Communications to the Editor

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OLIGOGLYCOSIDES OF SPIROCYCLIC NORTRITERPENOIDS RELATED TO EUCOSTEROL

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Seven compounds, scillascilloside D-1, E-1 ∿-5, and G-1 were obtained in pure state from the fresh bulbs of Scilla scilloides and characterized. They are oligoglycosides of the spirocyclic nortriterpenoids related to eucosterol.

KEYWORDS ——— Scilla scilloides; nortriterpenoid glycoside; eucosterol derivative; isolation; structure determination; MS; ^{13}C-NMR; GLC; GC-CIMS

Previously[2,3] it was reported that, from the AcOEt soluble fraction of MeOH extractives of the fresh bulbs of Scilla scilloides, four spirocyclic nortriterpenoids (1∿4) related to eucosterol (5) in Eucomis spp.[4] and two novel tetranortriterpene spirocyclic-γ-lactones (6,7) were isolated and characterized. 3 was identical to one of the five aglycons obtained by Parrilli et al.[5] from the methanolysate of a glycoside fraction of Muscari comosum. These compounds have been supposed to be contained mainly as their glycosides. This paper deals with isolation of seven kinds of the expected glycosides and with their structure elucidation.

The AcOEt insoluble and H_2O soluble portion of the MeOH extractives[3] was separated by successive and repetitive column chromatographies on Amberlite XAD-2 (H_2O → MeOH), Sephadex LH-20 (MeOH), silica gel ($CHCl_3$-MeOH-H_2O), Diaion HP20AG (80% MeOH-H_2O), and Lichroprep RP-8 (70∿80% MeOH-H_2O) followed by preparative HPLC on TSK Gel LS-410 (70∿80% MeOH-H_2O) to afford seven glycosides named scillascilloside D-1 (8), -E-1 (9), -G-1 (10), -E-2 (11), -E-3 (12), -E-4 (13), and -E-5 (14). They decomposed under acidic conditions and even on being left to stand for several weeks in a crystalline form as well as in a neutral solution at room temperature.

D-1 (8), needles (n-BuOH-MeOH-H_2O), mp 204-205°C,[6] $[\alpha]_D$ -62.5°,[7] $C_{52}H_{84}O_{22}\cdot 3H_2O$, MS[7] m/z: 1083 $(M+Na)^+$, 937 (1083-146(methyl pentosyl unit)), 775 (937-162(hexosyl unit)). Neither a peak at 921 (1083-162) nor one at 951 (1083-132 (pentosyl unit)) was detected. It showed the IR absorptions[7] due to hydroxy group at 3350 cm^{-1} and isolated ketone at 1728 cm^{-1}. On acid hydrolysis (2N H_2SO_4), it afforded glucose (glc), rhamnose (rha), arabinose (ara) and a complex mixture of decomposition products due to aglycon. Enzymatic hydrolysis (crude hesperidinase) of 8 failed to give the expected aglycon but it provided two glycosides (15), needles, mp 230-232°C, MS m/z: 937 $(M+Na)^+$, 775 (937-162) and (16), powder, MS m/z: 643 $(M+Na)^+$. The ^{13}C-NMR[7] spectral data (Tables I, II) of 8 showed four anomeric carbon signals, and they were quite similar to those of 1 in regard to the aglycon, except for the signals ascribable to C_2, C_3 and C_4, which were shifted owing to glycosylation at C_3.[8] All the above data indicated that 8 and 15 are 3-O-tetra- and -triglycoside of 1 possessing a linear sequence, -glc-ara-glc-rha and -glc-ara-glc, respectively, while 16 is a monoglucoside. Since permethylation of 8 and 15 by the Hakomori[9] or Kuhn[10] method provided no single product, probably due to carbonyl at C_{24},[11] 8 was reduced with $NaBH_4$ to afford a pair of 24-hydroxy epimers (ratio 2:3),[12] 8a, powder (more polar on RP-HPTLC), MS m/z: 1085 $(M+Na)^+$, and 8b, needles, mp 199-202°C, MS m/z: 1085 $(M+Na)^+$. 8a was permethylated by the Hakomori method, and the homogeneous product, powder, MS m/z: 1244 $(M)^{\cdot+}$, was methanolyzed to afford methyl 2,3,4-tri-O-Me-rha.pyranoside (p); 3,4-di-O-Me-ara.p; 3,4,6-tri-O-Me-glc.p and 2,3,4-tri-O-Me-glc.p as identified by GLC. One of the two reduction ($NaBH_4$)

