8,12;8,20-Diepoxy-8,14-secopregnane Glycosides from the Aerial Parts of *Asclepias tuberosa*

Tsutomu WARASHINA* and Tadataka NORO

Institute for Environmental Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan.
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Further study of constituents from the aerial parts of *Asclepias tuberosa* afforded twenty-two new steroidal glycosides along with tuberoside B_5 and G_5. These glycosides were confirmed to contain 8,12;8,20-diepoxy-8,14-secopregnanes, tuberogenin and its congeners, as their aglycones. The structure of each of these compounds was elucidated based on the interpretation of NMR and MS measurements and from chemical evidence.

Key words *Asclepias tuberosa*; Asclepiadaceae; 8,12;8,20-diepoxy-8,14-secopregnane glycoside; tuberoside; tuberogenin; 2,6-dideoxyhexopyranose

In the course of researching phytochemicals in Asclepiadaceous plants, we have reported the isolation and structural determination of pregnane glycosides from *Asclepias* spp., *Cynanchum* spp., *Metaplexis* spp., and *Araujia* spp.[1—8] In the preceding paper, we reported on 8,12;8,20-diepoxy-8,14-secopregnane glycosides from the aerial parts of *Asclepias tuberosa* L.[9] *A. tuberosa* L. is a plant indigenous to North America and distributed widely. Its roots are known as "pleurisy root" and used to treat pleurisy and bronchitis. In the present paper, we describe the isolation and structural determination of twenty-two same type glycosides from a more hydrophobic fraction of the methanol extract of the aerial parts of this plant.

Details of the extraction of the aerial parts of *A. tuberosa* were given in the previous paper.[9] The residue from the 80% MeOH in water soluble-fraction of the ether layer was subjected to silica gel column chromatography and semi-preparative HPLC to give compounds **1**—**24**. Compounds **4** and **14** were the known pregnane glycosides identified as tuberoside B_5 and G_5, respectively.[9]

The aglycones of these compounds except for **6** and **7** were identified as tuberogenin (**1a**),[9] 15β-acetoxytuberogenin,[9] 5,6-didehydrotuberogenin (**9a**),[9,10] or 15β-hydroxytuberogenin (**10a**)[9] based on the NMR spectroscopic data and acid hydrolysis. The component monosaccharides of each sugar moiety were determined as D-cymarose, D-oleandrose, D-digitoxose and/or D-canarose (see Experimental). Moreover, the compounds **2**, **3**, **5**—**10**, **15**—**17**, and **18** were identified as shown in Chart 1. The sugar sequences were previously determined in pregnane glycosides, consistent with their NMR spectroscopic data in the literature.[2,9]

Tuberoside M_5 (**1**) was considered to have the molecular formula, $C_{41}H_{66}O_{13}$, based on high resolution (HR)-FAB-MS [*m*/*z*: 789.4399 $[M+Na]^+$]. Because the NMR spectra of **1** showed three sets of anomeric proton and carbon signals at δ 97.5, 98.5, and 100.4 and δ 4.53, 5.01, and 4.55 in addition to the signals due to 3-*O*-glycosylated tuberogenin (glycosylation shifts[11]: C-2 (−2.1 ppm), C-3 (+6.0 ppm), C-4 (−3.7 ppm)), **1** was presumed to be tuberogenin 3-*O*-triglycoside. Moreover, the NMR spectra and acid hydrolysis suggested that the sugar moiety of **1** consisted of one digitoxose and one oleandrose along with a terminal oleandrose, which retained β-forms as judged from the *J* value of each anomeric proton signal (*J*=9.5, 2.0 Hz). The sequence of the sugar moiety was determined based on the measurements of the rotating frame nuclear Overhauser effect (ROE) difference spectra on irradiating the anomeric proton signal of each sugar in **1**. ROEs were found between δ 4.53 (H-1′ of β-D-oleandropyranose) and 3.59 (H-3 of the aglycone), δ 5.01 (H-1″ of β-D-digitoxopyranose) and 3.19 (H-4′ of β-D-oleandropyranose), and δ 4.55 (H-1‴ of β-D-oleandropyranose) and 3.22 (H-4″ of β-D-digitoxopyranose). Thus, **1** was established to be tuberogenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)β-D-oleandropyranoside.

The compounds **2**, **3**, **5**, **8**—**13**, **15**—**23**, and **24** were also glycosylated at the C-3 position of each aglycone, based on observations of glycosylation shifts in their ^{13}C-NMR spectra.

In HR-FAB-MS, tuberoside B_7 (**6**) and B_8 (**7**) were suggested to have the molecular formulae $C_{49}H_{80}O_{17}$ and $C_{49}H_{78}O_{17}$, respectively which were an O atom and an H_2O unit smaller than tuberoside B_2 (**25**).[9] The ^{13}C- and ^{1}H-NMR spectroscopic data of **6** and **7** were similar to those of **25**. However, **6** exhibited a methylene carbon signal for C-15 at δ 35.1 the same as **1**, replacing a hydroxy methine signal found in **25**. Acid hydrolysis of **6** afforded **6a**, whose configuration was determined as shown in Chart 2 by the difference ROE experiments. Moreover, **7** had olefin signals at C-5, C-6 and H-6 (δ 137.7, 118.9, 5.24) in its NMR spectra. Thus, the aglycones of **6** and **7** were identified as 2α-hydroxy-tuberogenin and 5,6-didehydro-2α-hydroxy-tuberogenin, and the structures of **6** and **7** were established as shown in Chart 1.

HR-FAB-MS showed the molecular formulae of tuberoside P_5 (**11**) and P_6 (**12**) to be $C_{48}H_{78}O_{16}$ and $C_{48}H_{76}O_{16}$, respectively, and the two were presumed to be 8:12;8:20-diepoxy-8,14-secopregnane 3-*O*-tetraglycosides, whose aglycones were identified as **1a** and **9a**, respectively, based on the NMR spectroscopic data and acid hydrolysis. The sugar moieties of these compounds were considered to have the same structure, owing to the similarity of their NMR spectroscopic data. Acid hydrolysis of **11** afforded cymarose, digitoxose, and oleandrose. The ^{13}C- and ^{1}H-NMR spectra of **11** revealed that the sugar moiety consisted of one β-D-cymaropyranosyl group, one β-D-digitoxopyranosyl group, and two β-D-oleandropyranosyl groups. The ^{13}C- and ^{1}H-NMR signal assignments of each sugar are shown in Tables 2 and 3, based on ^{1}H–^{1}H shift correlation spectroscopy (COSY), ^{1}H-detected

* To whom correspondence should be addressed. e-mail: warashin@u-shizuoka-ken.ac.jp

