7,7-Dimethylaporphine Alkaloids from the Stem of *Guatteriopsis friesiana*

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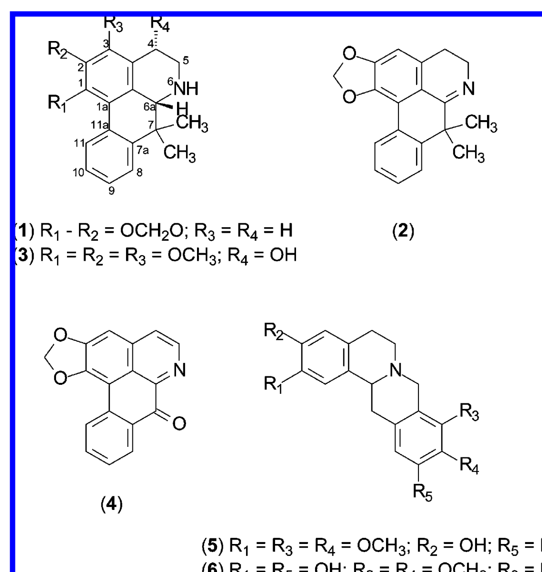
Phytochemical investigation of a methanolic extract of the stem of *Guatteriopsis friesiana* afforded two new 7,7-dimethylaporphine alkaloids, 6,6a-dihydrodemethoxyguadiscine (**1**) and guatteriopsiscine (**3**), together with demethoxyguadiscine (**2**), liriodenine (**4**), corypalmine (**5**), and coreximine (**6**). Their structures were elucidated on the basis of spectroscopic methods (UV, IR, EIMS, HRESIMS, 1D/2D NMR). The absolute configurations of **1** and **3** were determined from the circular dichroism curves. The presence of 7,7-dimethylaporphine alkaloids in this species is important for the chemotaxonomy of *Guatteriopsis*. Antimicrobial activity of compounds **1**−**5** was investigated, and **4** showed activity against *Rhodococcus equi*, with a MIC value of 10 $\mu g \cdot mL^{-1}$.

The family Annonaceae contains about 135 genera and more than 2500 species that are widely distributed in tropical and subtropical South and Central America, Africa, Asia, and Australia.[1] Several Annonaceae species are known for their edible fruits and medicinal properties.[2] In Brazil, there are 26 genera with about 260 species.[3] Despite the importance of the members of this family in folk medicine, the number of species that have been chemically investigated is small (ca. 150 species belonging to 41 genera).[4] Plants of this family are rich sources of aporphine and isoquinoline alkaloids.[5−11] *Guatteriopsis* is a small genus in the family, comprising about five species (*G. friesiana*, *G. hispida*, *G. blepharophylla*, *G. ramiflora*, and *G. kuhlmannii*), most of them distributed throughout South America.[4,12] *Guatteriopsis friesiana* W.A. Rodrigues (Annonaceae) is a small tree known as "envireira" that occurs in the Brazilian and Colombian Amazon basin.[4,12] Only one phytochemical study of this species is available; it describes components of the essential oil from leaves with antimicrobial activity.[4]

As part of our continuing investigation of compounds of Amazonian annonaceous plants, two novel 7,7-dimethylaporphine alkaloids (**1** and **3**), together with demethoxyguadiscine (**2**), liriodenine (**4**), corypalmine (**5**), and coreximine (**6**), were obtained from the stem of *G. friesiana*. The antimicrobial activity of compounds **1**−**5** was investigated (Table 1).



