Four New Triterpenoid Saponins from the Roots of *Bupleurum rigidum*

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Five triterpenoid saponins [buddlejasaponin IV, sandrosaponins VII (**1**), VIII (**2**), IX (**3**), and X (**4**)] were isolated from an *n*-BuOH extract of the roots of *Bupleurum rigidum*. Sandrosaponins VII−X (**1**−**4**) are new compounds, and their structures were established by 1D and 2D NMR techniques, FABMS, and chemical methods.

The roots of several *Bupleurum* species (Apiaceae) are important sources of crude drugs in asian medicine. These species have been used in various prescriptions in Chinese traditional medicine for the treatment of chronic hepatitis, kidney problems, and autoimmune diseases.[1] Studies on the triterpenoidal saponins of *Bupleurum* species were initiated by Shibata et al.[2] and Kubota and Hinoh.[3]

We have recently reported on the isolation and structural elucidation of new saikosaponins[4] obtained from the aerial parts of *Bupleurum rigidum* L. We now report on the investigation of the roots of the title plant, which led to isolation of four new saponins (**1**−**4**) and a known saponin (buddlejasaponin IV).

Results and Discussion

The powdered dried roots of *B. rigidum* were extracted initially with acetone and then with 60% methanol. The methanol extract was dried and partitioned between H_2O and *n*-butanol, and the butanol extract was subjected to Si gel column chromatography to afford compounds **1**−**4** and a previously known saponin. The ^{1}H and ^{13}C NMR spectra of this substance were identical to those published data, and comparison with an authentic sample demonstrated that it was 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 (buddlejasaponin IV).[4,5]

Compound **1** gave a FABMS compatible with a molecular formula $C_{48}H_{78}O_{19}$. The ^{1}H NMR spectrum of **1** contained signals typical for a saponin, with olefinic protons at 5.93 and 5.38 ppm, and five methyl singlets in the upfield region. Three anomeric doublets ($J_{1,2}$ ca.8 Hz) demonstrated the presence of *β*-anomers for the three sugar units present, labeled A−C, from low to high field. In addition, a methyl doublet at 1.25 ppm suggested the presence of a 6-deoxy sugar in the molecule **1**.

The ^{13}C NMR spectrum of **1** contained 48 singlets, with two of them olefinic (134.2 and 130.5 ppm), and three sugar anomeric carbons (105.3, 104.8, and 103.5 ppm) being observed. The presence of only five methyl singlets and signals for five oxygen-linked carbons greater than those corresponding to the carbohydrate residues indicated that three methyl groups were oxidized. Assignment of all the proton and carbon signals was achieved through a combination of COSY, TOCSY, HMQC, and HMBC 2D NMR experiments. The values of the chemical shifts deduced for **1** are included in Table 1. The hydroxyl groups at positions 3 and 16 are equatorial, because the coupling constants of H-3 ($J_{2a,3}$ = 11.9; $J_{2e,3}$ = 5.0 Hz) and H-16 ($J_{15a,16}$ = 10.0; $J_{15e,16}$ = 5.3 Hz) were consistent with axial configurations for both these protons.

