Journal of Natural Products
Vol. 51, No. 3, pp. 586-587, May-Jun 1988

4′,4‴-DIMETHYLCUPPRESSUFLAVANONE FROM *EUPATORIUM SUBHASTATUM*

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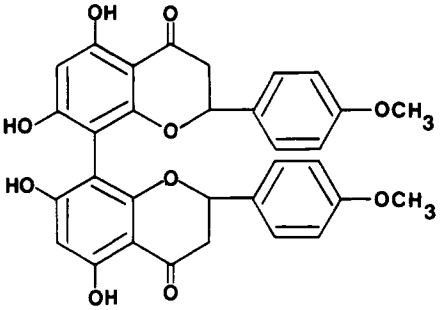
We have previously reported the isolation of several flavonoids and other polyphenolic compounds from *Eupatorium subhastatum* Hook. et Arn. (Asteraceae) (1,2). This species is a popular medicinal plant in Argentina, widely used as an anti-inflammatory, a digestive, and in the treatment of eye diseases (3,4). Preliminary pharmacological tests of eriodyctiol from the same plant species have demonstrated its antioxidant and free radical scavenging properties in chemiluminescence assays in vivo and in vitro. This compound shows a protective action against liver damage in situations of oxidative stress (5).

We report here the isolation and characterization of a biflavanone (I-5,II-5, I-7,II-7-tetrahydroxy-I-4′,II-4′-dimethoxy I-8,II-8-biflavanone) **1** from the C_6H_6 extract of *E. subhastatum*.



1

This compound showed the uv spectral profile (280 sh, 290, 320 sh) and diagnostic shifts typical of a naringenin flavanone skeleton but with a higher ε (26261) at 290 nm. Red color with $FeCl_3$ supported this observation.

The ^{1}H-nmr spectrum showed two doublets at δ 6.90 and δ 7.40 indicating a 4′ monosubstituted B ring; only one signal at δ 5.65 (singlet), corresponding to A ring protons, was assigned to H-6. No H-8 signal (δ 5.90–6.10) was present (6). The flavanone signals appeared at δ 5.25 (quartet, H-2) and at δ 2.75 (multiplet, H-3). One signal corresponding to two methoxyl groups appeared at δ 3.85.

Ms revealed a molecular ion at *m/z* 570 corresponding to the molecular formula $C_{32}H_{26}O_{10}$ indicative of a biflavanone and also some characteristic fragments at *m/z* 286 $[M/2]^+$, 134 $[B_1]^+$, 119 $[B_1 - Me]^+$, and 152 $[A_1]^+$. Permethylation (7) of the compound was carried out in order to confirm the data obtained by ms. Ms of the permethylated compound showed a molecular ion at *m/z* 626, corresponding to the molecular formula $C_{36}H_{34}O_{10}$, by introduction of four methyl groups in **1**. The most characteristic feature in this spectrum is the formation of two fragments at *m/z* 412 and 355, which indicated the presence of a I-8,II-8 flavanone linkage (8,9).

The analysis of these data led us to conclude that the isolated compound is a biflavanone to which structure **1** was assigned.

This is the first report on the isolation and identification of a biflavanone in the family Asteraceae. As biflavonoids are characteristic compounds of primitive plant families such as the Cupressaceae and Podocarpaceae, we would like to point out the presence of this atypical biflavanone in the more evolved family Asteraceae.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Shimadzu uv 240 spectrophotometer with a PR1 graphic printer. ^{1}H-nmr spectra were recorded on a Varian FT80

A spectrometer using CD_3OD as solvent and TMS as internal standard. Ms spectra were recorded on a Varian CH7A Data System 166. Cc was performed using Sephadex LH20. Preparative tlc was performed using Si gel HF 254.

PLANT MATERIAL.—*E. subhastatum* was collected in the Chaco Province, Argentina. A voucher specimen is deposited at the University Herbarium Museo de Botánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

EXTRACTION AND ISOLATION.—Air-dried, finely ground, aerial parts of *E. subhastatum* (1.1 kg) were extracted with EtOH (5 × 8 liters) at room temperature. The EtOH extract was taken to dryness under vacuo, suspended with hot H_2O, and extracted in a continuous apparatus with C_6H_6. This extract was chromatographed on a Sephadex LH 20 column using C_6H_6, CH_2Cl_2, and MeOH as the developing solvents. Fractions eluted with CH_2Cl_2-MeOH (99:1) showing a major spot by contrast under uv 254 light were purified by preparative tlc on Si gel using C_6H_6-EtOAc (9:1) as solvent. The uppermost band was scraped and eluted with MeOH. A white yellow precipitate appeared upon concentration, yielding the biflavonyl compound **1** (26 mg).

4′,4‴-DIMETHYLCUPRESSUFLAVANONE [**1**].—This compound did not melt below 300°. Positive reaction with $FeCl_3$ red color. Uv (MeOH) λ max nm 280 sh, 290 (ε 26261), 320 sh, (NaOMe) 250 sh, 325, ($AlCl_3$) 307, 370, ($AlCl_3$/HCl) 307, 370, (NaOAc) 280 sh, 321, (H_3BO_3) 290, 320 sh; ^{1}H nmr (CD_3OD) δ 7.40 (4H, dd, H-2′, H-2‴, H-6′, H-6‴), 6.95 (4H, dd, H-3′, H-3‴, H-5′, H-5‴), 5.65 (2H, s, H-6, H-6″), 5.25 (2H, dd, H-2, H-2″), 3.85 (6H, s, 2OMe), 2.75 (4H, m, H-3, H-3″); ms *m/z* (%) 570 (5), 540 (appears as 270 $[M-2Me/2]^+$ (2), 354 (3), 336 (2), 327 (2), 302 (3), 286 (100), 285 (9), 258 (9), 152 (18), 134 (9), 124 (24), 119 (35), 108 (14).

METHYLATION OF **1**.—Compound **1** (12 mg), dissolved in Me_2CO, was refluxed with 1.2 g K_2CO_3 and 0.1 ml Me_2SO_4 for 24 h. The reaction mixture was filtered, and the supernatant was concentrated and chromatographed by tlc on Si gel using C_6H_6-EtOAc (7:3). The major band (R_f 0.52), bearing pale blue fluorescence under uv 366 light, was scraped off and eluted with MeOH affording the tetramethylderivative of **1**. Uv (MeOH) λ max nm 230, 280, 315 sh, no change with NaOMe, $AlCl_3$, NaOAc; ms *m/z* (%) 626 (3), 622 (5), 477 (1.8), 462 (3.4), 447 (1), 434 (5.5), 431 (1.5), 416 (1), 412 (1), 397 (2), 355 (2), 341 (8.3), 327 (7.2), $[M/2]^+$ 313 (100), 311 (5), 298 (20.5), 283 (6.5), 180 (88), 134 (91.4), 108 (11.3).

ACKNOWLEDGMENTS

We would like to thank Prof. Benjamín Frydman, Department of Organic Chemistry, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, for recording the ^{1}H-nmr spectrum. This investigation was supported by IQUIMEFA (CONICET-UBA) PID N° 3-922802/85 and 3-922803/85.

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Received 14 September 1987