Tropane Alkaloids from *Erythroxylum caatingae* PLOWMAN

by **Steno L. de Oliveira**[a]), **Josean F. Tavares**[a]), **Marianna V. S. Castello Branco**[a]), **Hellane F. S. Lucena**[a]), **José M. Barbosa-Filho**[a]), **Maria de F. Agra**[a]), **Silene C. do Nascimento**[b]), **Jaciana dos S. Aguiar**[b]), **Teresinha G. da Silva**[b]), **Carlos A. de Simone**[c]), **João X. de Araújo-Júnior**[c])[d]), and **Marcelo S. da Silva***[a])

[a]) Departamento de Ciências Farmacêuticas, Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, C. P. 5009, 58051-970, João Pessoa, Paraíba, Brazil (phone: +55 83 3216 7427; fax: +55 83 3216 7365; marcelosobral@ltf.ufpb.br)

[b]) Departamento de Antibióticos, Universidade Federal de Pernambuco, 50670-901, Recife, PE, Brazil

[c]) Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, 57072-970, Maceió, AL, Brazil

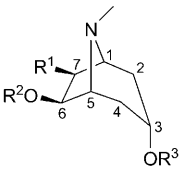
[d]) ESENFAR, Universidade Federal de Alagoas, 57072-970, Maceió, AL, Brazil

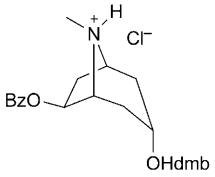
Three tropane alkaloids, **1**–**3**, were isolated from *Erythroxylum caatingae*, *i.e.*, 6β-benzoyloxy-3α-[(4-hydroxy-3,5-dimethoxybenzoyl)oxy]tropane (**1**), a new tropane alkaloid, along with the known alkaloids 3α,6β-dibenzoyloxytropane (**2**) and 6β-benzoyloxy-3α-[(3,4,5-trimethoxybenzoyl)oxy]tropane (catuabine B; **3**). Their structures were determined by 2D- (^{1}H and ^{13}C) NMR. By LC/ESI-MS/MS analysis of the fractions of alkaloids **1**–**3**, it was possible to detect five more alkaloids, **4**–**8**, two of these, **4** and **8**, possibly being new natural products. X-Ray crystallography of the chloride derivate of **1**, *i.e.*, 6β-benzoyloxy-3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)tropane hydrochloride (**1a**) confirmed the structure of **1**. Cytotoxicity was tested against the cell lines HEp-2, NCI-H292, and KB for the MeOH extract and alkaloid **3**, and antitumor activity was tested against Sarcoma 180 only for the MeOH extract.

Introduction. – The family Erythroxylaceae comprises four genera and *ca.* 240 species with pantropical distribution, with its main centers of diversity and endemism in Venezuela, Brazil, and Madagascar. The Erythroxylaceae consist of the genera *Aneulophus*, *Erythroxylum*, *Nectaropetalum*, and *Pinacopodium*, where *Erythroxylum* is the largest and most representative genus, with *ca.* 230 species and wide distribution in the tropical regions of the world, and South America is the center of diversity and endemism. *Erythroxylum caatingae* is a species with distribution restricted to the Northeast Brazil, being only recorded for the states of Bahia, Ceara, Paraíba, Pernambuco, and Rio Grande do Norte [1].

Tropane alkaloids occur frequently in the families Convolvulaceae, Erythroxylaceae, Proteaceae, Rhizophoraceae, and Solanaceae and have occasionally been reported in plants of the families Brassicaceae, Euphorbiaceae, and Oleaceae [2]. Recently, 17 tropane alkaloids were reported from *E. vacciniifolium*, collected in Paraíba (Brazil) [3][4]. Studies with the $CHCl_3$ extract of *E. pervillei* and *E. rotundifolium* revealed cytotoxicity against the multidrug-resistant (MDR) cell line KB-V1 in the presence of vinblastine [2–5]. Recently, we described a new trachylobane diterpene and its cytotoxicity against V79 cells and rat hepatocytes [6]. As part of a continuing investigation of new bioactive molecules from plants of Paraíba (Brazil), we describe here the isolation and structural identification of three tropane alkaloids,

among which 6β-benzoyloxy-3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)tropane (**1**) is a new tropane alkaloid, whereas 3α,6β-dibenzoyloxytropane (**2**) and 6β-benzoyloxy-3α-(3,4,5-trimethoxybenzoyloxy)tropane (catuabine B; **3**) are known compounds. The hydrochloride of **1**, *i.e.*, **1a**, was obtained by acidification with HCl. Furthermore, it was possible to detect, in the fractions of alkaloids **1**–**3**, five additional alkaloids, **4**–**8**, partially characterized by LC/ESI-MS/MS. Cytotoxicity of the MeOH extract and alkaloid **3** against HEp-2, NCI-H292, and KB cells, and *in vivo* antitumor activity of the MeOH extract of *E. caatingae* in the presence of methotrexate were determined.

