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Saikosaponins from roots of *Bupleurum gibraltaricum* and *Bupleurum spinosum*

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Abstract

Two new saikosaponins have been identified in the butanolic fraction of the ethanol extract of the roots of *Bupleurum spinosum*: 3β,16α,23,28-tetrahydroxyoleana-11,13(18)-dien-30-oic acid 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside, and 3β,16α,23,28,30-pentahydroxyoleana-11,13(18)-diene 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside. 3β,16β,23-trihydroxy-13,28-epoxyolean-11-ene 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside was also isolated, and this structure agreed with the one proposed for bupleuroside I, but their spectroscopic data have not been described until now. From the same fraction of the roots of *Bupleurum gibraltaricum*, the known compound buddlejasaponin IV has been isolated as the predominant component (90%). Structures were elucidated using spectroscopic analysis, specially 2D-NMR experiments.

Keywords: *Bupleurum spinosum*; *Bupleurum gibraltaricum*; Umbelliferae; Saikosaponins; Bupleuroside I

1. Introduction

Our investigations of the aerial parts of *Bupleurum gibraltaricum* L. (Barrero et al., 1998a), and *Bupleurum spinosum* L. (Barrero et al., 1998b), were extended to the butanolic fraction of the ethanol extract of the roots. In the last few years these studies have gained importance because extracts of the *Bupleurum genus* (Umbelliferae) have been shown to contain many saikosaponins which exhibit a broad range of biological activities (Kyo et al., 1999; Zong et al., 1998; Benito et al., 1998).

2. Results and discussion

The roots of *Bupleurum gibraltaricum* and *B. spinosum* were macerated in EtOH. The crude residues were suspended in H_2O–MeOH and extracted with *tert*-butylmethyl ether and subsequently with *n*-BuOH.

The main component of the butanol fraction of the roots of *B. gibraltaricum* was identified as buddlejasaponin IV, first isolated from *Buddleja japonica* (Yamamoto et al., 1991), by comparison of their physical and spectroscopic data with those reported in the literature.

Silica gel column chromatography and semi-preparative HPLC separations of the *n*-BuOH fraction of the roots of *B. spinosum* led to the isolation of two new saikosaponins, **1** and **2**, bupleuroside I (**3**) (Matsuda et al., 1997), together with the known saikosaponin a (Kubota and Hinoh, 1968) and saikosaponin b_3 (Ishii et al., 1980).

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The molecular formula of compound **1**, $C_{48}H_{76}O_{20}$, was deduced from its HRFAB-MS ($[M + Na]^+$, m/z 995.4838). The UV spectrum, with absorption maxima at 243, 257 and 262 nm, indicated the existence of a conjugated heteroannular diene. The IR spectrum showed absorption bands due to hydroxyl (3409 cm^{-1}), carbonyl (1720 cm^{-1}), and C=C double bond (1636 cm^{-1}) groups, respectively. DQF-COSY, HOHAHA, ROESY, HMQC, and HMBC 2D NMR experiments allowed the complete assignment of the proton and carbon NMR signals for the triterpene unit and sugar moiety (Tables 1–4). The data of ^1H and ^{13}C NMR spectra were identical to those of 2″-*O*-β-D-glucopyranosylsaikosaponin b_2 (**4**) (Lin et al., 1992) for the sugar moiety, while for the aglycone, an additional signal for a carboxyl group (δ 181.2 in the ^{13}C NMR spectrum) and the absence of the signal due to one of the methyl groups were observed. Considerable chemical shift changes were evidenced for the carbons of the E ring also: upfields shifts of −5.3 and −4.5 ppm for C-19 and C-21, respectively, and downfield shift of +11.3 ppm for C-20. These changes suggested that the carboxyl group was attached to C-20. The stereochemistry of C-20 in **1** was determined by studying the γ-effects on the ^{13}C NMR signals for C-19 and C-21 caused by the presence of oxygen substituent on C-30 (Pistelli et al., 1993; Luo et al., 1987). Furthermore, ^{13}C NMR δ values for the carbons in the E ring were in agreement with those reported for related saikosaponins having a carboxyl ester group in C-30 (Li et al., 1999). It was thus concluded that the structure for compound **1** was 3β,16α,23,28-tetrahydroxyoleana-11,13(18)-dien-30-oic acid 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside.

