CHAPTER IV

RESULTS AND DISCUSSION

The dried stem of *Strychnos vanprukii* Craib was extracted with 95% ethanol. The ethanol extract was dissolved in aqueous methanol and sequentially partitioned with hexane and chloroform to yield hexane, chloroform and aqueous methanol extract. Three compounds (SVH-1 to SVH-3) were obtained from the hexane extract, seven compounds (SVC-1 to SVC-7) from the chloroform extract and six compounds (SVM-1 to SVM-6) from the aqueous methanol extract.

The structure determination of all isolated compounds was performed by interpretation of their spectroscopic data (UV, IR, MS and NMR) and then confirmed by comparison with literature values.

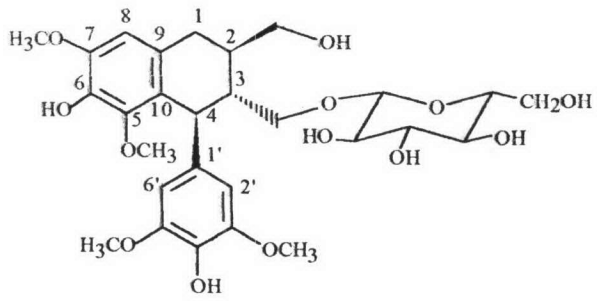
Identification of Compound SVM-1 (lyoniresinol 3a-*O*-β-glucopyranoside)

SVM-1 was obtained as amorphous powder. The compound developed an intense blue color upon spraying with $FeCl_3/HClO_4$ reagent, followed by heating for 5 minutes. Its IR spectrum (Figure 3) exhibited a strong OH absorption at 3366 cm^{-1} together with aromatic bands appearing at 1613, 1516 and 1461 cm^{-1}. The UV spectrum (Figure 2) showed absorption maxima at 205 and 280 nm. The molecular formula of $C_{28}H_{38}N_2O_{13}$ was confirmed by the molecular ion peak at *m/z* 582 in the mass spectrum (Figure 4). The fragment at *m/z* 420, 402 and 371 could be explained as arising from the subsequently loss of glucose, water and hydroxymethyl group in the molecule (Scheme 14). The NMR spectra of compound SVM-1 possessed signals characteristic of an aryl-tetralin type lignan.

In the ^{1}H-NMR spectrum (Figure 5 and Table 14), two signals due to three aromatic protons appeared at δ 6.42 (2H, s, H-2′, 6′) and 6.57 (1H, s, H-8) ppm, suggesting the presence of tetra- and penta- substituted benzene ring. In the non-aromatic part of the compound, the spectrum displayed signals of 3 methine protons at δ 1.70 (1H, *m*, H-2), 2.10 (1H, *m*, H-3) and 4.42 (1H, *d*, *J* = 6.1 Hz, H-4) ppm, together with 3 signals of methylene protons at δ 2.67 (2H, *dd*, H-1), 3.52-3.70 (2H, *dd*, H-2a), 3.43-3.47 (1H, *m*, H-3a) and 3.87-3.91 (1H, *m*, H-3a) ppm.

The APT spectrum (Figure 6 and Table 14) displayed 28 carbons, including one glucose unit. The signals of the aglycone consist of 22 peaks, corresponding to 9 quaternary carbon atoms at δ 147.5 (C-5), 148.6 (C-7), 130.2 (C-9), 126.4 (C-10), 139.3 (C-1′), 148.9 (C-3′, 5′), 134.5 (C-4′) ppm, 6 methine carbon atoms at δ 107.8 (C-8), 106.9 (C-2′, 6′), 40.6 (C-2), 46.7 (C-3), 42.7 (C-4) ppm and 3 methylene carbon signals at δ 33.8, 66.2 and 71.6 ppm, which were assigned as those of C-1, C-2a and C-3a, respectively. The NMR data showed the presence of 4 methoxy group located at C-5, C-7, C-3′ and C-5′. On the basis of chemical shift analysis indicated that the glucose moiety was located at C-3a.

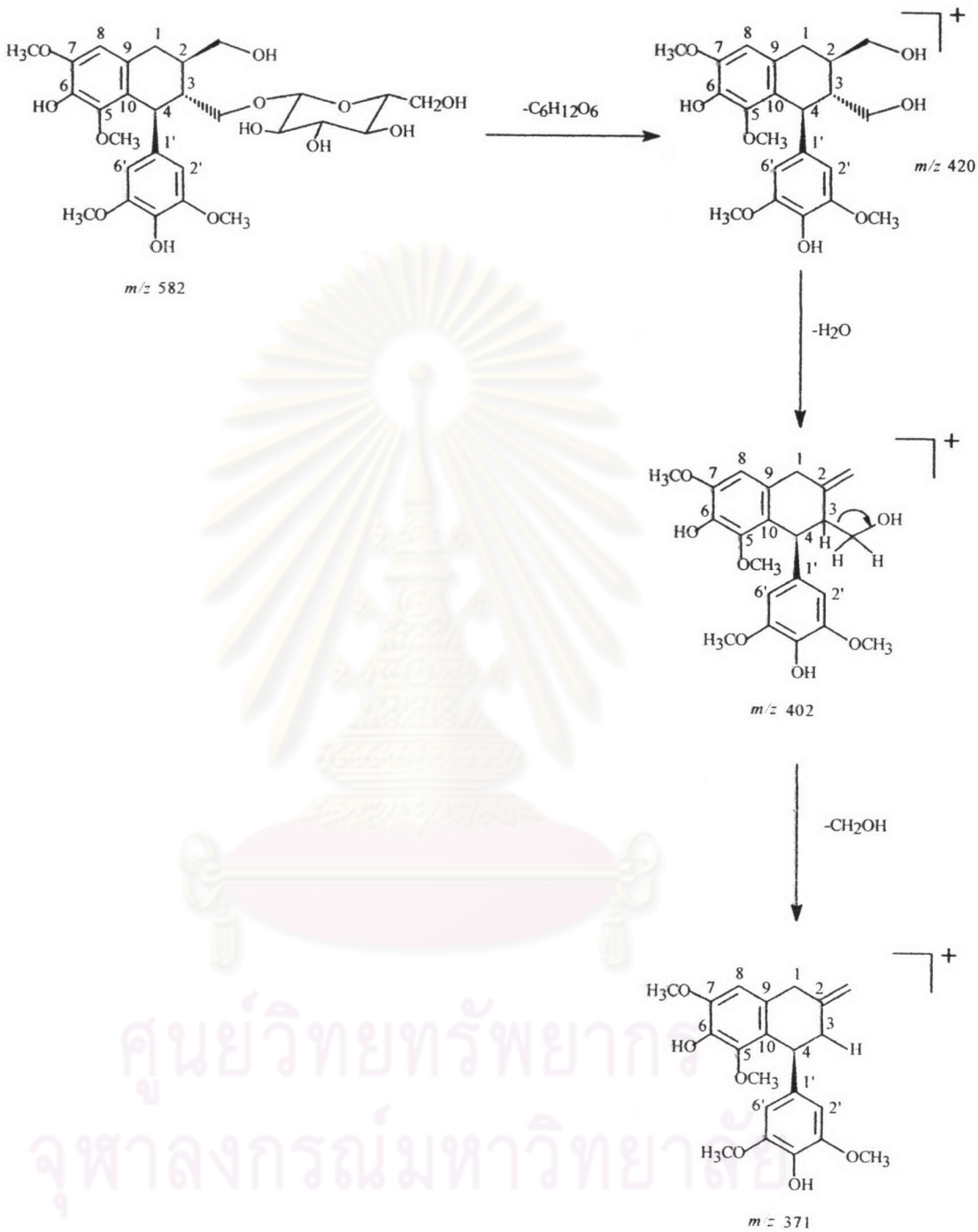
Therefore, it was concluded that SVM-1 is (+)-lyoniresinol 3a-*O*-β-glucopyranoside (Ohashi *et al.*, 1994), the structure of which is shown below.



(+)-lyoniresinol 3a-*O*-β-glucopyranoside

Table 14 Comparison of NMR spectral data of of compound SVM-1 and (+)-lyoniresinol 3a-*O*-β-glucopyranoside (CD_3OD)

position	SVM-1		(+)-lyoniresinol 3a-*O*-β-glucopyranoside (Ohashi *et al.*, 1994)	
	δ_H	δ_C	δ_H	δ_C
1	2.68 *m*	33.8 t	2.67 *m*	33.8 t
2	1.70 *m*	40.6 d	1.70 *m*	40.7 d
2a	3.56-3.70 *m*	66.2 t	3.52-3.70 *m*	66.4 t
3	2.08 *m*	46.7 d	2.10 *m*	46.7 d
3a	3.43-3.47 *m* 3.87-3.91 *m*	71.5 t	3.43-3.47 *m* 3.87-3.91 *m*	71.6 t
4	4.42 *d* (6.2)	42.8 d	4.42 *d* (6.1)	42.7 d
5	-	147.6 s	-	147.7 s
6	-	-	-	139.0 s
7	-	148.6 s	-	148.7 s
8	6.57 *s*	107.8 d	6.57 *s*	108.0 d
9	-	130.2 s	-	130.3 s
10	-	126.4 s	-	126.5 s
1′	-	139.3 s	-	139.4 s
2′	6.42 *s*	106.9 d	6.42 *s*	107.1 d
3′	-	149.0 s	-	149.1 s
4′	-	-	-	134.6 s
5′	-	149.0 s	-	149.1 s
6′	6.42 *s*	106.9 d	6.42 *s*	107.1 d
Glu1	4.28 *d* (7.7)	104.8 d	4.28 *d* (8.9)	104.9 d
2	3.23-3.32	75.1 d	3.20-3.33	75.3 d
3	3.23-3.32	78.2 d	3.20-3.33	78.3 d
4	3.23-3.32	71.7 d	3.20-3.33	71.8 d
5	3.23-3.32	77.9 d	3.20-3.33	78.0 d
6	3.65 3.82	62.8 t	3.65 3.83	62.9 t
5-OCH_3	3.34 *s*	60.2 q	3.36 *s*	60.3 q
7-OCH_3	3.84 *s*	56.6 q	3.86 *s*	56.7 q
3′, 5′-OCH_3	3.74 *s*	56.9 q	3.75 *s*	57.0 q



Scheme 14 Mass fragmentation of compound SVM-1

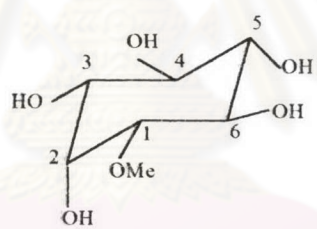
Identification of Compound SVM-2 (bornesitol)

Compound SVM-2 was obtained as colorless crytals. The IR spectrum (Figure 14) exhibited absorption bands at 3500-3000 cm^{-1} (hydroxyl group).

