A Novel Polyoxygenated Spirostanol Trisdesmoside from the Rhizomes of *Helleborus orientalis*

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A novel polyoxygenated spirostanol glycoside (**1**) was isolated from the rhizomes of *Helleborus orientalis* and its structure was determined by spectroscopic analysis, including extensive 1D and 2D NMR data, and the results of acid hydrolysis. The new glycoside (**1**) is unique in structure having oxygen atoms at C-1, C-3, C-21, C-23, and C-24 of the spirostanol skeleton and bearing a new acetylated tetraglycoside, a diglycoside, and a monosaccharide at C-1, C-21, and C-24 of the aglycon, respectively.

Helleborus orientalis Lam. (Ranunculaceae), especially its rhizomes, are known to contain several bufadienolide glycosides and were used as a cardiotonic agent.[1] Since an extract prepared from the rhizomes produces harmful side effects on the heart such as heart block and arrhythmia, nowadays this plant is cultivated only for ornamental purposes. Plants containing cardiac glycosides, for example, *Digitalis purpurea*, concomitantly produce spirostanol and/or furostanol glycosides. We have now made a phytochemical screening of *H. orientalis* rhizomes paying attention to the steroidal glycoside constituents and isolated a novel polyoxygenated spirostanol glycoside (**1**). This communication deals with the structural determination of **1** on the basis of spectroscopic analysis, including extensive 1D and 2D NMR data, and the results of acid hydrolysis.

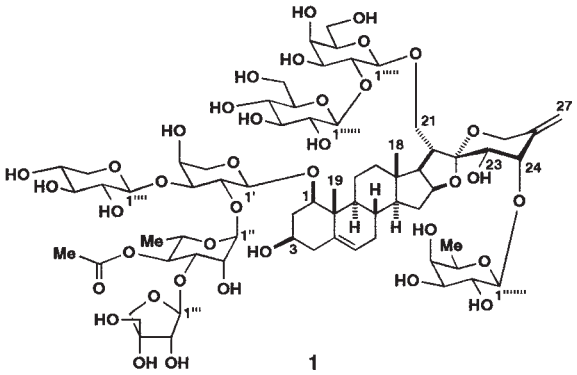

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Chart 1.

A concentrated MeOH extract of *H. orientalis* rhizomes was passed through a porous-polymer resin (Diaion HP-20) column, and the 80% MeOH eluate portion, in which steroidal glycosides were enriched, was subjected to column chromatography over silica gel and ODS to yield **1** (0.0033% based on fresh weight).