Compound **1** was obtained as a light brown, amorphous powder having the molecular formula $C_{19}H_{19}NO_2$ as deduced from its HRESIMS (observed *m/z* 294.1470 [M + H]$^+$) and NMR data. The ^{1}H NMR spectrum was similar to those of 1,2-disubstituted-6,6a-dihydro-7,7-dimethylaporphine alkaloids. The ^{1}H NMR spectrum showed four adjacent aromatic hydrogens at δ 8.11−8.07 (1H, m, H-11), 7.46−7.42 (1H, m, H-8), and 7.31−7.26 (2H, m, H-9 and H-10), which were ascribed to the hydrogens of the unsubstituted D ring of the aporphine nucleus. A singlet at δ 6.56 was attributed to H-3, while a pair of doublets at δ 6.07 and 5.92 (J = 1.3 Hz) was consistent with two hydrogens of a methylenedioxy group. Singlets at δ 1.52 (3H, 7-CH$_3$) and 0.92 (3H, 7-CH$_3$) indicated the presence of a 7,7-dimethylaporphine group in the molecule.[5,6] Two pairs of signals at δ 3.41−3.37 (1H, m)/2.98 (1H, td, J = 11.9 and 3.0 Hz) and δ 2.93−2.89 (1H, m)/2.64−2.58 (1H, m) attributed to methylene groups H-5 and H-4, respectively, and one singlet at δ 3.81 (1H, H-6a) were consistent with the hydrogens of the 6,6a-dihydro-7,7-dimethylaporphine skeleton.[5,6]

The ^{13}C NMR data together with DEPT 135 and one-bond ^{1}H−^{13}C correlations observed in the gHSQC experiments of **1** indicated the presence of 19 carbons, comprising 12 aromatic carbons between δ 146.5 and 107.9, one methine at δ 61.0, one quaternary at δ 38.5, three methylenes at δ 100.5, 43.3 and 29.5, and two methyl groups at δ 23.3 and 21.5, consistent with structure **1**. The long-range ^{1}H−^{13}C correlations observed in the gHMBC experiment allowed assignment of carbon atoms and confirmed the substitution at C-7 by the correlation observed between methyl groups at δ 1.52 and 0.92 with C-6a, C-7, and C-7a. Thus, **1** was elucidated as a novel 7,7-dimethylaporphinoid alkaloid and was named 6,6a-dihydrodemethoxyguadiscine. The absolute configuration of the chiral center C-6a of **1** was established as *R* as determined from the circular dichroism curve (CD), which showed a negative Cotton effect at 232 nm.[13]

Compound **2** was obtained as a yellow, amorphous powder and had the molecular formula $C_{19}H_{17}NO_2$ as deduced from its HRESIMS (observed *m/z* 292.1284 [M + H]$^+$) and NMR data. The ^{1}H NMR spectrum also resembled that of a 1,2-disubstituted-6,6a-

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Table 1. MIC Values for Alkaloid Fraction and Compound **4** from the Stem of *Guatteriopsis friesiana*

alkaloid fraction and compound **4**	MIC (μg·mL^{-1})										
	B. subtilis	*C. albicans*	*E. faecium*	*E. hirae*	*E. coli*	*M. luteus*	*P. aeruginosa*	*R. equi*	*S. choleraesuis*	*S. aureus*	*S. epidermides*
$CHCl_3$ alkaloid fraction	375	[c]	375	375	750	375	375	375	375	750	750
4	500	[c]	150	600	[c]	150	[c]	10	[c]	[c]	
chloramphenicol[a]	20		70	120	40	50	850	40	60	20	40
nystatin[b]		50									

[a] Drug reference for bacteria. [b] Drug reference for fungus. [c] >1000 μg·mL^{-1}.

dehydro-7,7-dimethylaporphine. The ^{1}H NMR spectrum showed four adjacent aromatic hydrogens at δ 8.28−8.24 (1H, m, H-11), 7.57−7.52 (1H, m, H-8), and 7.35−7.28 (2H, m, H-9 and H-10), which were ascribed to the hydrogens of the unsubstituted D ring of the aporphine nucleus, similar to structure **1**. One singlet at δ 6.68 was attributed to H-3, while the singlet at δ 6.11 (2H, s) was consistent with two hydrogens of a methylenedioxy group. One singlet at δ 1.52 (6H) indicated the presence of a 7,7-dimethylaporphine group in the molecule.[7] Multiplets at δ 2.62−2.58 (2H, H-4) and 3.66−3.62 (2H, H-5) were consistent with the hydrogens of the 6,6a-dehydro-7,7-dimethylaporphine skeleton.[7] Thus, the compound was identified as demethoxyguadiscine, which was previously isolated from *Hornschuchia obliqua* (Annonaceae).[7] However, the UV, IR, and HRESIMS were not reported, and some of the ^{1}H and ^{13}C NMR data have been revised. In ref 7, the ^{1}H NMR signals for methyl groups (δ 1.92) and H-4 (δ 3.06) were more downfield than observed in this work and other literature data for this type of alkaloid.[5,6] The same thing was observed in the ^{13}C NMR spectra for C-2 (δ 155.9), C-3b (δ 113.8), C-5 (δ 41.3), C-6a (δ 180.0), and C-7a (δ 140.3). In this paper we present the complete physical constant data for **2** (see the Experimental Section).

