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A NOVEL REARRANGED CHOLESTANE GLYCOSIDE WITH A δ-LACTONE RING SYSTEM FROM *ORNITHOGALUM SAUNDERSIAE* BULBS

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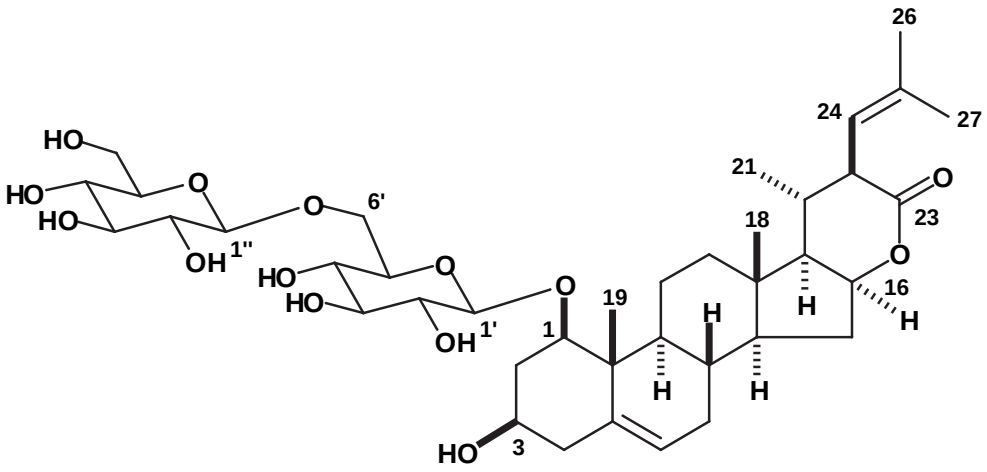
Abstract - The bulbs of *Ornithogalum saundersiae* have been revealed to contain a variety of cholestane glycosides. Further chemical investigation of the MeOH extract of *O. saundersiae* bulbs have resulted in the isolation of a novel 24(23→22)*abeo*-cholestane glycoside with a δ-lactone system (**1**). The structure of **1** was elucidated on the basis of extensive spectroscopic analysis and the result of acid hydrolysis.

INTRODUCTION

Ornithogalum saundersiae (Liliaceae) is a perennial plant native to Natal, Swaziland, and the eastern Transvaal. Although the plant has no medicinal folkloric background applied to treating tumors and is now cultivated for ornamental purposes, we have isolated a series of cholestane glycosides with potent cytotoxic and antitumor activities,[1] some of which are unique in structure having a novel side-chain rearranged cholestane skeleton.[2] Our further chemical investigation of the methanolic bulb extract of *O. saundersiae* resulted in the isolation of a novel 24(23→22)*abeo*-cholestane diglycoside with a δ-lactone ring system (**1**, 0.00027 %, fresh weight). This paper deals with the structural elucidation of **1** on the basis of extensive spectroscopic analysis and the result of acid hydrolysis.

RESULTS AND DISCUSSION

Compound (**1**), $[\alpha]_D$ −18.0° (*c*=0.1, MeOH), was obtained as an amorphous solid. The molecular formula was determined to be $C_{39}H_{60}O_{14}$ by negative-ion FAB-MS (*m/z* 751 $[M - H]^-$), positive-ion FAB-MS (*m/z* 775 $[M + Na]^+$), and elemental analysis. The IR spectrum of **1** indicated strong absorption band for hydroxyl groups (3388 cm^{-1}) and an ester carbonyl group (1718 cm^{-1}), the latter was further supported by the ^{13}C-NMR resonance at δ 175.5 (C). The ^{1}H-NMR spectrum displayed signals arising from two tertiary methyl groups at δ 1.22 and



