

Additional Coumarins from *Kielmeyera reticulata*

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Do extrato hexânico e da fase extraída com diclorometano do extrato metanólico de *Kielmeyera reticulata* foram isoladas três novas 4-fenilcumarinas, 5,7-diidroxi-6-(2'-hidroxi-3'-metil-3'-butenil)-8-(4''-cinamoil-3''-metil-1''-oxobutil)-4-fenilcumarina, 7-hidroxi-8-(4''-cinamoil-3''-metil-1''-oxobutil)-2'-(2-hidroxiisopropil)diidrofurano (5',4':5,6)-4-fenilcumarina e 5,7-diidroxi-8-(4''-cinamoil-3''-metil-1''-oxobutil)-4-fenilcumarina, além das substâncias já conhecidas, 7-hidroxi-8-(4''-cinamoil-3''-metil-1''-oxobutil)-2',2'-dimetilpirano(6',5':5,6)-4-*n*-propilcumarina, 5-hidroxi-6-(4''-cinamoil-3''-metil-1''-oxobutil)-2',2'-dimetilpirano(6',5':7,8)-4-*n*-propilcumarina, δ -tocotrienol e 2,3-metilenodioxixantona.

Three new 4-phenylcoumarins, 5,7-dihydroxy-6-(2'-hydroxy-3'-methyl-3'-butenyl)-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-4-phenylcoumarin, 7-hydroxy-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2'-(2-hydroxyisopropyl)-dihydrofurano(5',4':5,6)-4-phenylcoumarin, and 5,7-dihydroxy-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-4-phenylcoumarin, along with the known 4-*n*-propylcoumarins, 7-hydroxy-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2',2'-dimethylpyrano(6',5':5,6)-4-*n*-propylcoumarin, 5-hydroxy-6-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2',2'-dimethylpyrano(6',5':7,8)-4-*n*-propylcoumarin, the xanthone, 2,3-methylenedioxyxanthone and δ -tocotrienol were isolated from the organic extracts of *Kielmeyera reticulata* stems.

Keywords: *Kielmeyera reticulata*, Guttiferae, Clusiaceae, 4-phenylcoumarins, 4-propylcoumarins, neoflavonoids

Introduction

Kielmeyera reticulata is a wild shrub belonging to Guttiferae (Clusiaceae). Its stems were collected in sand dunes (restinga) on the Bahia state coast, Brazil. A previous work reported on the isolation of five prenylated 4-phenylcoumarins from this species.¹ Studies of this genus showed that species from the "cerrado" (savanna) of the Brazilian Central plateau are rich in xanthones,²⁻⁷ while species from the "restinga" (sand dunes) are rich in prenylated 4-phenyl and 4-alkylcoumarins.^{1,8} Only two species showed xanthones such as prenylated 4-phenyl and 4-alkylcoumarins.^{9,10}

Experimental

General procedures

UV spectra were obtained on Cary 1-E, Varian in MeOH

and MeOH/NaOH. EIMS were taken with direct probe insert at 70 eV on HP 5973. NMR spectra were obtained from Gemini 300-Varian in CDCl₃ solution with TMS as internal reference standard. Rotations were determined with 241 Perkin Elmer polarimeter.

Plant material

Stems of *K. reticulata* Saad were collected in the sand dunes of Parque Metropolitano do Abaeté, Salvador, Bahia, Brazil, in January 1992. A voucher specimen, 027415, is deposited in the "Alexandre Leal Costa" Herbarium, Instituto de Biologia, Universidade Federal da Bahia, Salvador, Bahia, Brazil.

Extraction and isolation

The dried stems (3.4 kg) were first extracted with hexane and after with methanol. The hexane extract (37.1 g) was submitted to silica gel (40-63 μ m, Merck) column chromatography using hexane/EtOAc gradient. Several

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fractions were rechromatographed on silica gel CC using hexane/EtOAc gradient to give **1** (276.9 mg), **2** (123.0 mg), 7-hydroxy-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2',2'-dimethylpyrano(6',5':5,6)-4-*n*-propylcoumarin (17.8 mg), 5-hydroxy-6-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2',2'-dimethylpyrano(6',5':7,8)-4-*n*-propylcoumarin (26.9 mg) and δ -tocotrienol (288.3mg). The methanol extract was submitted to partition with MeOH/H₂O/CH₂Cl₂ yielding a CH₂Cl₂ phase (32.1 g) that was submitted to silica gel column chromatography using hexane/acetone gradient. Several fractions were rechromatographed on silica gel CC using hexane/acetone gradient to give **3** (30.4 mg) and 2,3-methylenedioxyxanthone (6.7 mg).

