$CHCl_3$-EtOAc-MeOH, 2:2:1, and $CHCl_3$–iPrOH, 9:1, and reverse phase (Stratocrom C-18, Whatman), H_2O-EtOH, 6:4].

Full details on the isolation and identification of the compounds are available on request to the author.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Italian Ministry of Education, Ministero della Pubblica Istruzione.

LITERATURE CITED

1. G.K. Phokas, *Farmakeftikon Deltion*, **1**, 9 (1971).
2. A. Evidente, I. Iasiello, and G. Randazzo, *J. Nat. Prod.*, **47**, 1061 (1984).
3. A. Evidente, I. Iasiello, and G. Randazzo, *J. Nat. Prod.*, **47**, 1003 (1984).
4. W.C. Wildman, in: *The Alkaloids*. Ed. by R.H.F. Manske, Vol. XI, Academic Press, New York, 1968, p. 355.
5. A.M. Duffield, R.T. Aplin, H. Budzikiewicz, C. Djerassi, C.F. Murphy, and W.C. Wildman, *J. Am. Chem. Soc.*, **87**, 4902 (1965).
6. H.M. Fales and W.C. Wildman, *J. Am. Chem. Soc.*, **82**, 197 (1960).

Received 2 May 1985

THE FLAVONOIDS OF *AUREOLARIA VIRGINICA*

MOSTAFA K. MESBAH, CHUNG K. CHU,* and SAMIA L. DOSS

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Georgia, Athens, Georgia 30602

Aureolaria virginica L. (Scrophulariaceae) is a wild, annual herb distributed over most of the southern United States (1). The plant is usually found in dry, open woods where it is parasitic upon the roots of oaks (2). No chemical work has previously been reported on this plant. During our search for the pharmacologically active compounds, the flavonoids apigenin, apigenin-7-*O*-glucoside, kaempferol-3-*O*-rhamnoside, quercitrin, and quercetin-3-*O*-arabinopyranoside were isolated from the leaves.

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *A. virginica* were collected in August 1984, from the Oconee Forest, School of Forest Resources, University of Georgia, Athens, Georgia. The plant was identified by Nancy C. Coile, Herbarium, Department of Botany, University of Georgia. A voucher specimen is deposited in the Medicinal Chemistry and Pharmacognosy Department, College of Pharmacy, University of Georgia.

EXTRACTION, SEPARATION AND IDENTIFICATION.—The leaves (700 g) were cold extracted with 85% MeOH followed by 50% MeOH. The combined methanolic extracts were concentrated under reduced pressure until only the H_2O remained. The aqueous layer was then extracted with *n*-hexane followed by $CHCl_3$ and finally EtOAc. Paper chromatography indicated that only the EtOAc and $CHCl_3$ fractions contained flavonoids. The material from the $CHCl_3$ fraction (10.2 g) was chromatographed over a silica gel column (5×70 cm) using $CHCl_3$-MeOH mixtures. Apigenin (31 mg) was obtained from the $CHCl_3$-MeOH (96:4) fraction. THe EtOAc fraction (8.2 g) was chromatographed over a polyamide column (5×80 cm) eluted with MeOH-H_2O (3:13) with increasing amounts of MeOH to 100%. The flavonoids isolated from this column were apigenin-7-*O*-glucoside (95 mg), kaempferol-3-*O*-rhamnoside (25 mg), and a mixture of quercitrin and quercetin-3-*O*-arabinopyranoside (105 mg). This mixture was further separated by droplet counter current chromatography (dccc) using the upper layer of $CHCl_3$-MeOH-H_2O (7:13:8) as the mobile phase. All the flavonoids were purified before spectral analysis on columns of Sephadex LH20 (100×2 cm) eluted with MeOH. The flavonoid structures were determined by standard methods of uv, ^1H nmr, ^{13}C nmr, and mass spectroscopy (3-5). Sugars were identified from their gas liquid chromatograms after hydrolysis and trimethylsilylation (6). Further confirmation of the structures was made by comparison of the aglycones with reference compounds.

LITERATURE CITED

1. W.H. Duncan and L.E. Foot, "Wild Flowers of the Southeastern United States," The University of Georgia Press, Athens, 1975, p. 176.

