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5,7,3′,4′-TETRAHYDROXY-6-METHOXYFLAVANONE FROM *EUPATORIUM SUBHASTATUM*

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Key Word Index—*Eupatorium subhastatum*; Compositae; 5,7,3′,4′-tetrahydroxy-6-methoxyflavanone; acacetin; hispidulin; eupatorin, protocatechuic acid.

Abstract—5,7,3′,4′-Tetrahydroxy-6-methoxyflavanone has been isolated for the first time from *Eupatorium subhastatum*, besides acacetin, hispidulin, eupatorin and protocatechuic acid. Structures were assigned on the basis of spectroscopic analysis.

INTRODUCTION

Eupatorium subhastatum Hooker et Arnott [1], a perennial herb widely distributed in the northeastern region of the Argentine Republic, is a medicinal plant [2, 3]. In a previous paper [4] we reported the isolation of eriodictyol (5,7,3′,4′-tetrahydroxy flavanone), eupafolin (5,7,3′,4′-tetrahydroxy-6-methoxyflavone), quercetin, kaempferol, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside and quercetin 3-rutinoside. Preliminary pharmacological tests of eriodictyol *in vitro* and *in vivo* chemiluminiscence assays, have demonstrated its antioxidant and free radical scavenging properties, showing a protective action against liver damage in situations of oxidative stress [5]. Reexamination of the extracts of this plant using more sensitive techniques led to the identification of acacetin (5,7-dihydroxy-4′-methoxyflavone), protocatechuic acid, hispidulin (5,7,4′-trihydroxy-6-methoxyflavone), eupatorin (5,3′-dihydroxy-6,7,4′-trimethoxyflavone) and 5,7,3′,4′-tetrahydroxy-6-methoxyflavanone (**1**) a new flavanone.

RESULTS AND DISCUSSION

Compound **1** was isolated from the CH_2Cl_2 extract by Polyclar column chromatography eluted with CH_2Cl_2–MeOH (9:1) and crystallized from MeOH (mp 218–220°). The UV spectra were typical of a flavanone; the shifts induced by addition of different reagents ($AlCl_3$, NaOAc, and NaOMe) [6, 7] led us to conclude that the compound had hydroxyl groups at C5, C7 and C4′. Its ^1H NMR spectrum showed flavanone signals: protons at C2 appeared as a quartet centred at 5.3 ppm and the protons at C3 gave a multiplet at 2.0 ppm. Other ^1H NMR signals showed the presence of 3′,4′disubstituted aromatic ring (B ring), a singlet at 6.02 due to the proton at C8 and one methoxyl group. The structure of **1** was further confirmed by MS. Its spectrum showed a $[M]^+$ at 318 (3%), and a molecular ion at 303 (92%) which was indicative of the loss of a C6 methoxyl group. Ions at 183 (17%) corresponding to $[A_1]^+$, 169 (9%) $[A_1 - 15]$, from the A ring and 136 (100%) $[B_1^+]$ corresponding to B ring were also present.

Dehydrogenation of **1** [8] gave 5,7,3′4′-tetrahydroxy-6-methoxyflavone. This was identified by cochromatography in different solvents with an authentic sample of the compound isolated from the same plant [4]. Though flavanones are commonly found in the Compositae, this is the first report of 5,7,3′,4′-tetrahydroxy-6-methoxyflavanone from a natural source.

EXPERIMENTAL

Mps were taken with a Mettler FP2. ^1H NMR spectra were measured using TMS as int. standard.

Eupatorium subhastatum H. et A. was collected in Chaco Province, Argentina. A voucher specimen is deposited in the University Herbarium (Museo de Botánica, Universidad de Buenos Aires, Argentina).

The air-dried and finely ground (1.1 kg) aerial parts of the plant were extracted with EtOH (5 × 8 l) at room temp. The ethanolic extract was taken to dryness under vacuo, suspended with hot H_2O and extracted successively in a continuous liquid–liquid apparatus with C_6H_6, CH_2Cl_2 Et_2O and EtOAc. The CH_2Cl_2 extract was chromatographed on a Polyclar AT column eluted with C_6H_6, CH_2Cl_2 and MeOH. The fractions eluted with CH_2Cl_2–MeOH 19:1 afforded acacetin which was purified by preparative TLC on Silica gel 60 F 254 (Merck) using C_6H_6–EtOAc (7:3). Fractions eluted with CH_2Cl_2–MeOH (9:1) afforded **1** (5,7,3′,4′-tetrahydroxy6-methoxyflavanone) by crystallization from MeOH. Eupafolin, previously isolated from the Et_2O extract of this plant [4] crystallized from fractions eluted with CH_2Cl_2–MeOH (7:3). After filtration the supernatant was purified by preparative TLC on Silica gel 60 F 254 (Merck) using C_6H_6–EtOAc (7:3) afforing hispidulin and eupatorin. Protocatechuic acid and eriodictyol (previously isolated from the Et_2O extract of this plant) [4] crystallized from the fractions eluted with CH_2Cl_2–MeOH (3:2) and (1:1) respectively.

