Pregnane Glycosides from the Roots of *Asclepias tuberosa*[1)]

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Sixteen glycosides of pregnanes, including ikemagenin, lineolon, and a new pregnane, 3β,8β,14β,15β,16α-pentahydroxy-5α-pregnan-20-one, termed pleurogenin, were isolated from the roots of *Asclepias tuberosa*. Among ikemagenin and lineolon glycosides, one (1) was a known glycoside, and eight (2—7, 10, 13) were glycosides with new combinations of ikemagenin or lineolon and known sugar sequences composed of D-cymarose, D-oleandrose, D-thevetose and D-glucose. The structures of four new glycosides of ikemagenin (8, 9, 11, 12) and three of pleurogenin (14—16) were determined. The new glycosides have sugar sequences ranging from tetraoside to heptaosides.

Key words pregnane glycoside; 3β,8β,14β,15β,16α-pentahydroxy-5α-pregnan-20-one; *Asclepias tuberosa*; Asclepiadaceae; pleurisy root; ikemagenin

During our investigations on the constituents of Asclepiadaceous plants, the isolations and structure determinations of pregnane glycosides from *Asclepias fruticosa*,[2)] *Marsdenia tomentosa*,[3)] *Hoya carnosa*[4)] and *Tylophora tanakae*[5)] were reported. In the preceding paper, we described the isolation of cardenolide glycosides including Δ^5-calotropin 3′-*O*-glucoside and two Δ^5-calotropin derivatives having a spiro-type linkage of thiazolidinone at the 3′-carbon, from the roots of *Asclepias tuberosa* L., which was used to treat pleurisy and bronchitis in North America and called "pleurisy root."[1)] This paper deals with the pregnane glycosides from these roots.

When the roots were percolated with MeOH, the pregnane glycosides and cardenolide glycosides were roughly partitioned in the benzene- and $CHCl_3$-soluble fractions, respectively, from the MeOH extract. Prior to the isolation of individual pregnane glycoside, a portion of the benzene fraction was subjected to acid hydrolysis, in order to identify the component sugars and pregnanes. The sugars were identified as D-oleandrose (Ole), D-cymarose (Cym), D-digitoxose (Dgt), D-canarose (Can), D-thevetose (Thv), and D-glucose (Glc) along with two bioses, strophanthobiose (β-D-glucosyl-D-cymarose) and glucosyl-oleandrose by direct comparisons with authentic sugars on TLC, ^{1}H-NMR and optical rotation data. All sugar linkages in the glycosides were assigned to be in the β-form based on the coupling constants of the anomeric protons as shown in Table 3. From the pregnane fraction in the hydrolysate, two pregnanes (**a-1**, **a-2**) were obtained, and **a-1** was identified as ikemagenin by direct comparison with an authentic sample.[2)]

Pregnane **a-2** was suggested to have the molecular formula, $C_{21}H_{34}O_6$, based on high resolution (HR)-FAB-MS. The NMR spectra showed the presence of an acetyl side-chain at C-17 by the signals at δ_C 32.1 (q), 213.4 (s), and δ_H 2.28 (3H, s). The methine carbon signal at δ 70.9 was assigned to C-17 by 3-bond correlation with the H-21 proton signal in the heteronuclear multiple bond connectivity (HMBC) spectrum. The proton signal of H-17 (δ 3.12, d, J=5 Hz) was confirmed by the ^{1}H–^{13}C shift correlation spectroscopy (COSY) spectrum. The spin system from H-17 was observed at H-16 (δ 4.92, dd, J=6, 5 Hz) and then H-15 (δ 4.80, d, J=6 Hz) in the ^{1}H–^{1}H COSY spectrum. Corresponding tertiary carbon signals were observed at δ 81.8 and δ 81.9, respectively, suggesting the presence of hydroxy groups at C-15 and C-16, along with one at C-14. The presence of two quaternary carbons bearing oxygen was observed at δ 83.7 and 77.0, and the former signal was assigned to be C-14 by 3-bond correlations with H-17 and H-18, while the latter one was C-8, based on the coupling pattern of H-9 (br d, J=11 Hz). The three hydroxy groups at C-8, 14 and C-15 were assigned to have a β-orientation from the nuclear Overhauser effect (NOE) between H-9 and H-15. A 17*R*-configuration (17β-acetyl) and a 16α-OH configuration were confirmed by NOEs between H-21 and H-18, H-16, as well as the coupling constant (J=5 Hz) between H-16β and H-17α. The stereochemistry at C-5 was assigned to be 5α-H, based on shielding of the C-19 signal to δ 13.4. The remaining hydroxy group was considered to be 3β-OH, since a methine proton signal was observed at δ 3.86 in axial mode. Pregnane **a-2**, 3β,8β,14β,15β,16α-pentahydroxy-5α-pregnan-20-one, is a new compound and was called pleurogenin.

Isolation of the glycosides from the benzene extract was carried out using silica gel and octadecyl silica (ODS) column chromatography and preparative HPLC, to afford sixteen pregnane glycosides along with a small amount of free cardenolides.[1)] The aglycone of **1**—**12** was assigned to be ikemagenin based on the NMR data. Glycoside **1** was identical to compound **3** isolated from *Cynanchum caudatum*.[6)] The sugar sequences in **2**—**7** and **10** were considered to be those represented in Chart 1 based on the difference (DIF)-NOE and/or HMBC methods. They are previously known sequences in the glycosides of pregnanes except ikemagenin, obtained from Asclepiadaceae plants,[6,7)] and their structures were finally determined by comparison of the NMR signals from the sugar moieties.

