A New Sesquiterpene from *Ximenia americana* LINN.

by **Mônica R. S. de Araújo**[a]), **Francisco J. Q. Monte***[a]), and **Raimundo Braz-Filho**[b])

[a]) Departamento de Química Orgânica e Inorgânica, Centro de Ciências, Universidade Federal do Ceará, CEP: 60.451-700 Fortaleza, Ceará, Brazil (phone: +85-33669977; e-mail: fmonte@dqoi.ufc.br)
[b]) Setor de Química de Produtos Naturais – LCQUI-CCT Universidade Estadual do Norte Fluminense, CEP: 28.015-620, Campus- RJ, Brazil

The new sesquiterpene **1**, named ximonicane, and the known stigmastane steroid **2** were isolated from the EtOH extract of the stems of *Ximenia americana*. Spectroscopic methods, including 2D-NMR techniques, have been used to characterize **1** and assign all the signals of the ^{1}H- and ^{13}C-NMR spectra of **1**. Ximonicane did not inhibit the growth of HL-60 (human leukemia), HTC-8 (human colon), and MDA-MB-435 (human breast cancer) cell lines.

Introduction. – This paper describes partial results of our phytochemical studies on Brazil medicinal plants. *Ximenia americana* LINN. (Olacaceae) is a plant widely distributed in Northeast Brazil where it trivially is called ameixa do mato. The barks have been used in folk medicine as astringent in excessive menstruation and to cicatrisation of ulcers and their seed as purgative [1][2]. Recent investigations have revealed that the constituents of the plant show moluscicide, antirheumatic, and antioxidant effects [3][4]. This specimen which hasn't received previous phytochemical attention, has been examined, and the stem etanolic extract afforded the new sesquiterpene **1**, as well as the known stigmastane steroid **2**. The structures were elucidated on the basis of spectroscopic studies (IR, MS, and NMR). The complete assignment of the ^{1}H- and ^{13}C-NMR signals of **1** were achieved by 1D- (^{1}H, ^{13}C and DEPT) and 2D-shift-correlated (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMQC, ^{1}H,^{13}C-HMBC and ^{1}H,^{1}H-NOESY) NMR experiments. The new compound **1** did not inhibit the growth of HL-60 (human leukemia), HTC-8 (human colon), and MDA-MB-435 (human breast cancer) cell lines [5].

Results and Discussion. – Dried material of *X. americana* was extracted with EtOH, and the lipid extract obtained was subjected to repeated chromatography to give the compounds **1** and **2**.

Compound **1** was obtained as an amorphous solid from EtOH, with a melting point of 102.5 – 107.0°. The IR spectrum disclosed absorption bands due to a conjugated CO group ($\tilde{\nu}_{max}$ 2500 and 1685 cm^{-1}) with a C=C bond ($\tilde{\nu}_{max}$ 1635 cm^{-1}). The ^{13}C-HBBD and DEPT NMR spectra indicated the presence of 15 C-atoms divided into four non hydrogenated C-atoms (one sp^3, two olefinic, and one CO group (δ(C) 173.71) as suggested by the IR spectrum (1685 cm^{-1})), three CH groups (two sp^3 and one sp^2 olefinic CH groups), six CH_2 groups (five sp^3 and one sp^2 olefinic group), and two Me groups, requiring a total of 21 H-atoms ($C_{15}H_{21}$). These data indicated a molecular

© 2009 Verlag Helvetica Chimica Acta AG, Zürich

formula of $C_{15}H_{22}O_2$, including one COOH group, which was apparent from IR and ^{13}C-NMR spectra. The MS with a molecular ion peak of *m/z* 234 is in accord with the molecular formula above. Based on these data, five degrees of unsaturation could be attributed to two C=C bonds, one carbonyl group, and two ring systems. The ^{1}H-NMR spectrum exhibited a signal at δ(H) 7.01 (*t*, $J = 8.2$, 1 H). The chemical shift, multiplicity, and the coupling constant were compatible with an olefinic H-atom (H−C(9)) attached at the β C-atom of an α,β-unsaturated CO group and coupled to a CH_2 group (CH_2(10)) [6–8]. This was confirmed by the HMBC spectrum of **1**, which disclosed long range correlations of the signal (δ(H) 7.01) attributed for H−C(9) with the signals of a CH_2 group at δ(C) 28.58 (^2J) and a CO group at δ(C) 173.71 (^3J). Additional confirmation was obtained from the ^{1}H,^{1}H-COSY spectrum, in which H−C(9) (δ(H) 7.01) was coupled with two H-atoms occurring at δ(H) 2.50 and 2.42 (CH_2(10)), with the corresponding C-atom at δ(C) 28.58 (CH_2(10)), as revealed by the HMQC spectrum. In addition, the H-atom at δ(H) 7.01 showed HMBC connectivity (^3J) with another CH_2 group at δ(C) 23.79 (CH_2(7)) (*Table*). Together, these data allowed to establish the partial structure **A** (*Fig. 1*).

