Pergamon

PII: S0031-9422(97)00731-0

Phytochemistry, Vol. 47, No. 7, pp. 1367–1374, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

XANTHONES, TRITERPENES AND A BIPHENYL FROM *KIELMEYERA CORIACEA*

D. A. GARCIA CORTEZ, M. C. M. YOUNG,* A. MARSTON,† J.-L. WOLFENDER† and K. HOSTETTMANN†‡

Departamento de Farmacia e Farmacologia, Universidade Estadual de Maringa, CEP 87020-900, Maringa, PR, Brazil; * Instituto de Botanica, CEP 01061-970, São Paulo, SP, Brazil; † Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015 Lausanne, Switzerland

(*Received* 23 *June* 1997)

Key Word Index—*Kielmeyera coriacea*: Guttiferae; xanthones; biphenyl; triterpenes; LC-UV; LC-MS; antifungal activity.

Abstract—Analysis of the dichloromethane extracts of *Kielmeyera coriacea* leaves and stems by high performance liquid chromatography coupled with a photodiode array detector (LC-UV) and thermospray liquid chromatography-mass spectrometry (TSP/LC-MS) revealed the presence of several xanthones. Phytochemical investigation of these extracts resulted in the isolation and identification of ten xanthones, one biphenyl and two triterpenes. Their structures were established by chemical and spectroscopic methods (UV, EI-MS, D/CI-MS, ^{1}H NMR, ^{13}C NMR, HMQC and HMBC). One xanthone and two triterpenes are new compounds. Four xanthones and the biphenyl exhibited antifungal activity against the plant pathogenic fungus *Cladosporium cucumerinum*, while two prenylated xanthones inhibited the growth of *Candida albicans*.

INTRODUCTION

Kielmeyera coriacea L. (Guttiferae), known as Pau-Santo, is a tree indigenous to the central Brazilian plateau [1]. Since many species of the Guttiferae contain prenylated xanthones, a number of which possess significant antimicrobial properties [2, 3], a detailed investigation of this plant was undertaken. Previous phytochemical investigations of the plant led to the isolation of xanthonolignoids [4], osajaxanthone [5], a biphenyl [6] and tri-oxygenated xanthones [7]. The dichloromethane extracts of both the leaves and stems of *K. coriacea* exhibited antifungal properties in bioautographic assays with *Cladosporium cucumerinum* and *Candida albicans* [8, 9]. Subsequent isolation yielded ten known compounds (**1**–**8**, **10**, **11**), and three new compounds (**9**, **13**, **14**).

RESULTS AND DISCUSSION

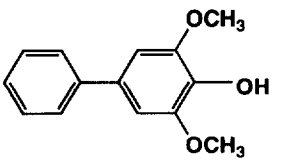
Preliminary analysis of the dichloromethane (Fig. 1) and the methanol extracts of the aerial parts of *K. coriacea* by HPLC with photodiode array detection (LC-UV) showed the presence of xanthones. Combined with HPLC using a thermospray mass spectrometry interface (TSP/LC-MS), it was possible to localize 10 different xanthones (**2**–**11**) (see corresponding single ion traces in Fig. 2) in the dichloromethane extracts of the leaves and stems. The xanthones, one biphenyl (**1**) and a mixture of triterpenes (**13** and **14**) were isolated after chromatography on silica gel and a combination of gel filtration on Sephadex LH-20, semi-preparative HPLC on a cyano column and crystallization (see Experimental).

The biphenyl, aucuparin (**1**) [10, 11], together with the xanthones 2-hydroxy-1-methoxyxanthone (**2**) [12], 3-hydroxy-2,4-dimethoxyxanthone (**3**) [6], 4-hydroxy-2,3-dimethoxyxanthone (**4**) [7], swertinin (**5**) [13], 6-hydroxy-1,3,5-trimethoxyxanthone (**6**) [14] and kielcorin (**10**) [4] were identified by comparison of their ^{1}H NMR, ^{13}C NMR, EI- and D/CI-MS with literature data.

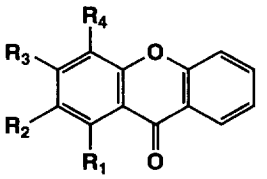
The TSP/LC-MS analysis of the extracts revealed also the presence of prenylated xanthones **7**, **8**, **9** and **11** (see selected ion traces in Fig. 2). The compounds 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (**7**) [15], 1,3,5-trihydroxy-2-(3-methylbut-2-enyl) xanthone (**8**) [16] and jacareubin (**11**) [16] were identified by comparison of spectral data (^{1}H NMR, ^{13}C NMR and D/CI-MS) with literature values.

