CHAPTER 3

RESULTS AND DISCUSSION

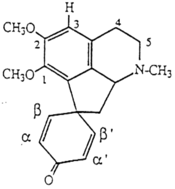
3.0 RESULTS AND DISCUSSION

In the following paragraphs, the structural elucidation of thirteen compounds isolated from the leaves and the bark of *Desmos dumosus* shall be discussed. The structures were established using various spectroscopic methods namely ^{1}H NMR, ^{13}C NMR, IR, MS and UV. In certain instances 2D NMR were also employed.

3.1 COMPOUNDS ISOLATED FROM THE LEAVES

Eleven compounds were isolated from the leaves; nine known alkaloids A, B, C, D, E, F, G, H, J, K and L, and two flavones, M and N.

Alkaloid A: Pronuciferine 37



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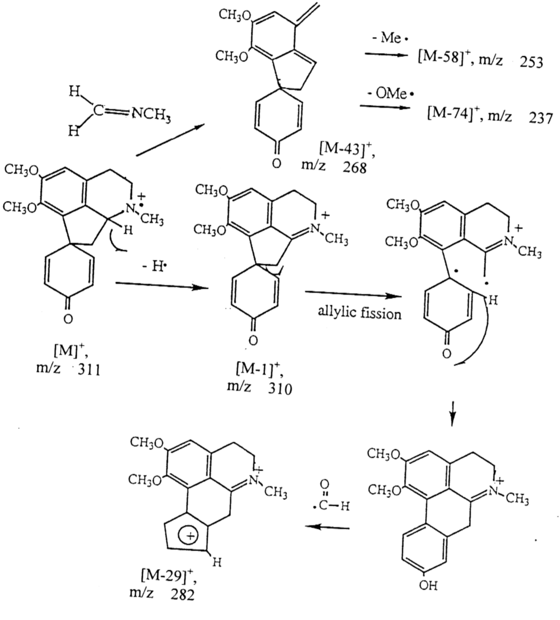
Alkaloid A was isolated as white crystals, m.p. 127-129°C. The IR spectrum exhibited absorptions of a conjugated carbonyl (1662 cm^{-1}) while the UV spectrum showed absorptions at 230nm (log ε 4.46) and 280nm (3.47), which were characteristic of a proaporphine skeleton[26].

The ^{1}H NMR spectrum of alkaloid A showed two singlets of methoxyl protons at δ 3.60 and δ 3.81. A singlet at δ 6.64 may be assigned to H-3, which suggested that C-1 and C-2 were substituted with the methoxyl groups. Another singlet appeared at δ 2.04, which is attributable to a N-CH_3 group. Furthermore, two sets of an AB doublet doublets were observed at δ 6.87 and δ 7.01 which were associated with the protons β and β' respectively, which came in resonance at low field because they were β to a carbonyl group. This suggests the existence of the vinyl protons of ring D in a proaporphine system. The proton β' was seen to be coupled with proton α' while proton β was coupled with proton α (d,J=10Hz) in the COSY spectrum (figure 3). The corresponding α and α' protons, came into resonance at δ 6.42 and δ 6.28, which were further upfield.

The mass spectrum (EI) showed a molecular ion peak at m/z 311 which corresponded to a molecular formula of $C_{19}H_{21}O_3N$. Other mass fragmentation peaks observed were m/z 310 $[M-1]^+$, 282 $[M-29]^+$, 268 $[M-43]^+$, 253 $[M-58]^+$, 237 $[M-74]^+$, 225 $[M-86]^+$. These findings (IR, ^1H NMR, COSY and MS) confirmed that alkaloid A is pronuciferine **37**.



Scheme 12: Mass fragmentations of pronuciferine 37

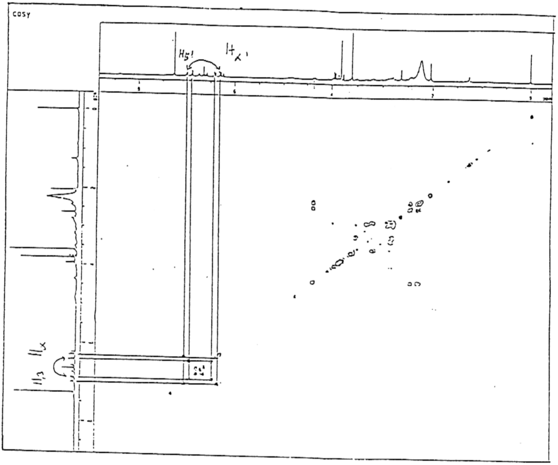
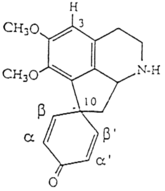


