A Trachylobane Diterpenoid from *Xylopia aethiopica*

Silvère Ngouela,[†] Barthélémy Nyassé,*[,†] Etienne Tsamo,[†] Marie-Christine Brochier,[‡] and Christophe Morin*[,‡]

Département de Chimie Organique, Faculté des Sciences de Yaoundé, B.P. 812, Yaoundé, Cameroon, and Département de Chimie, Université de Grenoble, B.P. 53 X, 38402 Grenoble, France

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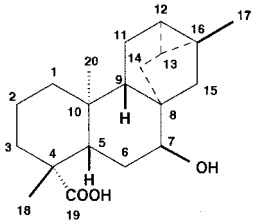
A new trachylobane derivative identified as 7α-hydroxytrachyloban-19β-oic acid (**1**) has been isolated from the bark of *Xylopia aethiopica* and its structure elucidated by various NMR techniques and molecular modeling.

Xylopia aethiopica A. Rich (Annonaceae) is a medicinal plant widely distributed in the western coast of Africa,[1] and decoctions of its bark are used in the treatment of bronchitis, bile-borne diseases, and dysentery.[2] Fractionation of a hexane extract of *X. aethiopica* bark has revealed the presence of a diterpene that was identified as a new trachylobane derivative (**1**). This work has led also to the assignment of complete ^{1}H- and ^{13}C-NMR data for **1**.

The molecular formula of **1** was established as $C_{20}H_{30}O_3$ (M^+ 318), and the presence of a hydroxyl group was shown by losses of 18 amu [*m/z* 300 $(M - H_2O)^+$ and 285 $(M - CH_3 - H_2O)^+$]. ^{13}C-NMR spectroscopy revealed a carboxy carbonyl group (δ_C 180.6) and a secondary alcohol (δ_C 75.3), but no other functionalities, which suggested that **1** was pentacyclic (five quaternary carbons, five methines, seven methylenes, and three methyl groups).



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Further information about the proton spin systems was gained from a COSY experiment, beginning the analysis at the highfield resonance (δ 0.62, H-12) belonging to a signal for a cyclopropane proton, that showed correlations with a methylene group (δ 1.80 and 2.02, H-11), a methyl group (δ 1.23, H-17), and a double doublet (δ 0.90, H-13). The latter was assigned to another cyclopropane proton that showed correlations with the same methyl group but with a different methylene (δ 1.32 and 2.12, H-14). Starting from the other end of the ^{1}H-NMR spectrum, the broad triplet (δ 3.84, H-7) attributed to the methine of a secondary alcohol was shown to belong to a −CH−CH_2−CHOH unit. Other isolated proton spin systems were, respectively, composed of a trimethylene unit, a methylene, and three methyl groups attached to quaternary centers.

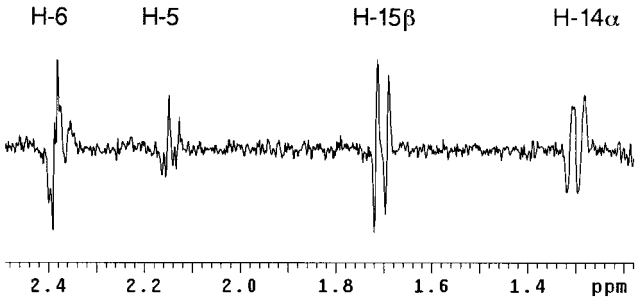

Figure 1. Long-range ^{13}C−^{1}H couplings observed after selective irradiation of C-7 (δ_C 75.3).

