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DITERPENES FROM *GUAREA TRICHILIOIDES*

MAYSA FURLAN,* MÁRCIA NASSER LOPES, JOÃO BATISTA FERNANDES† and JOSÉ RUBENS PIRANI‡

Instituto de Química, Universidade Estadual Paulista, CP. 355, 14800-900, Araraquara-SP, Brazil; † Departamento de Química, Universidade Federal de São Carlos, CP. 676, 13565-905, São Carlos-SP, Brazil; ‡ Instituto de Biociências, Departamento de Botânica, Universidade de São Paulo, Brazil

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Key Word Index—*Guarea trichilioides*; Meliaceae; diterpenoids; labdanes; clerodanes.

Abstract—Chemical investigation of the leaves of *Guarea trichilioides* afforded six diterpenoids, including four labdane and two clerodane derivatives. The following four are new: 3-oxolabd-8(17),12*Z*,14-triene, 3α-hydroxylabd-8(17),12*Z*,14-triene, 3β-hydroxylabd-8(17),12*Z*,14-triene and (−)-2-oxo-13-hydroxy,3,14-clerodandiene.

INTRODUCTION

Chemical studies of the Meliaceae have shown this family to be a rich source of tetranortriterpenoids [1]. In our earlier work on chemical constituents of *Guarea trichilioides*, we isolated nine cycloartane derivatives and one tetranortriterpenoid [2, 3]. As part of our continuing chemical investigations on this family, six diterpenoids **1–4** were isolated, four of which are new. The structural assignments, based on physical and spectral data analysis, are presented and discussed.

RESULTS AND DISCUSSION

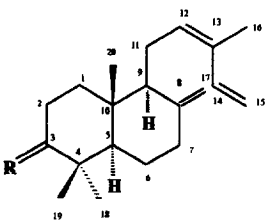
The crude petrol ether extract from the leaves of the *G. trichilioides* afforded (following chromatographic purification), the compounds **1a–4**. Based on spectroscopic properties, the compounds **1a–c** were shown to possess a labd-8(17),12*Z*,14-triene skeleton. However, these compounds differed by their substituent patterns at C-3. Structures were determined as follows.

Compound **1a** was shown to have a molecular formula of $C_{20}H_{32}O$ by analysis of mass spectral and ^{13}C NMR data. The presence of a conjugated double bond side chain was indicated by the ^1H broad triplet at δ5.27 ($J_{12,11}$ = 6.0 Hz) assigned to H-12, by the doublet of doublets at δ6.77 ($J_{14,15a}$ = 10.9, $J_{14,15b}$ = 17.9 Hz) assigned to the olefinic proton H-14 and by two doublets at δ5.07 ($J_{15a,14}$ = 10.9 Hz) and 5.16 ($J_{15b,14}$ = 17.9 Hz) assigned to the terminal methylenic protons of C-15. Carbon signals (δ19.7, 113.3 and 131.5) assigned to C-16, C-15 and C-14, respectively, showed the presence of a *cis* diene moiety [4]. The exocyclic methylenic protons of C-17 were observed by a characteristic pair of broad singlets at δ4.45 and 4.84. Carbon signals at δ147.9 and 108.0, assigned to C-8 and C-17, confirmed this proposition. The ^1H and ^{13}C spectrum of **1b** (Table 1) differed from that of **1a** by the configuration at C-3. That **1a** and **1b** each contained one hydroxyl moiety was determined by the doublet of doublets at δ3.25 assigned to H-3α ($J_{3\alpha,2\alpha}$ = 4.5 and $J_{3\alpha,2\beta}$ = 11.3 Hz) for **1a** and by the multiplet at δ3.39 assigned to H-3β, for **1b**. The carbon signals at δ78.8 and 76.0 were assigned to C-3 in **1a** and **1b**, respectively. The stereochemistry at C-3 was supported by the γ-effect of the axial OH group at the C-1 and C-5 in the ^{13}CNMR data (Table 1) [5, 6]. The fragmentation at *m/z* 270, 255 and 189 corroborated their epimeric relationship.