The ^{1}H-NMR spectrum (Figures 15-16 and Table 15) displayed 6 methine proton signals at δ 2.80 (H-1), 3.91 (H-2), 3.33 (H-3), 3.08 (H-4), 2.90 (H-5), 3.41 (H-6) ppm and one methoxy singlet at δ 3.28 ppm.

The ^{13}C-NMR (Figure 17 and Table 15) and DEPT spectrum (Figure 18) consist of 7 peaks, corresponding to 6 methine carbons at δ 81.7 (C-1), 68.3 (C-2), 71.7 (C-3), 72.5 (C-4), 75.3 (C-5), 71.8 (C-6) ppm and one methoxy at δ 56.7 ppm. The steriochemistry of this compound was deduced from its coupling constants (J_{ae} = 2-3 Hz and J_{aa} = 8-14 Hz).

From the above NMR data, together with the information from ^{1}H-^{1}H COSY (Figures 19-20), HMQC (Figures 21-22) and HMBC (Figures 23-24) experiments, compound SVM-2 was identified as bornesitol (Onocha *et al*, 1995).



bornesitol

Table 15 Comparison of NMR spectral data of compound SVM-2 and bornesitol (DMSO)

position	SVM-2		Bornesitol (Onocha *et al*,1995)
	δ_H	δ_C	δ_C
1	2.80 *dd* (7.0, 2.8)	81.7	82.8
2	3.91 *dd* (3.5, 2.8)	68.3	69.7
3	3.08 *ddd* (9.6, 5.8, 2.8)	71.7	73.1
4	3.33 *ddd* (9.6, 9.3, 4.6)	72.5	74.0
5	2.90 *ddd* (14.0, 9.3, 4.3)	75.3	76.3
6	3.41 *ddd* (14.0, 7.0, 4.6)	71.8	73.3
OCH_3	3.28 *s*	57.7	57.7
2-OH	4.48 *d* (3.5)	-	-
3-OH	4.42 *d* (5.8)	-	-
4-OH	4.53 *d* (4.6)	-	-
5-OH	4.61 *d* (4.3)	-	-
6-OH	4.58 *d* (4.6)	-	-

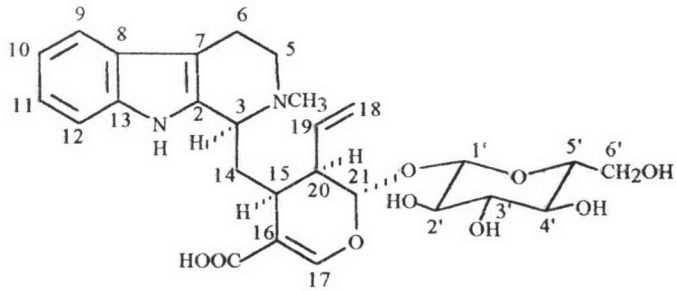
Identification of Compound SVM-3 (palicoside)

Compound SVM-3 was obtained as colorless crytals. It gave mass spectrum (Figure 28) with a peak at *m/z* 531 consistent with the formula $C_{27}H_{34}N_2O_9$ and a fragment peak at *m/z* 369 due to the loss of glucose unit. The UV spectrum (Figure 26) showed absorption maxima at 220 and 272 nm. The IR spectrum (Figure 27) exhibited absorption bands at 3406 (hydroxyl group) and 1643 (carbonyl group) cm^{-1}.

The ^{1}H-NMR spectrum (Figures 29-31 and Table 16) shows four aromatic proton signals of the A ring of the indole system, and characteristic signals of the secologanin unit i.e. vinyl protons at δ 5.24 and δ 5.18 ppm (H_2-18) and δ 5.76 (H–19), one hemiacetal proton at δ 5.49 ppm (*d*, *J* = 7.6 Hz, H-21), one anomeric proton of a β-linked glucose at δ 4.57 ppm (*d*, *J* = 7.9 Hz, H-1′) and one olefinic proton of the acrylic ester moiety at δ 7.48 ppm (H-17).

The ^{13}C-NMR spectrum (Figure 32) shows 27 carbon signals (Table 16), consistent with the biosynthetic pattern of indole alkaloids possessing a secologanin moiety as follows: 10 carbon atoms from tryptamine at δ 134.7 (C-2), 56.1 (C-3), 45.3 (C-5), 16.0 (C-6), 105.3 (C-7), 126.7 (C-8), 117.5 (C-9), 118.2 (C-10), 120.5 (C-11), 110.9 (C-12), 135.9 (C-13) ppm and 17 carbon atoms from secologanin at δ 35.4 (C-14), 30.8 (C-15), 112.7 (C-16), 151.8 (C-17), 117.8 (C-18), 135.7 (C-19), 44.1 (C-20), 95.9 (C-21), 168.6 (C-22) ppm, including the glucose moiety at δ 98.8 (C-1′), 73.1 (C-2′), 77.3 (C-3′), 70.0 (C-4′), 76.7 (C-5′), 61.1 (C-6′) ppm. The NMR data also shows the presence of N_b-methyl group as a proton singlet at δ 2.43 ppm and a carbon peak at δ 39.8 ppm.

The complete carbon chemical shift assignment of SVM-3 was found to be fully in agreement with those of the previously reported palicoside (Morita *et al*, 1989). This compound was first found in *Palicourea marcgravii* (Rubiaceae) and also found in the African *Strychnos mellodora*. Palicoside displayed antimycotic properties against *Candida albicans, C. glabrata* and *Aspergillus niger* in the presence of a specific glucosidase isolated from *Strychnos mellodora*. This glucoalkaloid could be converted into akagerine, which is a monoterpenoid alkaloid with an N_a-C-17 linkage (Brandt et al., 2001)



palicoside

Table 16 Comparison of NMR spectral data of compound SVM-3 and palicoside (CD_3OD)

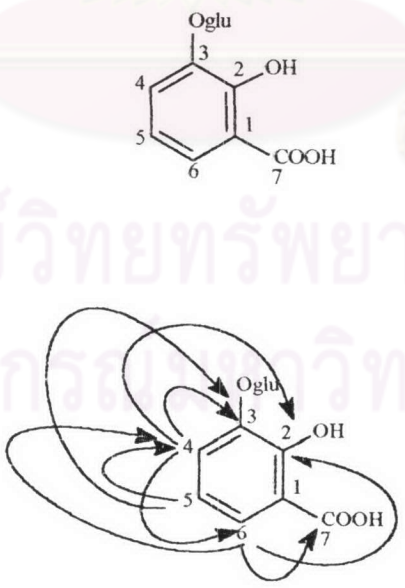
position	SVM-3		Palicoside (Morita *et al*, 1989	
	δ_H	δ_C	δ_H	δ_C
2	-	134.7 *s*	-	134.7 *s*
3	3.79 *d* (9.2)	56.1 *d*	3.77 *d* (10.2)	56.1 *d*
5	2.91-3.12 *m*	45.3 *t*	2.91-3.12 *m*	45.1 *t*
6		16.0 *t*		15.9 *t*
7	-	105.3 *s*	-	105.2 *s*
8	-	126.7 *s*	-	126.6 *s*
9	7.23 *d* (7.5)	117.5 *d*	7.23 *d* (7.5)	117.4 *d*
10	6.92 *t* (7.5)	118.2 d	6.91 *t* (7.5)	118.1 d
11	6.99 *t* (7.5)	120.5 *d*	6.99 *t* (7.5)	120.3 *d*
12	7.35 *d* (7.5)	110.9 *d*	7.34 *d* (7.5)	110.8 *d*
13	-	135.9 *s*	-	135.8 *s*
14	1.90 *t* (11.6) 1.66 *t* (11.0)	35.4 *t*	1.89 *t* (12.0) 1.65 *t* (10.8)	35.3 *t*
15	3.16 *m*	30.8 *d*	3.16 *m*	30.6 *d*
16	-	112.7 *s*	-	112.5 *s*
17	7.48 *s*	151.8 *d*	7.48 *s*	151.8 *d*
18	5.24 *d* (17.5) 5.18 *d* (10.5)	117.8 *t*	5.24 *d* (17.5) 5.18 *d* (10.5)	117.8 *t*
19	5.76 *dd* (17.7,10.7)	135.7 *d*	5.75 *dd* (17.5,10.5)	135.6 *d*
20	-	44.1 *d*	-	44.0 *d*
21	5.49 *d* (8.9)	95.9 *d*	5.48 *d* (8.8)	95.9 *d*
22	-	168.6 *s*	-	168.4 *s*
N-CH_3	2.43 *s*	39.8 *q*	2.43 *s*	39.8 *q*
1′	4.57 *d* (7.9)	98.8 *d*	4.56 *d* (7.8)	98.7 *d*
2′	3.02-3.36 *m*	73.1 *d*	3.01-3.36 *m*	73.0 *d*
3′	3.02-3.36 *m*	77.3 *d*	3.01-3.36 *m*	77.2 *d*
4′	3.02-3.36 *m*	70.0 *d*	3.01-3.36 *m*	70.0 *d*
5′	3.02-3.36 *m*	76.7 *d*	3.01-3.36 *m*	76.6 *d*
6′	3.63 *d* (11.4) 3.38 *d* (11.7)	61.1 *t*	3.63 *d* (11.4) 3.37 *d* (11.7)	61.0 *t*

Identification of Compound SVM-4 (pyrocatechuic 3-*O*-β-glucopyranoside)

Compound SVM-4 was obtained as amorphous powder. The UV spectrum (Figure 44) showed absorption maxima at 207 and 303 nm. The IR spectrum (Figure 45) exhibited absorption bands at 3329 (hydroxyl group) and 1696 (carbonyl group) cm^{-1}. The mass spectrum (Figure 46) showed the molecular ion peak at *m/z* 316, consistent with the formula $C_{13}H_{16}O_9$.

