NEW POLYHYDROXYLATED STEROIDAL SAPONINS FROM THE TUBERS OF *BRODIAEA CALIFORNICA*

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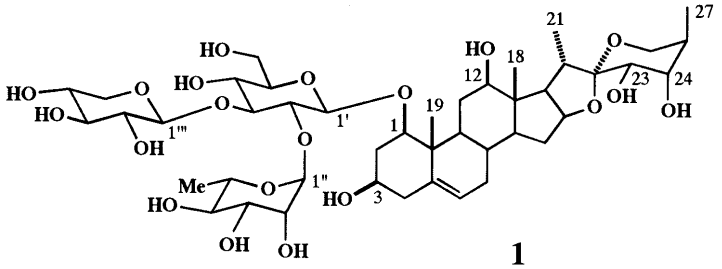
New polyhydroxylated steroidal saponins (**1**, **2**) were isolated from the tubers of *Brodiaea californica*. The structures were determined by spectroscopic analysis and acid-catalyzed hydrolysis. The bisdesmosidic saponin (**2**) is unique in structure, and is the first representative of a steroidal saponin bearing 6-deoxy-D-gulopyranose among both the steroidal and triterpene saponins reported up to the present.

KEY WORDS *Brodiaea californica*; Liliaceae; polyhydroxylated steroidal saponin; bisdesmosidic saponin; 6-deoxy-D-gulopyranose

Our previous chemical analysis disclosed that the plants belonging to the subfamily Allioideae in the Liliaceae contained certain amounts of steroidal saponins.[1] As a continuation, we have investigated the tubers of *Brodiaea californica*, an Allioideae plant native to northern California, resulting in the isolation of two new polyhydroxylated steroidal saponins (**1**, **2**). This paper briefly reports the structural elucidation of the new saponins based on spectroscopic analysis and acid hydrolysis.

The MeOH extract of *B. californica* tubers (fresh weight, 3.0 kg) yielded **1** (24.0 mg) and **2** (125 mg) after repeated column chromatography on Diaion HP-20, silica gel, and ODS silica gel.



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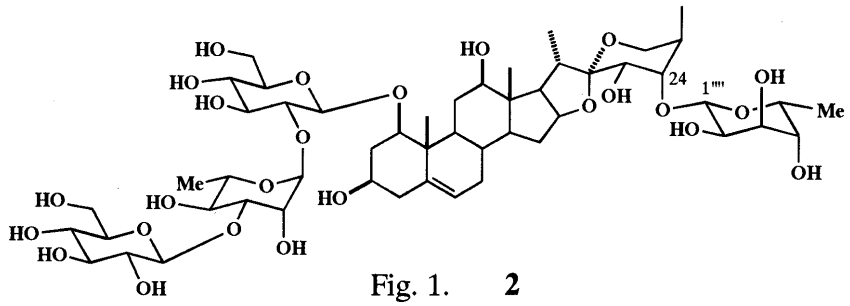

Fig. 1. **2**

The molecular formula of **1** was analyzed as being $C_{44}H_{70}O_{20}$ by negative-ion FABMS showing an $[M]^-$ ion at *m/z* 918 and elemental analysis. The fundamental structure of **1** was suggested to be a spirostanol triglycoside by the ^{1}H-NMR signals[2] at δ 1.34 and 1.22 (each 3H, s), 1.39 (3H, d, J = 7.1 Hz), and 1.08 (3H, d, J = 7.4 Hz) assignable to steroid methyls, and δ 6.17 (1H, d, J = 1.4 Hz), 4.82 (d, J = 7.7 Hz) and 4.70 (1H, d, J = 7.4 Hz) due to anomeric protons, and by a quaternary ^{13}C-NMR signal at δ 113.4.[3] Acid hydrolysis of **1** with 1 M HCl in dioxane-H_2O (1 : 1) yielded D-glucose, D-xylose, and L-rhamnose in a ratio of 1 : 1 : 1.[4] The ^{13}C-NMR spectrum of **1** showed 44 resonance lines, 17 of which were due to the three monosaccharides, and three

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anomeric carbons were observed at δ 105.1, 101.6, and 100.5. This implied a $C_{27}H_{42}O_7$ molecular formula for the aglycone portion, suggesting that the aglycone was a spirostanol into which five oxygen atoms were introduced. Analysis of the ^1H-^1H COSY spectrum combined with the HOHAHA data, followed by inspection of the HMQC spectrum, deduced three main fragments constituting the aglycone as well as four quaternary carbons and two angular methyls. The connectivities of the main fragments and the two angular methyls were established by interpretation of the HMBC spectrum optimized for $^nJ_{C,H}$ = 8 Hz, leading to a spirost-5-ene structure with oxygen atoms at C-1, C-3, C-12, C-23, and C-24.