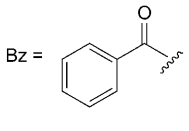


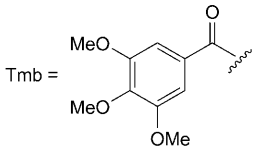


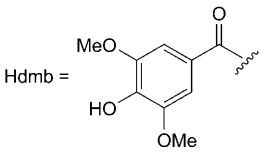
1a

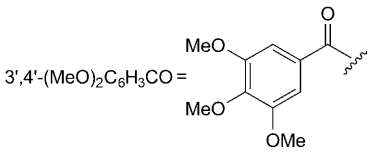
	R^1	R^2	R^3
1[a])	H	Bz	Hdmb
2[a])	H	Bz	Bz
3[a])	H	Bz	Tmb
4[b])	H	Me_2C=CHCO	Hdmb
5[b])	OH	OH	Bz
6[b])	H	OH	Bz
7[b])	H	OH	Tmb
8[b])	H	Bz	3',4'-$(MeO)_2C_6H_3CO$

[a]) Compounds identified by NMR.
[b]) Compounds partially identified by LC/ESI-MS/MS









Results and Discussion. – Compound **1** was isolated as an amorphous solid, with a melting point of 220–223°. HR-MS exhibited a molecular ion at *m*/*z* 442.1848 ([*M*+H]$^+$; calc. 442.1866), compatible with the molecular formula $C_{24}H_{27}NO_7$. The IR spectrum showed absorptions at 3425 (OH), and 1724 and 1277 cm^{-1} characteristic of an ester group. The ^{13}C-NMR spectrum of **1** showed signals at δ(C) 60.1 (C(1)), 65.7 (C(5)), and 40.1 (MeN), characteristics of a tropane skeleton. In addition, the signals at δ(C) 67.3 and 79.7 are compatible with the tropane skeleton substituted at C(3) and C(6) [7]. The HSQC spectrum showed direct correlations between δ(C) 60.1 (C(1)) and δ(H) 3.45 (H–C(1)), and 65.7 (C(5)), and 3.43 (H–C(5)). The presence of two COO groups was supported by the signals at δ(C) 165.5 and 166.0. The ^{1}H-NMR

spectra revealed signals of two O-bearing CH groups at δ(H) 5.34 (H_β–C(3)) and 5.93 (H_α–C(6)), two O-bearing CH groups at δ(H) 3.45 (H–C(1)) and 3.43 (H–C(5)), three O-bearing CH_2 groups at δ(H) 2.29 and 2.37 (H_{ax}–C(2) and H_{ax}–C(4), resp.), 1.82 and 2.03 (H_{eq}–C(2) and H_{eq}–C(4), resp.), and 2.33 and 2.82 (H_α–C(7) and H_β–C(7), resp.), and a MeN group at δ(H) 2.46. The complete assignment of all the C- and H-atoms is compiled in *Table 1*. The relative configuration of **1** was established by the signal at δ(H) 5.34 (br. *t*, H–C(3)) with a coupling constant of $J = 5.0$ Hz indicating the α-orientation of the substituent at C(3) [3]. On the other hand, the relative configuration of the substituent at C(6) was established by the analysis of multiplicity and of the coupling constants of H–C(6). H–C(6) (δ(H) 5.93) showed two couplings (*dd*, $J = 3.0, 7.5$) with the two H-atoms with signals at δ(H) 2.82 and 2.33 (H_α–C(7) and H_β–C(7), resp.) and did not show any coupling with the signal of H–C(5) (δ(H) 3.43). This observation is in line with the β-orientation of the substituent, and a dihedral angle of 90° between H-atoms H–C(5) and H_α–C(6) [3]. These data were corroborated by X-ray crystallography of **1a** (*Figs. 1* and *2*). The chemical shifts δ(H) 8.01 (*dd*, $J = 1.0, 7.5$, H–C(2′′,6′′)), 7.43 (*t*, $J = 7.5$, H–C(3′′,5′′)), and 7.55 (*t*, $J = 7.5$, H–C(4′′)) are characteristic of a benzoyloxy (BzO) group. The chemical shifts at δ(H) 7.32 (*s*, H–C(2′,6′)) and

Table 1. *NMR Data for Compound* **1**[a])