The ^{1}H-NMR (Figure 47 and Table 17) spectrum exhibited three aromatic protons at δ 7.22 (*d*, *J* = 7.4 Hz, H-4), 6.70 (*t*, *J* = 7.4 Hz, H-5) and 7.54 (*d*, *J* = 7.4 Hz, H-6) ppm and an anomeric proton of glucose at δ 4.86 ppm.

The APT spectrum (Figure 48 and Table 17) displayed 13 carbon signals, including those of a glucose unit. The signals of the aglycone consist of 7 peaks, corresponding to 4 quaternary carbon atoms at δ 120.8 (C-1), 152.2 (C-2), 146.9 (C-3), 176.0 (C-7) ppm, 3 methine carbon signals at δ 121.1 (C-4), 118.4 (C-5), 125.4 (C-6) ppm, HMBC spectrum (Figure 54) indicated that glucose moiety was located at C-3. Compound SVM-4 was thus identified as pyrocatechuic 3-*O*-β-glucoside (Sakushima *et al*, 1995).



HMBC correlation of compound SVM-4

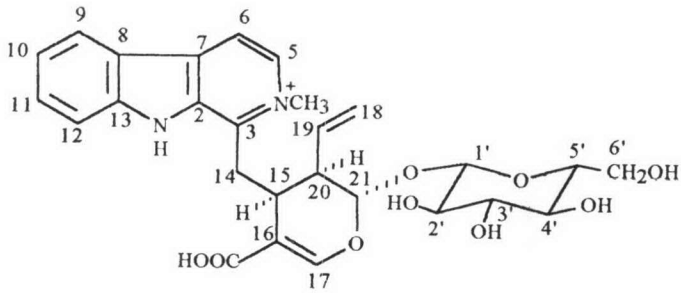
Table 17 Comparison of NMR spectral data of SVM-4 and pyrocatechuic glucopyranoside (CD_3OD)

position	SVM-4		HMBC (H/C correlations)[a]	pyrocatechuic glucopyranoside ($CD_3OD + D_2O$ 1 : 1) (Sakushima *et al*, 1995)
	δ_H	δ_C		δ_C
1	-	120.8	-	115.6
2	-	152.2	-	152.2
3	-	146.9	-	146.5
4	7.22 *d* (7.5)	121.1	C-2, C-3, C-6	122.7
5	6.70 *t* (7.5)	118.4	C-3, C-4	120.1
6	7.54 *d* (7.5)	125.4	C-2, C-4, C-7	125.2
7	-	176.0	-	173.7
1′	4.86 *d* (7.8)	103.5	C-3	102.4
2′	3.41-3.90 *m*	75.7	-	74.3
3′	3.41-3.90 *m*	77.5	-	77.0
4′	3.41-3.90 *m*	72.7	-	70.8
5′	3.41-3.90 *m*	78.6	-	77.6
6′	3.41-3.90 *m*	62.5	-	61.9

Identification of Compound SVM-5

Compound SVM-5 was obtained as amorphous powder which showed an intense blue fluorescence under UV light. The UV spectrum (Figure 55) showed absorption maxima at 204, 253, 309 and 373 nm. The IR spectrum (Figure 56) exhibited absorption bands at 3367 (hydroxyl group), 1636 (carbonyl group) cm^{-1}. It gave mass spectrum (Figure 57) with a molecular ion peak at *m/z* 527 consistent with the formula $C_{27}H_{31}N_2O_9$ and a fragment peak at *m/z* 365 due to the loss of glucose unit.

In the NMR spectrum (Figures 58-59 and Table 18), the presence of a β-carbolinium structure was confirmed by the presence of six aromatic protons, four of which corresponded to an unsubstituted indole moiety at δ 8.31 (1H, *d*, *J* = 7.1 Hz, H-9), 7.41 (1H, *t*, *J* = 7.1 Hz, H-10), 7.75 (1H, *t*, *J* = 8.1 Hz, H-11) and 7.82 (1H, *d*, *J* = 8.1 Hz, H-12) ppm and two more deshielded doublets at δ 8.31 (1H, *d*, *J* = 7.1 Hz, H-5) and 8.40 (1H, *d*, *J* = 7.1 Hz, H-6) ppm whose chemicals shifts were in accordance with a pyridinium ring. The presence of a quaternary N_b-methyl group (^1H: 4.43 (s), ^{13}C: 45.2 ppm) could also be detected. The deshielded value for the methyl protons are similar to those of melinonine F and normelinonine F (δ 4.20 and 4.55, ppm, respectively) (Brandt *et al*, 1999). Several resonances suggested the presence of a seco-iridoid moiety similar to secologanin. A singlet at δ 7.31 ppm corresponded to the olefinic H-17 whereas a doublet at δ 5.76 ppm could be assigned to for the hemiacetalic H-21. The vinyl group was identified by a couple of doublets at δ 5.12 (*J* = 10.3 Hz) and 5.11 (*J* = 17.8 Hz) ppm corresponding to the methylenic H_2-18 protons, which are respectively *cis*- and *trans*- coupled to the unique H-19 (doublet of doublets at δ 5.97 ppm). A doublet at δ 4.75 ppm with a coupling constant of 7.9 Hz indicated the β-configuration of the glucose moiety which located at C-21. Therefore, compound SVM-5 was identified as 3,4,5,6-tetradehydropalicoside. This compound was first found in *Strychnos mellodora*, another *Strychnos* species classified in the same section with *Strychnos vanprukii* (Brandt *et al*, 1999).



3,4,5,6-tetradehydropalicoside

Table 18 Comparison of NMR spectral data of compound SVM-5 and 3,4,5,6-tetradehydropalicoside (CD_3OD)

position	SVM-5		3,4,5,6-tetradehydropalicoside (Brandt *et al*, 1999)	
	δ_H	δ_C	δ_H	δ_C
2	-	-	-	-
3	-	-	-	-
5	8.31 *d* (6.7)	135.8 *d*	8.34 *d*	135.9 *d*
6	8.40 *d* (6.7)	116.8 *d*	8.34 *d*	117.1 *d*
7	-	-	-	-
8	-	-	-	-
9	8.31 *d* (7.1)	124.0 *d*	8.24 *d* (8.0)	124.3 *d*
10	7.41 *t* (7.1)	123.9 *d*	7.33 *t* (8.0)	123.1 *d*
11	7.75 *t* (8.1)	132.7 *d*	7.61 *t* (8.2)	132.8 *d*
12	7.82 *d* (8.1)	114.3 *d*	7.66 *d* (8.2)	114.1 *d*
13	-	-	-	-
14	3.62 *m* 3.56 *m*	32.2 *t*	3.62 *m* 3.56 *m*	32.0 *t*
15	3.45 *m*	36.5 *d*	3.45 *m*	36.0 *d*
16	-		-	
17	7.31 *s*	150.5 *d*	7.44 *s*	155.0 *d*
18	5.12 *d* (10.3) 5.11 *d* (17.8)	119.9 *t*	5.31 *d* (10.8) 5.23 *d* (17.9)	120.3 *t*
19	5.97 *dd* (17.8,10.3)	135.1 *d*	5.97 *dd* (17.9,10.8)	135.6 *d*
20	2.75 *d* (8.9)	45.2 *d*	2.72 *d* (8.9)	45.0 *d*
21	5.76 *d* (7.4)	97.1 *d*	5.95 *d* (8.9)	97.2 *d*
22	-		-	
N-CH_3	4.43 *s*	45.7 *q*	4.35 *s*	45.0 *q*
1′	4.75 *d* (7.9)	100.6 *d*	4.78 *d* (7.9)	100.8 *d*
2′	3.15 *d* (7.9)	74.1 *d*	3.15 *d* (7.9)	75.1 *d*
3′	3.35 *m*	77.5 *d*	3.35 *m*	78.0 *d*
4′	3.22 *m*	71.7 *d*	3.22 *m*	72.2 *d*
5′	3.35 *m*	77.9 *d*	3.35 *m*	79.1 *d*
6′	3.92 *d* (10.8) 3.60 *d* (10.8)	63.0 *t*	3.92 *d* (10.8) 3.60 *d* (10.8)	63.0 *t*

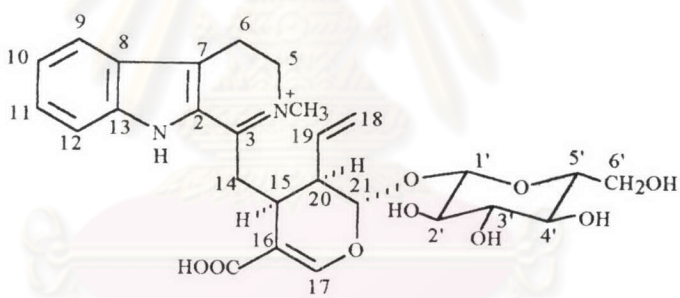
Structure elucidation of compound SVM-6 (3,4-dehydropalicoside)

Compound SVM-6 was obtained as amorphous powder. The UV spectrum (Figure 60) showed absorption maxima at 209, 360 nm. The IR spectrum (Figure 61) exhibited absorption bands at 3371 (hydroxyl group) and 1645 (carbonyl group) cm^{-1}.

