Cardenolide and Oxypregnane Glycosides from the Root of *Asclepias incarnata* L.

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Twenty-nine new oxypregnane glycosides were obtained along with two known cardenolides, frugoside and gofruside, and three known 12-*O*-acylated pregnane glycosides from the roots of *Asclepias incarnata* L.(Asclepiadaceae). By detailed studies of the ^{1}H- and ^{13}C-NMR spectra, the structures were determined to be tri- to penta glycosides of isolineolon, 12-*O*-acetyllineolon, ikemagenin, 12-*O*-benzoylisolineolon, and two new 12-*O*-acylated pregnanes.

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

In previous papers,[1,2] we reported the isolation and structural elucidation of oxypregnane glycosides from the aerial part of *Asclepias incarnata* L. Further studies of the root of this plant have resulted in the isolation of 29 new pregnane glycosides together with two known cardenolide glycosides, frugoside[3,4] and gofruside,[3] and three known 12-*O*-acylated pregnane glycosides (**1**, **6**, **23**).[1,5,6] The sugar sequences of these pregnane glycosides were suggested to be similar to those of compounds acquired from the aerial part of this plant in comparison with the ^{1}H- and ^{13}C-NMR spectral data.[1,2]

The MeOH extract obtained from the dried root of *A. incarnata* L. was suspended in water. The suspension was then extracted with diethyl ether and partitioned into an ether soluble fraction and a water soluble fraction. These fractions were chromatographed on a silica gel column to give a fraction of pregnane glycosides from which 29 new oxypregnane glycosides (**2**—**5**, **7**—**22**, **24**—**32**) were obtained.

In order to acquire the component aglycones and sugars, the fraction containing the pregnane glycosides from silica gel column chromatography was subjected to acid hydrolysis. The obtained aglycones were identified as isolineolon (**35**),[7] 12-*O*-acetyllineolon (**34**),[1] ikemagenin (**36**),[8] 12-*O*-nicotinoyllineolon (**33**)[5] and 12-*O*-benzoylisolineolon (**39**)[9] in view of the ^{1}H- and ^{13}C-NMR spectral data and/or the analysis of HPLC with the authentic samples. In addition, two new acylated pregnanes were obtained (**37**, **38**).

Compound **37** showed a $[M+Na]^{+}$ ion peak at *m/z* 519, which was larger by 2 mass units than that of **36**. In comparing the ^{13}C-NMR spectral data of **37** with those of **36**, signals of the cinnamoyl group were observed, but, in addition, two sp^3 carbon signals were seen at δ 45.9 and 25.4 instead of two sp^2 carbon signals at C-5 and C-6. Thus, **37** was considered to be a 5,6-dihydro derivative of ikemagenin. On the basis of ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H-detected heteronuclear multiple quantum coherency (HMQC) spectra, assignments of the proton signals were done as shown in the Experimental. In the nuclear Overhauser effect (NOE) difference experiments, irradiation at the H-3 signal (δ 3.92) showed a NOE on the H-5 signal (δ 1.16), and irradiation at the H-19 signal (δ 1.27) exhibited a NOE on the H-4axial signal (δ 1.71). Based on these findings, the orientation of H-5 was confirmed as α, and finally, **37** was determined to be 5α,6-dihydroikemagenin.

The ^{1}H- and ^{13}C-NMR spectra of compound **38** was similar to those of sibirigenin.[7,10] The chemical shift of the C-20 signal was consistent with that of sibirigenin, and H-17 signal was observed as a double-doublet signal whose *J* value was 9.5 and 5.5 Hz. This multiplicity and the *J* value of the H-17 signal were the same as those of **35**. Therefore, **38** was considered to be 12-*O*-acylated isolinelon. Moreover, alkaline hydrolysis of **38** afforded tiglic acid (see Experimental). Hence, **38** was concluded to be 12-*O*-tigloylisolineolon.

Table 1. ^{13}C-NMR Spectral Data of the Aglycone Moiety

	1	**2**	**3**	**4**	**7**	**8**	**9**
Carbon No.							
C-1	39.0	39.0	39.2	39.0	38.2	39.0	39.0
-2	29.9	29.9	30.0	29.9	29.6	29.9	29.9
-3	77.7	77.7	77.9	77.7	76.7	77.7[a)]	77.7
-4	39.3	39.3	39.4	39.4	35.0[a)]	39.3	39.4
-5	139.5	139.5	139.3	139.5	45.4	139.2	139.2
-6	119.2	119.2	119.7	119.2[a)]	25.3	119.4	119.3
-7	35.2	35.2	37.3	35.2	34.6[a)]	35.9	35.8
-8	74.6[a)]	74.6	74.5	74.6	76.5	74.4	74.4
-9	44.8	44.8	45.6	44.8	47.5	45.1	45.2
-10	37.6	37.5	37.6	37.6	36.7	37.6	37.7
-11	25.0	24.9	28.5	25.0	24.0	24.6	24.8[a)]
-12	74.3[a)]	73.3	74.0	73.4	73.9	77.6[a)]	78.6
-13	56.1	55.7	56.7	55.9	56.3	55.0	55.1
-14	87.5	87.5	86.7	87.5	87.6	86.6	86.6
-15	34.2	34.1	36.2	34.2	33.9	36.7	36.7
-16	22.2	21.8	24.7	22.0	22.4	24.6	24.7[a)]
-17	60.2	60.5	58.7	60.5	60.5	59.2	59.3
-18	15.9	15.6	11.6	15.8	16.2	12.6	12.7
-19	18.3	18.2	18.5	18.2	13.1	18.4	18.4
-20	209.8	209.6	216.8	209.3	209.4	214.3	214.1
-21	32.4	32.3	32.3	32.2	32.3	31.6	31.6
Ester moiety							
-1′	164.5	167.0	–	165.9	165.9	167.8	166.6
-2′	–	20.8	–	119.3[a)]	119.4	129.4	133.6[b)]
-3′	153.8	–	–	144.9	144.8	137.7	129.1
-4′	127.0	–	–	135.1	135.1	12.3	130.0
-5′	137.1	–	–	128.6	128.6	14.3	131.2[b)]
-6′	[c)]	–	–	129.3	129.3	–	130.0
-7′	151.1	–	–	130.6	130.5	–	129.1
-8′	–	–	–	129.3	129.3	–	–
-9′	–	–	–	128.6	128.6	–	–

Measured in pyridine-d_5 solution at 35 °C. *a*),*b*): Interchangeable in each column. *c*): Overlapping with the pyridine-d_5 signal.

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Table 2. ^{13}C-NMR Spectral Data of the Sugar Moiety

