

## SECONDARY METABOLITES OF *Ranunculus bulbosus*

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*Ranunculus* is a large genus with a worldwide distribution. In Algeria it consists of about 55 genera and 130 species [1]. In continuation of our studies on Algerian medicinal plants [2, 3], we report here a phytochemical study of aerial parts of *Ranunculus bulbosus* [3]. This species was collected during the flowering phase in the Oued Seguin region east of Algeria in June 2006.

Hexadecanoic acid (**1**),  $\beta$ -sitosterol (**2**), and anemonin (**3**) were isolated from the ethyl acetate extract. The structural elucidation was performed mainly by RX, MS, and 1D and 2D NMR spectral data.

The dried and powdered aerial parts of *R. bulbosus* (350 g) were extracted three times with 70% MeOH at room temperature for 24 h. The MeOH extract was evaporated to dryness. The residue was dissolved in boiling water; after filtration, the filtrates were concentrated and re-extracted several times with EtOAc and *n*-BuOH, resulting in 2.3 g of the EtOAc and 7.3 g of the *n*-BuOH extracts, respectively. The solvents were evaporated, and the residual EtOAc and *n*-BuOH extracts were dissolved in small volumes of MeOH. The EtOAc phase (2.3 g) was fractionated on a silica gel chromatographic column eluting with *n*-hexane–EtOAc mixtures of increasing polarities to obtain ten fractions (A–J). Fractions A, B, and I were concentrated under reduced pressure to give three precipitates, which were filtered and washed with EtOAc and MeOH, yielding three crystalline compounds **1** (50 mg), **2** (35 mg), and **3** (65 mg).

The structures of the pure compounds were improved using UV, <sup>1</sup>H NMR, <sup>1</sup>H–<sup>1</sup>H COSY, <sup>13</sup>C–<sup>1</sup>H HETCOR, <sup>13</sup>C–<sup>1</sup>H COLOC, and MS and RX mass spectrometry.

**Hexadecanoic Acid (1).** C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>. IE<sup>+</sup> showed M<sup>+</sup> at *m/z* 256. IR (KBr, cm<sup>-1</sup>): 2962 (CH<sub>3</sub>), 2918–2848 (CH<sub>2</sub>), 1705 (C=O acid). <sup>1</sup>H NMR (CDCl<sub>3</sub>,  $\delta$ , ppm, J/Hz): 0.90 (3H, t, J = 7.1, CH<sub>3</sub>), 1.18 (2H, m, CH<sub>2</sub>), 1.55 (2H, m, CH<sub>2</sub>-3), 2.27 (2H, t, J = 7.5, CH<sub>2</sub>-2). <sup>13</sup>C NMR (CDCl<sub>3</sub>,  $\delta$ , ppm): 14.55 (CH<sub>3</sub>), 23.10 (CH<sub>2</sub>-3), 25.09–32.33 (CH<sub>2</sub>-4→CH<sub>2</sub>-15), 34.25 (CH<sub>2</sub>-2), 179.31 (C=O acid).

**$\beta$ -Sitosterol (2)** see [4].

**Anemonin (3).** C<sub>10</sub>H<sub>8</sub>O<sub>4</sub>. IE<sup>+</sup> showed M<sup>+</sup> at *m/z* 192. IR (KBr, cm<sup>-1</sup>): 3107, 1772, 1118, 1022. <sup>1</sup>H NMR (CDCl<sub>3</sub>,  $\delta$ , ppm, J/Hz): 2.42 (2H, m, CH<sub>2</sub>-5), 2.57 (2H, m, CH<sub>2</sub>-6), 6.14 (2H, d, J = 5.7, CH-2), 7.75 (2H, d, J = 5.7, CH-3). <sup>13</sup>C NMR (CDCl<sub>3</sub>,  $\delta$ , ppm): 23.88 (C-5, C-6), 90.33 (C-4, C-7), 121.17 (C-2, C-9), 153.30 (C-3, C-8), 170.93 (C-1, C-10) [5]. The X-ray see Fig. 1.

Compounds **2**, **3** were isolated for the first time for this species.

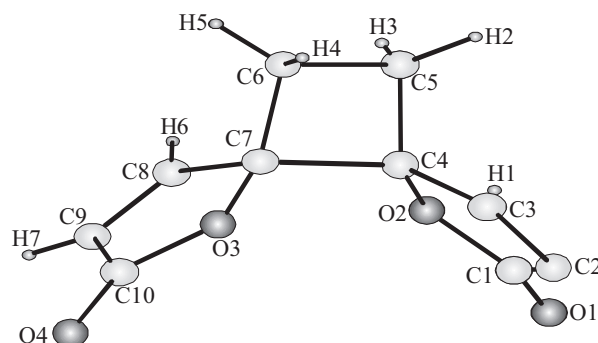


Fig. 1. X-ray of the compound **3**.

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