Position	δ(C)	δ(H)	HMBC	^{1}H,^{1}H-COSY
1	60.12	3.45 (*m*)	Me	
2_{ax}	34.59	2.29 (*m*)	H–C(7)	H–C(1)
2_{eq}		1.82 (br. *d*, $J = 15.0$)		
3_β	67.28	5.34 (br. *t*, $J = 5.0$)	H–C(2)/H–C(4)	
4_{ax}	33.25	2.37 (*m*)		
4_{eq}		2.03 (br. *d*, $J = 15.0$)		
5	65.74	3.43 (*m*)	Me/H–C(3)/H–C(7)	H–C(4)
6_α	79.69	5.93 (*dd*, $J = 3.0, 7.5$)	H–C(7)	
7_α	36.73	2.82 (*dd*, $J = 7.5, 14.0$)		
7_β		2.33 (*m*)		
1′	121.15		H–C(2′)/H–C(6′)	
2′	106.55	7.32 (*s*)	H–C(6′)	
3′	146.97		MeO/H–C(2′)	
4′	139.60			
5′	146.97		MeO/H–C(6′)	
6′	106.55	7.32 (*s*)	H–C(2′)	
7′	165.46		H–C(3)	
1′′	130.28		H–C(3′′)/H–C(5′′)	
2′′	129.46	8.01 (*dd*, $J = 1.0, 7.5$)	H–C(4′′)/H–C(6′′)	H–C(3′)
3′′	128.34	7.43 (*t*, $J = 7.5$)	H–C(5′′)	H–C(2′)
4′′	132.92	7.55 (*t*, $J = 7.5$)	H–C(2′′)/H–C(6′′)	
5′′	128.34	7.43 (*t*, $J = 7.5$)	H–C(3′′)	H–C(6′)
6′′	129.46	8.01 (*dd*, $J = 1.0, 7.5$)	H–C(2′′)/H–C(4′′)	H–C(5′)
7′′	166.00		H–C(2′′)/H–C(6′′)	
MeN	40.07	2.46 (*s*)		
MeO	56.50	4.00 (*s*)		

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

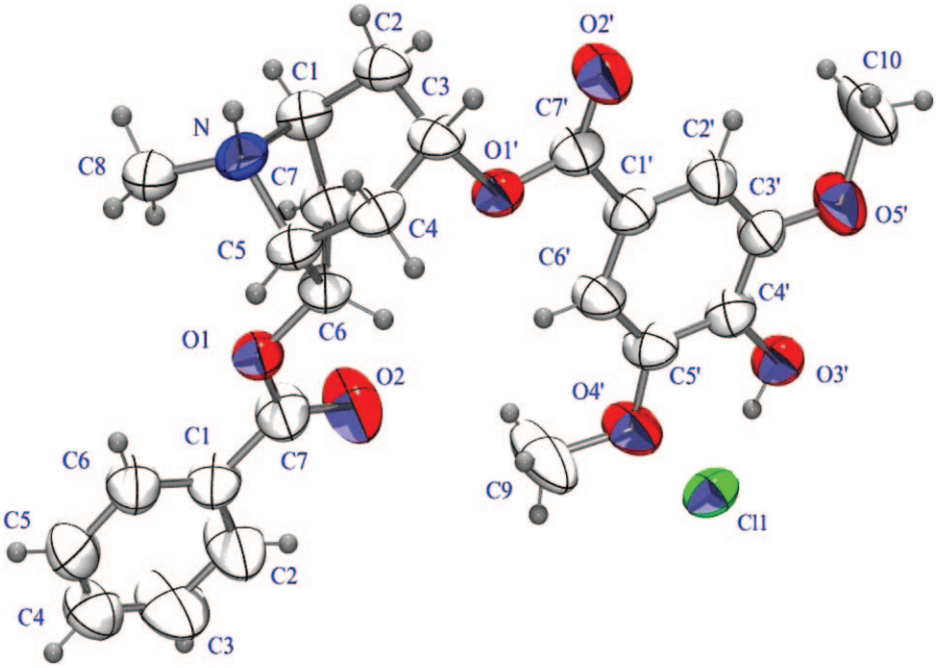


Fig. 1. *An ORTEP3 projection of the molecule* **1a**, *showing the atom numbering and displacement ellipsoids at the 50% probability level*

