Chemistry of Natural Compounds, Vol. 46, No. 5, 2010

SECONDARY METABOLITES FROM *Centaurea lippii*

N. Mezache,[1] D. Bendjeddou,[1] D. Satta,[1] R. Mekkiou,[1] S. Benayache,[2] and F. Benayache[1]*

UDC 547.972

The genus *Centaurea* is known to contain a variety of secondary metabolites of various types, especially sesquiterpene lactones and flavonoids, which have been shown to be biologically active [1–3].

Centaurea lippii L. (Asteraceae), synonyms *Volutaria lippii* (L.) Cass.; *Volutarella lippii* (L.) Cass.; *Amberboa lippii* (L.) DC., is widely distributed in all Mediterranean areas [4]. Previous phytochemical investigation of this species has led to the isolation of sesquiterpene lactones [5]. The present work concerned the phytochemical study of the chloroform and the *n*-butanolic soluble parts of the aqueous ethanol extract of the leaves and flowers of the Algerian species.

Centaurea lippii was collected during the flowering phase in May 1998, in the East of Algeria, and was authenticated by Prof. A. Kaabeche (Biology Department, University of Setif Algeria).

Air-dried and powdered leaves and flowers (1267 g) of *Centaurea lippii* were macerated with EtOH for 24 h three times. After filtration, the filtrates were combined, concentrated at room temperature, and diluted with 800 mL H_2O. After remove of chlorophyll, the remaining aqueous solution was extracted successively with $CHCl_3$, EtOAc, and *n*-BuOH to obtain the extracts: chloroform (12 g), ethyl acetate (1 g), and *n*-BuOH (16 g).

The chloroform extract was chromatographed on a 70–230 mesh silica gel column eluted with a gradient of chloroform–acetone to yield 8 fractions from which the most important compound **1** was isolated and purified by preparative TLC on silica gel using chloroform–acetone–EtOAc (1:1:3) as eluent.

The *n*-BuOH extract was applied to a polyamide SC6 column eluted with a gradient of toluene–MeOH to yield 9 fractions. Three flavonoid glycosides **2–4** contained in several fractions were isolated by preparative TLC using water–MeOH–methyl-ethyl ketone–acetylacetone (13:3:3:1) as solvent. Purification of each compound for spectral analysis was carried out using MeOH over Sephadex LH-20 column [6].

The structures were elucidated by IR, UV, ^{1}H NMR, ^{13}C NMR, and MS analysis. All these data were in good agreement with the respective literature data [6–10].

Compound 1. $C_{20}H_{26}O_7$, mp 143°C. IR spectrum (KBr, ν, cm^{-1}): 1759 (γ-lactone), 1705 (C=O conjugated ester), 3356 (-OH). ^{1}H NMR (250 MHz, $CDCl_3$ + 2 drops of CD_3OD, δ, ppm, J/Hz): 4.78 (1H, m, H-1), 2.20 (1H, m, H-2), 2.25 (1H, m, H-2′), 2.55 (1H, m, H-3), 2.00 (1H, m, H-3′), 4.81 (1H, d, $J_{5\text{-}6}$ = 9.7, H-5), 5.25 (1H, t, $J_{6\text{-}5} = J_{6\text{-}7}$ = 9.7, H-6), 3.12 (1H, m, H-7), 5.15 (1H, m, H-8), 2.50 (1H, m, H-9), 2.55 (1H, m, H-9′), 5.67 (1H, d, $J_{13\text{-}7}$ = 2.7, H-13), 6.14 (1H, d, $J_{13'\text{-}7}$ = 3.3, H-13′), 1.47 (1H, s, H-14), 3.99 (1H, d, $J_{15\text{-}15'}$ = 13.9, H-15), 4.23 (1H, d, $J_{15'\text{-}15}$ = 13.9, H-15′), 6.00 (1H, s, H-18), 6.27 (1H, s, H-18′); 4.52 (1H, dd, $J_{19\text{-}20}$ = 6.8, $J_{19\text{-}20'}$ = 3.3, H-19), 3.47 (1H, dd, $J_{20\text{-}20'}$ = 11.3, $J_{20\text{-}19}$ = 6.8, H-20), 3.75 (1H, dd, $J_{20'\text{-}20}$ = 11.3, $J_{20'\text{-}19}$ = 3.3, H-20′).

^{13}C NMR (82.5 MHz, $CDCl_3$+ 2 drops CD_3OD, δ): 129.7 (C-1), 26.0 (C-2), 34,4 (C-3), 135.3 (C-4), 127.9 (C-5), 77.1 (C-6), 52.9 (C-7), 73.0 (C-8), 48.5 (C-9), 139.8 (C-10), 144.5 (C-11), 170.6 (C-12), 125.4 (C-13), 16.6 (C-14), 60.3 (C-15), 165.00 (C-16), 132.00 (C-17), 126.9 (C-18), 70.7 (C-19), 65.7 (C-20). Mass spectrum (FAB^+, 70 eV), *m/z*: 379 $[M + H]^+$, 247 [MH^+ – (1′,2′-OH-Et) acrylic acid], 229 [247 – H_2O].

