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COUMARINS FROM *KIELMEYERA RETICULATA*

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Key Word Index—*Kielmeyera reticulata*; Guttiferae; stems; 4-phenylcoumarin; neoflavonoid.

Abstract—Five new prenylated 4-phenylcoumarins were isolated from the hexane extract of the stems of *Kielmeyera reticulata*, 7-hydroxy-8-(4-cinnamoyl-3-methyl-1-oxobutyl)-4-phenyl-2′,2′-dimethyl 2H,6H-benzo[1,2-b:3,4-b′]-dipyran-2-one, 7-hydroxy-8-(4-hydroxy-3-methyl-1-oxobutyl)-4-phenyl-2′,2′-dimethyl-2H,6H-benzo[1,2-b:3,4-b′]-dipyran-2-one, 5-hydroxy-6-(4-cinnamoyl-3-methyl-1-oxobutyl)-4-phenyl-2′,2′-dimethyl-2H,6H-benzo [1,2-b:3,4-b′]-dipyran-2-one, 5,7-dihydroxy-6-(4-cinnamoyl-3-methyl-1-oxobutyl)-8-(3-methyl-2-butenyl)-4-phenyl-2H-1-benzopyran-2-one, and 5,7-dihydroxy-6-(4-hydroxy-3-methyl-1-oxobutyl)-8-(3-methyl-2-butenyl)-4-phenyl-2H-1-benzopyran-2-one. These compounds were identified from spectral evidence and compared with literature data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The majority of Guttiferae are trees or shrubs generally confined to the tropics. In Brazil, there are 21 genera and ca 183 species [1], being the genus *Kielmeyera* endemic to South America [2]. Previous work on some species of this genus found in the cerrado (savanna) of the Central Brazilian plateau, describe xanthones as the principal constituents [3–11]. On the contrary, in *Kielmeyera reticulata*, a wild shrub that was collected in sand dunes (restinga) on the coast of Bahia, Brazil, only prenylated 4-phenylcoumarins were found. The structures of these compounds resemble those that were previously isolated from species of the genus *Mammea* [12–14], the mammea coumarins, which exhibit considerable insecticidal [15] and antibacterial properties [16], and inhibit the growth of Sarcoma 180 cells [17]. Recently, increasing interest has been given to pyranocoumarins due to their potential as anti-HIV agents [18, 19].

RESULTS AND DISCUSSION

Five new 4-phenylcoumarins, **1**–**5**, were isolated from the hexane extract of the stems of *K. reticulata* after chromatographic purification. These compounds are yellow-greenish amorphous solids. Both compounds of each pair of coumarins, **1** and **2**, and **4** and **5**, showed related spectral data and differed from one another only in the presence of a cinnamoyl group. The molecular formulae of these compounds were determined by EI mass spectrometry and confirmed by ^1H and ^{13}C NMR. Complete structural assignments were made by analogy with literature data [15, 16] and by a combination of NOISE, DEPT, ^1H-^1H COSY, ^1H-^{13}C COSY (J = 140.0 Hz), ^1H-^{13}C COSY (J = 9.0 Hz) and UV data.

The ^1H NMR spectrum of compound **1** (Table 1) showed the presence of a 3-H singlet, a 4-phenyl group, a 2,2-dimethylchromene ring system and one hydroxyl hydrogen (exchangeable with D_2O) bonded to an acyl side-chain, and a cinnamoyl group.

Compound **2** gave similar ^1H NMR data to compound **1**, except for the lack of cinnamoyl group signals and the expected differences in the signals of the acyl side-chain.

