Steroidal Glycosides from the Aerial Part of *Asclepias incarnata* L. II.

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Thirty new steroidal glycosides were obtained from the aerial part of *Asclepias incarnata* L. (Asclepiadaceae). These glycosides were confirmed to have lineolol, isolinolol, 12-*O*-acetyllineolol, 12-*O*-(*Z*)-cinnamoyllinolol, metaplexigenin, 15 β -hydroxylinolol, 15 β -hydroxyisolinolol, 16 α -hydroxyisolinolol, 12-*O*-tigloyl-16 α -hydroxyisolinolol as the aglycone and 2,6-dideoxyhexopyranose as the sugar sequence by spectroscopic methods and chemical evidence.

Key words *Asclepias incarnata*; Asclepiadaceae; pregnane glycoside; 2,6-dideoxyhexopyranose

In connection with a study on steroidal glycosides in the Asclepiadaceae family, we previously reported on oxypregnane glycosides which were obtained from the aerial part of *Asclepias incarnata* L.¹⁾ In this paper, the structural elucidation of 30 new pregnane glycosides is described as a part of our ongoing research.

Extraction and isolation of the aerial part of *A. incarnata* L. were described in our previous report,¹⁾ and a pregnane glycoside fraction afforded 30 new compounds **1**–**30**.

The procedure to acquire the component aglycones and monosaccharides of oxypregnane glycosides was also described earlier,¹⁾ and the afforded aglycones were identified as lineolol,^{1–4)} isolinolol,^{1,3)} 12-*O*-acetyllineolol,¹⁾ 15 β -hydroxylinolol,¹⁾ 15 β -hydroxyisolinolol¹⁾ and metaplexigenin.^{1,5)} The component sugars were identified to be cymarose, oleandrose, digitoxose, canarose, thevetose and glucose. As the absolute configuration of each monosaccharide, cymarose, oleandrose, digitoxose and canarose were believed to have D-form by optical rotation values. Thevetose and glucose were also thought to have D-form based on GC analysis following the reaction with D-cysteine methyl ester hydrochloride (see Experimental).^{1,6,7)}

Compound **1** was suggested to have the molecular formula, C₄₇H₇₆O₁₉, based on FAB-MS [positive FAB-MS ion at *m/z* 945 [M+H]⁺]. In the ¹H- and ¹³C-NMR spectra of **1**, four anomeric proton and carbon signals were observed at δ 5.44 (1H, dd, *J*=9.5, 2.0 Hz), 5.14 (1H, dd, *J*=9.5, 2.0 Hz), 4.74 (1H, dd, *J*=9.5, 2.0 Hz), 4.79 (1H, d, *J*=8.0 Hz) and δ 96.4, 99.7, 102.0, 105.1 along with the signals due to the aglycone which was identified as 15 β -hydroxylinolol on acid hydrolysis with 0.05 N HCl. In the ¹³C-NMR spectrum, glycosylation shifts⁸⁾ of C-2 (−2.2 ppm), C-3 (+6.1 ppm) and C-4 (−4.1 ppm) relative to 15 β -hydroxylinolol (**35**) revealed that **1** was glycosylated at the C-3 position. Therefore, **1** was considered to be 15 β -hydroxylinolol 3-*O*-tetraoside. Acid hydrolysis indicated that the sugar moiety consisted of digitoxose, cymarose, canarose and thevetose. The ¹H-NMR spectrum showed two characteristic H-3 signals of digitoxopyranose and cymaropyranose at δ 4.01 (1H, q, *J*=3.0 Hz) and 4.60 (1H, q, *J*=3.0 Hz), and the H-3 signal at δ 4.01 had a correlation with the carbon signal at δ 77.8 in the ¹H-detected heteronuclear multiple quantum coherence (HMQC) spectrum. In addition, this carbon signal revealed a long-range correlation to the methoxyl proton signal at δ 3.58 (3H, s) in the ¹H-detected heteronuclear multiple-bond connectivity (HMBC) spectrum. Accordingly, the signal at δ

4.01 was assigned to H-3 of cymaropyranose and the remaining signal at δ 4.60 belonged to H-3 of digitoxopyranose. The assignments of the proton signals of the sugar moiety were carried out based on the ¹H–¹H correlation spectroscopy (COSY) spectrum starting from anomeric proton signals, and judged from the coupling constant of each anomeric proton signal, each monosaccharide had β -linkage (Table 4). The sugar sequence of **1** was suggested by the nuclear Overhauser effect (NOE) difference spectra and the HMBC spectrum. Irradiation at δ 5.44 (H-1' of β -D-digitoxopyranose) caused enhancement at δ 3.84 (H-3 of the aglycone). Similarly, NOEs were observed as follows by irradiation of the anomeric proton signals, δ 5.14 (H-1'' of β -D-cymaropyranose) and 3.48 (H-4' of β -D-digitoxopyranose); δ 4.74 (H-1''' of β -D-canaropyranose) and 3.38 (H-4'' of β -D-cymaropyranose); δ 4.79 (H-1'''' of β -D-thevetopyranose) and 3.39 (H-4''' of β -D-canaropyranose). The HMBC spectrum displayed ³*J*_{COCH} between δ 96.4 (C-1' of β -D-digitoxopyranose) and δ 3.84 (H-3 of the aglycone); δ 99.7 (C-1'' of β -D-cymaropyranose) and δ 3.48 (H-4' of β -D-digitoxopyranose); δ 102.0 (C-1''' of β -D-canaropyranose) and 3.38 (H-4'' of β -D-cymaropyranose); δ 105.1 (C-1'''' of β -D-thevetopyranose) and δ 3.39 (H-4''' of β -D-canaropyranose). Consequently, **1** was proved to be 15 β -hydroxylinolol 3-*O*- β -D-thevetopyranosyl-(1→4)- β -D-canaropyranosyl-(1→4)- β -D-cymaropyranosyl-(1→4)- β -D-digitoxopyranoside.

Compound **2** had the molecular formula, C₅₀H₈₀O₂₀, on FAB-MS [positive FAB-MS ion at *m/z* 1023 [M+Na]⁺ and 1001 [M+H]⁺]. The aglycone moiety was identified as metaplexigenin by acid hydrolysis and the ¹³C-NMR spectral data. As the ¹H- and ¹³C-NMR spectral data of the sugar moiety included four anomeric proton and carbon signals at δ 5.45 (1H, dd, *J*=9.5, 2.0 Hz), 5.16 (1H, dd, *J*=9.5, 2.0 Hz), 4.68 (1H, dd, *J*=9.5, 2.0 Hz), 4.95 (1H, dd, *J*=8.0 Hz) and δ 96.4, 99.7, 101.9, 104.1, **2** was thought to be metaplexigenin 3-*O*-tetraoside in view of the glycosylation shifts around C-3 of the aglycone. Acid hydrolysis of **2** afforded digitoxose, cymarose, oleandrose and thevetose, and the above anomeric proton and carbon signals were assigned to each monosaccharide as presented in Tables 3 and 4 on the basis of the 2D-NMR experiments (¹H–¹H COSY, HMQC and HMBC). In the NOE difference experiments irradiating at each anomeric proton signal, NOEs were observed between δ 5.45 (H-1' of β -D-digitoxopyranose) and 3.85 (H-3 of the aglycone); δ 5.16 (H-1'' of β -D-cymaropyranose) and 3.50 (H-4' of β -D-digitoxopyranose); δ 4.68 (H-1''' of β -D-oleandropyranose)

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and 3.39 (H-4'' of β -D-cymaropyranose); δ 4.95 (H-1''' of β -D-thevetopyranose) and 3.67 (H-4''' of β -D-oleandropyranose). Thus, the sugar sequence of **2** was determined to be 3-*O*- β -D-theveopyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranoside, which was confirmed by observation of $^3J_{\text{COCHS}}$ between the C-1 and H-4 signals of the monosaccharides in the HMBC experiment.