To connect the fragments, use was made of observed long-range heteronuclear HMBC correlations. Those observed from quaternary carbons were useful in linking independent proton spin systems. Thus, connectivities between the carbon at δ 23.3 (C-16) and the protons of the methyl group (δ 1.23, H-17), the cyclopropane ring (δ 0.62, H-12 and δ 0.90, H-13) and, significantly, the isolated methylene unit (δ 1.72 and 1.96, H-15), were observed. From another quaternary carbon at δ 46.1 (C-8), correlations involved one of the cyclopropane protons (δ 0.90, H-13) but also the methylene at δ 2.40 (H-6) adjacent to the secondary alcohol. The methine (δ 2.15, H-5) of the CH−CH_2−CHOH spin system was coupled with a quaternary carbon atom (δ 43.7, C-4) which, as for the carboxyl group (δ 180.6, C-19) was also connected to one end of the trimethylene unit. The other end of this trimethylene showed a correlation with the last quaternary carbon (δ 39.4, C-10), which was itself connected to the remaining methylene group, that is, the one not involved in any of the above connectivities. To confirm the above findings, additional experiments were performed using selective excitations of given carbons under decoupled conditions followed by polarization transfer to protons.[3,4] These experiments were optimized for long-range couplings ($J_{C,H}$ = 10 Hz) and a typical spectrum is given in Figure 1. As an example, the irradiation of the carbon at δ 75.3 (C-7) bearing the secondary alcohol resulted in polarization transfer to protons located, respectively, at δ_H 2.40 (H-6), 2.15 (H-5), 1.72 (H-15β), and 1.32 (H-14α). Similar responses were observed with C-5 and the H-1, H-3, H-6, H-7, and CH_3-18 and CH_3-20 protons, and also with C-8 and H-6, H-9, H-11, H-13, H-14, and H-15. These data resulted in structure **1**, which corresponds to a trachylobane having an oxidized methyl group and a hydroxylated B-ring.

* Correspondence may be addressed to B. N. [Phone: (237) 315907. Fax: (237) 235388. E-mail: bnyasse@uycdc.uninet.cm] or to C. M. [Phone: (33) 476 514250. Fax: (33) 476 514382. E-mail: Christophe.Morin@ujf-grenoble.fr].

† Faculté des Sciences de Yaoundé.

‡ Université de Grenoble.

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The stereochemistry of the polar substituents remained to be defined. The X-ray crystallographic data of trachylobanic acid[5] were used to construct the basic pentacyclic system contained in **1**, and molecular dynamics simulations were performed on all four isomers (i.e., with the carboxylic and the hydroxyl groups being placed independently as α or β substituents), which allowed interatomic distances to be determined. Strong NOESY and ROESY responses between H-5 and the methyl group attached to C-4 on one hand and H-7 with H-14 on the other hand were observed, and these results were rationalized by placing both the methyl and the hydroxyl groups in the α configuration (because trachylobane derivatives belong to the *ent* series, an α substituent lies above the plane). Depending on the configuration at position C-4, different chemical shifts have been observed for this carbon atom,[6−11] and our assignments agree well with literature assignments for a β-positioned carboxylic acid.

Thus, the structure of **1** was established in this investigation as 7α-hydroxytrachyloban-19β-oic acid, a new trachylobane derivative.

Experimental Section

General Experimental Procedures. A Perkin−Elmer 241 polarimeter was used for the determination of the optical rotation, and the IR spectrum was recorded on a Perkin−Elmer 397 spectrophotometer. HRMS data were obtained on a 70S EQ Micromass spectrometer by the Service Central d'Analyses du CNRS (Vernaison, France). NMR experiments were conducted at 298 K on a UNITY$^+$ 500 Varian spectrometer equipped with a 5-mm indirect probe, two radio frequency channels with waveform generators, and a Sun Sparc IPX computer. COSY spectra were recorded using a 2048 × 1024 acquisition matrix and processed using a 4096 × 4096 transformed matrix with zero-filling in both dimensions with a 1865-Hz spectral width; a sine squared function was used prior to Fourier transformation. HMQC spectra without proton decoupling were recorded using a 2048 × 256 acquisition matrix and processed using a 4096 × 2048 transformed matrix with zero-filling in both dimensions. Spectral widths were 1865 Hz in F_1 and 12 570 Hz in F_2; sine multiplication was performed prior to Fourier transformation. HMBC spectra were recorded in a similar way except for spectral width in F_2, which was 25 000 Hz. The delay was optimized with a coupling constant value of ${}^nJ_{(CH)} = 5$ Hz. NOESY and ROESY spectra were obtained in the phase-sensitive mode with different mixing times (4096 × 256 acquisition matrix). The spectral width was 3190 Hz in both dimensions, with a 4096 × 2048 transformed matrix for processing. The selective irradiations of individual ^{13}C sites, followed by polarization transfer to protons were obtained by modifications of the methods proposed by Blechta et al.[3] and Nishida et al.,[4] with the pulse sequence being in the reversed-detection mode; broadband WALTZ homodecoupling was thus applied during the preparation delay (3.0 s), and a half-Gaussian selective excitation (91 ms) was applied to a selected ^{13}C site under decoupled conditions. This was followed by polarization transfer to protons, and the polarization transfer delay ($d = 0.5/{}^nJ_{CH}$) was chosen (3 ms to 100 ms) to cover a wide range of experimental values; spectral width was 1865 Hz, and a slight exponential broadening (lb = 0.6) was applied prior to Fourier transformation. Compound **1** was dissolved in pyridine-d_5, and chemical shifts (δ) were referenced from residual solvent shifts: 7.19 ppm (^{1}H) and 149.9 ppm (^{13}C). Molecular dynamic simulations to search for a global minimal energy structure were performed using the Discover module of Insight II (Biosym/MSI).

