Communications to the Editor

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26-O-ACYLATED FUROSTANOL SAPONINS PARDARINOSIDE A AND B FROM THE BULBS OF LILIUM PARDARINUM

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The new steroidal saponins pardarinoside A and B were isolated from the bulbs of Lilium pardarinum. The former is significantly bitter. Their respective structures were determined as 22-O-methyl-26-O-acetyl-(25R)-5α-furost-3β, 14α, 17α, 22, 26-pentaol 3-O-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside and 22-O-methyl-26-O-acetyl-(25R)-5α-furost-3β, 17α, 22, 26-tetraol 3-O-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside. Mostly, the ^{13}C-NMR chemical shifts of the saponins were used in making the structural assignments.

KEYWORDS —— Lilium pardarinum; Liliaceae; steroidal saponin; 26-acylated furostanol; pardarinoside A; pardarinoside B; bitter principle; bulb; ^{13}C-NMR

The occurrence of steroidal saponins in several species in the family Liliaceae is well documented.[1] Some of them are potentially of commercial importance as sources of steroid hormones. But there has been no systematic exploration of the steroidal glycoside in the genus Lilium, except for our recent studies on the constituents of Lilium henryi in which we reported a glucoside of a cholestane derivative.[2] Previously, we have isolated several phenolic glycosides as the bitter ingredients from the bulbs of L. pardarinum.[3] Additional attention to the compounds having a bitter taste has resulted in finding novel steroidal glycosides, pardarinoside A (**1**) and pardarinoside B (**2**). This is a brief account of the isolation and structural elucidation of **1** and **2**.

The chloroform-soluble part of the methanolic bulb extract of L. pardarinum[3] was repeatedly chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O, Et_2O-MeOH and EtOAc-MeOH-H_2O solvent systems as the eluents to provide **1** and **2**.

Pardarinoside A (**1**), 663 mg, $C_{42}H_{70}O_{16}$, $[\alpha]_D$ -56.4°(methanol), was obtained as a white amorphous powder. The IR spectrum proved the presence of hydroxyl group(s) (3420 cm^{-1}) and an acetoxyl group (1720 cm^{-1}). The latter was also indicated by the

^{1}H-NMR (δ 2.05, 3H, s) and the ^{13}C-NMR (δ 170.8 and 20.8) spectra. The ^{1}H-NMR spectrum (C_5D_5N) displayed two anomeric protons (δ 5.07, d, J = 7.2 Hz and 4.81, br s) and the ^{13}C-NMR spectrum (C_5D_5N) confirmed the sugar moiety and showed its sequence to be identical with α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside.[4] The ^{13}C-NMR spectrum showed a total of 27 carbons, except for two sugar unit carbons, a methoxyl carbon and acetoxyl carbons. The ^{1}H-NMR spectrum showed signals for two tertiary methyl groups at δ1.09 and 0.97, each s, and two secondary methyl groups at δ 1.34, d, J = 7.1 Hz, (21-Me) and 0.99, d, J = 6.6 Hz, (27-Me). From these findings, **1** seemed to be a steroidal saponin, spirostane or furostane glycoside. The lack of the characteristic IR absorptions of the spiroketal side chain[5] and the appearence of the methoxyl signal at δ 47.2 in the ^{13}C-NMR spectrum, which is typical of the C-22 methoxyl furostane derivatives,[6] suggested that **1** was a furostane glycoside, but it was negative to the Ehrlich reagent.[7] Detailed inspection of the ^{13}C-NMR spectrum enabled us to elucidate the structure of **1** without chemical degradation,[8] and the Distortionless Enhancement by Polarization Transfer (DEPT) was paticularly helpful for the spectral editing. The ^{13}C-NMR chemical shifts of the C-2, 3, 4, 5 and 19 in **1** resembled to those of tigogenin 3-O-glycoside; the C-3 configuration was β and the relationship between the A and B ring was A/B trans. There were two quaternary carbon signals bearing hydroxyl groups (δ 91.3 and 88.6), and the signals assignable to C-7 (δ 27.1), C-9 (δ 46.6), C-12 (δ 27.0) and C-21 (δ10.6) were displaced upfield, while the C-18 (δ 21.0) moved downfield, compared with the signals of tigogenin 3-O-glycoside. In addition, the ^{13}C-NMR chemical shifts of the D and E rings were related to those of ophiogenin,[9] except for the C-22 position. Thus, the presence of 14α and 17α hydroxyl groups was evident. The distribution of the acetyl group on the molecule was concluded to be the C-26 hydroxyl position because of the signals present at δ 4.14, dd, J = 10.8, 6.0 Hz and 4.07, dd, J = 10.8, 6.5 Hz, assignable to acylated hydroxy methylene protons in the ^{1}H-NMR spectrum; the signal appeared at δ 3.52 as a broad signal in compound **1a** as mentioned later. Compound **1** was treated with 1.5% sodium methoxide in methanol for 30 min, and after the neutralization of the reaction mixture, the crude product was further subjected to acid treatment using 0.2 N hydrochloric acid (H_2O-dioxane, 1:1), 50°C, 5 min to give the corresponding spirostane glycoside (**1a**). The structure was assigned as shown in chart 1 by the spectroscopic data,[10] and the C-25 configuration was found to be R by the IR, ^{1}H-, and ^{13}C-NMR spectra.[5,8] Thus, **1** was 22-O-methyl-26-O-acetyl-(25R)-5α-furost-3β, 14α, 17α, 22, 26-pentaol 3-O-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside.

