Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November–December, 2011]

PHYTOCHEMICAL STUDY OF *Halimium halimifolium*

L. Zaiter,[1] M. Bouheroum,[2] L. Hammoud,[1] D. Sarri,[3] S. Benayache,[2] F. Leon,[4] I. Brouard,[4] J. Bermejo,[4] and F. Benayache[1*]

UDC 547.918

Halimium halimifolium (L.) Willk. (Cistaceae) (synonyms: *Helianthemum halimifolium* Willk.; *Halimium lepidotum* Spach.) is a shrub which grows on both coastal and inland sandy soils in the Mediterranean region [1]. It is medium sized, with crown height between 60 and 120 cm. Flowers are large and yellow. Leaves are elliptical, white tomentose on both surfaces, covered with white hairs, trichomes, which provide high leaf reflectance [2].

This plant is used in folk medicine in the form of an infusion as an anticontraction and treat gastrointestinal pains. Previous chemical study led to the isolation after acid hydrolysis of the flavonoids quercetin, quercetin 3-methyl ether, kaempferol, and myricetin [3].

This work is concerned with the phytochemical study of the chloroform-soluble part of the aqueous–MeOH extract of the aerial parts. *H. halimifoliu*m was collected in April 2000 from El-Kala, northeast of Algeria. Air-dried aerial parts (flowers and leaves) (1195 g) were macerated at room temperature with MeOH–H_2O (7:3, v/v) for 48 h three times. After filtration, the filtrates were combined, concentrated at room temperature, diluted with 475 mL H_2O, filtered to remove chlorophyll, and successively extracted (3 × 300 mL) with $CHCl_3$, EtOAc, and *n*-butanol. The organic layers were dried with Na_2SO_4, to give, after removal of solvents under reduced pressure, $CHCl_3$ (2.1 g), EtOAc (23.2 g), *n*-butanol (32.9 g) extracts.

The $CHCl_3$ extract was chromatographed on a silica gel 60 (230–400 mesh) column eluted with a gradient of petroleum ether and ethyl acetate to yield 28 fractions (F_1–F_{28}) obtained by combining the eluates on the basis of TLC analysis. Fraction F_7 (57 mg) (80:20) was submitted to preparative TLC on silica gel 60, HF_{254} ($CHCl_3$, 100%, two elutions) to give **1** (36 mg). Fraction F_{13} (390 mg) (75:25) submitted to preparative TLC on silica gel ($CHCl_3$–MeOH, 95:5) gave **2** (42 mg) and **3** (45 mg). Compound **3** was purified by crystallization in MeOH with a small amount of $CHCl_3$.

The structures of the isolated compounds were elucidated by UV, 1D and 2D NMR (COSY, ROESY, HSQC, HMBC), and MS analysis. All the results were in good agreement with the literature data [4–6].

Compound 1. White crystals, mp 136–138°C. The Liebermann–Burchard test indicated its steroidal nature. The EI-MS spectrum presented a molecular ion at *m/z* 414 according to the molecular formula $C_{29}H_{50}O$. The J_{mod} spectrum recorded in $CDCl_3$ showed 29 signals from which those relative to a quaternary carbon atom at δ_C 140.7 and two CH groups at δ_C 121.7 and 71.8 were characteristic of C-5, C-6, and C-3 respectively, of β-sitosterol [4].

Myricetin 3,7,3′,4′-Tetramethyl Ether (2) [5, 7, 8]. $C_{19}H_{18}O_8$: UV spectrum (MeOH, λ_{max}, nm): 266, 296, 346; + NaOH: 264, 294, 362; + $AlCl_3$: 276, 304, 346, 399; + $AlCl_3$/HCl: 276, 304, 346, 399; + NaOAc: 264, 302, 344; + NaOAc/H_3BO_3: 265, 302, 344. PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.87 (3H, s, OCH_3-7), 3.88 (3H, s, OCH_3-3), 3.94 (3H, s, OCH_3-3′), 4.00 (3H, s, OCH_3-4′), 6.36 (1H, d, J = 2.2, H-6), 6.45 (1H, d, J = 2.2, H-8), 7.33 (1H, d, J = 2.0, H-6′), 7.35 (1H, d, J = 2.0, H-2′), 12.57 (1H, s, OH-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 55.8 (OCH_3-7), 56.1 (OCH_3-3′), 60.3 (OCH_3-3), 61.1 (OCH_3-4′), 92.2 (C-8), 98.0 (C-6), 105.1 (C-6′), 106.7 (C-10), 108.6 (C-2′), 126.0 (C-1′), 137.9 (C-4′), 139.4 (C-3), 149.2 (C-5′), 152.1 (C-3′), 155.0 (C-2), 156.8 (C-9), 162.0 (C-5), 165.6 (C-7), 178.8 (C-4).

