Glycosides of 14,15-Seco- and 13,14 : 14,15-Disecopregnanes from the Roots of *Tylophora tanakae*

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Five new glycosides of 14,15-seco- and 13,14 : 14,15-diseco-type pregnanes, including a new pregnane, 2α-hydroxyhirundigenin, were isolated, in addition to one known glycoside, cynatratoside B, from the roots of *Tylophora tanakae* MAXIM. Their structures were elucidated by spectral and chemical means.

Key words 14,15-secopregnane glycoside; *Tylophora tanakae*; Asclepiadaceae; 2α-hydroxyhirundigenin; tylophoside

Tylophora tanakae MAXIM. is an Asclepiadaceae and is indigenous to the Ryukyu Islands. In the preceding paper of this series, we described the isolation of phenanthroindolizidine alkaloids,[1] and presented evidence that two of them showed oviposition-promoting activity against *Ideopsis similis*.[2] On the other hand, the isolation of novel 14,15-seco-type pregnanes was reported from African *Tylophora sylvatica*.[3] During our studies of the constituents of *T. tanakae*, this paper deals with the structural determination of 14,15-seco- and 13,14 : 14,15-disecopregnane glycosides isolated from the roots.

When the roots of *T. tanakae* were extracted with MeOH and the MeOH extract was partitioned with $CHCl_3$–H_2O, six glycosides (**1**—**6**) were observed in the $CHCl_3$ layer along with phenanthroindolizidine alkaloids, tylophorine and isotylocrebrine.[1] Each glycoside was isolated using a silica gel column, an octadecyl silica (ODS) column, and HPLC.

The high-resolution (HR)-FAB-MS of **1** afforded a $[M+Na]^+$ peak at *m/z* 801.4037, suggesting the molecular formula $C_{41}H_{62}O_{14}$. Based on the NMR considerations, **1** was assignable as a glycoside of a 13,14 : 14,15-diseco-type pregnane such as glaucogenins. In fact, carbon signals due to the aglycone moiety were in good agreement with those of glaucogenin C glycosides.[4] In the ^{1}H-NMR signals due to the sugar moiety, signals of three 6-methyl and two methoxy groups were observed, in addition to three anomeric protons with doublet of doublets or broad doublet patterns, suggesting **1** to be a trioside composed of two 2,6-dideoxy-3-*O*-methylhexoses and one 2,6-dideoxyhexose.

In order to confirm the pregnane and sugars, **1** was subjected to acid hydrolysis. The pregnane obtained was identified as glaucogenin C,[4d] based on its physical constants, a HR-FAB-MS peak at *m/z* 360.1938 ($C_{21}H_{28}O_5$), and the ^{1}H- and ^{13}C-NMR spectra. From the H_2O layer of the hydrolysate, two sugars, oleandrose and digitoxose were obtained. Digitoxose was confirmed to be D-type, based on its optical rotation ($[\alpha]_D$ +55.2°). On the other hand, the rotation value of oleandrose was almost zero (+0.3°), suggesting that oleandrose was a 1 : 1 mixture of D- and L-forms. Anomeric proton signals were observed at δ 4.82 and 5.55 (each dd, J=10, 2 Hz), and at δ 5.25 (br d, J=3 Hz). The former two signals were assignable as axial anomeric protons of β-linked D-oleandrose and D-digitoxose, respectively, while the latter one was due to an equatorial anomeric proton of α-linked L-oleandrose based on the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum. The sequence of the three sugars was determined by nuclear Overhauser effects (NOEs) between H-$1_{D\text{-ole}}$/H-3α, H-$1_{D\text{-digt}}$/H-$4_{D\text{-ole}}$, and H-$1_{L\text{-ole}}$/H-$4_{D\text{-digt}}$ observed in the difference (DIF) of NOE measurement. In the heteronuclear multiple bond connectivity (HMBC) spectrum, 3-bond correlations were observed between H-$1_{D\text{-ole}}$/C-3, H-$4_{D\text{-ole}}$/C-$1_{D\text{-digt}}$, H-$4_{D\text{-digt}}$/C-$1_{L\text{-ole}}$, H-3α/C-$1_{D\text{-ole}}$, H-$1_{D\text{-digt}}$/C-$4_{D\text{-ole}}$, and H-$1_{L\text{-ole}}$/C-$4_{D\text{-digt}}$. Glycoside **1** was thus determined to be glaucogenin C 3-*O*-α-L-oleandrosyl-(1→4)-β-D-digitoxosyl-(1→4)-β-D-oleandroside, and was named tylophoside A.

