CHAPTER IV

DISCUSSION

Structure elucidation of the isolated alkaloids

Two alkaloids from the stem of *Strychnos minor* Dennst. will be described for their molecular structures as follows.

A-1

The alkaloid A-1 gives gray-brown colour with ferric chloride-perchloric acid spraying reagent. The ultraviolet absorption spectrum is typical for an indole chromophore in producing λ_{max} at 209.9, 222.2, 255.2 and 289.2 nm (Verpoorte, 1986). The IR spectrum shows an intense band at 1635 cm^{-1} which is indicative of the presence of a tertiary amide carbonyl function on the indole moiety. The mass spectrum shows a molecular ion peak at *m/z* 398 which corresponding to the molecular formular of $C_{22}H_{26}N_2O_5$. The presence of an acetyl substituted group is confirm by a peak at *m/z* 43. The mass spectrum shows the indole moiety fragments at *m/z* 176 and 190, of which a methoxyl and a hydroxyl groups are proposed to adding to the aromatic part. The presence of these two oxygenated substitutions can be confirmed by the peak at *m/z* 174 and 162. The characteristic features of some important ions of indole moiety in the mass fragmentations are shown in figure 11.

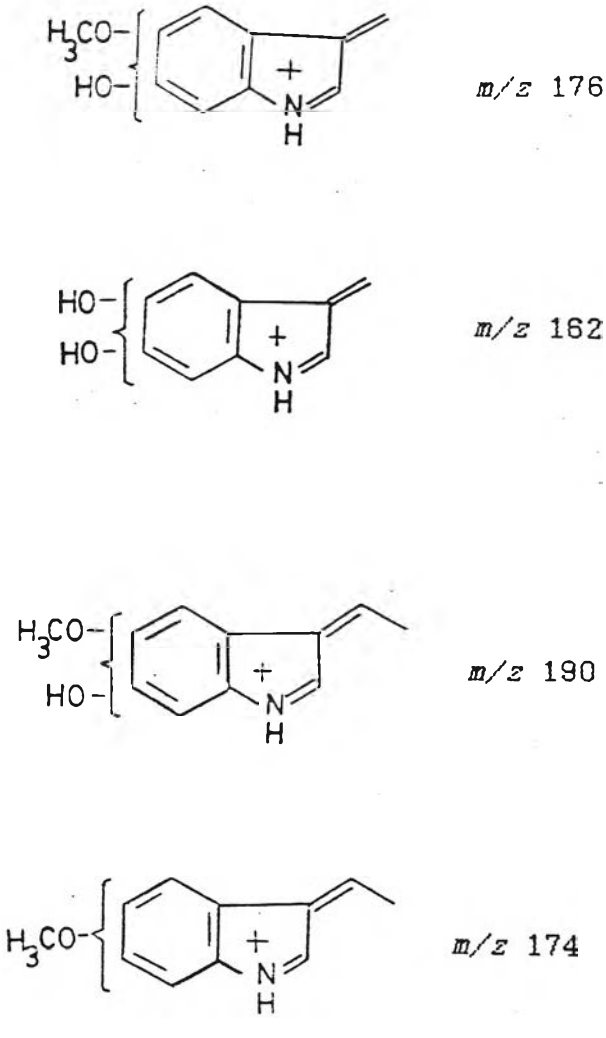
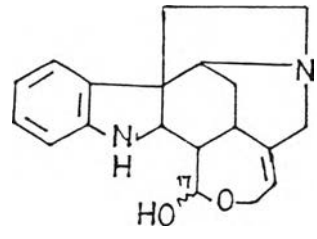


Figure 11 : Some important indole fragment ions in the mass spectrum of A-1

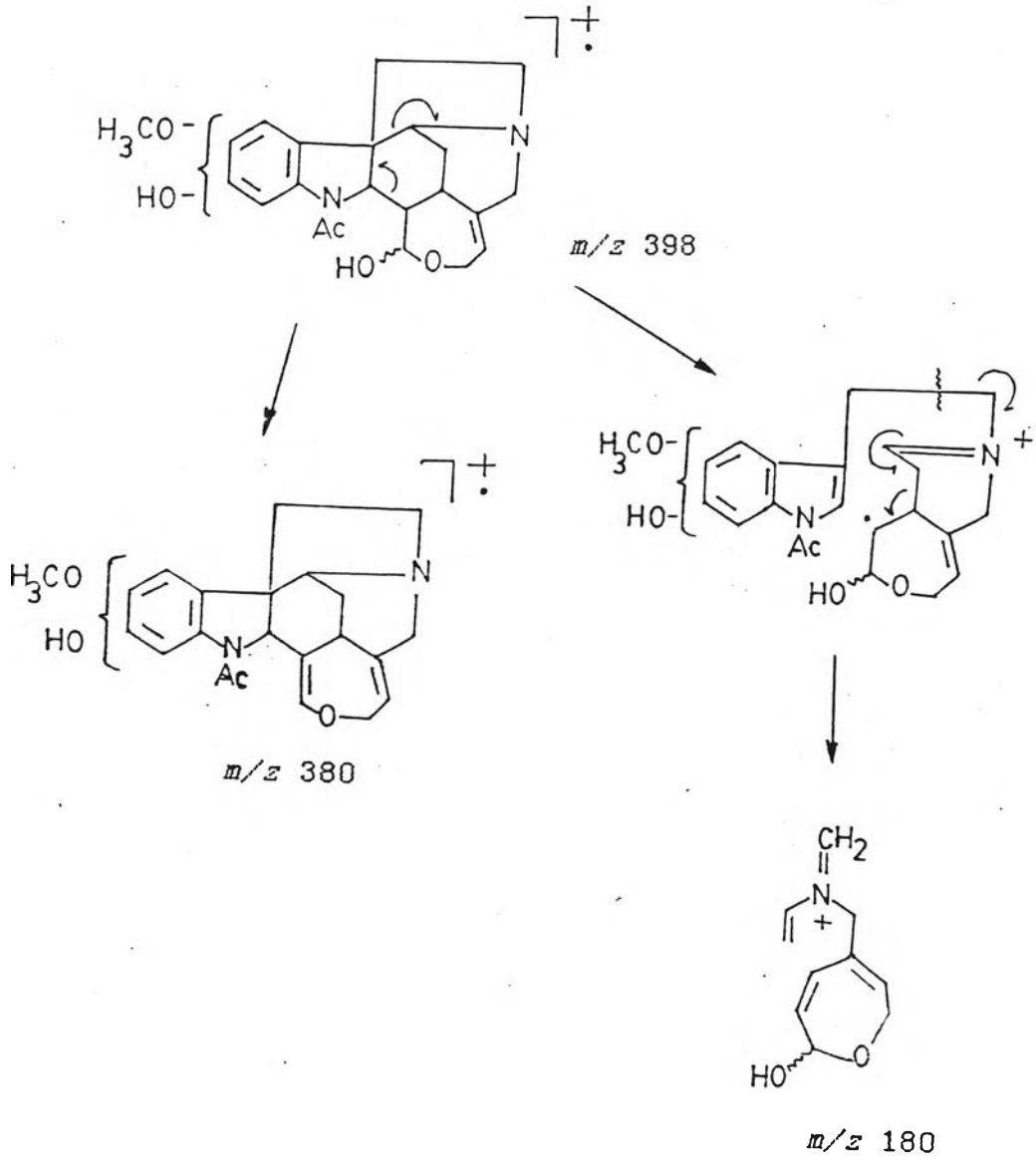
The mass fragment at *m/z* 180 indicates the presence of Wieland-Gumlich aldehyde derivative (50) (Hesse, 1973), while the mass fragment at *m/z* 380 indicates the hydroxyl substituted at C-17 of the skeleton.

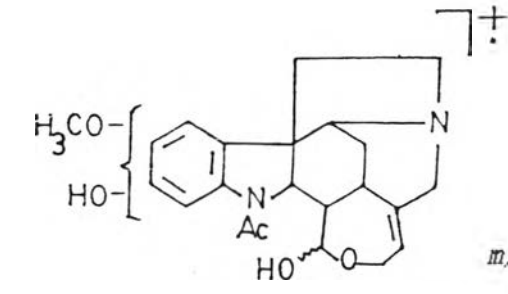


50 Wieland-Gumlich aldehyde

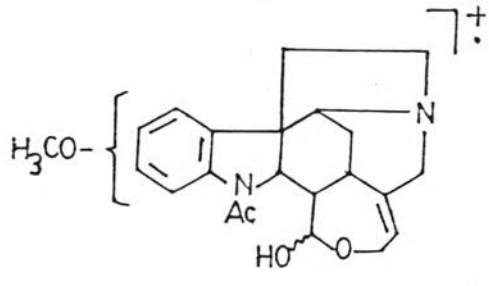
The other important fragments are observed as the very high relative intensities at *m/z* 382, 369, 353, 352 and 339 and recognized as a closely related fragments of Wieland-Gumlich aldehyde skeleton with an acetyl, a hydroxyl and a methoxyl substitutions.