Glycoside **8** was considered to have the molecular formula, $C_{68}H_{102}O_{26}$, based on HR-FAB-MS. The NMR spectra suggested that the sugar moiety in **8** was composed of 2 moles each of Dgt and Cym, along with one each of Can and terminal Glc. In DIF-NOE, the H-1 signals in Glc, outer Cym, outer Dgt, Can, and inner Cym showed correlations to H-4 in outer Cym, outer Dgt, Can, inner Cym, and inner Dgt, respectively. The signal of H-3 showed a response by irradiation of H-1 in inner Dgt. Furthermore, the H-4 signals of outer Cym, outer Dgt, Can, inner Cym, and inner Dgt showed cross peaks to the C-1 signals of Glc, outer Cym,

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Table 1. NMR Spectral Data for the Aglycone Moieties of **1**—**16** and **a-2** [δ ppm in Pyridine-d_5, J in Hz]

No.	**1**—**12**		**13**		**14**—**16**		**a-2**	
	C[a]	H[b]	C	H	C	H	C	H
1	38.9		39.0		38.3		38.5	
2	29.8		29.9		29.6		32.0	
3	77.6	3.85 (m)	77.7	3.86 (m)	76.6	3.86 (m)	70.7	3.86 (m)
4	39.2		39.3		34.5		38.9	
5	139.4		139.5		45.0	0.98 (m)	45.5	1.12 (m)
6	119.1	5.30 (br d, 4)	119.3	5.32 (br d, 4)	25.3		25.4	
7	35.1		34.5		34.9	2.11 (br d, 14, β)	35.0	2.13 (dt, 14,3, β)
8	74.5		74.5		77.0		77.0	
9	44.8		45.1		50.7	1.34 (br d, 11)	50.8	1.39 (br d, 11)
10	37.5		37.5		36.7		36.8	
11	24.9		29.3		18.3		18.4	
12	73.3	5.26 (dd, 12,4)	69.0	3.98 (dd, 12,4)	40.4	1.96 (br t, 12), 1.82 (br d, 12)	40.5	1.62 (td, 12,4), 2.12 (br d, 12)
13	55.8		57.9		47.5		47.5	
14	87.4		87.4		83.6		83.7	
15	34.1		35.3		81.9	4.77 (d, 5)	81.9	4.80 (d, 6)
16	21.9		22.1		81.8	4.91 (t, 5)	81.8	4.92 (dd, 6,5)
17	60.5		61.4		70.9	3.10 (d, 5)	70.9	3.12 (d, 5)
18	15.7	2.00 (s)	14.7	1.95 (s)	19.3	1.39 (s)	19.3	1.41 (s)
19	18.1	1.35 (s)	18.3	1.38 (s)	13.2	1.19 (s)	13.4	1.26 (s)
20	209.2		210.4		213.4		213.4	
21	32.1	2.27 (s)	32.0	2.42 (s)	32.1	2.27 (s)	32.1	2.28 (s)

a) 12-*O*-Cinnamoyl residue: δ 165.8 (C-1′), 119.2 (C-2′), 144.8 (C-3′), 135.0 (C-4′), 128.5 (C-5′, 9′), 129.2 (C-6′, 8′), 130.4 (C-7′). *b*) 12-*O*-Cinnamoyl residue: δ 6.78 (d, J=16 Hz, H-2′), 7.98 (d, J=16 Hz, H-3′), 7.34 (m, H-6′, 7′, 8′), 7.61 (br d, J=8 Hz, H-5′, 9′).

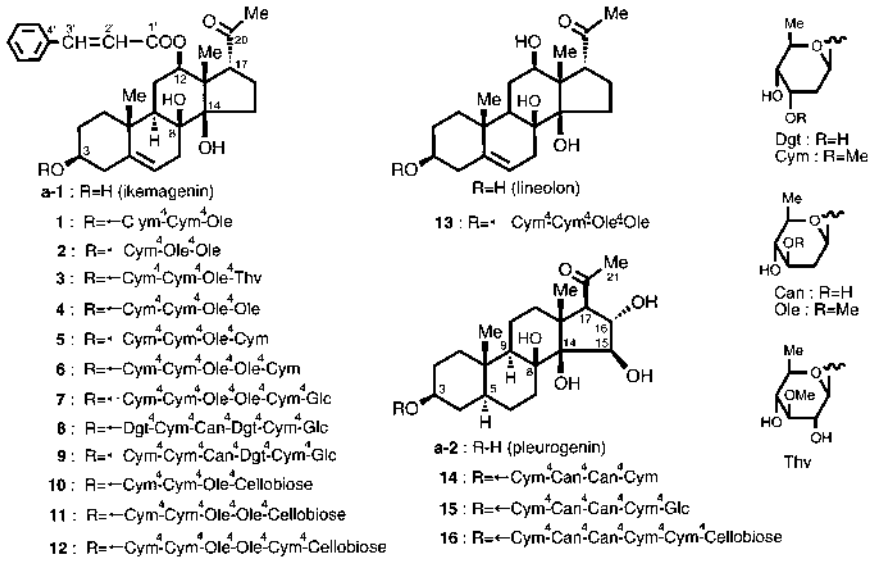

Chart 1

outer Dgt, Can, and inner Cym, respectively. Thus, **8** was determined to be ikemagenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranoside.

