# A NOVEL 16,23-EPOXY-5β-CHOLESTANE GLYCOSIDE WITH POTENT INHIBITORY ACTIVITY ON PROLIFERATION OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES FROM *ORNITHOGALUM SAUNDERSIAE* BULBS

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A novel 16,23-epoxy-5β-cholestane triglycoside (**1**) was isolated from the bulbs of *Ornithogalum saundersiae* (Liliaceae). The structure was determined by extensive spectroscopic analysis. The conformation of the E-ring part of **1** was studied through molecular mechanics and molecular dynamics calculation methods. Compound **1** potently inhibited proliferation of peripheral blood lymphocytes provided from a chronic renal failure patient without causing any cytotoxicity in the lymphocytes and HL-60 human leukemia cells.

**KEY WORDS** *Ornithogalum saundersiae*; Liliaceae; 16,23-epoxy-5β-cholestane triglycoside; conformational analysis; peripheral blood lymphocyte; immunosuppressive agent

Combined use of several immunosuppressive agents, such as prednisolone, cyclosporin A and FK-506 is clinically applied to kidney transplant recipients. However, a significantly large proportion of the chronic renal failure (CRF) patients who needed renal transplantation, compared with healthy subjects, showed a marked decrease in lymphocyte response to prednisolone.[1] During our search for new immunosuppressive agents in place of prednisolone from natural sources, we have found that a novel 16,23-epoxy-5β-cholestane triglycoside (**1**) (1.30 g) isolated from *Ornithogalum saundersiae* (7.7 kg) showed potent inhibitory activity on proliferation of peripheral blood lymphocytes (PBL) provided from a CRF patient. This paper reports the structural elucidation of **1** including the conformation of the E-ring part and its inhibitory activity on proliferation of PBL.

Compound **1**, $[\alpha]_D$ -64.0° (MeOH), was obtained as an amorphous solid. The molecular formula was determined to be $C_{45}H_{74}O_{17}$ by neg. FAB-MS showing an $[M - H]^-$ ion at *m/z* 885 and elemental analysis (Calcd: C, 59.72; H, 8.46. Found: C, 59.78; H, 8.33). The $^1$H-NMR spectrum displayed signals arising from two tertiary methyl groups at δ 1.01 and 1.00 (each s), two secondary methyl groups at δ 1.75 (d, $J$ = 6.2 Hz) and 1.35 (d, $J$ = 6.5 Hz), two methyl groups on a double bond at δ 1.81 and 1.73 coupled to an olefinic proton at δ 5.79 with small $J$ values of 1.1 Hz and 0.5 Hz >, respectively, and three anomeric protons at δ 6.14 (d, $J$ = 1.2 Hz), 5.72 (d, $J$ = 7.5 Hz) and 4.93 (d, $J$ = 7.4 Hz). The $^{13}$C-NMR spectrum showed 45 resonance lines; 27 of them could be due

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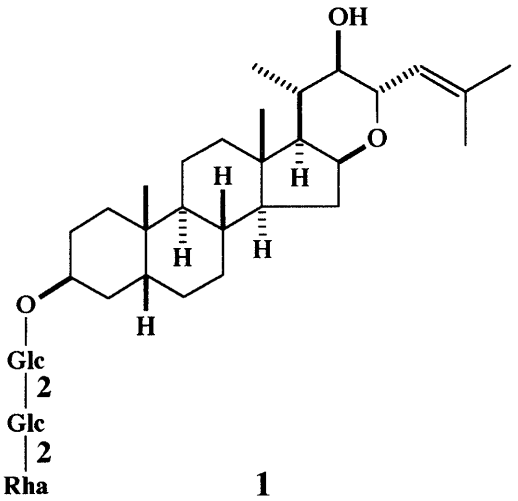
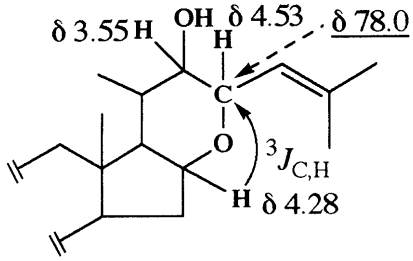
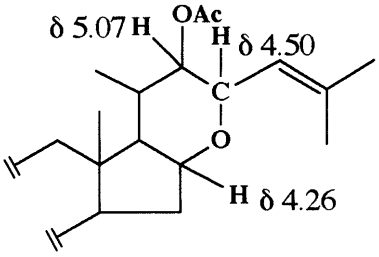
to the aglycone part and 18 to three monosaccharides. The existence of a trisubstituted double bond was apparently shown by a pair of $^{13}C$ signals at δ 136.2 (C) and 125.9 (CH). Acid hydrolysis of **1** gave D-glucose and L-rhamnose in a ratio of 2 : 1.[2] Thus, **1** was predicted to be a cholestane triglycoside.




