Pessoine and Spinosine, Two Catecholic Berbines from *Annona spinescens*[1]

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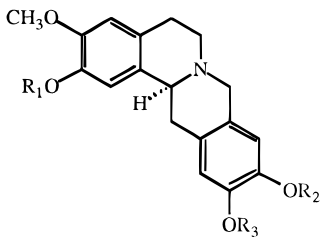
The trunk bark and roots of *Annona spinescens* have been investigated for their alkaloid content. Two new berbine alkaloids, pessoine (**1**) and spinosine (**2**), have been isolated from the bark, and their structures were elucidated by spectroscopic methods. Eight known isoquinoline alkaloids were also obtained. The trypanocidal and antileishmanial activities of these isolated compounds have been investigated.

The Annonaceae represent one of the largest families of the order Magnoliales, comprising about 120 genera and more than 2000 species.[2] The family is distributed in tropical and subtropical regions, occurring predominantly as aromatic trees, shrubs, or climbers.[3−5] *Annona spinescens* Mart. is a climber attaining 5 m in length, which grows along the east coast of Brazil.[6] In the present study, the plant material was collected in João Pessoa, Paraiba, Brazil. The extracts containing the crude alkaloids (0.29% from trunk bark and 0.05% from roots) were obtained by conventional methods. Seven known isoquinoline alkaloids were isolated from the roots (Table 1), together with a nonalkaloidal compound, methyl sinapate, a cinnamic acid derivative. From the trunk bark were isolated two noraporphines, a benzyltetrahydroisoquinoline, and two new catecholic alkaloids with a berbine skeleton, (−)-pessoine (**1**) and (−)-spinosine (**2**).

The EIMS of pessoine (**1**) showed a molecular ion peak at *m*/*z* 313, a strong peak at *m*/*z* 178 (93% of base peak), and a fragmentation pattern indicative of a berbine skeleton bearing two hydroxy groups on ring D (*m*/*z* 136) and one hydroxy and one methoxy group on ring A (*m*/*z* 178).[11] Chemical proof of this partial structure was obtained by acetylation of **1** with Ac_2O-pyridine, which afforded the tri-*O*-acetyl derivative (**1a**).

This unusually low degree of methylation present in the structure of **1** was confirmed from the ^{1}H-NMR spectrum, which exhibited a single methoxy signal at δ 3.75. Four aromatic proton singlets at δ 6.44, 6.49, 6.52, and 6.67 and the absence of any C-8 methylene resonance near δ 4.35 were indicative of a substitution at C-2, C-3, C-10, and C-11,[12,13] which was confirmed by the generation of xylopinine (**3**)[14] after methylation with CH_2N_2.

The homonuclear 2D NMR spectrum (COSY-DQF)[15] established the existence of three spin-systems in the aliphatic region of **1**, namely, an isolated AB system at δ 3.50 and 3.74 (CH_2-8); an AMX system (δ 2.64, 3.09, and 3.43), which could be assigned to CH_2-13 and CH-14; and a set of two multiplets (δ 2.55 and 3.01) for the



1 R_1=R_2=R_3=H
2 R_1=CH_3; R_2=R_3=H
3 R_1=R_2=R_3=CH_3

CH_2-5 and CH_2-6 methylene groups. The positions of the aromatic protons were obtained by ^{1}H-^{13}C correlations (HMBC, HMQC) with the vicinal aromatic and aliphatic carbons. The signals at δ 6.67 and 6.49 were attributed to H-1 and H-4, respectively, and those at δ 6.44 and 6.52 to H-9 and H-12. The existence of correlations between H-1 (δ 6.67) and C-14 (δ 59.2), and between H-4 (δ 6.49) and C-5 (δ 27.7) indicated the position of both aromatic protons, without ambiguity. The position of the methoxy group was further established using ^{1}H-^{13}C correlations and NOE-difference experiments.[16] The NOE enhancement observed on H-4 (δ 6.49) by saturating the methoxy protons at δ 3.75 indicated the position of the methoxy group at C-3 (Figure 1).

