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Characterization of crude extracts of *Ancistrocladus heyneanus* stem using high performance liquid chromatography/mass spectrometry and NMR spectroscopy

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ABSTRACT

New monomeric Naphthyl-iso-quinoline alkaloids, Yaoundamine A and Ancistrocline were isolated and identified from Ancistrocladus heyneanus stem using HPLC/MS and NMR. – Owing to the complexity of the extracts, the application of reverse phase HPLC resulted in a partially incomplete separation of the Naphthyl-iso-quinoline alkaloids. By performing HPLC/MS experiments, in combination of parallel isolation studies and structural elucidation using conventional methods, two alkaloids present in the plant could be identified. A. heyneanus stem were collected from the forests of a deep valley of Matheran, Mumbai in Maharashtra, India.

Keywords:-Nathyl-iso-quinoline alkaloid, Yaoundamine, Ancistrocline, Ancistrocladus heyneanus.

INTRODUCTION

The structurally unique naphthyl-iso-quinoline alkaloids produced by plants of Anaistrocladaceae family are different from all other 2500 iso-quinoline isolated from plants. This is because their unusual structure is rotationally hindered by biaryl axis between the two molecular parts and may also be because of their unprecedented biosynthetic origin. 4

Unlike most of the alkaloids which originate from amino acid residue, naphthyl-iso-quinoline alkaloids originate from acetate unit. Naphthyl-iso-quinoline alkaloids are grouped according to the position of biaryl linkage with the largest group being 5-1" linkage (e.g. Ancistrocladidine) and the recently isolated 5-8" linkage alkaloids e.g. Michellamine. The Naphthyl-iso-quinoline alkaloids are structurally unusual on account of the methyl group at the 3-position and oxygenation at the 8- and / or 6- position of Iso-quinoline ring which points to polyketide origin. This postulate has been supported by extensive studies conducted by Bringman and Pokorny (1995).

Our interest in this plant was fuelled by discovery that one of the species found in Africa i.e. *A. korupensis* contains a *Naphthyl-iso-quinoline alkaloid* having high levels of Michellamine B, which is active against HIV and it is being considered as, potential anti AIDS source. Apart from Michellamine B, *A. korupensis* has also shown presence of many other important *Naphthyl-iso-quinoline alkaloids* such as: Yaoundamine A and B a new anti-malarial Naphthyl-iso-quinoline alkaloids. Though Ancistrocladus found in Africa and South East Asia comprises of more than 15 species, In India we get only one species i.e. *A. heyneanus*. The notable morphologically of Ancistrocladus i.e. it has long tapering dark green leaves, it is a woody climber that has helped us in identifying and collecting this rare plant from the Hills Of Western Ghat of Maharashtra India. Our interest was to see whether this Indian species also has anti-HIV alkaloid Michellamine B or not.

MATERIALS AND METHODS

Ancistrocladus plants were collected from the forests of a deep valley of Matheran, a hill station about 100km from Mumbai in Maharashtra, India. There was a single mature plant growing the steep cliffs. The stem from this plant was collected in December .Collected stems – were dried in sun light to remove the moisture. Dried stems were powdered and then sieved through mesh having 0.02-0.04 mm pore size, to obtain a uniform particle sized powder and stored in clean plastic containers in refrigerator till further use. .

Extraction of Alkaloid in Organic Solvent (D***ichloromethane and Methanol)-*** to 500g dried stem powder of Ancistrocladus, 500 ml dichloromethane was added and extraction was carried our using Soxhlet apparatus for 6. The Dichloromethane was discarded. This step was repeated once more and then to residual powder 250ml dichloromethane and 250ml methanol was added and mixed thoroughly. The solvent was concentrated to dryness under reduced pressure to get the crude extract. Crude extract was mixed with 90% methanol + n hexane in a separating funnel. Two layers formed i.e. Methanol and Hexane. Methanol layer was separated and used for isolating alkaloids from it. The whole process was repeated till extraction was done using 5kg of stem powder.

Separation of alkaloids present in the Methanol extract – was done by three different methods I,e, (i) TLC, (ii) Column Chromatography and (iii) HPTLC.

TLC: For separation of alkaloids by TLC following different solvents for mobile phase were tried in different combinations

(i) Ethyl acetate: Methanol (7: 4)
(ii) Ethyl acetate: Methanol: Water (7: 4 :1)
(iii) Ethyl acetate: Methanol: 17% Ammonia (8: 1 :1)
(iv) Chloroform : Methanol : Water (7: 4 :1)
(v) Chloroform : Methanol (4: 6)
(vi) Chloroform : Methanol (9: 1)
(vii) Chloroform : Acetone : Toluene (25: 35: 40) (25: 35: 40)
(viii) Acetone : Hexane (2: 1)

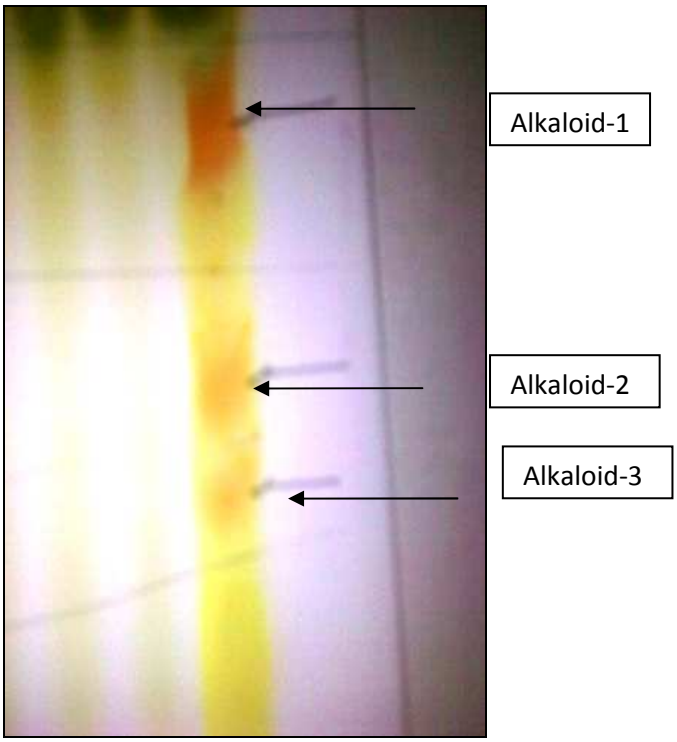


Figure – 1: TLC of stem extract of *A. heyneanus* carried out using Ethyl Acetate: Methanol: Ammonia solution (8:1;1) as mobile phase, showing three orange colored spots of alkaloids when sprayed with Dragendorf's reagent

Prior to TLC the developing chamber was saturated with the appropriate mobile phase. 20 x 20 cm TLC sheets (Silica60F254,MERCK) were used. Bands of Methanol extract was loaded onto TLC with the help of micropipette and then the TLC plate was dried with an air drier and placed in the saturated chamber. It took approximately 45

minutes for the run to get completed. The TLC plate was removed and air dried again. The separation of the alkaloids was satisfactorily achieved when Ethyl acetate: Methanol :17% Ammonia (8:1:1) was used as mobile phase; showing three orange colored spots that suggested presence of three alkaloids in the stem extract.