Compound **2** revealed an $[M + Na]^+$ ion peak at m/z 981.5033 in the HRFAB-MS spectrum, consistent with the molecular formula $C_{48}H_{78}O_{19}$. It showed hydroxyl (3446 cm^{-1}) and C=C double bond (1650 cm^{-1}) groups absorptions in its IR spectrum. The ^{13}C NMR spectroscopic data for the triterpene unit (Table 1) agreed with the structure of the disaccharide saponin **5** (3β,16α,23,28,30-pentahydroxyoleana-11,13(18)-diene 3-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside) (Pistelli et al., 1993). However, ^{13}C and ^1H signals of the sugar moiety (Tables 2 and 4) were consistent with those expected if the trisaccharide present in **1**, **3**, and **4** was joined to the triterpenic unit of **2** at C-3. Besides, the FAB-MS spectrum showed ion peaks at m/z 819.5 (M + Na − Glc) and 657.3 (M + Na − Glc − Glc). Thus, **2** was identified as 3β,16α,23,28,30-pentahydroxyoleana-11,13(18)-diene 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside.

Table 1
^{13}C NMR data of the aglycone moieties of compounds **1**–**5** in C_5D_5N[a]

C	**1**	**4**[b]	**2**	**5**[c]	**3**
1	38.5	38.5	38.4	38.3	38.6
2	26.2	26.3	26.2	26.1	26.2
3	82.2	82.2	82.1	81.6	81.9
4	43.7	43.8	43.7	43.6	43.8
5	47.5	47.5	47.4	47.3	47.3
6	18.3	18.4	18.2	18.8	17.6
7	32.3	32.4	32.3	32.3	31.6
8	41.2	41.2	41.1	41.0	42.2
9	54.1	54.1	54.0	54.0	53.1
10	36.5	36.6	36.5	36.4	36.1
11	126.9	126.4	126.2	126.1	132.2
12	126.1	126.3	126.4	126.4	131.2
13	137.5	136.2	136.3	136.7	84.0
14	42.1	42.0	42.0	41.9	45.6
15	32.0	32.1	32.0	31.9	36.3
16	67.8	67.8	67.8	67.8	64.1
17	45.5	45.4	45.9	45.8	47.0
18	131.3	133.1	132.9	132.9	52.2
19	33.9	39.2	34.0	33.9	37.7
20	44.0	32.7	38.3	38.2	31.6
21	31.1	35.6	30.2	30.2	34.7
22	24.2	24.6	24.2	24.2	25.8
23	64.3	64.3	64.2	64.0	64.0
24	13.0	13.2	13.0	13.1	13.0
25	18.8	18.9	18.8	18.8	18.7
26	17.3	17.4	17.3	17.2	20.1
27	21.9	22.0	22.0	21.9	20.8
28	65.1	64.8	65.0	64.9	73.0
29	21.5	25.2	21.2	21.1	33.7
30	181.2	32.7	73.5	73.4	23.8

[a] **1**: 125 MHz; **2**–**3**: 100 MHz.
[b] **4**:125 MHz (Lin et al., 1992).
[c] **5**: 50 MHz (Pistelli et al., 1993).

Table 2
^{13}C NMR data of the sugar units of compounds **1**–**4** in C_5D_5N[a]

Sugar	C	**1**	**2**	**3**	**4**[b]
fuc	1	105.4	105.4	105.3	105.4
	2	71.2	71.2	71.2	71.3
	3	86.8	86.7	86.8	86.9
	4	71.8	71.7	71.8	71.8
	5	71.0	70.9	70.9	71.0
	6	17.3	17.3	17.3	17.4
glc_1	1	104.7	104.7	104.7	104.8
	2	86.1	86.1	86.2	86.2
	3	77.8	77.8	77.8	77.8
	4	70.9	70.9	70.9	71.0
	5	78.5	78.5	78.5	78.5
	6	62.1	62.0	62.0	62.1
glc_2	1	107.2	107.1	107.2	107.2
	2	76.6	76.6	76.6	76.6
	3	77.8	77.8	77.8	77.7
	4	70.6	70.5	70.6	70.6
	5	79.1	79.1	79.1	79.2
	6	62.4	62.3	62.3	62.4

[a] **1**: 125 MHz; **2** and **3**: 100 MHz.
[b] **4**: 125 MHz (Lin et al., 1992).

