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ISOLATION FROM *COUTAREA HEXANDRA* AND SYNTHESIS OF EXOSTEMIN AND ITS METHYL ETHER §

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ABSTRACT: The structure of a new neoflavonoid, isolated from *Coutarea hexandra*, has been established as 5,7,8,4'-tetramethoxy-4phenylcoumarin and confirmed by synthesis. The corresponding 8-O-demethylcoumarin, exostemin, has also been isolated and synthesized.

INTRODUCTION

Coumarins with a substituted 4-phenyl ring are quite rare in nature, the first example being melannein, isolated from *Dalbergia baroni* (Leguminosae) (1).
The second example, exostemin (1) isolated from *Exostemma caribaeum* (Rubiaceae) (2), showed a ring substitution pattern, which was unusual in the neoflavonoid series. Compound **1** and its methyl ether **2** were synthesized by Mukerjee et al. and the correct structure of exostemin was questioned (3), but finally reconfirmed by SánchezViesca (4). Recently 4-arylcoumarins have been isolated from *Coutarea latiflora* and *C. hexandra* (Rubiaceae) (5-8) but no metabolite with 8-oxygenation was reported. We have now isolated from the benzene extract of the caulis of *C. hexandra* (Jacq) Schum two minor products, which were assigned the structures **1** and **2** and synthesized with procedures different from those reported in (3). The neoflavonoid **2** is reported of the first time from a natural source.

RESULTS AND DISCUSSION

The first product, $C_{19}H_{18}O_6$, was determined to be a tetramethoxy-4-phenylcoumarin based on spectral evidence. The B ring substitution pattern was disclosed by the A_2X_2 system of four aromatic protons in the ^1H NMR spectrum, whereas the remaining signal of an isolated proton was attributed to the H-6 or H-8 of A ring. Biogenetic considerations suggested the compound to be a 5,7-dimethoxy-4-arylcoumarin with an extra -OMe group in the 6 or 8 position. The mass spectrum showed an intense (M^+ - Me) ion, which was not present in the mass spectra of 5,7,4'trimethoxy or 5,7,3',4'-tetramethoxy-4-phenyl coumarin (6). Loss of 15 mu from the molecular ion in the mass fragmentation of coumarins was correlated to the presence of a 6-OMe substituent, through the formation of the para-quinonoid oxonium ion a (9); however an 8-OMe

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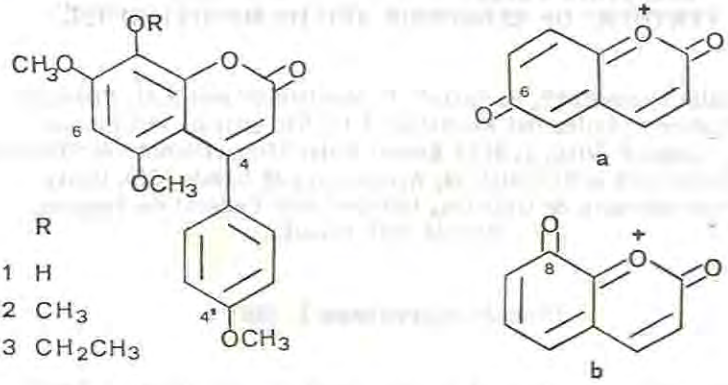
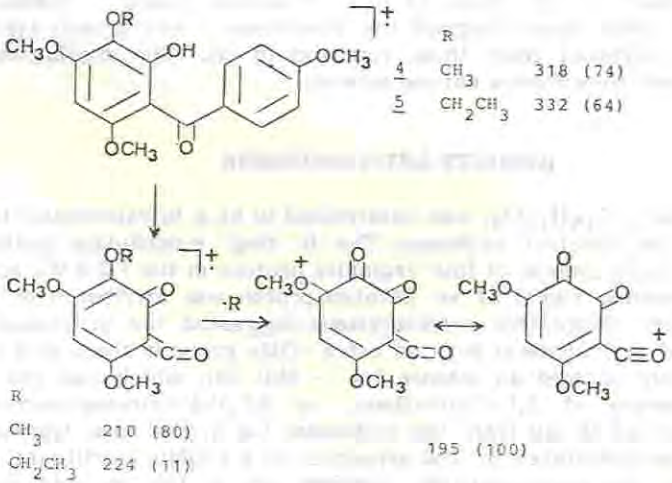
substituent might give the same loss yielding a fragment with the ortho-quinonoid structure b.



