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Structure of a Novel 22-Homo-23-norcholestane Trisaccharide from *Ornithogalum saundersiae*

Minpei KURODA, Yoshihiro MIMAKI and Yutaka SASHIDA*
Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-03, Japan
Tamotsu NIKAIDO and Taichi OHMOTO
Scool of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan

Abstract: A novel 22-homo-23-norcholestane trisaccharide, which showed an unique inhibitory pattern on cyclic AMP phosphodiesterase, was isolated from the bulbs of *Ornithogalum saundersiae*. The structure was determined by extensive 2D NMR analysis and hydrolysis.

The cyclic AMP phosphodiesterase inhibition test provides a useful means for the screening of biologically active compounds present in natural sources.[1] Previously, we have reported the structural elucidation of several new cholestane glycosides with potent inhibitory activity on cyclic AMP phosphodiesterase isolated from the methanolic bulb extract of *Ornithogalum saundersiae*.[2] Further analysis of the extract led to the isolation of a novel 22-homo-23-norcholestane trisaccharide (1). This communication refers to the structural determination of the new constituent and its unique inhibitory pattern on cyclic AMP phosphodiesterase.

Compound 1 (90 mg from 16 kg of the fresh plant material) was obtained as a white amorphous powder, $C_{46}H_{72}O_{19}$, , $[\alpha]_D$ -78.0° (MeOH). The spectral features [3] and the result of acid hydrolysis of 1,[4] suggested 1 to be a cholestanol trisaccharide. The ^{1}H-^{1}H COSY spectrum combined with the HSQC spectrum measured in CD_3OD revealed the structural fragments composed of 1 (Fig. 1). In the HSQC spectrum of 1, the ^{1}H signal at δ 4.87 (1H, d, J = 8.4 Hz) was correlated to the ^{13}C signal at δ 91.4 (CH), which was observed at δ 90.9 in C_5D_5N. Upon addition of a small amount of CD_3OD in C_5D_5N, the signal at δ 90.9 was split to appear as duplicate signals at δ 90.75 and 90.65.[5] On the other hand, the signal at δ 4.70 (1H, s) showed $^1J_{C\text{-}H}$ correlation with the signal at δ 109.4 (CH). In the HMBC spectrum, the signals at δ 4.70 and 3.32 (3H, s, OCH_3) showed long-range correlation peaks with the signals at δ 55.6 (OCH_3) and 109.4, respectively. These data led to the presence of a hemiacetal group and an acetal group in 1.

The molecular formula of 1 required 11 degrees of unsaturation. The presence of the three rings of the saccharide residue and two double bonds accounted for 5 degrees, and therefore, the aglycone moiety must contain a six-ring system. The connectivities of segments A, B, C and D, and two methyl groups through the quaternary carbons were shown by interpretation of the HMBC spectrum (Fig. 2). Cross peaks between the ^{13}C resonance at δ 91.4 (C-23) and the ^{1}H resonance at δ 4.31 (H-16), and between δ 86.4 (C-20) and δ 4.70 (H-18), resulted in the formations of a six-membered hemiacetal ring between C-16 and C-23, and a five-membered acetal ring between C-18 and C-20.

The configuration of the C-3 hydroxyl group bearing saccharide moiety was determined to be β from the multiplicity of the H-3 proton ($W_{1/2}$ = 20.6 Hz). The NOE correlations, H-8/H-18, H-21/H12β, H-15β/H-23

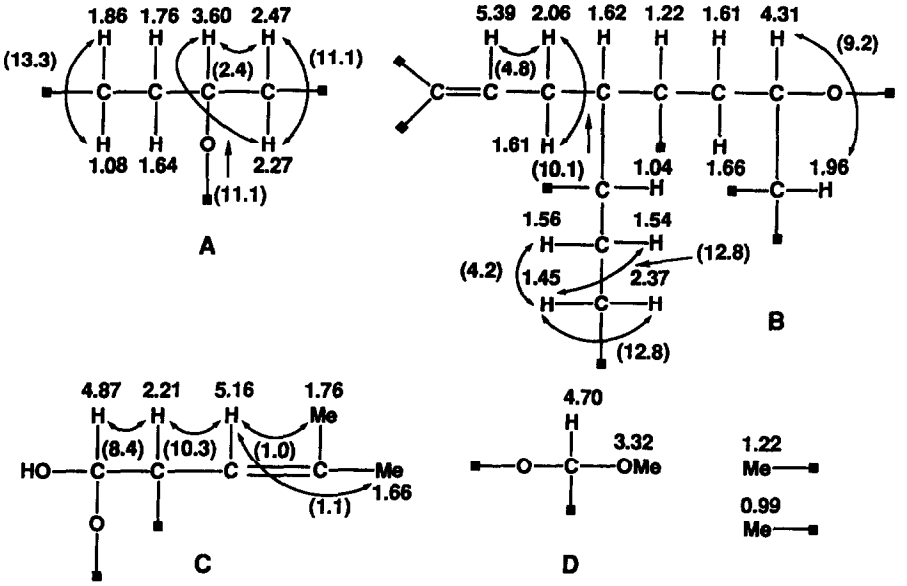

Fig. 1. Structural fragments of the aglycone moiety of **1** shown by the ^{1}H-^{1}H COSY spectrum in CD_3OD. *J* values (Hz) are given in parentheses.