The EI mass spectrum of **9** gave a molecular ion $[M]^+$ at m/z 330. The UV spectrum of **9** closely resembled that of **7**, suggesting a xanthone with a similar substitution pattern (1,2,3,7-tetra-oxygen-

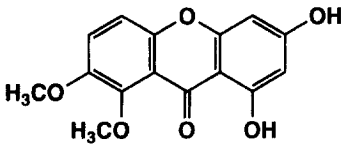
‡ Author to whom correspondence should be addressed.



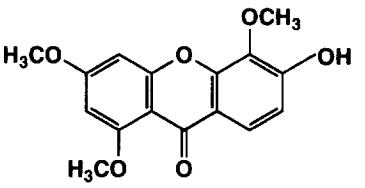
1



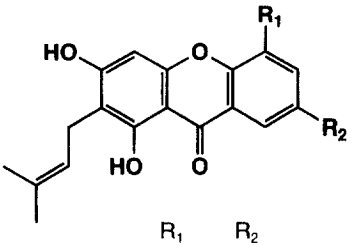
	R_1	R_2	R_3	R_4
2	OCH_3	OH	H	H
3	H	OCH_3	OH	OCH_3
4	H	OCH_3	OCH_3	OH



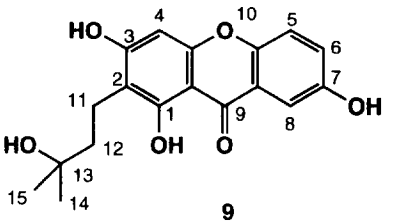
5



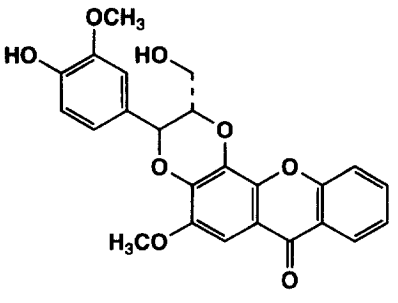
6



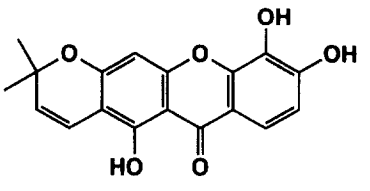
	R_1	R_2
7	H	OH
8	OH	H



9



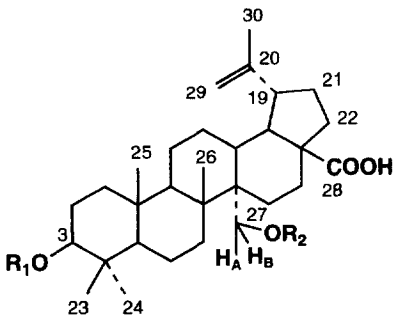
10



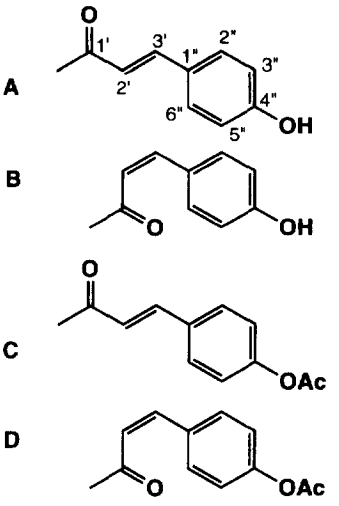
11

ation). The aromatic region of the ^1H NMR spectrum was almost identical to that of **7**. Signals for two methyl groups at δ 1.26 (s) and two methylene protons which appeared respectively as multiplets at δ 2.78 (H-12) and δ 1.71 (H-11) were also observed. Comparison of the ^{13}C NMR spectra of **9** and **7** (Table 1) showed that the only difference was due to the side chain, compatible with a hydroxy substituent at C-13 in **9**. In the spectrum of **9**, there were signals for two methylene carbons at C-12 (δ 42.06) and C-11 (δ 16.92), for the quaternary carbon connected to a tertiary hydroxy group at δ 69.83 (C-13), and methyl carbons at δ 29.14 (C-14 and 15). Compound **9** differed from **7** in that the olefinic group was replaced by a 2,3-dihydro,3-hydroxy moiety. Thus **9** was 1,3,7-trihydroxy-2-(3-hydroxy-3-methylbutyl)-xanthone.

The compound with a retention time of 31.3 min in the HPLC analyses of the dichloromethane extracts (Fig. 1) gave a ^{13}C NMR spectrum (Table 2) which revealed a mixture of two isomers (**13**/**14**). The IR spectrum of the compound mixture **13**/**14** showed the presence of an acid moiety. The molecular formula $C_{39}H_{54}O_6$ was determined from mass spectrometry ($[M + NH_4]^+$, D/CI MS (NH_3), *m/z* 636) and ^{13}C NMR



	R_1	R_2
12	H	H
12Ac	Ac	Ac
13	H	A
14	H	B
13Ac	Ac	C
14Ac	Ac	D



spectral data, including DEPT. In the EI-MS, a fragment at *m/z* 454 ($C_{30}H_{46}O_3$) corresponded to the loss of a coumaric acid ($C_9H_8O_3$) moiety from the molecular ion. The EI-MS of the mixture **13/14** also revealed two main ions at *m/z* 207 (a) and 189 (b). These fragment ions, which arise from the cleavage of the C-9 (11) and C-8 (14) bonds, are typical of 3-hydroxy triterpenes [17] (Fig. 3).

