Sweet Pregnane Glycosides from *Telosma procumbens*

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An intensely sweet polyoxypregnane glycoside, telosmoside A_{15} (15), was isolated from an Asian Asclepiadaceae plant, *Telosma procumbens*, collected in Vietnam. This is the first time a sweet pregnane glycoside has been found, and its sweetness intensity is 1000 times greater than that of sucrose. From the same plant, 17 other new glycosides were isolated, having the same aglycone; they are named telosmosides A_1—A_{14} (1—14) and A_{16}—A_{18} (16—18). Some of these glycosides are also sweet, but others are tasteless or bitter. Chemical structures of the 18 glycosides were determined, and the structure–taste relationship was discussed.

Key words *Telosma procumbens*; polyoxypregnane glycoside; sweetener; Asclepiadaceae; telosmoside

Telosma procumbens (Hance) MERR. is found in thickets and secondary forests at low altitudes in the Philippines, Vietnam and China. In the Phillipines, an infusion or a decoction of the leaves is used to cleanse or treat wounds, scabies, and ulcers.[1] The leaves are also applied to the forehead for the treatment of headache. In Vietnamese folk medicine, the whole plant is used as a substitute for licorice, due to its sweet taste. It is also used as an expectorant and antitussive. However, to date, no chemical studies on the plant have been reported. Our interest in the sweetness of this plant resulted in the finding of sweet pregnane glycosides, which are a new class of intense sweetener from natural sources. The present paper describes the isolation and structural elucidation of eighteen new polyoxyprenane glycosides named telosmosides A_1—A_{18} (**1**—**18**).

In order to identify the component sugars of the glycosides, a crude glycoside fraction was hydrolyzed under a mild acidic condition. The component sugars were identified as D-cymarose (Cym), D-oleandrose (Ole), D-digitoxose (Dig), D-thevetose (The), 6-deoxy-3-*O*-methyl-D-allose (Alm) and D-glucose (Glc), by comparing them with corresponding authentic samples on TLC and optical rotations. All sugar linkages of these glycosides were assigned to be in the β-form based on the coupling constant of anomeric protons in ^{1}H-NMR spectra (Table 3).

The molecular formula of telosmoside A_1 (**1**) was determined as $C_{49}H_{82}O_{17}$ by FAB-MS. Anomeric carbon signals observed at δ 95.9, 101.8 and 104.0 in the ^{13}C-NMR spectrum, revealed the presence of three monosaccharide units in **1**. The ^{1}H-NMR spectrum of **1** showed three anomeric proton signals, which were observed as two double doublets (δ 5.19, J=9.6, 1.5 Hz, δ 4.66, J=9.8, 1.5 Hz) and one doublet (δ 4.87, J=8 Hz), along with three 6-methyl proton signals (δ 1.41, 1.52, 1.63) and three methoxy proton signals (δ 3.46, 3.53, 3.83). On mild acid hydrolysis, **1** afforded aglycone **19** and cymarose, oleandrose and thevetose as the component sugars.

The negative FAB-MS spectrum of **19** showed a peak at *m/z* 493. The molecular formula was thus suggested to be $C_{28}H_{46}O_7$. The ^{13}C-NMR of **19** showed 28 carbon signals including 21 signals ascribable to a C_{21}-steroid skeleton and seven signals due to two acyl groups (Table 1). The proton and carbon signals of **19** were assigned by distortionless enhancement by polarization transfer (DEPT), proton–proton chemical shift correlation spectroscopy (^{1}H–^{1}H COSY) and heteronuclear multiple quantum coherence (HMQC) experiments. The ^{1}H-NMR signals at δ 1.65 and 0.80 were correlated with methyl carbon signals at δ 9.5 and 12.2, respectively, indicating the presence of two tertiary methyl groups ascribable to C-18 and C-19 methyls, respectively. The doublet of methyl group at δ 1.41 (J=6.2 Hz) was correlated with the methyl carbon signal at δ 15.3 and assigned to the C-21 methyl. This assignment was further supported by observing the coupling between this signal and the proton quartet signal of H-20 at δ 4.93 in the ^{1}H–^{1}H COSY experiment. The ^{1}H-NMR signals at δ 4.93 (1H, q, J=6.2 Hz) and 4.96 (1H, dd, J=11.5, 4.7 Hz) were deshielded downfield due to the esterifying moieties, and correlated with the methine carbon signals at δ 74.6 and 74.5 ascribed to C-20 and C-12, respectively. The large coupling constant of H-12 indicated the α (axial)-configuration of this proton. The methyl singlet at δ 2.23 was correlated with the methyl carbon signal at δ 22.2 and assigned to the C-2′ methyl of acetyl group. The two methyl signals at δ 0.82 (t, J=7.4 Hz) and 1.21 (d, J=7.1 Hz), in addition to the methyl carbon signals at δ 11.6 and 16.4, were assigned to C-4″ and C-5″ methyls of the 2-methylbutyryl group. In the ^{13}C-NMR spectrum of **19**, chemical shifts of all signals ascribable to a steroid nucleus and acetyl group at C-12 were almost the same as those of isotomentosin (**20**).[2a)] The difference between **19** and isotomentosin is only the acyl group at C-20. Finally, the locations of the acetyl and 2-methylbutyryl groups were assigned to C-12 and C-20 respectively, thanks to the heteronuclear multiple bond connectivity (HMBC) spectrum. Long-range correlations were observed between C-1′ of acetyl group (δ 171.3) and aglycone H-12 (δ 4.96), and between C-1″ of 2-methylbutyryl group (δ 175.8) and aglycone H-20 (δ 4.93). From these evidences, the structure of **19** was determined as 12-*O*-acetyl-20-*O*-2-methylbutyryltomentogenin,[3)] an isomer of tomentonin,[4)] and **19** was named telosmogenin I.

Glycosylation shifts of the aglycone carbon signals of **1** compared with those of **19** were observed at C-2 (−2.3 ppm), C-3 (+6.0 ppm) and C-4 (−4.2 ppm). Therefore, the sugar moiety must link to the C-3 hydroxyl group of **19**. An HMBC spectrum of **1** was measured to confirm the linkages of the sugar chain and two acyl groups. There were correlations between aglycone C-3 (δ 76.5) and Cym H-1 (δ 5.19), between Cym C-4 (δ 83.5) and Ole H-1 (δ 4.66), between

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Table 1. ^{13}C-NMR Spectral Data for the Aglycone Moieties of **1**—**20** (Pyridine-d_5, δ)

C	**19**	**20**[2)]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1	37.1	37.1	36.9	36.9	36.9	36.9	36.9	36.7	36.7	36.7	36.9	36.7	36.7	36.7	36.9	36.7	36.7	36.7	36.7	36.6
2	32.2	32.2	29.9	29.9	29.9	29.9	29.9	29.9	29.9	29.9	29.9	29.9	29.9	30.0	29.9	29.9	29.9	29.9	29.9	29.9
3	70.5	70.4	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.5
4	38.9	39.0	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.7	34.6
5	44.8	44.8	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.3	44.2
6	28.8	28.8	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.6
7	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.9	26.8
8	40.3	40.3	40.2	40.3	40.2	40.3	40.2	40.3	40.3	40.2	40.3	40.3	40.3	40.3	40.2	40.2	40.3	40.2	40.3	40.2
9	45.7	45.7	45.6	45.7	45.6	45.7	45.6	45.7	45.7	45.6	45.7	45.7	45.7	45.7	45.6	45.6	45.7	45.6	45.7	45.6
10	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.7	35.6
11	27.9	27.8	27.8	27.9	27.8	27.8	27.8	27.8	27.8	27.8	27.8	27.9	27.8	27.9	27.8	27.8	27.8	27.8	27.8	27.8
12	74.5	75.0	74.4	74.4	74.4	74.5	74.5	74.5	74.5	74.5	74.5	74.5	74.5	74.5	74.4	74.5	74.5	74.5	74.5	74.5
13	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.6	55.5
14	87.8	87.9	87.7	87.8	87.7	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8	87.8
15	30.8[a)]	30.8[a)]	30.8[a)]	30.9[a)]	30.8[a)]	30.8[a)]	30.8[a)]	30.8[a)]	30.9[a)]	30.8[a)]	30.8[a)]	30.9[a)]	30.8[a)]	30.9[a)]	30.8[a)]	30.8[a)]	30.8[a)]	30.8[a)]	30.9[a)]	30.7[a)]
16	33.8[a)]	34.2[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]	33.8[a)]
17	87.3	87.4	87.2	87.3	87.2	87.3	87.3	87.3	87.3	87.3	87.3	87.3	87.3	87.3	87.2	87.3	87.3	87.3	87.3	87.2
18	9.5	9.6	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
19	12.2	12.1	12.1	12.1	12.0	12.1	12.0	12.1	12.1	12.0	12.0	12.0	12.1	12.1	12.0	12.1	12.0	12.0	12.0	12.0
20	74.6	74.3	74.6	74.6	74.6	74.5	74.6	74.5	74.6	74.6	74.6	74.6	74.6	74.6	74.6	74.6	74.6	74.6	74.6	74.6
21	15.3	15.3	15.2	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.3	15.2
C-1′	171.3	171.1	171.2	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3	171.3
C-2′	22.2	22.1	22.2	22.3	22.2	22.2	22.3	22.2	22.3	22.3	22.2	22.3	22.2	22.3	22.2	22.2	22.3	22.2	22.2	22.2
C-1″	175.8	167.2	175.7	175.8	175.7	175.8	175.8	175.8	175.8	175.8	175.8	175.8	175.7	175.8	175.7	175.8	175.8	175.8	175.8	175.8
C-2″	41.2	129.5	41.1	41.2	41.1	41.1	41.2	41.2	41.2	41.2	41.2	41.2	41.2	41.2	41.1	41.2	41.1	41.2	41.2	41.1
C-3″	27.0	137.5	27.0	27.0	27.0	27.0	27.1	27.0	27.1	27.0	27.0	27.1	27.0	27.1	27.0	27.0	27.0	27.0	27.0	27.0
C-4″	11.6	14.2	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.5
C-5″	16.4	12.1	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.3

a) Signal assignments may be interchangeable in each column.