5,7-dihydroxy-6-(2'-hydroxy-3'-methyl-3'-butenyl)-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-4-phenylcoumarin, (1)

C₃₄H₃₂O₈. Amorphous yellow-greenish solid. NMR: see Tables 1 and 2. EIMS *m/z* (rel. int.): 568 [M]⁺ (1), 349 (99), 293 (59), 131 (100), 103 (54). [α]_D²⁴ -61.0° (c 0.410 CHCl₃).

7-hydroxy-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2'-(2-hydroxyisopropyl) dihydrofuran-(5',4':5,6)-4-phenylcoumarin, (2)

C₃₄H₃₂O₈. Amorphous yellow-greenish solid. NMR: see Tables 1 and 2. EIMS *m/z* (rel. int.): 420 [M-C₆H₅C₂H₂CO₂H]⁺ (7), 365 (9), 361 (8), 347 (8), 293 (9), 131 (100), 59 (30). [α]_D²⁴ +92.3° (c 0.370 CHCl₃).

5,7-dihydroxy-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-4-phenylcoumarin, (3)

C₂₉H₂₄O₇. Amorphous yellow-greenish solid. NMR: see

Tables 1 and 2. EIMS *m/z* (rel. int.): 484 [M]⁺ (2), 336 (39), 321 (100), 281 (44), 131 (63). [α]_D²⁴ +21.74° (c 0.460 CHCl₃).

Results and Discussion

From the hexane extract of *K. reticulata* two new 4-phenylcoumarins, **1** and **2**, two 4-*n*-propylcoumarins, 7-hydroxy-8-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2',2'-dimethylpyrano(6',5':5,6)-4-*n*-propylcoumarin, 5-hydroxy-6-(4''-cinnamoyl-3''-methyl-1''-oxobutyl)-2',2'-dimethylpyrano(6',5':7,8)-4-*n*-propylcoumarin, previously isolated from *K. argentea*,⁸ and δ -tocotrienol¹¹ were isolated. The CH₂Cl₂ phase of the methanol extract yielded a new 4-phenylcoumarin, **3**, and 2,3-methylenedioxyxanthone.¹⁰

Compounds **1**, **2**, and **3** are yellow-greenish amorphous solids and their molecular formulae and structures were established by UV, EI mass spectrometry, ¹H and ¹³C NMR. Complete structural assignments were made by analogy with literature data¹²⁻¹⁴ and by a combination of DEPT, ¹H-¹³C COSY one bond and multiple bonds. ¹H NMR spectra of **1**, **2**, and **3** (Table 1) showed characteristic signals of a H-3 singlet, a 4-phenyl group, and one hydroxyl hydrogen (exchangeable with D₂O) bonded to an acyl side chain, and a cinnamoyl group. The spectrum of **1** showed the additional presence of two broad singlets at δ 4.81 and δ 4.92 assigned to terminal double bond hydrogens, a broad doublet at δ 4.27 assigned to the oxymethine hydrogen H-2', a doublet at δ 4.18 assigned to oxymethylene hydrogens H-4'', and at δ 3.39 and δ 3.26, two pairs of doublets of doublets assigned to H-2''a and H-2''b respectively. The hydrogen H-1'a at δ 3.04 was a doublets of doublets (14.6 and 1.4 Hz) while H-1'b at δ 2.73, was partially overlapped with the multiplet of H-3''.