In HR-FAB-MS, **2** gave a $[M+Na]^+$ peak at *m/z* 801.4039, which was the same as that of **1**. On acid hydrolysis of **2**, glaucogenin C[4d] as an aglycone, plus oleandrose, digitoxose and cymarose as component sugars were identified on TLC. Three anomeric protons were observed at δ 4.81 (dd, J=10, 2 Hz), 5.43 (dd, J=10, 2 Hz), and 5.08 (dd, J=4, 2 Hz). Based on the ^{1}H–^{1}H COSY spectrum, the former two were assignable to those of oleandrose and digitoxose, respectively, and the latter to that of cymarose. Chemical shifts of C-2 (δ 32.2) and C-5 (δ 67.1) in the cymarose of **2**, as well as the signal of the anomeric proton, were consistent with the values in the literature of L-cymaroside.[4,5] The sugar sequence was determined to be cymarosyl-(1→4)-digitoxosyl-(1→4)-oleandroside, based on 3-bond correlations between H-1_{ole}/C-3, H-4_{ole}/C-1_{digt}, H-1_{digt}/C-4_{ole} and H-1_{cym}/C-4_{digt} in HMBC spectrum. The glycoside of glaucogenin C having the same sugar sequence as **2** was described as cynatratoside B.[4d] In comparison of the chemical shifts and coupling patterns in the NMR spectra, **2** was identical with cynatratoside B.

Glycoside **3** afforded a $[M+Na]^+$ peak at *m/z* 831.4149, suggesting the molecular formula $C_{42}H_{64}O_{15}$, which was CH_2O larger than **1**. The ^{1}H- and ^{13}C-NMR spectra of **3** indicated that the aglycone of **3** was glaucogenin A (2α-hydroxyglaucogenin C).[4a] Based on the signals due to three anomeric protons with doublet of doublets or broad doublet patterns, three methoxy and three 6-methyl groups, the sugar moiety was composed of three 2,6-dideoxy-3-*O*-methylhexoses. On hydrolysis of **3** in the same manner as **1**, glaucogenin A and two sugars, cymarose and sarmentose, were identified. Optical rotations of the sugars were observed as

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Table 1. ^{1}H-NMR Spectral Data for **1**—**6** [in Pyridine-d_5, 500 MHz]