Compounds **3**–**6** were suggested to have molecular formulae, $\text{C}_{48}\text{H}_{78}\text{O}_{18}$, $\text{C}_{48}\text{H}_{78}\text{O}_{18}$, $\text{C}_{48}\text{H}_{78}\text{O}_{19}$ and $\text{C}_{48}\text{H}_{78}\text{O}_{19}$, respectively, based on FAB-MS spectra. Compounds **3**–**6** possessed the same tetrasaccharide sugar chains attached to the C-3 position of the aglycone as that of **2**, according to the consistence of the NMR spectral data of each sugar moiety with that of **2**. The aglycone moieties of **3**–**6** were identified to be lineolon on **3**, isolineolon on **4**, 15 β -hydroxylineolon on **5** and 15 β -hydroxyisolineolon on **6** by acid hydrolysis. Hence, these arguments led us to conclude that the structures of **3**–**6** were as shown in Chart 1.

The molecular formula of **7** was $\text{C}_{56}\text{H}_{90}\text{O}_{25}$ by observation of positive FAB-MS ion at m/z 1185 $[\text{M}+\text{Na}]^+$ which was larger by 162 mass units than that of **2**. Since **7** was hydrolyzed to metaplexigenin, digitoxose, cymarose, olean-drose, thevetose and glucose after acid hydrolysis with 0.05 N HCl, the difference of mass units originated in the additional glucose unit. Thus, **7** was thought to be metaplexigenin 3-*O*-pentaoside. The ^1H - and ^{13}C -NMR spectra were similar to those of **2**, but one more anomeric proton and carbon signal

Table 1. ^{13}C -NMR Spectral Data of the Aglycone and Ester Moieties

	1	2	3	4	6	12 ⁺	35
Carbon No.							
Aglycone moiety							
C- 1	39.0	39.0	39.0	39.1	39.0	38.8	39.3
- 2	30.0	29.9	30.0	30.0	30.0	28.8	32.2
- 3	77.8	77.7	77.7	77.7	77.7	78.3	71.7
- 4	39.4	39.3	39.4	39.4	39.4	38.8	43.5
- 5	138.9	139.4	139.6	139.3	138.4	141.2	139.8
- 6	119.8	119.1	119.4	119.7	120.2	117.4	119.1
- 7	35.4	34.7	34.5	37.3	35.0	34.5	35.4
- 8	74.7	74.3	74.6	74.5	74.9	74.8	74.8
- 9	45.3	44.6	45.2	45.6	45.9	44.1	45.3
-10	37.6	37.6	37.5	37.6	37.6	37.3	37.6
-11	29.2	24.8	29.4	28.5	28.5	24.2	29.3
-12	68.6	73.6	69.0	74.0	73.5	72.1	68.7
-13	58.2	57.9	58.0	56.7	56.3	55.0	58.2
-14	86.3	89.4	87.5	86.7	84.7	86.6	86.4
-15	71.7	33.7	35.4	36.2	74.9	33.2	71.7
-16	34.5	32.9	22.2	24.7	36.0	21.2	34.5
-17	59.5	92.4	61.5	58.7	56.3	59.8	59.4
-18	15.2	10.4	14.8	11.6	12.5	14.8	15.2
-19	18.6	18.2	18.4	18.5	18.7	18.8	18.8
-20	209.7	210.1	210.5	216.8	215.3	209.6	209.8
-21	32.1	27.6	32.1	32.3	31.9	32.2	32.1
Ester moiety							
C- 1'	—	169.8	—	—	—	169.8	—
- 2'	—	20.8	—	—	—	20.7	—

Measured in pyridine- d_5 solution at 35 °C. +: Measured in CDCl_3 solution at 35 °C.

Table 2. ^{13}C - and ^1H -NMR Spectral Data of the Aglycone and Ester Moieties of Compounds **15**, **26** and **27**

	15	15 ⁺	26	27
Carbon and Proton No.				
Aglycone moiety				
C,H- 1	39.1	38.7	39.0	39.0
- 2	29.9	29.0	29.9	29.9
- 3	78.0 3.88 (m)	78.1 3.58 (m)	77.7 3.85 (m)	77.6 3.84 (m)
- 4	39.3	39.0	39.3	39.3
- 5	139.3	139.4	139.5	139.5
- 6	119.7 5.43 (br s)	118.6 5.36 (br s)	119.1 5.26 (br s)	119.2 5.28 (br s)
- 7	36.1	35.4	34.2	35.8
- 8	74.5	73.8	74.5	74.3
- 9	45.9	44.7	44.9	45.9
-10	37.7	37.2	37.5	37.7
-11	28.7	27.7	24.7	24.8
-12	75.3 4.42 (dd, 12.0, 4.0)	74.0 ^{a)} 3.80 (m)	73.4 5.12 (dd, 11.5, 4.0)	79.5 5.60 (dd, 12.0, 4.0)
-13	56.1	54.9	55.6	54.3
-14	86.4	86.4	87.5	86.1
-15	47.0	47.4	35.1	46.1
-16	73.3 5.37 (m)	73.9 ^{a)} 4.55 (m)	21.8	73.0 5.41 (m)
-17	70.2 4.13 (d, 4.5)	68.3 3.53 (d, 3.0)	60.6 3.47 (*)	70.5 3.51 (d, 5.5)
-18	11.6 1.60 (s)	10.5 1.19 (s)	15.6 1.78 (s)	12.7 1.58 (s)
-19	18.7 1.42 (s)	18.3 1.05 (s)	18.2 1.35 (s)	18.7 1.34 (s)
-20	214.5	216.3	209.5	212.1
-21	32.7 2.36 (s)	33.3 2.34 (s)	32.2 2.25 (s)	32.1 2.30 (s)
Ester moiety				
C,H- 1'	—	—	165.2	167.7
- 2'	—	—	120.6 6.11 (d, 12.5)	129.5
- 3'	—	—	143.3 7.05 (d, 12.5)	137.6 7.13 (br q, 6.5)
- 4'	—	—	135.1	14.3 1.70 (d, 6.5)
- 5'	—	—	130.5 7.88 (br d, 8.0)	12.3 1.97 (br s)
- 6'	—	—	128.3 7.36 (t, 8.0)	—
- 7'	—	—	129.3 7.28 (br t, 8.0)	—
- 8'	—	—	128.3 7.36 (t, 8.0)	—
- 9'	—	—	130.5 7.88 (br d, 8.0)	—

Measured in pyridine- d_5 solution at 35 °C. +: Measured in CDCl_3 solution at 35 °C. *: Overlapping with other signals. a) Interchangeable in each column.

