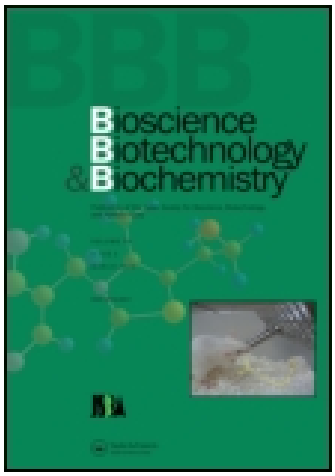

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Short Communication

A New Cytotoxic Cholestane Bisdesmoside from *Ornithogalum saundersiae* Bulbs

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Bioassay-guided fractionation of the MeOH extract of *Ornithogalum saundersiae* bulbs led to the isolation of a new cholestane bisdesmoside with potent cytotoxic activities toward leukemia HL-60 and MOLT-4 cells. The structure was deduced mainly from spectroscopic information.

Key words: cholestane bisdesmoside; *Ornithogalum saundersiae*; cytotoxic activity; HL-60 cells; MOLT-4 cells

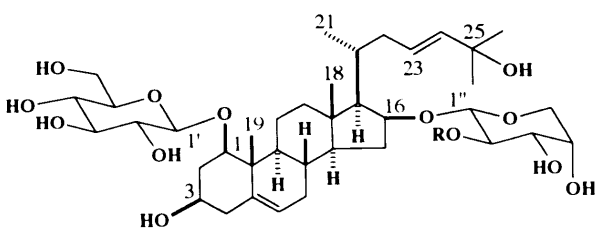
Ornithogalum saundersiae (Liliaceae) is a perennial plant which is native to Natal, Swaziland, and the eastern Transvaal. We have previously found that the MeOH extract of *Ornithogalum saundersiae* bulbs exhibited extremely potent cytotoxic activity toward human promyelocytic leukemia HL-60 cells with an LC_{50} value of 0.031 μg/ml.[1] A series of chromatographic fractionations of the MeOH extract while monitoring the cytotoxic activity toward HL-60 cells led to the isolation of a new cytotoxic cholestane bisdesmoside (**1**, 124 mg, 0.00078% of fresh plant material).

Compound **1** of $[\alpha]_D$ −8.0° (MeOH) was analyzed as being $C_{48}H_{72}O_{17}$ by negative-ion FAB-MS (*m/z* 920 $[M]^-$), ^{13}C-NMR combined with DEPT (135°) spectrum[2] and elemental analysis.[3] The presence of a 3,4,5-trimethoxybenzoyl ester group in **1** was indicated by the IR (ν_{max} 1705 cm^{-1}), UV [λ_{max} 266 nm (log ε=4.00)], ^{1}H-NMR [δ 7.63 (2H, s), 3.94 (3H, s) and 3.80 (3H×2, s)] and ^{13}C-NMR [δ 166.0 (C=O), 153.7 (C×2), 143.2 (C), 126.5 (C), 108.1 (CH×2), 60.8 (Me) and 56.3 (Me×2)] spectra, and by alkaline methanolysis of **1** with 3% NaOMe in MeOH, yielding methyl 3,4,5-trimethoxybenzoate and a deacyl derivative (**1a**, $C_{38}H_{62}O_{13}$).[4] We attempted to resolve the structure of **1a** first. Preliminary inspection of the ^{1}H-NMR data for **1a** assigned signals attributable to five methyls at δ 1.50, 1.49, 1.24 and 0.90 (each 3H, s) and 0.92 (3H, d, *J*=6.8 Hz), a *trans*-olefinic group at δ 6.08 and 5.96 coupled to each other (*J*=15.7 Hz), an olefinic proton at δ 5.57 (br. d, *J*=5.2 Hz), and two anomeric protons of pyranoses at δ 4.90 (d, *J*=7.7 Hz) and 4.56 (d, *J*=6.7 Hz).[2] The presence of a tertiary hydroxyl group in **1a** was suggested by the ^{13}C-NMR signal at δ 70.0 (C), and by the IR spectrum of an acetyl derivative (**1b**) of **1a** that had been prepared by treating **1a** with Ac_2O in pyridine, showing the absorption band of a hydroxyl group at ν_{max} 3400 cm^{-1}. Acid hydrolysis of **1a** with 1 M hydrochloric acid gave D-glucose and L-arabinose in a ratio of 1:1.[5] The ^{13}C-NMR data for **1a** showed 38 resonance lines, 11 of which could be assigned to one glucose and one arabinose unit each, and two anomeric carbons were observed at δ 107.6 and 101.3. This implied a $C_{27}H_{44}O_4$ composition for the aglycone portion, possessing six degrees of unsaturation, two of which were due to two double bonds. Consequently, the aglycone of **1a** was assumed to have the usual C_{27} steroid skeleton with a four-ring system.