H	1	2	3	4	5	6
1	0.96 (td, 13, 3)	0.96 (td, 13, 3)	1.22 (t, 12)	1.24 (t, 12)	1.25 (t, 12)	1.24 (t, 12)
	1.84 (dt, 13, 3)	1.84 (dt, 13, 3)	2.45 (dd, 12, 4)	2.47 (dd, 12, 4)	2.35 (dd, 12, 4)	2.35 (dd, 12, 4)
2	1.70 (m)	1.70 (m)	3.99 (ddd, 12, 10, 4)	4.02 (12, 10, 4)	4.02 (ddd, 12, 10, 4)	4.02 (ddd, 12, 10, 4)
	2.10 (m)	2.11 (m)				
3	3.77 (m)	3.77 (m)	3.56 (m)	3.59 (m)	3.55 (m)	3.56 (m)
6	5.42 (br d, 4)	5.41 (br d, 3)	5.41 (br d, 4)	5.42 (br d, 4)	5.49 (br d, 3)	5.48 (br d, 3)
15	3.96 (dd, 10, 9)	3.96 (dd, 10, 9)	3.95 (dd, 10, 9)	3.95 (t, 9)	3.80 (dd, 10, 4)	3.81 (dd, 10, 4)
	4.25 (dd, 9, 7)	4.25 (dd, 9, 7)	4.23 (dd, 9, 7)	4.24 (dd, 9, 7)	4.33 (dd, 10, 2)	4.33 (dd, 10, 2)
16	5.46 (ddd, 10, 8, 7)	5.46 (ddd, 10, 8, 7)	5.44 (ddd, 10, 9, 7)	5.45 (td, 9, 7)	4.77 (ddd, 8, 4, 2)	4.78 (ddd, 8, 4, 2)
17	3.56 (d, 8)	3.57 (d, 8)	3.54 (d, 9)	3.56 (d, 9)	2.91 (d, 8)	2.92 (d, 8)
18	6.48 (s)	6.48 (s)	6.47 (s)	6.47 (s)	3.76 (d, 9)	3.77 (d, 8)
					4.71 (d, 9)	4.71 (d, 8)
19	0.87 (s)	0.87 (s)	0.91 (s)	0.92 (s)	0.98 (s)	0.98 (s)
21	1.56 (s)	1.56 (s)	1.55 (s)	1.55 (s)	1.52 (s)	1.53 (s)
	β-ole	β-ole	β-cym	β-cym	β-cym	β-cym
1′	4.82 (dd, 10, 2)	4.81 (dd, 10, 2)	5.18 (dd, 9, 2)	5.19 (dd, 10, 2)	5.16 (dd, 9, 2)	5.16 (dd, 9, 2)
3′	3.59 (m)	3.57 (m)	3.91 (br q, 3)	3.92 (br q, 3)	3.89 (br q, 3)	3.88 (br q, 3)
4′	3.54—3.58[a]	3.53—3.57[a]	3.45 (dd, 10, 3)	3.47 (dd, 9, 3)	3.44 (dd, 10, 3)	3.43 (dd, 10, 3)
5′	3.54—3.58[a]	3.53—3.57[a]	4.22 (dq, 10, 6)	4.24[a]	4.22 (dq, 10, 6)	4.19 (dq, 10, 6)
6′	1.45 (d, 6)	1.46 (d, 6)	1.31 (d, 6)	1.33 (d, 6)	1.29 (d, 6)	1.29 (d, 6)
	digt	digt	sar	dign	sar	sar
1″	5.55 (dd, 10, 2)	5.43 (dd, 10, 2)	5.06 (br d, 4)	5.16 (br d, 3)	5.01 (br d, 3)	4.98 (br d, 3)
3″	4.60 (br q, 3)	4.46 (br q, 3)	4.03 (br q, 3)	3.82 (br d, 9)	4.02 (br q, 3)	3.97 (br q, 3)
4″	3.54 (dd, 9, 3)	3.45 (dd, 9, 3)	3.74 (br s)	4.09 (br s)	3.73 (br s)	3.67 (br s)
5″	4.39 (dq, 9, 6)	4.13 (dq, 9, 6)	4.64 (br q, 6)	4.25[a]	4.63 (br q, 6)	4.60 (br q, 6)
6″	1.45 (d, 6)	1.39 (d, 6)	1.39 (d, 6)	1.46 (d, 6)	1.39 (d, 6)	1.34 (d, 6)
	α-ole	α-cym	β-cym	β-cym	β-cym	β-cym
1‴	5.25 (br d, 3)	5.08 (dd, 4, 2)	5.14 (dd, 10, 2)	5.13 (dd, 10, 2)	5.13 (dd, 10, 2)	5.14 (dd, 10, 2)
3‴	3.82 (m)	3.72 (br q, 3)	3.76 (br q, 3)	3.74 (br q, 3)	3.76 (br q, 3)	4.13 (br q, 3)
4‴	3.52 (t, 9)	3.60 (dd, 9, 3)	3.55 (dd, 9, 3)	3.56 (dd, 9, 3)	3.55 (dd, 10, 3)	3.68 (dd, 10, 3)
5‴	4.41 (dq, 9, 6)	4.47 (dq, 9, 6)	4.11 (dq, 9, 6)	4.11 (dq, 9, 6)	4.10 (dq, 10, 6)	4.27 (dq, 10, 6)
6‴	1.52 (d, 6)	1.45 (d, 6)	1.51 (d, 6)	1.53 (d, 6)	1.51 (d, 6)	1.62 (d, 6)
						glc
1⁗						4.94 (d, 8)
2⁗						3.99 (dd, 8, 9)
3⁗						4.22 (t, 9)
4⁗						4.17 (t, 9)
5⁗						3.97 (m)
6⁗						4.38 (dd, 12, 5)
						4.55 (dd, 12, 2)
OMe	3.35 (s)	3.39 (s)	3.38 (s)	3.45 (s)	3.37 (s)	3.34 (s)
	3.57 (s)	3.55 (s)	3.47 (s)	3.47 (s)	3.47 (s)	3.54 (s)
			3.64 (s)	3.55 (s)	3.63 (s)	3.62 (s)

a) Overlapping with other signals. β-ole=β-D-oleandrose, α-ole=α-L-oleandrose, β-cym=β-D-cymarose, α-cym=α-L-cymarose, digt=β-D-digitoxose, sar=α-L-sarmentose, dign=α-L-diginose, glc=β-D-glucose.