An ES-MS-MS measurement of compound SVC-3 (Figure 62) gave a molecular formula of $C_{27}H_{33}N_2O_9$, which is one hydrogen less than that of palicoside. The ^{1}H-NMR spectrum (Figure 63 and Table 19) shows four aromatic proton signals of the unsubstituted A ring of the indole system at δ 7.67 (1H, *d*, *J* = 8.2 Hz, H-9), 7.16 (1H, *t*, *J* = 7.2 Hz, H-10), 7.41 (1H, *t*, *J* = 7.5 Hz, H-11) and 7.56 (1H, *d*, *J* = 8.5 Hz, H-12) ppm and the presence of a quaternary N_b-methyl group at δ 3.69 ppm. Similar to compound SVM-3 and SVM-5, the presence of a seco-iridoid moiety identical to secologanin was evident from several characteristic signals (Brandt et al., 1999). The vinyl group appeared as two doublets at δ 5.28 (*J* = 10.8 Hz, H-18_{cis}) and 5.22 (*J* = 17.9 Hz, H-18_{trans}) ppm coupled to the more downfield H-19 (doublet of doublets at δ 5.92 ppm). An olefinic proton signal at δ 7.29 ppm (*s*) and a hemiacetal proton resonance at δ 5.68 (*d*, *J* = 7.6 Hz) ppm correspond to H-17 and H-21, respectively. The anomeric proton of the glucose moiety which appeared as a doublet (*J* = 8.2 Hz, H-1′) at δ 4.76 ppm was indicative with the β-configuration of the sugar linkage.

The ^{13}C-NMR (Figure 64 and Table 19) spectrum shows 27 carbon signals including those of 8 aromatic carbons, one carboxyl carbon (δ 174.4 ppm), two vinyl carbons (δ 135.7 and 120.3 ppm), one acetal carbon (δ 96.8 ppm), and one glucose unit including an anomeric carbon (δ 100.6 ppm). Carbons of the secologanin part (C-16 to C-21 and C-1′ to C-6′) show similar chemical shifts as in palicoside (Morita et al, 1989). The deshielded value for the quaternary N_b-methyl protons (δ 3.69) are consistent with those of melinonine F and normelinonine F (Brandt et al, 1999). In addition to a carboxyl carbon, another downfield carbon signal at δ 170.1 ppm was observed. In the HMBC spectrum (Figure 72), long-range correlations between this carbon signal and three proton signals of H-5 (δ 4.07 ppm), H-15 (δ 3.45 ppm) and N-CH_3 (δ 3.69 ppm) were observed, indicating that the signal at δ 170.1 ppm should be

assigned to C-3 and that a double bond exists between C-3 and N-4. The structure of compound SVM-6 was thus elucidated as 3,4-dehydropalicoside. The signals of H-14 and C-14 were missing from the corresponding ^{1}H and ^{13}C NMR spectra recorded in CD_3OD. This characteristic phenomenon can be explained by the proton-deuterium exchange caused by an equilibrium between the imine and enamine form (Kitajima *et al*, 2000). The stereochemistry of H-15, H-20 and H-21 in the seco-iridoid unit was determined by comparison of their chemical shifts and coupling constants with those of compound SVM-5 and desoxy-cordifoline (Brandt *et al*., 1999). Their similarities indicated the configurations for H-15, H-20 and H-21 of compound SVM-6 to be α, α and β, respectively. These are the configurations commonly deduced from the proposed biosynthetic pathway of these alkaloids.



3,4-dehydropalicoside

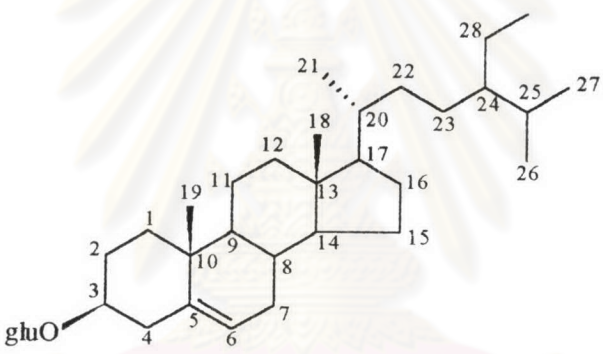
Table 19 NMR spectral data of compound SVM-6 (in CD_3OD)

position	δ_H	δ_C	HMBC	COSY
2				
3		170.1 *s*		
5	4.09 *t* (8.0)	55.1 *t*	C-3, C-7	H-6
6	3.29 *m*	20.1 *t*		H-5
7		124.0 *s*		
8		125.4 *s*		
9	7.67 *d* (8.2)	122.5 *d*	C-13	H-10
10	7.16 *t* (7.2)	122.7 *d*	C-8, C-12	H-9, H-11
11	7.41 *t* (7.5)	129.7 *d*	C-9, C-10, C-13	H-10, H-12
12	7.56 *d* (8.5)	114.6 *d*	C-8	H-11
13		142.8 *s*		
14				
15	3.5 *m*	36.9 *d*	C-3, C-16, C-17, C-20, C-21	H-20
16		114.7 *s*		
17	7.28 *s*	150.8 *d*	C-15, C-16, C-21	
18	5.28 *m* 5.22 *m*	120.3 *t*	C-20	H-19
19	5.92	135.7 *d*		H-18, H-20
20	2.74	45.2 *d*	C-19, C-21	H-15, H-19, H-21
21	5.67 *d* (7.6)	96.8 *d*	C-1′	H-20
22		174.4 *s*		
N-CH_3	3.69 s	43.3 *q*	C-5, N-CH_3	
1′	4.76 *d* (8.2)	100.6 *d*	C-21	
2′	3.16-3.41 *m*	74.7 *d*		
3′	3.16-3.41 *m*	78.0 *d*		
4′	3.16-3.41 *m*	71.7 *d*		
5′	3.16-3.41 *m*	78.5 *d*		
6′	3.95 3.50	63.0 *t*		

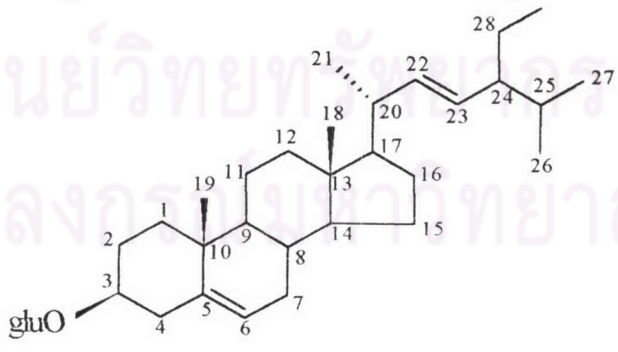
Identification of Compound SVC-1

Compound SVC-1 was obtained as colorless needles. This compound gave purple coloration upon spraying with 10% H_2SO_4 in 95% ethanol. The NMR data of SVC-1 were in full agreement with the published values of β-sitosterol and stigmasterol glucoside mixture.

In the ^{1}H-NMR spectrum (Figure 74), the signals at δ 5.03, 5.17 and 5.33 could be assigned to H-22 and H-23 of stigmasterol glucoside and H-6 of β-sitosterol and stigmasterol glucosides, respectively. The intergration value for H-6 was twice that of either H-22 or H-23. Therefore, it could be deduced that SVC-1 was a mixture of β-sitosterol and stigmasterol glucosides in the ratio of 1:1.



β-sitosterol glucoside



stigmasterol glucoside

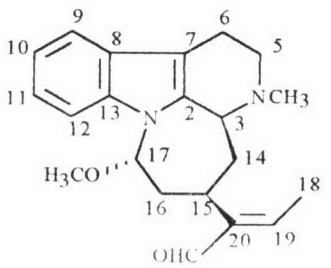
Identification of Compound SVC-2 (17-*O*-methylakagerine)

Compound SVC-2 was obtained as amorphous powder and gave orange color to Dragendorff reagent. The UV spectrum (Figure 75) showed absorption maxima at 204, 283 nm. The IR spectrum (Figure 76) exhibited absorption bands at 3377 (hydroxyl group), 1672 (unsaturated carbonyl group) and 751 (indole) cm^{-1}. The mass spectrum (Figure 77)showed a peak at *m/z* 338, consistent with the molecular formular $C_{21}H_{26}N_2O_2$.

The ^{1}H-NMR spectrum (Figures 78-79 and Table 20) showed aldehydic proton at δ 9.29 ppm, ethylidene side chain at δ 6.52 (H-19) and 2.05 (H-18) ppm and four aromatic protons at δ 7.49 (H-9), 7.12 (H-10), 7.18 (H-11) and 7.33 (H-12).

Compound SVC-2 is a tetracyclic indole alkaloid possessing a perhydroazepine ring coupled to tetrahydro-β-carboline by an original N_1-C_{17} bond. The ^{13}C-NMR (Figure 80) and DEPT (Figure 81) spectrum displayed resonances of the carboline ring at δ 60.6 (C-3), 50.0 (C-5), 19.5 (C-6), 126.5 (C-8), 118.4 (C-9), 121.5 (C-10), 119.4 (C-11), 108.5 (C-12) ppm and perhydroazepine ring at δ 36.8 (C-14), 29.7 (C-15), 36.3 (C-16), 83.0 (C-17) ppm, together with the ethylidene side chain at δ 15.2 (C-18), 150.7 (C-19), 147.7 (C-20) ppm and aldehydic carbon at δ 194.9 (C-21) ppm. The NMR data also showed the presence of N_b-methyl group (proton singlet at δ 2.53 ppm and carbon peak at δ 42.5 ppm) and methoxy group substituted at C-17 (proton singlet at δ 3.08 ppm and carbon peak at δ 55.2 ppm)

The data obtained from spectroscopic data of SVC-2 were in full agreement with the published spectral values of 17-*O*-methylakagerine, which was first found from *Strychnos elaeocarpa* and showed clonic and tonic convalsions similar to strychnine (Rolfsen *et al.*, 1978).



