ent-Trachylobane Diterpenoids from *Xylopia langsdorffiana*

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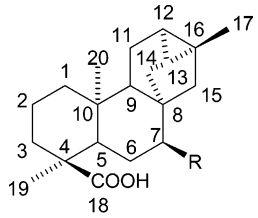
Received December 1, 2005

Two new diterpenes of the *ent*-trachylobane type were isolated from the stems of *Xylopia langsdorffiana*, *ent*-7α-acetoxytrachyloban-18-oic acid (**1**) and *ent*-7α-hydroxytrachyloban-18-oic acid (**2**). The structures of these isolates were deduced by spectroscopic data interpretation. X-ray crystallography of **1** was used to confirm its structure. The cytotoxic activity of **1** against V79 fibroblasts and rat hepatocytes was investigated.

The genus *Xylopia* (Annonaceae) comprises about 160 species.[1] Various species of this genus are used in folk medicine for rheumatism[2] and as antimicrobial agents.[3] *ent*-Kaurane and *ent*-trachylobane diterpenes are encountered in this genus,[4,5] with the latter group of compounds being rare and little-studied biologically thus far.[6−8] *Xylopia langsdorffiana* St-Hil. & Tul. is a tree, 5−7 m high and popularly known in Northeast Brazil as "pimenteira da terra".[3] Vasorelaxant and hypotensive activities were reported recently for 8(17),12*E*-labdatrien-18-oic acid, a labdane-type diterpene isolated from this plant.[9] As part of our continuing investigations of new bioactive molecules from plants of Brazil, we report herein the isolation and structure elucidation of two new *ent*-trachylobanes, **1** and **2**, from *X. langsdorffiana* stems as well as their cytotoxicity assay against V79 fibroblasts and hepatocytes.



1 R = $OCOCH_3$
2 R = OH

Compound **1** was obtained as a crystalline solid, mp 230−233 °C, $[\alpha]^{26}_D$ +25 (*c* 1.01, $CHCl_3$). The HREIMS of **1** showed a molecular ion peak at *m*/*z* 360.2309, compatible with the molecular formula, $C_{22}H_{32}O_4$, having 7 degrees of unsaturation in the molecule. The IR spectrum showed absorptions at 3200, 1700, and 1725 cm^{-1}, characteristic of COOH and ester groups. The presence of two CO groups was supported by the ^{13}C NMR spectrum, which shows signals at δ_C 185.1 and 171.4 ppm. The latter was assigned to a $OCOCH_3$ group as a result of the appearance of a typically deshielded CH_3 group at δ_H 2.04 (s, 3H) ppm in the ^1H NMR spectrum, which exhibited a characteristic cross-peak with the CO signal at δ_C 171.4 in the HMBC spectrum. With two CO groups accounting for 2 degrees of unsaturation and in the absence of any other signals at sp^2 regions of the NMR spectra, the possibility of a trachylobane ring system with 5 degrees of unsaturation was considered. Evidence for a cyclopropane ring was provided by the ^1H and ^{13}C NMR spectroscopic correlations at δ_H/δ_C 0.64/20.7 and 0.90/24.2 (H/C-12 and H/C-13, respectively).[10] Furthermore, by comparison of the NMR data of **1** with those of similar structures,[11] we concluded that it belongs to the *ent*-trachylobane diterpene compound class.

The carbonyl signal of the COOH group at δ_C 185.1 showed cross-peaks with resonances at δ_H 2.18 (H-5) and 1.15 (CH_3-19) in the HMBC spectrum, suggesting the position of this carboxylic acid unit at C-4. The location of the $OCOCH_3$ group at C-7 was also deduced from the HMBC correlations between the protons at δ_H 2.18 (H-5), 1.60 (H-9), 1.45 (H-14), and 1.35 (H-15) and the oxygenated carbon at δ_C 78.9.

