Short Communication

A New Cytotoxic Cholestanol 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 cholestanol bisdesmoside with potent cytotoxic activities toward leukemia HL-60 and MOLT-4 cells. The structure was deduced mainly from spectroscopic information.

Key words: cholestanol 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. 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 cholestanol bisdesmoside (1, 124 mg, 0.00078% of fresh plant material).

Compound 1 of [α]_D 8.0 (MeOH) was analyzed as being C_{41}H_{42}O_{14}, by negative-ion FAB-MS (m/z 920 [M]−). 13C-NMR combined with DEPT (135) spectrum and elemental analysis. The presence of a 3,4,5-trimethoxybenzoyl ester group in 1 was indicated by the IR (v_{max} 1705 cm⁻¹), UV (λ_{max} 266 nm (log ε = 4.00)), 1H-NMR [δ 7.63 (2H, s), 3.94 (3H, s) and 3.80 (3H×2, s)] and 13C-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 (la, C_{37}H_{34}O_{12}). We attempted to resolve the structure of 1a first. Preliminary inspection of the 1H-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 anomic protons of pyranoses at δ 4.90 (d, J = 7.7 Hz) and 4.56 (d, J = 6.7 Hz). The presence of a tertiary hydroxy group in 1a was suggested by the 13C-NMR signal at δ 70.0 (C), and by the IR spectrum of an acetyl derivative (Ib) of 1a that had been prepared by treating 1a with Ac_{2}O in pyridine, showing the absorption band of an hydroxyl group at v_{max} 3400 cm⁻¹. Acid hydrolysis of 1a with 1M hydrochloric acid gave D-glucose and L-arabinose in a ratio of 1:1. The 13C-NMR data for 1a showed 38 resonance lines, 11 of which could be assigned to one glucose and one arabinose unit each, and two anomic carbons were observed at δ 107.6 and 101.3. This implied a C_{37}H_{34}O_{12} 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 1H-1H COSY, HOHANHA and HMBC spectra of 1a gave confirmative evidence for sequential assignment of the 1H-NMR signals and the corresponding one-bond coupled 13C signals, giving rise to some fundamental structural features of 1a: three oxygen atoms, and two double bonds were located at C-4, C-3, and C-16, and at C-5 (J) and C-23 (J), respectively, on the steroid skeleton. Further information was obtained from the HMBC data. The quarternary carbon signal at δ 42.8 showed 2 or 3 1J_{C,H} correlation peaks with 4(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 quarternary carbon at δ 42.3 was assigned to C-13, which was correlated to 11(eq)-H at δ 2.76 (br. d, J = 12.0 Hz), 15a-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 quarternary 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 13C assignment of the saccharide part of 1a, which was composed of a D-glucose and an L-arabinose, was performed by 1H-1H COSY combined with the HMOC 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 (1^3C; δ _H 4.90, d, J = 7.7 Hz) and L-arabinopyranose (1^3C; δ _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 anomic 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 1H-NMR parameters of the 1-
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 ($J_L$=12.0 Hz, $J$=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 1α with those of the related polyhydroxylated cholestanol glycosides previously reported by us. 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, 15β-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 $J_{HH}$ value. A combination of molecular mechanics and molecular dynamics calculations in force field Discover-ff91 was performed on two possible compounds of C-20S and C-20R. The obtained minimum energy conformation of the C-20R model showed $-175^\circ$ for the H$_1$-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. 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-20R configuration was evident.

Compound 1 was a 3,4,5-trimethoxybenzoyl ester of 1a. The ester linkage at the arabinose C-2 hydroxyl 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 (23E)-cholesta-5,23-diene-3β,24,25-trietrol 1-O-β-d-glucopyranoside 16-O-(2-O-3,4,5-trimethoxybenzoyl-3,4,5-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.

References and Notes

1) The assay was carried out according to a modification of the method of Sargent and Taylor: J. M. Sargent and C. G. Taylor, Br. J. Cancer, 60, 206-210 (1989).
2) NMR spectra were measured in a mixed solvent of pyridine-d$_6$ and methanol-d$_4$ (11:1) to remove exchangeable protons and minimize signal overlap.
3) Compound 1: amorphous solid, [α]$_D$ = $-8.0$ (c 0.10, MeOH); negative-ion FAB-MS $m/z$: 290 [M]$,^-$, 276 [M-trimethoxybenzoyl + H]$^+$; UV $I_{max}$ (MeOH) nm (log ε): 266 (4.0); IR $v_{max}$ (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$_{27}$H$_{20}$O$_{5}$: C, 60.24; H, 8.00%.
4) Compound 1a: amorphous solid, [α]$_D$ = $+2.0$ (c 0.10, MeOH); negative-ion FAB-MS $m/z$: 726 [M$^-$]; IR $v_{max}$ (KBr) cm$^{-1}$: 3400 (OH), 2925 (CH). Anal. Found: C, 59.42; H, 8.73%. Calcd. for C$_{45}$H$_{24}$O$_{5}$: C, 59.82; H, 8.72%.
5) The monosaccharides were identified by converting them to the 1-L(+)-N-acetyl-a-methylbenzylamine-1-deoxyalditol acetate derivatives which were then analyzed by HPLC; R. Oshima, Y. Yamauchi, and J. Kumanoto, 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').
8) Discover 2.95 Program, Biosym Technol. Inc., San Diego, CA, U.S.A.