Elution of alkaloids was done from these TLC plates only, by scraping out the alkaloid bands from TLC. Scraping of each band was taken separately in centrifuge tubes to which water was added and centrifuged for 10 minutes at 5000 rpm to remove silica. The supernatant was collected.

HPTLC (High Performance Thin Layer Chromatography): was performed at ANCHROM HPTLC Laboratories, Mulund, Mumbai; because it functions as an open system on a planar column (plate) and sample loading is automated, therefore, a uniform band gets applied, which reduces the discrepancies that are encountered in manual loading in TLC. To run HPTLC, 20 x 10 cm Silica Gel 60 F_{254} HPTLC plates (MERCK) were used. Uniform bands of both organic and aqueous stem extracts were loaded onto the plates with the help of a Linomat 5 Applicator. Bands were applied maintaining a distance of 10 mm from each other and a distance of 15 mm from the edge of the plate. After sample application, the plates were dried with an air drier and then were carefully placed in the twin trough chamber. Four combinations of mobile phases were used. (Wagner *et al*)

1. Ethyl acetate: Diethylamine: Toluene (2:1:7)
2. Ethyl acetate: Methanol: Water (10:1.35:1)
3. Ethyl acetate: Methanol: Ammonia [17%] (8: 1.75:0.75)
4. Toluene : Chloroform: Ethanol (4:4:1)

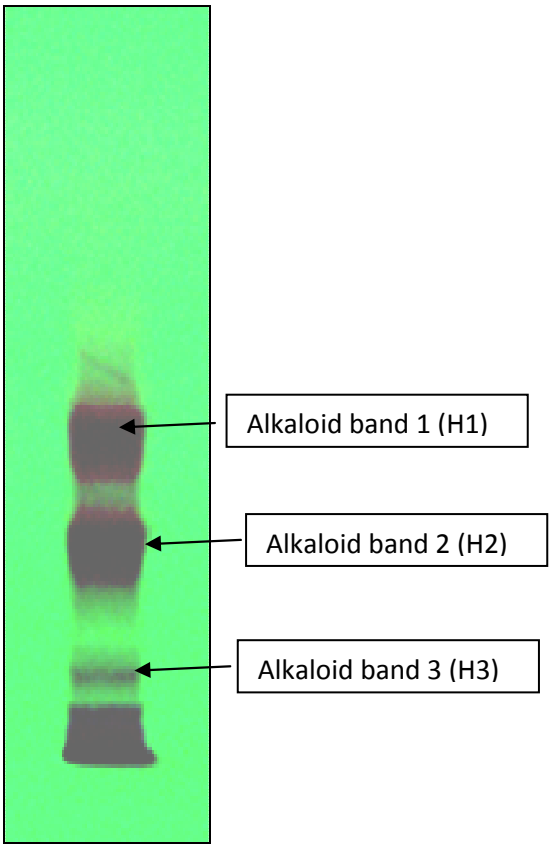


Figure - 2: HP**TLC of leaf extract of *A. heyneanus* carried out using Methanol: Ethyl Acetate: Ammonia solution (1:8:1) as mobile phase, showing three orange colored spots of alkaloids when sprayed with Dragendorf's reagent**

However, for aqueous extract mobile phase 1 was not used. Prior to placing plate in, the twin trough chambers were saturated with the mobile phase by placing a filter paper soaked in the solvent system. After the solvent system covered a stipulated distance, the plates were removed and air dried. These developed plates were then visualized under the UV chamber (Figure ~~4.6~~) at 254 and 366 nm. The plates were then derivatized with Dragendorrf's reagent and again visualized at 254 and 366 nm

For Elution of Alkaloids Separated by HPTLC the bands were cut into strips and then into very small pieces and transferred to test tubes containing methanol. The tubes were sonicated for 45 minutes. The strips were then removed from methanol methanol was then dried at 70^0 C using a water- bath.

Analysis of Alkaloids: by COLUMN CHROMATOGRAPHY was also tried. For this 75g stem power was taken in a filter paper pouch and placed in the cylindrical part of Soxhlet apparatus. 500 ml dichloromethane was added in the round bottom flask of Soxhlet apparatus by pouring it through the cylindrical part. The flask was heated to boiling of dichloromethane to complete the one cycle of Soxhlet. Then dichloromethane was removed from the Soxhlet apparatus. The same stem powder was then extracted in the mixture of dichloromethane and methanol (1:1). The stem extract in the mixture of dichloromethane and methanol (1:1) was extracted with hexane followed by 90% methanol. This 90% methanol fraction contains the alkaloid fraction, and was used for column chromatography through a column of [Silica gel used 60-120 mesh (Merck)] using Ammonia: Methanol: Ethyl acetate (1:1:8) as mobile phase.

The eluted material was again subjected to TLC. That showed three spots of alkaloids, which were scratched out and collected separately in the mixture of dichloromethane and methanol. These elutes were tested for anti-malarial test, anti-tumor test and anti-microbial activities (GIVE REFERENCE HERE) .

Analysis of alkaloids: was done by LC/MS (Liquid Chromatography/ Mass Spectroscopy) and NMR (Nuclear Magnetic Resonance)

As mentioned above three alkaloids (Alkaloid-1, Alkaloid-2, and Alkaloid-3) that were separated by TLC and later eluted in methanol were finally concentrated to dryness and used for LC-MS analysis. [Alkaloid-1 was not used because of the pigment interference during elution made it a complex mixture] 10 ml of dried material of both the alkaloids was separately dissolved in 5 ml mixture of water and methanol (20:80) by sonicating them at room temperature and used for LC-MS analysis to find out their molecular weight and elemental composition off the compound.

Liquid Chromatography (LC) and Mass Spectrometry (MS) of the sample – The LC-MS analysis was performed using Shimadzu LC 2010 with mass detector API-2000. HPLC grade water (Merck) and HPLC grade acetonitrile (Merck) were mixed in the ratio of (1:1) and degassed by vacuum degasser. This degassed mixture of water and Acetonitrile was used as the mobile phase. Quaternary gradient pump was used to carry the mobile phase from the mobile phase reservoir to the detector via injector port and HPLC column flow rate of mobile phase was set as 1.0 ml/minute.

