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# ISOLATION OF 8,3′-DIHYDROXY-5,7,4′-TRIMETHOXY-4-PHENYLCOUMARIN FROM *COUTAREA HEXANDRA*

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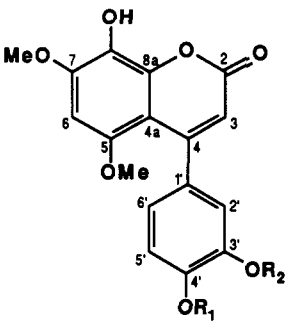
ABSTRACT.—A new natural product of the neoflavonoid series has been isolated by means of hplc from the MeOH extract of defatted stems of *Coutarea hexandra*, a plant growing in northeastern Brazil. This compound has been identified as 8,3′-dihydroxy-5,7,4′-trimethoxy-4-phenylcoumarin on the basis of spectral data.

Many 4-arylcoumarins (1–5) and 4-arylcoumarin glycosides (6) previously have been discovered in *Coutarea hexandra* (Jacq.) Schum. (Rubiaceae), a plant growing in northeastern Brazil and belonging to the same genus as *Coutarea latiflora* that has been used in folk medicine as an antimalarial and antidiabetic agent (7,8). Now, by investigating the MeOH extract of stems of this plant, we have isolated a minor constituent identified as 8,3′-dihydroxy-5,7,4′-trimethoxy-4-phenylcoumarin [**1**], a new natural product of the neoflavonoid series. The structure of this compound has been established on the basis of spectral data.




**1** $R_1$=Me, $R_2$=H
**2** $R_1$=$R_2$=H
**3** $R_1$=$R_2$=Me

The $^1$H-nmr spectrum of **1** showed two peaks (both s, 1H) at δ 6.58 and 5.92 due, respectively, to H-6 and H-3 of a tetrasubstituted coumarin, and signals at δ 6.80 (d, $J$ = 2 Hz, H-2′), 6.96 (d, $J$ = 7.5 Hz, H-5′), and 6.75 (dd, $J$ = 7.5 and 2 Hz, H-6′) ascribable to the 3H ABX system of the 4-phenyl radical 3′,4′-disubstituted (4–6). The peaks at δ 3.99 and 3.51 have been attributed to the methoxyl groups placed, respectively, at C-7 and C-5, while the signal at δ 3.93 is ascribable to the OMe bonded at C-4′ on the basis of 0.15 ppm downfield shift of H-5′ signal and 3.1 ppm upfield shift of C-5′ peak in comparison with homologous peaks of compound **2** (4) and on the basis of the positive response in the nOe difference spectra experiments between signals at δ 6.96 (H-5′) and 3.93. Also the assignment to H-6 of the signal at δ 6.58 and the 3′,4′ substitution of the 4-phenyl radical have been established by means of nOe difference spectra because the peak at δ 6.58 showed a very clear enhancement when both methoxyl signals at δ 3.99 and 3.51 were saturated, while the irradiation at δ 5.92 (H-3) led to the enhancement of the signals at δ 6.80 (H-2′) and 6.75 (H-6′), indicating a spatial relationship for protons H-3, H-2′, and H-6′. The $^{13}$C-nmr spectrum of **1**, apart from the methoxyl peaks at δ 56.5, 56.8, and 57.0, exhibited fifteen signals ascribable to a 4-arylcoumarin skeleton 8-oxygenated, divided by DEPT into five CH groups (C-3, C-6, C-2′, C-5′, and C-6′) and ten quaternary carbons, in agreement with the spectral data of **2** and **3** (4,5). Thus, on the basis of these results, **1** has been identified as 8,3′-dihydroxy-5,7,4′-trimethoxy-4-phenylcoumarin.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc was carried out on a Waters Model 6000 A equipped with a refractive index detector. Uv spectrum was registered on a Hitachi Perkin-Elmer instrument. Ms was taken on a Kratos MS-902 mass spectrometer by direct inlet at 70 eV. $^1H$ and $^{13}C$ nmr were obtained on a Bruker MW-250 Spectrospin spectrometer in $CD_3OD$ solution. The DEPT experiments were performed using polarization transfer pulses of 90° and 135°, respectively, to obtain in the first case only CH groups and in the second positive signals for CH and Me and negative ones for $CH_2$ groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. Determination of nOe difference spectra was performed on a sample previously degassed by bubbling Ar through the solution for 40 min.

PLANT MATERIAL.—Stems of *C. hexandra* were collected in northeastern Brazil (Patacube, Fortaleza) and identified by José Elias de Paula, Universidad Federal de Brasilia, Brazil. A voucher specimen of the plant is preserved in the Herbarium of this University.

EXTRACTION AND ISOLATION.—Air-dried stems of *C. hexandra* (2.0 kg), defatted with $C_6H_6$, were extracted with MeOH at room temperature. The MeOH extract (30 g), dispersed in $H_2O$, was shaken with $CHCl_3$. The $CHCl_3$ layer, dried in vacuo and fractionated by means of hplc on a $C_{18}$ μ-Bondapak column (30 cm × 8 mm i.d., flow rate 2.5 ml/min), with MeOH-$H_2O$ (50:50) as eluent, yielded **1** (20 mg, Rt = 17.5 min), which crystallized from EtOH as fine needles: mp 197–198°; uv λ max (MeOH) nm 270, 325, 425; ir ν max ($CHCl_3$) 3543, 3034, 2940, 2846, 1714, 1615, 1515, 1464, 1436, 1340, 1240, 1196, 1159, 1130, 1088, 1033, 931, 869, 808, 763; ms *m/z* $[M]^+$ 344, 316, 301, 273; hrms *m/z* 344.0891 ($C_{18}H_{16}O_7$ requires 344.0896); $^{13}C$ nmr ($CD_3OD$) δ 162.8 (C-2), 112.5 (C-3), 158.4 (C-4), 104.2 (C-4a), 152.9 (C-5 or C-7), 94.7 (C-6), 152.2 (C-7 or C-5), 129.5 (C-8), 145.2 (C-8a), 134.2 (C-1′), 115.7 (C-2′), 146.7 (C-3′), 149.2 (C-4′), 112.5 (C-5′), 119.7 (C-6′).

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