In the ^{1}H NMR spectrum (Table 3) of **13/14**, one doublet centered at δ 4.74 (1H), one multiplet at δ 4.61 (1H) and a vinyl methyl singlet at δ 1.69 agreed with the values expected for the protons of an isopropylidene group at C-19. Two doublets centered at δ 4.43 (1H) and at δ 4.35 (1H) were characteristic for the methylene protons H_A-27 and H_B-27, and one double doublet at δ 3.20 for H-3. The ^{1}H NMR spectrum also showed signals (H-2′ and H-3′) indicating the presence of both *trans*- and *cis*-*p*-coumaroyl moieties (Table 3). In the HMBC spectrum of **13/14** (correlations shown in Fig. 4), the methyl protons at δ 0.93 and δ 0.73 were correlated to the C-3 methine carbon with an oxygen function (δ 78.88) and to C-5 (δ 55.33). The methyl protons at δ 0.83 and δ 0.95 were correlated respectively with quaternary carbons C-10 (δ 37.45) and C-8 (δ 41.51), and the methylene protons at H_A-27 and H_B-27 with C-8. A comparison of the chemical shifts (**13/14**) of the triterpene carbons with cylicodiscic acid (**12**) [17] showed the latter to be the parent triterpene. Thus, **13/14** is a mixture of *cis* and *trans*-*p*-coumaroyl esters of cylicodiscic acid. Acetylation of **13/14** gave a mixture of diacetates **13Ac/14Ac** ($C_{43}H_{58}O_8$), *m/z* 720 $[M+NH_4]^+$ (D/CI-MS, NH_3). Signals for one aliphatic acetate (δ 2.03, C-3) and two aromatic acetates (δ 2.23, 2.30, C-4″—one for each isomer) were observed in the ^{1}H NMR spectrum. The H-3 proton double doublet at δ 3.20 was shifted downfield on acetylation, appearing under the peaks for protons H_A-27 and H_B-27 (δ 4.48 m). This confirmed esterification by the cinnamoyl moiety at the hydroxymethyl group and not at C-3. The ^{13}C NMR of the aglycone part of **13Ac/14Ac** was very similar to that of acetylated cylicodiscic acid **12Ac** [17]. The hydrolysis of compounds **13** and **14** yielded cylicodiscic acid (**12**) and *cis*- and *trans*-*p*-coumaric acids, identified by TLC comparison with standards.

Compounds **1**, **2**, **3**, **7** and **11** were responsible for the antifungal activity of the extract against *Cladosporium cucumerinum*. Prenylated xanthones **7** and **11** were also fungicidal against *Candida albicans* (Table 4).

Whereas simple prenylated xanthones [7] and biphenyl compounds [6] have been previously isolated from *Kielmeyera coriacea*, this is the first report of the occurrence of a cinnamic ester of a triterpene from the plant.

EXPERIMENTAL

General

Mps: uncorr. IR: KBr. UV: MeOH. ^{1}H and ^{13}C NMR spectra were measured at 500, 200 and 125.65, 50 MHz, respectively, in $CDCl_3$ (with a drop of DMSO-d_6 in certain cases), acetone-d_6 or pyridine-d_5. EIMS: 70 eV. TLC: silica gel precoated Al sheets (Merck), Diol, RP-18 HPTLC plates (Merck). CC: silica gel 60 (70–230 mesh, Merck), Sephadex LH-20 (Pharmacia). Analytical HPLC was carried out on a Hewlett Packard HP 1090 instrument equipped with photodiode array detector, using Nova-Pak C-18 columns (150 × 3.9 mm, Waters) at a flow rate of 1 ml/min. Semi-prep. HPLC was performed on a cyano column (7 μm, 250 × 16 mm, Knauer) at a flow rate of 10 ml/min. Bioassays were performed as described in previous papers [8, 9], with 100 μg of crude extracts and 0.01, 0.1, 1 and 10 μg of pure compounds.