Figure 3: COSY spectrum of pronuciferine **37**

Alkaloid B: stepharine **39**



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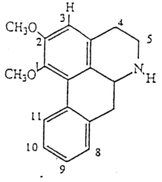
Alkaloid B was isolated as a white amorphous solid. Its mass spectrum revealed a molecular peak at m/z 297 (EI) which gave possibly to a molecular formula of $C_{18}H_{19}O_3N$. The IR spectrum showed a conjugated carbonyl absorption at 1665 cm^{-1} while the UV spectrum showed absorptions at 230 nm and 280 nm which were characteristic of a proaporphinic skeleton[26].

The ^1H NMR spectrum showed signals of four vinyl protons which appeared at δ 6.57 and δ 6.58 for α and α' respectively and δ 6.73 and δ 6.75 for β and β' respectively. A singlet at δ 6.21 was assigned for H-3, which indicated the presence of substituents at C-1 and C-2. The C-1 and C-2 were substituted with two methoxyl groups, which resonated as two singlets at δ 3.98 and δ 3.99 respectively. These findings (IR, UV, MS and ^1H NMR) suggested that alkaloid B is a proaporphine.

Comparison of the spectral data with the literature[14, 52] values confirmed that alkaloid B is stepharine **39**.

Alkaloid C: nornuciferine **40**



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Alkaloid C was isolated as white needles ($CHCl_3$), m.p. 245-246°C. It showed IR bands at 3688 cm^{-1} and 1596 cm^{-1}. The UV spectrum showed two maximum absorptions at 216 nm (log ε 4.69) and 272 nm (3.70) indicative of a 1,2-dioxygenated aporphine[12].

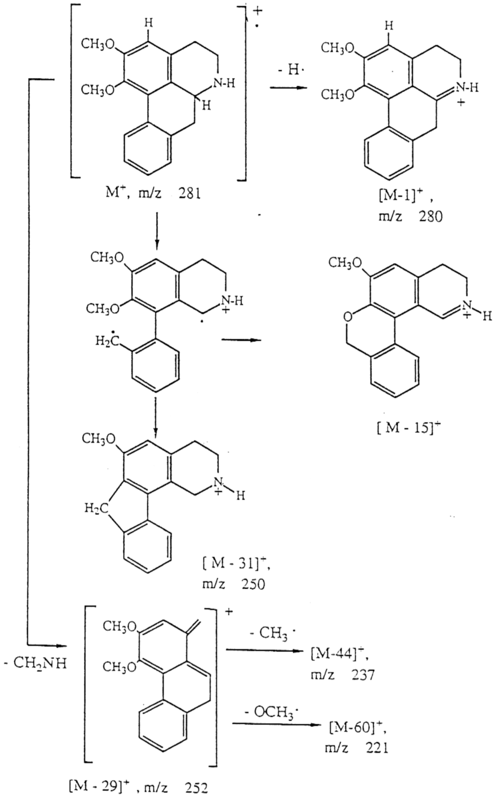
The molecular ion peak at m/z 281 (EI) established the molecular formula as $C_{18}H_{19}O_2N$.

The presence of a base peak at m/z 280 suggested that alkaloid C is an aporphine[10].

Findings from the ^1H NMR spectrum further supported the aporphinic nature of alkaloid C. A proton doublet was observed at δ 8.30 (J=8.90Hz), which may be attributed to H-11. It is very deshielded since it is experiencing the anisotropic effect from the facing ring A. Signals indicated that H-11 has ortho and meta proton neighbours (H-10 and H-9). In addition, this spectrum included a three proton multiplet near δ 7.20 (H-8, H-9, H-10), characteristic for an unsubstituted ring D.

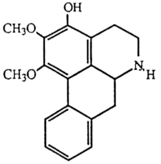
The presence of two methoxy singlets and a proton singlet, suggested that C-1, C-2 and C-3 were attached to the two methoxyl and a proton respectively. The peaks observed were at δ 3.60 (C-1-OMe), δ 3.67 (C-2-OMe) and δ 6.58 (H-3). The C-1 methyl protons were more up field than those of C-2 because of the neighbouring aromatic ring effect of ring D.