products of **15**, powder, MS m/z: 939 $(M+Na)^+$, 777 (939-162), provided, in the same way as above, methyl 2,3,4,6-tetra-O-Me-glc.p, 3,4-di-O-Me-ara.p, and 2,3,4-tri-O-Me-glc.p. Four anomeric proton signals at 4.93 (d, J=7 Hz), 5.12 (d, J=7 Hz), 5.32 (br s) and 6.32 (br s) in the ^{1}H-NMR spectrum[7] of **8** were attributed to those of the most common β-D-glc.p, for the first two doublets, α-L-rha.p and α-L-ara.p, respectively. Therefore, **8** is regarded as 15-deoxoeucosterol 3-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside.

E-1 (**9**), needles (aq. MeOH), mp 221-223°C, $[\alpha]_D$ -62.3°, $C_{58}H_{94}O_{27}\cdot 3H_2O$, MS m/z: 1245 $(M+Na)^+$, IR: 3350, 1720 cm^{-1}, liberated, on acid hydrolysis, the same monosaccharides as those from **8**. The ^{13}C-NMR data were identical to those of **8** in regard to the aglycon (Table I), but showed five anomeric carbon signals (Table II). These data and the fragment ions at m/z 1099 $((M+Na)^+-146)$, 1083 $((M+Na)^+-162)$ suggest that **9** is a 3-O-glycoside of **1**, where the sugar moiety is branched-chain pentasaccharide having the rha and glc units at terminals. $NaBH_4$-reduction of **9** gave a 24-hydroxy derivative (**9a**), needles, mp 224-226°C, MS m/z: 1247 $(M+Na)^+$, and the less polar 24-epimer (**9b**), powder, mp 198-220°C, MS m/z: 1247 $(M+Na)^+$. **9b** was incubated with gastric juice of Achatina fulica to afford **8b**. GLC analysis of the methanolysate of **9a** permethylate, MS m/z: 1448 $(M)^{\cdot+}$, indicated that **9** has a terminal glc unit which is combined with the sugar moiety of **8** to form a branched-chain pentasaccharide -glc-ara-glc(glc)-rha. The anomeric proton signals in the ^{1}H-NMR spectrum of **9** at 6.32 (br s), 5.30 (br s), 5.16, 4.99 and 4.93 (all d, J=7 Hz) were assigned to those of α-L-ara.p, α-L-rha.p, and three units of β-D-glc.p, respectively. Accordingly, **9** is 15-deoxoeucosterol 3-O-α-L-rhamnopyranosyl(1→2)-(β-D-glucopyranosyl(1→3))-β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside.

G-1 (**10**), powder (n-BuOH-MeOH-H_2O), mp 240-245°C, $[\alpha]_D$ -46.8 , $C_{70}H_{114}O_{37}\cdot 5H_2O$, FAB-MS[13] (glycerol) m/z: 1569 $(M+Na)^+$, 441 (aglycon-17), IR: 3350, 1725 cm^{-1}, has galactose (gal) besides glc, rha and ara, and was suggested by ^{13}C-NMR to be heptaglycoside of **1** (Tables I, II). **10** was treated with hesperidinase to give **9**. The less polar reduction ($NaBH_4$) product **10b**, powder, mp 241-247°C, FAB-MS (triethanolamine (TEA)) m/z: 1571 $(M+Na)^+$, 1697 $(M+TEA)^+$, was subjected to permethylation (powder, MS m/z: 1856 $(M)^{\cdot+}$), methanolysis, acetylation and subsequent GC-CIMS[14] indicating that **10b** permethylate has methyl 2,3,4,6-tetra-O-Me-glc.p, 2,3,4-tri-O-Me-rha.p, 3,4-di-O-Me-2-OAc-ara.p, 2,3,4-tri-O-Me-6-OAc-glc.p, 2,4,6-tri-O-Me-3-OAc-gal.p, 2,4,6-tri-O-Me-3-OAc-glc.p, and 4,6-di-O-Me-2,3-di-OAc-glc.p. These results and the J(C1-H1) values in the ^{13}C-NMR data of **10** (Table II) indicated it has the structure given in the Chart.

