CHAPTER IV

Results and Discussion

Isolation and Identification of Isolated Compounds

In the present investigation, three different parts of a crude alcoholic extract obtained from the stems of *Strychnos nitida* were separated by chromatographic techniques and afforded eight isolated compounds. Four compounds were obtained from the acid-base extract of the crude alkaloid mixture, three from the methanol extract and the last one from the hexane extract. The structural elucidation of the compounds was performed on the basis of spectral analysis. The results obtained are summarized in Table 4.1. The detailed discussion on the identification of individual compounds is also given.

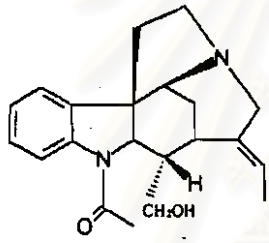
Table 4.1 Results obtained from isolation and identification of the compounds.

Isolated compound	Identified compound	% Yield of plant material
SN-1	retuline	0.005
SN-2	11-methoxyretuline	0.0002
SN-3	normacusine B	0.000025
SN-4	3-hydroxy-19(Z)-normacusine B	0.000028
SN-5	(±) lyoniresinol	0.00012
SN-6	(+) and (-) lyoniresinol β-D-glucopyranoside	0.01
SN-7	loganin	0.014
SN-8	β-sitosterol	0.03

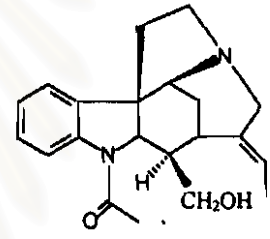
SN-1

SN-1 was obtained as a major component of the acid-base extract. The compound gave an orange coloration upon spraying with $FeCl_3/HClO_4$ reagent. Its UV spectrum showing maxima at 209, 253 nm. and a shoulder between 270 and 290 nm. revealed the characteristic of an indoline chromophore. The IR spectrum showed an intense band at 1640 cm^{-1}, indicated a tertiary amide carbonyl function, as well as an indoline band at 1598 cm^{-1}. ^1H and

^{13}C-NMR spectra of SN-1 suggested that the compound was one of the two isomeric alkaloids of the strychnan type, retuline (21) or isoretuline (22). The molecular peak at *m/z* 338 in the mass spectrum corresponded to the molecular formula $C_{21}H_{26}N_2O_2$ of the two isomers.

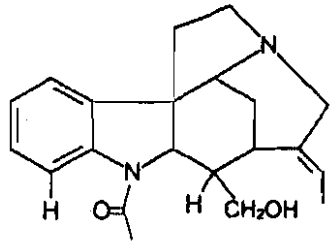


21 retuline

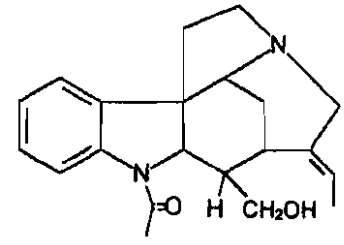


22 isoretuline

The complexity of the ^1H-NMR spectrum indicated the existence of two rotamers arising from the rotation of N_a-acetyl residue . According to data obtained from signal-integrating, the ratio of the two rotamers was suggested to be 5:2. The differentiation between both forms was also observed in ^{13}C-NMR spectrum where the resonance of each carbon appeared as a pair of signals with different intensity. The structures (105) and (106) of the two rotamers with different orientation of N_a-acetyl group are shown below.



105



106

In the ^{1}H-NMR spectrum, the non-substituted aromatic part of the indole nucleus was inferred from three signals, together integrating for eight protons (four for each rotameric form) observed in the aromatic region. The most downfield signal, the doublet at δ 8.12 (J=8.1 Hz), was assigned for the deshielded H-12 of the structure (105) in accordance with its chemical shift and coupling. This signal was equivalent to one proton of the dominant rotamer, the structure (105) was thus attributed to the major form. The assignment of the other signals at δ 7.23 (m, 2H : 1H for each rotamic form) and δ 7.09 (m,5H : 2 for the major form and 3 for the minor one) could be accomplished with assistance of 2D-^{1}H-^{1}H- and ^{13}C-^{1}H COSY spectra. The resonance of H-12 in the structure (106) of the minor form was observed as a part of the multiplet at δ 7.09, indicated by the correlation with the signal assigned for C-12 in the ^{13}C-^{1}H COSY spectrum.

The presence of the ethylidine side chain was deduced from somewhat ill defined quartet at δ 5.44 (2H : 1H for each form) and two half-overlapped doublets at δ 1.72 and δ 1.70, corresponding to the methyl protons at C-18 of the major and the minor forms, respectively. The N_a-acetyl group in the indole part was evident from two deshielded methyl singlets at δ 2.31 (3H of the major form) and δ 2.41 (3H of the minor one).

The 2D ^{13}C-^{1}H COSY spectrum provided the distinguishment between two groups of the remaining signals: one due to the protons of methines (H-2, H-3, H-15 and H-16) and the other to those of methylenes (H-5, H-6, H-14, H-17 and H-21).

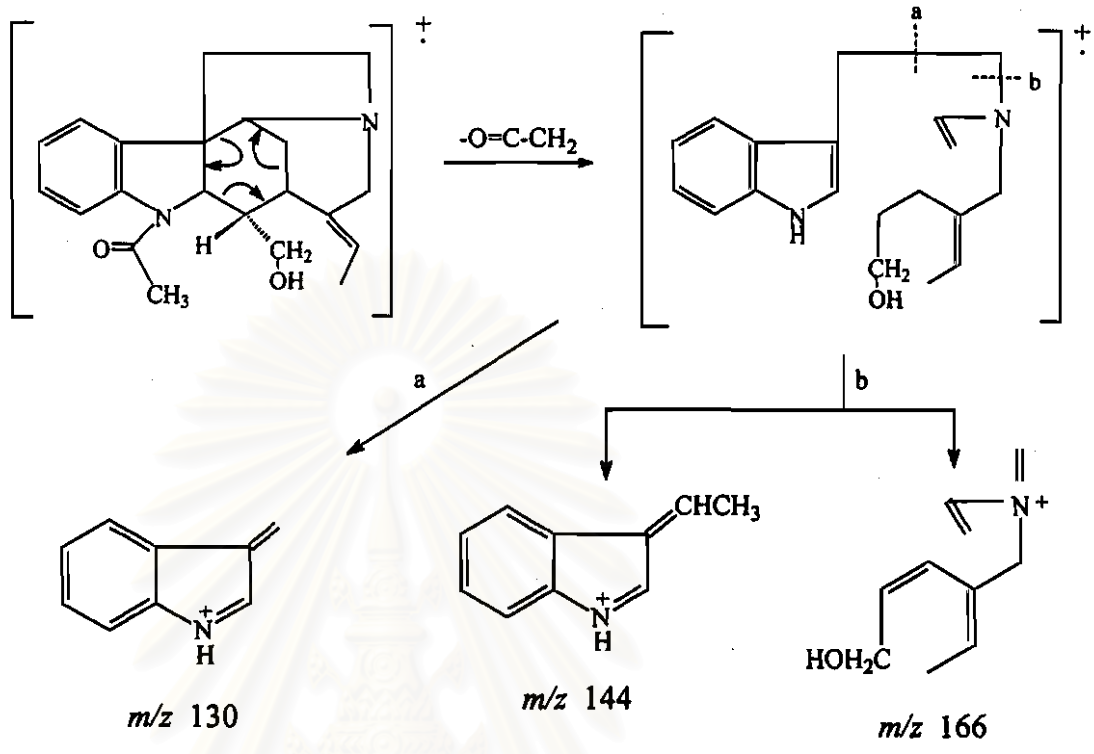
In the former group a pair of homologous signals with the most low field position was two doublets at δ 4.62 (J=7.9 Hz) and δ 4.13 (1H, J=7.5 Hz). As their chemical shifts were in agreement with a linkage to a deshielded *N*-atom, the signals were attributable to the proton of the aminomethine C-2. The more downfield signal was assigned for the deshield H-2 of the minor form, which was affected by the carbonyl function, and the other was for H-2 of the major one. The latter assignment was confirmed by the correlation in the NOESY spectrum between such doublet (δ 4.13) and the singlet at 2.31 which corresponded to the methyl protons of N_a-acetyl group. The doublet at δ 4.62 showed coupling with the small multiplet at δ 2.93 in the H-H COSY spectrum, the

multiplet was thus ascribable for H-16 of the minor rotameric form. On the basis of the same consideration, the resonance of H-16 of the major form was recognized as a part in the overlapped signals at δ 2.79. The homologous signals at δ3.88 (1H of the major form) and δ 3.83 (1H of the minor form), the chemical shifts of which were in agreement with a linkage to a N-atom, were assigned for the proton of the aminomethine C-3. The partially overlapped signals at δ 3.06 (1H of the major form) and δ 3.04 (1H 0f the minor form), the last two signals of the former group, were thus attributed to the proton of the remaining methine C-15. The signals in the latter group, which corresponded to the protons of methylene carbons, could be assigned according to their chemical shifts and their couplings with the signals mentioned before.

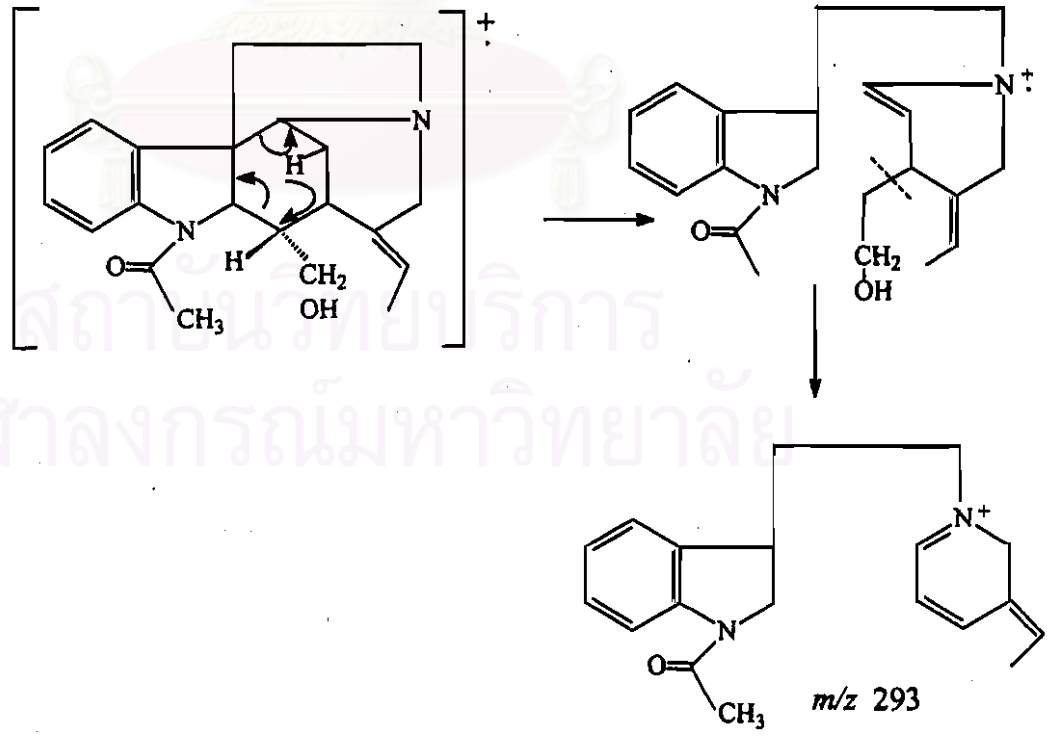
The carbon signals in the spectra obtained from various ^{13}C-NMR techniques were in agreement with numbers and types of the carbons in the proposed structure. The spectra indicated, for each rotamer, the presence of one carbonyl (C-22), four quaternary (C-7, C-8, C-13, C-20), and five protonated (C-9, C-10, C-11, C-12, C-19) carbons in the aromatic region. The aliphatic region contained four methines (C-2,C-3, C-15, C-16), five methylenes (C-5, C-6, C-14, C-17, C-21) and two methyl carbons (C-18, C-23). The assignment of the signals were mainly based on the C-H COSY spectrum for those due to protonated carbons and on the HMBC spectrum for those represented quaternary ones.

The proposed structure for SN-1 was also confirmed by the mass spectrum. The typical indole peaks at *m/z* 130, and 144 were evident. The peaks at *m/z* 185 (143+42) indicated that the indolic nitrogen N_a was acetylated. The presence of the primary alcoholic function was pointed out by the peak at *m/z* 307 (M-31). The important peaks at *m/z* 166 and 293 could explain as the fragments from the non-indole part of the strychnan skeleton. The proposed fragmentation of SN-1 is shown in Scheme 4.1.

The stereochemistry of the compound remained to be considered. The orientation of the ethylidine side chain was proposed to be *E* owing to the correlation between H-18 and H-15 as well as H-18 and H-16 in the NOESY spectrum. The proposed relative configurations of C-2, C-7, C-3 and C-15: 2β,7β, 3α and 15α were those commonly accepted from the biogenetical hypothesis[195] . The coupling constants observed between H-2 and H-16 (*ca.*7.5 Hz) favored a 16βH configuration of retuline (*cf* $J_{2,16} \approx 10$ Hz of

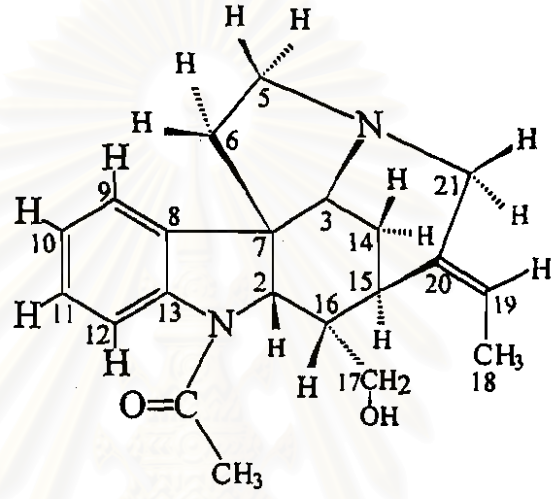


Scheme 4.1 (a) Proposed mass fragmentation of SN-1



Scheme 4.1 (b) Proposed mass fragmentation of SN-1

isoretuline series)[99, 160]. The chemical shifts of C-14 which appeared at a comparative low field (δ *ca* 19.0, *cf* δ *ca* 28 of isoretuline series)[196] pointed to the same deduction. Therefore SN-1 was unambiguously identified as retuline, a known member of the strychnan-type alkaloids. The ^{1}H and ^{13}C-NMR assignments of SN-1 are shown in Table 4.2 and Table 4.3; respectively.



Retuline (SN-1)

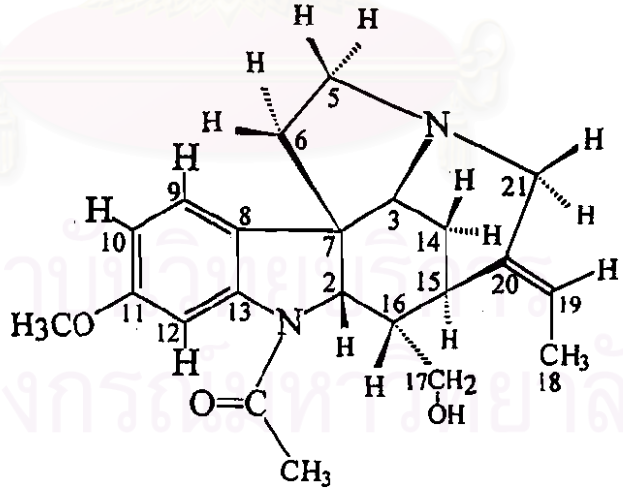
SN-2

SN-2 was obtained as colorless prismatic crystals. The compound gave a red color with $FeCl_3/HClO_4$ reagent. Its UV spectrum showed maxima at 227, 249 nm. corresponding to an indoline chromophor. The IR spectrum exhibited an OH absorption at 3400 cm^{-1} and a carbonyl band at 1650 cm^{-1}.

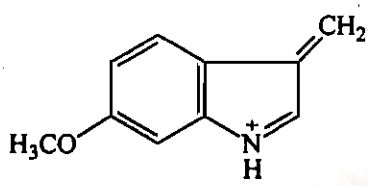
Its ^{1}H-NMR spectrum was similar to that of SN-1 except for a deshielded methyl singlet at δ 3.80 and for an AMX pattern in the aromatic area (δ_A6.95 ; δ_M 6.61; δ_X 7.80; J_{AM} = 8.2 Hz, J_{MX} *ca* 2 Hz). These information suggested a structure of retuline derivative in which a methoxy substituent was present at C-10 or C-11 on the benzene ring of the indole nucleus. This proposal was also substantiated by the mass spectrum

which displayed a molecular peak at *m/z* 368 as well as other important peaks at m/z 160(130+30), 174(144+30), 166, 215(185+30) and 323(293+30) (Scheme 4.2). The location of the methoxyl group was assigned at C-11, based on the ^{13}C-NMR spectrum of SN-2 which was interpreted according to Wenkert's results on isoretuline[196]. Introduction of a methoxy group on C-10 of isoretuline would have left almost unchanged chemical shifts of C-2 (δ 117). The absence of any carbon signal of SN-2 in this area did not favor the hypothesis of C-10 substitution.

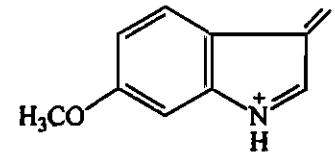
The stereostructure of the compound was finally refined. The coupling constant observed between H-16 and H-2 (*ca* 7.6 Hz) and the chemical shift of C-14 (δ *ca* 19) indicated that the compound belonged to the retuline series with H-16β[99, 160]. SN-2, therefore, was concluded to be 11-methoxy retuline , a known indole base of the strychnan-type. The ratio between two rotamers of the compound as deduced from proton signal integrating was suggested to be 3 : 1. The orientations of N_a acetyl group in the major and minor forms followed those of SN-1 . The ^{1}H and ^{13}C NMR assignments of SN-2 are shown in Table 4.4 and Table 4.5, respectively.



