CHAPTER V

DISCUSSION

Structure Elucidation of the Isolated Alkaloids

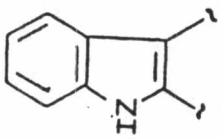
Six alkaloids have been isolated and identified in the stem bark of Strychnos ignatii Berg. They will be described according to their structure types as follows :-

1. Monomeric indole alkaloids

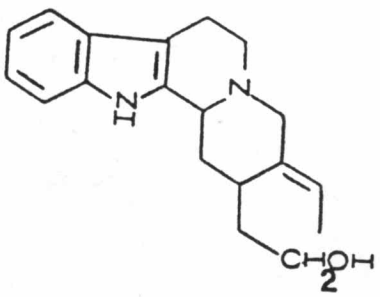
Two types of monomeric indole alkaloids are isolated from S.ignatii Berg. The classical strychnan type exemplifies by strychnine 54 and brucine 55, while the corynanthean type represents by geissoschizol 161 and polyneuridine 24.

On the structure elucidations, ultraviolet absorption spectra as well as mass spectra provide essential informations which lead to a clear consideration between the two isolated alkaloid types (106, 107)

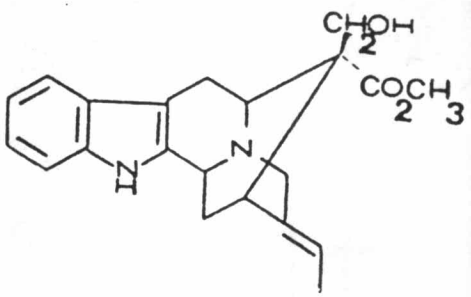
Indole and indoline chromophores (see page 141) are responsible for the characteristic UV absorption spectra of the corynanthean and strychnan alkaloids, respectively. Since, the indole chromophore shows UV absorption maxima at 226, 281 and 290 nm. (106,108) while the indoline chromophore shows the absorption bands at 214, 258 and 295 nm (106, 109) (see Figure 38 page 142).



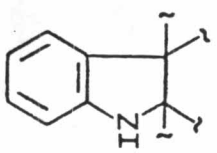
indole moiety



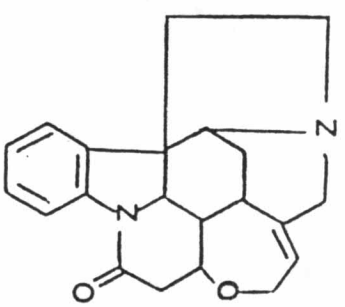
Geissoschizol 161



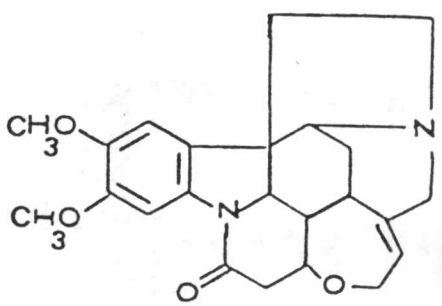
Polyneuridine 24



indoline moiety



Strychnine 55



Brucine 54

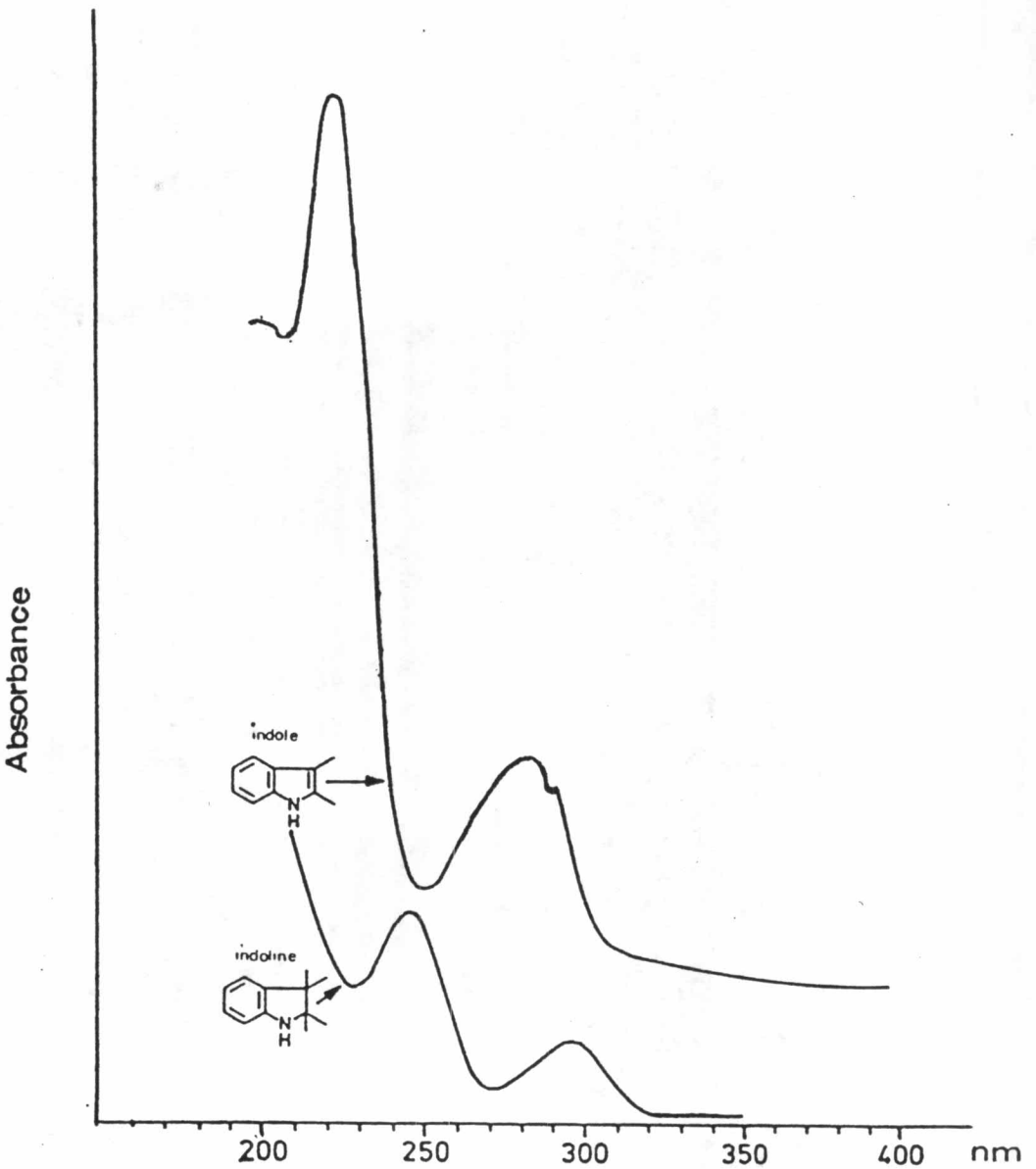


Figure 38 UV spectra of indole and indoline chromophores (not normalized for concentration) (106)

The mass spectral data of indole alkaloids are the important information in the identification of alkaloid types. As for a nonsubstituted indole alkaloids, the usual fragments can be recognized at m/z 130, 143 and 144 amu. (36) (see Figure 39 page 144). The corynanthean alkaloids would produce fragments at m/z 156, 169 and 170 amu. (Figure 40 page 145) (107), while the usual fragments for the strychnan alkaloid are at m/z 120 and 121 amu. (Figure 41 page 146) (107).

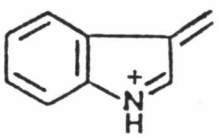
2. Bisindole alkaloid

Two bisindole alkaloids have been isolated from the stem bark of Strychnos ignatii Berg., however they could not be fully characterized due to the insufficient amount available. Their colour reactions with the ferric chloride-perchloric acid reagent appeared as a characteristic blue colour. Their mass spectral data display fragment ions belong to the longicaudatine type (B_5) of bisindole alkaloids (110).

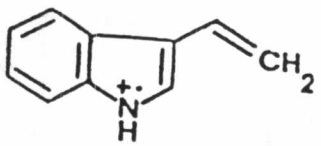
The structure elucidations of the individual alkaloids are following discussed.

Figure 39 Important mass fragment ions of indole alkaloids (36)

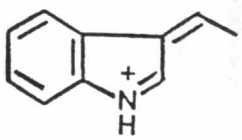
Substituent in Benzene ring	Indole peaks (m/z)		
	A	B	C
None	130	143	144
Dimethoxy	190	203	204



A

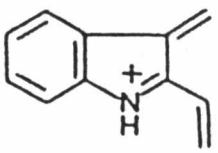


B

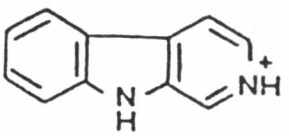


C

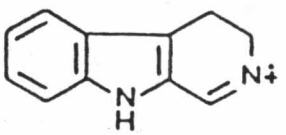
Figure 40 The characteristic feature of some important ions in the mass fragmentations of corynanthean type compounds (107).



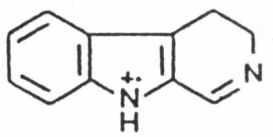
m/z 156



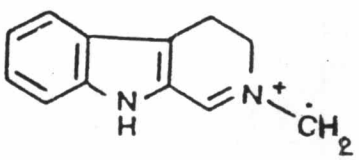
m/z 169



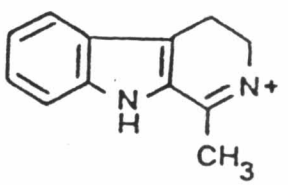
m/z 170



m/z 170

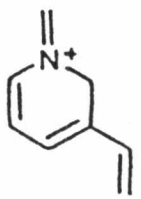


m/z 184

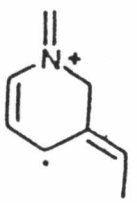


m/z 184

Figure 41 The characteristic feature of some ions in the mass fragmentation of strychnan type compounds (107)



