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TRITERPENOID SAPONINS AND FLAVONOID GLYCOSIDES FROM *BUPLEURUM FALCATUM* SUBSP. *CERNUUM*

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Key Word Index—*Bupleurum falcatum* subsp. *cernuum*; Umbelliferae; triterpenoid and flavonoid glycosides.

Abstract—A new triterpenoid saponin, 16α,23,28,30-tetrahydroxyolean-11,13(18)-dien-3β-yl-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside, has been isolated from *Bupleurum falcatum* subsp. *cernuum*. Its structure was mainly elucidated by spectral analysis. Among known compounds, three other saponins and three flavonoid glycosides were also isolated from the plant.

INTRODUCTION

Plants belonging to the genus *Bupleurum* are well known traditional Chinese drugs and have been accepted for medical use in China for more than two thousand years. Triterpene glycosides of the oleanane series (the so called saikosaponins) are considered as the major bioactive components of the crude drug, mainly used for its anti-inflammatory and anti-hepatotoxic activity [1, 2].

In the course of our investigations on the chemical constituents of some *Bupleurum* species of the Italian flora, we have taken into consideration *Bupleurum falcatum* subsp. *cernuum*; we report here on the isolation and structure elucidation of a new triterpenoid saponin, 16α,23,28,30-tetrahydroxyolean-11,13(18)-dien-3β-yl-β-D-glucopyranosyl-(1→3)β-D-fucopyranoside (**1**). Saikosaponins b_2, b_3 and b_4 and the flavonoid glycosides rutin, avicularin (**2**) and guaijaverin (**3**) have also been found in the plant.

RESULTS AND DISCUSSION

The residue obtained on evaporation of the methanolic extract of the whole plant was dissolved in water and the aqueous solution extracted with ethyl acetate and successively with *n*-butanol. Working-out of the butanolic extract led to isolation of saponin **1** along with the three known saikosaponins b_2, b_3 and b_4 [3, 4].

Elemental analysis of compound **1** afforded values consistent with the formula $C_{42}H_{68}O_{14}$. The substance exhibited IR bands at 3480 (OH) and 1640 (C=C) cm^{-1}; its UV adsorptions at 243, 252 and 260 nm indicated its heteroannular diene nature [5]; the FAB mass spectrum (glycerol, positive ion mode) gave quasi molecular ion species at *m/z* 797 $[M+H]^+$ and 819 $[M+Na]^+$, in agreement with the formula indicated above. The spectral data are reported in the Experimental section.

Concerning the most representative signals in the NMR spectra of the aglycone moiety, the ^1H NMR spectrum, in methanol-d_4, showed five tertiary methyl singlets between δ0.60 and 1.15; the olefinic signals at δ5.49 and 6.36 were assigned to H-11 and H-12. The ^{13}C signals of the olefinic carbons were located at δ126.7, 127.1, 131.9 and 137.7. These data, and a comparison with the NMR spectra of saikosaponin b_2 [4], permitted the deduction that compound **1** is, very probably, a glycoside of an olean-11,13(18)-diene.

The ^{13}C NMR spectrum also showed four signals (δ74.1, 69.0, 65.0 and 62.7) that, on the basis of DEPT experiments, were attributed to one CHOH and three CH_2OH groups. When the spectrum was recorded in pyridine-d_5, and a careful comparison was made between the data obtained from **1** and the spectra of both saikosaponin S24 [6] and saikosaponin BK2 [7], it was possible to deduce that the four hydroxyl groups were attached to C-16, C-23, C-28 and C-30. Indeed, in the spectrum of saponin **1**, the signals originated by the carbon atoms of rings A, B, C and D, and of their substituents resonate at the same frequency as those reported for saikosaponin S24, while the signals due to the carbons of ring E and of its substituents are coincident with those of saikosaponin BK2. The presence of five acylable hydroxyl groups in the free aglycone was confirmed by acetylation of the hydrolysed product: a pentaacetate was obtained, exhibiting five distinct acetyl singlets in the ^1H NMR spectrum.

The sugars contained in **1** appeared to be glucose and fucose, as deduced by TLC analysis after acid hydrolysis.

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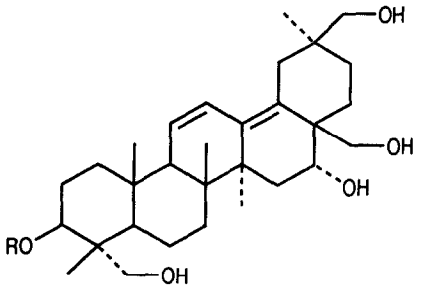
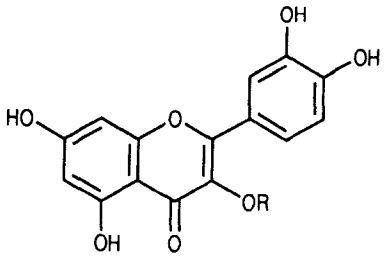


1 R= β-D-Glc*p*-(1→3)-β-D-Fuc*p*



2 R= α-L-Ara*f*
3 R= α-L-Ara*p*

The β-configurations of the two sugar units were indicated by the shifts of the two anomeric C-atoms and by the δ and *J* values of the anomeric protons (doublets with *J* =7.1 Hz). In addition, the site of the glycosylation appeared to be C-3 as shown by the significant downfield shift observed in the ^{13}C NMR spectrum and comparison with analogous saponins [4, 6, 7].