Ole C-4 (δ 83.0) and The H-1 (δ 4.87), between C-1′ of acetyl group (δ 171.2) and aglycone H-12 (δ 4.87), and between C-1″ of 2-methylbutyryl group (δ 175.7) and aglycone H-20 (δ 4.83). The ^{13}C-NMR spectral data ascribable to the sugar moiety of **1** were almost superimposable on those of stephanosides C and G with the sugar chain (-Cym^4-Ole^4-The) previously isolated from *Stephanotis japonica*.[2a,5)] The structure of **1** was thus determined to be telosmogenin I 3-*O*-β-D-thevetopyranosyl-(1–4)-β-D-oleandropyranosyl-(1–4)-β-D-cymaropyranoside.

The NMR spectral data of telosmosides A_2—A_{18} (**2**—**18**) indicated that they were 3-*O*-glycosides of telosmogenin I (**19**), and each of them differed from the others in the sugar moiety at C-3.

Telosmoside A_2 (**2**) had the molecular formula $C_{55}H_{92}O_{22}$ based on FAB-MS. The enzymatic hydrolysis of **2** with β-glucosidase gave a deglucosyl derivative which was identified as **1** on TLC. The sugar sequence of **2** was determined to be 3-*O*-β-D-glucopyranosyl-(1–4)-β-D-thevetopyranosyl-(1–4)-β-D-oleandropyranosyl-(1–4)-β-D-cymaropyranoside, based on the agreement of the ^{1}H- and ^{13}C-NMR spectral data for the sugar moiety of **2** with the marstomentosides B, D, F, J, N, O and P from *Marsdenia tomentosa*.[2)] Accordingly, the structure of **2** was concluded as shown.

FAB-MS of telosmoside A_3 (**3**) afforded a $[M-H]^-$ peak at *m*/*z* 1266 ($C_{61}H_{101}O_{27}$), 162 mass units more than that of **2**, suggesting the presence of an additional glucose unit in the molecule. The ^{13}C-NMR spectral comparison of **3** with **2** showed a glycosylation shift of +9.6 ppm for the first glucose C-4 (δ 81.6) in **3** compared with that of **2**, which indicated the site of glycosylation. Further, the sugar sequence of **3** was confirmed by an FAB-MS analysis which showed ions at *m*/*z* 1104 $[M-Glc]^-$, 942 $[M-(Glc\text{-}Glc)]^-$, 781 $[M-(Glc\text{-}Glc\text{-}The)]^-$, 637 $[M-(Glc\text{-}Glc\text{-}The\text{-}Ole)]^-$, 493 $[M-(Glc\text{-}Glc\text{-}The\text{-}Ole\text{-}Cym)]^-$. Therefore, the sugar moiety in **3** was assigned as 3-*O*-β-D-glucopyranosyl-(1–4)-β-D-glucopyranosyl-(1–4)-β-D-thevetopyranosyl-(1–4)-β-D-oleandropyranosyl-(1–4)-β-D-cymaropyranoside.

Telosmoside A_4 (**4**) had the molecular formula $C_{49}H_{82}O_{16}$ based on FAB-MS. Compound **4** appeared to be a 3-*O*-trioside, based on three methoxy signals at δ 3.45, 3.54 and 3.62, three 6-methyl signals at δ 1.39, 1.40 and 1.55, and three anomeric protons at δ 4.75, 5.10 and 5.21 in the ^{1}H-NMR spectrum, which indicated the presence of three 2,6-dideoxy-3-*O*-methylhexose units. On mild acid hydrolysis, **4** afforded **19**, and cymarose and oleandrose as the component sugars. The chemical shift of an anomeric proton of oleandrose was observed at a higher field (δ 4.73—4.89) than that of cymarose (δ 5.08—5.27) in pyridine-d_5.[2a,6)] Therefore, two double doublet signals at δ 5.10 and 5.21 were assigned to the anomeric protons of cymarose, and the remaining double doublet signal at δ 4.75 to the anomeric proton of oleandrose. The sugar sequence of **4** was deduced to be 3-*O*-β-D-oleandropyranosyl-(1–4)-β-D-cymaropyranosyl-(1–4)-β-D-cymaropyranoside, based on the long-range correlations in the HMBC spectrum of **4** observed between aglycone C-3 (δ 76.5) and Cym_1 H-1 (δ 5.21), between Cym_1 C-4 (δ 83.4) and Cym_2 H-1 (δ 5.10), and between Cym_2 C-4 (δ 83.1) and Ole H-1 (δ 4.75). This oligosaccharide chain was confirmed by a comparison with that of cynanchoside C_2[7)] isolated from *Cynanchum caudatum* and that of compounds 1, 2 and 3 from *Asclepias incarnata*.[8)]

The molecular formula of telosmoside A_5 (**5**) was suggested as $C_{61}H_{102}O_{26}$ by observation of an FAB-MS ion at

Table 2. ^{13}C-NMR Spectral Data for the Sugar Moieties of **1**—**18** (Pyridine-d_5, δ)