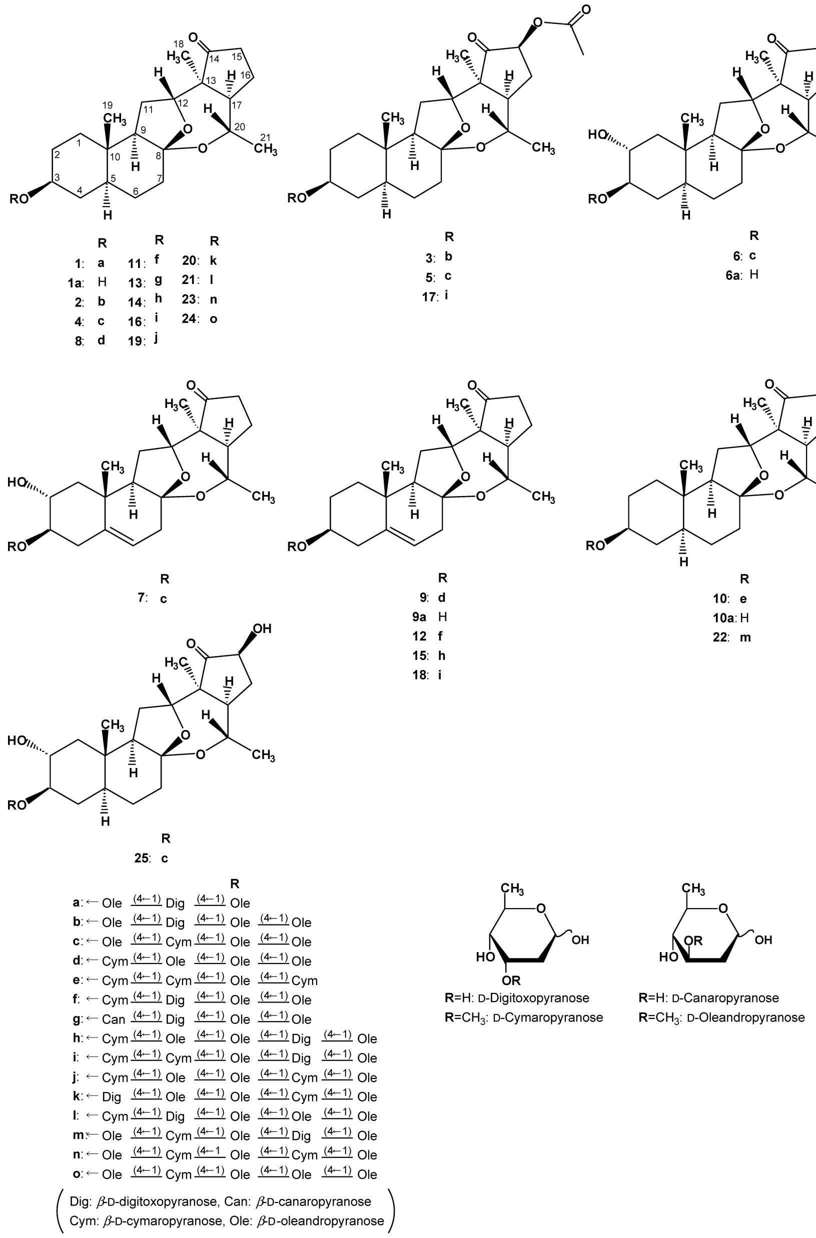

Chart 1. The Structures of Compounds **1**—**25**

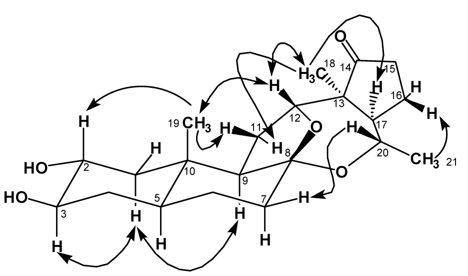

Chart 2. Important ROEs in Compound **6a**

heteronuclear multiple quantum coherency (HMQC) measurements and homoneuclear Hartmann–Hahn (HOHAHA) experiments on irradiating the anomeric proton of each β-D-oleandropyranose. Comparison of the NMR spectroscopic data of **11** with those of **2** indicated the substitution of β-D-cymaropyranose for the inner β-D-oleandropyranose in **2**. Hence, the sugar sequence of **11** was presumed to 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside, which was confirmed based on ROE difference experiments on irradiating each anomeric proton signal. The structures of **11** and **12** are shown in Chart 1.

Tuberoside Q_5 (**13**) was also considered to be a tuberogenin 3-*O*-tetraglycoside, its molecular formula determined as $C_{47}H_{76}O_{16}$ based on HR-FAB-MS. The NMR spectroscopic data of the sugar moiety in **13** were similar to those of **2** and **11**, but the characteristic H-4 signal for the β-D-canaropyranosyl group was observed at δ 2.97 (1H, t, J=9.0 Hz) in the higher field, instead of the signals for the inner β-D-oleandropyranosyl group in **2** and β-D-cymaropyranosyl group in **11**. Based on HOHAHA experiments and COSY measurements, the signal at δ 4.58 (1H, dd, J=9.5, 2.0 Hz) was assigned to the anomeric proton of β-D-canaropyranose. In addition, given the observation of a ROE between this anomeric proton and H-3 of the aglycone, this β-D-canaropyranosyl group was considered to be attached at the C-3 position of the aglycone. Therefore, the structure of **13** was determined as shown in Chart 1.

The molecular formula of tuberoside R_5 (**19**) was proposed to be $C_{56}H_{92}O_{19}$, which was larger than **14** by a CH_2 unit, based on HR-FAB-MS. The NMR spectra suggested that **19** was tuberogenin 3-*O*-pentaglycoside, and its sugar moiety was similar to that of **14**. However, this sugar moiety was composed of two β-D-cymaropyranosyl groups, two β-D-oleandropyranosyl groups and a terminal β-D-oleandropyranosyl group. The presence of the anomeric carbon and proton signals of the terminal β-D-oleandropyranose at δ 101.4 and 4.50 (1H, dd, J=9.5, 2.0 Hz) suggested that this β-D-oleandropyranose was attached at the C-4 position of β-D-cymaropyranose, and this was confirmed by the observation of a ROE between H-1′′′′′ of the terminal β-D-oleandropyranose (δ 4.50) and H-4′′′′ of β-D-cymaropyranose (δ 3.23). The whole sugar linkage of **19** was also determined on the basis of the results of ROE difference and HOHAHA experiments upon irradiating each anomeric proton. The structure of **19** is presented in Chart 1.

Tuberoside S_5 (**20**), T_5 (**21**), U_1 (**22**), V_5 (**23**), and W_5 (**24**) were also considered to be 8:12;8:20-diepoxy-8,14-secopregnane 3-*O*-pentaglycosides whose aglycones were identified as **1a** for **20**, **21**, **23** and **24**, and **10a** for **22**.

HR-FAB-MS revealed the molecular formula of tuberoside S_5 (**20**) to be $C_{55}H_{90}O_{19}$, which was consistent with that of **14**. Acid hydrolysis and the NMR spectroscopic data also suggested that the sugar moiety consisted of one β-D-cymaropyranosyl group, one β-D-digitoxopyranosyl group, two β-D-oleandropyranosyl groups, and one terminal β-D-oleandropyranosyl group. On comparison of the NMR spectroscopic data for the sugar moiety in **20** with that in **14** and **19**, the sugar sequence from the second sugar to the terminal one was deduced to be the same as for **19**. In addition, an anomeric proton signal due to the β-D-digitoxopyranosyl group was exhibited at δ 4.92 (1H, dd, J=9.5, 2.0 Hz), which showed a ROE to H-3 of the aglycone. Thus, it was assumed that the β-D-digitoxopyranosyl group functioned at the C-3 position of the aglycone, and the above array was attached at the C-4 position of this β-D-digitoxopyranosyl group. The whole sugar sequence was also confirmed by the ROE difference experiments on irradiating each anomeric proton. The structure of **20** was established as shown in Chart 1.

HR-FAB-MS showed tuberoside T_5 (**21**) to have the molecular formula $C_{55}H_{90}O_{19}$, which was the same as **14** and **20**, and larger than **11** by the 2,6-dideoxy-3-*O*-methyl-hexose unit. On comparing the NMR spectroscopic data with those of **11**, **21** seemed to have one extra β-D-oleandropyranosyl group. Moreover, on comparison of the NMR spectroscopic data with those of **8**, **21** was indicated to have the β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl array as a part of the sugar sequence. Thus, **21** was determined as tuberogenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside. The ROE difference spectra on irradiating the anomeric proton of each sugar supported this linkage.

HR-FAB-MS indicated tuberoside U_1 (**22**), V_5 (**23**), and W_5 (**24**) to have the molecular formulae $C_{55}H_{90}O_{20}$, $C_{56}H_{92}O_{19}$, and $C_{56}H_{92}O_{19}$, respectively. The sugar moiety of **22** consisted of one β-D-cymaropyranose, one β-D-digitoxopyranose, two β-D-oleandropyranoses and a terminal β-D-oleandropyranose. On comparing the NMR signals for the sugar moiety with those of **16**, **22** seemed to have the β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl array in its sugar sequence. Moreover, the ^{1}H- and ^{13}C-NMR signals at δ 4.53 (1H, dd, J=9.5, 2.0 Hz), 97.5 and 3.17 (1H, t, J=9.0 Hz) were assigned to H-1, C-1 and H-4 of the remaining β-D-oleandropyranosyl group. Because the chemical shifts of these H-1 and C-1 signals of the β-D-cymaropyranosyl group were consistent with those of **5**, the whole sugar sequence of **22** was presumed to be 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside. The sugar moieties of **23** and **24** were composed of two β-D-cymaropyranoses, three β-D-oleandropyranoses, and one β-D-cymaropyranose and four β-D-oleandropyranoses, respectively. Comparing the NMR signals for the sugar moieties with those of **19**, **21** and **22**, the sugar sequences of **23** and **24** were deduced as shown in Chart 1. The sugar linkages of these compounds were confirmed based on the ROE difference spectra upon irradiating each anomeric proton. Hence, the structures of **22**, **23**, and **24** were described as shown in Chart 1.

To our knowledge, *Asclepias tuberosa* is the first plant to afford 8:12;8:20-diepoxy-8:14-secopregnane-type glycosides. These compounds are thus suggested to be unique constituents of *A. tuberosa*. Abe and Yamauchi had already reported some pregnane glycosides whose aglycones were ikemagenin, lineolon and pleurogenin from the roots of this plant,[12] but these pregnane glycosides were not discovered in the aerial parts in our investigations. We also plan to search for these components in the roots of this plant, and confirm whether 8:12;8:20-diepoxy-8:14-secopregnane-type glycosides are present or not. Though cardenolides are considered to be characteristic constituents of *Asclepias* spp. together with pregnane glycosides, we could find no cardenolides in the more hydrophobic fraction of the methanol extract of the aerial parts of *A. tuberosa*, the same as previously.[9] Recently, pregnane glycosides in Asclepiadaceous plants were reported to have biological activities *in vitro* and *in vivo*.[13—19] We continue to be interested in the activity of novel secopregnane-type glycosides.