This compound was identified as cnicin [7].

1) Laboratoire de Phytochimie et Analyses Physico-Chimiques et Biologiques, Departement de Chimie, Universite Mentouri, Constantine, Route de Ain El Bey, 25 000 Constantine, Algeria, 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, 25 000 Constantine, Algeria. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 675–676, September–October, 2010. Original article submitted May 28, 2009.

0009-3130/10/4605-0801

Compound 2. $C_{21}H_{19}O_{10}$, mp 231–232°C. UV (MeOH, λ_{max}, nm): 273, 344; +NaOH: 275, 391; +$AlCl_3$: 258, 285, 362; +$AlCl_3$/HCl: 258, 285, 360; +NaOAc: 275, 385; +NaOAc/H_3BO_3: 273, 346. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 8.20 (2H, d, J = 8.9, H-2′, H-6′), 6.88 (2H, d, J = 8.9, H-3′, H-5′), 6.04 (1H, s, H-3), 6.05 (1H, s, H-8), 5.10 (1H, d, J = 7.4, H-1″ glucose), 3.9–3.3 (sugar protons).

This compound was characterized as apigenin 6-*C*-glucoside (isovitexin) [6].

Compound 3. $C_{21}H_{20}O_{12}$, mp 238–240°C; UV (MeOH, λ_{max}, nm): 275, 341; +NaOH: 269, 404; +$AlCl_3$: 260, 284, 367; +$AlCl_3$/HCl: 260, 383, 366; +NaOAc: 270, 354, 407; +NaOAc/H_3BO_3: 255, 273, 346. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.55 (1H, d, J = 2.5, H-2′), 7.43 (1H, dd, J = 8.7, 2.5, H-6′), 6.75 (1H, d, J = 8.7, H-5′), 6.24 (1H, d, J = 2.1, H-8), 6.20 (1H, d, J = 2.1, H-6), 5.28 (1H, d, J = 7.4, H-1″ glucose), 4.63–3.3 (sugar protons).

This compound was characterized as quercetin 3-*O*-glucoside (isoquercitrin) [8, 9].

Compound 4. $C_{27}H_{30}O_{15}$, mp 187–189°C; UV (MeOH, λ_{max}, nm): 267, 351; +NaOH: 274, 327, 402; +NaOH (after 5 minutes): 274, 327, 402; +$AlCl_3$: 273, 350, 397; +$AlCl_3$/HCl: 273, 327, 350; +NaOAc: 272, 299, 360; +NaOAc/H_3BO_3: 271, 299, 350. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 8.00 (2H, d, J = 8.8, H-2′, H-6′), 6.83 (2H, d, J = 8.8, H-3′, H-5′), 6.35 (1H, d, J = 2.4, H-8), 6.15 (1H, J = 2.4, H-6), 5.05 (1H, d, J = 7.4, H-1″ glucose), 4.45 (1H, d, J = 1,7, H-1″ rhamnose), 3.9–3.3 (10H, sugar protons); 1.10 (3H, d, J = 6.2, CH_3 rhamnose). Mass spectrum (FAB$^+$, 70 eV), *m/z*: 577 [MH – H_2O]$^+$, 431 [MH – H_2O – Rha]$^+$, 287 [MH – Rha – Glu]$^+$, 153 [A_1 + H]$^+$.

This compound was characterized as kaempferol 3-*O*-rutinoside (nicotiflorin) [10].

REFERENCES

1. E. Koukoulitsa, H. Skaltsa, A. Karioti, C. Demetzos, and K. Dimas, *Planta Med.*, **68**, 649 (2002).
2. E. Yesilada, I. Guerbuez, E. Bedir, I. Tatli, and I. A. Khan, *J. Ethnopharmacol*., **95**, 213 (2004).
3. M. Shoeb, M. Jaspars, S. M. Mac Manus, S. Celik, L. Nahar, P. Kong-Thoo-Lin, and S. D. Sarker, *J. Nat. Med.*, **61**, 164 (2007).
4. P. Quezel and S. Santa, *Nouvelle Flore de l′Algerie et des Regions desertiques Meridionales*, Tome **II**, ed. CNRS, Paris, 1963, p. 1034.
5. A. G. Gonzalez, B. G. Marrero, and J. L. Breton, *Anal. Quim*., 799 (1976).
6. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer, New York, 1970.
7. A. Rustaiyan, A. Nikhejad, and Y. Aynechi, *Planta Med.*, **44**, 185 (1982).
8. E. O. P. De Rijke, W. M. A. Niessen, F. Ariese, C. Gooijer, and U. A. T. Brinkman, *J. Chromatogr. A*, **1112**, 31 (2006).
9. J. Kagan, *Phytochemistry*, **7**, 1205 (1968).
10. K. Kazuma, N. Noda, and M. Suzuki, *Phytochemistry*, **62**, 229 (2003).