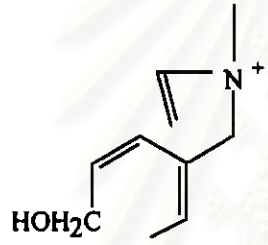
11-Methoxyretuline (SN-2)



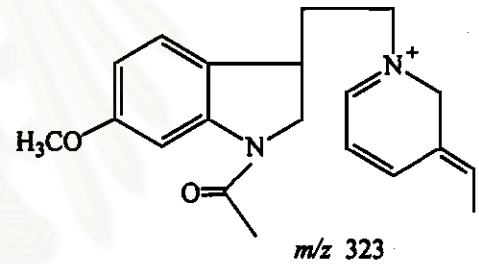
m/z 160



m/z 174



m/z 166



m/z 323

Scheme 4.2 Some important mass fragments of SN-2

Table 4.2 ^{1}H-NMR assignment of SN-1

Proton position	Chemical shift (ppm)	
	Major form	Minor form
2	4.13 (d: J=7.5 Hz)	4.62 (d: J=7.9 Hz)
3	3.88 (br s)	3.83 (br S)
5α	3.25 (dd: *J*=10, 8 Hz)	3.20 (t: *J*=10, 9 Hz)
5β	2.79 (m)	2.85 (m)
6α	1.64 (m)	1.64 (m)
6β	1.95 (m)	1.95 (m)
9	7.06 (m)	7.13 (d: *J*=7.4 Hz)
10	7.06 (m)	7.06 (m)
11	7.22 (m)	7.22 (m)
12	8.11 (d: *J*=8.1 Hz)	7.09 (d:*J*~8 Hz)
14α	1.59 (d: *J*~13-14 Hz)	1.56 (d:*J*~14-15 Hz)
14β	1.95 (d: *J*~13-14 Hz)	1.90 (d:*J*~14-15 Hz)
15	3.06 (br s)	3.04 (br s)
16	2.79 (m)	2.93 (m)
17α	3.41 (dd: *J*=10.9, 5.9 Hz)	3.37 (dd: *J*=11, 5.6 Hz)
17β	3.16 (dd: *J*=10.9, 8.3 Hz)	3.09 (dd: *J*=11, 8.9 Hz)
18	1.72 (d: *J*=7.1 Hz)	1.70 (d: *J*=8 Hz)
19	5.44 (q: *J*=7.1 Hz)	5.45 (q: *J*=8 Hz)
21α	3.79 (d: *J*=16 Hz)	3.75 (d: *J*~16 Hz)
21β	2.82 (d: *J*=16 Hz)	2.84 (d: *J*~16 Hz)
23	2.31 (s)	2.41 (s)

Table 4.3 ^{13}C-NMR assignment of SN-1

Carbon position	Chemical shift (ppm)	
	Major form	Minor form
2	64.5	64.2
3	57.6	58.1
5	51.8	52.0
6	40.8	41.1
7	51.8	50.5
8	135.5	137.8
9	120.8	122.1
10	124.4	124.0
11	128.3	127.9
12	117.2	114.3
13	142.9	142.1
14	19.0	19.2
15	29.8	29.1
16	42.6	41.6
17	61.0	61.5
18	13.4	13.3
19	121.8	122.0
20	136.6	136.3
21	55.2	55.2
22	168.8	168.8
23	23.2	23.9

Table 4.4 ^{1}H-NMR assignment of SN-2

Proton position	Chemical shift (ppm)	
	Major form	Minor form
2	4.12 (d: *J*=7.6 Hz)	4.59 (d: *J*=7.9 Hz)
3	3.82 (br s)	3.76 (br s)
5α	3.24 (dd: *J*=8.6, 8.5 Hz)	3.17 (dd: *J*~8,8 Hz)
5β	2.77 (m)	2.77 (m)
6α	1.60 (m)	1.60 (m)
6β	1.92 (m)	1.92 (m)
9	6.95 (d: *J*=8.2 Hz)	7.02 (d: *J*=8.2 Hz)
10	6.61 (dd: *J*=8.2, 2.1 Hz)	6.58 (dd: *J*=8.2, 1.5 Hz)
12	7.80 (d: *J*=2.1 Hz)	6.67 (d: *J*=1.5 Hz)
14α	1.60 (d: *J*~13)	1.60 (d: *J*~13)
14β	1.92 (d: *J*~13)	1.92 (d: *J*~13)
15	3.05 (br s)	3.03 (br s)
16	2.77 (m)	2.92 (m)
17α	3.44 (dd: *J*=10.9, 6.1 Hz)	3.39 (dd: *J*=11, 5.5 Hz)
17β	3.23 (dd: *J*=10.9, 8.6 Hz)	3.15 (dd: *J*=11, ~9 Hz)
18	1.72 (d: *J*=7.0 Hz)	1.70 (d: *J*=6.1 Hz)
19	5.45 (q: *J*=7.0 Hz)	5.43 (q: *J*=6.1 Hz)
21α	3.78 (d: *J*=15.7 Hz)	3.74 (d: *J*~15-16 Hz)
21β	2.81 (d: *J*=15.7 Hz)	2.83 (d: *J*~15-16 Hz)
23	2.31 (s)	2.41 (s)
11-OCH_3	3.80 (s)	3.81 (s)

Table 4.5 ^{13}C-NMR assignment of SN-2

Carbon position	Chemical shift (ppm)	
	Major form	Minor form
2	65.1	64.7
3	57.5	58.1
5	51.7	51.9
6	40.8	41.1
7	51.2	49.9
8	127.3	129.9
9	121.1	122.4
10	110.3	107.6
11	160.0	159.8
12	103.5	102.7
13	144.0	143.1
14	19.0	19.1
15	30.0	29.2
16	42.6	41.5
17	61.2	61.6
18	13.4	13.4
19	121.9	122.2
20	136.6	136.3
21	55.1	55.1
22	169.0	168.7
23	23.3	24.0
O-CH_3	55.6	55.6

SN-3

SN-3 was obtained as colorless needle crystals in very small amounts. The compound developed a grey coloration upon spraying with $FeCl_3/HClO_4$ reagent. Its UV maxima at 226, 285 and 295 nm was typical for an indole chromophore. No carbonyl band was observed in IR spectrum. Analyses of ^1H and ^{13}C NMR spectra of SN-3 suggested the structure of normacusin B , a known corynanthean-type alkaloid in the sarpagine group. The molecular formula of $C_{19}H_{22}N_2O$ was confirmed by the molecular peak at m/z 294, displayed in the mass spectrum.

The ^1H-NMR spectrum suggested a non-substituted aromatic part of the indole nucleus by exhibiting 3 signals due to 4 aromatic protons at δ 7.44 (d, 1H, *J*= 7.5Hz), δ7.30 (d, 1H, *J*= 7.4Hz), and δ 7.09 (m, 2H). The last one could be observed from the expanded ^1H spectrum (figure 20.2) as consisting of 2 subsignals centered at δ 7.08 (ddd, *J*= 7.5,7.3,1.2 Hz) and δ 7.13 (ddd, *J*= 7.4,7.3,1.2Hz). The doublet at δ 7.30 was assigned for H-12 in the indole moiety owing to its correlation with the singlet at δ 8.18 (1H), attributed to the indolic NH proton, in the NOESY spectrum.

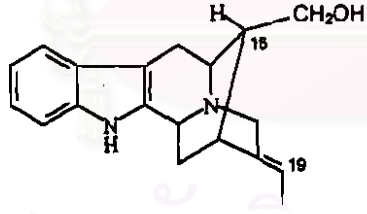
In the non-indole part, the presence of the ethylidine side chain was inferred from a quartet at δ 5.29 (1H, *J*=6.7 Hz) and a doublet at δ 1.59 (3H, *J*=6.7 Hz). The former also showed weak coupling with a multiplet (4H) at δ 3.50. Two of four protons related to this multiplet were thus attributable to geminal protons of the aminomethylene C-21. This assignment was collaborated by the chemical shifts of the signal, which was in agreement with a linkage to *N*-atom.

In addition, other two pairs of geminal protons could be recognized. One was deduced from two signals at δ 1.69 (1H, ddd, *J*=12.5,3.4,3.1 Hz) and δ1.98 (1H, ddd, 12.5,11.3,2.1 Hz) the other from those at δ 2.64 (d, 1H, *J*=15.3 Hz) and δ 3.05 (dd., 1H, *J*=15.3, 5.2 Hz). According to the chemical shifts and couplings, the former and the latter sets of signals were assigned for geminal protons of methylenes C-14 and C-6, respectively. The unassigned two protons of the aforementioned multiplet at δ 3.05, indicated by the HMQC spectrum as being the geminal protons, were arbitrary assigned for the protons of the last methylene C-17. The chemical shifts of the signal which was in consonance with a linkage to *O*-atom was supportive of the assignment.

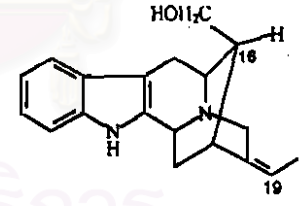
The remaining signals, each equivalent for one proton of the methine could be assigned in accordance with their chemical shifts and their couplings with the signals of the geminal protons mentioned before.

The mass spectrum of SN-3 also corresponded to the structure of normacusine B. The peak at *m/z* 263, indicating the loss of 31 mass units from the molecular ion, suggested the presence of a primary alcohol. The β-carboline fragments at *m/z* 168 and 169, which were characteristic of a sarpagine group alkaloid, were evident. The biogenesis of these two fragments was proposed as shown in Scheme 4.3.

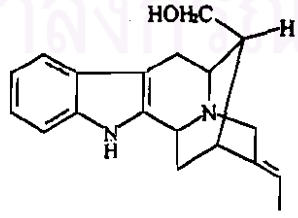
The discrimination of normacusine B (107) from its stereoisomers (108-110) was a crucial point in the structure elucidation of SN-3. The compound was proposed to possess 19 *E* configuration according to its NOESY spectrum where the correlation between H-18 and H-15 was observed and that between H-19 and H-15 could not be detected. This proposal was corraborated by the chemical shifts of C-15 (δ 27.6) and C-21 (δ 55.8) which were upfield and downfield, respectively, owing to the γ effect of the methyl group at C-19, as compared with those data reported for koumidine (108) ($\delta_{C\text{-}15}$=35.1, $\delta_{C\text{-}21}$= 54.6)[197].



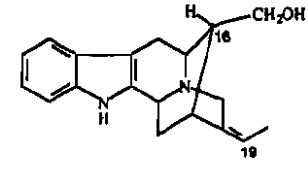
(107) normacusine B (tombozine)



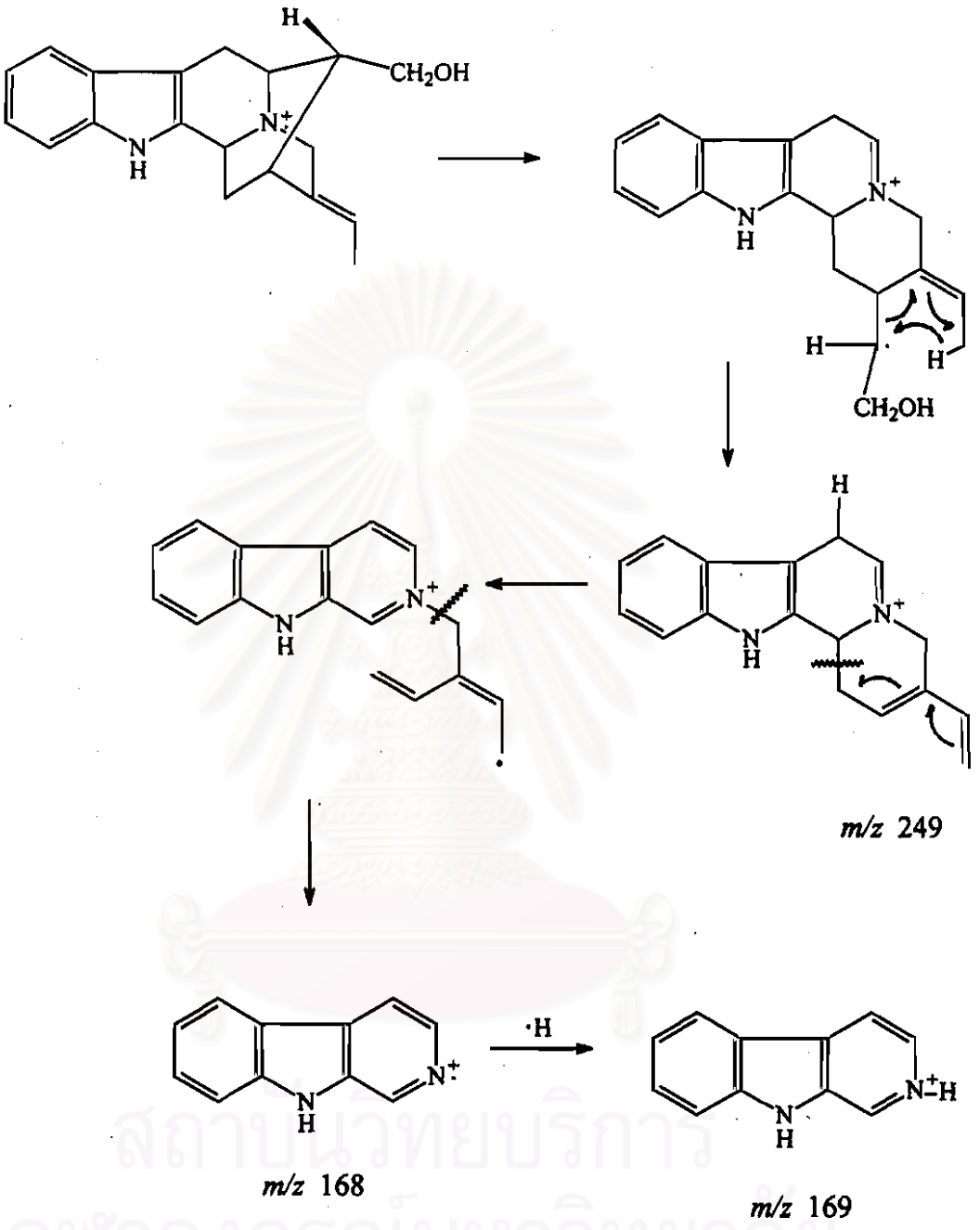
(108) koumidine



(109) 16 epinormacusine B
(19 *E* koumidine)



(110) 19*E* normacusine B



Scheme 4.3 Proposed mass fragmentation of SN-3

Table 4.6 ^{1}H-NMR assignment of SN-3

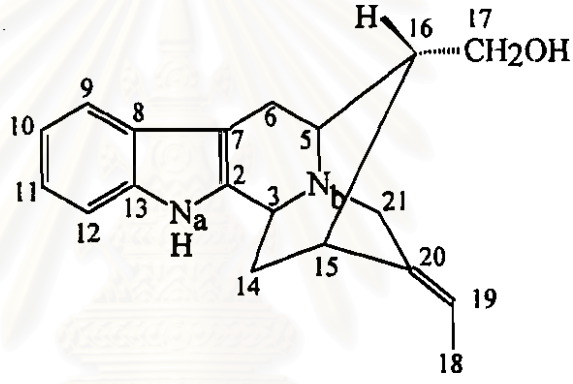
Proton position	Chemical shift (ppm)
1 (-NH)	8.18 (br s)
3	4.11 (d: *J*=10 Hz)
5	2.78 (dd: *J*=5.2, 6.1 Hz)
6α	3.05 (dd: *J*=15.3, 5.2 Hz)
6β	2.64 (d: *J*=15.3 Hz)
9	7.44 (d: *J*=7.5 Hz)
10	7.08 (ddd: *J*=7.5, 7.3, 1.2 Hz)
11	7.13 (ddd: *J*=7.4, 7.3, 1.2 Hz)
12	7.30 (d: *J*=7.4 Hz)
14α	1.98 (ddd: *J*=12.5, 11.3, 2.1 Hz)
14β	1.69 (ddd: *J*=12.5, 3.4, 3.1 Hz)
15	2.75 (m)
16	1.81 (qd: *J*~7, ~7, 6, 3 Hz)
17α	3.45-3.54
17β	3.45-.354
18	1.59 (d: *J*=6.7 Hz)
19	5.29 (q: *J*=6.7 Hz)
21α	3.45-3.54
21β	3.45-3.54

Table 4.7 ^{13}C-NMR assignment of SN-3

Carbon position	Chemical shift (ppm)
2	137.9
3	50.4
5	54.4
6	26.9
7	104.6
8	127.7
9	118.1
10	119.4
11	121.4
12	110.9
13	136.4
14	34.4
15	27.6
16	44.2
17	65.0
18	12.7
19	116.8
20	135.4
21	55.8

The configuration of H-16 could be determined on the basis of coupling constant analysis. The value observed between H-16 and H-5 (*ca* 6 Hz) of SN-3 was in agreement with a 16βH configuration. The correlations between H-16 and H-6β and between H-18 and H-17, displayed in the NOESY spectrum, confirmed this configuration.

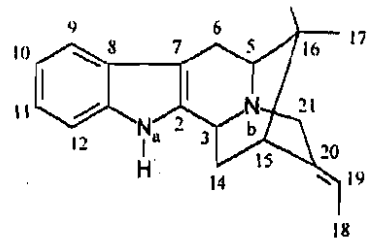
All information mentioned above was in consonance with the structure of normacussine B with 16βH and 19(*E*) configuration . SN-3, therefore, was concluded to be normacusine B. The ^{1}H and ^{13}C-NMR assignments of the compound are shown in Tables 4.6 and 4.7, respectively.



Normacusine B (SN-3)

SN-4

SN-4 was obtained in low yield as a white amorphous substance which gave a grey coloration with $FeCl_3/HClO_4$ reagent. The compound exhibited UV maxima at 236, 285 and 310 nm which were indicative of an indole chromophor. No carbonyl absorption was detectable in the IR spectrum. Its ^{1}H and ^{13}C NMR spectra were somewhat similar to those of SN-3 suggesting the presence of sarpagan as the basic skeleton in the molecule of SN-4 .



Sarpagan

In the ^{1}H-NMR spectrum, four aromatic protons were evident from three signals at δ 7.22 (1H, d, J=8.2 Hz), δ 7.08 (1H,m) and δ 6.89 (2H). The indolic NH proton was observed as a broad singlet at δ 9.00 (1H). This signal showed the correlation with the doublet at δ 7.22 in the NOESY spectrum, the latter was thus attributed to H-12 in the indole nucleus. The other aromatic signals at δ 7.08 and δ 6.89 could be assigned, according to their coupling, for H-11 and for H-9 as well as H-10 respectively.