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
	Cym	Cym	Cym	Cym$_1$	Cym$_1$	Dig	Dig	Dig	Dig	Dig	Dig	Dig	Dig$_1$	Dig	Dig	Dig	Dig	Dig
1	95.9	96.0	96.0	96.0	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9
2	37.2	37.3	37.2	37.2	37.2$^{a)}$	38.9	39.1	39.1	39.2	39.1	39.0	39.1	39.0	39.1	39.1	39.1	39.1	39.0
3	77.8	77.9	77.8	77.8	77.7	67.5	67.5	67.5	67.5	67.6	67.5	67.6	67.5$^{a)}$	67.5	67.5	67.5	67.5	67.5
4	83.5	83.5$^{a)}$	83.5$^{a)}$	83.4	83.4$^{b)}$	83.3	83.4$^{a)}$	83.4$^{a)}$	83.7$^{a)}$	83.4$^{a)}$	83.4$^{a)}$	83.4$^{a)}$	83.4$^{b)}$	83.4$^{a)}$	83.4	83.6$^{a)}$	83.4$^{a)}$	83.3$^{a)}$
5	68.8	68.9	68.9	68.9	68.9$^{c)}$	68.5	68.6	68.6	68.5	68.6	68.5	68.6	68.5	68.5	68.5	68.5	68.5	68.5
6	18.8$^{a)}$	18.7$^{b)}$	18.7$^{b)}$	18.6$^{a)}$	18.6$^{d)}$	18.6$^{a)}$	18.7$^{b)}$	18.7$^{b)}$	18.7$^{b)}$	18.7$^{b)}$	18.7$^{b)}$	18.7$^{b)}$	18.5$^{c)}$	18.7$^{b)}$	18.7$^{a)}$	18.7$^{b)}$	18.7$^{b)}$	18.6$^{b)}$
OMe	58.8	58.8	58.8	58.8	58.9													
	Ole	Ole	Ole	Cym$_2$	Cym$_2$	Cym	Cym	Cym	Ole	Cym	Cym	Cym	Dig$_2$	Cym	Cym	Cym	Cym	Cym
1	101.8	101.9	101.8	100.5	100.4	99.7	99.7	99.7	101.4	99.8	99.7	99.7	99.8	99.7	99.7	99.7	99.7	99.7
2	37.5	37.6	37.6	36.9	37.0$^{a)}$	36.9	36.9	36.9	37.4	36.9	36.9	36.9	38.7	36.9	36.9	36.9	36.9	36.9
3	79.2	79.3	79.2	78.0	78.0	77.7	77.7	77.7	79.3	77.7	77.7	77.7	67.4$^{a)}$	77.7	77.7	77.7	77.7	77.6
4	83.0	83.2$^{a)}$	83.4$^{a)}$	83.1	83.1$^{b)}$	83.0	83.1$^{a)}$	83.1$^{a)}$	83.3$^{a)}$	83.2$^{a)}$	83.1$^{a)}$	83.2$^{a)}$	83.2$^{b)}$	83.2$^{a)}$	83.1	83.1$^{a)}$	83.1$^{a)}$	83.2$^{a)}$
5	71.9	71.9$^{c)}$	71.9$^{c)}$	69.1	69.0$^{c)}$	69.1	69.0	69.0	72.0	69.0	69.0	69.0	68.5	69.0	69.0	69.0	69.0	69.0
6	18.6$^{a)}$	18.6$^{b)}$	18.6$^{b)}$	18.6$^{a)}$	18.5$^{d)}$	18.4$^{a)}$	18.5$^{b)}$	18.4$^{b)}$	18.7$^{b)}$	18.5$^{b)}$	18.4$^{b)}$	18.5$^{b)}$	18.5$^{c)}$	18.4$^{b)}$	18.4$^{a)}$	18.6$^{b)}$	18.4$^{b)}$	18.4$^{b)}$
OMe	57.2	57.3	57.3	58.8	58.8	58.8	58.9	58.8	57.3	58.9	58.9	58.9		58.8	58.8	58.8	58.9	58.8
	The	The	The	Ole	Ole	Ole	Ole$_1$	Ole	The	Ole$_1$	Ole$_1$	Ole$_1$	Ole$_1$	Ole$_1$	Ole$_1$	Ole$_1$	Ole$_1$	Ole
1	104.0	104.0	103.9	102.1	101.8	102.1	102.0	101.9	103.9	102.0	101.9	102.0	101.4	101.9	101.9	101.9	101.9	101.8
2	75.1	74.9	74.9$^{d)}$	37.3	37.4	37.2	37.6$^{c)}$	37.4	74.9	37.7$^{c)}$	37.7$^{c)}$	37.6	37.6$^{d)}$	37.7	37.7$^{b)}$	37.5	37.7$^{d)}$	37.4
3	88.0	86.3	86.3	81.4	79.4	81.3	79.0	79.3	86.3	79.0	79.0	79.0	78.9	78.9	78.9	78.9	78.9	79.2
4	75.9	83.1$^{a)}$	83.2$^{a)}$	76.3	83.5$^{b)}$	76.2	82.6$^{a)}$	83.2$^{a)}$	83.1$^{a)}$	82.8$^{a)}$	82.7$^{a)}$	82.7$^{a)}$	82.7$^{b)}$	82.7$^{a)}$	82.7	82.6$^{a)}$	82.6$^{a)}$	83.0$^{a)}$
5	72.7	72.0$^{c)}$	71.5$^{c)}$	72.9	71.5	72.9	71.7	71.9$^{c)}$	72.0	71.8$^{d)}$	71.6	71.6$^{c)}$	71.6	71.7$^{c)}$	71.6$^{c)}$	71.6$^{c)}$	71.6$^{c)}$	71.9$^{c)}$
6	18.5$^{a)}$	18.6$^{b)}$	18.6$^{b)}$	18.5$^{a)}$	18.8$^{d)}$	18.6$^{a)}$	18.7$^{b)}$	18.8$^{b)}$	18.7$^{b)}$	18.7$^{b)}$	18.7$^{b)}$	18.7$^{b)}$	18.7$^{c)}$	18.7$^{b)}$	18.7$^{a)}$	18.4$^{b)}$	18.6$^{b)}$	18.7$^{b)}$
OMe	60.9	60.6	60.6	57.0	57.3	57.0	57.3$^{d)}$	57.2	60.6	57.4$^{e)}$	57.3	57.3	57.3	57.3	57.3	57.4$^{d)}$	57.4$^{e)}$	57.2
		Glc	Glc$_1$		Glc$_1$		Ole$_2$	Glc	Glc	Ole$_2$	Ole$_2$	Ole$_2$	Ole$_2$	Ole$_2$	Ole$_2$	Ole$_2$	Ole$_2$	The
1		104.8	104.5		104.3		100.3	104.5	104.8	100.1	100.0	100.0	100.0	100.1	100.0	100.0	100.0	103.8
2		75.8	74.8$^{d)}$		74.8		37.3$^{c)}$	75.7	75.8	37.4$^{c)}$	37.5$^{c)}$	37.6	37.3$^{d)}$	37.5	37.5$^{b)}$	37.5	37.5$^{d)}$	74.8
3		78.6	76.2		76.3		81.6	78.7	78.6	79.3	79.4	79.6	79.4	79.2	79.5	79.7	79.5	86.2
4		72.0$^{c)}$	81.6		81.7		76.3	72.0$^{c)}$	72.0	82.9$^{a)}$	83.2$^{a)}$	83.4$^{a)}$	83.2$^{b)}$	82.9$^{a)}$	83.1	83.4$^{a)}$	83.1$^{a)}$	83.2$^{a)}$
5		78.1	76.8		76.9		73.0	78.2	78.1	71.6$^{d)}$	72.1	72.1$^{c)}$	72.0	72.1$^{c)}$	71.9$^{c)}$	71.5$^{c)}$	71.9$^{c)}$	71.5$^{c)}$
6		63.1	62.4$^{e)}$		62.5$^{e)}$		18.7$^{b)}$	63.1	63.1	18.7$^{b)}$	18.9$^{b)}$	18.9$^{b)}$	18.9$^{c)}$	18.7$^{b)}$	18.8$^{a)}$	18.8$^{b)}$	18.3$^{b)}$	18.6$^{b)}$
OMe							57.0$^{d)}$			57.4$^{e)}$	57.3	57.3	57.3	57.4	57.4	57.2$^{d)}$	57.3$^{e)}$	60.6
			Glc$_2$		Glc$_2$					Ole$_3$	The	Glc	The	Ole$_3$	The	Glc$_1$	Alm	Glc$_1$
1			104.9		105.0					100.3	104.1	104.5	104.0	100.1	103.9	104.2	101.9	104.4
2			75.3		75.3					37.6$^{c)}$	75.2	75.7	75.2	37.6	74.9	74.8	72.6	74.9
3			78.4		78.5					81.6	88.1	78.7	88.1	79.6	86.3	76.3	83.3$^{a)}$	76.3
4			71.9$^{c)}$		72.0					76.3	76.0	72.0$^{c)}$	76.0	83.4$^{a)}$	83.4	81.6	83.1$^{a)}$	81.6
5			78.2		78.3					73.0	72.8	78.1	72.8	71.6$^{c)}$	72.0$^{c)}$	76.8	69.5	76.7
6			62.3$^{e)}$		62.4$^{e)}$					18.8$^{b)}$	18.5$^{b)}$	63.1	18.6$^{c)}$	18.9$^{b)}$	18.7$^{a)}$	62.4$^{e)}$	18.9$^{b)}$	62.4$^{d)}$
OMe										57.0$^{e)}$	60.9		60.9	57.2	60.6		61.7	
														Glc	Glc	Glc$_2$	Glc	Glc$_2$
1														104.5	104.7	104.9	106.5	104.8
2														75.5	75.8	75.2	75.4	75.2
3														78.6	78.6	78.4	78.3	78.4
4														71.9$^{c)}$	72.0$^{c)}$	72.0$^{c)}$	72.0$^{c)}$	71.9$^{c)}$
5														78.1	78.1	78.2	78.3	78.2
6														63.1	63.1	62.3$^{e)}$	63.0	62.2$^{d)}$

a—*e*) Signal assignments may be interchangeable in each column.

m/*z* 1250, which was 324 mass units more than that of **4**. Acid hydrolysis of **5** afforded cymarose, oleandrose and glucose as the component sugars. A comparison of ^{13}C-NMR spectra of **5** and **4** indicated the presence of an additional set of carbon signals in **5** assignable to one terminal cellobiose, which was in agreement with those of **3**. Finally, the sugar sequence of **5** was confirmed by an HMBC experiment and the structure of **5** was accordingly concluded as shown.

Telosmoside A_6 (**6**) had the molecular formula $C_{48}H_{80}O_{16}$ based on FAB-MS. In the ^{1}H-NMR spectrum of **6**, three anomeric proton signals were observed as double doublets (δ 4.72, J=9.8, 1.5 Hz, δ 5.14, J=9.6, 1.5 Hz, δ 5.44, J=9.4, 1.5 Hz), along with three 6-methyl proton signals (δ 1.33, 1.43, 1.52) and two methoxy proton signals (δ 3.44, 3.53). Therefore, the sugar moiety was suggested to be composed of two units of 2,6-dideoxy-3-*O*-methylhexose and one of 2,6-dideoxy-hexose. On mild acid hydrolysis, **6** afforded **19** and cymarose, oleandrose and digitoxose as the component sugars. The sugar linkages of the sugar moiety at C-3 were determined by means of an HMBC experiment. Long-range correlations were all clearly observed between aglycone C-3 (δ 76.5) and Dig H-1 (δ 5.44), between Dig C-4 (δ 83.3) and Cym H-1 (δ 5.14), and between Cym C-4 (δ 83.0) and Ole H-1 (δ 4.72). Consequently, the structure of **6** was established to be telosmogenin I 3-*O*-β-D-oleandropyranosyl-(1–4)-β-D-cymaropyranosyl-(1–4)-β-D-digitoxopyranoside.