+48.4° (cymarose) and −15.9° (sarmentose), indicating them to be D- and L-forms, respectively. Since two anomeric protons observed as doublet of doublets (δ 5.18, J=9, 2 Hz; δ 5.14, J=10, 2 Hz) were assignable for D-cymarose based on the ^{1}H–^{1}H COSY spectrum, the glycosidic linkages of two D-cymarose were assignable as β. On the other hand, a small coupling constant of L-sarmentose indicated the glycosidic linkage to be α. The sugars were arrayed as cymarosyl–sarmentosyl–cymaroside by NOEs between H-3α/H-1$_{cym\text{-}1}$, H-1$_{sar}$/H-4$_{cym\text{-}1}$, and H-1$_{cym\text{-}2}$/H-4$_{sar}$ as well as by 3-bond correlations between H-3α/C-1$_{cym\text{-}1}$, H-4$_{cym\text{-}1}$/C-1$_{sar}$, H-4$_{sar}$/C-1$_{cym\text{-}2}$, H-1$_{cym\text{-}1}$/C-3, H-1$_{sar}$/C-4$_{cym\text{-}1}$, and H-1$_{cym\text{-}2}$/C-4$_{sar}$ in HMBC spectrum. The structure of **3** was therefore determined to be glaucogenin A 3-*O*-β-D-cymarosyl-(1→4)-α-L-sarmentosyl-(1→4)-β-D-cymaroside, and **3** was named tylophoside B.

A quasi-molecular peak of **4** was observed at *m*/*z* 831.4149, ($C_{42}H_{64}O_{15}$+Na), suggesting that **4** has the same molecular formula as **3**. Since the NMR signals of the aglycone moiety were almost the same as those of **3**, **4** seemed to be composed of glaucogenin A. Three 2,6-dideoxy-3-*O*-methylhexoses were considered to be two cymarose and one diginose, based on the ^{1}H–^{1}H COSY spectrum. Two sugars were further identified with authentic cymarose and diginose on TLC after the hydrolysis of **4**. The sequence of sugars was determined to be cymarosyl–diginosyl–cymaroside by 3-bond correlations between H-1$_{cym\text{-}1}$/C-3, H-4$_{cym\text{-}1}$/C-1$_{dign}$, H-4$_{dign}$/C-1$_{cym\text{-}2}$ in the HMBC spectrum. Since two units of cymarose in **4** showed almost the same chemical shifts and coupling patterns in the ^{1}H- and ^{13}C-NMR spectra as those of D-cymarose in **3** and the literature,[4,5] they seemed to be in the D-series with β-linkages, while diginose is in the L-series[6] with α. Thus, **4** was tentatively assigned to be glaucogenin A 3-*O*-β-D-cymarosyl-(1→4)-α-L-diginosyl-(1→4)-β-D-cymaroside, and was thus named tylophoside C.

In HR-FAB-MS, **5** afforded a $[M+Na]^+$ peak at *m*/*z* 833.4297, suggesting the molecular formula $C_{42}H_{66}O_{15}$.

Table 2. ^{13}C-NMR Spectral Data for **1**—**6** [in Pyridine-d_5, 125 MHz]

C	**1**	**2**	**3**	**4**	**5**	**6**	
1	36.5	36.5	44.7	44.7	45.3	45.3	
2	30.0	30.0	69.9	69.9	69.9	69.9	
3	77.5	77.5	85.4	85.3	85.6	85.6	
4	39.0	39.0	37.5	37.5	37.5	37.5	
5	140.6	140.6	139.7	139.8	139.3	139.3	
6	120.4	120.4	120.7	120.7	122.7	122.7	
7	28.4	28.4	28.5[a)]	28.4	26.7	26.7	
8	40.7	40.7	40.2	40.2	37.6	37.7	
9	53.2	53.2	53.0	53.0	44.1	44.1	
10	38.6	38.6	39.4	39.4	39.1	39.1	
11	23.9	23.9	23.8	23.8	20.9	20.9	
12	29.9	29.9	30.0	30.0	32.0	32.0	
13	114.3	114.3	114.3	114.3	59.7	59.7	
14	175.4	175.4	175.3	175.3	108.7	108.7	
15	67.8	67.8	67.7	67.7	75.0	75.0	
16	75.5	75.5	75.5	75.5	81.3	81.4	
17	56.2	56.2	56.1	56.1	65.8	65.8	
18	143.8	143.8	143.8	143.8	77.2	77.2	
19	17.9	17.9	18.8	18.9	19.1	19.1	
20	118.5	118.5	118.5	118.5	118.5	118.5	
21	24.7	24.7	24.7	24.7	22.6	22.6	
	β-ole	β-ole	β-cym	β-cym	β-cym	β-cym	
1′	98.1	98.1	97.7	97.5	97.7	97.7	
2′	38.0	37.9	36.9	35.2[a)]	36.9	36.9	
3′	79.2	79.2	77.6	77.4	77.6	77.6	
4′	83.1	83.0	81.9	82.0	81.9	81.9	
5′	71.7	71.7	69.6	69.5	69.5	69.5	
6′	18.8[a)]	18.8	18.2	18.3	18.2	18.2	
	digt	digt	sar	dign	sar	sar	
1″	98.5	98.4	99.4	101.0	99.4	99.4	
2″	39.9	38.4	28.4[a)]	32.4	28.5	28.5	
3″	67.8	67.8	75.7	73.8	75.7	75.7	
4″	82.3	80.7	77.2	74.6	77.2	77.0	
5″	69.2	69.1	63.1	67.6	63.1	63.0	
6″	18.6[a)]	18.3	16.9	17.8	16.9	16.8	
	α-ole	α-cym	β-cym	βcym	β-cym	β-cym	glc
1‴ (1⁗)	100.2	98.3	100.5	99.4	100.6	100.4	106.5
2‴ (2⁗)	35.7	32.2	35.6	35.3[a)]	35.6	36.4	75.3
3‴ (3⁗)	78.8	76.5	78.8	78.9	78.8	77.9	78.3
4‴ (4⁗)	76.8	72.7	74.1	74.1	74.1	83.1	71.8
5‴ (5⁗)	68.9	67.1	70.9	71.0	70.9	69.4	78.3
6‴ (6⁗)	18.4	18.5	18.8	18.7	18.8	18.5	63.0
OMe	57.0	56.7	56.2	55.3	56.2	56.2	
	57.4	57.3	58.0	57.3	58.0	58.3	
			58.3	57.9	58.3	58.6	