E-2 (**11**), powder (n-BuOH-MeOH-H_2O), mp 210-216°C, $[\alpha]_D$ -38.1°, $C_{57}H_{92}O_{28}\cdot 3H_2O$, IR: 3350, 1720 cm^{-1}, MS m/z: 1247 $(M+Na)^+$, afforded glc and ara on acid hydrolysis. Comparison of its ^{13}C-NMR data with those of **1**~**4** (Tables I, II) implies that **11** is a 3-O-pentaglycoside of **3** having the glc and ara units. Reduction ($NaBH_4$) of **11** yielded **11a**, powder, mp 216-222°C, MS m/z: 1249 $(M+Na)^+$, and **11b**, powder, mp 214-217°C, MS m/z: 1249. The less polar **11b** and crude hesperidinase afforded a tetraglycoside (**18**), powder, mp 185-189°C, MS m/z: 1087 $(M+Na)^+$, 955 (1087-132). The EI-Mass spectrum of pertrimethylsilyl(TMS) ether of **11a** showed fragment peaks at m/z: 349 $(ara(OTMS)_3)^+$, 451 $(glc(OTMS)_4)^+$, 1105 $((ara+2glc)(OTMS)_9)^+$, 1383 $((2ara+2glc)(OTMS)_{11}+H_2)^+$ and 1759 $((2ara+3glc)(OTMS)_{14})^+$, suggesting a branched-chain pentasaccharide moiety, -glc-ara-glc(glc)-ara. Permethylation and methanolysis of **11b** and **18** yielded methyl 2,3,5-tri-O-Me-ara.furanoside(f), 2,3,4,6-tetra-O-Me-glc.p, 3,4-di-O-Me-ara.p, 2,3,4-tri-O-Me-glc.p and 4,6-di-O-Me-glc.p for **11b**, and methyl 2,3,5-tri-O-Me-ara.f, 3,4,6-tri-O-Me-glc.p, 3,4-di-O-Me-ara.p and 2,3,4-tri-O-Me-glc.p for **18**. The modes of sugar linkages in **11** are regarded as α-L-ara.f, α-L-ara.p and β-D-glc, on the basis of J(C1-H1) values (Table II) of the anomeric carbons of **11b**. Therefore, the structure of **11** is as shown in the Chart.

E-3 (**12**), powder (n-BuOH-MeOH-H_2O), mp 218-221°C, $[\alpha]_D$ -51.9°, $C_{60}H_{96}O_{29}\cdot 5H_2O$, MS m/z: 1304 $(M+Na+H)^{\cdot+}$, 1244 $(1304-60(AcOH))^{\cdot+}$(base peak), IR: 3400, 1735 cm^{-1}, showed in the ^{1}H-NMR spectrum a 3H singlet at 2.00 ppm due to acetoxy methyl protons. On deacetylation with 0.3N NaOMe-MeOH, **12** gave an enone (**19**), powder, mp 210-214°C, MS m/z: 1244 $(M+Na+H)^{\cdot+}$, IR: 3400, 1690, 1612 cm^{-1}, ^{1}H-NMR :

