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TRITERPENOID SAPONINS FROM *BUPLEURUM FRUTICOSUM*

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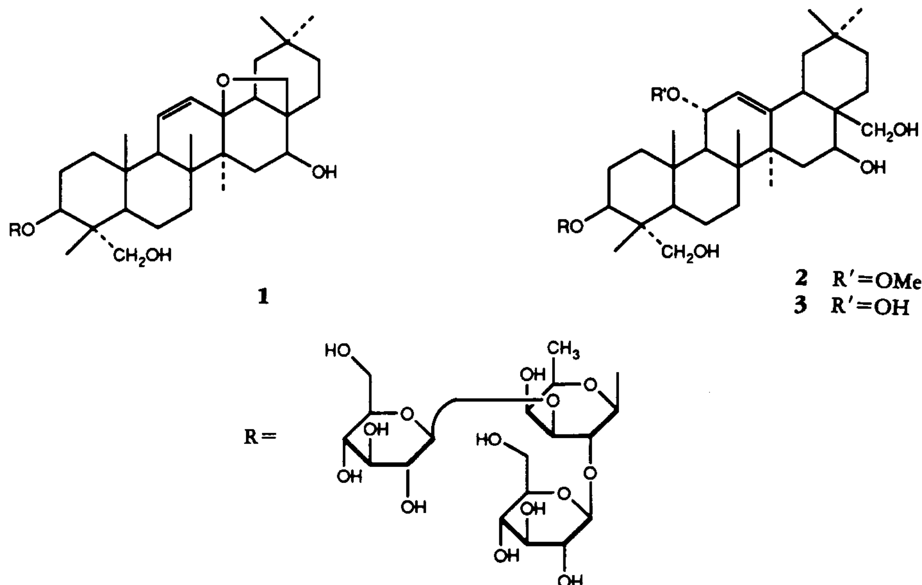
ABSTRACT.—Three triterpenoid saponins **1–3** of the oleanane series, containing one fucose and two glucose units, were isolated from the MeOH extract of the roots of *Bupleurum fruticosum*. Their structures were elucidated by means of fabms and a combination of homo- and heteronuclear 2D nmr techniques. Two of the three saponins are new.

Bupleuri Radix [roots of *Bupleurum* spp. (Umbelliferae)] is a well known crude drug used in oriental traditional medicine as an anti-inflammatory and anti-hepatotoxic agent (1,2). Previous studies on *Bupleurum fruticosum* L. deal only with the composition and biological activity of the essential oil (3,4), and no evidence is available in the literature concerning other constituents, for instance, triterpenoids.

This paper deals with the isolation of three saponins **1**, **2**, and **3** and their structure elucidation, mainly performed by means of 1D and 2D nmr techniques, which permitted assignment of all ^{1}H and ^{13}C signals, providing unambiguous information about the aglycone structure and the nature, position of the glycosidic linkage, and sequence of the monosaccharides in the sugar moiety.

RESULTS AND DISCUSSION

The plant roots were extracted with MeOH at room temperature. After evaporation



of the solvent, the aqueous solution of the residue was washed with Et_2O and extracted with *n*-BuOH. The *n*-BuOH extract was concentrated to dryness to give a crude saponin fraction (1.9% with respect to the dried drug), which was chromatographed to afford saponins **1**, **2**, and **3**.

The fabms (in negative ion mode) permitted assignment of the following molecular formulas: **1**, $C_{48}H_{78}O_{18}$; **2**, $C_{49}H_{82}O_{19}$; **3**, $C_{48}H_{80}O_{19}$.