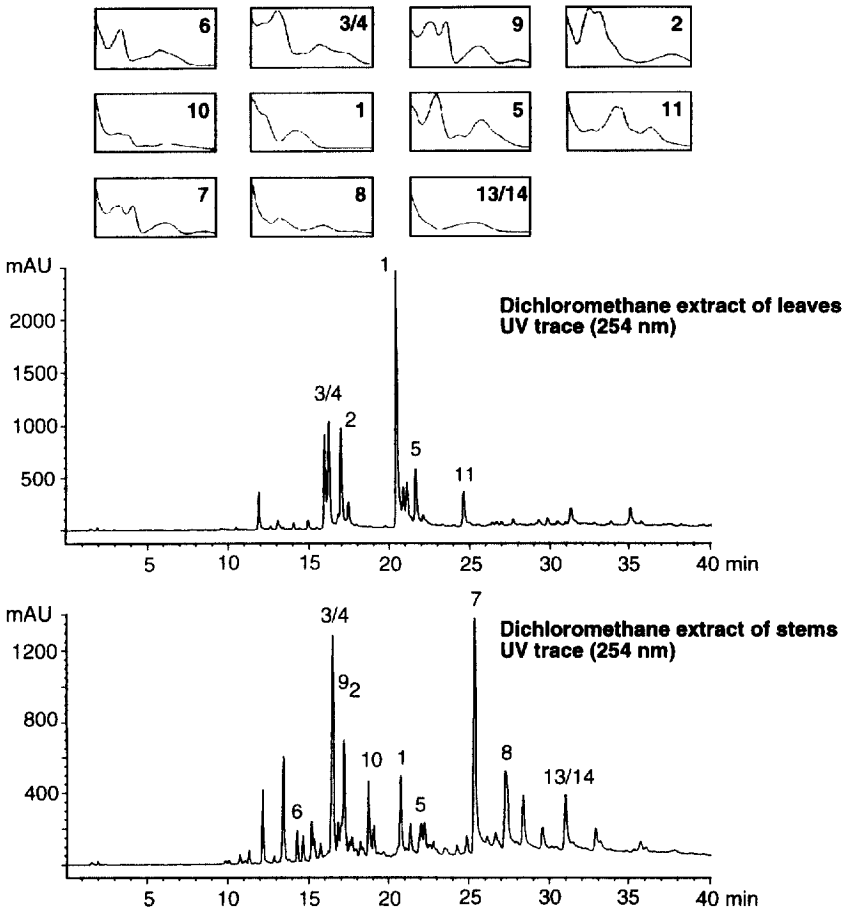


Fig. 1. Comparison of the LC-UV traces of dichloromethane extracts of leaves and stems of *Kielmeyera coriacea*. Conditions: Nova Pak C-18 column (4 μm, 150 × 3.9 mm); step gradient of acetonitrile-water (containing 0.05% TFA): 10% → 100% CH_3CN over 40 min; flow rate 1 ml/min; detection UV 254 nm.

Plant material

Kielmeyera coriacea L. was collected in Mogiguau (São Paulo, Brazil) in July 1995, and a voucher specimen (no. SP 298463) has been deposited at the State Botanical Institute, São Paulo, Brazil.

Extraction and isolation

Leaves and stems were extracted separately at room temp. with solvents of increasing polarity: CH_2Cl_2 and MeOH. The CH_2Cl_2 extract (33 g) of the leaves was submitted to CC on silica gel (63–200 μm) using step gradient elution CH_2Cl_2–EtOAc (98:2, 95:5, 90:10, 80:20, 50:50), EtOAc, EtOAc–MeOH (90:10, 50:50) and MeOH and sixteen frs were collected. HPLC analysis revealed the presence of peaks with xanthone-like UV spectra for frs B-F. Frs A, B, D, E and F were submitted to gel filtration on Sephadex LH-20 [$CHCl_3$–MeOH (50:50) and MeOH] and gave five compounds [**1** (49 mg), **2** (2 mg), **4** (9 mg), **5** (2 mg) and **11** (21 mg)]. Fr. C was purified by semi-prep. HPLC on a cyano column with hexane–isoPrOH (85:1) to give compound **3** (2 mg). The CH_2Cl_2 extract (42 g) of the stems was fractionated by the same procedure. HPLC analysis of frs B–J gave typical UV spectra for xanthones, while frs A and K contained other aromatic compounds. Frs A–K were submitted to gel filtration on Sephadex LH-20 [$CHCl_3$–MeOH (50:50) and MeOH]. Eleven compounds [**1** (10 mg), **2** (2 mg), **3** (2 mg), **4** (18 mg), **5** (1 mg), **6** (5 mg), **7** (146 mg), **8** (14 mg), **9** (8 mg) and **13/14** (50 mg)] were obtained from fr. A and frs C–K. Fraction B gave compound **10** (2 mg) after semi-prep HPLC on a cyano column with hexane–isoPrOH (85:1).

LC-TSP-MS-analyses

A Finnigan MAT TSQ-700 triple quadrupole instrument equipped with TSP 2 interface was used for data acquisition and processing, under the following conditions: source temperature 280°, vaporizer 100°, aerosol 300°, filament off and positive ion mode. Spectra (150–900 amu) were recorded every 3 s. Aqueous buffer (0.5 M NH_4OAc) was added post-column (0.2 ml/min) to help ionization.