Acacetin (5,7-*dihydroxy-4′-methoxyflavone*). From the Polyclar column by elution with (CH_2Cl_2–MeOH (19:1), and purified by prep. TLC; UV: deep purple, UV/NH_3: no change. R_f HOAc 15%: 0.06, R_f TBA: 0.94. UV $\lambda_{\max}^{\text{MeOH}}$ nm: 267, 290 sh, 325; NaOMe: 275, 295 sh, 360; $AlCl_3$: 275, 300, 340, 380 sh; $AlCl_3$/HCl: 275, 300, 340, 350 sh; NaOAc: 275, 295 sh, 355; NaOAc/H_3BO_3: 265, 295 sh, 340.

5,7,3′,4′-*Tetrahydroxy-6-methoxyflavanone* (1). Crystallized from the fractions eluted with CH_2Cl_2–MeOH (9:1) from the Polyclar column. Mp 218–220° (MeOH). UV: pale yellow blue; UV/NH_3:no change; PC Whatman No. 1, R_f HOAc: 15% 0.37, R_f TBA: 0.90; UV λ_{max}^{MeOH} nm: 227 sh, 286, 321 sh; NaOMe: 247, 321; $AlCl_3$: 307, 366; $AlCl_3$/HCl: 307, 366; NaOAc: 250 sh, 286 sh, 323; NaOAc/H_3BO_3: 250 sh, 286 sh, 323. ^1H NMR (DMSO-d_6): 7.0 (*m*, 3H, H2′, 5′,6′); 6.02 (*s*, 1H, H8); 5.3 (*m*, 1H, H2); 3.98 (*s*, 3H, OCH_3); 2.9 (*m*, 2H, H3). MS, *m/z* (%): 318 (3); 303 (92); 287 (3); 183 (17); 169 (9%); 151 (85); 136 (100); 107 (17).

Hispidulin (5,7,4′-*trihydroxy-6-methoxyflavone*). Was eluted with CH_2Cl_2–MeOH (7:3) and then purified by prep. TLC on Silicagel 60 F 254 (Merck) using C_6H_6–EtOAc (7:3) as solvent. UV: deep purple, UV/NH_3 yellow-green, PC Whatman No. 1, R_f HOAc 15% = 0.08; R_f TBA: 0.95. UV λ_{max}^{MeOH} nm: 272, 335; NaOMe: 276, 324; $AlCl_3$: 262 sh, 280 sh, 300 sh, 360, 390 sh; $AlCl_3$/HCl: 262 sh, 277 sh, 300 sh, 352; NaOAc: 274, 370; NaOAc/H_3BO_3: 274, 370. ^1H NMR (DMSO-d_6): 7.95 (*d*, 2H, $J = 9$ Hz, H2′, 6′); 7.05 (*d*, 2H, $J = 9$ Hz, H3′,5′); 6.70 (*s*, 1H, H8); 6.60 (*s*, 1H, H3); 3.72 (*s*, 3H, OMe). MS, *m/z* (%), 300 $[60, M]^+$ 299 [5, M − 1]; 285 [45, M − 15]; 257 [45, M − 43]; 182 $[1, A_1]^+$; 167 (20); 139 (30); 121 (10); 118 (20).

Eupatorin (5,3′-*dihydroxy-6,7,4′-trimethoxyflavone*). Was isolated from the fractions eluted with CH_2Cl_2–MeOH (7:3) by stepped chromatography on Silica gel 60 F 254 (Merck) using C_6H_6–EtOAc (7:3). UV: deep purple, UV/NH_3: no change, PC Whatman No. 1, R_f HOAc 15% = 0.09, TBA = 0.85 UV λ_{max}^{MeOH} nm: 240; 250 sh, 272, 338; NaOMe: 240 sh, 266, 326, 376; $AlCl_3$: 260, 284, 290 sh, 370; $AlCl_3$/HCl: 258, 282, 364; NaOAc: 268, 336; NaOAc/H_3BO_3: 250 sh, 272, 338. MS, *m/z* (%): 344 $[1, M]^+$; 330 [15, M − 15]; 315 [10, M − 30]; 301 [10, M − 43]; 196 (3); 182 (3); 167 (20); 153 (15); 151 (1); 148 (15).

Protocatechuic acid (3,4-*dihydroxybenzoic acid*). Mp 191–193°. UV λ_{max}^{MeOH} nm 222 sh, 252, 292; NaOMe: 225 sh, 270, 298. The structure was confirmed by chromatographic comparison with an authentic sample in different solvents.

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