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TRITERPENOID SAPONINS FROM *BUPLEURUM SMITHII* VAR. *PARVIFOLIUM*

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Key Word Index—*Bupleurum smithii* var. *parvifolium*; Umbelliferae; triterpene saponins; saikosaponin o.

Abstract—Four triterpenoidal saponins, prosaikogenin A and saikosaponins b_1, n and o, were isolated from the roots of the title plant for the first time. Saikosaponin O is a new compound, which was identified as 3β,16β,23,28-tetrahydroxyolean-11,13(18)-diene-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside.

INTRODUCTION

Many plants belonging to the genus *Bupleurum* have been used as traditional Chinese herbal drugs. *B. chinense* and *B. scorzonerifolium* have been recorded in the Chinese pharmacopoeia. Some saikosaponins from *Bupleurum* L. are considered as the major bioactive components of the drugs, mainly used for their anti-inflammatory, antihepatotoxic and immune activities [1]. *B. smithii* var. *parvifolium* Shan et Y. Li is abundantly distributed in the northwest region of China, but no evidence is available in the literature concerning its constituents. This paper deals with the isolation and identification of four triterpenoid saponins (**1**–**4**), prosaikogenin A and saikosaponins b_1, n and o, from the roots of the plant. Saikosaponin o is a new compound. Its structure was mainly elucidated by spectral analysis. Saikosaponin o was identified as 3β,16β,23,28-tetrahydroxyolean-11,13(18)-diene-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside.

RESULTS AND DISCUSSION

A crude saponin was afforded from the plant by methods described in the Experimental. The crude saponin was separated by repeated chromatography to give saponins **1**–**4**.

In the ^{13}C NMR spectra of compounds **1**, **2** and **3**, signals were found to be identical with those of the known compounds, prosaikogenin A [2], saikosaponin b_1 [2] and saikosaponin N [3], respectively. They were identified with authentic samples by co-TLC on silica gel [chloroform–methanol–H_2O (7:1:0.1) and (8:2:0.2)].

The new saponin **4**, a white powder, mp 223–225°, gave positive Liebermann–Burchard and Molich reactions. The ^1H NMR spectrum showed that the substance had six angular methyl groups (δ 0.79, 0.84, 0.91, 0.97, 1.03 and 1.07) as do the known oleanane saikosaponins. It was suggested to have a heteroannular diene at C-11, C-13(18) on the basis of the observation of the strong UV absorption at 242, 250 and 260 nm. This was also supported by an IR absorption at 1642 cm^{-1}, ^1H NMR signals at δ 6.41 (1H, *dd*, J = 10.5 Hz) and 5.68 (1H, *d*, J = 10.5 Hz) and four ^{13}C NMR signals (δ 136.5, 132.9, 127.2 and 125.5).

On TLC acid hydrolysis [4], **4** furnished an aglycone which was identical with an authentic sample, saikogenin A (**5**) [olean-11,13(18)-diene-3β,16β,23,28-tetrol]. The resulting sugar was identified as glucose.

A comparison of the ^{13}C NMR data for **4** with those for saikogenin A [2] showed that the signals for C-3 and C-23 of **4** undergo a downfield shift (9.1 ppm) and an upfield shift (2.8 ppm), respectively, on going from sakogenin A to **4**. These can be considered as glycosidation shifts and, therefore, the sugar moiety was determined to be linked to saikogenin A via the C-3 hydroxyl group. This conclusion was further supported by analysis of the ^{13}C NMR data for **4**. The signals for the genin of **4** were coincident with those of saponins **1**–**3**.

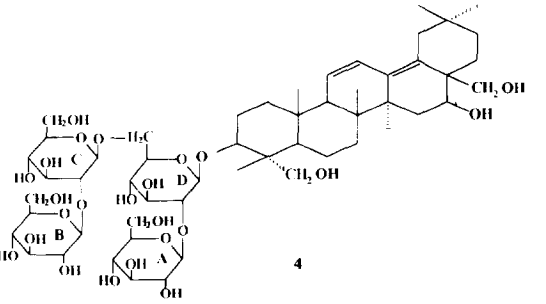
Four anomeric carbon signals and four sets of anomeric proton signals were observed at δ 106.7, 105.9, 103.7 and 103.2 (J_{CH} = 155.1, 161.2, 156.2 and 163.1 Hz) and 5.41 (1H, *d*, J = 7.7 Hz), 5.25 (1H, *d*, J = 7.7 Hz), 5.18 (1H, *d*, J = 7.7 Hz) and 5.13 (1H, *d*, J = 6.8 Hz). The FAB-mass spectrum showed the molecular ion at m/z [1159 $(M+K)]^+$, [1143 $(M+Na)]^+$, [1121 $(M+1)]^+$ and fragment ions at m/z 819 $[M-162-162+Na]^+$, 493 $[M-162\times4-H_2O+$

K]^{1}. These results indicated that **4** was a tetraglucoside of saikogenin A and a β-anomeric configuration for each of the glusose moieties was determined.