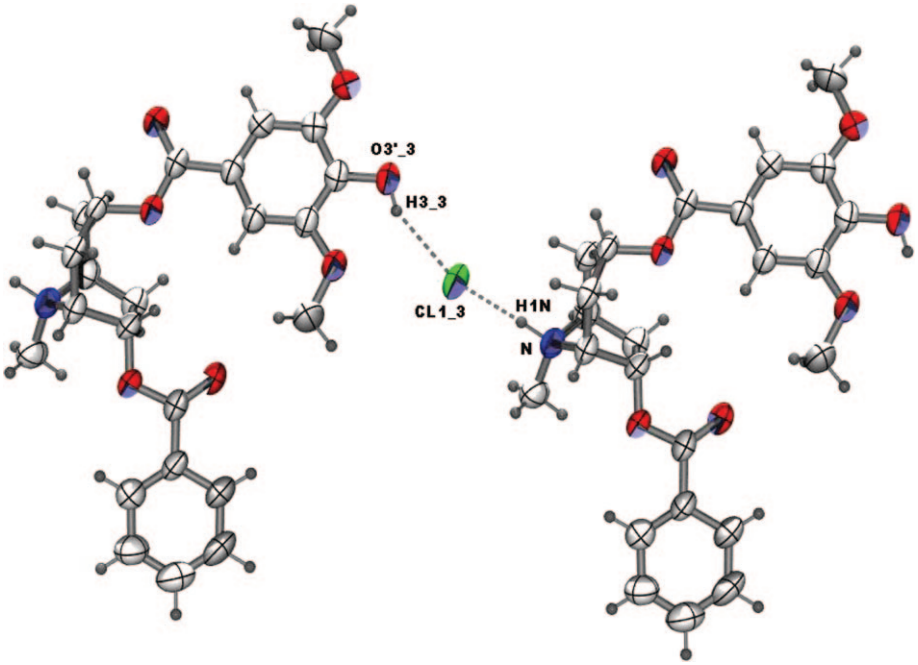


Fig. 2. *H-Bonding interactions in the crystal packing*

4.00 (2 MeO) are characteristic of a 3′,5′-dimethoxybenzoyloxy (Hdmb) group. The HMBC spectrum showed correlations between C(3′) and C(5′) (δ(C) 147.0) and MeO groups (δ(H) 4.00). Therefore, compound **1** was identified as 6β-benzoyloxy-3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)tropane, a new tropane alkaloid.

Compound **1a** was obtained as white crystals, with a melting point of 196–198°. Analysis of the ^{1}H- and ^{13}C-NMR data showed chemical shifts compatible with a 3,6-disubstituted tropane skeleton. Unlike alkaloid **1**, derivative **1a** showed chemical shifts for H–C(1) at δ(H) 4.11 and H–C(5) at δ(H) 4.12. These chemical shifts are compatible with those of cocaine hydrochloride [8] (*Table 2*). The structure of **1a** was established by X-ray crystallography (*Figs. 1* and *2*).

Table 2. *NMR Data for Compounds* **1a**, **2**, *and* **3**[a])

Position	**1a**		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1	67.18	4.11 (br. *s*)	59.92	3.42 (*m*)	60.03	3.43 (*m*)
2_{ax}	33.02	2.40 (br. *d*, *J* = 15.5)	34.65	2.26 (*m*)	34.66	2.26 (*m*)
2_{eq}				1.82 (br. *d*, *J* = 15.0)		1.83 (br. *d*, *J* = 15.0)
3_{β}	64.48	5.46 (br. *t*, *J* = 4.5)	67.57	5.34 (br. *t*, *J* = 5.0)	67.65	5.34 (br. *t*, *J* = 5.0)
4_{ax}	34.14		33.16	2.29 (*m*)	33.31	2.33 (*m*)
4_{eq}		2.20 (br. *d*, *J* = 16.0)		2.06 (br. *d*, *J* = 15.0)		2.03 (br. *d*, *J* = 15.0)
5	63.10	4.12 (br. *s*)	65.85	3.39 (br. *s*)	65.74	3.42 (*m*)
6_{α}	35.40	6.09 (*dd*, *J* = 3.0, 8.0)	80.10	5.87 (*dd*, *J* = 3.0, 7.5)	79.84	5.92 (*dd*, *J* = 3.0, 7.0)
7_{α}	74.85	3.19 (*dd*, *J* = 8.0, 15.0)	36.16	2.78 (*dd*, *J* = 7.5, 14.5)	36.69	2.80 (*dd*, *J* = 7.0, 15.0)
7_{β}		2.51 (*m*)		2.33 (*m*)		2.30 (*m*)
		Hdmb		Bz		Tmb
1′	119.75		130.41		125.38	
2′, 6′	106.58	7.35 (*s*)	129.50	8.11 (*dd*, *J* = 1.5, 8.0)	106.63	7.39 (*s*)
3′, 5′	147.12		128.58	7.49 (*t*, *J* = 8.0)	153.13	
4′	140.25		133.00	7.58 (*m*)	153.13	
7′	164.98		165.70		165.32	
		Bz		Bz		Bz
1″	128.51		130.37		130.31	
2″, 6″	129.44	7.98 (br. *d*, *J* = 7.5)	129.48	8.03 (*dd*, *J* = 1.5, 8.0)	129.48	8.02 (*dd*, *J* = 1.5, 8.5)
3″, 5″	128.67	7.49 (*t*, *J* = 7.5)	128.32	7.43 (*t*, *J* = 8.0)	128.35	7.42 (*t*, *J* = 8.0)
4″	133.89	7.63 (*t*, *J* = 7.0)	132.89	7.53 (*m*)	132.94	7.55 (*t*, *J* = 7.5)
7″	165.10		166.37		166.05	
MeN	40.34	3.10 (*s*)	40.11	2.60 (*s*)	40.16	2.60 (*s*)
m-MeO					56.31	3.98 (*s*)
p-MeO	56.41	4.00 (*s*)			60.89	3.92 (*s*)

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

Compound **2** was isolated as white crystals, with a melting point of 119–121°. The MS showed a molecular-ion peak of *m*/*z* 366.1 ($[M+H]^+$), compatible with the molecular formula $C_{22}H_{23}NO_4$. The ^{1}H- and ^{13}C-NMR spectra exhibited chemical-shift values similar to those for **1** (*Table 1*), the chemical shifts of which are in line with a tropane skeleton disubstituted at C(3) and C(6). The principal difference between the

NMR data of compounds **1** and **2** is the type of substituent at C(3). The signals at δ(H) 8.11 (*dd*, $J = 1.5$, 8.0, H–C(2′,6′)), 7.49 (*t*, $J = 8.0$, H–C(3′,5′)), and 7.58 (*t*, $J = 7.5$, H–C(4′)) are compatible with a BzO group at C(3) (*Table 2*). The HMBC spectrum showed long-range correlations between signals at 165.7 with 5.34 (H_β–C(3)), and 166.4 with 5.87 (H–C(6)), defining the chemical shifts of the CO groups C(7′) and C(7″), respectively. Thus, compound **2** was identified as 3α,6β-dibenzoyloxytropane.