Table 3
^{1}H NMR data of the aglycone moieties of compounds **1**–**4** in C_5D_5N[a]

H	**1**	**4**[b]	**2**	**3**
3	4.25 *m*	4.20 *m*	4.25 *m*	4.20 *m*
11	5.75 *d* (9.6)	5.66 *d* (9.0)	5.68 *d* (10.8)	6.02 *bd* (11.8)
12	6.74 *dd* (9.6, 2.0)	6.65 *dd* (9.0, 2.0)	6.76 *dd* (10.8, 2.7)	5.65 *dd* (11.8, 2.4)
16	4.89 *bs*	4.75 *bs*	4.83 *bs*	4.33 *m*
19α	3.30 *d* (15.0)	2.60 *d* (12.0)	2.89 *d* (14.8)	1.78 *m*
19β	2.85 *d* (15.0)	1.83 *d* (12.0)	2.34 *d* (14.8)	1.24 *m*
23*a*	3.74 *d* (11.0)	3.65 *m*	3.77 *d* (10.1)	3.66 *d* (10.7)
23*b*	4.32 *d* (11.0)	4.29 *m*	4.33 *m*	4.19 *m*
24	1.03 *s*	0.96 *s*	1.02 *s*	0.86 *s*
25	0.98 *s*	0.94 *s*	0.98 *s*	0.96 *s*
26	0.85 *s*	0.82 *s*	0.87 *s*	1.37 *s*
27	1.67 *s*	1.61 *s*	1.70 *s*	1.08 *s*
28*a*	3.82 *m*	3.70 *d* (13.0)	3.82 *m*	3.32 *d* (6.6)
28*b*	4.37 *m*	4.14 *m*	4.37 *m*	4.39 *d* (6.6)
29	1.58 *s*	0.98 *s*	1.23 *s*	0.89 *s*
30*a*	–	0.93 *s*	3.65 *m*	0.89 *s*
30*b*	–	0.93 *s*	3.80 *m*	0.89 *s*

[a] **1**: 500 MHz; 2 and 3: 400 MHz.
[b] **4**: 500 MHz (Lin et al., 1992).

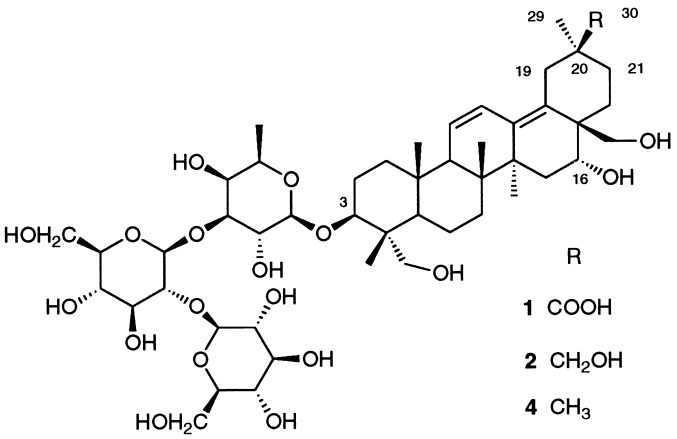
Compound **3** had a molecular formula $C_{48}H_{78}O_{18}$, as deduced from its HR-FAB-MS spectrum with an ion peak at 965.5085 $[M + Na]^+$. The FAB-MS also showed peaks at 803.5 (M + Na − Glc) and 641.3 (M + Na − Glc − Glc). The IR spectrum exhibited hydroxyl and C=C double bond bands at 3398 and 1645 cm^{-1}, respectively. Again, the ^{13}C and ^1H NMR data (Tables 1–4) suggested that **3** was a saikosaponin with the same sugar moiety of **1** and **2** (Tables 2 and 4). However, in this case, the data indicated that the aglycone at C-3 was the same as that of saikosaponin a (Kubota and Hinoh, 1968) (3β,16β,23-trihydroxy-13,28-epoxyolean-11-ene 3-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside). Thus, **3** had the structure of 3β,16β,23-trihydroxy-13,28-epoxyolean-11-ene 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside. Matsuda et al. (1997) have isolated from the roots of *B. scorzonerifolium* WILLD, a