m/z 120

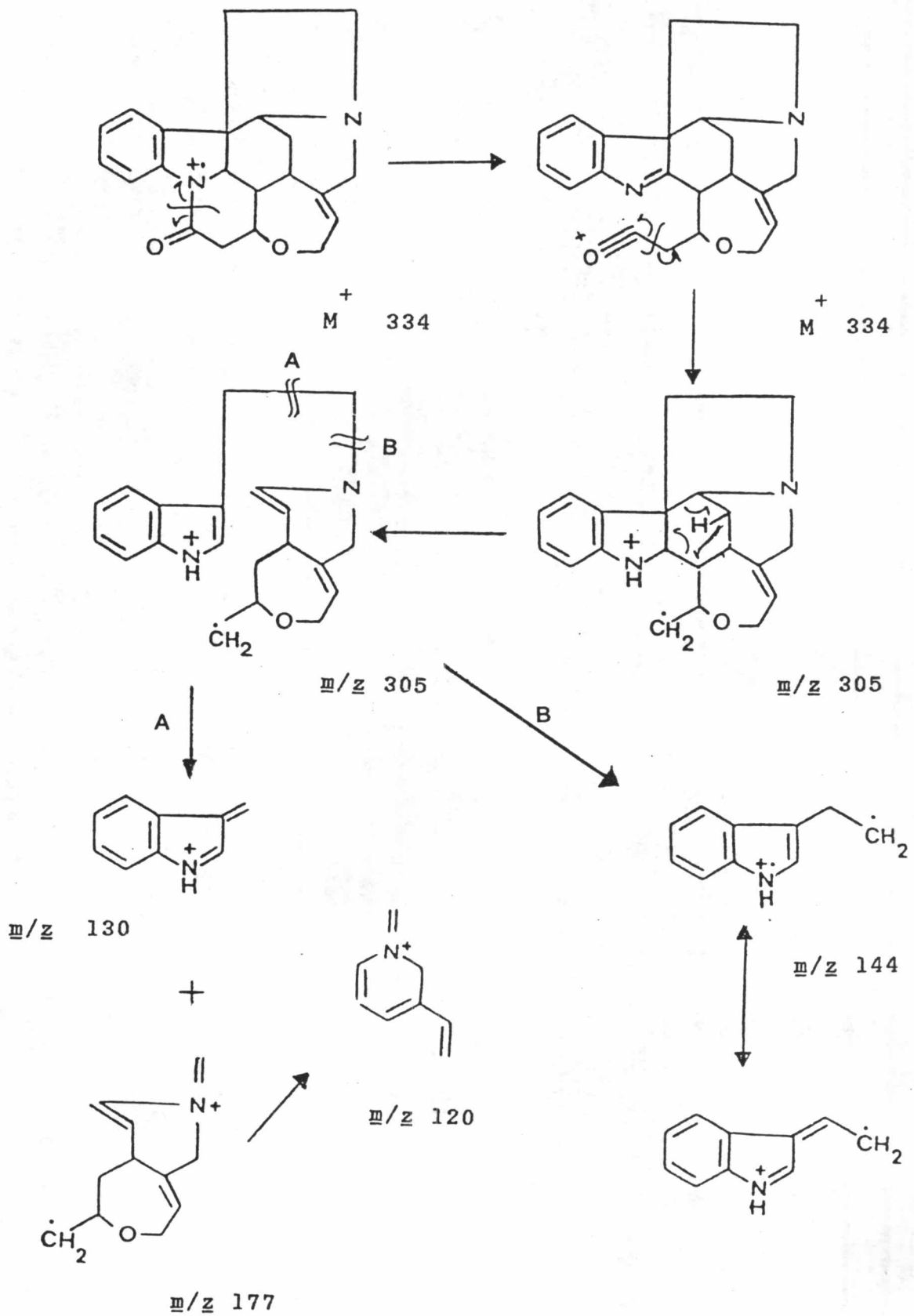


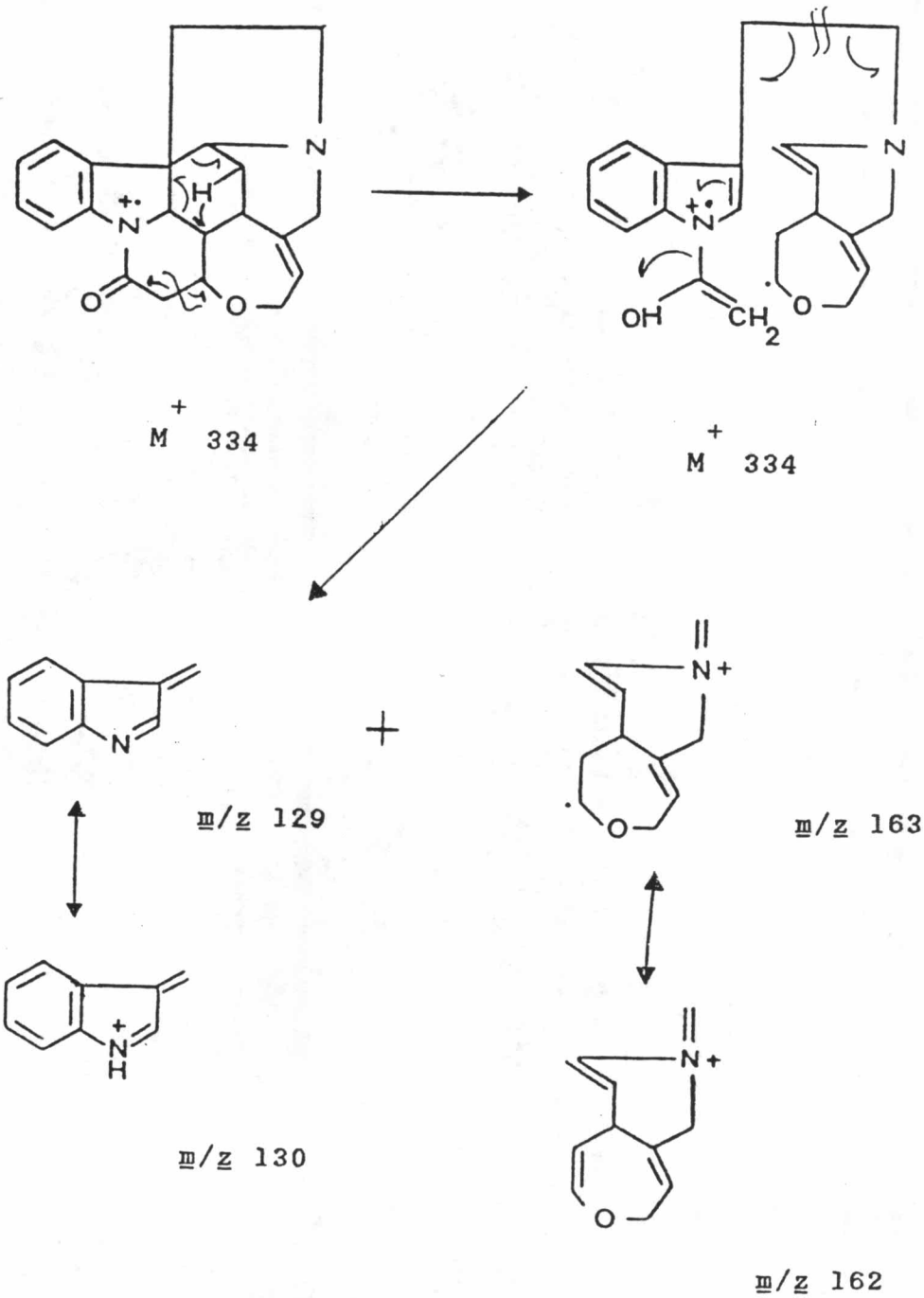
m/z 121

S1 Strychnine 54

The alkaloid S1 gives pink colour upon spraying with the ferric chloride-perchloric acid reagent. The UV absorption maxima at 210, 253, 277 and 280 nm (Figure 17 page 218) indicates the presence of an indoline chromophore. Its mass spectrum (Figure 19 page 220) displays a molecular ion at m/z 334 (100%) corresponding to the molecular formular of $C_{21}H_{22}N_2O_2$ and the indole fragments at m/z 144, 143 and 130 indicate the non-substituted pattern in the aromatic ring of the indole moiety (see Figure 39 page 144). The mass fragments at m/z 305 ($M^+ - CO$), 177, 163, 162 and 120 indicate the presence of strychnan skeleton (107) which agree well with the proposed fragmentation pattern as shown in page 148-149.

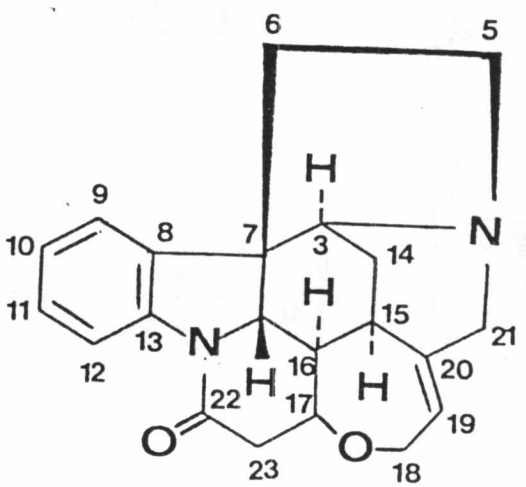
The IR spectrum (Figure 18 page 219) shows an intense band at ν_{max} 1665 cm^{-1} which is indicative of the presence of a lactam carbonyl function. A band at 760 cm^{-1} is indicating the presence of an 1,2-disubstituted aromatic in the structure of S1 (110).


M+ 334
M+ 334
A
B
m/z 305
m/z 305
A
B
m/z 130
m/z 144
m/z 120
m/z 177


M+ 334
M+ 334
m/z 129
m/z 130
m/z 163
m/z 162

The ^{1}H NMR spectrum [Figure 20 page 221] shows a deshielded aromatic proton of H-12 at δ 8.08 ppm (1H,d, $J_{12,11}$ = 8 Hz), H-9 at δ 7.27-7.22 (1H, m) and H-10, H-11 at δ 7.21-7.05 ppm (2H, m). The presence of one olefinic proton which is assigned to H-19 indicated by a triplets centred on δ 5.91 ppm (1H, ill defined t). These ^{1}H NMR spectral data are superimpossible which those of strychnine (101) while the ^{13}CNMR spectrum of S1 [Figure 21 page 222] indicates the presence of 21 carbon atoms as well as strychnine 54. The ^{13}C NMR chemical shifts of S1 are set out in full agreement with those of the published data [102] of strychnine 54. The assignments of these chemical shifts are set out in page 129.

In consequence of the colour reaction, UV,IR, MS, ^{1}H NMR and ^{13}C NMR spectroscopy, the alkaloid S1 is finally identified as strychnine 54 of which the structure is shown below.



S1 STRYCHNINE

S2 Brucine 55

The alkaloid S2 gives a yellow colour with the ferric chloride-perchloric acid reagent. Its UV spectrum exhibits the presence of indoline chromophore (λ_{max} 218, 261 and 298 nm) as those of strychnine (S1) 54 [Figure 22 page 223].

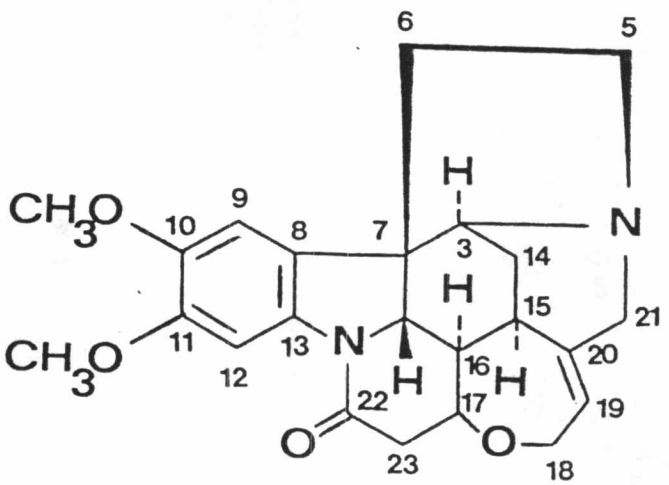
The mass spectrum (Figure 24 page 225) shows a molecular ion peak at m/z 394 (100%) which corresponding to $C_{23}H_{26}N_2O_4$. The usual indole fragments m/z 144, 143 and 130 are now shifted by 60 mass units to m/z 204, 203 and 190, respectively. These shifts are indicative of the presence of dimethoxyl groups adding to the aromatic part of the indole moiety (Figure 39 page 44).

IR spectrum of S2 showes the presence of a lactam carbonyl function at 1655 cm^{-1} while the band at 850 cm^{-1} is consistent with 1,2,4,5 - tetrasubstituted benzenoid indoline (Figure 23 page 224).

The ^1H NMR spectrum of S2 (Figure 25 page 226) give a signal at δ 5.89 ppm (1H, ill defined t) which is similar to that found in the spectrum of strychnine (S1) 54 thus indicates the presence of an ethylidine proton

The ^{13}CNMR spectrum of S2 (Figuer 26 page 227) shows carbon signals comparatively consistent with the assigned position for S1 excepted only two more downfield signals of C-10 and C-11 positions, accompanied with other two signals addition at 56.12 and 56.31.

Apparently, each mentioned down field signal would probably be attached with a methoxyl group. From the above evidence, S2 can be considered as a dimethoxy strychnine compound. Moreover, the colour reaction on tlc and the spectroscopic informations of S2 are in full agreement with those of brucine (10,11-dimethoxy strychnine) 55. Therefore it is concluded that S2 is brucine 55 where its structure is shown below.

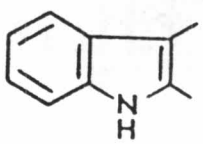


S2 BRUCINE

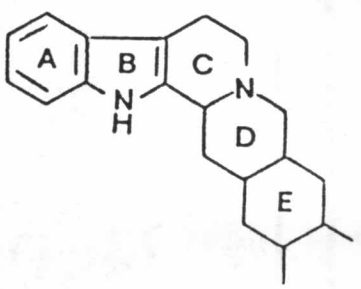
G1 Geissoschizol 161

This alkaloid (m.p. 216°C) gives a green colour with the ferric chloride-perchloric acid reagent. Its UV absorption spectrum (Figure 27 page 228) shows maxima at 220, 279, 286 and 312 nm which corresponds to the indole chromophore of the corynanthean type alkaloid.