Comparison of the spectral data with the literature[21] values confirmed that alkaloid C is nornuciferine **40**. The suggested mass fragmentation pattern for nornuciferine **40** is depicted in scheme 13.



Scheme 13: Mass fragmentations pattern of nornuciferine **40**

Alkaloid D: (-)-3-hydroxynornuciferine **41**



41

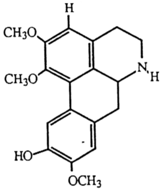
Alkaloid D was isolated as white needles ($CHCl_3$) with melting point of 163-164 °C. The molecular ion peak in EI mass spectrum appeared at m/z = 297, thus establishing a molecular formula of $C_{18}H_{19}O_3N$. The base peak, $[M-1]^+$ at m/z = 296 was indicative of an aporphine alkaloid[10].

The IR showed a broad band at 3521 cm^{-1}, proved the presence of a hydroxyl group. The UV spectrum showed maximum at 219 nm (log ε 4.39), 240 nm (3.90), 280 nm (4.14) and 292 nm (4.04), which are characteristic of a noraporphine alkaloid[12].

The ^1H NMR spectrum indicated that ring D is unsubstituted. It showed a three proton multiplet and a one proton doublet – doublet at δ 7.16-7.30 (H-8, H-9, H-10) and δ 8.28 (H-11) respectively. The presence of two methoxyl signals at δ 3.72 and δ 3.98 suggested that they are attached to C-1 and C-2 respectively. The absence of the C-3 proton signal suggested that it was substituted.

Comparison with the literature values[21], confirmed that alkaloid D is indeed (-)-3-hydroxynornuciferine **41** with a hydroxyl group attached to C-3.

Alkaloid E: norlirioferine **42**



42

Alkaloid E was isolated as white crystals ($CHCl_3$), m.p. 94-96°C and its mass spectrum showed a molecular ion peak at m/z 327 (EI) indicating a molecular formula of $C_{19}H_{21}O_4N$. The presence of a base peak $[M-1]^+$ at m/z 326 suggested that alkaloid E possessed an aporphine skeleton[10].

The UV spectrum showed maximum absorptions at λ_{max} 226 nm (log ε 4.30), 281 nm (3.83) and 310 nm (3.89), which were characteristic of a noraporphine[12]. In addition, the bathochromic shift in alkaline (NaOH) showed that the phenolic noraporphine was present[12]. This was further supported by the IR spectrum, which showed a sharp band at 3617 cm^{-1} indicative of a free hydroxyl group.

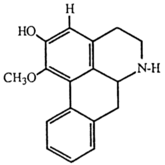
The ^1H NMR spectrum showed three methoxyl singlets. Methoxyl singlet of C-1 was the most shielded (δ 3.66) due to it being placed above the shielded area of the facing aromatic ring D. The other two methoxyl groups were less shielded and attributed to C-2 and C-9 (δ 3.88 and δ 3.89).

In addition, the ^1H NMR showed three singlets representing a proton each at δ 6.59, δ 6.80 and δ 8.08 which can be attributed to H-3, H-8 and H-11 respectively. Therefore, C-10 was proposed to be substituted by a hydroxyl group. The sharp singlet peak appeared at δ 2.04 was assigned to N-H.

In the ^{13}C NMR, are three methoxyl groups at δ 55.86, δ 56.06 and δ 60.19 corresponding to C-2, C-9 and C-1 respectively. It also shows three peaks of C-H aromatic at δ 110.85 (C-3), δ 111.33 (C-8) and δ 113.83 (C-11).

Thus it is suggested that compound E is norlirioferine **42** or 1,2,9-trimethoxy-10-hydroxynoraporphine. It is further confirmed by comparison with the literature spectral data [24].

Alkaloid F: asimilobine **43**



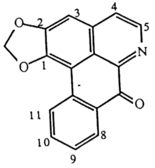
43

Alkaloid F was isolated as a brownish amorphous solid. The mass spectrum revealed a molecular ion peak at m/z 267 (EI) which gave a molecular formula of $C_{18}H_{19}O_3N$. The base peak at m/z 266 $[M-1]^+$ was indicative of an aporphine skeleton[10]. The mass fragmentations were m/z 266 $[M-1]^+$, 252 $[M-15]^+$, 250 $[M-17]^+$, 238 $[M-29]^+$, 236 $[M-31]^+$, 223 $[M-44]^+$, 207 $[M-60]^+$. The IR spectrum showed a band at 3521cm^{-1}, typical of a free hydroxyl group. The UV spectrum showed maximum at 229 nm (log ε 4.08) and 274 nm (4.08), which are characteristic of a noraporphine alkaloid[12].