FAB-MS of telosmoside A_7 (**7**) afforded a $[M-H]^-$ peak at *m*/*z* 1056 which was assignable for $C_{55}H_{91}O_{19}$ and 144

Table 3. ^{1}H-NMR Spectral Data of **1**—**18** (Pyridine-d_5, δ)

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Aglycon moiety									
3	3.76 (m)	3.83 (m)	3.80 (m)	3.80 (m)	3.82 (m)	3.82 (m)	3.83 (m)	3.83 (m)	3.83 (m)
12	4.87 (dd, 4.5, 11.6)	4.90 (dd, 4.8, 11.6)	4.90 (dd, 5.1, 10.7)	4.91 (dd, 3.9, 11.9)	4.94$^{b)}$	4.92 (dd, 4.4, 9.9)	4.94$^{b)}$	4.94 (dd, 4.8, 9.8)	4.92$^{b)}$
18	1.58 (s)	1.63 (s)	1.61 (s)	1.62 (s)	1.64 (s)	1.60 (s)	1.63 (s)	1.63 (s)	1.63 (s)
19	0.66 (s)	0.70 (s)	0.69 (s)	0.68 (s)	0.69 (s)	0.66 (s)	0.69 (s)	0.69 (s)	0.69 (s)
20	4.83 (q, 6.0)	4.87 (q, 5.9)	4.89 (q, 5.9)	4.89 (q, 6.2)	4.93 (q, 5.9)	4.87 (q, 6.0)	4.93$^{b)}$	4.92 (q, 6.0)	4.91$^{b)}$
21	1.35 (d, 6.0)	1.40 (d, 5.9)	1.39 (d, 5.9)	1.39 (d, 6.2)	1.39 (d, 5.9)	1.37 (d, 6.0)	1.40 (d, 6.1)	1.40 (d, 6.0)	1.41 (d, 6.1)
Acetyl moiety									
2′	2.18 (s)	2.23 (s)	2.21 (s)	2.22 (s)	2.23 (s)	2.20 (s)	2.23 (s)	2.23 (s)	2.23 (s)
2-Methylbutyryl moiety									
2″	2.37 (m)	2.39 (m)	2.37 (m)	2.39 (m)	2.39 (m)	2.39 (m)	2.42 (m)	2.42 (m)	2.39 (m)
3″	1.39 (m), 1.66 (m)	$^{a)}$	$^{a)}$	1.43 (m), 1.70 (m)	$^{a)}$	1.40 (m), 1.68 (m)	$^{a)}$	$^{a)}$	$^{a)}$
4″	0.79 (t, 7.5)	0.82 (t, 7.3)	0.81 (t, 7.4)	0.80 (t, 7.1)	0.81 (t, 7.3)	0.80 (t, 7.3)	0.81 (t, 7.3)	0.81 (t, 7.3)	0.82 (t, 7.3)
5″	1.16 (d, 7.1)	1.20 (d, 6.8)	1.19 (d, 6.8)	1.19 (d, 7.1)	1.20 (d, 7.1)	1.18 (d, 7.0)	1.20 (d, 7.1)	1.20 (d, 7.1)	1.20 (d, 7.0)
	Cym	Cym	Cym	Cym_1	Cym_1	Dig	Dig	Dig	Dig
1	5.19 (dd, 9.6, 1.5)	5.25 (dd, 9.5, 1.5)	5.23 (dd, 9.1, 1.5)	5.21 (dd, 9.6, 1.5)	5.27 (dd, 9.8, 1.5)	5.44 (dd, 9.4, 1.5)	5.46 (dd, 9.5, 1.2)	5.47 (dd, 9.3, 1.5)	5.47 (dd, 9.8, 1.5)
6	1.41 (d, 6.4)	1.43 (d, 5.8)	1.42 (d, 6.1)	1.40 (d, 6.1)	1.40 (d, 6.1)	1.43 (d, 6.2)	1.44 (d, 6.1)	1.44 (d, 6.1)	1.48 (d, 6.1)
OMe	3.53 (s)	3.58 (s)	3.56 (s)	3.54 (s)	3.55 (s)				
	Ole	Ole	Ole	Cym_2	Cym_2	Cym	Cym	Cym	Ole
1	4.66 (dd, 9.8, 1.5)	4.68 (dd, 9.5, 1.5)	4.67 (dd, 9.8, 1.5)	5.10 (dd, 9.6, 1.3)	5.11 (dd, 9.5, 1.5)	5.14 (dd, 9.6, 1.5)	5.16 (dd, 9.8, 1.5)	5.16 (dd, 9.5, 1.5)	4.72 (dd, 9.8, 1.5)
6	1.63 (d, 5.7)	1.65 (d, 6.1)	1.63 (d, 5.9)	1.39 (d, 6.2)	1.36 (d, 6.3)	1.33 (d, 6.1)	1.32 (d, 6.4)	1.30 (d, 6.1)	1.69 (d, 6.3)
OMe	3.46 (s)	3.49 (s)	3.46 (s)	3.62 (s)	3.63 (s)	3.53 (s)	3.56 (s)	3.56 (s)	3.47 (s)
	The	The	The	Ole	Ole	Ole	Ole_1	Ole	The
1	4.87 (d, 8.0)	4.85 (d, 7.8)	4.82 (d, 7.8)	4.75 (d, 9.6, 1.6)	4.67 (dd, 9.5, 1.5)	4.72 (dd, 9.8, 1.5)	4.68 (d, 9.8, 1.7)	4.66 (dd, 9.5, 1.5)	4.85 (d, 7.8)
6	1.52 (d, 6.2)	1.73 (d, 5.9)	1.69 (d, 5.9)	1.55 (d, 6.2)	1.69 (d, 5.6)	1.52 (d, 6.0)	1.43 (d, 6.1)	1.69 (d, 6.1)	1.73 (d, 6.1)
OMe	3.83 (s)	3.92 (s)	3.85 (s)	3.45 (s)	3.47 (s)	3.44 (s)	3.51 (s)	3.52 (s)	3.92 (s)
		Glc	Glc_1		Glc_1		Ole_2	Glc	Glc
1		5.11 (d, 7.8)	5.02 (d, 7.6)		5.06 (d, 8.0)		4.97$^{b)}$	5.10 (d, 7.8)	5.10 (d, 7.8)
6							1.57 (d, 6.1)		
OMe							3.50 (s)		
			Glc_2		Glc_2				
1			5.12 (d, 7.6)		5.18 (d, 7.8)				

mass units more than that of **6**. This suggested the presence of an additional 2,6-deoxy-3-*O*-methylhexose unit in the molecule of **7**. The NMR spectrum of **7** was very similar to that of **6**, except for a set of signals ascribable to an oleandrose unit attached to C-4 of the first oleandrose unit, due to a glycosylation shift of +6.4 ppm at its C-4 (δ 82.6). The sugar sequence of **7** was confirmed by an HMBC experiment, and its structure was concluded as shown.

FAB-MS of telosmoside A_8 (**8**) afforded a $[M-H]^-$ peak at *m/z* 1074 ($C_{54}H_{89}O_{21}$), 162 mass units more than that of **6**, suggesting the presence of an additional glucose unit in the molecule. Enzymatic hydrolysis of **8** with β-glucosidase produced **6**. Using a strategy similar to the one mentioned above, the structure of **8** was established.

Telosmoside A_9 (**9**) showed the molecular formula $C_{54}H_{90}O_{22}$ based on FAB-MS. In the ^{1}H- and ^{13}C-NMR spectra, four anomeric proton signals and four anomeric carbon signals were observed respectively. Thus **9** was determined to be a 3-*O*-tetraside, whose component sugars were revealed as one digitoxose, one oleandrose, one thevetose and one glucose from analysis of an acid hydrolysate. The sugar sequence of **9** was determined to be 3-*O*-β-D-glucopyranosyl-(1–4)-β-D-thevetopyranosyl-(1–4)-β-D-oleandropyranosyl-(1–4)-β-D-digitoxopyranoside based on an HMBC experiment.