Compound **1** was obtained as an amorphous solid, $[\alpha]_D$ -66.0° (MeOH). Its molecular formula was derived as $C_{68}H_{106}O_{38}$ by data from the positive-ion FABMS, which showed an $[M + Na]^+$ ion at m/z 1553, ^{13}C NMR spectrum with a total of 68 carbon signals, and the results of elemental analysis (Found: C, 51.60; H, 7.31%. Calcd for $C_{68}H_{106}O_{38} \cdot 3H_2O$: C, 51.51; H, 7.12%). The glycosidic nature of **1** was shown by strong IR absorptions at 3376 and 1042 cm^{-1}, and the ^1H NMR spectrum displayed signals for seven anomeric protons at δ 6.42 (br s), 5.93 (d, $J = 2.9$ Hz), 5.30 (d, $J = 7.7$ Hz), 5.13 (d, $J = 7.8$ Hz), 4.91 (d, $J = 6.7$ Hz), 4.89 (d, $J = 7.2$ Hz), and 4.57 (d, $J = 7.6$ Hz). In addition, the signals at δ_H 2.23 (3H, s) and δ_C 170.7 (C) and 21.1 (Me) were suggestive of an acetyl group. Acid hydrolysis of **1** with 0.2 M (1 M = 1 mol dm^{-3}) HCl in dioxane-H_2O (1 : 1) gave L-arabinose, D-apiose, D-fucose, D-galactose, D-glucose, L-rhamnose, and D-xylose as the carbohydrate moieties,[2] while the labile aglycon was decomposed under acid conditions. These data and preliminary inspection of the ^1H and ^{13}C NMR spectra indicated that **1** was a polyoxygenated spirostanol with seven monosaccharides and an acetyl group.[3] The ^1H–^1H COSY and 2D TOCSY spectra of **1** afforded two partial structures for the aglycon, (C-1—C-4) and (C-6—C-12/C-21). These partial structures, three quaternary carbons (C-5, C-10, C-13), and two tertiary methyl groups (C-18, C-19) were merged (A—E rings) on the basis of the HMBC information as shown Figure 1. Furthermore, a vicinal pair of oxymethine (C-23, C-24), an exomethylene (C-25, C-27), and an oxymethylene (C-26) group were identified and connected on the HMBC data to assemble ring F, which was linked to the C-22 acetalic carbon. Thus, the aglycon was shown to have a spirost-5-ene structure with oxygen atoms at C-1, C-3, C-21, C-23, and C-24. The NOE correlations in the phase-sensitive NOESY spectrum, H-8/Me-18 and Me-19, H-14/H-9, H-16 and H-17, H-16/H-17 and H-26ax, and Me-18/H-20 provided evidence for the usual B/C t*rans*, C/D *trans* and D/E *cis* ring fusions, and C-20α and C-22α orientations. The 1β and 3β configurations were shown by spin coupling constants of the H-1 and H-3 protons: H-1, δ 3.71 (dd, $J = 11.8$, 4.5 Hz); H-3, δ 3.86 (m, $W_{1/2} = 20.6$ Hz), and supported by NOEs between H-1 and H-9, and between H-1 and H-3. The NOEs from H-23 to H-20 and H-21b, and H-23 to H-24, and a small coupling constant between H-23 and H-24 ($J = 4.0$ Hz) allowed the assignments of the 23*S* and 24*S* configurations.[4] The sequences of the sugar moieties were solved by the concerted use of 1D TOCSY and 2D NMR experiments. Because of the selectivity of the multistep coherence transfer, 1D TOCSY method allowed a sub-spectrum of a single monosaccharide unit to be extracted from the crowded overlapped region. The isolated anomeric proton signals and methyl doublet signal of the rhamnosyl moiety, which resonated in an uncrowded region of the spectrum, were used for the starting points of the 1D TOCSY experiments. As a result, the sub-spectrum of each sugar residue, except for that of the apiofuranosyl moiety,[5] was obtained with high digital resolution. Subsequent analysis of the ^1H–^1H COSY spectrum resulted in the sequential assignments of all of the proton resonances for the individual monosaccharides. The HMQC spectrum correlated all

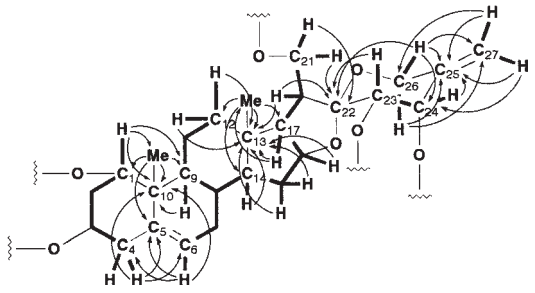

Figure 1. HMBC correlations of the aglycon moiety of **1**.

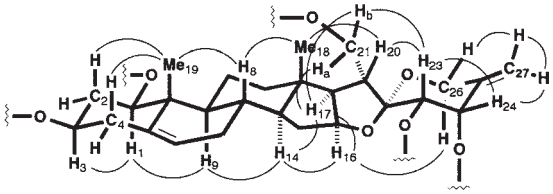

Figure 2. NOE correlations of the aglycon moiety of **1**.

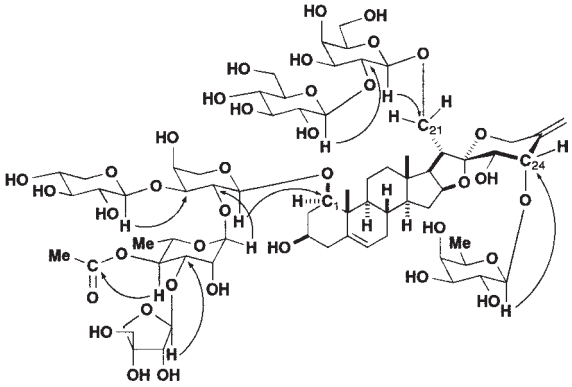

Figure 3. HMBC correlations of the sugar moiety of **1**.