The ^{13}C NMR spectroscopic data comparison of compound **1** with *ent*-7β-acetoxytrachyloban-18-oic acid[12] shows differences in the chemical shifts of C-5, C-6, C-9, C-15, and C-19. The chemical shift of the C-19 methyl group at δ_C 16.4 was compatible with the *ent*-trachyloban-18-oic acid[11,13] structure of compound **1**. Similarly, ^{13}C NMR chemical shifts at δ_C 27.8 (C-6) and 45.5 (C-15) ppm of compound **1** were consistent with those of 7α-hydroxytrachyloban-19-oic acid.[14] Moreover, long-range correlations in the HMBC spectrum between the H-7 signal at δ_H 4.65 ppm and the methine carbons at δ_C 42.8 and 48.9, along with the cross-peaks between H-12 and H-14 protons at δ_H 0.64 and 1.45, respectively, with the carbon at δ_C 48.9, confirmed unambiguously the assignments of the signals at δ_C 42.8 and 48.9 ppm to C-5 and C-9, respectively. The one- and two-dimensional ^1H /^{13}C NMR data are shown in Table 1. On the basis of the above evidence, the relative stereochemistry of the carbons at C-4 and C-7 is established. Therefore, the structure of compound **1**, a new diterpene, was proposed as *ent*-7α-acetoxytrachyloban-18-oic acid. This was confirmed from a single-crystal X-ray diffraction study on this isolate. Compound **2** was also obtained as a crystalline solid with mp 130−134 °C, $[\alpha]^{26}_D$ +16 (*c* 1.01 $CHCl_3$). The HREIMS of **2** showed a molecular ion peak at *m*/*z* 318.2129, compatible with the molecular formula, $C_{20}H_{30}O_3$, with 6 degrees of unsaturation. The IR spectrum showed absorptions at 3500, 3150, and 1695 cm^{-1}, indicating the presence of OH and COOH groups. The presence of only one CO group and the absence of any other sp^2 carbon in the molecule (NMR) along with signals at δ_H/δ_C 0.64/20.7 and 1.11/24.1 (H/C-12 and H/C-13, respectively), consistent with the presence of a cyclopropane ring, again suggested an *ent*-trachylobane skeleton. The

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10.1021/np050503o CCC: $33.50
Published on Web 05/28/2006

Table 1. NMR Data for Compounds **1** and **2**[a]

	1		**2**		HMBC		COSY		NOESY	
position	δ_C	δ_H	δ_C	δ_H	**1**	**2**	**1**	**2**	**1**	**2**
1	38.6	0.90 (m), 1.55 (m)	38.6	0.93 (m), 1.55 (m)		Me-20	H-1	H-1		
2	17.6	1.45 (m), 1.55 (m)	17.5	1.52 (m), 1.62 (m)						
3	37.2	1.65 (m), 1.70 (m)	37.0	1.65 (m), 1.75 (m)	H-5, Me-18					
4	46.9		45.7							
5	42.8	2.18 (d, J = 4.5 Hz)	42.1	2.23 (d, 4.6 Hz)	H-7, Me-20, Me-18	H-7, Me-20	H-6	H-6		
6	27.8	1.40 (m), 1.60 (m)	30.8	1.36 (m), 1.76 (m)	H-5	H-7, H-5				H-7
7	78.9	4.65 (b t, J = 4.0 Hz)	76.0	3.60 (br t, J = 4.1 Hz)	H-5, H-14, H-6	H-5, H-14	H-6	H-6		H-6
8	44.5		44.5							
9	48.9	1.60 (m)	48.0	1.52 (m)	H-7, Me-20, H-14			H-7, H-12		
10	37.9		37.9	-	H-11					
11	19.5	1.70 (m), 1.90 (m)	19.5	1.69 (m), 1.64 (m)						
12	20.7	0.64 (m)	20.7	0.64 (m)	Me-17	Me-17	H-13	H-13		
13	24.2	0.90 (m)	24.1	1.11 (m)	H-15, Me-17	H-14, Me-17	H-12	H-12		
14	32.8	1.45 (d, J = 4.8 Hz), 1.95 (m)	33.1	1.39 (d, J = 4.9 Hz), 1.99 (m)	H-7	H-7			H-14	H-14
15	45.5	1.35 (m), 1.55 (m)	45.5	1.32 (m), 1.49 (m)	H-7, H-14	H-14			H-7	H-7
16	23.3		23.3		H-14, H-11	H-11, H-14				
17	20.7	1.18 (s)	20.9	1.20 (s)	H-12, H-13				H-12	H-12
18	185.1		184.3		H-5, Me-18					
19	16.4	1.15 (s)	16.6	1.23 (s)	H-5					
20	15.2	1.01 (s)	15.3	1.02 (s)	H-5	H-5			H-1	
*CO*Me	171.4									
CO*Me*	21.6	2.06 (s)							H-15	H-15