Table 3. ¹³C-NMR Spectral Data of the Sugar Moiety

	1	2	7	8	9	14 ⁺	16	19	21 ⁺	23	27	28
Carbon No.												
	Dig	Dig	Dig	Cym	Cym	Can	Can	Can	Ole	Ole	Dig	Cym
C-1'	96.4	96.4	96.4	96.4	96.4	98.0	98.1	98.1	97.6	98.1	96.4	96.4
-2'	39.0	39.0	39.0	37.3 ^{a)}	37.3 ^{a)}	38.8	40.2	40.2	36.6	37.9 ^{a)}	39.0	38.8 ^{a)}
-3'	67.5	67.4	67.5	77.8 ^{b)}	78.0 ^{b)}	69.7	70.1	70.1	79.4 ^{a)}	79.4 ^{b)}	67.4	78.1
-4'	83.3 ^{a)}	83.3 ^{a)}	83.4 ^{a)}	83.4 ^{c)}	83.4 ^{c)}	88.6	88.5	88.5	82.7 ^{b)}	83.3 ^{c)}	83.5 ^{a)}	83.4 ^{b)}
-5'	68.6	68.6	68.6	69.0 ^{d)}	69.0 ^{d)}	70.3	70.9	70.9	71.3 ^{c)}	72.1 ^{d)}	68.7 ^{b)}	69.1
-OMe'	—	—	—	59.0 ^{e)}	58.9 ^{e)}	—	—	—	56.9 ^{d)}	57.4 ^{e)}	—	58.9
	Cym	Cym	Cym	Cym	Cym	Ole	Ole	Ole	Ole	Ole	Dig	Dig
C-1''	99.7	99.7	99.7	100.4	100.4	101.0	101.2	101.2	100.1	100.2	99.9	100.5
-2''	36.8	36.8	36.8	37.1 ^{a)}	37.1 ^{a)}	36.3 ^{a)}	37.6 ^{a)}	37.8 ^{a)}	36.6	37.9 ^{a)}	38.6 ^{c)}	37.3 ^{a)}
-3''	77.8	77.7	77.7	78.0 ^{b)}	77.8 ^{b)}	78.8 ^{b)}	79.1 ^{b)}	79.1 ^{b)}	79.3 ^{a)}	79.4 ^{b)}	67.4	67.4
-4''	83.2 ^{a)}	83.1 ^{a)}	83.3 ^{a)}	83.2 ^{c)}	83.3 ^{c)}	82.3	82.4 ^{c)}	82.4 ^{c)}	82.6 ^{b)}	83.3 ^{c)}	83.3 ^{a)}	83.3 ^{b)}
-5''	69.1	69.1	69.1	68.9 ^{d)}	68.8 ^{d)}	71.4	71.9 ^{d)}	71.9 ^{d)}	71.0 ^{c)}	71.8 ^{d)}	68.7 ^{b)}	68.7 ^{c)}
-OMe''	58.9	58.9	58.9	58.8 ^{e)}	58.8 ^{e)}	57.1 ^{c)}	57.5 ^{e)}	57.5 ^{e)}	56.7 ^{d)}	57.4 ^{e)}	—	—
	Can	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Dig	Dig
C-1'''	102.0	101.9	101.9	101.9	101.9	100.1	100.1	100.1	100.2	100.2	99.8	99.9
-2'''	39.9	37.4	37.6	37.6 ^{a)}	37.6 ^{a)}	36.5 ^{a)}	37.4 ^{a)}	37.5 ^{a)}	36.6	37.8 ^{d)}	38.7 ^{c)}	38.7 ^{a)}
-3'''	69.9	79.2	79.3	79.2	79.3	79.0 ^{b)}	78.9 ^{b)}	78.9 ^{b)}	79.0	79.4 ^{b)}	67.4	67.4
-4'''	88.0 ^{b)}	83.0 ^{a)}	83.2 ^{a)}	83.0 ^{c)}	83.2 ^{c)}	82.0	82.8 ^{c)}	82.8 ^{c)}	82.5 ^{b)}	83.2 ^{c)}	83.1 ^{a)}	83.1 ^{b)}
-5'''	71.4	72.1	72.1	72.1	72.1	71.4	71.9 ^{d)}	71.9 ^{d)}	71.2 ^{c)}	71.7 ^{d)}	68.6 ^{b)}	68.5 ^{c)}
-OMe'''	—	57.2	57.3	57.3	57.4	56.7 ^{c)}	57.4 ^{e)}	57.5 ^{e)}	56.5 ^{d)}	57.3 ^{e)}	—	—
	The	The	The	The	The	Cym	Cym	Cym	Cym	Cym	Ole	Ole
C-1''''	105.1	104.1	104.0	104.1	104.0	98.5	98.5	98.5	98.5	98.5	101.6	101.6
-2''''	74.6	75.2	75.0	75.3	75.0	35.4 ^{a)}	37.8 ^{a)}	37.3 ^{a)}	35.6	37.5 ^{a)}	37.0	37.0
-3''''	87.8 ^{b)}	88.2	86.4	88.2	86.3	77.1	78.1 ^{f)}	78.1	77.1	78.0 ^{f)}	81.4	81.4
-4''''	75.4	76.0	83.2 ^{a)}	76.0	83.2 ^{c)}	82.7	83.3	83.2	82.4 ^{b)}	83.1 ^{c)}	76.1	76.2
-5''''	73.1	72.9	72.1	72.9	72.1	68.7	69.2	69.2	68.7	69.2	73.1	73.0
-OMe''''	61.0	60.9	60.6	60.9	60.6	58.0	58.9	58.6	58.0	58.9	57.0	57.0
			Glc		Glc	Ole	Ole	Ole	Ole	Ole		
C-1'''''	—	—	104.8	—	104.8	101.5	101.9	101.9	101.5	101.9	—	—
-2'''''	—	—	75.9	—	75.9	35.4	37.1 ^{a)}	37.1 ^{a)}	35.4	37.1 ^{a)}	—	—
-3'''''	—	—	78.7	—	78.7	80.6	79.4 ^{b)}	79.3 ^{b)}	80.6	79.2 ^{b)}	—	—
-4'''''	—	—	72.1	—	72.1	75.4	83.3	83.2	75.5	82.9 ^{c)}	—	—
-5'''''	—	—	78.0	—	78.0	71.6	72.1 ^{d)}	72.1 ^{d)}	71.6	71.6 ^{d)}	—	—
-6'''''	—	—	63.2	—	63.2	—	—	—	—	—	—	—
-OMe'''''	—	—	—	—	—	56.3	57.2 ^{e)}	57.2 ^{e)}	56.3 ^{d)}	57.2 ^{e)}	—	—
						Glc	All		Glc			
C-1''''''	—	—	—	—	—	—	104.5	102.1	—	104.5	—	—
-2''''''	—	—	—	—	—	—	75.7	72.8	—	75.7	—	—
-3''''''	—	—	—	—	—	—	78.7	73.2	—	78.7	—	—
-4''''''	—	—	—	—	—	—	72.1	69.5	—	72.1	—	—
-5''''''	—	—	—	—	—	—	78.0 ^{f)}	75.6	—	78.1 ^{f)}	—	—
-6''''''	—	—	—	—	—	—	63.3	63.4	—	63.2	—	—
-6s	18.6	18.9	18.8	18.8	18.8	18.4	18.9	18.9	18.4×3	18.9	18.6×2	18.6×2
	18.4	18.7	18.7×2	18.7	18.7×2	18.2	18.7	18.7	18.2	18.8×3	18.5	18.5×2
	18.2	18.5	18.5	18.5×2	18.5	18.0×2	18.5	18.5	18.0	18.5	18.4	
	18.1	18.4				17.9	18.2×2	18.3×2				

Dig: β-D-digitoxopyranosyl, Cym: β-D-cymaropyranosyl, Ole: β-D-oleandropyranosyl, Can: β-D-canaropyranosyl, The: β-D-thevetopyranosyl, Glc: β-D-glucopyranosyl, All: β-D-allopyranosyl. Measured in pyridine-*d*₅ solution at 35 °C. +: Measured in CDCl₃ solution at 35 °C. a–f) Interchangeable in each column.

were shown at δ 5.12 (1H, d, $J=8.0$ Hz) and δ 104.8. This anomeric proton signal was assigned to H-1 of β-D-glucopyranose, because of its coupling constant and the ¹H–¹H COSY spectrum. In the NOE difference experiment, irradiation at this anomeric proton signal (δ 5.12) exhibited an enhancement at δ 3.86 (H-4''' of β-D-thevetopyranose). Moreover, enzymatic hydrolysis with cellulase (see Experimental) of **7** afforded **2**. This evidence suggested that the sugar sequence of **7** was 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranoside, and the structure of **7** was determined as shown in Chart 1.