the proton resonances with those of the corresponding one-bond coupled carbons, leading to the unambiguous assignments of the carbon shifts. Comparison of the carbon chemical shifts thus assigned with those of the reference methyl glycosides,[6] taking into account the known effects of *O*-glycosylation, indicated that **1** contained a β-D-apiofuranosyl unit (Api), a β-D-fucopyranosyl unit (Fuc), a β-D-glucopyranosyl unit (Glc), and a β-D-xylopyranosyl unit (Xyl) as the terminal glycosyl moieties, and an α-L-arabinopyranosyl unit (Ara), a β-D-galactopyranosyl unit (Gal), and an α-L-rhamnopyranosyl unit (Rha) as the substituted sugar moieties.[7] Finally, the $^3J_{C,H}$ correlation from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide revealed the exact sugar sequences. In the HMBC spectrum, the anomeric proton signals at δ 6.42 (Rha), 5.93 (Api), 5.30 (Glc), 5.13 (Fuc), 4.91 (Xyl), 4.89 (Gal), and 4.57 (Ara) showed correlations with the carbon signals at δ 72.7 (C-2 of Ara), 77.6 (C-3 of Rha), 81.3 (C-2 of Gal), 81.9 (C-24 of aglycon), 84.8 (C-3 of Ara), 69.6 (C-21 of aglycon), and 84.2 (C-1 of aglycon), respectively. In addition, a long-range correlation was observed from the Rha H-4 proton at δ 5.86 (dd, $J = 9.8$, 9.8 Hz) to the acetyl carbonyl carbon signal at δ 170.7. Accordingly, the structure of **1** was elucidated as depicted in Chart 1. Compound **1** is considered to be one of the most polar spirostanol saponins and is unique in structure having oxygen atoms at C-1, C-3, C-21, C-23, and C-24 of the spirostanol skeleton and bearing a new acetylated tetraglycoside, a diglycoside, and a monosaccharide at C-1, C-21, and C-24 of the aglycon, respectively. Cytotoxic evaluation of **1** against several tumor cell lines is in progress.

References and Notes

1 V. W. Wissner and H. Kating, *Planta Med.*, **26**, 228 (1974).

2 The identification of the monosaccharides, including their absolute configurations, was established by direct HPLC analysis of the sugar fraction of the acid hydrolysate using a combination of RI and optical rotary (OR) detectors.

3 ^{13}C NMR (C_5D_5N) of **1**: δ 84.2, 37.9, 67.9, 43.7, 139.4, 124.9, 31.8, 33.0, 50.2, 42.8, 23.8, 39.9, 40.8, 56.8, 32.3, 83.2, 57.8, 16.5, 15.0, 43.7, 69.6, 111.1, 71.3, 81.9, 143.4, 61.3, 113.9 (C-1—C-27), 100.7, 72.7, 84.8, 69.8, 66.9 (C-1′—C-5′), 100.7, 71.4, 77.6, 74.5, 66.6, 18.4 (C-1″—C-6″), 112.0, 77.8, 80.0, 74.9, 65.2 (C-1‴—C-5‴), 106.6, 74.4 78.3, 70.9, 67.0 (C-1⁗—C-5⁗), 103.4, 81.3, 75.2, 69.6, 76.8, 61.9 (C-1⁗′—C-6⁗′), 105.8, 76.5, 78.0, 71.1, 78.4, 62.2 (C-1⁗″—C-6⁗″), 106.0, 73.0, 75.2, 72.7, 71.4, 17.2 (C-1⁗‴—C-6⁗‴), 170.7 and 21.1 (Ac).

4 Since the aglycon of **1** could not be obtained by mild acid hydrolysis (0.2 M HCl, 95 °C, 30 min), the absolute configurations of the steroidal skeleton were chosen in keeping with those mostly encountered among plants.

5 The presence of a terminal β-D-apiofuranosyl group was confirmed by the five carbon signals at δ 112.0, 77.8, 80.0, 74.9, 65.2.

6 P. K. Agrawal, *Phytochemistry*, **31**, 3307 (1992).

7 The relatively large J values of the anomeric protons of the Ara, Fuc, Gal, Glc, and Xyl moieties ($J = 6.7$–7.8 Hz) indicated α anomeric orientation for the Ara, and β for the Fuc, Gal, Glc, and Xyl. For the Rha moiety, the large $^1J_{C,H}$ value (179.0 Hz) and three-bond coupled strong HMBC correlations from the anomeric proton to the C-3 and C-5 carbons (the dihedral angles between H-1 and C-3, and between H-1 and C-5 about 180 °), indicated that the anomeric proton was equatorial thus possessing an α-pyranoid anomeric form.