The structure elucidation of the non-indole part in the molecule was mainly based on examination of the 2D ^{1}H-^{1}H COSY spectrum where two substructures related to two mutually non-interacting spin systems could be observed. One corresponding to -CH_2-C=CH-CH_3 fragment was evident from a quartet at δ 5.29 (1H, *J*=6.1 Hz) and a doublet at δ 1.59 (3H, *J*=6.1 Hz), suggesting the presence of the ethylidine side chain, and from two doublets of geminal protons at δ 4.13 (1H, *J*=16.4 Hz) and δ 2.97 (1H, *J*=16.4 Hz), each of which displayed allylic connectivities with the aforementioned quartet. The chemical shifts of the last two signals of the germinal protons were in consonance with a linkage to *N*-atom; the fragment was thus postulated to be N_b-C(21)H_2-C(20)=C(19)H-C(18)H_3 of the sarpagan skeleton.

The other substructure could be assembled from three -CH-CH_2- fragments, indicated by three related spin systems. The first fragment was due to an ABX system which was discernible as two doublets, the AB part, at δ_A 2.02 and δ_B 1.88 (J_{AB}=13.3 Hz) and a singlet like signal, the X part, at δ_x 2.75 (J_{AX}=3.4 Hz, J_{BX} < 2 Hz). Since the chemical shifts of the three protons in this fragment indicated no deshielding effect, the fragment was suggested to be -C(14)H_2-C(15)H- unit.. The second fragment corresponded to two geminal protons, appeared as a doublet at δ 2.26 and a signal at δ 2.61, and to one

proton, the resonance of which was superimposed on the latter signal (δ 2.61). The chemical shifts of these three protons indicated the deshielding effect; the fragment thus probably arose from the tryptamine moiety of the molecule, corresponding to -N_b-C(5)H-C(6)H_2-C(7)= unit. The last CH-CH_2- unit was evident from an additional ABX system, the AB and the X parts of which appeared as two multiplets at δ 3.35 (2H) and at δ 1.67 (1H), respectively. The chemical shifts of the signal for the geminal protons of the last fragment was in agreement with the linkage to *O*-atom, therefore, this fragment was suggested to be -C(16)H-C(17)H_2-OH unit.

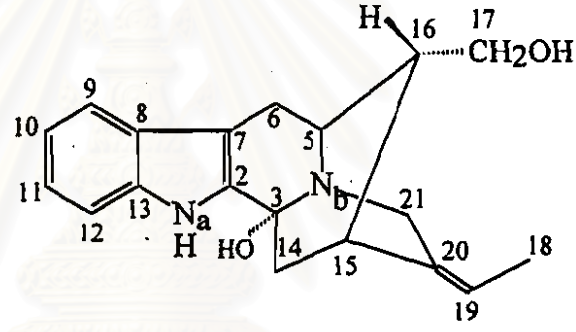
The means of establishing the second substructure from these three fragments was provided through the last mentioned signal at δ 1.67, assigned for H-16, which coupled with the signals of H-15 and H-5 of the first and the second fragments, respectively. The attachment between the two substructure of the non-indole part could be deduced according to the HMBC (8 Hz) spectrum which indicated correlation between H-19 of the former and C-15 of the latter.

The comparison of ^{1}H and ^{13}C- NMR spectra of SN-4 and normacusine B indicated the similar structures of the two compounds except for the proton attached to C-3 of normacusine B which could not be observed in the ^{1}H-NMR spectrum of SN-4. The 3-substituent in the molecule was suggested to be a hydroxyl group according to the chemical shift of a carbon signal assigned for C-3 in the ^{13}C spectrum (δ 82.1). Therefore the structure of 3 hydroxy-normacusune B (or its stereoisomers) was proposed for SN-4. The mass spectrum also supported this proposal. The molecular peak at *m/z* 310 corresponded to the formula of $C_{19}H_{22}N_2O_2$. The fragments arising from the loss of water at *m/z* 292 (310-18), and 293 (310-17) suggested the presence of the hydroxyl group in the molecule. The important fragments at *m/z* 184 (168+16) and 185 (169+16) implied the substitution of the hydroxyl group on the β-carboline unit of the sarpagan skeleton. Some important mass fragments of SN-4 are shown in Scheme 4.4.

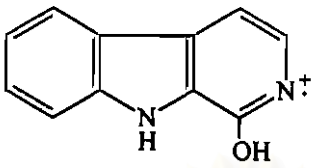
All the NMR signals were fully supportive of this structure. The complete carbon assignment could be achieved, mainly based on the HMQC spectrum for protonated carbons and on the HMBC spectrum for non-protonated ones.

The stereochemistry of the compound remained to be determined. The orientation of the ethylidine side chain was suggested to be *Z* owing to the correlation between H-15 and H-19 in the NOESY spectrum, together with the disagreeable chemical shifts of C-15 and C-21 comparing with those of normacusine B. The small coupling constant observed between H-16 and H-15 (*ca* 2 Hz) was in consonance with those of sarpagine group alkaloids with H-16β[198], indicating the β-configuration of H-16 in the structure of SN-4.

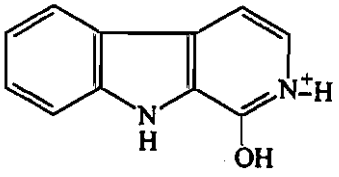
On the basis of all considerations mentioned above, SN-4 was identified as a new member of the sarpagine group, possessing the structure of 3 hydroxy-19(*Z*)-normacusine B . The ^{1}H- and ^{13}C-NMR assignments of the compound are shown in Table 4.8 and Table 4.9, respectively.



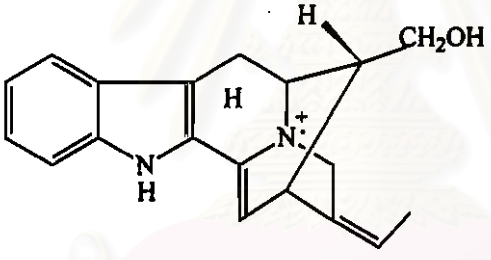
3-Hydroxy-19(*Z*)-normacusine B (SN-4)



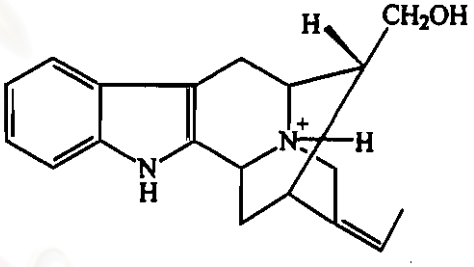
m/z 184



m/z 185



m/z 292



m/z 293

Scheme 4.4 Some important mass fragmentation of SN-4

Table 4.8 ^{1}H-NMR assignment of SN-4

Proton position	Chemical shift (ppm)
1 (-NH)	9.00 (br s)
5	2.61 (br s)
6α	2.61 (br s)
6β	2.26 (d: *J*=14.3 Hz)
9	6.89
10	6.89
11	7.08 (m)
12	7.22 (d: *J*=8.2 Hz)
14α	2.02 (dd: *J*=13.3, 3.4 Hz)
14β	1.88 (d: *J*=13.3 Hz)
15	2.75 (br s)
16	1.67 (m)
17α	3.35 (m)
17β	3.35 (m)
18	1.57 (d: *J*=6.1 Hz)
19	5.29 (q: *J*=6.1 Hz)
21α	2.97 (d: *J*=16.4 Hz)
21β	4.13 (d: *J*=16.4 Hz)

Table 4.9 ^{13}C-NMR assignment of SN-4

Carbon position	Chemical shift (ppm)
2	138.2
3	82.1
5	57.3
6	25.6
7	106.0
8	127.1
9	118.6
10	119.4
11	121.7
12	110.7
13	136.3
14	40.4
15	29.9
16	42.8
17	64.5
18	12.7
19	116.5
20	134.7
21	48.4

SN-5

SN-5 was obtained as a white amorphous substance in relatively low yield. The compound developed an intense blue color upon spraying with $FeCl_3/HClO_4$ reagent followed by heating for 5 minutes. Its IR spectrum showed a strong OH absorption at 3400 cm^{-1} together with aromatic bands, appearing in the range 1625-1470 cm^{-1}. On the basis of ^1H- and ^{13}C-NMR analyses, SN-5 was indicated to be a lignan of the anyltetralin type, namely lyoniresinol . The molecular formula of $C_{22}H_{28}O_8$ was confirmed by the molecular peak at *m/z* 420, displayed in the mass spectrum.

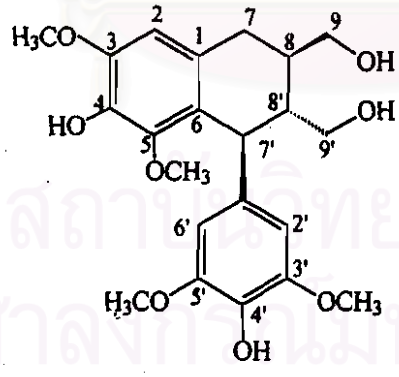
In the ^1H-NMR spectrum the presence of tetra- and penta-substituted benzene rings was deduced from two signals due to three aromatic protons at δ 6.37 (s, 2H) and δ 6.58 (s, 1H) respectively. This deduction was also supported through the HMQC spectrum where eight carbon signals related to nine quaternary carbons together with two signals due to three protonated ones were observed in the aromatic region. The singlet of two protons at δ 6.37 which correlated to one carbon signal at δ 106.9 also indicated the symmetrical substitution of two aromatic protons in one ring. This singlet was thus assigned for H-2' and H-6' in the structure of lyoniresinol and the other aromatic signals at δ 6.58 was attributable to H-2. Four methoxy groups substituted on the benzene moieties were inferred from the singlets of deshielded methyl protons at δ 3.85 (3H), δ 3.73 (6H) and δ 3.37 (3H).

In the non-aromatic part of the compound, the protons of three methines and three methylenes could be observed from the ^1H-NMR spectrum. The signals related to the methines included those appearing at δ 4.30 (d, 1H, *J*=5.5 Hz), δ 1.96 (m, 1H) and δ 1.62 (m, 1H). The first signal was ascribable for H-7' owing to its low field chemical shift and its correlation with the signal of H-2'/6' in the ^1H-^1H COSY spectrum. The second signal, which showed coupling with the first and the last, was assigned for H-8'. The last signal was thus attribute to H-8 of the remaining methine. The rest of signals due to the geminal protons of the methylenes could be assigned according to their chemical shifts and their couplings with the signals mentioned before.

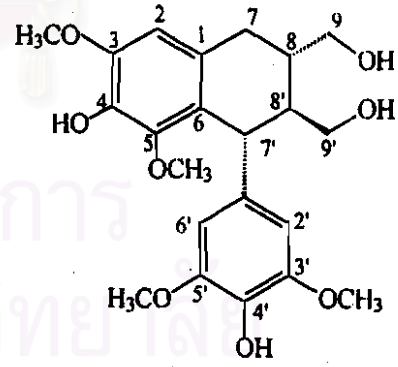
The completed ^{13}C assignment could be accomplished by analyses of the HMQC spectrum for the protonated carbons and of the HMBC spectrum for the quaternary ones.

The proposed structure of SN-5 was confirmed by mass spectral study. The fragment at *m/z* 402 could be explained as arising from the loss of water in the molecule of lyoniresinol. The subsequent loss of hydroxymethyl group led to the genesis of the fragment at *m/z* 371 (Scheme 4.5). Fragments at *m/z* 390, *m/z* 210, *m/z* 206, *m/z* 205 and *m/z* 167 arose by fragmentation involving the hydrogen transfer from a hydroxyl group to an aromatic ring via six-membered ring transition (Scheme 4.6). The loss of CH_2O caused the fragment at *m/z* 390. The rupture of the bond (a) generated the fragments at *m/z* 167 and *m/z* 206. The latter in turn readily aromatized to acquire a fairy stable species at *m/z* 205. The former had a more stable tropylium structure appeared at *m/z* 167.

All of the spectroscopic evidences of SN-5 were in agreement with the structure of lyoniresinol. The stereostructure of the compound was finally refined . The relative configurations at C-8 and C-8', which were proposed to be the same as that of lyoniresinol, were determined on the basis of coupling constant analysis [179] and confirmed by comparing such data with the reported values of lyoniresinol [199]. The melting point of the compound observed at 192°C as well as its optical inactivity ($[\alpha]_D = 0$) led to the conclusion that SN-5 was the racemic form of lyoniresinol. The ^1H and ^{13}C-NMR assignment of SN-5 are given in Table 4.10 and Table 4.11, respectively.