17-*O*-methylakagerine

Table 20 Comparison of NMR spectral data of of compound SVC-2 and 17-*O*-methylakagerine ($CDCl_3$)

position	SVC-2		17-*O*-methylakagerine	
	δ_H	δ_C	δ_H	δ_C
2	-	-	-	136.7 *s*
3	3.78 *d* (15)	60.6 *d*	-	60.8 *d*
5	3.06 *t* (6.3)	50.0 *t*	-	50.3 *t*
6	-	19.5 *t*	-	19.8 *t*
7	-	-	-	108.7 *s*
8	-	126.5 *s*	-	126.6 *s*
9	7.49 *d* (7.5)	118.4 *d*	7.00-7.60 *m*	118.4 *d*
10	7.10 *t* (7.5)	121.5 *d*	7.00-7.60 *m*	121.5 *d*
11	7.18 *t* (8.1)	119.4 *d*	7.00-7.60 *m*	119.4 *d*
12	7.33 *d* (8.1)	108.5 *d*	7.00-7.60 *m*	108.5 *d*
13	-	-	-	137.7 *s*
14	1.99 *m*	36.8 *t*	-	36.9 *t*
15	3.56 *t* (11.2)	29.7 *d*	-	29.7 *d*
16	2.30 *m*	36.3 *t*	-	36.5 *t*
17	5.66 *d* (3.9)	83.0 *d*	5.68 *m*	83.0 *d*
18	2.07 *d* (7.2)	15.2 *q*	2.05 *d* (8)	15.1 *q*
19	6.53 *q* (7.2)	150.7 *d*	6.52 *q* (8)	150.5 *d*
20	-	147.7 *s*	-	147.8 *s*
21	9.29 *s*	194.9 *d*	9.31 *d* (2)	194.8 *d*
N-CH_3	2.54 *s*	42.2 *q*	2.53 *s*	42.5 *q*
OCH_3	3.06 *s*	55.2 *q*	3.08 *s*	55.2 *q*

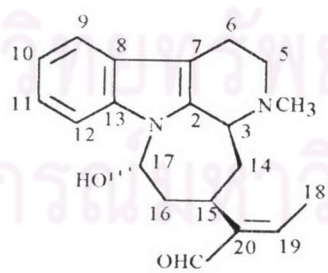
Identification of Compound SVC-3 (akagerine)

Compound SVC-3 was obtained as amorphous powder and gave orange color to Dragendorff reagent. The UV spectrum (Figure 82) showed absorption maxima at 211, 307 nm. The IR spectrum (Figure 83) exhibited absorption bands at 3354 (hydroxyl group), 1656 (unsaturated carbonyl group) and 754 (indole) cm^{-1}. The mass spectrum (Figure 84) showed a peak at *m/z* 326 $[M+2H]^+$, consistent with the formula $C_{20}H_{24}N_2O_2$.

Compound SVC-3 showed similar spectral data to SVC-2. However the methoxy signal was missing from NMR spectra of this compound. Furthermore, in the ^{1}H-NMR spectrum (Figures 85-86 and Table 21), the one proton multiplet at δ 5.68 ppm in SVC-2 had shifted to δ 6.24 ppm in SVC-3, whereas the ^{13}C-NMR spectrum (Figure 87 and Table 21) showed the upfield shift of the C-17 signal from δ 83.0 ppm in SVC-2 to δ75.7 ppm in SVC-3.

Therefore, it was concluded that compound SVC-3 is akagerine and this was confirmed by comparison of its ^{13}C-NMR chemical shift data with reported values (Rolfsen *et al.*, 1978).

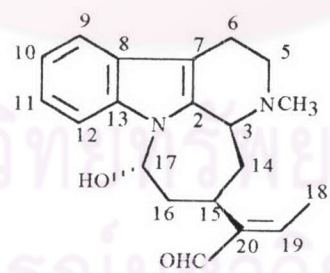
Akagerine was first found in the roots of *Strychnos usambarensis*. Previous pharmacological studies of this compound has revealed its cytotoxic and convulsant activities (Rolfsen *et al.*, 1978)



akagerine

Table 21 Comparison of NMR spectral data of SVC-3 and akagerine in $CDCl_3$

position	SVC-3		akagerine	
	δ_H	δ_C	δ_H	δ_C
2	-	-	-	136.7 *s*
3	3.82 *d* (10.8)	60.7 *d*	3.82 *d* (10.9)	60.5 *d*
5	3.07 *m* 2.72-2.78 *m*	50.0 *t*	3.07 *m* 2.72-2.78 *m*	49.8 *t*
6	2.72-2.78 *m*	19.6 *t*	2.72-2.78 *m*	19.4 *t*
7	-	-	-	107.9 *s*
8	-	126.6 *s*	-	126.4 *s*
9	7.45 *d* (7.4)	118.4 *d*	7.48 *d* (7.7)	118.2 *d*
10	7.08 *t* (7.4)	121.6 *d*	7.11 *t* (7.7)	121.3 *d*
11	7.16 *t* (7.8)	119.6 *d*	7.18 *t* (8.2)	119.3 *d*
12	7.28 *d* (7.8)	108.5 *d*	7.29 *d* (8.2)	108.3 *d*
13	-	136.1 *s*	-	136.1 *s*
14	1.96-2.33 *m*	36.6 *t*	2.19 *d* (10.9) 1.99 *d* (12.8)	37.7 *t*
15	3.66 *t* (11.7)	29.1 *d*	3.66 *t* (11.4, 1.4)	29.7 *d*
16	1.96-2.33 *m*	35.9 *t*	2.33 *m* 2.09 *m*	35.6 *t*
17	6.21 *d* (3.9)	75.7 *d*	6.24 *d* (3.7)	75.3 *d*
18	2.11 *d* (6.6)	15.2 *q*	2.07 *d* (7.2)	15.1 *q*
19	6.54 *q* (6.6)	150.8 *d*	6.55 *q* (7.2)	150.7 *d*
20	-	147.7 *s*	-	147.8 *s*
21	9.31 d (1.4)	194.9 *d*	9.31 d (1.4)	195.0 *d*
$N\text{-}CH_3$	2.53 s	41.9 *q*	2.53 s	41.7 *q*

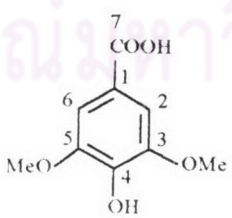


akagerine

Identification of Compound SVC-4 (syringic acid)

Compound SVC-4 which was obtained as an white amorphous powder, has a molecular formula of $C_9H_{10}O_5$ deduced from its molecular ion peak in the EIMS (Figure 91) at m/z 198. The fragment ions at *m/z* 181, 168 and 153 resulting from loss of hydroxyl, methoxyl and carboxyl moiety, respectively. The IR spectrum (Figure 90) displayed major absorption bands at 3372 cm^{-1} (hydroxyl group) and 1698 cm^{-1} (conjugated carbonyl group). UV spectrum (Figure 89) showed absorption maxima at 204, 252 and 286 nm.

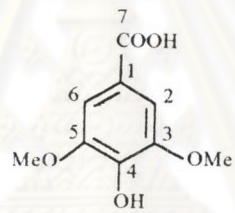
The ^{1}H-NMR spectrum of SVC-4 (Figure 92 and Table 22) showed a signal of two equivalent aromatic protons at δ 7.31 ppm (H-2, H-6) and another signal representing two equivalent methoxy groups at δ 3.87 ppm (3-OCH_3, 5-OCH_3). These data suggested SVC-4 as having the 1,3,4,5-tetrasubstituted symmetrical aromatic ring system with two methoxy, one hydroxy and one carboxy groups. Therefore, compound SVC-4 was identified as syringic acid (Inoshiri *et al*, 1987). This compound displayeded antioxidant activity (Kuo *et al*., 2002) and weak inhibitory activity against xanthine oxidase (Kong *et al*., 2002)



syringic acid

Table 22 Comparison of NMR spectral data of compound of SVC-4 and Syringic acid (CD_3OD)

position	Compound SVC-4		Syringic acid (Inoshiri *et al*, 1987)
	δ_H	δ_C	
1	-	121.9	122.0
2	7.31 *s*	108.3	108.3
3	-	148.8	148.7
4	-	-	142.2
5	-	148.8	148.7
6	7.31 *s*	108.3	108.3
7	-	169.9	169.2
3-OCH_3, 5-OCH_3	3.87 *s*	56.8	56.3

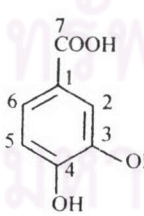


syringic acid

Identification of Compound SVC-5 (vanillic acid)

Compound SVC-5 which was obtained as an white amorphous powder, has a molecular formular of $C_8H_8O_4$ deduced from its molecular ion peak in the EIMS (Figure 96) at m/z 168. The presence of the fragment ions at *m/z* 151 and 123 due to the elimination of methoxyl and carboxyl moiety from the molecule, respectively. The IR spectrum (Figure 95) had bands at 3649 cm^{-1} (hydroxyl group) and 1681 cm^{-1} (conjugated carbonyl group). UV spectrum (Figure 94) showed absorption maxima at 210 and 263 nm.

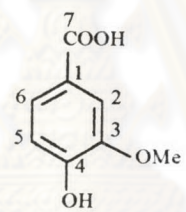
The ^{1}H-NMR spectrum (Figure 96 and Table 23) revealed three aromatic protons. The substitution pattern of aromatic ring was deduced from the *ortho* coupling of the proton signal at δ 7.55 (*dd*, J = 8.7, 1.8 Hz, H-6) ppm to the one at δ 6.83 (*d*, J = 8.7 , H-5) ppm and also *meta* coupling to another signal at δ 7.53 (*d*, J = 1.8 Hz, H-2) ppm. Therefore, the substituted groups could be assigned to positions 1, 3 and 4 of the ring with carboxy, methoxy and hydroxy groups. Compound SVC-5 was identified as vanillic acid (Sakushima *et al*, 1995)



vanillic acid

Table 23 Comparison of NMR spectral data of compound of SVC-5 and Vanillic acid (CD_3OD)

position	Compound SVC-5		Vanillic acid (Sakushima *et al*, 1995)
	δ_H	δ_C	
1	-	123.0 *s*	123.1 *s*
2	7.53 *d* (1.8)	113.8 *d*	113.9 *d*
3	-	148.6 *s*	148.6 *s*
4	-	152.7 *s*	152.7 *s*
5	6.83 *d* (8.7)	115.8 *d*	115.9 *d*
6	7.55 *dd* (8.7, 1.8)	125.3 *d*	125.3 *d*
7	-	170.0 *s*	170.0 *s*
3-OMe	3.88	56.4 *q*	56.4 *q*



vanillic acid

Structure Elucidation of Compound SVC-6 (strychnoside)

Compound SVC-6 was obtained as amorphous powder. The compound developed an intense blue color upon spraying with $FeCl_3/HClO_4$ reagent followed by heating at 105 °C for 5 minutes. Its IR spectrum (Figure 100) exhibited a strong OH absorption band at 3369 cm^{-1}, together with a peak at 1613 cm^{-1} indicating the presence of conjugated ester moiety in the molecule. The UV spectrum (Figure 99) showed absorption maxima at 206 and 277 nm. The molecular formula of $C_{46}H_{56}O_{21}$ was determined from the pseudomolecular ion peak at *m/z* 965 $[M+Na]^+$, in the mass spectrum (Figure 101). The NMR spectra of compound SVC-6 possessed signals characteristic of an aryl-tetralin type lignan.