Detailed interpretation of the ^{1}H–^{1}H COSY, HOHAHA and HMQC spectra of **1a** gave confirmative evidence for sequential assignment of the ^{1}H-NMR signals and the corresponding one-bond coupled ^{13}C signals,[6] giving rise to some fundamental structural features of **1a**: three oxygen atoms, and two double bonds were located at C-1, C-3, and C-16, and at C-5 (Δ^5) and C-23 (Δ^{23}), respectively, on the steroidal skeleton. Further information was obtained from the HMBC data. The quaternary carbon signal at δ 42.8 showed $^{2\text{ or }3}J_{C,H}$ correlation peaks with 4α(eq)-H at δ 2.52 (dd, *J*=12.0, 4.2 Hz) and an angular methyl at δ 1.24 (3H, s), and was assigned to C-10. The other quaternary carbon at δ 42.3 was assigned to C-13, which was correlated to 11α(eq)-H at δ 2.76 (br. d, *J*=12.0 Hz), 15α-H at δ 2.26 (m), 17-H at δ 1.14 (dd, *J*=10.8, 7.6 Hz), 20-H at δ 2.29 (m) and another angular methyl at δ 0.90 (3H, s). The deshielded quaternary carbon signal at δ 70.0 showed HMBC correlations with the two singlet methyls at δ 1.50 and 1.49, and with the *trans*-olefin protons at δ 6.08 and 5.96, accounting for the location of the tertiary hydroxyl group at C-25.

The ^{13}C assignment of the saccharide part of **1a**,[6] which was composed of a D-glucose and an L-arabinose, was performed by ^{1}H–^{1}H COSY combined with the HMQC spectra, indicating that each monosaccharide was directly attached to the aglycone without being substituted by another monosaccharide. The respective linkage positions of β-D-glucopyranose ($^4C_1$; $\delta_{1\text{-H}}$ 4.90, d, *J*=7.7 Hz) and α-L-arabinopyranose ($^4C_1$; $\delta_{1\text{-H}}$ 4.56, d, *J*=6.7 Hz) were revealed to be at C-1 and C-16 of the aglycone by observing three-bond C–H correlations from each anomeric proton across the glycosidic bond to the carbon of the aglycone; δ_H 4.90 to δ_C 83.0 (C-1) and δ_H 4.56 to δ_C 82.5 (C-16).

The C-1β and C-3β orientations of the oxygen atoms were confirmed by the ^{1}H-NMR parameters of the 1-



	R
1	**3,4,5-trimethoxybenzoyl**
1a	**H**

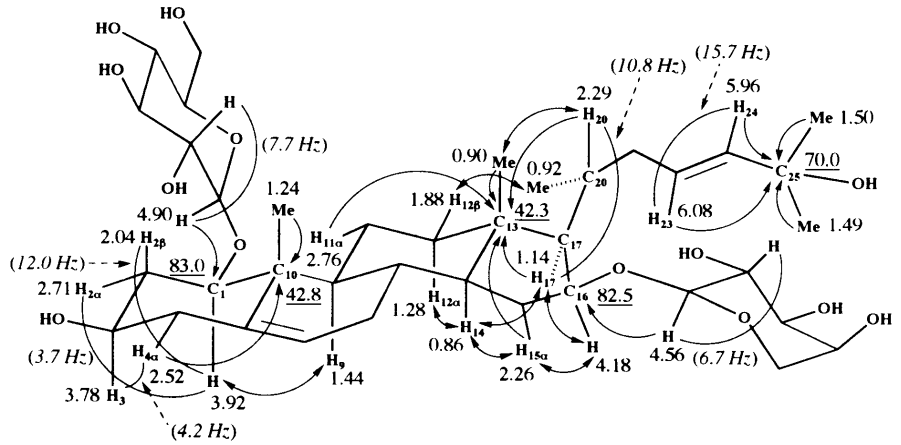


Fig. Important Spectral Data for **1a**.

Figures indicate ^{1}H-NMR shifts (ppm), and J value between protons indicated by the curved line is given in parentheses (Hz). Underlined figures indicate ^{13}C-NMR shifts. Arrows indicate HMBC correlations (from H to C), and resonance arrows indicate NOE correlations.