	1	**2**	**4**	**5**	**8**	**10**	**14**	**16**	**20**	**21**	**22**	**24**	**25**	**29**	**32**	**32′**
Carbon No.	Cym	Cym	Cym	Dig	Cym	Cym	Cym	Cym	Cym	Cym	Dig	Dig	Cym	Cym	Cym	Cym
C-1′	96.5	96.5	96.4	96.4	96.4	96.5	96.5	96.5	96.4	96.5	95.9	96.4	96.5	96.4	96.5	96.2
-2′	37.3[a]	37.3[a]	37.3[a]	38.7[a]	37.3	37.3	37.3[a]	37.3[a]	37.4[a]	37.7[a]	37.1	39.0	37.3[a]	37.3[a]	37.8[a]	35.4[a]
-3′	77.9[b]	77.9[b]	78.0[b]	67.6[b]	78.1	78.2[a]	78.0[b]	78.0[b]	78.0[b]	78.1[b]	66.6[a]	67.5	78.1[b]	78.0[b]	77.9	77.2
-4′	83.4[c]	83.4[c]	83.4[c]	83.5[c]	83.5[a]	83.5[b]	83.4[c]	83.4[c]	83.4[c]	83.4[c]	82.8[b]	83.4[a]	83.4[c]	83.4[c]	82.8[b]	82.8[b]
-5′	69.1[d]	69.1[d]	69.1[d]	68.6[d]	69.1	69.1	69.1[d]	69.0	69.1	69.1[d]	68.0	68.6	69.1[d]	69.1[d]	69.0	68.5
-OMe′	58.9	58.9	58.9[e]	–	58.9	58.9	58.9[e]	59.0[d]	58.9	58.9	–	–	58.9	58.9[e]	58.9	58.3
	Cym	Cym	Cym	Dig	Dig	Dig	Cym	Cym	Dig	Cym	Ole	Cym	Cym	Cym	Ole	Ole
C-1″	100.5	100.5	100.4	99.8	100.5	100.5	100.5[f]	100.5	100.5	100.5	100.4[c]	99.7	100.5	100.5	102.0	101.5
-2″	37.3[a]	37.3[a]	37.0[a]	39.1[a]	38.9[b]	38.8[c]	37.0[a]	37.2[a]	38.9	37.3[a]	36.3	36.8	37.1[a]	37.0[a]	37.3[a]	36.4[a]
-3″	78.0[b]	78.1[b]	78.2[b]	67.5[b]	67.5	67.5	78.1[b]	78.0[b]	67.5	77.8[b]	78.8	77.7	77.8[b]	77.8[b]	78.9	78.9
-4″	83.2[c]	83.2[c]	83.1[c]	83.1[c]	83.3[a]	83.2[b]	83.3[c]	83.2[c]	83.2[c]	83.2[c]	82.3	83.3[a]	83.2[c]	83.3[c]	83.3[b]	82.8[b]
-5″	69.0[d]	69.0[d]	69.4[d]	68.6[d]	68.7[c]	68.6	69.0[d]	69.0	68.5	69.0[d]	71.4	69.1	68.9[d]	68.9[d]	71.8	71.2
-OMe″	58.9	58.9	58.8[e]	–	–	–	58.9[e]	58.9[d]	–	58.9	56.8	58.9	58.9	58.8[e]	57.5[c]	56.6[c]
	Ole	Ole	The	Dig	Dig	Dig	Dig	Dig	Cym	Ole	Dig	Ole	Ole	Ole	Dig	Dig
C-1‴	102.2	102.2	106.2	99.8	99.9	99.9	100.6[f]	100.5	99.8	102.0	98.5	101.9	101.9	101.9	98.5	98.5
-2‴	37.0[a]	37.0[a]	75.1	38.6[a]	38.7[b]	38.7[c]	38.9	39.0	37.2[a]	37.4[a]	37.1	37.6	37.6[a]	37.6[a]	39.0	37.1
-3‴	81.4	81.4	87.9	67.5[b]	67.5	67.5	67.5	67.5	77.8[b]	79.1	66.7[a]	79.3	79.2	79.2	67.7	66.7
-4‴	76.3	76.2	75.9	83.1[c]	83.1[a]	83.1[b]	83.2[c]	83.2[b]	83.0[c]	82.7	82.7[b]	83.2[a]	83.1[c]	83.2[c]	83.5	82.5[b]
-5‴	73.0	73.0	72.8	68.7[d]	68.6[c]	68.6	68.6	68.5	69.1	71.7	68.3	72.0[b]	72.1	72.1	68.7	68.2
-OMe‴	57.0	57.0	60.9	–	–	–	–	–	58.9	57.3[e]	–	57.4	57.2	57.3	–	–
				Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	The	The	The	Ole	Ole
C-1⁗	–	–	–	101.4	101.6	101.4	101.6	101.4	102.2	100.3	100.3[c]	104.0	104.1	104.0	101.4	100.4
-2⁗	–	–	–	37.2	37.0	37.2	37.0	37.1	36.8	37.2[a]	35.3	75.0	75.3	75.0	37.2	36.0[a]
-3⁗	–	–	–	79.3	81.4	79.3	81.4	79.3	81.4	81.7	80.5	86.4	88.2	86.3	79.4	80.5
-4⁗	–	–	–	83.2[c]	76.2	83.1[b]	76.2	83.2[c]	76.2	76.4	75.3	83.2[a]	76.0	83.2[c]	83.2[b]	75.3
-5⁗	–	–	–	72.1	73.0	72.1	73.0	72.1	73.0	73.0	71.9	72.1[b]	72.9	72.1	72.1	71.8
-OMe⁗	–	–	–	57.3	57.0	57.3	57.1	57.2	57.0	57.1[e]	56.4	60.6	60.9	60.6	57.2[c]	56.4[c]
				Glc		Glc		Glc				Glc		Glc	Glc	
C-1⁗′	–	–	–	104.4	–	104.5	–	104.5	–	–	–	104.8	–	104.8	104.4	–
-2⁗′	–	–	–	75.7	–	75.7	–	75.7	–	–	–	75.9	–	75.9	75.7	–
-3⁗′	–	–	–	78.7	–	78.7	–	78.7	–	–	–	78.7	–	78.7	78.7	–
-4⁗′	–	–	–	72.1	–	72.1	–	72.1	–	–	–	72.1[b]	–	72.1	72.1	–
-5⁗′	–	–	–	78.2	–	78.1[a]	–	78.2[b]	–	–	–	78.0	–	78.0	78.2	–
-6⁗′	–	–	–	63.2	–	63.2	–	63.2	–	–	–	63.2	–	63.2	63.2	–
-6s	18.7	18.7	18.6×2	18.8	18.6×3	18.8	18.6×3	18.8	18.7	18.8	18.4	18.8	18.8	18.7×2	18.8	18.4
	18.6×2	18.6×2	18.5	18.7	18.5	18.6	18.4	18.6×2	18.6	18.7	18.2×2	18.7×2	18.6	18.6	18.7×2	18.2×2
				18.5×2		18.5×2		18.4	18.5×2	18.6	17.9	18.4	18.5×2	18.5	18.6	17.9
										18.5						

Cym: *β*-D-cymaropyranosyl, Ole: *β*-D-oleandropyranosyl, Dig: *β*-D-digitoxopyranosyl, The: *β*-D-thevetopyranosyl, Glc: *β*-D-glucopyranosyl. Measured in pyridine-d_5 solution at 35 °C except for **22** and **32′**. **22** and **32′** were measured in $CDCl_3$ solution at 35 °C. *a*—*f*): Interchangeable in each column.

The acquired sugars were fractionated to cymarose, oleandrose and digitoxose by silica gel column chromatography. The absolute configurations of these monosaccharides were believed to have a D-form based on their optical rotation values.[6,11,12] The absolute configurations of glucose and thevetose were determined to be a D-form based on GC analysis following their reaction with D-cysteine methyl ester hydrochloride (see Experimental).

Compounds **2** and **3** were suggested to have the molecular formulae, $C_{44}H_{70}O_{15}$ and $C_{42}H_{68}O_{14}$, respectively, based on the FAB-MS spectra. According to the consistency between the ^{1}H- and ^{13}C-NMR spectral data of the sugar moieties in **2** and **3**, these compounds were considered to have the same sugar sequence. In the ^{1}H- and ^{13}C-NMR spectra of **2**, three anomeric proton and carbon signals were observed at δ 5.37, 5.13, 4.77 and δ 96.5, 100.5, 102.2, together with signals due to the aglycone, which was identified as 12-*O*-acetyllineolon (**34**)[1] on acid hydrolysis with 0.05 N HCl. In comparison of the ^{13}C-NMR spectral data of **2** with that of 12-*O*-acetyllineolon,[1] glycosylation shifts were observed at the C-2, -3, and -4 positions [C-2 (−2.1 ppm), C-3 (+6.1 ppm), C-4 (−4.0 ppm)].[13] Therefore, **2** was glycosylated at the C-3 position, and **2** was considered to be 12-*O*-acetyllineolon 3-*O*-trioside. Acid hydrolysis of **2** showed the sugar moiety was composed of cymarose and oleandrose, and these sugars were identified as *β*-D-cymaropyranose and *β*-D-oleandropyranose as judged from the *J* values of the anomeric proton signals (J=9.5, 2.0 Hz). Moreover, the ^{1}H- and ^{13}C-NMR spectral data of the sugar moiety in **2** were consistent with those of cynanchoside C_2[14] and 12-*O*-nicotinoyllineolon 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**1**).[5] Thus, the sugar sequence of **2** was determined to be 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside and the structure of **2** was shown as presented in Chart 1. Because acid hydrolysis of **3** afforded isolineolon as the aglycone moiety, the structure of **3** was determined to be isolineolon 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside.

The following compounds, **4**, **5**, **7**—**22** and **24**—**32**, were also glycosylated at the C-3 position of each aglycone based on the observation of glycosylation shifts in the ^{13}C-NMR spectra.

