FLAVONOID GLYCOSIDES FROM *Pituranthos chloranthus*

A. Touil,[1] S. Rhouati,[1] and J. Creche[2]

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As part of our current work on the chemistry of some plants growing in the Hoggar region (southern of Algeria), *Pituranthos chloranthus* (Bent. et Hook.), which is an endemic species of the Sahara septentrional and central [1], was examined for its flavonoids. To our knowledge, this plant has not been investigated before.

Aerial parts of *P. chloranthus* were collected from Hoggar (Algeria) in 1999 and identified by Dr. Mohamed Kaabache (Department of Biology, University of Setif, Algeria). A voucher specimen has been deposited in the herbarium of the Laboratory of Natural Substances and Organic Synthesis, University of Constantine, under No. 10/ 1999/ OPC2.

Dried powder of aerial parts (350g) of flowering *P. chloranthus* was extracted with 70% MeOH solution. The MeOH extract was concentrated to dryness under reduced pressure. The residue was dissolved in dist. H_2O (300 ml) and filtered after 24 hrs. The filtrate was extracted successively with $CHCl_3$ (2.1g), EtOAc (1g), and *n*-BuOH (14g).

The *n*-BuOH extract (14g) was subjected to CC on polyamide MN-SC6 and eluted with a gradient of H_2O-MeOH with increasing polarity. Fifteen fractions of 150 ml were collected and analyzed by TLC on cellulose in 15% AcOH (system I) and BAW (*n*-BuOH–AcOH–H_2O, 4:1:5 top layer (system II)) as solvent systems and silica gel $60GF_{254}$ using CH_2Cl_2 –MeOH (3:1) (system III) in which some fractions were combined to get ten fractions.

Four flavonoids (**1**–**4**) were isolated from fractions 3, 4, 5, and 6 by preparative PC on Whatman 3MM and preparative TLC on silica gel $60GF_{254}$ using the above solvent systems. Purification of each compound for spectral analysis was carried out using a small column of polyamide MN-SC6 and solvent mixtures (toluene-MeOH) with increasing polarity and finally MeOH over a Sephadex LH-20 column [2]. The structures of these compounds were confirmed by UV, ^{1}H NMR, ^{13}C NMR, and MS, and all these data were in good agreement with the respective literature data [3, 4].

Acid Hydrolysis. The pure compounds were treated with 2M 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 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); detection: aniline malonate. So **1**, **4** gave glucose, **2** gave glucose and rhamnose, while **3** was acid resistant.

Compound 1, $C_{22}H_{22}O_{12}$; mp 154–157°C; R_f 0.36 (system I), 0.40 (system II), 0.74 (system III).

UV (λ_{max}, nm), MeOH: 357, 300sh, 267sh, 255; +NaOH: 415, 329, 271; +$AlCl_3$: 397sh, 361, 300, 267; +HCl: 398, 359, 300sh, 267; +NaOAc: 384, 320, 274; +H_3BO_3: 358, 305sh, 267sh, 255. FAB$^+$-MS(m/z): 479 $[M+H]^+$, 316 [M+H-glucosyl].

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.92 (1H, d, J = 2, H-2′), 7.61 (1H, dd, J = 8.5, J = 2, H-6′), 6.92 (1H, d, J = 8.5, H-5′), 6.35 (1H, d, J = 2, H-8), 6.17 (1H, d, J = 2, H-6), 5.34 (1H, d, J = 7.5, H-1″glucose), 3.96 (3H, s, OMe-3′), 3.15–3.80 (protons of glucose).

^{13}C NMR (62.89 MHz, CD_3OD, δ, ppm): 177.59 (C-4), 167.46 (C-7), 161.33 (C-5), 157.16 (C-2), 156.91 (C-9), 149.45 (C-4′), 147.00 (C-3′), 133.76 (C-3), 122.40 (C-1′), 121.60 (C-6′), 114.61 (C-2′), 112.78 (C-5′), 103.48 (C-10), 102.45 (C-1″), 98.21 (C-6), 94.04 (C-8), 77.00 (C-5″), 76.57 (C-3″), 74.42 (C-2″), 69.94 (C-4″), 61.02 (C-6″), 55.33 (OMe-3′). Identified as isorhamnetin-3-O-glucoside.

Compound 2, $C_{28}H_{32}O_{16}$; mp 172–174°C; R_f 0.55 (system I), 0.35 (system II), 0.30 (system III).

