Five New Nortriterpenoid Glycosides from the Bulbs of *Scilla scilloides*

Masateru Ono,*[,a] Tetsuya Ochiai,[a] Shin Yasuda,[a] Yoichiro Nishida,[b] Toshiharu Tanaka,[b] Masafumi Okawa,[c] Junei Kinjo,[c] Hitoshi Yoshimitsu,[d] and Toshihiro Nohara[d]

[a] *School of Agriculture, Tokai University; 5435 Minamiaso, Aso, Kumamoto 869–1404, Japan:* [b] *Aso Pharmaceutical Co., Ltd.; 91–1 Tsukure, Kikuyo, Kumamoto 869–1101, Japan:* [c] *Faculty of Pharmaceutical Sciences, Fukuoka University; 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan: and* [d] *Faculty of Pharmaceutical Sciences, Sojo University; 4–22–2 Ikeda, Nishi-ku, Kumamoto 860–0082, Japan.* Received February 5, 2013; accepted March 7, 2013

Five new norlanostane-type triterpenoid glycosides were isolated from the bulbs of *Scilla scilloides* Druce (Liliaceae). Their chemical structures were determined on the basis of spectroscopic data as well as chemical evidence.

Key words *Scilla scilloides*; triterpenoid; norlanostane; glycoside; Liliaceae

Scilla scilloides Druce is a perennial herb belonging to the Liliaceae family. The bulb of this plant has been used as a foodstuff and as a traditional medicine for promoting blood circulation, as an anti-inflammatory agent, and as an analgesic.[1)] Homoisoflavones and norlanostane- and lanostane-type triterpenoids are recognized chemical constituents of this bulb, as previously reported.[2–6)] In our prior studies,[7–9)] a new homostilbene, two new homoisoflavones, a new phenylpropanoid glycoside, five new norlanostane-type triterpenoid glycosides, and two new lanostane-type triterpenoid glycosides were isolated from the methanol (MeOH) extract of fresh bulbs of *S. scilloides* and were structurally characterized along with 16 known compounds comprising a homostilbene, seven homoisoflavones, a xanthone, a lignan, two alkaloids, three norlanostane-type triterpenoids, and a norlanostane-type triterpenoid glycoside. As part of an ongoing study of this plant, the isolation and structural characterization of five new norlanostane-type triterpenoid glycosides from the MeOH extract of *S. scilloides* are reported herein.

The MeOH extract of 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 using Diaion HP20, silica gel, and Chromatorex octadecyl silica (ODS) column chromatography as well as HPLC on ODS led to the isolation of five compounds (**1**–**5**).

Compound **1**, tentatively named scillanostaside H, was obtained as an amorphous powder. The negative-ion FAB-MS of **1** exhibited an $[M-H]^-$ ion peak at *m/z* 1045 along with fragment ion peaks at *m/z* 913 [1045−132 ($C_5H_8O_4$, pentosyl unit)]$^-$, 751 [913−162 ($C_6H_{10}O_5$, hexosyl unit)]$^-$, and 619 [751−132]$^-$. The molecular formula of **1** was determined as $C_{51}H_{82}O_{22}$ based on high-resolution (HR)-positive-ion FAB-MS. The ^{1}H-NMR spectrum of **1** was characterized by signals due to four tertiary methyl groups [δ 1.54 (s), 1.51 (s), 0.92 (s), 0.89 (s)], one secondary methyl group [δ 1.01 (d, J=7.0 Hz)], one primary methyl group [δ 1.05 (dd, J=7.0, 7.0 Hz)], and four anomeric protons [δ 6.36 (s), 5.19 (d, J=4.0 Hz), 5.09 (d, J=7.0 Hz), 4.96 (d, J=7.5 Hz)]. The ^{13}C-NMR spectrum of **1** exhibited signals due to one keto carbonyl carbon (δ 212.5), two olefinic carbons (δ 135.3, 134.6), and four anomeric carbons (δ 111.1, 106.1, 103.6, 101.4). A detailed analysis of these NMR spectral signals was performed using the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) techniques, and the planar structure of **1** could be determined as illustrated in Fig. 1.

On acidic hydrolysis, **1** afforded D-apiose, L-arabinose, and D-glucose, which were identified by optical rotation using chiral detection during HPLC analysis, along with several unidentified artificial aglycones. The ^{1}H- and ^{13}C-NMR spectral data of **1** indicated that the sugar moiety of **1** was composed of 1 mol each of α-L-arabinopyranose with $^1C_4$ conformation and β-D-apiofuranose and 2 mol of β-D-glucopyranose with $^4C_1$ conformation[9,10)] (Tables 1–4). The HMBC spectrum of **1** showed key correlations between H-1 of the first glucosyl unit (Glc) and C-3 of the aglycone moiety (Agl), H-1 of the arabinosyl unit (Ara) and C-6 of Glc, H-1 of the second glucosyl unit (Glc′) and C-2 of Ara, and H-1 of the apiosyl unit (Api) and C-2 of Glc′ (Fig. 1). Furthermore, the assigned ^{13}C-NMR spectral data of Agl and the sugar moiety were considerably similar to those of scillanostaside C[8)] and (23*R*)-17*α*,23-epoxy-3*β*,29-dihydroxy-27-nor-lanost-8-ene-15,24-dione 3-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside,[11)] respectively. The structure of **1** was thus concluded to be 15-deoxyeucosterol 3-*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (Fig. 2).