HPLC column used was Zorbax SBC18, where SB stands for stable bond and C18, stands for Octadesyl carbon. The dimension of HPLC column was 250mm×4.6mm, 5µm i.e., the length of the column 250mm, internal diameter of the column was 4.6mm and the particle size of the Silica material was 5µm. The temperature of HPLC column was 25°c. Injection volume of the dissolved sample was set to 100µl to inject the Alkaloid sample from sample vial.

Prior to use HPLC column was washed with a mixture of water and acetonitrile (80:20) for 30 minutes followed by another wash using a mixture of water and acetonitrile (30:70) for another 30 minutes. HPLC column was saturated with mobile phase (50:50) water and acetonitrile) for one hour. After that the stable base line alkaloid samples were injected in the chromatograph. Mass spectra obtained from Mass detector API-2000 was analyzed

NMR Analysis of the Samples - BRUKER NMR model No.400MHz was used for the analysis of NIQ alkaloids extracted from stem of *A. heyneanus* and separated by TLC. As mentioned above elutes of Alkaloid-2, and Alkaloid-3 were taken for NMR analysis. Both the elutes were dried at 50^0C in an oven to remove the solvents.

NMR tube was dried and placed in a 10-mL graduated cylinder. To remove any insoluble impurity from the alkaloid sample, a small piece of a Kimwipe was stuffed into a small Pasteur pipette which was placed on top of the NMR tube. To stuff the Kimwipe properly it was pushed in using a larger pipette.

5 mg of dried alkaloid-2 sample was dissolved in 0.5mL pure dry deuterated chloroform. This solution of alkaloid-2 was carefully transformed into the NMR tube (using a pipette) through the Kimwipe filter. Then NMR tube was capped. Same procedure was followed for the analysis of alkaloid -3. After NMR analysis alkaloid sample was recovered and stored in freezer.

The sample was taken in a glass tube placed between the pole faces of a magnet. A radio-frequency source was made to fall on the sample. A signal is detected if the nuclei in the sample resonate with the source. Energy is

transferred from the source via nuclei to the detector coil. The output from the detector is fed to the recorder after amplification.

RESULTS AND DISCUSSION

***Mass Spectrum* –** The plot of mass to charge value i.e. m/e values taken along the abscissa and their relative densities along the ordinate is called the mass spectrum. Mass spectrum is plot representing the m/e values of the various (parent as well as fragment ions) against their corresponding relative abundances. The molecular ion is called parent ion and usually designated as M+ m/e value of parent ion is equal to the molecular mass of the compound. It is positively charged molecule with an unpaired electron. The largest peak in the mass spectrum is called as base peak and its intensity is taken as 100.

***Mass Spectrum of Crude Extract* -** The MS of crude extract (Figure – 3) shows six different peaks i.e., m/e values as 421,407,392,391, 376 and 360; denoting the molecular masses of the fragments of the molecular ions of different compounds present in crude extracts.

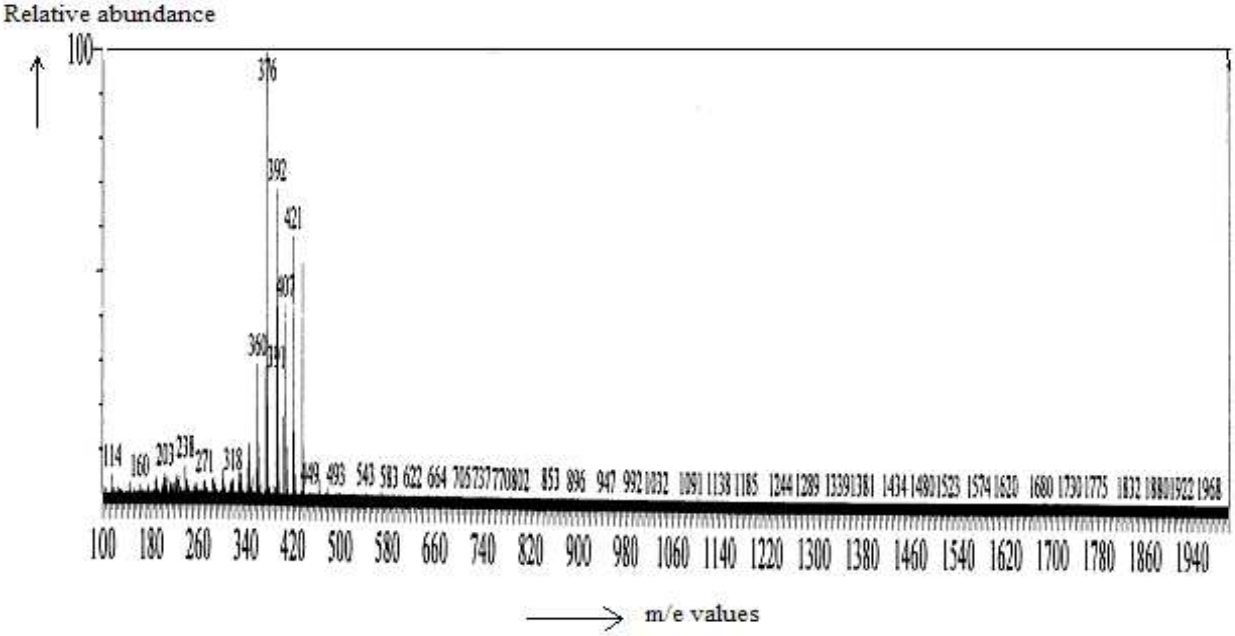


Figure – 3: Mass Spectrum of Crude extract of stem of *A. heyneanus*

***Mass Spectrum of Alkaloid separated as Band 2 by TLC* -** In the mass spectrum of alkaloid -2 from band-2 (Figure - 4) the m/e value of the molecular ion peak is 421. Hence, we can say that the molecular mass of alkaloid from band-2 is 421. Also there are some peaks in the mass spectrum indicating that the molecule undergoes fragmentation. The m/e values of the peaks are 420,407and 406. The m/e values of the fragmented peaks may be explained as below:-

The peak of m/e value 421is formed by bombardment of electron and formation of (M^+) molecular ion .The abundance of this peak is 3.5%.

$$M\ (421) + e^- \longrightarrow M+\ (421) + 2e^-$$

The peak of m/e value 420 is formed by the elimination of hydrogen (H.) radical (M^+-H) from molecular ion m/e421 and the abundance of this peak is 3.1%.

$$M^+\ (421) - 1 \longrightarrow m/e\ (420)$$

The peak of m/e value 407 is formed by the elimination of –CH2 radical (M^+-CH2) from molecular ion m/e 421and the relative abundance of this peak is 39%.

$$M^+\ (421) - 14 \longrightarrow m/e\ (407)$$

The peak of m/e value 406 is formed by the elimination of –CH3 radical (M^+-CH3) from molecular ion m/e (421) and the abundance of this peak is 100%.

$$M^+\ (421) - 15 \longrightarrow m/e\ (406)$$

According to Bringmannn et al (1992) the mass to charge value (m/e) of the molecular peak of extracts taken from the Ancistrocladus species was 421(M^+) and the abundance of this peak was 2.3%;

$$M\ (421)\ +e^- \longrightarrow M^+(421)\ +2e^-$$

Moreover, these peaks of m/e value 420,407 and 406 obtained by them also matches with results obtained (Figure – 5.3) as explained above.

