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FUROSTANOL GLYCOSIDES FROM *LILIUM CORDATUM*

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Key Word Index—*Lilium cordatum*; Liliaceae; furostanol glycosides; acetyl derivative.

Abstract—Two furostanol glycosides were isolated from the methanolic extract of the petals of *Lilium cordatum*. Their structures were established as 26-*O*-β-D-glucopyranosyl-(25*R*)-22ξ-methoxy-furost-5-en-3β, 26-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside and its 6′-*O*-acetyl derivative.

INTRODUCTION

In the previous paper [1], we reported two steroidal alkaloid glycosides (cordatine A and B) from the petals of *Lilium cordatum* (Thunb.) KOIDZ. (Liliaceae), which was used as a folk medicine in a mountain region of Kochi prefecture. In addition to this, we obtained two furostanol glycosides (compounds **1** and **2**), several flavonoids and phenolic glycosides from this petals. This paper deals with the structural elucidation of compounds **1** and **2**.

RESULTS AND DISCUSSION

The fractions previously obtained from the methanolic extract of the petals of *L. cordatum* were rechromatographed on silica gel and Sephadex LH-20 to afford compounds **1** and **2**, which exhibited no spiroketal absorptions in their IR spectra and were positive to Ehrlich reagent [2]. On boiling in aqueous acetone, **1** and **2** were converted to polar spots on TLC, while on boiling in methanol these were reversed. Both compounds showed three anomeric carbon signals and a methoxyl signal in the ^{13}C and ^{1}H NMR spectra.These results indicated **1** and **2** to be 22-methoxy furostanol glycosides and the more polar compounds (on TLC) to be the 22-hydroxy derivatives. It seems likely that the corresponding 22-hydroxy derivatives, existed in nature and, that they were methylated when the plant material was extracted with methanol [3].

The FAB-MS spectra of compounds **1** and **2** showed $[M+Na]^+$ at *m/z* 939 and 981, respectively. On hydrolysis with 2 N HCl–MeOH, both compounds yielded diosgenin together with glucose and rhamnose. Enzymic hydrolysis of **1** and **2** with almond emulsin gave D-glucose and the spirostanol glycosides **3** and **4** identified by TLC and IR [4]. Methylation of compound **3** by Hakomori's method [5] afforded the permethylate, which on methanolysis yielded methyl 2,3,4-tri-*O*-methyl L-rhammopyranoside and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside. Based on the above data, **3** was assumed to be prosapogenin A [6, 7] of dioscin and this was confirmed by direct comparison with an authentic sample. Accordingly, the structure of **1** was elucidated as 26-*O*-β-D-glucopyranosyl (25*R*)-22ξ-methoxy-furost-5-en-3β,26-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

The ^{1}H and ^{13}C NMR spectra of **2** and **4** displayed signals at δ 1.97 (3H, *s*) and 20.7 (*q*) and 170.9 (*s*) assignable to an acetoxyl group. Saponification of **4** afforded a product which was identified with **3** (prosapogenin A of dioscin). A comparative study of the ^{13}C NMR spectra of **3** and **4** allowed the assignment of the acetyl group. Thus the signals due to glucose C-6′ and C-5′ were shifted [8, 9] by +1.8 and −3.1 ppm, respectively, indicating that the acetyl group was linked to glucose C-6′. Therefore, compound **2** could be defined as 26-*O*-β-D-glucopyranosyl-(25*R*)-22ξ-methoxy-furost-5-en-3β,26-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-(6′-*O*-acetyl)-β-D-glucopyranoside.

EXPERIMENTAL

Mps: uncorr.; FAB- and EIMS: JEOL JMS D-300; ^{1}H and ^{13}C NMR: JEOL FX-200, C_5D_5N with TMS as an internal standard; CC: silica gel (Merck 60); TLC: Kiesel gel 60 F_{254} (Merck); The spots on TLC were detected by spraying 10% H_2SO_4 and Ehrlich reagent followed by heating. GLC: Shimadzu GC-6A, FID, glass column (3 mm × 2 m) neopentylglycol succinate, N_2 gas a carrier gas, initial 130° programme at 4°/min to final 190°.

