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A BIFLAVONOID FROM *PHYLLANTHUS SELLOWIANUS*

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Phyllanthus sellowianus Mueller Arg. (Euphorbiaceae) is a shrub native to South America. It is known by the common name of "sarandí blanco" and is used as an antidiabetic agent in folk medicine (1,2). In a previous paper we described the isolation and structure determination of phyllanthol from the less polar extract (3). In this paper, we report the isolation and identification of a biflavonoid from the Me_2CO extract of the stem bark of this plant which was identified as a 4′,4‴ di-*O*-methyl cupressuflavone.

A review of the literature on naturally occurring biflavonoids revealed that those belonging to the group of cupressuflavone (4,5) are a rare example of biflavonyl derived from apigenin with 8-8″ interflavonyl linkage. Ilyas *et al.* (6) have previously reported the presence of a 4′,4‴ di-*O*-methyl cupressuflavone in *Araucaria cunninghamii* and *Araucaria cookii*, but no convincing results are available in the literature concerning the spectroscopic analysis of this compound. Therefore, the spectroscopic data (uv, ir, ^{1}H nmr, ^{13}C nmr, and ms) are now reported.

The isolated compound appeared as a pale yellow powder, $C_{32}H_{22}O_{10}$, soluble in $CHCl_3$-MeOH (1:1) and did not melt below 300°. It showed the uv spectrum and diagnostic shifts similar to acacetin (7), but the molecular extinction coefficient values λ max (MeOH) 268 nm (ε 39,200) and 332 nm (ε 36,000) are clearly different (8). Therefore, we concluded that this compound contains two 5,7,4′-trioxygenated flavone units. The ir spectrum showed bands at 3370 cm^{-1} (hydroxyl) and 1625 cm^{-1} (chelated carbonyl), characteristic of 5-hydroxyflavones. The green color with $FeCl_3$ also supported the presence of the 5-hydroxyl group. The 90 MH_z ^{1}H-nmr spectrum in DMSO-d_6 indicated the singlets at 13.87 ppm (2-H) and 12.82 ppm (2-H), which were assigned to the hydroxyl groups at 5,5″ and 7,7″ positions. The doublet at 8.15 ppm (4-H) was attributed to 2′,6′ and 2‴,6‴ positions showing an *ortho* coupling with protons at 7.20 ppm (4-H) assigned to 3′,5′ and 3‴,5‴ positions. The singlet at 3.97 ppm (6-H) was assigned to methoxyl groups at 4′,4‴ positions. The singlet at 6.60 ppm (2-H) indicated that the structure should be of the 8-8″ biapigenyl type, because the signals for the 8 position protons generally appear slightly downfield up to 6.60 ppm (9). The ^{13}C-nmr spectrum was compared with literature data (10) that confirmed the structure assigned for the isolated compound. Hydrolysis with 2N HCl/MeOH confirmed that no sugar was present. The mass spectrum revealed a molecular ion at *m/z* 566 corresponding to the molecular formula $C_{32}H_{22}O_{10}$. Methylation with CH_2N_2 yielded a mixture of methyl ethers which were subjected to preparative tlc and yielded three bands. Only the major band was analyzed and identified by ms as a pentamethyl ether. The mass fragmentation pattern was in accordance with the proposed structure, significant peaks appearing at *m/z* 608 (M^+, 100%), 593, 578, 563, 548, 533, 476, 238, 135, 132.

In conclusion, the spectroscopic data revealed the structure to be a 4′,4‴ di-*O*-methyl cupressuflavone with the interflavonoid linkage at the 8,8″ positions.

Harborne *et al.* (10) mention the exis-

tence of biflavonoids in the family Euphorbiaceae. Though the cupressuflavone group is characteristic in the Cupressaceae family, our study demonstrates the presence of this kind of biflavonoid derivative in the Euphorbiaceae, thus, encouraging further investigations related to biflavonoids.