Compound **4** exhibited the molecular formula $C_{51}H_{74}O_{16}$

Table 3. ^{1}H-NMR Spectral Data of the Sugar Moiety

	1	**2**	**4**	**5**
Proton No.	Cym	Cym	Cym	Dig
H-1′	5.28 (dd, 9.5, 2.0)	5.27 (dd, 9.5, 2.0)	5.27 (dd, 9.5, 2.0)	5.46 (dd, 9.5, 2.0)
-3′	4.10 (q, 3.0)	4.09 (q, 3.0)	4.07[a]	4.63 (q, 3.0)
-4′	3.53[a]	3.52 (dd, 9.5, 3.0)	3.49 (dd, 9.5, 3.0)	3.51[a]
-5′	4.23 (dd, 9.5, 6.5)	4.22 (dq, 9.5, 6.5)	4.21 (dq, 9.5, 6.5)	4.29[a]
-6′	1.40 (d, 6.5)	1.39 (d, 6.5)	1.39 (d, 6.5)	1.42 (d, 6.5)
	Cym	Cym	Cym	Dig
H-1″	5.13 (dd, 9.5, 2.0)	5.13 (dd, 9.5, 2.0)	5.12 (dd, 9.5, 2.0)	5.36 (dd, 9.5, 2.0)
-3″	4.07 (q. 3.0)	4.06 (q, 3.0)	4.07[a]	4.62 (q, 3.0)
-4″	3.50[a]	3.50 (dd, 9.5, 3.0)	3.59 (dd, 9.5. 3.0)	3.44 (dd, 9.5, 3.0)
-5″	4.20 (dq, 9.5, 6.5)	4.19 (dq, 9.5, 6.5)	4.21 (dq, 9.5, 6.5)	4.25[a]
-6″	1.42 (d, 6.5)	1.42 (d, 6.5)	1.60 (d, 6.5)	1.33 (d, 6.5)
	Ole	Ole	The	Dig
H-1‴	4.78 (d, 6.5)	4.77 (dd, 9.5, 2.0)	4.76 (d, 8.0)	5.36 (dd, 9.5, 2.0)
-2‴			3.91[a]	
-3‴			3.61[a]	4.58 (q, 3.0)
-4‴			3.61[a]	3.41 (dd, 9.5, 3.0)
-5‴			3.72 (m)	4.27[a]
-6‴	1.58 (d, 6.5)	1.57 (d, 6.5)	1.58 (d, 6.5)	1.35 (d, 6.5)
				Ole
H-1⁗	—	—	—	4.72 (dd, 9.5, 2.0)
-2⁗	—	—	—	
-3⁗	—	—	—	3.63[a]
-4⁗	—	—	—	3.63[a]
-5⁗	—	—	—	3.65[a]
-6⁗	—	—	—	1.64 (d, 6.0)
				Glc
H-1⁗′	—	—	—	5.09 (d, 8.0)
-2⁗′	—	—	—	3.98 (t,8.0)
-3⁗′	—	—	—	4.20[a,b]
-4⁗′	—	—	—	4.17 (t, 8.0)[b]
-5⁗′	—	—	—	3.93 (m)
-6⁗′	—	—	—	4.52 (dd, 11.5, 2.5)
	—	—	—	4.33 (dd, 11.5, 5.5)
-OMe	3.63 (s)	3.62 (s)	3.89 (s)	3.52 (s)
	3.58 (s)	3.58 (s)	3.62 (s)	
	3.47 (s)	3.47 (s)	3.57 (s)	

Cym: β-D-cymaropyranosyl, Ole: β-D-oleandropyranosyl, Dig: β-D-digitoxopyranosyl, The: β-D-thevetopyranosyl, Glc: β-D-glucopyranosyl. Measured in pyridine-d_5 solution at 35 °C except for **20**—**22** and **32′**. **20**—**22** and **32′** were measured in $CDCl_3$ solution at 35 °C. *a*) Overlapping with other signals or H_2O signal. *b*) Interchangeable in each column.

on FAB-MS. Acid hydrolysis of **4** suggested that **4** consists of ikemagenin, cymarose and thevetose as the aglycone and sugar moieties. In the ^{13}C- and ^{1}H-NMR spectra of **4**, three anomeric carbon and three proton signals were observed at δ 96.4, 100.4, 106.2 and δ 5.27, 5.12, 4.76, two double-doublet signals at δ 5.27 and 5.12 were assigned to the anomeric protons of β-D-cymaropyranose, and the remaining doublet signal at δ 4.76 belonged to the anomeric proton of β-D-thevetopyranose. The sugar sequence of **4** was determined on the basis of difference in NOE experiments. Irradiation at the H-1′ signal of β-D-cymaropyranose (δ 5.27) exhibited a NOE to the H-3 signal of the aglycone (δ 3.85). Similarly, NOEs were observed as follows, δ 5.12 (H-1″ of β-D-cymaropyranose) and δ 3.49 (H-4′ of β-D-cymaropyranose), δ 4.76 (H-1‴ of β-D-thevetopyranose) and δ 3.59 (H-4″ of β-D-cymaropyranose). Thus, the structure of **4** was established as ikemagenin 3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. This oligosaccharide sugar chain was confirmed in stephanoside E and H from *Stephanotis lutchuenis var. japonica*.[15]

The molecular formula of compound **5** was $C_{61}H_{90}O_{23}$ based on FAB-MS. Because the ^{1}H- and ^{13}C-NMR spectra of **5** showed five anomeric proton and carbon signals at δ 5.46, 5.36×2, 4.72, 5.09 and δ 96.4, 99.8×2, 101.4, 104.4, along with signals due to ikemagenin, **5** was presumed to be ikemagenin 3-*O*-pentaoside. The ^{1}H- and ^{13}C-NMR spectral data of the sugar moiety in **5** were identified with those of lineolon 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside,[1] so that the structure of **5** was determined to be ikemagenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside.

The molecular formulae of compounds **7**—**9** were suggested to be $C_{56}H_{84}O_{18}$, $C_{52}H_{82}O_{18}$ and $C_{54}H_{80}O_{18}$, respectively, based on FAB-MS. Based on acid hydrolysis, ^{1}H- and ^{13}C-NMR spectral data, these compounds were supposed to be pregnane 3-*O*-tetraosides whose aglycones were 5α,6-dihydroikemagenin (**37**) on **7**, 12-*O*-tigloylisolineolon (**38**) on **8** and 12-*O*-benzoylisolineolon (**39**) on **9**, and the sugar moiety contained one β-D-cymaropyranose, two β-D-digitoxopyranose and one β-D-oleandropyranose. Compounds **7**—**9** possessed the same sugar moieties as ikemagenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside (**6**)[1] due

Table 3. (continued)

	8	10	14	16	20	21
Proton No.	Cym	Cym	Cym	Cym	Cym	Cym
H-1′	5.28 (dd, 9.5, 2.0)	5.27 (dd, 9.5, 2.0)	5.28 (dd, 9.5, 2.0)	5.27 (dd, 9.5, 2.0)	4.85 (dd, 9.5, 2.0)	4.85 (dd, 9.5, 2.0)
-3′	4.09 (q, 3.0)	4.09 (q, 3.0)	4.08 (q, 3.0)	4.07 (q, 3.0)	3.82 (q, 3.0)	3.81 (q, 3.0)
-4′	3.52 (dd, 9.5, 3.0)	3.52[a)]	3.50 (dd 9.5, 3.0)	3.50[a)]	3.23 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
-5′	4.22 (dq, 9.5, 6.5)	4.22[a)]	4.22 (dq, 9.5, 6.5)	4.21 (dq, 9.5, 6.5)	3.85 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)
-6′	1.38 (d, 6.5)	1.38 (d, 6.5)	1.40 (d, 6.5)	1.39 (d, 6.5)	1.22 (d, 6.5)	1.22 (d, 6.5)
Dig	Dig	Cym	Cym	Dig	Cym	
H-1″	5.32 (dd, 9.5, 2.0)	5.31 (dd, 9.5, 2.0)	5.12 (dd, 9.5, 2.0)	5.12 (dd, 9.5, 2.0)	4.83 (dd, 9.5, 2.0)	4.75 (dd, 9.5, 2.0)
-3″	4.64 (br s)	4.62 (q, 3.0)	4.09 (q, 3.0)	4.07 (q, 3.0)	4.22 (br s)	3.78 (q, 3.0)
-4″	3.48 (br d, 9.5)	3.47 (dd, 9.0, 3.0)	3.48 (dd, 9.5, 3.0)	3.48 (dd, 9.5, 3.0)	3.20 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
-5″	4.24 (dq, 9.5, 6.5)	4.24[a)]	4.17 (dq, 9.5, 6.5)	4.16 (dq, 9.5, 6.5)	3.77 (dq, 9.5, 6.5)	3.86 (dq, 9.5, 6.5)
-6″	1.38 (d, 6.5)	1.38 (d, 6.5)	1.33 (d, 6.5)	1.33 (d, 6.5)	1.22 (d, 6.5)	1.22 (d, 6.5)
	Dig	Dig	Dig	Dig	Cym	Ole
H-1‴	5.38 (dd, 9.5, 2.0)	5.37 (dd, 9.5, 2.0)	5.32 (dd, 9.5, 2.0)	5.30 (dd, 9.5, 2.0)	4.82 (dd, 9.5, 2.0)	4.45 (dd, 9.5, 2.0)
-2‴						
-3‴	4.64 (br s)	4.59 (q, 3.0)	4.64 (q, 3.0)	4.59 (q, 3.0)	3.81 (q, 3.0)	
-4‴	3.48 (br d, 9.5)	3.41 (dd, 9.5, 3.0)	3.51 (dd, 9.5, 3.0)	3.43 (dd, 9.5, 3.0)	3.22 (dd, 9.5, 3.0)	3.17 (t, 8.5)
-5‴	4.30 (dq, 9.5, 6.5)	4.28 (dq, 9.5, 6.5)	4.30 (dq, 9.5, 6.5)	4.27 (dq, 9.5, 6.5)	3.91 (dq, 9.5, 6.5)	3.31 (dq, 8.5, 6.0)
-6‴	1.40 (d, 6.5)	1.36 (d, 6.5)	1.46 (d, 6.5)	1.42 (d, 6.5)	1.22 (d, 6.5)	1.30 (d, 6.0)
	Ole	Ole	Ole	Ole	Ole	Ole
H-1⁗	4.80[a)]	4.72 (dd, 9.5, 2.0)	4.82[a)]	4.73 (dd, 9.5, 2.0)	4.50 (dd, 9.5, 2.0)	4.72 (dd. 9.5, 2.0)
-2⁗						
-3⁗	3.44[a)]	3.63[a)]	3.45[a)]	3.63[a)]		
-4⁗	3.41[a)]	3.63[a)]	3.42[a)]	3.65[a)]	3.15[a)]	
-5⁗	3.58 (dq, 8.5, 6.0)	3.64[a)]	3.60 (dq, 9.0, .6.5)	3.64[a)]	3.29 (dq, 9.0, 6.0)	3.31 (dq, 9.0, 6.0)
-6⁗	1.50 (d, 6.0)	1.64 (d, 6.0)	1.51 (d, 6.5)	1.65 (d, 6.0)	1.32 (d, 6.0)	1.35 (d, 6.0)
		Glc		Glc		
H-1⁗′	—	5.10 (d, 8.0)	—	5.10 (d, 8.0)	—	—
-2⁗′	—	3.99 (t, 8.0)	—	3.99 (t, 8.0)	—	—
-3⁗′	—	4.20[a,b)]	—	4.20[a,b)]	—	—
-4⁗′	—	4.17 (t, 8.0)[b)]	—	4.17[a,b)]	—	—
-5⁗′	—	3.94 (m)	—	3.93 (m)	—	—
-6⁗′	—	4.52 (dd, 11.5, 2.5)	—	4.52 (dd, 11.5, 2.5)	—	—
	—	4.33 (dd, 11.5, 5.5)	—	4.33 (dd, 11.5, 5.5)	—	—
-OMes	3.63 (s)	3.62 (s)	3.63 (s)	3.62 (s)×2	3.46 (s)	3.45 (s)
	3.46 (s)	3.53 (s)	3.62 (s)	3.52 (s)	3.45 (s)	3.44 (s)
			3.46 (s)		3.39 (s)	3.40 (s)×2