Telosmosides A_{10} (**10**), A_{11} (**11**) and A_{12} (**12**) were suggested to have the molecular formulae $C_{62}H_{104}O_{22}$, $C_{62}H_{104}O_{23}$ and $C_{61}H_{102}O_{24}$, respectively, based on FAB-MS. In the ^{1}H- and ^{13}C-NMR spectra of each compound, five anomeric proton signals and five anomeric carbon signals were observed. These compounds were then suggested to be 3-*O*-pentosides. On acid hydrolysis, **10**, **11** and **12** produced cymarose, oleandrose and digitoxose. Additionally, **11** gave thevetose while **12** afforded glucose as the component sugars. A comparative analysis of the ^{13}C-NMR spectra of these compounds and **7** showed that carbon signals of these compounds were almost superimposable on those of **7**, except for those due to the occurrence of one additional molecule of oleandrose, thevetose and glucose in **10**, **11** and **12**, respectively. The difference between these compounds is only at the terminal sugar unit of the oligosaccharide moiety. This was confirmed by the FAB-MS analysis of these compounds which showed the same ion at *m/z* 1056, corresponding to the loss of one terminal sugar unit in the molecule of each compound. Therefore, the sugar moieties of **10**, **11** and **12** were assigned as Ole-^{4}Ole-^{4}Ole-^{4}Cym-^{4}Dig-(aglycone), The-^{4}Ole-^{4}Ole-^{4}Cym-^{4}Dig-(aglycone) and Glc-^{4}Ole-^{4}Ole-^{4}Cym-^{4}Dig-(aglycone), respectively, and their structures were elucidated as shown in Chart 1.

Telosmoside A_{13} (**13**) showed the molecular formula

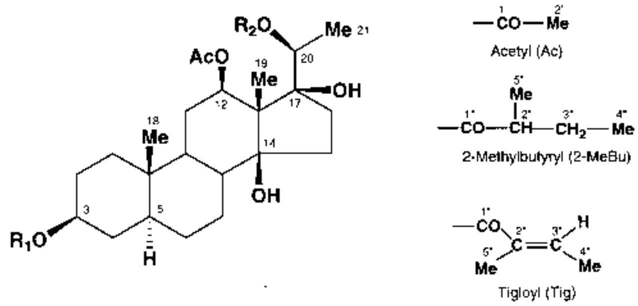
Table 3. (continued)

C	10	11	12	13	14	15	16	17	18
Aglycon moiety									
3	3.82 (m)	3.80 (m)	3.82 (m)	3.79 (m)	3.82 (m)	3.81 (m)	3.83 (m)	3.80 (m)	3.79 (m)
12	4.92[b)]	4.91[b)]	4.92[b)]	4.89 (dd, 4.6, 10.5)	4.92[b)]	4.92 (dd, 3.9, 11.3)	4.91 (dd, 5.1, 10.7)	4.92[b)]	4.91[b)]
18	1.64 (s)	1.60 (s)	1.64 (s)	1.60 (s)	1.62 (s)	1.61 (s)	1.61 (s)	1.62 (s)	1.61 (s)
19	0.69 (s)	0.68 (s)	0.70 (s)	0.67 (s)	0.68 (s)	0.70 (s)	0.68 (s)	0.68 (s)	0.67 (s)
20	4.91[b)]	4.90[b)]	4.90[b)]	4.88[b)]	4.91 (q, 5.8)	4.91 (q, 6.1)	4.90 (q, 5.7)	4.90[b)]	4.89 (q, 5.8)
21	1.37 (d, 6.1)	1.38 (d, 6.1)	1.42 (d, 5.7)	1.38 (d, 6.1)	1.40 (d, 5.8)	1.40 (d, 6.1)	1.39 (d, 5.7)	1.38 (d, 5.8)	1.36 (d, 5.8)
Acetyl moiety									
2′	2.23 (s)	2.20 (s)	2.23 (s)	2.20 (s)	2.21 (s)	2.21 (s)	2.21 (s)	2.21 (s)	2.21 (s)
2-Methylbutyryl moiety									
2″	2.40 (m)	2.39 (m)	2.42 (m)	2.42 (m)	2.41 (m)	2.40 (m)	2.42 (m)	2.39 (m)	2.40 (m)
3″	[a)]	[a)]	[a)]	[a)]	[a)]	1.42 (m), 1.72 (m)	[a)]	[a)]	[a)]
4″	0.81 (t, 7.3)	0.81 (t, 7.4)	0.82 (t, 7.4)	0.80 (t, 7.3)	0.81 (t, 7.3)	0.83 (t, 7.3)	0.81 (t, 7.3)	0.81 (t, 7.6)	0.80 (t, 7.3)
5″	1.21 (d, 7.1)	1.21 (d, 6.8)	1.21 (d, 7.1)	1.18 (d, 7.1)	1.20 (d, 7.1)	1.20 (d, 6.8)	1.19 (d, 6.8)	1.20 (d, 6.9)	1.19 (d, 6.8)
	Dig	Dig	Dig	Dig_1	Dig	Dig	Dig	Dig	Dig
1	5.48 (dd, 9.3, 1.5)	5.42 (dd, 9.3, 1.5)	5.47 (dd, 9.6, 1.5)	5.42 (dd, 9.5, 1.5)	5.45 (dd, 9.2, 1.5)	5.43 (dd, 9.5, 1.6)	5.44 (dd, 9.5, 1.5)	5.44 (dd, 9.5, 1.5)	5.44[b)]
6	1.42 (d, 6.5)	1.43 (d, 6.3)	1.46 (d, 6.1)	1.41 (d, 5.9)	1.44 (d, 6.1)	1.43 (d, 6.1)	1.44 (d, 6.1)	1.44 (d, 6.1)	1.42 (d, 5.8)
	Cym	Cym	Cym	Dig_2	Cym	Cym	Cym	Cym	Cym
1	5.17 (dd, 9.0, 1.5)	5.13 (dd, 9.5, 1.5)	5.17 (dd, 9.5, 1.5)	5.35 (dd, 9.5, 1.5)	5.16 (dd, 9.5, 1.5)	5.14 (dd, 9.5, 1.5)	5.15 (dd, 9.5, 1.5)	5.14 (dd, 9.5, 1.5)	5.13 (dd, 9.5, 1.5)
6	1.33 (d, 6.3)	1.30 (d, 6.1)	1.32 (d, 5.8)	1.34 (d, 5.9)	1.31 (d, 6.1)	1.31 (d, 6.1)	1.31 (d, 6.1)	1.30 (d, 6.1)	1.29 (d, 6.1)
OMe	3.57 (s)	3.54 (s)	3.54 (s)		3.56 (s)	3.56 (s)	3.55 (s)	3.55 (s)	3.53 (s)
	Ole_1	Ole_1	Ole_1	Ole_1	Ole_1	Ole_1	Ole_1	Ole_1	Ole
1	4.68 (dd, 9.8, 1.5)	4.64 (dd, 9.7, 1.5)	4.67 (dd, 9.8, 1.5)	4.68 (dd, 9.5, 1.7)	4.67 (dd, 9.8, 1.5)	4.65 (dd, 9.7, 1.2)	4.66 (dd, 9.5, 1.5)	4.64 (dd, 9.8, 1.8)	4.64 (dd, 9.5, 1.5)
6	1.41 (d, 6.1)	1.39 (d, 6.1)	1.41 (d, 5.7)	1.32 (d, 6.3)	1.42 (d, 5.3)	1.40 (d, 5.8)	1.39 (d, 5.6)	1.38 (d, 6.1)	1.62 (d, 6.1)
OMe	3.46 (s)	3.45 (s)	3.48 (s)	3.44 (s)	3.49 (s)	3.46 (s)	3.46 (s)	3.43 (s)	3.46 (s)
	Ole_2	Ole_2	Ole_2	Ole_2	Ole_2	Ole_2	Ole_2	Ole_2	The
1	4.91 (dd, 9.8, 1.7)	4.85 (dd, 9.8, 1.7)	4.88 (dd, 9.6, 1.5)	4.83 (dd, 9.7, 1.5)	4.87 (dd, 9.8, 1.5)	4.85 (dd, 9.5, 1.3)	4.86 (dd, 9.6, 1.5)	4.83 (dd, 9.8, 1.5)	4.83 (dd, 7.5)
6	1.46 (d, 6.1)	1.68 (d, 6.1)	1.72 (d, 5.6)	1.67 (d, 5.8)	1.42 (d, 5.3)	1.65 (d, 5.8)	1.69 (d, 5.8)	1.61 (d, 6.1)	1.69 (d, 5.9)
OMe	3.50 (s)	3.51 (s)	3.59 (s)	3.51 (s)	3.49 (s)	3.49 (s)	3.48 (s)	3.50 (s)	3.86 (s)
	Ole_3	The	Glc	The	Ole_3	The	Glc_1	Alm	Glc_1
1	4.92[b)]	4.91 (d, 7.5)	5.11 (d, 7.6)	4.90 (d, 7.6)	4.87 (d, 9.8, 1.5)	4.83 (d, 7.9)	5.03 (7.8)	5.23 (d, 8.1)	5.01 (d, 7.8)
6	1.58 (d, 6.1)	1.55 (d, 5.9)		1.56 (d, 6.1)	1.71 (d, 5.8)	1.71 (d, 6.1)		1.59 (d, 7.3)	
OMe	3.53 (s)	3.85 (s)		3.84 (s)	3.52 (s)	3.89 (s)		3.80 (s)	
					Glc	Glc	Glc_2	Glc	Glc_2
1					5.09 (d, 7.8)	5.06 (7.9)	5.13 (d, 7.6)	5.08 (d, 7.5)	5.11 (d, 7.5)

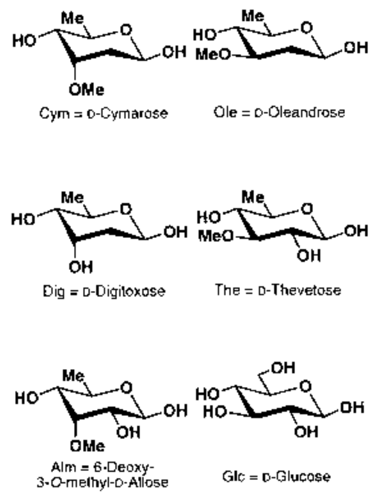
a) Overlapping with other signals. *b*) Overlapping with H_2O signal.