The ^{1}H-NMR (Figure 102) and APT (Figure 103 and Table 24) spectra of SVC-6 displayed peaks with chemical shifts similar to those of compound SVM-1. In addition, further signals corresponding to two syringyl units could also be detected at δ 121.3 (C-1″), 121.5 (C-1‴), 108.1 (C-2″, C-6″), 108.3 (C-2‴, C-6‴), 148.9 (C-3″, C-5″), 148.6 (C-3‴, C-5‴), 141.9 (C-4″), 141.7 (C-4‴), 167.9 (C-7″) and 167.4 (C-7‴) ppm.

The connectivity of the syringyl unit was established on the basis of chemical shift analysis. Comparison of the APT spectrum of SVC-6 with that of SVM-1 revealed the downfield shift of C-2 signal (+0.6 ppm) and upfield shift of C-1 and C-3 signals (-1.8 and -2.2 ppm, respectively) of the glucosyl moiety, together with the analysis of the HMBC spectrum (Figure 112), indicating that one syringyl unit is ester located at C-2 of the glucose moiety. Furthermore, the downfield shift of C-2a (+ 2.5 ppm) and upfield shift of C-2 (-2.6 ppm) were observed, indicating that another syringyl ester moiety is located at C-2a. Compound SVC-6 was therefore 2a-[(3,5-dimethoxy-4-hydroxy)-benzoyl]-(+)-lyoniresinol 3a-[2-[(3,5-dimethoxy-4-hydroxy)-benzoyl]-*O*-β-glucopyranoside, trivially named as strychnoside.

SVC-6

Table 24 NMR spectral data of compound SVC-6 (CD_3OD)

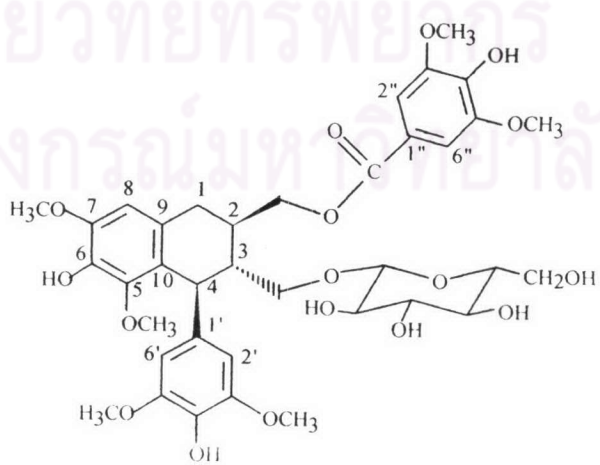
Position	δ_C	δ_H	HMBC (H/C correlations)[a]
1	33.4 *t*	2.59 *d* (7.5)	
2	37.8 *d*	1.84 *m*	
2a	68.3 *t*	4.13, 4.08	
3	47.0 *d*	2.14 *m*	
3a	71.3 *t*	3.48, 3.34	
4	42.2 *d*	4.24 *d* (6.1)	C-9, C-10, C-1′, C-2′, C-6′
5	147.6 *s*	-	
6	138.9 *s*	-	
7	148.6 *s*	-	
8	107.5 *d*	6.48 *s*	C-6, C-10
9	129.4 *s*	-	
10	125.4 *s*	-	
1′	139.2 *s*	-	
2′	106.6 *d*	6.27 *s*	C-4, C-3′, C-4′, C-6′
3′	148.9 *s*	-	
4′	134.5 *s*	-	
5′	148.9 *s*	-	
6′	106.6 *d*	6.27 *s*	C-4, C-2′, C-4′, C-5′
1″	121.3 *s*	-	
2″	108.1 *d*	7.21 *s*	C-1″, C-3″, C-4″, C-6″, C-7″
3″	148.9 *s*	-	
4″	141.9 *s*	-	
5″	148.9 *s*	-	
6″	108.1 *d*	7.21 *s*	C-1″, C-3″, C-4″, C-6″, C-7″
7″	167.9 *s*	-	
Glu1	102.9 *d*	4.61 *d* (8)	
2	75.8 *d*	5.02 *d* (8)	
3	76.0 *d*	3.74	
4	71.8 *d*	4.01	
5	78.1 *d*	3.32	
6	62.6 *t*	3.9 *m* 3.75 *m*	
1‴	121.3 *s*	-	
2‴	108.3 *d*	7.12 *s*	C-1‴, C-3‴, C-4‴, C-6‴, C-7‴
3‴	148.6 *s*	-	
4‴	141.7 *s*	-	
5‴	148.6 *s*	-	
6‴	108.3 *d*	7.12 *s*	C-1‴, C-3‴, C-4‴, C-6‴, C-7‴
7‴	167.4 *s*	-	
5-OCH_3	60.0 *q*	3.16 *s*	
7-OCH_3	56.5 *q*	3.81 *s*	
3′,5′-OCH_3	56.6 *q*	3.72 *s*	
3″,5″-OCH_3	56.8 *q*	3.81 *s*	
3‴,5‴-OCH_3	56.8 *q*	3.67 *s*	

Structure elucidation of compound SVC-7 (vanprukoside)

Compound SVC-7 was obtained as amorphous powder. The compound developed an intense blue color upon spraying with $FeCl_3/HClO_4$ reagent followed by heating at 105 °C for 5 minutes. Its IR spectrum (Figure 114) exhibited a strong OH absorption band at 3365 cm^{-1}, together with a peak at 1613 cm^{-1} indicating the presence of conjugated ester moiety in the molecule. The UV spectrum (Figure 113) showed absorption maxima at 208 and 277 nm. The molecular formular of $C_{37}H_{46}O_{17}$ was determined from the pseudomolecular ion peak at *m/z* 785 $[M+Na]^+$ and molecular ion peak at *m/z* 762 $[M]^+$, displayed in the mass spectrum (Figure 115). The NMR (Figure 116-118) spectra of compound SVC-7 possessed signals characteristic of an aryl-tetralin type lignan.

In the ^{1}H-NMR (Figure 116) and APT (Figure 117-118 and Table 25) spectra of SVC-7 showed chemical shifts similar to that of compound SVM-1 and accompanied with additional signals corresponding to syringyl substituent at δ 121.5 (C-1″), 108.1 (C-2″, 6″), 149.0 (C-3″, 5″), 142.1 (C-4″) and 168.2 (7″).

The acyloxy moiety was attached to the C-2a resulting from the basis of chemical shifts analysis. Comparison of the APT spectrum of SVC-7 with that of SVM-1 revealed the downfield shift of C-2a (+ 2.5 ppm) and upfield shift of C-2 (-2.6 ppm) indicating that the additional ester unit is a syringyl moiety located at C-2a. Consequently, the structure of compound SVC-7 was assigned as 2a-[(3,5-dimethoxy-4-hydroxy)-benzoyl]-(+)-lyoniresinol 3a-*O*-β-glucopyranoside, named vanprukoside.



SVC-7

Table 25 NMR spectral data of compounds SVC-7

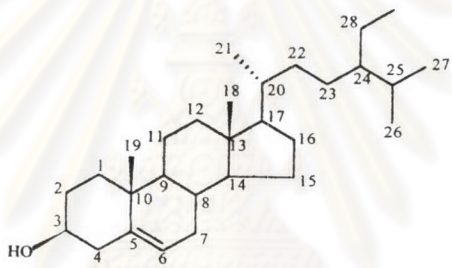
Position	δ_C	δ_H	HMBC (H/C correlations)[a]
1	33.5 *t*	2.74 *m*	
2	38.0 *d*	2.12 *m*	
2a	68.7 *t*	4.30 *m* 4.43 *m*	
3	46.6 *d*	2.27 *m*	
3a	71.8 *t*	3.57 *m* 3.98 *m*	
4	42.5 *d*	4.38 *d* (6.1)	C-9, C-10, C-1′, C-2′, C-6′
5	147.6 *s*	-	
6	139.2 *s*	-	
7	148.8 *s*	-	
8	107.9 *d*	6.59 *s*	C-6, C-10
9	129.5 *s*	-	
10	125.8 *s*	-	
1′	139.1 *s*	-	
2′	106.9 *d*	6.38 *s*	C-4, C-3′, C-4′, C-6′
3′	149.0 *s*	-	
4′	134.7 *s*	-	
5′	149.0 *s*	-	
6′	106.9 *d*	6.38 *s*	C-4, C-2′, C-4′, C-5′
1″	121.5 *s*	-	
2″	108.2 *d*	7.22 *s*	C-1″, C-3″, C-4″, C-6″, 7″
3″	149.0 *s*	-	
4″	142.1 *s*	-	
5″	149.0 *s*	-	
6″	108.1 *d*	7.22 *s*	C-1″, C-2″, C-4″, C-5″, 7″
7″	168.2 *s*	-	
Glu1	104.7 *d*	4.27 *d* (8.9)	
2	75.2 *d*	3.20-3.26 *m*	
3	78.1 *d*	3.20-3.26 *m*	
4	71.6 *d*	3.20-3.26 *m*	
5	77.9 *d*	3.20-3.26 *m*	
6	62.8 *t*	3.69 *m* 3.85 *m*	
5-OCH_3	-	3.34 *s*	
7-OCH_3	-	3.84 *s*	
3′,5′-OCH_3	-	3.71 *s*	
3″,5″-OCH_3	-	3.82 *s*	

[a] Correlation from H to the indicated carbons.

