PERGAMON

Phytochemistry 50 (1999) 139–142

PHYTOCHEMISTRY

Saikosaponins from roots of *Bupleurum scorzonerifolium*

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Received 7 August 1997

Abstract

Saikosaponin u and saikosaponin v, were isolated from the roots of *Bupleurum scorzonerifolium* and these saponins were identified as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranosyl]-3β,16α,23,28-tetrahydroxy-olean-11,13(18)-dien-30-oic acid-30-*O*-[pentito(1 → 1)-β-D-glucopyranosyl-(6→)] ester and 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranosyl]-3β,16α,23,28-tetrahydroxy-olean-11,13(18)-dien-30-oic acid-30-*O*-[pentito(1 → 1)-β-D-glucopyranosyl(6→)] ester, respectively.

Keywords: *Bupleurum scorzonerifolium*; Umbelliferae; Triterpenoid saponin; Saikosaponin u; Saikosaponin v

1. Introduction

Bupleurum scorzonerifolium Willd. is recorded in the Chinese Pharmacopoeia (1995 edition), and is used as an antiinflammatory and antihepatotoxic medicine; saikosaponins in its roots have previously been shown to possess antiinflammatory activity (Ocete, Risco, Zarzuelo, & Jimenez, 1989). This paper deals with the isolation and structure elucidation of two new saikosaponins.

2. Results and discussion

Saikosaponin u was obtained as white powder. A sodiated molecular ion was observed at *m/z* 1291 $[M + Na]^+$ in the positive FAB-MS. The ^1H NMR spectrum exhibited five diagnostic angular methyl signals (δ 0.82, 0.98, 1.02, 1.45 and 1.64), which indicated saikosaponin u was a triterpenoid saponin. Its UV spectrum also showed absorbances at 242, 251 and 261 nm, suggesting a heteroannular diene system at C-11, 12, 13 and 18 of the aglycone (Kobayashi & Ogihara, 1981). This was further confirmed by its proton NMR signals 6.65 (1H, d, $J_{11,12}$ = 10.2 Hz, H-11), 5.72 (1H, d, $J_{12,11}$ = 10.2 Hz, H-12) and the ^{13}C resonances at δ 137.6, 130.6, 127.1 and 126.0 corresponding to C-13, 18, 12 and 11, respectively. The DEPT spectrum of saikosaponin u also showed that the aglycone moiety possessed four hydroxyl groups at δ 82.1, 67.8 (CHOH) and at δ 64.2, 64.9 (CH_2OH) and a carbonyl group, at δ 178.6. The ^{13}C NMR signals of the aglycone moiety were in good agreement with those of saikosaponin b_2 (see Table 1 and Fig. 1) (Ishii, 1980), except for the signals at C-18, 19, 20, 21, 29 and 30. A comparison of the ^{13}C NMR data for their aglycone moieties showed that the signals for C-20 and C-18, 19, 21, 29 of saikosaponin u underwent a downfield shift (+11.5) and upfield shifts (−2.4, −5.8, −5.0 and −4.3), respectively, on going from saikosaponin b_2 to saikosaponin u and the methyl signal at δ 32.5 in saikosaponin b_2 was absent in saikosaponin u. This suggested that the carbonyl group was attached to C-20. This deduction was confirmed by HMBC experiments, which exhibited correlation of the carbonyl signal with the methyl protons (δ 1.45, 29-CH_3), indicating that the carbonyl group should be at C-30 (Wang, 1991). Therefore, the aglycone was ultimately determined as 3β,16α,23,28-tetrahydroxy-olean-11,13 (18)-dien-30-oic acid. This type of aglycone is novel in species of *Bupleurum*.