[a]In $CDCl_3$, at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR.

presence of a secondary OH group is supported by the ^1H /^{13}C NMR signals at δ_H/δ_C 3.60/76.0. The locations of the polar groups at C-4 and C-7 in compound **2** were determined from HMBC correlations as in **1** (vide supra). Moreover, the C-5 and C-9 chemical shifts in **2** were virtually identical to those of compound **1**. All the other ^{13}C NMR chemical shifts were practically identical to those of 7α-hydroxytrachyloban-19β-oic acid.[14] However, the chemical shift of the CH_3 at C-4 (Table 1) was significantly different and consistent with the inverted stereochemistry in this center of compound **2**. Thus, the structure of **2** was assigned as *ent*-7α-hydroxytrachyloban-18-oic acid, a new natural product that is an epimer of an analogue reported by Ngouela and associates.[14]

The in vitro cytotoxicity of **1** was tested against a permanent lung fibroblast cell line derived from Chinese hamsters (V79) and rat hepatocytes, using the MTT method,[15] and gave IC_{50} values of 224 and 231 μM, respectively. In a previous investigation, compound **1** exhibited an IC_{50} value of 200 μM against the K562 human leukemia cell line.[16]

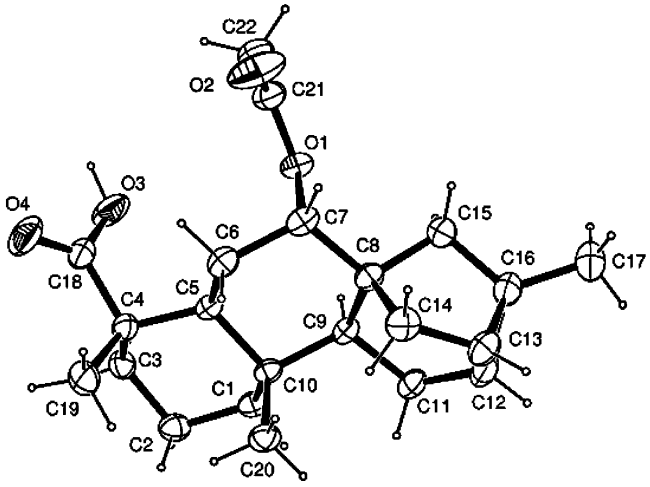

Figure 1. ORTEP diagram (50% probability ellipsoids) showing crystallographic atom numbering and solid-state conformation for compound **1**.

Experimental Section

General Experimental Procedures. Melting points were measured in a Geahaka model PF1500 version 1.0 apparatus and are uncorrected. Optical rotations were determined on a ADP 220 polarimeter, Bellingham-Stanley Ltd. IR spectra were obtained in KBr disks in a Bomem model MB 100M series spectrophotometer. ^1H and ^{13}C NMR spectra were obtained with a Bruker DRX-500 spectrometer, using $CDCl_3$ as solvent. HRMS were recorded in a VG-AutoSpec spectrometer, using a direct insertion probe and electron impact at 70 eV. Silica gel 60 (Merck) was used for column chromatography.

Plant Material. The stems of *Xylopia langsdorffiana* were collected in the municipality of Cruz do Espírito Santo, State of Paraíba, in July 2002. The plant material was identified by Prof. Maria de Fátima Agra, Head of the Botany Section of the Laboratório de Tecnologia Farmacêutica Prof. Delby Fernandes de Medeiros (LTF). A voucher specimen (AGRA 5541) is deposited at the Herbário Prof. Lauro Pires Xavier (JPB) of the Universidade Federal da Paraíba.