The ^1H NMR spectrum of compound **3** showed signals for the same groups as present in compound **1**, which were at different position in the aromatic coumarin ring; assignments are given in Table 1. In this compound the hydroxyl and acyl groups were located at C-5 and C-6, respectively, while in **1**, these groups were located at C-7 and C-8. Consequently, the 2,2-dimethylchromene rings are located at positions 5 and 6 in compounds **1** and **2**, and at positions 7 and 8 in compound **3**. The positions of the various substituents around the aromatic coumarin ring in both compounds were supported by other evidence. In **1** and **2**, the two methyl groups of the 2,2-dime-

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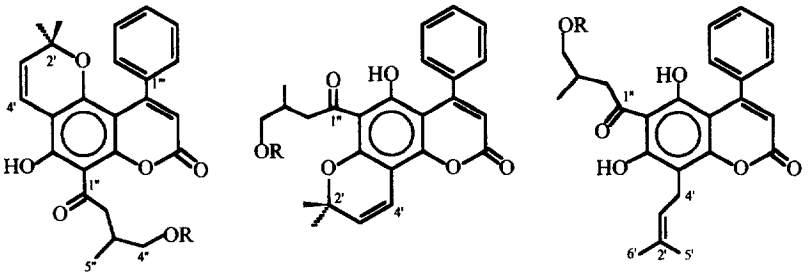

1 R = 1C-CO
2 R = H

3 R = 1C-CO

4 R = 1C-CO
5 R = H

thylchromene ring (δ 0.92 and δ 0.93, δ 0.95 and δ 0.97, respectively) are shielded due to the diamagnetic effect of the benzene ring at C-4, while in **3** (δ 1.55 and δ 1.57), this effect is absent. The signals at δ 102.9 and at δ 102.8 in the ^{13}C NMR spectra of compounds **1** and **3**, respectively, were attributed to C-4a. These assignments were corroborated by long-range correlations (Table 3) between these signals and those at δ 6.0 and δ 5.96 (H-3). The long-range correlations (Table 3) for the signal at δ 14.45 (OH-bonded) with carbons at δ 104.8 (C-8) and δ 106.4 (C-6) in compound **1**, showed that the hydroxyl group was located at C-7. In compound **3**, the signal at δ 14.65 showed correlations with carbons at δ 102.8 (C-4a) and δ 107.9 (C-6) and, consequently, the hydroxyl was located at C-5. In the UV spectrum of compound **3**, the long-wavelength band showed a large bathochromic shift (370 to 426 nm) after addition of alkali, characteristic of 6-acylcoumarins [12], while in the compound **1**, an 8-acylcoumarin, only a small bathochromic shift (376 to 391 nm) was observed.

Compounds **4** and **5** did not exhibit signals for

Table 1. ^1H NMR data for compounds **1–5** (300 MHz, $CDCl_3$)

H	1	2	3	4	5
3	6.01, 1H, *s*	6.02, 1H, *s*	5.96, 1H, *s*	6.00, 1H, *s*	5.90, 1H, *s*
3′	5.37, 1H, *d* (10.0 Hz)	5.40, 1H, *d* (10.1 Hz)	5.64, 1H, *d* (10.1 Hz)	5.06, 1H, *tl* (6.0 Hz)	5.07, 1H, *tl* (Hz)
4′	6.60, 1H, *d* (10.0 Hz)	6.62, 1H, *d* (10.1 Hz)	6.88, 1H, *d* (10.1 Hz)	3.27, 2H, *d* (8.0 Hz)	3.28, 2H, *d* (7.6 Hz)
5′	0.93, 3H, *s*	0.97, 3H, *s*	1.57, 3H, *s*	1.69, 3H, *s*	1.69, 3H, *s*
6′	0.92, 3H, *s*	0.95, 3H, *s*	1.55, 3H, *s*	1.64, 3H, *s*	1.63, 3H, *s*
2a″		2.71, 1H, *dd*	3.05, 1H, *dd*	3.31, 1H, *dd*	2.73, 1H, *dd*
	3.28–3.48	(9.1; 13.9 Hz)	(7.3; 17.0 Hz)	(6.0; 15.0 Hz)	(8.8; 14.0 Hz)
2b″	2H, *m*	3.82, 1H, *dd*	3.25, 1H, *dd*	3.45, 1H, *dd*	3.41, 1H, *dd*
		(4.3; 13.9 Hz)	(6.1; 17.0 Hz)	(6.0; 15.0 Hz)	(8.8; 11.3 Hz)
3″	2.71, 1H, *m*	2.50, 1H, *m*	2.65, 1H, *m*	2.71, 1H, *m*	2.48, 1H, *sl*
4a″		3.79, 1H, *dd*			
	4.17–4.29	(5.0; 10.5 Hz)	4.16, 2H, *d*	4.24, 2H, *m*	3.75, 2H, *m*
4b″	2H, *m*	3.42, 1H, *dd*	(6.1 Hz)		
		(9.0; 10.5 Hz)			
5″	1.18, 3H, *d*	1.01, 3H, *d*	1.06, 3H, *d*	1.18, 3H, *d*	0.99, 3H, *d*
	(6.8 Hz)	(6.7 Hz)	(6.7 Hz)	(6.0 Hz)	(6.6 Hz)
4-Phenyl	7.22, 2H, *m*	7.23, 2H, *m*	7.26, 2H, *m*	7.39, 2H, *m*	7.40, 2H, *m*
	7.37, 3H, *m*	7.40, 3H, *m*	7.38, 3H, *m*	7.52, 3H, *m*	7.54, 3H, *m*
4′-OH		1.83, 1H, *s*			
5-OH			14.65, 1H, *s*	5.97, 1H, *s*	
7-OH	14.45, 1H, *s*	14.67, 1H, *s*		14.39, 1H, *s*	14.61, 1H, *s*
2C	6.41, 1H, *d*		6.38, 1H, *d*	6.41, 1H, *d*	
	(16.0 Hz)		(16.0 Hz)	(16.0 Hz)	
3C	7.64, 1H, *d*		7.62, 1H, *d*	7.66, 1H, *d*	
	(16.0 Hz)		(16.0 Hz)	(16.0 Hz)	
Phenyl	7.37, 3H, *m*		7.38, 3H, *m*	7.39, 3H, *m*	
(cinnamoyl)	7.52, 2H, *m*		7.46, 2H, *m*	7.52, 2H, *m*	