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0031-9422/98/$ - see front matter
PII: S0031-9422(98)00451-8

Table 1. ^{13}C NMR and DEPT spectral assignments of aglycone moieties (500 MHz, C_5D_5N)

C	Saikosaponin u	Saikosaponin v	Saikosaponin b_2
1	38.4 CH_2	38.5	38.7
2	26.2 CH_2	26.1	25.9
3	82.1 CH	81.8	82.6
4	43.7 C	43.7	43.7
5	47.5 CH	47.5	48.2
6	18.2 CH_2	18.3	18.8
7	32.3 CH_2	32.4	32.5
8	41.1 C	41.2	41.4
9	54.0 CH	54.1	54.2
10	36.5 C	36.6	36.9
11	126.0 CH	126.0	126.3
12	127.1 CH	127.0	126.3
13	137.6 C	137.7	137.1
14	42.0 C	42.1	42.2
15	31.8 CH_2	31.9	32.8
16	67.8 CH	67.9	68.8
17	45.3 C	45.4	45.4
18	130.6 C (−2.4)	130.7	133.0
19	33.4 CH_2 (−5.8)	33.5	39.2
20	44.3 C (+11.5)	44.4	32.8
21	30.8 CH_2 (−5.0)	30.5	35.8
22	23.9 CH_2 (−1.0)	24.0	24.9
23	64.2 CH_2	64.3	65.3
24	13.1 CH_3	13.1	12.9
25	18.8 CH_3	18.9	18.6
26	17.2 CH_3	17.3	17.5
27	21.8 CH_3	21.9	22.3
28	64.9 CH_2	64.9	65.6
29	21.0 CH_3 (-4.3)	21.1	25.3
30	178.6 C	178.6	32.5

Acidic hydrolysis of saikosaponin u on TLC furnished fucose and glucose which were identical with authentic samples (Zhao, Li, & He, 1987). The signals at δ 4.97 (1H, d, $J = 8.35$ Hz), 4.99 (1H, d, $J = 7.95$ Hz), 5.09 (1H, d, $J = 7.49$ Hz), 5.12 (1H, d, $J = 7.78$ Hz), 1.45 (3H, d, $J = 6.31$ Hz) in ^1H NMR and δ 107.1, 105.3, 105.3, 104.7, 17.2 in ^{13}C NMR spectrum (see Table 2) showed that saikosaponin u was a tetraglycoside consisting of a fucose and three glucose residues with presumed β-anomeric configurations. TOCSY data also supported the above speculation

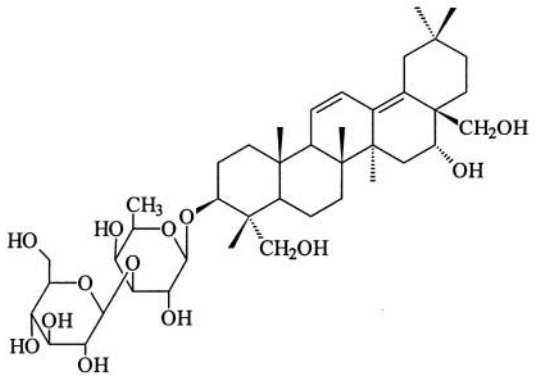

Fig. 1.

and showed that there was a fifth spin system with the lowest signal at δ 4.85. ^{13}C NMR and CH-COSY data suggested that the fifth spin system was a pentitol (substructure e).

One and two-dimensional NMR techniques (^1H NMR, ^{13}C NMR, DEPT, COSY, CH COSY, TOCSY) permitted assignments of the ^1H and ^{13}C signals of saikosaponin u (Tables 1 and 2). HMBC experiments showed correlations of the anomeric protons and 6-H of the glucose moiety nominated as d (glc-d) (see Fig. 2) with some ^{13}C signals (Table 3). The results provided unambiguous information about the positions of the glycosidic and ester linkage shown in Fig. 2.

Thus saikosaponin u was elucidated as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranosyl]-3β,16α,23,28-tetrahydroxy-olean-11,13(18)-dien-30-oic acid-30-*O*-[pentito(1 → 1)-β-D-glucopyranosyl-(6→)] ester.