Compound **3** was obtained as white needles having the molecular formula $C_{21}H_{25}NO_4$ as deduced from HRESIMS (observed *m/z* 356.1880 $[M + H]^+$) and NMR data. The HRESIMS/MS fragment at *m/z* 338.1798 $[(M + H) - 18]^+$ and the IR absorption at 3435 cm^{-1} both supported the existence of an OH group. The ^{1}H NMR spectrum pointed to a 1,2,3,4-tetrasubstituted-6,6a-dihydro-7,7-dimethylaporphine. The spectrum showed four adjacent aromatic hydrogen signals at δ 8.26−8.22 (1H, m, H-11), 7.44−7.39 (1H, m, H-8), and 7.30−7.24 (2H, m, H-9 and H-10), which were ascribed to hydrogens of the unsubstituted D ring of an aporphine nucleus, similar to structures **1** and **2**. Signals at δ 4.03, 3.97, and 3.71 (each 3H, s) typical of methoxy groups were placed on the A ring, and the signal at δ 4.80 (1H, t, $J = 2.0$ Hz, H-4) characteristic of an OH group was placed on the B ring of the aporphine nucleus. Singlets at δ 1.49 (3H, 7-CH_3) and 0.89 (3H, 7-CH_3) indicated the presence of a 7,7-dimethylaporphine group in the molecule.[5,6] Signals at δ 3.39 and 2.95 (each 1H, dd, $J = 12.4$ and 2.0 Hz, H-5 pseudoequatorial, and H-5 pseudoaxial) and δ 3.56 (H-6a) were consistent with hydrogens of the 4-hydroxy-6,6a-dihydro-7,7-dimethylaporphine skeleton.[5,6]

The ^{13}C NMR data together with DEPT 135 and one-bond ^{1}H−^{13}C correlations observed in the gHSQC experiments of **3** indicated the presence of 21 carbons: 12 aromatic, three methoxy, two methine, one quaternary, one methylene, and two methyl groups, consistent with structure **3**. Long-range ^{1}H−^{13}C correlations observed in the gHMBC experiment allowed assignment of carbon atoms and confirmed the substitution of C-7 by two methyl groups at δ 1.49 and 0.89 and the substitution at C-4 by the correlation observed between the hydrogen at δ 4.80 (H-4) and C-3, C-3a, and C-3b. Accordingly, structure **3** was elucidated as a 7,7-dimethylaporphinoid alkaloid type, named guatteriopsiscine.

The absolute configuration of the chiral center C-6a of **3** was established as *R* as determined from the circular dichroism curve, which showed a negative Cotton effect at 236 nm.[13,14] In the ^{1}H NMR spectrum, the H-4 triplet, together with its relatively upfield shift, denoted a *syn* relationship between H-4 and H-6a.[14] NOE experiments showed correlations of the signals at δ 3.56 (H-6a) with δ 1.49 (7-CH_3) and 2.95 (H-5 pseudoaxial), at δ 3.39 (H-5 pseudoequatorial) with δ 2.95 (H-5 pseudoaxial) and 4.80 (H-4), and at δ 4.80 (H-4) with δ 3.39 (H-5 pseudoequatorial), 2.95 (H-5 pseudoaxial), and 4.03 (3-OMe), confirming a *syn* relationship between H-4 and H-6a. Thus the absolute configuration of the chiral center C-4 of **3** was determined as *S*.

Compound **4** was obtained as yellow needles and identified as liriodenine according to literature data (mp, UV, and ^{1}H NMR).[10] The values for ^{13}C NMR data published for this compound in the literature showed some ambiguities.[11,15] The assignments for all carbons of **4** are shown in the Experimental Section.