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0.86, a secondary methyl group at δ 1.11 (d, *J*=6.4 Hz), two methyl groups on a double bond at δ 1.76 and 1.63, and two anomeric protons at δ 5.14 (d, *J*=7.6 Hz) and 4.81 (d, *J*=8.0 Hz). The presence of two trisubstituted C=C bonds in **1** was established by the ^{13}C-NMR spectrum combined with DEPT data [δ 139.1 (C), 135.3 (C), 125.0 (CH), and 121.8 (CH)]. Acid hydrolysis of **1** with 1 M HCl in dioxane-H_2O (1:1) gave D-glucose as the carbohydrate component, while the genuine aglycon was decomposed under acidic conditions. The ^{13}C-NMR spectrum showed 39 resonance lines, 12 of which could be assigned to two glucose units. This implied a $C_{27}H_{40}O_3$ molecular formular for the aglycon moiety, possessing eight degrees of unsaturation. Two C=C bonds and an ester carbonyl group accounted for three degrees. Consequently, the aglycon of **1** was assumed to have the C_{27} steroid skeleton with a five-ring system. The –CH(O-)-CH_2-CH(O-)-CH_2- fragment was shown to construct the A-ring part of **1** by analysis of the ^{1}H-^{1}H COSY, TOCSY, HMQC, and HMBC spectra. The three-proton singlet signal at δ 1.22 showed long-range correlations with the carbon signals at δ 83.8 (C-1), 139.1 (C-5), 50.4 (C-9), and 42.7 (C-10) in the HMBC spectrum, and was assigned to Me-19. Another methyl signal at δ 0.86 was attributable to Me-18, from which long-range correlations were observed to δ 39.9 (C-12), 41.7 (C-13), and 58.9 (C-17). These spectral data and comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of the reported cholestane glycosides reveled that C-1 – C-17 portion (A – D rings) of **1** was identical to that of the cholest-5-ene-1,3,16-triol derivatives,[3] including the orientations of the C-1 and C-3 oxygen atoms (1β-equatorial, 3β-equatorial) and ring junctions (B/C *trans*, C/D *trans*). The downfield-shifted proton signal at δ 4.92 (ddd, *J*=7.9, 7.9, 5.2 Hz) exhibited an HMBC correlation with C-13 and was assigned to H-16, which was associated with the carbon resonance at δ 79.1 by the HMQC spectrum. This indicated that C-16 was bonded to an oxygen atom. Tracing out the proton spin-coupling system from the distinctive H-16 signal through the COSY plots and interpretation of HMBC correlations allowed us to formulate the structure of the rearranged cholestane portion. The C-22 proton at δ 3.29 was coupled to not

Table 1. ^{1}H- and ^{13}C-NMR Data for Compound (**1**) [a)]

	H	C		H	C
1	3.89 dd (11.9, 4.3)	83.8	20	1.83	32.4
2α	2.69 br d (11.9)	38.0	21	1.11 d (6.4)	20.5
β	2.07 ddd (11.9, 11.9, 11.9)		22	3.29 dd (11.6, 9.7)	45.8
3	3.78 m (24.2, $W_{1/2}$)	67.9	23	—	175.5
4α	2.48 br dd (11.9, 4.5)	43.7	24	5.34 d (9.7)	121.8
β	2.60 br dd (11.9, 11.9)		25	—	135.3
5	—	139.1	26	1.76 s	26.0
6	5.49 br s	125.0	27	1.63 s	18.5
7α	1.87	31.6			
β	1.84		1'	4.81 d (8.0)	101.9
8	1.53	33.1	2'	3.90 dd (8.9, 8.0)	75.1
9	1.77	50.4	3'	4.06 dd (8.9, 8.9)	78.3
10	—	42.7	4'	3.84 dd (9.2, 8.9)	71.8
11α	2.95 br dd (13.4, 3.6)	24.0	5'	4.00 ddd (9.2, 7.3, 1.5)	77.6
β	1.54		6'	4.85 dd (11.9, 1.5)	70.7
12α	1.80 br d (13.1)	39.9		4.20 dd (11.9, 7.3)	
β	1.68 ddd (13.1, 13.1, 3.6)				
13	—	41.7	1"	5.14 d (7.6)	105.6
14	1.52	53.5	2"	3.94 dd (8.5, 7.6)	75.2
15α	2.29 m	34.0	3"	4.14 dd (8.8, 8.5)	78.3
β	1.51		4"	4.10 dd (9.2, 8.8)	71.7
16	4.92 ddd (7.9, 7.9, 5.2)	79.1	5"	3.96 ddd (9.2, 5.5, 2.2)	78.6
17	1.88 dd (8.8, 7.9)	58.9	6"	4.48 dd (11.9, 2.2)	62.8
18	0.86 s	15.2		4.29 dd (11.9, 5.5)	
19	1.22 s	14.7			