a) Signal assignments may be interchangeable in each column. β-ole=β-D-oleandrose, α-ole=α-L-oleandrose, β-cym=β-D-cymarose, α-cym=α-L-cymarose, digt=β-D-digitoxose, sar=α-L-sarmentose, dign=α-L-diginose, glc=β-D-glucose.

While no carbonyl carbon signal was observed, the 21-methyl group was linked to an acetal carbon (δ 118.4) as in **1**—**4**. Two quaternary carbon signals at δ 39.1 and δ 59.7 in distortionless enhancement by polarization transfer (DEPT) were assignable as C-10 and C-13, respectively, indicating the presence of a 13,14-bond in the usual steroid nucleus. The aglycone of **5** was considered to have a 14-hemiacetal-20-acetal structure such as hirundigenin,[7] based on two acetal carbon signals at δ 108.7 (C-14, s) and δ 118.5 (C-20, s). Since the multiplicities and chemical shifts due to ring A showed similarity to those of **3** and **4**, **5** was considered to be a 3-*O*-glycoside of 2α-hydroxyhirundigenin. On acid hydrolysis, **5** afforded cymarose and sarmentose along with a pregnane (**5a**). Due to carbon signals of a tetrasubstituted double bond (δ 103.9 (C-8), 152.9 (C-14)) and a trisubstituted double bond (δ 141.3 (C-5), 119.7 (C-6)), as well as the molecular formula, $C_{21}H_{28}O_5$, **5a** was assigned to be a 2α-hydroxy-derivative of anhydrohirundigenin.[7] The sugar moiety of **5** was considered to have the same structure as that of **3** based on the coincidence of ^{1}H- and ^{13}C-NMR spectra. Glycoside **5** was thus assigned to be 2α-hydroxyhirundigenin 3-*O*-β-D-cymarosyl-(1→4)-α-L-sarmentosyl-(1→4)-β-D-cymaroside, and was named tylophoside D.

HR-FAB-MS of **6** afforded a $[M+Na]^+$ peak at *m/z* 995.4843, ($C_{48}H_{76}O_{20}$+Na), suggesting that the molecular formula of **6** was larger than **5** for one hexose unit. On the acid hydrolysis of **6**, β-D-glucosyl-D-cymarose (strophanthobiose) was obtained in addition to cymarose, sarmentose and **5a**, while on hydrolysis with cellulase, **5** was detected on TLC and HPLC. The structure of **6** was therefore assigned as a glycoside with one additional glucose at C-4 (δ 74.1) of the terminal cymarose in **5**, based on the glycosylation shift of C-4 (+9.0 ppm) of the second cymarose in **6**. In the HMBC spectrum, cross peaks were observed between H-1$_{glc}$/C-4$_{cym\text{-}2}$ and H-4$_{cym\text{-}2}$/C-1$_{glc}$ as well as those observed in **5**. Glycoside **6** was thus determined to be 2α-hydroxyhirundigenin 3-*O*-β-D-glucosyl-(1→4)-β-D-cymarosyl-(1→4)-α-L-sarmentosyl-(1→4)-β-D-cymaroside, and was named tylophoside E.