Compound **2**, tentatively named scillanostaside I, was obtained as an amorphous powder, and it furnished the same monosaccharides as those obtained from **1** on acidic hydrolysis. The negative-ion FAB-MS of **2** was characterized by an $[M-H]^-$ ion peak at *m/z* 1061 accompanied by fragment ion peaks at *m/z* 929 [1061−132]$^-$, 767 [929−162]$^-$, and 635 [767−132]$^-$, all of which were 16 mass units greater than those of **1**. HR-positive-ion FAB-MS indicated that the molecular formula of **2** was $C_{51}H_{82}O_{23}$. The ^{1}H-NMR spectrum of **2** was analogous to that of **1**; in particular, the signals due to the sugar moiety were almost superimposable. Furthermore, the ^{13}C-NMR spectrum of **2** was also similar to that of **1**, with the exception of the appearance of a signal due to one additional oxymethine carbon and the disappearance of the signal due to one methylene carbon. Detailed assignment of these NMR spectral signals was performed with the aid of 2D-NMR techniques, as described for **1** (Tables 1–4). The ^{1}H–^{1}H COSY spectrum of **2** indicated correlation of the signal due to the oxygenated methine proton at δ 4.72 (br dd,

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: mono@agri.u-tokai.ac.jp

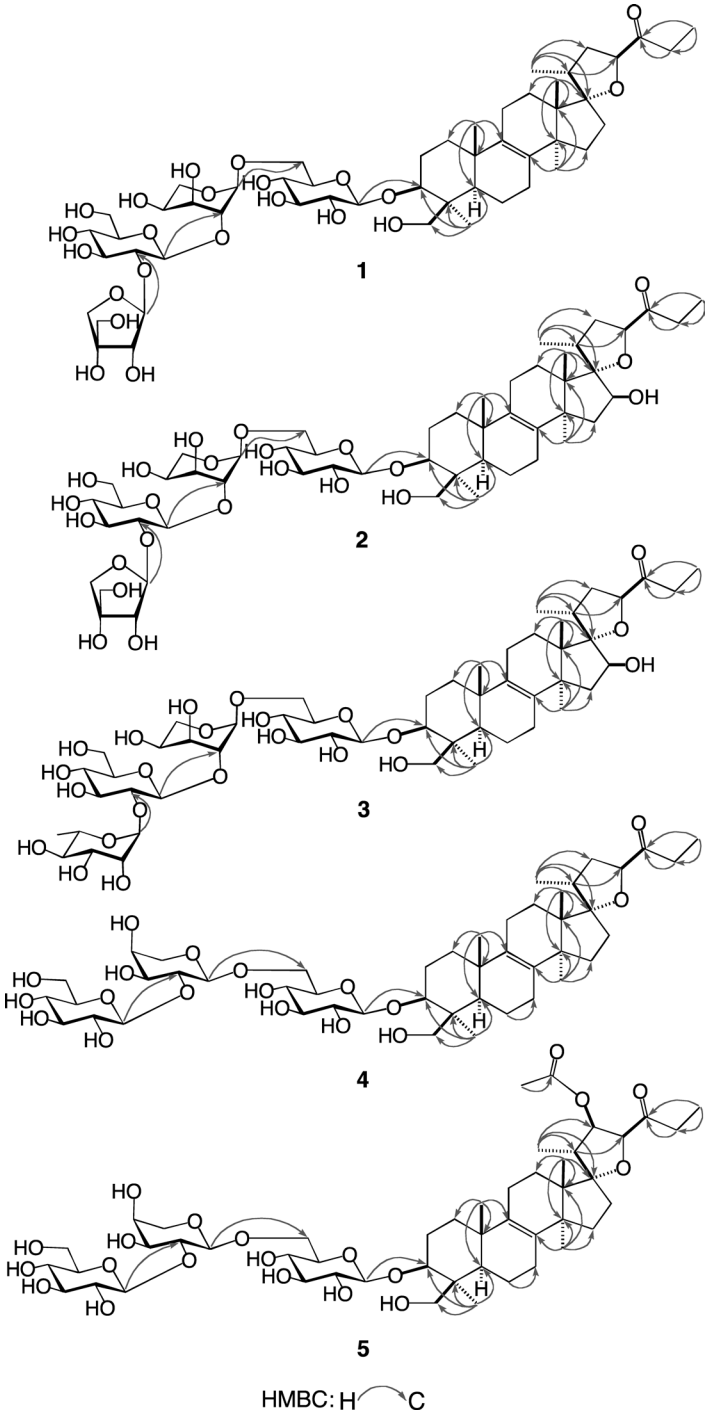

Fig. 1. ^{1}H–^{13}C Long-Range Correlations Observed in the HMBC Spectra of **1**–**5** (in Pyridine-d_5, 500 MHz)

