Three New Nortriterpene Glycosides and Two New Triterpene Glycosides from the Bulbs of *Scilla scilloides*

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Three new norlanostane-type triterpene glycosides, scillanostasides A, B, and C, and two new lanostane-type triterpene glycosides, scillanostasides D and E, were isolated from the bulbs of *Scilla scilloides* Druce (Liliaceae) along with one known norlanostane-type triterpene heptaglycoside, scillascilloside G-1. Their chemical structures were determined on the basis of spectroscopic data as well as chemical evidence.

Key words *Scilla scilloides*; triterpene; lanostane; glycoside; scillanostaside; Liliaceae

Scilla scilloides Druce is a perennial herb belonging to the Liliaceae family. The bulb of this plant has been used as a foodstuff, a traditional medicine for promoting blood circulation, an anti-inflammatory agent, and an analgesic.[1)] With regard to the chemical constituents of this bulb, the presence of homoisoflavones, norlanostane-type triterpenes, and lanostane-type triterpenes has been reported.[2—6)] In a previous paper,[7)] we reported the isolation and structural elucidation of a new homostilbene and two new homoisoflavones from the methanol (MeOH) extract of fresh bulbs of *S. scilloides* along with 13 known compounds consisting of a homostilbene, seven homoisoflavones, a xanthone, a lignan, and three norlanostane-type triterpenes. As part of an ongoing study of this plant, we describe the isolation and structural characterization of three new norlanostane-type triterpene glycosides and two new lanostane-type triterpene glycosides along with one known norlanostane-type triterpene glycoside from the MeOH extract.

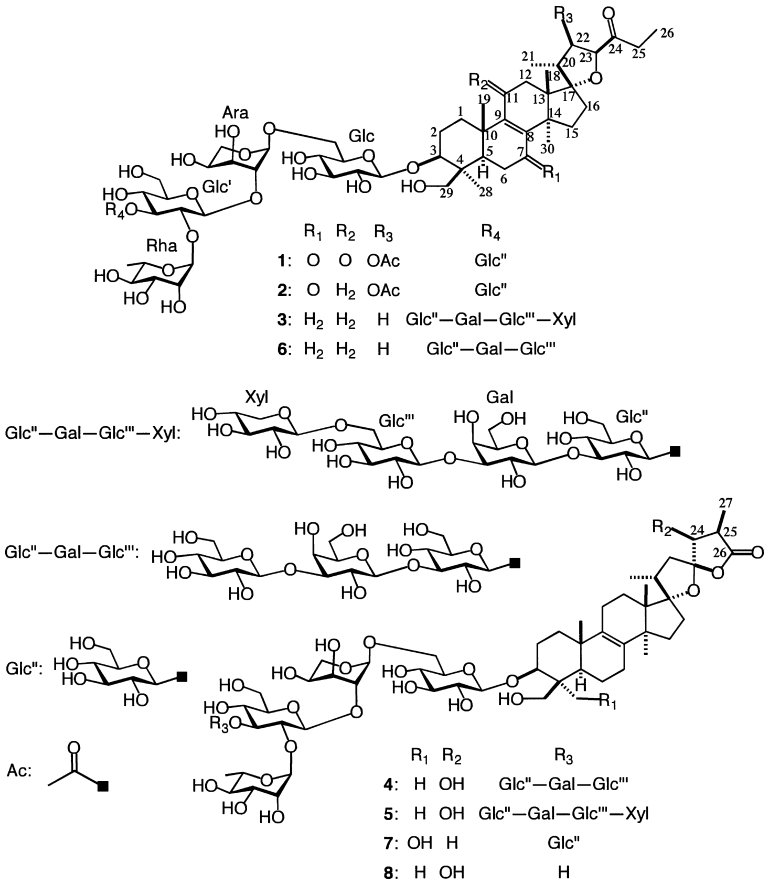

Fig. 1. Structures of **1**—**8**

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Table 1. ^{1}H-NMR Spectral Data for Aglycone Moiety of **1** and **2** (in Pyridine-d_5, 500 MHz)

	1	**2**
Ag-1a	3.13 ddd (4.0, 4.0, 13.5)	1.83 ddd (4.0, 4.0, 13.5)
1b	1.37 m	1.39 ddd (4.0, 13.5, 13.5)
2a	2.34 m	2.37 m
2b	2.20 m	2.09
3	3.63	3.62
5	1.87 dd (4.0, 13.0)	1.92 dd (7.0, 9.5)
6a	2.90	2.81
6b	2.86	2.77
11a		2.50
11b		2.29 m
12a	3.45 d (16.0)	2.47
12b	2.63 d (16.0)	1.58 m
15a	2.67	2.67
15b	2.08 m	2.06
16a	2.69	2.72
16b	1.97	2.11
18	1.01 s	0.89 s
19	1.49 s	1.15 s
20	2.38 q like (6.0)	2.41 q like (6.5)
21	1.02 d (6.0)	1.11 d (6.5)
22	5.40 d (5.5)	5.41 d (5.0)
23	4.99 d (5.5)	5.00 d (5.0)
25	2.52 q (7.0)	2.58 q (7.0)
26	1.08 t (7.0)	1.08 t (7.0)
28	1.52 s	1.50 s
29a	4.43 d (11.0)	4.42 d (11.5)
29b	3.87 d (11.0)	3.83 d (11.5)
30	1.93 s	1.74 s
$COCH_3$	1.97 s	1.95 s

δ in ppm from tetramethylsilane (TMS) (coupling constants (J in Hz) are given in parentheses).