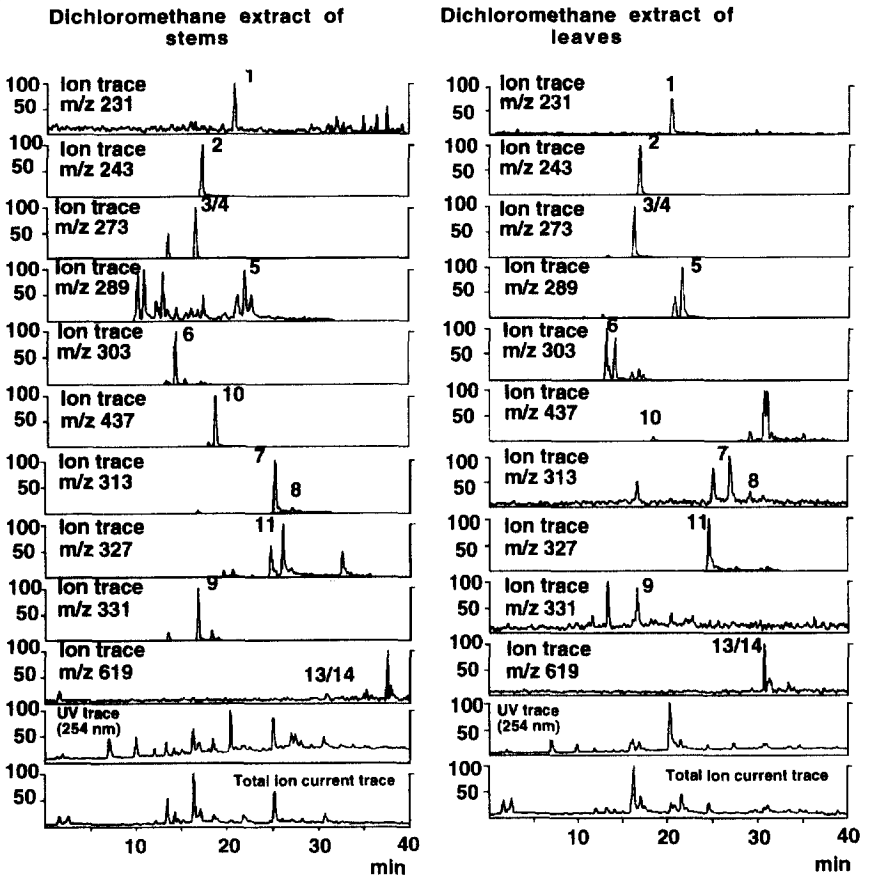
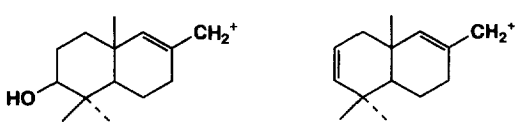


Fig. 2. LC-TSP-MS analyses of the dichloromethane extracts of the stems and leaves of *Kielmeyera coriacea*. Conditions: see Fig. 1.



(a) *m/z* 207 (b) *m/z* 189

Fig. 3. Major fragment ions in the EI mass spectrum of **13**/**14**.

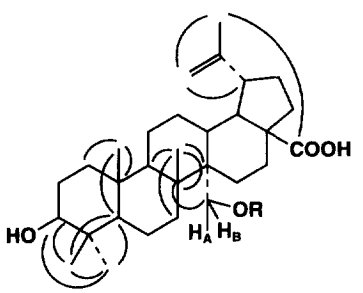


Fig. 4. Partial HMBC correlations of **13**/**14**.

Table 1. ^{13}C NMR spectral data of compounds **7**–**9** (50 MHz, $CDCl_3$/DMSO-d_6*)

C	7	8	9
1	160.18	159.94	159.45
2	110.23	110.25	110.61
3	163.23	163.49	162.70
4	93.36	93.36	92.93
4a	155.74	155.12	155.04
5	118.35	146.01	117.72
6	122.46	120.03	123.31
7	153.51	122.39	152.94
8	108.89	114.78	108.14
8a	120.96	121.11	120.24
9	180.37	180.28	179.69
9a	102.64	102.21	101.83
10a	149.61	144.92	148.90
11	21.29	21.07	16.92
12	123.90	123.35	42.06
13	131.40	130.59	69.83
14	25.78	25.63	29.14
15	17.84	17.71	29.14

* Addition of 1 drop of DMSO.