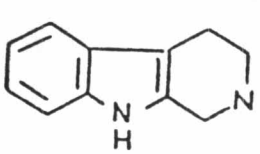
The mass spectrum (Figure 29 page 230) shows a molecular ion peak at m/z 296 (100%) which corresponding to $C_{19}H_{24}N_2O$, together with M-1 peak at m/z 295 a. The other noteworthy fragments are m/z 170,b and 169, c, accompanied by the satelite peak at 14 mass unit higher than b at m/z 184,d or lower than at m/z 156,e and the other peak at m/z 197,f. All of b,c,d,e and f are the characteristic fragmentation of a non-substituted Tetrahydro- β -carboline skeleton (107) which possesses by G1.



indole

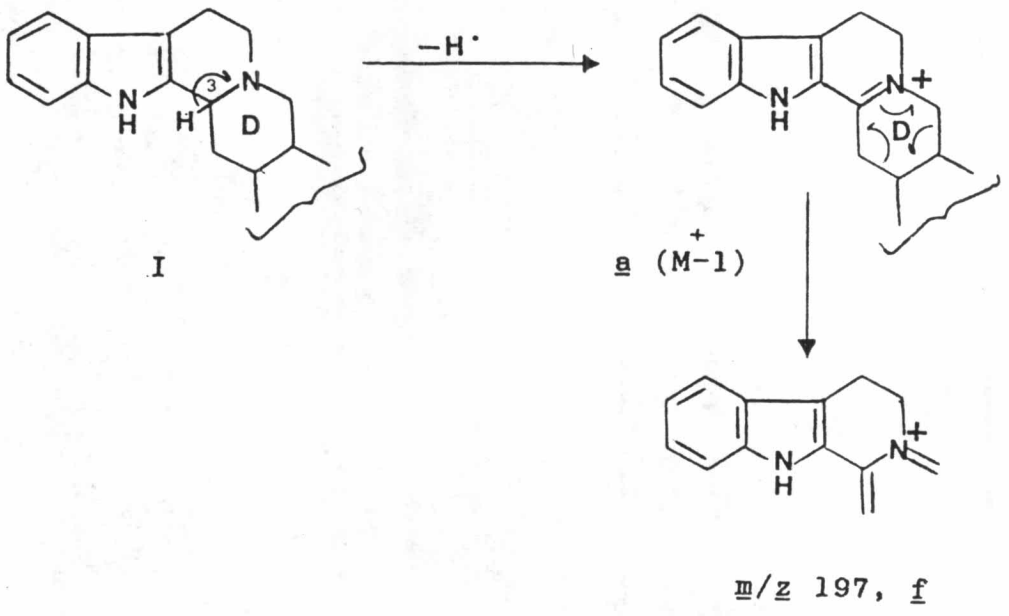


Corynanthean type



β - carboline

The pronounced $M^{+}-1$ peak a is produced by lossing of the hydrogen atom at C-3 while a retro-Diels-Alder fragmentation in the ring D of ion a would generate the m/z 197, f.

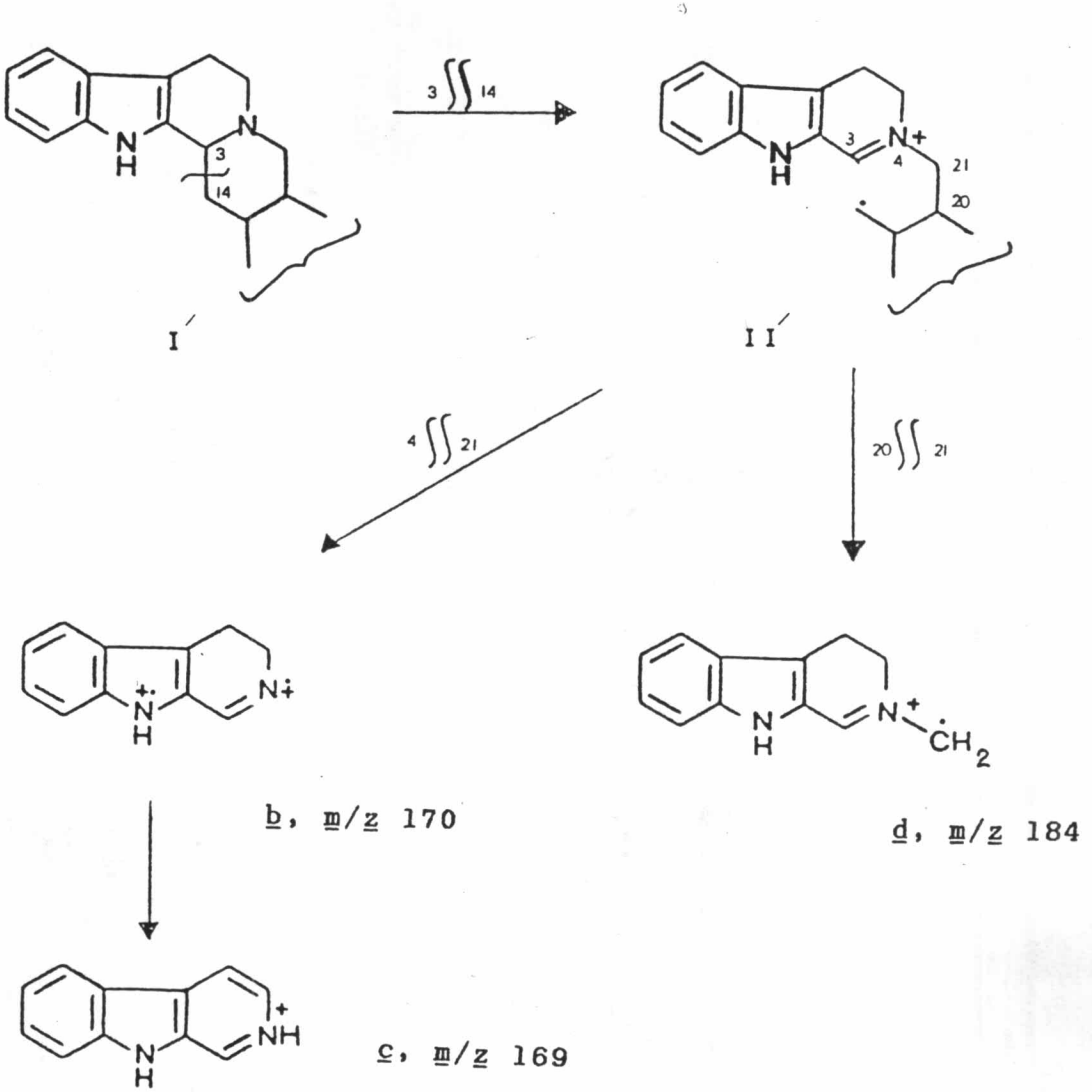


I

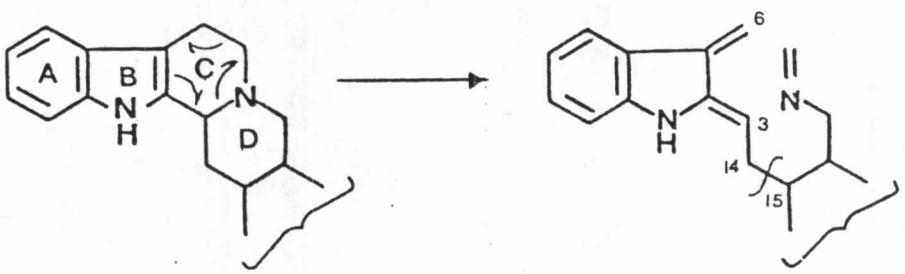
a $(M^{+}-1)$

m/z 197, f

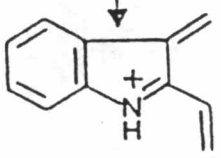
The geneses of the fragments species are demonstrated by first concerted homolysis cleavage of 3-14 bond of I′ (see below) gives intermediate II′ which further cleavages of 4-21 linkage to yield the stable dihydro β - carboline ion b, corresponding to m/z 170. Explusion of an additional hydrogen atom of b offers the β-carbolinium ion c (m/z 169). The next higher homolog of b is d (m/z 184) which is generated by the cleavage of the 20-21 bond of II′.



The proposed genesis of the m/z 156 species e involved a retro-Diels-Alder fragmentation of ring C of I′, g to generate two allylic center C-3 and C-6 (see arrow in g) followed by the homolytic fission of the allylic 14-15 bond in h.



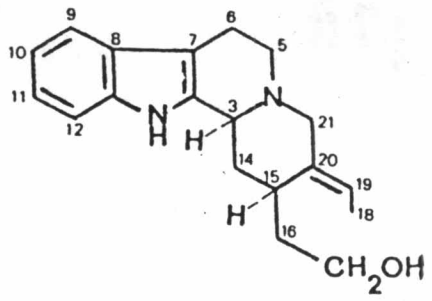
g , I′ h



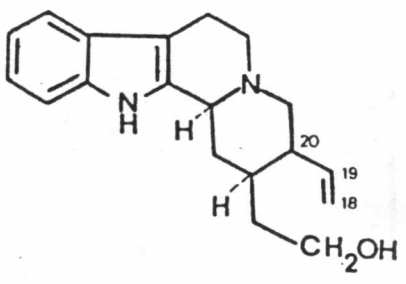
e m/z 156

The IR spectrum of G1 (Figure 28 page 232) shows the presence of primary hydroxyl as well as the NH vibrations at 3200-3100 cm^{-1} region. The medium intensity band at 2811 cm^{-1} and 2751 cm^{-1} of CH_2 systematic vibration corresponded to Bohlmann band (106,111). This band is an indication of the presence of a <u>trans</u>-quinolizidine C/D ring in G1 molecule.

From the above informations, it can be concluded that alkaloid G1 may assumed as an E-<u>seco</u> indole alkaloid. The possible structure of G1 might be geissoschizol <u>161</u> or its 18,19 dehydro isomer, normelinonine B <u>4</u>. As the possible candidate, normelinonine B <u>4</u> is ruled out as its mass spectrum must show the base peak at m/z 223 (100%) and m/z 225 (100%) (36,39).



Geissoschizol <u>161</u>



Normelinonine B <u>4</u>

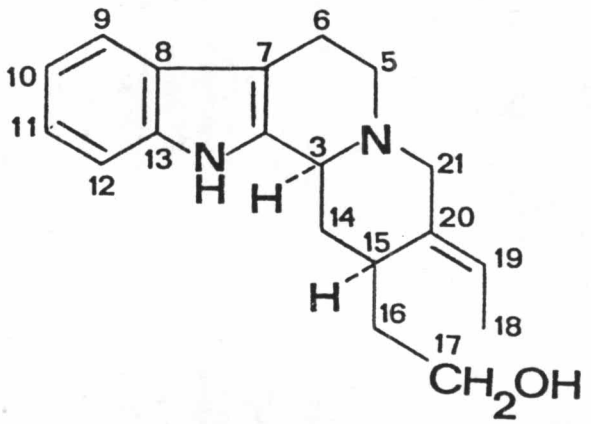
In addition, the 270 ^{1}H NMR spectral data of G1 (Figure 30 page 231) are in agreement with the proposed, geissoschizol 161 rather than normelinonine B 4. In geissoschizol 161, the spliting pattern of an ethylidine (C_{18}-C_{19}) side chain would appear as a doublet at δ 1.58 ppm (3H, d, J = 6.7 Hz, H-18, =CH-$\underline{CH}_3$) together with the low field quartet at δ 5.34 ppm (1H, q, J = 6.7 Hz H-19, =$\underline{CH}$-CH_3). For the normelinonine B 4 the C-18 terminal methylene (-CH=$\underline{CH}_2$) protons would appear at more downfield shift which lower field than those of the methyl protons of the vinyl function (=CH-$\underline{CH}_3$) of Geissoschizol (G1) 161.