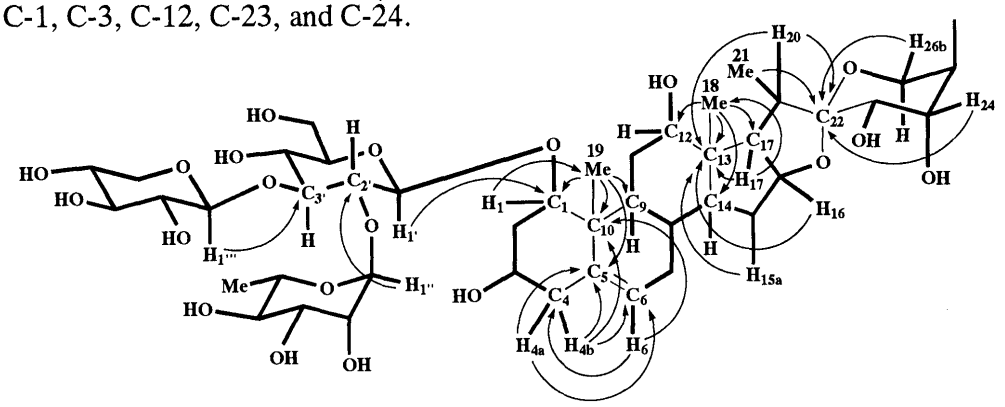

Fig. 2. HMBC Correlations of **1**

The NOEs in the phase-sensitive NOESY spectrum, 8-H/18-Me and 19-Me, 14-H/9-H, 16-H and 17-H, 16-H/17-H, and 18-Me/20-H, provided evidence for the usual B/C *trans*, C/D *trans*, and D/E *cis* ring fusions. The 1β, 3β, and 12β orientations were shown by the spin coupling constants of the 1-H, 3-H, and 12-H protons;

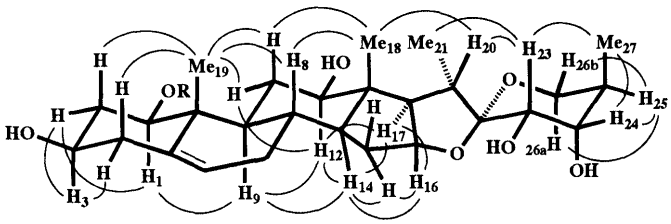

Fig. 3. NOE Correlations of the Aglycone Moiety of **1**

1-H: δ 3.75 (dd, J = 11.9, 4.1 Hz), 3-H: δ 3.68 (m, $W_{1/2}$ = 24.5 Hz), and 12-H: δ 3.73 (dd, J = 11.9, 3.5 Hz), supported by the NOEs, 9-H/1-H and 12-H, and 12-H/14-H and 17-H. The NOEs from 23-H to 20-H, 21-Me and 27-Me, and 24-H to 25-H and 27-Me, and small coupling constants between 23-H and 24-H (J = 3.0 Hz), and 25-H and 26-H_2 ($J_{25\text{-H},26a\text{-H}}$ = 2.1 Hz, $J_{25\text{-H},26b\text{-H}}$ = 0.5 Hz >), allowed assignment of the 22α, 23*S*, 24*S*, and 25*R* configurations.[5] Thus, the structure of the aglycone was revealed.

The presence of a terminal α-L-rhamnopyranosyl, a terminal β-D-xylopyranosyl, and a 2,3-branched β-D-glucopyranosyl units was shown by comparison of the ^{13}C-NMR resonances for each monosaccharide, which were assigned by means of combined use of the ^1H-^1H COSY, HOHAHA, and HMQC data, with those of authentic methyl glycosides.[3] In the HMBC spectrum, the anomeric proton signals at δ 6.17, 4.82, and 4.70 assigned to rhamnose, xylose, and glucose were correlated to the three-bond-coupled ^{13}C signals at δ 76.2 (C-2 of glucose), 88.4 (C-3 of glucose), and 85.0 (C-1 of aglycone), respectivly. The structure of the triglycoside, 2,3-branched glucose bearing rhamnose at C-2 and xylose at C-3, and its linkage to C-1 of the aglycone, were thus given. The data mentioned above allowed construction of the full structure of **1** as (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside}.

Compound **2** ($C_{51}H_{82}O_{25}$) was shown by the ^1H- and ^{13}C-NMR spectra to have the same polyhydroxylated sapogenol structure as **1**. The ^1H-NMR spectrum of **2** displayed four anomeric proton signals at δ 6.30 (d, J = 1.0 Hz), 5.70 (d, J = 8.3 Hz), 5.52 (d, J = 7.9 Hz), and 4.72 (d, J = 7.2 Hz). Acid hydrolysis of **2** with 1 M HCl in dioxane-H_2O (1 : 1) followed by HPLC analysis allowed identification of D-glucose and L-rhamnose in a ratio of 2 : 1, but the forth monosaccharide could not be identified through HPLC analysis. The triglycoside structure composed of two β-D-

glucoses and an α-L-rhamnose and its linkage position to the aglycone was established as *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside attached to C-1 of the aglycone by the observation of the ^{1}H-^{13}C long-range correlation from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide or aglycone, that is, from δ 6.30 (anomer of rhamnose) to δ 75.8 (C-2 of glucose), 5.52 (anomer of terminal glucose) to 82.4 (C-3 of rhamnose), and 4.72 (anomer of 2-substituted glucose) to 85.6 (C-1 of aglycone).