Extraction and Isolation. Dried stems of *X. langsdorffiana* (4 kg) were exhaustively extracted with 95% EtOH. The solvent was evaporated to yield a dark syrup (60 g), which was partitioned successively with hexane, chloroform, and ethyl acetate to yield 20, 16, and 12 g of crude residues, respectively. The hexane fraction was subjected to column chromatographic separation, using hexane and hexane with increasing amounts of ethyl acetate as eluents, and monitored by TLC. Altogether, 95 fractions of 100 mL each were collected and posted into 12 fractions (F-1−F-12). Fraction F-1 was recrystallized from methanol, yielding **1** (300 mg). Fraction F-4 was purified by preparative TLC with AcOEt−hexane (9:1) as developer to afford compound **2** (56 mg).

***ent*-7α-Acetoxytrachyloban-18-oic acid (1):** crystalline solid; mp 230−233 °C; $[\alpha]^{26}_D$ +25 (*c* 1.01 in $CHCl_3$); IR (KBr) 3200, 1700, 1725, 1239, 1133; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 360.2309 (calcd 360.2300 for $C_{22}H_{32}O_4$).**Crystallographic data for 1:** $C_{22}H_{32}O_4$; M = 359.47; orthorhombic, space group $P2_12_12_1$; a = 6.1359(2) Å, b = 16.9665(8) Å, c = 19.0101(8) Å; α = β = γ = 90°; V = 1979.04(1) Å^3; Z = 4; D_c = 1.21 g·cm^{-1}; λ(Mo Kα) = 0.71013 Å; $F(000)$ = 780; T = 293 K; colorless sheet, size 0.15 × 0.10 × 0.08 mm; 4505 independent measured reflections, refinement based on F^2 to give R_1 [$F^2 > 4\sigma(F^2)$] = 0.064; w_2 = 0.148 for 3250 observed reflections, and 239 parameters. The Flack absolute structure parameter was determined to be 1.08(2), and the refinement of the opposite enantiomer resulted in a value of 0.85(2). Unfortunately, the weak distinguishing value of the Flack parameter (which should be 0.0 for the correct enantiomer) could not be used to assign definitively the absolute stereochemistry. The positions of H atoms bonded to C were determined on the basis of stereochemical parameters, while those on

O were determined directly from a difference map. Hydrogen atoms on O were refined isotropically, while those on C rode on the carbon atom, and U_{iso} was set to 1.5 (methyl) or 1.2 (other) times the value of the equivalent isotropic displacement parameter of the attached atom. X-ray data collection were accomplished on an Enraf-Nonius KappaCCD area-detector diffractometer. The programs used in crystallographic study were as follows: data collection, COLLECT;[17] cell refinement, DENZO[18] and COLLECT; data reduction, DENZO and COLLECT; program used to solve structure, SHELXS97;[19] program used to refine structure, SHELXL97;[19] molecular graphics, ORTEP-3;[20] software used to prepare material for publication, WinGX-Routine.[21] The CCDC reference number is 270970. Copies of the available material can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CH21EZ, UK (fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

***ent*-7α-Hydroxytrachyloban-18-oic acid (2):** crystalline solid; mp 130−134 °C; $[\alpha]^{26}_{D}$ +16 (*c* 1.01 in $CHCl_3$); IR (KBr) 3500, 3150, 1695, 1232; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 318.2129 (calcd 318.2194 for $C_{20}H_{30}O_3$).

Cytotoxicity Bioassay. Cytotoxic activity of compound **1** was evaluated against V79 cells and rat hepatocytes using the MTT method, according to a previously described procedure.[15] Hepatocytes were isolated from 2-month-old Wistar male rats (200−250 g) by a two-step collagenase perfusion method.[22]

Acknowledgment. This work was supported by CNPq, CAPES, CENAUREM/UFC, and IMSEAR (Brazil). We thank Prof. J. Bhattacharyya, Visiting Professor (CAPES) at LTF, for revising the manuscript in English.

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NP050503O