Pardarinoside B (**2**), 263 mg, $C_{42}H_{70}O_{15}$, $[\alpha]_D$ -62.0° (methanol), was a white amorphous powder. The spectral data of **1** and **2** were essentially analogous to one another and suggestive of steroidal glycoside structure of the same type. In the ^{13}C-NMR spectrum, a quaternary carbon signal having a hydroxyl group appeared (δ 90.4) and the chemical shifts of the D and E rings were in good agreement with those of nolonin-type glycoside possessing a C-17α hydroxyl function.[6a] The corresponding spirostane glycoside (**2a**)[11] was formed from **2** by alkaline treatment followed by acid treatment as in **1**. Accordingly, **2** was characterized as 22-O-methyl-26-O-acetyl-(25R)-5α-furost-3β, 17α, 22, 26-tetraol 3-O-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside.

Compounds **1** and **2** are new, naturally occurring steroidal glycosides of furostane type. Both have a bitter taste and seem to contribute the bitter taste of the bulbs

Table I. ^{13}C-NMR Spectral Data for **1**, **1a**, **2** and **2a** (δ Values)[a)]

Carbon No.	1	1a	2	2a
1	37.6	37.6	37.3	37.3
2	30.0	30.0	29.9	30.0
3	77.0	76.9	77.0	77.0
4	34.5	34.5	34.4	34.5
5	44.6	44.5	44.6	44.6[h]
6	29.1	29.0[d]	29.0	29.0
7	27.1[b]	27.0	32.5[f]	32.5[i]
8	39.8	39.8	35.9	35.9
9	46.6	46.8	54.3	54.3
10	36.1	36.1	35.8	36.0
11	20.4	20.4	21.1	21.2
12	27.0[b]	27.0	32.2[f]	32.3[i]
13	48.9	48.5	45.7	45.4
14	88.6	88.7	52.7	52.8
15	40.2	40.2	31.5[f]	31.7[i]
16	90.9	90.7	90.3	90.1
17	91.3	90.9	90.4	90.1
18	21.0	21.0	17.3	17.4[j]
19	12.3	12.4	12.4	12.5
20	43.6	45.2	43.0	44.8[h]
21	10.6	9.8	10.4	9.7
22	113.0	109.5	113.2	109.8
23	30.9	32.2	30.5	32.1[i]
24	28.0	28.9[d]	27.9	28.8
25	33.3	30.4	33.2	30.5
26	69.3	66.8	69.2	66.7
27	16.9	17.3	16.8	17.3[j]
OMe	47.2	--	47.0	--
Ac	170.8	--	170.8	--
	20.8	--	20.8	--
1'	99.9	99.8	99.8	99.9
2'	79.7	79.7	79.6	79.6
3'	78.2[c]	78.2[e]	78.1[g]	78.1[k]
4'	72.0	72.0	71.9	72.0
5'	78.3[c]	78.3[e]	78.2[g]	78.3[k]
6'	62.9	62.8	62.8	62.8
1"	102.2	102.1	102.1	101.9
2"	72.6	72.6	72.5	72.6
3"	72.9	72.9	72.8	72.9
4"	74.2	74.1	74.1	74.0
5"	69.5	69.4	69.4	69.4
6"	18.7	18.7	18.6	18.6