Table 2. ^{1}H-NMR Spectral Data for Sugar Moiety of **1** and **2** (in Pyridine-d_5, 500 MHz)

	1	**2**
Glc-1	4.89 d (7.5)	4.92 d (8.0)
2	3.95	3.96 dd (8.0, 8.5)
3	4.13	4.13
4	4.14	4.14
5	3.98	4.00
6a	4.50	4.57 dd (3.0, 11.0)
6b	4.20	4.24
Ara-1	5.28 d (2.0)	5.33 d (2.5)
2	4.64	4.69 dd (2.5, 5.5)
3	4.64	4.66 dd (3.0, 5.5)
4	4.51	4.54
5a	4.33 dd (8.0, 11.0)	4.36 dd (8.0, 11.5)
5b	3.87	3.91 dd (3.5, 11.5)
Glc′-1	5.16 d (7.5)	5.20 d (8.0)
2	4.18	4.19
3	4.06	4.06
4	4.06	4.07
5	3.63	3.62
6a	4.21	4.22
6b	4.16	4.14
Rha-1	6.29 br s	6.32 d (1.5)
2	4.81 br s	4.81 dd (1.5, 3.0)
3	4.60 dd (3.0, 9.5)	4.61 dd (3.0, 9.5)
4	4.25	4.27 dd (9.5, 9.5)
5	4.84 dq (9.0, 6.5)	4.88 dq (9.5, 6.5)
6	1.72 d (6.5)	1.76 d (6.5)
Glc″-1	5.02 d (7.5)	5.01 d (7.5)
2	3.96	3.98
3	4.18	4.24 dd (9.0, 9.0)
4	4.06	4.07 dd (9.0, 9.0)
5	3.96	3.97
6a	4.51	4.52 dd (2.0, 11.5)
6b	4.23	4.23

δ in ppm from TMS (coupling constants (J in Hz) are given in parentheses).

The MeOH extract of the fresh bulbs of *S. scilloides* was suspended in H_2O and successively extracted with ethyl acetate (EtOAc) and *n*-butanol (BuOH). Repeated chromatography of the aqueous layer with Diaion HP20, silica gel, and Chromatorex octadecyl silica (ODS) column chromatography as well as HPLC on ODS and silica gel led to the isolation of six compounds (**1**—**6**).

Compound **6** was identified as scillascilloside G-1 on the basis of its physical and spectral data (Fig. 1).[5)]

Compound **1**, tentatively named scillanostaside A, was obtained as an amorphous powder. In negative-ion FAB-MS, **1** gave an $[M-H]^-$ ion peak at *m/z* 1307 along with fragment ion peaks at *m/z* 1247 [1307−60 ($C_2H_4O_2$, acetic acid unit)]$^-$, 1101 [1247−146 ($C_6H_{10}O_4$, methyl pentosyl unit)]$^-$, 1085 [1247−162 ($C_6H_{10}O_5$, hexosyl unit)]$^-$, 939 [1085−146]$^-$, and 777 [939−162]$^-$. High-resolution (HR)-positive-ion FAB-MS showed the molecular formula of **1** to be $C_{60}H_{92}O_{31}$. The ^{1}H-NMR spectrum of **1** indicated signals due to four tertiary methyl groups (δ 1.93, 1.52, 1.49, 1.01), two secondary methyl groups [δ 1.72 (d, J=6.5 Hz), 1.02 (d, J=6.0 Hz)], one primary methyl group [δ 1.08 (t, J=7.0 Hz)], one acetyl group (δ 1.97), and five anomeric protons [δ 6.29 (br s), 5.28 (d, J=2.0 Hz), 5.16 (d, J=7.5 Hz), 5.02 (d, J=7.5 Hz), 4.89 (d, J=7.5 Hz)]. The ^{13}C-NMR spectrum of **1** exhibited signals due to three keto carbonyl carbons (δ 208.0, 202.4, 202.0), one ester carbonyl carbon (δ 169.8), two olefinic carbons (δ 151.8, 151.1), and five anomeric carbons (δ 106.2, 104.2, 102.5, 102.0, 101.1). The ^{1}H- and ^{13}C-NMR spectra were similar to those of **6**, apart from the appearance of signals due to two carbonyl groups, one acetoxy group, and one oxygenated methine group and the loss of signals due to three methylene groups and two monosaccharide units. The NMR signals were assigned in detail with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and total correlation spectroscopy (TOCSY) techniques (Tables 1—4). In the HMBC spectrum of **1**, the keto carbonyl carbon at δ 202.0 showed long-range correlations with the methylene protons assignable to H_2-6 of aglycone moiety (Agl) at δ 2.90 and 2.86, with which the methine proton due to H-5 of Agl at δ 1.87 exhibited correlations in the ^{1}H–^{1}H COSY spectrum. Further, cross-peaks between the keto carbonyl carbon at δ 202.4 and the methylene protons due to H_2-12 of Agl at δ 3.45 and 2.63 were observed in the HMBC spectrum. In addition, the ester carbonyl carbon at δ 169.8 exhibited a long-range correlation with the oxygenated methine proton at δ 5.40 assignable to H-22 of Agl. The foregoing correlations indicated the presence of two carbonyl groups at C-7 and C-11 and an acetoxy group at C-22 of Agl. Thus, the planar structure of **1** was a pentaglycoside of 22-acetoxy-17,23-epoxy-3,28-dihydroxy-27-nor-lanost-8-ene-7,11,24-trione, as illustrated in Fig. 2. On acidic hydrolysis, **1** afforded L-rhamnose, L-arabinose, and D-glucose, which were identified by optical rotation using chiral detection in HPLC analysis, together with several unidentified artificial aglycones. The cou-

