A New Furostanol Saponin with Six Sugars from the Bulbs of *Allium sphaerosephalon* Structural Elucidation by Modern NMR Techniques

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A new bisdesmosidic furostanol saponin with six monosaccharides (**1**) was isolated from the bulbs of *Allium sphaerosephalon*. The structure was determined by the concerted use of modern NMR techniques. The corresponding spirostanol saponin (**2**) of **1** showed cytotoxic activity against leukemia MOLT-4 cells, while **1** was inactive.

Plants of the genus *Allium* have long been used as food and medicine, and are well known for their production of steroidal saponins, as well as sulfide compounds as the main secondary metabolites. As a continuation of our studies on the steroidal constituents of *Allium* plants with medicinal potential,[1] we have analyzed the bulbs of *Allium sphaerosephalon*, which is widely distributed from Great Britain, through Europe, to Iran, resulting in the isolation of a new bisdesmosidic furostanol saponin (**1**), which has a pentaglycoside moiety with a new sugar sequence.

This paper briefly describes the structural assignment of **1** by the concerted use of modern NMR techniques: phase-sensitive NOESY (pNOESY), phase-sensitive double quantum filter (pDQF) COSY, phase-sensitive HSQC (pHSQC), HMBC, and pHSQC-total correlation spectroscopy (TOCSY) spectra.

The plant material was extracted with hot MeOH. After removal of abundant saccharides in the crude MeOH extract by passage through a Diaion HP-20 column eluted with H_2O gradually enriched with MeOH, it was chromatographed on silica-gel using $CHCl_3$ - MeOH - H_2O system and ODS using MeOH - H_2O and MeCN-H_2O systems to give **1** (19.7 mg from 6.6 kg of fresh material).

Compound **1**, $[\alpha]_D$ –26.0° (pyridine, c = 0.10) analyzed for $C_{64}H_{106}O_{33}$ by negative-ion FAB-MS (m/z 1402 $[M]^-$) and elemental analysis (Found: C, 53.24; H, 7.35%. Calcd: C, 53.40; H, 7.70%), was suggested to be a 22-methoxyfurostanol saponin by Ehrlich's test, and the ^1H and ^{13}C NMR spectra [δ_H 3.28 (3H, s); δ_C 112.9 (C) and 47.4 (Me)].[2] The ^1H NMR spectrum displayed signals for six anomeric protons at δ 6.20 (br s), 5.15 (d, J = 7.7 Hz), 5.06 (d, J = 8.1 Hz), 4.95 (d, J = 7.4 Hz), 4.94 (d, J = 7.2 Hz), and 4.84 (d, J = 8.5 Hz).[3] Enzymatic hydrolysis of **1** with β-D-glucosidase gave D-glucose and the corresponding spirostanol saponin (**2**). Subsequent hydrolysis of **2** with 1 M (1 M = 1 mol dm^{-3}) HCl in dioxane - H_2O (1 : 1) yielded (25*R*)-spirost-5-en-3β-ol (diosgenin), D-glucose and L-rhamnose.[4] A three-proton doublet signal at δ 1.76 (J = 6.5 Hz) accounted for the presence of one L-rhamnopyranosyl residue. The data described above indicated that **1** was a 26-*O*-glucosyl-22α-methoxyfurostanol[5] based on proto-diosgenin,[2] and that the carbohydrate moieties were composed of one L-rhamnose and five D-glucoses including the sugar attached to C-26 of the aglycone.

The saccharide structures of several saponins have been established by detecting $^3J_{C,H}$ correlation from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide.[6] This method requires a complete sequential assignment of all the ^1H and ^{13}C NMR resonances of each monosacchride constituting the oligosaccharide moieties in the first process. The severe overlapping of the protons for the five glucoses (Figure 1) excluded a complete assignment in a straightforward way using a conventional homonuclear COSY spectrum in **1**. A modern NMR technique, pHSQC-TOCSY was found to be useful in solving the saccharide sequence of **1**, because it could correlate the anomeric protons with their respective skeleton carbon atoms. This allowed us to identify the terminal monosaccharides and predict the substituted positions of most of the inner monosaccharides without any knowledge of the sequential ^1H and ^{13}C NMR assignment. A part of the pHSQC-

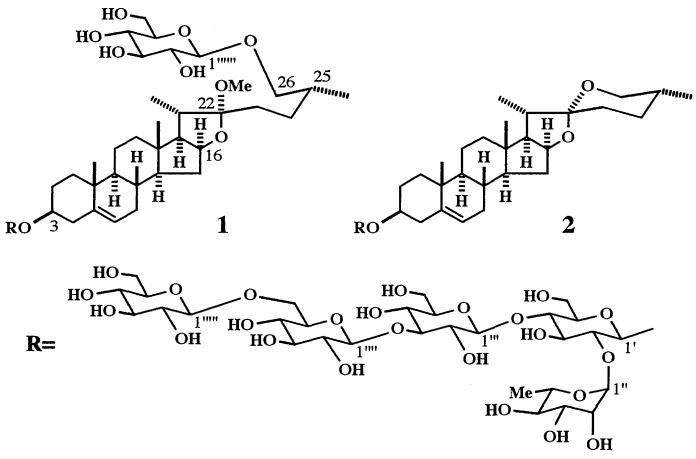

Figure 1. ^1H NMR spectrum of the sugar moieties of **1** (600 MHz).