Compound **3** was isolated as white crystals, with a melting point of 154–157°. The MS showed a molecular-ion peak of *m*/*z* 456.1 ($[M+H]^+$), compatible with the molecular formula $C_{25}H_{29}NO_7$. The ^{1}H- and ^{13}C-NMR spectra of this alkaloid also displayed chemical-shift values similar to those for alkaloids **1** and **2**, but the signals at 7.39 (*s*, 2 H), 3.98 (*s*, 6 H), and 3.92 (*s*, 3 H) indicated that the substituent at C(3) is a 3,4,5-trimethoxybenzoyloxy group (*Table 2*). The HMBC spectrum showed correlations between the signals of C(7′) (δ(C) 165.3) and H–C(3) (δ(H) 5.34), and between the signals of C(7″) (δ(C) 166.1) and H–C(6) (δ(H) 5.92). The relative configuration of **3** was assigned as in compound **1**. Thus, the structure of compound **3** is 6β-benzoyloxy-3α-(3,4,5-trimethoxybenzoyloxy)tropane (catuabine B).

The ^{1}H- and ^{13}C-NMR spectra of the fractions of alkaloids **1**–**3** showed the presence of other constituents in smaller proportions. In an attempt to identify these constituents, these fractions were analyzed by LC/ESI-MS/MS (*Table 3*). Supported by the fragmentation model of alkaloid **1** (*Scheme*), it was possible to partially identify alkaloids **4**–**8**. The selective MS^2 of the three fractions analyzed showed ions at *m*/*z* 140.1 and 122.2, suggesting the presence of disubstituted tropane alkaloids [9]. The chromatogram of total ions of fraction of **1**, showed three peaks, with the molecular-ion peaks at *m*/*z* 420.1, 442.1, and 366.1, where the largest component ion, with the peak at *m*/*z* 442.1, was identified as alkaloid **1**. The MS^2 experiment of the ion with the peak at *m*/*z* 420.1 resulted in the formation of ions with peaks at *m*/*z* 320.2 and 222.1, among others. These fragments [420.1 → 320.2] and [420.1 → 222.1] are compatible with the loss of the Me_2CCHCO and Hdmb substituents from the tropane ring, respectively, suggesting the molecular formula $C_{22}H_{29}NO_7$ for compound **4**. Similarly, for fraction of **2**, the chromatogram of total ions showed three peaks with molecular-ion peaks at *m*/*z* 278.1, 262.1, and 366.1, where the largest component ion, with the peak at *m*/*z* 366.1, was identified as alkaloid **2**. The MS^2 experiment of the ion with the peak at *m*/*z* 278.1 resulted in the formation of ions with peaks at *m*/*z* 156.1 and 138.1, among others. These fragments [278.1 → 156.1 → 138.1] are compatible with the loss of the BzO and OH substituents, respectively, suggesting the molecular formula $C_{15}H_{19}NO_4$ for compound **5**. The MS^2 experiment of ion with the peak at *m*/*z* 262.1 resulted in the formation of ions with peaks at *m*/*z* 140.1 and 122.2, among others. These fragments [262.1 → 140.1 → 122.1] are compatible with the loss of the BzO and OH substituents, suggesting the molecular formula $C_{15}H_{19}NO_3$ for compound **6**. The chromatogram of total ions of fraction of **3**, showed four peaks with molecular-ion peaks at *m*/*z* 262.1, 352.1, 426.1, and 456.1, where the largest component ion, with the peak at *m*/*z* 456.1, was identified as alkaloid **3**. The MS^2 experiment of the ion with the peak at *m*/*z* 352.1 resulted in the formation of ions with peaks at *m*/*z* 195.0 and 140.2, among others. The fragment [352.1 → 140.2] is compatible with the loss of the substituent Tmb, thus suggesting the molecular formula $C_{10}H_{11}NO_4$ for compound **7**. The MS^2 experiment of the ion with the peakt at *m*/*z* 426.1, resulted in the formation of ions with peaks at *m*/*z* 304.1 and 122.2,

among others. These fragments [426.1 → 304.1 → 122.2] are compatible with the loss of the BzO and Dmb substituents, suggesting the molecular formula $C_{24}H_{27}NO_6$ for compound **8**. Thus, of the alkaloids only partially characterized, **4** and **8** are possibly new natural products.