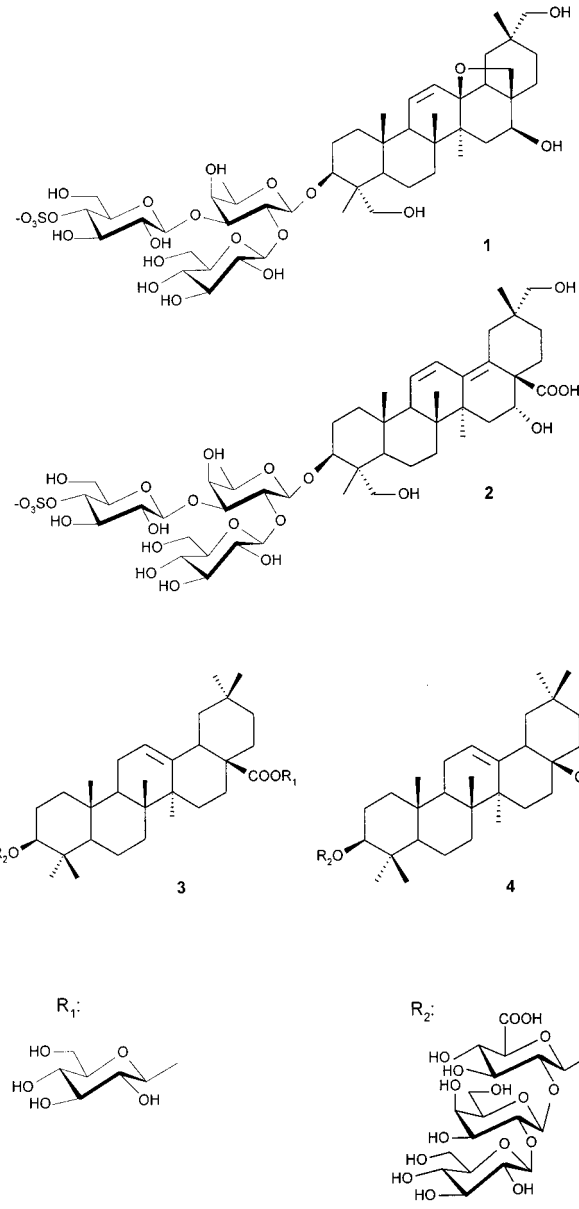

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Table 1. ^1H and ^{13}C NMR Chemical Shifts (δ) and Proton−Proton Coupling Constants (J, Hz) for the Aglycon and Sugar Moieties of Compounds **1**−**4**[a]