H and 3-H protons ($^3J_{1\text{-H}(\delta\,3.92)\text{-}2\beta(\text{ax})\text{-H}(\delta\,2.04)} = 12.0$ Hz, $^3J_{1\text{-H-}2\alpha(\text{eq})\text{-H}(\delta\,2.71)} = 3.7$ Hz, and $W_{1/2(3\text{-H})} = 27.0$ Hz). The usual steroidal ring-junctions, B/C *trans* and C/D *trans*, were ascertained by NOE correlations between 1α(ax)-H and 9-H, and 12α(ax)-H and 14-H, and by agreement of the ^{13}C-NMR assignments of the A-C ring carbons of **1a** with those of the related polyhydroxylated cholestane glycosides previously reported by us.[7] The β-configuration of C-16 bearing an oxygen atom and of C-17 attached to the side chain was verified by NOEs of 14-H/15α-H and 17-H, and 16-H/15α-H and 17-H. The stereochemistry at C-20 was examined by using molecular modeling, NOESY data and $^3J_{H,H}$ value. A combination of molecular mechanics and molecular dynamics calculations in force field Discover-cff91 was performed on two possible compounds of C-20*S* and C-20*R*.[8] The obtained minimum energy conformation of the C-20*R* model showed −175° for the H_{17}-C_{17}-C_{20}-H_{20} torsion angle. The experimental 17-H/20-H J value (10.8 Hz) almost corresponded to that (10.3 Hz) calculated through the application of the given dihedral angles to the advanced Karplus-type equation proposed by Altona *et al.*[9] Furthermore, the calculated conformation, in which 20-H lay toward 18-Me, fitted well with the clear NOEs observed between 20-H and 18-Me, and 21-Me and 12β(eq)-H. Thus, the C-20*R* configuration was evident.

Compound **1** was a 3,4,5-trimethoxybenzoyl ester of **1a**. The ester linkage at the arabinose C-2 hydroxy position of **1** was formed from a 3,4,5-trimethoxybenzoic acid, as was evident in the ^{1}H-NMR paramagnetic chemical shift due to acylation; the 2-H proton of the arabinose was deshielded by 1.75 ppm in comparison of the ^{1}H-NMR data for **1** with that of **1a** to appear at δ 6.05 (dd, J = 8.1, 7.0 Hz). From the foregoing data, the full structure of **1** was determined to be (23*E*)-cholesta-5,23-diene-1β,3β,16β,25-tetrol 1-*O*-β-D-glucopyranoside 16-*O*-(2-*O*-3,4,5-trimethoxybenzoyl-α-L-arabinopyranoside).

Compound **1** exhibited potent cytotoxic activity toward HL-60 cells, showing an LC_{50} value of 0.02 μM, which is almost as potent as the clinically applied anticancer agents, etoposide (LC_{50} 0.025 μM) and methotrexate (LC_{50} 0.012 μM). It was also cytotoxic toward human T-lymphocytic leukemia MOLT-4 cells with an LC_{50} value of 0.0042 μM, which is about ten times more potent than the values for etoposide (LC_{50} 0.054 μM), doxorubicin (LC_{50} 0.035 μM) and methotrexate (LC_{50} 0.048 μM). Deacyl derivative **1a** showed no activity toward either cell line (10 μM<), indicating that the aromatic acyl group linked to the saccharide part was essential for the exceptional activity. Some natural marine products with a steroidal skeleton such as cephalostatins are known to have potent cytotoxic activity[10]; however, **1** must be emphasized as a new class of cytotoxic natural product without close parallel to any other known secondary cytotoxic metabolites produced by higher plants.

References and Notes

1) The assay was carried out according to a modification of the method of Sargent and Tayler: J. M. Sargent and C. G. Tayler, *Br. J. Cancer*, **60**, 206–210 (1989).
2) NMR spectra were measured in a mixed solvent of pyridine-d_5 and methanol-d_4 (11 : 1) to remove exchangeable protons and minimize signal overlap.
3) Compound **1**: amorphous solid, $[\alpha]_D$ −8.0 (c 0.10, MeOH); negative-ion FAB-MS m/z: 920 $[M]^-$, 726 $[M-$trimethoxybenzoyl$+H]^-$; UV λ_{max} (MeOH) nm (log ε): 266 (4.00); IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2925 (CH), 1705 (C=O), 1585 and 1495 (aromatic ring). *Anal.* Found: C, 60.24; H, 8.01%. Calcd. for $C_{48}H_{72}O_{17}\cdot 2H_2O$: C, 60.24; H, 8.00%.
4) Compound **1a**: amorphous solid, $[\alpha]_D$ +2.0 (c 0.10, MeOH); negative-ion FAB-MS m/z: 726 $[M]^-$; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2925 (CH). *Anal.* Found: C, 59.42; H, 8.73%. Calcd. for $C_{38}H_{62}O_{13}\cdot 2H_2O$: C, 59.82; H, 8.72%.
5) The monosaccharides were identified by converting them to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives which were then analyzed by HPLC: R. Oshima, Y. Yamauchi, and J. Kumanotani, *Carbohydr. Res.*, **107**, 169–176 (1982).
6) ^{13}C-NMR assignments of **1a**: δ 83.0, 37.4, 68.1, 43.6, 139.5, 125.0, 31.8, 33.2, 50.4, 42.8, 24.0, 40.4, 42.3, 55.1, 37.3, 82.5, 62.0, 13.6, 14.8, 30.1, 18.2, 39.5, 125.8, 141.1, 70.0 and 30.6×2 (C-1–C-27), 101.3, 75.3, 78.4, 72.2, 78.1 and 63.4 (C-1′–C-6′), and 107.6, 72.6, 74.3, 69.2 and 66.6 (C-1″–C-5″).
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