Compounds **5** and **6** were obtained as light yellow needles and identified as corypalmine (discretinine)[16] and coreximine,[17] respectively. The ^{13}C NMR data for compound **6** are described in the literature for the first time. The structures of all isolated compounds were determined by extensive NMR studies, including DEPT, HSQC, HMBC, NOE, and NOESY, and MS analyses.

On the basis of morphological characters, *Guatteriopsis* belongs to Fries' *Guatteria* group, which consists of four genera: *Guatteria*, *Guatteriopsis*, *Guatteriella*, and *Heteropetalum*.[12] It is distinguishable from *Guatteria* by the presence of valvate petal whorls (nonoverlapping) instead of imbricate ones (overlapping).[12] Maia et al.[18] observed relationships among the genera *Guatteria* and *Guatteriopsis* based on analyses of the oil composition of these genera.[18] A subsequent study by Erkens et al.,[12] based on molecular phylogeny of *Guatteria* and its three smaller satellite genera *Guatteriopsis*, *Guatteriella*, and *Heteropetalum*, suggested that the species of these genera should be transferred to *Guatteria*, contributing to the study by Maia et al.[18]

In our study, we observed that 7,7-dimethylaporphine alkaloids are present in the stem of *G. friesiana*. This class of compounds is very common in species of *Guatteria* and could be considered a chemotaxonomic marker of this genus.[5,6] On the basis of the study of Maia et al.,[18] Costa et al.,[4] literature data,[5,6] and our study, the genus *Guatteriopsis* shows some of the chemotaxonomic characteristics of *Guatteria*, in agreement with the study by Erkens et al.[12] that classified this species within the genus *Guatteria*.

The results of an antimicrobial activity assay (Table 1) indicated that only compound **4** had significant antimicrobial activity. This compound showed activity for five of the microorganisms evaluated, displaying activity against *Rhodococcus equi, Enterococcus faecium*, and *Micrococcus luteus*, with MIC values of 10, 150, and 150 μg·mL^{-1}, respectively. It should be emphasized that compound **4** was 4 times more active against *R. equi* than was chloramphenicol (the reference drug). This strong activity could be interesting for veterinary medicine. *R. equi* is a Gram-positive, acid-fast, pleomorphic coccobacillus that is associated with severe pneumonia, pulmonary abscessation, and enteritis in animals. In Brazil, this infection is considered one of the most severe diseases, mainly in young horses, in which it causes high mortality, even with standard treatment with antimicrobial agents.[19] The activity observed for the $CHCl_3$ alkaloid fraction (Table 1) can be attributed to compound **4**, which was the only active compound. This compound is known for various biological activities, such as leishmanicidal,[8,9] antimicrobial.[20,21] and *in vitro* cytotoxicity properties against tissue-culture

cells of humans.[10,22] Although the antimicrobial activity of liriodenine (**4**) has been known for a long time, in our study it was evaluated against *R. equi* for the first time.

Experimental Section

General Experimental Procedures. Melting points were determined on a Quimis Q-340S23 micromelting point apparatus. Optical rotations were measured in $CHCl_3$ or MeOH solutions at room temperature on a Rudolph Research Autopol III automatic polarimeter. Circular dichroism spectra were measured in MeOH on a JASCO J-720 spectropolarimeter. UV spectra were obtained in CH_3OH on a Hewlett-Packard HP 8452A diode array spectrophotometer. IR spectra were acquired on a BIORAD FTS-3500 GX spectrophotometer. NMR data, 1D and 2D, were recorded at 293 K in $CDCl_3$ or $CDCl_3 + CD_3OD$ on Bruker Avance DRX 400 and Varian Inova 500 spectrometers. Chemical shifts (δ) are given in ppm relative to TMS (δ 0.00) as the internal standard. HRESIMS analyses were carried out on a Micromass Q-TOF mass spectrometer (Micromass, Manchester, UK). Silica gel 60 (70−230 mesh) was used for column chromatography, precoated silica gel plates (60 F_{254} Merck, 0.25 mm, aluminum) were used for analytical TLC, and precoated silica gel plates (60 PF_{254} Merck, 1 mm, glass) were used for preparative TLC. The spots were detected by spraying with Dragendorff's reagent or *p*-anisaldehyde reagent and then heating on a hot plate.