to good agreement of the ^{1}H- and ^{13}C-NMR spectral data of the oligosaccharide moieties. Thus, the structures of **7**—**9** were established as shown in Chart 1.

The FAB-MS spectra revealed that the molecular formulae of compounds **10**—**13** were $C_{62}H_{92}O_{23}$, $C_{62}H_{94}O_{23}$, $C_{58}H_{92}O_{23}$ and $C_{60}H_{90}O_{23}$. ^{1}H- and ^{13}C-NMR spectra and acid hydrolysis suggested that **10**—**13** possessed **36**—**39** as each aglycone moiety, and shared the same oligosaccharide sequence which consisted of one *β*-D-cymaropyranose, two *β*-D-digitoxopyranose, one *β*-D-oleandropyranose and one *β*-D-glucopyranose. As the ^{1}H- and ^{13}C-NMR spectral data of the sugar moieties in **10**—**13** agreed with those of lineolon 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-cymaropyranoside,[1)] the sugar sequences of **10**—**13** were determined to be 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-cymaropyranoside. Moreover, enzymatic hydrolysis with cellulase afforded **6**—**9** from **10**—**13**, respectively.

Compounds **14**—**19** had the molecular formulae, $C_{57}H_{84}O_{18}$, $C_{57}H_{86}O_{18}$, $C_{63}H_{94}O_{23}$, $C_{63}H_{96}O_{23}$, $C_{59}H_{94}O_{23}$ and $C_{61}H_{92}O_{23}$ as determined by FAB-MS. By acid hydrolysis, compounds **14**—**19** produced ikemagenin from **14** and **16**, **37** from **15** and **17**, **38** from **18** and **39** from **19** as the aglycone moieties. Cymarose, digitoxose and oleandrose were also yielded from **14**—**19**, and additionally, **16**—**19** afforded glucose. The sugar linkages of these compounds were determined by comparing the ^{1}H- and ^{13}C-NMR spectral data of the known compounds in the aerial part of this plant. The NMR spectral data of the sugar moiety in **14**, **15** and **16**—**19** were identified with those of isolineolon 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside[1)] and 15*β*-hydroxylineolon *β*-D-glucopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside,[1)] respectively. Therefore, the structures of **14**—**19** were determined as presented in Chart 1. Moreover, the enzymatic hydrolysis of **16** and **17** produced **14** and **15**, respectively, and the ^{1}H-NMR spectral data of the sugar moieties of the derivatives which were produced from **18** and **19** by enzymatic hydrolysis were consistent with those of **14**.

The molecular formula of compound **20** was considered to be $C_{57}H_{84}O_{18}$ on FAB-MS. On the basis of acid hydrolysis and the NMR spectral data, **20** was deduced to be ikemagenin 3-*O*-tetraoside whose sugar moiety consisted of two *β*-D-cymaropyranose, one *β*-D-digitoxopyranose and one *β*-D-oleandropyranose, the same as that of **14**. However, in the ^{1}H- and ^{13}C-NMR spectral measurement of pyridine-d_5 solu-

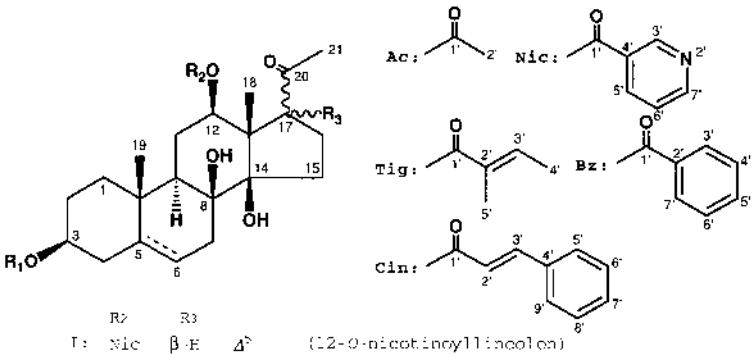
Table 3. (continued)

