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MELIACIN BUTENOLIDES FROM *TRICHILIA ESTIPULATA*

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Key Word Index—*Trichilia estipulata*; Meliaceae; stem bark; leaves; meliacin butenolides; dammaranes; coumarins; sterols.

Abstract—The stem bark of *Trichilia estipulata* afforded three novel meliacin butenolides, 7α-23-dihydroxy-3-oxo-24,25,26,27-tetranorapotirucall-1,14,20(22)-trien-21,23-olide, 7-deacetyl-23-hydroxyneotrichilenonelide and 7-deacetyl-21-hydroxyneotrichilenonelide, which were identified on the basis of spectroscopic analyses. Scopoletin, isofraxidin, 7-oxo-24β-, 7-oxo-24α-sitosterols and 3β-*O*-β-D-glucopyranosylsitosterol were also isolated. The known compounds, velozonol, carnaubadiol, velozona, carnauba-21-ol-3-one, isofouqueriol, isofouquerinone, sitostenone and sitosterol were isolated from the leaves.

INTRODUCTION

As a part of our continuous investigation into the chemical composition of Brazilian Meliaceae, we have recently reported the isolation of aryltetralin lignan glycosides from *Trichilia estipulata* [1]. Further studies with this species have now led to the isolation of three new meliacin butenolides.

RESULTS AND DISCUSSION

A methanol-soluble fraction of the dichloromethane extract of the stem bark was purified by repeated column chromatography on silica gel to give 3β-*O*-β-D-glucopyranosylsitosterol and two mixtures of meliacin butenolides (**1–4**).

The components of the major mixture were identified as **1** and **2** on the basis of the following data. The ^{1}H NMR spectrum (Table 1) showed signals (δ 5.46 *m*, 1H, 6.29 *br s*, 1 H; 6.63–6.65 *m*, 2 H) for a γ-hydroxybutyrolactone. This was corroborated by the ^{13}C NMR spectrum (Table 2) which showed signals for two hemiacetal carbons (δ 98.5, C-21 and 96.3, C-23) and two α-β-unsaturated-γ-lactones (δ 137.1, C-20; 170.1, C-21; 145.8/146.0, C-22 and 166.6, C-20; 119.7, C-22; 170.8, C-23). These signals indicated the presence of 21-hydroxy- and 23-hydroxy-20(22)-ene-21,23-γ-lactones. Acetylation of **1** and **2** with acetic anhydride in pyridine gave acetates **1a** and **2a**, which could be separated by preparative HPLC. Butenolide **1a** showed all the spectral data (Tables 1 and 2) of 7α,21-diacetoxy-3-oxo-24,25,26,27-tetranorapotirucall-1,14,20(22)-trien-21,23-olide, which has previously been isolated from *Picrolemma granatensis* (Simaroubaceae) as the deacetyl compound but it was also transformed into **1a** [2]. The mass spectrum of **2a** indicated a molecular formula $C_{30}H_{38}O_7$ ($[M]^+$ *m/z* 510), which strongly suggested an isomer of **1a**. The principal change observed in the ^{1}H NMR of **2a** was the deshielded resonances δ 6.94 and 6.88 for a γ-hydroxybutyrolactone. This was also supported by the ^{13}C NMR spectrum (Table 2), which agreed closely with published data for a 23-γ-hydroxybutenolide moiety related to that of nimocinolide (**5**) [3]. The structure of the new natural product was thus established as 7α,23-dihydroxy-3-oxo-24,25,26,27-tetranorapotirucall-1,14,20(22)-trien-21,23-olide (**2**).

The second mixture of butenolides exhibited spectral data similar to **1** and **2**. The ^{13}C NMR spectrum (Table 2) showed signals at δ 217.4 and 220.1, characteristic of a ring D15-one, as in the model 7-acetylneotrichilenone (**6**) [4], instead of a ring D14-ene. This mixture was submitted to preparative TLC affording **3** and **4**, in addition to a small

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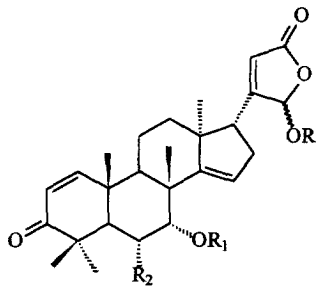
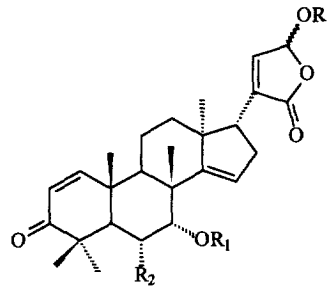
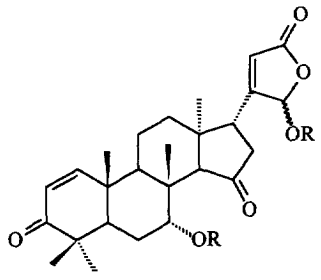


