Chemistry of Natural Compounds, Vol. 42, No. 2, 2006

AGLYCONE FLAVONOIDS OF *Centaurea tougourensis* FROM ALGERIA

A. Nacer,[1] A. Bernard,[2] J. Boustie,[2] R. Touzani,[3] and Z. Kabouche[1]

UDC 547.972

Plants of *Centaurea* (Asteraceae) genus are rich sources of flavonoids [1–5] and sesquiterpenes [6–10]. In Algerian folk medicine, *Centaurea* species are known for their antibacterial and antifungal activities. We report here six flavonoid aglycones (**1–6**) from the dichloromethane and ethyl acetate extracts of the endemic species *Centaurea tougourensis* [11].

The air dried aerial parts (1 kg) of *Centaurea tougourensis*, collected during flowering (April 2002) near Batna (North Eastern Algerian), were macerated at room temperature in a methanol solution (80%). The extract was concentrated under low pressure, diluted and filtered to remove chlorophyll, then successively extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol.

The dichloromethane extract was column chromatographed on silica gel (70–200 mesh), eluted with dichloromethane-methanol with increasing polarity. The major fraction was subjected to circular chromatography using a mixture of dichloromethane–methanol (98:2), leading to compounds **1**, **2**. Preparative silica TLC plates, eluted with dichloromethane–methanol (95:5), afforded the compound **3**.

The ethyl acetate extract was column chromatographed on silica gel (70–200 mesh) using the mixture ethyl acetate–methanol with increasing polarity. TLC purification on silica gel, eluted with dichloromethane–methanol (9:1), led to compounds **4–6**.

All compounds were identified by ^{1}H NMR, ^{13}C NMR, EI/MS, and UV analytical methods.

Compound 1, $C_{17}H_{14}O_7$, yellow needles (EtOAc), mp 227–229°C, UV spectrum (MeOH, λ_{max}, nm): 274, 346; + $AlCl_3$/HCl: 259, 286, 364; +NaOH: 268, 320, 397; + NaOAc: 265; 275, 370. ^{1}H NMR data (500 MHz, $CDCl_3$, δ, ppm, J/Hz), 4.00 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 6.50 (1H, s, H-8), 6.60 (1H, s, H-3), 7.02 (1H, d, J = 9, H-5′), 7.32 (1H, d, J= 2, H-2′), 7.47 (1H, dd, J = 2 and 9, H-6′). Mass spectrum (EI, 70 eV), *m/z*: 330 $[M]^+$. Characterized as 5,7,4′-trihydroxy-6,3′-dimethoxyflavone (jaceosidin) [12].

Compound 2, $C_{18}H_{16}O_7$, yellow cristals (EtOAc), mp 234–236°C, UV spectrum (MeOH, λ_{max}, nm): 339, 274; + $AlCl_3$/HCl: 361, 288, 258; + NaOH: 379, 320, 275; + NaOAc : 348, 276; ^{1}NMR data (500 MHz, $CDCl_3$, δ, ppm, J/Hz), 3.72 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 6.56 (1H, s, H-8), 6.86 (1H, s, H-3), 7.03 (1H, d, J = 9, H-5′), 7.47 (1H, d, J = 2, H-2′), 7.58 (1H, dd, J = 2 and 9, H-6′). Mass spectrum (EI, 70 eV), *m/z*: 344 $[M]^+$. Characterized as 5,7-dihydroxy-6,3′,4′-trimethoxyflavone (eupatilin) [13].

Compound 3, $C_{19}H_{18}O_7$, mp 189–190°C, ^{1}H NMR data (500 MHz, $CDCl_3$, δ, ppm, J/Hz), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 6.58 (1H, s, H-8), 6.60 (1H, s, H-3), 6.98 (1H, d, J = 9, H-5′), 7.35 (1H, d, J = 2, H-2′), 7.53 (1H, dd, J = 2 and 9, H-6′). ^{13}C NMR data (125 MHz, $CDCl_3$, δ, ppm): 90.6 (C-8), 104.5 (C-3), 111.2 (C-5′), 108.7 (C-2′), 120.1 (C-6′), 60.90 (OCH_3), 56.1 (OCH_3), 56.14 (OCH_3), 56.4 (OCH_3). Mass spectrum (EI, 70 eV), *m/z*: 358 $[M]^+$ Characterized as 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (3′-*O*-methyleupatorin) [14].

Compound 4, $C_{16}H_{12}O_7$, yellow needles, ^{1}H NMR data (500 MHz, CD_3OD, d, ppm, J/Hz), 3.98 (3H, s, OCH_3), 6.51 (1H, s, H-3), 6.52 (1H, s, H-8), 6.90 (1H, d, J = 9, H-5′), 7.36 (1H, d, J=2, H-2′), 7.38 (1H, dd, J = 2 and 9, H-6′). ^{13}C NMR

1) Laboratoire d′Obtention de Substances Therapeutiques (L.O.S.T), faculte des sciences, Universite Mentouri–Constantine, Campus Chaabat Ersas, 25000 Constantine, Algerie, fax: 213 31 81 88 59, e-mail: zkabouche@yahoo.com; 2) Laboratoire de Pharmacognosie et Mycologie, Faculte de Pharmacie, Universite de Rennes 1, 12 avenue du Professeur Leon Bernard, 35043 Rennes Cedex, France; 3) Universite Mohammed Premier, Faculte Pluridisciplinaire, Nador, Maroc. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 190-191, March-April, 2006. Original article submitted March 22, 2005.