Compound **8** had the molecular formula, C₄₉H₈₀O₁₉, on FAB-MS, and afforded 15β-hydroxylineolon as the aglycone

and cymarose, oleandrose and thevetose as the component sugars after acid hydrolysis. The ¹H- and ¹³C-NMR spectra indicated that **8** was 15β-hydroxylineolon 3-*O*-tetraglycoside, and the NMR spectral data of the sugar sequence were consistent with those of stephanoside A, F and I.⁹⁾ Thus, the structure of **8** was established as 15β-hydroxylineolon 3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

The molecular formula of **9** was determined as C₅₅H₉₀O₂₄ on FAB-MS. The ¹H- and ¹³C-NMR spectra of **9** resemble to those of **8** except for one more anomeric proton and carbon signal [δ 5.12 (1H, d, $J=8.0$ Hz) and δ 104.8]. Comparison of the NMR spectra of **9** with those of **7** indicated that these anomeric signals belonged to H-1 and C-1 of β-D-glucopyra-

Table 4. ¹H-NMR Spectral Data of the Sugar Moiety

	1	2	7	8	9	14 ⁺
Proton No.						
	Dig	Dig	Dig	Cym	Cym	Can
H-1'	5.44 (dd, 9.5, 2.0)	5.45 (dd, 9.5, 2.0)	5.46 (dd, 9.5, 2.0)	5.25 (dd, 9.5, 2.0)	5.25 (dd, 9.5, 2.0)	4.59 (dd, 9.5, 2.0)
-3'	4.60 (q, 3.0)	4.62 (br s)	4.62 (q, 3.0)	4.07 (q, 3.0)	4.07 (q, 3.0)	3.60 (m)
-4'	3.48 (dd, 9.5, 3.0)	3.50 (dd, 9.5, 3.0)	3.50 (dd, 9.5, 3.0)	3.49 (dd, 9.5, 3.0)	3.49 (*)	2.96 (t, 8.5)
-5'	4.24 (dq, 9.5, 6.5)	4.27 (dq, 9.5, 6.5)	4.27 (dq, 9.5, 6.5)	4.19 (dq, 9.5, 6.5)	4.19 (*)	3.31 (*)
-6'	1.40 (d, 6.5)	1.43 (d, 6.5)	1.43 (d, 6.5)	1.36 (d, 6.5)	1.35 (d, 6.5)	1.28 (d, 6.5)
	Cym	Cym	Cym	Cym	Cym	Ole
H-1''	5.14 (dd, 9.5, 2.0)	5.16 (dd, 9.5, 2.0)	5.16 (dd, 9.5, 2.0)	5.11 (dd, 9.5, 2.0)	5.10 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)
-3''	4.01 (q, 3.0)	4.02 (q, 3.0)	4.00 (*)	4.02 (q, 3.0)	4.01 (*)	3.38 (*)
-4''	3.38 (dd, 9.5, 3.0)	3.39 (dd, 9.5, 3.0)	3.38 (dd, 9.5, 3.0)	3.43 (dd, 9.5, 3.0)	3.43 (dd, 9.5, 3.0)	3.20 (t, 8.5)
-5''	4.16 (dq, 9.5, 6.5)	4.18 (dq, 9.5, 6.5)	4.17 (dq, 9.5, 6.5)	4.16 (dq, 9.5, 6.5)	4.15 (dq, 9.5, 6.5)	3.41 (*)
-6''	1.29 (d, 6.5)	1.32 (d, 6.5)	1.31 (d, 6.5)	1.37 (d, 6.5)	1.37 (d, 6.5)	1.33 (d, 6.5)
	Can	Ole	Ole	Ole	Ole	Ole
H-1'''	4.74 (dd, 9.5, 2.0)	4.68 (dd, 9.5, 2.0)	4.66 (dd, 9.5, 2.0)	4.69 (dd, 9.5, 2.0)	4.68 (dd, 9.5, 2.0)	4.65 (dd, 9.5, 2.0)
-3'''	3.96 (*)	3.58 (*)	3.55 (*)	3.57 (*)	3.54 (*)	3.34 (*)
-4'''	3.39 (t, 8.5)	3.67 (t, 8.5)	3.61 (t, 8.5)	3.67 (t, 8.5)	3.60 (*)	3.18 (t, 8.5)
-5'''	3.60 (*)	3.57 (*)	3.55 (*)	3.57 (*)	3.54 (*)	3.30 (*)
-6'''	1.68 (d, 6.5)	1.69 (d, 6.0)	1.66 (d, 6.0)	1.70 (d, 6.0)	1.66 (d, 6.5)	1.31 (d, 6.5)
	The	The	The	The	The	Cym
H-1''''	4.79 (d, 8.0)	4.95 (d, 8.0)	4.87 (d, 8.5)	4.95 (d, 8.0)	4.86 (d, 8.5)	4.95 (dd, 9.5, 2.0)
-2''''	3.93 (*)	3.91 (*)	3.89 (t, 8.5)	3.92 (*)	3.89 (t, 8.5)	—
-3''''	3.59 (*)	3.60 (*)	3.68 (t, 8.5)	3.60 (*)	3.68 (t, 8.5)	3.80 (q, 3.0)
-4''''	3.59 (*)	3.61 (*)	3.86 (t, 8.5)	3.60 (*)	3.86 (t, 8.5)	3.23 (dd, 9.5, 3.0)
-5''''	3.79 (dq, 9.0, 6.0)	3.72 (dq, 8.5, 6.0)	3.74 (dq, 8.5, 6.0)	3.73 (dq, 8.5, 6.0)	3.74 (dq, 8.5, 6.5)	3.90 (dq, 9.5, 6.5)
-6''''	1.52 (d, 6.0)	1.59 (d, 6.0)	1.75 (d, 6.0)	1.59 (d, 6.0)	1.75 (d, 6.5)	1.24 (d, 6.5)
	—	—	Glc	—	Glc	Ole
H-1'''''	—	—	5.12 (d, 8.0)	—	5.12 (d, 8.0)	4.50 (dd, 9.5, 2.0)
-2'''''	—	—	4.01 (t, 8.0)	—	4.01 (*)	—
-3'''''	—	—	4.20 (*)	—	4.20 (*)	3.15 (*)
-4'''''	—	—	4.20 (*)	—	4.19 (*)	3.12 (t, 8.5)
-5'''''	—	—	3.95 (m)	—	3.96 (m)	3.28 (*)
-6'''''	—	—	4.34 (dd, 11.5, 5.5)	—	4.34 (dd, 12.0, 5.5)	1.32 (d, 6.5)
	—	—	4.51 (dd, 11.5, 2.5)	—	4.51 (dd, 12.0, 2.5)	—
H-1'''''	—	—	—	—	—	—
-2'''''	—	—	—	—	—	—
-3'''''	—	—	—	—	—	—
-4'''''	—	—	—	—	—	—
-5'''''	—	—	—	—	—	—
-6'''''	—	—	—	—	—	—
-OMe	3.58 (s) 3.89 (s)	3.53 (s) 3.57 (s) 3.89 (s)	3.50 (s) 3.57 (s) 3.93 (s)	3.53 (s) 3.57 (s) 3.61 (s) 3.89 (s)	3.50 (s) 3.56 (s) 3.61 (s) 3.93 (s)	3.39 (s) 3.41 (s)x2 3.44 (s)

nose, which existed as the terminal sugar in the oligosaccharide sugar chain. In the NOE difference spectrum, irradiation at H-1 of β -D-glucopyranose (δ 5.12) exhibited an NOE to δ 3.86 (H-4''' of β -D-thevetopyranose). Moreover, enzymatic hydrolysis of **9** with cellulase afforded **8**. Thus, the sugar sequence was determined to be 3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-thevetopyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranoside. This oligosaccharide sugar chain was confirmed in the constituent from *Leptadenia hastata*.¹⁰⁾