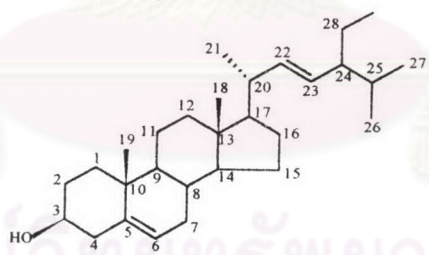
Identification of Compound SVH-1

Compound SVH-1 was obtained as colorless needles. This compound gave purple coloration upon spraying with 10% H_2SO_4 in 95% ethanol. The IR (Figure 127) and NMR (Figure 128) data of SVH-1 were in full agreement with the published values of β-sitosterol and stigmasterol mixture.

In the ^1H NMR spectrum (Figure 128), the signals at δ 5.03, 5.12 and 5.33 ppm could be assigned to H-22 and H-23 of stigmasterol and H-6 of both β-sitosterol and stigmasterol, respectively. The integrated peak areas of H-6 and either H-22 or H-23 were in the ratio of 2 : 1. Therefore, it could be deduced that SVH-1 was a 1: 1 mixture of β-sitosterol and stigmasterol. The ^{13}C-NMR data are shown in table 26.



β-sitosterol



stigmasterol

Table 26 Comparison of NMR spectral data of SVH-1 and mixture of β-sitosterol and stigmasterol ($CDCl_3$)

Carbon	Chemical shift (ppm)		
	β-sitosterol	Stigmasterol	SVH-1
	(Wolbis *et al.*, 2001)		
1	37.3	37.3	37.3
2	31.6	31.7	31.7
3	71.7	71.8	71.8
4	42.5	42.4	42.3
5	140.8	140.8	140.8
6	121.6	121.7	121.7
7	32.0	32.0	31.7, 31.9
8	32.0	32.0	31.7, 31.9
9	50.2	50.2	50.1
10	36.5	36.6	36.5
11	21.1	21.1	21.1, 21.2
12	39.8	39.7	39.8
13	42.3	42.4	42.3
14	56.7	56.9	56.8, 56.9
15	24.3	24.4	24.3, 24.4
16	28.3	29.0	28.2, 28.9
17	56.1	56.1	56.1
18	11.9	12.1	11.9
19	19.4	19.4	19.4
20	36.2	40.5	36.5
21	18.8	21.1	18.8
22	34.0	138.4	34.0
23	26.1	129.3	26.1
24	45.9	51.3	45.9
25	29.2	32.0	29.1
26	19.8	21.3	19.8
27	19.0	19.0	19.0
28	23.1	25.4	23.1
29	12.3	12.3	12.0, 12.2

Structure elucidation of Compound SVH-2 (7β-hydroxyisopimara-8,15-dien-14-one)

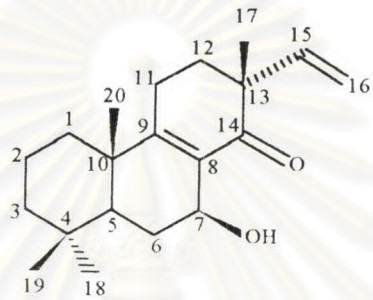
Compound SVH-2 was obtained as viscous oil. The mass spectrum (Figure 133) gave a molecular ion peak at *m/z* 302, corresponding to the molecular formula $C_{20}H_{30}O_2$. The FT-IR spectrum (Figure 132) shows absoption bands at 3442 (OH) , 3082 cm^{-1} (vinylidene group) and 1655 (C = O). The UV spectrum (Figure 131) in methanol showed absorption maxima at 242 nm.

The ^{1}H-NMR spectrum (Figures 134-135) showed exomethylene signals at δ 5.85 (*dd*, *J* = 17.6 and 10.8 Hz), 5.06 (*d*, *J* = 10.8 and 0.9 Hz) and 5.01 (*d*, *J* = 17.6 and 0.9 Hz) ppm which were assigned to H-15 and H-16, respectively. Furthermore, there are four methyl singlets at δ 0.89 (H_3-19), 0.94 (H_3-18), 1.06 (H_3-20), and 1.12 (H_3-17) ppm, together with those of seven methylene and 3 methine protons.

The APT spectrum (Figures 136-137 and Table 26) shows 20 carbon signals, supporting the assignment of SVH-2 as a diterpenoid derivative, These carbon resonances were classified as those of four methyl, seven methylene, three methine and 6 quaternary carbons, of which one is a keto carbon (δ 204.2). From HMQC spectrum (Figures 140-141) all protonated carbons were assigned (Table 26)

The HMBC correlations (Figures 142-143) between both signals at δ 5.01 and 5.06 ppm to the carbon signal at δ 48.7 ppm (C-13) confirmed the attachment of the vinylidene moiety to this carbon position. Furthermore, NOESY correlations between the methyl protons on the same face of the molecule at C-4 (Me-19), C-10 (δ Me-20) and C-13 (Me-17) proved that compound SVH-2 was an isopimarane diterpene. The ^3J coupling between the keto carbonyl carbon at δ 204.2 ppm and protons of H_2-12 (δ 1.75), H-15 (δ 5.85) and Me-17 can be observed, indicating the keto group to be at C-14. The long-range coupling between a single proton signal at δ 4.63 ppm (*ddd*, *J* = 4.4, 1.5, 1.4 Hz, H-7) with the carbon signals at δ 45.7 (C-5) and 132.1 (C-8) ppm, between H-6 (δ 1.84 ppm) and C-10 (δ 41.2 ppm), and consistency of these chemical shifts with those of the diterpenoids possessing a similar partial structure (Evidente *et al.*, 2002) indicates the location of a hydroxyl group at C-7 and suggests a fully substituted olefinic double bond between C-8 and C-9. The stereochemistry of compound SVH-2 was established by NOE experiment. The H-7

doublet of doublet of doublets at δ 4.63 ppm showed a correlation with the signal of the α-oriented H-5 (δ 1.54 ppm), hence confirming the configuration of 7-OH as β. Therefore, compound SVH-2 was elucidated as 7β-hydroxyisopimara-8,15-dien-14-one. This is the first time, to our knowledge, that isopimarane diterpenoid derivative was found in this genus.



7β-hydroxyisopimara-8,15-dien-14-one

Table 27 NMR spectral data of compounds SVH-2 (CD_3OD)

position	δ_C	δ_H	HMBC (H/C correlations)[a]
1	36.3 *t*	1.85 *m* 1.15 *m*	C-10 C-20
2	19.7 *t*	1.70 *tt* (13.7, 3.4) 1.54 *m*	
3	42.4 *t*	1.46 *m* 1.23 *m*	C-2, C-4, C-18, C-19
4	34.0 *s*		
5	45.7 *d*	1.54 *dd* (13.1, 1.6)	C-3, C-4, C-6, C-7, C-18, C-19, C-20
6	28.4 *t*	1.84 *ddd* (13.7, 1.6, 1.5) 1.63 *td* (13.7, 4.4)	C-5, C-7, C-10
7	63.1 *d*	4.63 *ddd* (4.4, 1.5, 1.4)	C-5, C-8
8	132.1 *s*		
9	169.8 *s*		
10	41.2 *s*		
11	23.2 *t*	2.37 *ddd* (8.1, 4.0, 1.4)	C-8, C-9, C-12, C-13
12	36.7 *t*	1.97 *dt* (13.4, 4.0) 1.75 *dt* (13.4, 8.1)	C-9, C-11, C-13, C-14, C-15
13	48.7 *s*		
14	204.2 *s*		
15	141.6 *d*	5.85 *dd* (17.6,10.8)	C-12, C-13, C-14, C-17
16	115.4 *t*	5.06 *dd* (10.8, 0.9) 5.01 *dd* (17.6, 0.9)	C-13, C-15
17	24.3 *q*	1.12 *s*	C-12, C-13, C-14, C-15
18	33.3 *q*	0.94 *s*	C-3, C-4, C-5, C-19
19	22.2 *q*	0.89 *s*	C-3, C-4, C-5, C-18
20	18.3 *q*	1.06 *s*	C-1, C-5, C-9, C-10

[a] Correlation from H to the indicated carbons.

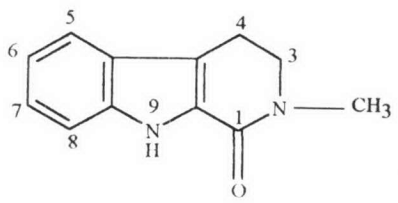
Identification of Compound SVH-3 (strychnocarpine)

Compound SVH-3 was obtained as yellow prism crystals. The UV spectrum (Figure 146) showed absorption maxima at 224 and 302 nm. The mass spectrum (Figure 148) showed a peak at *m/z* 200, consistent with the formular $C_{12}H_{12}N_2O$.

The ^{1}H-NMR spectrum (Figure 149 and Table 27) was in agreement with tetrahydro-1-oxo-carboline structure, with four aromatic proton signals at δ 7.56 (H-5), 7.12 (H-6), 7.28 (H-7), 7.43 (H-8) ppm, two set of correlated methylene proton triplets at δ 3.70 (H-3) and 3.07 (H-4) ppm. The N_b-methyl group appeared as a three proton singlet at δ 3.09 ppm.

The ^{13}C-NMR spectrum (Figure 150 and Table 27) shows further evidence for the N_b-methyl tetrahydro-1-oxo-carboline structure, with the signals due to two methylene carbons at δ 50.2 (C-3) and 20.7 (C-4) ppm, the N_b-methyl carbon at δ 34.2 ppm and the amide carbonyl carbon at δ 161.6 ppm.