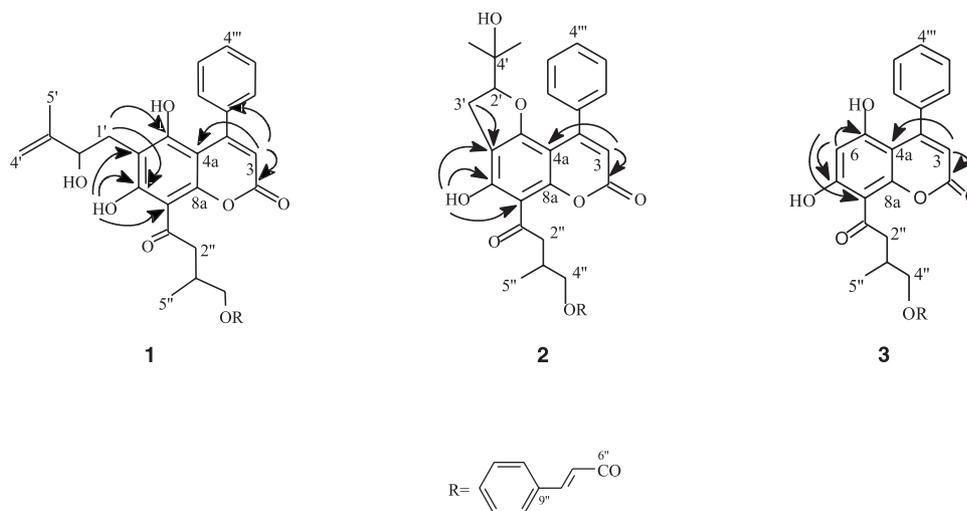


Figure 1. Structure of compounds **1**, **2**, and **3** and some important long range correlations observed in the ¹H-¹³C COSY spectra (COLOC).

Table 1. ¹H NMR data for compounds **1**, **2**, and **3** (300 MHz, CDCl₃)

| H | 1 | 2 | 3 |
|---------------------------|---------------------------|--------------------------|--------------------------|
| 3 | 5.97, 1H, s | 6.08, 1H, s | 6.00, 1H, |
| 6 | | | 6.22, 1H, s |
| 1'a | 3.04, 1H, dd (14.6, 1.4) | | |
| 1'b | 2.73, 1H, overlap | | |
| 2' | 4.27, 1H, bd (8.4) | 4.45, 1H, dd (9.7, 8.9) | |
| 3'a | | 2.87, 1H, dd (15.4; 8.9) | |
| 3'b | | 2.99, 1H, dd (15.4; 9.7) | |
| 4'a | 4.81, 1H, s | | |
| 4'b | 4.92, 1H, s | | |
| 5' | 1.75, 3H, s | 0.91, 3H, s | |
| 6' | | 0.98, 3H, s | |
| 2''a | 3.26, 1H, dd (16.2; 6.2) | 3.29, 1H, dd (16.3; 6.2) | 3.28, 1H, dd (16.5; 6.3) |
| 2''b | 3.39, 1H, dd (16.2; 7.1) | 3.46, 1H, dd (16.3; 7.2) | 3.41, 1H, dd (16.5; 6.8) |
| 3'' | 2.68, 1H, overlap | 2.71, 1H, m | 2.69, 1H, m |
| 4'' | 4.18, 2H, d (5.9) | 4.24, 2H, m | 4.21, 2H, m |
| 5'' | 1.13, 3H, d (6.7) | 1.18, 3H, d (7.0) | 1.16, 3H, d (6.8) |
| 7'' | 6.38, 1H, d (16.2) | 6.42, 1H, d (16.1) | 6.41, 1H, d (16.0) |
| 8'' | 7.61, 1H, d (16.2) | 7.64, 1H, d (16.1) | 7.65, 1H, d (16.0) |
| Phenyl (Cinnamoyl) | 7.36, 3H, m 7.49, 2H, m | 7.44, 3H, m 7.53, 2H, m | 7.36, 3H, m 7.52, 2H, m |
| 4-phenyl | 7.28, 2H, m 7.36, 3H, m | 7.30, 2H, m 7.38, 3H, m | 7.36, 2H, m 7.46, 3H, m |
| 7-OH | 14.62, 1H, s | 14.10, 1H, s | 14.00, 1H, s |

Values in parentheses indicate coupling constants *J* (Hz).

The ¹³C NMR spectrum (Table 2) confirmed the presence of the groups above and their positions on the 4-phenylcoumarin nucleus were unequivocally assigned by ¹H-¹³C long range correlations (COLOC). Thus, the correlations between the signal at δ 5.97 (H-3) with the carbon signals at δ 159.4, 139.7 and 102.3 permitted to assign these resonances to C-2, C-1'', and C-4a, respectively. The correlations of the signal at δ 14.62 (OH bonded) with the carbon signals at δ 166.7 (C-7), 110.1 (C-6) and 103.9 (C-8) made possible to place this hydroxyl group at C-7. The group 2'-hydroxy-3'-methyl-3'-butenyl was located at C-6 due to the observed correlations between the signal at δ 3.04 (H-1') with the carbons at δ 166.7 (C-7), 161.1 (C-5), and 110.1 (C-6). Therefore, the 4''-cinnamoyl-3''-methyl-1''-oxobutyl group was placed at C-8. This assumption was corroborated by the observation of a low bathochromic shift (387 to 392 nm) of the longer wavelength band in the UV spectrum after alkali addition.¹³