Plant Material. The stems of *G. friesiana* was collected in the experimental farm at the Universidade Federal do Amazonas (UFAM), in the vicinity of Manaus, state of Amazonas, Brazil, in January 2005, and identification was made by Prof. Dr. Antônio Carlos Webber of UFAM. A voucher specimen (No. 7341) has been deposited at the Herbarium of the Department of Biology, UFAM.

Extraction and Isolation. Powdered, air-dried stem material of *G. friesiana* (2.2 kg) was extracted successively with hexane (4 × 5 L), and then MeOH (4 × 5 L), at room temperature. Removal of the solvents under reduced pressure gave 2.9 g of hexane and 78.4 g of MeOH extracts. TLC tests of the MeOH extract revealed the presence of alkaloids. The MeOH extract (75.0 g) was redissolved in $CHCl_3$ (250 mL) and extracted with 3% aqueous HCl (7 × 250 mL). Following this, the aqueous solution was adjusted with NH_4OH to pH 12 and extracted with $CHCl_3$ (7 × 250 mL). The organic fractions were combined, and the solvent was evaporated under vacuum to yield the $CHCl_3$ alkaloid fraction (1.0 g).

The alkaloid fraction (0.8 g) was initially subjected to silica gel column chromatography (CC), having been previously treated with a 10% $NaHCO_3$ solution[8] and eluted with increasing concentrations of CH_2Cl_2 in petroleum ether, followed by EtOAc in CH_2Cl_2 and MeOH in EtOAc, affording 67 fractions of 30 mL each. The eluted fractions were evaluated and pooled after TLC analysis, yielding 12 subfractions. Subfraction 3 (182 mg) was purified by preparative TLC eluted with petroleum ether−acetone, 70:30 (two times), to yield **1** (56.0 mg) and **2** (18.0 mg). Subfraction 4 (92.9 mg) was purified by preparative TLC eluted with petroleum ether−acetone 70:30 (three times) to yield **3** (22.8 mg) and **4** (11.0 mg). Subfraction 5 (239 mg) was further purified by silica gel CC (treated with a 10% $NaHCO_3$ solution),[8] using the same methodology as above, yielding 34 fractions. The fractions were pooled, based on the TLC analysis, into seven subfractions. Subfraction 5.2 (49.5 mg) was purified by preparative TLC eluted with petroleum ether−acetone, 70:30 (three times), to yield again compound **3** (23.9 mg). Subfraction 5.3 (100 mg) was purified by preparative TLC eluted with CH_2Cl_2−MeOH, 95:05 (two times), to yield compound **5** (3.2 mg). Subfraction 7 (62.0 mg) was purified by preparative TLC eluted with CH_2Cl_2−MeOH, 90:10 (two times), to yield compound **6** (2.8 mg).

6,6a-Dihydrodemethoxyguadiscine (1): light yellow, amorphous powder; $[\alpha]^{25}_D$ −50.7 (*c* 1.415, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.30), 216sh (4.26), 234sh (4.05), 274 (4.07), and 317sh (3.53) nm; IR (KBr) ν_{max} 2966, 2921, 2837, 2790, 1630, 1613, 1570, 1499, 1459, 1420, 1382, 1358, 1304, 1279, 1227, 1150, 1111, 1047, 1011, 940, 850, 813, 758, 687, 640, 586, 522, 502, and 438 cm^{-1}; CD $\Delta\varepsilon$ MeOH (λ nm) +13.9 (214), −43.9 (232), +7.8 (272); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.11−8.07 (1H, m, H-11), 7.46−7.42 (1H, m, H-8), 7.31−7.26 (2H, m, H-9 and H-10), 6.56 (1H, s, H-3), 6.07 and 5.92 (each 1H, d, *J* = 1.3 Hz, OCH_2O), 3.81 (1H, br s, H-6a), 3.41−3.37 (1H, m, H-5 pseudoequatorial), 2.98 (1H, td, *J* = 11.9 and 3.0 Hz, H-5 pseudoaxial), 2.93−2.89 (1H, m, H-4 pseudoequatorial), 2.64−2.58 (1H, m, H-4 pseudoaxial), 2.30 (1H, br s, NH), 1.52 (3,H, s, 7-CH_3), and 0.92 (3H, s, 7-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 146.5 (C-2), 144.7 (C-7a), 142.1 (C-1), 129.6 (C-11a), 128.0 (C-3a), 127.9 (C-9), 127.3 (C-11), 126.5 (C-10), 126.4 (C-3b), 123.8 (C-8), 116.0 (C-1a), 107.9 (C-3), 100.5 (OCH_2O), 61.0 (C-6a), 43.3 (C-5), 38.5 (C-7), 29.5 (C-4), 23.3 (7-CH_3) and 21.5 (7-CH_3); HRESIMS *m/z* 294.1470 $[M + H]^+$ (calcd for $[C_{19}H_{19}NO_2 + H]^+$, 294.1494).