$C_{61}H_{102}O_{23}$ based on FAB-MS and was also determined to be a 3-*O*-pentoside. Comparison of ^{13}C-NMR spectra of **13** and **11** revealed that **13** differed from **11** by the replacement of a cymarose in **11** with a digitoxose in **13**. The sugar sequence of **13** was confirmed by the FAB-MS analysis which showed ions at *m/z* 1042 $[M-The]^-$, 898 $[M-(The\text{-}Ole)]^-$, 753 $[M-(The\text{-}Ole\text{-}Ole)]^-$, 623 $[M-(The\text{-}Ole\text{-}Ole\text{-}Dig)]^-$. Accordingly, the structure of **13** was concluded as shown in Chart 1.

Molecular formulae of telosmosides A_{14} (**14**), A_{15} (**15**) and A_{16} (**16**) were suggested to be $C_{68}H_{114}O_{27}$, $C_{68}H_{114}O_{28}$ and $C_{67}H_{112}O_{29}$, respectively, based on FAB-MS. In the ^{1}H- and ^{13}C-NMR spectra of each compound, six anomeric proton signals and six anomeric carbon signals were observed respectively, suggesting that these compounds were 3-*O*-hexosides. A comparative analysis of the ^{13}C-NMR spectra of these compounds and **10**, **11** and **12**, showed that carbon signals of these compounds were similar to those of **10**, **11** and **12**, except for those ascribable to an additional glucose unit in **14**, **15** and **16**, respectively. On acid hydrolysis of **15**, cymarose, oleandrose, digitoxose, thevetose and glucose were obtained as the component sugars. The ^{13}C-NMR spectral data and the coupling constant of each anomeric proton signal suggested that the sugar moiety included one digitoxopyranose, one cymaropyranose, two oleandropyranose, one thevetopyranose and one glucopyranose. Enzymatic hydrolysis of **15** with β-glucosidase afforded **11**. The sugar sequence of **15** was then determined by an HMBC experiment. Long-range correlations were observed between the aglycone C-3 (δ 76.5) and Dig H-1 (δ 5.43), between Dig C-4 (δ 83.4) and Cym H-1 (δ 5.14), between Cym C-4 (δ 83.1) and Ole_1 H-1 (δ 4.65), between Ole_1 C-4 (δ 82.7) and Ole_2 H-1 (δ 4.85), between Ole_2 C-4 (δ 83.1) and The H-1 (δ 4.83), and between The C-4 (δ 83.4) and Glc H-1 (δ 5.06). Further, the sugar sequence of **15** also was confirmed by a FAB-MS analysis which showed ions at *m/z* 1216 $[M-Glc]^-$, 1056 $[M-(Glc\text{-}The)]^-$, 912 $[M-(Glc\text{-}The\text{-}Ole)]^-$, 767 $[M-(Glc\text{-}The\text{-}Ole\text{-}Ole)]^-$, 623 $[M-(Glc\text{-}The\text{-}Ole\text{-}Ole\text{-}Cym)]^-$, 493 $[M-(Glc\text{-}The\text{-}Ole\text{-}Ole\text{-}Cym\text{-}Dig)]^-$. Based on this evidence, the structure of **15** was determined as shown in Chart 1. From the result of acid hydrolysis and spectral analyses of the ^{1}H-, ^{13}C-NMR, DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC



	R_1	R_2	Taste
1 :	-Cym^4-Ole^4-The	2-MeBu	N
2 :	-Cym^4-Ole^4-The^4-Glc	2-MeBu	B
3 :	-Cym^4-Ole^4-The^4-Glc^4-Glc	2-MeBu	N
4 :	-Cym^4-Cym^4-Ole	2-MeBu	N
5 :	-Cym^4-Cym^4-Ole^4-Glc^4-Glc	2-MeBu	N
6 :	-Dig^4-Cym^4-Ole	2-MeBu	N
7 :	-Dig^4-Cym^4-Ole^4-Ole	2-MeBu	N
8 :	-Dig^4-Cym^4-Ole^4-Glc	2-MeBu	S
9 :	-Dig^4-Ole^4-The^4-Glc	2-MeBu	S
10 :	-Dig^4-Cym^4-Ole^4-Ole^4-Ole	2-MeBu	S
11 :	-Dig^4-Cym^4-Ole^4-Ole^4-The	2-MeBu	S
12 :	-Dig^4-Cym^4-Ole^4-Ole^4-Glc	2-MeBu	S
13 :	-Dig^4-Dig^4-Ole^4-Ole^4-The	2-MeBu	S
14 :	-Dig^4-Cym^4-Ole^4-Ole^4-Ole^4-Glc	2-MeBu	S
15 :	-Dig^4-Cym^4-Ole^4-Ole^4-The^4-Glc	2-MeBu	S
16 :	-Dig^4-Cym^4-Ole^4-Ole^4-Glc^4-Glc	2-MeBu	S
17 :	-Dig^4-Cym^4-Ole^4-Ole^4-Alm^4-Glc	2-MeBu	S
18 :	-Dig^4-Cym^4-Ole^4-The^4-Glc^4-Glc	2-MeBu	S
19 :	-H	2-MeBu	
20 :	-H	Tig (isotomentosin)	



S: sweet, N: tasteless, B: bitter

Chart 1

spectra, the structures of **14** and **16** also were determined, using the same procedure as for **15**.

FAB-MS of telosmoside A_{17} (**17**) afforded a $[M-H]^-$ peak at *m/z* 1378 ($C_{68}H_{113}O_{28}$), which was the same as that of **15**. Acid hydrolysis of **17** afforded cymarose, oleandrose, digitoxose, 6-deoxy-3-*O*-methylallose and glucose as the component sugars. The anomeric proton signal of thevetose was observed in the field to be higher by 5.00 ppm in comparison with that of 6-deoxy-3-*O*-methylallose.[2a,9] ^{1}H-NMR spectral comparison of **17** and **15** showed that **17** differed structurally from **15** in its sugar moiety, with a 6-deoxy-3-*O*-methylallose (δ 5.23, d, J=8.1 Hz) in **17** instead of a thevetose (δ 4.83, d, J=7.9 Hz) in **15**. Hence, the structure of **17** was elucidated as shown in Chart 1.

Telosmoside A_{18} (**18**) had the molecular formula $C_{67}H_{112}O_{30}$ based on FAB-MS. Acid hydrolysis of **18** afforded cymarose, oleandrose, digitoxose, thevetose and glucose as the component sugars. Comparison of ^{1}H- and ^{13}C-NMR spectra of **18** and **16** revealed that **18** differed from **16** only by the replacement of a second oleandrose (Ole_2) in **16** with a thevetose in **18**. The structure of **18** was established as shown in Chart 1.

Of these pregnane glycosides, telosmoside A_2 (**2**) tastes bitter, and some (**1**, **3**—**7**) are tasteless, whereas the others (**8**—**18**) are sweet. Telosmoside A_{15} (**15**), a major compound obtained in a large amount (1.7 g) was evaluated as 1000 times sweeter than sucrose. This is the first finding of intensely sweet pregnane glycosides in nature. Because of the small yield, the taste of other glycosides was not evaluated quantitatively. However, our preliminary sensory test of these compounds suggested that the taste correlated with the aglycone (to be reported elsewhere) and sugar moieties. Regarding the sugar moieties, the number of sugar units and the linkage of them seemed to play an important role in determining the intensity of sweetness. As far as our isolated compounds are concerned, more than four sugar units and a digitoxose unit attached directly to the aglycone seem to be necessary for sweet taste. Further study on the structure–taste relationship of pregnane glycosides is in progress.