The other signals are assigned, an indolic NH proton at δ 10.72 ppm (1H, br S, NH) together with four aromatic protons resonated at 7.33 ppm (1H,d,J = 7.4 Hz, H-9), δ 7.27 ppm (1H, d, J = 7.0 Hz, H-12) and 7.02-6.89 ppm (2H, m, H-10, H-11). The signal assigned to the C-3 proton resonated as triplets at δ 4.27 ppm (1H, t, H-3).

The 19 carbon atoms of G1 are determined from ^{13}C NMR spectrum (Figure 31 page 232) and the assignments of the chemical shifts are closely related to its congeners, especially geissoschizine 1 (35,105).

The alkaloid G1 has the trans-quinolizidine C/D ring configuration as same as geissoschizol 161 which is previously indicated by the presence of "Bohlmann band" (2800-2700 cm-1 region) in the IR spectrum (112).

From the above spectroscopic information, the alkaloid G1 is in full agreement with the known alkaloid geissoschizol 161, thus G1 is identified as geissoschizol 161. The structure of geissoschizol (G1) is shown below.



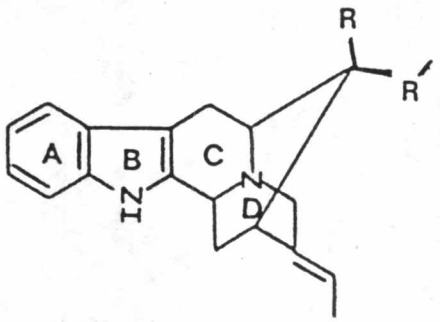
G1 GEISSOSCHIZOL

G2 Polyneuridine 24

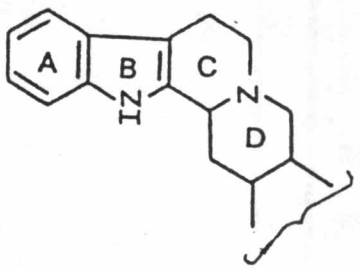
The UV absorption spectrum [Figure 32 page 233] of alkaloid G2 is typical for an indole chromophore in producing λ_{max} at 225, 272, 279 and 288 nm which is superimposable to geissoschizol (G1) 161. However, the difference is evidenced by their colour reactions with the ferric chloride - perchloric acid reagent of which black grey to G2 as compare with green to geissoschizol G1 161.

The IR spectrum of G2 (Figure 33 page 234) shows the presence of hydroxyl group as well as the secondary amine (NH_2) vibrations at 3155-3600 cm^{-1}. The sharp band at 1720 cm-1 indicates the presence of a carbonyl group of the carbomethoxy function, while a 1,2-disubstituted aromatic ring is recognized by 720 cm^{-1} band. The absence of Bohlmann band in the IR spectrum (ν_{max} 2800-2700 cm-1) (111) indicates the presence of a cis-quinolizidine function.

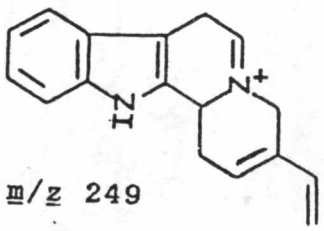
Its mass spectrum (Figure 34 page 235) reveals an abundant parent molecular ion at m/z 352, corresponding to $C_{21}H_{22}N_2O_3$. The other fragments are recognized as a closely related fragments of sarpagine group alkaloids (107) by producing the very intense fragments at m/z 249, 182, 169 and 168 species. (see page 161).



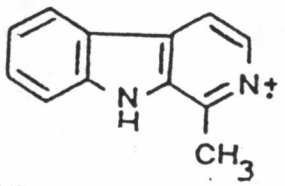
Sarpagine Type



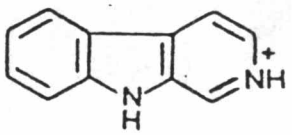
E-seco indole



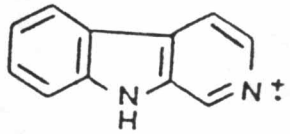
m/z 249



m/z 182

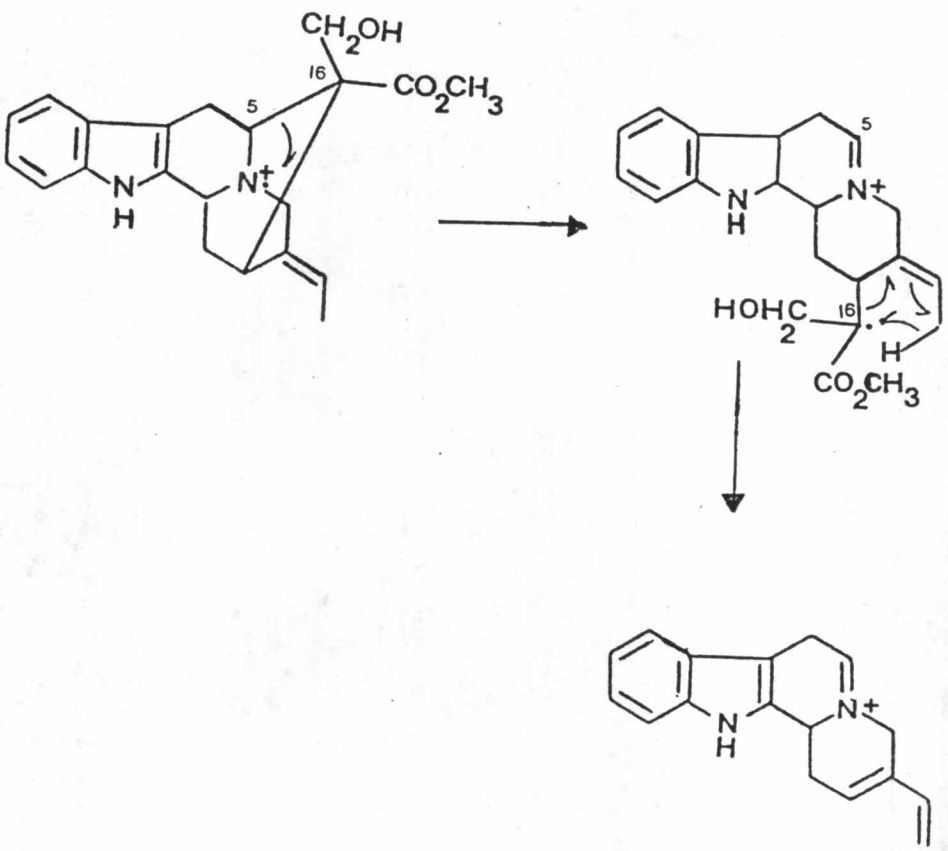


b′ m/z 169



c′ m/z 168

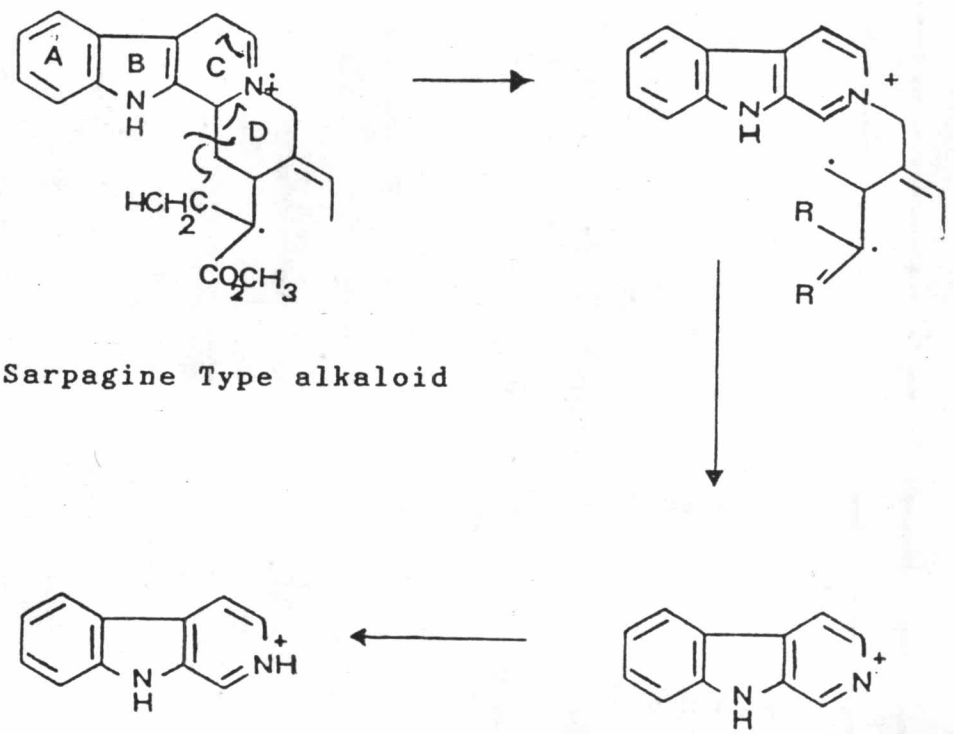
The strong fragment at m/z 249 i is generated by the homolytic cleavage of the C_5 -C_{16} bond which loose the C_{16} carbon bridge of the sarpagine skeleton follows by the transfering of C-18 hydrogen atom in a six-membered cyclic transition state.



i m/z 249

Like other E-seco indole alkaloids such as geissoschizol (G1) 161, the fragments at m/z 169 b´ and 168 C´ of the sarpagine alkaloid as well as G2 are related to the β -carboline ion b (m/z 170) and the β -carbolinium ion C (m/z 169), respectively.

The reason for one mass unit shifts between the two sets of the fragments has been clearified by the presence of an additional bond in the ring C of sarpagine moiety before the β -carboline ion and the β -carbolinium ion can be produced.



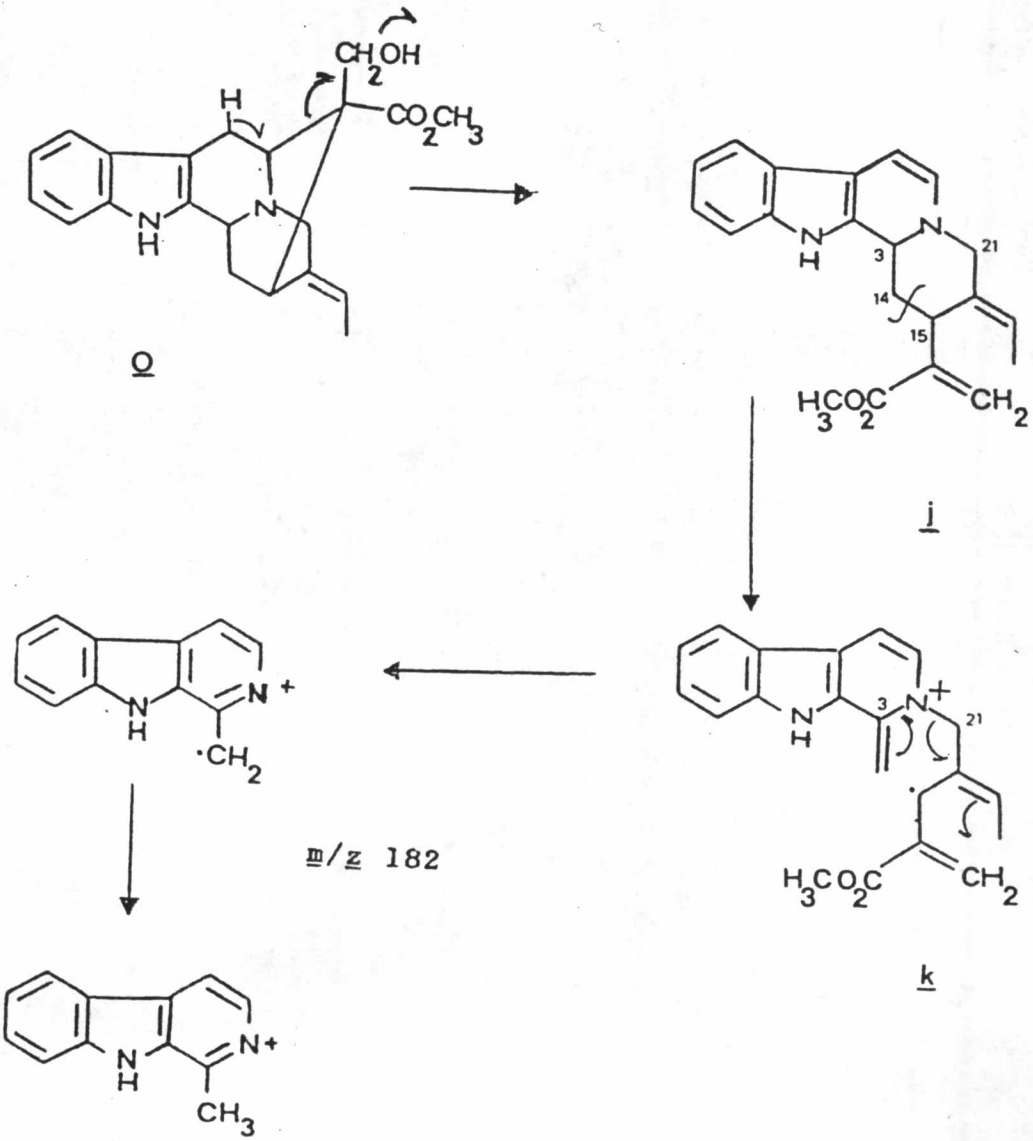
Sarpagine Type alkaloid

b´ m/z 169

c´ m/z 168

The fragment at m/z 182 would be accommodated readily by the cleavage at C$_{14}$-C$_{15}$ bond and N$_b$-C$_{21}$ bonds, both of which are activated the allylic bond coupled further loss of one hydrogen as in j or k.