Pessoine (**1**) is, therefore, an isomer of artavenustine (2-methoxy-3,10,11-trihydroxyberbine) isolated from the

Table 1. Alkaloids Isolated from *Annona spinescens* (% w/w Yields from Total Alkaloids)

trunk bark	roots
tetrahydroprotoberberines	noraporphines
(−)-pessoine (**1**) (10%)	(−)-anonaine[7] (11.5%)
(−)-spinosine (**2**) (4.7%)	(−)-norushinsunine[7] (8.3%)
	(+)-nordomesticine[7] (4.3%)
	(+)-norbracteoline[8] (3.1%)
noraporphines	aporphine
(−)-norushinsunine[7] (5.1%)	(+)-bracteoline[7] (5.9%)
(−)-anonaine[7] (10%)	
benzyltetrahydroisoquinoline	oxoaporphine
(+)-reticuline[10] (2.0%)	liriodenine[7] (9.3%)
	proaporphine
	(−)-stepharine[9] (2.1%)

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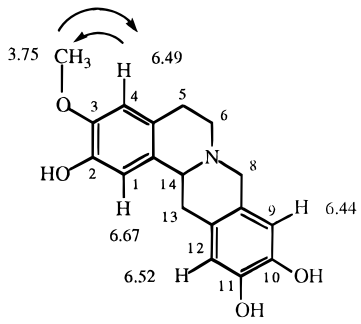

Figure 1. ^{1}H-NMR chemical shifts and NOEs of pessoine (**1**).

stem bark of *Artabotrys venustus*.[16] The absolute configuration has been determined as 14*S* (H-14α) from the (−)-optical rotation.[17]

The UV spectrum of spinosine (**2**) showed typical absorptions of a phenolic berbine skeleton at 208, 287, and 320 nm[17] and a bathochromic shift after addition of NaOH. The presence of two phenolic groups was indicated by the preparation of the di-*O*-acetylated derivative **2a**. The MS of **2** showed a molecular ion peak at *m*/*z* 327, a base peak at *m*/*z* 192, and a fragmentation pattern indicative of a berbine skeleton bearing two methoxy groups on ring A and two hydroxy groups on ring D.[11] The phenol positions at C-10 and C-11 were indicated by the ^{1}H-NMR data and chemical correlation with the known xylopinine (**3**). The absolute 14*S* configuration (H-14α) of (−)-spinosine (**2**), 10,11-dihydroxy-2,3-dimethoxyberbine, has been deduced from its optical rotation.[17] Spinosine (**2**) seems to be identical with alkaloid E, the first catecholic berbine obtained from the trunk bark of *Polyalthia oligosperma*,[18] but the structure of alkaloid E, isolated and partially described as its acetylated derivative, has never been confirmed, and its stereochemistry was not determined. The natural compound **2** is isolated and described here for the first time.

Pessoine (**1**) and spinosine (**2**) are new members of the tiny group of catecholic isoquinoline alkaloids, considered as unstable intermediates in the biosynthesis of their methoxy analogues. Their present isolation is probably due to the extraction procedure using percolation with MeOH followed by liquid-liquid separation, instead of the traditional Soxhlet extraction, which usually results in a degradation of the *ortho*-diphenolic compounds initially present in the plant.

Pessoine (**1**), anonaine, acetylnordomesticine, norbracteoline, acetylbracteoline, liriodenine, stepharine, and methyl sinapate were evaluated for their trypanocidal activities in vitro (Table 2) under the conditions described previously.[19] The most significant activity was observed for anonaine and acetylbracteoline. Liriodenine and anonaine showed significant activity against promastigote forms of *Leishmania* species (Table 3).

Experimental Section

General Experimental Procedures. EIMS were obtained on a Nermag R1010C spectrometer. UV spectra were recorded on a Philips PU8700 series UV/vis spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 200 MHz and 50 MHz, respectively, on a Bruker AC-200 P spectrometer, and the ^{1}H-^{1}H (COSY-DQF) and ^{1}H-^{13}C (HMQC and HMBC) correlation spectra and NOEs at 400 MHz on a Bruker ARX-400 spectrometer. Optical rotations were determined using a Schmidt-Haensch Polartronic I polarimeter.