The ^{13}C NMR spectrum was in agreement with a 5,7,8-trimethoxy substitution of the ring A, only one lower field OMe resonance (61 vs 56.5, 56 and 55)) being present (10). In the 5,6,7-trimethoxy derivative both 5- and 6-OMe would give 2 downfield signals (vide infra). The new product was thus assigned the structure (1) of the methyl derivative of exostemin (2). For a chemical confirmation of this assignment, 5,7,8,4'-tetramethoxy-4-phenylcoumarin was synthesized via Perkin cyclization of 2-hydroxy-3,4,6,4'-tetramethoxybenzophenone (3). Condensation of 1,2,3,5-tetramethoxybenzene and anisoyl chloride in dry Et_2O gave compound 4 (50%) but also the homologous 5 (18%). The structures of the two compounds were confirmed by the deshielded resonances of the 3-O-substituents in the ^{13}C NMR spectra and by a mass fragmentation, which can be rationalized as in the scheme 1. Both the benzophenones 4 and 5 gave

Scheme 1: Mass fragmentation of 3-O-substituted benzophenones; m/z (rel.int:)



the corresponding 4-arylcoummarins **2** and **3**. Notably in the mass spectrum of **3** a significant loss of 29 mu from the molecular ion was observed. The synthetic 5,7,8,4'-tetramethoxy-4-phenylcoumarin (**2**) was identical in all respects with the natural product. The isomeric 5,6,7,4'-tetramethoxy-4-phenylcoumarin was prepared in low yield by condensation of 3,4,5-trimethoxyphenol and ethyl p-methoxybenzoyl acetate in the presence of BCl_3, whereas no significant product was obtained when HCl gas or conc. H_2SO_4 were used. 5,6,7,4'-tetramethoxy-4-phenylcoumarin showed very distinct features from those of 5,7,8,4'-tetramethoxy analogues, particularly two downfield OMe resonances in the ^{13}C NMR spectrum (10).

The second product, $C_{18}H_{16}O_6$, was a monohydroxy-trimethoxy-4-phenylcoumarin and gave **1** by methylation, showing the same oxygenation pattern. Moreover the fragment (M^+ - Me) in the mass spectrum was not significant. The hydroxy group was thus placed at C-8 and the compound was assigned the structure **3**. 8-Hydroxy-5,7,4'-trimethoxy-4-phenylcoumarin (**1**, exostemin), has been previously isolated from *E. caribaeum* (5), but a direct comparison was not possible. However reaction of 5,7,4'-trimethoxy-8-ethoxy-4-phenylcoumarin (**3**) with BCl_3 gave **1**, which was identical with the natural product isolated from *C. hexandra*.

EXPERIMENTAL

General experimental procedures: ^1H NMR was at 60 MHz and ^{13}C NMR was at 25.16 MHz. MS were recorded by direct inlet at 70 eV.

Isolation: Collection and identification of the plant are reported in our previous work (6). A voucher specimen of the plant is conserved at the Herbarium of Departamento de Quimica of Maceió (Brazil) under the cypher 1262. Extraction of caulis (6.5 kg) of *C. hexandra* with hot C_6H_6 gave a residue of 29.5 g, a portion of which (9.5 g) was chromatographed on Si gel with $CHCl_3$ - MeOH mixtures. The first of the fractions eluted with $CHCl_3$ - MeOH (99:1) gave on further purification (Si gel, C_6H_6 - EtOAC 9:1) 3'-hydroxy-5,7,4'-trimethoxy-4-phenylcoumarin (6), β-sitosterol, an unidentified terpenoid and a minor product (**2**, 45 mg), which was finally purified on plc (Si gel, C_6H_6 - EtOAC 85:15). The second fraction obtained with $CHCl_3$ - MeOH (99:1) gave (Si gel C_6H_6 EtOAC 4:1) 4'-hydroxy-5,7-dimethoxy-4-phenylcoumarin (6) and another minor product (**1**, 25 mg), also purified on plc (C_6H_6 - EtOAc 4:1, two runs).