The fragmentation patterns described above are shown as follows :

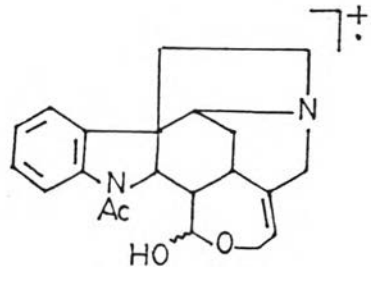

H3CO-
HO-
N
Ac
HO
O
m/z 398
H3CO
HO
m/z 380
CH2
m/z 180



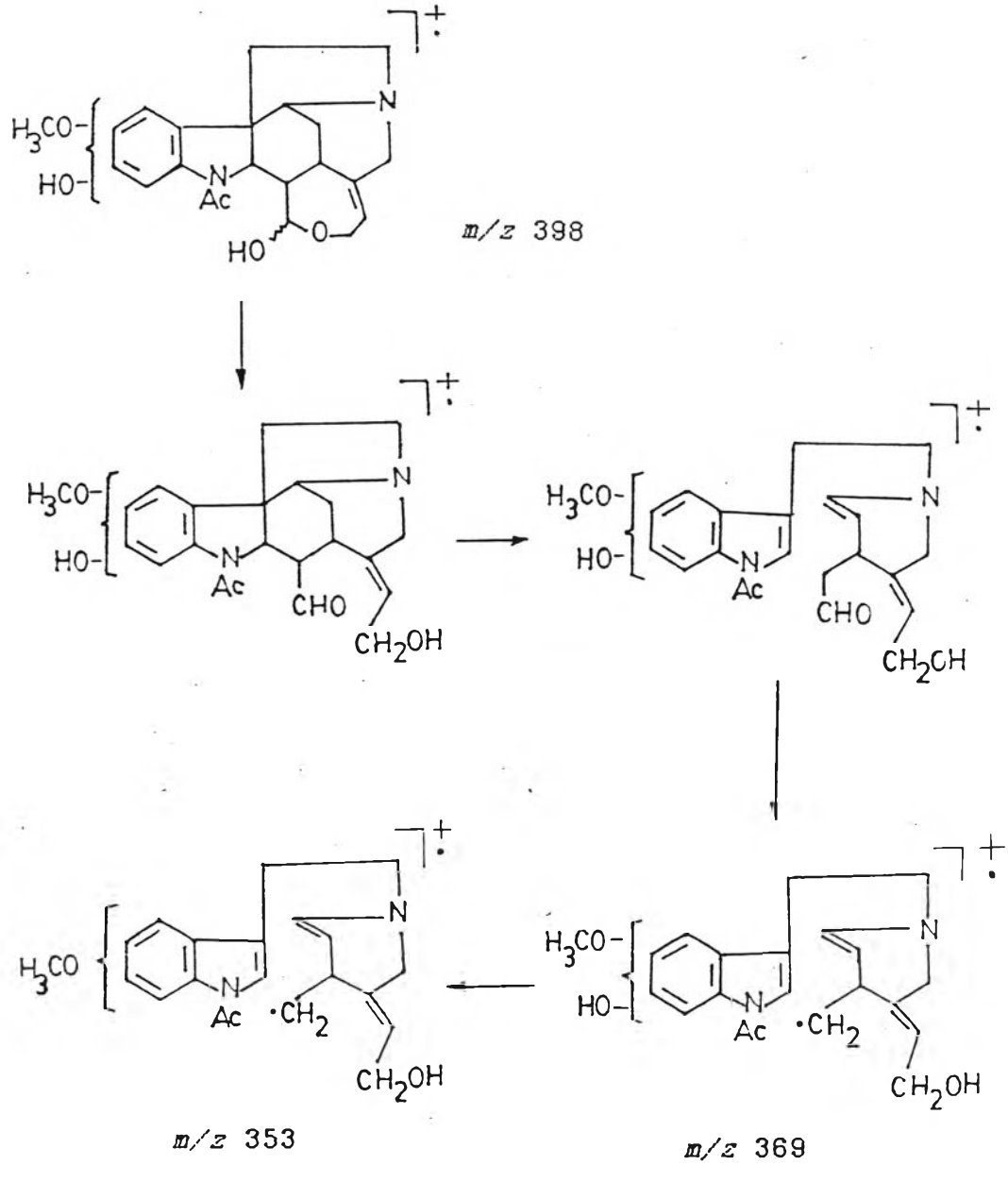
m/z 398



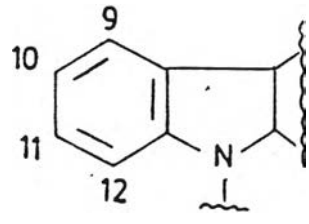
m/z 382



m/z 352

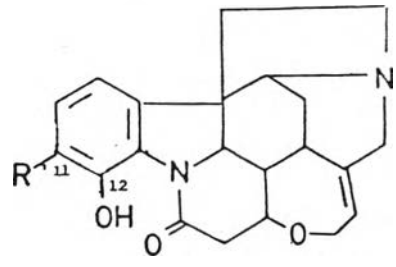

H3CO-
HO-
N
Ac
HO
O
m/z 398
CHO
CH2OH
CH2CH
·CH2
m/z 353
m/z 369

In the ^1H NMR spectrum, there are two three-proton singlets, one at δ 3.87 ppm, probably are aromatic methoxyl protons whereas one at δ 2.45 ppm, probably due to a N_a-acetyl protons. The two adjacent aromatic protons are observed as doublets (J = 8 Hz) at δ 6.73 and δ 6.57 ppm, from which a 11,12-, 9,10- or 9,12-substitution of indole nucleus can be expected.



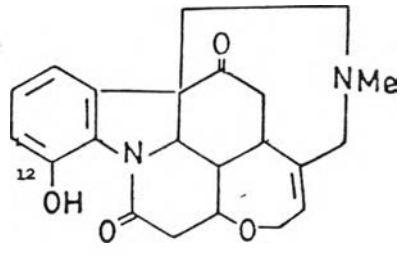
The substitution at 9,10- and 9,12- are less likely in the view of the fact that no base with a substituted at the position 9 has been discovered in strychnan type alkaloid of *Strychnos* species base on chemotaxonomical evidence (Bisset, and Khalil, 1976). Moreover, the 11,12-substitution is quite common for *Strychnos* alkaloids such as 12-hydroxy-11-methoxy-strychnine (139).

Furthermore, the singlet at δ 10.18 ppm also agrees with the presence of a phenolic hydroxyl group. This phenolic hydroxyl group is shifted as far down-field as which found in 12-hydroxy-strychnine (84) and vomicine (148), probably due to the hydrogen bonding with the acetyl group at N_a. The rotation of acetyl group is common for all other N_a-acetyl strychnan alkaloids, but the ^1H NMR



139 12-OH-11-OMe-strychnine ; R = OCH_3

84 12-OH-strychnine ; R = H



148 Vomicine

spectrum of this alkaloid shows rather clear and sharp signals for all protons. It can be explained by the hydrogen bonding between the hydroxyl group and the acetyl group that reduces the chance of possessing the rotation. From these spectral informations, it can be assumed that the neighbouring functional group of the N_a-acetyl function must be the hydroxyl group, not methoxyl group. The methoxyl group and hydroxyl group must be placed at position 11 and 12 respectively.

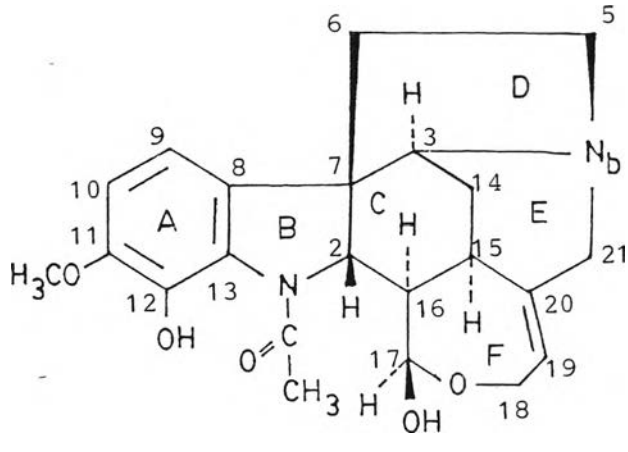
The 22 carbon atoms of the alkaloid are confirmly detected in ^{13}C NMR spectrum. The assignments of each carbon and proton for their chemical shift are shown in table 7 and 8.