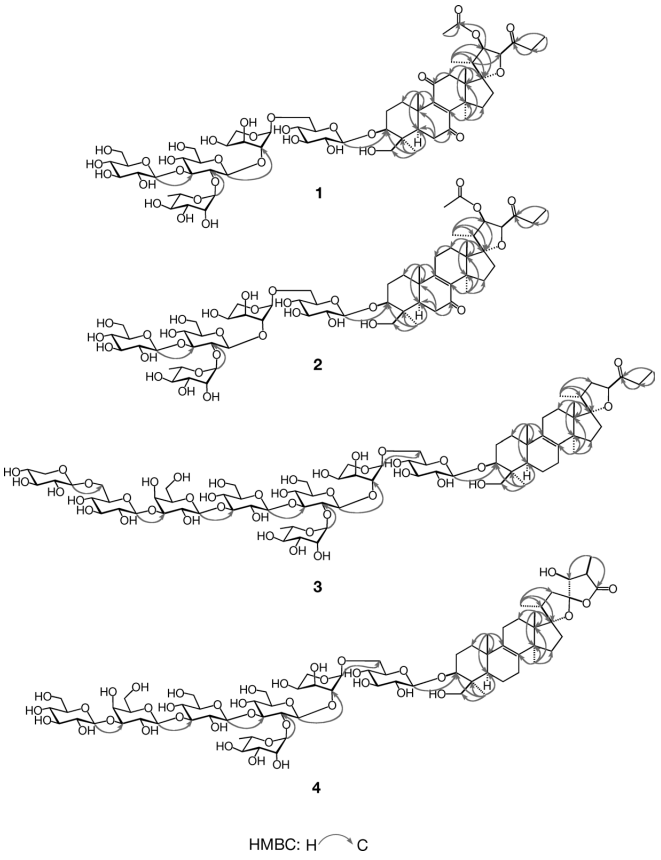

Fig. 2. ^{1}H–^{13}C Long-Range Correlations Observed in the HMBC Spectra of **1**—**4** (in Pyridine-d_5, 500 MHz)

Table 3. ^{13}C-NMR Spectral Data for Aglycone Moiety of **1**—**6** (in Pyridine-d_5, 125 MHz)

	1	**2**	**3**	**4**	**5**	**6**
1	34.4	35.0	35.7	35.7	35.7	35.7
2	27.2	27.1	27.4	27.4	27.4	27.4
3	87.4	87.8	88.9	88.9	89.0	88.9
4	44.0	44.1	44.3	44.4	44.3	44.4
5	51.0	50.9	51.7	51.7	51.7	51.8
6	37.4	37.5	18.7	18.7	18.6	18.7
7	202.0	198.3	26.8	26.8	26.8	26.9
8	151.1	139.5	135.2	135.0	135.0	135.4
9	151.8	164.5	134.5	134.7	134.7	134.6
10	39.8	39.6	36.7	36.8	36.8	36.8
11	202.4	23.6	21.0	20.9	20.9	21.1
12	47.5	24.7	25.2	24.9	24.9	25.3
13	50.2	48.8	48.8	48.7	48.7	48.9
14	51.9	50.4	50.8	50.7	50.7	50.8
15	34.1	34.2	32.0	31.8	31.8	32.1
16	38.6	39.6	39.6	37.7	37.7	39.7
17	96.0	96.2	97.0	99.2	99.1	97.1
18	20.0	19.4	19.2	18.7	18.6	19.3
19	17.5	18.3	19.5	19.5	19.5	19.5
20	49.3	49.5	43.6	43.8	43.8	43.7
21	14.8	15.4	17.2	18.6	18.6	17.2
22	81.5	82.0	36.8	38.4	38.4	36.9
23	85.0	85.0	81.5	117.0	116.9	81.6
24	208.0	208.9	212.6	77.4	77.4	212.5
25	33.2	33.4	32.2	41.2	41.2	32.3
26	7.5	7.5	7.7	178.7	178.7	7.7
27				8.8	8.8	
28	22.9	22.5	23.0	23.1	23.0	23.1
29	63.1	62.9	63.1	63.1	63.1	63.1
30	28.6	27.5	26.3	25.9	25.9	26.4
CO	169.8	169.9				
CH_3	20.7	20.8				

δ in ppm from TMS.

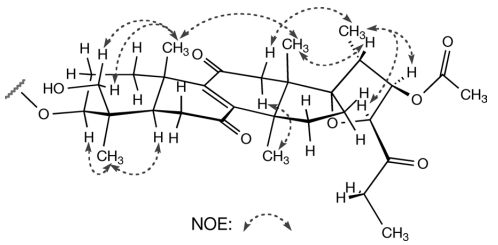

Fig. 3. Key NOE Correlations Observed in the NOESY Spectrum of **1** (in Pyridine-d_5, 500 MHz)

pling constants of the signals due to the anomeric protons in the ^{1}H-NMR spectrum and the chemical shifts[8,9] of the signals due to arabinosyl and rhamnosyl units in the ^{13}C-NMR spectrum suggested that all monosaccharide units were of the pyranose form. Furthermore, the mode of glycosidic linkages of glucopyranosyl units were β in $^{4}C_1$ conformation and those of arabinopyranosyl and rhamnopyranosyl units were α in $^{1}C_4$ conformation. The HMBC spectrum of **1** showed key correlations between H-1 of the first glucosyl unit (Glc) and C-3 of Agl; H-1 of the second glucosyl unit (Glc′) and C-2 of the arabinosyl unit (Ara); H-1 of the rhamnosyl unit (Rha) and C-2 of Glc′; and H-1 of the third glucosyl unit (Glc″) and C-3 of Glc′ (Fig. 2). In addition, the ^{13}C-NMR spectral data of the sugar moiety of **1** were considerably similar to those of scillasaponin B (**7**) (Fig. 1).[10] These data indicated that the sugar chain attached to the hydroxyl group at C-3 of Agl of **1** was identical to that of **7**. The stereochemistry of Agl was defined on the basis of the nuclear Overhauser effect spectroscopy (NOESY) and the ^{13}C-NMR spectra of **1**. In the NOESY spectrum, key nuclear Overhauser effect (NOE) correlations were observed between H-3 and H_3-28; H-5 and H_3-28; Hb-12 and H_3-21; Ha-12 and H_3-30; H_3-18 and H_3-19; H_3-18 and H-20; H_3-19 and H_2-29; H_3-21 and H-22; and H_3-21 and H-23 (Fig. 3). Moreover, the resonances due to C-20—C-26 in the ^{13}C-NMR spectrum were superimposable on those of (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-ene-24-one.[11] The structure of **1** was thus concluded to be (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-ene-7,11,24-trione 3-*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **2**, tentatively named scillanostaside B, was obtained as an amorphous powder. The molecular formula of **2** was determined to be $C_{60}H_{94}O_{30}$ by HR-positive-ion FAB-MS. The ^{1}H-NMR spectrum of **2** was similar to that of **1**, especially the signals due to the sugar moiety were almost superimposable. Furthermore, the ^{13}C-NMR spectrum of **2** was also quite similar to that of **1**, except for the loss of signal due to one carbonyl carbon and the appearance of an additional signal due to one methylene carbon. As in the case of **1**, these ^{1}H- and ^{13}C-NMR signals were examined in detail, and the structure of **2** was deduced to be a deoxy-derivative at C-11 of **1** (Fig. 2). This assumption was confirmed by the observation of NOE correlations similar to those of **1** in the