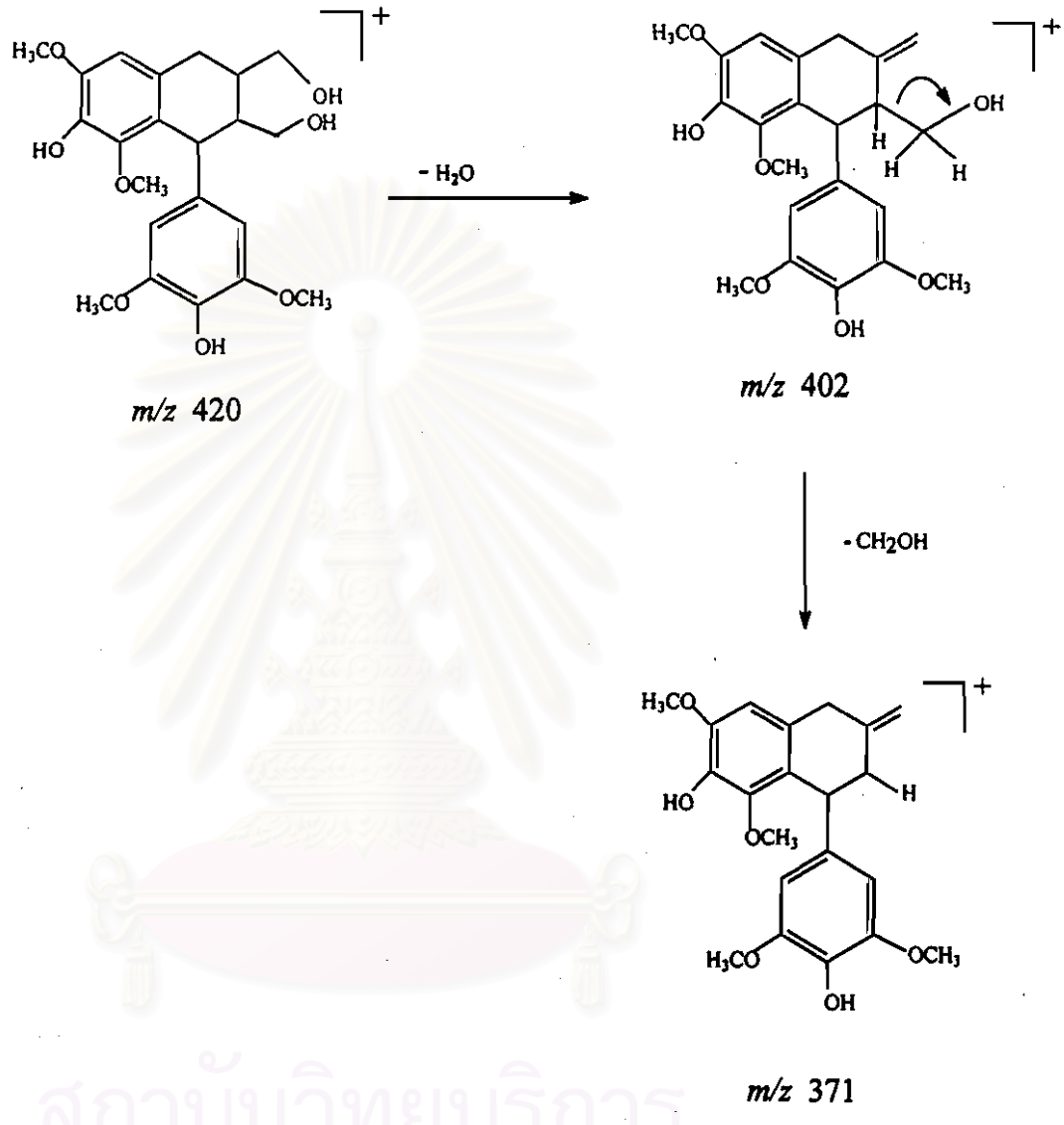


(+) Lyoniresinol

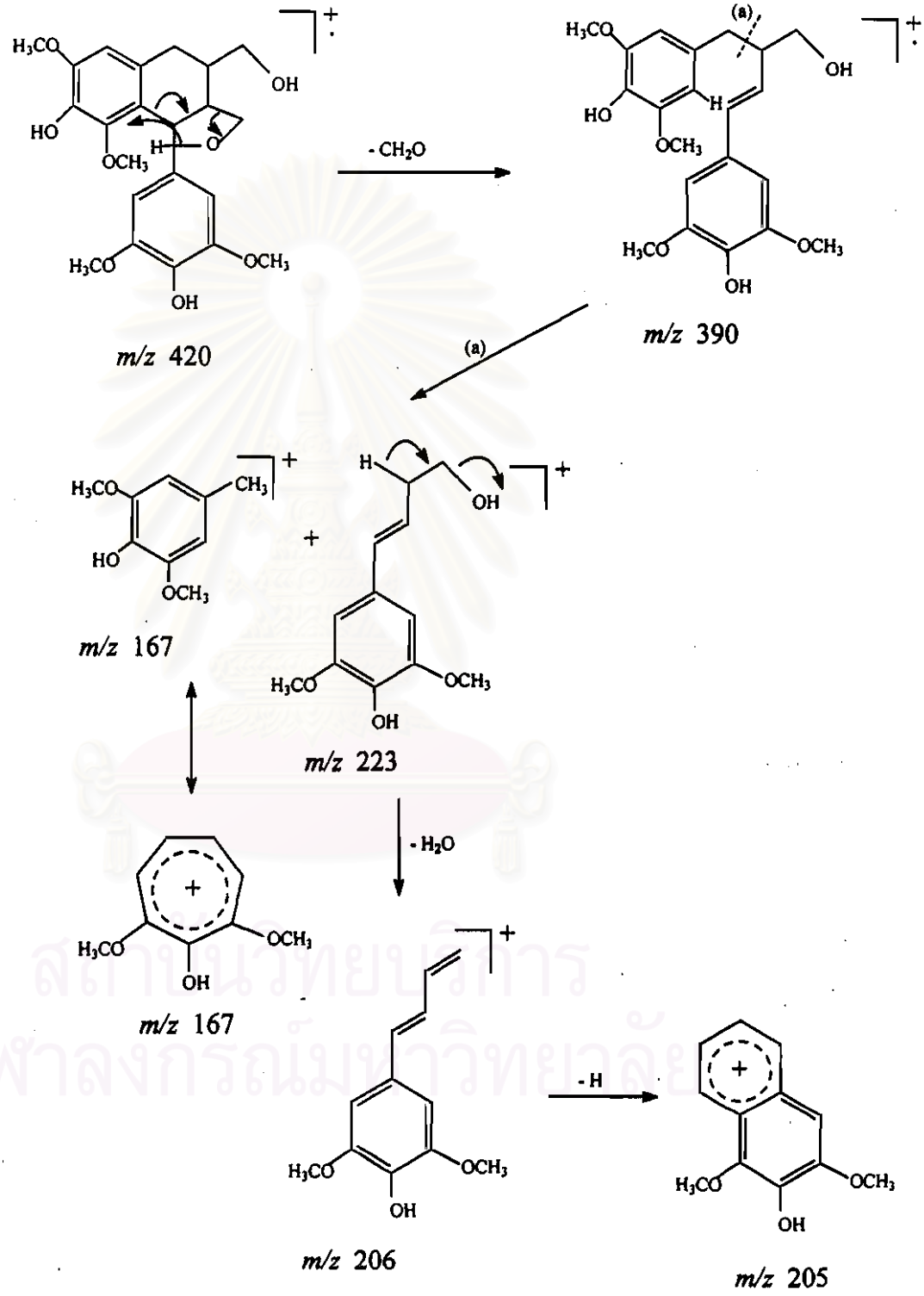


(-) Lyoniresinol

SN-5



Scheme 4.5 Mass fragmentation involving loss of water of SN-5



Scheme 4.6 Mass fragmentation involving hydrogen transfer of SN-5

Table 4.10 ^{1}H-NMR assignment of SN-5

Proton position	Chemical shift (ppm)
2	6.58 (s)
7a	2.69 (dd : *J*=15.1, 4.9 Hz)
7b	2.56 (dd : *J*=15.1, 11.3 Hz)
8	1.62 (m)
9a	3.58 (dd : *J*=10.7, 5.2 Hz)
9b	3.48 (dd : *J*=10.7, 6.7 Hz)
2'	6.37 (s)
6'	6.37 (s)
7'	4.30 (d : *J*=5.5 Hz)
8'	1.96 (ddd : *J*=8.2, 5.5,5.3, 5.3 Hz)
9'a	3.49 (d : *J*=5.3 Hz)
9'b	3.49 (d : *J*=5.3 Hz)
3-OCH_3	3.85 (s)
5-OCH_3	3.37 (s)
3'-OCH_3	3.73 (s)
5'-OCH_3	3.73 (s)

Table 4.11 ^{13}C-NMR assignment of SN-5

Carbon position	Chemical shift (ppm)
1	130.19
2	107.80
3	148.68
4	138.91
5	147.71
6	126.26
7	33.57
8	40.91
9	66.80
1'	139.32
2'	106.91
3'	148.99
4'	134.56
5'	148.99
6'	106.91
7'	42.30
8'	48.90
9'	60.15
3-OCH_3	56.62
5-OCH_3	60.15
3'-OCH_3	56.78
5'-OCH_3	56.78

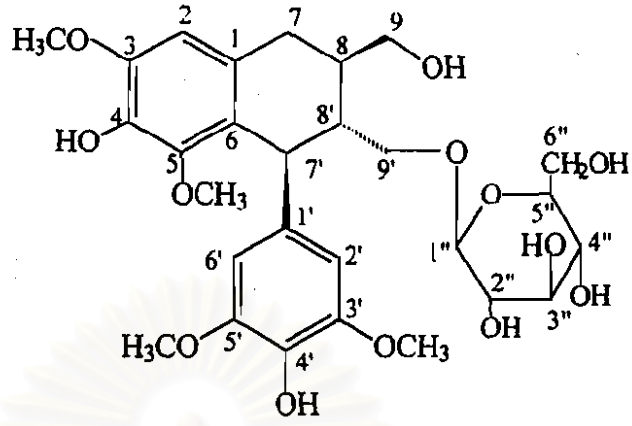
SN-6

SN-6, a white amorphous substance, exhibited the same characteristic as SN-5 upon detecting with $FeCl_3/HClO_4$ reagent. Its IR spectrum showed a strong OH absorption at 3400 cm^{-1} . The ^{1}H-NMR spectrum of the compound was similar to that of SN-5 except for some additional signals in the range of δ 3.00 - δ 4.60. The observation of two proton signals corresponding to H-7' of SN-5 (the doublets at δ 4.23 and δ 4.42) and the presence of two sets of allied carbon signals in the ^{13}C-NMR spectrum suggested the existence of two isomeric compounds. The data obtained from integration of proton signals confirmed this suggestion.

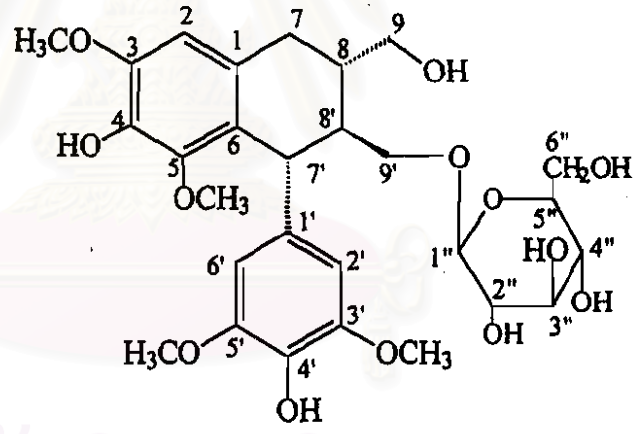
In the ^{1}H-NMR spectrum the presence of two doublets at δ 4.13 and δ 4.28, indicative of anomeric protons, along with the complicated signals in δ 3.10 -δ 3.95 interval was reminiscent of the sugar moiety. The 12 carbon signals correlated with these proton resonances were observed at chemical shifts which were in agreement with sugar carbons. All the information strongly suggested that SN-6 was a mixture of two isomeric compounds, which were the hexose-containing derivatives of lyoniresinol.

The location of the glycosyl substituent was determined on the basis of carbon chemical shifts analysis. The comparison of such values related to the carbons of lyoniresinol and aglycone part of SN-6 indicated only one significant difference which was due to the downfield shifts of C-9' in both cases of the two isomers, comparing with that of lyoniresinol (δ 64.2 of lyoniresinol; δ 71.6 and δ 72.0 of SN-6). This carbon position was thus attributable to the site of glycosylation in both forms of SN-6.

The identification of the hexose sugars in the molecules of two glycosides was hardly accomplished by ^{1}H-NMR analysis owing to the overlapping signals of the sugar protons in the range of δ 3.10 - δ 3.95. The more convenient alternative dealt with the ^{13}C-NMR determination. The ^{13}C-NMR data of SN-6 were indicated to be in consistent with the published values of (+) and (-) lyoniresinol -9'-β-D-glucopyranosides [199], the compound, therefore, was concluded to be a mixture of these two isomers . The ^{1}H- and ^{13}C-NMR assignments of the glycosides are given in Table 4.12 and Table 4.13, respectively.



(+) Lyoniresinol-9'-β-D-glucopyranoside



(-) Lyoniresinol-9'-β-D-glucopyranoside

SN-6

Table 4.12 ^{1}H-NMR assignment of SN-6

Proton position	Chemical shift (ppm)	
	(+) form	(-) form
2	6.57 (s)	6.57 (s)
7a	2.67 (m)	2.67 (m)
7b	2.67 (m)	2.67 (m)
8	1.70 (m)	1.70 (m)
9a	3.52-3.70	3.52-3.70
9b	3.52-3.70	3.52-3.70
2'	6.42 (s)	6.41 (s)
6'	6.42 (s)	6.41 (s)
7'	4.42 (d : J=6.1 Hz)	4.23 (d : J=6.4 Hz)
8'	2.10 (m)	2.10 (m)
9'a	3.43-3.47	3.52-3.60
9'b	3.87-3.91	3.87-3.91
3-OCH_3	3.86 (s)	3.86 (s)
5-OCH_3	3.36 (s)	3.33 (s)
3'-OCH_3	3.75 (s)	3.76 (s)
5'-OCH_3	3.75 (s)	3.76 (s)
1"	4.28 (d : J=7.6 Hz)	4.13 (d : J=7.6 Hz)
2"	3.14-3.30	3.14-3.30
3"	3.14-3.30	3.14-3.30
4"	3.14-3.30	3.14-3.30
5"	3.14-3.30	3.14-3.30
6"a	3.60-3.72	3.60-3.72
6"b	3.80-3.85	3.80-3.85

Table 4.13 ^{13}C-NMR assignment of SN-6

Carbon position	Chemical shift (ppm)	
	(+) form	(-) form
1	130.2	130.2
2	107.8	107.8
3	148.6	148.6
4	138.9	
5	147.5	147.5
6	126.4	126.2
7	33.8	33.8
8	40.6	41.2
9	66.2	66.2
1'	139.3	139.4
2', 6'	106.9	107.1
3', 5'	148.9	148.9
4'	134.5	134.5
7'	42.7	43.2
8'	46.7	46.6
9'	71.6	72
3-OCH_3	56.6	56.6
5-OCH_3	60.2	60.1
3',5'-OCH_3	56.9	56.9
1"	104.8	104.2
2"	75.1	75.0
3"	78.2	78.1
4"	71.5	71.5
5"	77.9	77.9
6"	62.8	62.7

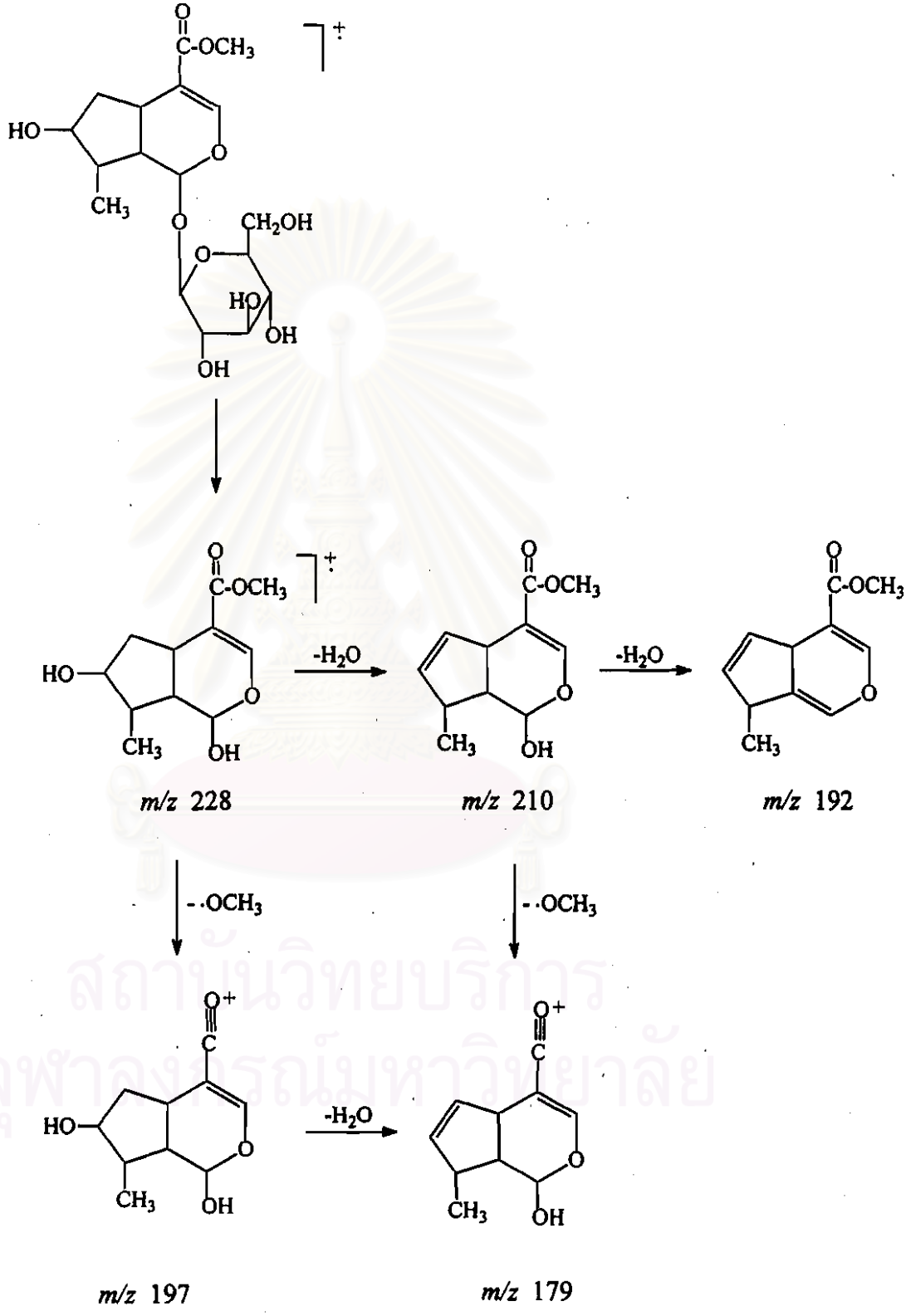
SN-7

SN-7 was obtained as colorless needle crystals in substantial quantities. The compound gave a reddish violet coloration with $FeCl_3/HClO_4$ reagent. Its UV spectrum exhibited absorption maximum at 238 nm, indicative of a carbonyl enol ether chromophore which was typical for an iridoid [182]. The IR spectrum showed the OH absorption at 3400 cm^{-1} as well as the bands at 1700, 1640 and 1290 cm^{-1}, characteristic of the α,β-unsaturated ester carbonyl function. According to ^1H- and ^{13}C-NMR analysis, SN-7 was proposed to be loganin, a known iridoid glycoside with ten carbons skeleton.

The ^1H-NMR spectrum of SN-7 exhibited characteristic signals for an iridoid glycoside: a signal due to the olefinic proton, ascribable for H-3, at δ 7.38 (d, 1H, *J*=1.2 Hz) , a signal of methyl protons, attributable to H-10, at δ 1.09 (d, 3H, *J*=7.0 Hz) and signals for hemiacetalic protons at δ 4.65 (d, 1H, *J* = 7.9 Hz) and δ 5.27 (d, 1H, *J* = 4.4 Hz) which were assignable according to their chemical shifts and couplings, for H-1 and anomeric H-1, respectively. The presence of carbomethoxyl substitution at C-4 was inferred from a downfield methyl singlet at δ 3.69 and the signal of the olefinic H-3 (δ7.38) which was highly deshielded.

The remaining signals could be considered as involving two mutually non-interacting spin systems : one due to the protons of the cyclopentane ring and the other related to those of the sugar moiety. On the basis of chemical shift and coupling analysis, all signals were assigned as shown in Table 4.14. The carbon assignment of SN-7, based on the analysis of HMQC and HMBC spectral data, is given in Table 4.15. The configurations of asymmetric carbons in the molecule of SN-7 were proved to be in agreement with those of loganin as deduced from the data obtained from the NOESY spectrum.

The mass spectrum of SN-7 also corresponded to the structure of loganin. The fragment at *m/z* 228 arose from the loss of the glucosyl moiety while the other important fragments were generated from the process involving the loss of water as shown in Scheme 4.7.



Scheme 4.7 Proposed mass fragmentation of SN-7

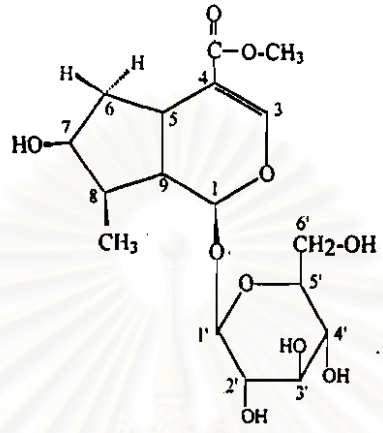
Table 4.14 ^{1}H-NMR assignment of SN-7

Proton position	Chemical shift (ppm)
1	5.27 (d : *J*=4.4 Hz)
3	7.38 (d : *J*=1.2 Hz)
5	3.11 (ddd : *J*=9.2, 7.9, 7.6 Hz)
6α	1.62 (ddd : *J*=13.2, 7.6, 4.9 Hz)
6β	2.23 (ddd : *J*=13.2, 7.9, 1.4 Hz)
7	4.04 (ddd : *J*=4.9, 4.9, 1.4 Hz)
8	1.87 (m)
9	2.03 (ddd : *J*=9.2, 9.2, 4.4 Hz)
10	1.09 (d : *J*=7.0 Hz)
O=C-OCH_3	3.69 (s)
1'	4.65 (d : *J*=7.9 Hz)
2'	3.20 (dd : *J*=9.0, 7.9 Hz)
3'	3.37 (t: *J*=9.0 Hz)
4'	3.28 (dd)
5'	3.31 (m)
6'a	3.67 (dd : *J*=11.9, 5.5 Hz)
6'b	3.89 (dd : *J*=11.9, 1.8 Hz)

Table 4.15 ^{13}C-NMR assignment of SN-7

Carbon position	Chemical shift (ppm)
1	13.4
3	152.1
4	114.0
5	32.1
6	42.7
7	75.0
8	42.1
9	46.5
10	13.4
11	169.5
11-OCH_3	51.6
1'	100.0
2'	74.7
3'	78.0
4'	71.6
5'	78.3
6'	62.7

According to all information mentioned above and the ^{1}H and ^{13}C- NMR spectral data of SN-7 which were in full consistence with the published values of loganin [182]. SN-7 was identified as loganin.



