)

(Fig. 38)

Chemical shift (δ , ppm)	Proton position	multiplicity	Coupling constant (J, Hz)
11.63	- OH	singlet	
9.9	- CHO	singlet	
8.1	- NH	singlet	
7.9	4 (H)	singlet	
7.83	5 (H)	doublet	J = 8.5
6.91	8 (H)	doublet	J = 2.1
6.9	6 (H)	double doublet	J = 8.5, 2.1
5.31	14 (H)	triplet	J = 1.4
3.9	- OCH_3	singlet	
3.62	13 (2H)	doublet	J = 6.5
1.89	16 (3H)	singlet	
1.77	17 (3H)	singlet	

 $^1\text{H NMR}$ (90 MHz, in CDCl_3 and DMSO-d_6)

(Fig. 39)

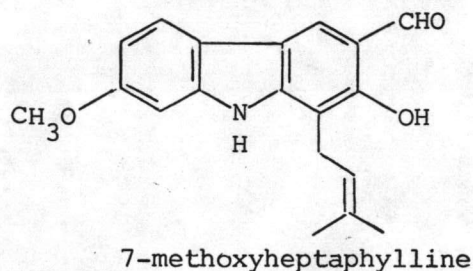
Proton position	Chemical shift (δ , ppm)		The change of chemical shift
	CDCl_3	DMSO-d_6	
H_4	7.86	8.16	0.3
H_5	7.86, 7.75	7.90, 7.81	0.05
H_6	6.89, 6.80	6.84, 6.75	0.05
H_7	6.89	6.99	0.1

Mass spectrum

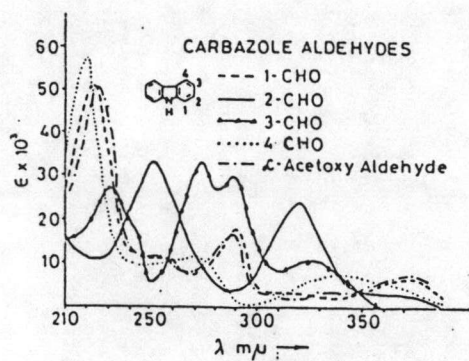
m/e (%)	309(M ⁺ , 26.16), 294(3.94), 266(3.75), 255(3.42)
	254(19.59), 253(6.28), 238(2.79), 225(3.35),
	210(2.95), 226(1.94), 182(1.29), 154(1.31)

It was analysed for the molecular formula C₁₉H₁₉NO₃.

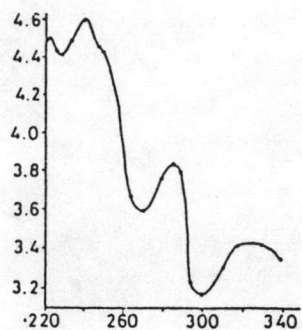
All these information, suggested that a compound VI is a new naturally occurring carbazole alkaloids, 7-methoxyheptaphylline. Its structure was elucidated on the basis of ultraviolet, infrared, nuclear magnetic resonance and mass spectra.

UV spectra

The UV spectrum of carbazole is characteristic and differs from its carbocyclic analog, fluoresecne, due to the contribution of the lone pair of electrons of the heterocyclic nitrogen to the π -electronic state of the molecule as a whole. The presence of formyl, methoxy and methyl function at different position of the carbazole nucleus could modify the spectrum to a great extent so as to provide a diagnostic pattern.⁽⁴⁰⁾ The significant and diagnostic spectra of substituted carbazoles have been recorded in the case of formyl and methoxycarbazoles.⁽⁴²⁾ (Fig. 13) Formyl, methoxy and pyranocarbazoles (Fig. 13) gives rise to characteristic spectra

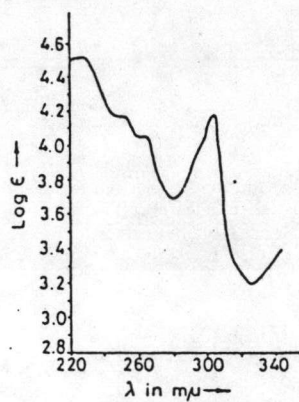


Formylcarbazoles

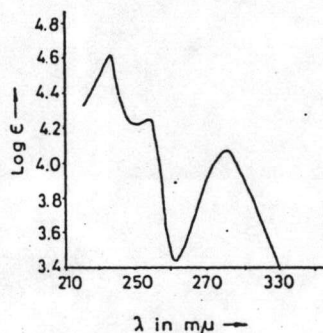


1-Methoxycarbazole

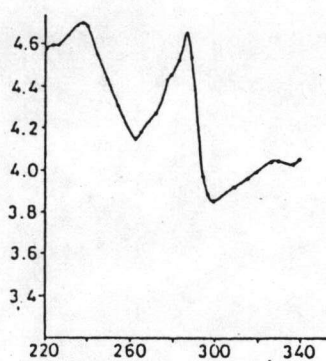
Alkaloids



3-Methoxy-6-methoxycarbazole



2-Methoxycarbazole



Girinimbine (Pyranocarbazole)

