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Brodioside B, a Novel (20*R*,22*S*)-Spirostanol Tetrasaccharide from *Brodiaea californica* Tubers

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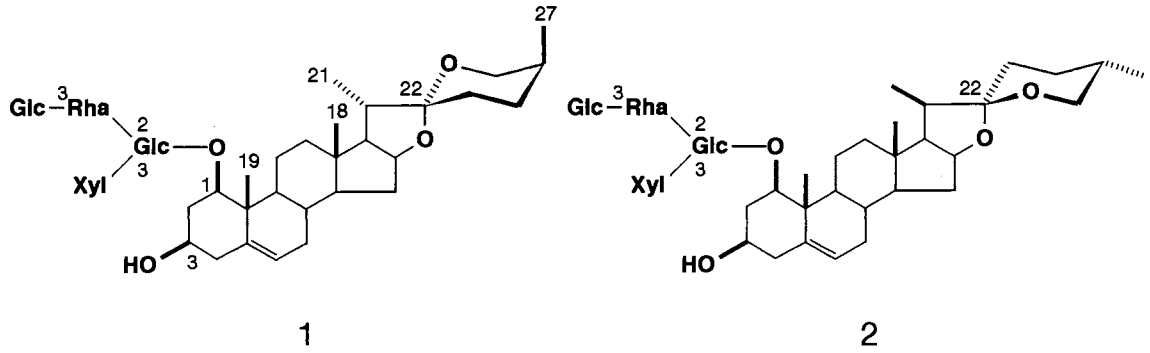
A novel (20*R*,22*S*)-spirostanol tetrasaccharide, named brodioside B, was isolated from *Brodiaea californica* together with a new (25*S*)-ruscogenin tetrasaccharide, named brodioside A. Their structures were determined by extensive 2D NMR analysis and hydrolysis. Brodiosides A and B were active as a cyclic AMP phosphodiesterase inhibitor.

The genus *Brodiaea* with fifteen species is classified to the subfamily Allioideae in Liliaceae along with the genera *Dichelostemma*, *Ipheion*, and *Triteleia*.[1] The Allioideae plants expected to produce steroidal saponins as one of the main secondary methabolites.[2] *Brodiaea californica* is indigenous to north California and no chemical work appears to have been done on the plant. Our attention to the steroidal constituents in *B. californica* tubers has resulted in finding a novel (20*R*,22*S*)-spirostanol tetrasaccharide together with a new (25*S*)-ruscogenin tetrasaccharide. This paper briefly refers to the structural elucidation of the new saponins based on extensive 2D NMR analysis and the products formed on acid-catalysed hydrolysis.

The MeOH extract of the fresh tubers of *B. californica* (3. 0 kg) was chromatographed on silica-gel with a gradient mixture of CH_2Cl_2 - MeOH to give three fractions (I - III). Fraction III contained steroidal saponins and aboundant saccharides, from which the saccharides were removed by passing through a Diaion HP-20 column eluting with H_2O gradually enriched with MeOH. The saponin fraction thus prepared was subjected to column chromatography on silica-gel with $CHCl_3$ - MeOH - H_2O (30 : 10 : 1) to give a mixture of compounds **1** and **2**, which were separated by preparative HPLC.[3]

Compound **1** was obtained as an amorphous powder.[4] The ^{1}H NMR spectrum of **1** (pyridine-d_5) showed two three-proton singlet signals at δ 1.45 and 0.90, indicating the presence of two angular methyl groups, as well as two three-proton doublet signals at δ 1.10 (J = 6.9 Hz) and 1.06 (J = 7.1 Hz) assignable to secondary methyl groups, and four anomeric proton signals at δ 6.50 (br s), 5.67 (d, J = 7.8 Hz), 4.88 (d, J = 7.6 Hz), and 4.72 (d, J = 7.8 Hz). Acid hydrolysis of **1** with 1M HCl (dioxane - H_2O, 1 : 1) afforded (25*S*)-spirost-5-ene-1β,3β-diol [(25*S*)-ruscogenin],[5] and D-glucose, D-xylose and L-rhamnose in relations of 2 : 1 : 1.[6] The ^{13}C NMR assignments of the aglycone moiety of **1** exhibited a close similarity to those of the reported (25*S*)-ruscogenin 1-*O*-glycosides. The above data led to prove **1** to be (25*S*)-ruscogenin 1-*O*-tetrasaccharide.[5]

