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NEW NEOFLAVONOIDS FROM *COUTAREA LATIFLORA*

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Some species of the genera *Coutarea* and *Exostema* (Rubiaceae), trees native to Central and South America, are reputed to show antimalarial (1) or antidiabetic activity (2). The stem barks of these species are called "copalchi." In the course of our search for constituents of several different copalchi (3), the stem bark of *Coutarea hexandra* D.C. afforded a neoflavonoid, the structure of which was elucidated by spectroscopic methods (4). It was identified as 5,2′,5′-trihydroxy-7-methoxy-4-phenylcoumarin (**1**).

RESULTS AND DISCUSSION

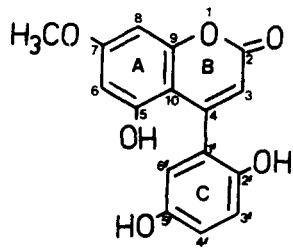
From the species *C. latiflora*, a new compound showing a striking violet fluorescence has been isolated. The molecular formula was determined by high resolution ms and was found to be $C_{16}H_{10}O_6$. TMS-derivatization revealed the presence of two OH-functions. The ^{1}H-nmr signal at 3.86 ppm, showing three protons, could be attributed to a methoxy function. This was confirmed by the presence of a corresponding ^{13}C-nmr signal at 56.02 ppm. The ^{1}H-nmr singlet at 6.07 ppm was interpreted to be a vinylic proton in a lactone structure (=CH-COOR) (5). The isolated compound exhibited a similarity of several spectral data with those of the 4-phenylcoumarin **1** (4). Thus, by considering additionally the strong fluorescence, a substituted 4-phenylcoumarin structure with an oxygen bridge between C-5 and C-2′, causing the fluorescence (6), could be suggested. The positions of the OH- and OCH_3-substituents were determined by interpreting the ^{1}H-nmr spectral data. The two one-proton doublets with J=2.2 Hz at 6.74 and 6.71 ppm could be assigned to two aromatic *meta*-protons. Because of the lack of any further measurable coupling constant, these doublets must be attributable to C_6-H and C_8-H, and the three one-proton singlets were attributable to C_3-H, $C_{3'}$-H, and $C_{6'}$-H. From the behavior of the uv spectrum (upon the addition of $AlCl_3$-solution, a bathochromic shift resulted, reversible upon the addition of HCl) (7), we inferred that the compound must have been *ortho*-dihydroxy-substituted. Thus, structure **2** could be suggested.

Two further compounds with striking fluorescences have been isolated from *C. latiflora*. The molecular formulas were found to be $C_{17}H_{12}O_6$ (**3**) and $C_{18}H_{14}O_7$ (**4**). After interpretation of the spectral data, the same 4-phenyl-5,2′-oxido-coumarin structure could be assigned for both compounds. With the data available to us at the moment, it is not possible to decide about the positions of the OH- and OCH_3-substituents.

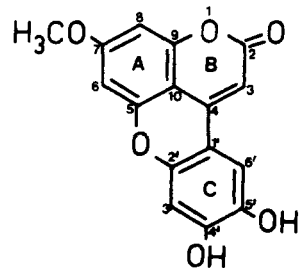
To the best of our knowledge, the isolation of the 4-phenyl-5,2′-oxido-coumarin structure from plant material has not been described before. A report about this structure was given by Bhanu *et al.* (8) who isolated several compounds of this type when synthesizing xanthones from 4-phenylcoumarins. Concerning the biosynthesis, H_2O-elimination from 5,2′-dihydroxy-substituted 4-phenylcoumarins seems likely to be the step leading to 4-phenyl-5,2′-oxido-coumarins; the isolation of **1** from the same genus *Coutarea* supports this presumption.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Plant material was received from Curarina Arzneimittel GmbH (Eurasburg/Obb., GFR) and identified as bark of *Coutarea latiflora* DC. (A



1



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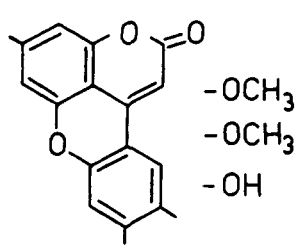

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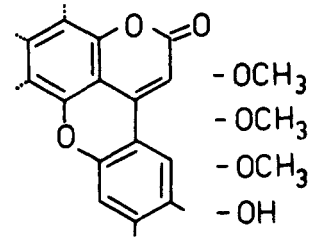

4

voucher specimen is deposited at Lehrstuhl für Pharmakognosie, Hamburg.)