One- and two-dimensional NMR techniques (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HETCOR and TOCSY) permitted assignments of all ^{1}H and ^{13}C signals of the sugars. HMBC experiments showed correlation of the H-1 of glucose A, B, C and D with C-2, C-2 and C-6 of glucose D, C, D, and C-3 of the genin, respectively (Table 1). These results provided unambiguous information about the positions of the glycosidic linkage and permitted us to conclude that glucoses linked together at C-2, C-6, and the sugar chain bound to C-3 of the sapogenin. The ^{13}C NMR data of the sugar moiety indicated the D-configuration of the glucoses.

Consequently, the structure of saponin **4** was determined to be 3β,16β,23,28-tetrahydroxyolean-11,13(18)-diene-3-*O*-β-D-glucopyranosyl-(1$\rightarrow$2)]-β-D-glucopyranosyl-(1$\rightarrow$6)-[β-D-glucopyranosyl-(1$\rightarrow$2)-β-D-glucopyranoside, and it was named as saikosaponin o.

Since Kubota and Hinoh [5] suggested that saikosaponin b is an artefact derived from saikosaponin d during the isolation process, compound **4** may be an artefact originating from its corresponding saikosaponin.



EXPERIMENTAL

Mps (uncorr.) were measured with an X_4 micro melting point apparatus. IR spectra were determined in a KBr pellet on a Perkin-Elmer 983G IR spectrometer. UV spectra were recorded on a Shimadzu UV 260 spectrometer. ^{1}H and ^{13}C NMR spectra of compounds **1–3** were recorded on a VXR at 300 MHz, and all the

Table 1. NMR chemical shifts of **4** (in pyridine-d_5)

Aglycone		Sugar	^{13}C (by ^{13}C NMR, HETCOR and DEPT)	^{1}H (by COSY and TOCSY)	HMBC
1	38.2	Glu-D			
2	26.1	1	103.7	5.130 (*d*, *J* = 6.8 Hz)	82.2 (genin (C-3)
3	82.2	2	83.7	4.210	
4	43.7	3	78.1	4.195	
5	47.7	4	71.2	4.175	
6	18.2	5	76.3	4.050	
7	32.3	6	70.0	4.625, 4.325	
8	40.4	Glu-C			
9	54.4	1	103.2	5.175 (*d*, *J* = 7.7 Hz)	70.0 (Glu D-6)
10	36.4	2	84.7	4.085	
11	127.2	3	78.0	4.275	
12	125.5	4	71.1	4.175	
13	136.5	5	78.4	3.860	
14	44.2	6	62.4	4.465, 4.305	
15	34.8	Glu-B			
16	76.6	1	106.7	5.250 (*d*, *J* = 7.7 Hz)	84.7 (Glu C-2)
17	44.3	2	76.5	4.080	
18	132.9	3	78.1	4.160	
19	38.4	4	70.9	4.215	
20	32.6	5	78.7	3.850	
21	35.1	6	62.1	4.495, 4.345	
22	29.9	Glu-A			
23	64.6	1	105.9	5.410 (*d*, *J* = 7.7 Hz)	83.7 (Glu D-2)
24	12.9	2	76.8	4.095	
25	18.8	3	78.1	4.205	
26	17.0	4	71.4	4.275	
27	21.9	5	78.2	3.910	
28	63.9	6	62.6	4.485, 4.410	
29	24.8				
30	32.3				

NMR spectra of **4** were recorded with a Bruker AM-500 instrument in pyridine-d_5. FAB-MS were recorded on a VAB-HS(VG) instrument. For CC silica gel (Marine Chemical Plant, Qing Dao) and Sephadex LH-20, RP-18 (Chemical Reagent Factory, Tian Jin) were used. TLC was performed on RP-18 precoated layer (Merck).

Plant material. Plant material of *B. smithii* var. *parvifolium* Shan et Y. Li were collected in Datong County of Qinghai Province, China, and identified by Director and Pharmacist Shen Yuan, Beijing Institute of Drug Control.

Extraction and separation. The powdered roots of the plant (7.3 kg) were extracted with 50% EtOH containing pyridine [6] at room temp. The extract was concd under red. pres. and diluted with H_2O. The aq. soln was defatted with petrol and subjected to CC on macroporous polymer resin D101, eluting with H_2O and 80% MeOH. The 80% MeOH eluate was concd to dryness, affording a crude saponin (75 g). The crude saponin was fractionated by silica gel CC using $CHCl_3$–MeOH (1:0→1:1) as eluent to give Frs 1–7.

Fr. 4 was subjected to repeated CC on silica gel, eluting with $CHCl_3$–[MeOH–Me_2CO (1:1)] (1:0→0:1) and $CHCl_3$–MeOH–H_2O (7:1:0.1), respectively, to afford the saponin fr. The saponin fr. was purified on a Sephadex LH-20 column, using MeOH as mobile phase to give a fr. that was further sepd on a RP-18 column with MeOH–H_2O (4:1) to yield pure **1** (60 mg).