Table 2. ^{13}C NMR spectral data of compounds **12**–**14** and **12Ac**–**14Ac** [$CDCl_3$ or pyridine-d_5 (**12**)]

C	**13***	**14***	**13Ac†**	**14Ac†**	**12†**	**12Ac**[a]
1	39.01	39.01	38.49	38.49	39.28	38.57
2	24.18	24.18	23.66	23.66	26.02	23.73
3	78.88	78.88	80.67	80.67	78.20	80.79
4	37.48	37.48	37.81	37.81	39.28	37.89
5	55.33	55.33	55.50	55.50	56.22	55.77
6	18.20	18.20	18.10	18.10	18.99	18.20
7	35.16	35.16	35.13	35.13	35.27	35.17
8	41.51	41.51	41.55	41.55	42.15	41.62
9	51.70	51.70	51.77	51.77	50.65	51.72
10	37.45	37.45	37.37	37.37	37.88	37.50
11	20.93	20.93	20.94	20.94	21.71	21.05
12	25.14	25.14	25.08	25.08	27.80	25.19
13	39.14	39.14	38.67	38.67	39.58	39.37
14	45.45	45.45	45.50	45.50	47.00	45.30
15	29.68	29.68	29.69	29.69	28.40	30.57
16	32.54	32.54	31.78	31.78	33.80	32.61
17	56.05	56.05	57.40	57.40	57.40	56.21
18	49.49	49.49	49.31	49.31	50.65	49.54
19	46.85	46.85	46.41	46.41	48.19	46.97
20	150.05	150.05	149.48	149.48	152.73	149.93
21	30.42	30.42	30.04	30.04	30.00	30.57
22	36.71	36.71	35.68	35.68	36.32	36.66
23	27.90	27.90	27.89	27.89	28.60	27.96
24	15.43	15.43	16.64	16.64	16.41	16.60
25	16.63	16.63	16.57	16.57	16.95	16.60
26	16.55	16.55	16.37	16.37	17.34	16.60
27	63.33	63.33	63.06	63.06	60.21	63.49
28	181.06	181.06	182.15	182.15	178.20	182.24
29	109.94	109.94	110.36	110.36	109.22	110.03
30	19.50	19.50	19.44	19.44	19.72	19.49
1′	166.88	167.72	167.06	166.85		
2′	115.80	116.92	118.43			
3′	144.63	143.81	143.68	142.88		
1″	127.04	126.68	122.17	121.32		
2″, 6″	115.14	115.96	119.72			
3″, 5″	130.01	132.35	129.28	131.27		
4″	158.44	157.38				
OCOMe			22.34			21.05
			22.19	22.19		20.94
			22.32	22.32		
			22.16	22.16		
OCOMe			171.47			170.69
			170.92	170.92		170.65
			167.06	167.06		
			166.85	166.85		

* Assigned by DEPT (125.65 MHz), HMQC, HMBC.
† Assigned by DEPT (50 MHz).
[a] Values from Ref. [17].

Table 3. ^{1}H NMR spectral data of compounds **13** and **14** (assigned by ^{1}H–^{1}H COSY and HMBC) [500 MHz, $CDCl_3$], **13Ac** and **14Ac** [200 MHz, $CDCl_3$]

H	13	14	13Ac	14Ac
1α,β	1.64/2.36 *m*	1.64/2.36 *m*		
2α,β	1.49/1.61 *m*	1.49/1.61 *m*		
3	3.20 *dd* (11; 3.5)	3.20 *dd* (11; 3.5)	4.48 *m*	4.48 *m*
5	0.96 *m*	0.96 *m*		
6α,β	1.31/1.51 *m*	1.31/1.51 *m*		
7α,β	1.44/1.53 *m*	1.44/1.53 *m*		
9	1.23 *m*	1.23 *m*		
11α,β	2.40/1.42 *m*	2.40/1.42 *m*		
12α,β	1.36/1.85 *m*	1.36/1.85 *m*		
13	2.22 *m*	2.22 *m*		
15	1.64 *m*	1.64 *m*		
16α,β	1.25/2.31 *m*	1.25/2.31 *m*		
18	1.64 *m*	1.64 *m*		
19	3.01 *m*	3.01 *m*	3.00	3.00
21α,β	1.42/1.98 *m*	1.42/1.98 *m*		
22α,β	1.44/1.97 *m*	1.44/1.97 *m*		
23	0.93 *s*	0.93 *s*		
24	0.73 *s*	0.73 *s*		
25	0.83 *s*	0.83 *s*		
26	0.95 *s*	0.95 *s*		
27_A	4.43 *d* (12.5)	4.43 *d* (12.5)	4.48 *m*	4.48 *m*
27_B	4.35 *d* (12.5)	4.35 *d* (12.5)	4.48 *m*	4.48 *m*
29 E	4.74 *d* (6.5)	4.74 *d* (6.5)	4.73 *br s*	4.73 *br s*
29 Z	4.61 *m*	4.61 *m*	4.61 *br s*	4.61 *br s*
30	1.69 *s*	1.69 *s*	1.67 *s*	1.70 *s*
2′	6.29 *d* (16)	5.83 *d* (12.5)	6.39 *d* (16)	5.88 *d* (12.8)
3′	7.60 *d* (16)	6.86 *d* (12.5)	7.64 *d* (16)	6.93 *d* (12.8)
2″,6″	6.83 *d* (9)	6.85 *d* (8.5)	7.10 *d* (8.6)	7.13 *d* (8.4)
3″,5″	7.43 *d* (9)	7.66 *d* (8.5)	7.56 *d* (8.6)	7.70 *d* (8.4)
OCOMe			2.03 *s*	2.03 *s*
			2.23 *s*	2.23 *s*
			2.30 *s*	2.30 *s*