 0009-3130/06/4202-0230

data (125 MHz, $CDCl_3$, δ, ppm): 60.9 (OCH_3), 95.5 (C-3), 103.2 (C-8), 114.0 (C-2′), 116.6 (C-5′), 120.1 (C-6′). Mass spectrum (EI, 70 eV), *m/z*: 316 $[M]^+$. Characterized as 5,7,3′,4′-tetrahydroxy-6-methoxyflavone (nepetin) [15].

Compound 5, $C_{15}H_{10}O_5$, mp 347°C, UV spectrum (MeOH, λ_{max}, nm): 336, 268; + $AlCl_3$/HCl: 382, 350, 300, 277; + NaOH: 391, 325, 275; + NaOAc: 336, 268. ^{1}H NMR data (250 MHz, CD_3OD, δ, ppm, J/Hz): 6.20 (1H, d, J = 2, H-6), 6.35 (1H, d, J = 2, H-8), 6.62 (1H, s, H-3), 6.90 (2H, dd, J = 9 and 2, H-5′, H-3′), 7.87 (2H, dd, J = 9 and 2, H-2′, H-6′). Mass spectrum (EI, 70 eV), *m/*z: 270 $[M]^+$. Characterized as 5,7,4′-trihydroxyflavone (apigenin) [16].

Compound 6, $C_{15}H_{10}O_6$, UV spectrum (MeOH, λ_{max}, nm): 336, 268; + $AlCl_3$/HCl: 393, 356, 303, 276; + NaOH: 397, 325, 275; + NaOAc: 342, 268. ^{1}H NMR data (250 MHz, CD_3OD, δ, ppm, J/Hz): 6.20 (1H, d, J = 2, H-6), 6.40 (1H, d, J = 2, H-8), 6.90 (2H, dd, J = 9 and 2, H-5′, H-3′), 8.05 (2H, dd, J = 9 and 2, H-2′, H-6′). Mass spectrum (EI, 70 eV), *m/z*: 286 $[M]^+$. Characterized as 3,5,7,4′-tetrahydroxyflavone (kaempferol) [15].

ACKNOWLEDGMENT

The authors would like to thank the National Health Research Agency, Oran, Algeria (ANDRS) and F.N.R. for financial support.

REFERENCES

1. G. Flamini, E. Antognoli, and I. Morelli, *Phytochemistry*, **57**, 459 (2001).
2. R. E. Negrete, N. Backhouse, B. Bravo, S. Erazo, R. Garcia, and S. Avendano, *Plantes Med. Phytother.*, **21**, 168 (1987).
3. A. Kery, H. A. Twaij, and N. K. Al-Khazraji, *Herba Hungarica,* **24**, 183 (1985).
4. I. Fernandez, B. Garcia, F. J. Grancha, and J. R. Pedro, *Phytochemistry*, **28**, 2405 (1989).
5. H. Skaltsa, D. Lazari, C. Panagouleas, E. Georgiadou, B. Garcia, and M. Sokovic, *Phytochemistry*, **55** (8), 903 (2000).
6. V. Vajs, N. Todorovic, M. Ristic, V. Tesevic, B. Todorovic, P. Janackovic, P. Marin, and S. Milosavljevic, *Phytochemistry*, **52**, 383 (1999).
7. E. Koukoulitsa, H. Skaltsa, A. Karioti, and D. K. Demetzos, *Planta Med.* **68**, 649 (2002).
8. R. E. Negrette, N. Backhouse, S. Avendano, and A. San Martin, *Plantes Med. Phytother.* **18**, 226 (1984).
9. A. G. Gonzalez, V. Darias, G. Alonso, and E. Estevez, *Planta Med.*, **40**, 179 (1980).
10. P. Quezel and S. Santa, *Nouvelle Flore de l'Algerie et des Regions Désertiques et Meridionales*, C.N.R.S., Paris 1962, p. 1023.
11. S. Oksuz, H. Ayyildiz, and C. Johansson, *J. Nat. Product*, **47**, 902 (1984).
12. H. Roeler and T. J. Mabry, *Phytochemistry*, **10**, 450 (1971).
13. S. M. Adekenov, G. M. Kadirberlina, V. I. Sadykova, T. I. Kupriyanova, and A. D. Kagarlitskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **3**, 65 (1986).
14. Z. F. Ahmed, H. Rimpler, F. M. Hammouda, A. M. Rizk, and S. L. Ismail, *Phytochemistry*, **9**, 1595 (1970).
15. V. A. Bandyukova, K. Khalatov, and K. I. Alimov, *Khim. Prir. Soedin.*, 324 (1969).