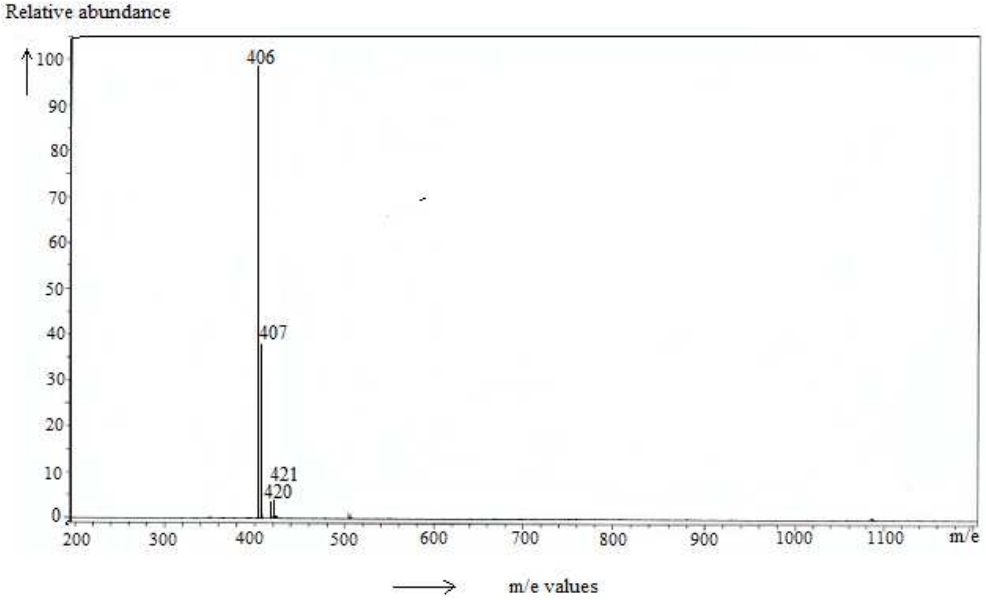


Figure – 4: Mass spectrum of Alkaloid from band -2 of TLC

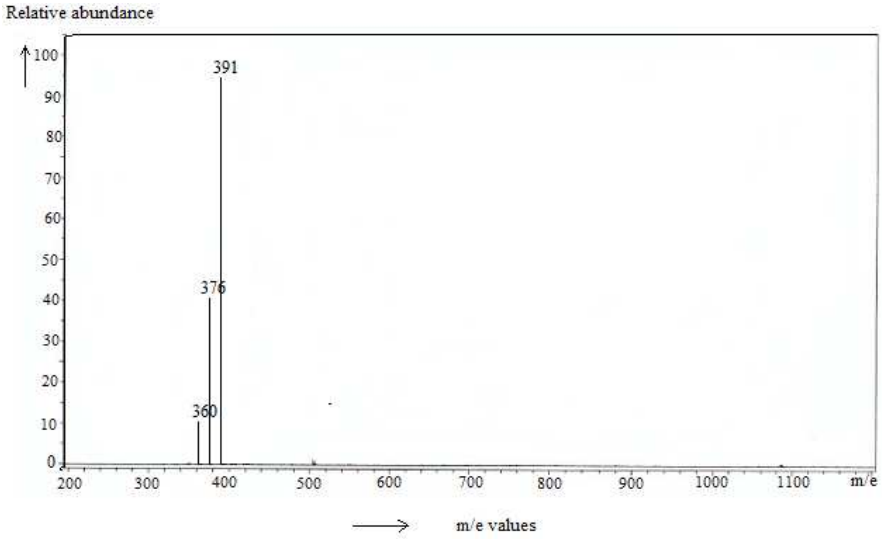


Figure - 5 : Mass spectrum of Alkaloid from band -3 of TLC

Mass Spectrum of Alkaloid separated as Band 3 by TLC - The mass spectrum alkaloid from band-3 (Figure - 5) the m/e value of the molecular ion peak is 391. Hence, we can say that the molecular mass of alkaloid from band-3 is 391.Also there are some other peaks in the mass spectrum are observed due to the fragmentation of molecules. The m/e value of these peaks are 376and 360. The m/e value of fragmented peaks is explained below:

The peak of m/e value 391 is formed by the bombardment of electron and formation of (M^+) molecular ion. The abundance of this peak is 100%.

$$M\ (391) + e- \longrightarrow M^+\ (391) + 2e-$$

The peak m/e value 376 is formed by the elimination of –CH3 radical (M^+-CH3) from molecular ion m/e 391and the abundance of this peak is 40%.

$$M^+\ (391) - 15 \longrightarrow m/e\ (376)$$

The peak of m/e value 360 is formed by the elimination of –OCH3 radical (M^+-OCH3) from molecular ion m/e 391 and the abundance of peak is10%

M^+ (391) -31 ⟶ m/e (360)

From the mass spectrum of alkaloid-2 and alkaloid-3 it is conclude that the molecular weight of alkaloid-2 from band-2 is 421and alkaloid-3 from band-3 is 391. These molecular masses matches with those of Ancistrocline isolated by Bringmann et al (1992) from *Ancistrocladus tectorius* and Yaoundamine A isolated by Hallock et al. (1997) from *Ancistrocladus korupensis.*

NMR Analysis of Alkaloids 2 and 3 Separated by TLC - A further effort to analyze and confirm the identity of these two alkaloids by NMR was under taken.NMR characterization of two alkaloids (Alkaloid-2, and Alkaloid-3) that showed possibility of having NIQ alkaloid was done to find out their structures.

Alkaloid -2: Prior to understanding the NMR peaks, it is imperative to understand the typical chemical shifts which occur in ^{1}H NMR and ^{13}C NMR spectra. Hence, in table 1 and 2, Typical Chemical Shift in ^{1}H NMR ^{13}C NMR spectra is respectively presented.