The FAB-mass spectrum showed a fragment ion at *m/z* 617 derived from the loss of one glucose molecule and a peak at *m/z* 471 due to the subsequent loss of one fucose molecule. This revealed that fucose was the inner sugar unit in the disaccharide chain. The interglycosidic linkage between glucose and fucose was deduced from the downfield shift of fucose C-3′ (10 ppm), as compared with a model compound [8].

Since no acidic conditions were employed during extraction and isolation, we can reasonably assume that compound **1** is a primary constituent of the plant, and not an artifact derived from the allyl ether precursor [3, 4]. In addition to **1**, the three known saikosaponins b_2, b_3 and b_4 [3, 4] were also isolated and identified by comparison with authentic samples.

From the ethyl acetate extract, rutin and two other quercetin glycosides, avicularin (3-α-L-arabinofuranoside) (**2**) and guaijaverin (3-α-L-arabinopyranoside) (**3**) were isolated and identified. Their structures were confirmed by NMR spectral analysis and acid hydrolysis (Experimental). Both compounds have been isolated from *Andromeda polifolia* [9]; this is the first report of their presence in the genus *Bupleurum*.

EXPERIMENTAL

UV spectra were recorded in MeOH; IR spectra as nujol mulls. ^1H and ^{13}C NMR were recorded at 200 and 50 MHz, respectively, in MeOH-d_4 and pyridine-d_5 (TMS as an int. standard). Carbon multiplicities were determined by DEPT 90° or 135° pulse sequence. FAB-MS: positive ion mode, glycerol as matrix. TLC was carried out on silica gel, RP-8 and polyamide plates (Merck). Lobar RP-8 (Merck) was used with a Duramat pump.

Plant material. Bupleurum falcatum subsp. *cernuum* was collected in June 1989 in Italy (massiccio Catria, Marche country). A voucher specimen is deposited in the Herbarium of the Istituto di Botanica e Orto Botanico, Università di Urbino.

Extraction and isolation of saponins. The air-dried and powdered whole plant (265 g) was successively extracted with *n*-hexane and MeOH at room temp. The MeOH extract, on removal of the solvent under red. pres. yielded a viscous dark mass (30 g), which was taken-up in H_2O and extracted with EtOAc and *n*-BuOH. The organic layers were concd to dryness under red. pres. to give an EtOAc residue (6.2 g) and a butanolic residue (6.8 g), respectively. The latter was chromatographed, first on Sephadex LH-20 (MeOH), then on Lobar RP-8 with MeOH–H_2O (7:3), to yield compound **1** (18 mg) as pure product. The other fractions contained mixtures of saikosaponins b_2, b_3 and b_4, which were identified by co-TLC with authentic samples.

Saponin **1**. $[\alpha]_D^{20} = -14.2°$ (MeOH; *c* 0.97). (Found: C, 63.22; H, 8.35%; $C_{42}H_{68}O_{14}$ requires: C, 63.31; H, 8.54%). UV λ_{max} nm: 243, 252, 260; IR ν_{max} cm^{-1}: 3480, 2920, 1640, 900; ^1H NMR (MeOH-d_4): δ0.60, 0.63, 0.74, 0.85, 1.15 (3H each, *s*, Me × 5), 1.17 (3H, *d*, *J*=6.4 Hz, Me-fuc), 4.30 (1H, *d*, *J*=7.0 Hz, H-1′), 4.44 (1H, *d*, *J*=7.1 Hz, H-1″), 5.49 (1H, *dd*, *J*=11.0 Hz and *J*=3.9 Hz, H-11), 6.36 (1H, *d*, *J*=11.0 Hz, H-12); ^{13}C NMR (MeOH-d_4/pyridine-d_5): δ39.0/38.3 (CH_2-1), 26.1/26.1 (CH_2-2), 83.3/81.6 (CH-3), 43.9/43.6 (C-4), 48.1/47.3 (CH-5), 18.9/18.8 (CH_2-6), 31.9/32.3 (CH_2-7), 41.8/41.0 (C-8), 54.8/54.0 (CH-9), 37.3/36.4 (C-10), 127.1/126.1 (CH-11), 126.7/126.4 (CH-12), 137.7/136.7 (C-13), 42.4/41.9 (C-14), 32.9/31.9 (CH_2-15), 69.0/67.8 (CH-16), 45.7/45.8 (C-17), 131.9/132.9 (C-18), 39.9/33.9 (CH_2-19), 38.3/38.2 (C-20), 33.8/30.2 (CH_2-21), 23.9/24.2 (CH_2-22), 62.7/64.0 (CH_2-23), 12.8/13.1 (Me-24), 19.1/18.8 (Me-25), 17.5/17.2 (Me-26), 20.6/21.9 (Me-27), 65.0/64.9 (CH_2-28), 22.1/21.1 (Me-29), 74.1/73.4 (CH_2-30), 105.5/106.0 (CH-1′), 71.8/71.5 (CH-2′), 85.1/85.2 (CH-3′), 72.3/71.8 (CH-4′), 71.1/71.0 (CH-5′), 16.9/17.2 (Me-6′), 105.6/106.7 (CH-1″), 75.3/75.8 (CH-2″), 77.8/78.8 (CH-3″), 72.3/72.1 (CH-4″), 77.8/78.4 (CH-5″), 62.5/62.6 (CH_2-6″). Positive FAB-MS *m/z*: 819 $[M+Na]^+$, 797 $[M+H]^+$, 617 $[M+H-Glc]^+$, 471 $[M+H-Glc-Fuc]^+$, 779 $[M+H-H_2O]^+$, 766 $[M+H-CH_2OH]^+$, 748 $[M+H-H_2O-CH_2OH]^+$, 181, 179.