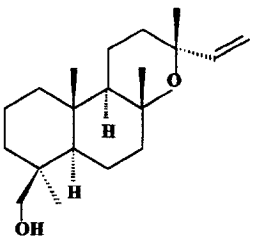
Compound **1c** was determined to have a molecular formula of $C_{20}H_{30}O$ by the analysis of the mass spectral data. The IR band at 1700 cm^{-1} indicated a carbonyl absorption. The absence of a proton signal between δ3.0 and 4.0 and the presence of a multiplet at δ2.60, attributed to H-2, indicated the presence of a ketone group at C-3. The carbon signal at δ216.0 and the indirect evidence based on the carbon signals belongs to C-2, C-4 and C-5, as well as the comparison by the literature data [6–9], reinforce this attribution. The fragmentation pattern is compatible with 3-oxo-labd-8(17),12*Z*,14-triene by the fragment ions at *m/z* 271 $[M - Me]^+$, 190 and 189 $[M - \text{side chain}]^+$.

The spectral properties of **2** allowed its identification as 19-hydroxymanoyloxide [10]. The analysis of ^1HNMR spectra of **3** and **4** revealed the features of clerodane type diterpenoids. The correlation of **3** and **4** with clerodane derivatives is due to the presence of three angular methyl groups and one methyl group as a doublet at δ0.93. The spectral and physical data of **3** have already been described in the literature [11]. Compounds **3** and **4** possessed the same side chain. This was evidenced by the presence of the signals at δ5.04 (1H, *dd*, J_{cis} = 10.0 and 1.3 Hz, H-15), 5.20 (1H, *dd*,

*Author to whom correspondence should be addressed.



	R
1a	β OH,α H
1b	α OH,β H
1c	O



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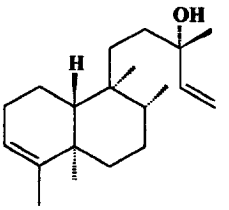

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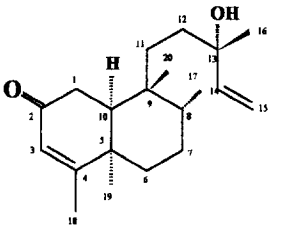

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Table 1. ^{13}C NMR spectral data (δ) for compounds **1a**, **1b**, **1c** and **4** (in $CDCl_3$, 50 MHZ)

C	1a	1b	1c	4
1	37.9* (*t*)	31.8 (*t*)	37.5* (*t*)	35.4 (*t*)
2	27.9 (*t*)	25.9 (*t*)	34.5 (*t*)	199.3 (*s*)
3	78.8 (*d*)	76.0 (*d*)	216.0 (*s*)	128.5 (*d*)
4	39.1 (*s*)	37.8 (*s*)	47.6 (*s*)	168.9 (*s*)
5	54.5 (*d*)	46.4 (*d*)	55.0 (*d*)	39.5 (*s*)
6	23.6 (*t*)	23.8 (*t*)	24.7 (*t*)	26.5 (*t*)
7	37.2* (*t*)	37.0 (*t*)	37.4* (*t*)	26.5 (*t*)
8	147.9 (*s*)	146.2 (*s*)	148.9 (*s*)	35.1 (*d*)
9	57.0 (*d*)	57.0 (*d*)	56.1 (*d*)	38.5 (*s*)
10	39.3 (*s*)	39.3 (*s*)	39.0 (*s*)	46.9 (*d*)
11	22.3 (*t*)	22.3 (*t*)	22.3 (*t*)	31.7 (*t*)
12	133.6 (*d*)	133.7 (*d*)	133.5 (*d*)	34.9 (*t*)
13	131.7 (*s*)	131.7 (*s*)	131.8 (*s*)	73.3 (*s*)
14	131.5 (*d*)	131.6 (*d*)	130.8 (*d*)	145.1 (*d*)
15	113.3 (*t*)	113.2 (*t*)	113.4 (*t*)	111.9 (*t*)
16	19.7 (*q*)	19.8 (*q*)	19.6 (*q*)	27.6 (*q*)
17	108.0 (*s*)	107.8 (*s*)	106.7 (*s*)	14.4 (*q*)
18	28.6 (*q*)	28.6 (*q*)	25.8 (*q*)	20.9 (*q*)
19	15.4 (*q*)	22.1 (*q*)	21.6 (*q*)	31.1 (*q*)
20	14.5 (*q*)	14.4 (*q*)	13.9 (*q*)	22.6 (*q*)

*May be interchanged.

Multiplicity in the DEPT spectrum is given after the respective chemical shifts: *s* (singlet), *d* (doublet), *t* (triplet) and *q* (quartet).

J_{trans} = 17.0 and 1.3 Hz, H-15) and 5.90 (1H, *dd*, J_{cis} = 10.0 and J_{trans} = 17.0, H-14). The ^{13}C NMR spectra (Table 1) showed signals at δ145.1 and 111.9 reinforcing this attribution. The presence of the hydroxyl group at C-13 was confirmed by the signal at δ73.3. The signals at δ1.25 in the ^{1}H NMR and δ27.6 in the ^{13}C NMR spectra can be assigned to the methyl group (C-16).