Isolation of saponins. The saponin fraction which had been previously obtained [1] by CC of the MeOH extract of the petals of *L. cordatum*, was rechromatographed over Sephadex LH-20 and silica gel to afford compounds **1** (442 mg) and **2** (43 mg).

Compound **1**. Amorphous, $[\alpha]_D^{26}$ −47.1 (MeOH; *c* 0.96). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no spiroketal side chain. Ehrlich reagt: positive. FABMS *m/z*: 939 $[M+Na]^+$, EIMS *m/z*: 576, 414, 396, 139 (base peak). R_f 0.27 (on TLC, $CHCl_3$–MeOH–H_2O; 14:6:1) 0.19 (on boiling aq. Me_2CO). ^{1}H NMR: 3.28 (OMe). ^{13}C NMR:

aglycone; 37.5, 30.1, 77.9[a]*, 39.0, 140.9, 121.6, 32.1, 31.7, 50.3, 37.1, 21.0, 39.7, 40.4, 56.6, 32.3, 81.3, 64.1, 16.2, 19.4, 40.7, 16.2, 112.6, 30.7, 28.1, 34.2, 75.0, 17.1 (C-1-C-27); 47.2 (OMe); sugar moiety; 100.3, 79.5, 77.8[a], 71.8, 78.1[a], 62.6[b] (3-*O*-glc C-1′-C-6′), 101.9, 72.4[c], 72.7[c], 74.0, 69.3, 18.6 (2′-*O*-rha C-1″-C-6″), 104.8, 75.0, 78.5[a], 71.7, 78.3[a], 62.8[b](26-*O*-glc C-1‴–C–6‴).

Compound **2**. Amorphous, $[\alpha]_D^{24}$ −75.8° (MeOH; *c* 0.91). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (C=O), no spiroketal side chain. Ehrlich reagt.: positive FABMS *m/z*.: 981 $[M+Na]^+$. R_f 0.38 (on TLC, $CHCl_3$–MeOH–H_2O, 14:6:1) 0.29 (on boiling in aq. Me_2CO). ^{1}H NMR: 1.97 (OAc), 3.27 (OMe). ^{13}C NMR: aglycone; 37.6, 30.2, 77.7, 39.1, 140.9, 121.7, 32.2, 31.7, 50.4., 37.1, 21.1, 39.8, 40.8, 56.6, 32.3, 81.3, 64.2, 16.3, 19.4, 40.5, 16.2, 112.7, 30.8, 28.2, 34.2, 75.1, 17.1 (C-1-C-27); 47.3 (OMe); sugar moiety; 100.6, 79.2, 77.7, 71.4[a], 74.8, 64.5 (3-*O*-glc C-1′-C-6′), 102.0, 72.4[b], 72.7[b], 74.0, 69.4, 18.5 (2′-*O*-rha C-1″-C-6″), 104.8, 75.1, 78.5[c], 71.3[a], 78.3[c], 62.9 (26-*O*-glc C-1‴-C-6‴), 20.7, 170.7 (OAc).

Acid hydrolysis of **1** *and* **2**. A soln of **1** (20 mg) in 2 N HCl–MeOH (5 ml) was refluxed for 2 hr, diluted wtih H_2O and extracted with $CHCl_3$. The organic layer was evapd to give a residue, which was crystallized from MeOH to afford an aglycone, colourless needles (5 mg), mp 206–207°, $[\alpha]_D^{27}$ −93.3° ($CHCl_3$; *c* 0.6), identical with diosgenin. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 980, 920, 900, 860 (900 > 920, 25*R* spiroketal side chain). The aq. layer was neutralized, evapd and examined by TLC ($CHCl_3$–MeOH–H_2O; 4:1:0.1) to detect the respective methylsides of D-glucose (R_f 0.20) and L-rhamnose (R_f 0.40). The same hydrolysate was obtained from **2** by use of the same procedure.