Experimental

General Procedures and Plant Materials The instrumental analysis and plant materials were described previously.[9]

Extraction and Isolation Extraction of the aerial parts of *A. tuberosa* is described in the previous paper.[9] The residue (34.8 g) from an 80% MeOH in water soluble-fraction was chromatographed on a silica gel column with a $CHCl_3$–MeOH (98 : 2—85 : 15) system to obtain seven fractions (A (6.94 g), B (3.47 g), C (4.67 g), D (2.18 g), E (4.24 g), F (2.92 g), and G (2.77 g)). Fraction B (3.47 g) was subjected to silica gel chromatography with a $CHCl_3$–MeOH (99 : 1—95 : 5) system again to acquire three factions (A′ (0.98 g), B′ (1.91 g), and C′ (0.28 g)). By recrystallization ($CHCl_3$–MeOH) or using semi-preparative HPLC (Develosil-ODS-15/30 50 mm i.d.×100 cm, Inertsil ODS-3 30 mm i.d.×50 cm, Shiseido Capcellpak-ODS-UG-80 30 mm i.d.×25 cm, YMC-ODS 20 mm i.d.×25 cm, and Cosmosil-PhA 20 mm i.d.×25 cm: 56—64% MeCN in water and 75—80% MeOH in water), fraction B′ (1.91 g) afforded the compounds **1** (6 mg), **2** (25 mg), **3** (4 mg) **4** (204 mg), **5** (3 mg), **6** (16 mg), **7** (2 mg), **8** (14 mg), **9** (6 mg), **10** (7 mg), **11** (16 mg), **12** (8 mg), **13** (2 mg), **14** (24 mg), **15** (5 mg), **16** (21 mg)

Table 1. [13]C-NMR Spectroscopic Data for the Aglycone Moiety of Compounds **1**, **5**—**7**, **9**, and **10**

	1	5	6	6a	7	9	10
Carbon No.							
-1	38.1	38.1	45.3	45.9	45.1	37.8	38.2
-2	28.3	28.3	69.2	71.9	69.3	28.9	28.4
-3	77.1	77.1	86.9	76.4	86.4	77.1	77.1
-4	33.9	34.0	33.9	35.3[a)]	37.5[a)]	38.2	33.9
-5	42.0	42.0	42.0	42.1	137.7	138.9	41.9
-6	25.7	25.6	24.9	24.9	118.9	118.5	25.6
-7	31.8	31.8	31.8	31.8	33.2	33.1	31.7
-8	106.9	107.2	106.6	106.5	105.8	106.3	107.1
-9	57.2[a)]	57.2	56.9	57.0[b)]	54.7	54.8	57.2
-10	35.3	35.3	36.6	37.2	37.3[a)]	36.5	35.3
-11	27.5	27.2	27.5	27.5	27.8	27.9	27.3
-12	81.1	80.0	81.1	81.1	81.0	81.0	79.9
-13	57.1[a)]	56.6	57.1	57.1[b)]	57.1	57.1	56.4
-14	220.7	214.3	220.6	220.7	220.6	220.8	219.2
-15	35.2	71.4	35.1	35.2[a)]	35.2	35.3	70.9
-16	21.6	28.8	21.6	21.6	21.5	21.6	30.0
-17	53.5	52.2	53.5	53.5	53.6	53.7	52.6
-18	19.6	20.2	19.6	19.6	19.7	19.7	20.2
-19	12.2	12.2	13.3	13.6	20.3	19.3	12.4
-20	65.5	67.0	65.5	65.6	65.8	65.7	67.5
-21	21.3	20.9[a)]	21.3	21.3	21.4	21.4	20.8
-Ac	—	170.0	—	—	—	—	—
	—	20.7[a)]	—	—	—	—	—

Measured in $CDCl_3$ solution at 35 °C. *a*, *b*) Signal assignments may be interchanged in each column.

17 (8 mg), **18** (14 mg), **19** (15 mg), **20** (4 mg), **21** (3 mg), **22** (5 mg) **23** (8 mg), and **24** (10 mg).

Tuberoside M_1 (**1**): Amorphous powder. $[\alpha]_D^{23}$ −59° (c=0.57, MeOH). FAB-MS *m/z*: 789 $[M+Na]^+$. HR-FAB-MS *m/z*: 789.4399 (Calcd for $C_{41}H_{66}O_{13}Na$: 789.4401). [13]C-NMR spectroscopic data of the aglycone moiety: shown in Table 1. [1]H-NMR spectroscopic data of the aglycone moiety: ($CDCl_3$ at 35 °C) δ: 4.50 (1H, dd, 8.0, 7.0, H-12), 3.89 (1H, dq, 10.5, 6.5, H-20), 3.59 (1H, m, H-3), 1.22 (3H, d, 6.5, H-21), 0.91 (3H, s, H-18), 0.82 (3H, s, H-19). [13]C- and [1]H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1′ and H-4′, and H-1‴ and H-4‴. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, and H-1‴ and H-4″.

Tuberoside A_5 (**2**): Amorphous powder. $[\alpha]_D^{21}$ −61° (c=1.29, $CHCl_3$). FAB-MS *m/z*: 933 $[M+Na]^+$. HR-FAB-MS *m/z*: 933.5206 (Calcd for $C_{48}H_{78}O_{16}Na$: 933.5188). The [13]C- and [1]H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. [13]C-NMR spectroscopic data of the sugar moiety: shown in Table 2. [1]H-NMR spectroscopic data of the sugar moiety ($CDCl_3$ at 35 °C) δ: 5.00 (1H, dd, 9.5, 2.0, H-1″), 4.71 (1H, dd, 9.5, 2.0, H-1⁗), 4.53 (1H, dd, 9.5, 2.0, H-1′), 4.50 (1H, dd, 9.5, 2.0, H-1‴), 4.20 (1H, br s, H-3″), 3.81 (1H, dq, 9.5, 6.0, H-5″), 3.41 (3H, s, –OMe), 3.40 (3H, s, –OMe), 3.38 (3H, s, –OMe), 3.34 (1H, dq, 9.0, 6.0, H-5‴), 3.31 (1H, dq, 9.0, 6.0, H-5⁗), 3.28 (1H, dq, 9.0, 6.0, H-5′), 3.20 (1H, dd, 9.5, 3.0, H-4″), 3.19 (1H, t, 9.0, H-4′), 3.16 (1H, t, 9.0, H-4‴), 3.14 (overlapping, H-4⁗), 1.35 (3H, d, 6.0, H-6⁗), 1.29 (3H, d, 6.0, H-6′), 1.28 (3H, d, H-6‴), 1.25 (3H, d, 6.0, H-6″).

Tuberoside A_3 (**3**): Amorphous powder. $[\alpha]_D^{23}$ −51° (c=0.34, $CHCl_3$). FAB-MS *m/z*: 991 $[M+Na]^+$. HR-FAB-MS *m/z*: 991.5232 (Calcd for $C_{50}H_{80}O_{18}Na$: 991.5242). The [13]C- and [1]H-NMR spectroscopic data of the aglycone moiety were consistent with those of **5**, but the carboxy carbon signal of the acetyl group was not detected. The [13]C- and [1]H-NMR spectroscopic data of the sugar moiety were in good agreement with those of **2**.