Six pregnane glycosides were isolated from the roots of *T. tanakae* and their structures were determined. Tylophorosides, glycosides of the 14,15-seco-type pregnanes from *T. sylvatica* in Africa, have unique butenolide structures,[3] while the pregnane glycosides from *T. tanakae* show a similar pregnane pattern to those from *Cynanchum* from an Asian source.[4] A glycoside similar to **1** was described as cynatratoside C, glaucogenin C 3-*O*-α-D-oleandrosyl-(1→4)-β-D-digitoxosyl-(1→4)-β-D-oleandroside,[4d] and the ^{13}C-NMR spectra of these two glycosides showed quite similar patterns.

Experimental

Melting points were taken on a hot stage apparatus without correction. ^{1}H- and ^{13}C-NMR spectra were recorded on a JNM-A500 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to internal tetramethylsilane (TMS), and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad, dd=doublet of doublets. HR-FAB-MS were recorded on a JEOL HX-110 spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter. For silica gel column chromatography and TLC, the following solvent systems were applied: 1, $CHCl_3$–MeOH–H_2O (10 : 1 : 1—7 : 3 : 1, bottom layer); 2, EtOAc–MeOH–H_2O (8 : 1 : 1.2—5 : 1 : 4, top layer); 3, benzene–acetone (10 : 1—1 : 1). For ODS column chromatography and HPLC, MeCN–H_2O (30—60%) was used. Spray reagent: 10% H_2SO_4.

Plant Materials *Tylophora tanakae* was cultivated in the farm of Fukuoka University. Fresh roots (2.8 kg) were harvested in November, 1998, and soaked in MeOH.

Extraction and Isolation of Pregnane Glycosides The roots were homogenized with MeOH, and the MeOH extract was partitioned with $CHCl_3$–H_2O. The $CHCl_3$ extract (10 g) was subjected to column chromatography on a silica gel column (solvent 1, 2) and an ODS column to isolate pregnane glycosides, and finally each glycoside was purified by HPLC (ODS; 40—65%). Glycoside **1**, 105 mg; **2** (cynatratoside B), 7 mg; **3**, 220 mg; **4**, 10 mg, **5**, 130 mg; **6**, 18 mg; tylophorine, 98 mg;[1a)] isotylocrebrine, 86 mg.[1a)]

Tylophoside A (**1**): Solid, $[\alpha]_D^{30}$ −8.8° (*c*=0.43, $CHCl_3$), HR-FAB-MS *m/z*: 801.4037 (Calcd for $C_{41}H_{62}O_{14}$ +Na: 801.4037). **1** (90 mg) was heated with 0.05 N HCl–50%dioxane (2.0 ml) for 30 min at 95 °C. The mixture was diluted with MeOH and deacidified with Amberlite IRA-410. The solution was concentrated *in vacuo* and diluted with H_2O, then extracted with $CHCl_3$. The $CHCl_3$ extract was purified by silica gel column chromatography (solvent 3; 10 : 1) and crystallized from MeOH to give prisms (glaucogenin C), mp 211—213 °C (MeOH), $[\alpha]_D^{20}$ +54.9° (*c*=0.45, $CHCl_3$), HR-FAB-MS *m/z*: 360.1938 (Calcd for $C_{21}H_{28}O_5$: 360.1937). ^{1}H-NMR: 0.92 (3H, s, H-19), 1.56 (3H, s, H-21), 3.57 (1H, br d, *J*=7 Hz, H-17), 3.81 (1H, m, H-3), 3.97 (1H, dd, *J*=10, 9 Hz, H-15a), 4.25 (1H, dd, *J*=9, 7 Hz, H-15b), 5.37