5,7,8,4'-Tetramethoxy-4-phenylcoumarin (**2**): mp 145-146° (MeOH), lit (4) mp 144 - 145° and (3) mp 142 - 143°; UV λ max (MeOH) 265 (log ε 4.16) and 319 nm (4.18); IR ν max ($CHCl_3$): 1720 sh, 1710, 1605, 1590, 1510, 1343, 1243, 1117, 1056, 831 cm^{-1}; ^1H NMR (Me_2CO-d_6): δ 7.20 (2H, d, J = 8.5 Hz, H-2',H-6'), 6.88 (2H, d, J = 8.5 Hz, H-3', H-5'), 6.54 (1H, s, H-6), 5.80 (1H, s,H-3), 3.90 (3H, s, 8-OMe), 3.76 + 3.74 (3H+3H, s +s, 7-OMe + 4'-OMe), 3.46 (3H, s, 5-OMe); ^{13}C NMR (Me_2CO-d_6): δ 160.4, 159.9 (2xs, C-2, C-4'), 156.8, 156.0 (2xs, C-4, C5), 154.5, 149.9 (2xs, C-7, C-8a), 132.8, 131.1 (C-8, C-1'), 129.3 (d, C2'+C6'), 113.3 (d,C-3'+ C-5'), 112.8 (d, C-3), 103.9 (s, C-4a), 94.0 (d, C6), 61.0 (q, 8OMe), 56.5, 56.0,55.4 (3xq, 5-OMe + 7-OMe + 4'-OMe); EIMS m/z (rel. int.): 342 (M^+, 100), 327 (M-Me, 48), 314 (M-CO, 6) 312 (2), 299 (M-MeCO, 46), 271 (M-43-CO, 15), 256 (9), 171 (M/2, 8), 157 (M-CO/2, 15), 149.5 (M-MeCO/2, 3), 135.5 (M-43-CO/2, 3); m*: 312.7 (342 → 327), 284.7 (314 → 299), 273.4 (327 → 299), 245.6 (299 271). (Found: C, 66.80; H,5.22; $C_{19}H_{18}O_6$ requires: C, 66.66; H,5.30).

Synthesis of **5:** to an icecold mixture of anhyd. $AlCl_3$ (12 g), dry Et_2O (50 ml) and 1,2,3,5-tetramethoxybenzene (5 g) was added a suspension of p-anisoyl chloride (6 g) in dry Et_2O (50 ml). The mixture was kept at room temp for 48 hr and then treated with crushed ice and conc. HCl (25 ml). The Et_2O layer was separated and the aqueous phase extracted with CH_2Cl_2. The residue of the combined organic layers was dissolved in 10% NaOH aq. and CH_2Cl_2. The alkaline extracts were acidified with conc. HCl and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were washed with sat. $NaHCO_3$ aq. and evaporated under vacuo. The residue (5.7 g) by repeated cc on Si gel with C_6H_6-EtOAc mixtures gave (in order of elution) **5** (1.5 g, 18%) and **4** (4.0 g, 50%).

2-Hydroxy-3,4,6,4'-tetramethoxybenzophenone (**4**): Mp 92-94° (Et_2O); UV λ max (MeOH): 285 nm (log ε 4.25); λ max ($AlCl_3$) 311 nm; IR ν max ($CHCl_3$): 3520, 1622sh, 1597, 1578, 1512, 1505 cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.39 (1H, s, exchg. D_2O, 2-OH), 7.53 (2H, d, J = 8.5 Hz, H-2', H-6'), 6.79 (2H, d, J = 8.5 Hz, H-3', H-5'), 5.98 (1H, s, H-5), 3.88 + 3.81 (3H + 6H, s + s, 3-OMe + 4-OMe + 4'OMe), 3.52 (3H, s, 6-OMe); ^{13}C NMR ($CDCl_3$): δ 63.7 (q, 3-OMe), 58.8, 58.4, 58.2 (3xq, 4-OMe + 6-OMe + 4'-OMe); EIMS m/z (rel. int): 318 (M^+, 74), 317 (5), 303 (4),210 (M - ring B, 80),195 (210 - Me, 100), 167 (195 - CO, 22).