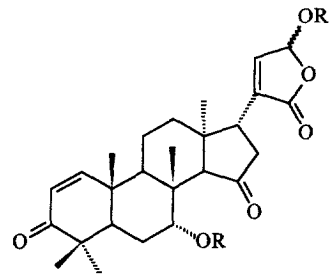
1: $R = R_1 = R_2 = H$
1a: $R = R_1 = Ac$, $R_2 = H$



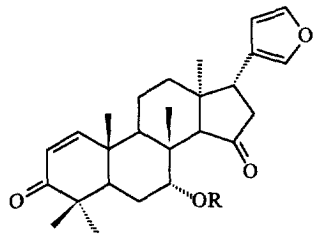
2: $R = R_1 = R_2 = H$
2a: $R = R_1 = Ac$, $R_2 = H$
5: $R = H$, $R_1 = Ac$, $R_2 = OH$



4: R = H
4a: R = Ac



3: R = H
3a R = Ac



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amount of **3**, that could not be separated. The ^1H and ^{13}C NMR spectra of **3** showed deshielded resonances (δ 146.0 C-22 and δ 6.94 H-22) for a 23-γ-hydroxybutenolide moiety. The new natural product is therefore 7-diacetyl-23-hydroxyneotrichilenonelide (**3**). The ^1H and ^{13}C NMR spectra of **4** with a trace of **3**, in addition to the signals described above for **3**, revealed significant upfield shifts for H-22 (δ 6.16) and C-22 (δ 120.1). From these data **4** was characterized as 7-deacetyl-21-hydroxyneotrichilenonelide. As a further confirmation of the assignments, the above mixture was acetylated and subsequently purified by preparative HPLC to yield two pure acetate derivatives, whose UV, IR, ^1H NMR and mass spectra were consistent with structures **3a** and **4a**. However, they were obtained in amounts insufficient for ^{13}C NMR.

From the medium polar fraction of the dichloromethane extract of the stem bark, two known coumarins, scopoletin [5] and isofraxidin [6], and a mixture of 7-oxo-24β- and 7-oxo-24α-sitosterols [7, 8] were obtained.

The hexane extract of the leaves afforded the dammarane triterpenoids, velozonol [9], carnaubadiol [10], velozona and carnauba-21-ol-3-one [11], and the sterols, sitostenone and sitosterol. From the dichloromethane extract, isofouqueriol [12] and isofouquerione [13] were obtained. The occurrence of meliacin butenolides is a feature of *Trichilia* [14].

EXPERIMENTAL

General

IR: KBr. ^1H and ^{13}C NMR: 200 and 50 MHz, respectively, with TMS as standard.

Plant material

Trichilia estipulata L. was collected in Cachoeiro do Itapemirim, ES, Brazil. A voucher is deposited

Table 1. ^{1}H NMR chemical shifts for compounds **1** (C_2D_6CO, 200 MHz), **1a**, **2a**, **3**, **4**, **3a** and **4a** ($CDCl_3$, 200 MHz)

H	1*	1a	2*	2a	3	4*	3[a]	4a
1	6.78 *d*(10)	7.09 *d*(10)	6.78 *d*(10)	7.13 *d*(10)	7.10 *d*(10)	7.60 *d*(10)	7.15 *d*(10)	7.12 *d*(10)
2	5.38 *d*(10)	5.84 *d*(10)	5.38 *d*(10)	5.38 *d*(10)	5.80 *d*(10)	5.78 *d*(10)	5.88 *d*(10)	5.89 *d*(10)
7	3.67 *m*	5.37 *m*	3.65 *m*	5.34 *m*	3.98 *m*	3.79 *m*	4.92 *m*	3.90 *m*
14							2.46 *s*	2.46 *s*
15	5.20 *m*	5.23 *m*	5.19 *m*	5.24 *m*				
17					3.66 *m*	3.54 *m*	3.60 *m*	3.51 *m*
21	5.46 *m*	6.85 *br s*				5.88 *m*		6.90 *br s*
22	6.62 *m*	5.99 *br s*	6.64 *m*	6.94 *m*	6.94 *br s*	6.16 *br s*	6.95 *br s*	6.13 *br s*
23			6.29 *br s*	6.88 *m*	6.15 *m*		6.94 *br s*	
7-Ac		1.94 *s*		1.94 *s*			2.10 *s*	2.10 *s*
21-Ac		2.15 *s*						2.20 *s*
23-Ac				2.14 *s*			2.24 *s*	
Me	0.80 *s*	1.19 *s*	0.80 *s*	1.18 *s*	1.15 *s*	1.15 *s*	0.89 *s*	0.89 *s*
	0.79 *s*	1.15 *s*	0.79 *s*	1.17 *s*	1.14 *s*	1.11 *s*	1.14 *s*	1.10 *s*
	0.72 *s*	1.05 *s*	0.72 *s*	1.05 *s*	1.07 *s*	1.07 *s*	1.05 *s*	1.05 *s*
	0.67 *s*	0.91 *s*	0.67 *s*	0.90 *s*	0.85 *s*	1.06 *s*	1.07 *s*	1.06 *s*
	0.62 *s*		0.62 *s*			0.84 *s*	1.17 *s*	1.12 *s*