Table 4. ^{13}C-NMR Spectral Data for Sugar Moiety of **1**—**6** (in Pyridine-d_5, 125 MHz)

	1	**2**	**3**	**4**	**5**	**6**
Glc-1	106.2	106.1	105.9	106.1	106.0	106.0
2	75.2	75.3	75.2	75.3	75.3	75.3
3	78.1	78.2	78.1	78.2	78.2	78.2
4	72.6	72.4	72.4	72.5	72.5	72.5
5	75.4	75.8	75.5	75.5	75.5	75.5
6	68.7	68.6	68.5	68.6	68.6	68.5
Ara-1	101.1	101.2	101.1	101.2	101.2	101.1
2	77.6	77.6	77.5	77.5	77.5	77.6
3	71.6	71.6	71.6	71.5	71.6	71.5
4	66.7	66.8	66.8	66.8	66.8	66.8
5	62.8	62.8	62.8	62.8	62.8	62.8
Glc′-1	102.5	102.3	102.3	102.4	102.4	102.4
2	76.9	76.9	76.9	76.8	77.0	76.9
3	88.9	89.1	88.9	89.0	88.9	89.0
4	69.1	69.2	69.1	69.0	69.1	69.1
5	77.8	77.7	77.8	77.7	77.7	77.7
6	61.8	61.8	61.7	61.7	61.7	61.8
Rha-1	102.0	102.0	101.8	101.9	101.9	101.9
2	72.1	72.2	72.0	72.1	72.1	72.1
3	72.5	72.5	72.4	72.5	72.5	72.5
4	74.0	74.1	73.9	74.1	74.0	74.1
5	69.7	69.7	69.6	69.7	69.7	69.7
6	18.7	18.7	18.6	18.7	18.7	18.7
Glc″-1	104.2	104.3	103.7	103.8	103.7	103.7
2	74.9	74.9	73.5	73.6	73.6	73.6
3	78.3	78.4	88.1	88.2	88.2	88.2
4	71.4	71.4	69.4	69.5	69.5	69.5
5	78.5	78.6	77.9	77.9	77.9	77.9
6	62.3	62.3	62.0	62.0	62.0	62.1
Gal-1			105.2	105.3	105.4	105.3
2			71.6	71.6	71.7	71.7
3			83.9	84.2	84.0	84.3
4			69.2	69.5	69.3	69.5
5			76.8	76.9	76.8	76.8
6			61.9	62.0	62.0	62.0
Glc‴-1			105.6	106.0	105.7	106.1
2			75.2	75.7	75.3	75.7
3			77.4	78.3	77.4	78.3
4			71.4	71.7	71.4	71.6
5			76.6	78.5	76.7	78.5
6			70.0	62.6	70.0	62.6
Xyl-1			105.6		105.7	
2			74.7		74.8	
3			78.1		78.2	
4			70.9		71.0	
5			66.9		67.0	

δ in ppm from TMS.