EXPERIMENTAL

PLANT MATERIAL.—The material was collected in February 1978, in Concepción del Uruguay, Argentina. A voucher specimen is deposited at the University Herbarium, Museo de Botánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

GENERAL EXPERIMENTAL PROCEDURES.—Chemical structure of the isolated compound was elucidated using uv, ir, ^{1}H nmr, ^{13}C nmr, and ms techniques. Uv spectra were recorded on a Shimadzu UV-240 spectrophotometer. Ir spectra were recorded on a Beckman Infrared Spectrophotometer. ^{1}H-nmr and ^{13}C-nmr spectra were obtained on a Varian FT 90 A with TMS as internal standard. Electron impact mass spectra were made on a Varian mat CH-7A Data System 166.

EXTRACTION AND ISOLATION.—The powdered, air-dried stem bark (900 g) of *P. sellowianus* was extracted successively with C_6H_6, CH_2Cl_2, Me_2CO, and MeOH. The Me_2CO extract (4.22 g) was chromatographed on a Polyclar AT NE 62466 column which was eluted with CH_2Cl_2 containing increasing amounts of MeOH. Elution with CH_2Cl_2-MeOH (4:6) yielded 4′,4‴ di-*O*-methyl cupressuflavone (20 mg).

4′,4‴ DI-*O*-METHYL CUPRESSUFLAVONE.—Pale yellow powder, soluble in $CHCl_3$-MeOH (1:1) which did not melt below 300°; uv dark purple; uv/NH_3 dark purple; pc, Whatman 3MM, HOAc 15%, Rf 0.30; uv pale yellow; uv/NH_3 yellow; tlc Si gel HF_{254}, toluene-ethyl formate-formic acid (3:4:3), Rf 0.58; uv orange; uv/NH_3 orange; tlc Si gel HF_{254}, EtOAc-butanone-HOAc-H_2O (5:3:1:1), Rf 0.60; uv λ max (MeOH) nm 268, 290 sh, 300 sh, 332; (NaOMe) 280, 298 sh, 374; ($AlCl_3$) 236 sh, 260 sh, 279, 307, 336, 338; ($AlCl_3$/HCl) 236 sh, 260 sh, 279, 307, 336, 388; (NaOAc) 268, 280 sh, 300 sh, 330; (NaOAc/H_3BO_3) 268, 280 sh, 300 sh, 330; ir ν max cm^{-1} 3370, 1625; ^{1}H nmr (DMSO-d_6) δ 13.87 (s, 2H, OH-5,5″), 12.82 (s, 2H, OH-7,7″), 8.15 (d, 4H, H-2′,6′,2‴,6‴), 7.20 (d, 4H, H-3′,5′,3‴,5‴), 6.97 (s, 2H, H-3,3″), 6.60 (s, 2H, H-6,6″), 3.97 (s, 6H, OCH_3-4′,4‴); ^{13}C nmr (DMSO-d_6) δ 161.2 (C-2,2″), 105.0 (C-3,3″), 185.4 (C-4,4″), 158.7 (C-5,5″), 86.5 (C-6,6″), 160.0 (C-7,7″), 100.0 (C-8,8″), 157.5 (C-9,9″), 107.5 (C-10,10″), 121.2 (C-1′,1‴), 131.2 (C-2′,2‴,6′,6‴), 112.5 (C-3′,3‴,5′,5‴), 162.0 (C-4′,4‴), 55.0 (C-4′,4‴ OCH_3); ms *m/z* (rel. int. %) 566 (M^+) (4.5%), 551 (M-CH_3) (1.2%), 536 (1.2%), 432 (2.7%), 283 (2.5%), 149 (2.3%), 135 (8.9%), 132 (7.6%), 57 (100%). Penta methyl ether derivative ms *m/z* (rel. int. %) 608 (M^+) (100%), 593 (M-CH_3) (75.0%), 578 (48.0%), 563 (35.0%), 548 (26.0%), 533 (19.0%), 476 (7.9%), 238 (34.0%), 135 (13.1%), 132 (28.1%).

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