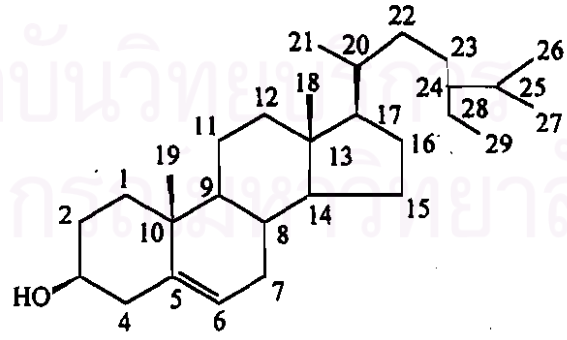
Loganin (SN-7)

SN-8

SN-8 was isolated from the hexane extract as colorless needle crystals. The compound gave a positive green color to the Liebermann-Burchard's reagent, suggesting a steroid nucleus in the molecule. The data obtained from IR, NMR and mass spectra of SN-8 were in full agreement with the published values of β-sitosterol [201]. Therefore, it was concluded that SN-8 was β-sitosterol, a well known phytosterol widely distributed through the plant kingdom. The 13C-NMR assignment of the compound is given in Table 4.16.

Table 4.16 ^{13}C-NMR assignments of SN-8

Carbon position	δC (ppm)	Carbon position	δC (ppm)
1	37.2	16	28.3
2	31.7	17	56
3	72	18	12
4	42.3	19	19.4
5	140.5	20	36.2
6	122	21	18.8
7	32	22	33.9
8	32	23	26.1
9	50.1	24	45.9
10	36.4	25	29.1
11	21	26	19.8
12	39.8	27	19
13	42.3	28	23
14	56.8	29	11.9
15	24.3		



β-sitosterol (SN-8)

Bioactivity Determination

In addition to the structure elucidation, the determination on biological activity of the isolated compounds has been carried out. The activity investigated included brine shrimp lethality, antimicrobial activity, antiviral activity and cardioactivity. The results and discussion dealt with the determination for each activity are given as follows.

1) Brine Shrimp Lethality

The results obtained from the determination of brine shrimp lethality are shown in Table 4.17

Table 4.17 Brine shrimp bioassay results

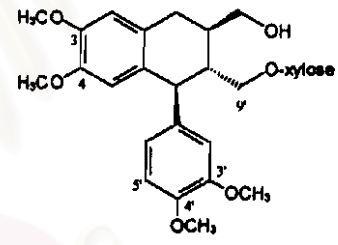
Compound	Percent death at 24 hour			$BSLC_{50}$
	1 μg/ml	10 μg/ml	100 μg/ml	(μg/ml)
SN-1	0	0	0	>100
SN-2	0	0	0	>100
SN-3	0	2	13	>100
SN-4	0	2	11	>100
SN-5	0	9	17	>100
SN-6	0	0	7	>100
SN-7	0	0	0	>100

As indicated in the table , no compounds exhibited distinguished lethal toxicity against the brine shrimp. SN-1, SN-2 and SN-7 had no lethal effects at the maximum test concentration (100 μg/ml). However, it was observable in the experiment that the alkaloids SN-1 and SN-2 exerted non-lethal activity at concentrations of 10 and 100 μg/ml, indicated by the abnormal movement of the brine shrimp.

The compounds which showed lethal toxicity were the sarpagine-group alkaloids, SN-3 and SN-4 as well as the lignans, SN-5 and SN-6. Nevertheless the $BSLC_{50}$ values of these compounds, as determined by using the probit analysis method described by FINNEY[202], were considered to be very low (>100 μg/ml). The known active natural products which structurally related to the compounds investigated, podophyllotoxin and strychnine sulfate, were reported to possess the $BSLC_{50}$ values of 2.4 and 77.2 μg/ml, respectively[191].

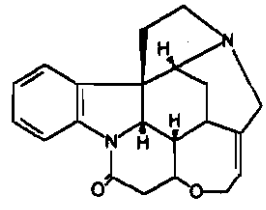
Podophyllotoxin with the high $BSLC_{50}$ is well known of its cytotoxic effect. Several other lignans are also described of their antitumor or cytotoxic activity[179,180]. Many of these compounds share the following features. The five-membered lactone ring, a 3,4,5-trimethoxy phenyl group, a methylenedioxy group and two substituted phenyl groups separated by a four carbon chain. There is no doubt that the podophyllotoxin derivatives dominate the group of cytotoxic lignans. However (+) dimethylisolariciresinol-9'-xyloside , which almost lack all of the features mentioned, has been reported of its antitumour activity[180]. The structure of this compound is very similar to those of SN-5 and especially SN-6. The difference is attributed to the substituents at C-4, C-5, C-4', C-5' and C-9'. However it is inconvenient to make a discussion on the structure-activity relationships because there is no report of the $BSLC_{50}$ value of (+)dimethylisolariciresinol-9'-xyloside and in some cases, the results obtained from the brine shrimp bioassay do not correspond to those from antitumour test[203].

Podophyllotoxin



(+) Dimethylisolariciresinol-9'-xyloside

In the case of strychnan-type alkaloids, as strychnine sulfate has been indicated to be lethally toxic against brine shrimp[191], the absence of activity of SN-1 and SN-2, the bases of the retuline group, may be presumed as resulting from the lack of two additional cyclic rings in the molecule of strychnine .

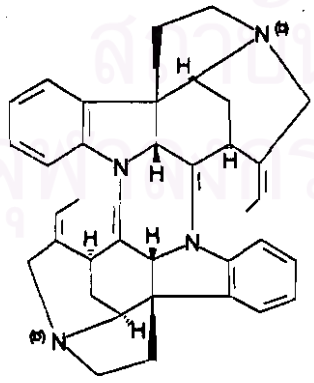


Strychnine

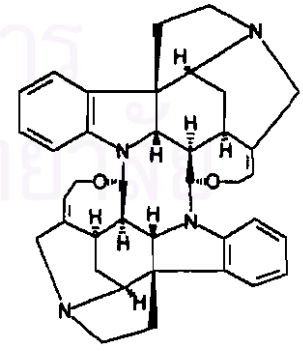
2) Antimicrobial activity

The compounds investigated for this activity were SN-1, SN-2, SN-6 and SN-7. The tests for SN-3, SN-4 and SN-5 were not performed because of limited quantities of the compounds.The activity was tested by means of the agar-diffusion method. The results obtained indicated that all of the tested compounds possessed no activity against any of the test microorganisms at maximum test concentrations (8 mg/ml for SN-1, SN-2 and SN-7; 4 mg/ml for SN-6).

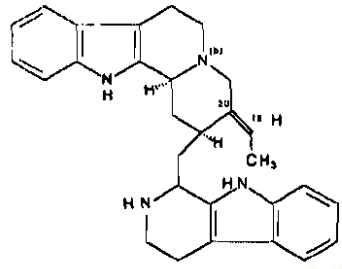
In view of *Strychnos* alkaloids, the compounds which have been reported of a pronounced antimicrobial activity were the bases with usambarane skeleton (tetrahydrousambarensine) of the corynanthean type[170] ,*eg.* bisnor-dihydrotoxiferine and caracurine V , the two bis-tertiary alkaloids, as well as diploceline also exhibited this activity[53,170,171]. No monomeric members of the strychnan type have been so far reported of antimicrobial effect. The results obtained from SN-1 (retuline) and SN-2 (11-methoxy retuline) also showed no evidence to the contrary. However some observation was provided. While SN-1, retuline, showed no activity against any microorganisms, bisnor-dihydrotoxiferine ,which could be regarded as a retuline derivative, exhibited the activity against all strains of the microorganisms used in this experiment[170]. Therefore it was probable that the dimeric feature of the alkaloids was responsible for the antimicrobial activity. The antimicrobial activity of caracurine V , the dimer of the diabloine group, was supportive of this hypothesis.



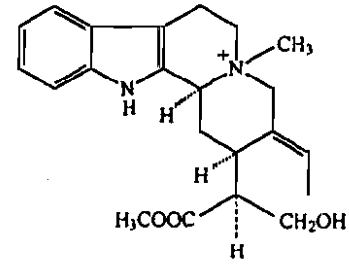
Bisnordihydrotoxiferene



Caracurine V



Tetrahydrousambarensine



Diploceline

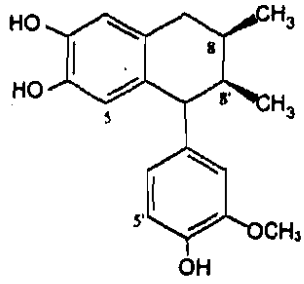
In the case of lignans, nor-isoguaiacin and dihydroguaiaretic acid have been reported to exert inhibitory effects against the growth of *Streptococcus* species, *Staphylococcus aureus* and *Bacillus subtilis* [203]. Noriso-guaiacin also showed activity against *Pseudomonas aeruginosa* [203]. The structure of these two compounds were similar to that of SN-6 which possessed no activity. The marked differences due to the substituted groups at C-8, C-8', C-5 and C-5'. On the basis of limited information obtained, the lack of activity of SN-6 could be hypothetically proposed as resulting from one or more following features:

(1) the sugar and/or hydroxyl substitution at C-9'/C-9 which produced an increase in polarity as well as steric effect in the molecule

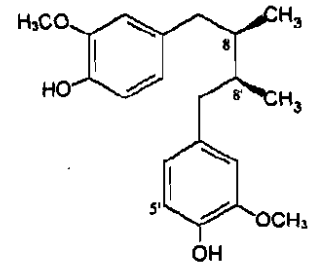
(2) the different relative configuration of the substituents at C-8 and C-8' (*cis cf trans* configurations in the molecule of the active compounds and SN-6 respectively,

(3) the different pattern of substitution on the two aromatic rings.

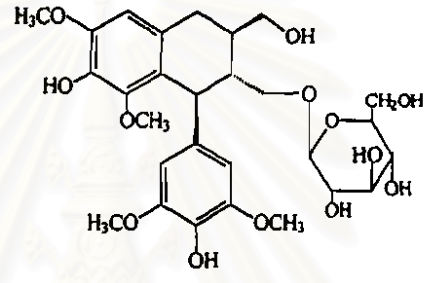
However in order to prove this hypothesis, much more information was still required, It was a pity that SN-5, the free lignan aglycone, was isolated from the plant in so small amounts that the test for the compound could not be performed, otherwise the useful information for better understanding in the structure activity relationship might be obtained.



Nor-isoguaiacin



Dihydroguaiaretic acid



SN-6

3) Antiviral activity

Table 4.18 Results of antiviral testing

Compound	Concentration tested (μg/ml)	Antiviral activity[a]	
		HSV-1	HSV-2
SN-1	100	++	+
SN-2	100	++	++
SN-4	50	+	+++
SN-5	20	+	+
SN-6	100	+++	++
SN-7	100	+	+

[a] plaque reduction test
++++, 100% reduction; +++, more than 50% reduction;
++, approximately 50% reduction; +, less than 50% reduction.

The results obtained indicated that each compound exhibited some antiviral activity. Among the compounds tested at the concentration of 100 μg/ml, SN-6 was the most active against HSV-1. The ED_{50} value of the compound was less than 50 μg/ml as

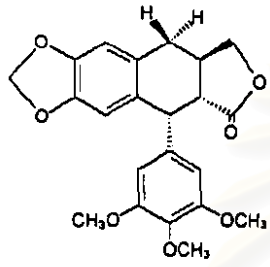
deduced from the degree of positive result (+++). On the basis of the same consideration, the ED_{50} values of SN-1 and SN-2 were 100 μg/ml while that of SN-7 was less than 100 μg/ml. In the case of HSV-2, SN-2 and SN-6 exhibited the same activity (ED_{50} = 100 μg/ml), being more active than SN-1 and SN-7. However, the activity of all the compounds was relatively low as compared with the ED_{50} values of acyclovir which were 0.06 μg/ml for HSV-1 and 0.5μg/ml for HSV-2.

There were some difficulties in comparing the activity of SN-4 and SN-5 with those of the other compounds owing to the different concentration tested. However, in the case of SN-4 the activity against HSV-2 was rather distinguished. Despite the lower concentration the compound exhibited higher activity than those tested at concentration of 100 μg/ml. (The ED_{50} of the compound was less than 50 μg/ml). This compound is a new alkaloid of the sarpagine group to which another isolated compound, SN-3, belongs. However SN-3 has not been submitted to antiviral test because of its limited yield obtained.

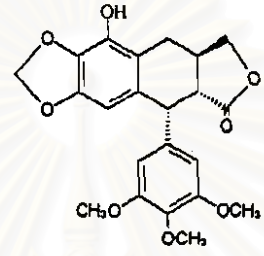
The compound SN-5 of the lignan group showed relatively low activity. However it should be realized that the concentration tested of the compound was only at 20 μg/ml. Pharmacological study on antiviral activity of podophyotoxin derivatives indicated that in all cases tested, the glucoside derivatives exhibited less antiviral activity than their respective aglycones [179,180]. On the basis of this finding, SN-5 was expected to be more active, at the same concentration, than SN-6 which showed significant activity against both of HSV-1 and HSV-2. However this proposal still required more information for leading to the conclusion.

As already mentioned, most of reports on antiviral activity of lignans dealt with podophyllotoxin derivatives. The pharmacological study of these compounds indicated that deoxypodophyllotoxin and β-peltatin were the most potent and approximately equal in effectiveness [180]. The C-7 hydroxy was proved not to be essential for antiviral effect while replacement of a furan ring instead of the lactone ring resulted in a ten-fold reduction in activity. The maintenance of the two phenyl groups in a specific configuration relative to each other was important, evident from a much decrease in activity of dihydroanhydropodorhizol as compared to that of the corresponding cyclic analogue, deoxypodophyllotoxin . The antiviral activity of SN-5 and SN-6 observed in this experiment might be considered as an additional evidence for some points of the structure-

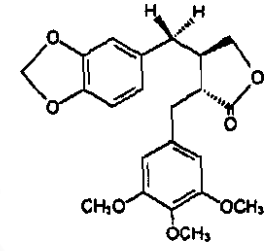
activity relationships mentioned above such as in cases of the unnecessary of the hydroxyl group at C-7 (equivalent to C-2' in the structure of SN-5 and SN-6) and the importance of the certain relative configuration of the two aromatic rings. In addition it was shown that the presence of the cyclic ester or ether in the molecule might not be an essential requirement for antiviral activity. (Nonetheless it was expected to have some effect in quantitative point of view).



Deoxypodophyllotoxin



β-Peltatin



Dihydroanhydropodorhizol

4) Cardioactivity

Effects of all tested compounds at concentration of 1×10^{-5} M on the isolated auricles of rat are shown in Figures 65-68.

The alkaloids SN-1 and SN-2 increased the amplitude of beats of the right auricle in some extent. A little increase in the rate was also observed for SN-1 but none for SN-2. The two compounds affected neither the amplitude of contraction nor the rate of the left auricle.

The lignan SN-6 caused a little increase in the amplitude of beats but a marked increase in the rate of the right auricle. The compound also increased the amplitude of contraction of the left auricle but no effect on the rate was observed.

A little increase in the rate and amplitude of contraction of the right auricle and in the amplitude of contraction of the left auricle was observed for the iridoid SN-7. The compound had no effect on the rate of the left auricle.

This test on the isolated auricles of rat was performed in order to primarinary examine the cardioactivity of the compounds. The concentration of 1×10^{-5} M used in the test was logical for the determination. The results obtained indicated that SN-6 was the most intereting substance in this point of view. More useful information was expected to be obtained from the detailed investigation on this activity of the compound.

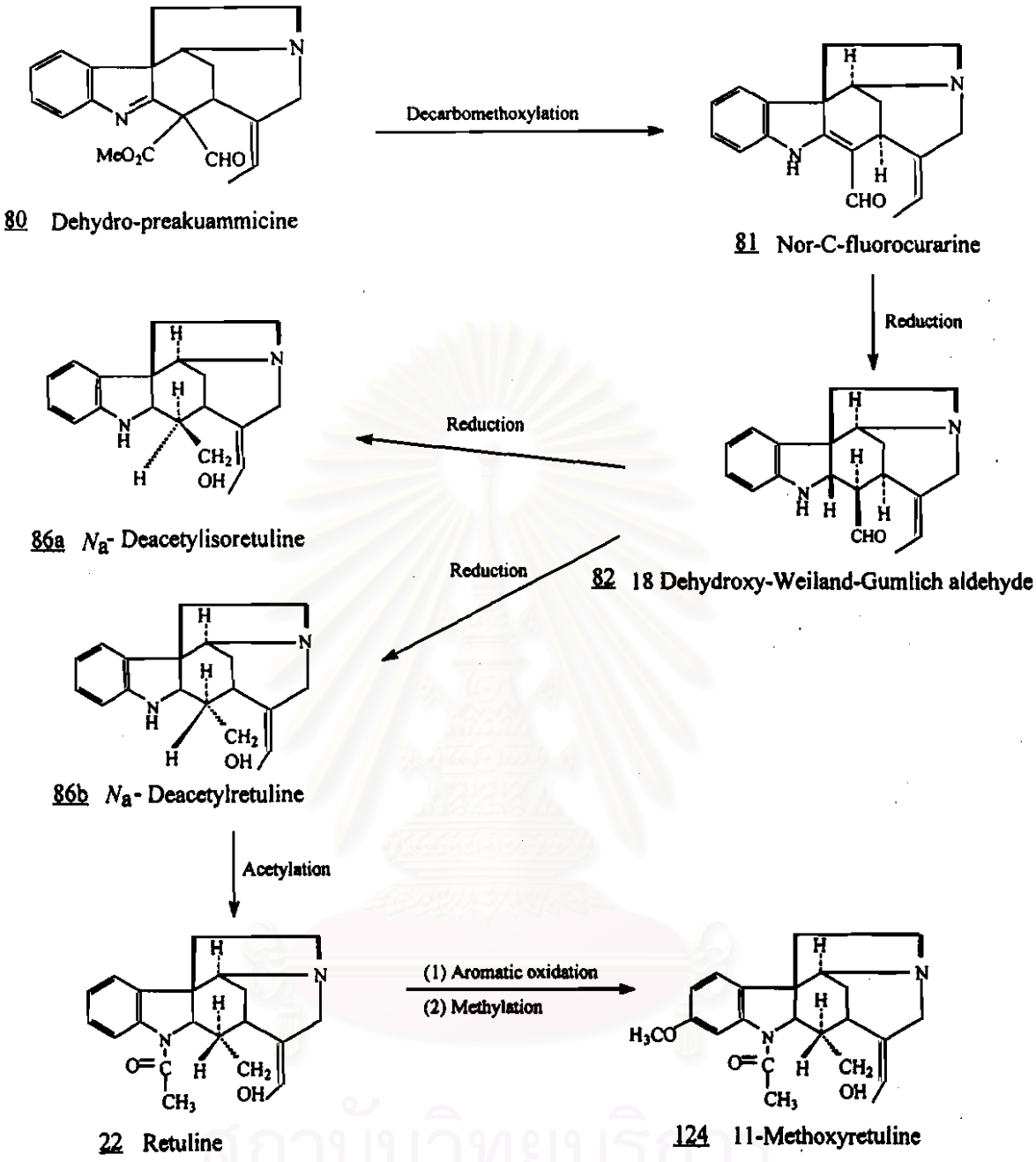
Biogenetic Considerations of Isolated Compounds

The Alkaloids

It is currently accepted that the terpenoid indole alkaloids of various types are derived from the central intermediate, strictosidine. The further important intermediates in the pathway to the bases of the corynanthean type have been postulated to be the hypothetical intermediate (78) and 4,21 dehydrogeissoschizine (79) while the formation of the strychnan type requires some additional ones which are dehydropreakuammicine (80), nor-C-fluorocurarine (81) and 18-deoxy Weiland-Gumlich aldehyde (82). A summary account of the proposed biogenetic pathway of these alkaloids is also given in Chapter II under the heading of 'Biosynthesis of *Strychnos* Alkaloids'.