Fig. 13 Ultraviolet absorption spectra of carbazoles alkaloids

which have been extensively utilized in the structure determination of carbazole and related alkaloids. The behaviour of compounds containing both formyl and other contributing substituents is interesting Murraganine and Indizoline which have a formyl group at the 3- and a methoxy at the 1-position show spectra characteristic of the 3-formyl carbazole chromophore. On reduction of the formyl group, UV spectra similar to that of 1-methoxycarbazole are obtained. (42)

The UV spectrum of 7-methoxyheptaphylline (Fig. 33) closely resembles the spectrum of heptaphylline, which is characteristic of 3-formyl carbazole. (21,23) (Fig. 13)

IR spectra

Usually the aromatic aldehyde peaks of carbazole alkaloids appear in the region 1680 cm^{-1} , with chelation as in heptaphylline, the carbonyl absorption moves to higher waves-length. The aromatic aldehyde band at 2870 cm^{-1} is also discernible in some cases. (42)

The IR spectrum of 7-methoxyheptaphylline in KBr (Fig. 34) showed -OH- and -NH stretching as same as Heptaphylline. The peak a at 2860 cm^{-1} and 1630 cm^{-1} show a carbonyl stretching of aldehyde. The $-\text{OCH}_3$ of aromatic was showed at rang $1160\text{-}1300\text{ cm}^{-1}$ and $1000\text{-}1060\text{ cm}^{-1}$.

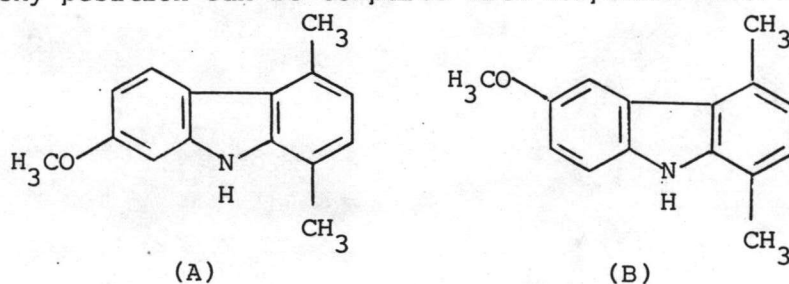
NMR spectra

The NMR spectra of the carbazole alkaloids have been very helpful in structure elucidation. The carbazole -NH- being acidic it absorbs near $\delta 10.0$ but with substitution the signal for the -NH- proton has been found to be shifted. The signal for the -NH- proton

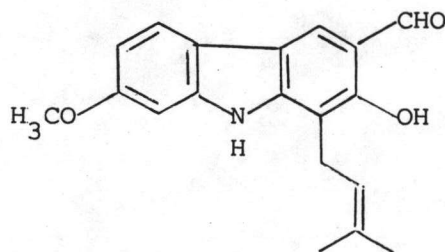
has been registered at 7.6 to 12.01 in different carbazole alkaloids. The signal for the protons on C-4 and C-5 appear at lower field (around 7.17 to 7.5) as these two protons are phenanthranic and are mutually deshielded. The other aromatic protons of carbazole occur at higher frequency as complete multiplets. Resonance are shielded or deshielded according to the environment of the proton in question. (42)

The position of methoxy group on the aromatic ring were determined by using the evidence from the known spectral data of similar compounds and by using solvent effect. The following information supported, compound VI is 7-methoxyheptaphylline.

1. ^{13}C NMR spectrum of methoxy position of pyridocarbazole structure were reported by A. Ahond and C. Poupat, (70) and chemical shift are showed in Table 12. The difference of chemical shift of 6 and 7 methoxy position can be compared with compound VI (7-methoxy position).



pyridocarbazole



compound VI

(7-Methoxyheptaphylline)

Table 12 The comparison of chemical shift between 6- and 7-methoxy position of pyridocarbazole and compound VI