	22	**24**	**25**	**29**	**32**	**32′**
Proton No.	Dig	Dig	Cym	Cym	Cym	Cym
H-1′	4.93 (dd, 9.5, 2.0)	5.47 (dd, 9.5, 2.0)	5.28 (dd, 9.5, 2.0)	5.28 (dd, 9.5, 2.0)	5.28 (dd, 9.5, 2.0)	4.85 (dd, 9.5, 2.5)
-3′	4.22 (br s)	4.62 (q, 3.0)	4.09 (q, 3.0)	4.09 (q, 3.0)	4.05 (q, 3.0)	3.79 (q, 3.0)
-4′	3.20 (dd, 9.5, 3.0)	3.50[a)]	3.51[a)]	3.51[a)]	3.51[a)]	3.21 (dd, 9.5, 3.0)
-5′	3.80 (dq, 9.5, 6.5)	4.28 (dq, 9.5, 6.5)	4.22 (dq, 9.5, 6.5)	4.22[a)]	4.23 (dq, 9.5, 6.5)	3.87 (dq, 9.5, 6.5)
-6′	1.24 (d, 6.5)	1.43 (d, 6.5)	1.38 (d, 6.5)	1.39 (d, 6.5)	1.44 [a)]	1.22 (d, 6.5)
	Ole	Cym	Cym	Cym	Ole	Ole
H-1″	4.50 (dd, 9.5, 2.0)	5.16 (dd, 9.5, 2.0)	5.12 (dd, 9.5, 2.0)	5.12[a)]	4.70 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)
-3″	3.32[a)]	4.00[a)]	4.02 (q, 3.0)	4.02[a)]	3.59[a)]	3.35[a)]
-4″	3.19 (t, 8.5)	3.39 (dd, 9.5, 3.0)	3.44 (dd, 9.5, 3.0)	3.43 (dd, 9.5, 3.0)	3.54[a)]	3.19 (t, 8.5)
-5″	3.32[a)]	4.17 (dq, 9.5, 6.5)	4.17 (dq, 9.5, 6.5)	4.16 (dq, 9.5, 6.5)	3.52[a)]	3.28 (dq, 8.5, 6.0)
-6″	1.28 (d, 6.5)	1.31 (d, 6.5)	1.39 (d, 6.5)	1.37 (d, 6.5)	1.44[a)]	1.28 (d, 6.0)
	Dig	Ole	Ole	Ole	Dig	Dig
H-1‴	5.01 (dd, 9.5, 2.0)	4.66 (br d, 9.5)	4.69 (dd, 9.5, 2.0)	4.68 (dd, 9.5, 2.0)	5.48 (dd, 9.5, 2.0)	5.00 (dd, 9.5, 2.0)
-2‴						
-3‴	4.22 (br s)	3.55[a)]	3.58[a)]	3.57[a)]	4.61 (q, 3.0)	4.23 (q, 3.0)
-4‴	3.21 (dd, 9.5, 3.0)	3.61 (t, 8.0)	3.67 (t, 8.5)	3.59[a)]	3.45 (dd, 9.5, 3.0)	3.22 (dd, 9.5, 3.0)
-5‴	3.83 (dq, 9.5, 6.5)	3.55[a)]	3.58[a)]	3.57[a)]	4.29 (dq, 9.5, 6.5)	3.82 (dq, 9.5, 6.5)
-6‴	1.27 (d, 6.5)	1.66 (d, 6.0)	1.70 (d, 6.0)	1.66 (d, 6.5)	1.44[a)]	1.27 (d, 6.5)
	Ole	The	The	The	Ole	Ole
H-1⁗	4.55 (dd, 9.5, 2.0)	4.87[a)]	4.95 (d, 8.0)	4.87 (d, 8.0)	4.73 (dd, 9.5, 2.0)	4.55 (dd, 9.5, 2.0)
-2⁗		3.89 (t, 8.5)	3.91[a)]	3.89[a)]		
-3⁗	3.15[a)]	3.68 (t, 8.5)	3.60[a)]	3.68 (t, 8.5)	3.65[a)]	3.16 (m)
-4⁗	3.12 (t, 8.5)	3.86 (t, 8.5)	3.60[a)]	3.86 (t, 8.5)	3.65[a)]	
-5⁗	3.32[a)]	3.74 (dq, 8.5, 6.0)	3.72 (dq, 8.5, 6.0)	3.74 (dq, 8.5, 6.5)	3.65[a)]	3.32 (dq, 8.5, 6.0)
-6⁗	1.31 (d, 6.5)	1.75 (d, 6.0)	1.59 (d, 6.0)	1.75 (d, 6.5)	1.65 (d, 6.0)	1.31 (d, 6.0)
		Glc		Glc	Glc	
H-1⁗′	—	5.12 (d, 8.0)	—	5.12 (d, 8.0)	5.10 (d, 8.0)	—
-2⁗′	—	4.01 (t, 8.0)	—	4.02[a)]	3.99 (t, 8.0)	—
-3⁗′	—	4.20[a)]	—	4.20[a)]	4.19[a,b)]	—
-4⁗′	—	4.20[a)]	—	4.20[a)]	4.17[a,b)]	—
-5⁗′	—	3.95 (m)	—	3.95 (m)	3.93 (m)	—
-6⁗′	—	4.51 (dd, 11.5, 2.5)	—	4.51 (dd, 11.5, 2.5)	4.52 (dd, 11.5, 2.5)	—
	—	4.33 (dd, 11.5, 5.5)	—	4.34 (dd, 11.5, 5.5)	4.33 (dd, 11.5, 5.5)	—
-OMes	3.41 (s)	3.93 (s)	3.89 (s)	3.93 (s)	3.58 (s)	3.40 (s)×2
	3.40 (s)	3.57 (s)	3.62 (s)	3.62 (s)	3.56 (s)	3.45 (s)
		3.50 (s)	3.58 (s)	3.57 (s)	3.51 (s)	
			3.53 (s)	3.50 (s)		

tion (see Table 2 and Experimental), chemical shifts of the anomeric proton signals were slightly different from those of **14**. Assignments of the proton signals of the sugar moiety are shown in Table 3 based on the ^{1}H–^{1}H COSY spectrum starting from the anomeric proton signals. In the NOE difference experiments with irradiation at the anomeric proton signals, NOEs were observed as follows, δ 4.85 (H-1′ of β-D-cymaropyranose) and 3.57 (H-3 of the aglycone), δ 4.83 (H-1″ of β-D-digitoxopyranose) and 3.23 (H-4′ of β-D-cymaropyranose), δ 4.82 (H-1‴ of β-D-cymaropyranose) and 3.20 (H-4″ of β-D-digitoxopyranose), δ 4.50 (H-1⁗ of β-D-oleandropyranose) and 3.22 (H-4‴ of β-D-cymaropyranose). Consequently, **20** was proved to be ikemagenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside.

The NMR spectra of compounds **21** and **22** were suggested to be ikemagenin 3-*O*-tetraosides. Acid hydrolysis afforded cymarose and oleandrose from **21** and digitoxose and oleandrose from **22** as the component sugars. According to identification of the NMR spectral data of each sugar moiety with those of calotroposide E[16] and lineolon 3-*O*-β-oleandropyranosyl-(1→4)-β-digitoxopyranosyl-(1→4)-β-oleandropyranosyl-(1→4)-β-digitoxopyranoside,[17] the structures of **21** and **22** were shown as presented in Chart 1.

Compounds **24**, **29**—**31** and **25**—**28** were supposed to be pregnane 3-*O*-pentaosides and pregnane 3-*O*-tetraosides, respectively, by observation of anomeric proton and carbon signals in the NMR spectra. Acid hydrolysis of **24**—**31** yielded **36** from **25** and **29**, **38** from **24**, **27** and **30**, **39** from **28** and **31** and **34** from **26**; in addition, monosaccharides were obtained as follows: digitoxose, cymarose, oleandrose, thevetose and glucose from **24**, cymarose, oleandrose, and thevetose from **25**—**28**, and cymarose, oleandrose, thevetose and glucose from **29**—**31**. Because the ^{1}H- and ^{13}C-NMR spectral data of the oligosaccharide moieties in these compounds were identified with those of pregnane glycosides from the aerial part of this plant,[2] metaplexigenin 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, 15β-hydroxylineolon 3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and 15β-hydroxylineolon 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, the structures of **24**—**31** were determined to be as shown in Chart 1. Also **25**, **27** and **28** were obtained from **29**—**31** by enzymatic hydrolysis, respectively.



	R2	R3		
I:	Nic	β-H	Δ5	(12-O-nicotinoyllineolon)
II:	Ac	β-H	Δ5	(12-O-acetyllineolon)
III:	H	α-H	Δ5	(isolineolon)
IV:	Cin	β-H	Δ5	(ikemagenin)
V:	Cin	β-H	5α,6-H	(5α,6-dihydroikemagenin)
VI:	Tig	α-H	Δ5	(12-O-tigloylisolineolon)
VII:	Bz	α-H	Δ5	(12-O-benzoylisolineolon)

R1

a: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Cym-(1→4)-β-D-Ole
b: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Cym-(1→4)-β-D-The
c: (agly.-(1→3)-β-D-Dig-(1→4)-β-D-Dig-(1→4)-β-D-Dig-(1→4)-β-D-Glc
d: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Dig-(1→4)-β-D-Dig-(1→4)-β-D-Ole
e: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Dig-(1→4)-β-D-Dig-(1→4)-β-D-Ole-(1→4)-β-D-Glc
f: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Cym-(1→4)-β-D-Dig-(1→4)-β-D-Ole
g: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Cym-(1→4)-β-D-Dig-(1→4)-β-D-Ole-(1→4)-β-D-Glc
h: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Dig-(1→4)-β-D-Cym-(1→4)-β-D-Ole
i: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Ole-(1→4)-β-D-Ole
j: (agly.-(1→3)-β-D-Dig-(1→4)-β-D-Glc-(1→4)-β-D-Dig-(1→4)-β-D-Ole
k: (agly.-(1→3)-β-D-Dig-(1→4)-β-D-Ole-(1→4)-β-D-Dig-(1→4)-β-D-Ole-(1→4)-β-D-Glc
l: (agly.-(1→3)-β-D-Dig-(1→4)-β-D-Cym-(1→4)-β-D-Ole-(1→4)-β-D-The-(1→4)-β-D-Glc
m: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Cym-(1→4)-β-D-Ole-(1→4)-β-D-The
n: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Cym-(1→4)-β-D-Ole-(1→4)-β-D-The-(1→4)-β-D-Glc
o: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Ole-(1→4)-β-D-Dig-(1→4)-β-D-Ole-(1→4)-β-D-Glc
p: (agly.-(1→3)-β-D-Cym-(1→4)-β-D-Ole-(1→4)-β-D-Dig-(1→4)-β-D-Ole