and H-23/H-24 in the phase-sensitive NOESY spectrum provided evidences for the 16*S**, 17*R**, 18*R**, 20*S**, 22*S** and 23*R** configurations (Fig. 3). The half-chair conformation of the six-membered hemiacetal ring was indicated by the NOE correlation between H-15β and H-23, and by the large *J* value between H-22 and H-23 (*J* = 8.4 Hz) (Fig. 4).

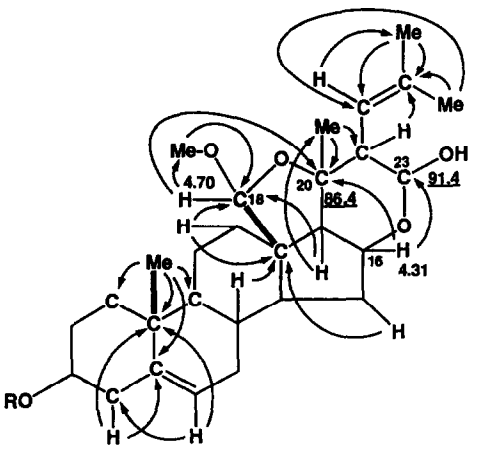

Fig. 2. ^{1}H-^{13}C long-range correlations of the aglycone moiety of **1**.

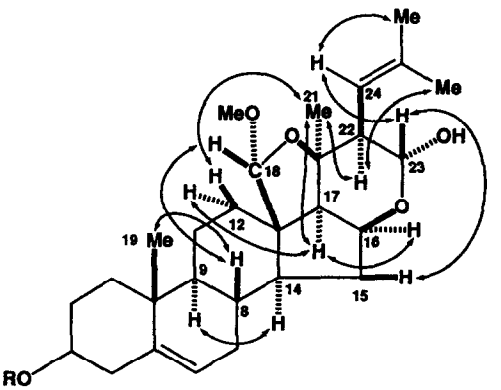

Fig. 3. NOE correlations of the aglycone moiety of **1**.

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 ^{1}H-^{1}H COSY and HSQC spectra, with those of reference methyl glycosides.[6] The ^{1}H-^{13}C long-range correlation from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide confirmed the sugar sequence (Fig. 5). The data presented above led to construct the full structure of **1**, which contains a new steroidal skeleton, 22-homo-23-norcholestane skeleton.

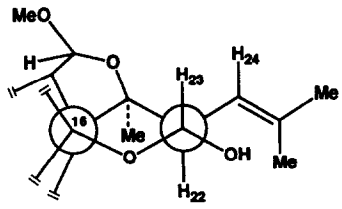

Fig. 4. $^{3}J_{H22\text{-}H23} = 8.4$ Hz
$^{3}J_{H22\text{-}H24} = 10.3$ Hz

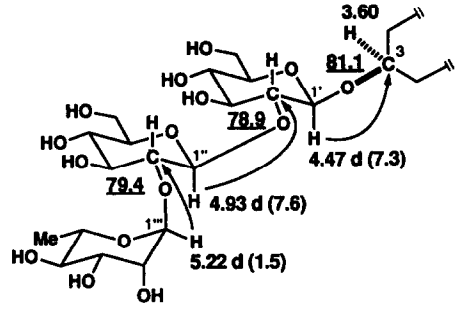

Fig. 5. ^{1}H-^{13}C long-range correlations of the saccharide moiety of **1**.

Inhibitory activity of **1** on cyclic AMP phosphodiesterase was assayed from a sample concentration of 0.02 mg/ml to 0.20 mg/ml at an interval of 0.02 mg/ml.[7] The activity increased when increasing the sample concentration from 0.02 mg/ml to 0.08 mg/ml, however, it decreased from 0.10 mg/ml with the minimum activity (16.4 %) at 0.16 mg/ml, and in turn, increased from 0.18 mg/ml (Fig. 6). This phenomenon should encourage further detailed studies of inhibitory effects of the cholestanes from the *Ornithogalum* plants on phosphodiesterase.