Table – 1: Typical Chemical Shifts in ^{1}H NMR spectra

S.N.	Type of protons ^{1}H		Chemical shift in ppm
1	Primary aliphatic	R- CH_3	0.9
2	Hydroxy	R-OH	1 – 5.5
3	Ester CH	H-C-COOR	2 – 2.2
4	Acid CH	H-C-COOH	2 – 2.5
5	Acetylinic	C≡C-H	3 – 3.5
6	Ether CH	H-C-OR	3.3 – 4
7	Alcohol CH	H-C-OH	3.4 – 4
8	Phenolic	Ar-OH	4 – 12
9	Vinylic	C=C-H	4.6 – 5.8
10	Aromatic CH	Ar-H	6 – 9.0
11	Aldehydic CH	RCHO	9 – 10
12	Carboxylic OH	RCOOH	10.5 – 12
13	Enolic	C=C-OH	15 – 17

Table –2: Typical Chemical Shifts in ^{13}C NMR spectra

S.N.	Carbon environment	Chemical shift in ppm
1	C=O (in ketones)	205 – 220
2	C=O (in aldehyde)	190 – 200
3	C=O (in acids and esters)	170 – 185
4	C in aromatic rings	125 – 150
5	C=C (in alkenes)	115 – 140
6	RCH2OH	50 – 65
7	RCH2Cl	40 – 45
8	RCH2NH2	37 – 45
9	R3CH	25 – 35
10	CH3CO-	20 – 30
11	R2CH2	16 – 25
12	RCH3	10 – 15

From the **^{1}H NMR** (Fig. 6) it is observed that there are five aryl protons, methoxy groups (δ4.60), an arymethyl group (δ 2.29) and doublet for H-4 proton. This arrangement of groups is similar to those observed for monomeric naphthyl-isoquinolines