Tuberoside B_3 (**5**): Amorphous powder. $[\alpha]_D^{23}$ −47° (c=0.38, $CHCl_3$). FAB-MS *m/z*: 1005 $[M+Na]^+$. HR-FAB-MS *m/z*: 1005.5400 (Calcd for $C_{51}H_{82}O_{18}Na$: 1005.5399). [13]C-NMR spectroscopic data of the aglycone and sugar moieties: shown in Tables 1 and 2. [1]H-NMR spectroscopic data of the aglycone moiety ($CDCl_3$ at 35 °C) δ: 5.32 (1H, dd, 9.5, 2.5, H-15), 4.54 (1H, dd, 8.0, 7.0, H-12), 3.92 (1H, dq, 10.5, 6.5, H-20), 3.60 (1H, m, H-3), 2.53 (1H, dt, 16.5, 8.5, H-16), 2.10 (3H, s, –OAc), 1.17 (3H, d, 6.5, H-21), 0.98 (3H, s, H-18), 0.84 (3H, s, H-19). [1]H-NMR spectroscopic data of the sugar moiety ($CDCl_3$ at 35 °C) δ: 4.96 (1H, dd, 9.5, 2.0, H-1″), 4.72 (1H, dd, 9.5, 2.0, H-1⁗), 4.53 (1H, dd, 9.5, 2.0, H-1′), 4.45 (1H, dd, 9.5, 2.0, H-1‴), 3.89 (1H, dq, 9.5, 6.0, H-5″), 3.78 (1H, q, 3.0, H-3″), 3.43 (3H, s, –OMe), 3.40 (6H, s, –OMex2), 3.38 (3H, s, –OMe), 3.31 (2H, dq, 9.0, 6.0, H-5‴ and H-5⁗), 3.28 (1H, dq, 9.0, 6.0, H-5′), 3.21 (1H, dd, 9.5, 3.0, H-4″), 3.17 (2H, t, 9.0, H-4′ and H-4‴), 3.13 (1H, td, 9.0, 1.5, H-4⁗), 1.35 (3H, d, 6.0, H-6⁗), 1.29 (6H, d, 6.0, H-6′ and H-6‴), 1.24 (3H, d, 6.0, H-6″).

Tuberoside B_7 (**6**): Amorphous powder. $[\alpha]_D^{21}$ −47° (c=0.99, $CHCl_3$). FAB-MS *m/z*: 963 $[M+Na]^+$. HR-FAB-MS *m/z*: 963.5294 (Calcd for $C_{49}H_{80}O_{17}Na$: 963.5293). [13]C-NMR spectroscopic data of the aglycone and sugar moieties: shown in Tables 1 and 2. [1]H-NMR spectroscopic data of the aglycone moiety ($CDCl_3$ at 35 °C) δ: 4.50 (1H, dd, 8.0, 7.0, H-12), 3.98 (1H, dq, 10.5, 6.0, H-20), 3.62 (1H, m, H-2), 3.29 (overlapping H-3), 1.22 (3H, d, 6.0, H-21), 0.92 (3H, s, H-18), 0.85 (3H, s, H-19). The [1]H-NMR spectroscopic data of the sugar moiety were almost similar to those of **5**, but the signals due to the first β-D-oleandropyranose were observed as follows ($CDCl_3$ at 35 °C) δ: 4.47 (1H, dd, 9.5, 2.0, H-1′), 3.37 (overlapping, H-5′), 3.20 (1H, t, 9.0, H-4′), 1.31 (3H, d, 6.0, H-6′).

Tuberoside B_8 (**7**): Amorphous powder. $[\alpha]_D^{18}$ −70° (c=0.23, $CHCl_3$). FAB-MS *m/z*: 961 $[M+Na]^+$. HR-FAB-MS *m/z*: 961.5144 (Calcd for $C_{49}H_{78}O_{17}Na$: 961.5137). [13]C-NMR spectroscopic data of the aglycone moiety: shown in Table 1. [1]H-NMR spectroscopic data of the aglycone moiety ($CDCl_3$ at 35 °C) δ: 5.24 (1H, br s, H-6), 4.51 (1H, dd, 8.0, 7.0, H-12), 3.97 (1H, dq, 10.5, 6.0, H-20), 3.71 (1H, m, H-2), 3.26 (overlapping H-3), 1.23 (3H, d, 6.0, H-21), 1.03 (3H, s, H-19), 0.94 (3H, s, H-18). The [13]C- and [1]H-NMR spectroscopic data of the sugar moiety were in good agreement with those of **6**.

Tuberoside N_5 (**8**): Amorphous powder. $[\alpha]_D^{19}$ −51° (c=0.57, MeOH). FAB-MS *m/z*: 947 $[M+Na]^+$. HR-FAB-MS *m/z*: 947.5350 (Calcd for $C_{49}H_{80}O_{16}Na$: 947.5344). The [13]C- and [1]H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. [13]C-NMR spectroscopic data of the aglycone moiety: shown in Table 2. The [1]H-NMR spectroscopic data of the sugar moiety ($CDCl_3$ at 35 °C) δ: 4.84 (1H, dd, 9.5, 2.0, H-1′), 4.72 (1H, dd, 9.5, 2.0, H-1⁗), 4.66 (1H, dd, 9.5, 2.0, H-1‴), 4.44 (1H, dd, 9.5, 2.0, H-1″), 3.86 (1H, dq, 9.5, 6.0, H-5′), 3.77 (1H, q, 3.0, H-3′), 3.43 (3H, s, –OMe), 3.41 (3H, s, –OMe), 3.40 (3H, s, –OMe), 3.38 (3H, s, –OMe), 3.32 (overlapping, H-5‴), 3.31 (1H, dq, 9.0, 6.0, H-5⁗), 3.29 (1H, dq, 9.0, 6.0, H-5″), 3.21 (1H, dd, 9.5, 3.0, H-4′), 3.17 (1H, t, 9.0, H-4‴), 3.15 (1H, t, 9.0, H-4″), 3.13 (1H, t, 9.0, H-4⁗), 1.34 (3H, d, 6.0, H-6⁗), 1.32 (3H, d, 6.0, H-6‴), 1.28 (3H, d, 6.0, H-6″), 1.22 (3H, d, 6.0, H-6′).

Tuberoside N_6 (**9**): Amorphous powder. $[\alpha]_D^{24}$ −82° (c=0.50, MeOH). FAB-MS *m/z*: 945 $[M+Na]^+$. HR-FAB-MS *m/z*: 945.5178 (Calcd for $C_{49}H_{78}O_{16}Na$: 945.5188). [13]C-NMR spectroscopic data of the aglycone moiety: shown in Table 1. [1]H-NMR spectroscopic data of the aglycone moiety: ($CDCl_3$ at 35 °C) δ: 5.21 (1H, br s, H-5), 4.51 (1H, dd, 8.0, 7.0, H-12), 3.97 (1H, dq, 10.5, 6.5, H-20), 3.53 (1H, m, H-3), 2.58 (1H, dt, 19.0, 4.0, H-7), 1.23 (3H, d, 6.5, H-21), 0.98 (3H, s, H-19), 0.93 (3H, s, H-18). The [13]C- and [1]H-NMR spectroscopic data of the sugar moiety were consistent with those of **8**, but the C-1′ signal was observed at δ 96.1.

Tuberoside O_1 (**10**): Amorphous powder. $[\alpha]_D^{23}$ −9.1° (c=0.66, MeOH). FAB-MS *m/z*: 963 $[M+Na]^+$. HR-FAB-MS *m/z*: 963.5281 (Calcd for $C_{49}H_{80}O_{17}Na$: 963.5293). [13]C-NMR spectroscopic data of the aglycone and sugar moieties: shown in Tables 1 and 2. [1]H-NMR spectroscopic data of the aglycone moiety ($CDCl_3$ at 35 °C) δ: 4.52 (1H, dd, 8.0, 7.0, H-12), 4.22 (1H, dd, 8.0, 7.0, H-15), 3.96 (1H, dq, 10.5, 6.5, H-20), 3.60 (overlapping, H-3), 1.19 (3H, d, 6.5, H-21), 0.97 (3H, s, H-18), 0.85 (3H, s, H-19). [1]H-NMR spectroscopic data of the sugar moiety ($CDCl_3$ at 35 °C) δ: 4.88 (1H, dd, 9.5, 2.0, H-1⁗), 4.84 (1H, dd, 9.5, 2.0, H-1′), 4.75 (1H, dd, 9.5, 2.0, H-1″), 4.45 (1H, dd, 9.5, 2.0, H-1‴), 3.86 (1H, dq, 9.5, 6.0, H-5″), 3.84 (1H, dq, 9.5, 6.0, H-5′), 3.79 (1H, q, 3.0, H-3′), 3.78 (1H, q, 3.0, H-3″), 3.62 (1H, q, 3.0, H-3⁗), 3.60 (1H, dq, 9.5, 6.0, H-5⁗), 3.44 (3H, s, –OMe), 3.43 (3H, s, –OMe), 3.42 (3H, s, –OMe), 3.40 (3H, s, –OMe), 3.29 (1H, dq, 9.0, 6.0, H-5‴), 3.21 (3H, dd, 9.5, 3.0, H-4′, H-4″ and H-4⁗), 3.16 (1H, t, 9.0, H-4‴), 1.29 (3H, d, 6.0, H-6⁗), 1.28 (3H, d, 6.0, H-6‴), 1.21 (6H, d, 6.0, H-6′ and H-6″).

Tuberoside P_5 (**11**): Amorphous powder. $[\alpha]_D^{19}$ −39° (c=1.57, MeOH). FAB-MS *m/z*: 933 $[M+Na]^+$. HR-FAB-MS *m/z*: 933.5174 (Calcd for $C_{48}H_{78}O_{16}Na$: 933.5188). The [13]C- and [1]H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. [13]C- and [1]H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1‴ and H-4‴, and H-1⁗ and H-4⁗. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, and H-1⁗ and H-4‴.