Table 2. *In Vitro* Activity of *Annona spinescens* Constituents against *Trypanosoma cruzi* (Trypomastigote Forms)

compd	activity (% lysis), (concentration 250 μg/mL)
pessoine (**1**)	55
liriodenine	50
anonaine	99
stepharine	65
acetylbracteoline	80
acetylnordomesticine	60
norbracteoline	62
methyl sinapate	44
gentian violet[a]	100
control	0

[a] Reference compound.

Table 3. *In Vitro* Activity of *Annona spinescens* Constituents against *Leishmania* Species (Promastigote Forms)

	concentration (μg/mL) resulting in total lysis of parasites		
compd	*L. brasiliensis* (2903)	*L. amazonensis* (LV 79)	*L. donovani* (PP-75)
liriodenine	100[b]	100	100
		50[b]	25[b]
anonaine	50	25	100
		10[b]	10[b]
glucantime[a]	>100	>100	>100

[a] Reference compound. [b] More than 90% lysis.

Plant Material. Aerial parts of *Annona spinescens* (Annonaceae) were collected in July 1994, along the Paraiba River, João Pessoa, Paraiba, Brazil. Voucher specimens are deposited at the Prof. Lauro Pires Xavier Herbarium (JPB-No. 18.329) and were identified by Prof. Carlos Alberto B. de Miranda of the Department of Science of Nature, University of Paraiba, Brazil.

Extraction and Isolation. Trunk bark (341 g) of *A. spinescens* was defatted with petroleum ether and extracted with MeOH. The MeOH extract was diluted with H_2O and extracted with hexane and then CH_2Cl_2. The hydroalcoholic solution was basified with concentrated aqueous NH_4OH and extracted with CH_2Cl_2. The organic layer afforded 1 g (0.29%) of crude alkaloids, which were separated by column chromatography on Si gel, eluting with CH_2Cl_2−MeOH (99:1). The previously reported alkaloids obtained were characterized spectroscopically and by direct comparison with authentic samples. Roots of *A. spinescens* (1.5 kg) were treated in the same manner, affording 1.1 g (0.05%) of crude alkaloids, which were purified as before. Physical and spectral data of the known alkaloids obtained were in good agreement with published values. Pessoine (**1**) and spinosine (**2**) were further purified by TLC on Si gel, eluting with CH_2Cl_2−MeOH (98:2).

Pessoine (1): amorphous; $[\alpha]^{20}_D$ −160° (*c* 0.2, MeOH); UV (EtOH) λ max (log ϵ) 214 (4.8), 288 (4.5) nm; (EtOH + OH^-) 192 (4.5), 218 (4.9), 303 (4.5) nm; ^{1}H NMR ($CDCl_3$-CD_3OD, 95:5, 400 MHz) δ 2.55, 3.01 (2H each, m, CH_2-5 and 6), 2.64 (1H, dd, J = 16.4 and 12 Hz, H-13ax), 3.09 (1H, dd, J = 16.4 and 4.1 Hz, H-13eq), 3.43 (1H, dd, J = 4.1 and 12 Hz, H-14), 3.50, 3.74 (2H, 2d, J = 15 Hz, CH_2-8), 3.75 (3H, s, OMe), 6.44 (1H, s, H-9), 6.49 (1H, s, H-4), 6.52 (1H, s, H-12), 6.67 (1H, s, H-1); ^{13}C NMR ($CDCl_3$-CD_3OD, 95:5, 50 MHz) δ 27.7 (C-5), 34.8 (C-13), 51.0 (C-6), 55.0 (MeO-3), 57.4 (C-8), 59.2 (C-14), 110.5 (C-4), 111.3 (C-1), 111.7 (C-9), 114.9

(C-12), 124.3 (C-8a), 124.6 (C-12a), 124.7 (C-4a), 129.2 (C-14a), 143.0 (C-10), 143.3 (C-11), 144.0 (C-2), 145.0 (C-3); EIMS *m/z* 313 (M^+, 38), 178 (93), 136 (47), 83 (100).