In consequence of melting point, colour reaction, UV, IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The alkaloid A-1 is undoubtedly identified as 12-hydroxy-11-methoxy-diaboline.

The stereochemistry of the alkaloid is the other point of interest. There are six carbon atoms that are characterized as the chiral centers , C-2, 3, 7, 15, 16 and 17. All of their stereochemistry, except for C-17, are known (structure no. 118 next page). The stereochemistry of C-17 can be observed from the ^{1}H NMR spectrum. In the ^{1}H NMR, the very small coupling constant between H-16,17 (2 Hz) indicated the presence of *cis* conformation between H-16,17. On the other hand, the ring F of diaboline derivatives is a 7-membered cyclic ether

and behaves like a sugar molecule. The configuration of 17-OH can be pointed to the axial position within the boat form and the equatorial position within the chair conformation.

The melting point, mass spectrum, ^{1}H, and ^{13}C spectral data of A-1 are unambiguously identical with those previous publish of henningsoline (Biemann et al., 1965; Ohiri, Verpoorte and Baerheim Svendsen 1984; Thepenier et al., 1988). Thus alkaloid A-1 can be identified as the known alkaloid henningsoline (12-hydroxy -11-methoxy- diaboline)

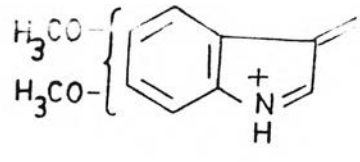


118 Henningsoline

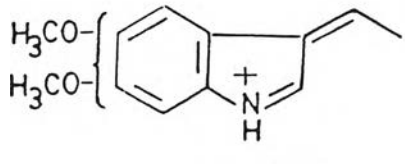
AA-1

The alkaloid AA-1 gives grayish-brown colour with ferric chloride-perchloric acid spraying reagent and its UV absorption maxima (235.0, 250.8, 282.3 and 318.5 nm) typifies for an indole chromophore (Verpoorte, 1986) such as those of henningsoline (118).

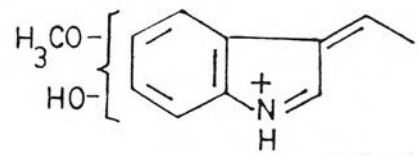
The mass spectrum shows a rather confused molecular ion peaks, probably because of the impurities. The important fragment is at *m/z* 180 which indicates the alkaloid of Wieland-Gumlich aldehyde skeleton (50). The indole moiety fragments at *m/z* 176 and 190 are shift by 46 mass units from the usual indole fragments. These shifts are indicative of the presence of a methoxyl and a hydroxyl groups. Another possibility is the dimethoxyl substitutions which usually give the indole fragments at *m/z* 190 and 204. However, the *m/z* 204 mass fragment is not observed in the spectrum. The *m/z* 176 fragment is produceed by lossing of the $-CH_2$ from methoxyl groups.



m/z 190



m/z 204



m/z 176

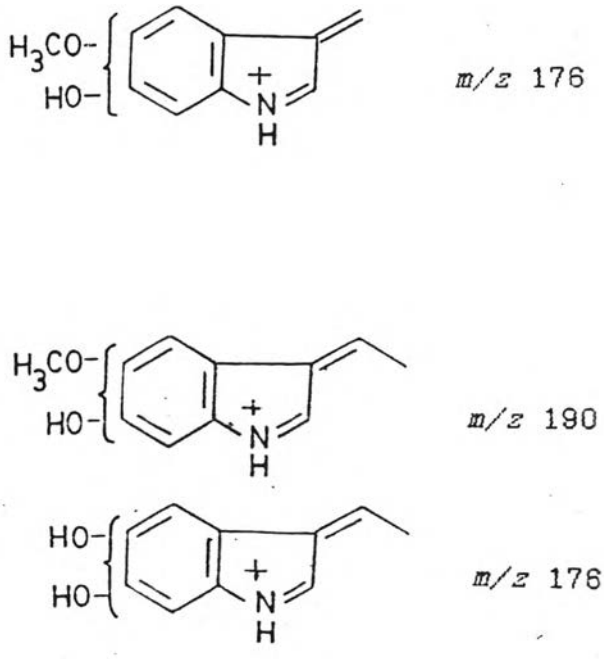
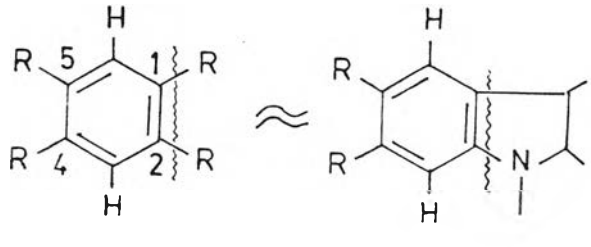


Figure 12 : Some important indole fragment ions in the mass spectrum of AA-1

The presence of an acetyl group is indicated by the mass fragment at *m/z* 43 together with the intense band at 1635 cm^{-1} in the IR spectrum. This vibration band (1635 cm^{-1}) usually is an indicative of an amide carbonyl function. Thus the acetyl group is placed at N_a of the indole moiety.

The 90 MHz ^1H NMR spectrum shows three-proton singlet at δ 2.433 ppm verifying the presence of an acetyl protons in the molecular structure. Other unclear six-proton singlet at δ 3.87 ppm should represent the two aromatic methoxyl protons. But the two aromatic methoxyl groups are unlikely due to the presence of a phenolic hydroxyl group at 10.078 ppm. Thus the substitutions on the aromatic part must be a methoxyl group and a hydroxyl group.

Moreover, the 90 ^1H NMR spectrum shows two superimposed aromatic singlet protons at 7.266 ppm. Usually, a two-proton singlet would be recognized as the two para-aromatic protons or two protons of 1,2,4,5-tetrasubstituted aromatic nucleus as follows.



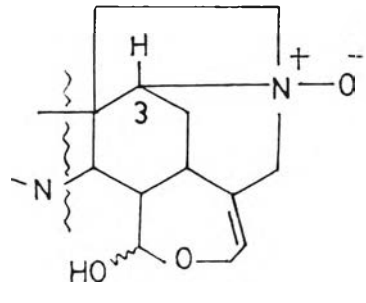
Because of low resolution of the 90 MHz ^1H NMR data, the assignment of proton signals espectially in the aromatic and methoxyl regions would mislead of the interpretation. When the 300 MHz ^1H NMR is considered (figure 25), the aromatic proton signals are observed as the two very nearly superimposed doublets which indicated that the two substituted functional groups must be placed at the ortho, not para, positions. The 11,12-substitution pattern is quite common for *Strychnos* alkaloids.