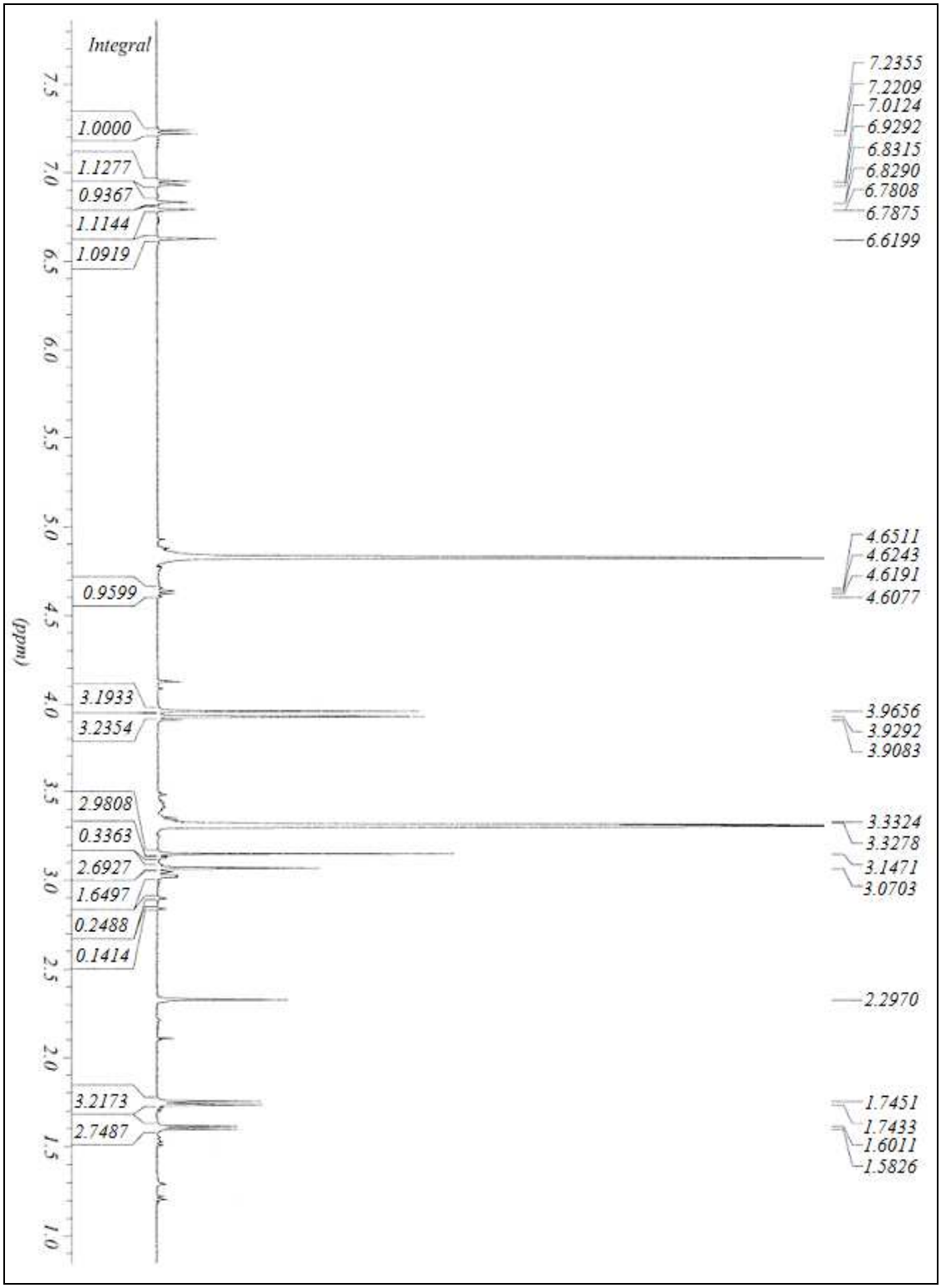


Figure - 6: ^{1}H NMR spectrum of Alkaloid from Band 2

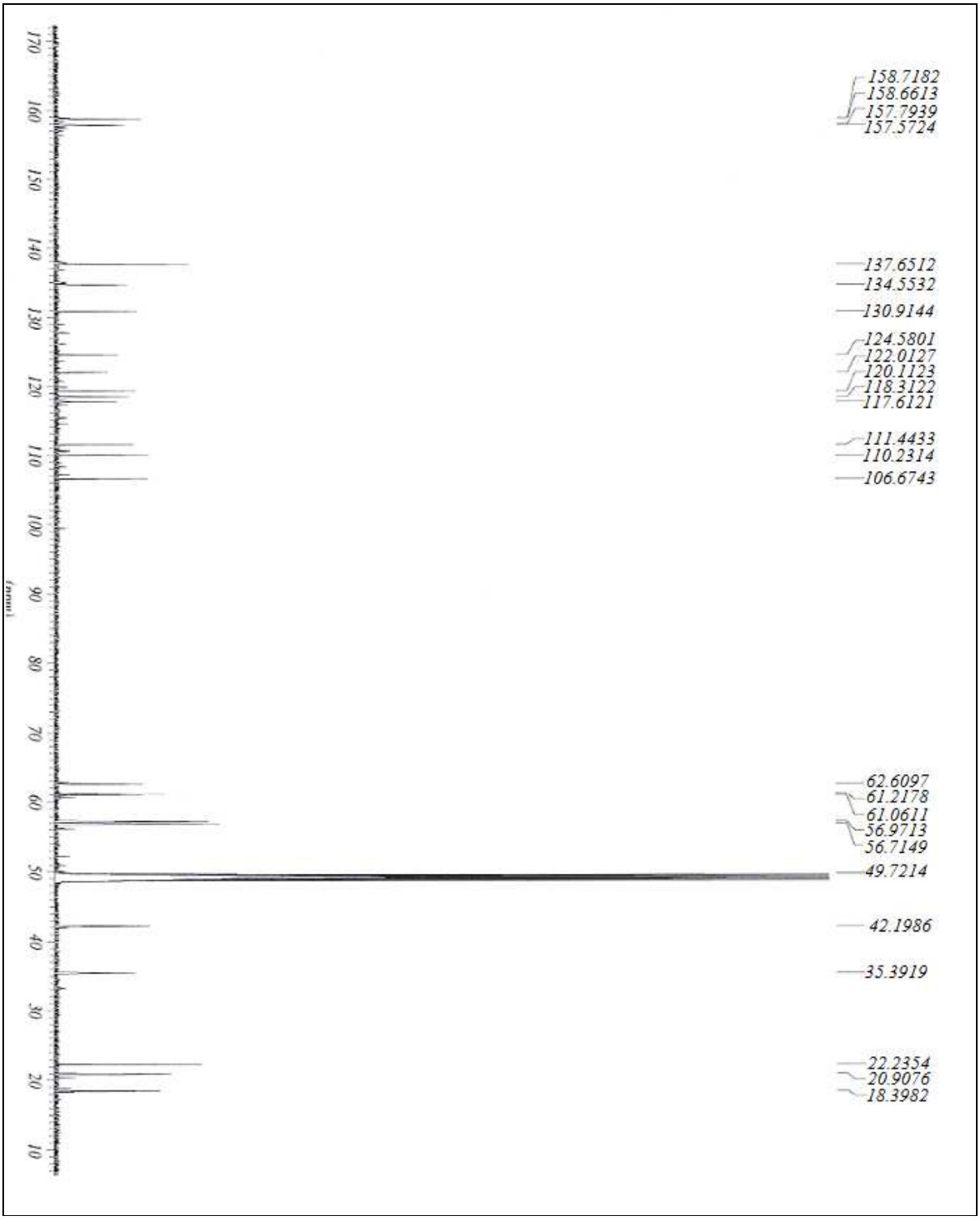


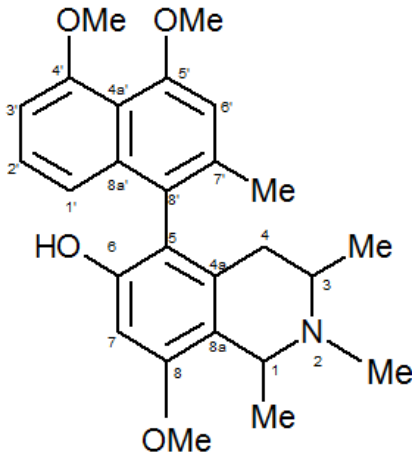
Figure - 7: ^{13}C NMR spectrum of alkaloid from Band 2

The ^{13}C NMR spectrum (Figure -7) indicates presence of 3 methyl groups distinguished individually, later shown peak intensity for two methyl groups at δ20.9 and δ61.2 shows one methylene and five methine resonance quaternary carbons. The correlation from δ3.32 (H-4ax) and δ3.14 (H-4eq) to δ120.1 (C-5) and δ157.7 (C-8a) and δ122.0(C-7') to δ106.6(C-7) establishes that C-8' of naphthalene ring is attached to C-5 in the isoquinoline ring. Quaternary carbons at δ157.7 in place of sp3 methine carbon also support the analysis.

Table - 3: Compiled data of ^{1}H and ^{13}C observations obtained for alkaloid-2

Position	^{1}H		^{13}C	
1,3	1.74(m)	Alkyl proton	49.7	Methylidine carbon
4ax	3.32(dd)	Alkyl proton	35.3	Methylene carbon
4eq	3.14(dd)	Alkyl proton		Methylene carbon
4a	--		130.9	Methine carbon
5	--		120.1	Methine carbon
6	--		111.4	Methine carbon
7	6.61(s)	Aryl proton	106.6	Methylidine carbon
8	--		157.5	Methine carbon
8a	--		157.7	Methine carbon
1'	6.82(d)	Aryl proton	118.3	Methylidine carbon
2'	6.92(q)	Aryl proton	117.6	Methylidine carbon
3'	7.22(d)	Aryl proton	110.2	Methylidine carbon
4'	--		158.7	Methine carbon
4a'	--		134.5	Methine carbon
5'	--		158.6	Methine carbon
6'	6.78(s)	Aryl proton	106.6	Methylidine carbon
7'	--		122.0	Methine carbon
8'	--		124.5	Methine carbon
8a'	--		137.6	Methine carbon
C1-CH3, C3-CH3	1.58(dd)	Alkyl proton	20.9	Methyl carbon
C2-NCH3	3.07(s)	Alkyl amine	42.1	Methyl amine carbon
C8-OCH3	3.96(s)	Alkyl oxy	61.0	Methoxy carbon
C4'-OCH3, C4'-OCH3	4.60(s)	Alkyl oxy	61.2	Methoxy carbon
C7'-CH3	2.29(s)	Alkyl proton	22.2	Methyl carbon

Structure of the compound on the basis of data presented in table 3, would be as follows:



Ancistrocline

NMR ANALYSIS OF ALKALOID -3

NMR analysis of alkaloid – 3, is presented in figure 8 and 9 and in table .4 and 5.