Acid hydrolysis of compound **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 hr, then 5 ml H_2O was added and the aglycone was removed by extracting with 10 ml $CHCl_3$. The aq. layer was neutralized with Amberlite IRA 400 (OH^- type) and evaporated to dryness. The sugar samples were directly analysed by TLC; glucose and fucose were identified by comparison with authentic samples.

Acetylation of aglycone **1a**. The genin (5 mg) obtained by hydrolysis, was acetylated with Ac_2O and pyridine to give genin pentaacetate (5 mg). IR ν_{max} cm^{-1}: 1700; ^1H NMR ($CDCl_3$): δ2.03, 2.05, 2.06, 2.07, 2.09 (3H each, 5×COMe).

Isolation of flavonoids. The EtOAc residue was chromatographed on Sephadex LH-20 using MeOH–$CHCl_3$ (9:1) to give 5 fractions. Fr. 2, after precipitation, afforded compound **2** (13 mg). The flavonoid mixture contained in frs 3–5 was subjected to CC over polyamide eluted with toluene–EtOAc–MeOH (3:1:1) to give compounds **3** (21 mg) and rutin (74 mg). This last compound was identified by comparison of its R_f with an authentic sample.

Compound **2**. UV λ_{max} nm: 369; MeOH/$AlCl_3$ nm: 436; MeOH/$AlCl_3$/HCl nm: 436; ^1H NMR (MeOH-d_4): δ3.40–4.24 (5H, *m*, pentose), 5.36 (1H, *d*, *J*=0.9 Hz, H-1″), 6.10 (1H, *d*, *J*=2.1 Hz, H-8), 6.28 (1H, *d*, *J*=2.1 Hz, H-6), 6.79 (1H, *d*, *J*=8.2 Hz, H-5′), 7.47 (1H, *dd*, *J*=8.5 Hz and *J* =2.0 Hz, H-6′), 7.65 (1H, *d*, *J*=2.0 Hz, H-2′); ^{13}C NMR (MeOH-d_4) aglycone: δ179.9 (C-4), 165.9 (C-7), 163.0 (C-5), 159.3 (C-9), 158.5 (C-2), 149.8 (C-4′), 146.3 (C-3′), 134.9 (C-3), 123.1 (C-6′), 123.0 (C-1′), 116.8 (C-5′), 116.4 (C-2′), 105.6 (C-10), 99.8 (C-6), 94.7 (C-8); sugar: δ109.5 (C-1″), 83.3 (C-2″), 78.6 (C-3″), 87.9 (C-4″), 62.5 (C-5″).

Compound **3**. UV λ_{max}: identical to compound **2**; ^1H NMR (MeOH-d_4): δ3.51–4.1 (5H, *m*, pentose), 5.06 (1H, *d*, *J*=6.6 Hz, H-1″); ^1H and ^{13}C NMR (MeOH-d_4) aglycone: δ see compound **5**; sugar: δ104.6 (C-1″), 72.9 (C-2″), 74.1 (C-3″), 69.1 (C-4″), 67.0 (C-5″).

Hydrolysis of flavonoids **2–3**. Each glycoside (1 mg) was refluxed in 1 M HCl (5 ml) for 2 hr. The aglycone was extracted with EtOAc and identified as quercetin by co-TLC with an authentic sample and by UV spectral analysis with the usual shifts reagents. The aq. layer was neutralized with Amberlite IRA 400 (OH^-) and evapd to dryness. The residue was identified by co-TLC with $CHCl_3$–MeOH–H_2O (6:4:1), detection with TTC: only arabinose was shown to be present.

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