2. M.V. Walcott, "Wild Flowers of America," Crown Publishers, New York, 1953, p. 55.
3. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970.
4. J.B. Harborne and T.J. Mabry, "The Flavonoids: Advances in Research," Chapman and Hall, New York, 1982.
5. K.R. Markham, "Techniques of Flavonoid Identification," Academic Press, New York, 1982.
6. C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).

Received 30 May 1985

FLAVONOL DIGLYCOSIDES FROM *MELIA AZEDARACH*

J.A. MARCO,* O. BARBERÁ, J.F. SANZ, and J. SÁNCHEZ-PARAREDA

Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad de Valencia, Burjasot, Valencia, Spain

Melia azedarach L. is an ornamental tree, very common in the last century in Spanish gardens and promenades, although not so frequent today. It originated in China and the Himalaya country and was introduced to the Mediterranean region. It is the only *Melia* species to be found in Europe. Few reports on the genus *Melia* have appeared in the literature, and only two of them refer to flavonoid constituents. Subramanian *et al.* (1) isolated three known flavonol monoglycosides and a new one, myricetin-3′-*O*-L-arabinoside, from *M. azadirachta*. The same authors later reported (2) the isolation of quercetin-3-*O*-L-rhamnoside and quercetin-3-*O*-β-rutinoside (rutin) from *M. azedarach* and *Soymida febrifuga*. *M. azedarach* is the only plant from the Meliaceae family, which comprises about 50 genera and 800 species, investigated in relation to its flavonoid content (3). We now report the isolation in high yield of two flavonol diglycosides from the leaves of *M. azedarach*: quercetin-3-*O*-β-rutinoside (rutin) and kaempferol-3-*O*-β-rutinoside. This is, to our knowledge, the second report on the isolation of flavonoid diglycosides in the Meliaceae. Interestingly, we did not find detectable amounts of quercetin-3-*O*-L-rhamnoside (2), whereas the Indian workers did not report the isolation of kaempferol-3-*O*-β-rutinoside, a fact which may be related to the different habitat of both plant specimens.

EXPERIMENTAL

PLANT MATERIAL.—*M. azedarach* has been authenticated by Prof. J. Mansanet, of the Botany Department at the Faculty of Biology in Valencia. A voucher specimen is deposited in the herbarium of this Department. Leaves of the plant were collected in July at Buñol, Valencia, Spain, then air-dried and finely ground.

EXTRACTION AND ISOLATION.—The plant material (10 g) was extracted at room temperature with 80% aqueous MeOH (250 ml, 36 h) and 50% aqueous MeOH (250 ml, 36 h) (4). The extracts were mixed, concentrated with a rotary evaporator to remove most of the MeOH and extracted several times with $CHCl_3$ and EtOAc. The $CHCl_3$ extract did not contain detectable amounts of flavonoid aglycones. The EtOAc extract weighed 40 mg and showed two major spots on tlc plates. These were separated by column chromatography on Polyamide Macherey-Nagel SC6, 0.05-0.16 mm (elution with $CHCl_3$-MeOH-MeCOEt, 6:2:1). The two, tlc-pure, main fractions were percolated through a Sephadex LH-20 column (elution with MeOH). This gave kaempferol-3-*O*-β-rutinoside (14 mg, 0.14%) and rutin (8 mg, 0.08%), identified by spectroscopic (uv, ^1H and ^{13}C nmr, fabms) and direct (pc, tlc, mp) comparison with authentic samples. The aqueous extract that remained after the two extractions also contained some of these two compounds, but only trace amounts of other flavonoids.

Full details of the isolation and identification of the compounds can be obtained from the senior author.

LITERATURE CITED

1. S.S. Subramanian and A.G.R. Nair, *Indian J. Chem.*, **10**, 452 (1972).
2. A.G.R. Nair and S.S. Subramanian, *Indian J. Chem.*, **13**, 527 (1975).
3. M.M. Rao, P.J. Gupta, E.M. Krishna, and P.P. Singh, *Indian J. Chem.*, **17B**, 178 (1979).
4. K.R. Markham, *Techniques of Flavonoid Identification*, London, Academic Press, 1982, Chapt. 2.

Received 7 June 1985