(agly.: aglycone, Dig: digitoxopyranosyl, Cym: cymaropyranosyl, Ole: oleandropyranosyl, The: thevetopyranosyl, Glc: glucopyranosyl)

1:	I	a	11:	V	e	21:	IV -i
2:	II	-a	12:	VI	-e	22:	IV -j
3:	III	-a	13:	VII	e	23:	IV -k
4:	IV	b	14:	IV	f	24:	VI -l
5:	IV	-c	15:	V	-f	25:	IV -m
6:	IV	-d	16:	IV	-g	26:	II -m
7:	V	-d	17:	V	-g	27:	VII -m
8:	VI	d	18:	VI	g	28:	VII -m
9:	VII	-d	19:	VII	-g	29:	IV n
10:	IV	-e	20:	IV	h	30:	VI n

31:	VII	n	
32:	IV	o	
32':	IV	-p	
33:	I	H	
34:	II	H	
35:	III	-H	
36:	IV	-H	
37:	V	-H	
38:	VI	-H	
39:	VII	-H	

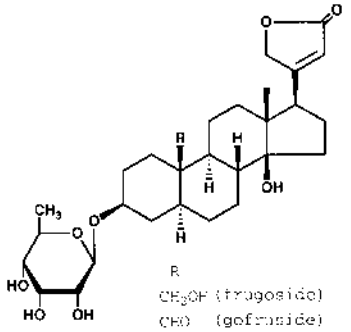


Chart 1

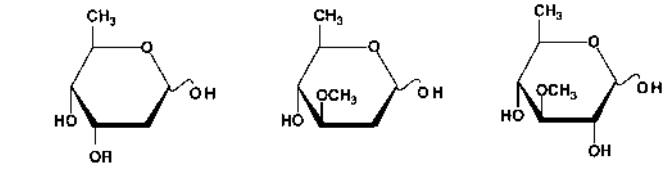


Chart 2

Compound **32** was considered to be ikemagenin 3-*O*-pentaoside whose molecular formula was $C_{63}H_{94}O_{23}$, the same as that of **16** on the basis of acid hydrolysis and FAB-MS. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **32** with those of **16** revealed that the sugar sequence of **32** was composed of one *β*-D-cymaropyranose, two *β*-D-oleandropyranose, one *β*-D-digitoxopyranose and one *β*-D-glucopyranose, in which *β*-D-glucopyranose existed as the terminal sugar. On enzymatic hydrolysis of **32** with cellulase, **32** yielded **32′**. The ^{1}H- and ^{13}C-NMR spectra suggested that **32′** was ikemagenin 3-*O*-tetraoside which lost the terminal *β*-D-glucopyranose in the sugar sequence of **32**. The ^{1}H–^{1}H COSY experiment of **32′** starting from each anomeric proton signal enabled us to assign the proton signals due to each monosaccharide in Table 3. On the NOE difference experiments involving irradiation at each anomeric proton signal, NOEs were observed between *δ* 4.85 (H-1′ of *β*-D-cymaropyranose) and 3.56 (H-3 of the aglycone), *δ* 4.44 (H-1″ of *β*-D-oleandropyranose) and 3.21 (H-4′ of *β*-D-cymaropyranose), *δ* 5.00 (H-1‴ of *β*-D-digitoxopyranose) and 3.19 (H-4″ of *β*-D-oleandropyranose), *δ* 4.55 (H-1⁗ of *β*-D-oleandropyranose) and 3.22 (H-4‴ of *β*-D-digitoxopyranose). Then, the sugar sequence of **32′** was

determined to be 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside. Furthermore, in the NOE difference experiment of **32**, irradiation of the anomeric proton signal of *β*-D-glucopyranose (δ 5.10) showed a NOE on the H-4'''' signal of *β*-D-oleandropyranose (δ 3.65). Based on the above evidence, the structure of **32** was elucidated as presented in Chart 1.

Experimental

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

Extraction and Isolation The root of *Asclepias incarnata* L. (870 g) were extracted twice with MeOH under reflux. The extract was concentrated under reduced pressure and the residue was suspended in H_2O. This suspension was extracted with Et_2O. The H_2O layer was passed through a Mitsubishi Diaion HP-20 column, and adsorbed material was eluted with 50% MeOH in water, 70% MeOH in water and MeOH. The Et_2O layer, 70% MeOH eluate and MeOH eluate of the HP-20 column were concentrated, respectively. These residues were then rechromatographed on a silica gel column with a $CHCl_3$–MeOH (98 : 2—85 : 15) system and semi-preparative HPLC (Develosil-ODS, PhA, C-8 and YMC-ODS: 45—62.5% MeCN in water and 75—82.5% MeOH in water) to give compounds **1** (4 mg), **2** (8 mg), **3** (5 mg), **4** (4 mg), **5** (11 mg), **6** (24 mg), **7** (7 mg), **8** (3 mg), **9** (3 mg), **10** (28 mg), **11** (4 mg), **12** (9 mg), **13** (9 mg), **14** (23 mg), **15** (5 mg), **16** (206 mg), **17** (10 mg), **18** (13 mg), **19** (10 mg), **20** (3 mg), **21** (7 mg), **22** (5 mg), **23** (16 mg), **24** (4 mg), **25** (22 mg), **26** (6 mg), **27** (6 mg), **28** (11 mg), **29** (16 mg), **30** (15 mg), **31** (10 mg), and **32** (6 mg) in addition to frugoside (83 mg) and gofruside (51 mg). All compounds were obtained as amorphous powders.

Compound **2**: $[\alpha]_D^{25}$ −20.9° (*c*=0.75, MeOH). FAB-MS *m/z*: 861 $[M+Na]^+$. ^{13}C- and ^1H-NMR: shown in Tables 1—3.

Compound **3**: $[\alpha]_D^{25}$ +39.7° (*c*=0.53, MeOH). FAB-MS *m/z*: 797 $[M+H]^+$, 819 $[M+Na]^+$. ^{13}C-NMR: shown in Table 1. The ^{13}C- and ^1H-NMR spectra of the sugar moiety were in good agreement with those of **2**.

Compound **4**: $[\alpha]_D^{25}$ +19.3° (*c*=0.29, MeOH). FAB-MS *m/z*: 965 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.24), 223 (4.18), 278 (4.42). ^{13}C- and ^1H-NMR: shown in Tables 1—3.

Compound **5**: $[\alpha]_D^{25}$ +7.7° (*c*=1.13, MeOH). FAB-MS *m/z*: 1213 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.13), 222 (4.06), 278 (4.32). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **7**: $[\alpha]_D^{25}$ +12.7° (*c*=0.64, MeOH). FAB-MS *m/z*: 1067 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.21), 223 (4.15), 278 (4.39). ^{13}C-NMR: shown in Table 1. The ^{13}C- and ^1H-NMR spectra of the sugar moiety were in good agreement with those of **8**.

Compound **8**: $[\alpha]_D^{25}$ +32.9° (*c*=0.31, MeOH). FAB-MS *m/z*: 995 $[M+H]^+$, 1017 $[M+Na]^+$. ^{13}C- and ^1H-NMR: shown in Tables 1—3.

Compound **9**: $[\alpha]_D^{25}$ +30.0° (*c*=0.32, MeOH). FAB-MS *m/z*: 1039 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 230 (4.14), 274 (3.28), 280 (3.23). ^{13}C-NMR: shown in Table 1. The ^{13}C- and ^1H-NMR spectra of the sugar moiety were in good agreement with those of **8**.

Compound **10**: $[\alpha]_D^{25}$ +12.3° (*c*=1.20, MeOH). FAB-MS *m/z*: 1227 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 216 (4.14), 222 (4.07), 278 (4.32). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **11**: $[\alpha]_D^{25}$ +13.1° (*c*=0.42, MeOH). FAB-MS *m/z*: 1229 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.25), 278 (4.37). The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **7** and **10**.

Compound **12**: $[\alpha]_D^{25}$ +24.1° (*c*=0.89, MeOH). FAB-MS *m/z*: 1179 $[M+Na]^+$. The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **8** and **10**.

Compound **13**: $[\alpha]_D^{25}$ +24.7° (*c*=0.88, MeOH). FAB-MS *m/z*: 1201 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 228 (4.10), 274 (3.42). The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **9** and **10**.

Compound **14**: $[\alpha]_D^{25}$ +17.1° (*c*=0.97, MeOH). FAB-MS *m/z*: 1079 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 216 (4.19), 222 (4.12), 278 (4.36). The ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **15**: $[\alpha]_D^{25}$ +15.8° (*c*=0.49, MeOH). FAB-MS *m/z*: 1081 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.18), 222 (4.12), 278 (4.36). The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **7** and **14**.