Coupling constants (Hz) in parentheses.
* Obtained from mixture

in the Herbarium of Instituto de Biociências, USP, São Paulo.

Isolation of compounds

Ground stem bark (530 g) were extracted with hexane, then CH_2Cl_2 and, finally, with MeOH. The CH_2Cl_2 extract (7.9 g) was submitted to vacuum chromatography over silica gel using CH_2Cl_2, CH_2Cl_2–EtOAc (1:1), EtOAc and MeOH. The MeOH fr. was subjected to CC over silica gel. Elution with a CH_2Cl_2–Me_2CO gradient afforded two mixts. of limonoids and 3*β*-*O*-*β*-D-glucopyranosylsitosterol (30 mg). After spectroscopic analysis, the mixt. (28 mg) containing **1** and **2** was allowed to react overnight with excess Ac_2O in pyridine. Work-up as usual yielded the 7,21- and 7,23-diacetates, which were separated by prep. HPLC

Table 2. ^{13}C NMR chemical shifts for compounds **1** (C_2D_6CO, 50 Mhz), **2**, **1a**, **3** and **4** ($CDCl_3$, 50 MHz) and a model compounds **5** and **6**

C	1	2	1a	2a	5**	6	3	4*
1	158.3	157.7	157.5	158.0	157.4	157.8	158.2	158.6
2	125.6	124.5	125.7	125.5	126.3	125.9	125.5	125.8
3	204.9	203.9	205.1	204.5	206.0	204.4	205.2	205.4
4	45.1	44.2	44.1	44.1	40.5	44.1	44.2	45.2
5	44.4	43.7	38.3	38.3	50.7	45.2	45.0	45.8
6	24.3	26.2	24.2	23.4	68.2	22.3	24.2	25.8
7	71.6	70.8	74.3	74.4	78.9	73.3	71.6	69.6
8	44.2	43.4	46.1	46.1	45.5	41.1	41.1	42.2
9	41.0	42.0	46.1	47.5	37.1	47.1	47.0	46.2
10	40.1	39.5	39.8	39.9	43.4	39.6	40.1	39.9
11	16.2	15.6	16.4	16.4	16.4	17.6	16.2	17.6
12	32.9	33.0	39.8	39.2	32.7	34.5	34.0	35.1
13	47.6	46.7	47.6	47.5	47.5	42.1	42.2	42.3
14	160.6	159.3	158.2	158.2	157.2	61.3	61.5	62.4
15	119.7	118.2	118.8	118.3	119.5	218.6	217.4	220.1
16	33.3	33.2	33.3	33.9	34.0/34.1	43.2	44.2	43.7
17	53.7	52.0	52.8	50.6	50.0 51.0	38.0	36.7	37.1
18	15.0	15.5	13.6	19.0		27.9	27.7	27.9
19	18.9	19.3	19.6	20.7		19.3	19.1	19.2
20	166.6	137.1	166.7	138.4	137.5/137.6	122.9	137.7	166.7
21	98.5	170.1	93.3	171.0	169.5/169.1	140.3	170.2	99.2
22	119.7	145.8/146.0	120.2	143.3	145.6/145.8	110.8	146.0	120.1
23	170.8	96.3	169.0	92.3/92.4	96.8/96.9	143.0	96.5/96.2	171.7
28	20.9	20.6	20.6	21.4		27.4	27.1	27.3
29	27.6	26.7	27.1	27.5		21.1	21.5	21.3
30	26.3	26.9	27.0	27.0		18.2	18.9	18.5
-OCOCH3				170.0 169.0	171.9	169.3		
-OCO*CH3*				21.3 21.0	21.2	21.1		

Multiplicity obtained by DEPT or J-MOD experiments.
* Obtained from mixture
** Me = unassigned (27.2, 20.9, 20.3, 19.3 and 16.6).