Tuberoside P_6 (**12**): Amorphous powder. $[\alpha]_D^{19}$ −58° (c=0.77, MeOH). FAB-MS *m/z*: 931 $[M+Na]^+$. HR-FAB-MS *m/z*: 931.5048 (Calcd for

Table 2. ^{13}C-NMR Spectroscopic Data for the Sugar Moiety of Compounds **1**, **2**, **5**, **6**, **8**, **10**, **11**, **13**, **15**, **16**, **19**—**23**, and **24**

	1	**2**	**5**	**6**	**8**	**10**	**11**	**13**	**15**	**16**	**19**	**20**	**21**	**22**	**23**	**24**
Carbon No.																
	Ole	Ole	Ole	Ole	Cym	Cym	Cym	Can	Cym	Cym	Cym	Dig	Cym	Ole	Ole	Ole
C-1′	97.5	97.5	97.5	99.9	95.8	95.8	95.8	97.6	96.0	95.8	95.8	95.6	95.8	97.5	97.5	97.5
-2′	36.6	36.7	36.7	36.4	35.9$^{a)}$	35.7	35.6	38.9	35.9	35.7	35.8	37.2	35.7$^{a)}$	36.4	36.4	36.4$^{a)}$
-3′	79.1	79.2$^{a)}$	79.2$^{a)}$	78.8	77.2	77.1	77.1	69.8	77.1	77.1	77.1	66.6	77.2	79.1$^{a)}$	79.1$^{a)}$	79.3$^{b)}$
-4′	82.8$^{a)}$	82.7$^{b)}$	82.3$^{b)}$	81.8	82.8$^{b)}$	82.6$^{a)}$	82.6	88.5	82.8$^{a)}$	82.7$^{a)}$	82.8$^{a)}$	82.9$^{a)}$	82.7$^{b)}$	82.2$^{b)}$	82.7$^{b)}$	82.7$^{c)}$
-5′	71.2	71.2	71.2$^{c)}$	71.4	68.4	68.5$^{b)}$	68.5	70.4	68.4	68.5$^{b)}$	68.4	67.9	68.5	71.2	71.2	71.2$^{d)}$
-6′	18.4$^{b)}$	18.4$^{c)}$	18.4$^{d)}$	18.4$^{a)}$	18.4$^{c)}$	18.4$^{c)}$	18.4$^{a)}$	18.4$^{a)}$	18.4$^{b)}$	18.4$^{c)}$	18.4$^{b)}$	18.4$^{b)}$	18.5$^{c)}$	18.4$^{c)}$	18.4$^{c)}$	18.5$^{e)}$
	Dig	Dig	Cym	Cym	Ole	Cym	Dig	Dig	Ole	Cym	Ole	Ole	Dig	Cym	Cym	Cym
C-1″	98.5	98.5	98.4	98.4	101.4	99.7	99.5	99.2	101.4	99.7	101.5$^{c)}$	100.2	99.5	98.5$^{d)}$	98.5$^{d)}$	98.4
-2″	37.1	37.1	35.6$^{e)}$	35.6$^{b)}$	36.5$^{d)}$	35.7	36.8	36.6	36.5$^{c)}$	35.7	36.5$^{d)}$	36.6$^{c)}$	36.8	35.7	35.7$^{e)}$	35.6$^{f)}$
-3″	66.8	66.7	77.1	77.1	79.3$^{e)}$	77.1	66.6	66.5	79.2$^{d)}$	77.1	79.2$^{e)}$	79.1$^{d)}$	66.7	77.2	77.2$^{f)}$	77.2
-4″	82.6$^{a)}$	82.6$^{b)}$	82.7	82.6	82.6$^{b)}$	82.6$^{a)}$	82.6	82.2$^{b)}$	82.8$^{a)}$	82.6$^{a)}$	82.4$^{f)}$	82.3$^{e)}$	82.6$^{b)}$	82.8$^{b)}$	82.6$^{b)}$	82.6$^{c)}$
-5″	68.2	68.2	68.7	68.8	71.1	68.3$^{b)}$	67.8	68.4	71.0	68.3$^{b)}$	71.0	71.4$^{f)}$	67.8	68.8	68.7	68.7
-6″	18.2$^{b)}$	18.4$^{c)}$	18.4$^{d)}$	18.2$^{a)}$	18.4$^{c)}$	18.3$^{c)}$	18.2$^{a)}$	18.0$^{a)}$	18.4$^{b)}$	18.2$^{c)}$	18.4$^{b)}$	18.4$^{b)}$	18.4$^{c)}$	18.4$^{c)}$	18.4$^{c)}$	18.4$^{c)}$
	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole
C-1‴	100.4	100.3$^{d)}$	101.4	101.4	100.3$^{f)}$	101.4	100.3$^{b)}$	100.3$^{c)}$	100.2	101.4	100.1	100.2	100.3$^{d)}$	101.5	101.5$^{g)}$	101.4
-2‴	35.3	36.3	36.4	36.4	36.4$^{d)}$	36.4	36.3	36.2	36.4$^{c)}$	36.4	36.4$^{d)}$	36.4$^{c)}$	36.5$^{e)}$	36.7	36.7	36.7
-3‴	80.5	79.1$^{a)}$	79.1$^{a)}$	79.2	79.2$^{e)}$	79.0	79.1	79.0	79.0$^{d)}$	78.9	79.0$^{e)}$	79.0$^{d)}$	79.2$^{f)}$	78.9$^{a)}$	78.9$^{a)}$	79.2$^{b)}$
-4‴	75.3	82.2	82.2$^{b)}$	82.3	82.4$^{b)}$	82.3$^{a)}$	82.2	82.1$^{b)}$	82.7$^{a)}$	82.5$^{a)}$	82.3$^{f)}$	82.2$^{e)}$	82.5$^{b)}$	82.5$^{b)}$	82.2$^{b)}$	82.4$^{c)}$
-5‴	71.8	71.2	71.0$^{c)}$	71.0	71.0	71.1	71.2	71.3	71.4	71.2	71.4	71.3$^{f)}$	71.2	71.2	71.2	71.2$^{d)}$
-6‴	17.9	18.2	18.2$^{d)}$	18.1$^{a)}$	18.2$^{c)}$	18.2$^{c)}$	18.2$^{a)}$	17.9$^{a)}$	18.2$^{b)}$	18.2$^{c)}$	18.2$^{b)}$	18.2$^{b)}$	18.2$^{c)}$	18.2$^{c)}$	18.2$^{c)}$	18.4$^{e)}$
		Ole	Ole	Ole	Ole	Cym	Ole	Ole	Dig	Dig	Cym	Cym	Ole	Dig	Cym	Ole
C-1⁗	—	100.2$^{d)}$	100.2	100.2	100.1$^{f)}$	98.2	100.2$^{b)}$	100.2$^{c)}$	98.5	98.5	98.5	98.5	100.2$^{d)}$	98.4	98.4$^{d)}$	100.3$^{g)}$
-2⁗	—	35.5	35.5$^{e)}$	35.5$^{b)}$	35.6$^{a)}$	34.0	35.5	35.5	37.1	37.1	35.6	35.6	36.3$^{e)}$	37.1	35.6$^{e)}$	36.5$^{a)}$
-3⁗	—	80.7	80.8	80.8	80.8	77.6	80.7	80.7	66.8	66.7	77.1	77.1	79.1$^{f)}$	66.7	77.1$^{f)}$	79.1$^{b)}$
-4⁗	—	75.5	75.6	75.5	75.6	72.5	75.5	75.5	82.4	82.4$^{a)}$	82.7$^{a)}$	82.7$^{a)}$	82.2$^{b)}$	82.6$^{b)}$	82.2$^{b)}$	82.2$^{c)}$
-5⁗	—	71.8	71.7	71.7	71.7	71.1	71.7	71.8	68.2	68.2	68.7	68.7	71.2	68.2	68.7	71.0$^{d)}$
-6⁗	—	18.0	18.0$^{d)}$	18.0	18.0	18.2$^{c)}$	18.0$^{a)}$	17.8$^{a)}$	18.2$^{b)}$	18.2$^{c)}$	18.2$^{b)}$	18.2$^{b)}$	18.2$^{c)}$	18.2$^{c)}$	18.2$^{c)}$	18.2$^{e)}$
									Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole
C-1′′′′′	—	—	—	—	—	—	—	—	100.4	100.4	101.4$^{c)}$	101.5	100.1$^{d)}$	100.4	101.4$^{g)}$	100.1$^{g)}$
-2′′′′′	—	—	—	—	—	—	—	—	35.4	35.3	35.4	35.4	35.6$^{a)}$	35.4	35.4	35.5$^{f)}$
-3′′′′′	—	—	—	—	—	—	—	—	80.5	80.5	80.6	80.7	80.8	80.5	80.7	80.8
-4′′′′′	—	—	—	—	—	—	—	—	75.3	75.3	75.5	75.5	75.6	75.3	75.5	75.6
-5′′′′′	—	—	—	—	—	—	—	—	71.9	71.8	71.6	71.6	71.7	71.8	71.6	71.7
-6′′′′′	—	—	—	—	—	—	—	—	17.9	17.9	18.0	18.0	18.0	17.9	18.0	18.0
-OMes	56.4	56.8	58.0	58.1	58.1	58.3	57.8	56.9	58.1	58.3	58.0×2	58.1	57.9	58.1	58.1	58.1
	56.3	56.3×2	56.7	56.6×2	56.8	57.9	56.8	56.4	56.7×2	57.9	56.8	57.0	56.9	56.5	58.0	56.8
	—	—	56.4	56.3	56.7	57.1	56.3	—	56.4	56.5	56.7	56.7	56.8	56.4×3	56.6	56.7
	—	—	56.3	—	56.3	56.5	—	—	—	56.4	56.2	56.3	56.3	—	56.4	56.4
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	56.3	56.3