The other fragments of G2 at m/z 293 (M^+ - 59; M^+ - CO_2CH_3), m/z 321 (M^+ - 31; M^+ - OCH_3 or M^+ - CH_2OH) are superimpossable with those ions of akuammidine 23 as well as polyneuridine (16-epimer akuammidine) 24 (113,114).



Since akuammidine 23 and polyneuridine 24 are stereoisomers, their mass spectra especially in the direct inlet system show less significant for the identification (107, 113,114). However, the most outstanding difference between mass spectra of these two molecules can be obtained by only using all-glass pre-heated inlet system (107, 113). Indeed, polyneuridine 24 shows a prounounced fragment at m/z 334 which is due to the loss of water ($M^+ -18$) from the molecular ion. The propose mechanism of this implied by the arrow in O which is consistent with the assigned C-16 stereochemistry of polyneuridine 24 while the inserted stereochemistry at C-16 of akuammidine 23 would be strearically impossible. However, only the available EI direct inlet system mass spectrometer is routinely used instead of the all-glass pre-heated inlet system mass spectrometer, thus the $M^+ -18$ ion (if it is present) could not be obtained.

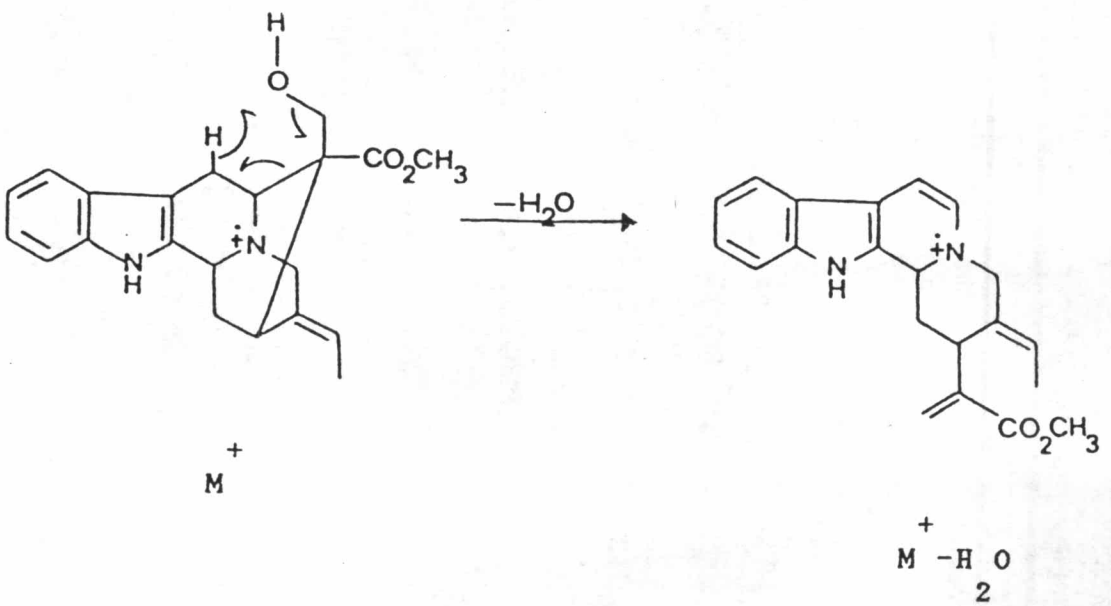


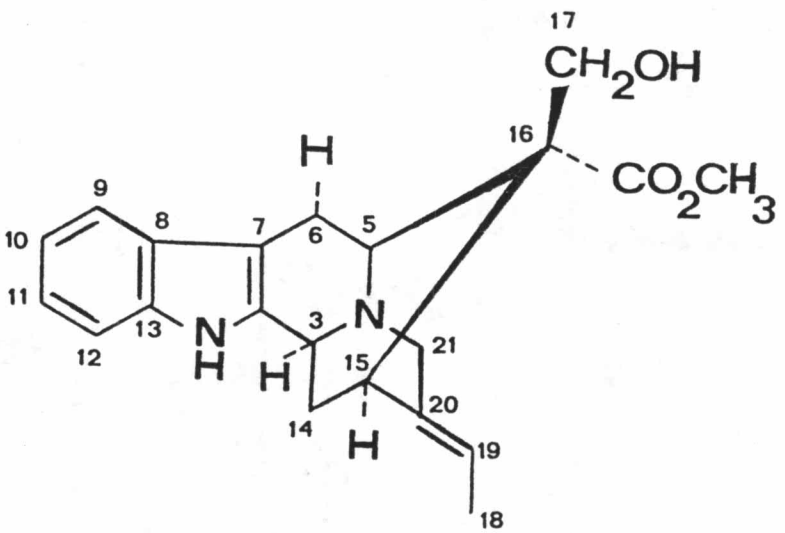
Table 5 ^{1}H NMR chemical shifts of G2 comparision with those of Akuammidine 23 and Polyneuridine 24 (105).

	G2	23	24
H-3	4.07 dd	4.27 d(d)	4.09 dd
H-5	4.28 d	3.08 m	4.32 d(d)
H-6	3.10 dd	2.96 dd	3.12 dd
H-6	2.95 d(d)	3.30 dd	2.98 d(d)
H-18	1.59 d(d)	1.65 d(dd)	1.59 d(dd)
H-19	5.28 br q	5.40 br q	5.26 br q
>NH	8.05 s	7.97 br s	8.20 br s
$-COOCH_3$	3.72 s	2.95 s	3.72 s

The alternative method to distinguish the difference between polyneuridine 24 and akuammidine 23 is by using ^{1}H NMR spectrometry (105). The ^{1}H NMR spectra of both compounds show marked differences in chemical shifts of H-5 and H-6 β as well as the shielding or deshielding protons of the methoxy carbonyl functions. The chemical shift of these proton assignments from the both published data of polyneuridine 24 and akuammidine 23 are compared with those from G2 (see Table 5 page 166).

From Table 5, the 270 MHz ^{1}H NMR spectrum of G2 (Figure 35 page 236) shows the presence of a broad singlet at δ 8.05 ppm (1H, brS, NH) due to the NH proton. The presence of two signals resonated at 4.28 ppm (d, 1H, J = 5.68 Hz) and δ 2.95 ppm (d, 1H, J = 16 Hz) are assigned to H-5 and H-6 β , respectively. Accompanied by three deshielding acetyl protons resonated at δ 3.72 ppm (3H, S) are in agreement with those of the spectral data for polyneuridine 24 (105). Four aromatic protons are observed at δ 7.48 ppm (d, 1H ; H-9), δ 7.33 ppm (dd, 1H ; H-12) and δ 7.15 ppm (m, 2H; H-11, H-10). One proton quartets centred at δ 5.28 ppm indicates an ethylidine (= $\underline{CH}$-CH_3) function while the methyl protons of the corresponding function (=CH-$\underline{CH}_3$) are observed as doublets at δ 1.59 ppm. The last two sets of signals indicates the presence of an exocyclic ethylidine function (-CH=CH_3).

From the above significant informations, G2 can be identified as polyneuridine 24. The structure of polyneuridine is demonstrated below.

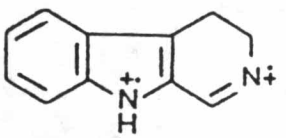


G2 POLYNEURIDINE

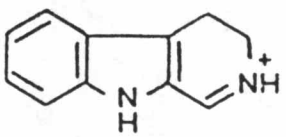
B1 Longicaudatine 119

The alkaloid B1 immediately gives blue colour with the ferric chloride - perchloric acid reagent. The characteristic blue colour gradually changes to grey after a few days as similar to that produced by corynanthean type alkaloids. This leads to presume that a part of the molecule of B1 may possess β-carboline moiety.

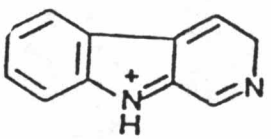
Its mass spectrum (Figure 36 page 237) possesses an abundant molecular ion peak at m/z 568 which may corresponding to the formular $C_{38}H_{40}N_4O$. Only a few peaks are observed in the upper mass range while most fragments accumulate in the lower mass range of the mass spectrum. The mass spectrum can be used to proved the actual presence of the two halves of an asymmetric dimer. The cluster of fragment ions center at m/z 251, 250 and 249 indicating of the indoloquinolizidine nucleus bearing an unsaturated two carbon side chain in B1 (34, 107, 109) molecule. These fragment ions are further break down to the corresponding β-carboline ions at m/z 171, 170 and 169.



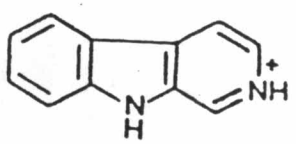
m/z 170



m/z 171

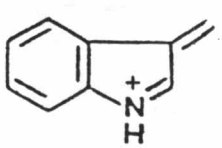


m/z 169

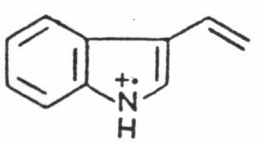


m/z 169

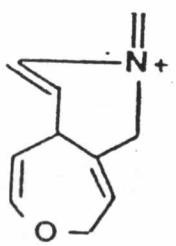
The fragments at m/z 130 and 144 are indicative of the presence of an unsubstituted indole nucleus. The fragments at m/z 162, 122 and 121 are probably generated from the non-indolic strychnan portion of the molecule (34, 110, 115) emphasizing the presence of strychnan type nulceus.