Scheme. *Proposed Fragmentation of Alkaloid* **1**

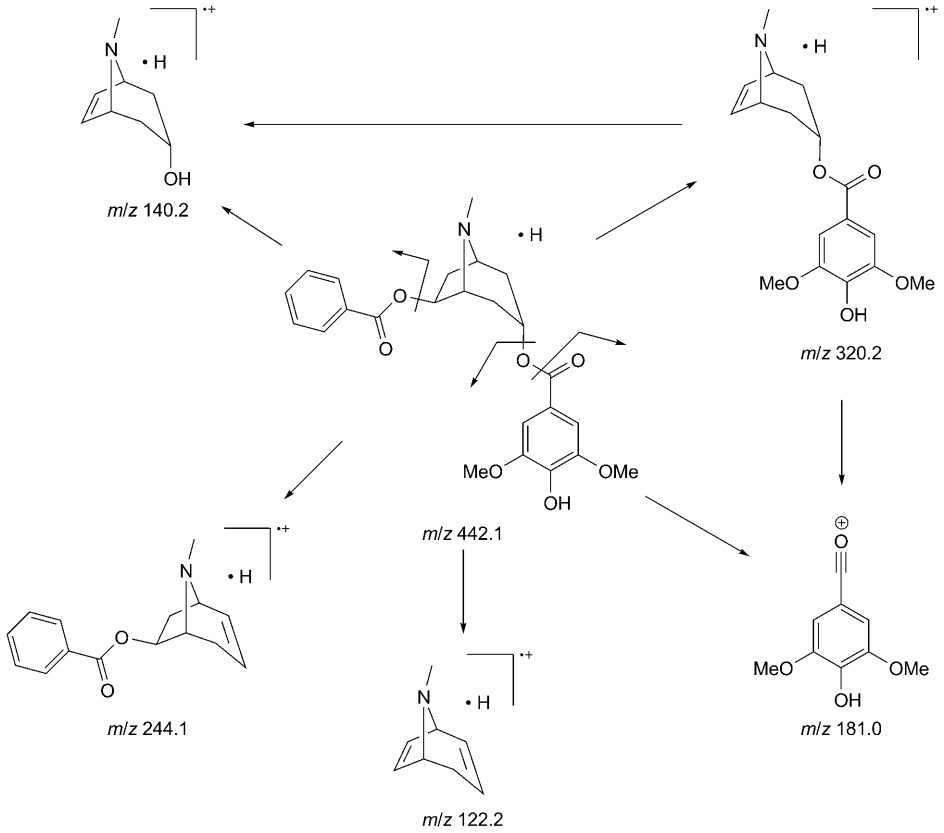


Table 3. *LC/MS/MS Data for Each Ion Analyzed and Its Fragmentations*

Alkaloid	Peak No.	M^+	MS^2 Fragments
1	1	420	320, 222, 181, 140, 122
	2	442	320, 244, 181, 140
	3	366	244, 122
2	1	278	156, 138, 94
	2	262	140, 122, 91
	3	366	244, 122
3	1	262	140, 105
	2	352	195, 140, 122
	3	426	244, 165, 140, 122
	4	456	244, 195, 167

The cytotoxic activities of the MeOH extract of *Erythroxylum caatingae* stems and alkaloid **3** were evaluated against human cancer cell lines (HEp-2, NCI-H292, and KB). Alkaloid **3** exhibited significant cytotoxicity only against NCI-H292 ($IC_{50} = 50$ μg/ml), and the MeOH extract of *E. caatingae* stems was inactive against all cell lines tested ($IC_{50} > 50$ μg/ml).

The effects of MeOH extract of *E. caatingae* stems on mice transplanted with Sarcoma 180 are shown in *Table 4*. There was a significant reduction in tumor weight in animals treated with different doses. These reductions gave tumor inhibition percentages of 59.4 to 66.4%. The MeOH extract tested showed IC_{50} values greater than 10 μg/ml for all tumor cell lines tested, suggesting that the *in vivo* anticancer properties were not related to direct antiproliferative effects.

Table 4. In vivo *Antitumor Activity against Sarcoma 180 Using MeOH Extract of* Erythroxylum caatingae *Stems*[a])

Compound	Dose [mg/kg]	Weight of tumor [g]	Inhibition [%]
MeOH extract of stems	100	0.698 ± 0.05	59.4
	200	0.577 ± 0.10	66.4
	400	0.614 ± 0.11	64.3
Methotrexate	2.5	0.659 ± 0.08	61.7
Control	–	1.718 ± 0.20	–

[a]) Data are presented as means ± SEM for seven animals. *: $P < 0.05$ compared with control group.

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Experimental Part

General. M.p.: *Microquimica* digital melting-point apparatus, model *MQAPF-302*, with the Pt block in a *REICHERT Kofler*-type light microscope, model *R3279*, with a temp. that varies from 0 to 350°; values uncorrected. IR Spectra: *BOMEM SERIE 100 MB* spectrometer, in the range of 4000–400 cm^{-1}, with KBr pellets (0.5 mg of the sample/100 mg of KBr). NMR Spectra: *VARIAN-NMR SYSTEM* spectrometer, operating at 500 MHz for ^1H and at 125 MHz for ^{13}C; recorded in $CDCl_3$, with TMS as internal standard. LC/ESI-MS/MS: *HPLC Agilent 1200RRLC* and *Bruker HCT Ultra* mass spectrometer. X-Ray crystallography: *Enraf-Nonius Kappa-CCD* diffractometer.