The ^{1}H-nmr spectra indicated that compounds **1**, **2**, and **3** had identical sugar moieties. In addition, the fabms of each saponin exhibited a fragment ion (at *m/z* 471 for **1**, at *m/z* 503 for **2**, at *m/z* 489 for **3**) corresponding to the molecular mass of the aglycone and consistent with the loss of two hexose and one deoxyhexose moieties (470 mass units) from the quasi-molecular ion. Analysis of the ^{1}H (Experimental) and ^{13}C (Table 1) nmr of saponin **1** suggested the identity of the aglycone moiety with saikogenin F and C-3 as the site of the glycosidic linkage (5). Finally, acid hydrolysis of the

TABLE 1. ^{13}C-nmr Chemical Shifts and DEPT Data of Aglycone Moieties.[a]

Carbon	Compound					
	1[b]		**2**[c]		**3**[b]	
	^{13}C	DEPT	^{13}C	DEPT	^{13}C	DEPT
C-1	38.29	CH_2	39.88	CH_2	41.64	CH_2
C-2	25.77	CH_2	26.22	CH_2	26.72	CH_2
C-3	83.99	CH	82.51	CH	84.38	CH
C-4	44.40	C	43.53	C	44.57	C
C-5	47.85	CH	47.92	CH	48.33	CH
C-6	18.06	CH_2	17.68	CH_2	18.53	CH_2
C-7	31.85	CH_2	32.96	CH_2	33.71	CH_2
C-8	42.92	C	40.80	C	41.30	C
C-9	52.72	CH	53.00	CH	55.09	CH
C-10	36.00	C	37.66	C	38.11	C
C-11	130.80	CH	76.60	C	67.39	C
C-12	135.83	CH	122.29	CH	127.66	CH
C-13	85.31	C	148.04	C	146.00	C
C-14	46.45	C	43.42	C	44.76	C
C-15	34.94	CH_2	36.67	CH_2	34.72	CH_2
C-16	65.08	CH	66.00	CH	68.50	CH
C-17	47.00	C	43.67	C	43.67	C
C-18	53.67	CH	43.78	CH	44.25	CH
C-19	39.03	CH_2	46.76	CH_2	47.00	CH_2
C-20	32.00	C	30.91	C	31.69	C
C-21	35.73	CH_2	34.04	CH_2	36.52	CH_2
C-22	26.12	CH_2	25.74	CH_2	25.74	CH_2
C-23	64.69	CH_2	64.00	CH_2	64.38	CH_2
C-24	12.30	Me	13.04	Me	13.06	Me
C-25	18.50	Me	17.68	Me	17.01	Me
C-26	19.88	Me	18.14	Me	18.14	Me
C-27	20.21	Me	26.06	Me	26.06	Me
C-28	73.11	CH_2	68.00	CH_2	68.00	CH_2
C-29	33.65	Me	33.07	Me	33.63	Me
C-30	23.80	Me	23.80	Me	24.18	Me
			51.94	OMe		

[a]In ppm, TMS as internal standard.
[b]Recorded in MeOH-d_4.
[c]Recorded in pyridine-d_5.

saponins gave glucose and fucose as sugar components. These results permitted us to conclude that the sugar moiety linked at C-3 in all three substances was a trisaccharide formed by one fucose and two glucose units.

Structures of the aglycone of saponins **2** and **3** were deduced from the ^{1}H- and ^{13}C-nmr spectra. Based on a comparison of its spectral data, the aglycone of **2** appeared to be identical with that contained in saikosaponin b_4 (6). The ^{1}H- and ^{13}C-nmr spectra showed that the signals of the aglycone of **3** were almost superimposable with those of the aglycone of **2**, the only significant differences being the absence of a methoxyl group and a shift of the carbinolic C-11 signal. Thus, the structural difference between the aglycones of **2** and **3** was the presence, at position 11, of a $CHOCH_3$ in the former and a CHOH group in the latter.

