Notes

A Sulfated Saponin from *Bupleurum rigidum*

Sandra Sánchez-Contreras,[†] Ana María Díaz-Lanza,*[,†] Lidia Fernández Matellano,[†] Manuel Bernabé,[‡] Evelyne Ollivier,[§] Guy Balansard,[§] and Robert Faure[⊥]

Laboratorio de Farmacognosia, Departamento de Farmacología, Facultad de Farmacia, Universidad de Alcalá de Henares, Carretera Madrid-Barcelona, Km-33,600, 28871-Alcalá de Henares, Madrid, Spain, Departamento de Química Orgánica Biológica, Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006-Madrid, Spain, Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université d'Aix-Marseille II, 27, Boulevard Jean Moulin, 13385 Marseille Cedex 13, France, and URA 14111 Université d'Aix-Marseille II, Avenue Escadrille Normandie Niemen, 13397 Marseille Cedex 13, France

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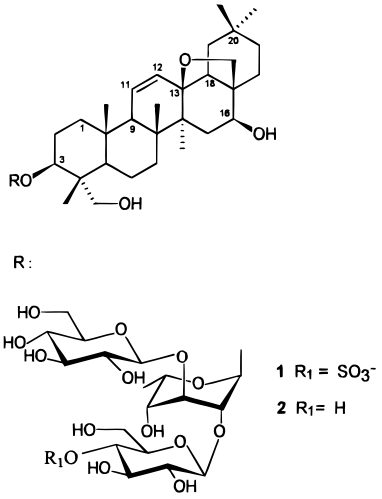
A new sulfated triterpene glycoside with the sulfate group located in an unusual position in the carbohydrate moiety, was isolated from the MeOH extract of the aerial parts of *Bupleurum rigidum*. This compound was identified by a combination of chemical degradation and spectral methods as 3*β*,-16*β*,23-trihydroxy-13,28-epoxyolean-11-en-3*β*-yl-*β*-D-glucopyranosyl-(1→2)[4-sulfate-*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside (sandrosaponin I) (**1**). In addition, the known compound 3*β*,16*β*,23-trihydroxy-13,28-epoxyolean-11-en-3*β*-yl-*β*-D-glucopyranosyl-(1→2)[*β*-D-glucopyranosyl-(1→3)]*β*-D-fucopyranoside (**2**) was isolated in the present investigation.

Many plants belonging to the genus *Bupleurum* (Umbelliferae) have been used as traditional oriental herbal drugs. Saikosaponins from *Bupleurum falcatum* are considered the major bioactive components of these drugs, which are used mainly for their antiinflammatory, antihepatotoxic, and immunostimulant activities.[1] About 40 *Bupleurum* species have been studied chemically, and some 60 saikosaponin derivatives, as well as a number of coumarins, fatty acids, flavonoids, lignans, polyacetylenes, and steroids have been isolated from other plants of this genus.[2−9]

Bupleurum rigidum is distributed abundantly in the Mediterranean zone of Spain, but no literature reports concerning its constituents have yet appeared. This paper reports on the isolation and structure identification of two triterpenoid saponins **(1** and **2)** of *B. rigidum*, of which one contains a sulfate group.

The MeOH extract of *B. rigidum* was fractionated by Si gel column chromatography to give several fractions, of which two were further purified, yielding two saponins (**1** and **2**). The molecular formula of **1** was established as $C_{48}H_{78}O_{21}SNa$ by HRFABMS. Acid hydrolysis of compound **1**, followed by extraction with ether, gave an ether-soluble portion and a water-soluble portion. From the ether-soluble portion, saikogenin F was isolated and identified on TLC comparison with an authentic sample.[6] The water-soluble portion showed the presence of glucose and fucose. Acid hydrolysis of **1**, followed by treatment with barium chloride[10] demonstrated that a sulfate group was present in this compound. The ^{1}H NMR spectrum for compound **1** showed two ethylene proton signals at 5.94 and 5.38 ppm (J = 10.7 Hz), three doublets in the region of the anomeric protons of sugars, and six singlets (three protons each),



demonstrating the presence of six methyl groups (Table 1). Assignment of most of the protons was achieved using 2D COSY and TOCSY (mixing time = 80 ms) experiments. In this way, six aglycon protons were observed at low field, corresponding to oxygen-bearing carbons. The observation of a signal at 4.17 ppm (J = 10.3, 5.9 Hz) suggested the presence of an equatorial hydroxyl group at C-16. Two −OCH_2− groups were also detected (δ 3.90−3.04 and 3.79−3.26). The ^{13}C NMR spectrum of **1** showed the presence of 48 carbons. The chemical shifts of two of these (134.2 and 130.6 ppm) were consistent with the presence of a double bond in the structure of **1**. The assignment of the carbon and proton signals was accomplished by a combination of HMQC and HMBC experiments, which also provided information on the connectivities of seven quaternary carbons.