Table I. ^{13}C-NMR Data[7] of Aglycons

C No.	1	2	3	8	9	10	11	12	13	14(=19)
1	35.7	35.9	35.7	35.7	35.7	35.7	35.7	35.7	35.8	35.7
2	28.9	29.0	28.7	27.4	27.4	27.4	27.3	27.4	27.6	27.5
3	79.8	79.9	73.9	88.9	89.0	88.9	82.3	88.9	89.2	88.9
4	43.1	43.1	46.9	44.4	44.4	44.3	48.1	44.3	44.4	44.4
5	51.5	51.6	44.1a	51.8	51.8	51.8	43.7a	51.8	51.8	51.7
6	18.9	19.0	19.0	18.7	18.7	18.7	18.8	18.7	18.8	18.7
7	26.8	26.9	26.5	26.9	26.9	26.9	26.8	27.0	26.9	26.9
8	134.4	134.5	134.6	135.3	135.3	135.2	135.4	135.0	135.1	135.3
9	134.9	135.2	135.1	135.9	135.9	135.9	136.0	135.9	135.9	135.8
10	37.0	37.1	37.0	36.8	36.8	36.7	36.7	36.7	36.9	36.9
11	21.0	21.2	21.1	21.2	21.1	21.1	21.2	21.1	21.2	21.0
12	25.2	25.4	25.3	25.4	25.4	25.4	25.4	25.2	25.3	25.5
13	48.7	50.0	48.7	48.9	48.8	48.9	48.9	49.8	49.9	48.8
14	50.7	50.3	50.7	50.8	50.8	50.8	50.9	50.4	50.4	51.2
15	31.9	32.6	32.0b	32.3a	32.4	32.3	32.3	32.5	32.6	32.4
16	39.6	40.4	39.6	39.7	39.7	39.7	39.7	40.1	40.1	41.1
17	96.8	97.3	96.9	97.1	97.1	97.0	97.1	97.4	97.5	100.6
18	19.2	19.6	19.3	19.4	19.3	19.3	19.4	19.3	19.4	19.5
19	20.0	20.4	19.9	19.6	19.5	19.5	19.7	19.5	19.7	19.5
20	43.6	52.3	43.6a	43.8	43.7	43.7	43.6a	49.5	49.5	47.4
21	17.2	16.2	17.2	17.3	17.3	17.3	17.3	15.4	15.4	17.3
22	36.8	80.6	36.8	36.8	36.8	36.7	36.9	82.0	82.1	117.9
23	81.4	87.9	81.4	81.6	81.6	81.6	81.6	85.0	85.0	153.0
24	211.8	210.6	211.9	212.5	212.6	212.5	212.5	208.8	208.9	194.3
25	32.2	33.7	32.2b	32.4a	32.4	32.3	32.3	33.3	33.3	31.5
26	7.7	7.6	7.7	7.8	7.7	7.7	7.7	7.6	7.6	8.0
30	23.3	23.3	62.9	23.2	23.2	23.1	62.6	23.1	23.2	23.1
31	64.2	64.3	63.3	63.2	63.2	63.1	64.2	63.1	63.2	63.2
32	26.2	26.6	26.2	26.4	26.4	26.4	26.3	26.4	26.5	26.3
CH_3COO-								20.8	20.9	
								169.8	169.9	

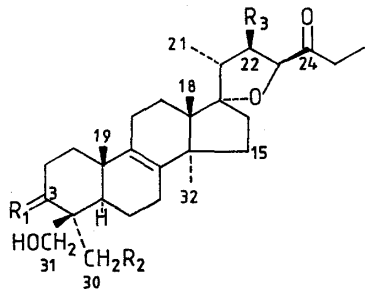
a, b: may be reversed in each vertical column.

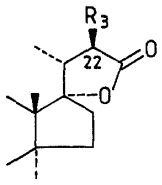
Table II. ^{13}C-NMR Data of Anomeric Carbons

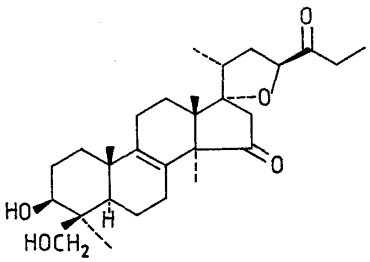
Sugar	8	9	10	11	12	13	14(=19)
α-L-rha.p	101.9	102.0	102.0 (174)		102.0		102.0
α-L-ara.p	100.9	101.1	101.0 (167)	101.7 (166)*	101.1	101.8 (164)	101.1
α-L-ara.f				111.2 (173)*		111.2 (170)	
β-D-glc.p	106.0	106.0	106.1 (158)	105.5 (158)*	106.0	106.1 (160)	106.1
β-D-glc.p	103.0	102.4	102.4 (167)	103.5 (161)*	102.5	103.5 (160)	102.5
β-D-glc.p		104.3	103.7 (161)	104.5 (157)*	104.3	104.4 (161)	104.3
β-D-glc.p			105.3 (164)				
β-D-gal.p			106.1 (158)				

The J(C1-H1) values (Hz) are given in parentheses.

* those of 11b.