The presence of one methoxyl group in its ^{1}H NMR spectrum was indicative of a 1,2-dioxygenated pattern with the hydroxyl and methoxyl (δ 3.58) at position 2 and 1 [26, 27] respectively. A proton singlet at δ 6.71 was assigned for the proton at C-3. The presence of the H-3 as a singlet peak indicated that the C-1 and C-2 were substituted.

The multiplet corresponding to three protons (H-8, 9, 10) near δ 7.16-7.30 in the ^{1}H NMR spectrum showed that ring D was unsubstiuted. The H-11 appeared as doublet of doublet at δ 8.29. After comparison with the literature spectral data, alkaloid F was in fact asimilobine **43** or 2-hydroxy-1-methoxynoraporphine [42, 25].

Alkaloid G: liriodenine **44**



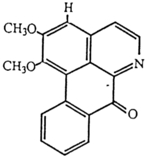
44

Alkaloid G was isolated as yellow amorphous. Its intense deep yellow color and its red coloration on treatment with acid suggested that it is an oxoaporphines. This was also supported by its UV and IR spectral data. The former showed absorption bands at 237nm (log ε 4.10), 270nm (3.80), 313nm (3.41) and 410nm (3.42), indicating a highly conjugated system. The IR spectrum showed an intense absorption at 1662cm^{-1}, indicative of a conjugated carbonyl group. In addition, a peak at 969 cm-1, characteristic of a methylenedioxy group, was also observed. The mass spectrum gave a molecular ion peak at m/z 275 (EI) thus complying with the molecular formula of $C_{17}H_9O_3N$.

The ^{1}H NMR spectrum of alkaloid G exhibited the characteristic AB pair of doublets significant of H-4 and H-5 (J~5.00Hz) at δ 7.82 and δ 8.92 respectively. The aromatic region also showed multiplets of two protons between δ 7.61 –7.79 (H-9, H-10) and two sets of doublet-doublet representing two protons at δ 8.62 (J=7.80Hz, J'=1.10Hz, H-8) and δ 8.72 (J=8.70Hz, J'=1.80Hz, H-11), indicating an unsubstituted ring D. The proton at C-3 appeared at δ 7.24 as a singlet. A singlet peak at δ 6.40 corresponded to two protons indicating the presence of methylenedioxy group in ring A located at C-1 and C-2.

By comparison of the spectroscopic data and the literature values[28, 29], it is confirmed that alkaloid G is liriodenine **44**.

Alkaloid H: lysicamine **45**



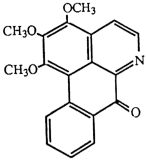
45

Alkaloid H was isolated as a yellow amorphous solid. The mass spectrum revealed an $[M+1]^+$ peak at m/z 292 (APCI) which corresponded to the molecular formula of $C_{18}H_{13}O_3N$.

In the UV spectrum, maxima were observed at 236nm (log ε 3.77), 267nm (3.69), 310nm (3.23) and 395nm (3.04) indicating the presence of a highly conjugated oxoaporphine system. The IR spectrum showed a conjugated ketone peak at 1665 cm^{-1}.

The ^1H NMR spectrum showed two distinct methoxyl peaks at δ 4.02 and δ 4.11 which were most probably positioned at C-1 and C-2. No methylenedioxy signals were observed. The proton at C-3 appeared at δ 7.24 as a singlet. The AB pair of doublets significant of H-4 and H-5 was exhibited at δ 7.80 and δ 8.80 respectively. Multiplets corresponding to two aromatic protons of ring D were revealed at δ 7.58-7.78 which were attributable to H-9 and H-10. A very downfield signal (dd, J=8.50Hz, J=1.10Hz) which appeared at δ 9.17 was assigned to H-11. In addition, a doublet-doublet was observed at δ 8.60 (J=7.80Hz, J=1.70Hz) which belonged to H-8 that experienced a deshielding effect from the neighboring C-7 carbonyl. Hence the molecule can be deducted to be lysicamine **45** and spectra were in full agreement with the literature values [30, 31, 32].

