COMPARATIVE PHYTOCHEMICAL STUDY OF THE BUTANOLIC EXTRACTS OF TWO ALGERIAN *Phlomis* SPECIES

A. Kabouche[1], Z. Kabouche[1], E. Seguin[2], F. Tillequin[3], and C. Bruneau[4]

UDC 547.92

The genus *Phlomis* [1], commonly called "Djaada," is known in algerian folk medicine for its antiinflammatory and antirhcumatic activities, as well as the chinese species *P. umbrosa* [2] and the korean species *P. rotata* [3]. Only one product, an iridoid named lamiid [4], has recently been reported from a bulgarian *P. herba venti*.

Air dried aerial parts of *P. herba-venti* and *P. crinita* (*Lamiaceae*), collected from Djebel El-Ouach at Constantine (North Algerian) at an altitude of 800 m in May 2000, were macerated in a methanolic solution (70%), the residue was filtered, concentrated, then successively extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol. The butanolic extract was concentrated under reduced pressure and column chromatographed on silica gel G-60 with a gradient of EtOAc–MeOH with increasing polarity. Preparative TLC of the *P. herba-venti* fraction on silica gel led to compounds **1–5** while compounds **4–6** were found in *P. crinita*. The purification of compounds **1–6** was carried out in a Sephadex LH-20 column with a toluene-MeOH gradient of increasing polarity.

The structures of the pure compounds were improved using UV, ^{1}H NMR, ^{13}C NMR, DIC/NH_3 and ES/MS analytical methods. Multiple-pulse 2D NMR experiments (^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{13}C–^{1}H HETCOR, and ^{13}C–^{1}H COLOC) were used for the structure elucidation of compounds **1** and **6**.

Compound 1, $C_{29}H_{34}O_{15}$, mp 208°C (dec.) (H_2O), identified as verbascoside [5];

Compound 2, $C_{15}H_{10}O_5$, mp 347°C, identified as apigenin [6];

Compound 3, $C_{21}H_{20}O_{10}$, mp 226–228°C identified as apigenin 7-O-glucoside or apigetrin [7];

Compound 4, $C_{15}H_{10}O_6$, mp 329–331°C (dec.) (EtOH), identified as luteolin [8];

Compound 5, $C_{21}H_{20}O_{11}$, mp 239–242°C (MeOH), identified as luteolin 7-O-glucoside [8];

Compound 6, chrysoeriol-7-β-D-(3″E-*p*-coumaroyl) glucoside, $C_{31}H_{28}O_{13}$, UV spectrum (MeOH, λ_{max}, nm): 270, 294sh, 318, 355sh; IR spectrum (KBr, ν, cm^{-1}): 2980, 2934, 1732, 1650, 1452, 1375, 1290, 1169, 1128, 1080, 1035, 1013. Mass spectrum, ES$^+$, *m/z*: 609 $[M+H]^+$, 579$[M+H-OCH_3]^+$, 301[M+H-*p*-coumaroylglucoside]$^+$. P^1 NMR (DMSO-d_6, 400 MHz, δ, ppm, J/Hz): 3.46 (1H, t, J = 9, H-4″); 3.50 (1H, dd, J = 9.4, H-2″); 3.53 (1H, m, H-6″$_B$); 3.62 (1H, m, H-5″); 3.72 (1H, m, H-6″$_A$); 3.85 (3H, s, OCH_3); 5.06 (1H, d, J = 9.4, H-1″); 5.25 (1H, t, J = 7.8, H-3″); 6.42 (1H, d, J = 15.7, CHCO); 6.46 (1H, d, J = 2, H-6); 6.79 (1H, d, J = 8.6, H-5′); 6.81 (2H, d, J = 9, H-2‴, H-6‴); 6.86 (1H, d, J = 2, H-8); 7.0 (1H, s, H-3); 7.48 (1H, d, J = 2, H-2′); 7.55 (1H, d, J = 2, H-6′); 7.56 (2H, d, J = 9, H-3‴ and H-5‴); 7.58 (1H, d, J = 15.7, CH=C).

The verbascoside's structure was confirmed by its peracetylation leading to the nonaacetyl derivative **7**.