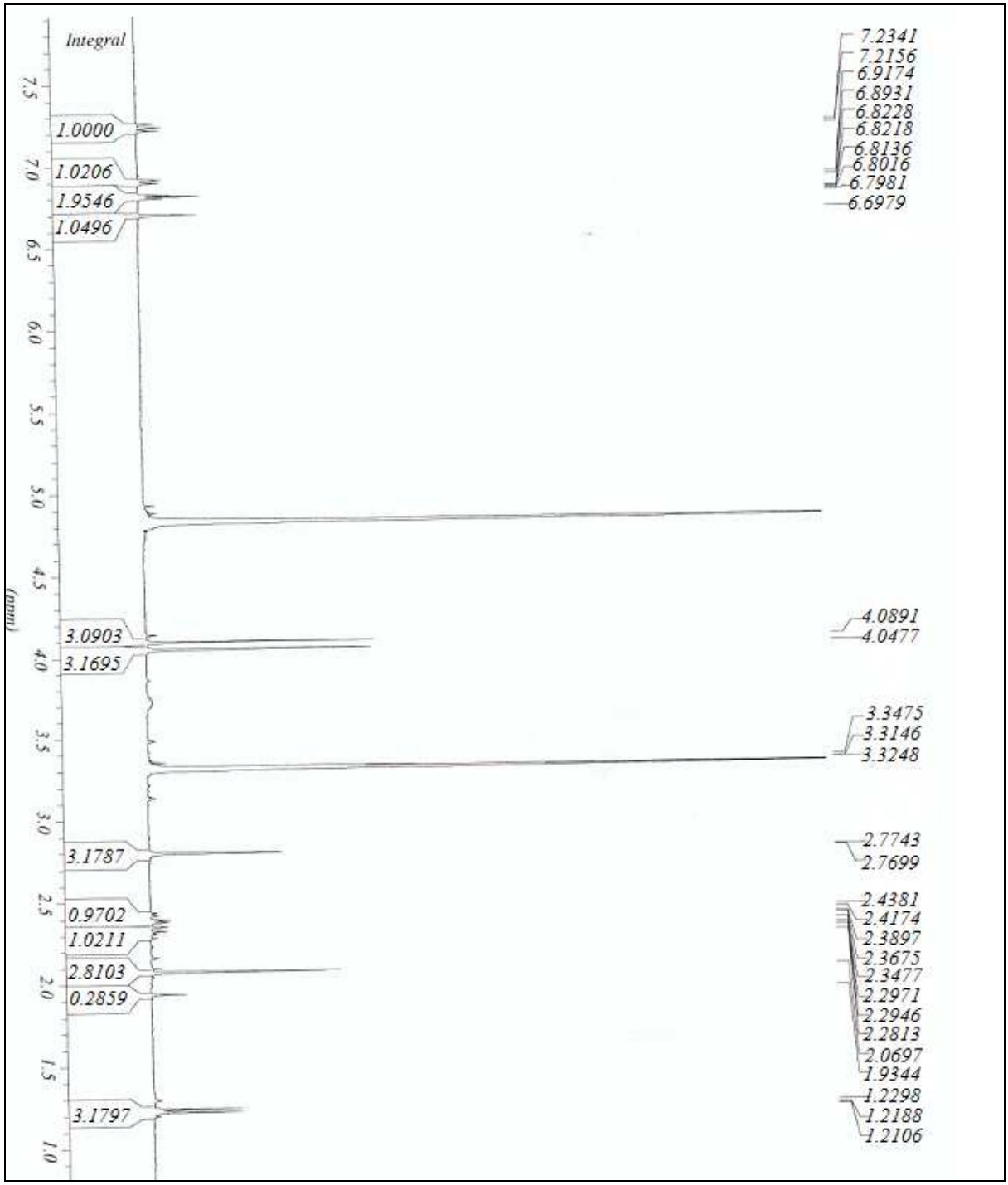


Figure – 8: ^{1}H NMR spectrum of Alkaloid from Band -3

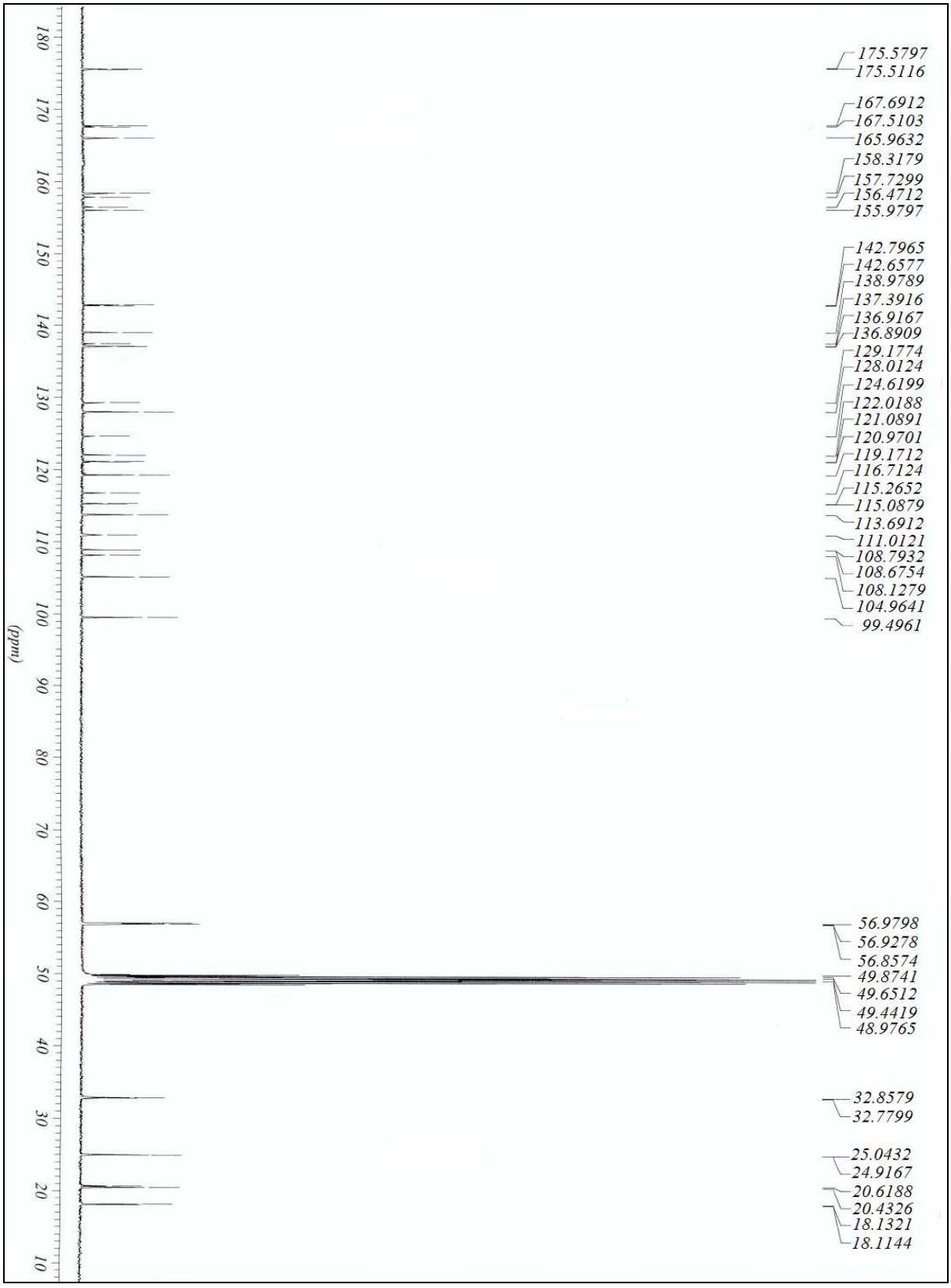
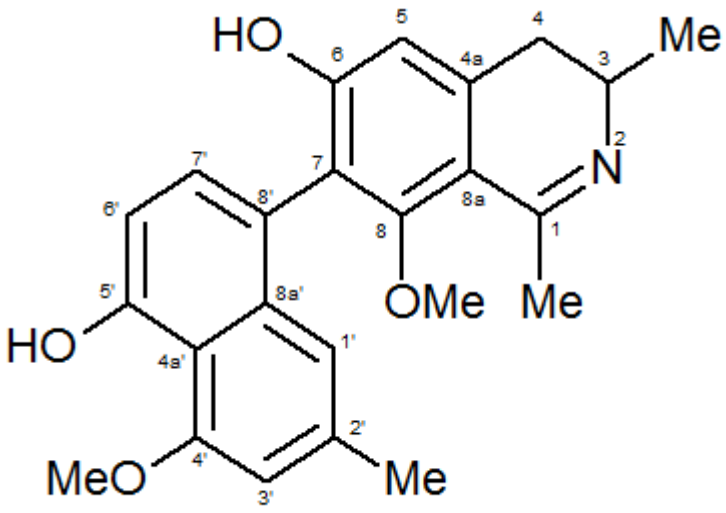


