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FLAVONOIDS OF *Bupleurum plantagineum*

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Bupleurum (Apiaceae) species are used in traditional medicine to treat various diseases. Bupleuri Radix (roots of Bupleurum) is one of the most frequently prescribed crude herbs in the prescriptions of traditional Chinese medicine for the treatment of inflammatory and autoimmune diseases [1]. It is used in at least 66% of the formulations/prescriptions in traditional Chinese medicine [2]. *Bupleurum* species have been reported to possess anti-inflammatory [3], antioxidant, and hepatoprotective effects [4–8]. Saikosaponins are the principal secondary metabolite of *Bupleurum* [2, 4–8] in addition to polyacetylenes, terpenoids, and two flavonoids (tamarixetin 3-robinobioside and tamarixetin 3-galactoside) [9] and polysaccharides [10].

Bupleurum plantagineum (Apiaceae), an endemic species [11], was collected around Cap Carbon at Bejaia (Eastern Algerian) in may 2004 and authenticated by Prof. Gerard De Belair (Annaba, Algeria). A voucher specimen was deposited in the Herbarium of the Laboratory of Therapeutic Substances (LOST) at Mentouri University (LOST/Bp/05/04).

Air-dried and powdered aerial parts (1 kg) of *Bupleurum plantagineum* were macerated in a methanolic solution (70%). The extract was successively concentrated to dryness (under low pressure); the residue was dissolved in boiling water and extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol, successively.

The butanolic extract was column chromatographed on polyamid SC6, eluting with toluene–methanol with increasing polarity. Whatman 3MM paper chromatography using 15% AcOH and BAW [*n*-BuOH–AcOH–H_2O, 4:1:5 (upper phase)] and TLC on polyamid DC6, eluting with H_2O–MeOH–methylethylketone–acetylacetone (13:3:3:1), followed by a column flash chromatography over Sephadex LH-20 in MeOH, led to three pure flavonoids **1–3**, which were identified using UV, ^{1}H NMR, ^{13}C NMR, and MS analysis [12–14].

Acid Hydrolysis. The pure compounds were treated with 2 M HCl at 100°C for 1 h. The hydrolysates were extracted with EtOAc, and the aglycones were identified by their UV spectra in methanol and by comparison of their R_f with authentic samples.

Sugars were identified in the aqueous residue by comparison with authentic samples on silica gel TLC impregnated with 0.2 M NaH_2PO_4, solvent Me_2CO–H_2O (9:1), and revealed with aniline malonate.

Compound 1, $C_{15}H_{10}O_7$, yellow needles (acetone), mp >300°C. This compound was characterized as quercetin [12–14].

Compound 2, $C_{27}H_{30}O_{16}$, mp 250–254°C. UV (MeOH, λ_{max}, nm): 257, 300 sh, 356; +NaOH: 272, 325, 407; + $AlCl_3$: 275, 290, 350 sh; +HCl: 268, 285 sh, 350, 390; +NaOAc: 271, 385; +H_3BO_3: 263, 378. FAB$^+$-MS, *m/z* 611 [M + H]$^+$. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.80 (1H, d, J = 2.0, H-2′), 7.75 (1H, dd, J = 9.0, J = 2.0, H-6′), 6.85 (1H, d, J = 9.0, H-5′), 6.30 (1H, d, J = 2.0, H-8), 6.20 (1H, d, J = 2.0, H-6), 5.12 (1H, d, J = 7.0, H-1″ glucose), 4.55 (1H, d, H-1‴rhamnose), 3.20–3.90 (10H, protons of rutinose), 1.1 (3H, d, J = 6.2, H-6‴rhamnose). Acid hydrolysis of **2** produced quercetin, D-glucose, and L-rhamnose. Compound **2** was identified as quercetin-3-*O*-rutinoside [12–14].

Compound 3, $C_{28}H_{32}O_{16}$. UV (MeOH, λ_{max}, nm): 254, 265 sh, 306 sh, 356; +NaOH: 271, 330, 414; +$AlCl_3$: 267, 300 sh, 368, 401 sh; +HCl: 267, 302 sh, 360, 400; +NaOAc: 271, 320, 398; +H_3BO_3: 254, 267 sh, 305 sh, 360. FAB$^+$-MS, *m/z* 625 [M + H]$^+$. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.50 (1H, d, J = 2.0 H-2′), 7.34 (1H, dd, J = 8.5, J = 2.0, H-6′), 6.82 (1H, d, J = 8.5, H-5′), 6.44 (1H, d, J = 2.0, H-8), 6.15 (1H, d, J = 2.0, H-6), 5.30 (1H, d, J = 7.5, H-1″glucose), 4.35 (1H, d, J = 2.0, H-1‴rhamnose), 3.25–3.85 (10H, protons of rutinose), 3.87 (3H, s, 3′-OMe), 0.90 (3H, d, J = 6.5, H-6‴rhamnose).

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Acid hydrolysis of **3** produced isorhamnetin, D-glucose, and L-rhamnose. Compound **3** was identified as isorhamnetin-3-*O*-rutinoside [12–14].

The flavonoids **1–3** are reported for the first time from the the genus *Bupleurum* (Apiaceae).

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