Plant Material. The stems of *Erythroxylum caatingae* were collected in Picui, Paraíba, Brazil. The botanical material was identified by *M. F. A.* A dried specimen was deposited with the Herbario Prof. *L. P. Xavier* (JPB), Universidade Federal da Paraíba, under the identification label AGRA 5666.

Extraction and Isolation. The crude MeOH extract (500 g) was dissolved in H_2O and defatted with hexane. The defatted aq. extract was acidified with 3% HCl by mechanical mixing and filtered through *Celite*, yielding a residue that was discarded and an acidic soln. This acidic soln. was extracted with $CHCl_3$ (3×500 ml) resulting in an acidic $CHCl_3$ phase and an aq. phase that was neutralized to pH 7.0 with NH_4Cl. The aq. phase at pH 7.0 was then extracted with $CHCl_3$ to yield an aq. phase and basic $CHCl_3$

phase *FA1* (4.0 g), which was submitted to column chromatography (CC) utilizing SiO_2 as the stationary phase, and $CHCl_3$ and MeOH as the eluents alone or in binary mixtures with increasing polarity. The result were 55 fractions of 100 ml. The 55 fractions were monitored by TLC, eluted with various solvent systems ($CHCl_3$ and/or $CHCl_3$/MeOH in order of increasing polarity) in chambers pre-saturated with NH_3 vapor, revealed with *Dragendorff*'s reagent, and placed in 21 groups based on their R_f values. *Frs. 25*, *45*, and *46* were submitted to repeated recrystallization with acetone and Et_2O, to yield compounds **1**, **2**, and **3**. Compound **1** was acidified with HCl to yield the alkaloid hydrochloride, referred to as **1a**.

6β-Benzoyloxy-3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)tropane (=*(3*S*,6*S*)-8-Methyl-6-[(phenylcarbonyl)oxy]-8-azabicyclo[3.2.1]oct-3-yl 4-Hydroxy-3,5-dimethoxybenzoate*; **1**). White crystals. IR (KBr): 3425 (OH), 1724 (C=O), 1277. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 442 ($[M+H]^+$), 320 ($[M-BzOH]^+$), 244 ($[M-HdmbOH]^+$), 181 $[M-BzOH-C_8H_{13}ON]^+$), 140 ($[M-HdmbOH-C_7H_5O]^+$).

3α,6β-Dibenzoyloxytropane (=*(3*S*,6*S*)-8-Methyl-8-azabicyclo[3.2.1]octane-3,6-diyl Dibenzoate*; **2**). White crystals. IR (KBr): 1724 (C=O), 1600–1585 (C=C). ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 366 ($[M+H]^+$), 244 ($[M-BzOH]^+$), 122 ($[M-BzOH-C_8H_{14}N]^+$).

Catuabine B (=*6β-Benzoyloxy-3α-(3,4,5-trimethoxybenzoyloxy)tropane*=*(3*S*,6*S*)-8-Methyl-6-[(phenylcarbonyl)oxy]-8-azabicyclo[3.2.1]oct-3-yl 3,4,5-Trimethoxybenzoate*; **3**). White crystals. IR (KBr): 1709 (C=O), 1600–1585 (C=C), 1281. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 456 ($[M+H]^+$), 334 ($[M-BzOH]^+$), 244 ($[M-BzOH-3\,MeO]^+$), 195 ($[M-BzOH-C_8H_{14}N]^+$).

X-Ray Crystallographic Analyses[1]). X-Ray diffraction data collections were performed on an *Enraf-Nonius Kappa-CCD* diffractometer (95-mm CCD camera on κ-goniostat) using graphite monochromated MoK_α radiation (0.71073 Å), at r.t. Data collections were carried out using the COLLECT software [10] up to 50° in 2θ. Final unit-cell parameters were based on 9048 reflections. Integration and scaling of the reflections, and correction for *Lorentz* and polarization effects were performed with the HKL DENZO-SCALEPACK system of programs [11]. The structure of the compound was solved by direct methods with SHELXS-97 [12]. The models were refined by full-matrix least squares on F^2 using SHELXL-97 [13]. The program ORTEP-3 [14] was used for graphic representation and the program WINGX [15] to prepare materials for publication. All H-atoms were located by geometric considerations placed (d(C–H)=0.93–0.98 Å) and refined as riding with U_{iso}(H)=1.5 U_{eq}(C–methyl) or 1.2 U_{eq}-(other). An ORTEP-3 diagram of the molecule is shown in *Fig. 1*, and *Table 5* contains the main crystallographic parameters.