Alkaloid J: *O*-methylmoschatoline **46**



46

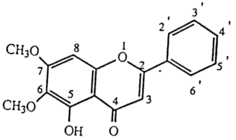
Alkaloid J was isolated as orange needles ($CHCl_3$) with m.p. 182-184°C. Its IR spectrum showed a strong absorption at 1662 cm^{-1} which indicated the presence of a conjugated carbonyl group. The UV spectrum with λ_{max} 274nm (log ε 4.34), 315nm (3.67) and 436 nm (3.50) indicated that it possessed a 7-oxodibenzo [d, e, g] quinoline skeleton[34]. The $[M+1]^+$ peak at m/z 322 (APCI) indicated a molecular formula of $C_{19}H_{15}NO_4$.

The ^{1}HNMR spectrum displayed three singlets at δ 4.08, δ 4.10 and δ 4.19, which is the characteristic of the methoxyl groups ($-OCH_3$). The AB pair of doublets aromatic protons were at δ 8.91 (1H, J=5.40Hz, H-5) and δ 8.16 (1H, J=5.40Hz, H-4). The multiplet peaks at δ 7.54-7.75 were for the two aromatic protons, which are allocated at position C-9 and C-10. The H-8 signal appeared at δ 8.52 (dd, J=7.80Hz, J'=1.80Hz) and H-11 at δ 9.10 (1H, dd, J=8.30Hz, J'=1.20Hz). The signal of H-11 was more downfield due to the presence of the methoxyl groups at ring A which deshielded the proton.

In the ^{13}C NMR spectrum, the methoxyl groups on C-1, C-2 and C-3 showed signals at δ 61.10, δ 61.57 and δ 61.91 respectively. C-4 resonated at δ 119.27 while C-5, being adjacent to nitrogen, was more deshielded and resonated at δ 144.66. A small peak corresponding to a conjugated carbonyl was observed at δ 182.75.

Comparison of the observed data with the literature [33, 34, 43] values confirmed that alkaloid J is *O*-methylmoschatoline **46**.

Flavone K: 5-hydroxy-6,7-dimethoxyflavone **47**



47

Flavone K was isolated as yellow crystals from chloroform, with m.p. 205-207°C. The mass spectrum revealed an $[M+1]^+$ peak at m/z 299 (APCI) corresponding to a molecular formula of $C_{17}H_{14}O_5$.

In its IR spectrum, a sharp band at 1665 cm^{-1} was observed indicative of a highly conjugated carbonyl group. The UV spectrum showed maximum at 221 nm (log ε 4.33), 274 nm (4.74), and 313 nm (4.27) implying a highly unsaturated chromophore.

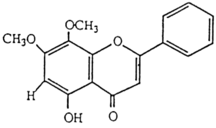
The ^1H NMR spectrum showed a very downfield shift at δ 12.61 reminiscent of a hydroxyl proton involved in intramolecular hydrogen bonding usually between a hydroxyl on C-5 and the C-4 carbonyl of a flavone.

The presence of a singlet at δ 6.50 showed that both C-6 and C-7 were substituted. The methoxyl protons resonated at δ 3.90 and δ 3.86 respectively.

Ring B protons resonated as two groups of multiplets. H-6' and H-2' signals appeared at ~ δ 7.78-δ 7.83 while H-3', H-4' and H-5' resonated at δ 7.48. The H-3 singlet appeared at δ 6.65.

Comparison with literature[48, 49] data it is confirmed that compound K is in fact the known 5-hydroxy-6, 7-dimethoxyflavone **47**.

Flavone L: 5-hydroxy-7, 8-dimethoxyflavone **48**



48

Flavone L was obtained as yellow crystals ($CHCl_3$) melting at m.p. 210-212°C. The mass spectrum revealed an $[M+1]^+$ peak at m/z 299 (APCI), which is consistent with a molecular formula of $C_{17}H_{14}O_5$.

Its UV spectrum showed maximum at 216 nm (log ε~4.18), 273nm (4.18) and 320nm (3.91), characteristic of a flavone[20]. The IR spectrum indicated the presence of a conjugated carbonyl group at 1665cm^{-1}.

The ^1H NMR spectrum gave a singlet at δ 6.42 which may be attributed to H-6. The signal is more shielded than that of H-3, which resonated at δ 6.60 (1H, s). This is because H-6 is ortho to a methoxyl and a hydroxyl, which are electron donating groups (figure 4). On the other hand, H-3 was ortho to the carbonyl group which is an electron withdrawing group thus resulting in a more downfield signal (figure 5).