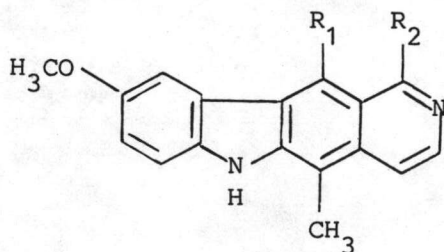
Carbon position	Chemical shift (δ , ppm)			multiplicity
	(A)	(B)	Compound VI	
C ₁₁	118.6	124.8	117.5, 117.3	singlet
C ₉	138.8	134.4	134.13	singlet
C ₅	123.3	106.3	124.02	doublet
C ₆	108.0	153.5	108.94	doublet
C ₇	158.5	113.4	159	singlet
C ₈	94.8	110.9	95.7	doublet
C ₁₂	121.6	121.3	117.5, 117.3	singlet
C ₁₀	140.4	139.6	141.48	singlet
OCH ₃	55.6	56.1	55.7	quarlet

The chemical shift of structure (A) was very similar to chemical shift of 7-methoxyheptaphylline, but the chemical shift of structure (B) was very difference, especially C₁₁, C₅ and C₈ position, suggested that the substitution of methoxy is on 7-position.

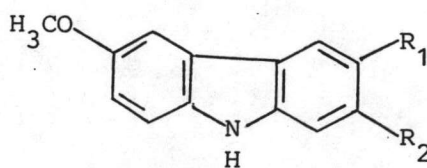
The chemical shift of 6-methoxy structure were also have been reported by Ahond and Poupat⁽⁷⁰⁾ (Table 13).

Table 13 The comparison of chemical shift of the same 6-methoxy position of carbazoles structure.

Carbon position	Chemical shift (δ , ppm)			
	(B)	(D)	(E)	(F)
C ₁₁	124.8	123.5	123.0	121.7
C ₉	134.4	137.1	137.1	135.6
C ₅	106.3	107.7	104.4	103.2
C ₆	153.5	153.0	153.4	153.4
C ₇	113.4	115.0	115.0	114.2
C ₈	110.9	111.0	111.5	103.2
C ₁₂	121.3	121.5	121.2	123.7
C ₁₀	139.6	141.1	141.5	139.3
OCH ₃	56.1	55.6	55.6	56.2



(D), R₁ = CH₃, R₂ = H



(F), R₁ = et, R₂ = -(CH₂)₄-

(E), R₁ = H, R₂ = CH₃

2. The consideration of solvent effect in NMR spectroscopy.

G. Van Binst^(71,72) have tried improved the determination of position of methoxy substituents on the indole nucleus of alkaloids by a NMR study of chemical shift of aromatic proton. The comparison compounds were synthesized as 1,2,3,4-tetrahydro-6, 7-methoxycarbazole and determined by ¹H NMR spectra. They can showed in Table 14.

Table 14 Chemical shift of aromatic proton of 1,2,3,4-tetrahydro-6, 7-methoxycarbazole in CDCl₃ and DMSO-d₆ (Hz).

Compounds	Solvent	Middle of the peak pattern				J		
		H ₅	H ₆	H ₇	H ₈	J _o	J _m	J _p
1,2,3,4-tetrahydro-6-methoxycarbazole	CDCl ₃	415.5	-	404.0	426.0	8.5	2.5	0.8
1,2,3,4-tetrahydro-6-methoxycarbazole	DMSO	409.5	-	396.5	427.5	8.6	2.6	0.7
1,2,3,4-tetrahydro-7-methoxycarbazole	CDCl ₃	435.0	401.3	-	399.5	8.8	2.2	0.3
1,2,3,4-tetrahydro-7-methoxycarbazole	DMSO	430.0	393.5	-	406.5	8.4	2.4	-

The difference pattern of ¹H NMR spectra of 6 and 7 methoxy position showed in Fig. 40 Proton ortho to the methoxy group are shielded and appear at lower fields. The solvent effect on the chemical shift of the aromatic proton is rather weak. The proton in position 8 is the most sensitive and is always shifted to lower fields.⁽⁷¹⁾

The spectrum of ¹H NMR of compound VI (Fig. 39) shows the change of chemical shift of H₄ and H₈ in DMSO-d₆. They are move to down field. While H₅ and H₆ do not change. This evidence showed that

the structure have the methoxy group on 7-position. This phenomena occurred due to the forming of hydrogen bond between the DMSO-d₆ and proton donor (OH, NH), especially with amino group. The effect of DMSO-d₆ can changed the chemical shift H₈ to down field.

Our studies showed that the sovent effect on compound VI corresponded to 1,2,3,4-tetrahydroxy-7-methoxy carbazole (Table 14, Fig. 40).

3. The consideration physical properties of 6-methoxy-heptaphylline.

B.S. Joshi, D.H. Gawad and V.N. Kamat (21) reported that 6-methoxyheptaphylline, a new carbazole alkaloid from *Clausena indica* Oliv., afforded a bright yellow crystalline substance, with m.p. 173-174°C.⁽²¹⁾ While the new naturally occurring 7-methoxyheptaphylline afforded m.p. 159-160°C.