The signals in the ¹H NMR spectrum of **2** (Table 1) at δ 4.45, 2.87 and 2.99, in addition to the signals at δ 93.3 (C-2'), 72.1 (C-4'), 25.3 (C-5') and 23.9 (C-6') in the ¹³C NMR spectrum (Table 2), suggested the presence of the 2'-(2-hydroxyisopropyl) dihydrofuran system. This group was confirmed by ¹H-¹³C long range correlations and by the presence of an abundant ion at *m/z* 59 (C₃H₇O⁺) in the mass spectrum, which is characteristic of that system.¹⁴ The positions of the groups in aromatic coumarin ring were deduced by long range correlations (COLOC). The

Table 2. ¹³C NMR data for compounds **1**, **2**, and **3** (75 MHz, CDCl₃)

| C | 1 | 2 | 3 |
|------------------|--------|--------|--------|
| 2 | 159.4 | 159.2 | 158.8 |
| 3 | 111.9 | 111.7 | 111.8 |
| 4 | 156.8* | 155.4* | 157.7* |
| 4a | 102.3 | 99.3 | 101.2 |
| 5 | 161.1 | 162.9 | 160.4 |
| 6 | 110.1 | 110.6 | 101.0 |
| 7 | 166.7 | 164.0 | 168.4 |
| 8 | 103.9 | 105.7 | 104.8 |
| 8a | 156.4* | 157.7* | 155.0* |
| 1' | 28.8 | - | - |
| 2' | 76.9 | 93.3 | - |
| 3' | 146.2 | 27.3 | - |
| 4' | 110.6 | 72.1 | - |
| 5' | 18.4 | 25.3 | - |
| 6' | - | 23.9 | - |
| 1'' | 204.6 | 205.4 | 204.4 |
| 2'' | 48.5 | 49.1 | 48.5 |
| 3'' | 30.0 | 30.8 | 30.9 |
| 4'' | 68.9 | 69.4 | 68.9 |
| 5'' | 17.3 | 17.9 | 17.3 |
| 6'' | 167.1 | 167.4 | 167.2 |
| 7'' | 117.9 | 118.7 | 117.9 |
| 8'' | 144.8 | 145.2 | 144.9 |
| 9'' | 134.3 | 135.1 | 134.4 |
| 10''-14'' | 128.1 | 128.7 | 128.8 |
| 11''-13'' | 128.8 | 129.4 | 128.9 |
| 12'' | 130.3 | 130.7 | 130.2 |
| 1''' | 139.7 | 138.6 | 137.4 |
| 2'''-6''' | 127.1 | 128.7 | 127.3 |
| 3'''-5''' | 127.6 | 129.4 | 128.1 |
| 4''' | 128.2 | 128.00 | 129.6 |

* This signals may be interchangeable

correlations observed between the signal at δ 6.08 (H-3) with the signals at δ 159.2 and δ 99.3 permitted the assignment of these resonances to carbons C-2 and C-4a, respectively. The correlations of the resonance at δ 14.10 (OH bonded) with the signals at δ 105.7 (C-8), 110.6 (C-6) and 164.0 (C-7) and those at δ 2.87 (H-3'a) and 2.99 (H-3'b) with δ 110.6 (C-6) made evident the location of the bonded hydroxyl at C-7, the 2''-(2-hydroxyisopropyl)-dihydrofuran ring at C-5 and C-6 and the 4''-cinnamoyl-3''-methyl-1''-oxobutyl group at C-8. The UV spectrum of **2** after alkali addition showed a bathochromic shift (348 to 379 nm) of the longer wavelength band. The observed shift was lower than the obtained with a similar compound with the acyl side chain at C-6 (348 to 428 nm).¹⁵ In conclusion, the UV data corroborated with the location of the acyl side chain at C-8.

The long-range correlation observed in the ¹H-¹³C COSY spectrum of **3**, between the signal at δ 6.00 (H-3) and the carbon signal at δ 101.2 allowed the assignment of this signal to C-4a. The correlations of the hydrogen signal at δ 6.22 with the carbon signals at δ 160.4 (C-5), 168.4 (C-7) and 104.8 (C-8) allowed the placement of this hydrogen at C-6 and consequently the 4''-cinnamoyl-3''-methyl-1''-oxobutyl side chain was placed at C-8. The acyl side chain at C-8 was confirmed by the low bathochromic shift (382 to 388 nm) of the longer wavelength band in the UV spectrum after alkali addition.

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