J=7.0, 7.0 Hz) with the signals due to the methylene protons at δ 2.23 (dd, J=7.0, 12.5 Hz) and 2.00, with which the signal due to the methylene carbon at δ 44.4 exhibited cross-peaks in the HMQC spectrum. In addition, a long-range correlation between the signal due to the methylene carbon at δ 44.4 and the signal due to the methyl protons at δ 1.56 (s) assignable to H_3-30 of Agl was observed in the HMBC spectrum (Fig. 1). On the basis of the foregoing data, it was proposed that **2** is a derivative of **1**, in which a hydroxyl group is attached at C-16 of Agl of **1**. The stereochemistry was defined on the basis of the nuclear Overhauser and exchange spectroscopy (NOESY) spectrum, in which key nuclear Overhauser effect (NOE) correlations were observed between H-3 of Agl and H_3-28 of Agl, H-5 of Agl and H_3-28 of Agl, H-16 of Agl and H_3-30 of Agl, H_3-18 of Agl and H-20 of Agl, H_3-18 of Agl and H_3-19 of Agl, H_3-19 of Agl and Ha,b-29 of Agl, and H_3-21 of Agl and H-23 of Agl (Fig. 3). Furthermore, the ^{1}H-NMR spectral profile of Agl of **2** exhibited downfield shifts of H_3-18 (0.31 ppm) and H-20 (1.12 ppm) relative to the corresponding peaks of **1**; these shifts were attributed to deshielding effects of the hydroxyl group at C-16. Based on these effects, it was deduced that the hydroxyl group at C-16 assumed the β-configuration. Consequently, the structure of **2** was concluded to be (23*S*)-17α,23-epoxy-3β,16β,29-trihydroxy-27-nor-lanost-8-ene-24-one 3-*O*-β-