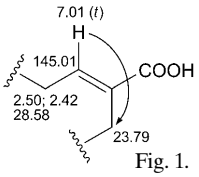

Fig. 1. *Partial structure* **A** *of compound* **1**

On the other hand, the HMBC spectrum of **1** showed the correlations of two Me *singlet*s at δ(H) 1.01 and 0.97 with the saturated C-atoms at δ(C) 33.97 (C(4), ^2J), 40.25 (CH_2(3), ^3J) and 52.01 (H−C(5), ^3J). These correlations determined the partial structure **B** (*Fig. 2*, *Table*).

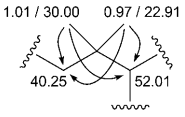

Fig. 2. *Partial structure* **B** *of compound* **1**

The signals corresponding to the remaining five C-atoms, related to a third isoprene unit, allowed to form the structural unit **C**, also using the HMBC spectrum. In this way, the following correlations were observed: *a*) the olefinic CH_2 group at δ(C) 111.63 (CH_2(12)) correlated with two CH_2 H-atoms at δ(H) 2.45 and 2.31 (CH_2(2), ^3J) and one CH group at δ(H) 2.50 (H−C(11), ^3J); the signals δ(C) 34.16 (CH_2(2)) and 40.16 (H−C(11)), respectively, were deduced from the HMQC spectrum; *b*) the CH_2 group CH_2(2) (δ(H) 2.45 and 2.31) correlated with the saturated CH_2(3) group (δ(C) 40.25, ^2J); c) the CH_2(3) group (δ(H) 1.74 and 1.58, δ(C) 40.25) correlated with the CH_2(2)

Table. *NMR Spectral Data of Compound* **1** (^{1}H: 500 MHz; ^{13}C: 125 MHz[a]); in $CDCl_3$)

	^{1}H,^{13}C-HMQC		^{1}H,^{13}C-HMBC		^{1}H,^{1}H-COSY	^{1}H,^{1}H-NOESY
	δ(C)	δ(H)	^2J	^3J		
1	154.71	–	H–C(11), CH_2(12)	CH_2(3), H–C(5)		
2	34.16	2.31, 2.45 (2*m*)	CH_2(3)	H–C(11), CH_2(12)	H–C(3), H_a–C(2), H_b–C(2)	H–C(12)
3	40.25	1.74 (*dd*, *J* = 10.8, 8.9), 1.58 (*t*, *J* = 10.8)	CH_2(2)	H–C(5), Me(13); Me(14)	H–C(2), H_a–C(3), H_b–C(3)	
4	33.97	–	CH_2(3), H–C(5), Me(13); Me(14)	CH_2(2), H–C(11)		
5	52.01	1.79–1.84 (*m*)	CH_2(6), H–C(11)	CH_2(3), CH_2(7), Me(13); Me(14)	H–C(6), H–C(11)	Me(13), H_{ax}–C(3), H–C(11), H_{ax}–C(6)
6	27.37	1.50, 1.68 (2*m*)	H–C(5), CH_2(7)		H_a–C(6), H_b–C(6)	CH_2(7), H–C(5)
7	23.79	2.35, 2.45 (2*m*)	CH_2(6)	H–C(9)	H–C(6)	CH_2(6)
8	132.34	–	CH_2(7), H–C(9)	CH_2(6)		
9	145.01	7.01 (*t*, *J* = 8.2)		CH_2(7), H–C(11)	H_a–C(9), H_b–C(9)	H–C(11)
10	28.58	2.42, 2.50 (2*m*)	H–C(9)		H–C(9), H–C(11)	H–C(9), H–C(12)
11	40.16	2.50 (*q*, *J* = 9.2)	H_a–C(10)	CH_2(2), CH_2(6), CH_2(12)		H–C(9), H–C(2), Me(14), H–C(5)
12	111.63	4.82 (*s*), 4.87 (*s*)		H–C(11), CH_2(12)		H–C(3), H_{eq}–C(10)
13	30.00	1.01 (*s*)		CH_2(3), H–C(5), Me(14)		H_{ax}–C(3), H–C(5)
14	22.91	0.97 (*s*)		CH_2(3), H–C(5), Me(13)		H_{ax}–C(6), H_{eq}–C(3), H–C(11)
15	173.71	–		CH_2(7), H–C(9)		

[a]) Multiciply deduced by comparative analysis of ^{1}H- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (*J* in parentheses) obtained from ^{1}H-NMR spectrum. ^{1}H,^{1}H-COSY and ^{1}H,^{1}H-NOESY spectra were also used in these assignments.

group (δ(C) 34.16, ^2J). These and some other significant long-range ^{1}H,^{13}C correlations are indicated by arrows and supported the partial structure **C** (*Fig. 3*, *Table*).