Table 2. ^{13}C NMR data for compounds **1**–**5** (75 MHz, $CDCl_3$)

C	1	2	3	4	5
2	159.2	160.1	159.9	158.9	160.0
3	112.6	112.2	113.4	112.8	112.4
4	156.5	157.3	156.8	154.6	155.5
4a	102.9	102.8	102.8	101.1	101.3
5	157.0	157.2	164.8	156.5	156.2
6	106.4	106.5	107.9	105.2	105.6
7	164.0	164.6	155.5	167.4	167.6
8	104.8	104.7	102.2	113.2	113.4
8a	157.0	157.2	158.7	157.9	158.0
2′	79.7	79.8	80.7	134.7	134.6
3′	127.3	127.4	127.0	121.3	121.3
4′	115.8	115.8	116.1	22.1	22.1
5′	28.0	28.0	28.8	17.9	17.0
6′	28.0	28.0	28.7	26.2	26.2
1″	205.4	206.7	206.0	205.5	206.6
2″	49.3	49.4	49.1	49.3	49.4
3″	30.8	34.0	30.0	30.7	33.7
4″	69.4	68.7	69.3	69.4	68.5
5″	17.9	17.0	17.8	18.4	17.0
1‴	140.5	140.4	139.8	137.4	137.5
2‴–6‴	127.7	127.6	127.6	128.0	128.0
3‴–5‴	128.1	128.2	128.2	130.1	130.0
4‴	128.3	128.4	128.8	130.7	130.7
1C	167.4		167.3	167.1	
2C	118.6		118.4	118.7	
3C	145.2		145.5	145.2	
4C	135.1		134.9	135.1	
5C–9C	128.7		128.6	128.6	
6C–8C	129.3		129.5	129.4	
7C	130.7		130.9	130.8	