Measured in $CDCl_3$ solution at 35 °C. *a*—*g*) Signal assignments may be interchanged in each column. Cym: β-D-cymaropyranose, Ole: β-D-oleandropyranose, Dig: β-D-digitoxopyranose, Can: β-D-canaropyranose.

Table 3. ^{1}H-NMR Spectroscopic Data for the Sugar Moiety of Compounds **1**, **11**, **13**, **19**—**23**, and **24**

	1	**11**	**13**	**19**	**20**	**21**	**22**	**23**	**24**
Proton No.									
	Ole	Cym	Can	Cym	Dig	Cym	Ole	Ole	Ole
H-1′	4.53 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.58 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.92 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.53 (dd, 9.5, 2.0)	4.53 (dd, 9.5, 2.0)	4.53 (dd, 9.5, 2.0)
-3′	3.35*	3.79 (q, 3.0)	3.56 (m)	3.77 (q, 3.0)	4.20 (br s)	3.79*	3.35*	3.35*	
-4′	3.19 (t, 9.0)	3.22 (dd, 9.5, 3.0)	2.97 (t, 9.0)	3.21 (dd, 9.5, 3.0)	3.19 (dd, 9.5, 3.0)	3.22 (dd, 9.5, 3.0)	3.17 (t, 9.0)	3.17 (t, 9.0)	3.17 (t, 9.0)
-5′	3.28 (dq, 9.0, 6.0)	3.84 (dq, 9.5, 6.0)	3.28 (dq, 9.0, 6.0)	3.86 (dq, 9.5, 6.0)	3.79 (dq, 9.5, 6.0)	3.84 (dq, 9.5, 6.0)	3.28 (dq, 9.0, 6.0)	3.38*[a)]	3.28 (dq, 9.0, 6.0)
-6′	1.29 (d, 6.0)	1.22 (d, 6.0)	1.27 (d, 6.0)	1.22 (d, 6.0)	1.24 (d, 6.0)	1.21 (d, 6.0)	1.29 (d, 6.0)[a)]	1.29 (d, 6.0)	1.29 (d, 6.0)
	Dig	Dig	Dig	Ole	Ole	Dig	Cym	Cym	Cym
H-1″	5.01 (dd, 9.5, 2.0)	4.83 (dd, 9.5, 2.0)	4.82 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)	4.49 (dd, 9.5, 2.0)	4.83 (dd, 9.5, 2.0)	4.95 (dd, 9.5, 2.0)	4.95 (dd, 9.5, 2.0)	4.95 (dd, 9.5, 2.0)
-3″	4.22 (br s)	4.21 (br s)	4.23 (br s)	3.34*	3.35*	4.20 (br s)	3.78 (q, 3.0)	3.78 (q, 3.0)	3.78 (q, 3.0)
-4″	3.22 (dd, 9.5, 3.0)	3.20 (dd, 9.5, 3.0)	3.23 (dd, 9.5, 3.0)	3.15 (t, 9.0)	3.14 (t, 9.0)	3.19 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
-5″	3.83 (dq, 9.5, 6.0)	3.79*	3.91 (dq, 9.5, 6.0)	3.29 (dq, 9.0, 6.0)	3.29*	3.79*	3.89 (dq, 9.5, 6.0)	3.89 (dq, 9.5, 6.0)	3.89 (dq, 9.5, 6.0)
-6″	1.26 (d, 6.0)	1.23 (d, 6.0)	1.27 (d, 6.0)	1.30 (d, 6.0)[a)]	1.31 (d, 6.0)[a)]	1.22 (d, 6.0)	1.23 (d, 6.0)	1.23 (d, 6.0)	1.23 (d, 6.0)
	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole	Ole
H-1‴	4.55 (dd, 9.5, 2.0)	4.50 (dd, 9.5, 2.0)	4.51 (dd, 9.5, 2.0)	4.65 (dd, 9.5, 2.0)	4.65 (dd, 9.5, 2.0)	4.49 (dd, 9.5, 2.0)	4.45 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)	4.44 (dd, 9.5, 2.0)
-3‴	3.17*	3.37*	3.38*	3.34*	3.34*	3.38*[a)]	3.34*	3.35*	
-4‴	3.12 (t, 9.0)	3.17 (t, 9.0)	3.17 (t, 9.0)	3.18 (t, 9.0)	3.18 (t, 9.0)	3.16 (t, 9.0)	3.19 (t, 9.0)	3.18 (t, 9.0)	3.15 (t, 9.0)
-5‴	3.32 (dq, 9.0, 6.0)	3.33 (dq, 9.0, 6.0)	3.34 (dq, 9.0, 6.0)	3.30 (dq, 9.0, 6.0)	3.31*	3.32 (dq, 9.0, 6.0)	3.27 (dq, 9.0, 6.0)	3.38*[a)]	3.29 (dq, 9.0, 6.0)
-6‴	1.31 (d, 6.0)	1.29 (d, 6.0)	1.29 (t, 6.0)	1.28 (d, 6.0)[a)]	1.28 (d, 6.0)[a)]	1.28 (d, 6.0)	1.28 (d, 6.0)[a)]	1.28 (d, 6.0)	1.28 (d, 6.0)
		Ole	Ole	Cym	Cym	Ole	Dig	Cym	Ole
H-1⁗	—	4.72 (dd, 9.5, 2.0)	4.72 (dd, 9.5, 2.0)	4.95 (dd, 9.5, 2.0)	4.95 (dd, 9.5, 2.0)	4.66 (dd, 9.5, 2.0)	5.00 (dd, 9.5, 2.0)	4.95 (dd, 9.5, 2.0)	4.66 (dd, 9.5, 2.0)
-3⁗	—	3.17*	3.17*	3.80 (q, 3.0)	3.80*	3.35*[a)]	4.23 (br s)	3.80 (q, 3.0)	
-4⁗	—	3.13 (t, 9.0)	3.13 (t, 9.0)	3.23 (dd, 9.5, 3.0)	3.22 (dd, 9.5, 3.0)	3.17 (t, 9.0)	3.22 (dd, 9.5, 6.0)	3.24 (dd, 9.5, 3.0)	3.17 (t, 9.0)
-5⁗	—	3.31 (dq, 9.0, 6.0)	3.31 (dq, 9.0, 6.0)	3.90 (dq, 9.5, 6.0)	3.90 (dq, 9.5, 6.0)	3.33*	3.82 (dq, 9.5, 6.0)	3.89 (dq, 9.5, 6.0)	3.32 (dq, 9.0, 6.0)
-6⁗	—	1.35 (d, 6.0)	1.35 (d, 6.0)	1.24 (d, 6.0)	1.24 (d, 6.0)	1.32 (d, 6.0)	1.26 (d, 6.0)	1.24 (d, 6.0)	1.32 (d, 6.0)
				Ole	Ole	Ole	Ole	Ole	Ole
-1′′′′′	—	—	—	4.50 (dd, 9.5, 2.0)	4.50 (dd, 9.5, 2.0)	4.72 (dd, 9.5, 2.0)	4.55 (dd, 9.5, 2.0)	4.50 (dd, 9.5, 2.0)	4.71 (dd, 9.5, 2.0)
-3′′′′′	—	—	—	3.16*	3.17*	3.17*	3.17*		
-4′′′′′	—	—	—	3.12 (t, 9.0)	3.12 (t, 9.0)	3.13 (t, 9.0)	3.12 (t, 9.0)	3.12 (t, 9.0)	3.13 (t, 9.0)
-5′′′′′	—	—	—	3.28 (dq, 9.0, 6.0)	3.29*	3.31 (dq, 9.0, 6.0)	3.32 (dq, 9.0, 6.0)	3.37*[a)]	3.31*
-6′′′′′	—	—	—	1.32 (d, 6.0)	1.32 (d, 6.0)	1.34 (d, 6.0)	1.31 (d, 6.0)	1.32 (d, 6.0)	1.35 (d, 6.0)
-OMes	3.40 (s)	3.43 (s)	3.41 (s)	3.44 (s)	3.44 (s)	3.42 (s)	3.43 (s)	3.44 (s)	3.43 (s)
	3.39 (s)	3.41 (s)	3.40 (s)	3.43 (s)	3.41 (s)	3.41 (s)	3.39 (s)×2	3.43 (s)	3.41 (s)
	—	3.40 (s)	—	3.41 (s)	3.40 (s)	3.40 (s)×2	3.38 (s)	3.40 (s)	3.40 (s)
	—	—	—	3.39 (s)×2	3.39 (s)	—	—	3.39 (s)	3.38 (s)×2
	—	—	—	—	—	—	—	3.38 (s)	—