2-Hydroxy-4,4,4'-trimethoxy-3-ethoxybenzophenone (**5**). Mp 98 - 100° (Et_2O-hexane); UV λ max (MeOH) 285 nm (log ε 4.30); λ max ($AlCl_3$): 311nm; IR ν max ($CHCl_3$): 3520, 1622sh, 1602, 1580sh, 1512, 1504 cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.06 (1H, s, exchg D_2O, 2-OH), 7.60 (2H, d, J = 8.5Hz, H-2', H-6'), 6.82(2H, d, J = 8.5Hz, H-3', H-5'), 5.98 (1H, s, H-5); 4.01 (2H, q, J = 7Hz, OCH_2), 3.87 + 3.80 (3H + 3H, s + s, 4-OMe + 4'-OMe), 3.52 (3H, s, 6-OMe), 1.34 (3H,t,J = 7Hz, Me); ^{13}C NMR ($CDCl_3$): δ 71.8 (3-OCH_2), 58.8, 58.4, 58.2 (4-OMe + 6-OMe + 4'-OMe), 18.4 (Me); EIMS m/z (rel. int.) 332 (M^+, 64), 331 (2), 317 (1), 303 (9), 224 (M-ring B, 11), 209 (7), 196 (224 -CO, 16), 195 (224 - CH_2Me, 100), 167 (195 - CO, 57), 153 (5), 150 (6), 135 (M - ring A, 35), 107 (135 - CO, 6) 77 (16); m*: 302.7 (332 → 317), 276.5 (332 → 303), 171.4 (224 → 196), 169.7 (224→195), 151.1 (303 →195), 115.5 (332→196), 84.8 (135→107), 55.4 (107→77).

5,7,8,4'Tetramethoxy-4-phenylocumarin (**2**): 2-Hydroxy-3,4,6,4'-tetramethoxybenzophenone (1.0 g), fused AcONa (0.19 g) and Ac_2O (2 ml) were treated under reflux for 24 hr. The reaction mixture was treated with ice-water and filtered to give a solid, which on Si gel with C_6H_6-EtOAc mixtures afforded 5,7,8,4'-tetramethoxy-4-phenylcoumarin (2, 0.42g, 39%), mp 144 - 145° (MeOH), identical with the natural product (mmp 144-145°) and 2-acetoxy-3,4,6,4'-tetramethoxybenzophenone (0.53 g, 47%), mp 130-131° (MeOH); IR ν max ($CHCl_3$): 1758 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.99 (3H, s, COMe).

5,7,4'-Trimethoxy8-ethoxy-4phenylcoumarin (**3**): 2-hydroxy-4,6,4'trimethoxy-3-ethoxybenzophenone (0.66 g), KOAc (0.35 g) and Ac_2O (0.6 ml) were heated under reflux for 24 hr. AC_2O was added after 6 and 9 hr (0.2 ml each). The mixture was poured into ice-water and extracted with $CHCl_3$. The residue on Si gel with $CHCl_3$ gave **2** (0.61 g, 85%), mp 164 -165° (MeOH); UV λmax (MeOH) 266 (log ε 4.14) and 313nm (4.10); IR ν max ($CHCl_3$): δ 1712, 1608, 1592, 1511, 1346, 1250, 1118, 1065, 835 cm^{-1}; ^1H NMR (Me_2COd_6): 7.21(2H, d, J = 8.5Hz, H-2', H-6'), 6.89 (2H, d, J = 8.5Hz, H-3', H-5'), 6.54 (1H, s, H-6), 5.80 (1H, s, H-3), 4.01 (2H, q, J = 7.5Hz, OCH_2), 3.88 + 3.77 (3H + 3H, s + s, 7-OMe + 4'-OMe), 3.46 (3H, s, 5OMe), 1.34 (3H, t, J = 7.5Hz, Me); ^{13}C NMR (Me_2COd_6): δ 160.4, 160.3 (2xs, C-2, C-4'), 157.4, 156.8 (2xs, C-4, C-5), 154.6 (C-7), 150.7 (C-8a), 132.4, 130.5 (2xs, C-8, C-1'), 129.4 (d,C-2'+ C-6'), 113.41 (d,C-3'+ C-5'), 112.8 (d, C-3), 103.0 (s, C-4a), 94.1 (d, C-6), 69.6 (t, 8-OCH_2), 56.6, 56.1, 55.4 (5-OMe + 4'-OMe), 15.62 (Me);EIMS m/z (rel. int.): 356 (M^+, 100), 341 (M-Me, 3), 328 (M-CO,6), 327 (M-CH_2Me, 70), 313 (M-MeCO, 31), 299 (16), 285 (M-43-CO, 6), 271 (12), 270 (5), 178 (M/2, 6), 164 (M-C0/2, 5), 156.5 (M-MeCO/2,

3), 142.5 (M-43-CO/2, 4).