Figure - 9 : ^{13}C NMR spectrum of alkaloid from Band -3

Table - 4: Compiled data of ^{1}H and ^{13}C observations obtained for alkaloid-3

Position	^{1}H		^{13}C	
1	--		175.5	Methine carbon
3	4.08(m)	Alkyl proton	49.6	Methylidine carbon
4ax	2.43(dd)	Alkyl proton	32.8	Methylene carbon
4eq	2.76(dd)	Alkyl proton		Methylene carbon
4a	--		142.7	Methine carbon
5	6.69(s)	Aryl proton	115.0	Methylidine carbon
6	--		155.9	Methine carbon
7	--		124.6	Methine carbon
8	--		165.9	Methine carbon
8a	--		108.7	Methine carbon
1'	6.91(d)	Aryl proton	122.0	Methylidine carbon
2'	--		137.3	Methine carbon
3'	6.80(d)	Aryl proton	104.9	Methylidine carbon
4'	--		157.7	Methine carbon
4a'	--		113.6	Methine carbon
5'	--		155.9	Methine carbon
6'	6.81(d)	Aryl proton	111.0	Methylidine carbon
7'	7.22(d)	Aryl proton	129.1	Methylidine carbon
8'	--		122.0	Methine carbon
8a'	--		138.9	Methine carbon
C1-CH3	2.41(d)	Alkyl proton	32.8	Methyl carbon
C3-CH3	2.00(d)	Alkyl proton	18.1	Methyl carbon
C8-OCH3	3.32(s)	Alkyl oxy	56.9	Methoxy carbon
C2'-CH3	2.07(s)	Alkyl proton	25.0	Methyl carbon
C4'-OCH3	4.04(s)	Alkyl oxy	56.9	Methoxy carbon

From the above ^{1}H NMR (Figure - 8) it is observed that there are five aryl protons, methoxy groups (δ4.04), an arymethyl group (δ2.07) and doublet for H-4 proton. These features match to the feature of monomeric naphthyl-isoquinolines. Only one methyl resonance was observed and two extra methyl singlets were present at δ2.07 and δ3.32. Another signal which appeared at δ2.41 and integrated for three protons slowly exchange with duterated solvent. This phenomenon suggests that the compound contains an imine linkage at C1/ C2 and therefore a 3, 4-dihydroisoquinoline ring system. A proton resonance at δ4.08 which corresponded to methane carbon at δ49.6 (C-3) showed correlation to carbon signals at δ175.5, 32.8 (C-4) and 142.7 (C-4a) thus linkage the signal at δ175.5 with C1.The correlation from δ2.43(H-4ax) and 2.76(H-4eq) to δ115.0 (C-5) and 108.7 (C-8a) and from δ6.69(H-5) and 7.33(H-7') to 124.6(C-7) established that C-8' of naphthalene ring is attached to C-7 in the dihydro-isoquinoline ring. The methoxy proton at δ4.04 (C-4'-OMe) correlated with a carbon resonance at δ157.7 (C-4') while the methyl singlet at δ3.32 (C-8-OMe) in the ^{1}H NMR spectrum has a correlation to at δ165.9 (C-8). The ^{13}C NMR spectrum indicates the presence of three methyl groups, one methylene and six methine resonance a quaternary carbon. Quaternary carbons at δ175.5 in place of sp3 methine carbon also support the analysis.
Structure of the compound on the basis of above data:



Yaoundamine A

Table – 5: ^{1}H and ^{13}C NMR assignments for Yaoundamine A (*as per Hallock et al, Tetrahedron, vol. 53, No. 24, PP.8121-8128, 1997*)

Position	^{1}H	^{13}C
1	--	171.6
3	3.72(m)	49.7
4ax	2.69(dd, 13.2, 16.1)	36.1
4eq	2.90(dd,4.4, 16.1)	
4a	--	141.8
5	6.34(s)	115.6
6	--	155.8
7	--	123.8
8	--	165.3
8a	--	108.9
1'	6.96(d)	120.1
2'	--	137.1
3'	6.76(d)	107.3
4'	--	157.7
4a'	--	114.8
5'	--	155.8
6'	6.77(d, 7.7)	110.2
7'	7.13(d, 7.7)	131.7
8'	--	123.4
8a'	--	137.3
C1-CH3	2.53(d, 1.0)	30.7
C3-CH3	1.41(d, 6.3)	18.8
C8-OCH3	3.10(s)	60.9
C2'-CH3	2.34(s)	22.3
C4'-OCH3	4.07(s)	56.8

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