Experimental

General Procedure Optical rotations were recorded on a Union PM-101 automatic digital polarimeter. NMR spectra were recorded on JEOL JNM A400 and JNM-ECP 500 spectrometers in pyridine-d_5 using tetramethylsilane (TMS) as an internal standard. MS were obtained on a JEOL JMS-SX102 spectrometer by the direct inlet method. HPLC was carried out using D-ODS-5 and Polyamine II (20 mm i.d.×25 cm, YMC) columns with a TOSOH HLC 803D pump and a TOSOH RI-8000 differential refractometer as detector. For column chromatography, Kieselgel 60 (70—230 mesh, Merck), LiChroprep RP-18 (Merck) and Diaion HP-20 (Mitsubishi) were used. For TLC, Silica gel 60 precoated plate, F-254 (Merck) were used. HPTLC was carried out using RP-18 precoated plate F-254 s and NH_2 F-254 s (Merck). Spots on TLC were visualized by spraying 10% H_2SO_4 followed by heating.

Extraction and Isolation of Compounds 1—18 The dried stem (1.5 kg) of *T. procumbens* collected in Phu Yen Province, Vietnam, in March 1998, was extracted with MeOH under reflux, and the MeOH extract was evaporated to dryness. The residue (164 g) was suspended in H_2O, passed through a Diaion HP-20 column using water, 25% MeOH, 50% MeOH, 80% MeOH and MeOH, successively, as eluting solvents. The MeOH eluate (30 g) was chromatographed over silica gel column with CH_2Cl_2–MeOH (98 : 2 to 85 : 15) system to give 15 fractions, then subjected to repeated Lichroprep RP-18 CC (40—80% CH_3CN) and preparative HPLC (ODS, 45—85% CH_3CN and Polyamine II, 86—94% CH_3CN) to afford telosmosides A_1 (**1**, 314 mg), A_2 (**2**, 387 mg), A_3 (**3**, 69 mg), A_4 (**4**, 39 mg), A_5 (**5**, 8 mg), A_6 (**6**, 107 mg), A_7 (**7**, 75 mg), A_8 (**8**, 13 mg), A_9 (**9**, 10 mg), A_{10} (**10**, 8 mg), A_{11} (**11**, 180 mg), A_{12} (**12**, 116 mg), A_{13} (**13**, 65 mg), A_{14} (**14**, 47 mg), A_{15} (**15**, 1709 mg), A_{16} (**16**, 362 mg), A_{17} (**17**, 202 mg), and A_{18} (**18**, 36 mg).

Telosmoside A_1 (**1**): An amorphous white powder, $[\alpha]_D^{30}$ $-9.6°$ (c=2.80, MeOH). Negative HR-FAB-MS m/z: 941.5432 (Calcd for $C_{49}H_{81}O_{17}$: 941.5474). Negative FAB-MS m/z: 942 $[M-H]^-$, 781 $[M-The]^-$, 493 $[M-(The\text{-}Ole\text{-}Cym)]^-$.

Telosmoside A_2 (**2**): An amorphous white powder, $[\alpha]_D^{31}$ $-2.2°$ (c=1.79, MeOH). Negative HR-FAB-MS m/z: 1103.5986 (Calcd for $C_{55}H_{91}O_{22}$: 1103.6002). Negative FAB-MS m/z: 1104 $[M-H]^-$, 942 $[M-Glc]^-$.

Telosmoside A_3 (**3**): An amorphous white powder, $[\alpha]_D^{31}$ $-0.7°$ (c=1.49, MeOH). Negative HR-FAB-MS m/z: 1265.6538 (Calcd for $C_{61}H_{101}O_{27}$: 1265.6530). Negative FAB-MS m/z: 1266 $[M-H]^-$, 1104 $[M-Glc]^-$, 942 $[M-(Glc\text{-}Glc)]^-$, 781 $[M-(Glc\text{-}Glc\text{-}The)]^-$, 637 $[M-(Glc\text{-}Glc\text{-}The\text{-}Ole)]^-$, 493 $[M-(Glc\text{-}Glc\text{-}The\text{-}Ole\text{-}Cym)]^-$.

Telosmoside A_4 (**4**): An amorphous white powder, $[\alpha]_D^{30}$ $+1.2°$ (c=0.87, MeOH). Negative HR-FAB-MS m/z: 925.5546 (Calcd for $C_{49}H_{81}O_{16}$: 925.5525). Negative FAB-MS m/z: 926 $[M-H]^-$, 781 $[M-Ole]^-$, 493 $[M-(Ole\text{-}Cym\text{-}Cym)]^-$.

Telosmoside A_5 (**5**): An amorphous white powder, $[\alpha]_D^{21}$ $+2.0°$ (c=0.51, MeOH). Negative HR-FAB-MS m/z: 1249.6537 (Calcd for $C_{61}H_{101}O_{26}$: 1249.6581). Negative FAB-MS m/z: 1250 $[M-H]^-$, 1088 $[M-Glc]^-$.

Telosmoside A_6 (**6**): An amorphous white powder, $[\alpha]_D^{30}$ $-6.8°$ (c=1.76, MeOH). Negative HR-FAB-MS m/z: 911.5330 (Calcd for $C_{48}H_{79}O_{16}$: 911.5368). Negative FAB-MS m/z: 912 $[M-H]^-$, 767 $[M-Ole]^-$, 623 $[M-(Ole\text{-}Cym)]^-$, 493 $[M-(Ole\text{-}Cym\text{-}Dig)]^-$.

Telosmoside A_7 (**7**): An amorphous white powder, $[\alpha]_D^{30}$ $-8.3°$ (c=1.92, MeOH). Negative HR-FAB-MS m/z: 1055.6105 (Calcd for $C_{55}H_{91}O_{19}$: 1055.6155). Negative FAB-MS m/z: 1056 $[M-H]^-$, 912 $[M-Ole]^-$, 493 $[M-(Ole\text{-}Ole\text{-}Cym\text{-}Dig)]^-$.

Telosmoside A_8 (**8**): An amorphous white powder, $[\alpha]_D^{30}$ $-2.3°$ (c=0.89, MeOH). Negative HR-FAB-MS m/z: 1073.5950 (Calcd for $C_{54}H_{89}O_{21}$: 1073.5897). Negative FAB-MS m/z: 1074 $[M-H]^-$, 912 $[M-Glc]^-$, 623 $[M-(Glc\text{-}Ole\text{-}Cym)]^-$.

Telosmoside A_9 (**9**): An amorphous white powder, $[\alpha]_D^{30}$ $-6.0°$ (c=0.67, MeOH). Negative HR-FAB-MS m/z: 1089.5826 (Calcd for $C_{54}H_{89}O_{22}$: 1089.5845). Negative FAB-MS m/z: 1090 $[M-H]^-$, 928 $[M-Glc]^-$, 623 $[M-(Glc\text{-}The\text{-}Ole)]^-$.

Telosmoside A_{10} (**10**): An amorphous white powder, $[\alpha]_D^{21}$ $+7.6°$ (c=0.53, MeOH). Negative HR-FAB-MS m/z: 1199.6992 (Calcd for $C_{62}H_{103}O_{22}$: 1199.6941). Negative FAB-MS m/z: 1200 $[M-H]^-$, 1056 $[M-Ole]^-$.

Telosmoside A_{11} (**11**): An amorphous white powder, $[\alpha]_D^{31}$ $-7.0°$ (c=1.85, MeOH). Negative HR-FAB-MS m/z: 1215.6879 (Calcd for $C_{62}H_{103}O_{23}$: 1215.6890). Negative FAB-MS m/z: 1216 $[M-H]^-$, 1056 $[M-The]^-$, 912 $[M-(The\text{-}Ole)]^-$.

Telosmoside A_{12} (**12**): An amorphous white powder, $[\alpha]_D^{30}$ $-5.0°$ (c=2.38, MeOH). Negative HR-FAB-MS m/z: 1217.6680 (Calcd for $C_{61}H_{101}O_{24}$: 1217.6683). Negative FAB-MS m/z: 1218 $[M-H]^-$, 1056 $[M-Glc]^-$, 912 $[M-(Glc\text{-}Ole)]^-$, 767 $[M-(Glc\text{-}Ole\text{-}Ole)]^-$.

Telosmoside A_{13} (**13**): An amorphous white powder, $[\alpha]_D^{31}$ $-16.3°$ (c=1.41, MeOH). Negative HR-FAB-MS m/z: 1201.6740 (Calcd for $C_{61}H_{101}O_{23}$: 1201.6734). Negative FAB-MS m/z: 1202 $[M-H]^-$, 1042 $[M-The]^-$, 898 $[M-(The\text{-}Ole)]^-$, 753 $[M-(The\text{-}Ole\text{-}Ole)]^-$, 623 $[M-(The\text{-}Ole\text{-}Ole\text{-}Dig)]^-$.