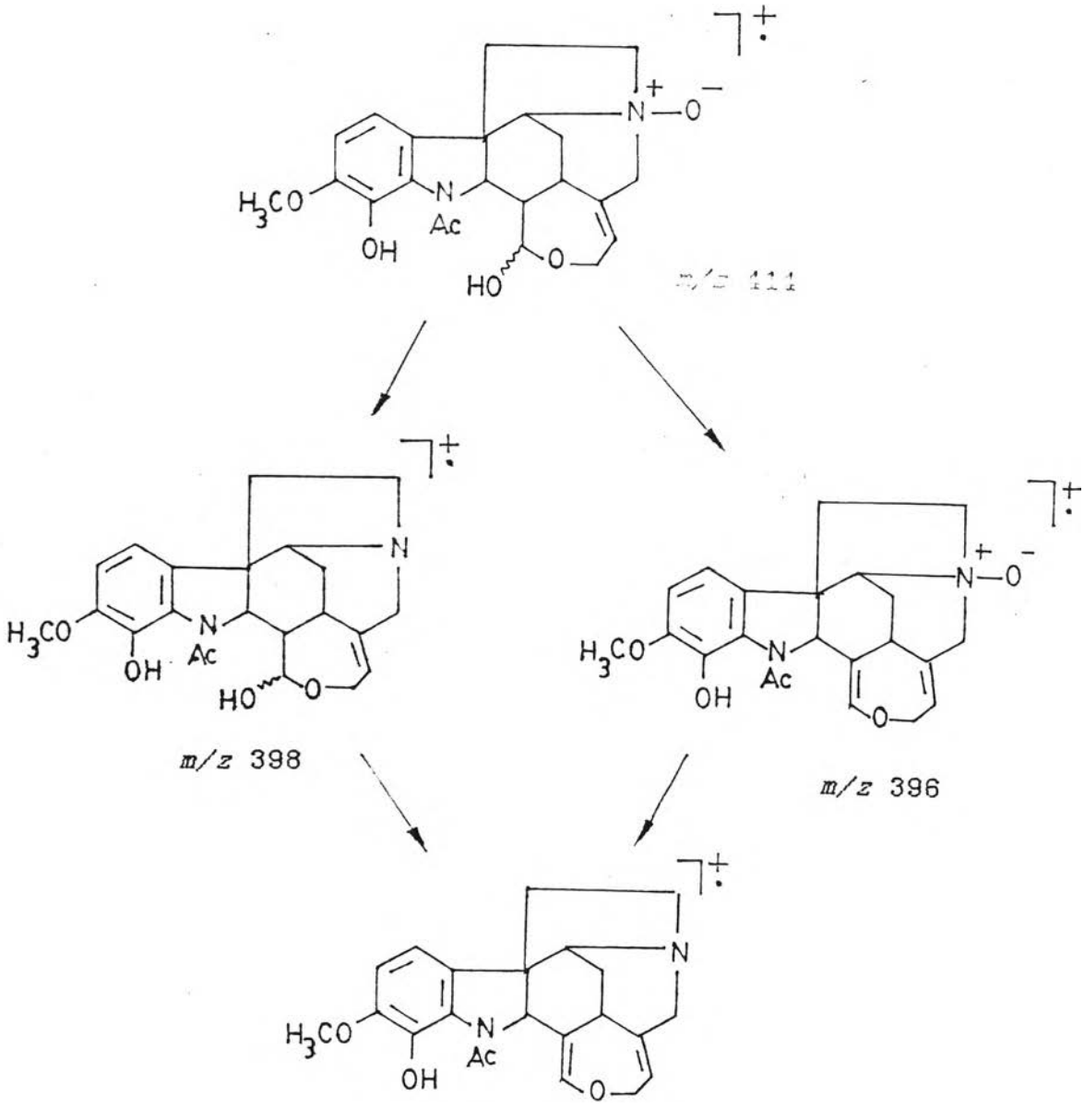
The signal of the phenolic hydroxyl group is shift so far down-field (10.07 ppm), probably due to the hydrogen bonding with the acetyl group at N_b as that of A-1. This information shows that the hydroxyl group and the methoxyl group must be placed at 12 and 11 position, respectively.

In this compound, the hydrogen bond between 12-hydroxyl and acetyl group that suppressing the rotation of the acetyl group is not so efficient. The ^1H NMR spectra show rather board and excess signals, espectially the estimated six-proton singlet at δ 3.87 ppm of 90 MHz ^1H NMR spectrum. When 300 MHz spectrum is observed, that signal is clearly split into two singlets. This is possibly due to the rotamer formation in the magnetic field of AA-1.

Most of the ^1H NMR signals of the alkaloid AA-1 is similar to those of henningsoline (118) except for the chemical shifts of some protons. Many of the signals of

AA-1 show farther down-field chemical shifts, especially for the H-3 than that of henningsoline or other alkaloids with the same main skeleton. Usually the chemical shift of the H-3 is at about 3.8 ppm, while it is 4.37 ppm (300 MHz ^{1}H NMR spectrum) in this alkaloid. The formation of N_b-oxide is agreed for this phenomenon (see part of structure bellow). The N_b-oxide is a major cause in producing a different pattern of the aromatic protons of this alkaloid from A-1 too. The signal of H-9 is shifted a little. Furthermore the N_b-oxide can be used to explain the molecular ion and other related fragments in mass spectrum. All known *Strychnos* alkaloids which possess N_b-oxide function, usually show intense peak at *m/z* M^+-16, not at M^+ (Baser, 1978). The mass spectrum of alkaloid AA-1 should behave the same. It shows a small peak at the *m/z* 414 which is corresponding to the molecular formular $C_{22}H_{26}N_2O_6$. Some important fragments are shown in the next page.




m/z 398
m/z 396
m/z 380

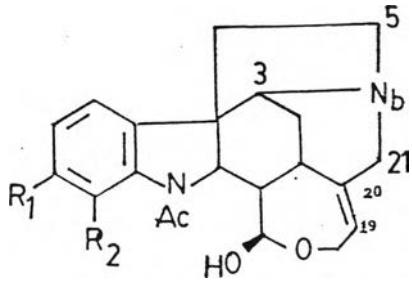
The 22 carbon atoms and the N_b-oxide of this alkaloid AA-1 are confirmed by ^{13}C NMR. The assignment of each carbon can be set out by comparison with those of the alkaloid henningsoline (118), and diaboline (117). It is noticed that the chemical shifts of the neighbouring carbon atoms of N_b-oxide, such as C-3, 5 and 21, are shifted to more down-field than those of the mentioned compounds (table 6). Furthermore, the chemical shifts of C-20 is shifted up-field and that of C-19 down-field. This may be explained by the effect of the positively charged nitrogen on the π-electrons of 19,20-double bond, causing shielding at C-20 and deshielding at C-19 (Verpoorte, Hylands, and Bisset, 1977).

From all of the above informations, the alkaloid AA-1 is in agreement with that of 12-hydroxyl-11-methoxydiaboline N_b-oxide (149). The assignment of each proton and carbon for their chemical shift are set out in the table 7 and 8.

Table 6 : Some ^{13}C chemical shift assignment of AA-1 (149), A-1 (118), and diaboline (117)

carbon	chemical shift (ppm)		
	AA-1	A-1	diaboline*
C-3	81.760	58.887	59.4
C-5	69.300	51.574	51.9
C-19	133.720	126.334	127.7
C-20	138.069	143.020	143.0
C-21	71.980	53.416	53.2

* Wenkert, Cheung, and Gottlieb, 1978

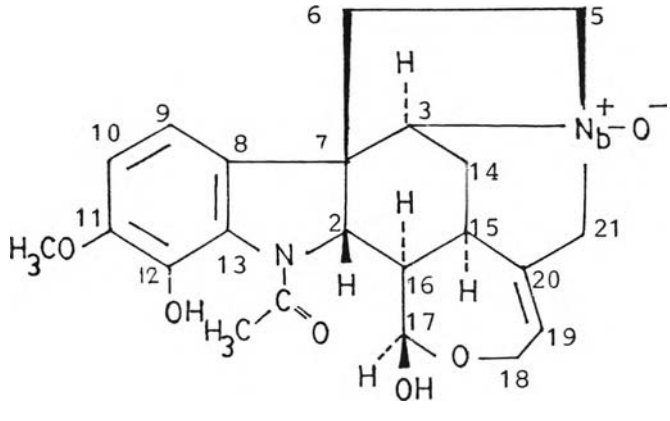


117 Diaboline ; $R_1 = R_2 = H$

118 A-1 ; $R_1 = OCH_3$, $R_2 = OH$

149 AA-1 ; $R_1 = OCH_3$, $R_2 = OH$, N_b-oxide

According to the regidity of the molecule, the stereochemistry of this new alkaloid is base on the structure of henningsoline (118). Only the 17-OH can be placed either at α- or β-position. The ^1H NMR spectrum shows a very small coupling constant between H-16 and H-17 (2 Hz). Thus the 17-OH should be placed at the β-position. The molecular structure and stereochemistry of 12-hydroxy-11-methoxy-diaboline N_b-oxide or henningsoline N_b-oxide (149) are shown below.