Compounds **10**–**15** had the molecular formulae, C₅₅H₉₀O₂₀, C₅₅H₉₀O₂₀, C₅₇H₉₂O₂₁, C₅₇H₉₂O₂₂, C₅₅H₉₀O₂₁ and C₅₅H₉₀O₂₁ on FAB-MS, respectively. Acid hydrolysis of **10**–**15** afforded canarose, oleandrose and cymarose as the component sugars and lineolone on **10**, isolineolone on **11**, 12-O-acetyllineolone on **12**, metaplexigenin on **13** and 15 β -hydroxylneolone on **14**, as the aglycone moiety and a new aglycone was obtained from **15**. Based on the consistence of the ¹³C- and ¹H-NMR spectral data of the sugar moieties in these

compounds, they had the same oligosaccharide sugar sequence. Evidence for the sugar sequence and their linkage sites was derived from the consequences of the NOE difference experiments irradiating at each anomeric proton signal and the HMBC experiment in **14**. NOEs were observed between δ 4.59 (H-1' of β -D-canaropyranose) and 3.54 (H-3 of the aglycone); δ 4.44 (H-1'' of β -D-oleandropyranose) and 2.96 (H-4' of β -D-canaropyranose); δ 4.65 (H-1''' of β -D-oleandropyranose) and 3.20 (H-4'' of β -D-oleandropyranose); δ 4.95 (H-1'''' of β -D-cymaropyranose) and 3.18 (H-4''' of β -D-oleandropyranose); δ 4.50 (H-1''''' of β -D-oleandropyranose) and 3.23 (H-4'''' of β -D-cymaropyranose). In HMBC spectrum of **14**, ³J_{COCH}s were shown between δ 78.3 (C-3 of the aglycone) and 4.59 (H-1' of β -D-canaropyranose); δ 101.0 (C-1'' of β -D-oleandropyranose) and 2.96 (H-4' of β -D-canaropyranose); δ 100.1 (C-1''' of β -D-oleandropyranose) and 3.20 (H-4'' of β -D-oleandropyranose); δ 98.5 (C-1'''' of β -D-cymaropyranose) and 3.18 (H-4''' of β -D-oleandropyranose); δ 101.5 (C-1''''' of β -D-oleandropyranose) and 3.23 (H-4'''' of β -D-cymaropyranose).

Table 4. (Continued)

	16	19	21 ⁺	23	27	28
Proton No.						
	Can	Can	Ole	Ole	Dig	Cym
H-1'	4.91 (dd, 9.5, 2.0)	4.87 (dd, 9.5, 2.0)	4.54 (dd, 9.5, 2.0)	4.82 (dd, 9.5, 2.0)	5.46 (dd, 9.5, 2.0)	5.27 (br d, 9.5)
-3'			3.35 (*) ^{a)}		4.63 (br s)	4.09 (q, 3.0)
-4'	3.32 (t, 8.5)	3.31 (t, 9.0)	3.15 (t, 9.5)		3.51 (dd, 9.5, 3.0)	3.52 (dd, 9.5, 3.0)
-5'			3.30 (*)		4.29 (dq, 9.5, 6.5)	4.21 (dq, 9.5, 6.5)
-6'	1.44 (d, 6.5)	1.43 (*)	1.30 (d, 6.0) ^{b)}	1.46 (*) ^{a)}	1.39 (d, 6.5) ^{a)}	1.38 (d, 6.5)
	Ole	Ole	Ole	Ole	Dig	Dig
H-1''	4.77 (dd, 9.5, 2.0)	4.76 (dd, 9.5, 2.0)	4.67 (dd, 9.5, 2.0)	4.90 (dd, 9.5, 2.0) ^{b)}	5.37 (dd, 9.5, 2.0)	5.32 (br d, 9.5)
-3''			3.35 (*) ^{a)}		4.63 (br s)	4.63 (br s)
-4''		3.50 (*)	3.15 (t, 9.5)		3.45 (dd, 9.5, 3.0)	3.48 (br d, 9.5)
-5''		3.58 (*)	3.32 (*)		4.26 (dq, 9.5, 6.5)	4.24 (dq, 9.5, 6.5)
-6''	1.39 (d, 6.5) ^{a)}	1.37 (d, 6.0)	1.30 (d, 6.0) ^{b)}	1.45 (*) ^{a)}	1.33 (d, 6.5)	1.38 (d, 6.5)
	Ole	Ole	Ole	Ole	Dig	Dig
H-1'''	4.86 (d, 9.5, 2.0)	4.85 (dd, 9.5, 2.0)	4.65 (dd, 9.5, 2.0)	4.89 (dd, 9.5, 2.0) ^{b)}	5.37 (dd, 9.5, 2.0)	5.38 (br d, 9.5)
-3'''			3.32 (*) ^{a)}		4.63 (br s)	4.63 (br s)
-4'''		3.50 (*)	3.18 (t, 9.5)		3.48 (dd, 9.5, 3.0)	3.48 (br d, 9.5)
-5'''			3.31 (*)		4.29 (dq, 9.5, 6.5)	4.30 (dq, 9.5, 6.5)
-6'''	1.44 (d, 6.5)	1.43 (*)	1.29 (d, 6.0) ^{b)}	1.45 (*) ^{a)}	1.43 (d, 6.5) ^{a)}	1.40 (d, 6.5)
	Cym	Cym	Cym	Cym	Ole	Ole
H-1''''	5.28 (dd, 9.5, 2.0)	5.27 (dd, 9.5, 2.0)	4.95 (dd, 9.5, 2.0)	5.28 (dd, 9.5, 2.0)	4.79 (dd, 9.5, 2.0)	4.80 (dd, 9.5, 2.0)
-2''''						
-3''''	4.04 (q, 3.0)	4.02 (q, 3.0)	3.80 (q, 3.0)	4.04 (q, 3.0)	3.44 (*)	3.45 (*)
-4''''	3.44 (dd, 9.5, 3.0)	3.44 (dd, 9.5, 3.0)	3.23 (dd, 9.5, 3.0)	3.44 (dd, 9.5, 3.0)	3.41 (t, 8.5)	3.41 (t, 8.5)
-5''''	4.19 (*)	4.18 (*)	3.90 (dq, 9.5, 6.5)	4.19 (*)	3.57 (dq, 8.5, 6.0)	3.57 (dq, 8.5, 6.0)
-6''''	1.38 (d, 6.5) ^{a)}	1.39 (d, 6.0)	1.24 (d, 6.5)	1.39 (d, 6.5)	1.49 (d, 6.0)	1.49 (d, 6.0)
	Ole	Ole	Ole			
H-1'''''	4.69 (dd, 9.5, 2.0)	4.68 (dd, 9.5, 2.0)	4.50 (dd, 9.5, 2.0)	4.69 (dd, 9.5, 2.0)	—	—
-2'''''					—	—
-3'''''		3.62 (*)	3.15 (*)		—	—
-4'''''	3.72 (t, 8.5)	3.64 (*)	3.12 (t, 8.5)	3.72 (t, 8.5)	—	—
-5'''''	3.65 (dq, 8.5, 6.0)	3.64 (*)	3.28 (*)	3.65 (dq, 8.5, 6.0)	—	—
-6'''''	1.72 (d, 6.0)	1.67 (d, 6.0)	1.32 (d, 6.0)	1.72 (d, 6.0)	—	—
	Glc	All		Glc		
H-1'''''	5.11 (d, 8.0)	5.50 (d, 8.0)	—	5.11 (d, 8.0)	—	—
-2'''''	4.00 (t, 8.0)	3.92 (dd, 8.0, 2.5)	—	—	—	—
-3'''''	4.19 (*)	4.69 (t, 2.5)	—	4.20 (*)	—	—
-4'''''	4.19 (*)	4.17 (*)	—	4.20 (*)	—	—
-5'''''	3.94 (m)	4.43 (*)	—	3.94 (m)	—	—
-6'''''	4.34 (dd, 11.5, 5.5)	4.32 (dd, 11.5, 5.5)	—	4.34 (dd, 11.5, 5.5)	—	—
	4.51 (dd, 11.5, 2.5)	4.46 (dd, 11.5, 3.0)	—	4.51 (dd, 11.5, 2.5)	—	—
-OMe	3.52 (s)	3.52 (s)	3.38 (s)	3.51 (s)	3.46 (s)	3.46 (s)
	3.53 (s)	3.53 (s)	3.39 (s)	3.52 (s)		3.62 (s)
	3.55 (s)	3.55 (s)	3.40 (s)	3.53 (s)		
	3.57 (s)	3.56 (s)	3.41 (s)	3.56 (s)		
			3.44 (s)	3.57 (s)		

Measured in pyridine-*d*₅ solution at 35 °C. +: Measured in CDCl₃ solution at 35 °C. *a, b*) Interchangeable in each column. *: Overlapping with other signals.