Telosmoside A_{14} (**14**): An amorphous white powder, $[\alpha]_D^{21}$ $-7.5°$ (c=3.05, MeOH). Negative HR-FAB-MS m/z: 1361.7479 (Calcd for $C_{68}H_{113}O_{27}$: 1361.7469). Negative FAB-MS m/z: 1362 $[M-H]^-$, 1200 $[M-Glc]^-$, 1056 $[M-(Glc\text{-}Ole)]^-$, 912 $[M-(Glc\text{-}Ole\text{-}Ole)]^-$, 623 $[M-(Glc\text{-}Ole\text{-}Ole\text{-}Ole\text{-}Cym)]^-$.

Telosmoside A_{15} (**15**): An amorphous white powder, $[\alpha]_D^{31}$ $+3.7°$ (c=1.35, MeOH). Negative HR-FAB-MS m/z: 1377.7405 (Calcd for $C_{68}H_{113}O_{28}$: 1377.7419). Negative FAB-MS m/z: 1378 $[M-H]^-$, 1216 $[M-Glc]^-$, 1056 $[M-(Glc\text{-}The)]^-$, 912 $[M-(Glc\text{-}The\text{-}Ole)]^-$, 767 $[M-(Glc\text{-}The\text{-}Ole\text{-}Ole)]^-$, 623 $[M-(Glc\text{-}The\text{-}Ole\text{-}Ole\text{-}Cym)]^-$, 493 $[M-(Glc\text{-}The\text{-}Ole\text{-}Ole\text{-}Cym\text{-}Dig)]^-$.

Telosmoside A_{16} (**16**): An amorphous white powder, $[\alpha]_D^{30}$ $+6.0°$ (c=2.51, MeOH). Negative HR-FAB-MS m/z: 1379.7207 (Calcd for $C_{67}H_{111}O_{29}$: 1379.7211). Negative FAB-MS m/z: 1380 $[M-H]^-$, 1218 $[M-Glc]^-$, 1056 $[M-(Glc\text{-}Glc)]^-$, 912 $[M-(Glc\text{-}Glc\text{-}Ole)]^-$, 767 $[M-(Glc\text{-}Glc\text{-}Ole\text{-}Ole)]^-$, 623 $[M-(Glc\text{-}Glc\text{-}Ole\text{-}Ole\text{-}Cym)]^-$.

Telosmoside A_{17} (**17**): An amorphous white powder, $[\alpha]_D^{30}$ $+2.0°$ (c=1.51, MeOH). Negative HR-FAB-MS m/z: 1377.7415 (Calcd for $C_{68}H_{113}O_{28}$: 1377.7419). Negative FAB-MS m/z: 1378 $[M-H]^-$, 1216 $[M-Glc]^-$, 1056 $[M-(Glc\text{-}Alm)]^-$, 912 $[M-(Glc\text{-}Alm\text{-}Ole)]^-$, 623 $[M-(Glc\text{-}Alm\text{-}Ole\text{-}Ole\text{-}Cym)]^-$, 493 $[M-(Glc\text{-}Alm\text{-}Ole\text{-}Ole\text{-}Cym\text{-}Dig)]^-$.

Telosmoside A_{18} (**18**): An amorphous white powder, $[\alpha]_D^{31}$ $+4.7°$ (c=1.93, MeOH). Negative HR-FAB-MS m/z: 1395.7123 (Calcd for $C_{67}H_{111}O_{30}$: 1395.7160). Negative FAB-MS m/z: 1396 $[M-H]^-$, 1234 $[M-Glc]^-$, 1272 $[M-(Glc\text{-}Glc)]^-$.

Acid Hydrolysis of Crude Glycosides The crude fraction containing pregnane glycosides (2.5 g), was heated at 95 °C with 80 ml of 0.05 N HCl–50% aq.dioxane for 2 h, and the mixture was then evaporated *in vacuo*. The residue was partitioned with CH_2Cl_2/H_2O and the H_2O layer was neutralized with Amberlite MB-3. The H_2O layer was then concentrated and passed through a silica gel column, using CH_2Cl_2–MeOH–H_2O (98 : 2 : 0 to 7 : 1 : 1.2, lower layer) as eluting solvents to afford six sugars, cymarose, oleandrose, digitoxose, thevetose, 6-deoxy-3-*O*-methylallose and glucose. Each sugar was identified by comparison with the authentic samples on TLC and optical rotation. Optical rotation was determined after dissolving the sugars in H_2O and allowing them to stand for 24 h; cymarose: $[\alpha]_D^{30}$ $+40.5°$ (c=1.16) (lit. $+54.9°$),[10] oleandrose: $[\alpha]_D^{30}$ $-10.2°$ (c=2.92) (lit. $-12.0°$),[10] digitoxose: $[\alpha]_D^{30}$ $+50.8°$ (c=0.63) (lit. $+50.2°$),[10] thevetose: $[\alpha]_D^{30}$ $+31.1°$ (c=1.03) (lit. $+35.5°$),[10] 6-deoxy-3-*O*-methylallose: $[\alpha]_D^{30}$ $+13.3°$ (c=0.15) (lit. $+10°$),[2a] glucose: $[\alpha]_D^{30}$ $+45.1°$ (c=0.71) (lit. $+52.0°$).[10]

Acid Hydrolysis of 1 Compound **1** (50 mg) was treated in the same way as described above. The CH_2Cl_2 extract (40 mg) was separated by HPLC (ODS-5, 50% CH_3CN) to afford **19** (telosmogenin I) (20 mg), $[\alpha]_D^{28}$ $-21.5°$ (c=1.58, $CHCl_3$). Negative HR-FAB-MS m/z: 493.3160 (Calcd for $C_{28}H_{45}O_7$: 493.3165). ^{1}H-NMR (C_5D_5N) δ: 0.80 (3H, s, CH_3-19), 0.82 (3H, t, J=7.4 Hz, CH_3-4″), 1.21 (3H, d, J=7.1 Hz, CH_3-5″), 1.41 (3H, d, J=6.2 Hz, CH_3-21), 1.42 (1H, m, H-3″a), 1.65 (3H, s, CH_3-18), 1.71 (1H, m, H-3″b), 2.23 (3H, s, CH_3-2′), 2.40 (1H, m, H-2″), 3.83 (1H, m, H-3), 4.93 (1H, d, J=6.2 Hz, H-20), 4.96 (1H, dd, J=11.5, 4.7 Hz, H-12). ^{13}C-NMR: Table 1. Cymarose, oleandrose and thevetose were identified in the H_2O layer by comparison with authentic samples on TLC with solvent 1 (CH_2Cl_2 : MeOH=15 : 1).

Acid Hydrolysis of 2—18 Each compound (*ca.* 2 mg) in 0.05 N HCl–50% aq. dioxane (4 drops) was heated at 95 °C for 2 h. After hydrolysis, the reaction mixture was passed through Amberlite MB-3 and the eluate was evaporated *in vacuo* to dryness. A portion of the residue was analyzed by HPLC to identify the aglycone (**19**) [condition: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min, 50% CH_3CN in H_2O; t_R (min), telosmogenin I (**19**) 7.0] Subsequently, sugar components in the remaining residue were identified by comparison with authentic samples on TLC using solvents 1 and 2 (EtOAc : MeOH=9 : 1).

Enzymatic Hydrolysis of 2, 8, 12, 14 and 15 with β-Glucosidase A suspension (0.5 ml) of each compound (*ca.* 2 mg) in 0.3 M NaOAc buffer solution adjusted to pH 5.5 was added to a solution (0.5 ml) of β-glucosidase (6 mg) and kept at 37 °C for 3—4 d. The mixture was extracted with EtOAc and the solvent was evaporated to dryness. The residue was identified by comparison with authentic samples on TLC. Compounds **2**, **8**, **12**, **14** and **15** produced **1**, **6**, **7**, **10** and **11**, respectively.

Sensory Evaluation[11] of Telosmoside A_{15} (15) The taste panel consisted of ten experienced tasters from Maruzen Pharmaceuticals Co., Ltd. The tasters determined the intensity of sweetness of telosmoside A_{15} (**15**) in 7% ethanol–water solution. The relative sweetness of compound **15** compared to a 3.2—9.6% solution (w/v) of sucrose was determined by tasting its solutions at different concentrations and selecting the concentration at which the taste was approximately closest to that of the sucrose solution.

Analysis of the results indicated that panel members recognized that compound **15** at a concentration of 0.008% was equivalent in sweetness intensity to sucrose at 8% (w/v). Therefore, the relative sweetness of compound **15** was determined to be 1000 times greater than that of sucrose, respectively.

Acknowledgments We would like to thank Dr. Kenji Mizutani of Maruzen Pharmaceuticals Co., Ltd. for the taste evaluation, Dr. Yoshikazu Hiraga of Department of Chemistry, Graduate School of Science Hiroshima University for two dimentional NMR measurements, and Dr. Fumiko Abe of the Faculty of Pharmaceutical Sciences, Fukuoka University, for providing

samples of authentic sugars.

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