a) Spectra were measured in pyridine-d_5-methanol-d_4 (11:1). Chemical shift values are in ppm from TMS, and *J* values (in Hz) are presented in parenthesis.

only H-20, but also the olefinic proton assigned to H-24 at δ 5.34 with a *J* value of 9.7 Hz. The H-24 proton, in turn, showed long-range correlations with two methyl carbons at δ 26.0 and 18.5 (C-26 and C-27). A long-range correlation from H-22 to the olefinic carbon at δ 135.3 was also observed. These information confirmed that a 2-methyl-1-propenyl group was attached at C-22. The ester carbonyl group was deposited at C-23 by the observation of long-range correlations from H-20, H-22, and H-24 to the signal at δ 175.5. Although a three-bond correlation from H-16 to C-23 was not observed in the conventional HMBC experiments using duration optimized for the $^{3}J_{C,H}$ of 1, 2, 3.3, 5, and 8 Hz, the selective PFG-HMBC experiment,[4] which was performed to increase the sensitivity using ^{1}H-selective Gaussion inversion pulse of 2ms with the ^{1}H carrier set to the H-16 resonance, allowed the observation of a $^{3}J_{C,H}$ correlation from H-16 to C-23 clearly and confirmed the formation of a δ-lactone between C-16 and C-23. NOE correlations from H-14 to H-17, H-16 to H-17 and H-22, H-17 to Me-21 and H-22, Me-18 to H-20, H-20 to H-24, and H-22 to Me-27 observed in the phase-sensitive NOESY spectrum provided evidence for the D/E *cis* ring junction, and 16β, 17β, 20α, and 22β configurations. Thus, the structure of the aglycon moiety of **1** was revealed to be 1β,3β,16β-trihydroxy-22β-(2-methylprop-1-enyl)-24- norchol-5-en-23-oic acid δ-lactone.