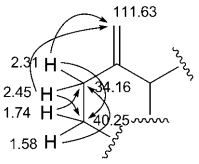

Fig. 3. *Partial structure* **C** *of compound* **1**

From the partial structures **A**, **B**, and **C**, the bicyclic structure of ximonicane was proposed to be that of compound **1** (*Fig. 4*), a new sesquiterpene isolated from *X. americana:* (4a*R*,9a*R*)-2,3,4,4a,5,6,9,9a-octahydro-4,4-dimethyl-1-methylidene-1*H*-benzocycloheptene-7-carboxylic acid.

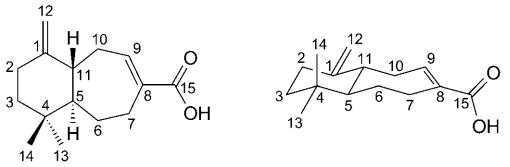

Fig. 4. *Structure of* **1**

The linkage of these partial structures (**A**, **B**, and **C**) to each other was based on additional data extracted from the long-range ^{1}H,^{13}C-HMBC and cross peaks in the ^{1}H,^{1}H-NOESY spectra. Thus, in the HMBC were observed obvious connectivities between: *a*) CH_2(7) (δ(C) 23.79) and C(8) (δ(C) 132.34) of **A** and the H-atoms of CH_2(6) (δ(H) 1.68 and 1.50) of **B**; *b*) CH_2(6) (δ(C) 27.37) of **B** and the H-atoms of CH_2(7) (δ(H) 2.45 and 2.35) of **A**; *c*) H_a–C(10) (δ(H) 2.50) of **A** and the C-atom of H–C(11) (δ(C) 40.16) of **C**; *d*) H–C(11) (δ(H) 2.50) of **C** and C(9) (δ(C) 145.01) of **A**; *e*) C(4) (δ(C) 33.97) of **B** and the H-atoms of CH_2(2) (δ(H) 2.45 and 2.31), CH_2(3) (δ(H) 1.74 and 1.58), and H–C(11) (δ(H) 2.50) of **C**, and *f*) CH_2(3) (δ(C) 40.25) of **C** and the H-atoms of H–C(5) (δ(H) 1.79–1.84) and Me(13) (δ(H) 1.01)/Me(14) (δ(H) 0.97) of **B**. In the NOESY experiment, the following correlations were found: *a*) CH_2(7) (2.45 and 2.35) of **A** with CH_2(6) (δ(H) 1.68 and 1.50) of **B**; *b*) H–C(9) (δ(H) 7.01) of **A** with H–C(11) (δ(H) 2.50) of **C**; *c*) H_{eq}–C(10) (δ(H) 2.42) of **A** with H–C(12) (δ(H) 4.82) of **C**; *d*) Me(13) (δ(H) 1.01) of **B** with H_{ax}–C(3) (δ(H) 1.74) of **C**, and *e*) Me(14) (δ(H) 0.97) of **B** and CH_2(3) (δ(H) 1.74 and 1.58) of **C** (*Table*).

The configuration of **1**, particularly the *trans*-fused ring, was assigned on the basis of the NOESY spectrum which clearly showed NOE correlations between Me(13) (δ(H) 1.01) and H–C(5) (δ(H) 1.79–1.84), as well as between Me(14) (δ(H) 0.97) and H–C(11) (δ(H) 2.50). In addition, NOEs were observed between H–C(5) (δ(H) 1.79–1.84) and H_{ax}–C(3) (δ(H) 1.74), H–C(11) (δ(H) 2.50), and H_{ax}–C(6) (δ(H) 1.50) (*Table*).

The steroid **2** (*Fig. 5*) was identified by comparison of IR, MS, ^{1}H- and ^{13}C NMR data with those of the literature [9][10].

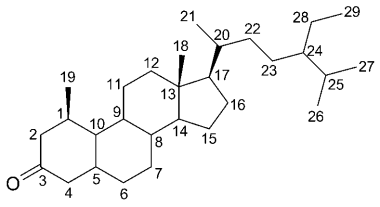

Fig. 5. *Structure of* **2**

Experimental Part

General Procedure. Column chromatography (CC): *Merck* Si gel *60* (0.004–0.0063). Analytical TLC: *Sigma* Si gel *60* F_{254} (0.002–0.0050); compounds were detected by spraying with 0.75M H_3PO_4 soln. containing 5% vanillin in EtOH, followed by heating. M.p.: *Kofler* melting point apparatus; uncorrected. IR Spectra: *Perkin-Elmer 2000* series FT-IR spectrometer, in KBr. ^{1}H- and ^{13}C-NMR spectra: *Bruker DPX 300* and *DRX 500* spectrometers in $CDCl_3$; with TMS as an internal standard. DEPT and all 2D experiments (COSY, HMBC, HMQC and NOESY) with standard *Bruker* pulse sequences. EI-MS: at 70 eV on a *Shimadzu QP5050* mass spectrometer.

