Chemistry of Natural Compounds, Vol. 44, No. 5, 2008

FIVE GLYCOSYLATED FLAVONOIDS FROM THE ANTIBACTERIAL BUTANOLIC EXTRACT OF *Pituranthos scoparius*

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UDC 547.972

We have recently reported the antibacterial activity of the essential oils of the stems and the seeds of the endemic species *Pituranthos scoparius* (Coss. & Dur) Schinz (=*Deverra scoparia* Coss.& Dur), commonly known as "guezzah" [1]. It is a Saharian species [2] used in traditional medicine for the treatment of asthma and rheumatism [3, 4] and in food as a flavoring [3]. Two isocoumarins have been reported from the roots of *Pituranthos scoparius* [5]. To our knowledge, the aerial parts of this plant have not been the subject of any study. We report here, for the first time, the identification and the antibacterial activity of the flavonoids of the aerial parts of *Pituranthos scoparius.*

Pituranthos scoparius, an endemic species of the *Pituranthos* genus (Apiaceae), was collected from Ghardaia in April 2003. The plant material was authenticated by Prof. Gerard De Belair (University of Annaba, Algeria) and a voucher specimen (ZKNAPs04/01) was deposited at the Herbarium of the Faculty of Sciences, University Mentouri-Constantine and at the Musee Botanique de la ville d'Angers, France (MBAng2005.13).

Air-dried and powdered aerial parts (1 kg) of *Pituranthos scoparius* 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 polyamid SC6 with a gradient of toluene–MeOH with increasing polarity. Fraction F_3 was chromatographed on silica gel preparative TLC plates eluted with the system ethylacetate–formic acid–H_2O (8:1:1), affording compounds **1**–**3**. Fractions F_5 and F_7 were subjected to preparative TLC on polyamid DC6 using H_2O–MeOH–methylethyl ketone–acetylacetone (13:3:3:1) and flash column chromatography purification with Sephadex LH-20 eluted with methanol, leading to two compounds **4**, **5**.

Acid Hydrolysis. The pure compounds were treated with 2 M HCl at 100°C for 1 h. The hydrolysates were extracted with EtOAc and the aglycones were identified by their UV spectra in methanol and by comparison of their R_f with authentic samples.

Sugars were identified in the aqueous residue by comparison with authentic samples on silica gel TLC impregnated with 0.2 M NaH_2PO_4, solvent Me_2CO–H_2O (9:1) revealed with aniline malonate.

Compound 1, $C_{21}H_{20}O_{10}$, mp 227–228°C identified as apigenin 7-*O*-glucoside or apigetrin [6].

Compound 2, $C_{21}H_{20}O_9$, mp 284°C identified as apigenin 7-*O*-rhamnoside [6, 7].

Compound 3, $C_{28}H_{32}O_{16}$, mp 171–173°C. UV (λ_{max}, nm), MeOH: 356, 300sh, 254; +NaOH: 412, 329, 275; + $AlCl_3$: 396sh, 361, 300, 267; +HCl: 402, 359, 302sh, 267; +NaOAc: 382, 320, 273; +H_3BO_3: 359, 305sh, 270sh, 259. FAB^+-MS (*m/z*): 624 $[M+H]^+$.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.92 (1H, d, J = 2.0, H-2′), 7.68 (1H, dd, J = 8.5, J = 2.0, H-6′), 6.92 (1H,d, J = 8.5, H-5′), 6.40 (1H, d, J = 2.0, H-8), 6.25 (1H, d, J = 2.0, H-6), 5.25 (1H, d, J = 7.5, H-1″glucose), 4.50 (1H, d, J = 1.2, H-1‴rhamnose), 3.95 (3H, s, OMe-3′), 1.12 (3H, d, J = 6.5, H-6‴ rhamnose), 3.20–3.90 (protons of rutinose), identified as isorhamnetin-3-*O*-rutinoside.

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0009-3130/08/4405-0639

TABLE 1. Antibacterial Activity of the Butanolic Extract of *Pituranthos scoparius*

Microorganism	Inhibition zone, mm	MIC, μg/mL
	Extract (128 μg/mL)	
Escherichia coli ATCC 25922	30	0.03
Enterobacter	22	128
Klebsiella pneumoniae	18	32
Pseudomonas aeruginosa ATCC27853	30	0.125
Staphyloccocus aureus	30	8
Streptoccocus α-hemolytic	24	64

Standard: ampicillin.

Compound 4, $C_{22}H_{22}O_{12}$, mp 154–155°C. UV (λ_{max}, nm), MeOH: 355, 300sh, 267sh, 255; +NaOH: 417, 329, 272; +$AlCl_3$: 398sh, 361, 300, 267; +HCl: 400, 359, 300sh, 267; +NaOAc: 382, 320, 274; +H_3BO_3: 358, 305sh, 267sh, 255. FAB$^+$-MS (*m/z*): 479 $[M+H]^+$.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.90 (1H, d, J = 2, H-2′), 7.59 (1H, dd, J = 8.5, J = 2, H-6′), 6.90 (1H, d, J = 8.5, H-5′), 6.31 (1H, d, J = 2, H-8), 6.14 (1H, d, J = 2, H-6), 5.30 (1H, d, J = 7.5, H-1″glucose), 3.92 (3H, s, OMe-3′), 3.14–3.78 (protons of glucose), identified as isorhamnetin-3-*O*-glucoside.

Compound 5, $C_{27}H_{30}O_{15}$, mp 148–151°C. UV (λ_{max}, nm) MeOH: 329, 273; +NaOH: 402, 333, 271; + $AlCl_3$: 392sh, 347, 305, 278; +HCl: 390sh, 343, 304, 278; +NaOAc: 363, 341, 278; +H_3BO_3: 363, 347, 279.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.98 (2H, d, J = 8.5, H-2′, H-6′), 6.93 (2H, d, J = 8.5, H-3′, H-5′), 6.70 (1H, s, H-3), 5.20 (1H, m, H-1″glucose), 4.90 (1H, m, H-1‴glucose), 2.50 – 3.90 (protons of two glucoses), identified as apigenin-6,8-di-C-glucoside (vicenin-2).

Thus, apigenin-7-*O*-glucoside (**1**, apigetrin) and apigenin-7-*O*-rhamnoside (**2**) are isolated for the first time from the genus, while compounds **3–4** were reported from *P. triradiatus* and *P. tortuosus* [8, 9] and the Algerian species *P. chlorantus,* from which apigenin-6,8-di-C-glucoside (**3**) was also recently isolated [10].

As reported in Table 1, the butanolic extract of *Pituranthos scoparius, using* the disk diffusion method [11, 12], showed good antibacterial activity against the microrganisms *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC27853,* and *Staphyloccocus aureus*. This extract inhibited remarkably the growth of *Escherichia coli ATCC 25922* (30 mm), *Pseudomonas aeruginosa ATCC27853* (30 mm), and *Staphyloccocus aureus* (30 mm) at the respective concentration levels 0.03 μg/mL, 0.125 μg/mL, and 8 μg/mL.

ACKNOWLEDGMENT

The authors thank the National Health Research Agency, Oran Algeria, (A.N.D.R.S) and F.NR for financial support.

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