The identity of sugar units, the β-pyranosidic form, and sequence in the trisaccharide bound at C-3 in saponins **1**, **2**, and **3** were deduced by a combination of 2D proton-proton correlation spectroscopy (COSY), 2D homonuclear Hartman-Hahn (HOHAHA) (7), 2D ^{1}H-^{13}C one bond (HETCOR), and proton-detected multiple bond correlation (HMBC) (8,9) nmr experiments. Moreover, COSY and HOHAHA permitted assignment of all spin correlations from H-1 to H-6 of the fucose and glucose units and, together with results obtained from HETCOR and from literature data (10), permitted us to establish that the two glucose units were terminal and bound to C-2 and C-3 of fucose (the sugar directly linked to the aglycone). These deductions were confirmed by HMBC experiments (Table 2), which showed correlation of the fucose H-1 with the aglycone C-3, of the H-1 of a glucose unit with a fucose C-2, and of the H-1 of

TABLE 2. 1D and 2D nmr Experiments for Sugar Moiety (MeOH-d_4).

Sugar	δ ^{1}H[a]	δ ^{13}C[b]	DEPT	H-H COSY	HMBC[c]
Fucose					
1	4.50 (d, J = 7.5 Hz)	104.65	CH	3.94	83.99
2	3.94 (dd, J = 7.5 and 9.8 Hz)	76.70	CH	3.81/4.50	
3	3.81 (dd, J = 3.4 and 9.8 Hz)	85.20	CH	3.94/3.91	
4	3.91 (dd, J = 3.4 and 0.9 Hz)	72.86	CH	3.81/3.68	
5	3.68 (m)	70.98	CH	3.91/1.28	
6	1.28 (d, J = 6.5 Hz)	16.50	Me	3.68	
Glucose					
1′	4.88 (d, J = 7.8 Hz)	103.18	CH	3.15	85.20
2′	3.15 (dd, J = 7.5 and 9.0 Hz)	75.77	CH	4.88/3.34	
3′	3.34[d]	77.70	CH	3.15/3.12	
4′	3.12 (dd, J = 9.0, and 9.0 Hz)	72.06	CH	3.34/3.29	
5′	3.29[d]	77.79	CH	3.12/3.56/3.83	
6′	3.56 (dd, J = 11.2 and 4.5 Hz)	62.93	CH_2	3.83/3.29	
	3.83 (dd, J = 11.2 and 2.5 Hz)			3.56/3.29	
Glucose					
1″	4.62 (d, J = 7.3 Hz)	105.60	CH	3.34	76.70
2″	3.34[d]	75.01	CH	4.62/3.35	
3″	3.35[d]	77.79	CH	3.34/3.36	
4″	3.36 (dd, J = 9.5 and 9.5 Hz)	71.00	CH	3.35/3.38	
5″	3.38 (m)	78.20	CH	3.36/3.69/3.82	
6″	3.69 (dd, J = 11 and 5.6 Hz)	62.13	CH_2	3.38/3.82	
	3.82 (dd, J = 11 and 2.5 Hz)			3.69/3.38	

[a]Assignments confirmed by combination of COSY and HOHAHA results.
[b]Assignments confirmed by 2D-^{13}C,^{1}H direct cross correlation spectroscopy (HETCOR).
[c]Selected data connectivities observed across the glycosidic linkages.
[d]Overlapping.

another glucose unit with the fucose C-3. Thus, the sugar sequence in the trisaccharide bound to C-3 of the aglycones of **1**, **2**, and **3** is as indicated.

A question remains to be answered at this point: do both minor saponins derive from the major one during extraction and isolation and are they, therefore, artifacts? Although the presence in saponins **2** and **3** of the same trisaccharide might indicate that both compounds are artifacts, we prefer to consider that saponin **3** (if not even **2**) is an authentic product contained as such in the plant. Our belief is based on three observations: (i) the ratio **1**:**2**:**3** was constant when comparison was made among various extractions; (ii) no diene-type artifacts were isolated from the extracts, as observed by Ding *et al.* (11); (iii) to our knowledge, artifacts like **3** have not been isolated from *Bupleurum* previously.

While our research work was completed and this manuscript was still in preparation, Yamamoto *et al.* (12) reported on the constituents of an extract of *Buddleja japonica*, describing the isolation and structure elucidation of buddlejasaponin IV, which appears to be identical to saponin **1**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr measurements were performed on a Bruker AMX 500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. The samples were prepared by dissolving 10 mg of sample in 0.4 ml of pyridine-d_5 or in 0.4 ml of MeOH-d_4.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a sodium lamp operating at 589 nm. Fabms was recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe atoms of energy of 2-6 KV).

