Alkaloids from the Bark of *Guatteria hispida* and Their Evaluation as Antioxidant and Antimicrobial Agents

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Phytochemical investigation of the bark of *Guatteria hispida* afforded three new alkaloids, 9-methoxy-*O*-methylmoschatoline (**1**), 9-methoxyisomoschatoline (**2**), and isocerasonine (**3**), along with 10 known alkaloids, 8-oxopseudopalmatine (**4**), *O*-methylmoschatoline (**5**), lysicamine (**6**), liriodenine (**7**), 10-methoxyliriodenine (**8**), nornuciferine (**9**), anonaine (**10**), xylopine (**11**), coreximine (**12**), and isocoreximine (**13**). The major compounds, **2**, **6**, **12**, and **13**, showed significant antioxidant capacity in the $ORAC_{FL}$ assay. Compounds **5**, **6**, and **7** were active against *S. epidermidis* and *C. dubliniensis*, with MIC values in the range 12.5−100 μg mL^{-1}.

The family Annonaceae, comprising about 135 genera and more than 2500 species,[1] is a large family of tropical and subtropical trees and shrubs. Members of this family are known for their edible fruits and for their medicinal properties.[2] Previous chemical and pharmacological investigations on some species revealed the presence of bioactive compounds exhibiting pharmacological activities such as cytotoxicity against human tumor cell lines,[3−5] antimicrobial activity,[5−9] and antiparasitic properties against *Leishmania* sp.,[3,7,10] *Plasmodium falciparum*,[5,11] and *Trypanosoma* sp.[3,12] Despite their importance in folk medicine, the number of species that have been chemically investigated is still very small. *Guatteria hispida* (R. E. Fr.) Erkens & Maas (Annonaceae) is a small rare tree that occurs in the Brazilian Amazon, mainly in the state of Amazonas, and is popularly known as "envireira" and "envira da folha peluda".[13,14] Previous phytochemical studies on this species described chemical constituents of essential oils from the leaves that showed antimicrobial activity.[8]

In our search for antioxidant and antimicrobial compounds from Amazonian annonaceous plants, three new (**1**−**3**) and 10 known alkaloids (**4**−**13**) were obtained by systematic bioguided procedures from the bark of *G. hispida*. Their structures were established on the basis of spectrometric data, including 1D (^{1}H and ^{13}C) and 2D (HSQC and HMBC) NMR experiments as well as HR-MS analysis. Antioxidant capacity and antimicrobial activity were demonstrated for the pure compounds.

The MeOH extract was subjected to an acid−base extraction with CH_2Cl_2. The CH_2Cl_2 fraction containing the alkaloids was fractionated as described in the Experimental Section, leading to the isolation of the compounds **1**−**13**.

Compound **1** was obtained as an orange, amorphous powder with the molecular formula $C_{20}H_{17}NO_5$, as determined by HR-ESIMS (observed *m/z* 352.1322 $[M + H]^+$) and NMR data. The IR, UV, and NMR spectroscopic data of **1** were similar to those reported for *O*-methylmoschatoline (**5**),[15] with the exception of the substitution of a methoxy group at C-9. The substitution at C-9 was established by the strong long-range $^1H-^{13}C$ correlation of H-11 with C-9 and by the correlation of the OCH_3 hydrogens at δ 3.99 with the same carbon (C-9) (Table 1). Additionally, a spin system was found consisting of three hydrogens at δ 9.04 (1H, d 9.1 Hz), 8.03 (1H, d 3.1 Hz), and 7.32 (1H, dd 9.1 and 3.1 Hz), in accordance with the pattern of substitution shown for **1** (Table 1). Therefore, compound **1** was established as a new oxoaporphine alkaloid and was named 9-methoxy-*O*-methylmoschatoline.

Compound **2** was obtained as a red, amorphous powder with the molecular formula $C_{19}H_{15}NO_5$. The IR, UV, and NMR data of **2** were very similar to those of **1**, except for the absence of an OCH_3 group, which was replaced by an OH at C-3 in structure **2**. The OH at C-3 was established on the basis of the long-range $^1H-^{13}C$ correlation of H-4 with the C-3 signal at δ 165.3, which showed no correlation with any of the three remaining OCH_3 groups (Table 1). Therefore, compound **2** was established as a new oxoaporphine alkaloid, named 9-methoxyisomoschatoline.