The compound crystallized with one Cl-atom that forms N–H1N···Cl1^i and O3′–H3···Cl1^{ii} H-bonding interactions, where: [$i=x+1/2, y+1/2, z$; $ii=x, y, z$] and H1N···Cl1^i=2.065(2) Å ; N–H1N···Cl1^i=178° and H3···Cl1^{ii}=2.477(1) Å; O3′–H3···Cl1^{ii}=140° (*Fig. 2*).

Cytotoxicity Assay. NCI-H292 (human lung mucoepidermoid carcinoma cell line), HEp-2 (human larynx epidermoid carcinoma cell line), and KB (human mouth epidermoid carcinoma cell line) cells were obtained from the *Adolph Lutz* Institute (São Paulo, Brazil) and were maintained in DMEM (= *Dulbecco*'s Modified *Eagle*'s Medium) supplemented with 10% fetal bovine serum (FBS), 1000 IU/ml of penicillin, and 250 μg/ml of streptomycin, and 1% of 200 mM glutamine at 37° with 5% CO_2. Cytotoxicity was evaluated with the colorimetric MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [16][17]. Cell suspensions were diluted to 10^5 cells/ml and were distributed in 96-well culture plates (220 μl in each well), which were incubated for 24 h at 37° in a humidified incubator with 5% CO_2. After 24 h, 22 μl of MeOH extract of *Erythroxylum caatingae* stems and alkaloid **3** were added, and the plates were incubated again at 37°. At the end of this period, the culture medium with excess MTT was removed, and 100 μl of DMSO were added to each well to dissolve the formazan crystals. The optical density (OD) of the wells was measured at 595 nm with an ELISA plate reader and compared to the control.

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[1]) Crystallographic data have been deposited with the *Cambridge Crystallographic Data Center* as supplementary publication No. CCDC-711367. Copies of available material can be obtained, free of charge, upon request through 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).

Table 5. *Crystal Data and Structure Refinement for Compound* **1a**

Empirical formula	$C_{24}H_{28}ClNO_7$
Formula weight [g mol^{-1}]	477.92
Temp. [K]	295(2)
Crystal dimensions [mm]	$0.19 \times 0.17 \times 0.13$
Crystal system	Monoclinic
Space group	$C2$
Unit cell dimensions	
a [Å]	22.5640(13)
b [Å]	14.8870(5)
c [Å]	8.3820(5)
β [°]	97.135(2)
V [Å^3]	2793.8(2)
Z	4
Λ(MoK_α) radiation [Å]	0.71073
p_{calc} [Mg m^{-3}]	1.136
μ(MoK_α) [mm^{-1}]	0.174
F_{000}	1008
θ Range for data collection [°]	2.5–27.5
Index range	$-23 \le h \le 29,\ -15 \le k \le 19,\ -8 \le l \le 10$
Reflections collected	9939
Independent reflections [R_{int}]	5809 [0.024]
Reflections with $I > 2\sigma(I)$	4374
Number of parameters refined	299
R [$F^2 > 2\sigma(F^2)$]	0.085
Goodness-of-fit on F^2	1.07
Residual electron density [e A^{-3}]	0.93

Animals. Female Swiss albino mice (*Mus musculus*), 60-d-old and weighing 25 ± 5 g, were obtained from the animal house of the Departamento de Antibioticos, UFPE, Brazil. The animals were housed under standard environmental conditions of temp., humidity, and under a light-dark cycle of 12 h. The mice were fed animal house diet (LABINE Purina, Brazil) and given water *ad libidum*. The animals were treated according to the ethics principles of animal experimentation of COBEA (Colegio Brasileiro de Experimentacão Animal), Brazil. The Animal Studies Committee of the Universidade Federal de Pernambuco approved the experimental protocols (No. 23076.012173/2007-77).

Antitumor Assay. Sarcoma 180 tumor cells were maintained in the peritoneal cavities of the Swiss mice in the Laboratorio de Bioensaios para Pesquisa de Farmacos from the Departamento de Antibioticos, UFPE, Brazil. Ascitic tumor cells (suspension of 5×10^6 cells) were injected subcutaneously in the axillary region of healthy animals previously weighed, and the mice were divided into experimental groups ($n = 7$) [18]. Twenty-four hours after inoculation, MeOH extract of *E. caatingae* stems (100, 200, and 400 mg/kg) was dissolved in normal saline soln. with cremophor EL (2%) and administered intraperitoneally for 7 d in mice transplanted with Sarcoma 180 tumor. Methotrexate (2.5 mg/kg) was used as the positive control. The negative control received the vehicle only. On day 8, the mice were weighed and euthanized. The tumors were dissected and weighed. The percentage of tumor inhibition was calculated in accordance with [19] as: $TWI = C - T/C \times 100$, where C is the weight of the control tumor and T is the weight of the treated tumor.

Statistical Analysis. Data are reported as means $\pm$ standard error of the mean (SEM) of n experimental animals. Statistical differences were compared by analysis of variance (ANOVA) followed by *Tukey*'s multiple comparison test. Differences were considered statistically significant if $P < 0.05$.

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