of β -D-cymaropyranose). Hence, the sugar sequence was decided to be 3-*O*- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranoside. The ¹³C-NMR spectral data of the aglycone moiety of **15** was similar to that of **6**, but the chemical shifts of the signals due to the D ring were different. The ¹H- and ¹³C-NMR spectra displayed the carbinyl proton and carbon signals at δ 5.37 (1H, m) and δ 73.3. In the ¹H-¹H COSY spectrum, this carbinyl proton signal had a correlation with the signal at δ 4.13 (H-17). Accordingly, these signals were assigned to H-16 and C-16 of the aglycone. Because irradiation at δ 4.42 (H-12) showed an NOE to δ 4.13 (H-17) in the NOE difference experiment, the orientation of H-17 was suggested to be α . Similarly, irradiation at δ 5.37 (H-16) showed an NOE to δ 2.36 (H-21), and then H-16 was considered to have β -orientation. In addition, in the NOE difference experiments of

15 β -hydroxyisolineolone,¹⁾ an NOE was observed between the signals due to H-15 α and H-16 α [δ 2.61 (1H, dt, *J* = 14.0, 9.5 Hz)]. And in the ¹H-¹H COSY spectrum, the signal at δ 2.32 (1H, ddd, *J* = 14.0, 7.0, 5.0 Hz) was assigned to H-16 β . In the ¹H-NMR spectrum, the *J* value of H-17 α and H-16 α was observed by 9.5 Hz, and the *J* value of H-17 α and H-16 β was observed by 5.0 Hz. On the other hand, in the ¹H-NMR spectrum of **15**, the *J* value of H-17 signal observed by 4.5 Hz suggested that H-16 had β -orientation and hydroxyl group attached at C-16 had α -orientation. Thus, the aglycone moiety of **15** was determined to be 16 α -hydroxyisolineolone. Moreover, the *J* value of H-17 signal of 16 α -hydroxycalotropin¹¹⁾ shown by 4 Hz supported the β -orientation of H-16 in **15**.

The FAB-MS spectra revealed that the molecular formulae of compounds **16**–**18** were C₆₁H₁₀₀O₂₅, C₆₁H₁₀₀O₂₅ and C₆₁H₁₀₀O₂₆, respectively. The NMR spectra and acid hydroly-

Chart 2

sugar sequences of **20**–**22** were similar to those of **10**, **11** and **14**, but β -D-oleandropyranose attached at the C-3 position of the aglycone instead of β -D-canaropyranose. This sugar linkage was supported by the results of the NOE difference experiment irradiating at each anomeric proton signal on **21**. Hence, the structures of **20**–**22** were elucidated as in Chart 1.

Compounds **23**–**25** were believed to be pregnane 3-O-hexaosides whose aglycones were also decided to be lineolol, isolineolol and 15 β -hydroxylineolol, respectively, by acid hydrolysis. The sugar moiety consisted of four β -D-oleandropyranose, one β -D-cymaropyranose and one β -D-glucopyranose based on the NMR spectral data and acid hydrolysis. Moreover, the NMR spectra and enzymatic hydrolysis suggested that β -D-glucopyranose was attached to the C-4 position of the terminal β -D-oleandropyranose of the sugar sequence in **20**–**22**. Accordingly, compounds **23**–**25** are shown in Chart 1.

The FAB-MS spectrum of **26** indicated that the molecular formula of **26** was C₅₅H₈₀O₁₈ [positive FAB-MS ion at m/z 1051 [M+Na]⁺]. The ¹H- and ¹³C-NMR spectral data of **26** were similar to those of ikemagenin 3-O- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranoside (**37**).¹⁾ As to the ester moiety, the ¹³C-NMR spectrum showed six aromatic carbon signals, two sp^2 carbon signals and one carbonyl carbon signal the same as that of **37**. But the ¹H-NMR spectrum revealed that the J values of the sp^2 proton signals were 12.5 Hz. From this evidence, the ester moiety was deduced to be (Z)-cinnamic acid. That was supported by alkaline hydrolysis with 2 N NaOH of the aglycone moiety of **26** (see Experimental). Thus, the structure of **26** was established as 12-O-(Z)-cinnamoyllineolol 3-O- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranoside.

Compound **27** had the molecular formula, C₅₁H₈₀O₁₉, on FAB-MS. The sugar chain was the same as that of **26** and **37**, based on the ¹³C- and ¹H-NMR spectral data. In the ¹H-NMR spectrum, H-17 was observed as a doublet signal at δ 3.51 and its J value was revealed as 5.5 Hz. Moreover, the ¹³C-NMR spectral data of the aglycone moiety were similar to those of **15** except for the acylation shifts around C-12. In the

NOE difference experiments, irradiation of the H-12 α signal [δ 5.60 (1H, dd, J =12.0, 4.0 Hz)] showed an NOE to the H-17 signal [δ 3.51 (1H, d, J =5.5 Hz)]. Accordingly, the orientation of H-17 was suggested to be α , and the aglycone of **27** was considered to be 12-O-acylated 16 α -hydroxyisolineolol. As the acyl group attached to C-12-OH of 16 α -hydroxyisolineolol showed one carbonyl carbon signal, two sp^2 carbon signals, two methyl carbon signals and one sp^2 proton signal, two methyl proton signals in ¹³C- and ¹H-NMR spectra, the acyl group was presumed to be tigloyl group or angeloyl group. Alkaline hydrolysis of the aglycone moiety of **27** afforded tiglic acid, thus, the acyl group was decided as tigloyl group. On the above evidence, the aglycone was determined to be 12-O-tigloyl-16 α -hydroxyisolineolol, and the structure of **27** was proved as shown.

The FAB-MS spectrum of compound **28** indicated that the molecular formula of **28** was C₅₆H₈₂O₁₈. The ¹³C-NMR spectra of the aglycone moiety of **28** were consistent with those of **26**, and the ¹³C- and ¹H-NMR spectral data of the sugar moiety were similar to those of ikemagenin 3-O- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranoside (**38**).¹⁾ Thus, the structure of **28** was elucidated as in Chart 1.

The aglycone moieties of compounds **29** and **30** were suggested to be 12-O-tigloyl-16 α -hydroxyisolineolol in good agreement of the ¹³C- and ¹H-NMR spectral data with those of **27**. The sugar chains of **29** and **30** were the same as those of **2** and **10**, respectively, according to the comparison with the ¹³C- and ¹H-NMR spectral data. Therefore, **29** and **30** were characterized as 12-O-tigloyl-16 α -hydroxyisolineolol 3-O- β -D-thevetopyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranoside and 12-O-tigloyl-16 α -hydroxyisolineolol 3-O- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranoside, respectively.

Experimental

General Procedure Instrumental analyses were carried out as described previously.¹⁾

Extraction and Isolation Procedures for extracting and isolating pregnane glycosides were described.¹⁾ The pregnane glycoside fraction isolated afforded compounds **1** (7 mg), **2** (11 mg), **3** (10 mg), **4** (7 mg), **5** (15 mg), **6** (5 mg), **7** (6 mg), **8** (13 mg), **9** (7 mg), **11** (55 mg), **12** (6 mg), **13** (6 mg), **14** (52 mg), **15** (11 mg), **16** (8 mg), **17** (13 mg), **18** (22 mg), **19** (11 mg), **20** (11 mg), **21** (12 mg), **22** (20 mg), **23** (6 mg), **24** (4 mg), **25** (9 mg), **26** (7 mg), **27** (4 mg), **28** (7 mg), **29** (4 mg), **30** (6 mg).

Compound **1**. Amorphous powder. [α]_D²⁷ -1.6° (c =0.67, MeOH). FAB-MS m/z : 945 [M+H]⁺, 967 [M+Na]⁺. ¹³C- and ¹H-NMR: see Tables 1, 3 and 4.

Compound **2**. Amorphous powder. [α]_D²⁴ -7.7° (c =1.10, MeOH). FAB-MS m/z : 1001 [M+H]⁺, 1023 [M+Na]⁺. ¹³C- and ¹H-NMR: see Tables 1, 3 and 4.