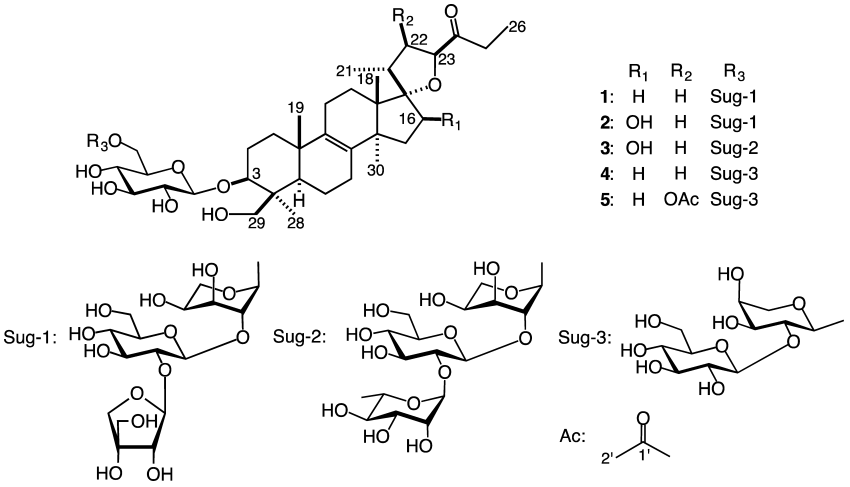

Fig. 2. Structures of **1**–**5**

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

	1	**2**	**3**
1a	1.68[a)]	1.71 m	1.71 m
1b	1.15 ddd (3.5, 14.5, 14.5)	1.15[a)]	1.15[a)]
2a	2.26 m	2.28 m	2.28 m
2b	1.98[a)]	2.07[a)]	2.04[a)]
3	3.57 dd (4.0, 12.0)	3.59 dd (4.5, 11.5)	3.58 dd (4.5, 12.0)
5	1.26 d (12.5)	1.28 d (12.5)	1.28 d (12.5)
6a	1.80[a)]	1.79 m	1.80 m
6b	1.47[a)]	1.49 m	1.49 m
7a	2.04[a)]	2.08[a)]	2.07[a)]
7b	1.98[a)]	2.08[a)]	2.07[a)]
11a	2.13[a)]	2.18 m	2.15 m
11b	1.96[a)]	2.02[a)]	2.04[a)]
12a	2.41 ddd (8.5, 8.5, 13.0)	2.47[a)]	2.48[a)]
12b	1.45[a)]	1.56[a)]	1.57[a)]
15a	1.66[a)]	2.23 dd (7.0, 12.5)	2.23 dd (8.0, 12.5)
15b	1.38 dd (9.5, 9.5)	2.00[a)]	2.00[a)]
16a	2.16[a)]	4.72 br dd (7.0, 7.0)	4.73[a)]
16b	1.61[a)]		
18	0.89 s	1.20 s	1.21 s
19	0.92 s	0.94 s	0.94 s
20	2.04[a)]	3.16 dq (7.0,7.0)	3.16 dq (7.0, 7.0)
21	1.01 d (7.0)	1.16 d (7.0)	1.16 d (7.0)
22a	2.00[a)]	2.54[a)]	2.53[a)]
22b	1.76 dd (7.5, 12.0)	1.89 dd (7.5, 12.0)	1.92 dd (7.5, 11.5)
23	4.61 dd (7.5, 10.5)	4.75 dd (7.5, 11.0)	4.75[a)]
25a	2.56[a)]	2.51[a)]	2.51[a)]
25b	2.53[a)]	2.51[a)]	2.51[a)]
26	1.05 dd (7.0, 7.0)	1.00 dd (7.5, 7.5)	1.00 dd (7.5, 7.5)
28	1.54 s	1.55 s	1.55 s
29a	4.41 d (11.0)	4.42 d (11.0)	4.43 d (11.0)
29b	3.64 br d (11.0)	3.64 br d (11.0)	3.64 d (11.0)
30	1.51 s	1.56 s	1.57 s

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses). *a*) Signals were overlapped with other signals.

D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (Fig. 2).

Compound **3**, tentatively named scillanostaside J, was obtained as an amorphous powder. Acidic hydrolysis of **3** afforded L-rhamnose, L-arabinose, and D-glucose. The negative-ion FAB-MS of **3** exhibited an $[M-H]^-$ ion peak at *m/z* 1075 along with fragment ion peaks at *m/z* 929 [1075−146 ($C_6H_{10}O_4$, deoxyhexosyl unit)]$^-$, 767 [929−162]$^-$, and 635 [767−132]$^-$. The molecular formula of **3** was determined to be $C_{52}H_{84}O_{23}$ using HR-positive-ion FAB-MS. The ^{1}H-NMR spectrum of **3**, which was analogous to that of **2**, showed signals due to four tertiary methyl groups, two secondary methyl groups, one primary methyl group, and four anomeric protons. The ^{13}C-NMR spectrum of **3**, which was also similar

Table 2. ^{1}H-NMR Spectral Data for Sugar Moiety of **1**–**3** (in Pyridine-d_5, 500 MHz)

	1	**2**	**3**
Glc-1	4.96 d (7.5)	4.97 d (7.5)	4.97 d (7.0)
2	3.96 dd (7.5, 8.5)	3.98 dd (7.5, 8.5)	3.98 [a]
3	4.18[a]	4.18[a]	4.18[a]
4	4.18[a]	4.19[a]	4.18[a]
5	4.02 ddd (4.0, 4.0, 8.5)	4.04 ddd (4.0, 4.0, 9.5)	3.99[a]
6a	4.51[a]	4.52[a]	4.50 dd (4.0, 10.5)
6b	4.24[a]	4.27[a]	4.23 dd (4.5, 10.5)
Ara-1	5.19 d (4.0)	5.21 d (3.5)	5.34 d (3.0)
2	4.53[a]	4.54[a]	4.64[a]
3	4.50[a]	4.53[a]	4.67[a]
4	4.51[a]	4.53[a]	4.63[a]
5a	4.32[a]	4.35[a]	4.40 dd (7.5, 11.0)
5b	3.80 dd (2.5, 12.0)	3.82 dd (3.0, 11.5)	3.94 dd (3.5, 11.0)
Glc′-1	5.09 d (7.0)	5.11 d (7.5)	5.17 d (7.5)
2	4.12[a]	4.12 dd (7.5, 9.0)	4.24 dd (7.5, 8.5)
3	4.13[a]	4.15[a]	4.18[a]
4	4.13[a]	4.15[a]	4.18[a]
5	3.68 m	3.71 m	3.69 m
6a	4.35[a]	4.38[a]	4.34 dd (2.5, 12.0)
6b	4.25[a]	4.27[a]	4.27 dd (4.0, 12.0)
Api-1	6.36 s	6.38 d (1.5)	
2	4.77 s	4.79 d (1.5)	
4	4.77 d (9.5)	4.79[a]	
4	4.32[a]	4.35[a]	
5a	4.25[a]	4.26[a]	
5b	4.24[a]	4.23 d (11.5)	
Rha-1			6.38 s
2			4.75[a]
3			4.66[a]
4			4.31 dd (9.0, 9.0)
5			4.90 dq (9.0, 6.0)
6			1.76 d (6.0)

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses). Glc, glucopyranosyl; Ara, arabinopyranosyl; Api, apiofuranosyl; Rha, rhamnopyranosyl. *a*) Signals were overlapped with other signals.

to that of **2**, was characterized by signals assignable to one keto carbonyl carbon, two olefinic carbons, and four anomeric carbons. These signals were assigned by cross-comparison with 2D-NMR data as in the case of **1**, and the data assignments for Agl and the sugar moiety of **3** were quite similar to those of **2** and (23*R*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-ene-15,24-dione 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside, respectively[11] (Tables 1–4). Thus, **3** was determined to be a derivative of **2**, in which the apiofuranosyl unit in **2** was replaced by an α-L-rhamnopyranosyl unit.