Lobar RP 8 reversed-phase (40-63 μm Merck) and Sephadex LH-20 (Pharmacia) were used for cc. Tlc was performed on RP 8 reversed-phase precoated layers (Merck).

PLANT MATERIAL.—The roots of *B. fruticosum* were collected in June 1989, near Sassari, Sardinia. A voucher specimen is deposited in the Herbarium of the Istituto di Botanica Farmaceutica, Università di Sassari, Italy.

EXTRACTION AND ISOLATION OF COMPOUNDS **1–3**.—Powdered air-dried roots (2630 g) were extracted with MeOH at room temperature. The extract was concentrated under reduced pressure and the residue washed with Et_2O. The light brown powder (63 g) was dissolved in H_2O and successively partitioned with *n*-BuOH. The *n*-BuOH solution was evaporated to afford a crude saponin mixture (50.4 g). This mixture was chromatographed on a Sephadex LH-20 column, using MeOH as mobile phase. The saponin fractions (200 mg) [monitoring by tlc on Si gel using $CHCl_3$-MeOH-H_2O (6:4:1) as eluent; Komarowsky spray] was rechromatographed on a Lobar RP 8 column with MeOH-H_2O (7:3) to yield three pure compounds **1** (100 mg), **2** (13 mg), and **3** (30 mg).

Saponin **1**.—3β,16β,23-Trihydroxy-13,28-epoxyolean-11-en-3β-yl-[β-D-glucopyranosyl(1↦2)]-[β-D-glucopyranosyl(1↦3)]-β-D-fucopyranoside: $C_{48}H_{78}O_{18}$; scales; mp 255–258°; $[\alpha]^{22}D$ +28.84 (c = 1.38, pyridine); RP 8 tlc R_f 0.22; fabms m/z $[M-H]^-$ 941, $[M-H-470]^-$ 471; ^1H nmr aglycone (MeOH-d_4) δ ppm 0.75 (3H, s, Me-24), 0.94 (3H, s, Me-30), 0.97 (3H, s, Me-25), 1.00 (3H, s, Me-29), 1.06 (3H, s, Me-27), 1.12 (3H, s, Me-26), 1.20 (1H, m, H-5), 1.24 (3H, m, H_a-15, H_b-15, H_a-7), 1.31 (2H, H_a-1 and H_b-1), 1.34 (1H, H_a-2), 1.48 (1H, H_a-21), 1.49 (1H, H_a-6), 1.56 (2H, H_b-6 and H_b-7), 1.61 (1H, H_b-21), 1.82 (1H, H-9), 1.83 (1H, H_a-19), 1.91 (1H, H-18), 1.94 (1H, H_b-19), 2.02 (1H, H_b-2), 2.13 (2H, H_a-22 and H_b-22), 3.13 (1H, d, J = 10.7 Hz, H_a-28), 3.28 (1H, d, J = 11.2 Hz, H_a-23), 3.65 (1H, H-3), 3.72 (1H, d, J = 10.7 Hz, H_b-28), 3.81 (1H, d, J = 11.2 Hz, H_b-23), 4.21 (1H, dd, J = 9.5 and J = 3.5 Hz, H_a-16), 5.40 (1H, dd, J = 3.5 and 12.0 Hz, H-11), 5.98 (1H, d, J = 3.5 Hz, H-12); for sugar ^1H and ^{13}C nmr (MeOH-d_4) see Table 2.