Measured in $CDCl_3$ solution at 35 °C. *: Overlapping with other signals. *a*) Signal assignments may be interchanged in each column. Cym: β-D-cymaropyranose, Ole: β-D-oleandropyranose, Dig: β-D-digitoxopyranose, Can: β-D-canaropyranose.

$C_{48}H_{76}O_{16}Na$: 931.5031). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **9**. The ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety were in good agreement with those of **11**, but the C-1′ signal was observed at δ 96.1.

Tuberoside Q_5 (**13**): Amorphous powder. $[\alpha]_D^{24}$ −52° (c=0.22, $CHCl_3$). FAB-MS *m/z*: 919 $[M+Na]^+$. HR-FAB-MS *m/z*: 919.5046 (Calcd for $C_{47}H_{76}O_{16}Na$: 919.5031). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric protons of β-D-canaropyranose and β-D-oleandropyranoses as follows: H-1′ and H-4′, H-1‴ and H-4‴, and H-1⁗ and H-4⁗. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, and H-1⁗ and H-4‴.

Tuberoside G_6 (**15**): Amorphous powder. $[\alpha]_D^{24}$ −62° (c=0.53, MeOH). FAB-MS *m/z*: 1075 $[M+Na]^+$. HR-FAB-MS *m/z*: 1075.5814 (Calcd for $C_{55}H_{88}O_{19}Na$: 1075.5818). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **9**. ^{13}C-NMR spectroscopic data of the sugar moiety: shown in Table 2. ^{1}H-NMR spectroscopic data of the sugar moiety ($CDCl_3$ at 35 °C) δ: 5.00 (1H, dd, 9.5, 2.0, H-1⁗), 4.84 (1H, dd, 9.5, 2.0, H-1′), 4.66 (1H, dd, 9.5, 2.0, H-1‴), 4.55 (1H, dd, 9.5, 2.0, H-1⁗′), 4.44 (1H, dd, 9.5, 2.0, H-1″), 4.23 (1H, br s, H-3⁗), 3.86 (1H, dq, 9.5, 6.0, H-5′), 3.83 (1H, dq, 9.5, 6.0, H-5⁗), 3.78 (1H, q, 3.0, H-3′), 3.44 (3H, s, –OMe), 3.41 (3H, s, –OMe), 3.40 (3H, s, –OMe), 3.38 (3H, s, –OMe), 3.32 (overlapping, H-5⁗′), 3.29 (2H, dq, 9.0, 6.0, H-5″ and H-5‴), 3.22 (1H, dd, 9.5, 3.0, H-4⁗), 3.21 (1H, dd, 9.5, 3.0, H-4′), 3.19 (1H, t, 9.0, H-4‴), 3.14 (1H, t, 9.0, H-4″), 3.12 (1H, t, 9.0, H-4⁗′), 1.31 (3H, d, 6.0, H-6⁗′), 1.31 (3H, d, 6.0, H-6″ or H-6‴), 1.28 (3H, d, 6.0, H-6‴ or H-6″), 1.26 (3H, d, 6.0, H-6⁗), 1.22 (3H, d, 6.0, H-6′).

Tuberoside I_5 (**16**): Amorphous powder. $[\alpha]_D^{23}$ −27° (c=1.07, MeOH). FAB-MS *m/z*: 1077 $[M+Na]^+$. HR-FAB-MS *m/z*: 1077.5975 (Calcd for $C_{55}H_{90}O_{19}Na$: 1077.5974). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. ^{13}C-NMR spectroscopic data of the sugar moiety: shown in Table 2. ^{1}H-NMR spectroscopic data of the sugar moiety ($CDCl_3$ at 35 °C) δ: 5.00 (1H, dd, 9.5, 2.0, H-1⁗), 4.84 (1H, dd, 9.5, 2.0, H-1′), 4.75 (1H, dd, 9.5, 2.0, H-1″), 4.55 (1H, dd, 9.5, 2.0, H-1⁗′), 4.44 (1H, dd, 9.5, 2.0, H-1‴), 4.23 (1H, br s, H-3⁗), 3.85 (1H, dq, 9.5, 6.0, H-5″), 3.84 (1H, dq, 9.5, 6.0, H-5′), 3.83 (1H, dq, 9.5, 6.0, H-5⁗), 3.79 (1H, q, 3.0, H-3′), 3.78 (1H, q, 3.0, H-3″), 3.44 (3H, s, –OMe), 3.43 (3H, s, –OMe), 3.40 (6H, s, –OMex2), 3.32 (1H, dq, 9.0, 6.0, H-5⁗′), 3.27 (1H, dq, 9.0, 6.0, H-5‴), 3.22 (1H, dd, 9.5, 3.0, H-4⁗), 3.20 (overlapping, H-4′ and H-4″), 3.19 (1H, t, 9.0, H-4‴), 3.12 (1H, t, 9.0, H-4⁗′), 1.31 (3H, d, 6.0, H-6⁗′), 1.28 (3H, d, 6.0, H-6‴), 1.26 (3H, d, 6.0, H-6⁗), 1.22 (3H, d, 6.0, H-6″), 1.20 (3H, d, 6.0, H-6′).

Tuberoside I_3 (**17**): Amorphous powder. $[\alpha]_D^{24}$ −33° (c=0.79, MeOH). FAB-MS *m/z*: 1135 $[M+Na]^+$. HR-FAB-MS *m/z*: 1135.6038 (Calcd for $C_{57}H_{92}O_{21}Na$: 1135.6029). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **5**. The ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety were in good agreement with those of **16**.

Tuberoside I_6 (**18**): Amorphous powder. $[\alpha]_D^{21}$ −41° (c=0.43, MeOH). FAB-MS *m/z*: 1075 $[M+Na]^+$. HR-FAB-MS *m/z*: 1075.5839 (Calcd for $C_{55}H_{88}O_{19}Na$: 1075.5818). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **9**. The ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety were in good agreement with those of **16**, but the C-1′ signal was shown at δ 96.1.

Tuberoside R_5 (**19**): Amorphous powder. $[\alpha]_D^{24}$ −36° (c=1.42, MeOH). FAB-MS *m/z*: 1091 $[M+Na]^+$. HR-FAB-MS *m/z*: 1091.6124 (Calcd for $C_{56}H_{92}O_{19}Na$: 1091.6131). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1″ and H-4″, H-1‴ and H-4‴, and H-1⁗′ and H-4⁗′. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, H-1⁗ and H-4‴, and H-1⁗′ and H-4⁗.

Tuberoside S_5 (**20**): Amorphous powder. $[\alpha]_D^{24}$ −50° (c=0.34, MeOH). FAB-MS *m/z*: 1077 $[M+Na]^+$. HR-FAB-MS *m/z*: 1077.5995 (Calcd for $C_{55}H_{90}O_{19}Na$: 1077.5974). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1″ and H-4″, and H-1‴ and H-4‴. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, H-1⁗ and H-4‴, and H-1⁗′ and H-4⁗.

Tuberoside T_5 (**21**): Amorphous powder. $[\alpha]_D^{24}$ −40° (c=0.30, MeOH). FAB-MS *m/z*: 1077 $[M+Na]^+$. HR-FAB-MS *m/z*: 1077.5994 (Calcd for $C_{55}H_{90}O_{19}Na$: 1077.5974). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1‴ and H-4‴, H-1⁗ and H-4⁗, and H-1⁗′ and H-4⁗′. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, H-1⁗ and H-4‴, and H-1⁗′ and H-4⁗.