Compound **3** was obtained as a red, amorphous powder and showed strong blue UV fluorescence, as well as a blue spot when sprayed with Dragendorff's reagent, characteristic of 8-oxoprotoberberines.[16] The molecular formula was established as $C_{20}H_{19}NO_5$ from the HR-ESIMS and NMR data. The IR, UV, and NMR spectra of **3** were similar to those described for 8-oxopseudopalmatine (**4**), with the exception of an OH replacing an OCH_3 group at C-3. The OH at C-3 was established on the basis of the long-range $^1H-^{13}C$ correlation of H-1 with C-3 at δ 146.8, which showed no correlation with any of the three OCH_3 groups (Table 2). Therefore, compound **3** was established as a new 13,13a-didehydro-8-oxoprotoberberine alkaloid, named isocerasonine.

The extensive analysis of ^{1}H, $^{13}C\{^1H\}$ spectra and one-bond and long-range $^1H-^{13}C$ NMR correlation from HSQC and HMBC experiments allowed complete and unambiguous ^{1}H and ^{13}C NMR chemical shift assignments for compounds **1**−**3** (Tables 1 and 2). The complete and unequivocal NMR assignments (Table 2) and the IR, UV, and MS data for 8-oxopseudopalmatine (**4**) are described here. All of the other compounds isolated from *G. hispida* were identified by comparison of their spectroscopic data with those

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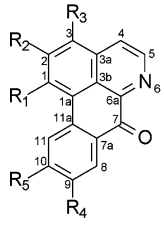
Table 1. NMR Data (400 MHz) for Compounds **1** and **2**

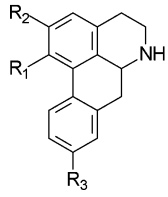
	1			**2**		
position	δ_C mult.[a]	δ_H mult. (J in Hz)[a]	$^1H-^{13}C$ HMBC[a,c]	δ_C[b]	δ_H mult. (J in Hz)[b]	$^1H-^{13}C$ HMBC[b,c]
1	155.5, qC			161.9		
1a	116.0, qC			103.2		
2	147.6, qC			143.9		
3	147.7, qC			165.3		
3a	131.1, qC			136.1		
3b	122.2, qC			123.7		
4	119.3, CH	8.23 d (5.3)	3, 3b, 5	123.9	8.57 d (5.1)	1a, 3, 3b, 5
5	144.5, CH	8.98 d (5.3)	3a, 4, 6a	141.9	8.71 d (5.1)	3a, 4, 6a
6a	145.4, qC			144.9		
7	182.6, qC			184.1		
7a	133.0, qC			131.7		
8	110.4, CH	8.03 d (3.1)	7, 9, 10, 11a	109.5	7.82 d (3.0)	1a, 7, 9, 10, 11a
9	159.3, qC			158.1		
10	122.7, CH	7.32 dd (9.1 and 3.1)	8, 11a	124.0	7.22 dd (9.3, 3.0)	8, 9, 11a
11	129.4, CH	9.04 d (9.1)	1a, 7a, 9	129.1	8.94 d (9.3)	1a, 7, 7a, 8, 9, 10a
11a	127.9, qC			132.6		
1-OCH_3	60.9	4.06 s	1	61.1	4.04 s	1
2-OCH_3	61.5	4.11 s	2	60.5	3.95 s	2
3-OCH_3 (OH)	61.9	4.18 s	3			
9-OCH_3	55.7	3.99 s	9	55.7	3.86 s	9

[a] The experiments were obtained at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$. [b] In CD_3OD. [c] Long-range $^1H-^{13}C$ HMBC correlations, optimized for 8 Hz, are from hydrogens stated to the indicated carbon.