Demethoxyguadiscine (2): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 208 (4.55), 229sh (4.35), 256 (4.55), 272sh (4.32), 295 (4.14), 338 (3.91), and 350 (3.87) nm; IR (KBr) ν_{max} 2962, 2923, 2848, 1630, 1594, 1570, 1495, 1454, 1422, 1382, 1317, 1274, 1227, 1081, 1049, 946, 901, 843, 820, 760, 595, 558, and 517 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.28−8.24 (1H, m, H-11), 7.57−7.52 (1H, m, H-8), 7.35−7.28 (2H, m, H-9 and H-10), 6.68 (1H, s, H-3), 6.11 (2H, s, OCH_2O), 3.66−3.62 (2H, m, H-5), 2.62−2.58 (2H, m H-4), 1.52 (6H, s, 7-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 170.5 (C-6a), 149.6 (C-2), 144.3 (C-7a), 142.2 (C-1), 133.0 (C-3a), 128.5 (C-9), 128.3 (C-11a), 127.4 (C-11), 126.5 (C-10), 124.9 (C-8), 117.6 (C-3b), 116.5 (C-1a), 106.9 (C-3), 101.0 (OCH_2O), 46.9 (C-5), 42.8 (C-7), 27.6 (7-CH_3), 26.3 (C-4); HRESIMS *m/z* 292.1284 $[M + H]^+$ (calcd for $[C_{19}H_{17}NO_2 + H]^+$, 292.1337).

Guatteriopsiscine (3): white needles ($CHCl_3$−MeOH, 2:1); mp 177−178 °C; $[\alpha]^{25}_D$ −84.4 (*c* 0.73, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 219 (4.51) and 278 (4.26) nm; IR (KBr) ν_{max} 3435, 3308, 3079, 2966, 2948, 2876, 2831, 1606, 1581, 1466, 1404, 1340, 1259, 1205, 1169, 1115, 1070, 1045, 1025, 1000, 955, 900, 823, 756, 641, and 551 cm^{-1}; CD $\Delta\varepsilon$ MeOH (λ nm) +73.4 (215), −107.0 (236), +13.7 (276); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.26−8.22 (1H, m, H-11), 7.44−7.39 (1H, m, H-8), 7.30−7.24 (2H, m, H-9 and H-10), 4.80 (1H, t, *J* = 2.0 Hz, H-4), 3.56 (1H, br, s, H-6a), 3.39 (1H, dd, *J* = 12.4 and 2.0 Hz, H-5 pseudoequatorial), 2.95 (1H, dd, *J* = 12.4 and 2.0 Hz, H-5 pseudoaxial), 4.03 (3H, s, 3-OCH_3), 3.97 (3H, s, 2-OCH_3), 3.71 (3H, s, 1-OCH_3), 1.49 (3H, s, 7-CH_3) and 0.89 (3H, s, 7-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 151.3 (C-3), 151.1 (C-1), 145.4 (C-2), 144.7 (C-7a), 130.8 (C-3b), 130.1 (C-11a), 128.0 (C-11), 127.4 (C-9), 126.4 (C-3a), 126.3 (C-10), 123.4 (C-8), 121.9 (C-1a), 61.4 (C-6a), 61.4 (3-OCH_3), 60.9 (2-OCH_3), 60.8 (C-4), 60.5 (1-OCH_3), 50.0 (C-5), 37.7 (C-7), 23.2 (7-CH_3), and 20.8 (7-CH_3); HRESIMS *m/z* 356.1880 $[M + H]^+$ (calcd for $[C_{21}H_{25}NO_4 + H]^+$, 356.1862).