The proposed for biogenetic pathway of retuline (SN-1) and 11-methoxyretuline (SN-2), the two retuline group alkaloids obtained from the present work, followed that of the strychnan-type alkaloids. Reduction of nor-*C*-fluorocurarine (81), the starting point for the formation of these bases, could afford 18-dehydroxy-Weiland-Gumlich aldehyde (82) from which two 16-epimeric compounds, N_a-deacetyl isoretuline (86a) and N_a-decetyl retuline (86b) are derived by reduction. Retuline (22) can be considered as N_a-acetylated product of the epimer. The proposed biogenetic pathway to retuline is depicted in Scheme 4.8.

Like retuline, 11-methoxy retuline (124) is postulated to be derived from the 16α reduced product of 18-dehydroxy-Weiland-Gumlich aldehyde, N_a-deacetyl retuline. The 11-methoxy substitution is presumed to occur after the acetylation *ie.* in the molecule of retuline rather than in that of its N_a-deacetyl precursor. This proposal is based on the fact that the aromatic substitution of N_a-deacetyl retuline should take place at C-10 or C-12 rather than C-11 owing to the indolic amino group which behaves as *ortho*-or *para*-director in accordance with its electron-donating property. In the case of retuline in which the indolic amino group is acetylated, the ability in donating electron of nitrogen is reduced because of the carbonyl function, the *meta*-substitution at C-11 thus more possibly occurs. This is substantiated by the finding that no *N*a-deacetyl bases of the retuline group with C-11 monosubstitution are known; tsilamnibine, the only one monosubstituted retuline derivatives of N_a-deacetyl series, possesses C-10 substitution [101]. In fact only two members of the strychnan type have been so far reported as N_a-deacetyl bases with C-11 substitution.



80 Dehydro-preakuammicine

81 Nor-C-fluorocurarine

86a N_a- Deacetylisoretuline

82 18 Dehydroxy-Weiland-Gumlich aldehyde

86b N_a- Deacetylretuline

22 Retuline

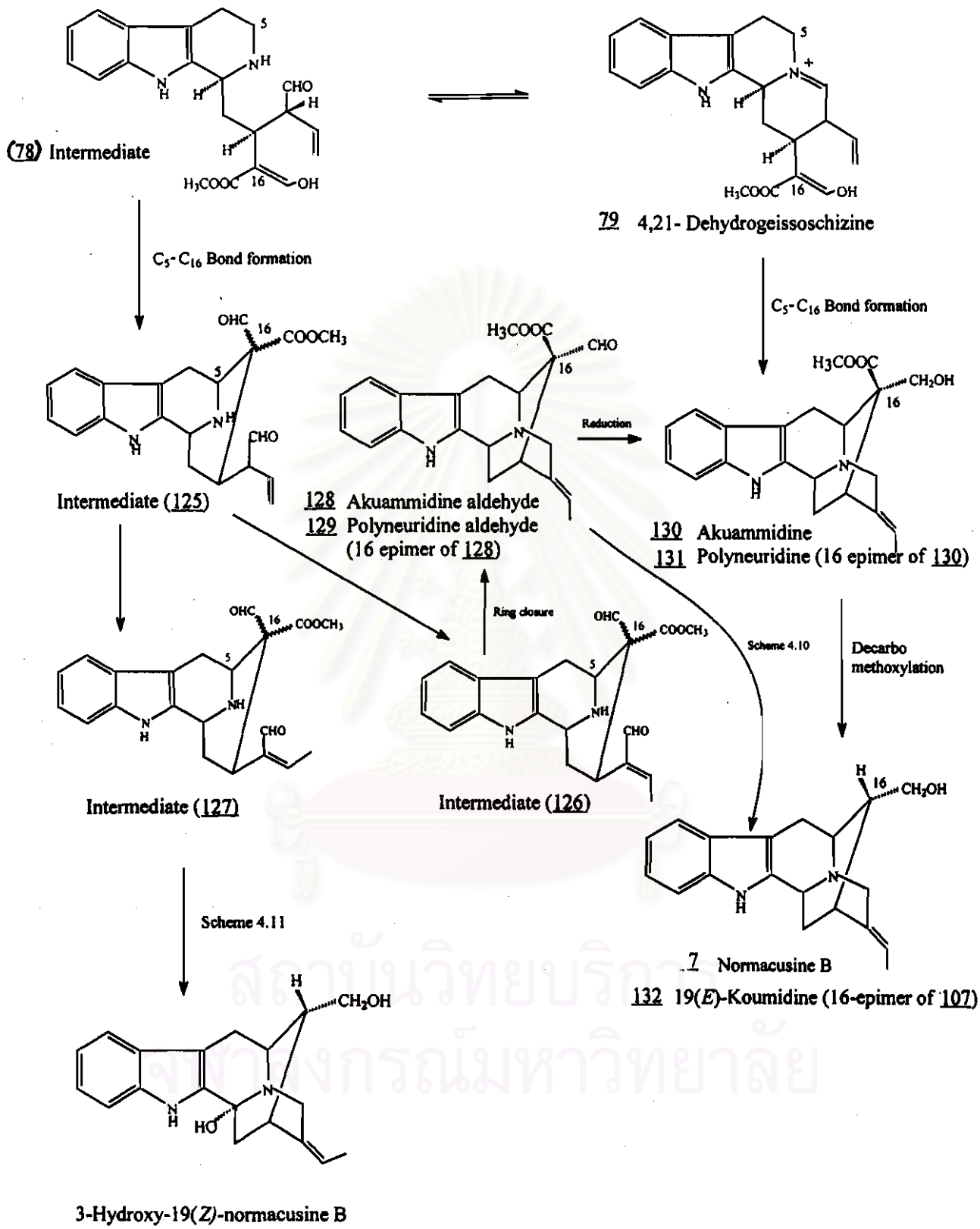
124 11-Methoxyretuline

Scheme 4.8 Tentative biogenetic pathway to the retuline group alkaloids of *Strychnos nitida* G.Don.

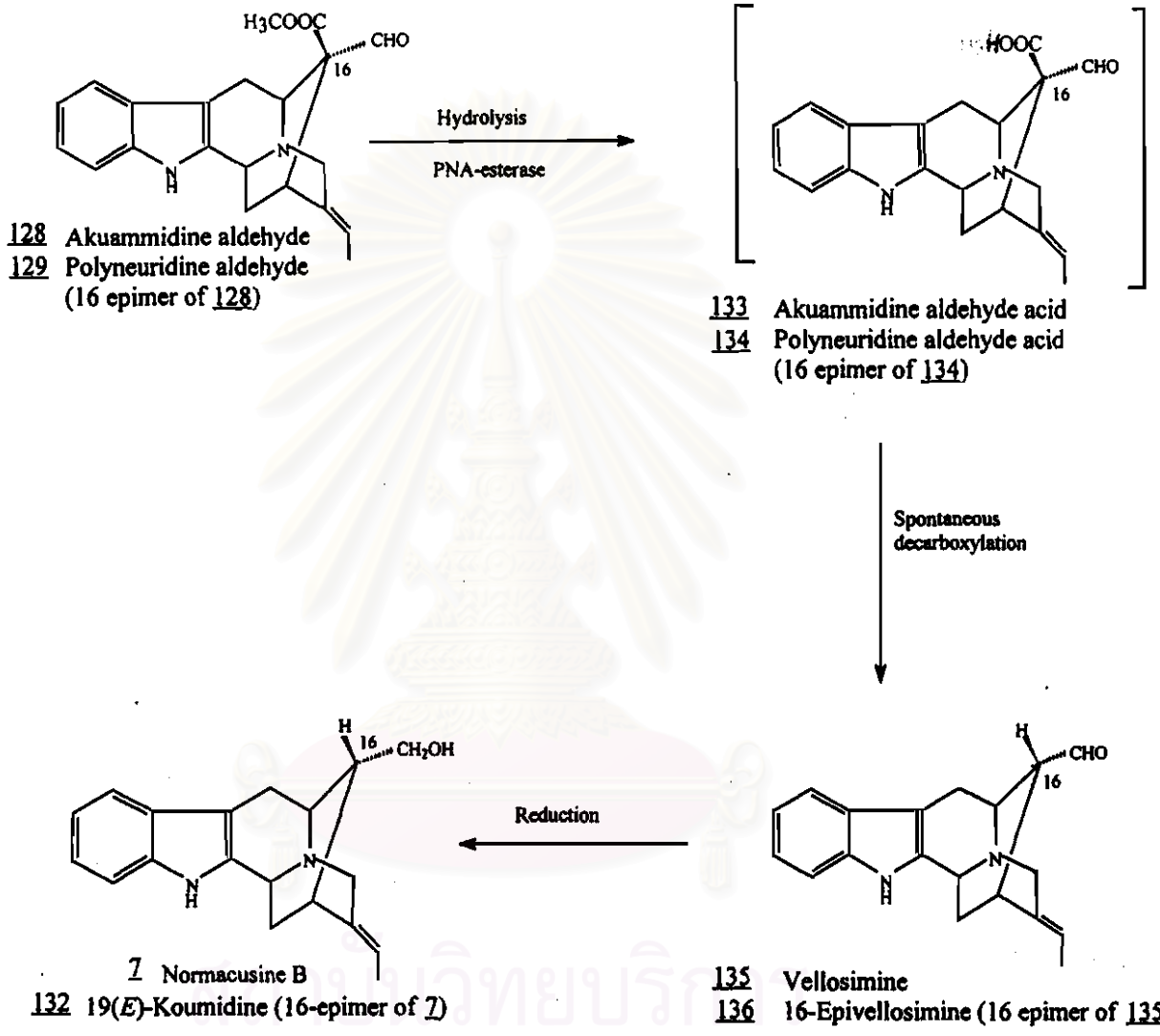
They are 11-methoxy Wieland-Gumlich aldehyde and its 17-*O*-methyl derivative of the diaboline group, both of which have been isolated from *Strychnos angolensis* [114].The assumption that 11-methoxy retuline is derived directly from retuline is also in agreement with the co-occurrence of these two compounds in plants. This situation is found in case of 11-methoxyisoretuline and isoretuline as well. In conclusion, the formation of 11-methoxy retuline (124)can be considered to be derived from retuline by aromatic oxidation at C-11 followed by methylation (Scheme 4.8)

Besides retuline and 11-methoxy retuline, the other isolated alkaloids includes normacusine B (SN-3) and 3-hydroxy-19(*Z*)-normacusine B (SN-4) of the sarpagine group. The tentative biogenetic pathway to normacusine B could be demonstrated by a rather complicated grid as shown in Scheme 4.9. The starting point of the grid is postulated to be the hypothetical intermediate (78) from which the intermediate (125) is derived by intramolecular C-C bond formation between C-5 and C-16. Double bond migration from C-18 to C-19 in the molecule of the latter leads to the formation of two geometrical isomers, the intermediate 126 and 127 with 19-(*E*) and 19-(*Z*) configurations, respectively. Cyclization of the 19-(*E*)- intermediate 126 gives rise to akuammidine aldehyde (128) and polyneuridine aldehyde (129), both of which could be reduced to afford akuammidine (130) and polyneuridine, respectively. Decarbomethoxylation of akuammidine finally leads to the production of normacusine B (7).

The alternative route to the formation of normacusine B from the cyclization product of the intermediate (126) is also proposed in accordance with the isolation of enzyme polyneuridine aldehyde esterase (PNA-esterase) from cell suspension cultures of *Rauwalfia serpentina* Benth [205] (Scheme 4.10). Polyneuridine aldehyde (129) and akuammidine aldehyde (128) have been proved to be exclusive substrates of the enzyme, and products of the reaction have been pointed out by the experiment to be vellosimine (135) and 16-epivellosimine (136). The loss of one carbon from the monoterpenoid unit of the substrate might be explained by the postulated intermediacy of the unstable hydrolysis product, polyneuridine aldehyde acid (133) (or akuammidine aldehyde acid (134)), which can undergo decarboxylation to afford vellosimine (135) and its epimer (136). Vellosimine (135) can be regarded as another parent of normacusine B, its reduced product.



Scheme 4.9 Tentative biogenetic pathway to the sarpagine groups alkaloids of *Strychnos nitida* G. Don.



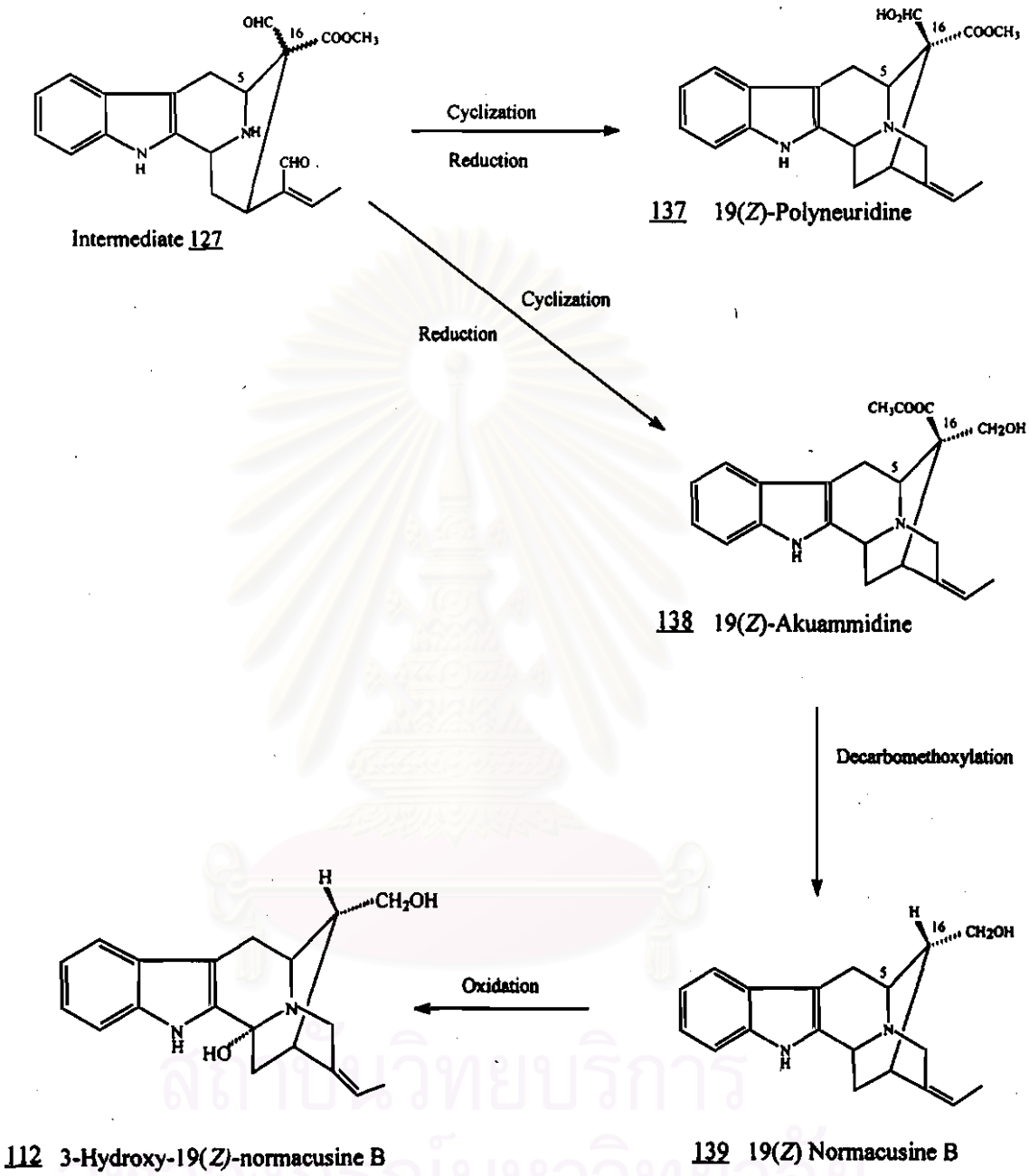
128 Akuammidine aldehyde
129 Polyneuridine aldehyde
(16 epimer of 128)