The IR (1.670 and 1.610 cm^{-1}) spectra of **4** revealed the presence of a β-substituted enone system, which was corroborated by the signals at δ5.88 (1H, under H-14 signals, H-3) and 1.95 (3H, *d*, J = 1.2 Hz, H-18) in the ^{1}H NMR spectrum. These data together with ^{13}C NMR signals at δ35.4, 199.3, 128.5 and 168.9 indicated that **4** had an enone system in the A-ring. The low resolution MS showed a molecular ion at *m/z* 304 and peaks at *m/z* 205, 124 and 109 confirming this proposal. The AB-ring junction was demonstrated to be *cis* by the chemical shift of the angular methyl (C-19) at δ31.1 [12, 13, 14].

EXPERIMENTAL

Instrumentation and chromatography materials. Silica gel (Merck 230–400 mesh) was used for all column chromatography unless otherwise stated and solvents were redistilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded at 200 MHz and 50 MHz, respectively, using $CDCl_3$ as a solvent.

Plant material. *G. trichilioides* leaves were collected in Cabo Frio, Rio de Janeiro, with a voucher deposited in the IB-USP Herbarium, São Paulo-SP, Brazil.

Extraction and isolation of constituents. The dried and powdered leaves of *G. trichilioides* (1.0 kg) were extracted with hexane (4.0 l) and CH_2Cl_2 (6.0 l) . The resulting green hexane and CH_2Cl_2 extract was filtered and concd in vacuo to afford 1.0 g and 2.0 g of a green gum, respectively. The hexane residue was applied to a silica gel column (300 g), 70–230 mesh, and eluted with hexane containing increasing amounts of EtOAc [hexane (1.0 l), hexane–EtOAc (98:2, 2.0 l; 95:5, 2.0 l; 9:1, 2.0 l; 1:1, 1.0 l and EtOAc (1.0 l)] to give 125 fractions (100 ml). The fractions (32–35) containing **1c** were combined and evaporated *in vacuo* to give 13.2 mg of material. This material was concentrated to a minimum amount of $CHCl_3$ and further purified by preparative TLC, developed with C_6H_6–EtOAc (9:1) to give pure **1c** (6.7 mg). Fractions (40–44) containing **1a** and **1b** were combined to give 72.0 mg of material. This material, following the same methodology below, was purified by preparative TLC, developed with C_6H_6–EtOAc (8:2) to give 56 mg of a mixture of **1a** and **1b**. The separation of **1a** and **1b** was performed on chromatographic silica gel (Merck 60H, 5–40 mm, 60 g) eluted with C_6H_6–EtOAc (9:1, 1.5 l), to give pure **1a** (8.3 mg) and **1b** (34 mg).

The CH_2Cl_2 extract was applied to a silica gel column (100 g, 1 × 15 cm) and eluted with CH_2Cl_2 containing increasing amounts of EtOAc [CH_2Cl_2 (2 l), CH_2Cl_2–EtOAc (99:1, 2 l, 98:2, 2 l; 95:5, 1 l; 1:1, 1.0 l)] to give 80 fractions of 100 ml. Fraction 5 (750 mg) was applied to a chromatographic silica gel column (Merck 60, 100 g) eluted with hexane and increasing amounts of diethyl ether [hexane (0.5 l), hexane–ether 99:1, 0.5 l; 98:2, 0.5 l; 95:5, 0.5 l; 99:1, 0.5 l; 9:1, 0.5 l; 1:1, 0.5 l] to give 100 fractions of 20 ml. Fractions 13–15 (24.3 mg) were combined and purified by preparative TLC, developed with $CHCl_3$–MeOH, 98:2, using 1% of HOAc to give pure **4** (10 mg).