	1		**2**		**3**		**4**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.83-0.89	39.3	1.87-1.00	39.1	1.60-0.97	40.0	1.58-1.00	40.0
2	1.96-1.79	26.4	1.98-1.82	26.8	1.96-1.70	27.0	2.00-1.69	27.0
3	3.6 ($J_{2a,3}$=11.9; $J_{2e,3}$=5.0)	84.2	3.64 ($J_{2a,3}$=11.6; $J_{2e,3}$=4.9)	84.4	3.18 ($J_{2a,3}$=11.6; $J_{2e,3}$=3.9)	91.4	3.19 ($J_{2a,3}$=11.6; $J_{2e,3}$=4.8)	91.4
4	----	44.4	----	44.4	----	40.8	----	40.8
5	1.17	48.0	1.25	48.2	0.75 ($J_{5,6a}$=11.6)	57.1	0.75 ($J_{5,6a}$=10.5)	57.1
6	1.54-1.52	18.2	1.57-1.40	18.8	1.55-1.37	19.4	1.54-1.35	19.4
7	1.53-1.21	32.1	1.49-1.39	32.9	1.46-1.30	34.0	1.47-1.30	34.0
8	----	43.0	----	42.3	----	40.4	----	40.4
9	1.89	54.0	2.04	54.7	1.56	49.1	1.56	49.1
10	----	37.0	----	37.2	----	37.9	----	37.9
11	5.93 ($J_{11,12}$=10.5)	134.2	5.58 ($J_{11,12}$=10.7)	127.2	1.90-1.88	24.6	1.89-1.87	24.6
12	5.38 ($J_{9,12}$=3.1)	130.5	6.46 ($J_{9,12}$=3.1)	126.5	5.25	123.8	5.20 ($J_{11a,12}$=3.6)	123.8
13	----	85.7	----	138.0	----	144.8	----	144.8
14	----	46.4	----	41.9	----	43.0	----	43.0
15	1.59-1.43	36.0	1.93-1.44	32.0	1.90-1.88	28.9	1.89-0.99	28.9
16	4.16 dd, ($J_{15a,16}$=10.0; $J_{15e,16}$=4.9)	65.4	4.04 ($J_{15a,16}$=$J_{15e,16}$=3.1)	69.3	2.04-1.70	24.0	1.82-1.56	24.0
17	----	47.8	----	45.5	----	49.1	----	49.1
18	1.81	52.3	----	132.2	2.85	42.6	2.88	42.6
19	1.85-1.23	32.9	2.77-2.17	34.2	1.16-1.70	47.2	1.65-1.08	47.2
20	----	37.6	----	45.5	----	31.6	----	31.6
21	1.54-1.13	21.6	2.01-1.57	30.8	1.38-1.21	34.9	1.35-1.13	34.9
22	2.10-1.28	25.5	2.01-1.65	24.1	1.72-1.60	33.2	1.71-1.50	33.2
23	3.77-3.26	64.4	3.78-3.26	64.5	3.78-3.26	28.7	1.05 (s)	28.7
24	0.72 (s)	12.6	0.72 (s)	12.7	1.06 (s)	17.1	1.86 (s)	17.1
25	0.94 (s)	18.8	0.94 (s)	19.0	0.86 (s)	16.2	0.93 (s)	16.2
26	1.09 (s)	20.2	0.73 (s)	17.6	0.95 (s)	17.8	0.84 (s)	17.8
27	1.05 (s)	21.2	1.25 (s)	22.1	0.79 (s)	26.4	1.13 (s)	26.4
28	3.90-3.05 (d, J=7.5)	73.3	3.77-3.28	65.1	----	178.1	----	178.1
29	3.24 (br.s.)	74.6	1.10 (s)	21.8	0.91 (s)	33.5	0.87 (s)	33.5
30	0.90 (s)	19.7	----	183.0	0.93 (s)	24.0	0.94 (s)	24.0
	Glucose 1(A)		Glucose 1(A)		Glucose 1 (A)			
1	4.85 ($J_{1,2}$=7.9)	103.5	4.87 ($J_{1,2}$=7.9)	103.4	5.37 ($J_{1,2}$=7.9)	95.7		
2	3.13 ($J_{2,3}$=9.1)	76.1	3.12 ($J_{2,3}$=9.1)	46.2	3.32	74.0		
3	3.34 ($J_{3,4}$=9.1)	78.3	3.34 ($J_{3,4}$=9.1)	78.3	3.40	78.7		
4	3.14 ($J_{4,5}$=9.5)	72.4	3.12 ($J_{4,5}$=9.2)	72.3	3.34	71.0		
5	3.27	78.4	3.27	78.3	3.34	78.7		
6a	3.81	63.6	3.80	63.6	3.80	62.5		
6b	3.54		3.55		3.37			
	Glucose 2 (B)		Glucose 2 (B)		Galactose (B)		Galactose (B)	
1	4.58 ($J_{1,2}$=7.9)	105.3	4.65 ($J_{1,2}$=7.9)	105.0	4.72 ($J_{1,2}$=6.8)	104.5	4.72 ($J_{1,2}$=6.8)	104.5
2	3.32 ($J_{2,3}$=9.1)	75.3	3.42	75.3	3.76	83.6	3.76	83.5
3	3.32 ($J_{3,4}$=9.2)	78.2	3.65 ($J_{3,4}$=9.2)	76.9	3.70	74.8	3.70	74.8
4	3.30	71.3	4.14 ($J_{4,5}$=9.4)	77.5	3.88	69.6	3.88	69.6
5	3.28	78.0	3.43	76.2	3.47	76.4	3.47	76.4
6a	3.83	62.4	3.86	62.3	3.69	62.0	3.69	62.0
6b	3.66		3.75		3.68		3.68	
	Fucose (C)		Fucose (C)		Glucose 2 (C)		Glucose 2 (C)	
1	4.58 ($J_{1,2}$=7.9)	104.8	4.47 ($J_{1,2}$=8.0)	104.8	4.62 ($J_{1,2}$=7.6)	106.3	4.61 ($J_{1,2}$=7.6)	106.1
2	3.32 ($J_{2,3}$=9.6)	76.4	3.93 ($J_{2,3}$=9.7)	46.4	3.28	76.0	3.27	77.9
3	3.77	85.7	3.78	85.7	3.39	77.9	3.35	77.5
4	3.87	72.7	3.87	72.7	3.36	71.2	3.36	71.4
5	3.65	71.2	3.65	71.4	3.38	79.0	3.35	78.9
6[a]	1.25 ($J_{5,6}$=6.5)	16.9	1.26 ($J_{5,6}$=6.4)	16.9	3.95	62.5	3.93	62.2
6b					3.75		3.74	
					Glucuronic (D)		Glucuronic (D)	
1					4.46	105.1	4.45 ($J_{1,2}$=7.8)	105.0
2					3.48	83.6	3.47	83.5
3					3.75	77.9	3.70	78.2
4					3.53	72.9	3.49	72.8
5					3.64	76.4	3.55	76.5
6						178.1		176.8

[a] When two values are shown, the first is the equatorial proton, and the second the axial one.

On acid hydrolysis, glucose and fucose were obtained as the only sugars. As only a methyl doublet was observed in the ^1H NMR spectrum, it was apparent that two units of glucose and one of fucose form part of saponin **1**, with A

and B being glucopyranose units and C the fucopyranose residue. The unequivocal sequence assignment of the sugars was deduced from the HMBC spectrum, where cross-peaks H-1A/C-2C, H-1B/C-3C, and H-1C/C-3 (aglycon) were observed. Compound **1** was thus assigned the structure 3*β*,16*β*,23,29-tetrahydroxy-13,28-epoxyolean-11-en-3*β*-yl *β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside and was given the trivial name sandrosaponin VII.