Plant Material. X. americana was collected in Acarape County, Brazil. The plant material was identified in the Departamento de Biologia do Centro de Ciências da Universidade Federal do Ceará, Brazil. A voucher (No. 040411) specimen is deposited with the Herbarium Prisco Bezerra in that University.

Extraction and Isolation. The air-dried stems (3.67 kg) of *X. americana* were powdered and extracted at r.t. with hexane and EtOH. The EtOH extract yielded a viscous material (51.5 g), of which a part (42.5 g) was subjected to CC on SiO_2 (75 g) using hexane, CH_2Cl_2, AcOEt, and MeOH as solvents. The CH_2Cl_2 fraction (12.68 g) was subjected to flash SiO_2 CC eluted with hexane/AcOEt mixtures of increasing polarity. The hexane/AcOEt 15% fraction (618.0 mg) was chromatographed in flash SiO_2 column eluted with hexane/$CHCl_3$ mixtures in increasing polarity. The hexane/$CHCl_3$ 80% fraction, after removal of the solvent, yielded **1** (*(4a*R*,9a*R*)-2,3,4,4a,5,6,9,9a-Octahydro-4,4-dimethyl-1-methylidene-1*H*-benzocycloheptene-7-carboxylic acid*; 61.5 mg). Concentration of the hexane extract yielded material (4.0 g), which was subjected to CC on SiO_2 (6.5 g) using hexane/$CHCl_3$ mixtures of increasing polarity. The hexane/$CHCl_3$ 40% fraction after removal of the solvent, yielded **2** (*(1β,17β)-17-(5-ethyl-6-methylheptan-2-yl)-1-methylestran-3-one*).

Data of **1**. Amorphous solid. M.p. 102.5–107.0°. IR (KBr): 2500, 1685, 1635. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS (70 eV): 234 (M^+).

Cytotoxicity Assays. The HL-60 (human leukemia), HCT-8 (human colon), and MDA-MB-435 (human breast cancer) cell lines were cultured at 37° in *RPMI 1640* medium supplemented with 10% (*v*/*v*) fetal bovine serum in a atmosphere of 5% CO_2. The cells were cultured in 96-microwell plates for 72 h in the presence of tested compounds (0.09 to 25 μg/ml). After incubation, the culture medium was replaced by 150 μl of fresh medium containing 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (0.5 mg/ml), and the plates were further incubated for 3 h at 37° with 5% CO_2. Then, the plates were centrifuged and the pellet was resuspended in 150 μl of DMSO. The absorbance was measured using a multiplate reader (*DTX 880 Multimode Detector*, *Beckman Coulter, Inc.*, Fullerton, California, EUA). The drug effect was quantified as the percentage of control absorbance of reduced dye at 595 nm [5]. The dose response curve was plotted for each tested sample, and the concentration giving the average inhibitory concentration (IC_{50}) was calculated.

This work was supported by research fellowships and by grants from Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq) and Centro de Aperfeiçoamento de Pessoal do Ensino Superior (CAPES), Brazil.

REFERENCES

[1] R. Braga, 'Plantas do Nordeste', Especialmente do Ceará, ESAM, Fortaleza, 3ª Ed., 1976, p. 66.
[2] M. P. Correa, 'Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas', Ministério da Agricultura, Brasília,1984, p. 90.
[3] J.-P. Mevy, J.-M. Bessiere, S. Greff, G. Zombre, J. Viano, *Biochem. Syst. Ecol.* **2006**, *34*, 549.
[4] V. T. Uchoa, J. S. Luna, 'A. C. C. M. Argolo', 29ª RA-SBQ, Águas de Lindóia, São Paulo, 2006.
[5] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[6] A. A. Amonkar, T. G. McCloud, C. Chang, J. A. Saenz-Renauld, J. M. Cassady, *Phytochemistry* **1985**, *24*, 1803.
[7] H. Stuppner, E. P. Muller, H. Wagner, *Phytochemistry* **1991**, *30*, 305.
[8] F. J. Q. Monte, S. M. A. Papa, C. R. Sousa, R. Braz-Filho, *Rev. Latinoam. Quím.* **2003**, *31*, 89.
[9] F. W. Wehrli, T. Nishida, 'Progress in the Chemistry of Organic Natural Products', Springer-Verlag, Wien, 1979, p. 104.
[10] E. Breitmaier, W. Voelter, 'Carbon-13 NMR Spectroscopy', VCH, Weinheim, 1979, p. 349.

Received June 3, 2008