Compound **2** [7] gave also (25*S*)-ruscogenin, and D-glucose, D-xylose and L-rhamnose in a ratio of 2 : 1 : 1 on acid hydrolysis, however, in the ^{13}C NMR spectrum of **2**, signals due to the E- and F-ring carbons did not coincide with those of **1**. The significant differences were also observed in the shift values of the 18-Me, 21-Me,



and 27-Me protons between the ^{1}H NMR spectra of **1** and **2**. These data suggested that the aglycone of **2** might be different in the stereostructure from that of **1** with respect to the E- and F-ring parts. The phase-sensitive NOESY spectrum provided certain information for the stereostructure assignment. The ^{1}H signals were assigned by the ^{1}H-^{1}H COSY spectrum combined with the HOHAHA spectrum prior to the inspection of the NOESY spectrum.[8] The 17-H showed clear NOE correlations with 14-H, 16-H, and 20-H, indicating the D/E *cis* ring junction, and C-20*R* configuration, which were supported by an intense NOE between 12β(eq)-H and 21-Me. The 20-H, in turn, showed an NOE with 23(eq)-H, giving evidence for C-22*S* (Fig. 1). The C-25*S* configuration was corroborated by the fact that **2** was transformed into (25*S*)-ruscogenin on acid treatment.

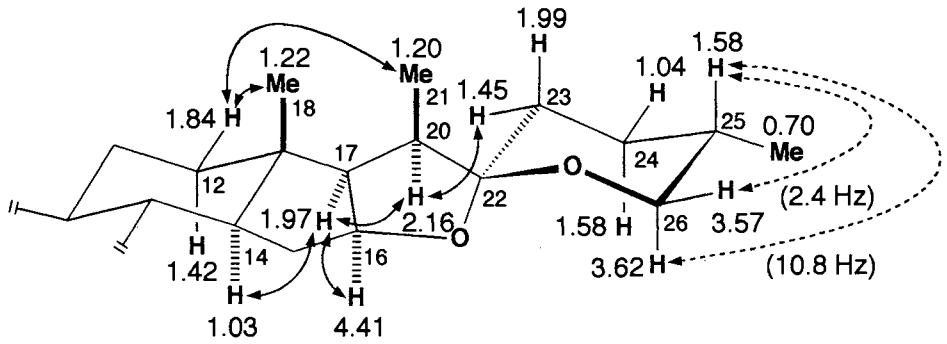


Fig. 1. ^{1}H NMR chemical shifts (ppm) and NOE correlations of **2** in pyridine-d_5 - methanol-d_4 (10 : 1).[8]

The chair-form conformation of the F-ring was confirmed by the ^{1}H NMR parameters of the 26-H$_2$ protons ($^3J_{26ax\text{-}H\,(\delta\,3.62)\,-\,25\text{-}H}$ = 10.8 Hz and $^3J_{26eq\text{-}H\,(\delta\,3.57)\,-\,25\text{-}H}$ = 2.4 Hz) (Fig. 1). Thus, the structure of the aglycone moiety of **2** was concluded to be (20*R*,22*S*,25*S*)-spirost-5-ene-1β,3β-diol.

The sequence of the tetrasaccharide moiety of **2** was determined by the following NMR analysis. All ^{1}H signals due to the monosaccharides could be assigned by a combined use of ^{1}H-^{1}H COSY and HOHAHA spectra (Table 1). Assignments of the ^{13}C signals of each saccharide were achieved by tracing out the one-bond ^{1}H-^{13}C connectivities through the use of the HMQC spectrum. Comparison of the ^{13}C shifts thus assigned with those of reference methyl glycosides,[9] together with the known *O*-glycosylation shift data, indicated that **2** contained a terminal β-D-glucopyranosyl unit, a terminal β-D-xylopyranosyl unit, a 3-substituted α-L-

rhamnopyranosyl unit, and a 2,3-disubstituted β-D-glucopyranosyl unit. The ^{1}H-^{13}C long-range correlation from each anomeric proton traversing the glycosidic linkage to carbon of another substituted monosaccharide or aglycone confirmed the new oligoside structure as shown in Fig. 2. Compound **1** was shown to have the same oligoside structure as **2** by the comparison of the ^{13}C NMR spectra between **1** and **2**.