149 Henningsoline N_b-oxide

Table 7 : The ^{1}H NMR chemical shift assignment of A-1 and AA-1

proton position	chemical shift (ppm)	
	A-1	AA-1
H-2	4.22 (d, $J_{2,16}$=11 Hz)	4.28 (d, $J_{2,16}$=11 Hz)
H-3	3.86 (s)	4.37 (s)
H-5α	3.34 (dd, $J_{5\alpha,5\beta}$=10 Hz; $J_{5\alpha,6\alpha}$=7 Hz)	3.9-4.0 (m)
H-5β	2.81 (m, $J_{5\beta,5\alpha}$=10 Hz; $J_{5\beta,6\alpha}$=12 Hz; $J_{5\beta,6\beta}$=6 Hz)	
H-6α	1.72 (ddd, $J_{6\alpha,6\beta}$=12 Hz; $J_{6\alpha,5\beta}$=12 Hz; $J_{6\alpha,5\alpha}$=7 Hz)	0.8-1.0 (m)
H-6β	1.92 (dd, $J_{6\beta,6\alpha}$=12 Hz; $J_{6\beta,5\beta}$=6 Hz)	
H-9	6.73 (d, $J_{9,10}$=8 Hz)	6.78 (d, $J_{9,10}$=8 Hz)
H-10	6.57 (d, $J_{10,9}$=8 Hz)	6.73 (d, $J_{10,9}$=8 Hz)
H-14α	1.40 (td, $J_{14\alpha,14\beta}$=14 Hz; $J_{14\alpha,15}$=2 Hz; $J_{14\alpha,3}$=2 Hz)	1.28 (td, $J_{14\alpha,14\beta}$=14 Hz)
H-14β	2.21 (td, $J_{14\beta,14\alpha}$=14 Hz; $J_{14\beta,15}$=4 Hz; $J_{14\beta,3}$=4 Hz)	1.98 (td, $J_{14\beta,14\alpha}$=14 Hz)

Table 7 (continue)

proton position	chemical shift (ppm)	
	A-1	AA-1
H-15	3.40 (s)	3.54 (s)
H-16	1.57 (td, $J_{16,2}$=11 Hz; $J_{16,15}$=2 Hz; $J_{16,17}$=2 Hz)	1.60 (td, $J_{16,2}$=11 Hz)
H-17	5.30 (d, $J_{17,16}$=2 Hz)	5.31 (d, $J_{17,16}$=2 Hz)
H-18α	3.68 (dd, $J_{18\alpha,18}$ =14 Hz; $J_{18\alpha,19}$=7 Hz)	3.77 (dd, $J_{18\alpha,18}$ =14 Hz; $J_{18\alpha,19}$=7 Hz)
H-18β	4.84 (dd, $J_{18\beta,18\alpha}$=14 Hz; $J_{18\beta,19}$=5 Hz)	4.86 (dd, $J_{18\beta,18\alpha}$=14 Hz; $J_{18\beta,19}$=5 Hz)
H-19	5.89 (t)	6.25 (t)
H-21α	3.70 (d, $J_{21\alpha,21\beta}$=15 Hz)	4.13 (d, $J_{21\alpha,21\beta}$=12 Hz)
H-21β	2.72 (d, $J_{21\beta,21\alpha}$=15 Hz)	3.94 (d, $J_{21\beta,21\alpha}$=12 Hz)
$NCOCH_3$	2.45 (s)	2.43(s), 2.50*(s)
11-OCH_3	3.87 (s)	3.87 (s), 3.88*(s)
12-OH	10.18 (s)	10.07 (s)

s = singlet, d = doublet, t = triplet, m = multiplet,

td = triplet of doublet,

dd = doublet-doblet, ddd = doublet-doublet-doublet,

J = coupling constant, * = rotamer

Table 8 : The ^{13}C NMR chemical shift assignment of A-1 and AA-1

carbon position	chemical shift (ppm)	
	A-1	AA-1
C-2	66.905	65.846
C-3	58.887	81.760
C-5	51.574	69.300
C-6	38.193	34.800
C-7	53.036	54.167
C-8	131.246	125.615
C-9	112.141	112.326
C-10	110.407	110.479
C-11	150.225	151.000
C-12	137.982	136.758
C-13	130.018	129.548
C-14	25.191	27.709
C-15	28.712	29.318
C-16	45.885	46.539
C-17	93.667	92.420
C-18	55.203	54.167
C-19	126.334	133.720
C-20	143.020	138.069
C-21	53.416	71.980
$NCOCH_3$	22.861	23.061
$NCOCH_3$	172.654	172.810
OCH_3	56.612	56.550

Biogenetic discussion on the isolated alkaloids

Both alkaloids isolated in this investigation are classified as the diaboline group of strychnan type alkaloid. According to the biogenetical proposal, all of the terpenoid indole alkaloids are derived from the central intermediate, strictosidine (36). Strictosidine (36) is derived to the strychnan type alkaloid *via* 4,21-dehydrocorynantheine (51), 4,21-dehydrogeissoschizine (52), dehydroakuammicine (49) and nor-C-fluorocurarine (54). The summarization of this pathway is previously presented in chapter I.

The hydroxylation of nor-C-fluorocurarine (54) gives rise to 18-hydroxy-nor-C-fluorocurarine (150). This compound is clearly to be the precursor of Wieland-Gumlich aldehyde (open form) (151) and Wieland-Gumlich aldehyde (close form) (50). Diaboline (117) is the N_a-acetyl derivative of Wieland-Gumlich aldehyde (close form) (50) and seemingly the end product of the biosynthetic pathway in many *Strychnos* species (Bisset, 1980).

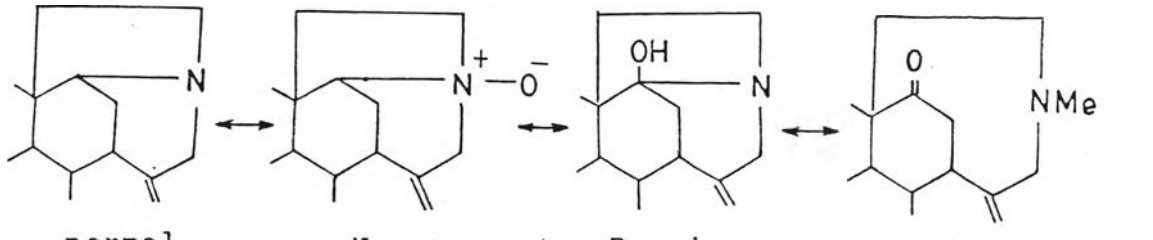
Up till now, the alkaloids with the skeleton of Wieland-Gumlich aldehyde (50) and diaboline (117) have been shown only two different substitution patterns; 11-methoxy and 12-hydroxy-11-methoxy substitutions. These substitutions are proceeded through the aromatic-oxidation (Bisset, 1980). It is interesting to prove that the aromatic-oxidation reaction is taken place on somewhere of

the biosynthetic pathway. The fact is that 11-methoxy and 12-hydroxy-11-methoxy substitutions have not been found in any intermediate compounds, and also in other *Strychnos* alkaloids except for some groups of strychnan type such as strychnine group (17) and spermostrychnine group (18). This information indicates that the aromatic-oxidation occures on the substituted indole alkaloid after the strychnan skeleton is completely formed. Thus the steps before forming of the two isolated alkaloids must involve the aromatic-oxidation of Wieland-Gumlich aldehyde (close form) (50). Though it could not be concluded that the substitution reaction or the condensation with an acetyl group to become diaboline derivatives (15) is taken place in the very last step.

One of the alkaloid isolated from *Strychnos minor* Dennst. is in the form of *N*-oxide. The *N*-oxide alkaloids are often present in several plants, including the *Strychnos* species. The control experiments indicate that they are not artefact, but the possibility of their occurrence has not yet been investigated. The studies on the *N*-oxide alkaloids with pyrrolizidine skeleton suggested that the *N*-oxide alkaloids would be the compound extremely well suited for transportation or reservation (Borstel and Hartmann, 1986; Toppel, Witte, and Hartmann, 1988).

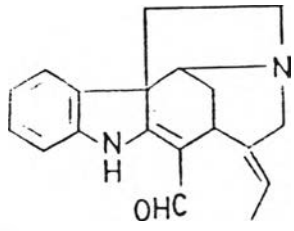
In strychnan type alkaloid not only *N*-oxide but also pseudo and *N*-methyl-*sec*-pseudo series have been

detected. There is a tendency on going from the roots to the leaves of the plants for the conversion of the normal series base to become the corresponding pseudo series *via* the *N*-oxides (Bisset and Phillipson, 1976; Bisset, 1980).

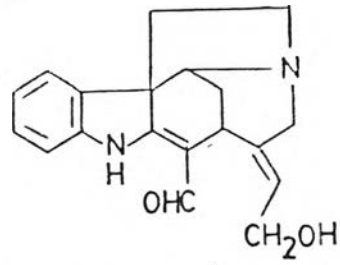


normal series — *N*-oxide — Pseudo or 3-OH series — *N*-Methyl-*sec*-pseudo series

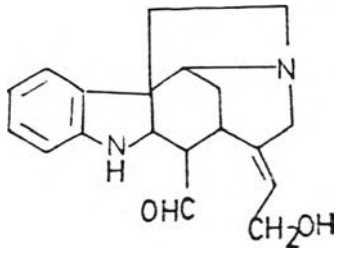
For conclusion, the biogenetical proposal of henningsoline (118) and henningsoline N_b-oxide (149) can be shown as follows in figure 13.