a) Measured in C_5D_5N (100.6 MHz). b - k) Assignments may be interchanged.

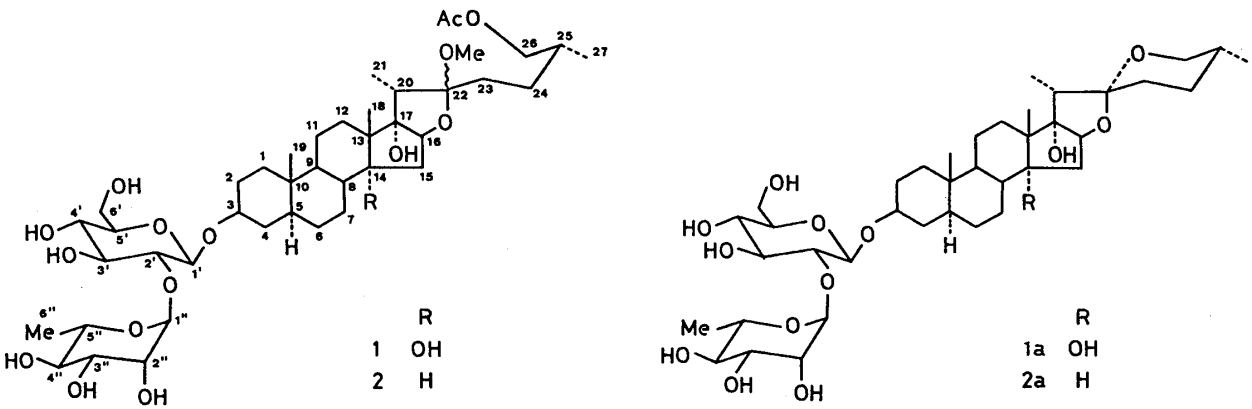

Chart 1

of L. pardarinum as do the phenolic glycosides isolated previously. Compound **1** is much more bitter than **2** and it is found that the introduction of a hydroxyl group at the C-14 position enhances the bitter taste.

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10) A white amorphous powder, $C_{39}H_{64}O_{14}$, secondary ion mass spectrometry (SI-MS); m/z 779 $[M + Na]^+$, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 975, 915, 890, 860 (intensity 915 < 890, 25R spiroketal), ^{1}H-NMR (C_5D_5N): δ 5.05 (1H, d, J = 7.4 Hz, H-1'), 4.81 (2H, H-1", 16, overlapping), 1.28 (3H, d, J = 7.2 Hz, H-21), 1.10 (3H, s, H-18 or 19), 0.95 (3H, s, H-18 or 19), 0.68 (3H, d, J = 5.9 Hz, H-27).
11) A white amorphous powder, $C_{39}H_{64}O_{13}$, SI-MS; m/z 740 $[M]^+$, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 975, 915, 895, 860 (intensity 915 < 895, 25R spiroketal), ^{1}H-NMR (C_5D_5N): δ 5.07 (1H, d, J = 7.2 Hz, H-1'), 4.80 (1H, br s, H-1"), 1.23 (3H, d, J = 7.2 Hz, H-21), 0.96 (3H, s, H-18 or 19), 0.91 (3H, s, H-18 or 19), 0.69 (3H, J = 5.5 Hz, H-27).

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