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NEW APORPHINE ALKALOIDS FROM *GUATTERIA FOLIOSA*[1]

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ABSTRACT.—Four new alkaloids were obtained from *Guatteria foliosa*, namely, the noraporphines (−)-3-methoxyputerine [**1**] and (+)-norguattevaline [**2**], the more highly oxidized (+)-3-methoxyguattescidine [**3**], and the oxoaporphine 3-methoxyoxoputerine [**4**]. Among several other known alkaloids also found in this same plant, (−)-3-hydroxynornuciferine, (−)-isoguattouregidine, and argentinine exhibited significant activity against *Trypanosoma cruzi*.

Guatteria foliosa Benth. (Annonaceae), commonly known as "Sayakasi" by the local Chimane Indians, was collected in the "Alto-Beni," a tropical region of Bolivia. This species, previously known to occur in Guyana, Brazil, and Venezuela, has been described by Fries in his revision of the genus *Guatteria* (2), and belongs to the section *Trichostemon* together with *G. maypurensis, G. trichostemon,* and *G. polyantha*. This tree is used by indigenous people as an insect repellent. Its chemical composition is investigated here for the first time.

Preliminary studies carried out by IBBA (Instituto Boliviano de Biologia de Altura) and ORSTOM (Institut Français de Recherche Scientifique pour le Développement en Coopération) on *Guatteria foliosa* extracts demonstrated an interesting antiparasitic activity against different strains of *Leishmania* spp., responsible for leishmaniasis, and against *Trypanosoma cruzi*, the causative factor of Chagas's disease. In these studies, four extracts were prepared from the stem bark and roots, namely two petroleum ether and two alkaloidal extracts. Biological activity was measured in vitro at 100 μg/ml on the promastigote form of three species of *Leishmania (L. braziliensis, L. amazonensis, L. chagasi)*, and on the epimastigote form of three strains of *Trypanosoma cruzi (T. c. tulahuen,* c8cl1, and *tehuentepec)* (Table 1) (3).

From the studies, it became clear that the neutral extracts were inactive, and that the antiprotozoal activity is due to the alkaloids that induce complete lysis of the parasites. Fractionation of the alkaloid extract of *Guatteria foliosa* stem bark resulted in the isolation of 13 substances, comprised of two neutral compounds and 11 isoquinoline alkaloids.

RESULTS AND DISCUSSION

The powdered stem bark of *G. foliosa* was extracted by percolation with MeOH. Neutral compounds were extracted with hexane and then with CH_2Cl_2. The alkaloids were subsequently extracted with CH_2Cl_2 after alkalinization and then subjected to chromatographic separation.

Two known cinnamic acid derivatives, methyl ferulate and methyl sinapate, were isolated in low yield from the less polar fractions of the "alkaloid" extract.

Of the 11 isoquinoline alkaloids isolated, seven were identified as known compounds, namely, the aporphines (−)-elmerrillicine (4), (−)-norstephalagine (5,6), (−)-

[1]Part 98 in the series "Alcaloïdes des Annonacées." For part 97, see Nghia *et al.* (1).

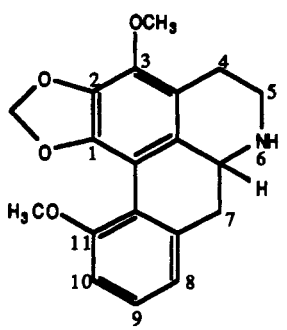
TABLE 1. In Vitro Inhibitory Effects of *Guatteria foliosa* Extracts on *Leishmania* (Promastigote) and *Trypanosoma cruzi* (Epimastigote).

	Extract[b]	Organism/Activity[a]					
		Leishmania[c]			*Tryp. cruzi*[d]		
		L.b.	*L.a.*	*L.c.*	*T.c. tula.*	*T.c. c8cl1*	*T.c. tehue.*
Roots	Petroleum Ether	0	0	0	0	0	0
	Alkaloidal Extract	+++	+++	+++	+++	+++	+++
Stem bark	Petroleum Ether	0	0	0	0	0	0
	Alkaloidal Extract	+++	+++	+++	+++	+++	+++

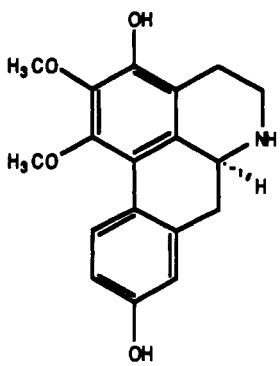
[a]0: no lysis; +++: total lysis of parasites.
[b]Concentration=100 μg/ml.
[c]*L.b.=L. braziliensis; L.a.=L. amazonensis; L.c.=L. chagasi.*
[d]*T.c. tula.=T.c. tulahuen; T.c. tehue.=tehuentepec.*