(Hypersil silica 5 μm, 27 × 0.7 cm, with hexane–CH_2Cl_2–Me_3CN, 10:9:1) affording **1a** (2 mg) and **2a** (3.4 mg). The second mixt. of limonoids was purified by prep. TLC (silica gel, CH_2Cl_2–MeOH, 49:1) to yield 6 mg of pure **3** and a mixt. of **3** and **4**. This mixt. was acetylated as described above yielding, after final purification by prep. HPLC as described above, acetates **3a** (0.5 mg) and **4a** (0.5 mg).

The concd CH_2Cl_2-sol. fr. was subjected to CC over silica gel, eluting with a hexane–EtOAc–MeOH gradient, affording scopoletin (8 mg), isofraxidin (3 mg) and a mixt. of 7-oxo-24β- and 7-oxo-24α-sitosterol (21 mg).

Ground leaves (648 g) were extracted in the same way as stem bark. The hexane extract was chromatographed on silica gel (hexane–CH_2Cl_2–EtOAc–MeOH gradient) yielding sitostenone (90 mg), sitosterol (20 mg) and two frs. Fr. 1 (37 mg) was a mixt. of velozonol and carnaubadiol. Fr. 2 (21 mg) was a mixt. of velozona and carnauba-21-ol-3-ona. These triterpenoids were identified in mixt. by comparison with published data. The CH_2Cl_2 extract was chromatographed as described above to give isofouquerriol and isofouquerione.

*7α,21-Diacetoxy-3-oxo-24,25,26,27-tetranorapotirucall-1,14,20(22)-trien-21,23-olide (**1a**)*

Viscous oil. $[\alpha]_D$ +56° (25°, $CHCl_3$; *c* 0.017). UV [$CHCl_3$ λ_{max} nm (ϵ)]: 251 (2792). IR ν_{max} film KBr cm^{-1}: 2933, 1763, 1740, 1663, 1455, 1030, 880. ^1H NMR ($CDCl_3$, 200 MHz): Table 1. ^{13}C NMR ($CDCl_3$, 50 MHz): Table 2. MS *m/z* (rel. int.): 450 $[M-60]^+$ (32), 391(9), 390(25), 375(16), 135(15), 120(6), 93(19), 92(2), 91(17), 69(15), 43(100).

*7α,23-Diacetoxy-3-oxo-24,25,26,27-tetranorapotirucall-1,14,20(22)-trien-21,23-olide (**2a**)*

Viscous oil. $[\alpha]_D$ +30° (25°, $CHCl_3$; *c* 0.010). UV [$CHCl_3$ λ_{max} nm (ϵ)]: 245(2448). IR ν_{max} film KBr cm^{-1}. 2928, 1770, 1730, 1664, 820. ^1H NMR ($CDCl_3$, 200 MHz): Table 1. ^{13}C NMR ($CDCl_3$, 50 MHz): Table 2. MS *m/z* (rel. int.): 510 $[M]^+$ (1), 450(3), 331(1), 301(12), 241(15), 149(17), 136(3), 121(6), 93(8), 92(2), 91(10), 43(100).

*7-Deacetyl-23-hydroxyneotrichilenonelide (**3**)*

Viscous oil. IR ν_{max} film KBr cm^{-1}: 3427, 2931, 1734, 1658, 1127, 1036. ^1H NMR ($CDCl_3$, 200 MHz): Table 1. ^{13}C NMR ($CDCl_3$, 50 MHz): Table 2. MS *m/z* (rel. int.): 424 $[M-18]^+$ (1), 259(16), 184(32), 156(13), 149(36), 136(24), 120(29), 105(30), 95(19), 55(64), 43(100).

*23-Acetoxyneotrichilenonelide (**3a**)*

Viscous oil. $[\alpha]_D$ −9.0° ($CHCl_3$; *c* 0.0033). UV [$CHCl_3$ λ_{max} nm (ϵ)]: 251(2105). IR ν_{max} film KBr cm^{-1}: 2924, 1740, 1734, 1658, 1559, 1460, 1227, 1021. ^1H NMR ($CDCl_3$, 200 MHz): Table 1. MS *m/z* (rel. int.): 526 $[M]^+$ (2), 483(14), 330(12), 261(15), 202(12),156(35), 154(34), 148(100).

*21-Acetoxyneotrichilenonelide (**4a**)*

Viscous oil. $[\alpha]_D$ −6.0° ($CHCl_3$; *c* 0.0033). UV [$CHCl_3$ λ_{max} nm (ϵ)]: 251(3684). IR ν_{max} film KBr cm^{-1}: 2927, 1744, 1671, 1558, 1459, 1208, 1033. ^1H NMR ($CDCl_3$, 200 MHz): Table 1. MS *m/z* (rel. int.): 526 $[M]^+$ (2), 484(4), 483(14), 466(9), 467(5), 451(1), 424(1), 407(3), 406(6), 391(2), 330(13), 271(2), 137(32), 148(100).

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