In HR-FAB-MS, **9** was suggested to have the molecular formula, $C_{69}H_{104}O_{26}$, one CH_2 greater than **8**. Based on the NMR data, the sugar moiety of **9** was composed of 3 moles of Cym and one mole each of Dgt, Can and terminal Glc, indicating the substitution of Cym for inner Dgt in **8**. The same correlations as in **8** were observed in DIF-NOE except for the NOE between Cym (inner) and H-3. In the HMBC spectrum, cross-peaks were observed between the H-4 signals and the corresponding C-1 signals in the connecting sugars with 3-bond relations. Consequently, **9** was identified as ikemagenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

The molecular formula of **11** was considered to be $C_{70}H_{106}O_{28}$, based on the HR-FAB-MS data, and the sugar moiety was composed of 2 moles each of Cym, Ole and Glc. In the same DIF-NOE and HMBC procedures as in **8** or **9**, the sugar moiety of **11** was determined to be the cellobioside of **4**, that is, β-cellobiosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Glycoside **12** ($C_{77}H_{118}O_{31}$) was suggested to be a heptao-

Table 2. ^{13}C-NMR Spectral Data for the Sugar Moieties (**8**, **9**, **11**, **12**, **14**—**16**) [δ ppm in Pyridine-d_5][a)]

C	8	9	11	12	14	15	16
Sug-1	Dgt(1)	Cym(1)	Cym(1)	Cym(1)	Cym(1)	Cym(1)	Cym(1)
1	96.3	96.4	96.4	96.4	95.8	95.8	95.8
2	38.9	37.2	37.2[b)]	37.8[b)]	37.2	37.2	37.3
3	67.3[b)]	77.9[b)]	78.0[c)]	78.0	77.9	77.9	77.5[b)]
4	83.1	83.3	83.3	83.3 (Cym(2)-1)	83.6 (Can(1)-1)	83.6	83.6
5	69.4	69.4[c)]	68.8	68.9[c)]	68.9	68.9	68.9
6	17.9[c)]	17.9[d)]	18.4[d)]	18.4[d)]	17.6	17.5[b)]	17.6[c)]
Sug-2	Cym(1)	Cym(2)	Cym(2)	Cym(2)	Can(1)	Can(1)	Can(1)
1	99.6 (Dgt(1)-4)	100.4 (Cym(1)-4)	100.4 (Cym(1)-4)	100.4	101.9	101.9 (Cym(1)-4)	101.9
2	36.8	37.0	37.0[b)]	37.6[b)]	39.8	39.8	39.8
3	77.7	77.8[b)]	77.7[c)]	77.7	69.7	69.7	69.7[d)]
4	83.3	83.1[e)]	83.1	83.1 (Ole(1)-1)	88.1 (Can(2)-1)	88.1 (Can(2)-1)	88.1
5	69.1[d)]	69.0[c)]	69.0	69.0[c)]	70.8	70.8	70.8
6	18.0[c)]	18.0[d)]	18.5[e)]	18.5[d)]	18.0	18.0[b)]	17.9[e)]
Sug-3	Can	Can	Ole(1)	Ole(1)	Can(2)	Can(2)	Can(2)
1	101.9 (Cym(1)-4)	101.9 (Cym(2)-4)	101.9 (Cym(2)-4)	101.9 (Cym(2)-4)	101.3 (Can(1)-4)	101.3 (Can(1)-4)	101.3
2	39.7	39.8	37.5[b)]	37.2[b)]	39.4	39.4	39.4
3	69.7	69.7	78.9	79.1[e)]	69.7	69.6	69.6[d)]
4	88.0	88.0 (Dgt-1)	82.6	82.7	87.6 (Cym(2)-1)	87.4 (Cym(2)-1)	87.5
5	70.9	70.9	72.0[e)]	71.8[d)]	71.2	71.2	71.2
6	18.3[c)]	18.3[d)]	18.6[d)]	18.5[d)]	18.4	18.1[b)]	18.0[c)]
Sug-4	Dgt(2)	Dgt	Ole(2)	Ole(2)	Cym(2)	Cym(2)	Cym(2)
1	99.7 (Can-4)	99.8 (Can-4)	99.9	100.1	99.7 (Can(2)-4)	99.6 (Can(2)-4)	99.7
2	38.4	38.4	37.5[b)]	36.9[b)]	35.5	36.3	36.7[e)]
3	67.4[b)]	67.2	79.7	79.0[e)]	78.5	77.6	77.8[b)]
4	82.5	82.5 (Cym(3)-1)	83.6 (Glc(1)-1)	82.6	73.7	82.5 (Glc-1)	82.5
5	68.5	68.8[c)]	71.5[e)]	71.5[f)]	71.4	69.9	69.5
6	18.5[c)]	18.5[d)]	18.8[d)]	18.6[d)]	18.6	18.6[b)]	18.5[c)]
Sug-5	Cym(2)	Cym(3)	Glc(1)	Cym(3)		Glc	Cym(3)
1	99.8 (Dgt(2)-4)	99.7	104.2 (Ole(2)-4)	98.4		106.5 (Cym(2)-4)	100.3
2	36.4	36.4	75.2	36.8[b)]		75.3	36.5[e)]
3	77.8	77.7[b)]	76.2	78.1		78.3	77.9[b)]
4	82.8	82.8[e)] (Glc-1)	81.7 (Glc(2)-1)	83.3 (Glc(1)-1)		71.8	83.0
5	69.0[d)]	69.0	76.9	69.6		78.4	69.3
6	18.6[c)]	18.5[d)]	62.5	18.7[d)]		63.0	18.6[c)]
Sug-6	Glc	Glc	Glc(2)	Glc(1)			Glc(1)
1	106.4 (Cym(2)-4)	106.5 (Cym(3)-4)	104.9 (Glc(1)-4)	106.1 (Cym(3)-4)			106.1
2	75.3	75.3	74.7	74.9			74.9
3	78.3	78.3	78.2	76.5			76.5
4	71.8	71.8	71.6	81.3 (Glc(2)-1)			81.3
5	78.4	78.4	78.4	76.4			76.4
6	63.0	63.0	62.5	62.4			62.4
Sug-7				Glc(2)			Glc(2)
1				104.9			104.9
2				74.7			74.7
3				78.2			78.2
4				71.6[f)]			71.5
5				78.5			78.4
6				62.4			62.4
OMe	58.6	58.6	57.2 (×2)	57.3	58.1	58.7 (×2)	58.0
	58.9	58.8	58.8 (×2)	57.4	58.7		58.8
		58.9		58.6			58.9
				58.8 (×2)			

a) Proton signals coupled *via* 3-bonds are shown in parentheses. *b*—*f*) Signal assignments may be interchangeable.