1 : R_1= α-OH, R_2=R_3=H

2 : R_1= β-OH, R_2=H, R_3=OH

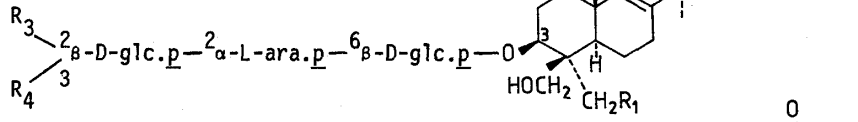
3 : R_1= α-OH, R_2=OH, R_3=H

4 : R_1= O, R_2=R_3= H

6 : R_1= β-OH, R_2=R_3=H

7 : R_1= β-OH, R_2=H, R_3=OH

5 (eucosterol)



8 : R_1=R_2=R_4=H, R_3=α-L-rha.p-

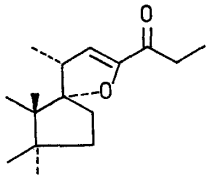
9 : R_1=R_2=H, R_3=α-L-rha.p-, R_4=β-D-glc.p-

10 : R_1=R_2=H, R_3=α-L-rha.p-, R_4=β-D-glc.p-^{3}β-D-gal.p-^{3}β-D-glc.p-

11 : R_1=OH, R_2=H, R_3=α-L-ara.f-, R_4=β-D-glc.p-

12 : R_1=H, R_2=OAc, R_3=α-L-rha.p-, R_4=β-D-glc.p-

13 : R_1=H, R_2=OAc, R_3=α-L-ara.f-, R_4=β-D-glc.p-



14 : R_1=H
R_3=α-L-rha.p-
R_4=β-D-glc.p-

Chart

6.08 (d, J=3.2 Hz, olefinic H). It was assumed to be the 22-en-24-one formed by elimination of the 22-acetoxy group from **12**. The structure of **19** and β-orientation of the 22-acetoxy group in **12** were confirmed by comparison of the ^{13}C-NMR data (Table I) of **12**, **19** and **2**,[15] as well as by the ^{1}H-NMR spectrum of **12**, which showed a doublet at 5.41 (J=5 Hz) assignable to 22α-H. **12** gave, on acid hydrolysis, glc, ara and rha, and the ^{13}C-NMR data (Tables I, II) were in good agreement with those of **9** except for signals due to the side chain. Therefore, **12** is considered to be the 22β-acetoxy derivative of **9**.

E-4 (**13**), powder (n-BuOH-MeOH-H_2O), mp 208-213°C, $[\alpha]_D$ -41.6°, $C_{59}H_{94}O_{29}\cdot 5H_2O$, MS m/z: 1289 $(M+Na)^+$, 1229 $((M+Na)-60)^+$ (base peak), IR: 3400, 1735 cm^{-1}, was hydrolyzed with acid to yield glc and ara. It showed the same ^{13}C-NMR data as those of **12** in regard to the aglycon (Table I), and the carbon signals of its sugar moiety were almost the same as those of **11** except for a few which were slightly shifted owing to participation of 30-hydroxy group in **11**. The identity of sugar parts of **13** and **11** was proved chemically in the same manner as for **11**, by using a deacylated 24-hydroxy epimer derived from **13**, powder, mp 197-203°C, MS m/z: 1249 $((M+Na+H)-CH_3CO)^+$.

E-5 (**14**), powder (n-BuOH-MeOH-H_2O), mp 211-215°C, $[\alpha]_D$ -60.7°, $C_{58}H_{92}O_{27}\cdot 6H_2O$, MS m/z: 1244 $(M+Na+H)^{\cdot +}$, afforded glc, ara and rha on hydrolysis. It was identified with **19** by direct comparison of their IR and ^{13}C-NMR spectra (Tables I, II).

Recently, Parrilli *et al.*[16] reported two oligoglycosides of eucosterol from *Muscari comosum*. Glycosides **8**∿**14** are different from, but closely related to, them in their structures of both aglycons and sugar moieties. A chemotaxonomical relation between *Scilla* and *Muscari* genera is noted.

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6) Melting points were determined on a micro melting point apparatus MP-S_3 (Yanagimoto) and are uncorrected. Melting was accompanied by decomposition, except in **8b**, **9a**, **b**, **10b** and **11a**, **b**.
7) Optical rotations and IR spectra were measured in a pyridine solution (c= 1.3∿4.7) and in a KBr pellet, respectively. ^{1}H- and ^{13}C-NMR spectra were taken with a JEOL FX-100 (100 and 25 MHz, respectively) in a pyridine-d_5 solution (δ (ppm) from tetramethylsilane). Mass spectra (MS) were recorded on a JEOL JMS-DX-300/JMA 3500 by FD-mode unless otherwise specified.
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