Cytotoxic Evaluation of Pungencine: A New Tropane Alkaloid from the Roots of *Erythroxylum pungens* O. E. SCHULZ

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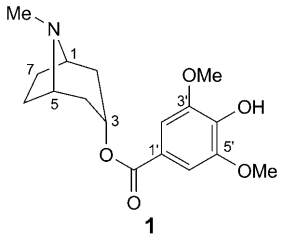
A new tropane alkaloid as well as a new substitution group, (3-*endo*,8-*anti*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 4-hydroxy-3,5-dimethoxybenzoate, called pungencine (**1**), was isolated from the roots of *Erythroxylum pungens* O. E. SCHULZ. The structure was elucidated by spectral analyses, including ^{1}H- and ^{13}C-NMR and 2D-NMR techniques (^{1}H,^{1}H-COSY, NOESY, DEPT, HMQC, and HMBC) and HR-ESI-MS. Furthermore, compound **1** was tested for cytotoxicity against several cell lines (Jurkat, HL-60, U937, K562, KG-1, and U266) and was determined not to inhibit cell viability at 10 μM.

Introduction. – The genus *Erythroxylum* (Erythroxylaceae), which is distributed throughout the tropics [1], comprises *ca.* 240 species of tropical trees and shrubs [2]. *Erythroxylum pungens* O. E. SCHULZ (Erythroxylaceae), vulgarly known as 'rompe-gibao', is a tree found in the caatinga region of Brazil, specially in the Brazilian states of Bahia, Ceará, Maranhão, Pernambuco, and Piauí [3]. The enormous variety of *Erythroxylum* species in Brazil and the potential activity of tropane alkaloids prompted us to focus on the alkaloid content of the roots of *Erythroxylum pungens* O. E. SCHULZ. Thus, we isolated the alkaloids from the roots of *E. punges*, for which no prior investigation regarding chemical analysis had been reported, and characterized a crystalline compound, pungencine (**1**). The cytotoxic evaluation of **1** against several cell lines (Jurkat, HL-60, U937, K562, KG-1, and U266) was also tested.



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Recently, our research group has published a review on ^{13}C-NMR data of tropane alkaloids from the *Erythroxylum* genus and of ecdysteroids in *Vitex* (Verbenaceae) species [4][5]. This type of investigation permits an efficient way to identify a specific type of compounds present in plants. Furthermore, these studies contribute to establish the chemotaxonomy of tropane alkaloids in the *Erythroxylum* genus.

Results. – The crystal-containing fractions from *E. pungens* yielded an optically active compound **1** with the quasi-molecular ion in the HR-ESI-MS at *m*/*z* of 322.1655 ($[M+H]^+$, $C_{17}H_{24}NO_5^+$). Analyses of the ^{1}H-NMR, ^{1}H,^{1}H-COSY, NOESY, HMBC, and HMQC data were used to characterize the new tropane alkaloid, pungicine (**1**). The following ^{1}H-NMR resonances were attributable to a tropane ring system: δ(H) 2.37 (*d*, $J=15.1$, H_{exo}–C(2,4)), 1.89 (*d*, $J=15.1$, H_{endo}–C(2,4)), 2.16 (br. *s*, CH_2(6,7)), 3.88 (br. *s*, H–C(1,5)), and 5.18 (*t*-like, $J=4.47$, H–C(3)). The ^{1}H,^{13}C-HMBCs H–C(2′,6′)/C(3′,5′) and H–C(2′,6′)/C(4′) were used to assign the aromatic ring, and the NOESY correlations between MeN and H–C(6) and H–C(7) corroborated the position of the Me group (*Fig.*). All data are summarized in the *Table*.

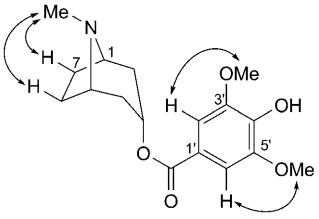

Fig. 1. *Selected NOESY correlations and relative configuration of* **1**. Arrows denote the principal NOESY correlations.

Pungencine (**1**) was tested for cytotoxicity against several cell lines (Jurkat, HL-60, U937, K562, KG-1, and U266) and was determined not to inhibit cell viability at 10 μM.