Compound **4**, tentatively named scillanostaside K, was obtained as an amorphous powder, and afforded L-arabinose and D-glucose upon acidic hydrolysis. The negative-ion FAB-MS of **4** was characterized by an $[M-H]^-$ ion peak at *m/z* 913 accompanied by fragment ion peaks at *m/z* 751 $[913-162]^-$ and 619 $[751-132]^-$. Based on HR-positive-ion FAB-MS, the molecular formula of **4** was deduced to be $C_{46}H_{74}O_{18}$. The ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of **1**; the signals due to Agl of the respective molecules were almost superimposable, with the exception of the disappearance of the signals due to the apiosyl unit in the case of **4**. As in the case of **1**, these ^{1}H- and ^{13}C-NMR spectral signals were examined in detail, and the structure of **4** was deduced to be a prosapogenol of **1** (Fig. 2), in which the apiosyl unit of **1** was cleaved (Tables 3–6). The coupling constants of the signals due to the anomeric and methine protons of the sugar moiety in the ^{1}H-NMR spectral data indicated that all of the monosaccharide units were of the pyranose form, and further, the modes of the glycosidic linkages of the two glucopyranosyl units were β in the $^4C_1$ conformations, and that of the arabinopyranosyl unit was α in the $^4C_1$ conformation, which was different from that of the arabinopyranosyl unit in **1**–**3**. Comparison of the data for **4** with the ^{13}C-NMR spectral data of methyl β-D-glucopyranoside and methyl α-L-arabinopyranoside from the literature[12] indicated glycosylation shifts[13,14] in the data of **4** at C-6 (+6.7 ppm) of Glc and C-2 (+8.8 ppm) of Ara. Furthermore, key correlations were observed between H-1 of Glc and C-3 of Agl, H-1 of Ara and C-6 of Glc, and H-1 of Glc' and C-2 of Ara in the HMBC spectrum, as illustrated in Fig. 2. Consequently, the structure of **4** was found to be 15-deoxyeucosterol 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **5**, tentatively named scillanostaside L, was obtained as an amorphous powder, and its negative-ion FAB-MS exhibited an $[M-H]^-$ ion peak at *m/z* 971 along with fragment ion peaks at *m/z* 911 $[971-60\ (CH_3COOH)]^-$, 809 $[971-162]^-$, and 677 $[809-132]^-$. The molecular formula of **5** was determined to be $C_{48}H_{76}O_{20}$ using HR-positive-ion FAB-MS. Acidic hydrolysis of **5** furnished the same monosaccharides

Table 3. ^{13}C-NMR Spectral Data for Aglycone Moiety of **1**–**5** (in Pyridine-d_5, 125 MHz)

	1	2	3	4	5
1	35.7	35.7	35.7	35.9	35.9
2	27.5	27.5	27.5	27.5	27.6
3	89.0	89.0	89.0	88.8	88.8
4	44.4	44.3	44.3	44.4	44.4
5	51.8	51.8	51.8	51.9	51.9
6	18.7	18.7	18.7	18.7	18.8
7	26.9	26.9	27.0	26.9	27.0
8	135.3	135.2	135.3	135.1	135.1
9	134.6	134.6	134.7	134.6	134.8
10	36.8	36.8	36.8	36.8	36.8
11	21.1	20.9	20.9	21.1	21.1
12	25.3	25.6	25.6	25.3	25.3
13	48.9	48.4	48.4	48.9	49.9
14	50.8	48.0	48.0	50.8	50.5
15	32.1	44.4	44.4	32.1	32.6
16	39.7	80.2	80.2	39.8	40.1
17	97.1	99.2	99.2	97.1	97.5
18	19.3	18.9	19.0	19.3	19.4
19	19.5	19.5	19.5	19.6	19.6
20	43.7	37.1	37.2	43.7	49.5
21	17.2	17.0	17.0	17.2	15.4
22	36.9	37.5	37.5	36.9	82.1
23	81.6	82.1	82.1	81.6	85.0
24	212.5	212.4	212.4	212.5	208.8
25	32.3	32.2	32.3	32.3	33.4
26	7.7	7.7	7.7	7.7	7.6
28	23.1	23.1	23.2	23.2	23.2
29	63.2	63.1	63.2	63.2	63.2
30	26.4	27.3	27.3	26.4	26.5
1′					169.9
2′					20.8

δ in ppm from TMS.

as those obtained from **4**. The ^{1}H- and ^{13}C-NMR spectral data of **5** were similar to those of **4**, except for the appearance of signals due to one each of acetoxy group and one oxygenated methine group and the disappearance of the signals due to one methylene group. Furthermore, the NMR spectral data of the sugar moiety and Agl were quite similar to those of **4** and 22*R*-acetoxy-15-deoxyeucosterol glycoside,[15] respectively (Tables 3–6). On the basis of these data, **5** was determined to be 22*R*-acetoxy-15-deoxyeucosterol 3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside.