3-hydroxynornuciferine (7) and (−)-isoguattouregidine (8), the oxoaporphine atherospermidine (9), the aminoethylphenanthrene argentinine (10), and the tetrahydroprotoberberine (−)-coreximine (11). Their structures were confirmed by comparison of their spectroscopic data with those already published (12–15). The ^{13}C-nmr spectrum of isoguattouregidine is described here for the first time.

The remaining four alkaloids are new and were named (−)-3-methoxyputerine [**1**], (+)-norguattevaline [**2**], (+)-3-methoxyguattescidine [**3**], and 3-methoxyoxoputerine [**4**], respectively.



1



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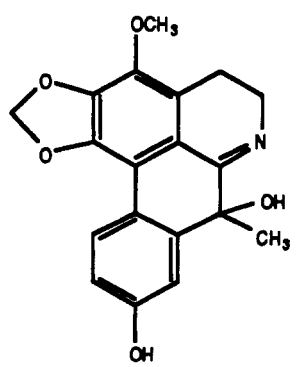

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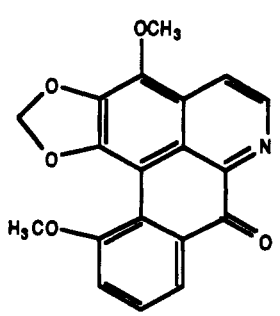

4

Following a classical separation, **1** was obtained as a mixture with (−)-elmerrillicine and (−)-norstephalagine. (−)-Elmerrillicine was separated from the non-phenolic bases by partition between 1N NaOH and Et_2O. Subsequently, (−)-3-methoxyputerine [**1**] and (−)-norstephalagine were further separated by prep. hplc under reversed-phase conditions. (−)-3-Methoxyputerine [**1**] presented a molecular weight of 325 daltons, corresponding to $C_{19}H_{19}NO_4$. The ^{1}H-nmr spectral data were closely comparable to those of (−)-elmerrillicine. The main difference lay in the replacement of the OH group at C-11 by a second OMe group appearing at 3.89 ppm. This difference was confirmed by the upfield shift of the OCH_2O group and by relevant observations in the ^{13}C-nmr spectrum. The negative specific rotation was indicative of a 6a*R* configuration (16).

(+)-Norguattevaline [**2**] is a phenolic noraporphine substituted with two methoxy groups as shown by its uv and nmr spectra (17,18). The molecular formula, $C_{18}H_{19}NO_4$, indicated the presence of a second hydroxy group. In the ^{1}H-nmr spectrum, an AMX system of three protons pointed to the presence of a monosubstituted ring D. The resonance of H-11 as a doublet at 8.04 ppm was consistent with substitution at C-9 (18). The singlet at 3.67 ppm was typical of a methoxy group at the C-1 position (18). The ^{13}C-nmr chemical shift of the two methoxy groups at 59.8 and 60.7 ppm showed that the second methoxy group is also located on ring A, either at C-2 or C-3 (12–15). The exact position of the substituents was determined by nOe nmr experiments. Irradiation of the C-1 methoxy singlet at 3.67 ppm showed the expected enhancement of the 8.04 ppm signal representing H-11. On the other hand, irradiation of the methoxy group at 3.94 ppm had no effect on the aliphatic protons at C-4, which is in agreement with the location of the OMe at C-2.

Alkaloid **3**, (+)-3-methoxyguattescidine, was purified by prep. hplc, and was obtained as an amorphous dextrorotatory compound. The molecular formula $C_{19}H_{17}NO_5$ was established from the hrms. The uv spectrum, typical of an aporphine, showed bathochromic shifts upon both the addition of base and acid, indicating the phenolic nature of this alkaloid and the presence of an imine function (17,18). The ^{1}H-nmr spectrum displayed the typical AX system of a 1,2-methylenedioxy group (18), an AMX system of three protons assigned to a C-9 monosubstituted ring D, and a singlet at 4.02 ppm typical of a methoxy group at C-3 in a trisubstituted ring A. The phenolic OH was thus located at C-9. In the aliphatic region, only four protons were observed instead of seven in a *sensu stricto* aporphine. They were assigned to H-4 and H-5 of a 6,6a-dehydroaporphine disubstituted at C-7 as confirmed by the ^{13}C-nmr spectrum. On the other hand, the ^{1}H-nmr spectrum showed a singlet at 1.47 ppm consistent with a methyl group located at C-7, and geminal to an hydroxy group. It has been previously shown that the alkaloid ring system is not planar; the biphenyl moiety is twisted by about 20°, with the C-7 hydroxy group pseudoequatorial and the *C*-methyl pseudoaxial (20).