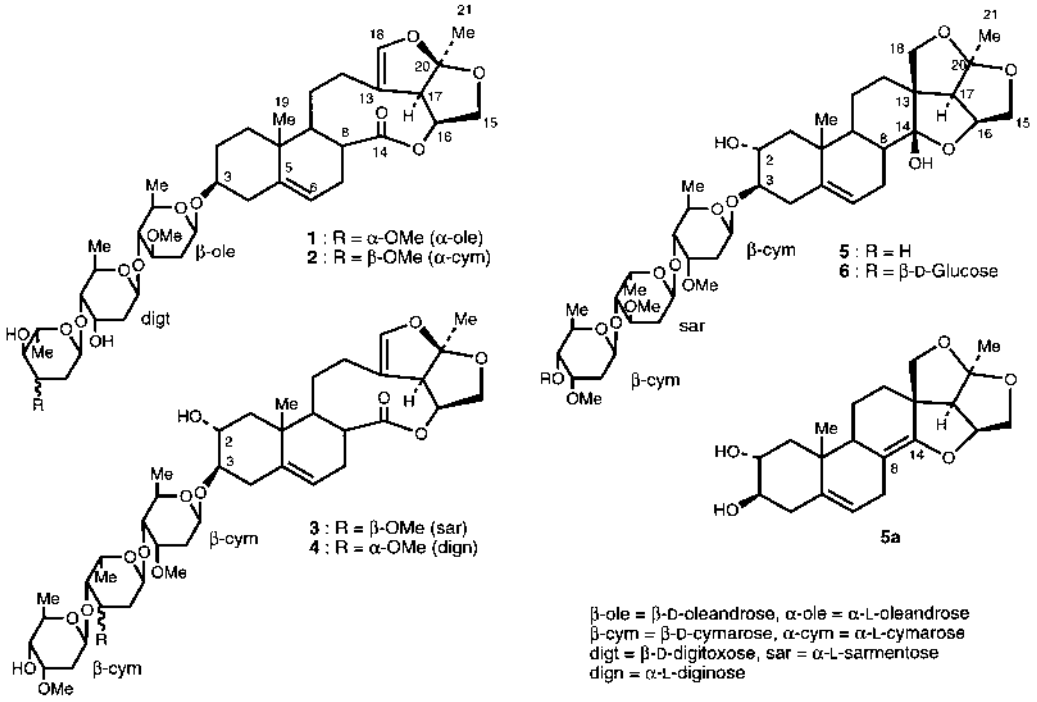

Chart 1

(1H, br d, J=5 Hz, H-6), 5.46 (1H, dt, J=9, 7 Hz, H-16), 6.48 (1H, s, H-18). ^{13}C-NMR: 18.0 (q, C-19), 23.9 (t, C-12), 24.7 (q, C-21), 28.4, 30.0 (t, C-7, 11), 32.3 (t, C-2), 36.7 (t, C-4), 38.6 (s, C-10), 40.7 (d, C-8), 43.0 (t, C-1), 53.3, 56.2 (d, C-9,17), 67.8 (t, C-15), 71.1 (d, C-3), 75.5 (d, C-16), 114.4 (s, C-13), 118.5 (s, C-20), 119.7 (d, C-6), 141.6 (s, C-5), 143.8 (d, C-18), 175.5 (s, C-14). The H_2O layer, after extraction with $CHCl_3$, was chromatographed on a silica gel column with solvent 3 (5 : 1) to isolate two sugars which were identified as oleandrose and digitoxose on TLC (solvents 1, 2, 3). Oleandrose: $[\alpha]_D^{21}$ +0.3° (c=1.01, H_2O, 24h), digitoxose: $[\alpha]_D^{24}$ +55.2° (c=0.30, H_2O, 24h).

Tylophoside B (**3**): Prisms (MeOH), mp 202—204 °C, $[\alpha]_D^{30}$ −6.3° (c= 0.40, $CHCl_3$), HR-FAB-MS m/z: 831.4149 (Calcd for $C_{42}H_{64}O_{15}$ +Na: 831.4143). On the hydrolysis of **3** (129 mg) using the same procedure as **1**, a pregnane (glaucogenin A) was obtained as needles (hexane–EtOAc), mp 234—236 °C, $[\alpha]_D^{20}$ +44.4° (c=0.60, $CHCl_3$), HR-FAB-MS m/z: 376.1888 (Calcd for $C_{21}H_{28}O_6$: 376.1886). ^{1}H-NMR: 0.98 (3H, s, H-19), 1.55 (3H, s, H-21), 3.55 (1H, br d, J=8 Hz, H-17), 3.79 (1H, ddd, J=11, 9, 6 Hz, H-3), 3.96 (1H, dd, J=10, 9 Hz, H-15a), 4.12 (1H, ddd, J=12, 9, 5 Hz, H-2), 4.25 (1H, dd, J=9, 7 Hz, H-15b), 5.41 (1H, br d, J=5 Hz, H-6), 5.30 (1H, dt, J= 9, 7 Hz, H-16), 6.45 (1H, s, H-18). ^{13}C-NMR: 19.1 (q, C-19), 23.9 (t, C-12), 24.7 (q, C-21), 28.4, 30.0 (t, C-7, 11), 40.0 (s, C-10), 40.3 (d, C-8), 40.4 (t, C-4), 45.5 (t, C-1), 53.1, 56.2 (d, C-9, 17), 67.8 (t, C-15), 72.3 (d, C-2), 75.5 (d, C-16), 76.7 (d, C-3), 114.4 (s, C-13), 118.5 (s, C-20), 120.0 (d, C-6), 140.8 (s, C-5), 143.7 (d, C-18), 175.4 (s, C-14). From the H_2O layer, two sugars were isolated and identified as cymarose and sarmentose on TLC (solvents 1, 2, 3). Cymarose: $[\alpha]_D^{23}$ +48.4° (c=1.15, H_2O, 24h), sarmentose: $[\alpha]_D^{24}$ −15.9° (c=0.34, H_2O, 24h).