To the best of our knowledge, **1**–**5** are new compounds; notably, the conformation of the *α*-L-arabinopyranosyl unit in **4** and **5** was different from that in **1**–**3**.

Experimental

All instruments and materials used were the same as cited in a previous report[16] 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 (SCK2005) 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 (Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan), eluted with H_2O, MeOH, and acetone. The MeOH eluate (76.7 g) was further subjected to Diaion HP20 column chromatography using gradient mixtures of H_2O–MeOH (50% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, 100% MeOH) as eluents to give fractions 1–4. Fraction 2 (31.4 g) was chromatographed over silica gel column (Merck, Art. 1.09385; Merck, Darmstadt, Germany) using gradient mixtures of $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0) as eluents to give fractions 2.1–2.6. Fraction 2.2 (5.08 g) was subjected to silica gel column chromatography using gradient mixtures of $CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0) as eluents to afford fractions 2.2.1–2.2.20. Fraction 2.2.7 (155 mg) was subjected to Chromatorex ODS column (Fuji Silysia Chemical, Ltd., Aichi, Japan) chromatography using gradient mixtures of H_2O–MeOH (60% MeOH, 65% MeOH, 70% MeOH, 75% MeOH, 80% MeOH, 90% MeOH, 100% MeOH) as eluents to afford fractions 2.2.7.1–2.2.7.6. Fractions 2.2.7.3 (30 mg) and 2.2.7.4 (23 mg) were each subjected to HPLC [Nacalai Tesque, Inc., Kyoto, Japan, Cosmosil 5C18 AR-II, 20 mm i.d.×250 mm (column 1)] with 80% MeOH as eluent to give **5** (7 mg) from fraction 2.2.7.3 and **4** (12 mg) from fraction 2.2.7.4. Fraction 2.2.8 (848 mg) was subjected to Chromatorex ODS column chromatography using gradient mixtures of H_2O–MeOH (60% MeOH, 65% MeOH, 70% MeOH, 75%

Table 4. ^{13}C-NMR Spectral Data for Sugar Moiety of **1**–**5** (in Pyridine-d_5, 125 MHz)

	1	2	3	4	5
Glc-1	106.1	106.1	106.1	106.0	106.0
2	75.3	75.3	75.4	75.5	75.5
3	78.3	78.3	78.3	78.4	78.4
4	72.4	72.4	72.7	72.0	72.0
5	75.6	75.6	75.4	76.1	76.1
6	68.8	68.8	68.6	69.2	69.2
Ara-1	101.4	101.4	100.9	102.3	102.3
2	79.8	79.6	78.2	81.0	81.0
3	72.1	72.1	71.5	73.1	73.1
4	67.0	66.9	66.4	67.8	67.8
5	63.3	63.3	62.2	65.0	65.0
Glc′-1	103.6	103.6	103.1	105.9	105.9
2	79.6	79.8	77.7	75.9	75.9
3	78.4	78.4	79.3	78.3	78.3
4	71.2	71.2	71.4	71.2	71.2
5	78.1	78.1	78.2	78.7	78.7
6	62.0	62.0	62.1	62.3	62.3
Api-1	111.1	111.1			
2	77.9	77.9			
3	80.3	80.3			
4	75.3	75.3			
5	65.9	65.9			
Rha-1			101.9		
2			72.3		
3			72.6		
4			74.2		
5			69.7		
6			18.7		

δ in ppm from TMS. Glc, glucopyranosyl; Ara, arabinopyranosyl; Api, apiofuranosyl; Rha, rhamnopyranosyl.