Tuberoside U_1 (**22**): Amorphous powder. $[\alpha]_D^{21}$ −38° (c=0.54, MeOH). FAB-MS *m/z*: 1093 $[M+Na]^+$. HR-FAB-MS *m/z*: 1093.5917 (Calcd for $C_{55}H_{90}O_{20}Na$: 1093.5923). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **10**. ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1′ and H-4′, H-1‴ and H-4‴, and H-1⁗′ and H-4⁗′. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, H-1⁗ and H-4‴, and H-1⁗′ and H-4⁗.

Tuberoside V_5 (**23**): Amorphous powder. $[\alpha]_D^{23}$ −38° (c=0.77, MeOH). FAB-MS *m/z*: 1091 $[M+Na]^+$. HR-FAB-MS *m/z*: 1091.6135 (Calcd for $C_{56}H_{92}O_{19}Na$: 1091.6131). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1′ and H-4′, H-1‴ and H-4‴, and H-1⁗′ and H-4⁗′. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, H-1⁗ and H-4‴, and H-1⁗′ and H-4⁗.

Tuberoside W_5 (**24**): Amorphous powder. $[\alpha]_D^{24}$ −48° (c=0.54, $CHCl_3$). FAB-MS *m/z*: 1091 $[M+Na]^+$. HR-FAB-MS *m/z*: 1091.6127 (Calcd for $C_{56}H_{92}O_{19}Na$: 1091.6131). The ^{13}C- and ^{1}H-NMR spectroscopic data of the aglycone moiety were consistent with those of **1**. ^{13}C- and ^{1}H-NMR spectroscopic data of the sugar moiety: shown in Tables 2 and 3. HOHAHAs were exhibited on irradiation of the anomeric proton of each β-D-oleandropyranose as follows: H-1′ and H-4′, H-1‴ and H-4‴, H-1⁗ and H-4⁗, and H-1⁗′ and H-4⁗′. ROEs were observed on irradiating each anomeric proton as follows: H-1′ and H-3, H-1″ and H-4′, H-1‴ and H-4″, H-1⁗ and H-4‴, and H-1⁗′ and H-4⁗.

All compounds were named with reference to the previous report.[9)]

Acid Hydrolysis of the Pregnane Glycoside Fraction The fraction of pregnane glycosides eluted with a $CHCl_3$–MeOH (98 : 2) system on a silica gel column (295 mg) was heated at 60 °C for 2.5 h with dioxane (4 ml) and 0.1 M H_2SO_4 (1 ml) to obtain the aglycones and sugars. After hydrolysis, this reaction mixture was diluted with H_2O and extracted with EtOAc. The H_2O layer was passed through an Amberlite IRA-60E column and the eluate was concentrated to dryness. The residue was subjected to silica gel CC with a $CHCl_3$–MeOH–H_2O (7 : 1 : 1.2 bottom layer and 7 : 1.5 : 1.2 bottom layer) system to obtain cymarose, oleandrose, digitoxose and canarose. As to the absolute configuration, the monosaccharides were believed to have D-forms based on their optical rotation values.

D-Cymarose: $[\alpha]_D^{20}$ +49° (c=0.65, 24 h after dissolution in H_2O). (lit: $[\alpha]_D^{21}$ +51.6° (c=1.02, H_2O)[20)]).

D-Oleandrose: $[\alpha]_D^{20}$ −11° (c=2.46, 24 h after dissolution in H_2O). (lit: $[\alpha]_D$ −11° (c=1.1 H_2O)[21)]).

D-Canarose: $[\alpha]_D^{20}$ +15° (c=0.56, 24 h after dissolution in H_2O). (lit: $[\alpha]_D$ +25° (c=1.4, H_2O)[22)]).

Because the fraction containing D-digitoxose was not completely purified, the optical rotation value ($[\alpha]_D^{20}$ +27° (c=2.18, 24 h after dissolution in H_2O) (lit: $[\alpha]_D^{26}$ +48.4° (c=0.90, H_2O)[23)]) was not consistent with data in the literature. But, to date, all of the digitoxoses in pregnane glycosides from *Asclepias* spp. were reported to have the D-form, thus, digitoxose from the pregnane glycoside fraction of this plant was also believed to have the D-form.

Acid Hydrolysis of Compound 6 Compound **6** (9 mg) dissolved in dioxane (1 ml) and 0.1 M H_2SO_4 (0.25 ml) was heated at 60 °C for 1.5 h. The following procedures were described above. The EtOAc layer was concentrated to dryness. Purification of the residue by HPLC (YMC-ODS 10 mm i.d.×25 cm, 22.5% MeCN in water) afforded 2α-hydroxy-tuberogenin (**6a** (2 mg)).

2α-Hydroxy-tuberogenin (**6a**): Amorphous powder. $[\alpha]_D^{23}$ −47° (c=0.16, MeOH). FAB-MS *m/z*: 365 $[M+H]^+$, 387 $[M+Na]^+$. HR-FAB-MS *m/z*: 365.2305, 387.2137 (Calcd for $C_{21}H_{33}O_5$: 365.2328, $C_{21}H_{32}O_5Na$: 387.2147). ^{1}H-NMR spectroscopic data of the sugar moiety ($CDCl_3$ at 35 °C) δ: 4.51 (1H, dd, 8.5, 7.0, H-12), 3.89 (1H, dq, 10.5, 6.5, H-20), 3.60

(1H, m, H-2), 3.39 (1H, m, H-3), 2.36 (1H, br dd, 19.0, 9.0, H-15), 2.23 (1H, ddd, 19.0, 12.0, 9.0, H-15), 2.11 (1H, br d, 13.5, H-7β), 2.00 (overlapping, H-11α), 1.89 (1H, dd, 11.0, 6.5, H-17), 1.89 (1H, dd, 13.0, 8.5, H-11β), 1.86 (1H, dd, 12.0, 4.5, H-1β), 1.79 (1H, br dd, 14.0, 9.0, H-16β), 1.74 (1H, d, 8.0, H-9), 1.22 (3H, d, 6.5, H-21), 0.99 (1H, t, 12.0, H-1α), 0.93 (3H, s, H-18), 0.88 (3H, s, H-19).

The procedures for the detection of the component sugars were described in the previous report.[9] From the residue of the H_2O layer, cymaritol acetate and oleandritol acetate were identified using GC analysis. GC conditions: column, Supelco SP-2380^{TM} capillary column 0.25 mm i.d.×30 m; carrier gas, N_2; column temperature 200 °C; t_R, 6.8 min (cymaritol acetate), 7.7 min (oleandritol acetate).

Acid Hydrolysis of Compounds 1—3, 5, 7—13, 15—23, and 24 The compounds **1**—**3, 5, 7**—**13, 15**—**23**, and **24** (*ca.* 0.5 mg) were each dissolved in dioxane (80 μl) and 0.1 M H_2SO_4 (20 μl). The solutions were heated at 60 °C for 1 h. The following procedures were described previously.[9] The residue from each compound was analyzed using HPLC and GC to identify the aglycone and sugars through a comparison with authentic samples. HPLC conditions: column, YMC-ODS-AM 4.6 mm i.d.×25 cm; flow rate, 1.0 ml/min; 40% MeCN in water; t_R, 12.2 min (tuberogenin (**1a**)), 12.8 min (5,6-didehydrotuberogenin (**9a**)), 25% MeCN in water; t_R, 15.2 min (15β-hydroxytuberogenin (**10a**)); **1a** was detected in **1**, **2**, **8**, **11**, **13**, **16**, **19**, **20**, **21**, **23** and **24**. **9a** and **10a** were found in **9**, **12**, **15**, and **18** and in **10** and **22**. The residues of **3**, **5**, and **17** were acetylated with Ac_2O and pyridine (100 μl each) overnight at room temp. 3-*O*-Acetyl-15β-acetoxytuberogenin was detected in the reaction mixture of **3**, **5**, and **17** by HPLC. HPLC conditions: column, YMC-ODS-AM 4.6 mm i.d.×25 cm; flow rate, 1.0 ml/min; 60% MeCN in water; t_R, 13.2 min (3-*O*-acetyl-15β-acetoxytuberogenin). Detection of the aglycone of **7** could not be performed without authentic samples. GC conditions were described above. t_R, 6.8 min (cymaritol acetate), 7.7 min (oleandritol acetate), 9.6 min (digitoxitol acetate), 10.7 min (canaritol acetate). Cymaritol acetate was detected in **5**, **7**—**12**, **15**—**23** and **24**. Digitoxitol acetate was found in **1**—**3**, **11**—**13**, **15**—**18**, **20**, **21** and **22**. Canaritol acetate was observed in **13**. Oleandritol acetate was identified in all compounds.

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