Table 1. NMR Data of Compound **1**

	^{13}C		^{1}H		
position	δ (ppm)	multiplicity[a]	δ (ppm)	multiplicity	stereochemistry
1	40.1	CH_2	1.01	dt, J= 13, 4 Hz	α
			1.64	broad d, J = 13 Hz	β
2	19.8	CH_2	1.45	m	α
			2.25	tq, J = 13, 3 Hz	β
3	39.0	CH_2	1.16	dt,J = 13, 3 Hz	α
			2.45	broad t,J = 13 Hz	β
4	43.7	C			
5	48.0	CH	2.15	dd, J = 13, 3 Hz	
6	30.9	CH_2	2.40	m	α, β
7	75.3	CH	3.84	broad t, J = 3 Hz	
8	46.1	C			
9	47.1	CH	1.88	m	
10	39.4	C			
11	19.6	CH_2	1.80	ddd, J= 13, 6, 2 Hz	α
			2.02	m	β
12	21.2	CH	0.62	broad d, J = 9 Hz	
13	24.7	CH	0.90	dd, J = 8, 3 Hz	
14	33.1	CH_2	1.32	broad d, J = 11 Hz	α
			2.12	d, J =11 Hz	β
15	46.6	CH_2	1.72	d, J =11 Hz	β
			1.96	d, J = 11 Hz	α
16	23.3	C			
17	21.0	CH_3	1.23	s	
18	29.3	CH_3	1.19	s	
19	180.6	C			
20	13.0	CH_3	1.40	s	

[a] Values are from DEPT experiments.

Plant Material. *X. aethiopica* was collected at Edéa, Province du Littoral, Cameroon, in February 1994. A voucher specimen (no. 55011) is deposited at the National Herbarium, Yaoundé, Cameroon.

Extraction and Isolation. The air-dried and finely powdered stem bark of *X. aethiopica* (5 kg) was extracted with hexane using a Soxhlet apparatus. The crude extract (80 g) obtained after evaporation of the solvent was chromatographed over 70−230 mesh Si gel (900 g), using hexanes−EtOAc mixtures of increasing polarity and collecting 250-mL fractions. All fractions were monitored by TLC (hexanes−EtOAc). Fractions 82−200 eluted with hexanes−EtOAc 9:1 and showing the same composition by TLC (hexanes−EtOAc, 85:15) were pooled, and the solvents were evaporated leaving an oily residue (5 g). This residue was further purified by column chromatography on Si gel (200 g) using an isocratic system (hexanes−EtOAc, 85:15). The main fractions gave, upon evaporation to dryness, crystals of **1**, which were recrystallized from MeOH to give pure **1** (350 mg) as colorless needles: mp 128−129 °C; $[\alpha]^{27}_D$ −48° (*c* 1.7, pyridine); IR (KBr) ν 3500 cm^{-1} (OH) 3150, 1685 cm^{-1} (COOH); ^{1}H- and ^{13}C-NMR data, see Table 1; EIMS *m/z* 318, [M$^+$], 22, 300 [(M − H_2O)$^+$] (67), 285 [(M − H_2O − CH_3)$^+$] (16), 260 (16), 69 (100); HREIMS *m/z* 318.2183, calcd for $C_{20}H_{30}O_3$, 318.2195.

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