3β-*Hydroxy-labd*-8(17),12*Z*,14-*triene* (**1a**). Oil, $[\alpha]_D^{25}$ + 13.5 ($CHCl_3$; *c* 1.04): IR ν_{max}^{film} cm^{-1}: 3500, 1640, 890; MS *m/z* (rel. int.): 288 $[M]^+$ (7), 270 $[M - H_2O]^+$. (39), 255 $[270 - CH_3]^+$ (10), 189 $[270 -$ side chain$]^+$ (73), 94 (100), ^1H NMR: δ 0.73 (3H, *s*, H-20); 0.76 (3H, *s*, H-18); 1.00 (3H, *s*, H-19); 1.78 (3H, *d*, *J* = 1.0 Hz, H-16); 3.25 (1H, *dd*, $J_{3\alpha,2\alpha}$ = 4.5 and $J_{2\alpha,3\beta}$ = 11.3 Hz); 4.45 and 4.84 (2H, *br s*, H-17a and H-17b, respectively); 5.07 (1H, *d*, $J_{15a,14}$ = 10.9 Hz, H-15a) and 5.16 (1H, *d*, $J_{15b,14}$ = 17.9 Hz, H-15b); 5.27 (1H, *br t*, $J_{12,11}$ = 6.0 Hz, H-12); 6.77 (1H, *dd*, $J_{14,15a}$ = 10.9 and $J_{14,15b}$ = 17.9 Hz, H-14).

3α-*Hydroxy-labd*-8(17),12*Z*,14-*triene* (**1b**). Oil, $[\alpha]_D^{25}$ + 16.1 ($CHCl_3$; *c* 0.56): IR ν_{max}^{film} cm^{-1}; 3500, 1640, 890; MS *m/z* (rel. int.): 288 $[M]^+$ (26.8), 270 $[M - H_2O]^+$ (88.8), 255 $[270 -$ Me$]^+$ (82.4), 189 $[270 -$ side chain$]^+$ (17.9), 175 (100), ^1H NMR: δ 0.79 (3H, *s*, H-20); 0.85 (3H, *s*, H-18); 0.79 (3H, *s*, H-19); 1.74 (3H, *d*, *J* = 1.0 Hz, H-16); 3.39 (1H, *m*, H-3β); 4.45 and 4.80 (2H, *br s*, H-17a and H-17b, respectively); 5.03 (1H, *d*, $J_{15a,14}$ = 10.8 Hz, H-15a) and 5.10 (1H, *d*, $J_{15b,14}$ = 17.0 Hz, H-15b); 5.26 (1H, *br t*, *J* = 6.0 Hz, H-12); 6.80 (1H, *dd*, $J_{14,15b}$ = 17.0 and $J_{14,15a}$ = 10.8 Hz, H-14).

3-*Oxo-labd*-8(17),12*Z*,14-*triene* (**1c**). Oil, $[\alpha]_D^{25}$ + 7.4 ($CHCl_3$; *c* 2.03): IR ν_{max}^{film} cm^{-1}: 1700, 1640, 890; MS *m/z* (rel. int.): 286 $[M]^+$ (7), 271 $[M -$ Me$]^+$ (7), 205 (8), 190 (5), 189 (11), 95 (100), ^1H NMR: δ 0.81 (3H, *s*, H-20); 0.96 (3H, *s*, H-19); 1.03 (3H, *s*, H-18); 1.70 (3H, *d*, *J* = 1.0 Hz, H-16); 2.60 (2H, *m*, H-2); 4.50 and 4.84 (2H, *br s*, H-17a and H-17b, respectively); 5.00 (1H, *d*, $J_{15a,14}$ = 10.6 Hz, H-15a) and 5.16 (1H, *d*, $J_{15b,14}$ = 17.0 Hz, H-15b); 5.20 (1H, *br t*, *J* = 6.0 Hz, H-12); 6.70 (1H, *dd*, $J_{14,15a}$ = 10.6 and $J_{14,15b}$ = 17.0 Hz, H-14).

(−)-2-*Oxo*-13-*hydroxy*-3,14-*clerodandiene* (**4**). Oil, $[\alpha]_D^{25}$ − 38.9 ($CHCl_3$; *c* 0.54): IR ν_{max}^{film} cm^{-1}: 3595, 1670, 1610, 1480; MS *m/z* (rel. int.) : 304 $[M]^+$ (5), 286 $[M - H_2O]^+$ (6), 205 $[M -$ side chain$]^+$ (22), 85 (100), ^1H NMR: δ 0.81 (3H, *s*, H-20); 0.93 (3H, *d*, *J* = 6.0 Hz, H-17); 1.25 (3H, *s*, H-16); 1.28 (3H, *s*, H-19); 1.95 (3H, *d*, *J* = 1.2 Hz, H-18); 5.04 (1H, *dd*, $J_{15a,14}$ = 10.0 and 1.3 Hz, H-15a); 5.20 (1H, *dd*, $J_{15b,14}$ = 17.0 and 1.3 Hz, H-15b); 5.88 (1H, under H-14 signals, H-3); 5.90 (1H, *dd*, $J_{14,15a}$ = 10.0 and $J_{14,15b}$ = 17.0 Hz, H-14).

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