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

Table 4. Antifungal activities of compounds **1**, **2**, **3**, **7** and **11**

Compounds	Activity against *C. cucumerinum*[a]	Activity against *C. albicans*[a]
1	0.62	>50
2	1.50	>50
3	0.31	>50
7	6.25	10
11	6.25	10
Miconazole		0.001
Propiconazole	0.1	

[a] Minimum amount (μg) of compound needed to inhibit fungal growth on TLC plates.

1,3,7-*Trihydroxy*-2-(3-*methylbut*-2-*enyl*)-*xanthone* (**7**)

Yellow needles from MeOH: mp 217–218° (lit. [15] 218–220°). TLC (silica gel, $CHCl_3$–MeOH 20:1): R_f 0.28. HPTLC (RP-18, MeOH–H_2O 17:3): R_f 0.45. UV λ_{max} nm (log ε) (MeOH): 238 (17.57), 262 (16.08), 316 (8.08), 375 (3.00). ^{1}H NMR (200 MHz, Acetone-d_6): 13.26 (1H, *s* HO-1), 9.40 (2H, *br s*, HO-3 and HO-7), 7.58 (1H, *d*, J = 2.8 Hz, H-8), 7.42 (1H, *d*, J = 8.8 Hz, H-5), 7.32 (1H, *dd*, J = 9.0, 2.8 Hz, H-6), 6.49 (1H, *s*, H-4), 5.28 (1H, *t*, J = 7.4 Hz, H-12), 3.48 (1H, *d*, J = 7.4 Hz, H-11), 1.79 (3H, *s*, Me-15), 1.65 (3H, *s*, Me-14). ^{13}C NMR: Table 1. EIMs *m/z* (rel. int.): 312 M^+. (71), 297 (30), 270 (12), 269 (63), 258 (14), 257 (100).

1,3,5-*Trihydroxy*-2-(3-*methylbut*-2-*enyl*)-*xanthone* (**8**)

Yellow needles from MeOH: mp 279–280° (lit. [16] 280–281°). TLC (silica gel, $CHCl_3$–MeOH 20:1): R_f 0.18. HPTLC (RP-18, MeOH–H_2O 17:3): R_f 0.41. UV λ_{max} nm (log ε) (MeOH): 243 (6.82), 312 (3.22). ^{1}H NMR (200 MHz, $CDCl_3$/DMSO-d_6): 13.13 (1H, *s*, HO-1), 7.60 (1H, *dd*, J = 7.8, 1.6 Hz, H-8), 7.26 (1H, *dd*, J = 7.8, 1.6 Hz, H-6), 7.15 (1H, *t*, J = 7.8 Hz, H-7), 6.56 (1H, *s*, H-4), 5.23 (1H, *t*, J = 6.4 Hz, H-12), 3.29 (1H, *d*, J = 6.4 Hz, H-11), 1.77 (3H, *s*, Me-15), 1.66 (3H, *s*, Me-14). ^{13}C NMR: Table 1. EIMS *m/z*

(rel. int.): 312 M^+. (59), 297 (28), 269 (55), 258 (11), 257 (100).

1,3,7-*Trihydroxy*-2-(3-*hydroxy*-3-*methylbutyl*)-*xanthone* (**9**)

Yellow crystals from MeOH: mp 233–235°. TLC (silica gel, $CHCl_3$-MeOH 20:1): R_f 0.14. HPTLC (RP-18, MeOH-H_2O 17:3): R_f 0.70. IR ν_{max} (KBr) cm^{-1}: 3400, 1610, 1450, 1210. UV λ_{max} nm (log ε) (MeOH): 235 (6.88), 261 (6.07), 312 (3.09), 375 (1.16); +NaOMe: 244 (7.79), 273 (5.86), 348 (3.96); +NaOAc: 234 (8.86), 262 (4.86), 365 (2.45), unchanged on addition of H_3BO_3; +$AlCl_3$: 230 (6.10), 274 (4.84), 329 (3.13); +$AlCl_3$/H_3BO_3: 230 (5.04), 274 (3.91), 329 (2.72). ^1H NMR (200 MHz, Acetone-d_6): 13.26 (1H, *s*, HO-1), 9.00 (2H, *br s*, HO-3 and HO-7), 7.59 (1H, *d*, J = 3.0 Hz, H-8), 7.42 (1H, *d*, J = 8.6 Hz, H-5), 7.33 (1H, *dd*, J = 9.0, 2.8 Hz, H-6), 6.51 (1H, *s*, H-4), 2.78 (1H, *m*, H-12), 1.71 (1H, *m*, H-11), 1.26 (6H, Me-14 and Me-15). ^{13}C NMR: Table 1. EIMS *m/z* (rel. int.): 330 M^+. (96), 315 (20), 312 (61), 297 (67), 269 (59), 257 (100), 149 (44).