Liriodenine (4): yellow needles ($CHCl_3$−MeOH, 2:1); mp 279−280 °C (lit. 280−282 °C);[8] identified by comparison with literature data (co-TLC, mp, UV, IR, and ^1H NMR);[10,11] ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.5 (C-7), 151.6 (C-2), 147.9 (C-1), 145.3 (C-6a), 144.7 (C-5), 135.7 (C-3a), 133.8 (C-10), 132.8 (C-11a), 131.2 (C-7a), 128.8 (C-8), 128.5 (C-9), 127.3 (C-11), 124.3 (C-4), 123.1 (C-3b), 107.9 (C-1a), 103.2 (C-3), 102.4 (OCH_2O).

Corypalmine or Discretinine (5): yellow needles ($CHCl_3$−MeOH, 2:1); mp 237−238 °C (lit. 235−236 °C);[16] $[\alpha]^{25}_D$ −202.5 (*c* 0.04, MeOH); identified by comparison with literature data (co-TLC, mp, UV, IR, ^1H NMR and ^{13}C NMR).[16]

Coreximine (6): light yellow needles ($CHCl_3$−MeOH, 1:1); mp 240−241 °C; $[\alpha]^{25}_D$ −200.0 (*c* 0.075, MeOH); identified by comparison with literature data (co-TLC, mp, UV, IR, and ^1H NMR);[17] ^{13}C NMR ($CDCl_3$−CD_3OD, 1:1, 125 MHz) δ 147.0 (C-3), 146.9 (C-10), 145.4 (C-2), 145.1 (C-11), 130.1 (C-13b), 126.8 (C-12a), 125.6 (C-4a), 125.1 (C-8a), 115.4 (C-12), 112.4 (C-1), 111.8 (C-4), 109.5 (C-9), 60.2 (C-13a), 58.6 (C-8), 56.2 (3-OCH_3), 56.1 (10-OCH_3), 52.1 (C-6), 35.9 (C-13), 28.7 (C-5).

Antimicrobial Assays. Growth inhibitory activity of the extracts, fractions, and pure compounds was tested against 11 microorganisms (*Bacillus subtilis* ATCC 5061, *Candida albicans* ATCC 10231, *Enterococcus faecium* CCT 5079, *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 11775, *Micrococcus luteus* ATCC 4698, *Pseudomonas aeruginosa* ATCC 13388, *Rhodococcus equi* ATCC 6939, *Salmonella choleraesuis* ATCC 10708, *Staphylococcus aureus* ATCC 6538, and *Staphylococcus epidermides* ATCC 12228). These microorganisms were obtained from the Tropical Culture Collection (TCC) of the André Tosello Foundation, Campinas, São Paulo, Brazil.

The bacterial strains were grown overnight at 36 °C in nutrient agar (Merck), while *C. albicans* was grown in Saboraud dextrose agar. Inoculum for the assays was prepared by diluting a cell mass in 0.85% NaCl solution, adjusted to McFarland scale 0.5, and confirmed by spectrophotometry reading at 580 nm. Cell suspensions were diluted to 10^4 CFU mL^{-1} for use in the activity assays. Minimal inhibitory concentration (MIC) tests were carried out according to Eloff (1998),[23]

using Müller-Hinton broth on a tissue-culture test plate (96 wells). Each sample was tested in duplicate. The stock solutions of extracts, fractions, and pure compounds were diluted so that concentrations in the range 1.0−0.016 mg·mL^{-1} were obtained. Chloramphenicol and nystatin (Merck) were used as reference antibiotic controls in the range 0.25−0.002 mg·mL^{-1}. The inoculum was added to all wells, and the plates were incubated at 36 °C for 48 h. Antimicrobial activity was detected by adding 20 μL of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. The MIC was defined as the lowest concentration of the extractions, fractions, and pure compounds that inhibited visible growth, as indicated by TTC staining (dead cells are not stained by TTC).

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Supporting Information Available: 1D and 2D NMR, MS, IR, and UV spectra of **1** and **3**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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