133 Akuammidine aldehyde acid
134 Polyneuridine aldehyde acid
(16 epimer of 134)

7 Normacusine B
132 19(*E*)-Koumidine (16-epimer of 7)

135 Vellosimine
136 16-Epivellosimine (16 epimer of 135)

Scheme 4.10 Proposed biogenetic route from akuammidine aldehyde to normacusine B



Scheme 4.11 Tentative biogenetic route to 3-Hydroxy-19(*Z*)-normacusine B

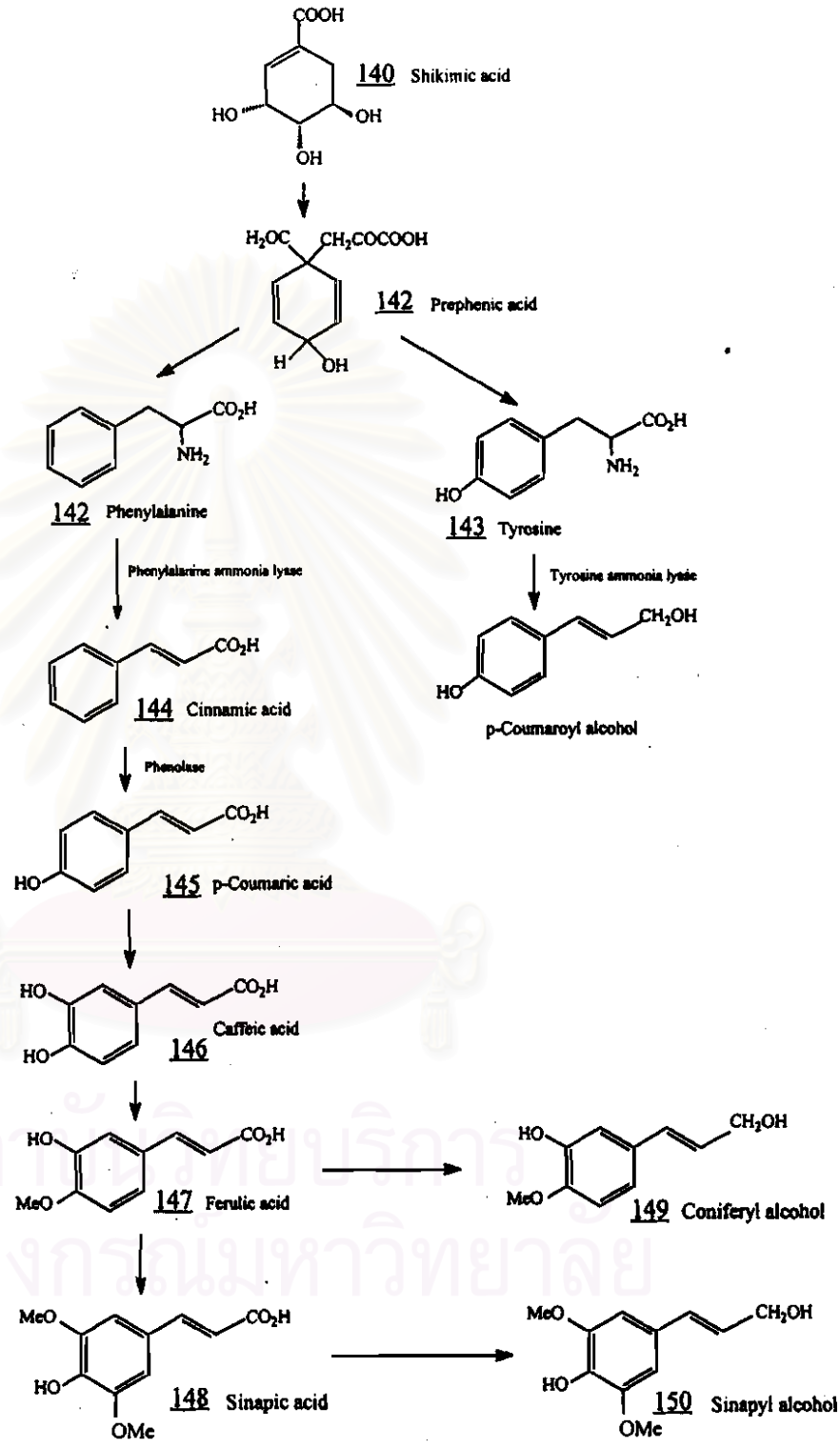
In addition to the process through the intermediates (125) and (126), the biogenetic route to normacusine B could be considered as starting more directly from 4,21-dehydrogeissoschizine. Intramolecular C-C bond formation between C-5 and C-16 and double bond migration from C-18 to C-19 could yield akuammidine (130) from which normacusine (7) is derived through decarbomethoxylation.

The biogenetic pathway to the other sarpagine group alkaloids, 3-hydroxy-19(*Z*)-normacusine B (112), could be considered as proceeding through progression of intermediate (78) → intermediate 125 → intermediate 127. The last intermediate can undergo cyclization and reduction to afford 19-(*Z*)-polyneuridine (137) and 19-(*Z*)-akuammidine (138) (Scheme 4.11). Decarbomethoxylation of the latter epimer gives rise to 19-(*Z*)-normacusine B (138) which could be oxidized to yield 3-hydroxy-19-(*Z*)-normacusine B (112).

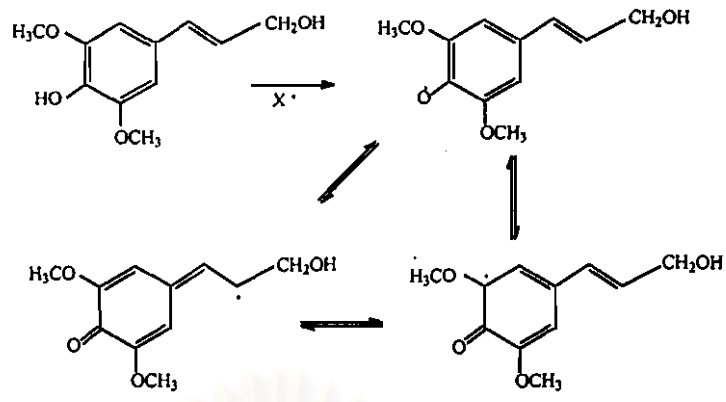
The lignans

The biosynthesis of lignans, similar to that of lignins, proceeds through the shikimic acid-cinnamic acid pathway (Scheme 4.12). Classical work of Davis has suggested the conversion of carbohydrates into shikimic acid (140) with subsequent branching to afford either phenylalanine (142) or tyrosine [143](206). The principal route to lignins and lignans follows the action of a lyase on phenylalanine (143) to give cinnamic acid (144) from which its derivatives with various patterns of aromatic substitution (145-148) can be produced by the function of phenolases and of methyl transferase. On catalyzation by dehydrogenase, these acids are converted into its corresponding alcohol called lignols (*eg.* 149, 150) Condensation of two or more units of lignols, induced by the action of peroxidase, finally affords lignins and lignans [179].

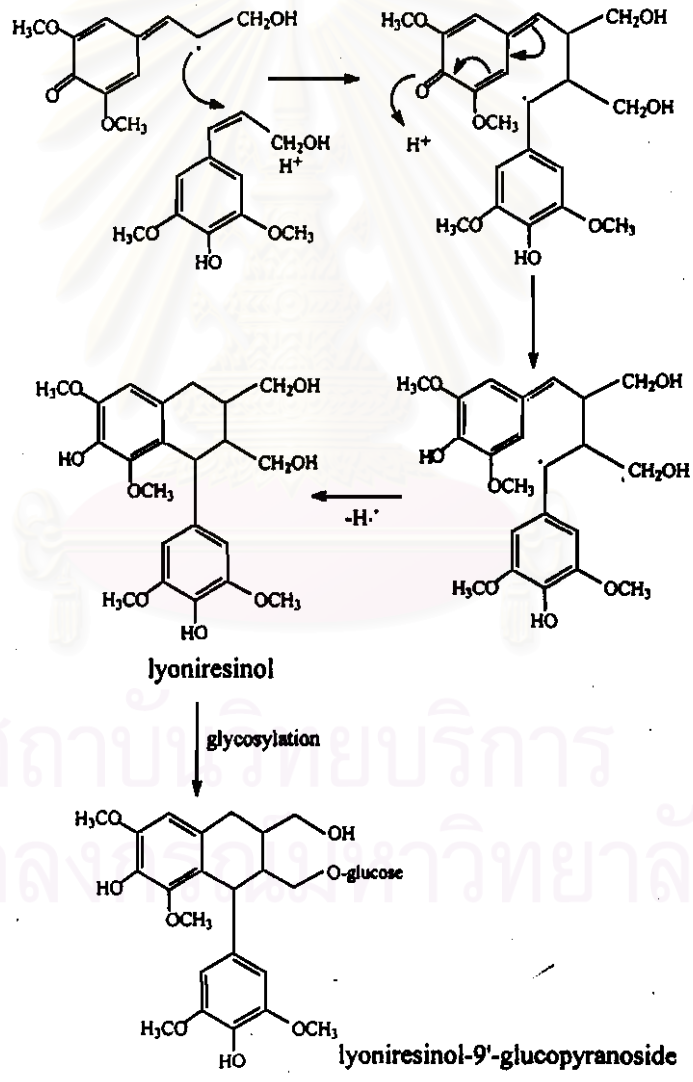
The lignans isolated from the stem of *Strychnos nitida* G.Don. include *rac*-lyonresinol and its 9'-glucopyranosides. These compounds can be considered as being derived from synapyl alcohol (150) through the quinone methide intermediate (151) which is a mesomeric form of the oxy radical generated by ionization of the phenolic hydroxyl group followed by a single electron transfer (Scheme 4.13). The proposed formation of lyoniresinol is demonstrated in Scheme 4.14. Glycosylation at the position 9' in the molecule of lyoniresinol gives rise to its (+) and (-) glucopyranosides .



Scheme 4.12 Shikimate-cinnamate pathway



Scheme 4.13 Formation of mesomeric radicals from synapyl alcohol.



Scheme 4.14 Proposed biogenetic route to lyoniresinol and lyoniresinol-9'-glucopyranoside

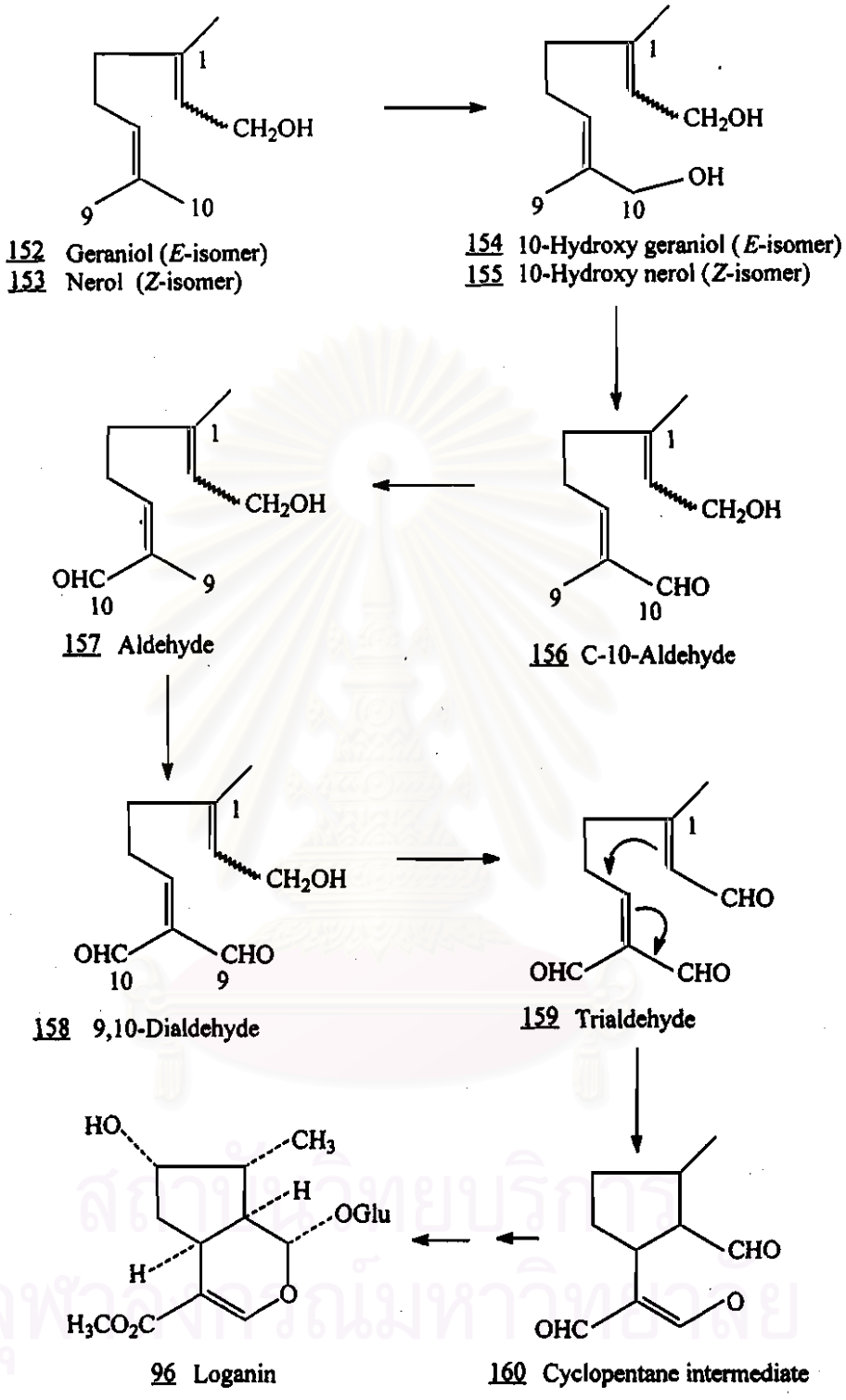
The Iridoid

Loganin, the iridoid obtained from this work, is biogenetically related to terpenoid indole alkaloids. It is known as a key precursor of the terpenoid building unit of the indole bases, secologanin. Many biosynthetic studies of these two iridoids as well as the alkaloids have been carried out [207-212]. The proposed biosynthetic pathway of loganin has been established inaccordance with feeding experiments and cell free determination [162]. However more detailed investigation are necessary for the elucidation of some problematic steps the mechanism of which is still unknown.

The results obtained from *in vivo* feeding experiments have suggested that both geriniol (152) and nerol (153), two isomeric monoterpene alcohol arising from mevalonic acid, are biogenetic precursors of loganin [165]. The allylic hydroxylation at C-10 to form 10-hydroxy derivative was postulated to be the first step beyond the stage of geraniol/nerol as evident from the incorporation of 10-hydroxygeraniol (154) and 10-hydroxynerol (155) into loganin [207]. The reaction was demonstrated to be catalyzed by a monoterpene hydroxylase which exclusively oxidized both substrates with a high degree of stereospecificity *ie.* the hydroxylation occurred only at the C-10 position (*E*-configuration) of the compounds [208].

The next step in the pathway leading to loganin was assumed to be enzymatic oxidation of 10-hydroxygeraniol or -nerol (154 or 155) to yield corresponding C-10 aldehyde (156). An isomerisation at this stage then would provide the aldehyde (157) which might be converted to the 9,10-dialdehyde by the functions of the mentioned monoterpene hydroxylase and alcohol dehydrogenases, respectively. Final oxidation at C-1 of the dialdehyde (158) could produce the trialdehyde (159) which on cyclization give rise to the cyclopentane intermediate (160) and, finally, loganin (96). The proposed formation of loganin from geraniol/neol is illustrated in Scheme 4.15.

The sequence from the intermediate to loganin has been studied in detail by several groups of researchers. Inouye *et al* demonstrated the precursor role of 7-deoxyloganic acid (161) in loganin biosynthesis by feeding experiments performed in *Lonicera japonica* [209] while the intermediacy of 7-deoxyloganin (162) was suggested by Battersby *et al* in accordance with the incorporation of the compound into loganin and



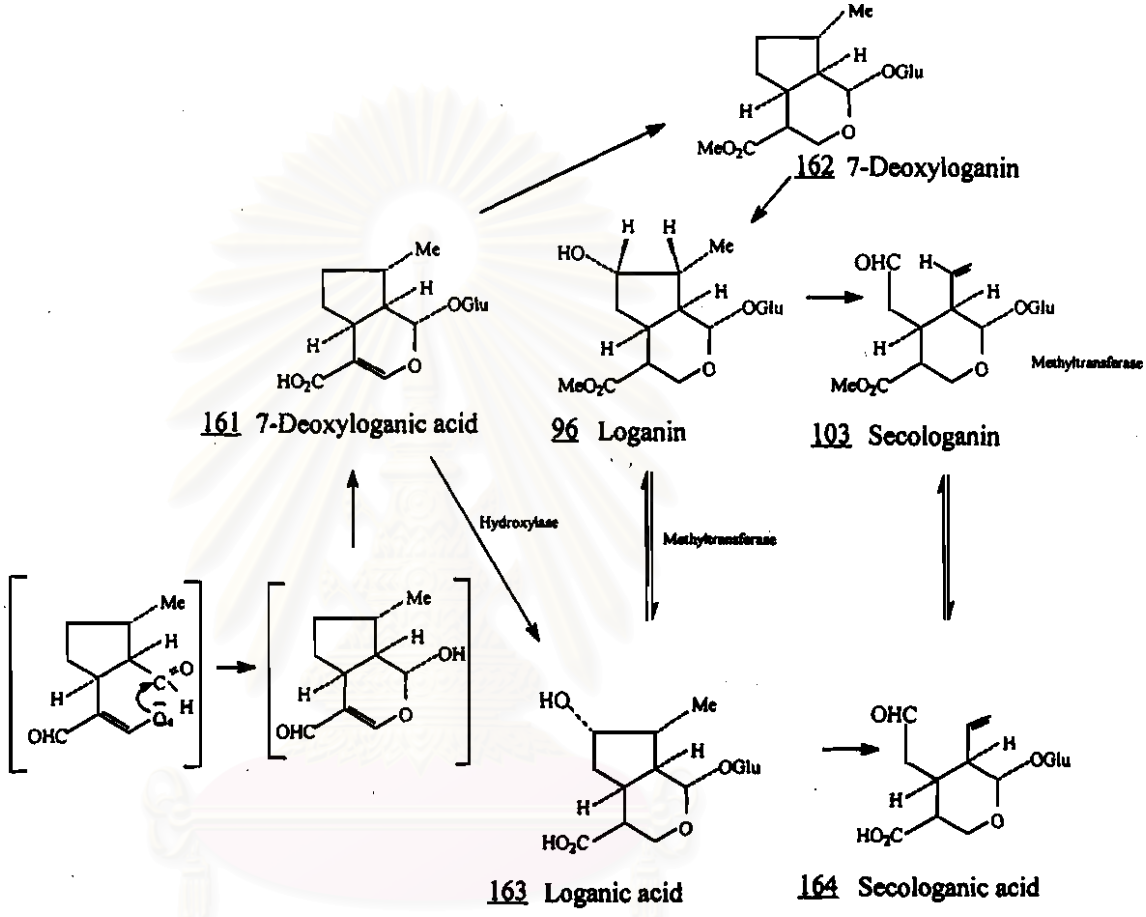
Scheme 4.15 Hypothetical pathway from geraniol/nerol to loganin.

several alkaloids in *Catharanthus roseus* [210]. These findings pointed to the involvement of hydroxylation at C-7 which should take place at the ester stage in the biosynthetic pathway to loganin.