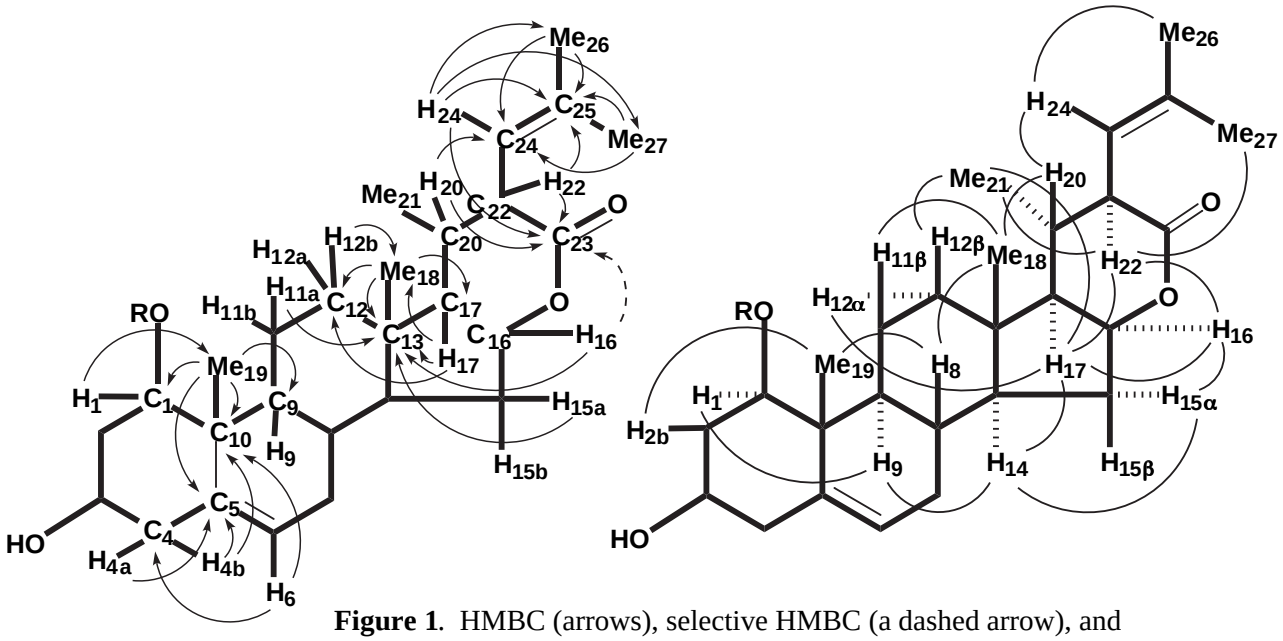

Figure 1. HMBC (arrows), selective HMBC (a dashed arrow), and NOE (curved lines) correlations of the aglycon moiety of **1**

Treatment of **1** with acetic anhydride in pyridine gave the corresponding octaacetate (**1a**). Seven acetyl groups were introduced into the sugar moiety and one acetyl group into the aglycon residue. When the ^{1}H-NMR spectrum of **1a** was compared with that of **1**, the signal due to the aglycon H-3 was shifted downfield by 0.90 ppm, whereas the H-1 signal appeared at the almost same position. This implied that the oxygen atom linked to C-3 was present as a free hydroxy group and C-1 was substituted by the diglycoside. The ^{13}C-NMR resonances for the diglycoside moiety composed of two β-D-glucopyranosyl units, which were assigned by the combined use of the ^{1}H-^{1}H COSY, TOCSY, and HMQC spectra, indicated that one glucose was present as the terminal unit, and another glucose unit was glycosylated at C-6. The above findings were consisted with the β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl group being attached at C-1 of the aglycon. This was ascertained by the observation of the HMBC correlation from each anomeric proton to the carbon of the linked site. From the data presented above, the structure of **1** was determined to the 1β-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-1β,3β,16β-trihydroxy-22β-(2-methylprop-1-enyl)-24-norchol-5-en-23-oic acid δ-lactone.

The structure of **1** differs from that of the 24(23→22)*abeo*-cholestane glycosides reported up to the present in having a δ-lactone ring system formed from the rearranged side-chain and a sugar moiety at C-1 of the aglycon.

EXPERIMENTAL

General Experimental Procedures. The selective PFG-HMBC spectrum was obtained with a JEOL JNM-A600 spectrometer. The other instruments and plant material used were the same as

described in the previous paper.[2]

Extraction and Isolation. The extraction and partition procedures were described in the previous paper.[2] Fraction V was subjected to ODS silica gel column chromatography (CC) eluting with MeOH-H_2O (3:1) and divided into three fractions (Va-Vc). Fraction Vb was subjected to silica gel CC eluting with $CHCl_3$-MeOH-H_2O (40:10:1), ODS silica gel CC with MeOH-H_2O (7:3), and to preparative HPLC using MeOH-H_2O (3:1) to furnish **1** (44.5 mg).