Detailed inspection of the ^{1}H-^{1}H COSY spectrum in conjunction with the HOHAHA data of **2** resulted in the identification of the spin-coupling system of the unidentified monosaccharide, suggesting that it was 6-deoxy-β-gulopyranose.[6] A certain amount of **2** was subjected to acid hydrolysis with 2.5 M HCl in dioxane-MeOH (1 : 1) followed by purification by column chromatography on silica gel, and preparative HPLC gave methyl 6-deoxy-β-D-gulopyranoside, identified by its specific rotation, and ^{1}H-NMR, and ^{13}C-NMR data.[7] This unusual monosaccharide was directly linked to the C-24 hydroxyl group of the aglycone with formation of a β-D-glycoside linkage, the evidence of which was obtained from a $^{3}J_{C,H}$ correlation peak between δ 5.70 (d, J = 8.3 Hz) and δ 82.3 (C-24) in the HMBC spectrum. Finally, the structure of **2** was formulated as (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-{*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside} 24-*O*-6-deoxy-β-D-gulopyranoside.

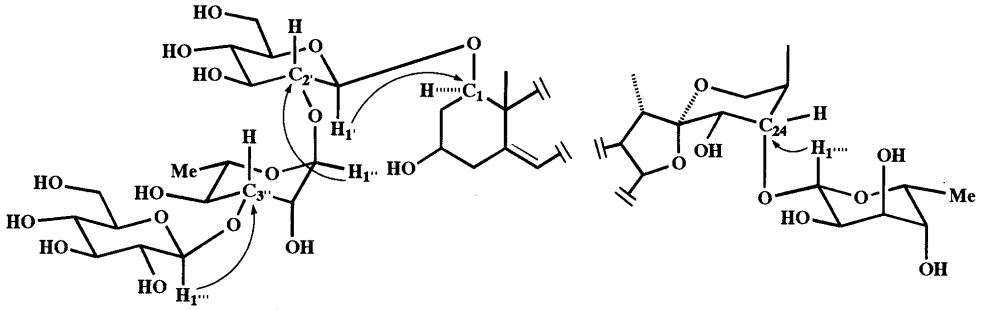

Fig. 4. HMBC Correlations of the Saccharide Parts of **2**

Compounds **1** and **2** are new polyhydroxylated spirostanol saponins, and **2** is especially unique in structure since it is the first representative of a steroidal saponin bearing 6-deoxy-D-gulopyranose among both the steroidal and triterpene saponins reported up to the present.

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2) 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.

3) Agrawal P. K., Jain D. C., Gupta R. K., Thakur R. S., *Phytochemistry*, **24**, 2479-2496 (1985).

4) 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).

5) A combination of molecular mechanics and molecular dynamics calculations in force field Discover-cff91 was performed on two possible compounds of 22α and 22β. The total energy of the most stable conformation calculated was almost identical between the 22α and 22β conformers. However, the spatial distance between 20-H and 23-H in the 22α conformer was 3.0 Å, and that in the 22β conformer was 3.7 Å. These calculated data and a clear NOE correlation between 20-H and 23-H led us to conclude that the 22β configuration should be ruled out.

6) ^{1}H-NMR signals due to the unidentified monosaccharide: δ 5.70 (1H, d, J = 8.3 Hz, 1''''-H), 4.50 (1H, dd, J = 8.3, 3.2 Hz, 2''''-H), 4.69 (1H, dd, J = 3.2, 3.2 Hz, 3''''-H), 4.03 (1H, br d, J = 3.2 Hz, 4''''-H), 4.53 (1H, br q, J = 6.6 Hz, 5''''-H), and 1.50 (3H, d, J = 6.6 Hz, 6''''-Me).

7) Methyl 6-deoxy-β-D-gulopyranoside: $[\alpha]_D$ -94.7° (MeOH). ^{1}H-NMR (methanol-d_4): δ 4.47 (1H, d, J = 8.2 Hz, 1-H), 3.55 (1H, dd, J = 8.2, 3.4 Hz, 2-H), 3.94 (1H, dd, J = 3.4, 3.4 Hz, 3-H), 3.46 (1H, dd, J = 3.4, 1.3 Hz, 4-H), 4.00 (1H, qd, J = 6.6, 1.3 Hz, 5-H), 1.22 (3H, d, J = 6.6 Hz, 6-Me), and 3.48 (3H, s, OMe). ^{13}C-NMR (methanol-d_4): δ 103.4 (C-1), 70.1 (C-2), 73.4 (C-3), 73.6 (C-4), 69.6 (C-5), 16.2 (C-6), and 56.9 (OMe). Mori M., Tejima S., Niwa T., *Chem. Pharm. Bull.*, **34**, 4037-4044 (1986).

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