Synthesis of 5,6,7,4'-tetramethoxy-4-phenylcoumarin: to a cooled soln of 3,4,5-trimethoxyphenol (0.37 g) and ethyl p-methoxybenzoylacetate (0.45 g) in CH_2Cl_2 (10 ml) was added BCl_3 1M in CH_2Cl_2 (5 ml). The mixture was left standing at 0° for 1 week, MeOH was added and evaporated under vacuo. The residue by cc and plc on Si gel with CH_2Cl_2-EtOAc 4:1 gave 5,6,7,4'-tetramethoxy-4-phenylcoumarin (70 mg, 9%), mp 141-142° (MeOH); UV λ max (MeOH): 320 nm (log ε 4.20); IR νmax($CHCl_3$): 1710, 1604, 1580sh, 1548, 1510, 1170, 1132, 1100, 1033, 1000, 965, 940, 888, 865, 832 cm^{-1}; ^1H NMR (Me_2COd_6): δ 7.30 (2H, d, J = 8.5Hz, H-2', H-6'), 6.94 (2H, d, J = 8.5Hz, H-3', H-5'), 6.80 (1H, s, H-8), 5.88 (1H, s, H-3), 3.94 (3H, s, 5-OMe); ^{13}C NMR (Me_2COd_6): δ 160.5 (s, C-2), 157.9 (s, C-4'), 155.7 (s, C-4), 152.5, 151.8 (2xs, C-5, C-7), 148.3 (s, C-8a), 132.1 (d, C-6), 131.4 (s, C-1'), 129.6 (d, C-2' + C-6'), 114.2 (d, C-3), 113.5 (d, C-3'+ C-5'), 103.2 (s, C-4a), 97.0 (s, C-8), 61.1, 60.90 (2xq, 5-OMe + 6-OMe), 56.6, 55.4 (2xq, 7-OMe + 4'-OMe); EIMS m/z (rel. int.): 342 (M^+, 100), 327 (M-Me, 36), 314 (M-CO, 10), 299 (M-MeCO, 11), 271 (M-43-CO, 3), 256 (5), 171 (M/2, 8), 163.5 (M-Me/2, 2), 157 (M-CO/2, 6), 149.5 (M-MeCO/2, 3); m*: 312.7 (342 → 327), 288.3 (242 → 314), 284.7 (314 → 299), 273.4 (327 → 299), 245.6 (299 → 271).

8-Hydroxy-5,7,4'-trimethoxy-4-phenylcoumarin (**1**). Mp 172-174° (MeOH), lit (4) mp 173-174° and (3) mp 195-196°; UV, IR and ^1H NMR spectra were coincident with those reported in the literature (3,4). ^{13}C NMR (Me_2CO-d_6): δ160.4, 160.0 (2xs, C-2, C-4'), 157.8, 156.2 (2xs, C-5, C-7), 155.2 (s, C-4), 151.4 (s, C-8a), 133.0 (s, C-8), 130.5 (s, C-1'), 129.4 (d, C-2' + C-6'), 113.4 (d, C-3' + C-5'), 112.8 (d, C-3), 104.4 (s, C-4a), 94.5 (s, C-6), 56.6, 56.4, 55.4 (5-OMe + 7-OMe + 4'-OMe); EIMS m/z (rel. int.): 328 (M^+, 100), 327 (3), 313 (M-Me, 9), 300 (M-CO, 7), 285 (M-MeCO, 15), 271 (3), 270 (3), 257 (M-43-CO, 4), 242 (3), 164 (M/2, 5), 150 (M-CO/2, 2), 142.5 (M-MeCO/2, 2); m*: 298.7 (328 313), 274.4 (328 → 300), 270.5 (300 → 285), 231.8 (285 → 257), 259.5 (313 → 285). (Found: C, 65.92; H, 4.80; $C_{18}H_{16}O_6$ requires C, 65.85; H, 4.91). Acetyl derivative (with AC_2O/pyridine: mp 201-202° (EtOH), loss of solvent at 186-188°, lit (4) mp 203-204° and (3) mp 187-188°.

Preparation of **1** from **3**: to a cooled solution of 5,7,4'-trimethoxy-8-ethoxy-4phenylcoumarin (**3**, 178 mg) in dry CH_2Cl_2 (8 ml) was added BCl_3, 1M in CH_2Cl_2 (1 ml). After 2 days at room temp the reaction mixture was evaporated under vacuo, MeOH added and left overnight. The residue on Si gel with CH_2Cl_2 - EtOAc 4:1 gave 8-hydroxy-5,7,4'-trimethoxy-4-phenylcoumarin (112 mg, 68%), identical in all respects with the natural compound **1** (mp 175 176°, mmp 173-174°).

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