NOESY spectrum of **2**. Consequently, the structure of **2** was found to be (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-ene-7,24-dione 3-*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **3**, tentatively named scillanostaside C, was obtained as an amorphous powder. In negative-ion FAB-MS, **3** gave an $[M-H]^-$ ion peak at *m/z* 1677 along with fragment ion peaks at *m/z* 1545 [1677−132 ($C_5H_8O_4$, pentosyl unit)]$^-$, 1531 [1677−146]$^-$, 1383 [1545−162]$^-$, 1237 [1383−146]$^-$, 1221 [1383−162]$^-$, 1075 [1221−146]$^-$, 1059 [1221−162]$^-$, 913 [1059−146]$^-$, 751 [913−162]$^-$, 619 [751−132]$^-$, and 457 [619−162]$^-$ (Fig. 4). HR-positive-ion FAB-MS showed the molecular formula of **3** to be $C_{75}H_{122}O_{41}$. The ^{1}H-NMR spectrum of **3**, which was analogous to that of **6**, showed signals due to four tertiary methyl groups (δ 1.54, 1.52, 0.94, 0.91), two secondary methyl groups [δ 1.72 (d, *J*=6.0 Hz), 1.04 (d, *J*=7.0 Hz)], one primary methyl group [δ 1.07 (t, *J*=7.5 Hz)], and eight anomeric protons [δ 6.16 (br s), 5.26 (br s), 5.18 (d, *J*=7.5 Hz), 5.16 (d, *J*=7.5 Hz), 5.09 (d, *J*=8.0 Hz), 4.96 (d, *J*=7.5 Hz), 4.93 (d, *J*=7.5 Hz), 4.84 (d, *J*=7.5 Hz)] (Table 5). The ^{13}C-NMR spectrum of **3**, which was also similar to that of **6** with additional signals due to one pentosyl unit, contained signals assignable to one keto carbonyl carbon (δ 212.6), two olefinic carbons (δ 135.2, 134.5), and eight anomeric carbons (δ 105.9, 105.6, 105.6, 105.2, 103.7, 102.3, 101.8, 101.1). These signals were assigned with the help of 2D-NMR techniques as in the case of **1**, and the assigned data of Agl were superimposable on those of **6** (Table 3). Thus, **3** was determined to be an octaglycoside of 15-deoxyeucosterol. On acidic hydrolysis, **3** afforded L-rhamnose, L-arabinose, D-xylose, D-glucose, and D-galactose. Moreover, the glycosidic linkages in glucopyranosyl, galactopyranosyl, and xylopyranosyl units were β and those in arabinopyranosyl and rhamnopyranosyl units were α based on the ^{1}H- and ^{13}C-NMR spectral data. In the HMBC spectrum of **3**, key correlations were observed between H-1 of Glc and C-3 of Agl; H-1 of Ara and C-6 of Glc; H-1 of Glc′ and C-2 of Ara; H-1 of Rha and C-2 of Glc′; H-1 of Glc″ and C-3 of Glc′, H-1 of galactosyl unit (Gal) and H-3 of Glc″; H-1 of firth glucosyl unit (Glc‴) and C-3 of Gal; H-1 of xylosyl unit (Xyl) and C-6 of Glc″ (Fig. 2). These data showed that for **3**, one β-D-xylopyranosyl unit may be attached to OH-6 of Glc‴ of **6**. This was confirmed by the following evidence. Comparing the chemical shifts of signals due to the sugar moieties between **3** and **6**, glycosylation shifts[12,13] were observed at C-5 and C-6 of Glc‴ with magnitudes −1.9 and +7.4 ppm, respectively, with the appearance of signals due to one terminal xylopyranosyl unit. The resonances of other signals were almost identical to those of **6**. In addition, the HR-positive-ion FAB-MS of the peracetate (**3a**) of **3** revealed fragment ion peaks at *m/z* 259.0812, 273.0966, 835.2495, and 1123.3357, which were assigned to the fragment ions of the 2,3,4-*O*-triacetylxylopyranosyl unit, the 2,3,4-*O*-triacetylrhamnopyranosyl unit, the 2,3,4-*O*-triacetylxylopyranosyl-(1→6)-*O*-(2,3,4-*O*-triacetyl)glucopyranosyl-(1→3)-*O*-(2,4,6-*O*-triacetyl)galactopyranosyl unit, and the 2,3,4-*O*-triacetylxylopyranosyl-(1→6)-*O*-(2,3,4-*O*-triacetyl)glucopyranosyl-(1→3)-*O*-(2,4,6-*O*-triacetyl)galactopyranosyl-(1→3)-*O*-(2,4,6-*O*-triacetyl)glucopyranosyl unit, respectively, whereas a fragment ion peak ($[C_{14}H_{19}O_9]^+$) generated from a 2,3,4,6-tetracaetylhexosyl unit was not detected. Thus, **3** was concluded to be 15-deoxyeucosterol 3-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→3)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **4**, tentatively named scillanostaside D, was obtained as an amorphous powder, and negative-ion FAB-MS showed an $[M-H]^-$ ion peak at *m/z* 1589 together with fragment ion peaks at *m/z* 1443 [1589−146]$^-$, 1427 [1589−162]$^-$, 1281 [1427−146]$^-$, 1265 [1427−162]$^-$, 1103 [1265−162]$^-$, 957 [1103−146]$^-$, 795 [957−162]$^-$, 663 [795−132]$^-$, and 501 [663−162]$^-$. The molecular formula of **4** was analyzed as $C_{71}H_{114}O_{39}$ using HR-positive-ion FAB-

Table 5. ^{1}H-NMR Spectral Data for Sugar Moiety of **3**—**6** (in Pyridine-d_5, 500 MHz)

	3	**4**	**5**	**6**
Glc-1	4.93 d (7.5)	4.94 d (8.0)	4.94 d (8.0)	4.96 d (8.0)
2	3.96	3.97	3.98	3.97
3	4.18	4.16	4.18	4.16
4	4.19	4.17	4.19	4.17
5	3.97	4.02	3.99	4.01
6a	4.52	4.53	4.53	4.53
6b	4.27	4.26	4.26	4.26
Ara-1	5.26 br s	5.28 d (2.5)	5.28 d (3.0)	5.30 br s
2	4.66	4.66	4.66	4.66
3	4.66	4.65	4.66	4.66
4	4.52	4.52	4.53	4.53
5a	4.35	4.35	4.36	4.36 dd (5.0, 11.5)
5b	3.90	3.89	3.90	3.90
Glc′-1	5.16 d (7.5)	5.15 d (7.5)	5.16 d (7.5)	5.16 d (7.5)
2	4.17	4.16	4.17	4.18
3	4.04	4.05	4.03	4.06
4	4.04	4.05	4.03	4.06
5	3.59	3.57	3.58	3.59
6a	4.21	4.19	4.19	4.20
6b	4.14	4.15	4.15	4.15
Rha-1	6.16 br s	6.20 br s	6.19 br s	6.21 br s
2	4.81 br s	4.81 br s	4.81 d (2.5)	4.81 br s
3	4.61 dd (2.0, 9.0)	4.61 dd (3.0, 8.5)	4.61 dd ((2.5, 9.0)	4.62
4	4.25	4.25	4.26	4.26 dd (9.0, 9.0)
5	4.86	4.86 dq (9.0, 6.5)	4.86 dq (9.0, 6.5)	4.87 dq (9.0, 6.5)
6	1.72 d (6.0)	1.73 d (6.5)	1.73 d (6.5)	1.74 d (6.5)
Glc″-1	4.96 d (7.5)	4.97 d (8.0)	4.97 d (7.5)	4.97 d (8.0)
2	3.91	3.91	3.91	3.92
3	4.11	4.11	4.11	4.11
4	3.90	3.91	3.90	3.90
5	3.91	3.91	3.90	3.91
6a	4.41	4.42	4.40	4.43 dd (3.5, 11.5)
6b	4.09	4.08	4.09	4.10 dd (5.0, 11.5)
Gal-1	5.09 d (8.0)	5.12 d (7.5)	5.08 d (7.5)	5.11 d (8.0)
2	4.59 dd (8.0, 9.0)	4.61 dd (7.5, 8.5)	4.61 dd (7.5, 9.0)	4.63
3	4.23	4.21	4.24	4.21
4	4.75 br d (3.0)	4.65	4.78	4.65
5	4.02	4.03	4.01	4.03
6a	4.37	4.37	4.37	4.37 dd (3.5, 11.0)
6b	4.21	4.23	4.21	4.23
Glc‴-1	5.18 d (7.5)	5.31 d (7.5)	5.21 d (7.5)	5.31 d (8.0)
2	3.96	4.00	3.96	4.01
3	4.16	4.25	4.17	4.24
4	3.99	4.19	4.01	4.18
5	3.89	3.96	3.90	3.97
6a	4.76	4.50 dd (2.0, 12.0)	4.79	4.50 dd (2.5, 11.5)
6b	4.10	4.32 dd (5.5, 12.0)	4.11	4.33 dd (5.5, 11.5)
Xyl-1	4.84 d (7.5)		4.88 d (7.5)	
2	3.97		3.99	
3	4.09		4.09	
4	4.17		4.17	
5a	4.27		4.29 dd (5.0, 11.5)	
5b	3.59		3.61	