Accordingly, the structure of **2** was estabilised as (20*R*,22*S*,25*S*)-spirost-5-ene-1β,3β-diol 1-*O*-{*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranoside}, and that of **1** as (25*S*)-ruscogenin 1-*O*-glycoside with the same saccharide sequence as **2**.

Wall *et al.* had reported the synthesis of the (20*R*,22*S*)-sapogenols,[10] however, to the best of our knowledge, brodioside B is the first (20*R*,22*S*)-spirostanol glycoside from a natural sourse.

Table 1. ^{1}H and ^{13}C NMR chemical shifts for oligoside unit of **2** in pyridine-d_5 - methanol-d_4 (10 : 1)

	^{1}H NMR		^{13}C NMR
1'	4.71 d	(8.0)	100.4
2'	4.11 dd	(8.8, 8.0)	75.5
3'	3.95 dd	(8.8, 8.8)	88.8
4'	3.80 dd	(8.8, 8.8)	70.1
5'	3.72 ddd	(8.8, 5.9, 2.1)	77.7
6'	4.43 dd	(11.5, 2.1)	63.2
	4.16 dd	(11.5, 5.9)	
1''	6.36 d	(1.6)	101.3
2''	4.90 dd	(3.1, 1.6)	71.8
3''	4.68 dd	(9.5, 3.1)	82.4
4''	4.41 dd	(9.5, 9.5)	73.1
5''	4.83 dq	(9.5, 6.1)	69.3
6''	1.66 d	(6.1)	18.9
1'''	5.52 d	(7.8)	106.2
2'''	4.02 dd	(9.1, 7.8)	76.0
3'''	4.15 dd	(9.1, 9.1)	78.1
4'''	4.07 dd	(9.1, 9.1)	71.6
5'''	4.04 ddd	(9.1, 5.1, 2.2)	78.2
6'''	4.41 dd	(11.5, 2.2)	62.5
	4.17 dd	(11.5, 5.1)	
1''''	4.85 d	(7.7)	105.4
2''''	3.88 dd	(8.3, 7.7)	74.6
3''''	4.02 dd	(8.3, 8.3)	78.2
4''''	4.04 ddd	(11.0, 8.3, 5.0)	70.5
5''''	4.22 dd	(11.0, 5.0)	67.2
	3.67 dd	(11.0, 11.0)	

J values in parentheses were expressed in Hz

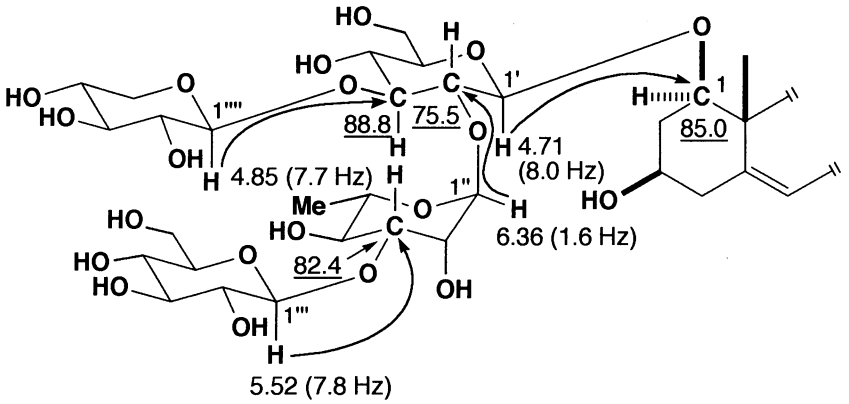


Fig. 2. ^{1}H-^{13}C long-range correlations of the saccharide moieties of **2** in pyridine-d_5 - methanol-d_4 (10 : 1). *J* values in the ^{1}H NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C NMR chemical shifts.