Compound **16**: $[\alpha]_D^{25}$ +15.1° (*c*=1.26, MeOH). FAB-MS *m/z*: 1241 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 216 (4.17), 222 (4.08), 278 (4.35). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **17**: $[\alpha]_D^{24}$ +13.3° (*c*=0.87, MeOH). FAB-MS *m/z*: 1243 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 202 (4.31), 217 (4.23), 222 (4.18), 278 (4.36). The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **7** and **16**.

Compound **18**: $[\alpha]_D^{25}$ +26.4° (*c*=1.33, MeOH). FAB-MS *m/z*: 1193 $[M+Na]^+$. The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **8** and **16**.

Compound **19**: $[\alpha]_D^{25}$ +27.1° (*c*=0.99, MeOH). FAB-MS *m/z*: 1215 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 229 (4.31), 273 (3.25). The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **9** and **16**.

Compound **20**: $[\alpha]_D^{25}$ +17.5° (*c*=0.28, MeOH). FAB-MS *m/z*: 1079 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.21), 223 (4.15), 278 (4.39). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. ^1H-NMR (pyridine(Py)-d_5 at 35 °C): δ 5.32 (dd, 9.5, 2.0, H-1″ of *β*-D-digitoxopyranose), 5.28 (dd, 9.5, 2.0, H-1′ of *β*-D-cymaropyranose), 5.16 (dd, 9.5, 2.0, H-1‴ of *β*-D-cymaropyranose), 4.76 (dd, 9.5, 2.0, H-1'''' of *β*-D-oleandropyranose). The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **21**: $[\alpha]_D^{24}$ +3.6° (*c*=0.65, MeOH). FAB-MS *m/z*: 1093 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.24), 223 (4.17), 278 (4.40). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **22**: $[\alpha]_D^{25}$ −2.1° (*c*=0.46, MeOH). FAB-MS *m/z*: 1065 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 202 (4.40), 217 (4.25), 222 (4.18), 277 (4.41). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **24**: $[\alpha]_D^{25}$ +20.1° (*c*=0.36, MeOH). FAB-MS *m/z*: 1209 $[M+Na]^+$. ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with those of **8**.

Compound **25**: $[\alpha]_D^{25}$ +10.3° (*c*=1.34, MeOH). FAB-MS *m/z*: 1109 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 216 (4.23), 222 (4.16), 278 (4.40). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **26**: $[\alpha]_D^{25}$ −17.9° (*c*=0.64, MeOH). FAB-MS *m/z*: 1021 $[M+Na]^+$. The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **2** and **25**.

Compound **27**: $[\alpha]_D^{25}$ +24.7° (*c*=0.60, MeOH). FAB-MS *m/z*: 1061 $[M+Na]^+$. The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **8** and **25**.

Compound **28**: $[\alpha]_D^{25}$ +24.2° (*c*=1.12, MeOH). FAB-MS *m/z*: 1083 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 229 (4.11), 278 (2.99), 280 (2.93). The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **9** and **25**.

Compound **29**: $[\alpha]_D^{25}$ +10.7° (*c*=0.61, MeOH). FAB-MS *m/z*: 1271 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.25), 222 (4.19), 278 (4.44). ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Compound **30**: $[\alpha]_D^{25}$ +22.1° (*c*=1.12, MeOH). FAB-MS *m/z*: 1223 $[M+Na]^+$. The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **8** and **29**.

Compound **31**: $[\alpha]_D^{25}$ +24.0° (*c*=1.01, MeOH). FAB-MS *m/z*: 1245 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 230 (4.16), 273 (2.93). The ^{13}C- and ^1H-NMR spectra of the aglycone moiety and the sugar moiety were in good agreement with those of **9** and **29**.

Compound **32**: $[\alpha]_D^{25}$ +1.4° (*c*=0.53, MeOH). FAB-MS *m/z*: 1241 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 217 (4.23), 222 (4.15), 278 (4.38). The ^{13}C- and ^1H-NMR: shown in Tables 2 and 3. The ^{13}C-NMR spectrum of the aglycone moiety was in good agreement with that of **4**.

Acid Hydrolysis of a Mixture of Pregnane Glycosides The fraction of pregnane glycosides eluted from the $CHCl_3$–MeOH (96 : 4) system on a silica gel column (600 mg) was heated at 60 °C for 5 h with dioxane (8 ml) and 0.2 N H_2SO_4 (2 ml) to obtain the aglycones and sugars. After hydrolysis, this reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was concentrated to dryness. Purification of the residue by HPLC (YMC-ODS, 67.5% MeOH in water) afforded two new 12-*O*-acylated pregnanes (**37** (5 mg) and **38** (2 mg)) along with isolineolon (**35**, 6 mg)[7)], 12-*O*-acetyllineolon (**34**, 7 mg)[1)], 12-*O*-nicotinoyllineolon (**33**, 2

mg),[5] ikemagenin (**36**, 30 mg)[8] and 12-*O*-benzoylisolineolon (**39**, 3 mg).[9]

35: ^{1}H-NMR (Py-d_5 at 35 °C): δ 5.43 (br s, H-6), 3.90 (m, H-3), 3.88 (dd, 9.5, 5.5, H-17), 3.77 (dd, 12.0, 4.5, H-12), 2.29 (s, H-21), 1.67 (dd, 13,5, 2.5, H-9), 1.57 (s, H-18), 1.48 (s, H-19).

37: $[\alpha]_D^{24}$ −16.5° (c=0.44, MeOH), FAB-MS *m/z*: 519 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 204 (4.25), 217 (4.24), 278 (4.40). ^{13}C-NMR (Py-d_5 at 35 °C): δ 209.5 (C-20), 165.9 (C-1′), 144.8 (C-3′), 135.1 (C-4′), 130.5 (C-7′), 129.3 (C-6′, 8′), 128.6 (C-5′, 9′), 119.4 (C-2′), 87.6 (C-14), 76.5 (C-8), 74.0 (C-12), 70.8 (C-3), 60.5 (C-17), 56.3 (C-13), 47.6 (C-9), 45.9 (C-5), 38.9 (C-1), 38.4 (C-4), 36.7 (C-10), 35.1 (C-7), 33.9 (C-15), 32.4 (C-21), 32.0 (C-2), 25.4 (C-6), 24.1 (C-11), 22.5 (C-16), 16.2 (C-18), 13.3 (C-19). ^{1}H-NMR (Py-d_5 at 35 °C): δ 7.97 (d, 16.0, H-3′), 6.78 (d, 16.0, H-2′), 5.86 (s, 14-OH), 5.22 (dd, 10.0, 6.0, H-12), 4.30 (s, 8-OH), 3.92 (m, H-3), 3.52 (t, 9.5, H-17), 2.28 (s, H-21), 2.00 (s, H-18), 1.71 (q, 11.5, H-4ax), 1.43 (dd, 12.0, 4.0, H-9), 1.27 (s, H-19), 1.16 (overlapping, H-5).

38: $[\alpha]_D^{24}$ +40.8° (c=0.20, MeOH). FAB-MS *m/z*: 447 $[M+H]^+$, 469 $[M+Na]^+$. ^{13}C-NMR ($CDCl_3$ at 35 °C): δ 217.4 (C-20), 167.8 (C-1′), 139.2 (C-5), 137.8 (C-3′), 128.8 (C-2′), 118.8 (C-6), 86.3 (C-14), 75.9 (C-12), 73.9 (C-8), 72.0 (C-3), 57.7 (C-17), 54.2 (C-13), 44.4 (C-9), 42.2 (C-4), 38.9 (C-1), 37.0 (C-10), 37.0, 35.6 (C-7, 15), 33.0 (C-21), 31.2 (C-2), 24.7, 24.0 (C-11, 16), 18.2 (C-19), 14.5 (C-4′), 12.1, 12.0 (C-18, 5′). ^{13}C-NMR (Py-d_5 at 35 °C): δ 214.3 (C-20), 167.8 (C-1′), 140.2 (C-5), 137.7 (C-3′), 129.4 (C-2′), 118.7 (C-6), 86.6 (C-14), 77.7 (C-12), 74.4 (C-8), 71.6 (C-3), 59.2 (C-17), 55.1 (C-13), 45.2 (C-9), 43.4 (C-4), 39.3 (C-1), 37.6 (C-10), 36.7, 35.9 (C-7, 15), 32.1 (C-2), 31.6 (C-21), 24.8, 24.6 (C-11, 16), 18.5 (C-19), 14.3 (C-4′), 12.7 (C-18), 12.3 (C-5′). ^{1}H-NMR (Py-d_5 at 35 °C): δ 7.15 (br q, 7.0, H-3′), 5.53 (s, 14-OH), 5.38 (br s, H-4), 5.09 (dd, 12.0, 4.0, H-12), 4.51 (s, 8-OH), 3.88 (m, H-3), 3.22 (dd, 9.5, 5.5, H-17), 2.25 (s, H-21), 1.99 (br s, H-5′), 1.72 (br d, 7.0, H-4′), 1.56 (s, H-18), 1.42 (s, H-19).