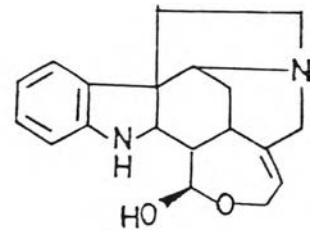
54 Nor-C-fluorocurarine



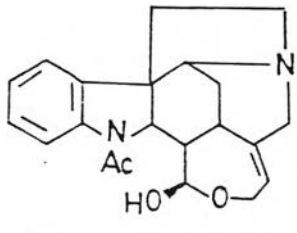
150 18-OH-nor-C-fluorocurarine



151 Wieland-Gumlich aldehyde (open form)



50 Wieland-Gumlich aldehyde (closed form)



117 Diaboline

b

a

b

a

A-1 ⟶ AA-1

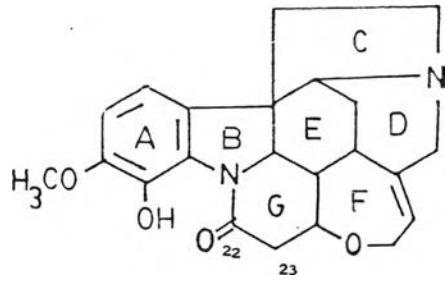
Figure 13 : The possible biosynthetic routes leading to henningsoline (A-1) (118), and henningsoline N_b-oxide (AA-1) (149)

a = aromatic-oxidation and methylation

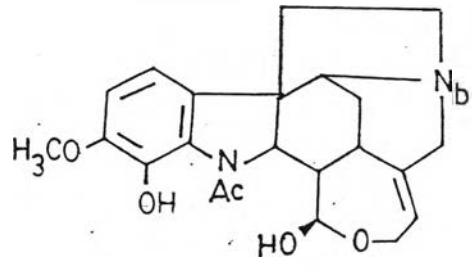
b = acetylation

Furthermore, the molecular structures and the substitution patterns of henningsoline (118) and henningsoline N_b-oxide (149) are similar to those of 12-hydroxy-11-methoxy-strychnine (139) except for the lack of lactamization of ring G. In *Strychnos minor* Dennst. henningsoline (118) and henningsoline N_b-oxide (149) are found together without the presence of 12-hydroxy-11-methoxy-strychnine (139). But for the latter alkaloid which possess lactam function is found commonly in *S. nux-vomica* L. (Baser, 1976) and *S. wallichiana* Steud. ex DC. (Bisset and Phillipson, 1973).

This finding is supporting the hypothesis that the extra two carbon atoms C-22 and C-23 of strychnine derivatives (17) must come from another pathway rather than the ring closure between C-17 and N_a-acetyl function of diaboline derivatives (15). Heimberger and Scott (1973) found that Wieland-Gumlich aldehyde (close form) (50) is a precursor of strychnine (1) by feeding experiment. The C-22 and C-23 of strychnine were proved to come from the condensation of an acetate unit at C-17 of Wieland-Gumlich aldehyde (open form) (151) to produce the intermediate prestrychnine (152) (Schlalter et al., 1969), protostrychnine (153) (Bisset, 1980) and final ring closure to yield the corresponding strychnine (1).



139 12-Hydroxy-11-methoxy-strychnine



118 Henningsoline

149 Henningsoline N_b-oxide ;N_b-oxide

Probably the enzymes that involve in the biosynthesis of strychnine derivative (17) are different from those of diaboline derivatives (15) and they are absent in *S. minor* Dennst. while it is present in *S. nux-vomica* L. and *S. wallichiana* Steud. ex DC.

The relationships between diaboline derivatives (15) and strychnine derivatives (17) are shown in figure 14.

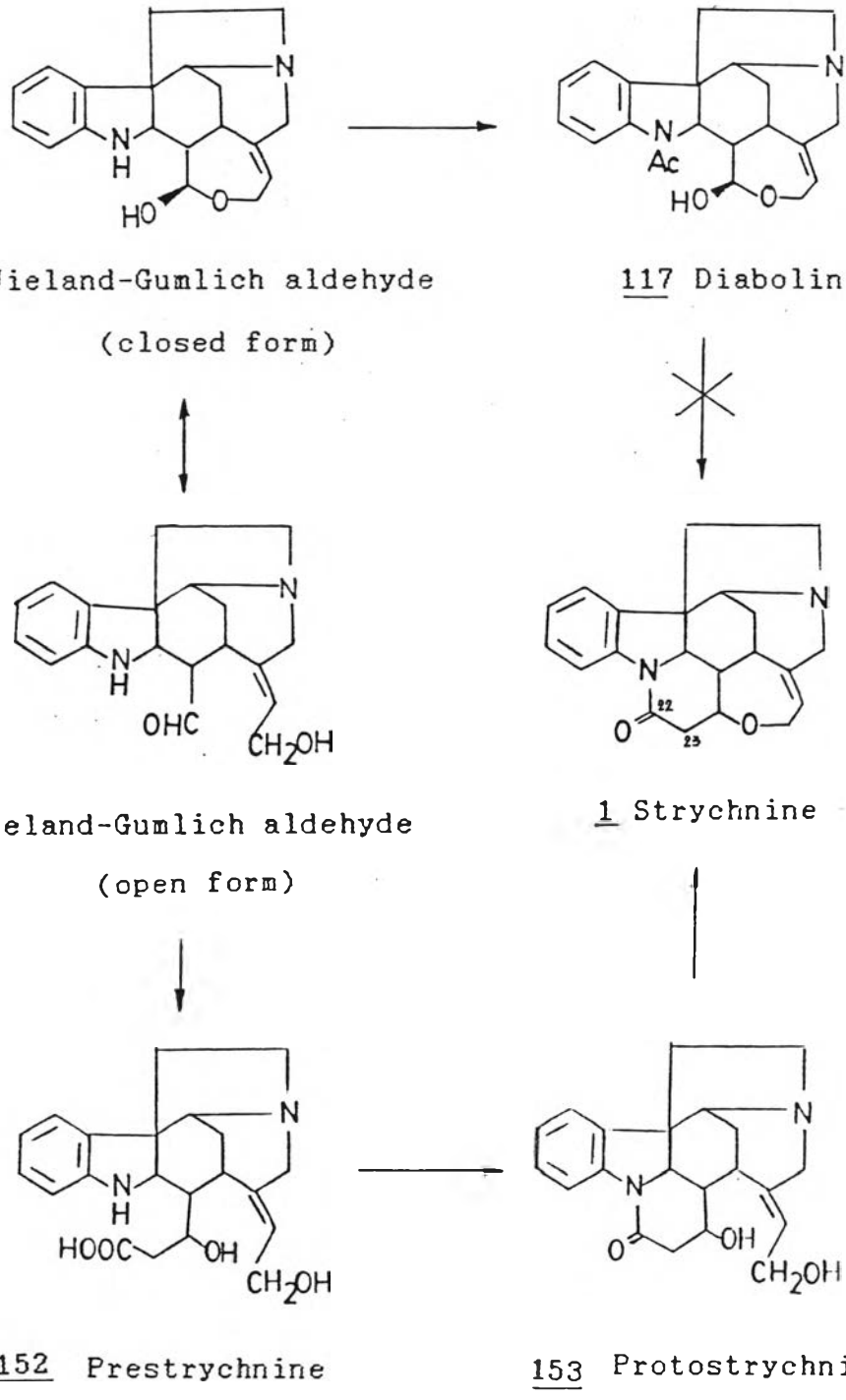


Figure 14 : Biosynthetic relationship between strychnine derivatives and diaboline derivatives.

Chemotaxonomic significance of the isolated alkaloids

The two isolated alkaloids are classified as the diaboline group. Henningsoline N_b-oxide (149) is the new compound while henningsoline (118) is found in *Strychnos minor* Dennst. as the fifth natural source. The occurrence of henningsoline (118) in natural source is shown in table 9.