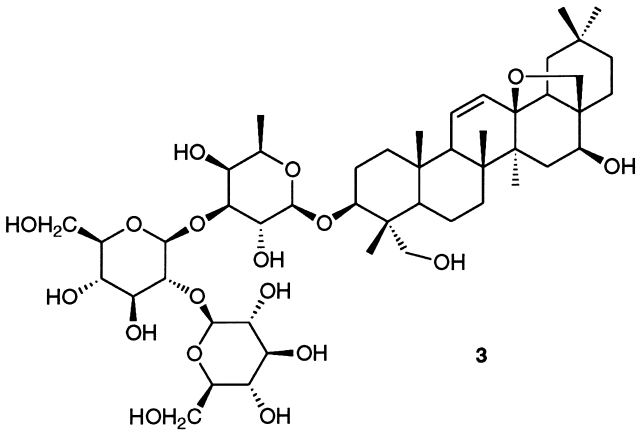
Table 4
^{1}H NMR data of the sugar units of compounds **1**–**4** in C_5D_5N[a]

Sugar	H	**1**	**2**	**3**	**4**[b]
fuc	1	4.98 *d* (7.8)	4.98 *d* (7.8)	4.98 *d* (7.8)	4.93 *d* (7.5)
	2	4.50 *dd* (7.8, 8.2)	4.52 *t* (8.0)	4.50 *dd* (7.8, 8.2)	4.46 *t* (8.0)
	3	3.83 *dd* (8.2, 2.1)	3.85 *dd* (8.0, 2.1)	3.80 *m*	3.80 *dd* (8.0, 2.0)
	4	4.23 *d* (2.1)	4.23 *d* (2.1)	4.23 *m*	4.16 *d* (2.0)
	5	3.64 *m*	3.64 *m*	3.64 *m*	3.64 *q* (6.0)
	6	1.40 *d* (6.2)	1.43 *d* (6.3)	1.46 *d* (6.3)	1.39 *d* (6.0)
glc_1	1	5.14 *d* (7.8)	5.14 *d* (7.8)	5.08 *d* (7.7)	5.06 *d* (7.5)
	2	3.93 *t* (8.0)	3.92 *m*	3.93 *m*	3.89 *t* (7.5)
	3	4.30 *m*	4.30 *m*	4.25 *m*	4.23 *m*
	4	4.20 *m*	4.20 *m*	4.15 *m*	4.13 *m*
	5	3.91 *m*	3.92 *m*	3.91 *m*	3.86 *m*
	6	4.31 *m*	4.31 *m*	4.31 *m*	4.25 *m*
	6′	4.35 *m*	4.35 *m*	4.35 *m*	4.35 *bd* (11.0)
glc_2	1	5.10 *d* (7.8)	5.11 *d* (8.2)	5.07 *d* (7.6)	5.03 *d* (8.0)
	2	4.05 *t* (7.9)	4.06 *t* (8.2)	4.05 *t* (7.9)	4.00 *t* (8.0)
	3	4.12 *t* (8.1)	4.12 *t* (8.2)	4.12 *m*	4.06 *t* (8.0)
	4	4.18 *m*	4.18 *m*	4.18 *m*	4.14 *m*
	5	3.77 *m*	3.77 *m*	3.77 *m*	3.73 *m*
	6	4.30 *m*	4.30 *m*	4.30 *m*	4.27 *bd* (12.0)
	6’	4.40 *dd* (11.7, 2.8)	4.40 *m*	4.40 *m*	4.41 *d* (12.0)

[a] **1**: 500 MHz; **2** and **3**: 400 MHz.
[b] **4**: 500 MHz (Lin et al., 1992).

compound named bupleuroside I, for which they measured only the hepatoprotective activity and proposed structure **3**, without any physical or spectroscopic data.





3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 141 polarimeter. UV spectra were recorded on a Spectronic 2000 UV–VIS Bausch and Lomb spectrometer and IR on a Perkin-Elmer 983G spectrometer. The FAB-MS and the HRFAB-MS were determined on a Micromass AutoSpec-Q instrument (thioglycerol, 1% NaI). NMR spectra were recorded on Bruker AMX 500 or Bruker ARX 400 spectrometers (δ values given in ppm relative to internal TMS and J values in Hz). Column chromatography was carried out using silica gel Chromagel 60 (35–70 μm). Analytical TLC was performed on layers of silica gel Merck 60G using a 7% phosphomolybdic acid solution (EtOH) to visualize the spots. Semi-preparative HPLC separations were carried out on a column of Spherisorb ODS 2 (15 cm × 1 cm) in a Hewlett-Packard Series 1100 instrument.