Compound **3**. Amorphous powder. [α]_D²⁷ +0.54° (c =1.01, MeOH). FAB-MS m/z : 965 [M+Na]⁺. ¹³C-NMR: see Table 1. The ¹³C- and ¹H-NMR spectra of the sugar moiety were in good agreement with those of **2**.

Compound **4**. Amorphous powder. [α]_D²⁷ +28.1° (c =0.80, MeOH). FAB-MS m/z : 943 [M+H]⁺, 965 [M+Na]⁺. ¹³C-NMR: see Table 1. The ¹³C- and ¹H-NMR spectra of the sugar moiety were in good agreement with those of **2**.

Compound **5**. Amorphous powder. [α]_D²⁷ -3.2° (c =0.96, MeOH). FAB-MS m/z : 959 [M+H]⁺, 981 [M+Na]⁺. The ¹³C- and ¹H-NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **1** and **2**.

Compound **6**. Amorphous powder. [α]_D²¹ +2.9° (c =0.50, CHCl₃). FAB-MS m/z : 981 [M+Na]⁺. ¹³C-NMR: see Table 1. The ¹³C- and ¹H-NMR

spectra of the sugar moiety were in good agreement with those of **2**.

Compound **7**. Amorphous powder. $[\alpha]_D^{27} -3.1^\circ$ ($c=0.66$, MeOH). FAB-MS m/z : 1185 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **2**.

Compound **8**. Amorphous powder. $[\alpha]_D^{27} -1.2^\circ$ ($c=1.34$, MeOH). FAB-MS m/z : 995 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **1**.

Compound **9**. Amorphous powder. $[\alpha]_D^{27} +4.7^\circ$ ($c=0.70$, MeOH). FAB-MS m/z : 1157 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **1**.

Compound **10**. Amorphous powder. $[\alpha]_D^{27} -22.7^\circ$ ($c=0.73$, MeOH). FAB-MS m/z : 1093 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **3** and **14**.

Compound **11**. Amorphous powder. $[\alpha]_D^{27} +2.7^\circ$ ($c=1.18$, MeOH). FAB-MS m/z : 1093 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **4** and **14**.

Compound **12**. Amorphous powder. $[\alpha]_D^{27} -40.2^\circ$ ($c=0.63$, MeOH). FAB-MS m/z : 1135 $[M+Na]^+$. ^{13}C -NMR: see Table 1. The ^{13}C - and 1H -NMR spectra of the sugar moiety were in good agreement with those of **14**.

Compound **13**. Amorphous powder. $[\alpha]_D^{27} -30.1^\circ$ ($c=0.53$, MeOH). FAB-MS m/z : 1151 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **2** and **14**.

Compound **14**. Amorphous powder. $[\alpha]_D^{24} -24.8^\circ$ ($c=0.98$, MeOH). FAB-MS m/z : 1087 $[M+H]^+$, 1109 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **1**.

Compound **15**. Amorphous powder. $[\alpha]_D^{27} -11.4^\circ$ ($c=1.23$, MeOH). FAB-MS m/z : 1109 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Table 2. The ^{13}C and 1H -NMR spectra of the sugar moiety were in good agreement with those of **14**.

Compound **16**. Amorphous powder. $[\alpha]_D^{21} -18.2^\circ$ ($c=0.94$, MeOH). FAB-MS m/z : 1255 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **3**.

Compound **17**. Amorphous powder. $[\alpha]_D^{27} +3.6^\circ$ ($c=0.86$, MeOH). FAB-MS m/z : 1255 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **4** and **16**.

Compound **18**. Amorphous powder. $[\alpha]_D^{27} -22.4^\circ$ ($c=0.72$, MeOH). FAB-MS m/z : 1271 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **1** and **16**.

Compound **19**. Amorphous powder. $[\alpha]_D^{27} -21.9^\circ$ ($c=1.18$, MeOH). FAB-MS m/z : 1271 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **1**.

Compound **20**. Amorphous powder. $[\alpha]_D^{27} -20.9^\circ$ ($c=0.97$, MeOH). FAB-MS m/z : 1107 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **3** and **21**.

Compound **21**. Amorphous powder. $[\alpha]_D^{27} +4.2^\circ$ ($c=1.23$, MeOH). FAB-MS m/z : 1107 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with those of **4**.

Compound **22**. Amorphous powder. $[\alpha]_D^{27} -22.3^\circ$ ($c=1.10$, MeOH). FAB-MS m/z : 1123 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **1** and **21**.

Compound **23**. Amorphous powder. $[\alpha]_D^{21} -15.0^\circ$ ($c=0.59$, MeOH). FAB-MS m/z : 1269 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **3**.

Compound **24**. Amorphous powder. $[\alpha]_D^{27} +4.7^\circ$ ($c=0.44$, MeOH). FAB-MS m/z : 1269 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **4** and **23**.

Compound **25**. Amorphous powder. $[\alpha]_D^{27} -16.6^\circ$ ($c=0.94$, MeOH). FAB-MS m/z : 1285 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **1** and **23**.

Compound **26**. Amorphous powder. $[\alpha]_D^{27} +16.2^\circ$ ($c=0.69$, MeOH). FAB-MS m/z : 1051 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ϵ): 272 (3.95). ^{13}C - and 1H -NMR: see Table 2. The ^{13}C - and 1H -NMR spectra of the sugar moieties were in good agreement with those of **27**.

Compound **27**. Amorphous powder. $[\alpha]_D^{24} +3.7^\circ$ ($c=0.34$, MeOH). FAB-MS m/z : 997 $[M+H]^+$, 1019 $[M+Na]^+$. ^{13}C - and 1H -NMR: see Tables 2—4.

Compound **28**. Amorphous powder. $[\alpha]_D^{27} +25.0^\circ$ ($c=0.41$, MeOH). FAB-MS m/z : 1065 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ϵ): 272 (4.02). ^{13}C - and 1H -

NMR: see Tables 3 and 4. The ^{13}C -NMR spectrum of the aglycone moiety was in good agreement with that of **26**.

Compound **29**. Amorphous powder. $[\alpha]_D^{20} +4.0^\circ$ ($c=0.36$, MeOH). FAB-MS m/z : 1063 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **27** and **2**.

Compound **30**. Amorphous powder. $[\alpha]_D^{27} -17.2^\circ$ ($c=0.62$, MeOH). FAB-MS m/z : 1191 $[M+Na]^+$. The ^{13}C - and 1H -NMR spectra of the aglycone and the sugar moieties were in good agreement with those of **27** and **14**.

Acid Hydrolysis of a Mixture of Pregnane Glycosides The procedure of acid hydrolysis of a mixture of pregnane glycosides (510 mg) was described in a previous report.¹⁾ Purification of the EtOAc layer by HPLC afforded lineolol (**31**, 12 mg), isolineolol (**32**, 2 mg), 12-*O*-acetyllineolol (**33**, 2 mg), metaplexigenin (**34**, 5 mg) and 15 β -hydroxylineolol (**35**, 28 mg) and 15 β -hydroxyisolineolol (**36**, 5 mg). The 1H - and ^{13}C -NMR spectral data of **33**, **35** and **36** were also recorded earlier.¹⁾

The H₂O layer afforded cymarose (13 mg), oleandrose (37 mg), digitoxose (57 mg), and canarose (9 mg).