Saikosaponin v was obtained as white powder. A sodiated molecular ion was observed at m/z 1129 $[M + Na]^+$ in the positive FAB-MS. Five angular methyl proton signals (δ 0.83, 0.91, 0.98, 1.44, 1.64) in the ^1H NMR spectrum and UV signals λ nm: 241, 250, 261, suggested that it was a triterpenoidal saponin with a heteroannular diene system at C-11 12, 13 and

Table 2. ^{13}C NMR, CH-COSY and TOCSY assignments of sugar moieties of steroidal saponins (500 MHz, C_5D_5N)

C	Saikosaponin u		Saikosaponin v
a1	105.3	4.99	106.0
a2	71.1	4.52	71.7
a3	86.8	3.85	85.4
a4	71.7	4.23	71.9
a5	70.9	3.69	71.1
a6	17.2	1.45	17.2
b1	104.7	5.12	105.4
b2	86.1	3.94	75.2
b3	77.7	4.32	78.8
b4	70.6	4.21	72.2
b5	79.1	3.79	78.8
b6	62.3	4.45	62.8
c1	107.1	5.09	
c2	75.1	4.08	
c3	78.4	3.93	
c4	71.8	4.04	
c5	78.4	4.19	
c6	62.1	4.40	
d1	105.3	4.97	106.7
d2	75.5	4.06	75.5
d3	76.5	4.04	76.6
d4	71.1	4.14	71.1
d5	77.8	4.30	78.5
d6	64.9	5.03, 4.78	65.2
e1	72.8	4.85, 4.44	72.8
e2	72.9	4.75	72.9
e3	74.1	4.53	74.1
e4	74.2	4.53	74.2
e5	64.9	4.47	65.2

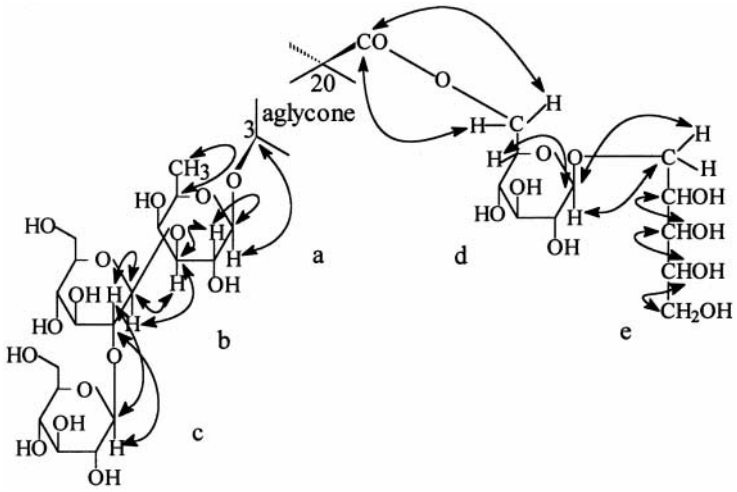

Fig. 2.

18. Its ^{1}H NMR and ^{13}C NMR data of the aglycone moiety was found to be coincident with those of saikosaponin u (see Table 1). This was further confirmed by an acidic hydrolysis of saikosaponin u and saikosaponin v on TLC (Zhao et al., 1987). Acidic hydrolysis of saikosaponin v gave fucose and glucose. The signals, δ 4.97 (1H, d, $J = 7.62$ Hz), 4.99 (1H, d, $J = 7.69$ Hz), 5.33 (1H, d, $J = 7.82$ Hz), 1.44 (3H, d, $J = 4.89$ Hz) in the ^{1}H NMR and those at δ 106.8, 106.0, 105.4, 17.2 in the ^{13}C NMR spectrum indicated that saikosaponin v was a triglycoside consisting of a fucose and two glucose residues with β-anomeric configurations. As shown by comparing the ^{13}C NMR data of the sugar moieties of saikosaponin v with those of saikosaponin u, there was no glycosidation at position 2 of the glucose moiety b (see Fig. 3) and the other data were identical (see Tables 1 and 2). Therefore, saikosaponin v was determined as 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranosyl]-3β,16α,23,28-tetrahydroxy-olean-11,13(18)-dien-30-oic acid-30-*O*-[pentito(1 → 1)-β-D-glucopyranosyl-(6→)] ester.