δ in ppm from TMS (coupling constants (J in Hz) are given in parentheses).

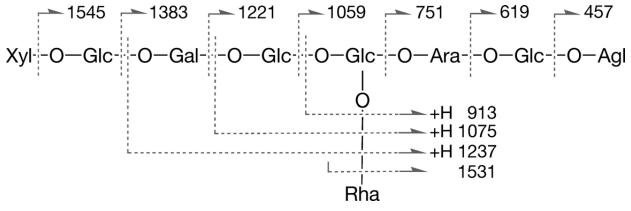

Fig. 4. Fragment Ions Observed in the Negative-Ion FAB-MS of **3**

MS. The ^{1}H-NMR spectrum of **4** showed signals due to four tertiary methyl groups (δ 1.54, 1.27, 0.93, 0.89), three secondary methyl groups [δ 1.73 (d, J=6.5 Hz), 1.50 (d, J=7.5 Hz), 1.09 (d, J=6.5 Hz)], and seven anomeric protons [δ 6.20 (br s), 5.31 (d, J=7.5 Hz), 5.28 (d, J=2.5 Hz), 5.15 (d, J=7.5 Hz), 5.12 (d, J=7.5 Hz), 4.97 (d, J=8.0 Hz), 4.94 (d, J=8.0 Hz)]. The ^{13}C-NMR spectrum of **4** was analogous to that of **6**; in particular, the signals due to the sugar moiety were almost superimposable, indicating signals due to one carboxyl carbon (δ 178.7), two olefinic carbons (δ 135.0, 134.7), one acetal carbon (δ 117.0), and seven anomeric car-

bons (δ 106.1, 106.0, 105.3, 103.8, 102.4, 101.9, 101.2). These NMR signals were assigned in detail with the aid of 2D-NMR techniques as done for **1**. The assigned ^{13}C-NMR spectral data of Agl were quite similar to those of scillasaponin D (**8**) (Fig. 1).[14] Consequently, the structure of **4** was concluded to be (23*S*,24*S*,25*R*)-17α,23-epoxy-24,29-dihydroxy-lanosta-8-en-23,26-olide 3-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→3)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **5**, tentatively named scillanostaside E, was obtained as an amorphous powder, and positive-ion FAB-MS showed an $[M-H]^-$ ion peak at *m/z* 1721, which was 132 mass units larger than that of **4**, along with fragment ion peaks at *m/z* 1589 $[1721-132]^-$, 1575 $[1721-146]^-$, 1427 $[1589-162]^-$, 1265 $[1427-162]^-$, 1119 $[1265-146]^-$, 1103 $[1265-162]^-$, 957 $[1103-146]^-$, 795 $[957-162]^-$, 663 $[795-132]^-$, and 501 $[663-162]^-$. The molecular formula of **5** was determined to be $C_{76}H_{122}O_{43}$ by HR-positive-ion FAB-MS. The ^{1}H- and ^{13}C-NMR spectral data of the sugar moiety and Agl were considerably similar to those of **3** and **4**, respectively. On the basis of these data, **5** was determined to be (23*S*,24*S*,25*R*)-17α,23-epoxy-24,29-dihydroxy-lanosta-8-en-23,26-olide 3-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→3)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

The study of the bulbs of *S. scilloides* resulted in the isolation and structural elucidation of three new norlanostane-type triterpene glycosides, named scillanostasides A, B, and C, and two new lanostane-type triterpene glycosides, scillanostasides D and E, along with one known norlanostane-type triterpene glycoside. Among them, each scillanostasides A and B had new aglycones; furthermore, the sugar moiety attached to C-3 of the aglycones of scillanostasides C and E was a new octasaccharide.

Experimental

All instruments and materials used were the same as cited in a previous report[15] unless otherwise specified.

Plant Material The bulbs of *S. scilloides* were cultivated in Kumamoto prefecture, Japan, and were harvested in August 2005, and identified by one of authors (T. Nohara). A voucher specimen has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