side. On comparing the NMR signals with those of **11**, **12** seemed to have one extra Cym. The presence of the β-cellobiosyl→Cym linkage and the array of Ole→Ole→Cym→Cym→ikemagenin in **12** were assigned by DIF-NOE and HMBC. Upon partial hydrolysis of **12** with cellulase, deglucosyl-**12** (**7**) and decellobiosyl-**12** (**6**) were observed on TLC. Therefore, the structure of **12** was determined to be ikemagenin 3-*O*-β-cellobiosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

HR-FAB-MS of **13** suggested the molecular formula, $C_{49}H_{80}O_{17}$, and no NMR signals due to a cinnamoyl residue were observed. The signal pattern of the aglycone moiety in **13** was similar to that of lineolon 3-*O*-glycoside.[8)] The chemical shifts and multiplicities due to the sugar moiety were identical with those of **4**, and **13** was assigned to be lineolon 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Since the signals due to the aglycone moieties in **14**—**16** were in good agreement with those of **a-2**, except for the deshielding of C-3 and shielding of C-2 and C-4, the sugar moieties were linked to the 3-OH of **a-2**. The molecular for-

Table 3. ^{1}H-NMR Spectral Data for the Sugar Moieties (**8**, **9**, **11**, **12**, **14**—**16**) [δ ppm in Pyridine-d_5, J in Hz]