Table 3. HMBC analysis of saikosaponin u

Proton signals	Carbon signals
*4.99 (fuc a 1-H)	*82.1 (C-3)
4.53 (fuc a 2-H)	105.3 (fuc a 1-C)
4.53 (fuc a 2-H)	86.6 (fuc a 3-C)
4.23 (fuc a 4-H)	86.6 (fuc a 3-C)
3.69 (fuc a 5-H)	105.3 (fuc a 1-C)
3.69 (fuc a 5-H)	71.5 (fuc a 4-C)
3.69 (fuc a 5-H)	17.2 (fuc a 6-C)
1.45 (fuc a 6-H)	70.6 (fuc a 5-C)
3.85 (fuc a 3-H)	104.7 (glc b 1-C)
*5.12 (glc b 1-H)	*86.6 (fuc a 3-C)
3.94 (glc b 2-H)	104.7 (glc b 1-C)
4.33 (glc b 3-H)	86.1 (glc b 2-C)
3.94 (glc b 2-H)	107.1 (glc c 1-C)
*5.09 (glc c 1-H)	*86.1 (glc b 2-C)
4.08 (glc c 2-H)	107.1 (glc c 1-C)
5.03 (glc d 6-H)	178.6 (C-30)
*4.78 (glc d 6-H)	*178.6 (C-30)
*4.44 (pentitol e 1-H)	*135.3 (glc d 1-C)
*4.97 (glc d 1-H)	*72.8 (pentitol e 1-C)
4.53 (pentitol e 3-H)	72.9 (pentitol e 2-C)
4.53 (pentitol e 4-H)	74.1 (pentitol e 3-C)
4.53 (pentitol e 4-H)	64.9 (pentitol e 5-C)

Although the structures were identified, the configurations of the three chiral centers of the pentitol in saikosaponin u or saikosaponin v were not established.

3. Experimental

3.1. General

UV spectra were taken in MeOH soln on a Shimadzu UV-260 spectrometer. The ^{1}H NMR, ^{13}C NMR, DEPT, HH-COSY, CH-COSY and HMBC spectra were recorded in pyridine-d_5 at 500 MHz for ^{1}H and 125 MHz for ^{13}C with a Bruker AM-500 spectrometer. FAB-MS were recorded on a ZABSTEC-MS instrument. D101 macroporous resins used for isolation were obtained from Tianjin Gujiao Factory, Tianjin.

3.2. Plant materials

The roots of *Bupleurum scorzonerifolium* Willd. were collected in Yunsho, Shanxi Province of China and were identified by Dr Shen Yuan, Beijing Institute of Drug Control. A voucher specimen has been deposited in the herbarium of the Department of Natural Medicines, Beijing Medical University.

3.3. Extraction and isolation

Powdered roots (7.5 kg) were extracted with 100 l 50% EtOH in water containing 0.5% pyridine