Extraction and Isolation The crushed fresh bulbs of *S. scilloides* (18.5 kg) were extracted with MeOH at room temperature, and the solvent was removed under reduced pressure to give a syrup (3521.7 g). The MeOH extract was suspended in H_2O and successively extracted with EtOAc and BuOH. The aqueous layer was chromatographed over Diaion HP20 column (H_2O, MeOH, acetone) to afford MeOH eluate fraction (fr.) and acetone eluate fr. The MeOH eluate fr. (76.7 g) was further subjected to Diaion HP20 column (H_2O, 50% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to give fractions (frs.) 1—4. Fr. 2 (31.4 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to give frs. 2.1—2.6. Fr. 2.3 (7.69 g) was subjected to Chromatorex ODS (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to afford frs. 2.3.1—2.3.6. HPLC (Nacalai Tesque, Inc., Kyoto, Japan, Cosmosil 5C18 AR-II, 20 mm i.d.×250 mm, 60% MeOH) of fr. 2.3.4 (2.63 g) afforded **1** (42 mg), **2** (100 mg), and frs. 2.3.4.1—2.3.4.4. A part (10.0 g) of fr. 2.4 (13.6 g) was subjected to Chromatorex ODS (70% MeOH, 80% MeOH, 90% MeOH, MeOH) to afford **6** (8.60 g) and frs. 2.4.1—2.4.5. Fr. 2.5 (1.68 g) was subjected to HPLC (Cosmosil 5C18 AR-II, 80% MeOH) to give frs. 2.5.1—2.5.4. Fr. 2.5.2 (281 mg) and fr. 2.5.4 (334 mg) were each subjected to HPLC [Nacalai Tesque, Inc., Kyoto, Japan, Cosmosil 5SL-II, 20 mm i.d.×250 mm, $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, 6 : 4 : 1)] to give **5** (78 mg) and frs. 2.5.2.1—2.5.2.3 from fr. 2.5.2, and **3** (77 mg) from fr. 2.5.4. HPLC (Cosmosil 5C18 AR-II, 70% MeOH) of fr. 2.5.2.2 (85 mg) afforded **4** (27 mg).

1: Amorphous powder. $[\alpha]_D^{25}$ −33.2° (*c*=4.4, pyridine). UV λ_{max} (MeOH) nm (log ε): 264 (3.65). Positive-ion FAB-MS *m/z*: 1331 $[M+Na]^+$. HR-positive-ion FAB-MS *m/z*: 1331.5508 (Calcd for $C_{60}H_{92}O_{31}Na$: 1331.5520). Negative-ion FAB-MS *m/z*: 1307 $[M-H]^-$, 1247 $[1307-60]^-$, 1161 $[1307-146]^-$, 1146, 1101 $[1247-146]^-$, 1085 $[1247-162]^-$, 939 $[1085-146]^-$, 777 $[939-146]^-$, 661, 529, 367. ^{1}H-NMR spectral data (in pyridine-d_5, 500 MHz) δ: see Tables 1 and 2. ^{13}C-NMR spectral data: see Tables 3 and 4.

2: Amorphous powder. $[\alpha]_D^{25}$ −33.3° (*c*=3.0, pyridine). UV λ_{max} (MeOH) nm (log ε): 254 (3.78). Positive-ion FAB-MS *m/z*: 1317 $[M+Na]^+$. HR-positive-ion FAB-MS *m/z*: 1317.5726 (Calcd for $C_{60}H_{94}O_{30}Na$: 1317.5725). Negative-ion FAB-MS *m/z*: 1233 $[M-H-60]^-$, 1087 $[1233-146]^-$, 1071 $[1233-162]^-$, 925 $[1071-146]^-$, 763 $[925-162]^-$, 661 $[763-132]^-$. ^{1}H-NMR spectral data (in pyridine-d_5, 500 MHz) δ: see Tables 1 and 2. ^{13}C-NMR spectral data: see Tables 3 and 4.

3: Amorphous powder. $[\alpha]_D^{31}$ −77.0° (*c*=1.1, pyridine). Positive-ion FAB-MS *m/z*: 1701 $[M+Na]^+$. HR-positive-ion FAB-MS *m/z*: 1701.7365 (Calcd for $C_{75}H_{122}O_{41}Na$: 1701.7360). Negative-ion FAB-MS *m/z*: 1677 $[M-H]^-$, 1545 $[1677-132]^-$, 1531 $[1677-146]^-$, 1383 $[1545-162]^-$, 1237 $[1383-146]^-$, 1221 $[1383-162]^-$, 1075 $[1221-146]^-$, 1059 $[1221-162]^-$, 913 $[1059-146]^-$, 751 $[913-162]^-$, 619 $[751-132]^-$, 457 $[619-162]^-$. ^{1}H-NMR spectral data (in pyridine-d_5, 500 MHz) δ: 2.58 (2H, m, H_2-25 of Agl), 1.54 (3H, s, H_3-28 of Agl), 1.52 (3H, s, H_3-30 of Agl), 1.07 (3H, t, *J*=7.5 Hz, H_3-26 of Agl), 1.04 (3H, d, *J*=7.0 Hz, H_3-21), 0.94 (3H, s, H_3-19 of Agl), 0.91 (3H, s, H_3-18 of Agl); sugar moiety: see Table 5. ^{13}C-NMR spectral data: see Tables 3 and 4.

4: Amorphous powder. $[\alpha]_D^{31}$ −67.3° (*c*=1.1, pyridine). Positive-ion FAB-MS *m/z*: 1613 $[M+Na]^+$. HR-positive-ion FAB-MS *m/z*: 1613.6830 (Calcd for $C_{71}H_{114}O_{39}Na$: 1613.6835). Negative-ion FAB-MS *m/z*: 1589 $[M-H]^-$, 1443 $[1589-146]^-$, 1427 $[1589-162]^-$, 1281 $[1427-146]^-$, 1265 $[1427-162]^-$, 1119 $[1265-146]^-$, 1103 $[1265-162]^-$, 957 $[1103-146]^-$, 795 $[957-162]^-$, 663 $[795-132]^-$, 501 $[663-162]^-$. ^{1}H-NMR spectral data (in pyridine-d_5, 500 MHz) δ: 4.42 (1H, d, *J*=11.0 Hz, Ha-29 of Agl), 3.65 (1H, d, *J*=11.0 Hz, Hb-29 of Agl), 3.31 (1H, dq, *J*=4.5, 7.5 Hz, H-25 of Agl), 2.70 (1H, d, *J*=14.5 Hz, Ha-22 of Agl), 2.52 (1H, dd, *J*=6.5, 14.5 Hz, Hb-22 of Agl), 2.20 (1H, dq-like, *J*=6.5, 6.5 Hz, H-20 of Agl), 1.54 (3H, s, H_3-28 of Agl), 1.50 (3H, d, *J*=7.5 Hz, H_3-27 of Agl), 1.27 (3H, s, H_3-30 of Agl), 1.09 (3H, d, *J*=6.5 Hz, H_3-21), 0.93 (3H, s, H_3-19 of Agl), 0.89 (3H, s, H_3-18 of Agl); sugar moiety: see Table 5. ^{13}C-NMR spectral data: see Tables 3 and 4.