Enzymic hydrolysis of **1** *and* **2**. A mixture of **1** (100 mg) and almond emulsin (30 mg) in AcOH–NaOAc buffer (5 ml, pH 4.5) was incubated at 37° for 9 hr and evapd under red. pres. The residue was subjected to silica gel CC ($CHCl_3$–MeOH–H_2O; 90:10:1) to afford compound **3** (74 mg), colourless needles, mp 249–251°, $[\alpha]_D^{18}$ −88.2° (MeOH; *c* 1.19), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 920, 900, 860 (900 > 920). ^{13}C NMR: 37.5, 30.2, 77.9[a], 39.0, 140.9, 121.7, 32.2, 31.7, 50.3, 37.1, 21.1, 39.9, 40.5, 56.7, 32.3, 81.1, 62.9, 16.3, 19.4, 42.0, 15.0, 109.2, 31.9, 29.3, 30.6, 66.9, 17.3 (C-1–C–27); sugar moiety; 100.4, 79.5, 77.8[a], 71.8, 77.9[a], 62.7 (glc C-1′-C-6′), 101.9, 72.5[b], 72.8[b], 74.1, 69.4, 18.6 (rha C-1″-C-6″). It was identical with prosapogenin A of dioscin by direct comparison with an authentic sample (TLC R_f 0.33, $CHCl_3$–MeOH–H_2O; 40:10:1 and ^{13}C NMR).

A mixture of **2** (23 mg) and almond emulsin (10 mg) in H_2O (8 ml) was incubated at 40° overnight and evapd *in vacuo* to dryness to give a residue. The MeOH-soluble portion was subjected to silica gel CC ($CHCl_3$–MeOH–H_2O; 9:1:0.1) to afford compound **4** (7 mg), amorphous, $[\alpha]_D^{27}$ −25.0° (MeOH; *c* 0.64), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (C=O), 980, 920, 900, 860 (900 > 920). R_f 0.48 ($CHCl_3$–MeOH–H_2O, 40:10:1). ^{13}C NMR: 37.6, 29.9, 77.8, 39.0, 140.9, 121.7, 32.1, 31.7, 50.3, 37.1, 22.1, 39.9, 40.5, 56.6, 32.2, 81.1, 62.9, 16.3, 19.4, 42.0, 15.0, 109.3, 31.8, 29.2, 30.6, 66.9, 17.3 (C–1–C–27); sugar moiety; 100.6, 79.2, 78.6, 71.6, 74.8, 64.5 (glc C–1′–C–6′), 102.1, 72.4[a], 72.8[a], 74.0, 69.5, 18.6 (rha C–1″–C–6″), 20.7, 170.9 (OAc).

Methylation of **3**. Compound **3** (70 mg) was methylated by the Hakomori method [5] and worked-up as usual. The methylate was obtained as a brown residue (92 mg). Hydrolysis with 2 N HCl–MeOH gave diosgenin and methyl 2,3,4-tri-*O*-methyl L-rhamnopyranoside and methyl 3,4,6-tri-*O*-methyl D-glucopyranoside identified by comparison with authentic samples by TLC (hexane–EtOAc, 2:3, benzene–Me_2CO, 5:1) and GLC.

Alkaline hydrolysis of **4**. Compound **4** (3 mg) was saponified with 3% KOH–MeOH (0.5 ml) for 30 min at room temp. The soln was neutralized with 1 N HCl–MeOH and evapd to dryness *in vacuo*. The residue was identified with **3** by TLC ($CHCl_3$–MeOH–H_2O, 40:10:1, R_f 0.33).

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*^{13}C NMR data: [a,b,c] Assignments are interchangeable between carbons marked with similar sign in a compound.