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Experimental Part

General. Thin-layer chromatography (TLC): silica gel plates (SiO_2); spots were detected by spraying with *Draggendorff*'s reagent. Column chromatography (CC): Al_2O_3. Optical rotations: *Perkin-Elmer-192* polarimeter equipped with an Na lamp (589 nm) and 10-cm microcell. NMR Spectra: *Bruker-DRX-500* spectrometer; in CD_3OD; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz; DEPT, ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments were performed with the UXNMR software package. HR-ESI-MS: *Waters Micromass LCT Premier* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. Roots of *E. pungens* were collected in Brazil, State of Paraiba, near the town São José de Espinharas, in March 2007. A voucher specimen (IPA-81029) was deposited with the Herbarium Dárdano de Andrade Lima (Empresa Pernambucana de Pesquisa Agropecuária (IPA) – Pernambuco State, Brazil.

Isolation and Extraction, and Characterization of Pungencine (= *(3*-endo,*8*-anti*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 4-Hydroxy-3,5-dimethoxybenzoate*; **1**). Dried root material (1829 g) was exhaustively extracted with 95% EtOH at r.t. Part of the EtOH extract (200 g) was submitted to alkaloid extraction. The whole fraction of tertiary alkaloids (50 g) was subjected to CC (basic Al_2O_3,

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp., $CDCl_3$) *of Pungencine* (**1**), *Including Results Obtained by Heteronuclear 2D Shift-Correlated HSQC and HMBC*[a]). δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC	
			^2J(C,H)	^3J(C,H)
H–C(1)	60.39	3.88 (br. *s*)		H–C(3), MeN
CH_2(2)	35.40	2.37 (*d*, J = 15.1, H_{exo}),	H–C(3)	
		1.89 (*d*, J = 15.1, H_{endo})	H–C(3)	
H–C(3)	66.42	5.18 (*t*-like, J = 4.47)	H_{endo}–C(2,4)	
CH_2(4)	35.40	2.37 (*d*, J = 15.1, H_{exo}),	H–C(3)	
		1.89 (*d*, J = 15.1, H_{endo})	H–C(3)	
H–C(5)	60.39	3.88 (br. *s*)		H–C(3), MeN
CH_2(6)	25.13	2.16 (br. *s*)		
CH_2(7)	25.13	2.16 (br. *s*)		
C(1′)	120.42		H–C(2′,6′)	
H–C(2′,6′)	106.72	7.21 (*s*)		
C(3′,5′)	147.13		H–C(2′,6′)	*Me*O–C(3′,5′)
C(4′)	140.26			H–C(2′,6′)
COO	165.54			H–C(3), H–C(2′,6′)
MeN	39.22	2.40 (*s*)		
*Me*O–C(3′,5′)	56.24	3.88 (*s*)		

[a]) The number of H-atoms bound to a C-atom was deduced by comparative analysis of {^{1}H}- and APT-^{13}C-NMR spectra. δ and J values from the 1D ^{1}H-NMR spectrum. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC, and ^{1}H,^{1}H-COSY experiments.

$CHCl_3 \rightarrow CHCl_3$/MeOH 95:5, 125 ml fractions (a total of 136), TLC monitoring (SiO_2; $CHCl_3$/MeOH 95:5)). Crystals of **1** (6 mg) appeared in the *Fractions 77–85*. $[\alpha]_D^{20} = -9.8$ (c = 0.1, MeOH). NMR: *Table*. HR-ESI-MS: 322.1655 ($[M+H]^+$, $C_{17}H_{24}NO_5^+$; calc. 322.1654).

Cytotoxicity Assay. The cytotoxicity assay was performed according to *Promega*'s *CellTiter-Blue®* cell viability assay kit [6]. Briefly, cells (Jurkat, HL-60, U937, K562, KG-1, and U266) were distributed into 96-well plates containing RPMI 1640 medium supplemented with 10% fetal-calf serum, and 10 units/ml of penicillin–streptomycin. The samples were incubated for 48 h, resazurin (= 7-hydroxy-3*H*-phenoxazin-3-one 10-oxide; 20 µl) was added to each well, and the cells were incubated for an additional 2 h prior to fluorescence measurements with a microplate fluorometer (λ_{ex} 560 nm, λ_{em} 590 nm). All experiments were performed in triplicate.

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