The coupling constants for the anomeric protons of the sugar moieties of **1** were characteristic of *β*-anomers ($J_{1,2}$ ca. 7.8 Hz). The sugar units were labeled **A**−**C**, from low to high-field, according to their anomeric protons. The high

* To whom correspondence should be adressed. Tel.: 91-885 46 45. Fax: 91-885 46 79. E-mail: tfamdl@farma.alcala.es.
[†] Universidad de Alcalá de Henares.
[‡] CSIC.
[§] Faculté de Pharmacie, Université d'Aix-Marseille II.
[⊥] URA, Université d'Aix-Marseille II.

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Table 1. ^{1}H- and ^{13}C NMR Data for the Aglycon and Sugar Moieties of Compound 1[a]

position	δ_H	δ_C
1	1.85−0.92	39.3
2	1.95−1.81	26.1
3	3.61	84.3
4		44.4
5	1.16	48.1
6	1.52−n.d.	18.2
7	1.53−1.21	32.1
8		43.0
9	1.89	54.0
10		37.1
11	5.94 ($J_{11,12}$ = 10.5)	134.2
12	5.37 ($J_{9,12}$ = 3.0)	130.6
13		85.7
14		46.5
15	1.58−1.44	36.0
16	4.16 (dd, $J_{15a,16}$ = 10.3;	65.4
17		47.6.
18	1.77	53.1
19	1.78−1.27	38.6
20		32.3
21	1.42−1.17	35.3
22	2.05−1.25	26.4
23	3.78−3.25	64.6
24	0.72 (s)	12.6
25	0.94 (s)	18.8
26	1.09 (s)	20.2
27	1.03 (s)	21.2
28	3.89−3.03 (d, J = 7.3)	73.4
29	0.97 (s)	33.9
30	0.92 (s)	24.1
	glucose 1 (A)	
1	4.85 ($J_{1,2}$ = 8.1)	103.5
2	3.13 ($J_{2,3}$ = 9.3)	76.2
3	3.35 ($J_{3,4}$ = 9.2)	78.2
4	3.12 ($J_{4,5}$ = 9.3)	72.4
5	3.27 ($J_{5,6a}$ = 2.2; $J_{5,6b}$ = 7.0)	78.2
6a	3.82 ($J_{6a,6b}$ = 12.0)	63.6
6	3.55	
	glucose 2 (B)	
1	4.65 ($J_{1,2}$ = 7.8)	104.9
2	3.42 ($J_{2,3}$ = 9.2)	75.3
3	3.64 ($J_{3,4}$ = 8.8)	76.8
4	4.16 ($J_{4,5}$ = 9.7)	77.4
5	3.43 ($J_{5,6a}$ = 2.1; $J_{5,6b}$ = 5.2)	76.1
6a	3.86 ($J_{6a,6b}$ = 12.0)	62.2
6b	3.78	
	fucose (C)	
1	4.46 (d, $J_{1,2}$ = 7.8)	104.7
2	3.92 ($J_{2,3}$ = 9.8)	76.4[b]
3	3.78	85.6[b]
4	3.87	72.5
5	3.65	71.2
6	1.25 ($J_{5,6}$ = 6.4)	16.8

[a] Protons are multiplets, unless otherwise stated (s, singlet; dd, double doublet; t, triplet). When two values are given, the first corresponds to the equatorial proton and the second to the axial one, with the exception of the C-23 hydroxymethyl group. [b] Values correspond to glycosylation points.

field region showed a doublet at ca. 1.28 ppm (three protons, J = 6.5 Hz), indicative of the presence of a 6-deoxy sugar. One of the carbohydrate protons was strongly deshielded, suggesting that its geminal hydroxyl group was substituted. From homonuclear 2D experiments, as well as selective decoupling, this proton was identified as H-4 of unit **B**. In addition, the observed coupling constants (Table 1) led to the identification of **A** and **B** as glucose residues ($J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ > 9 Hz), and **C** as a fucose unit ($J_{2,3}$, $J_{3,4}$ > 9 Hz, $J_{5,6}$ = 6.5 Hz).