27-*O*-cis,trans-p-*coumaroylcylicodiscic acid* (**13**/**14**)

Yellow amorphous solid. TLC (silica gel, $CHCl_3$–MeOH 20:1): R_f 0.26. HPTLC (RP-18, MeOH-H_2O 17:3): R_f 0.40. IR ν_{max} (KBr) cm^{-1}: 3600–2600, 3350, 1700, 1600, 1450, 1380. UV λ_{max} nm (log ε) (MeOH): 215 (10.00), 312 (16.48). ^1H NMR: Table 3. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): 454 $[M-164]^+$. (61), 393 (42), 207 (48), 147 (100). D/CIMS (NH_3, positive ion mode) *m/z*: 636 $[M+NH_4]^+$, 455, 388, 256. Acetylation (Ac_2O–pyridine) yielded the diacetate **13Ac**/**14Ac**. Yellow amorphous solid. ^1H NMR: Table 3. ^{13}C NMR: Table 2. D/CIMS (NH_3 positive ion mode) *m/z*: 720 $[M+NH_4]^+$, 678, 510, 388.

Hydrolysis of compounds **13** *and* **14**

The mixture of compounds **13** and **14** (5 mg) was hydrolysed with 0.5 M NaOH under reflux for 6 hr. The aglycone (**12**, 1.5 mg) was extracted with $CHCl_3$. The remaining soln was acidified with 0.1 M HCl and extracted with $CHCl_3$ to obtain *cis*- and *trans*-*p*-coumaric acids. The compounds were identified by TLC comparison with authentic samples.

Acknowledgements—This study was supported in part by a grant from the Conselho Nacional de Pesquisas, Brazil and in part by the Swiss National Science Foundation.

REFERENCES

1. Guillemonat, A. and Triaca, M., *Bulletin de la Société Chimique de France*, 1968, **3**, 950.
2. Sundaram, B. M., Gopalakrishnan, C., Subramanian, S., Shankaranarayanan, D. and Kameswaran, L., *Planta Medica*, 1983, **49**, 60.
3. Mahabusarakam, W., Wiriyachitra, P. and Phongpaichit, S., *Journal of the Scientific Society of Thailand*, 1986, **12**, 239.
4. Pinto, M. M. M., Mesquita, A. A. L. and Gottlieb, O. R., *Phytochemistry*, 1987, **26**, 2045.
5. Lopes, J. L. C., Lopes, J. N. C., Gilbert, B. and Bonini, S. E., *Phytochemistry*, 1977, **16**, 1101.
6. Pimenta, A., Mesquita, A. A. L., Camey, M., Gottlieb, O. R. and Magalhães, M. T., *Anais da Academia Brasilevia de Ciências*, 1964, **36**, 283.
7. Gottlieb, O. R., Magalhães, M. T., Camey, M., Mesquita, A. A. L. and Correa, D. de B., *Tetrahedron*, 1966, **22**, 1777.
8. Homans, A. L. and Fuchs, A., *Journal of Chromatography*, 1970, **51**, 327.
9. Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M. and Frenk, E., *Phytochemical Analysis*, 1991, **2**, 199.
10. Malterud, K. E. and Dugstad, E. K., *Zeitschrift für Naturforschung*, 1985, **40B**, 53.
11. Kokubun, T., Harborne, J. B., Eagles, J. and Waterman, P. G., *Phytochemistry*, 1995, **40**, 57.
12. Gottlieb, O. R. and Stefani, G. M., *Phytochemistry*, 1970, **9**, 453.
13. Rivaille, P., Massicot, J., Guyot, M. and Plouvier, V., *Phytochemistry*, 1969, **8**, 1533.
14. Iinuma, M., Tosa, H., Toriyama, N., Tanaka, T., Ito, T. and Chelladurai, V., *Phytochemistry*, 1996, **43**, 681.
15. Harrison, L. J., Leong, L.-S., Sia, G.-I., Sim, K.-Y. and Tan, H. T.-W., *Phytochemistry*, 1993, **33**, 727.
16. Gunasekera, S. P., Jayathilaka, G. S., Selliah, S. and Sultanbawa, M. U. S., *Journal of the Chemical Society, Perkin Transactions I*, 1977, 1505.
17. Tchivounda, H. P., Koudogbo, B., Besace, Y. and Casadevall, E., *Phytochemistry*, 1990, **29**, 3255.