***O*-Methylation of Pessoine (1).** Compound **1** (7 mg), dissolved in MeOH, was methylated with CH_2N_2 in Et_2O, yielding xylopinine (**3**) (4.5 mg, 57%), identical (TLC, ^{1}H NMR, MS) with an authentic sample.

Tri-*O*-acetylpessoine (1a). Pessoine (**1**) (7 mg) was acetylated with Ac_2O-pyridine 1:1, affording the tri-*O*-acetyl derivative **1a** (5 mg, 51%): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 2.20 (3H, s, OAc), 2.29 (6H, s, 2 × OAc), 3.78 (3H, s, OCH_3), 6.65, 6.82, 6.90, 6.95 (1H each, 4s, H-1, H-4, H-9, and H-12); EIMS *m/z* 439 (M^+, 85), 220 (11), 219 (37), 218 (19), 43 (100).

Spinosine (2): amorphous; $[\alpha]^{20}_D$ −35° (*c* 0.3, EtOH); UV (EtOH) λ max (log ϵ) 208 (4.7), 287 (4.0), 320 (3.5) nm; (EtOH + OH^-) 210 (4.8), 291 (4.0), 336 (3.6) nm; ^{1}H NMR ($CDCl_3$-CD_3OD, 95:5, 400 MHz) δ 3.78 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 6.44 (1H, s, H-9), 6.54 (2H, s, H-4, H-12), 6.63 (1H, s, H-1); ^{13}C NMR ($CDCl_3$-MeOD, 95:5, 50 MHz) δ 27.8 (C-5), 34.9 (C-13), 50.9 (C-6), 55.2 (MeO-2), 55.7 (MeO-3), 57.4 (C-8), 59.4 (C-14), 108.8 (C-1), 111.1 (C-4), 112.2 (C-9), 114.6 (C-12), 124.1 (C-8a), 124.6 (C-12a), 125.7 (C-4a), 128.4 (C-14a), 142.9 (C-10), 143.3 (C-11), 147.2 (C-2), 147.7 (C-3); EIMS *m/z* 327 (M^+, 78), 192 (100), 176 (22), 136 (48).

***O*-Methylation of Spinosine (2).** Spinosine (**2**) (5 mg), dissolved in MeOH, was methylated with CH_2N_2 in Et_2O, yielding xylopinine (**3**) (3 mg, 55%) identical (TLC, ^{1}H NMR, MS) with an authentic sample.

Di-*O*-acetylspinosine (2a). Spinosine (**2**) (7 mg) was acetylated with Ac_2O-pyridine 1:1, affording the di-*O*-acetyl derivative **2a** (4.2 mg, 48%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.20 (6H, s, 2 × OAc), 3.80 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 6.57, 6.60, 6.85, and 6.95 (1H each, s, H-1, H-4, H-9, and H-12); EIMS *m/z* 411 (M^+, 51), 410 (10), 220 (17), 190 (45), 43 (100).

Biological Assays. Cultures of *Leishmania* spp. and *Trypanosoma cruzi* were obtained from IBBA (Instituto Boliviano de Biologia de Altura), La Paz, and identified by isoenzyme analysis. Three species of *Leishmania* were used during these investigations, *L. brasiliensis* (2903), *L. amazonensis* (LV 79), and *L. donovani* (PP-75).

Promastigote forms of *Leishmania* spp., were grown at 28 °C in USMARU medium[20] containing 10% heat-inactivated (56 °C for 30 min) fetal bovine serum. The isolated compounds were dissolved in known volumes of DMSO and then in medium, from which aliquots were drawn. The viability of the parasites was estimated by direct observation after 48 h incubation at 28 °C, with an inverted microscope.[19] The base line drug was Glucantime (Rhône-Poulenc, France).

Trypomastigote (blood circulating) forms of *Trypanosoma cruzi* were obtained from blood samples of Balb C-infected mice, seven days after inoculation of the parasite. The isolated compounds were dissolved in a saline-glucose solution of Kreb's-Ringer at pH 7.4 containing 0.25% of DMSO. The solutions (0.1 mL) were incubated at 4 °C with the blood samples (0.9 mL) containing the parasites. After 24-h incubation, the parasites were counted using a Thoma cell.[21] The base line drug was gentian violet.

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References and Notes

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