^{13}C NMR values for the sugar units of **1** were determined in an analogous manner to those for the aglycon moiety (Table 1). From these values it could be deduced that C-2 and C-3 of the fucose residue were shifted downfield, as compared to those observed in model compounds,[11] and these were established as the glycosylation sites. The HMBC experiment demonstrated these conclusions unequivocally, showing cross-peaks at H-1**A**/C-3**C**, H-1**B**/C-2**C**, and also at H-1**C**/C-3 (aglycon). A 2D ROESY experiment (mixing time = 400 ms) showed cross-peaks at H-3/H-1a, H-3/H-2e, H-3/H-5, H-11/H-1e, H-12/H-18, H-18/H-28, H-16/H-19a, H-16/Me-27, H-28/H-15a, and also, in the sugar region cross-peaks between H-1**A**/H-2**C**, H-1**B**/H-3**C**, and H-1**C**/H-3 (aglycon) were observed, which gave additional information on the stereochemistry of the aglycon protons and the connectivities of the three carbohydrate units. Accordingly, compound **1** was established as 3*β*,16*β*,23-trihydroxy-13,28-epoxyolean-11-en-3*β*-yl-*β*-D-glucopyranosyl-(1→2)[4-sulfate-*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside, and has been accorded the trivial name sandrosaponin I.

Following analogous methodology, we also identified compound **2**, which is an already known compound.[9,12]

Experimental Section

General Experimental Procedures. UV spectra were run in MeOH, on a UV/vis Philips PU 8720 spectrophotometer. IR spectra were recorded in KBr on a Perkin−Elmer 681 spectrometer. NMR spectra were recorded in CD_3OD, on a Varian Unity 500 instrument at 25 °C. Chemical shifts refer to the MeOH-d_4 multiplet (^{1}H, 3.30 ppm; ^{13}C, 49.0 ppm). HRFABMS were carried out in a VG AutoSpec (Fisons). Analytical TLC was carried out on Merck Si gel F_{254} aluminum sheets, eluted with *n*-BuOH−AcOH−H_2O (4:1:5) and visualized with 1% vanillin in MeOH−H_2SO_4 (1:1). Carbohydrates were identified by chromatographic comparison with authentic samples of D-glucose and D-fucose.

Plant Material. *Bupleurum rigidum* was collected in San Andrés del Congosto, Guadalajara, Spain, in June 1995, and was identified by Dr. C. Bartolomé, Departamento of Biología Vegetal, Facultad de Ciencias, Universidad de Alcalá de Henares, Madrid, Spain. A voucher specimen has been deposited at the herbarium of the University of Alcalá.

Extraction and Isolation. The aerial parts of *B. rigidum* (900 g) were treated for 3 h at room temperature with $CHCl_3$ (9 L). Subsequently, the residue was extracted with 60% MeOH for 24 h. After removal of the MeOH under vacuum, the resulting aqueous solution was extracted with *n*-BuOH saturated with H_2O. The *n*-BuOH layer was concentrated to dryness under a vacuum, affording a saponin mixture (40 g, yield 4.4%), of which 7 g were chromatographed over a Si gel column, eluting with $CHCl_3$−MeOH−H_2O (80:20:1 → 55:37:7), to give two fractions containing the saikosaponins **1** (500 mg) and **2** (446 mg).

3*β*,16*β*,23-Trihydroxy-13,28-epoxyolean-11-en-3*β*-yl-*β*-D-glucopyranosyl-(1→2)[4-sulfate-*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside (sandrosaponin I, 1): amorphous powder; mp 282−285 °C (dec); $[\alpha]_D$ +47.8° (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (2.93), 251 (2.96), 260 (2.86) nm; IR (KBr) ν_{max} 3920, 3420, 2880, 1640, 1250, 1070, 630 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRFABMS $[M]^+$ *m*/*z* 1045.4654, calcd for $C_{48}H_{77}O_{21}SNa$ 1045.4679, $[M + Na]^+$ *m*/*z* 1067.4473, calcd for $C_{48}H_{77}O_{21}SNa_2$ 1067.4510.

3*β*,16*β*,23-Trihydroxy-13,28-epoxyolean-11-en-3*β*-yl-*β*-D-glucopyranosyl-(1→2) [*β*-D-glucopyranosyl-(1→3)]*β*-D-fucopyranoside (2):[9,12] $[\alpha]_D$ +53.6° (MeOH) [lit.[12] $[\alpha]_D$ +54.6° (MeOH)]; HRFABMS $[M]^+$ *m*/*z* 965.5086, calcd for $C_{48}H_{78}O_{18}Na$ 965.5078. This compound was identified by spectral date comparison to buddlejasaponin IV.[9,12]

Acid Hydrolysis of Compounds 1 and 2. An aliquot (2 mg) of each sample was refluxed with 10% HCl (4 mL) for 4 h. After extraction with ethyl ether to isolate saikogenin F, the aqueous solution was treated with $BaCl_2$ to give a white precipitate ($BaSO_4$); the determination was performed according to Akai et al.[10] The rest of the aqueous layer was neutralized (10% *N*,*N*-dioctylmethylamine in $CHCl_3$) and

concentrated under reduced presure. The sugars were directly analyzed by TLC. D-Glucose and D-fucose were identified on comparison with authentic samples.[13]

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