The FABMS of compound **2** gave a peak at *m*/*z* 1052 in accordance with the molecular formula $C_{48}H_{75}O_{23}S$. The ^{1}H NMR spectrum showed olefinic peaks at δ 6.46 and 5.58 ppm, chemical shifts low enough to suggest conjugation with a second fully substituted double bond. Three sugar anomeric doublets, labeled A−C, five methyl singlets, and a methyl doublet also appeared in the spectrum. The hydroxyl group at C-3 was assigned as equatorial ($J_{2a,3}$ = 11.9; $J_{2e,3}$ = 5.0), with the one at position 16 axial ($J_{15a,16} \approx J_{15e,16} \approx$ 3.1 Hz).

The ^{13}C NMR spectrum of **2** contained 48 singlets, with one of them being carboxylic (183.0 ppm) and four olefinic (138.0, 132.2 127.2, and 126.5 ppm). Analysis of the sugars after acid hydrolysis gave a composition identical to that found for compound **1**. A test for a sulfate group[6] was positive. The sulfate group was deduced to be located at O-4 of unit B, due to the strong deshielding observed for H-4 of that residue. Consequently, the structure 3*β*,16α,23,29-tetrahydroxy-11,13(18)-oleanedien-3*β*-yl-30-oic acid *β*-D-glucopyranosyl-(1→2)-[4-sulfate-*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside was deduced for compound **2**, which has been given the trivial name sandrosaponin VIII.

The ^{1}H NMR spectrum of compound **3** showed a triplet at 5.25 ppm (J = 3.5 Hz), typical for an olefinic proton of an oleanene system. The FABMS supported a molecular formula of $C_{54}H_{87}O_{24}$. Four doublets at 5.37, 4.72, 4.62, and 4.46 ppm (J = 7−8 Hz) suggested the presence of four sugar units with *β* configurations. Seven methyl singlets were also observed in the region 0.8−1.2 ppm. The ^{13}C NMR spectrum contained 54 singlets, with two of those at ca. 178 ppm, corresponding to two carboxyl groups, and two (123.8 and 144.8 ppm) representative of a double bond. The HMQC experiments allowed all the direct connectivities to be established and showed only one aglycon proton linked to an oxygen-bearing carbon, corresponding to position 3. The configuration of the different protons and carbons was inferred from ROESY and HMBC experiments, which led also to the assignment of the quaternary carbons (see Table 1).

Concerning the sugar units of **3**, labeled A−D from low to high field, hydrolysis and comparison with authentic samples led to identification of the sugars as glucose, galactose, and glucuronic acid. One of the anomeric carbons appeared at 95.7 ppm, suggesting a linkage to a carboxyl group.[7] In addition, comparison of the chemical shift and coupling constant values obtained for the four residues (Table 1) with standard values[8] suggested that position 2 of both the Gal*p* and Glc*p*A units were the glycosylation sites, with both having Glc*p* moiety terminal residues.

The unequivocal linkage sequence for **3** was provided by a HMBC experiment, which displayed the cross-peaks H-1A/C-28, H-1C/C-2B, H-1B/C-2D, and H-1D/C-3 (aglycon), which demonstrated that the saponin **3** was 3-*β*-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-oleanolic acid 28-*O*-*β*-D-glucopyranoside (sandrosaponin IX).

Compound **4** displayed a ^{1}H NMR spectrum very similar to that of compound **3**, with a triplet at 5.20 ppm (J = 3.6 Hz, olefinic) and seven methyl singlets in the high-field region. A significant difference was the absence of the anomeric doublet at 5.37 ppm. The ^{13}C NMR spectrum contained six carbons less (48 singlets) than **3**, and the chemical shifts values of the carbons were within the range of 1 ppm of those found for compound **3**, with the exception of C-28, which appeared at 183.0 ppm (cf. δ 178.1 for **3**). These observations indicated therefore the absence of any Glc*p* moiety at the carboxyl group. As additional proof, compound **3** was hydrolyzed with KOH, giving compound **4** and glucose. All these data allowed compound **4** to be assigned the structure 3-*β*-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyloleanolic acid (sandrosaponin X).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a sodium lamp operating at 598 nm. NMR spectra were recorded in CD_3OD, on a Varian Unity 500 instrument at 25 °C. Chemical shifts were referenced to the methanol-d_4 multiplet (^{1}H, 3.30 ppm; ^{13}C, 49.0 ppm). FABMS 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−AcOH−H_2O (4:1:5), and visualized with 1% vanillin in MeOH−H_2SO_4 (1:1). Sugars were identified by chromatographic comparison with authentic samples of D-glucose, d-glucuronic acid, and D-galactose.