Fr. 5 was further sepd by silica gel CC [$CHCl_3$–MeOH (1:0→0:1)] to yield 3 frs. The first was chromatographed by prep. silica gel TLC [$CHCl_3$–MeOH–H_2O (100:2:1.2)] and Sephadex LH-20 (MeOH) to give **2** (100 mg). The third was treated in the similar way to afford **3** (30 mg).

Fr. 7 was first subjected to CC on silica gel [$CHCl_3$–MeOH (5:1→0:1)], then on Sephadex LH-20 (MeOH) and RP-18 [MeOH–H_2O (4:1)] to yield **4** as a pure produce (45 mg).

Saponin **1**. Powder, mp 212–214°. IR ν_{max}^{KBr} cm^{-1}: 3410, 2937, 1626, 1363, 1070, 995. UV λ_{max}^{MeOH} nm: 242.0, 250.4, 259.8. Acidic hydrolysis of **1** by silica gel TLC gave **5** and fucose, which were identical with authentic samples. ^{13}C NMR (aglycone, No. 1–30, Fuc 1–6): 38.4, 26.1, 81.6, 43.7, 47.4, 19.0, 32.4, 40.6, 54.6, 36.6, 127.0, 125.6, 136.3, 44.3, 35.0, 76.6, 44.5, 133.3, 38.5, 32.8, 35.2, 30.1, 64.3, 13.2, 18.3, 17.6, 22.1, 64.0, 24.9, 32.5, 106.4, 73.0, 75.6, 72.9, 71.3, 17.2.

Saponin **2**. Powder, mp 235–238°. IR ν_{max}^{KBr} cm^{-1}: 3414, 2940, 1644, 1384, 1072. UV λ_{max}^{MeOH} nm: 241.8, 250.2, 259.3. ^1H NMR: 0.85 (6H, *s*), 0.91 (3H, *s*), 0.96 (6H, *s*), 1.07 (3H, *s*), 1.45 (3H, *d*, $J = 6.3$ Hz, Fuc-CH_3), 5.00 (1H, *d*, $J = 6.9$ Hz), 5.38 (1H, *d*, $J = 7.8$ Hz), 5.70 (1H, *d*, $J = 10.8$ Hz, 11-H), 6.50 (1H, *dd*, $J = 10.8$ Hz, 12-H). Acidic hydrolysis of **2** by silica gel TLC gave **5**, glucose and fucose, which were identical with authentic samples. ^{13}C NMR data, (aglycone No. 1-30, Fuc. 1-6, Glc 1-6): 38.4, 26.1, 81.7, 43.7, 47.4, 18.8, 32.3, 40.5, 54.5, 36.5, 127.1, 125.7, 136.4, 44.4, 34.9, 76.5, 44.3, 133.4, 38.4, 32.7, 35.2, 30.0, 64.1, 13.1, 18.2, 17.2, 22.0, 64.0, 24.8, 32.4, 106.0, 71.6, 85.3, 71.8, 71.0, 17.1, 106.7, 75.8, 78.4, 72.2, 78.7, 62.8.

Saponin **3**. Powder, mp 211–214°. IR ν_{max}^{KBr} cm^{-1}: 3406, 2937, 1626, 1383, 1042. UV λ_{max}^{MeOH} nm: 241.8, 250.4, 259.8. Acidic hydrolysis of **3** by silica gel TLC afforded **5**, glucose and fucose, which were identified with authentic samples by co-TLC. ^{13}C NMR data (aglycone No. 1-30, Glc 1-6, Rha. 1-6, Glc. 1-6): 38.4, 25.9, 82.0, 43.6, 47.3, 18.8, 32.3, 40.5, 54.5, 36.5, 127.1, 125.6, 136.4, 44.3, 34.9, 76.6, 44.4, 133.3, 38.2, 32.7, 35.2, 30.0, 64.3, 13.1, 18.5, 17.0, 22.0, 64.0, 24.8, 32.4, 105.8, 75.2, 76.6, 79.9, 75.5, 68.9, 102.9, 72.5, 72.7, 73.6, 70.6, 18.2, 105.1, 74.8, 78.4, 71.5, 78.5, 62.6.

Saponin **4**. Powder, mp 220–225°. IR ν_{max}^{KBr} cm^{-1}: 3390, 2923, 1642, 1449, 1363, 1160, 1073, 896. UV λ_{max}^{MeOH} nm: 242.0, 250.4, 259.8. FAB-MS, *m/z* (rel. int.) 1159 $[M + K]^+$ (57), 1143 $[M + Na]^+$ (100), 1121 $[M + 1]^+$ (36), 981 $[M - 162 + Na]^+$ (21), 819 $[M - 162 \times 2 + Na]^+$ (42), 493 $[M - 162 \times 4 - H_2O + K]^+$ (21), 253 [from the genin]$^+$ (26), ^1H NMR: 0.79, 0.84, 0.91, 0.97, 1.03, 1.07 (3H each, *s*, Me × 6), 5.68 (1H, *d*, $J = 10.5$ Hz, 11-H), 6.41 (1H, *dd*, $J = 10.5$ Hz, 12-H), anomeric proton signals of sugars, ^{13}C NMR and 2D NMR data: see Table 1.

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