5: Amorphous powder. $[\alpha]_D^{31}$ −56.0° (*c*=1.1, pyridine). Positive-ion FAB-MS *m/z*: 1745 $[M+Na]^+$. HR-positive-ion FAB-MS *m/z*: 1745.7228 (Calcd for $C_{76}H_{122}O_{43}Na$: 1745.7258). Negative-ion FAB-MS *m/z*: 1721 $[M-H]^-$, 1589 $[1721-132]^-$, 1575 $[1721-146]^-$, 1427 $[1589-162]^-$, 1265 $[1427-162]^-$, 1119 $[1265-146]^-$, 1103 $[1265-162]^-$, 957 $[1103-146]^-$, 795 $[957-162]^-$, 663 $[795-132]^-$, 501 $[663-162]^-$. ^{1}H-NMR spectral data (in pyridine-d_5, 500 MHz) δ: 4.42 (1H, d, *J*=11.0 Hz, Ha-29 of Agl), 3.65 (1H, d, *J*=11.0 Hz, Hb-29 of Agl), 3.30 (1H, dq, *J*=4.5, 7.0 Hz, H-25 of Agl), 2.70 (1H, d, *J*=14.5 Hz, Ha-22 of Agl), 2.52 (1H, dd, *J*=7.0, 14.5 Hz, Hb-22 of Agl), 2.20 (1H, dq-like, *J*=7.0, 7.0 Hz, H-20 of Agl), 1.54 (3H, s, H_3-28 of Agl), 1.50 (3H, d, *J*=7.0 Hz, H_3-27 of Agl), 1.27 (3H, s, H_3-30 of Agl), 1.09 (3H, d, *J*=7.0 Hz, H_3-21), 0.93 (3H, s, H_3-19 of Agl), 0.89 (3H, s, H_3-18 of Agl); sugar moiety: see Table 5. ^{13}C-NMR spectral data: see Tables 1 and 2.

6: Amorphous powder. $[\alpha]_D^{16}$ −41.0° (*c*=1.8, pyridine). Positive-ion FAB-MS *m/z*: 1569 $[M+Na]^+$. ^{1}H-NMR spectral data (in pyridine-d_5, 500 MHz) δ: 2.57 (2H, m, H_2-25 of Agl), 1.55 (3H, s, H_3-28 of Agl), 1.53 (3H, s, H_3-30 of Agl), 1.07 (3H, t, *J*=7.5 Hz, H_3-26 of Agl), 1.04 (3H, d, *J*=6.5 Hz, H_3-21), 0.94 (3H, s, H_3-19 of Agl), 0.91 (3H, s, H_3-18 of Agl); sugar moiety: see Table 5. ^{13}C-NMR spectral data: see Tables 3 and 4.

Acetylation of 3 Compound **3** (10 mg) in Ac_2O–pyridine (1 : 1, 1 ml) was left to stand at room temperature overnight. After removal of the reagent under a stream of N_2, the residue was partitioned between ether (1 ml×3) and H_2O (1 ml). The ether layer was concentrated to afford **3a** (8 mg).

3a: Amorphous powder. ^{1}H-NMR spectral data δ: 2.40 (3H, s), 2.38 (3H, s), 2.32 (3H, s), 2.28 (3H, s), 2.22 (3H, s), 2.21 (3H, s), 2.20 (6H, s), 2.17 (3H, s), 2.13 (3H, s), 2.12 (6H, s), 2.11 (6H, s), 2.10 (6H, s), 2.08 (3H, s), 2.05 (3H, s), 1.99 (3H, s), 1.98 (3H, s), 1.98 (3H, s), 1.96 (3H, s), 1.92 (3H, s). HR-positive-ion FAB-MS *m/z*: 1123.3357 (Calcd for $C_{47}H_{63}O_{31}$:

1123.3353), 835.2495 (Calcd for $C_{35}H_{47}O_{23}$: 835.2509), 273.0966 (Calcd for $C_{12}H_{17}O_7$: 273.0974), 259.0812 (Calcd for $C_{11}H_{15}O_7$: 259.0818).

Sugar Analysis Compounds **1** (5 mg) and **3** (15 mg) were each heated in 2 M HCl (**1**, 1 ml; **3**, 3 ml) at a temperature of 95 °C for 1 h. The reaction mixture was extracted with AcOEt. The aqueous layer was neutralized with Amberlite MB-3 (Organo Co.) and then evaporated under reduced pressure to give a monosaccharide fr. This fr. was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613, Showa Denko, 150 mm×6.0 mm; solvent, CH_3CN–H_2O (3 : 1); flow rate, 1.0 ml/min; column temperature, 70 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; and column oven, JASCO CO-2060. The retention time (t_R) and optical activity of each of the monosaccharides were detected as follows. L-Rhamnose [t_R, 3.7 min; optical activity, negative], L-arabinose [t_R, 5.7 min; optical activity, positive], and D-glucose [t_R, 6.8 min; optical activity, positive] for **1**; L-rhamnose [t_R, 3.6 min; optical activity, negative], D-xylose [t_R, 5.1 min; optical activity, positive], L-arabinose [t_R, 5.7 min; optical activity, positive], D-glucose [t_R, 6.8 min; optical activity, positive], and D-galactose [t_R, 7.2 min; optical activity, positive] for **3**. However, the ethyl acetate extract exhibited several spots by TLC, and the aglycones of **1** and **3** could not be obtained.

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