ISOLATION OF COMPOUND **2**.—The air-dried bark of *C. latiflora* was macerated with EtOH 20%, and after that the maceration was extracted with $CHCl_3$. Concentrating the $CHCl_3$ extract, compound **2** precipitated as a yellow, crystalline substance which, in uv light 366 nm, showed a bright violet fluorescence. Dried stem bark (1 kg) produced an amount of 30 mg.

ISOLATION OF COMPOUND **3** AND **4**.—The powdered, air-dried stem bark (16 kg) of *C. latiflora* was continuously extracted in a Soxhlet with toluene. The extract was concentrated under reduced pressure to a volume of 3.2 liters and then separated by means of preparative hplc (mobile phase: toluene-EtOAc-HCO_2H 80:20:10; sample volume, 80 ml). The fractions from the column were monitored by qualitative tlc and analytical hplc on silica gel. The fractions of interest were purified by low-pressure lc on silica gel (pre-packed column size B, LiChroprep Si 60 Merck). Dried stem bark (16 kg) produced 55 mg of the yellow compound **3** (bright violet fluorescence in uv light 366 nm) and 25 mg of the yellow compound **4** (bright yellow fluorescence in uv light 366 nm). For details of separation see reference (3).

PHYSICAL DATA.—Mp (uncorr.): **2**: 335-342° (decomp.); **3**: 258-265°; **4**: 340-345° (decomp.); ir spectra in KBr disc: **2**: 3300, 3480 cm^{-1} (OH); 1710, 1615 cm^{-1} (C=CCOOR); **3**: 3360 cm^{-1} (OH); 1695, 1620 cm^{-1} (C=CCOOR); **4**: 3350 cm^{-1} (OH); 1700, 1635 cm^{-1} (C=CCOOR); uv spectra λ max (MeOH): **2**: 260, 310, 371sh, 389 (+$AlCl_3$ 389sh, 403, +$AlCl_3$/HCl 260sh, 310, 370, 389sh); **3**: 260, 306, 371, 387; **4**: 261, 318, 380sh, 393. The ms were run on a Varian MAT CH 7, 70 eV, ion source temp. 250°, direct inlet; or high resolution: Varian MAT 311 A, 70 eV, ion source temp. 200° (peak matching); *m/z* (%): **2**: 298 (M^+, 100), 270 (M^+-CO, H.R., 49), 242 (70); **3**: 312 (M^+, 100), 284 (M^+-CO, H.R., 38), 269 (M^+-(CH_3+CO), H.R., 15), 256 (M^+-2 CO, H.R., 49); **4**: 342 (M^+, 100), 327 (92), 299 (47). TMS-derivatization: 0.5 mg sample +100 μl MSTFA (*N*-methyl-*N*-trimethylsilyl-trifluoroacetamide) 30 min at 100°. ^{1}H-nmr spectra were taken with TMS at internal standard using a Bruker WM 400 (400 MHz). Chemical shifts in δ (ppm) scale (DMSO-d_6 solution): **2**: 7.32 (1H, s), 6.76 (1H, s), 6.74 (1H, d, J=2.2 Hz), 6.71 (1H, d, J=2.2 Hz), 6.07 (1H:C_3-H, s), 3.86 (3H, s). **3**: 7.37 (1H, s), 6.99 (1H, s), 6.74 (1H, d, J=2.2 Hz), 6.71 (1H, d, J=2.2 Hz), 6.15 (1H:C_3-H, s), 3.92 (3H, s), 3.88 (3H, s). **4**: 7.38 (1H, s), 6.98 (1H, s), 6.90 (1H, s), 6.15 (1H:C_3-H, s), 3.94 (3H, s), 3.91 (3H, s), 3.79 (3H, s). ^{13}C-nmr spectra were determined with TMS as internal standard using a Bruker WM 360 (93.66 MHz) (**3**) and a Bruker WM 250 (62.89) (**2**,**4**). Chemical shifts in δ (ppm) scale (DMSO-d_6 solution): **2**: 56.02, 92.33, 95.97, 96.41, 99.72, 103.22, 106.13, 109.03, 141.17, 143.69, 146.44, 150.59, 152.25, 154.46, 160.91, 162.99; **3**: 55.84, 55.94, 92.91, 95.80, 96.24, 99.62, 100.46, 107.03, 108.53, 140.67, 144.16, 146.23, 150.38, 153.33, 154.35, 160.54, 162.91; **4**: 55.93, 56.35, 60.32, 92.50, 94.52, 99.74, 100.38, 106.71, 108.40, 131.07, 140.95, 144.16,

145.43, 145.60, 146.30, 153.35, 156.19, 160.16. ^{13}C-nmr spectra were taken at 60°.

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