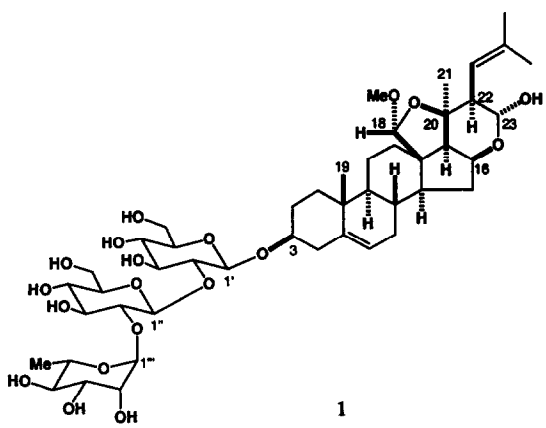

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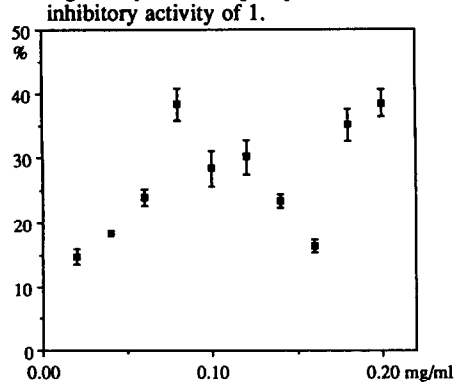
Fig. 6. Cyclic AMP phosphodiesterase inhibitory activity of 1.



Each value represents the mean± SE of triplicate or quadroplicate experiments.

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3. Some spectral data of **1**: negative-ion FABMS *m/z* 927 [M - H]$^-$, 783 [M - rhamnose]$^-$; IR ν_{max} (KBr): 3410 (OH), 2930 (CH) cm^{-1}; ^{1}H NMR (C_5D_5N - CD_3OD, 5 : 1): δ 6.27 (1H, br s, H-1‴), 5.78 (1H, d, *J* = 7.3 Hz, H-1"), 5.65 (1H, br d, *J* = 10.2 Hz, H-24), 5.44 (1H, d, *J* = 8.4 Hz, H-23), 5.41 (1H, br d, *J* = 4.5 Hz, H-6), 5.10 (1H, d, *J* = 7.5 Hz, H-1'), 4.75 (1H, s, H -18), 4.54 (1H, ddd, *J* = 9.2, 9.2, 6.7 Hz, H-16), 3.94 (1H, m, $W_{1/2}$ = 20.6 Hz, H-3), 3.37 (3H, s, OMe), 2.63 (1H, dd, *J* = 10.2, 8.4 Hz, H-22), 2.00 (1H, d, J = 9.2 Hz, H-17), 1.80 (3H, br s, H-26), 1.78 (3H, d, *J* = 6.2 Hz, H-6‴), 1.77 (3H, br s, H-27), 1.41 (3H, s, H-21), 0.89 (3H, s, H-19); ^{13}C NMR (CD_3OD): δ 38.3, 30.5, 81.1, 40.0, 142.2, 122.4, 33.0, 33.5, 51.4, 38.0, 23.8, 35.3, 58.6, 52.1, 30.2, 78.2, 54.0, 109.4, 19.9, 86.4, 27.7, 51.0, 91.4, 122.0, 136.4, 26.4, 18.9 (C-1 - C-27), 101.6, 78.9, 79.3, 72.1, 77.8, 62.9 (C-1' - C-6'), 102.0, 79.4, 79.3, 72.4, 78.0, 63.3 (C-1" - C-6"), 102.0, 72.2, 72.2, 74.2, 69.7, 18.3 (C-1‴ - C-6‴); ^{13}C NMR (C_5D_5N): δ 37.4, 30.1, 78.9, 39.4, 141.5, 121.3, 32.2, 32.3, 50.0, 37.2, 22.9, 34.5, 57.5, 51.1, 29.6, 77.1, 53.2, 108.5, 19.4, 85.7, 27.7, 50.7, 90.9, 122.8, 134.5, 26.4, 19.0 (C-1 - C-27), 101.3, 80.8, 79.3, 72.3, 77.7, 62.8 (C-1' - C-6'), 102.3, 78.8, 79.1, 71.8, 78.1, 63.0 (C-1" - C-6"), 102.1, 72.3, 72.6, 74.4, 69.8, 19.0 (C-1‴ - C-6‴).
4. Acid hydrolysis of **1** with 1N HCl (dioxane - H_2O, 1 : 1) gave D-glucose and L-rhamnose. No genuine aglycone could be obtained. The identification of the monosaccharides including their absolute configurations was achieved 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.* **1982**, 107, 169 - 176.
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