Table 9 : The occurrence of henningsoline (118) in natural source

plant	part	reference
Strychnos henningsii Gilg.	bark	Grossert et al., 1965
S. spinosa Lam	stem bark	Ohiri et al., 1984
S. cathayensis Merr.	seed	Renrong, and Lida, 1985
S. staudtii Gilg	root bark, stem bark, leaves	Thepenier et al., 1988

In America, almost all *Strychnos* species that contain alkaloids of diaboline group are in the Section Strychnos, only two spcies are in Section Breviflorae. In Africa, this type of bases has been found in several Sections, mostly in the Section Breviflorae. In Asia, the diaboline group have been mostly found in Section Strychnos and Lanigerae, only two species are in Section Rouhamon and Brevitubae (Table 10).

Table 10 : Distribution of diaboline derivatives in *Strychnos* species.

(Bisset, and Phillipson, 1976; Kisakürek, Leeuwenberg, and Hesse, 1983; Southon, and Buckingham, 1989)

America	50 Wieland-Gumlich aldehyde	117 Diaboline	154 Ethyl-diaboline	155 3-Hydroxy-diaboline	157 Henningsumine	159 Hemitoxiferine I	158 Jobertine	161 11-Methoxy-diaboline
Section Breviflorae								
S. castelneana Wedd.		/	/	/			/	
S. rubiginosa A.DC.								/
Section Strychnos								
S. amazonica Krukoff								/
S. brachiata Ruiz & Pavon	/							/
S. chlorantha Prog.		/			/			
S. diaboli Sandw.	/	/						
S. erichsonii Rich. Schomb.		/						
S. froesii Ducke	/	/						
S. gardneri A.DC.		/						/
S. jobertiana Baill.		/			/		/	
S. medeola Sagot ex Progel								/
S. mitscherlichii Rich. Schomb.	/	/						
S. panamensis Seem.		/						
S. pseudo-quina A.St.Hil.		/						
S. romeu-belenii Krukorff & Barneby								/
S. rondeletioides Spruce ex Benth.		/						
S. solerederi Gilg.	/	/						
S. solimoesana Krukoff		/						
S. toxifera Rob. Schomb.						/		

Table 10 (continue)

Africa	50 Wieland-Gumlich aldehyde	117 Diaboline	156 2,16-Dehydro-diaboline	157 Henningsamine	118 Henningsoline	160 *O*-Acetyl-henningsoline	161 11-Methoxy-diaboline	162 Condensamine	163 epi-17-O-methyl-11-methoxy-diaboline	164 11-methoxy-Wieland-Gumlich aldehyde	165 17-*O*-methyl-11-methoxy-Wieland-Gumlich aldehyde	167 11-Methoxy-2,16-dehydro-diaboline	166 12-Hydroxy-11-methoxy-henningsamine
Section Breviflorae													
S. afzelii Gilg	/	/											
S. angolensis Gilg	/						/		/	/	/		
S. dolichothyrsa Gilg ex Onochie et Hepper	/						/						
S. henningsii Gilg	/	/	/	/	/	/	/	/				/	
S. malacoclados C.H.Wright							/						
Secion Densiflorae													
S. Staudtii Gilg *					/		/	/					/
Section Lanigerae													
S. chrysophylla Gilg	/												
S. kasengaensis De Wild.	/												
Section Penicillatae													
S. matopensis S. Moore**	/	/					/						
S. longicaudata Gilg **	/												
Section Rouhamon													
S. potatorum L.f.		/											
Section Spinosae													
S. spinosa Lam.					/		/						

* Thepenier et al., 1988

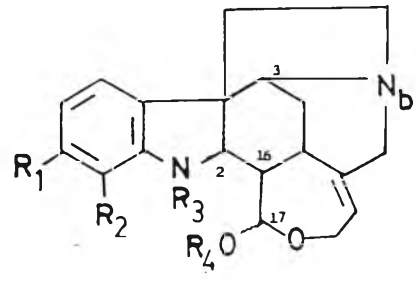
** Massiot et al., 1988

Table 10 (continue)

Asia	50 Wieland-Gumlich aldehyde	117 Diaboline	147 Methoxy-diaboline	157 Henningsamine	158 Jobertine	118 Henningsoline	161 11-Methoxy-diaboline	148 Henningsoline N_b-oxide
Section Brevitubae								
S. umbellata (Lour.) Merr.		/						
Section Lanigerae								
S. ledermannii Gilg et Bened		/		/				
S. minor Dennst.		/	/		/*	/**		/**
S. oleifolia A.W.Hill		/						
Section Rouhamon								
S. potatorum L.f.		/			/*			
Section Strychnos								
S. cathayensis Merr.		/				/	/	
S. ignatii Berg.	/	/						
S. lucida R.Br.		/						
S. nux-blanda A.W.Hill		/						
S. nux-vomica L.	/	/						

* paper did not show its stereochemistry

** present work



		R_1	R_2	R_3	R_4	
50	Wieland-Gumlich aldehyde	H	H	H	H	
117	Diaboline	H	H	Ac	H	
118	Henningsoline	OMe	OH	Ac	H	
147	Methoxy-diaboline	aromatic OMe		Ac	H	
149	Henningsoline N_b-oxide	OMe	OH	Ac	H	
154	Ethyl-diaboline	H	H	Ac	Et	
155	3-Hydroxy-diaboline	H	H	Ac	H	3-OH
156	2,16-Dehydro-diaboline	H	H	Ac	H	2,16-dehydro
157	Henningsamine	H	H	Ac	Ac	17-α-H
158	Jobertine	H	H	Ac	Ac	17-β-H
159	Hemitoxiferine I	H	H	Me	H	
160	*O*-Acetyl-henningsoline	OMe	OH	Ac	Ac	
161	11-Methoxy-diaboline	OMe	H	Ac	H	
162	Condensamine	OMe	H	Ac	Ac	
163	epi-17-*O*-methyl-11-methoxy-diaboline	OMe	H	Ac	Me	
164	11-methoxy-Wieland--Gumlich aldehyde	OMe	H	H	H	
165	17-*O*-methyl-11-methoxy-Wieland-Gumlich aldehyde	OMe	H	H	Me	
166	12-Hydroxy-11-methoxy-henningsamine	OMe	OH	Ac	Ac	
167	11-Methoxy-2,16-dehydro-diaboline	OMe	H	Ac	H	2,16-dehydro

From table 10, it can be noted that the diaboline derivatives with aromatic substitutions are found mostly in African *Strychnos*. Only the alkaloid 11-methoxy-diaboline (161) is found in several species of American *Strychnos*. For Asian species, only *S. cathayensis* Merr. and *S. minor* Dennst. are found to contain alkaloids with aromatic substitution on diaboline derivatives. *S. cathayensis* Merr. is in the section Strychnos and has been found to contain the alkaloid henningsoline (118) and 11-methoxy-diaboline (161). *S. minor* Dennst. used in the present work is in the Section Lanigerae. It is found to contain the alkaloid henningsoline (118) and a new alkaloid henningsoline N_b-oxide (149). All of these *Strychnos* species must have some relationships.

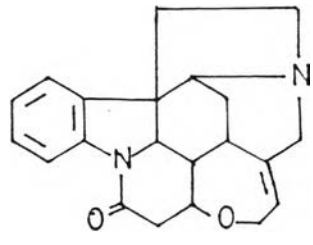
It has been known that the Section Strychnos of Asia continent is a rich source of strychnine derivatives (17). Although it contains some diaboline derivatives (15) but in only small amount. This finding is opposite to those of American species. *S. cathayensis* Merr. is the only one Asian species that contains diaboline derivatives (15) as the main compounds without the presence of strychnine derivatives (15). The chemotaxonomical relationships among the Section Strychnos of Asia, Africa and America are barred by the absence of this Section in Africa.

For the Asian Lanigerae, it has been only a screening investigation for their phytochemistry (Bisset,

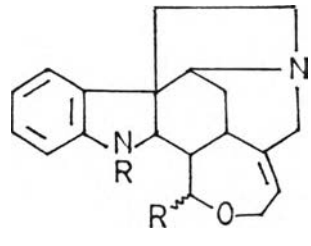
and Phillipson, 1976). Strychnine group (17), diaboline group (15) and also angustine group (13) were found in this plant's Section. Diaboline derivatives (15) was detected as a major compound in *S. ledermannii* Gilg et Bened and *S. oleifolia* A.W.Hill, and also *S. minor* Dennst. in this present work. It is strange that the same Section in Africa, some species are found to contain the spermostrychnine group (18) without the presence of diaboline group (15) (Kisakurek, Leewenberg, and Hesse, 1983).