1) Departement de chimie, Faculte des sciences, Universite Mentouri de Constantine, route de Ain el-Bey, 25000, Constantine, Algerie, tel/fax: 213 31 61 46 22, e-mail: ahmedtouil@yahoo.fr. 2) EA 2106 Biomolecules et Biotechnologie vegetales, Faculte des Sciences Pharmaceutiques, Universite de Tours, France. Published in Khimiya Prirodnykh Soedinenii, No. 1, pp. 85-86, January-February, 2006. Original article submitted May 6, 2005.

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UV (λ_{max}, nm), MeOH: 357, 300sh, 267sh, 254; +NaOH: 415, 329, 271; + $AlCl_3$: 397sh, 361, 300, 278sh, 267; +HCl: 398, 359, 302sh, 277sh, 267; +NaOAc: 384, 320, 274; +H_3BO_3: 359, 305sh, 270sh, 255. FAB^+-MS(m/z): 624 $[M+H]^+$, 316 [M+H-rutinosyl].

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.97 (1H, d, J = 1.8, H-2′), 7.62 (1H, dd, J = 8.5, J = 1.8, H-6′), 6.91 (1H, d, J = 8.5, H-5′), 6.34 (1H, d, J = 1.8, H-8), 6.16 (1H, d, J = 1.8, H-6), 5.19 (1H, d, J = 7.3, H-1″glucose), 4.54 (1H, d, J = 1.2, H-1‴), 3.95 (3H, s, OMe-3′), 1.12 (3H, d, J = 6.2, H-6‴ rhamnose), 3.25–3.80 (protons of rutinose). Identified as isorhamnetin-3-*O*-rutinoside.

Compound 3, $C_{27}H_{30}O_{15}$; mp 149–150°C; R_f 0.45 (system I), 0.04 (system II), 0.06 (system III).

UV (λ_{max}, nm) MeOH: 332, 273; +NaOH: 401, 328, 271; + $AlCl_3$: 392sh, 348, 305, 280; +HCl: 392sh, 345, 304, 280; +NaOAc: 363, 339, 276; +H_3BO_3: 363, 347, 280.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 8.01 (2H, d, J = 8.7, H-2′, H-6′), 6.91 (2H, d, J = 8.7, H-3′, H-5′), 6.78 (1H, s, H-3), 5.2 (1H, m, H-1″), 4.8 (1H, m, H-1‴), 2.50 – 3.90 (protons of two glucoses).

So **3** is identified as apigenin-6,8-di-C-glucoside (vicenin-2).

Compound 4, $C_{22}H_{22}O_{12}$; mp 315–317°C; R_f 0.32 (system I), 0.25 (system II), 0.76 (system III).

UV (λ_{max}, nm), MeOH: 349, 267sh, 255; +NaOH: 391, 321, 272; +$AlCl_3$: 395sh, 356, 300sh, 267; + HCl: 396sh, 355, 300sh, 268; +NaOAc: 369, 322, 274;+H_3BO_3: 355, 267sh, 256.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.71 (2H, m, H-2′, H-6′), 7.06 (1H, d, J = 9.1, H-5′), 6.25 (1H, d, J = 1.9, H-8), 6.10 (1H, d, J = 1.9, H-6), 5.16 (1H, d, J = 7.2, H-1″), 3.95 (3H, s, OMe-4′), 3.25–3.80 (protons of glucose; **4** is identified as tamarixetin-3-*O*-glucoside).

Thus, all these compounds are isolated from *P. chloranthus* for the first time, compounds **1**, **2** are isolated from other species *P. triradiatus* and *P. tortuosus* [5, 6], but to our knowledge compounds **3**, **4** are identified for the first time in the genus *Pituranthos*.

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REFERENCES

1. P. Ozenda, *Flore du Sahara Septentrional et Central*, C.N.R.S., Paris, 1958.
2. K. R. Markham, *Techniques of Flavonoid Identification*, Academic Press, London, 1982.
3. T. J. Mabry, K. R. Markham, and M. B. Tomas, *The Systematic Identification of Flavonoids*, Springer, New York, 1970.
4. J. B. Harborne and T. J. Mabry, *The Flavonoids: Advances in Research*, Chapman and Hall, London, 1982.
5. A. N. Singab, T. Khalifa, G. H. Mahran, Y. Okada, Y. Matsumaru, H. Nishino, and T. Okuyama, *Natural Medicines* (Tokyo), **52**(2), 191 (1998).
6. N. Shalaby, *Modelling, Measurement and Control, C: Energetics, Chemistry and Chemical Engineering, Earth, Resources, Environment, Biomedical Problems*, **57** (1), 17–35 (1998).