39: $[\alpha]_D^{24}$ +35.0° (c=0.33, MeOH). FAB-MS *m/z*: 491 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 230 (4.18), 273 (3.06). ^{13}C-NMR ($CDCl_3$ at 35 °C): δ 217.3 (C-20), 166.4 (C-1′), 139.1 (C-5), 133.3, 130.2 (C-5′, 2′), 129.6 (C-4′, 6′), 128.6 (C-3′, 7′), 118.8 (C-6), 86.4 (C-14), 77.2 (C-12), 73.9 (C-8), 72.0 (C-3), 57.8 (C-17), 54.3 (C-13), 44.4 (C-9), 42.1 (C-4), 38.9 (C-1), 37.1 (C-10), 37.1, 35.6 (C-7, 15), 33.0 (C-2), 31.1 (C-21), 24.7, 24.1 (C-11, 16), 18.2 (C-19), 12.2 (C-18). ^{13}C-NMR (Py-d_5 at 35 °C): δ 214.1 (C-20), 166.6 (C-1′), 140.2 (C-5), 133.6, 131.4 (C-5′, 2′), 130.0 (C-4′, 6′), 129.1 (C-3′, 7′), 118.7 (C-6), 86.6 (C-14), 78.6 (C-12), 74.4 (C-8), 71.6 (C-3), 59.3 (C-17), 55.1 (C-13), 45.2 (C-9), 43.4 (C-4), 39.3 (C-1), 37.7 (C-10), 36.7, 35.9 (C-7, 15), 32.1 (C-21), 31.7 (C-2), 24.8, 24.7 (C-11, 16), 18.5 (C-19), 12.7 (C-18). ^{1}H-NMR (Py-d_5 at 35 °C): δ 8.37 (br d, 7.5, H-3′, 7′), 7.60 (br t, 7.5, H-5′), 7.53 (br t, 7.5, H-4′, 6′), 5.57 (s, 14-OH), 5.39 (br s, H-6), 5.27 (dd, 12.0, 4.0, H-12), 4.59 (s, 8-OH), 3.89 (m, H-3), 3.27 (dd, 9.5, 5.5, H-17), 2.20 (s, H-21), 1.78 (dd, 13.5, 2.5, H-9), 1.66 (s, H-18), 1.44 (s, H-19).

The H_2O layer was passed through an Amberlite IRA-60E column and the eluate was concentrated to dryness. The residue was chromatographed on a silica gel with a $CHCl_3$–MeOH–H_2O (7 : 1 : 1.2 bottom layer) system to obtain cymarose (7 mg), oleandrose (12 mg), and digitoxose (25 mg). As to the absolute configuration of each monosaccharide, all of these monosaccharides were believed to have a D-form based on their optical rotation values.[6,11,12]

D-Cymarose: $[\alpha]_D^{26}$ +52.1° (c=0.67, 24 h after dissolution in H_2O). (lit: $[\alpha]_D^{21}$ +51.6° (c=1.02, H_2O)).[11]

D-Oleandrose: $[\alpha]_D^{26}$ −12.3° (c=1.15, 24 h after dissolution in H_2O). (lit: $[\alpha]_D$ −11° (c=1.1, H_2O)).[12]

D-Digitoxose: $[\alpha]_D^{26}$ +43.8° (c=0.71, 24 h after dissolution in H_2O). (lit: $[\alpha]_D^{24}$ +48.4° (c=0.90, H_2O)).[6]

This H_2O layer also produced a disaccharide, thevetopyranosyl-(1→4)-oleandropyranoside (6 mg). Part of this disaccharide (*ca*. 0.5 mg) was hydrolyzed with 0.05 N HCl–dioxane (1 : 1) at 95 °C for 1.5 h, then the residue was reacted with D-cysteine methyl ester hydrochloride, hexamethyldisilazane and trimethylsilylchloride using the same procedure described in a previous report.[1,2,18,19] After a series of reactions, the precipitate was centrifuged and the supernatant was subjected to GC analysis. GC conditions: column, GL capillary column TC-1 (GL Sciences, Inc., Tokyo, Japan) 0.32 mm×30 m, carrier gas N_2, 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.

Alkaline Hydrolysis of Compounds 37 and 38 Solutions of compounds **37** and **38** (*ca*. 0.3 mg) were hydrolyzed with 2 N NaOH aq. and dioxane (each 40 μl) at 60 °C for 2 h in N_2 atmosphere. After alkaline hydrolysis, the reaction mixture was diluted with H_2O, then 1 N HCl (*ca*. 100 μl) was added to them. Next, the ester moiety was extracted with diethylether. HPLC analysis suggested that cinnamic acid, tiglic acid and benzoic acid were yielded from **37** and **38**, respectively. Conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min, 50% MeOH in water+0.05% trifluoroacetic acid (TFA); t_R, cinnamic acid 18.0 min, 40% MeOH in water+0.05% TFA; t_R, tiglic acid 13.4 min.

Acid Hydrolysis of a Mixture of Pregnane Glycosides to Determine the Configuration of Glucose The fraction of pregnane glycosides eluted from the $CHCl_3$–MeOH (9 : 1) system formed a silica gel column (*ca*. 10 mg) which was heated at 95 °C for 1.5 h with dioxane and 0.05 N HCl (10 drops each). After hydrolysis, the reaction mixture was passed through an Amberlite IRA-60E column, and the eluate was evapolated under reduced pressure. The residue was partitioned with H_2O and EtOAc, then the H_2O layer was concentrated to dryness. This residue was stirred with D-cysteine methyl ester hydrochloride, hexamethyldisilazane and trimethylsilylchloride in pyridine, as described above. After the reactions, the supernatant was subjected to GC analysis. GC conditions: column, GL capillary column TC-1 (GL Sciences, Inc.) 0.32 mm×30 m, carrier gas N_2, column temperature 210 °C; t_R D-glucose 18.8 min, L-glucose 17.8 min. D-Glucose was detected from the mixture of pregnane glycosides.

Acid Hydrolysis of Compounds 2—5, 7—22 and 23—32 Solutions of compounds **2**—**5**, **7**—**22** and **23**—**32** (*ca*. 0.5 mg) in dioxane and 0.05 N HCl (2 drops each) were heated at 95 °C for 1.5 h. The following procedures after hydrolysis for the detection of the aglycone and the monosaccharides of each compound were described in previous papers.[1,2] The acquired aglycone and monosaccharides were analyzed with HPLC and GC, respectively. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min, 47.5% MeOH in water; t_R isolineolon (**35**) 9.4 min, 50% MeOH in water; t_R 12-*O*-acetyllineolon (**34**) 14.0 min, 70% MeOH in water; t_R ikemagenin (**36**) 12.8 min, 5α,6-dihydroikemagenin (**37**) 14.8 min, 12-*O*-tigloylisolineolon (**38**) 14.0 min, 12-*O*-benzoylisolineolon (**39**) 17.0 min; GC conditions: column, Supelco SP-2380™ capillary column 0.25 mm×30 m, carrier gas N_2, column temperature 200 °C; t_R cymaritol acetate 7.9 min, oleandritol acetate 8.9 min, digitoxitol acetate 11.5 min, column temperature 215 °C; t_R thevetitol acetate 11.5 min, column temperature 250 °C; t_R glucitol acetate 13.1 min.

Enzymatic Hydrolysis of Compounds 5, 10—13, 16—19, 23 and 29—32 Compounds **5**, **10**—**13**, **16**—**19**, **23** and **29**—**32** (*ca*. 2 mg) were dissolved in H_2O (0.7 ml), then cellulase (Sigma Chem. Co.) (*ca*. 20—30 mg) was added. The mixture was stirred at 40 °C for 3 or 4 d. After hydrolysis, the reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc extract of each compound, except for **18**, **19** and **32**, contained ikemagemin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-digitoxopyranoside,[1] **6**—**9**, **14**, **15**, **22**, **25**, **27** and **28**, whose structures were confirmed by comparison of their ^{1}H-NMR spectra and HPLC analysis with those of authentic sample. In the ^{1}H-NMR spectra of the products from **18** and **19** by enzymatic hydrolysis, signals due to the sugar moiety were consistent with those of **14**.

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