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FLAVONOIDS FROM *ACHYROCLINE TOMENTOSA*

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Continuing our phytochemical investigation on Argentine plants from the genus *Achyrocline* (Compositae) used in folk medicine (1-4), we have now investigated *Achyrocline tomentosa* Rusby (5), a perennial plant that grows in the Northern and Central regions of Argentina. It is known by the common name of "vira-vira" and is reputed to be an antitussive and is used for the treatment of influenza (6). In this paper, we report the isolation and identification of two flavonoids and the known β-sitosterol from the C_6H_6 extract of the aerial parts of this plant. The flavonoids isolated were identified as: 3,5-dihydroxy-7,8-dimethoxyflavone (**1**) and 5,7,4′-trihydroxy-8,3′-dimethoxyflavanone (**2**).

There is a noted uncertainty about the correct structure of gnaphaliin which Hänsel and Ohlendorf (7) isolated from *Gnaphalium obtusifolium* and for which they proposed the structure of 3,5-dihydroxy-7,8-dimethoxyflavone. Later, Wagner and co-workers (8) synthetized several 3,5,7,8-substituted flavones and reviewed the structure of gnaphaliin, for which they then proposed the structure of 5,7-dihydroxy-3,8-dimethoxyflavone.

The structure 3,5-dihydroxy-7,8-dimethoxyflavone, was assigned to **1** on the basis of the study of its uv, nmr, and ms spectra (9). The mp and uv (MeOH) data found were identical to those reported in the literature for this substance synthetized by Wagner *et al.* (8). This is, thus, the first report in the literature of this substance as a natural product.

The uv spectrum of compound **2** showed it to be a 5,7-dihydroxyflavanone. The ^{1}H-nmr spectrum gave a multiplet at 7.5 ppm characteristic of a dioxygenated B ring; a double doublet at 5.6 ppm corresponding to the proton attached to C2, and a multiplet at 2.9 due to the proton attached to C3. The two signals at 3.75 and 3.6 ppm, integrating for three protons each, corresponded to two methoxyl groups. The sustitution of C8 by a methoxyl group is indicated because of the position of the singlet at 6.0 ppm characteristic for an H on C6 (10).

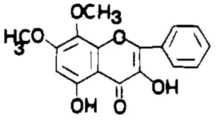
In order to determine the position of the hydroxyl and methoxyl groups on the B ring, alkaline degradation of the flavanone was performed and vanillic acid was obtained, indicating a 3′-methoxy-4′-hydroxy substitution (11).

There is no previous report in the literature of 5,7,4′-trihydroxy-8,3′-dimethoxyflavanone from a natural source.

EXPERIMENTAL

PLANT MATERIAL.—The material was collected in March 1983, in Cordoba Province, Argentina. A voucher specimen is deposited at the University Herbarium Museo de Botanica, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires.

GENERAL EXPERIMENTAL PROCEDURES.—Chemical structures of the compounds were elucidated using ^{1}H-nmr, uv, and ms techniques. Uv spectra were recorded on a Shimadzu UV-240 spectrophotometer. ^{1}H-nmr spectra were obtained on a Varian FT80A with TMS as internal standard. Electron impact mass spectra were made on a Varian mat CH-7A Data System 166.



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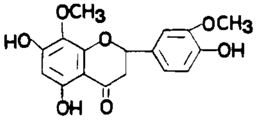

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EXTRACTION AND ISOLATION.—Air-dried, aerial parts were extracted successively with C_6H_6, $CHCl_2$, Me_2CO, and MeOH. The C_6H_6 extract (23 g) was chromatographed over a flash column of Silica gel PF 254 using C_6H_6 as the developing solvent. Two major fractions were collected. Fraction 1 was chromatographed and purified on Sephadex LH 20 using C_6H_6 affording 3,5-dihydroxy-7,8-dimethoxyflavone (23 mg). Fraction 2 was chromatographed and purified by the same procedure. Two bands were collected, A and B. Band A afforded β-sitosterol (15 mg) and Band B yielded 5,7,4'-trihydroxy 8,3'-dimethoxyflavanone (64 mg).

3,5-DIHYDROXY-7,8-DIMETHOXYFLAVONE (**1**.—Yellow crystals (MeOH) mp 202-203° (lit. 204-205°) (8). Uv: purple; uv/NH_3 yellow. Pc, Whatman 3, *t*-BuOH-HOAc-H_2O, 3:1:1 (TBA) Rf: 0.94. Uv λ max (MeOH) 250 sh, 271, 296, 308, 372; NaOMe 255, 266, 330 sh, 417; $AlCl_3$ 240 sh, 250, 276, 310 sh, 340, 430; $AlCl_3$/HCl 240 sh, 250, 276, 310 sh, 340, 431; NaOAC 252 sh, 269, 310 sh, 400; NaOAc/H_3BO_3 252 sh, 270, 310 sh, 375. ^{1}H nmr (DMSO-d_6) d 8.00 (m, 5H, H2', H6', H3', H4', H5 '), d 6.70 (s, 1H, H6), d 4.00 (s, 3H, OCH_3), and d 3.87 (s, 3H, OCH_3). Ms *m/z* (rel. int. %) 314 (M^+) (15%), 299 (M-CH_3) (36%), 286 (1%), 271 (2%), 256 (2%), 182 (4%), 153 (40%), 105 (89%), 102 (7%), 89 (25), 77 (100%).

5,7,4'-TRIHYDROXY- 8, 3-DIMETHOXYFLAVANONE (**2**).—Uv: purple; uv/NH_3: pale yellow. Pc, Whatman 3, TBA Rf: 0.97. Uv λ max (MeOH) 242 sh, 290, 325 sh. NaOME 245, 325; $AlCl_3$ 310, 380 sh; $AlCl_3$/HCl 310, 380 sh; NaOAc 250 sh, 290 sh, 327; NaOAc/H_3BO_3 250 sh, 327. ^{1}H nmr (DMSO-d_6) d 7.50 (m, 3H, H2', H5',H6'), d 6.00 (s , 1H, H6), d 5.6 (dd, 1H, H2), d 3.75 (s, 3H, OCH_3), d 3.60 (s, 3H, OCH_3), and a 2.95 (m, 2H, H3). Ms *m/z* (rel. int. %) 332 (M+) (11%), 317 (M-CH_3) (5%), 302 (2%), 183 (7%), 150 (100%), 135 (40%).

ALKALINE DEGRADATION OF **2**.—Compound (**2**, 8 mg) in 0.5 ml of MeOH was refluxed with 2 ml of a solution of 30% NaOH for 3 h. The reaction mixture was cooled, acidified with concentrated HCl, and extracted with Et_2O (3 × 10 ml). Vanillic acid was identified in the Et_2O extract by direct comparison with an authentic sample (tlc, Si gel 60 F254 using C_6H_6-dioxane-HOAc, 90:25:4).

β-SITOSTEROL.—Identified by direct comparison (mp, ir, ms) with an authentic sample.

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