In addition to the studies mentioned above, Guarnaccia *et al*, who reported the occurrence of loganic acid (163) and secologanic acid (164) in *C. roseus*, demonstrated that loganic acid (163) was converted *in vivo* into loganin (96) and secologanic acid (164), respectively and on the other hand loganin was a precursor of secolaganic acid (164) and secologanin (103), respectively as well [211]. On the basis of these results, carboxyl methylation steps in the sequence from loganic acid to secologanin was proposed to be reversible (illustrated in Scheme 4.16) and a direct conversion of loganic acid to secologanic acid could be possible.

The isolation of the enzyme responsible for carboxyl methylation, loganic acid methyl transferase from seeding of *C. roseus*, provided more evidence for loganin/secologanin biosynthesis [212]. The enzyme showed high substrate specificity with either loganic acid (163) or secologanic acid (164) whereas 7-deoxyloganic acid (161) was not converted into corresponding methyl ester. The results strongly indicated that hydroxylation at the 7-position in the molecule of 7-deoxyloganic acid occurred before the methylation steps during loganin biosynthesis. If so, the progression of 7-deoxyloganic acid (161) → loganic acid (163) → loganin 96 would be more probable than the sequence: 7-deoxyloganic acid 161 → 7-deoxyloganin 162 → loganin (96).

In the case of the *Strychnos*, the occurrence of both loganic acid and 7-deoxyloganin, the proposed intermediates in loganin biosynthesis, can be detected in plants of this genus. The former have been isolated from the wood of *S. ligustrina* [187] while the latter from the fruits of *S. nux-vomica* [136]. In both cases, the co-occurrence of loganin in the same plant part has been encountered. These findings have suggested that the loganin biosynthesis in *Strychnos* could proceed *via* either loganic acid or 7-deoxyloganin. Both *S. lucida* and *S. nux-vomoca* are Asian species belonging to the section *Strychnos*. The different biosynthetic pathway to loganin in the *Strychnos* might be due to the different enzymatic systems in species level or plant part level.



Scheme 4.16 Biogenetic relationship at the iridoid and secoiridoid stage

Chemotaxonomic Consideration of Isolated Compounds

The Alkaloids

The alkaloids isolated from the stems of *Strychnos nitida* belong to two types of terpenoid indole alkaloids: the strychnan type with retuline (SN-1) and and 11-methoxy retuline (SN-2) of the retuline group, and the corynanthean type with normacusine B (SN-3) and 3 hydroxy-19(*Z*)-normacusine (SN-4) belonging to the sarpagine group. The distribution of the former type in the family Loganiaceae is restricted to the genus Strychnos. Only a few of its members can be found in some genera of the Apocynaceae.

In general , the strychnan type of alkaloids is considered as a characteristic of *Strychnos* species. The corynanthean type , in contrast, has a widespread distribution throughout three plants families, Apocynaceae, Loganiaceae and Rubiaceae, all of which are the major sources of terpenoid indole alkaloids. The distribution of alkaloids in the sub-type level is similar to those of the types to which they belong. The sarpagine group alkaloids can be found nonspecifically in the Loganiaceae and the Apocynaceae, while the alkaloids of the retuline group is rather specific to the genus *Strychnos*.

In view of individual alkaloids, retuline has been previously reported as a constituent of some African *Strychnos* species belonging to various botanical sections while 11-methoxy retuline has been formerly found from only one African species. The occurrences of the two compounds in *Strychnos* species are shown in Table 4.19.

Table 4.19 The occurence of retuline and 11-methoxy retuline in the *Strychnos*.

Compounds	Sources			
	plant parts	species	sections	locations
retuline	stem bark; root bark	*S. henningsii*	Breviflora	Africa
	stem bark & root bark	*S. kasengaensis*	Lanigerae	Arica
	root bark	*S. variabilis*	Rouhamon	Africa
	stem bark	*S. camptoneura*	Scyphostrychnos	Africa
11-methoxy retuline	stem bark	*S. kasengaensis*	Lanigerae	Africa

As can be deduced from the table , the isolation of retuline and 11-methoxy retuline from *Strychnos nitida* reported from the present investigation is the first evidence

which indicates the presence of the two compounds in the Asian species as well as in the section Strychnos. None of the American species have been reported of these alkaloids.

All the previous reports of retuline and 11-methoxy retuline deal with the African species which are the main source of retuline froup alkaloids in both monomeric and dimeric forms. The member of the retuline group can be found in American species as well ; most of them belong to the group of dimeric alkaloids, frequently found in the section Strychnos. Only a few of monomeric alkaloids have been isolated from the American species; the majority of the bases are the members of *N*-methyl-*sec*-pseudo series found in the sections Strychnos and Breviflorae. It is notable that this series of the retuline group are so far encountered in the American species only, and the bases of pseudo series have never been found in any species of the three continents.

The occurrence of the retuline group alkaloids is rare in *Strychnos* species of Asia and Australia. Only one representatives of monomeric and of dimeric alkaloids, N_a-deacetylretuline and bisnordihydrotoxiferine, have been reported to be isolated from *S. potatorum* and *S. wallichiana,* respectively [61,120]. The obtained number of the report of the dimeric alkaloids, however, might not be indicated the trend of the real existence. Since most of these bases naturally occur in a quaternized form, thus present in an aqueous part of the plant extract, but almost all of the chemical works on the Asian *Strychnos* species have been carried out on the non-aqueous extract containing tertiary alkaloids. On the other hand the result obtained for the monomeric alkaloid is more reliable since almost all of the compounds so far found are the tertiary bases. The isolation of retuline and 11-methoxy retuline from *S. nitida*, therefore, provides an interesting point. This is the second observation for the monomeric alkaloid of the retuline group found in the Asian *Strychnos* species.

According to previous reports of alkaloids found in Asian *Strychnos* species [3-6] together with the results obtained from the present work of *S. nitida*, it may be suggested that the section Strychnos of Asia consists of three group of species which are different in chemical evolution. The first group comprises 6 species which are *S. nux-vomica, S. nux-blanda, S. ignatii, S. wallichiana, S. lucida* and *S. rupicola.* These species contain large amounts of alkaloids, most of which are the bases of the strychnine group, indicative of a strongly developed strychnan-type pathway. This group can be regarded as the leader of

advancement as deduced from the high potential of alkaloid production in both quantitative and quanlitative points of view.

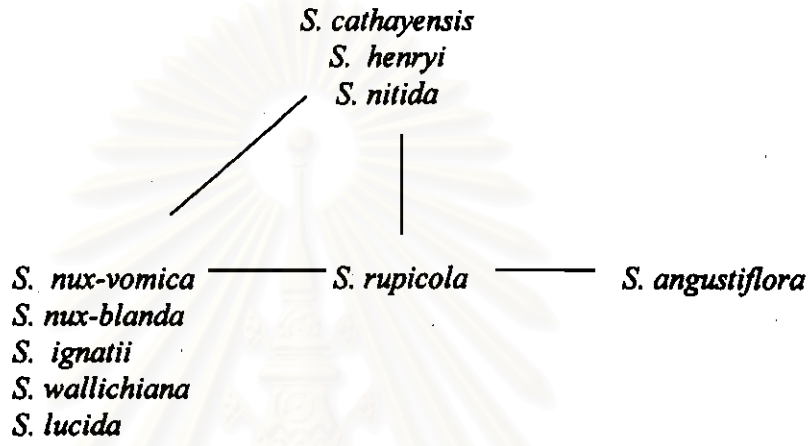
The second group includes *S. nitida, S. cathayensis* and *S. heryi*, all of which contained at most only trace of alkaloids as indicated by Bisset and Phillipson's on their investigation of the leaf sample [3]. In consideration of their chemical evolution, *S. nitida* and *S. cathayensis* are used as representatives of the group since they are the species from which identified alkaloids have been obtaied. The existence of retuline and 11-methoxy retuline as the major alkaloids in *S. nitida* and of diaboline, 11-methoxy diaboline and henningsoline in *S. cathayensis* [112] probably points to their incapability in producing more complex alkaloids. The metabolic process of the strychnan-type production in the plant is somehow unable to further the next step beyond the early stage of the retuline-group or diaboline-group formation. On the basis of the alkaloid content and the information obtained from *S. nitida* and *S. cathayensis* , this group of species can be considered as more primative than the former.

The last group is represented by *S. angustiflora* which produces alkaloid in a moderate content [3]. The species is differentiated from those six species of the first group by the presence of the yellow angustine- type alkaloids in its leaves. Morphologically, this member of section Strychnos could be considered as related to section Rouhamon, evident from certain of its floral character. The occurrence of angustine bases in *S. potatorum*, the only one Asian species of section Rouhamon, is also in agreement with such correlation. According to the degree of metabolic evolution determined by the numbers and sites of cyclization together with bond migration, angustine-type alkaloids are regarded to be less advance than the bases of the strychnan type. This may imply the evolution of the plants in which the alkaloids found.

The relationships among the species belonging to these three groups of section Strychnos can be deduced from their alkaloids. The affinity between the first and the second is suggested by the same developed biosynthetic pathway to strychnan-type bases. One species of the first group, *S. rupicola*, also produce angustine derivatives. This species contains small amounts of alkaloids however it is included in the first group owing to its ability in producing the bases of the strychnine group. *S. rupicola*, therefore, may be considered as a connecting link between the first and the last groups with different

developed pathway of alkaloid biosynthesis, the former with the pathway towards the typical *Strychnos* alkaloids and the latter with the other towards those of the angustine type.

On the basis of the above consideration, the Asian species of the section Strychnos are related as shown in the following diagram:



The demonstrated relationships is established under the limitation of the present information obtained. A lot of research still remains to be undertaken. The detailed investigation of *S. henryi*, which belongs to the same group as *S. nitida* and *S. cathayensis*, and the determination on the nature of minor alkaloids present in the alkaloid-rich species together with the comparative studies of the alkaloids encountered in different plant parts will be provide a more comprehensive picture of this relationship.

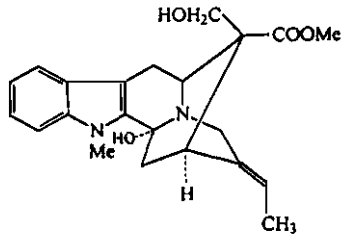
Besides the retuline group alkaloids, *S. nitida* contain the bases of sarpagine group, normacusine B and 3-hydroxy-19(*z*) normacusine B. The former is a known alkaloid obtained from some species of *Strychnos* ; and also from those of various genera of the Apocynaceae. The occurrence of the alkaloid in *Strychnos* species is shown in the table below:-

Table 4.20 The occurrence of normacusine B in the *Strychnos* .

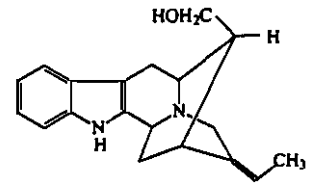
S. species	plant parts	sections	location
S. dolichothyrsa	root bark	Breviflorae	Africa
S. malacoclados	root bark	Breviflorae	Africa
S. mostueoides	root bark	Penicillatae	Africa
S. rubiginosa	root bark, stem bark	Lanigerae	Asia
S. potatorum	root bark	Rouhamon	Africa
S. lucida	root	Strychnos	Asia
S. nux-vomica	root bark, leaves	Strychnos	Asia
S. trinervis	root	Strychnos	America

As can be seen from the table, normacusine B has been isolated from *Strychnos* species of various botanical sections. However only one American species has been reported as its sources. Other tertiary alkaloids of the sarpagine group have never been reported to be found in American species. But quaternary bases, such as macusine A,B and C, are commonly found in these plants [50,64,65]. Therefore few reports of normacusine B and other terrtiary bases of the sarpagine group from the American *Strychnos* species might be a result of unadequate studies on the organic-soluble parts of the plants rather than the real existence.

The other sarpagine-group alkaloid of *S. nitida*, 3-hydroxy-19(*Z*)normacusine B is a new member of the group. The alkaloid has two characteristics, the 3-hydroxy substitution and 19(*Z*) configuration, each of which has never been encountered previously in any structure of sarpagine-group alkaloids isolated from *Strychnos* species. Sarpagine derivatives with 3-hydroxy substitution, exemplified by 3-hydroxyvoachalotine , can be found in some species of the Apocynaceae [213]and those with 19 (*Z*) configuration, represented by koumidine , have been reported from *Gardneria* species of the Loganiaceae [197]. However, besides 3-hydroxy 19 (*Z*)-normacusine B, no other alkaloids of the sarpagine group have been reported to possess both of these characters.



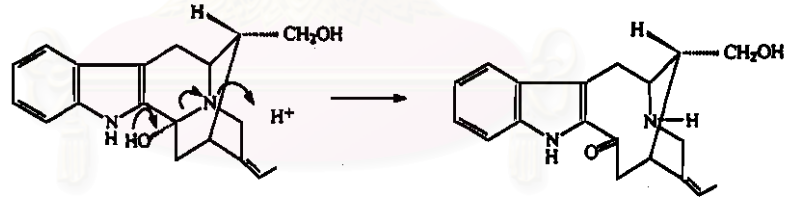
3-Hydroxyvoachalotine



Koumidine

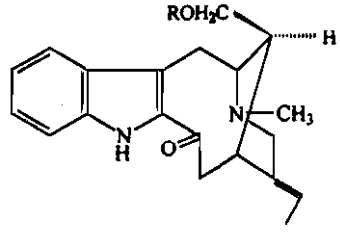
The 19 (*Z*) configuration of the sarpagan skeleton is relatively rare. The isolation of 3-hydroxy-19(*Z*) normacusine B from *S. nitida* has provided the first evidence which indicates the ability of *Strychnos* species to produce the alkaloid with this configuration.

The 3 hydroxy derivatives of the sarpagine group are related to derivatives of vobasine which have the keto group at C-3 (Scheme 4.17). In *Strychnos* species, the members of the so-called vobasine group alkaloids can be found. These alkaloids are erichsonine , 16-epiaffinine and 16-epi-*O*-acetyl affinine ; all have been isolated from American species of the section *Strychnos, S. erichsonii* [70]. The occurrence of these bases in such species and of 3-hydroxy-19(*Z*) normacusine B in *S. nitida* of Asia probably point to the linkage between species belonging to the section Strychnos of the two continents.

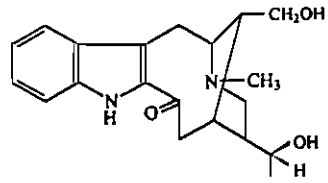


3-Hydroxy-19(*Z*)-normacusine B

Scheme 4.17 Relationship between 3-Hydroxy-19(*Z*)-normacusine B and the vobasine - group alkaloid



16 Epiaffinine, R = H
16 Epi-O-acetylaffinine , R = Ac



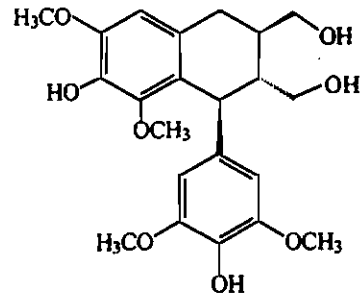
Erichsonine

The Lignans

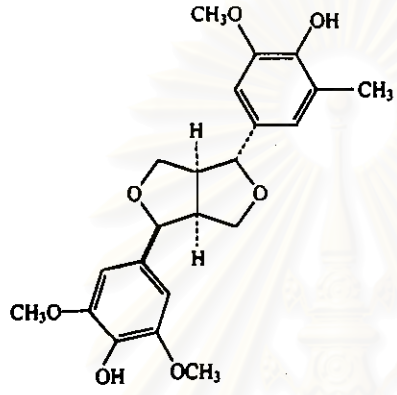
The information obtained from phytochemical study of *Strychnos* species has suggested the rare occurrence of lignans in the *Strychnos*. Only two species have been previously reported to contain few representatives of lignans. Such compounds are lirioresinol A and lirioresinol B, the two isomeric furofurans isolated from the stem bark of an African species, *S. dinklagei*[142] and lyoniresinol, the oxygenated tetrahydronaphthalene derivative found in the stem of an Asian species, *S. thorelli*[8]. Both *Styrchnos* species containing lignans are members of the section Lanigerae.

The present investigation has indicated the presence of lignans in the stem of *S. nitida* . The lignans detected from the plant sample includes the racemic form of lyoniresinol and the two isomeric glycosides, (+) and (-) lyoniresinol β-D-glucopyranoside. This is the second report of lyoniresinol from *Strychnos* species (the previous report had not describe the absolute configuration of the compound), but the first one of lignans from the section *Strychnos*.

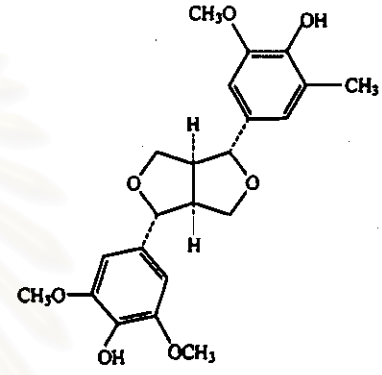
The lignans so far obtained from *Strychnos* species are members of two types: the furofuran type with lirioresinol A and lirioresinol B, and the tetrahydronaphthalene or tetralin type with lyoniresinol and its glycosides. Interestingly, in spite of different structural skeleton the members of two types can be regarded to be derived from the same biogenetic precursor, synapyl alcohol (150). The difference is only due to linking pattern between the two molecules of the precursor.



Lyoniresinol



Lirioresinol A



Lirioresinol B

The Iridoids

The iridoids isolated from the stem of *S. nitida* is loganin. This compound is defined as an intermediate in the biogenetic pathway of terpenoid indole alkaloids. The compound, therefore, is expected to be found in other plants containing these bases.