Plant Material. Roots of *Bupleurum rigidum* were collected in Guadalajara, Spain, in March 1997, and were identified by Dr. C. Bartolomé, Departamento de Biología Vegetal, Facultad de Ciencias, Universidad de Alcalá de Henares, Madrid, Spain. A voucher specimen has been deposited at the herbarium of the Alcalá University, No AH25638.

Extraction and Isolation. Dried roots of *B. rigidum* (600 g) were powdered and extracted with acetone at room temperature. The acetone extract was concentrated to dryness under a vacuum, affording a mixture containing polyacetylenes (14.86 g), which was not further investigated. Subsequently, the mixture was extracted with 60% MeOH for 24 h. After removal of the MeOH under a vacuum, the resulting aqueous solution was extracted with *n*-BuOH saturated with H_2O.

The *n*-BuOH extract (13 g) was submitted to column chromatography on Si gel and eluted with $CHCl_3$−MeOH−H_2O (80:20:0.5→70:30:3→55:37:5) to afford 20 fractions (B1−B20). Four fractions (fractions 17−20) were separated and studied further. All were chromatographed on Si gel using mixtures of MeOH−H_2O of increasing polarity as eluents. Fraction 17 (0.847 g) afforded buddlejasaponin IV (6 mg), fraction 18 (1.77 g) afforded sandrosaponin VII (**1**, 8.9 mg), fraction 19 (0.266 g) afforded sandrosaponin VIII (**2**, 4.2 mg), and fraction 20 (0.617 mg) afforded sandrosaponins IX (**3**, 57.5 mg) and X (**4**, 9 mg).

3*β*,16*β*,23-Trihydroxy-13,28-epoxiolean-11-en-3*β*-yl *β*-D-Glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside (Buddlejasaponin IV). This compound was identified by spectral data comparison with literature values and also using an authentic sample.[4,5]

3*β*,16*β*,23,29-Tetrahydroxy-13,28-epoxyolean-11-en-3*β*-yl *β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside (Sandrosaponin VII, 1): amorphous powder; $[\alpha]_D$ + 23° (*c* 0.2 MeOH); ^{1}H and ^{13}C NMR see Table 1; FABMS *m*/*z* 981.7 $[M + Na]^+$.

3*β*,16α,23,29-Tetrahydroxy-11,13(18)-dien-3*β*-yl-30-oic acid *β*-D-glucopyranosyl-(1→2)-[4-sulfate-*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranoside (Sandrosaponin VIII, 2): amorphous powder; $[\alpha]_D$ + 6.6° (*c* 0.2 MeOH); ^{1}H and ^{13}C NMR see Table 1; FABMS *m*/*z* 1053 $[M]^+$.

3*β*-*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-

glucopyranoside (Sandrosaponin IX, 3): amorphous powder; $[\alpha]_D + 5.29°$ (*c* 0.15 MeOH); ^1H and ^{13}C NMR see Table 1; FABMS *m*/*z* 1141.5 $[M]^+$.

3*β*-*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl oleanolic acid (Sandrosaponin X, 4): amorphous powder; $[\alpha]_D + 17.7°$ (*c* 0.10 MeOH); ^1H and ^{13}C NMR see Table 1; FABMS *m*/*z* 979.7 $[M]^+$.

Alkaline Hydrolysis of Compound 3. The saponin (2 mg) in KOH 10% (2 mL) was heated at 100 °C in a sealed tube for 75 min. After acidification with HCl (pH 5), the monodesmoside was extracted with *n*-BuOH. Comparison with compound **4** demonstrated that both compounds were identical. The aqueous solution contained glucose, identified by TLC comparison with an authentic sample.

Acid Hydrolysis of Compounds 1−4. Each sample (1−2 mg) was refluxed with 10% HCl (4 mL) for 4 h. After extraction with diethyl ether, the aqueous solution was treated with $BaCl_2$ to give a white precipitate ($BaSO_4$), according to Akai et al.[6] 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. Glucose, fucose, galactose, and glucuronic acid were identified by TLC comparison with authentic samples.

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