Two oxoaporphines were isolated during the purification of (−)-3-methoxyputerine [**1**] and (−)-norstephalagine by prep. hplc, and correspond to oxidation products. Their uv spectra were characteristic of oxoaporphine alkaloids; they exhibited two uv absorption bands above 350 nm, diagnostic of extensive conjugation (21). The first of these was identified as the known atherospermidine (9). The second, 3-methoxyoxoputerine [**4**], exhibited in the mass spectrum a molecular ion at *m/z* 335 corresponding to the elemental formula $C_{19}H_{13}NO_5$. The ^{1}H-nmr spectrum was typical of an oxoaporphine; all the signals were shifted downfield, there were no aliphatic protons, and the methylenedioxy group resonated as a singlet at 6.24 ppm. The aromatic region showed protons H-4 and H-5 at 8.18 ppm and 8.90 ppm, respectively, and three protons of an 11-monosubstituted ring D. The C-3 and C-11 positions were occupied by two methoxy groups.

TABLE 2. In Vitro Study on the Promastigote Form of *Leishmania* Species.

Compound[a]	*L. donovani* (pp-75)[b]	*L. braziliensis* (2903)[b]	*L. amazonensis* (H-142)[b]	*L. amazonensis* (LV 79)[b]
Isoguattouregidine	+++	++	+++	+++
Argentinine	+	++	+	+
Coreximine	++	++	+	+
Pentamidine.....	+++	+++	+++	+++
Glucantime	+++	+++	+++	+++

[a]Concentration=100 μg/ml.

[b]0: no lysis; +: parasites less mobile than controls; ++: modified or immobilized parasites; +++: total lysis of parasites.

Alkaloids isolated and purified in sufficient amount were tested on four strains of *Leishmania* spp. (Table 2), under the same conditions as the crude extracts (Table 1) (3). Due to the small amount of pure compounds available, only the three major alkaloids could be tested. Isoguattouregidine was the only alkaloid showing any significant activity against *Leishmania donovani* and *L. amazonensis*.

On the other hand, most of the alkaloids were available in sufficient quantities to be tested against *Trypanosoma cruzi*. They were evaluated in the bloodstream form (trypomastigote) isolated from a blood sample of *T. cruzi* infected mice (Table 3), as described elsewhere (22). This parasite stage is encountered in the bloodstream during the acute stage of the disease. The three alkaloids exhibiting significant activity (% lysis $\geq$ 60%) against *T. cruzi* were identified as the noraporphine, 3-hydroxynornuciferine, the 7-hydroxy-7-methylaporphine, isoguattouregidine, and the aminoethylphenanthrene, argentinine. The substantial differences among the three structures did not allow for the elaboration of any structure-activity relationships.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Philips PU 8700 spectrophotometer. All ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ (δ ppm) on a Bruker AC 200 P spectrometer operating at 200 and 50 MHz, respectively. Eims spectra were obtained on a Kratos MS-80 spectrometer.

PLANT MATERIAL.—Stem bark of *Guatteria foliosa* was collected by A. Fournet (AF 390) in September 1987, at Fatima de Chimane, Beni, Bolivia (altitude 350 m). The botanical identification of this species was performed by Prof. P.J.M. Maas and Dr. A.M. Polak, at the University of Utrecht, Netherlands. Voucher specimens are deposited in the National Herbarium of Bolivia (La Paz) and in the herbarium of the Rijksuniversiteit-Utrecht (nos. 792 and 848).

TABLE 3. In Vitro Study on the Trypomastigote Form of *Trypanosoma cruzi*, Strain Y.

Compound[a]	Activity (% lysis)
Elmerrillicine	0
3-Methoxyputerine [**1**]	35.7±2.0
Norguattevaline [**2**]	2.55±2.7
3-Hydroxynornuciferine	68.2±1.7
Isoguattouregidine	92±1.9
3-Methoxyguattescidine [**3**]	0
3-Methoxyoxoputerine [**4**]	46.6±1.1
Argentinine	81.3±3.7
Coreximine	0
Gentian violet	100

[a]Concentration=250 μg/ml.