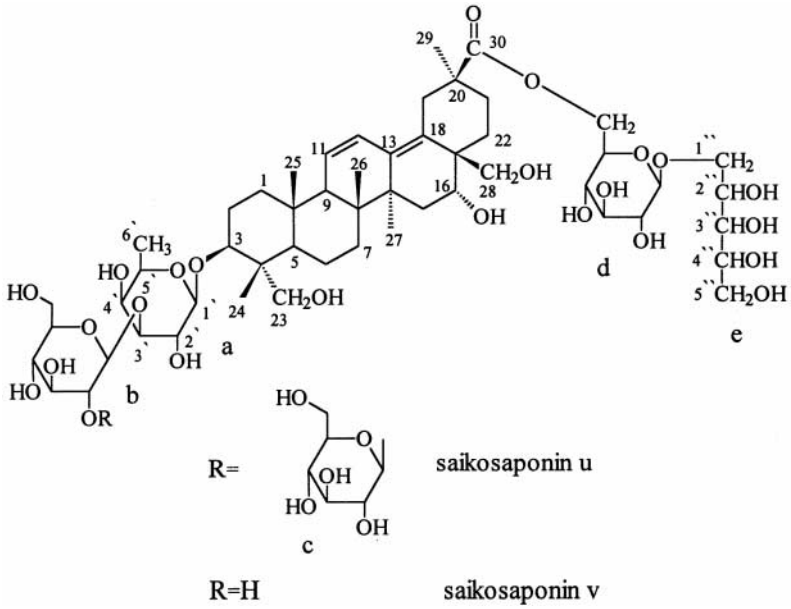

Fig. 3.

(Hideaki, Shigeo, & Shoji, 1978). The extract was concentrated under reduced pressure and diluted with 1 l water. The aqueous solution was subjected to a column contained 1 kg D101 macroporous resin and eluted successively with H_2O 2 l, 70% MeOH 1 l and 80% MeOH 1 l, respectively. Both the 70% and the 80% MeOH extract were combined, evaporated and then the residue was dissolved in MeOH and discolored by simply passing through a short Al_2O_3 column (250 g) and then eluting with MeOH. All the MeOH eluent was evaporated and the residue was dissolved in water and extracted with 1.5 l *n*-BuOH. The crude saponin (15 g) was obtained from the *n*-BuOH extract. The crude saponin was chromatographed on silica gel (500 g) column to give frs 1–21. Fr. 21 (200 mg) was subjected to repeated chromatography on a RP-18 (20 g) column eluted with 66% MeOH in H_2O. Finally, 20 mg of saikosaponin u and 15 mg of saikosaponin v were obtained.

3.4. Acid hydrolysis of saponin on TLC

As the saponins were obtained only in a small amount (20 mg), the acid hydrolysis was performed on TLC (Zhao et al., 1987). 1 mg saponin was dissolved in 1 ml MeOH and loaded on two TLC plates. In a container, the two plates were suspended over a solution of 10 ml 6 N HCl at a temperature of 60°C for 30 min. After hydrolysis, HCl, absorbed by the silica gel on the plates, was evaporated. Then the plates were chromatographed with two development systems, for aglycones $CHCl_3$:CH_3OH:H_2O = 10:1:0.1 and for sugars $CHCl_3$:CH_3OH:H_2O:HOAc = 30:12:4:4.6, respectively.

3.5. Saikosaponin u

White powder, m.p. 276–278°C. UV λ_{max}^{MeOH} nm: 242, 251, 261; FAB-MS *m/z*: 1291 $[M + Na]^+$, ^1H NMR (C_5D_5N) δ: 0.82, 0.98, 1.02, 1.45, 1.64 (each 3H, s, 5 × CH_3), 1.45 (3H, d, J = 6.31 Hz), 4.97 (1H, d, J = 8.35 Hz), 4.99 (1H, d, J = 7.95 Hz), 5.09 (1H, d, J = 7.49 Hz), 5.12 (1H, d, J = 7.78 Hz), 5.72 (1H, d, J = 10.2 Hz), 6.65 (1H, d, J = 10.2 Hz); The ^{13}C NMR data, see Tables 1 and 2.

3.6. Saikosaponin v

White powder, m.p. 265–267°C. UV λ_{max}^{MeOH} nm: 241, 250, 261; FAB-MS *m/z*: 1129 $[M + Na]^+$, ^1H NMR (C_5D_5N) δ: 0.83, 0.91, 0.98, 1.44, 1.64 (each 3H, s, 5 × CH3), 1.44 (3H, d, J = 4.89 Hz), 4.97 (1H, d, J = 7.62 Hz), 4.99 (1H, d, J = 7.69 Hz), 5.33 (1H, d, J = 7.82 Hz), 5.71 (1H, d, J = 10.6 Hz), 6.63 (1H, d, J = 10.6 Hz); for the ^{13}C NMR data, see Tables 1 and 2.

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