Table 3. ^1H-^{13}C COSY ($J = 9.0$ Hz) for compounds **1**, **3** and **5**

δ(H)	δ(C)
Compound **1**	
5.37(2′)	79.7(2′); 106.4(6)
6.0(3)	102.9(4a); 140.5(1‴); 159.2(2)
6.60(1′)	157.0(5)
14.45(7-OH)	104.8(8); 106.4(6); 164.0(7)
Compound **3**	
5.64(2′)	80.7(2′); 102.2(8); 127.0(3′)
5.96(3)	102.8(4a); 139.8(1‴); 159.9(2)
6.88(1′)	80.7(2′); 116.1(4′); 158.7(8a)
14.65(5-OH)	102.8(4a); 107.9(6); 164.8(5)
Compound **5**	
3.28(1′)	113.4(8); 158.0(8a); 167.6(7)
5.90(3)	101.3(4a); 137.5(1‴); 160.0(2)
14.61(7-OH)	105.6(6); 113.4(8); 167.6(7)

a 2,2-dimethylchromene ring, but they indicated the presence of 2,2-dimethylallyl group at C-8, an acyl group at C-6 and two hydroxyl groups at C-5 and C-7. For compound **5**, the long-range correlations (Table 3) of the signal at δ 14.61 (OH-bonded) with the signals at δ 105.6 (C-6), δ 113.4 (C-8), and δ 167.6 (C-7), and those correlations of the signal at δ 3.28 (4′) with δ 113.4 (C-8), δ 158.0 (C-8a), and δ 167.6 (C-7) suggested placement of the acyl group at C-6, OH-bonded at C-7 and the 3,3-dimethylallyl group at C-8. The large bathochromic shift (332 to 385 nm) after the addition of alkali in the UV spectrum confirmed the assignments proposed.

EXPERIMENTAL

UV: MeOH and MeOH–NaOH. EIMS: Direct probe insert at 70 eV. NMR: Gemini 300-Varian.

Plant material

Kielmeyera reticulata Saad was collected in the sand dunes of Parque Metropolitano da Lagoa do Abaeté, Salvador, Bahia, Brasil, in January 1992. A voucher specimen, No. 027415, is deposited in the "Alexandre Leal Costa" Herbarium, Instituto de Biologia, Universidade Federal da Bahia, Salvador, Brasil.

Extraction and isolation

Dried stems were extracted with hexane. The extract (37.09 g) was concd under red. pres. and then submitted to silica gel CC using a hexane–EtOAc gradient. Some frs. were rechromatographed by silica gel CC using a hexane–EtOAc gradient to give **1** (85 mg), **2** (118,5 mg), **3** (163,0 mg), **4** (16,0 mg), and **5** (464,7 mg).

Compound **1**. 7-hydroxy-8-(4-cinnamoyl-3-methyl-1-oxobutyl)-4-phenyl-2′,2′-dimethyl-2H,6H-benzo [1,2-b:3,4-b′]-dipyran-2-one. $C_{34}H_{30}O_7$. Amorphous yellow-greenish solid. NMR: Tables 1 and 2. EIMS *m/z* (rel. int): 550 $[M]^+$ (3), 535 (13), 402 (19), 388 (26), 387 (100), 331 (20), 148 (28), 147 (43), 131 (36), 103 (33). $[\alpha]_D^{24}$ −7.14° ($CHCl_3$).

Compound **2**. 7-hydroxy-8-(4-hydroxy-3-methyl-1-oxobutyl)-4-phenyl-2′,2′-dimethyl-2H,6H-benzo[1,2-b:3,4-b′]-dipyran-2-one. $C_{25}H_{24}O_6$. Amorphous yellow-greenish solid. NMR: Tables 1 and 2. EIMS *m/z* (rel. int): 402 $[M-H_2O]^+$ (30), 403 (8), 388 (28), 387 (100), 359 (6), 331 (16). $[\alpha]_D^{24}$ −1.92 ($CHCl_3$).

Compound **3**. 5-hydroxy-6-(4-cinnamoyl-3-methyl-1-oxobutyl)-4-phenyl-2′,2′-dimethyl-2H,6H-benzo [1,2-b:3,4-b′]-dipyran-2-one. $C_{34}H_{30}O_7$. Amorphous yellow-greenish solid. NMR: Tables 1 and 2. EIMS *m/z* (rel. int): 550 $[M]^+$ (7), 402 (31), 388 (19), 387 (82), 347 (10), 331 (28), 147 (52), 131 (100), 103 (56). $[\alpha]_D^{24}$ −6.60° ($CHCl_3$).