Compound (**1**)**:** amorphous solid; $[\alpha]^{26}_{D}$ –18.0° (*c*=0.1, MeOH); FT-IR (film) ν_{max} 3388 (OH), 2965, 2922 (CH), 2853, 1718 (C=O), 1653, 1450, 1377, 1312, 1274, 1159, 1072, 1034 cm^{-1}; negative-ion FAB-MS *m/z* 751 $[M - H]^-$; positive-ion FAB-MS *m/z* 775 $[M + Na]^+$; *Anal*. Calcd for $C_{39}H_{60}O_{14}$: C; 60.06, H; 8.14. Found: C; 60.02, H; 8.49; ^{1}H-NMR (pyridine-d_5) δ 5.49 (1H, br s, H-6), 5.38 (1H, d, *J*=9.6 Hz, H-24), 5.22 (1H, d, *J*=7.7 Hz, H-1"), 4.92 (1H, ddd, *J*=7.9, 7.9, 5.2 Hz, H-16), 4.85 (1H, d, *J*=7.7 Hz, H-1'), 3.93 (1H, dd, *J*=11.9, 4.7 Hz, H-1), 3.85 (1H, m, $W_{1/2}$=21.1 Hz, H-3), 3.30 (1H, dd, *J*=11.3, 9.6 Hz, H-22), 1.92 (1H, dd, *J*=8.7, 8.7 Hz, H-17), 1.75 (3H, s, Me-26), 1.62 (3H, s, Me-27), 1.25 (3H, s, Me-19), 1.13 (3H, d, *J* =6.1 Hz, Me-21), 0.88 (3H, s, Me-18).

Acid Hydrolysis of 1. A solution of **1** (5 mg) in 1 M HCl (dioxane-H_2O, 1:1, 5 mL) was heated at 100 ¡C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo) column, and chromatographed on silica gel eluting with $CHCl_3$-MeOH (9:1; 1:1) to give an aglycon fraction (1.2 mg) and D-glucose (1.9 mg). TLC analysis of the aglycon fraction showed that it contained several unidentified artifactual sapogenols. D-Glucose was identified by HPLC analysis following its convention to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative.[2, 5] t_R (min): 23.10.

Acetylation of 1. Compound **1** (6.4 mg) was acetylated with acetic anhydride (2 mL) in pyridine (2 mL), and the crude acetate was chromatographed on silica gel eluting with hexane-Me_2CO (2:1) to yield the corresponding octaacetate (**1a**) (4.2 mg).

Compound (**1a**)**:** amorphous solid; FT-IR (film) ν_{max} 2962, 2925 and 2854 (CH), 1756 (C=O), 1456, 1414, 1376, 1260, 1093, 1030, 864; ^{1}H-NMR (pyridine-d_5) δ 5.74 (1H, dd, *J*=9.5, 9.5 Hz, H-3'), 5.68 (1H, dd, *J*=9.6, 9.6 Hz, H-3"), 5.63 (1H, br d, *J*=5.4 Hz, H-6), 5.46 (1H, dd, *J*=9.6, 8.0 Hz, H-2"), 5.44 (1H, dd, *J*=9.6, 9.6 Hz, H-4"), 5.43 (1H, dd, *J*=9.5, 8.0 Hz, H-2'), 5.42 (1H, d, *J*=9.7 Hz, H-24), 5.41 (1H, dd, *J*=9.5, 9.5 Hz, H-4'), 5.08 (1H, ddd, *J*=7.9, 7.9, 5.2 Hz H-16), 5.04 (1H, d, *J*=8.0 Hz, H-1"), 4.98 (1H, d, *J*=8.0 Hz, H-1'), 4.75 (1H, br m, $W_{1/2}$=24.1 Hz, H-3), 4.55 (1H, dd, *J*=12.1, 5.7 Hz, H-6a"), 4.41 (1H, dd, *J*=12.1, 2.6 Hz, H-6b"), 4.27 (1H, br d, *J*=11.6 Hz, H-6a'), 4.13 (1H, ddd, *J*=9.6, 5.7, 2.6 Hz, H-5"), 4.06 (1H, br dd, *J*=9.5, 5.5 Hz, H-5'), 3.94 (1H, dd, *J*=11.6, 5.5 Hz, H-6b'), 3.73 (1H, dd, *J*=11.8, 4.1 Hz, H-1), 3.44 (1H, dd, *J*=11.8, 9.9 Hz, H-22), 2.26, 2.11, 2.08 x 2, 2.07, 2.04 x 2 and 2.00 (each 3H, s, Ac), 1.81 (3H, s, Me-26), 1.68 (3H, s, Me-27), 1.27 (3H, d, *J*=6.3 Hz, Me-21), 1.12 (3H, s, Me-19), 0.90 (3H, s, Me-18).

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