1) Laboratoire de Phytochimie et Analyses Physico-Chimiques et Biologiques, Departement de Chimie, Universite Mentouri, Constantine, Route de Ain El Bey, 25000 Constantine, Algerie, fax: 213 31 81 88 83, e-mail: fbenayache@yahoo.fr; 2) Laboratoire de Valorisation des Ressources Naturelles, Departement de Chimie, Universite Mentouri, Constantine, Route de Ain El Bey, 25000 Constantine, Algerie; 3) Departement de Biologie, Faculte des Sciences, Universite Mohammed Boudiaf, M′Sila, 28000, M′Sila, Algerie; 4) 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. 6, pp. 889–890, November–December 2011. Original article submitted August 31, 2010.

Compound 3. The HR-EI-MS spectrum exhibited a molecular ion of *m/z* 166.094154 which agreed with the formula $C_{10}H_{14}O_2$ (calcd 166.099380).This spectrum also showed an ion of *m/z* 148.083282 corresponding to the loss of H_2O (calcd 148.088815), indicating the presence of a hydroxyl group in the compound. In the EI-MS spectrum, the presence of the signals at *m/z* 94; 77 and 51 led to the presence of an oxygenated aromatic ring. The ^{1}H NMR spectrum confirmed these observations by exhibiting an AB system at δ 6.90 and 6.62 (2H each, J = 8.2 Hz) corresponding to a *para*-substituted aromatic ring. This spectrum also showed the presence of an oxygenated methyne, two methylenes and a methyl group. In the COSY spectrum, the protons of the methyl group (δ 1.07, d, J = 6.5 Hz) showed correlation with the proton of the methyne group (δ 3.63, sextet, J = 6.5 Hz) which showed correlation with the protons of the methylene group (δ 1.58, m). The protons of this methylene group correlated with those of the second methylene group (δ 2.47, m). These last protons showed correlations with the aromatic quaternary carbon at δ 137.0 and the two aromatic methynes at δ 133.0 in the HMBC spectrum, indicating the substitution of this ring by the C_4 chain. The combination of all these data and the negative value of the optical rotation were in good agreement with the structure of (–)-betuligenol] [6].

(–)-Betuligenol (3). $C_{10}H_{14}O_2$. $[\alpha]_D^{25}$ –7° (0.00213, MeOH). PMR (400 MHz, CD_3OD + 2 drops $CDCl_3$, δ, ppm, J/Hz): 1.07 (3H, d, J = 6.5, H-1), 1.58 (2H, m, H-3), 2.47 (2H, m, H-4), 3.63 (1H, sextet, J = 6.5, H-2), 6.62 (2H, d, J = 8.2, H-3′, H-5′), 6.90 (2H, d, J = 8.2, H-2′, H-6′). ^{13}C NMR (100 MHz, CD_3OD + 2 drops $CDCl_3$, δ): 26.7 (C-1), 34.9 (C-4), 44.8 (C-3), 70.9 (C-2), 118.9 (C-3′, C-5′), 133.0 (C-2′, C-6′), 137.0 (C-1′), 158.4 (C-4′). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 166 (M^+, 35.01), 148 ($[M - H_2O]^+$, 27.50), 133 ($[M - H_2O - CH_3]^+$, 79.37), 107 ($[M - C_3H_7O]^+$, 100), 121 ($[M - C_2H_5O]^+$, 5.63), 94 ($[PhOH]^+$, 12.50), 77 (18.13), 65 (6.25), 51 (5.13).

All these compounds were isolated for the first time from this plant.

REFERENCES

1. P. Quezel, S. Santa, *Nouvelle Flore de l´Algerie et des Regions Desertiques Meridionales*, Tome **II**, ed. CNRS, Paris, 1963, p. 700.
2. M. Zunzunegui, M. C. Diaz Baradas, and F. Garcia Novo, *Plant Ecol*., **148**, 165 (2000).
3. I. Poetsch and H. Reznik, in: *Berichte der Deutsch. Bot. Ges.*, **85**, 209 (1972).
4. D. W. Ness, R. A. Norton, and M. Benson, *Phytochemistry*, **31**, 805 (1992).
5. E. Wollenweber, K. Mann, and J. N. Roitman, *Z. Naturforsch.,* **47C**, 638 (1992).
6. S. K. Chattopadhyay, S. Srivastava, K. V. Sashidhara, A. K. Tripathi, A. K. Bhattacharya, and A. S. Negi, *Bioorg. Med. Chem. Lett*., **14**, 1729 (2004).
7. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer, New York, 1970, p. 54.
8. B. Voirin, *Phytochemistry*, **22**, 2107 (1983).