Tylophoside C (**4**): Solid, $[\alpha]_D^{29}$ −14.4° (c=0.50, $CHCl_3$), HR-FAB-MS m/z: 831.4149 (Calcd for $C_{42}H_{64}O_{15}$ +Na: 831.4143). **4** was hydrolyzed under the same condition as **1**. Glaucogenin A from the $CHCl_3$ layer, and cymarose and diginose from the H_2O layer were identified on TLC (solvents 1, 2, 3).

Tylophoside D (**5**): Solid, $[\alpha]_D^{23}$ −38.4° (c=1.70, MeOH) , HR-FAB-MS m/z: 833.4297 (Calcd for $C_{42}H_{66}O_{15}$ +Na: 833.4310). On hydrolysis of **5** in the same procedure as in **1**, **5a** was obtained as fine prisms (hexane–EtOAc), mp 196—206 °C (dec.), $[\alpha]_D^{21}$ −13.8° (c=0.34, MeOH), HR-FAB-MS m/z: 360.1938 (Calcd for $C_{21}H_{28}O_5$: 360.1937). ^{1}H-NMR: 0.91 (3H, s, H-19), 1.58 (3H, s, H-21), 2.80 (1H, d, J=8 Hz, H-17), 3.81 (1H, dd, J=11, 4 Hz, H-15a), 3.82 (1H, m, H-3), 4.02 (1H, d, J=9 Hz, H-18a), 4.08 (1H, m, H-2), 4.10 (1H, d, J=9 Hz, H-18b), 4.26 (1H, br d, J=11 Hz, H-15b), 4.76 (1H, ddd, J=8, 4, 1 Hz, H-16), 5.39 (1H, br s, H-6). ^{13}C-NMR: 19.8 (q, C-19), 20.3, 25.8, 31.9 (t, C-7, 11, 12), 22.6 (q, C-21), 39.3 (s, C-10), 40.5 (t, C-4), 45.2 (d, C-9), 45.5 (t, C-1), 53.7 (s, C-13), 63.6 (d, C-17), 72.4 (t, C-15), 72.6 (d, C-2), 76.0 (d, C-3), 76.7 (t, C-18), 84.3 (d, C-16), 103.9 (s, C-8), 118.2 (s, C-20), 119.7 (d, C-6), 141.3 (s, C-5), 152.9 (s, C-14). From the H_2O layer, two sugars were isolated and identified as cymarose and sarmentose on TLC (solvents 1, 2, 3).

Tylophoside E (**6**): Solid, $[\alpha]_D^{23}$ −44.4° (c=0.72, MeOH), HR-FAB-MS m/z: 995.4843 (Calcd for $C_{48}H_{76}O_{20}$ +Na: 995.4828). On hydrolysis of **6** (3 mg) with cellulase (6 mg, Sigma Type-II) in H_2O–EtOH (3 : 1, 0.5 ml) at 37 °C for 6 h, and by extraction of the mixture with $CHCl_3$, **5** was detected on TLC and HPLC. On acid hydrolysis using the same procedure as in **1**, cymarose, sarmentose, strophanthobiose and **5a** were identified on TLC (solvents 1, 2, 3).

Solvent Systems and Approximate *Rf* Values for Sugars Solvent 1 (7 : 2 : 1): cymarose 0.62, sarmentose 0.56, diginose 0.55, oleandrose 0.52 and digitoxose 0.29. Solvent 1 (7 : 3 : 1): strophanthobiose 0.15. Solvent 2 (9 : 1 : 0.1): oleandrose 0.63, cymarose 0.60, sarmentose 0.54, diginose 0.52 and digitoxose 0.44. Solvent 2 (4 : 1 : 0.5): strophanthobiose 0.25. Solvent 3 (3 : 2): cymarose 0.66, oleandrose 0.62, diginose 0.51, sarmentose 0.48 and digitoxose 0.39.

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