3.2. Plant materials

B. gibraltaricum L. was collected in the Quéntar Mountain Range (Granada, Spain) in May 1995 and *B. spinosum* L. in Ketama (North of Morocco) in June 1993 and were identified by Professor F. Valle (Departamento de Botánica, Universidad de Granada, Spain) and Professor M. Ater (Laboratoire de Biologie, Université de Tétouan, Morocco), respectively. A voucher specimen of each one is available for inspection at the herbarium of both Universities.

3.3. Extraction and isolation

Maceration of the roots of *B. gibraltaricum* (1415 g) in EtOH afforded, after solvent evaporation, 70.6 g of extract. This was dissolved in MeOH/H_2O and extracted with *tert*-butylmethyl ether to yield 38.3 g of ethereal extract. The aqueous phase was then extracted with *n*-BuOH; solvent evaporation afforded a *n*-BuOH extract (19.2 g) and an aqueous phase (7.9 g). The roots of *B. spinosum* (1140 g) were macerated in EtOH (80.5 g) and the extract was partitioned in a similar way to obtain 61.2 g of ether extract, 12.2 g of aqueous phase, and 4.0 g of *n*-BuOH extract.

Ribitol was the major component in the aqueous phase of the extracts of both plants. The BuOH fraction of *B. gibraltaricum* was subjected to column chromatography on silica gel eluting with EtOAc–MeOH–H_2O (9:1:0.5 v/v) and was found to contain 90% buddlejasaponin IV (17.3 g).

The BuOH fraction (2 g) of *B. spinosum* was chromatographed on a silica gel column, eluting with $CHCl_3$–MeOH mixtures of increased polarity. Some fractions were subjected to semipreparative HPLC with a gradient H_2O–CH_3CN to give saikosaponin a (9 mg), saikosaponin **1** (22 mg), saikosaponin **2** (18 mg), saikosaponin **3** (7 mg), and saikosaponin b_3 (7 mg).

3β,16α,23,28-Tetrahydroxyoleana-11,13(18)-dien-30-oic acid 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (**1**): $C_{48}H_{76}O_{20}$; white powder; mp 210–213°C; $(\alpha)_D^{25}$ +17.1° (MeOH, *c* 0.1); UV λ_{max}^{MeOH} nm (log ε): 243 (3.9), 257 (4.1), 262 (3.8); IR ν_{max}^{KBr} cm^{-1}: 3409, 2925, 1720, 1636, 1448, 1384, 1307, 1258, 1163, 1074; HRFAB-MS: 995.4838 $[M + Na]^+$ (calcd. 995.4836); for the NMR data see Tables 1–4.

3β,16β,23,28,30-Pentahydroxyoleana-11,13(18)-diene 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (**2**): $C_{48}H_{78}O_{19}$: amorphous powder; $(\alpha)_D^{25}$ −27.5° (MeOH, *c* 0.08); IR ν_{max}^{film} cm^{-1}: 3446, 2924, 1650, 1023, 901; FAB-MS, *m/z*: 981.5 [M

+ Na]$^+$, 819.5 (981.5 − Glc), 657.3 (981.5 − Glc − Glc); HRFAB-MS: 981.5033 [M + Na]$^+$ (calcd. 981.5035); for the NMR data see Tables 1–4.

3β,16β,23-Trihydroxy-13,28-epoxyolean-11-ene 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (**3**): $C_{48}H_{78}O_{18}$: amorphous powder; $(\alpha)_D^{25}$ +15.7° (MeOH, *c* 0.06); IR ν_{max}^{film} cm^{-1}: 3398, 2928, 1645, 1448, 1384, 1307, 1260, 1163, 1046, 906; FAB-MS, *m*/*z* 965.5 [M + Na]$^+$, 803.5 (965.5 − Glc), 641.3 (965.5 − Glc − Glc); HRFAB-MS: 965.5085 [M + Na]$^+$ (calcd. for 965.5086); for the NMR data see Tables 1–4.

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