Both the two methoxyl groups of C-7 and C-8 showed signals at δ 3.90. The protons in ring B appeared as two groups of multiplets. H-2' and H-6' showed a multiplet at δ 7.88 and H-3', H-4' and H-5' at δ 7.52.

In addition, the hydroxyl proton on C-5 resonated at very low field (δ 12.09) indicating the presence of hydrogen bonding with the C-4 carbonyl.

Thus, comparison with literature[48, 49] values, the structure of this flavone was found to be 5-hydroxy-7, 8-dimethoxyflavone **48**.

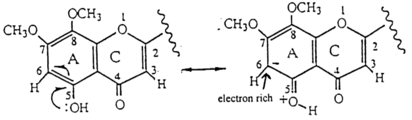


Figure 4: Electron donating effect of C-5 hydroxyl

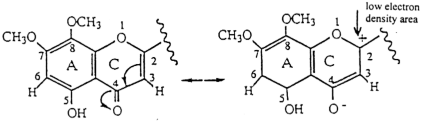
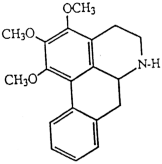


Figure 5: Electron withdrawing effect of carbonyl group on H-3

3.2 COMPOUNDS ISOLATED FROM THE BARK

Six compounds were isolated from the bark. They were four alkaloids and two flavones. However, four of the compounds were already discovered in the leaves; lysicamine **45**, *O*-methylmoschatoline **46**, 5-hydroxy-6,7-dimethoxyflavone **47** and 5-hydroxy-7,8-dimethoxyflavone **48**. Alkaloid M and N were found only in the bark.

Alkaloid M: *O*-methylisopiline **49**



49

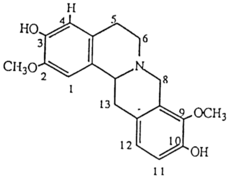
Alkaloid M exhibited an $[M+1]^+$ peak at m/z 312 (APCI) thus suggested a molecular formula of $C_{19}H_{21}O_3N$. The UV absorption at 213 nm (log ε ~ 3.97) and 272nm (3.76) were characteristic of a 1,2-disubstituted aporphine[26].

The ^1H NMR spectrum of this compound showed signals typical of an aporphine skeleton. It showed three singlets of methoxyl protons and δ 3.71, δ 3.92 and δ 3.89 which were attributed to C-1, C-2 and C-3 methoxyl respectively. The absence of H-3 singlet proved that the C-3 was substituted with a methoxyl group.

Aromatic protons signals of ring D appeared at δ 8.25 and δ 7.14-7.28. The signal of H-11 was observed as a doublet at δ 8.25 (J=7.80Hz) since it was ortho coupled to H-10 and it was the most downfield as compared to the other protons due to the anisotropic effect of the C-O single bond[9]. The other three protons signals appeared at δ ~ 7.14 -7.28 as multiplet.

Comparison with the literature [35, 38] spectral data confirmed that alkaloid M is the known *O*-methylisopiline **49**.

Alkaloid N: discretamine **50**



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Alkaloid N was isolated as white crystals from chloroform with m.p. 209-211°C. The UV spectrum showed two maxima at 207 nm (log ε 3.49) and 273 nm (3.44). A bathochromic shift was observed upon addition of alkali, thus suggesting that the other two constituents were phenolic OH groups. In addition, the IR spectrum showed a broad peak at 3246 cm^{-1}, indicating the presence of intramolecular hydrogen bonded hydroxyl group. The mass spectrum revealed an $[M+1]^+$ peak at m/z 328 (APCI) which corresponded to the molecular formula $C_{19}H_{21}NO_4$.

The ^1H NMR spectrum of alkaloid N showed that it was tetrasubstituted and that two of these substituents were methoxyl groups. Two singlets, which were attributable to methoxyl groups, appeared at δ 3.82 and δ 3.90 respectively. A half AB doublet pair (J=16.00Hz) at δ 4.21 was assigned to proton β at C-8. The large geminal coupling of the C-8 protons indicated that C-9 and C-10 were substituted[15].

Another signal corresponding to two protons of an AB system was assigned to H-11 and H-12. These peaks appeared at δ 6.82 (d, H-12) and δ 6.81 (H-11) with coupling constants of 8.79 Hz. The singlets at δ 6.68 and δ 6.71 could be attributed to H-1 and H-4 respectively.

Comparison of alkaloid N with the literature[36] confirmed that it is discretamine **50** or 3,10-dihydroxy-2, 9-dimethoxy tetrahydroberberine.