Steroidal saponins bearing oligoside composed of more than four monosaccharides at the C-1 hydroxyl group as **1** and **2** are very rare in nature.[11] Compounds **1** and **2** exhibited inhibitory activity on cyclic AMP

phosphodiesterase (**1**: IC_{50} 8.9 x 10^{-5} M; **2**: 10.0 x 10^{-5} M).[12]

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2) S. B. Mahato, A. N. Ganguly, and N. P. Sahu, *Phytochemistry*, **21**, 959 (1982).
3) Conditions for preparative HPLC to separate the stereoisomers: Tosoh HPLC system (pump: CCPM; detector: RI-8010); column: Kaseisorb LC ODS-120-5 (10 mm i.d. x 250 mm, ODS, 5μm); mobile phase: MeOH - H_2O (85 : 15), 1.0 ml min^{-1}; column temperature: 5°.
4) Compound **1**: 118 mg, $[\alpha]_D$ -62.0° (MeOH); negative-ion FABMS *m/z* 1031 [M - H]$^-$; ^{1}H NMR (pyridine-d_5) δ = 6.50 (1H, br s, 1"-H), 5.67 (1H, d, *J* = 7.8 Hz, 1'''-H), 5.56 (1H, br d, *J* = 5.7 Hz, 6-H), 4.88 (1H, d, *J* = 7.6 Hz, 1''''-H), 4.72 (1H, d, *J* = 7.8 Hz, 1'-H), 1.70 (3H, d, *J* = 6.1 Hz, 6"-Me), 1.45 (3H, s, 19-Me), 1.10 (3H, d, *J* = 6.9 Hz, 21-Me), 1.06 (3H, d, *J* = 7.1 Hz, 27-Me), and 0.90 (3H, s, 18-Me); ^{13}C NMR (pyridine-d_5) δ = 85.2, 38.3, 68.1, 43.7, 139.5, 124.9, 31.9, 33.1, 50.5, 42.8, 24.1, 40.4, 40.3, 57.0, 32.3, 81.2, 62.9, 16.9, 15.2, 42.5, 14.8, 109.7, 26.4, 26.2, 27.6, 65.0, and 16.3 (C-1 - C-27), 100.5, 75.0, 88.8, 70.2, 77.8, and 63.2 (C-1' - C-6'), 101.2, 71.9, 82.6, 73.3, 69.3, and 18.9 (C-1" - C-6"), 106.4, 76.2, 78.3, 71.7, 78.4, and 62.6 (C-1''' - C-6'''), and 105.4, 74.6, 78.3, 70.6, and 67.2 (C-1'''' - C-5'''').
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6) The identifications of the monosaccharides including their absolute configurations were achieved by converting them to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives followed by HPLC analysis; R. Oshima, Y. Yamauchi, and J. Kumanotani, *Carbohydr. Res.*, **107**, 169 (1982).
7) Compound **2**: 39.3 mg, $[\alpha]_D$ -36.0° (MeOH); negative-ion FABMS *m/z* 1031 [M - H]$^-$; ^{1}H NMR (pyridine-d_5) δ = 6.51 (1H, br s, 1"-H), 5.68 (1H, d, *J* = 7.6 Hz, 1'''-H), 5.57 (1H, br d, *J* = 6.0 Hz, 6-H), 4.89 (1H, d, *J* = 7.7 Hz, 1''''-H), 4.73 (1H, d, *J* = 7.8 Hz, 1'-H), 1.69 (3H, d, *J* = 6.1 Hz, 6"-Me), 1.46 (3H, s, 19-Me), 1.24 (3H, s, 18-Me), 1.21 (3H, d, *J* = 7.4 Hz, 21-Me), and 0.68 (3H, d, *J* = 5.1 Hz, 27-Me); ^{13}C NMR (pyridine-d_5) δ = 85.3, 38.4, 68.2, 43.8, 139.4, 124.9, 31.9, 32.4, 50.6, 42.8, 23.8, 41.3, 41.6, 57.6, 35.4, 83.3, 60.5, 16.5, 15.2, 46.3, 10.2, 106.9, 34.6, 29.6, 30.3, 67.8, and 17.3 (C-1 - C-27). Signals for the saccharide part agreed with those of **1** within ± 0.2 ppm.
8) The 2D NMR spectra were measured in pyridine-d_5 - methanol-d_4 (10 : 1) to minimize signal overlap.
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