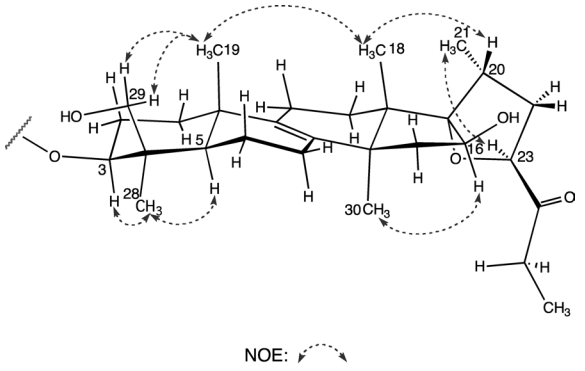

Fig. 3. Key NOE Correlations Observed in the NOESY Spectrum of **2** (in Pyridine-d_5, 500 MHz)

MeOH, 80% MeOH, 85% MeOH, 90% MeOH, 100% MeOH) as eluents to give fractions 2.2.8.1–2.2.8.11. HPLC (column 1) of fraction 2.2.8.8 (81 mg) with 75% MeOH as eluent furnished **1** (37 mg). Fraction 2.2.9 (2368 mg) was subjected to Chromatorex ODS column chromatography using gradient mixtures of H_2O–MeOH (60% MeOH, 65% MeOH, 70% MeOH, 75% MeOH, 80% MeOH, 85% MeOH, 90% MeOH, 100% MeOH) as eluents to furnish fractions 2.2.9.1–2.2.9.10. Fraction 2.2.9.5 (106 mg) was subjected to HPLC (column 1) with 65% MeOH as eluent to afford **2** (70 mg) and **3** (14 mg).

1: Amorphous powder. $[\alpha]_D^{25}$ −55.7° (c=1.6, pyridine). Positive-ion FAB-MS m/z: 1069 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 1069.5203 (Calcd for $C_{51}H_{82}O_{22}Na$: 1069.5196). Negative-ion FAB-MS m/z: 1045 $[M-H]^-$, 913 $[1045-132]^-$, 751 $[913-162]^-$, 619 $[751-132]^-$. ^{1}H-NMR spectral data: see Tables 1 and 2. ^{13}C-NMR spectral data: see Tables 3 and 4.

2: Amorphous powder. $[\alpha]_D^{25}$ −37.4° (c=2.0, pyridine). Positive-ion FAB-MS m/z: 1085 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 1085.5139 (Calcd for $C_{51}H_{82}O_{23}Na$: 1085.5144). Negative-ion FAB-MS m/z: 1061 $[M-H]^-$, 929 $[1061-132]^-$, 767 $[929-162]^-$, 635 $[767-132]^-$. ^{1}H-NMR spectral data: see Tables 1 and 2. ^{13}C-NMR spectral data: see Tables 3 and 4.

3: Amorphous powder. $[\alpha]_D^{25}$ −28.2° (c=1.2, pyridine). Positive-ion FAB-MS m/z: 1099 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 1099.5286 (Calcd for $C_{52}H_{84}O_{23}Na$: 1099.5301). Negative-ion FAB-MS m/z: 1075 $[M-H]^-$, 929 $[1075-146]^-$, 767 $[929-162]^-$, 635 $[767-132]^-$. ^{1}H-NMR spectral data: see Tables 1 and 2. ^{13}C-NMR spectral data: see Tables 3 and 4.

4: Amorphous powder. $[\alpha]_D^{24}$ −21.9° (c=2.4, pyridine). Positive-ion FAB-MS m/z: 937 $[M+Na]^+$. Negative-ion FAB-MS m/z: 913 $[M-H]^-$, 751 $[913-162]^-$, 619 $[751-132]^-$. HR-negative-ion FAB-MS m/z: 913.4786 (Calcd for $C_{46}H_{73}O_{18}$: 913.4797). ^{1}H-NMR spectral data: see Tables 5 and 6. ^{13}C-NMR spectral data: see Tables 3 and 4.

5: Amorphous powder. $[\alpha]_D^{24}$ −21.5° (c=1.2, pyridine). Positive-ion FAB-MS m/z: 995 $[M+Na]^+$. HR-positive-ion FAB-MS m/z: 995.4838 (Calcd for $C_{48}H_{76}O_{20}Na$: 995.4828). Negative-ion FAB-MS m/z: 971 $[M-H]^-$, 911 $[971-60]^-$, 809 $[971-162]^-$, 677 $[809-132]^-$. ^{1}H-NMR spectral data: see Tables 5 and 6. ^{13}C-NMR spectral data: see Tables 3 and 4.

Sugar Analysis Compounds **1** (5 mg), **2** (5 mg), **3** (5 mg), **4** (5 mg), and **5** (5 mg) were each heated in 2 M HCl (1 mL) at a temperature of 95°C for 2 h. The reaction mixture was extracted with EtOAc (1 mL). The aqueous layer was neutral-

Table 5. ^{1}H-NMR Spectral Data for Aglycone Moiety of **4** and **5** (in Pyridine-d_5, 500 MHz)