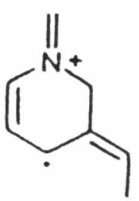
m/z 130



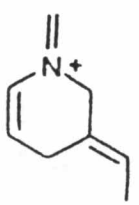
m/z 140



m/z 162



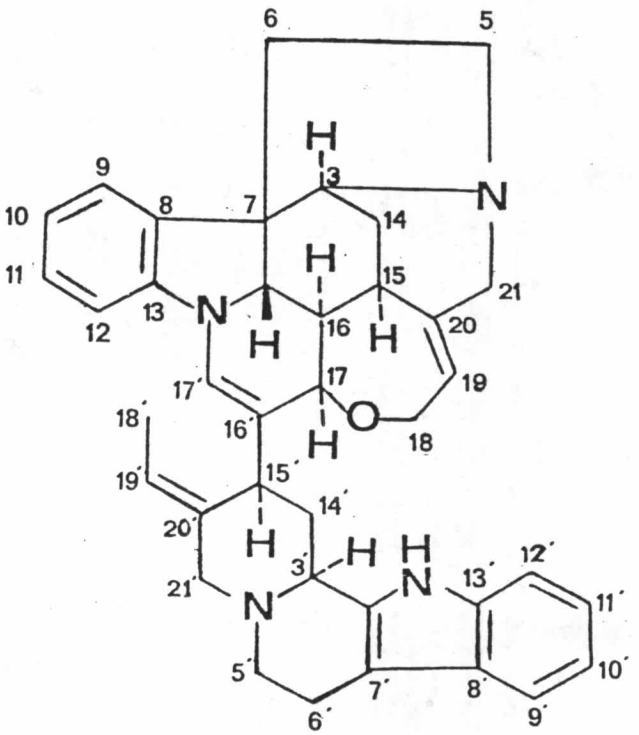
m/z 121



m/z 122

The other mass fragment patterns of B1 are very similar to those of longicaudatine 119 excepted for some difference intensities. Basing on the mass fragment data , it can lead to the conclusion that the alkaloid B1 is probably belongings to longicaudatine type (B_5) and probably is longicaudatine 119 itself.

It is unfortunate that only small quantity of B1 is available, the complete elucidation of B1 structure could not be carried out. However, it may be concluded on the basis of mass spectral data that B1 is probably the known longicaudatine 119 where its structure is shown below.



B1 LONGICAUDATINE

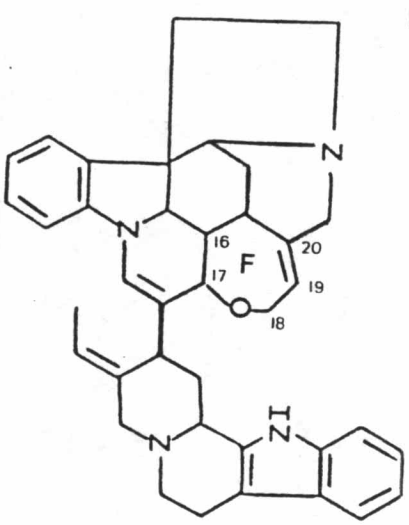
B2 Dihydrolongicaudatine

The trace of alkaloid B2 (1.6 mg) is isolated from the plant. This alkaloid gives violet-blue colour with the ferric chloride-perchloric acid reagent. Its mass spectrum (Figure 37 page 238) shows an abundant molecular ion peak at m/z 570 which is two mass units higher than longicaudatine 119. Its molecular weight indicates that the compound is probably a bisindole alkaloid.

Moreover, B2 exhibits the mass fragmentation pattern very similar to that of longicaudatine 119. The fragment ions are observed at m/z 251, 250, 249, 171, 170 and 169 which indicating the presence of the corynanthean type nucleus in the molecule. A pair of fragment ions centered at m/z 130 and 144 is assigned to the unsubstituted indole nucleus while the other pair of the fragment ions center at m/z 123 and 121 is also representative of the strychnan type nucleus of B2 molecule. Thus, B2 is probably a bisindole alkaloid which possesses an asymetrical corynanthean-strychnan combination similar to longicaudatine 119

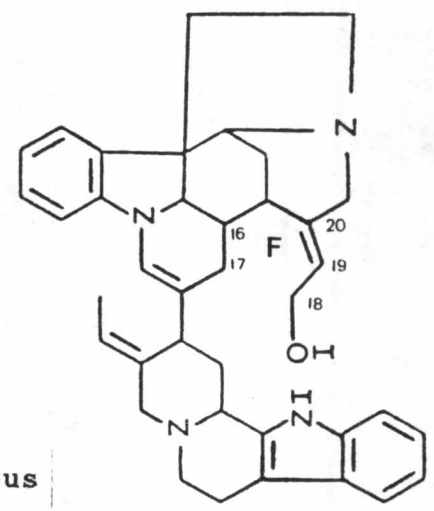
The molecular weight (M^+ = 570) is assumed as $C_{38}H_{42}N_4O$. On the basis of longicaudatine skeleton, an attempt to postulate the structure of B2 has been made (Figure 42 page 177).

The first case, the ether linkage (C$_{17}$ -O- C$_{18}$) of the ring F of strychnan type nucleus is broken and led to the formation of the corresponding alcohol derivative (m/z 570), longicaudatine Y 162. Longicaudatine Y 162 was first isolated from the root bark of Strychnos longicaudata Gilg (109). However, the mass spectrum of longicaudatine Y 162 possesses a characteristic peak at m/z 522 (M^+ -18, 6%) which represents an ion derived by lossing water from the molecular ion. The other noteworthy peaks at m/z 320 (85%) and 302 (90%) represent the corresponding ion fragments of the strychnan nucleus and the strychnan nucleus lossing water, respectively (109).



Strychnan nucleus

Corynanthean nucleus



Longicaudatine 119

Longicaudatine Y 162

The absence of these last two peaks in the mass spectrum of alkaloid B2 would rule out the possiblity of its being as longicaudatine Y 162

On the otherhand, two mass units higher than longicaudatine 119 indicate that the alkaloid B2 might be the dihydro-derivative of longicaudatine 119 which derives from the reduction of an olefinic function. The reduction might be located either at the position 19,20 of the strychnan part or at the Δ 16',17' or Δ 19',20' positions of the corynanthean part of the longicaudatine 119 molecule. The reduction of the Δ 19,20, Δ16',17' and Δ 19',20' would be led to the probablity structuces of dihydrolongicaudatine as 163,164 and 165 respectively (see Figure 42 page 177).

However, it is necessary to get more spectroscopic or other informations for a clear characterization of the alkaloid B2. The reinvestigation of this plant would be necessary to establish the structural formular of B2 alkaloid.

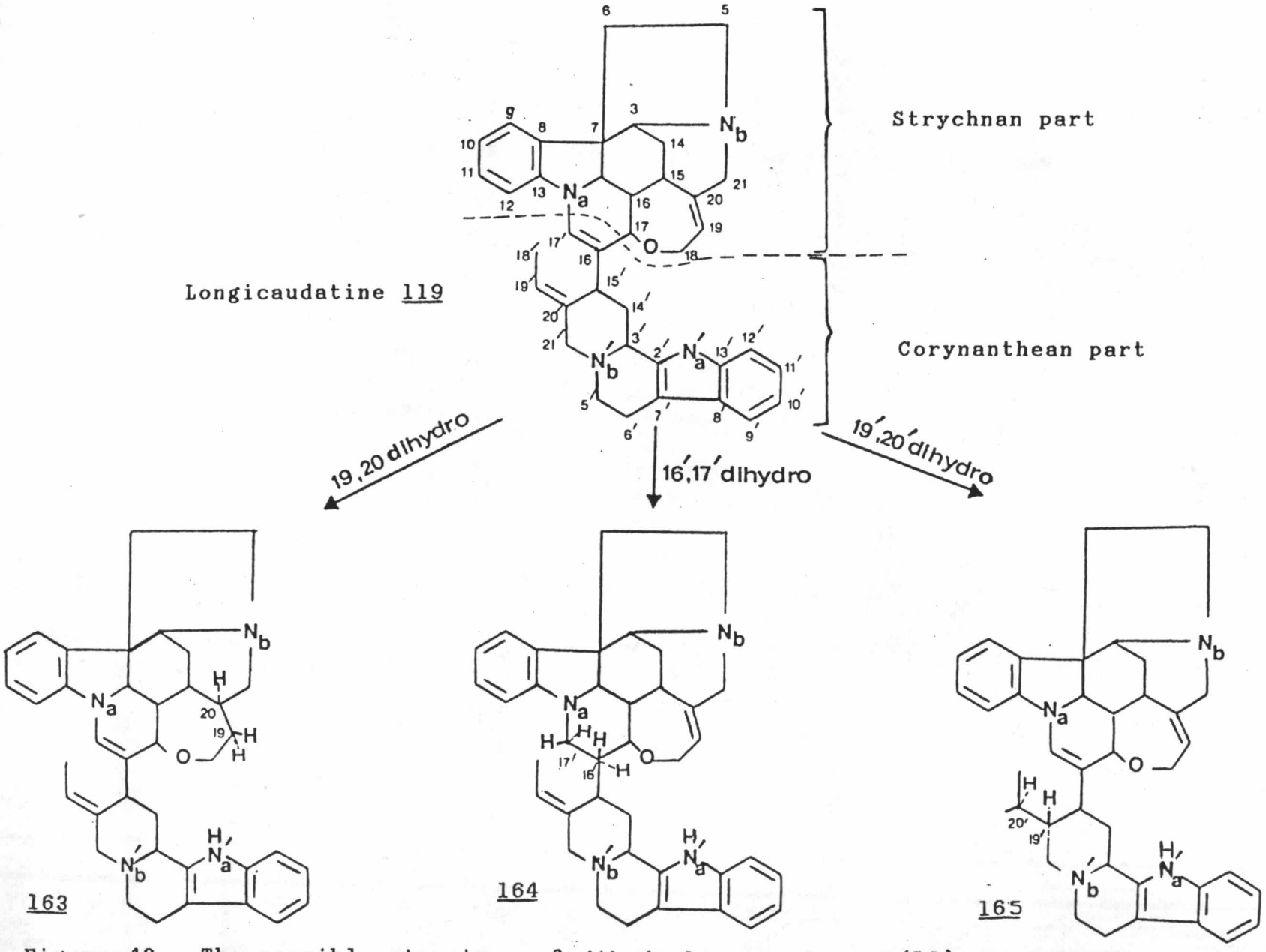


Figure 42 The possible structure of dihydrolongicaudatine (B2) 163-165

Biogenetic Discussion on the Isolated Alkaloids

The alkaloids which are isolated in this present work, can be classified on the basis of the biogenetic background as follows :-

Table 6 Classification of the isolated alkaloids

Code	Alkaloid	Class	Type
S1	Strychnine 54	Monomer	Strychnan
S2	Brucine 55		
G1	Geissoschizol 161	Monomer	Corynanthean
G2	Polyneuridine 24		
B1	Longicaudatine 119	Bisindole	Strychnan-Corynanthean
B2	Dihydrolongicaudatine 163-165		

Strychnine 54 and brucine 55, the representatives of the strychnan type alkaloids, are the main alkaloids of Strychnos ignatii Berg. These two alkaloids have been commonly found in other Asian Strychnos species (Section Strychnos) such as S.lucida R.Br., S.nux-vomica Linn., S.wallichiana Steud. ex DC. and also found in small amount in S.nux-blanda A.W.Hill and S.rupicola Pierre ex Dop (20) (See Table 3 page 55-64).

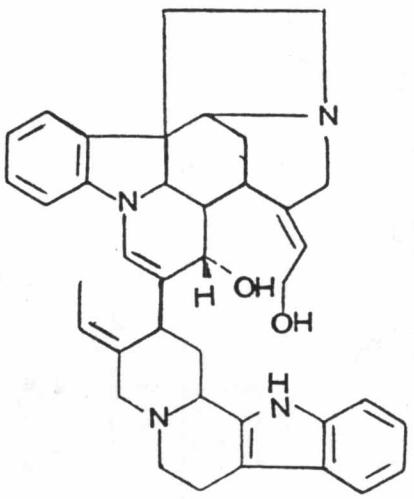
Geissoschizol 161 and polyneuridine 24 are just isolated for the first time from S.ignatii Berg. as well as from the family Loganiaceae. This is also the first report on the isolation of monomeric corynanthean type alkaloids in S.ignatii Berg.

Geissoschizol 161, a corynanthean possessing moiety has been isolated from Tabernaemontana laeta (Mert.) Miers (Peschiera laeta Mart.) (116) and from many Rauwolfia species (78,117) of the family Apocynaceae. This alkaloid also found in the callus culture of Tabernaemontana elegan Stapf. (118). Geissoschizol 161 is the representative of E-seco indole alkaloid and is regarded as the intermediate precursor of the other corynanthean type alkaloids (78,117) as well as the strychnan-corynanthean bisindole alkaloids (34). The biogenetic assumptions would be discussed later.