As to absolute configuration of each monosaccharide, all of these monosaccharides were believed to have D-form based on their optical rotation values.^{13–16)}

D-Cymarose: $[\alpha]_D^{21} +51.7^\circ$ ($c=1.29$, 24 h after dissolution in H₂O) (lit.: $[\alpha]_D^{21} +51.6^\circ$ ($c=1.02$, H₂O)).¹³⁾

D-Oleandrose: $[\alpha]_D^{21} -11.7^\circ$ ($c=1.87$, 24 h after dissolution in H₂O) (lit.: $[\alpha]_D^{21} -11^\circ$ ($c=1.1$, H₂O)).¹⁴⁾

D-Digitoxose: $[\alpha]_D^{21} +45.0^\circ$ ($c=1.04$, 24 h after dissolution in H₂O) (lit.: $[\alpha]_D^{24} +48.4^\circ$ ($c=0.90$, H₂O)).¹⁵⁾

D-Canarose: $[\alpha]_D^{21} +22.8^\circ$ ($c=0.71$, 24 h after dissolution in H₂O) (lit.: $[\alpha]_D^{21} +25^\circ$ ($c=1.4$, H₂O)).¹⁶⁾

This H₂O layer also brought about a disaccharide, thevetopyranosyl-(1 $\rightarrow$ 4)-oleandropyranoside. This disaccharide was hydrolyzed with 0.05 N HCl-dioxane (1 : 1) at 95 °C for 1.5 h, and then the residue was reacted with D-cysteine methyl ester hydrochloride following the procedure described.¹⁾ After a series of reactions, the supernatant was subjected to GC analysis. GC conditions: Column, GL Capillary Column TC-1 (GL Sciences Inc., Tokyo, Japan) 0.32 mm $\times$ 30 m, carrier gas N₂, column temperature 195 °C; t_R , D-thevetose 15.5 min, L-thevetose 14.0 min. The t_R for D-thevetose was obtained from its enantiomer (L-thevetose+L-cysteine). D-Thevetose was detected from this disaccharide.

Acid and Alkaline Hydrolysis of Compounds 26 and 27 The solutions of compounds **26** and **27** (ca. 0.5 mg) in dioxane and 0.05 N HCl (2 drops each) were heated at 95 °C for 1.5 h. The acquired aglycones were hydrolyzed with 2 N NaOH aq.-dioxane (each 40 μ l) at 60 °C for 2 h in N₂ atmosphere. After alkaline hydrolysis, the reaction mixture was diluted with H₂O and 1 N HCl (ca. 100 μ l) was added. The ester moiety was then extracted with diethylether. HPLC analysis suggested that (Z)-cinnamic acid and tiglic acid were yielded from the aglycone of **26** and **27**, respectively. [Conditions: Column, YMC-ODS 4.6 mm $\times$ 25 cm; flow rate, 1.0 ml/min, 50% MeOH in water+0.05% trifluoroacetic acid (TFA); t_R , (Z)-cinnamic acid 12.0 min, 40% MeOH in water+0.05% TFA; t_R , tiglic acid 13.4 min].

Acid Hydrolysis of Compounds 1—30 The solutions of compounds **1—29** and **30** (ca. 0.5 mg) in dioxane and 0.05 N HCl (2 drops each) were heated at 95 °C for 1.5 h. After hydrolysis, the procedures mentioned earlier were followed to obtain each aglycone and alditol acetate.¹⁾ The conditions of HPLC and GC analyses were recorded as follows. HPLC conditions: Column, YMC-ODS 4.6 mm $\times$ 25 cm; flow rate, 1.0 ml/min, 25% MeOH in water; t_R , 16 α -hydroxyisolineolol 14.4 min, 30% MeOH in water; t_R , 15 β -hydroxylineolol 12.0 min, 15 β -hydroxyisolineolol 12.8 min, 35% MeOH in water; t_R , lineolol 14.6 min, 42.5% MeOH in water; t_R , isolineolol 12.8 min, 50% MeOH in water; t_R , 12-*O*-acetyllineolol 14.4 min, 52.5% MeOH in water; t_R , metaplexigenin 15.6 min, 67.5% MeOH in water; t_R , 12-*O*-tigloyl-16 α -hydroxyisolineolol 9.0 min, 12-*O*-(Z)-cinnamoyllineolol 12.4 min.

GC conditions: Column, Supelco SP-2380TM capillary column 0.25 mm $\times$ 30 m, carrier gas N₂, column temperature 200 °C; t_R , cymaritol acetate 7.5 min, oleandritol acetate 8.5 min, digitoxitol acetate 10.9 min, canaritol acetate 12.4 min, column temperature 215 °C; t_R , thevetitol acetate 10.8 min, column temperature 250 °C; t_R , allitol acetate 9.4 min, glucitol acetate 12.4 min.

Acid Hydrolysis of Compound 19 to Determine the Configuration of Allose Acid hydrolysis of **19** (3 mg) with 0.05 N HCl and dioxane (5 drops each) and after hydrolysis, a reaction of resulting product with D-cysteine methyl ester hydrochloride, hexamethyldisilazane and trimethylsilylchloride succeeded in the same manner.¹⁾ GC analysis were carried out under the following conditions: Column, GL capillary column TC-1 (GL Sciences Inc.,

Tokyo, Japan) 0.32 mm×30 m, carrier gas N₂, column temperature 210 °C; *t*_R, D-allose 19.0 min, L-allose 17.9 min. The *t*_R for L-allose was obtained from its enantiomer (D-allose+L-cysteine). D-Allose was detected from **19**.

Acid Hydrolysis of a Mixture of Pregnane Glycosides to Determine the Configuration of Glucose A mixture of pregnane glycosides (*ca.* 10 mg) was heated at 95 °C for 1.5 h with 0.05 N HCl and dioxane (10 drops each). After hydrolysis, the described procedures to determine the configuration of glucose were followed.¹⁾ GC conditions: Column, GL Capillary Column TC-1 (GL Sciences Inc., Tokyo, Japan) 0.32 mm×30 m, carrier gas N₂, column temperature 210 °C; *t*_R, D-glucose 17.7 min, L-glucose 16.9 min. D-Glucose was detected from a mixture of pregnane glycosides.

Enzymatic Hydrolysis of Compounds 7, 9, 16—18, 23, 24 and 25 Enzymatic hydrolysis of compounds **7**, **9**, **16—18**, **23**, **24** and **25** (*ca.* 2 mg) was done as described.¹⁾ Each compound produced **2**, **8**, **10**, **11**, **14**, **20**, **21** and **22**, whose structures were confirmed by comparison of their ¹H-NMR spectra and HPLC analysis with those of authentic sample.

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References and Notes

- 1) Warashina T., Noro T., *Phytochemistry* (submitted).
- 2) Yamagishi T., Hayashi K., Mitsuhashi H., Inamori M., Matsushita K., *Tetrahedron Lett.*, **37**, 3527—3530 (1973).
- 3) Yamagishi T., Hayashi K., Mitsuhashi H., Inamori M., Matsushita K., *Tetrahedron Lett.*, **37**, 3531—3534 (1973).
- 4) Warashina T., Noro T., *Chem. Pharm. Bull.*, **42**, 322—326 (1994).
- 5) Mitsuhashi H., Nomura T., *Chem. Pharm. Bull.*, **13**, 274—280 (1965).
- 6) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **35**, 501—506 (1987).
- 7) Zhang D., Miyase T., Kuroyanagi M., Umehara K., Ueno A., *Chem. Pharm. Bull.*, **44**, 173—179 (1996).
- 8) Kasai R., Okihara M., Asakawa J., Mizutani K., Tanaka O., *Tetrahedron*, **35**, 1427—1432 (1979).
- 9) Yoshikawa K., Okada N., Kann Y., Arihara S., *Chem. Pharm. Bull.*, **44**, 1790—1796 (1996).
- 10) Aquino R., Peluso G., De Tommasi N., De Simone F., Pizza C., *J. Nat. Prod.*, **59**, 555—564 (1996).
- 11) Abe F., Mohri Y., Yamauchi T., *Chem. Pharm. Bull.*, **40**, 2917—2920 (1992).
- 12) Mitsuhashi T., Hayashi K., *Shoyakugaku Zasshi*, **39**, 1—27 (1985).
- 13) Tsukamoto S., Hayashi K., Kaneko K., Mitsuhashi H., *Chem. Pharm. Bull.*, **34**, 3130—3134 (1986).
- 14) Nakagawa T., Hayashi K., Wada K., Mitsuhashi H., *Tetrahedron*, **39**, 607—612 (1983).
- 15) Abe F., Mohri Y., Okabe H., Yamauchi T., *Chem. Pharm. Bull.*, **42**, 1777—1783 (1994).
- 16) Miyamoto M., Kawamatsu Y., Shinohara M., Nakadaira Y., Nakanishi K., *Tetrahedron*, **22**, 2785—2799 (1966).