According to the proposed biosynthesis of strychnan type alkaloids; strychnine (17), diaboline (15) and spermostrychnine groups (18) are derived from the same precursor, Wieland-Gumlich aldehyde (open form) (151) (Bisset, 1980). The *Strychnos* species of Section Lanigerae in present time between these two continents are possibly come from a same ancient unknown *Strychnos* species. In Africa, the *Strychnos* species can be divided in to two groups. One develops for the biosynthesis of alkaloids of spermostrychnine group (18) and another for those of diaboline group (15). These is the same as Asian *Strychnos* species which produce alkaloid of strychnine (17) and diaboline groups (15).

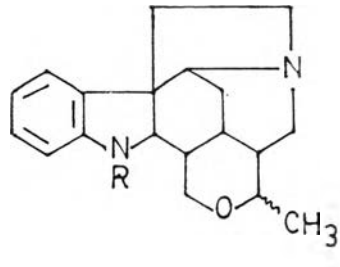
The Section Penicillatae is different from Section Lanigerae in view of alkaloid biosynthesis. Spermostrychnine group (18) was found in Asian species (Bisset and



17 Strychnine group



15 Diaboline group



18 Spermostrychnine group

Phillipson, 1976). while diaboline group (15) and strychnine group (17) (Bisset and Phillipson, 1971) were detected from African species.

The other two species of Asian *Strychnos* that have been found to contain diaboline derivatives (15) are *S. potatorum* L.f. of Section Rauhamon and *S. umbellata* (Lour.) Merr. of the Section Brevitubae. In Africa, the Section Rauhamon is a source of diaboline group (15) too, while not any of diaboline derivatives (15) have been found in Section Brevitubae but strychnine group (17) for instead (Bisset and Phillipson, 1971). The relationship of Section Brevitubae between Africa and Asia is probably similar to those of Section Lanigerae.

In Africa, the section Breviflorae is an available source of diaboline derivatives (15). This Section does not have plants distribute in Asia, only two American species have been found to contain diaboline group (15).

All of the informations point to the possibility of Section Lanigerae as an important source of diaboline derivatives (15) in Asia. While the Section Strychnos of America and Section Breviflorae of Africa provide the same type of compound. Base on the production of diaboline derivative (15), the Section Brevitubae, Rauhamon, Strychnos and Lanigerae of Asia; the Section Spinosae, Rauhamon, Penicillatae, Densiflorae and Breviflorae of Africa; as well as the Section Breviflorae and Strychnos

of America are chemotaxonomically related. The common Section Breviflorae is the chemotaxonomic linkage between the *Strychnos* species of America and Africa, while the Section Rauhamon and Lanigerae are represent the same for between Africa and Asia. It is noted that all of the Sections contain diaboline derivatives are closely related in the field of morphology suggested by Leewenberg (Bisset and Phillipson, 1971). All of these relationships are shown in figure 15.

The two isolated alkaloids from *S. minor* Dennst., one of the Asian Lanigerae, are similar to those present in several Sections of Africa. It is possible to suggest that this *Strychnos* species may be closely related to African *Strychnos* by chemical and morphological evidences.

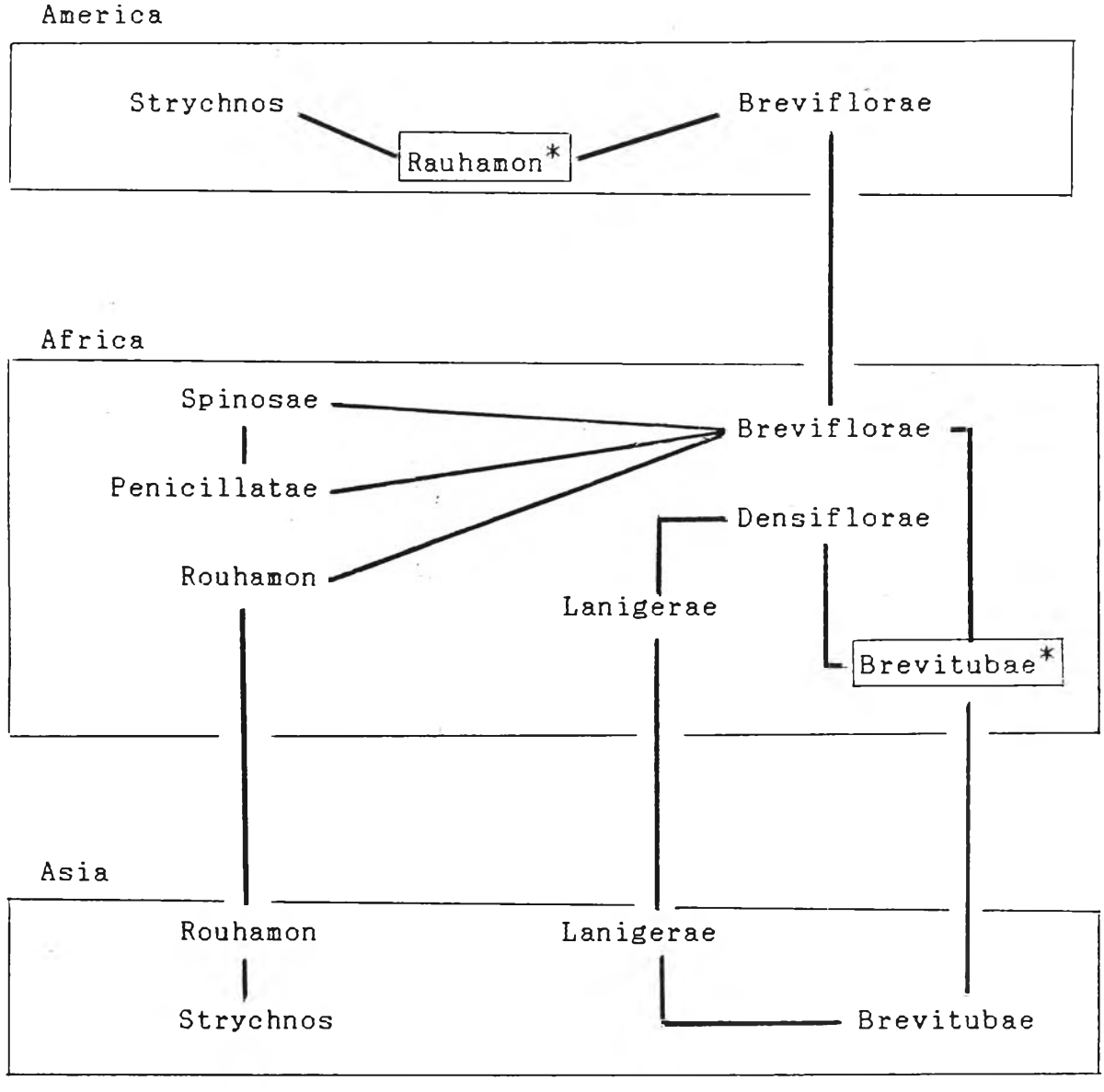


Figure 15 : Relationships among *Strychnos* Sections in each continent by the appearance of diaboline derivatives.

——— = close botanical relationships

* = having no report about diaboline derivatives

Proposed pharmacological activity of the isolated alkaloids

The activities of the two isolated alkaloids from *S. minor* Dennst., henningsoline (118) and henningsoline N_b-oxide (149), can be proposed due to the main skeleton diaboline (117). Diaboline (117) itself is a hypotensive agent (Singh and Kapoor, 1976), cytotoxic agent (Hokanson, 1976) and convulsive agent with a less activity than strychnine (1) (Ohiri et al.,1983).

The alkaloid henningsoline (118) was found to have cytotoxic activity too (Hokanson, 1976). Other activities may be the same as diaboline (117) but in different potency. The 12-OH substitution on the aromatic part is formed hydrogen-bond to the amide carbonyl. It effects in the increasing of the absorptive property and also the potency. The substitution on H-11 is found to decrease the convulsive activity (Ohiri et al., 1983), but no any data available about the hypotensive action. Furthermore the convulsive activity is decrease if the alkaloid is formed an *N*-oxide (Ohiri et al., 1983).

All of these informations indicate that the convulsant property of the alkaloid henningsoline (118) is increase and decrease by its aromatic substitutions and hypotensive action may be shown more or less equivalent to diaboline. The henningsoline N_b-oxide (149) may posscess a very weak convulsive agent, probably only cytotoxic and hypotensive actions may be present.

The indigenous medicinal use of *S. minor* Dennst. in Southern Thailand is mainly for the treatment of paralysis. It is suggested that this use may be correlated to the convulsive action of henningsoline (118). Possibly the convulsive activity of this main compound is not strong and found only as a CNS-stimulant. However, the individual alkaloid may be worth further investigation.