Compound **4**. 5,7-dihydroxy-6-(4-cinnamoyl-3-methyl-1-oxobutyl)-8-(3-methyl-2-bute-nyl)-4-phenyl-2H-1-benzo-pyran-2-one. $C_{34}H_{32}O_7$. Amorphous yellow-greenish solid. NMR: Tables 1 and 2. EIMS *m/z* (rel. int): 404 $[M-C_6H_5C_2H_2CO_2H]^+$ (38), 389 (11),

361 (38), 349 (21), 321 (9), 131 (100). $[\alpha]_D^{24}$ −12.17° ($CHCl_3$).

Compound **5**. 5,7-dihydroxy-6-(4-hydroxy-3-methyl-1-oxobutyl)-8-(3-methyl-2-bute-nyl)-4-phenyl-2H-1-benzopy-ran-2-one. $C_{25}H_{26}O_6$. Amorphous yellow-greenish solid. NMR: Tables 1 and 2. EIMS *m/z* (rel. int): 404 $[M-H_2O]^+$ (20), 389 (15), 349 (30), 361 (100), 327 (6). $[\alpha]_D^{24}$ +1.58° ($CHCl_3$).

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REFERENCES

1. Barroso, G. M., *Sitemática de Angiospermas do Brasil*, Vol. 1. EDUSP, SP. 1978, p. 139.
2. Sultanbawa, M. U. S., *Tetrahedron*, 1980, **36**, 1465.
3. Gottlieb, O. R., Magalhães, M. T., Camey, M., Mesquita, A. A. L. and Corrêa, D. B., *Tetrahedron*, 1966, **22**, 1777.
4. Gottlieb, O. R., Magalhães, M. T., Pereira, M. O. S., Mesquita, A. A. L., Corrêa, D. B. and Oliveira, G. G., *Tetrahedron*, 1968, **24**, 1601.
5. Gottlieb, O. R., Mesquita, A. A. L., Da Silva, E. M. and Melo, M. T., *Phytochemistry*, 1969, **8**, 65.
6. Gottlieb, O. R. and Stefani, G. M., *Phytochemistry*, 1970, **9**, 453.
7. Corrêa, D. B., Silva, L. G. F., Gottlieb, O. R. and Gonçalves, S. J., *Phytochemistry*, 1970, **9**, 447.
8. Gottlieb, O. R., Mesquita, A. A. L., de Oliveira, G. G. and de Melo, M. T., *Phytochemistry*, 1970, **9**, 2537.
9. Gottlieb. O. R. and Nagem, T. J., *Rev. Latinoam. Quim.*, 1977, **8**, 137.
10. Lopes, J. L. C., Lopes, J. N. C., Gilbert, B. and Bonini, S. E., *Phytochemistry*, 1977, **16**, 1101.
11. Nagem, T. J. and Silva, M. A., *Phytochemistry*, 1988, **27**, 2961.
12. Crombie, L., Games, D. E. and McCromick, A., *J. Chem. Soc. (C)*, 1967, 2545.
13. Carpenter, I., McGarry, E. J. and Scheinmann, F., *J. Chem. Soc. (C)*, 1971, 3783.
14. Crichton, E. G. and Waterman, *Phytochemistry*, 1978, **17**, 1783.
15. Crombie, L., Jones, R. C. and Palmer, C. J., *J. Chem. Soc., Perkin Trans. I*, 1987, 317.
16. Joshi, B. S., Kamat, V. N., Govindachari, T. R. and Ganguly, A. K., *Tetrahedron*, 1969, **25**, 1453.
17. Finnegan, R. A., Menkel, K. E. and Back, N., *J. Pharm. Sci.*, 1972, **61**, 1599.
18. Kashman, Y., Gustafson. K. R., Fuller, R. W., Cardellina, J. H., McMahon, J. B., Currens, M. J., Buckheit, R. W. Jr., Hughes, S. H., Cragg, G. M. and Boyd, M. R., *J. Med. Chem.*, 1992, **35**, 2735.
19. McKee, T. C., Fuller, R. W., Covington, C. D., Cardellina, J. H., Gulakowski, R. J., Krepps, B. L., McMahon J. B. and Boyd, M. R., *J. Nat. Prod.*, 1996, **59**, 754.