Polyneuridine 24 has been isolated from Aspidosperma polyneuron Mill Arg. (114) and several species of the family Apocynaceae (119-122). This alkaloid is also belonged to the sarpagine group alkaloid.

In this investigation of the bisindole type alkaloids, only the traces of asymmetrical bisindole alkaloids are found. They are longicaudatine 119 and its derivative, dihydrolongicaudatine 163-165. Both alkaloids are the combination products between the two different monomeric indole alkaloids of the diaboline (S_2) and E-seco indole (C_1) groups. They are exhibit similar behavior in producing the blue colour with the ferric chloride-perchloric acid spray reagent.

Longicaudatine 119 was first reported to be isolated from S. lucida R.Br. root bark and at the same time was isolated in a small amount from S. ignatii Berg. root bark (34). Later this alkaloid was found in associated with bisnordihydrotoxiferine 102 from S. wallichiana Steud. ex DC. (47). Longicaudatine 119 has occurred as a major constituent in S. longicaudata Gilg together with bisnor-C-alkaloid H 105 and other two longicaudatine derivatives, longicaudatine Y 162 and longicaudatine F 166 (109).



Longicaudatine F 166

Apart from those mentioned Strychnos species, longicaudatine 119 itself was widely occurred among African and Asian Strychnos species such as S.ngouniensis Pellegr., S. dolichothyrsa Gilg ex Onochie et Hepper, S. crysophylla Gilg, S. afzelii Gilg, S. urceolata Leeuwenberg and S. nux-vomica Linn. (35).

Although its dihydroderivative of longicaudatine, dihydrolongicaudatine 163-165 was isolated in this investigation, its absolute structural identification is hampered due to its inadequate quantity.

According to the current biogenetical proposal, indole alkaloids take root from the central intermediate, strictosidine 134. Strictosidine 134 is derived to the corynanthean and strychnan type or other indole alkaloid types via 4,21-dehydrocorynantheine aldehyde 146, 4,21-dehydrogeissoschizine 147 and geissoschizine 1. The summarization of the biogenetic pathway is previously presented in the biogenetic section (See Figure 10 page 75).

The knowledge of the interrelationships among the Strychnos alkaloids is far from complete since many postulated intermediates have not been isolated from the Strychnos plant materials. However, the possible intermediate, geissoschizine 1 and its congeners, geissoschizal 2 and de-carbomethoxygeissoschizine 3 have been isolated from the seedlings of Strychnos nux-vomica Linn. (37).

The isolation of geissoschizol 161 in this present investigation can therefore be looked upon as the indirect indication of the present of geissoschizine 1 in S.ignatii Berg. since the structure of geissoschizol 161 is closely related to geissoschizine 1 (103,117).

Geissoschizol 161 might be derived from the hypothetical intermediate, 4,21-dehydrogeissoschizine 147. The sequence of the biosynthetic pathway to geissoschizol 161 should be explained as involving the hydrogenation of the C$_{4}$-C$_{21}$ unsaturated position and follows by the decarbomethoxylation of 4,21-dehydrogeissoschizine 147. Therefore, the biosynthetic pathway to geissoschizol 161 (Figure 43 page 181) might be displayed by the two difference sequence routes , route A or route B assumptions.

At the first stage of the biosynthetic on the route A assumption, 4,21-dehydrogeissoschizine 147 is hydrogenated at its C$_{4}$-C$_{21}$ unsaturated position to give geissoschizine 1 and then 1 is decarbomethoxylation to give another intermediate, de-carbomethoxy geisoschizine 3. Finally, de-carbomethoxy geissoschizine 3 seems to directly reduced its C$_{16}$-C$_{17}$ unsaturated position to form geissoschizol 161. This route is well documented by the isolation of both geissoschizine 1 and de-carbomethoxy geissoschizine from S.nux-vomica seedlings (37) (See Figure 43 route A, page 181).

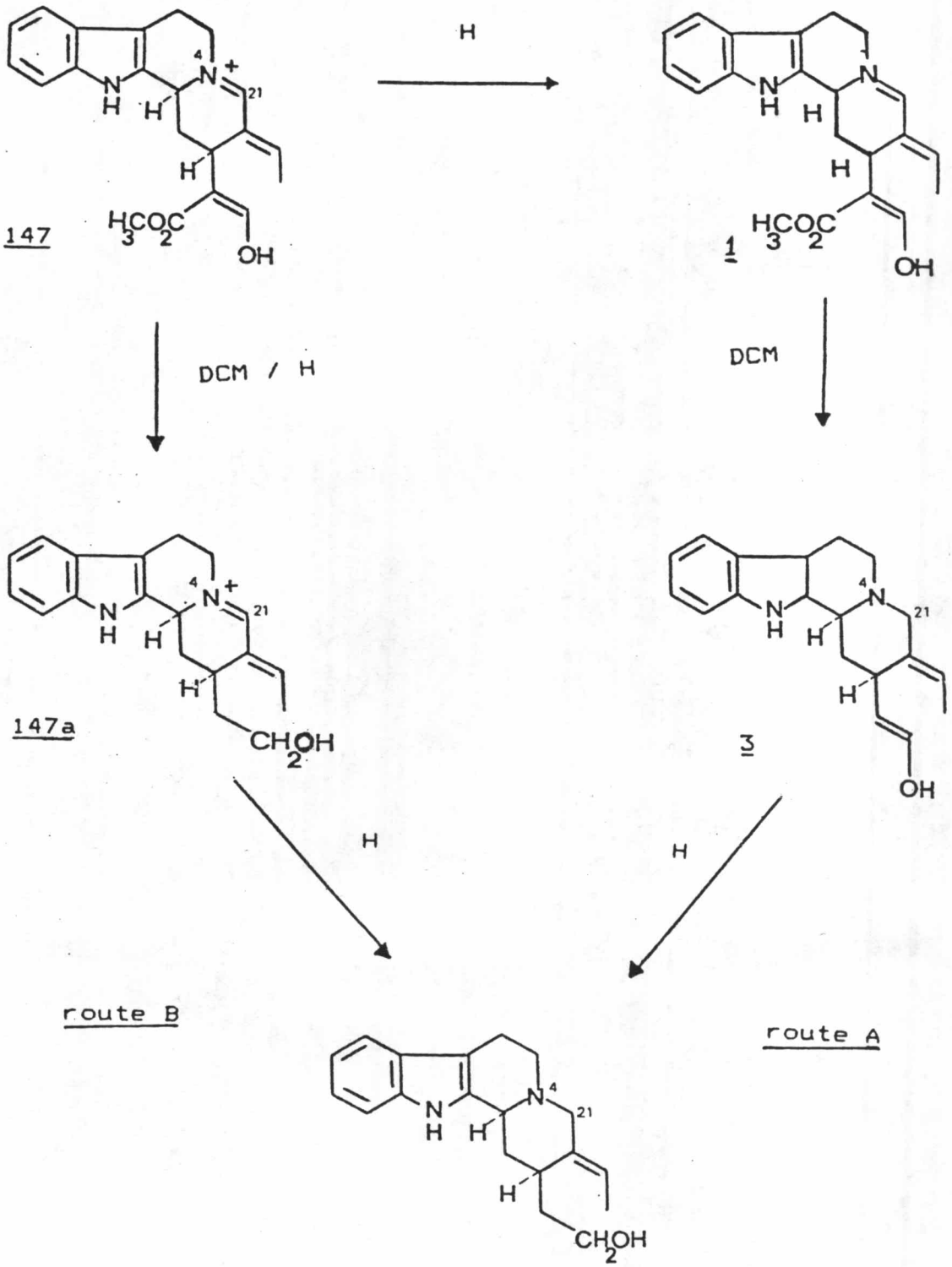


Figure 43 The possible biosynthetic routes leading to geissoschizol 161

DCM = Decarbomethoxylation ; H = Hydrogenation

On the other hand, the decarbomethoxylation of 4,21-dehydrogeissoschizine 147 might be postulated at the first stage on the route B assumption. The consequent substrate would be the corresponding 4,21-dehydrogeissoschizol 147a which would be hydrogenated to give geissochizol 161 (See Figure 43 route B, page 181).

However, the biogenetic route leading to geissoschizol 161 is still not clearly demonstrated and the more biosynthetic investigation would be necessary.

Cordell (77) indicated that the E-seco indole alkaloid (C_1) seems to be an intermediate precursor in forming other corynanthean type alkaloids such as the sarpagine group alkaloid (C_4). Geissoschizol 161 itself is the simplest E-seco indole alkaloid which could be regarded as the precursor of the sarpagine group alkaloids (117) (see Figure 44 page 18). In this investigation, the occurrance of geissoschizol 161 accompanied with polyneridine 24, an sarpagine group alkaloids is probably confirmed this proposed route. Polyneuridine 24 and akuammidine 24 seem to be the cyclized acetate product of geissoschizol 161, but alternatively they seem to be directly derived from 4,21-dehydrogeissoschizine 147 too (34).