H	**8**	**9**	**11**	**12**	**14**	**15**	**16**
H-3α	3.86 (m)[a)]	3.84 (m)[a)]	3.84 (m)[a,b')]	3.84 (m)[a,b')]	3.86 (m)	3.86 (m)	3.86 (m)[a')]
Sug-1	Dgt(1)	Cym(1)	Cym(1)	Cym(1)	Cym(1)	Cym(1)	Cym(1)
1	5.46 (dd, 10,2)[a')]	5.27 (dd, 10,2)[a')]	5.28 (dd, 10,2)[a',b')]	5.28 (dd, 10,2)[a,b)]	5.29 (dd, 10,2)	5.29 (dd, 10,2)	5.29 (dd, 10,2)[a)]
3	4.61 (br s)	4.08 (br q, 3)	4.09 (br q, 3)	4.09 (br q, 3)	4.07 (br q, 3)	4.07 (q, 3)	4.07 (br q, 3)
4	3.49 (dd, 9, 3)[b)]	3.51 (dd, 9, 3)[b)]	3.52 (dd, 9, 3)[c)]	3.54 (dd, 9, 3)	3.52 (dd, q, 3)[a)]	3.50 (dd, 9, 3)	3.52 (dd, 9, 3)[b)]
5	4.25 (dq, 9, 6)	4.21 (dq, 9, 6)	4.22 (dq, 9, 6)	4.23 (dq, 9, 6)	4.26 (dq, 9, 6)	4.24 (dq, 9, 6)	4.25 (dq, 9, 6)
6	1.43 (d, 6)	1.39 (d, 6)	1.40 (d, 6)	1.40 (d, 6)	1.45 (d, 6)	1.44 (d, 6)	1.45 (d, 6)
Sug-2	Cym(1)	Cym(2)	Cym(2)	Cym(2)	Can(1)	Can(1)	Can(1)
1	5.14 (dd, 10, 2)[b')]	5.11 (dd, 10, 2)[b')]	5.12 (dd, 10, 2)[c')]	5.13 (dd, 10, 2)	4.76 (dd, 10, 2)[a')]	4.76 (dd, 10, 2)	4.76 (dd, 10, 2)[b')]
3	4.01 (br q, 3)	4.01 (br q, 3)	4.02 (br q, 3)	4.03 (br q, 3)	3.93 (m)	3.93 (m)	3.92 (m)
4	3.38 (dd, 9, 3)[c)]	3.43 (dd, 9, 3)[c)]	3.46 (dd, 9, 3)[d)]	3.46 (dd, 9, 3)[c)]	3.27 (t, 9)[b)]	3.25 (t, 9)	3.26 (t, 9)[c)]
5	4.18 (dq, 9, 6)	4.17 (dq, 9, 6)	4.17 (dq, 9, 6)	4.18 (dq, 9, 6)	3.53 (dq, 9, 6)	3.53 (dq, 9, 6)	3.52 (dq, 9, 6)
6	1.30 (d, 6)	1.36 (d, 6)	1.39 (d, 6)	1.39 (d, 6)	1.40 (d, 6)	1.39 (d, 6)	1.39 (d, 6)
Sug-3	Can(2)	Can	Ole(1)	Ole(1)	Can(2)	Can(2)	Can(2)
1	4.72 (dd, 10, 2)[c')]	4.74 (dd, 10, 2)[c')]	4.69 (dd, 10, 2)[d')]	4.69 (dd, 10, 2)[c')]	4.82 (dd, 10, 2)[b')]	4.80 (dd, 10, 2)	4.79 (dd, 10, 2)[c')]
3	3.88 (m)	3.91 (m)	*	*	3.95 (m)	3.94 (m)	3.95 (m)
4	3.25 (t, 9)[d)]	3.26 (t, 9)[d)]	*	*	3.30 (t, 9)[c)]	3.25 (t, 9)	3.27 (t, 9)[d)]
5	3.50—3.55	3.50—3.55	*	*	3.63 (dq, 9, 6)	3.59 (dq, 9, 6)	3.60 (dq, 9, 6)
6	1.33 (d, 6)	1.34 (d, 6)	1.43 (d, 6)	1.43 (d, 6)	1.36 (d, 6)	1.31 (d, 6)	1.32 (d, 6)
Sug-4	Dgt(2)	Dgt	Ole(2)	Ole(2)	Cym(2)	Cym(2)	Cym(2)
1	5.27 (dd, 10, 2)[d')]	5.28 (dd, 10, 2)[d')]	4.89 (dd, 10, 2)	4.88 (dd, 10, 2)	5.05 (dd, 10, 2)[c')]	5.05 (dd, 10, 2)	5.07 (dd, 10, 2)[d')]
3	4.61 (br s)	4.61 (br q, 3)	*	*	3.75 (br q, 3)	4.12 (br q, 3)	4.04 (br q, 3)
4	3.42 (dd, 9, 3)[e)]	3.43 (dd, 9, 3)[e)]	3.68 (t, 9)[e)]		3.52 (dd, 9, 3)	3.64 (dd, 9, 3)	3.41 (dd, 9, 3)[e)]
5	4.30 (dq, 9, 6)	4.31 (dq, 9, 6)	*	*	4.16 (dq, 9, 6)	4.28 (dq, 9, 6)	4.19 (dq, 9, 6)
6	1.32 (d, 6)	1.33 (d, 6)	1.72 (d, 6)	1.43 (d, 6)	1.46 (d, 6)	1.56 (d, 6)	1.27 (d, 6)
Sug-5	Cym(2)	Cym(3)	Glc(1)	Cym(3)		Glc	Cym(3)
1	5.09 (dd, 10, 2)[e')]	5.10 (dd, 10, 2)[e')]	5.06 (d, 8)[e')]	5.278 (dd, 10, 2)		4.89 (d, 8)	5.04 (dd, 10, 2)[e')]
2	*	*	3.98 (dd, 8, 9)	*		3.96 (dd, 8, 9)	*
3	4.07 (br q, 3)	4.07 (br q, 3)	4.24 (t, 9)	4.07 (br q, 3)		4.21 (t, 9)	4.03 (br q, 3)
4	3.59 (dd, 9, 3)[f')]	3.60 (dd, 9, 3)[f')]	4.28 (t, 9)[f')]	3.60 (dd, 9, 3)[d)]		4.16 (t, 9)	3.58 (dd, 9, 3)[f')]
5	4.25 (dq, 9, 6)	4.25 (dq, 9, 6)	3.89 (m)	4.27 (dq, 9, 6)		3.95 (m)	4.22 (dq, 9, 6)
6	1.54 (d, 6)	1.54 (d, 6)	4.3, 4.5	1.62 (d, 6)		4.37 (dd, 12, 5)	1.59 (d, 6)
						4.56 (dd, 12, 2)	
Sug-6	Glc	Glc	Glc(2)	Glc(1)			Glc(1)
1	4.91 (d, 8)[f')]	4.91 (d, 8)[f')]	5.16 (d, 8)[f')]	4.87 (d, 8)[d')]			4.87 (d, 8)[f')]
2	3.98 (dd, 8, 9)	3.99 (dd, 8, 9)	4.07 (dd, 8, 9)	3.98 (dd, 8, 9)			3.98 (dd, 8, 9)
3	4.21 (t, 9)	4.22 (t, 9)	4.19 (t, 9)	4.23 (t, 9)			4.25 (t, 9)
4	4.15 (t, 9)	4.16 (t, 9)	4.16 (t, 9)	4.29 (t, 9)			4.26 (t, 9)[g)]
5	3.95 (m)	3.95 (m)	4.00 (m)	3.93 (m)			3.93 (m)
6	4.36 (dd, 12, 5)	4.37 (dd, 12, 4)	4.3, 4.5	4.3, 4.5			4.3, 4.5
	4.55 (dd, 12, 2)	4.56 (dd, 12, 2)					
Sug-7				Glc(2)			Glc(2)
1				5.18 (d, 8)			5.18 (d, 8)[g')]
2				4.09 (dd, 8, 9)			4.09 (dd, 8, 9)
3				4.19 (t, 9)			4.19 (t, 9)
4				4.17 (t, 9)			4.18 (t, 9)
5				4.00 (m)			4.00 (m)
6				4.3, 4.5			4.3, 4.5
OMe	3.51	3.52	3.49	3.50	3.46	3.52	3.50
	3.58	3.59	3.50	3.52	3.61	3.60	3.59
		3.62	3.58	3.53			3.60
			3.62	3.58			
				3.63			

* Overlapping with other signals. *a*—*g*) The signals showed response by irradiation of the corresponding *a'*—*g'*), respectively, in DIF-NOE.

mula of **14** was suggested to be $C_{47}H_{78}O_{18}$, based on HR-FAB-MS. The component sugars were 2 moles each of Cym and Can, and NOEs were observed between the H-1 signals of outer Cym, outer Can, inner Can/the H-4 signals of outer Can, inner Can, inner Cym, respectively. The linkages between four sugars were also confirmed by cross-peaks in the HMBC spectrum. The structure of **14** was, thus, assigned to be pleurogenin 3-*O*-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranoside.

The molecular formula of **15** was considered to be $C_{53}H_{88}O_{23}$, one hexose greater than **14**, and the NMR signals of **15** were observed to have almost similar chemical shifts as those of **14**, except for signals due to the extra Glc and deshielding of C-4 in the outer Cym. In the HMBC spectrum, cross-peaks were observed between H-1 of Glc/C-4 of outer Cym, and H-4 of outer Cym/C-1 of Glc, along with those in **14**. Therefore, **15** was assigned to be pleurogenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranoside.