EXTRACTION.—Extraction method for preliminary biological studies: Dried and powdered roots and stem bark (10 g) were macerated with petroleum ether (50 ml) for two days. The extractives were filtered and evaporated to dryness, affording the petroleum ether extracts. Other samples of the plant material (10 g) were triturated with NH_4OH and then macerated with $CHCl_3$ (50 ml) for two days, leading to the alkaloidal extracts.

Extraction method for alkaloids: powdered stem bark (4 kg) was extracted by percolation with MeOH. The MeOH extract was concentrated under reduced pressure, diluted with H_2O, and the neutral compounds were extracted with hexane (residue 12.23 g) and then with CH_2Cl_2 (residue 133.7 g). The aqueous layer, alkalinized with NH_4OH and extracted with CH_2Cl_2, afforded a CH_2Cl_2 extract (17.7 g, 4.8%) containing the total alkaloids.

ISOLATION.—The crude CH_2Cl_2 extract (17.7 g) was purified by the usual chromatographic methods on Si gel (cc and tlc) followed by reversed-phase prep. hplc when necessary. Si gel GF_{254} was used for tlc, Si gel 60 N and 60 H were used for cc. Exclusion chromatography was performed on Sephadex LH-20 using CH_2Cl_2-MeOH (2:1) as solvent. Liquid chromatography consisted of a programmable hplc pump (Waters 590) and a uv detector (Waters 484) connected to a data module (Waters). Separations were performed on μBondapak C-18, 10 μm, 125 Å column (25×100 mm)+ guard column. The solvent system was MeOH-H_2O-HOAc (50:45.5:0.5), with a flow rate of 20 ml/mn, and uv detection at 277 nm.

(−)-*3-Methoxyputerine* [**1**].—$C_{19}H_{19}NO_4$, 23 mg, amorphous; $[\alpha]_D$ −75° (EtOH); uv λ max (MeOH) (log ε) 217 (4.31), 276 (4.08) nm; eims *m/z* 325.1302 (M^+, 98) (325.1319 calcd), 324 (100), 323 (18), 310 (15), 296 (21), 295 (28), 294 (37); ^{1}H nmr (200 MHz, $CDCl_3$) δ 2.52–2.98 (5H, m, 2H-4, 2H-7, H-5ax), 3.38 (1H, dd, *J*=12 and 2 Hz, H-5eq), 3.75 (1H, dd, *J*=11 and 4 Hz, H-6a), 3.89 (3H, s, OCH_3-11), 4.03 (3H, s, OCH_3-3), 5.83 and 6.03 (2H, 2d, *J*=2 Hz, OCH_2O), 6.86 (1H, dd, *J*=8 and 2 Hz, H-8 or 10), 6.90 (1H, dd, *J*=8 and 2 Hz, H-8 or 10), 7.20 (1H, t, *J*=8 Hz, H-9); ^{13}C nmr (50 MHz, $CDCl_3$) δ 23.4 (C-4), 37.5 (C-7), 42.5 (C-5), 53.7 (C-6a), 55.7 (OCH_3-11), 59.4 (OCH_3-3), 100.2 (OCH_2O), 108.1 (C-1a), 110.6 (C-10), 118.2 and 119.7 (C-3a and C-11a), 120.1 (C-8), 128.0 (C-9), 129.5 (C-1b), 135.2 (C-2), 137.4 (C-7a), 140.3 and 144.5 (C-1 and C-3), 156.0 (C-11).

(+)-*Norguattevaline* [**2**].—$C_{18}H_{19}NO_4$, 38 mg, amorphous; $[\alpha]_D$ +35° (EtOH); uv λ max (EtOH) (log ε) 210 (4.49), 285 (4.27) nm; (OH^-) 213 (4.64), 308 (4.38) nm; eims *m/z* 313.1297 (M^+, 83) (313.1319 calcd), 312 (100), 298 (18); ^{1}H nmr (200 MHz, $CDCl_3$+CD_3OD) δ 3.67 (3H, s, OCH_3-1), 3.94 (3H, s, OCH_3-2), 6.68 (1H, d, *J*=3 Hz, H-8), 6.73 (1H, dd, *J*=3 and 9 Hz, H-10), 8.04 (1H, d, *J*=9 Hz, H-11); ^{13}C nmr (50 MHz, $CDCl_3$+CD_3OD) δ 21.0 (C-4), 34.7 (C-7), 41.3 (C-5), 53.2 (C-6a), 59.8 and 60.7 (OCH_3-1 and -2), 109.2 (C-1a), 114.1 and 114.4 (C-8 and C-10), 118.5 (C-3a), 123.2 (C-11a), 128.2 (C-11), 134.5 (C-1b), 139.6 (C-7a), 145.9 (C-2), 148.8 and 148.9 (C-1 and C-3), 155.5 (C-9).