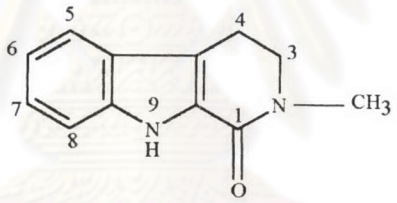
Compound SVH-3 was identified as strychnocarpine which was first isolated from the stem bark of *Strychnos elaeocarpa* and found to have a weak muscle relaxant effect together with a reduction of the 5-hydroxytryptamine (5-HT) depletion in mice brain, after 5-HT synthesis inhibition, indicating a stimulation of 5-HT recepters (Rolfsen *et al.*, 1980).



strychnocarpine

Table 28 Comparison of NMR spectral data of SVH-3 and strychnocarpine ($CDCl_3$)

position	SVH-3		strychnocarpine	
	δ_H	δ_C	δ_H	δ_C
1	-	161.6	-	161.1
3	3.70 *t* (7.2)	50.2	3.70 *t* (7)	49.6
4	3.07 *t* (7.2)	20.6	3.07 *t* (7)	20.1
4a	-	117.8	-	117.2
4b	-	125.2	-	124.9
5	7.56 *d* (7.7)	120.1	7.6-7.0 *m*	120.1
6	7.12 *t* (7.7)	120.0	7.6-7.0 *m*	119.5
7	7.28 *t* (7.8)	124.6	7.6-7.0 *m*	124.0
8	7.43 *d*(7.8)	112.3	7.6-7.0 *m*	112.6
8a	-	137.1	-	137.4
9a	-	126.9	-	137.4
N-CH_3	3.09 *s*	34.2	3.19 *s*	33.7



strychnocarpine

Determination of biological activities

The biological activities including antioxidant, anticholinesterase, antimicrobial and cytotoxic activities of the isolated compounds were determined. The compounds investigated for these activities were SVM-1, SVM-3, SVM-4, SVM-5, SVM-6, SVC-7, SVC-8, SVC-9 and SVH-2. Other compounds were not tested because of their limited quantities. The results for each activity are given below.

1. Antioxidant activity

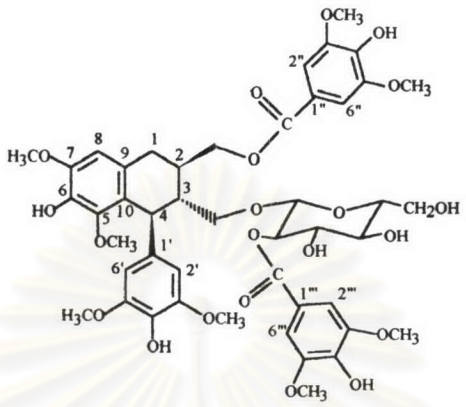
There is considerable recent evidence that free radicals induce oxidative damage to biomolecules and play an important role in cardiovascular disease, aging, cancer, inflammatory diseases, and a variety of other disorders. Antioxidants which scavenge free radicals are now known to possess preventive as well as therapeutic potential in free radical mediated disease conditions (Tiwari *et al.*, 1999). The search for potential pharmacological antioxidants is of great interest and plants are an important source of such compounds.

The compounds from *Strychnos vanprukii* stem investigated for this activity were SVM-1, SVM-3, SVM-4, SVM-5, SVM-6, SVC-4, SVC-5, SVC-6, SVC-7, and SVH-2. The results are shown in Table 28 was performed by spectrophotometric assay.

Table 29 Antioxidant activity (IC_{50}) of the isolated and reference compounds.

Compounds	Antioxidant activity (μM)
SVM-1	31.2
SVM-3	733.0
SVM-4	894.6
SVM-5	688.9
SVM-6	636.3
SVC-4	127.9
SVC-5	375.4
SVC-6	12.5
SVC-7	18.7
SVH-2	1644.7
Vitamin C	57.1

Three lignan compounds, strychnoside (SVC-6), vanprukoside (SVC-7) and lyoniresinol 3-*O*-β-glucopyranoside (SVM-1), exhibited the antioxidant activity more active than ascorbic acid which was used as reference compound. Compound SVC-6 showed the most active activity in these tested compounds.



SVC-6

2. Anticholinesterase activity

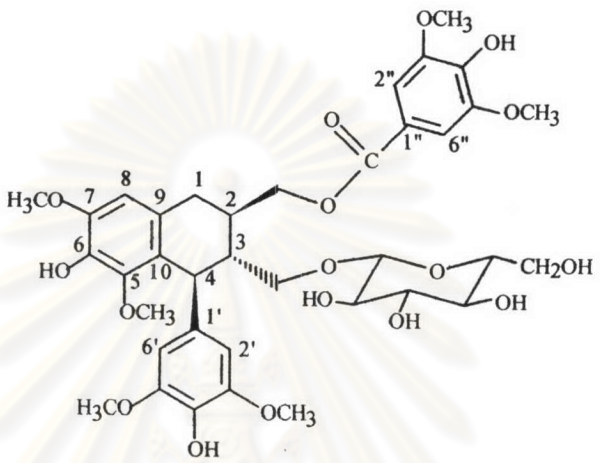
Alzheimer disease (AD) is a neurodegenerative disorder marked by progressively worsening cognitive deficiencies, behavioral disturbances, and distinctive neuropathology. The loss of the basal forebrain cholinergic system is one of the most significant aspects of neurodegeneration in the brains of AD patients, and it is thought to play a central role in producing cognitive impairment. Therefore, AD drug development strategies over the past two decades have focused on the augmentation of cholinergic transmission as a means of restoring cognitive function of these patients (Krall *et al.*, 1999). Acetylcholinesterase inhibitors (AchEIs), which enhance cholinergic transmission by reducing the enzymatic degradation of acetylcholine, are approved for the treatment of AD. In 1993, tacrine was approved by the Food and Drug Administration (FDA) in the United States for mild to moderate Alzheimer's dementia. However, its narrow therapeutic range and hepatoxicity limit its use. Galanthamine, an Amaryllidaceous alkaloid, has received the first approved in Austria (Ingkaninan *et al.*, 2000). Further search for other AchEIs is of great interest and natural products are an important potential source of such compounds.

In this study, compounds SVM-1, SVM-3, SVM-5, SVC-4, SVC-6, SVC-7 and SVH-2 were investigated on acetylcholinesterase inhibitory activity. The results are summerized in Table 29.

Table 30 Anticholinesterase activity (IC_{50}) of isolated compounds

Compounds	Anticholinesterase activity (μM)
SVM-1	128.0
SVM-3	201.9
SVM-5	167.8
SVC-4	531.1
SVC-6	113.6
SVC-7	18.1
SVH-2	633.4
galanthamine	4

All of the tested compounds showed acetylcholinesterase inhibitory activity less active than galanthamine. Compound SVC-7 (vanprukoside) displayed the most potent activity in these tested compounds.

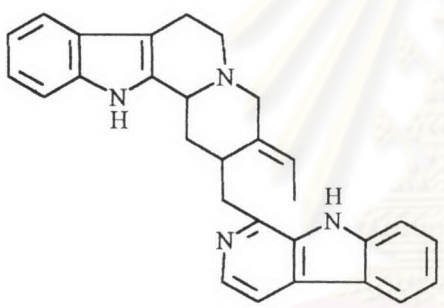


SVC-7

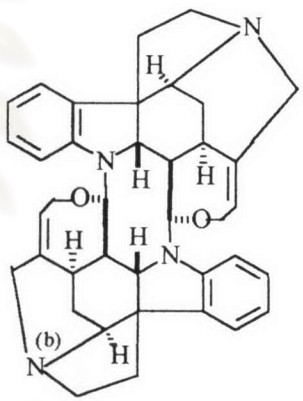
3. Antimicrobial activity

The compounds investigated for this activity against *S. aureus*, *E. coli* and *C. albicans* were SVM-1, SVM-3, SVM-4, SVM-6, SVC-6, SVC-7 and SVH-2. All of the tested compounds possessed no activity against any of the test microorganisms at maximum concentration of 800 μg/ml.

In view of *Strychnos* alkaloids, the compounds which displayed antimicrobial activity were the bases with usambarane skeleton such as usambarensine and dimeric alkaloids such as caracurine V. None of the tested compound possessed these skeletons. Most of the tested compounds are glycosides which may be result in inability to penetrate into cells.



usambarensine

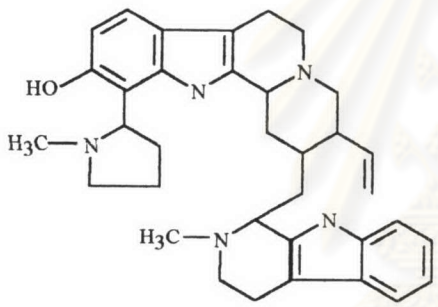


caracurine V

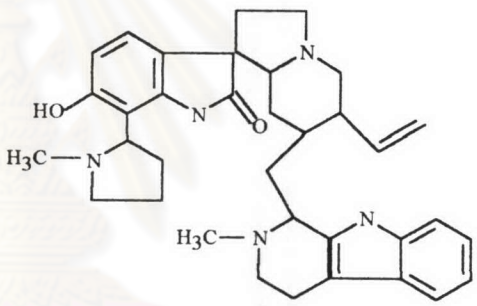
4. Cytotoxic activity

The compounds investigated for this activity were SVM-1, SVM-3, SVM-4, SVM-6, SVC-6, SVC-7 which are glycosides and diterpenoid SVH-2. All of the tested compounds exhibited no cytotoxic activity against melanoma B16 cell line at maximum concentration of 1,000 μg/ml.

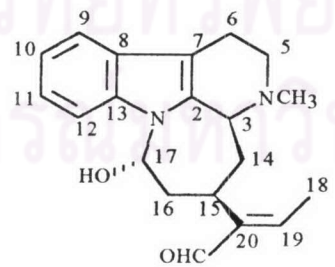
The *Strychnos* alkaloids which have been reported of a pronounced cytotoxic activity against melanoma B16 were dimeric alkaloids such as strychnopentamine and strychnophylline. Akagerine (SVC-3) was reported to have this activity against melanoma B 16 (Leclercq *et al*, 1986). The absence of activity of tested compounds could be resulting from the sugar which increase in polarity in the molecule.



Strychnophylline



strychnopentamine



akagerine (SVC-3)