The molecular formula of **16** was suggested to be

$C_{66}H_{110}O_{31}$ by HR-FAB-MS. The sugar moiety was composed of 3 moles of Cym and 2 moles each of Can and Glc. Correlations in NOE were observed between the H-1 signals of outer Glc, inner Glc, outer Cym, intermediate Cym, outer Can and inner Can/the H-4 signals of inner Glc, outer Cym, intermediate Cym, outer Can, inner Can and inner Cym, respectively. A response was also observed between H-3 of **a-2**/H-1 of inner Cym. The structure of **16** was, thus, determined to be pleurogenin 3-*O*-*β*-cellobiosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-cymaropyranoside.

Sixteen glycosides of pregnanes, including ikemagenin, lineolon and a new pregnane, pleurogenin, were isolated and their structures were elucidated. Since these glycosides foam to a lesser degree than spirostan or triterpenoid saponins, an expectorant effect can be expected when the roots are used to treat pleurisy or bronchitis.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a JNM-A500 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to the internal standard, tetramethylsilane (TMS), and the following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet, br s=broad singlet, dd=doublet of doublets. HR-FAB-MS were recorded on a JEOL HX-110 spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter. For silica gel column chromatography and TLC, the following solvent systems were applied: $CHCl_3$–MeOH–H_2O (7 : 1 : 1.6—7 : 2 : 1.2) (bottom layer, solvent 1), EtOAc–MeOH–H_2O (8 : 1 : 1.2—6 : 1 : 1.2) (top layer, solvent 2), benzene–acetone (3 : 1—1 : 1) (solvent 3). For ODS column chromatography and HPLC, MeCN–H_2O (2 : 3—3 : 2) (solvent 4) was used. Spray reagent: 10% H_2SO_4.

Extraction and Isolation The roots of *Asclepias tuberosa* L. collected in North Carolina in March, 1994, and purchased from Wilcox Natural Products, North Carolina (4.5 kg), were powdered and percolated with MeOH. The concentrated MeOH extract was dissolved in 50% MeOH and filtered. The filtrate was partitioned with benzene (extract 34.0 g) and then with $CHCl_3$ (extract 4.5 g). The benzene fraction was subjected to column chromatography on a silica gel column with solvent 2. A pregnane glycoside-rich aliquot obtained on initial chromatography (1.5 g) was subjected to hydrolysis. The remaining fraction was chromatographed successively on a silica gel column (solvents 1, 2, 3) and an ODS column (solvent 4) to afford 16 glycosides; **1**: 38 mg, **2**: 19 mg, **3**: 42 mg, **4**: 110 mg, **5**: 40 mg, **6**: 36 mg, **7**: 110 mg, **8**: 68 mg, **9**: 34 mg, **10**: 23 mg, **11**: 85 mg, **12**: 10 mg, **13**: 19 mg, **14**: 13 mg, **15**: 8 mg, **16**: 8 mg.

Hydrolysis of the Extract A glycoside-rich aliquot (1.5 g) from the benzene-soluble fraction was heated with 0.05 N HCl in 50% dioxane (20 ml) for 2 h at 95 °C. The mixture was deacidified with Amberlite IRA-410, diluted with H_2O and extracted with *n*-BuOH. The *n*-BuOH extract (880 mg) was chromatographed on a silica gel column with solvent 2 to give ikemagenin (**a-1**) which was further purified on a silica gel column with solvent 3 (230 mg), lineolon (10 mg), and pleurogenin (**a-2**, 8 mg). The H_2O layer, after extraction with *n*-BuOH, was chromatographed on a silica gel column with solvent 1, 2 and 3 to afford D-oleandrose: 54 mg, $[\alpha]_D^{20}$ −9.5° (*c*=2.7, H_2O, 24 h) (lit. −12.0°),[9] D-cymarose: 55 mg, $[\alpha]_D^{19}$ +56.7° (*c*=2.8, H_2O, 24 h) (lit. +54.9°),[9] D-canarose: 27 mg, $[\alpha]_D^{20}$ +22.3° (*c*=1.4, H_2O, 24 h) (lit. +22.8°),[8] D-digitoxose: 12 mg, $[\alpha]_D^{21}$ +37.7° (*c*=0.6, H_2O, 24 h) (lit. +50.2°),[9] D-thevetose: 6 mg, $[\alpha]_D^{20}$ +23.9° (*c*=0.3, H_2O, 24 h) (lit. +35.5°),[9] D-glucose: 50 mg, $[\alpha]_D^{20}$ +50.0° (*c*=0.2, H_2O, 24 h) (lit. +52.5°),[9] strophanthobiose: 20 mg, $[\alpha]_D^{24}$ +27.8° (*c*=1.0, H_2O, 24 h) (lit. +31.1°),[9] *β*-D-glucosyl-D-oleandrose: 33 mg, $[\alpha]_D^{24}$ +12.7° (*c*=0.17, H_2O, 24 h) (lit. +7.0°).[2]

Pleurogenin (**a-2**): Solid, $[\alpha]_D^{24}$ +30.0° (*c*=0.05, MeOH), FAB-MS (negative) *m/z*: 381.2275 (Calcd for $C_{21}H_{34}O_6$-1: 381.2277).