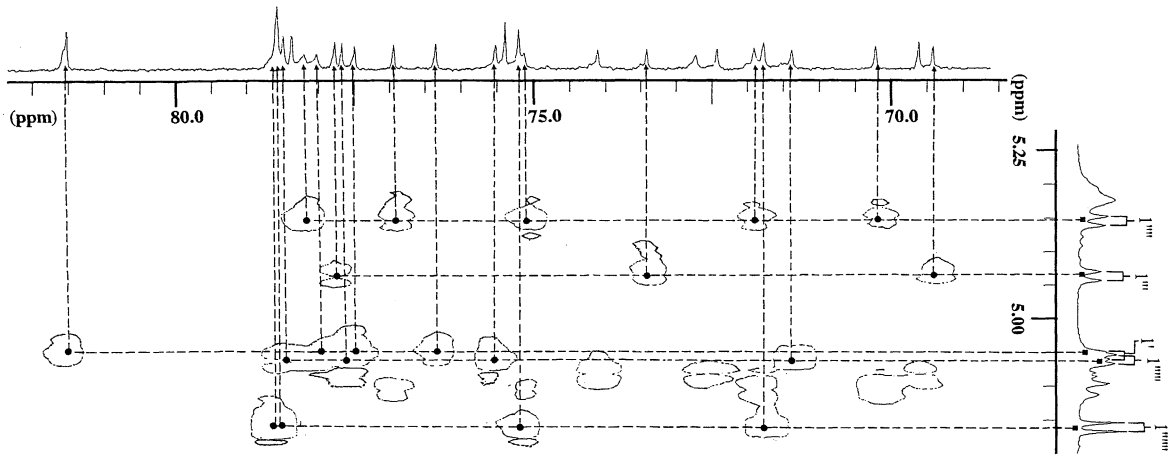

Figure 2. A part of the pHSQC-TOCSY spectrum of **1**.

TOCSY spectrum of **1** is shown in Figure 2. On the 1-H track (1"" in Figure 2) through the anomeric $^{1}H/^{13}C$ correlations at $\delta_{H/C}$ = 5.15/105.6, five relayed cross-peaks extending to C-6"" were observed, with ^{13}C chemical shifts of δ 78.2 (CH), 77.0 (CH), 75.2 (CH), 71.9 (CH) and 70.2 (CH_2). The signal at δ 70.2, which showed $^{1}J_{C,H}$ correlations with δ 4.90 (br d, J = 10.5 Hz) and 4.05 in the pHSQC spectrum and was shifted downfield by *ca.* 8 ppm compared with that of an authentic methyl β-D-glucopyranoside,[2,7] was assigned to C-6 of a 6-substituted glucose. The anomeric proton at δ 5.06 showed relayed correlation peaks with the ^{13}C signals at δ 104.7, 88.7, 77.7, 73.4, 69.4 and 61.7. The C-3 atom was readily assigned because of the downfield shift to δ 88.7, which is typical of a 3-substitution.[2,8] The anomeric protons at δ 6.20, 4.94 and 4.84 were correlated to δ 102.0, 74.1, 72.7, 72.4, 69.6 and 18.8, δ 104.9, 78.5, 77.8 75.5, 71.4 and 62.5, and δ 105.1, 78.6, 78.6, 75.2, 71.8 and 62.9, respectively, indicating the presence of one terminal rhamnose and two terminal glucoses. The anomeric proton at δ 4.95 showed cross-peaks at δ 100.1, 81.6, 78.0, 77.5, 76.4 and 61.6, the shift values of which suggested the existence of a 2,4-disubstituted glucose.[2] In the pDQF COSY spectrum, the anomeric proton at δ 4.95 showed a $^{3}J_{H,H}$ correlation with δ 4.20, which was correlated to δ 78.0 in the pHSQC, leading to the assignment of δ 78.0 as C-2 of the glucose. Further, δ 61.6 showed $^{1}J_{C,H}$ correlations with δ 4.48 and 4.42 assignable to 6-H_2, and in the pDQF COSY, each signal displayed a cross-peak with an isolated signal at δ 3.83 of 5-H, which, in turn, showed a correlation with δ 4.18 assignable to 4-H. The signal at δ 81.6 was assigned to C-4 by a $^{1}J_{C,H}$ correlation with δ 4.18. The above analysis provided evidence for the presence of a terminal rhamnose, two terminal glucoses, a 3-substituted glucose, a 6-substituted glucose and a 2,4-disubstituted glucose in **1**.

Finally, the HMBC correlations from each anomeric proton across the glycosidic bond to the carbon with another substituted monosaccharide or aglycone confirmed the sugar sequence. The signal at δ 6.20 (rhamnose), 5.15 (6-substituted glucose), 5.06 (3-substituted glucose), 4.95 (2,4-disubstituted glucose, 4.94 (terminal glucose) and 4.84 (terminal glucose) showed correlations with δ 78.0 (C-2 of 2,4-disubstituted glucose), 88.7 (C-3 of 3-substituted glucose), 81.6 (C-4 of 2,4-disubstituted glucose), 78.4 (C-3 of aglycone), 70.2 (C-6 of 6-substituted glucose) and 75.4 (C-26 of aglycone). Thus, one glucose was shown to be linked to the C-26 hydroxyl group of the aglycone, and the pentaglycoside moiety, *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranose linked to C-3 of the aglycone. The complete structure of **1** was formulated as 26-*O*-β-D-glucopyranosyl-22-*O*-methy-(25*R*)-furost-5-en-3β,22α,26-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}.

Compound **1** has a pentaglycoside moiety with a new sugar sequence, and the corresponding spirostanol saponin (**2**) showed cytotoxic activity against human T-lymphocytic leukemia MOLT-4 cells with an LC_{50} of 2.1 μM, while **1** was inactive (LC_{50} 10 μM <).[9]

References and Notes

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3 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.
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