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FLAVONOID AGLYCONES FROM *Centaurea maroccana*

S. Bicha, A. Bentamene,[1] O. Benaissa,[2] S. Benayache,[2] V. P. Garcia,[3] F. Leon,[3] I. Brouard,[3] J. Bermejo,[3] and F. Benayache[1*]

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The genus *Centaurea* (Compositae), which contains more than 700 species, is present in Algeria: 45 species, including 7 in the Sahara [1–3]. As part of our ongoing program of research on plants of this genus [4, 5], we report our results on *Centaurea maroccana* Vahl., an endemic species in the North of the Sahara [3]. In a previous study, we reported on the chemical composition of the ethyl acetate soluble part of the aqueous-MeOH extract of the aerial parts of this plant [6]. The purpose of the present work was the isolation and the structural elucidation of the constituents of the chloroform soluble part of the same extract.

Centaurea maroccana Vahl. was collected from the area of Biskra in the South of Algeria in April 2002 and authenticated by Prof. M. Kaabeche (Biology Department, University of Setif, Algeria). A voucher specimen (CCM12/04/02) has been deposited in the Herbarium of Nature and Life Sciences Department, Mentouri University of Constantine.

Air-dried aerial parts (2700 g) of *C. maroccana* were macerated at room temperature with MeOH–H_2O (80:20 v/v) for 24 h three times. After filtration, the filtrates were combined, concentrated at room temperature, diluted with 1100 mL H_2O, filtered to remove chlorophyll, and successively extracted with $CHCl_3$ and EtOAc. The organic layers were dried with Na_2SO_4 to give, after removal of solvents under reduced pressure, $CHCl_3$ (12.5 g) and EtOAc (20.0 g) extracts. The $CHCl_3$ extract (9.0 g) was chromatographed on a silica gel 60 (230–400 mesh) column (416 g) eluted with a gradient of chloroform–acetone (99:1 to 100% acetone) to yield 49 fractions (F_1–F_{49}) obtained by combining the eluates on the basis of TLC analysis. Fraction F_{21} (580 mg) (95:5) was submitted to preparative TLC on silica gel 60, HF_{254} ($CHCl_3$–CH_3COCH_3, 9:1) to give **1** (100 mg), **2** (90 mg), and **3** (220 mg). Fractions F_{26} (80 mg) (90:10) and F_{37} (100 mg) (85:15), which were submitted to preparative TLC on silica gel HF_{254} ($CHCl_3$–CH_3COCH_3, 9:1 and 8:2, respectively), gave **4** (20 mg) and **5** (25 mg), respectively.

The structures of the isolated compounds were elucidated by UV, ^1H NMR, and MS analysis [7, 8]. All the results were in good agreement with the literature data [9–14].

Compound 1. Yellow needles, mp 263°C. The mass spectrum (EI, 70 eV) exhibited a molecular ion of *m/z* 314, which agreed with the formula $C_{17}H_{14}O_6$. The UV spectrum, recorded in MeOH, showed characteristic bands of a flavonoid. The value of λ_{max} at 332 nm of the band I and the deep purple fluorescence under UV radiation at 365 nm were indicative of a flavone. Upon addition of $AlCl_3$ + HCl, the UV spectrum showed a bathochromic shift of 24 nm relative to the spectrum in MeOH, suggesting the presence of a free hydroxyl group at C-5 and an oxygen-containing group at C-6 [7]. After adding 2 N NaOH, the spectrum showed a bathochromic shift with an increase in intensity of band I, and no band shift between 320 and 335 nm, indicating the presence of a free hydroxyl group at C-4′ and the substitution of that at C-7. The PMR spectrum (250 MHz, $CDCl_3$) exhibited an AB system at δ 7.90 and 6.90 (J = 8.9 Hz) typical of the four protons H-2′, H-6′ and H-3′, H-5′of a *p*-substituted B ring of flavonoid. This spectrum also showed two 3H singlets at δ 3.97 and 3.85 relative to two methoxy groups and a 2H singlet at δ 6.60 attributed to H-3 and H-8 of the A ring. The combination of all these data led to 4′,5-dihydroxy-6,7-dimethoxyflavone (cirsimaritin) as the structure for **1** [9].

1) Laboratoire de Phytochimie et Analyses Physico-Chimiques et Biologiques, Universite Mentouri, Route de Ain El Bey, 25000 Constantine, Algeria, fax: 213 31 81 88 83, e-mail: fbenayache@yahoo.fr; 2) Laboratoire de Valorisation des Ressources Naturelles et Synthese de Substances Biologiquement Actives, Universite Mentouri, Route de Ain El Bey, 25000 Constantine, Algeria; 3) Instituto de Productos Naturales y Agrobiologia, C.S.I.C. Instituto Universitario de Bio-Organica "Antonio Gonzalez", Universidad de La Laguna, Av. Astrofisico F. Sanchez 3, 38206 La Laguna, Tenerife, Spain. Published in Khimiya Prirodnykh Soedinenii, No. 1, pp. 94–95, January–February, 2011. Original article submitted January 19, 2009.

Compound 2. Yellow needles, mp 330°C. The mass spectrum (EI, 70 eV) exhibited a molecular ion of *m/z* 330, which agreed with the formula $C_{17}H_{14}O_7$. The UV spectrum recorded in MeOH agreed with a flavonoid. The value of the λ_{max} of the band I at 344 and the deep purple fluorescence under UV radiation at 365 nm were indicative of a 3-OR flavonol. The UV spectrum obtained after addition of 2N NaOH indicated free hydroxyl groups at C-4′ and C-7 by exhibiting a bathochromic shift of the band I (56 nm) with an increase in intensity and the presence of a new band at λ_{max} 335 nm. The UV spectrum obtained upon addition of $AlCl_3$ + HCl was very similar to that of compound **1** in the same condition, which led to the presence of a free C-5 hydroxyl and an oxygen-containing group at C-6. The PMR (250 MHz, $CDCl_3$) spectrum exhibited an AB system at δ 8.00 and 7.00 (J = 8.8 Hz) typical of the four protons H-2′, H-6′ and H-3′, H-5′ of a *p*-substitued B ring. This spectrum also showed two 3H singlets at δ 4.00 and 3.85 relative to two methoxy groups and a 1H singlet at δ 6.60 attributed to H-8 of the A ring. Therefore this compound was characterized as 4′,5,7-trihydroxy-3,6-dimethoxyflavone (6-methoxyisokaempferide) [10].

Compound 3. $C_{16}H_{12}O_6$, yellowish powder, mp 291°C. This flavonoid was characterized as 4′,5,7-trihydroxy-6-methoxyflavone (hispidulin) [11].

Compound 4. $C_{15}H_{10}O_5$ yellow needles, mp 349°C. This compound was characterized as 4′,5,7-trihydroxyflavone (apigenin) [12, 13].

Compound 5. $C_{16}H_{12}O_6$, yellow needles, mp 299°C. This compound was characterized as 4′,5,7-trihydroxy-3-methoxyflavone (isokaempferide) [11, 14].

Compounds **1**, **2**, and **5** were isolated for the first time from *Centaurea maroccana* Vahl.

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