Ikemagenin 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside (**2**): Solid, $[\alpha]_D^{23}$ +7.2° (*c*=0.63, MeOH), FAB-MS *m/z*: 949.4910 (Calcd for $C_{51}H_{74}O_{15}$+Na: 949.4925). ^{1}H-NMR δ: 5.29 (1H, dd, *J*=10, 2 Hz, H-1_{Cym}), 4.72, 4.99 (1H each, dd, *J*=10, 2 Hz, H-$1_{Ole(1,2)}$), 3.59, 3.53, 3.48 (3H each, s, OC$\underline{H}_3$), 1.59, 1.48, 1.45 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole}$). ^{13}C-NMR δ: 100.2, 101.9 (C-$1_{Ole(2,1)}$), 96.3 (C-1_{Cym}), 58.7, 57.2, 56.9 (O$\underline{C}H_3$), 18.7, 18.59, 18.56 (C-$6_{Cym,Ole}$).

Ikemagenin 3-*O*-*β*-D-thevetopyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**3**): Solid, $[\alpha]_D^{23}$ +4.3° (*c*=2.10, MeOH), FAB-MS *m/z*: 1109.5664 (Calcd for $C_{58}H_{86}O_{19}$+Na: 1109.5661). ^{1}H-NMR δ: 5.12, 5.28 (1H each, dd, *J*=10, 2 Hz, H-$1_{Cym(2,1)}$), 4.69 (1H, dd, *J*=10, 2 Hz, H-1_{Ole}), 4.95 (1H, d, *J*=8 Hz, H-1_{Thv}), 3.89, 3.62, 3.58, 3.53 (3H each, s, OC$\underline{H}_3$), 1.70, 1.59, 1.39, 1.38 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole,Thv}$). ^{13}C-NMR δ: 96.3, 100.4 (C-$1_{Cym(1,2)}$), 101.8 (C-1_{Ole}), 104.0 (C-1_{Thv}), 60.8, 58.75, 58.84, 57.1 (O$\underline{C}H_3$), 18.7, 18.5, 18.43, 18.40 (C-$6_{Cym,Ole,Thv}$).

Ikemagenin 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**4**): Solid, $[\alpha]_D^{24}$ +11.8° (*c*=0.82, MeOH), FAB-MS *m/z*: 1093.5717 (Calcd for $C_{58}H_{86}O_{18}$+Na: 1093.5712). ^{1}H-NMR δ: 5.13, 5.28 (1H each, dd, *J*=10, 2 Hz, H-$1_{Cym(2,1)}$), 4.71, 4.99 (1H each, dd, *J*=10, 1 Hz, H-$1_{Ole(1,2)}$), 3.63, 3.59, 3.52, 3.49 (3H each, s, OC$\underline{H}_3$), 1.60, 1.48, 1.401, 1.399 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole}$). ^{13}C-NMR δ: 96.4, 100.4 (C-$1_{Cym(1,2)}$), 100.2, 101.9 (C-$1_{Ole(2,1)}$), 58.8, 58.7, 57.2, 57.0 (O$\underline{C}H_3$), 18.7, 18.6, 18.5, 18.4 (C-$6_{Cym,Ole}$).

Ikemagenin 3-*O*-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**5**): Solid, $[\alpha]_D^{24}$ +7.0° (*c*=0.63, MeOH), FAB-MS *m/z*: 1093.5717 (Calcd for $C_{58}H_{86}O_{18}$+Na: 1093.5712). ^{1}H-NMR δ: 5.12, 5.26, 5.28 (1H each, dd, *J*=10, 2 Hz, H-$1_{Cym(2,3,1)}$), 4.70 (1H, dd, *J*=10, 2 Hz, H-1_{Ole}), 3.62, 3.58, 3.53, 3.47 (3H each, s, OC$\underline{H}_3$), 1.54, 1.46, 1.393, 1.388 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole}$). ^{13}C-NMR δ: 96.4, 98.4, 100.4 (C-$1_{Cym(1,3,2)}$), 101.9 (C-1_{Ole}), 58.9, 58.8, 57.9, 57.2 (O$\underline{C}H_3$), 18.9, 18.6, 18.5, 18.4 (C-$6_{Cym,Ole}$).

Ikemagenin 3-*O*-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**6**): Solid, $[\alpha]_D^{19}$ +16.2° (*c*=1.84, MeOH), FAB-MS *m/z*: 1237.6500 (Calcd for $C_{65}H_{96}O_{21}$+Na: 1237.6498). ^{1}H-NMR δ: 5.12, 5.26, 5.27 (1H each, dd, *J*=10, 2 Hz, H-$1_{Cym(1,3,2)}$), 4.69, 4.90 (1H each, dd, *J*=10, 2 Hz, H-$1_{Ole(1,2)}$), 3.62, 3.58, 3.55, 3.51, 3.46 (3H each, s, OC$\underline{H}_3$), 1.53, 1.47, 1.44, 1.40, 1.39 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole}$). ^{13}C-NMR δ: 96.4, 98.5, 100.4 (C-$1_{Cym(1,3,2)}$),100.0, 101.9 (C-$1_{Ole(2,1)}$), 58.83, 58.76, 57.9, 57.3 (×2) (O$\underline{C}H_3$), 18.9, 18.7, 18.6, 18.5, 18.4 (C-$6_{Cym,Ole}$).

Ikemagenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**7**): Solid, $[\alpha]_D^{25}$ +11.1° (*c*=2.40, MeOH), FAB-MS *m/z*: 1399.7025 (Calcd for $C_{71}H_{108}O_{26}$+Na: 1399.7026). ^{1}H-NMR δ: 5.12, 5.26, 5.27 (1H each, dd, *J*=10, 1 Hz, H-$1_{Cym(2,3,1)}$), 4.69, 4.88 (1H each, dd, *J*=10, 2 Hz, H-$1_{Ole(1,2)}$), 4.92 (1H, d, *J*=8 Hz, H-1_{Glc}), 3.62, 3.58, 3.534, 3.530, 3.50 (3H each, s, OC$\underline{H}_3$), 4.56 (1H, dd, *J*=12, 2 Hz, H-$6a_{Glc}$), 4.38 (1H, dd, *J*=12, 5 Hz, H-$6b_{Glc}$), 1.62, 1.43 (×2), 1.40, 1.39 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole}$). ^{13}C-NMR δ: 96.4, 98.3, 100.4 (C-$1_{Cym(1,3,2)}$), 100.0, 101.9 (C-$1_{Ole(2,1)}$), 106.5 (C-1_{Glc}), 58.83, 58.77, 58.5, 57.4, 57.3 (O$\underline{C}H_3$), 18.63, 18.59 (×2), 18.5, 18.4 (C-$6_{Cym,Ole}$).

Ikemagenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-digitoxopyranoside (**8**): Solid, $[\alpha]_D^{23}$ +16.0° (*c*=1.29, MeOH), FAB-MS *m/z*: 1357.6566 (Calcd for $C_{68}H_{102}O_{26}$+Na: 1357.6557). NMR (see Tables 2 and 3).

Ikemagenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-digitoxopyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**9**): Solid, $[\alpha]_D^{24}$ +6.4° (*c*=1.70, MeOH), FAB-MS *m/z*: 1371.6713 (Calcd for $C_{69}H_{104}O_{26}$+Na: 1371.6714). NMR (see Tables 2 and 3).

Ikemagenin 3-*O*-*β*-cellobiosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**10**): Solid, $[\alpha]_D^{19}$ +9.4° (*c*=1.70, MeOH), FAB-MS *m/z*: 1273.5985 (Calcd for $C_{63}H_{94}O_{25}$+Na: 1273.5982). ^{1}H-NMR δ: 5.11, 5.28 (1H each, dd, *J*=10, 2 Hz, H-$1_{Cym(1,2)}$), 4.68 (1H, dd, *J*=10, 2 Hz, H-1_{Ole}), 5.06, 5.16 (1H each, d, *J*=8 Hz, H-$1_{Glc(1,2)}$), 3.62, 3.57, 3.49 (3H each, s, OC$\underline{H}_3$), 1.70, 1.39, 1.37 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole}$). ^{13}C-NMR δ: 96.4, 100.4 (C-$1_{Cym(1,2)}$), 101.8 (C-1_{Ole}), 104.2, 104.9 (C-$1_{Glc(1,2)}$), 58.9, 58.8, 57.2 (O$\underline{C}H_3$), 18.8, 18.5, 18.4 (C-$6_{Cym,Ole}$).

Ikemagenin 3-*O*-*β*-cellobiosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**11**): Solid, $[\alpha]_D^{24}$ +10.1° (*c*=1.22, MeOH), FAB-MS *m/z*: 1417.6780 (Calcd for $C_{70}H_{106}O_{28}$+Na: 1417.6769). NMR (see Tables 2 and 3).

Ikemagenin 3-*O*-*β*-cellobiosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**12**): Solid, $[\alpha]_D^{22}$ +15.7° (*c*=0.49, MeOH), FAB-MS *m/z*: 1561.7535 (Calcd for $C_{77}H_{118}O_{31}$+Na: 1561.7554). NMR (see Tables 2 and 3). A mixture of **12** (5 mg) and cellulase II (Sigma, 15 mg) in 20% EtOH was stirred for 6 h at 37 °C and extracted with *n*-

BuOH. On TLC (solvent 1 and 2), 2 spots at the same *Rf* values as **7** and **6** were observed.

Lineolon 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**13**): Solid, $[\alpha]_D^{22}$ +2.2° (*c*=0.95, MeOH), FAB-MS *m*/*z*: 963.5295 (Calcd for $C_{49}H_{80}O_{17}$+Na: 963.5293). ^{1}H-NMR *δ*: 5.12, 5.28 (1H each, dd, *J*=10, 2 Hz, H-$1_{Cym(2,1)}$), 4.71, 4.99 (1H each, dd, *J*=10, 2 Hz, H-$1_{Ole(1,2)}$), 3.62, 3.58, 3.52, 3.49 (3H each, s, OC$\underline{H}_3$), 1.59, 1.48, 1.39, 1.38 (3H each, d, *J*=6 Hz, H-$6_{Cym,Ole}$). ^{13}C-NMR *δ*: 96.4, 100.4 (C-$1_{Cym(1,2)}$), 100.2, 101.9 (C-$1_{Ole(2,1)}$), 58.8, 58.7, 57.2, 57.0 (O$\underline{C}H_3$), 18.7, 18.6, 18.5, 18.4 (C-$6_{Cym,Ole}$).

Pleurogenin 3-*O*-*β*-D-cymaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**14**): Solid, $[\alpha]_D^{22}$ −5.4° (*c*=0.73, MeOH), FAB-MS *m*/*z*: 953.5088 (Calcd for $C_{47}H_{78}O_{18}$+Na: 953.5086). NMR (see Tables 2 and 3).

Pleurogenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**15**): Solid, $[\alpha]_D^{25}$ −2.3° (*c*=0.39, MeOH), FAB-MS *m*/*z*: 1115.5613 (Calcd for $C_{53}H_{88}O_{23}$+Na: 1115.5614). NMR (see Tables 2 and 3).

Pleurogenin 3-*O*-*β*-cellobiosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-cymaropyranoside (**16**): Solid, $[\alpha]_D^{25}$ +2.7° (*c*=0.40, MeOH), FAB-MS *m*/*z*: 1421.6929 (Calcd for $C_{66}H_{110}O_{31}$+Na: 1421.6929). NMR (see Tables 2 and 3).

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