Twenty mg of verbascoside **1** was dissolved in anhydrous pyridine (2 ml) and stirred at 25°C during 48 hours. The evaporation of the solution afforded the verbascoside nonaacetate **7** (26 mg), $C_{47}H_{54}O_{24}$, as an amorphous powder. –56° (*c* 1.0, $CHCl_3$). UV spectrum (MeOH, λ_{max}, nm): 281. IR spectrum (KBr, ν, cm^{-1}): 1750, 1645, 1428. Mass spectrum (DIC/NH_3) *m/z*: 1020 $[M+NH_3]^+$. P NMR (400 MHz, δ, ppm, J/Hz): 7.76 (1H, d, J = 16, H-7‴), 7.40 (2H, dd, J = 8 and J = 2, H-6,

1) Laboratoire d'Obtention de Substances Therapeutiques (LOST), Faculte des Sciences, Universite Mentouri–Constantine, Campus Chaabet Ersas, 25000 Constantine, Algerie, fax: (21331) 63 53 52, e-mail: zkabouche@hotmail.com; 2) Equipe Pharmacomodulation d'antitumoraux d'origine naturelle et synthétique, Laboratoire de Pharmacognosie, Faculte de Pharmacie, 22, Boulevard Gambetta, F-76183 Rouen Cedex 1, France; 3) Laboratoire de Pharmacognosie de l'Universite Rene Descartes, U.M.R./C.N.R.S. N 8638, Faculte des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire, F-75006 Paris, France; 4) Universite de Rennes 1, U.M.R./C.N.R.S. N 6509, Campus de Beaulieu, 35042 Rennes cedex, France. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 159-160, March-April, 2004. Original article submitted January, 12, 2004

 0009-3130/04/4002-0188

H-6‴), 7.24 (1H, d, J = 8, H-5), 7.20 (1H, d, J = 8, H-5‴), 7.10 (1H, d, J = 1, H-2‴), 7.04 (1H, d, J = 1, H-2), 6.36 (1H, d, J = 16, H-8‴), 5.24 (1H, t, J = 9, H-4′), 5.14 (1H, dd, J = 10 and J = 8, H-2′), 5.10 (1H, dd, J = 10, J = 2, H-3″), 5.04 (1H, dd, J = 10 and J = 2, H-2″), 4.96 (1H, t, J = 9, H-4″, (4.84 (1H, d, J = 1, H-1″), 4.40 (1H, d, J = 8, H-1′), 4.17–4.11 (3H, m, H-8a, CH_2-6′), 3.89 (1H, t, J = 9, H-3′), 3.82 (1H, m, H-5″), 3.65 (3H, m, H-5′, H-8b, H-4″), 2.90 (2H, m, CH_2-7), 2.31–2.28 (4´3H, 4s, 4 Ar–OAc), 2.11–1.88 (5´3H, 5s, 5 R-OAc), 1.04 (3H, d, J = 8, CH_3-6″); ^{13}C NMR (75 MHz, DMSO-d_6, d, ppm, J/Hz): 17.3 (C-6″), 35.3 (C-7), 62.2 (C-6′), 67.2 (C-5″), 68.5 (C-4′), 69.7 (C-8), 69.7 (C-3″), 69.9 (C-2″), 70.5 (C-4″), 71.9 (C-5′), 72.0 (C-2′), 80.3 (C-3′), 99.0 (C-1″), 100.6 (C-1′), 117.9 (C-2‴), 122.7 (C-2), 123 (C-8‴), 123.0 (C-5), 124 (C-5‴), 126.4 (C-6), 127.2 (C-6‴), 132.8 (C-1‴), 137.5 (C-1), 140.5 (C-4), 141.7 (C-3), 142.5 (C-3‴), 143.8 (C-4‴), 144.3 (C-7‴), 164.8 (C-9‴).

Compounds **1–5** and **4–6** are isolated for the first time from *P. herba-venti* and *P. crinita*, respectively. Compound **6** was reported for a second time from a *Phlomis species* [9] but we report here the corrected P NMR and ^{13}C NMR values for this compound.

The authors would like to thank the National Health Research Agency, Oran Algeria (A.N.D.R.S), for financial support and Dr. Rachid Touzani (Ottawa, Canada) for helpful bibliographical complements.

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