Saponin **2**.—16α,23,28-Trihydroxy-11α-methoxyolean-12-en-3β-yl-[β-D-glucopyranosyl(1↦2)]-[β-D-glucopyranosyl(1↦3)]-β-D-fucopyranoside: $C_{49}H_{82}O_{19}$; white powder; mp 245–253; $[\alpha]^{22}D$ + 16.04 (c = 0.86 pyridine); RP 8 tlc R_f = 0.29; fabms m/z $[M-H]^-$ 973, $[M-H-470]^-$ 503; ^1H nmr aglycone (MeOH-d_4) δ ppm 0.77 (3H, s, Me-24), 0.94 (3H, s, Me-29), 0.96 (3H, s, Me-30), 1.10 (3H, s, Me-26), 1.14 (3H, s, Me-25), 1.19 (1H, H_a-19), 1.22 (1H, H_a-1), 1.28 (1H, H_a-15), 1.29 (1H, H-5), 1.32 (1H, H_a-7), 1.37 (3H, s, Me-27), 1.41 (1H, H_a-6), 1.42 (1H, H_a-21), 1.44 (1H, H_b-15), 1.55 (1H, H_b-6), 1.65 (1H, H-9), 1.72 (2H, H_b-7 and H_b-19), 1.74 (1H, H_b-21), 1.93 (1H, H_b-2), 2.13 (2H, H_a-22 and H_b-22), 2.17 (1H, H_b-1), 2.30 (1H, dd, J = 3.5 and J = 8.5 Hz, H-18), 3.30 (1H, H_a-23), 3.31 (1H, d, J = 8.7 Hz, H_a-28), 3.64 (1H, H-3), 3.80 (1H, H_b-23), 3.83 (1H, d, J = 8.75 Hz,

H_b-28), 4.19 (1H, dd, $J=3$ and 10 Hz, H_a-16), 4.35 (1H, dd, $J=5$ and 12.5 Hz, H-11), 5.23 (1H, d, $J=3.5$ Hz, H-12), 3.34 (3H, s, OMe).

Saponin **3**.—11α,16β,23,28-Tetrahydroxyolean-12-en-3β-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside: $C_{48}H_{80}O_{19}$; scales; mp 230 (dec); $[\alpha]^{22}D+16.34$ ($c=0.82$, MeOH); RP 8 tlc $R_f=0.50$; fabms *m/z* $[M-H]^-$ 959, $[M-H-470]^-$ 489; ^{1}H nmr aglycone (MeOH-d_4) δ ppm 0.77 (3H, s, Me-24), 0.94 (3H, s, Me-29), 0.96 (3H, s, Me-30), 1.10 (3H, s, Me-26), 1.14 (3H, s, Me-25), 1.19 (1H, H_a-19), 1.22 (1H, H_a-1), 1.28 (1H, H_a-15), 1.29 (1H, H-5), 1.32 (1H, H_a-7), 1.37 (3H, s, Me-27), 1.41 (1H, H_a-6), 1.42 (1H, H_a-21), 1.44 (1H, H_b-15), 1.55 (1H, H_b-6), 1.65 (1H, H-9), 1.72 (2H, H_b-7 and H_b-19), 1.74 (1H, H_b-21), 1.93 (1H, H_b-2), 2.13 (2H, H_a-22 and H_b-22), 2.17 (1H, H_b-1), 2.30 (1H, H-18), 3.30 (1H, H_a-23), 3.31 (1H, d, $J=8.75$ Hz, H_a-28), 3.64 (1H, H-3), 3.80 (1H, H_b-23), 3.83 (1H, d, $J=8.7$ Hz, H_b-28), 4.19 (1H, dd, $J=3$ and 10 Hz, H_a-16), 4.39 (1H, dd, $J=5$ and 12.5 Hz, H-11), 5.23 (1H, d, $J=3.7$ Hz, H-12).

ACID HYDROYLSIS OF SAPONIN **1**.—A mixture containing 1 ml of 1 N HCl, 1 ml of dioxane, and 10 mg of **1** was heated in a sealed tube at 90° for 4 h. H_2O (5 ml) was added, and the aglycone was removed by extracting with 10 ml of $CHCl_3$. The aqueous layer was neutralized with Amberlite IRA 400 (OH^- type) and evaporated to dryness. The sugar samples were directly analyzed by tlc. Glucose and fucose were identified by comparison with authentic samples.

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