(−)-*Isoguattouregidine*.—$C_{19}H_{19}NO_5$, 35 mg, amorphous; $[\alpha]_D$ −25° (EtOH); ^{13}C nmr (50 MHz, $CDCl_3$) δ 19.5 (C-4), 33.1 (CH_3-7), 45.4 (C-5), 60.3 and 61.1 (OCH_3-1 and -3), 73.1 (C-7), 111.4 (C-8), 114.7 (C-10), 117.6 (C-1a), 119.4 (C-1b and C-11a), 120.3 (C-3a), 128.7 (C-11), 143.5, 143.8, 144.7 (C-1, C-2, C-7a), 148.5 (C-3), 156.4 (C-9), 170.6 (C-6a).

(+)-*3-Methoxyguattescidine* [**3**].—$C_{19}H_{17}NO_5$, 7 mg, amorphous; $[\alpha]_D$ +37° (*c*=0.23, MeOH); λ max (EtOH) (log ε) 240 sh (3.98), 268 (4.30) nm; (OH^-) 203 (4.85), 267 (4.55), 371 (4.36) nm; (H^+) 213 (4.32), 280 (4.34) nm; eims *m/z* 339.1110 (M^+, 13) (339.1106 calcd), 325 (21), 324 (100); ^{1}H nmr (200 MHz, $CDCl_3$) δ 1.47 (3H, s, CH_3-7), 2.48 (1H, td, *J*=16 and 6 Hz, H-4ax), 3.02 (1H, dd, *J*=16 and 6 Hz, H-4eq), 3.25 (1H, td, *J*=16 and 6 Hz, H-5ax), 4.02 (3H, s, OCH_3-3), 4.08 (1H, dd, *J*=16 and 6 Hz, H-5eq), 6.05 and 6.16 (2H, 2d, *J*=2 Hz, OCH_2O), 6.81 (1H, dd, *J*=8 and 2 Hz, H-10), 7.32 (1H, d, *J*=2 Hz, H-8), 7.79 (1H, d, *J*=8 Hz, H-11); ^{13}C nmr (50 MHz, $CDCl_3$) δ 19.7 (C-4), 34.0 (CH_3-7), 45.6 (C-5), 59.9 (OCH_3-3), 73.1 (C-7), 101.4 (OCH_2O), 111.9 (C-8), 114.7 (C-10), 115.3 (C-1a and C-1b), 119.1 (C-11a), 124.2 (C-3a), 128.3 (C-11), 138.6, 140.4, 143.2, 143.6 (C-1, C-2, C-7a, and C-3), 156.5 (C-9), 170.6 (C-6a).

3-Methoxyoxoputerine [**4**].—$C_{19}H_{13}NO_5$, 10 mg, amorphous; uv λ max (EtOH) (log ε) 212 (4.13), 250 (3.91), 288 (4.06), 385 (3.22), 453 (3.43) nm; eims *m/z* 335.0771 (M^+, 100) (335.0793 calcd), 320 (29), 290 (10); ^{1}H nmr (200 MHz, $CDCl_3$) δ 4.04 (3H, s, OCH_3-11), 4.29 (3H, s, OCH_3-3), 6.24 (2H, s, OCH_2O), 7.28 (1H, dd, *J*=8 and 2 Hz, H-10), 7.53 (1H, t, *J*= 8 Hz, H-9), 8.18 (2H, m, H-4 and 8), 8.90 (1H, m, H-5).

BIOLOGICAL ASSAYS.—In vitro study on the promastigote form of *Leishmania* and on the epimastigote form of *Trypanosoma cruzi*: Test compounds were dissolved in DMSO. Each assay was performed three times. The viability of the parasites was estimated by direct observation after 24 h incubation at 28°, with an inverted microscope, as previously described (3). The control drugs were glucantime (Rhône-Poulenc, France), pentamidine (May and Baker, UK), and gentian violet.

In vitro study on the trypomatigote form of *Trypanosoma cruzi*: Test compounds were dissolved in DMSO. Each assay was performed three times. The parasites were counted after 24 h incubation at 4° as described (22). The control drug was gentian violet.

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