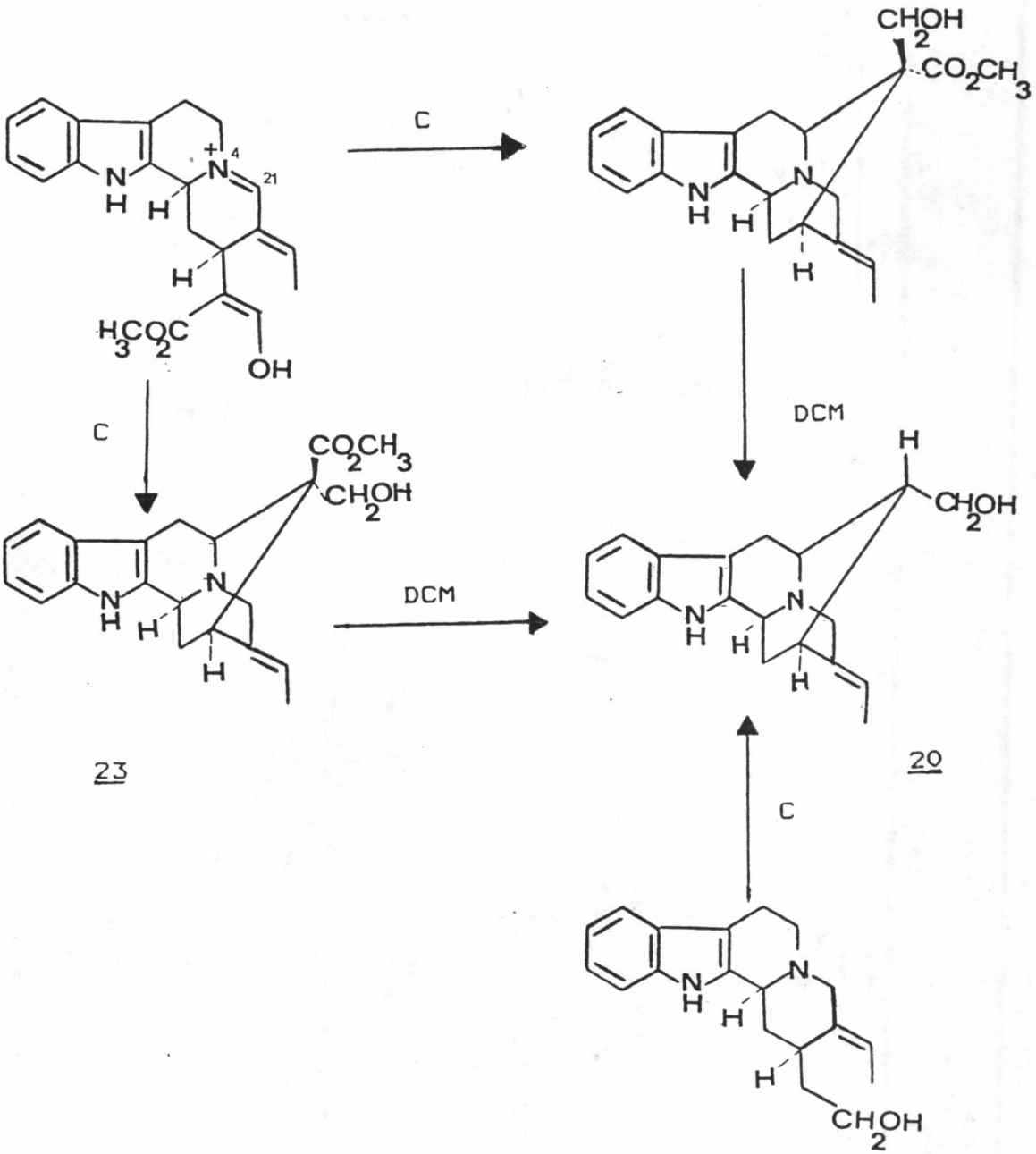


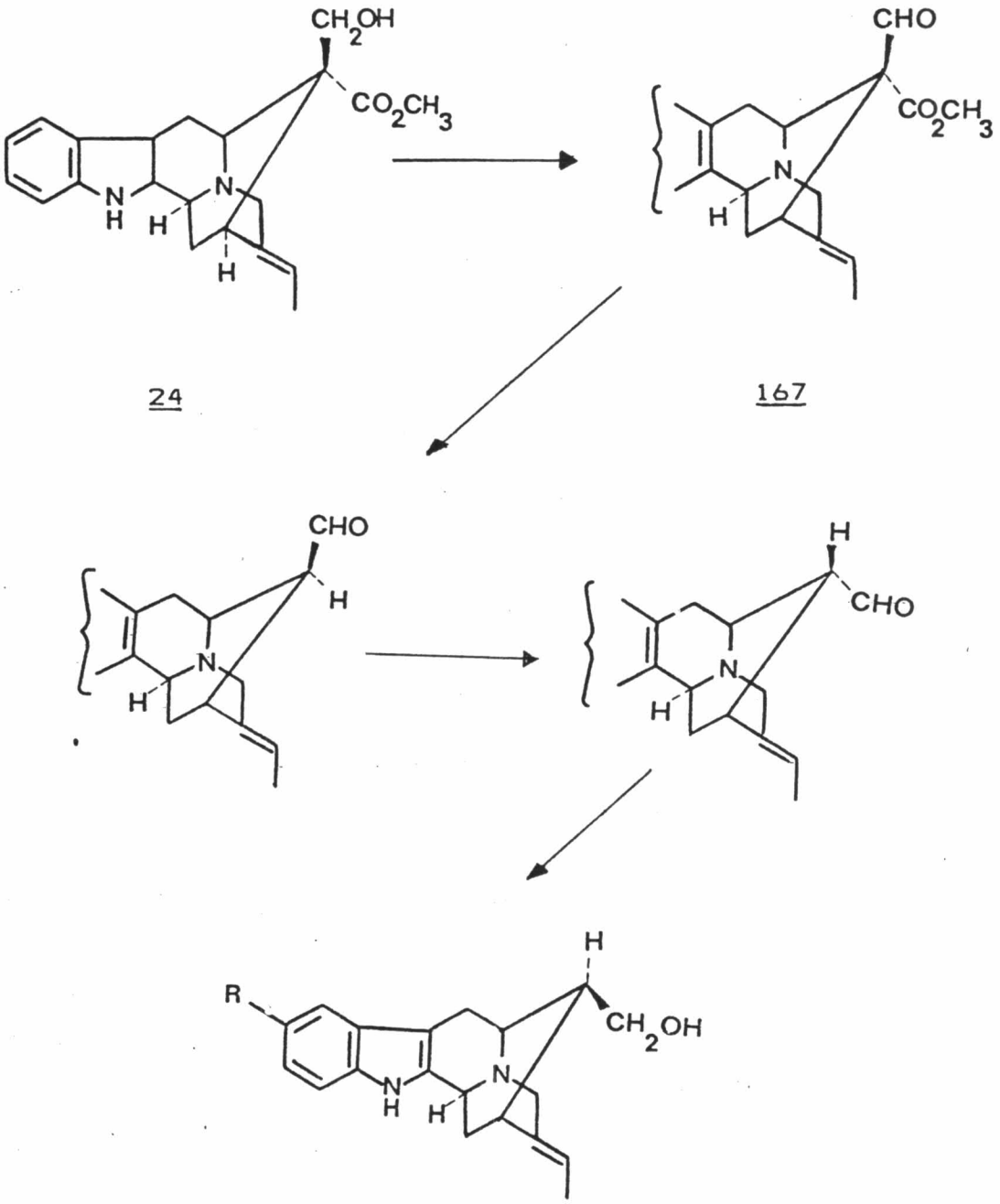
Figure 44 The formation of Sarpagine group (C_6) Alkaloid

C = Cyclization

DCM = Decarbomethoxylation

Akuammidine 23 the C_{16}-epimer of polyneuridine 24 is considered to be the precursor of normacusine B 20 and its 10-hydroxy congener, sarpagine 18 (123). The cell-free suspension of Rauwolfia serpentina Benth. (124), has been indicated that there is an enzyme, polyneuridine aldehyde esterase (PNA-esterase) catalysed at the stage of polyneuridine aldehyde 167 during the biosynthesis of sarpagine type alkaloids.

Polyneuridine 24 might be converted to polyneuridine aldehyde, which is the substrate specificity of PNA-esterase, therefore Polyneuridine 24 probably has a key role in the biosynthesis of sarpagine type alkaloids as shown in Figure 45 page 186.



20 Normacusine B ; R = H

18 Sarpagine ; R = OH

Figure 45 Polyneuridine 24 and Polyneuridine aldehyde 167 as the key role intermediate in the biosynthesis of sarpagine type alkaloids.

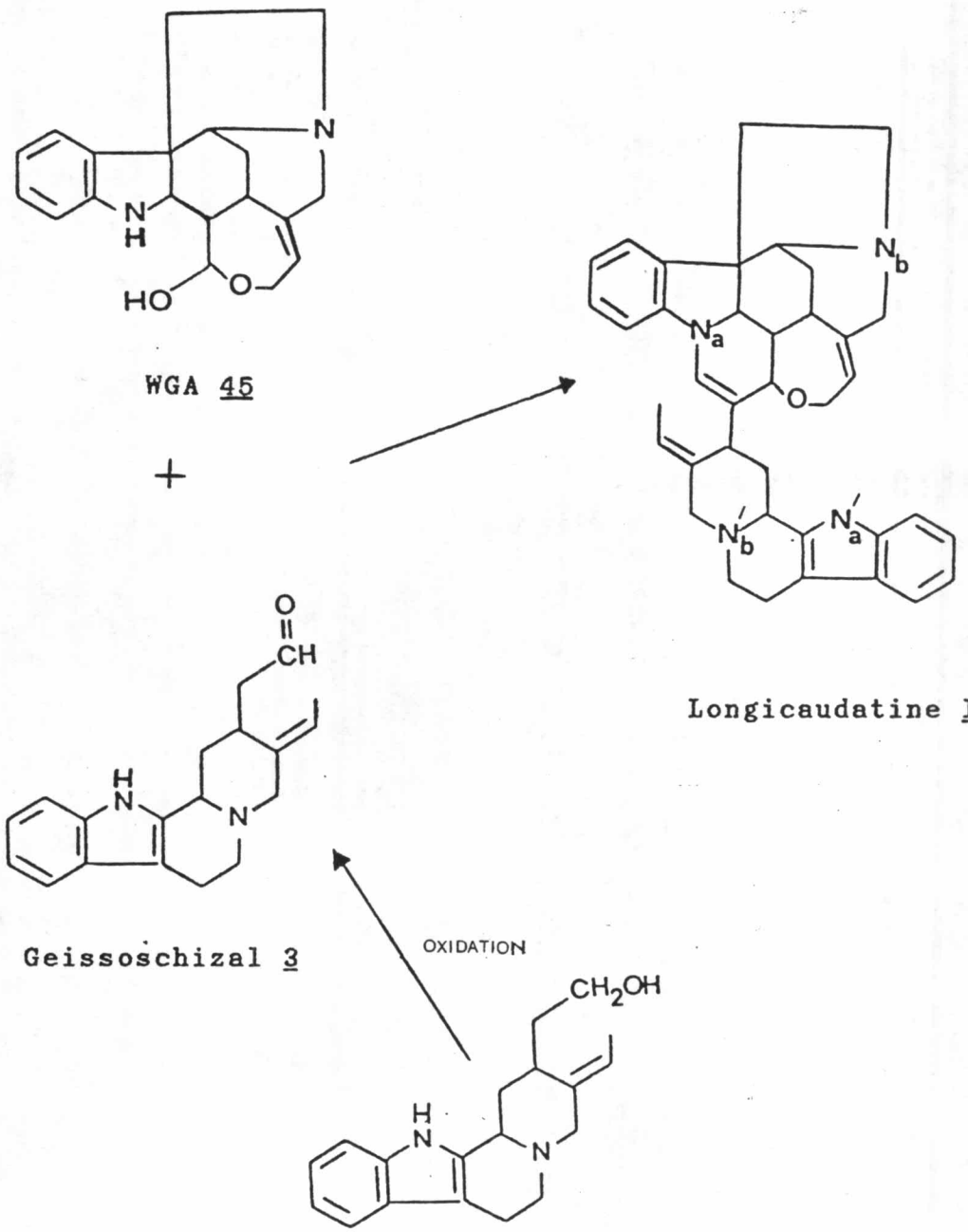


Figure 46 Geissoschizol 161, geissoshizal 3 and Wieland Gumlich aldehyde 45 are the possible precursors of longicaudatine 119

Chemotaxonomic Significance of the Isolated Alkaloids.

The chemotaxonomic comparision of the Asian and American species of the Section Strychnos is rater difficult due to the fact that up till now almost all of the chemical works on the American Strychnos species have been carried out either on the root barks or stem barks while a few investigations on these plant parts are taken in Asian Strychnos species. In addition, the investigation on American Strychnos species is concentrated on the monomeric and bisindole alkaloids of the quaternary bases, while on Asian Strychnos species, their tertiary bases are usually carried out.

Strychnine 54 and brucine 55 can be seen as the typical alkaloids from the Asian Strychnos seeds of the Section Strychnos. These two alkaloids are also found in the seeds of only one South American species S.panamensis Seemann (3) of the Section Strychnos. At the same time, it should be pointed out that the seeds from many south American species have not been investigated (3,22).

American Strychnos species contained numbers of symmetrical bisindole alkaloids of which 4 strychnan-strychnan types (B_1, B_2, B_3 and B_4) was found. Only one alkaloid of the alkaloid B_1 type, bisnor-dihydrotoxiferine 102 was found in S.wallichiana Steud. ex DC. (47) of the Asian Strychnos species and up till now, only one

type of asymmetrical bisindole alkaloid of Strychnan-corynanthean type (B_5), longicaudatine and its congeners are found in Asian Strychnos species.

The number of the detected alkaloids from the Section Strychnos belonged to Asian Strychnos species can be arranged according to their structural type as shown in Table 7 .

Table 7

Distribution of the Alkaloid Types Isolated from Asian Strychnos Species (Section Strychnos)

Plant	Alkaloid Contents											
	C-type			V-type	S-type			A-type	B-type	
	C 1	C 3	C 6	V 3	S 2	S 3	S 4	A 1	B 1	B 5
S. ignatii Berg.	1	-	1	-	1	-	15	-	-	2
S. lucida R.Br.	-	-	3	-	2	-	10	-	-	1
S. nux-vomica Linn.	4	1	3	-	2	3	22	1	-	-
S. nux-blanda A.W.Hill	-	-	-	-	1	-	8	-	-	-
S. rupicola Pierre ex Dop	-	-	-	3	-	-	5	-	-	-
S. wallichiana Steud. ex DC.	-	-	-	3	2	-	21	-	1	1

Further study should be recommened on surveying of the alkaloids belonging to the corynanthean type of monomeric indole alkaloids or another type of bisindole alkaloids in which the results would be beneficial in providing information for the chemo-botanical classification at the Tribe, Section, and Genus Levels.