	4	5
1a	1.70[a)]	1.71 ddd (4.5, 4.5, 13.0)
1b	1.22 m	1.24 m
2a	2.30 dddd (4.5, 4.5, 4.5, 13.5)	2.32 dddd (4.5, 4.5, 4.5, 14.5)
2b	2.03[a)]	2.04[a)]
3	3.70 dd (4.5, 11.5)	3.72 dd (4.5, 11.5)
5	1.31 brd (12.5)	1.33 dd (1.0, 11.5)
6a	1.82 brdd (6.0, 13.5)	1.83 brdd (6.0, 13.5)
6b	1.51 m	1.50[a)]
7a	2.05[a)]	2.10 m
7b	1.99[a)]	2.04[a)]
11a	2.18[a)]	2.19 ddd (1.0, 8.5, 16.5)
11b	1.96[a)]	1.98[a)]
12a	2.42 ddd (9.0, 9.0, 13.0)	2.38[a)]
12b	1.44 dd (9.0, 13.0)	1.44 dd (9.5, 11.5)
15a	1.68[a)]	1.76 m
15b	1.39 dd (10.0, 10.0)	1.51[a)]
16a	2.17[a)]	2.62 m
16b	1.64[a)]	2.01[a)]
18	0.91 s	0.91 s
19	0.95 s	0.96 s
20	2.05[a)]	2.41 q (7.0)
21	1.02 d (7.0)	1.09 d (7.0)
22a	2.00[a)]	5.43 d (5.5)
22b	1.78 dd (7.5, 12.0)	
23	4.62 dd (7.5, 10.5)	4.96 d (5.5)
25a	2.57[a)]	2.57[a)]
25b	2.53[a)]	2.56[a)]
26	1.07 dd (7.0, 7.0)	1.10 dd (7.0, 7.0)
28	1.58 s	1.60 s
29a	4.46 d (11.0)	4.47 d (11.5)
29b	3.66 brd (11.0)	3.67 brd (11.5)
30	1.53 s	1.59 s
2′		2.00 s

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses). *a*) Signals were overlapped with other signals.

Table 6. ^{1}H-NMR Spectral Data for Sugar Moiety of **4** and **5** (in Pyridine-d_5, 500 MHz)

	4	5
Glc-1	5.05 d (8.0)	5.06 d (8.0)
2	4.02 dd (8.0, 9.0)	4.02 dd (8.0, 9.0)
3	4.22 dd (9.0, 9.0)	4.22 dd (9.0, 9.0)
4	4.26 dd (9.0, 9.0)	4.26 dd (9.0, 9.0)
5	4.15[a)]	4.15[a)]
6a	4.64 dd (3.0, 12.5)	4.65 dd (2.5, 10.5)
6b	4.30[a)]	4.30[a)]
Ara-1	5.12 d (5.5)	5.13 d (5.5)
2	4.59 dd (5.5, 7.5)	4.59 dd (5.5, 7.5)
3	4.36 dd (3.0, 7.5)	4.37 dd (2.5, 7.5)
4	4.40 ddd (3.0, 3.0, 4.5)	4.40 ddd (2.5, 2.5, 4.5)
5a	4.32[a)]	4.31[a)]
5b	3.76 dd (2.5, 12.5)	3.76 dd (2.5, 12.0)
Glc′-1	5.11 d (8.0)	5.11 d (8.0)
2	4.03 dd (8.0, 9.0)	4.04 dd (8.0, 9.0)
3	4.13 dd (9.0, 9.0)	4.13 dd (9.0, 9.0)
4	4.18 dd (9.0, 9.0)	4.18 dd (9.0, 9.0)
5	3.81 ddd (2.5, 5.0, 9.0)	3.81 ddd (2.5, 4.5, 9.0)
6a	4.48 dd (2.5, 11.5)	4.49 dd (2.5, 11.5)
6b	4.32[a)]	4.32[a)]

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses). Glc, glucopyranosyl; Ara, arabinopyranosyl. *a*) Signals were overlapped with other signals.

ized with Amberlite MB-3 column (Organo Co., Tokyo, Japan, 13 mm i.d.×230 mm) and then evaporated under reduced pressure to give a monosaccharide fraction. This fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (Showa Denko, Tokyo, Japan, 6.0 mm i.d.×150 mm; solvent, CH_3CN–H_2O (4 : 1); flow rate, 1.0 mL/min; column temperature, 70°C; detector, JASCO OR-2090 plus (JASCO Co., Tokyo, Japan); 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. D-apiose [t_R 5.7 min; optical activity, positive], L-arabinose [t_R, 8.1 min; optical activity, positive], and D-glucose [t_R, 11.8 min; optical activity, positive] for **1** and **2**: L-rhamnose [t_R, 4.8 min; optical activity, negative], L-arabinose [t_R, 8.1 min; optical activity, positive], and D-glucose [t_R, 11.8 min; optical activity, positive] for **3**; L-arabinose [t_R, 8.1 min; optical activity, positive] and D-glucose [t_R, 11.8 min; optical activity, positive] for **4** and **5**. D-Apiose was prepared by the acidic hydrolysis of benzyl β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (icariside F_2).[10] However, the EtOAc extract exhibited several spots by TLC, and the aglycones of **1**–**5** could not be obtained.

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