**1**




**1**



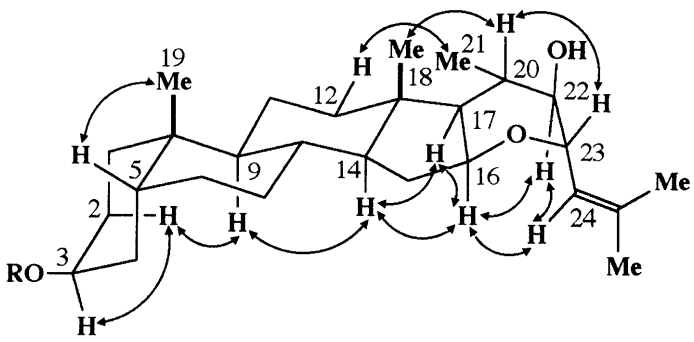

**1a**

Compound **1** required 9 degrees of unsaturation, and three monosaccharides and a double bond consumed 4 degrees. Consequently, **1** must possess a pentacyclic steroidal aglycone. Sequential assignments of the $^1H$-NMR of the steroid nucleus of **1** were performed through detailed interpretation of the $^1H$-$^1H$ COSY spectrum, indicating the presence of the oxygen atoms at the C-3, C-16, C-22, and C-23 positions, and a double bond at C-24. On comparison of the $^1H$-NMR spectrum of the corresponding decaacetate (**1a**) of **1** with that of **1**, the signal due to 22-H was shifted downfield by 1.52 ppm through *O*-acetylation; however, those due to 16-H and 23-H were almost unaffected, suggesting the formation of a six-membered ring between C-16 and C-23 in the aglycone of **1**. This was well supported by the detection of $^3J_{C,H}$ coupling from 16-H (δ 4.28) to C-23 (δ 78.0) in the HMBC spectrum.

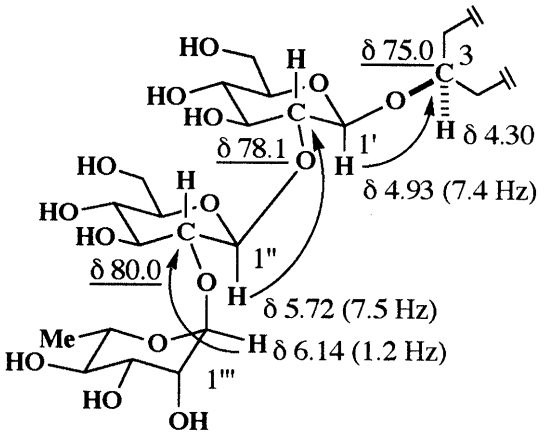
An analysis of the phase-sensitive NOESY spectrum made the relative stereochemistry assignable. The NOE correlations from 5-H (δ 2.42) to 19-Me (δ 1.01), and 9-H (δ 1.36) to 2α(ax)-H (δ 1.59) and 14-H (δ 0.94) were consistent with A/B *cis* and B/C *trans* ring junctions. The 14-H in turn showed NOEs with 16-H and 17-H (δ 1.14), indicating 16α-H and 17α-H orientations. Further NOEs from 20-H (δ 2.14) to 18-Me (δ 1.00) and 23-H (δ 4.53), 21-Me to 12β(eq)-H (δ 1.19), and 22-H (δ 3.55) to 21-Me and 16-H, confirmed C/D *trans* and D/E *cis* junctions, and 20*S**, 22*R** and 23*S** configurations. The orientation of the C-3 oxygen atom was determined to be a β-form from an NOE between 3-H and 2α(ax)-H and by $W_{1/2}$ value (9.0 Hz) of 3-H.

The presence of a terminal α-L-rhamnopyranosyl unit and two 2-substituted β-D-glucopyranosyl units in the molecule was shown by comparison of the $^{13}C$-NMR resonances for each monosaccharide, which were assigned by a combined use of $^1H$-$^1H$ COSY and HMQC spectra with those of reference methyl glycosides.[3] The $^1H$-$^{13}C$ long-range correlation from each anomeric

proton across the glycosidic bond to the carbon of another substituted monosaccharide or the aglycone confirmed the sugar sequence. From the data presented above, the structure of **1** was elucidated.




**NOE Correlations**




**HMBC Correlations**

The E-ring conformation was shown to be almost a boat-form through molecular mechanics calculations using the MM2 force field as implemented in Macro-model 4.0. The starting geometries were generated by a systematic Monte Carlo conformational search. The most stable conformer thus found was taken as starting structures for molecular dynamics calculations *in vacuo* at 296 K with a path length of 100ps and following by minimizing random structures sampled after multiple 1ps intervals. In this run, three conformers were obtained; the most stable conformer, whose boltzmann population was 99.7% at 296 K, showed 176.8° for the $H_{20}$-$C_{20}$-$C_{22}$-$H_{22}$ torsion angle and 150.9° for the $H_{22}$-$C_{22}$-$C_{23}$-$H_{23}$ torsion angle. The observed proton coupling constants, $^3J_{20\text{-H},\ 22\text{-H}}$ = 11.5 Hz and $^3J_{22\text{-H},\ 23\text{-H}}$ = 7.5 Hz, almost corresponded to those (10.8 Hz and 7.1 Hz) calculated through the application of the given dihedral angles to the advanced Karplus-type equation proposed by Altona *et al.*[4]

Compound **1** potently inhibited proliferation of PBL provided from a CRF patient ($IC_{50}$ 3.1 μM) without causing any cytotoxicity in the lymphocytes and HL-60 human leukemia cells ($IC_{50}$ 10 μM <).[5] Thus, the potentiality of **1** as a new immunosuppresive agent is evident.

## REFERENCES AND NOTES

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2) The monosaccharides were identified by converting them to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives followed by HPLC analysis; Oshima R., Yamauchi Y., Kumanotani J., *Carbohydr. Res.*, **107**, 169 - 176 (1982).

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4) Haasnoot C. A. G., Leeuw F. A. A. M. De, Altona C., *Tetrahedron*, **36**, 2783 - 2792 (1980).

5) Prednisolone used as a positive control inhibited proliferation of PBL from healthy subjects with $IC_{50}$ value of 0.17 μM, which, however, varies by individual.

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