Chart 1



	R_1	R_2	R_3	R_4	R_5
1	OCH_3	OCH_3	OCH_3	OCH_3	H
2	OCH_3	OCH_3	OH	OCH_3	H
5	OCH_3	OCH_3	OCH_3	H	H
6	OCH_3	OCH_3	H	H	H
7	OCH_2O		H	H	H
8	OCH_2O		H	H	OCH_3



	R_1	R_2	R_3
9	OCH_3	OCH_3	H
10	OCH_2O		H
11	OCH_2O		OCH_3

reported in the literature; *O*-methylmoschatoline (**5**),[15] lysicamine (**6**),[17] liriodenine (7),[6,17] 10-methoxyliriodenine (**8**),[17] nornuciferine (**9**),[15] anonaine (**10**),[15] xylopine (**11**),[18] coreximine (**12**),[6] and isocoreximine (**13**).[19]

G. hispida was originally placed in the genus *Guatteriopsis* (*Guatteriopsis hispida* R.E. Fries) and was recently transferred to *Guatteria* on the basis of molecular phylogeny of *Guatteria* and the closely related genera *Guatteriopsis*, *Guatteriella*, and *Heteropetalum* by Erkens and Maas.[13] Our results support the reassignment of this species to *Guatteria* because of the similarity of the newly isolated compounds, which are found in many species of *Guatteria*.

The major alkaloids **2**, **5**, **6**, **7**, **9**, **12**, and **13** were tested for antioxidant capacity using the oxygen radical absorbance capacity ($ORAC_{FL}$) assay (Table 3) and also screened for antimicrobial activity (Table 4). Compounds **2**, **6**, **12**, and **13** showed a significant antioxidant capacity (Table 3), and the other compounds tested showed less antioxidant activity in the test conditions. The oxoaporphine alkaloids **5**, **6**, and **7** were active against *S. epidermidis* (strain 6ep) and *C. dubliniensis* (strains ATCC 777 and ATCC 778157) with minimal inhibitory concentration (MIC) values in the range 12.5 and 100 μg mL^{-1} (Table 4), whereas the other alkaloids tested were inactive (MIC > 100 μg mL^{-1}). Alkaloid **5** was more active than the positive control (chloramphenicol) against *S. epidermidis* and was similar in activity to the positive control (ketoconazole) against *C. dubliniensis* (strains ATCC 777) (Table 4). Compounds **6** and **7** showed activity similar to that of chloramphenicol against *S. epidermidis*. The MeOH extract and the CH_2Cl_2 neutral and alkaloid fractions also showed antimicrobial activity against the same microorganisms, with the best results for the CH_2Cl_2 alkaloid fraction. Similar results were found for the antioxidant capacity and are probably due to the presence of a high concentration of the alkaloids.

Experimental Section

General Experimental Procedures. UV spectra were obtained in CH_3OH on a UV−Vis Agilent HP 8453 spectrophotometer. IR spectra were acquired on a Bomem MB-100 spectrophotometer. 1D and 2D NMR experiments were acquired in $CDCl_3$, $CDCl_3$ + CD_3OD, or CD_3OD at 293 K on a Bruker Avance 400 NMR spectrometer operating at 9.4 T, observing ^1H and ^{13}C at 400 and 100 MHz, respectively. The spectrometer was equipped with a 5 mm multinuclear direct detection probe with *z*-gradient. One-bond and long-range $^1H-^{13}C$ correlation (HSQC and HMBC) experiments were optimized for an average coupling constant $^1J_{(C,H)}$ and $^{LR}J_{(C,H)}$ of 140 and 8 Hz, respectively. All ^1H and ^{13}C NMR chemical shifts (δ) are given in ppm relative to the TMS signal at 0.00 ppm as internal reference, and the coupling constants (J) are in Hz. The HR-ESIMS measurements were carried out on a hybrid quadrupole reflector orthogonal time-of-flight high-resolution Micromass Q-TOF mass spectrometer, equipped with an electrospray source. Silica gel 60 (70−230 mesh) was used for column chromatography, while silica gel 60 F_{254} were used for analytical (0.25 mm) and preparative (1.00 mm) TLC. Compounds were visualized by exposure under $UV_{254/366}$ light, spraying *p*-anisaldehyde reagent followed by heating on a hot plate, and spraying with Dragendorff's reagent.

Plant Material. The bark of *Guatteria hispida* was collected in November 2008 in the Adolpho Ducke Forest Reservation [coordinates: S from 02°54′26″ to 03°00′22″; W from 59°52′40″ to 59°58′40″], located 26 km northeast of the city of Manaus, Amazonas, Brazil.[14] The species was identified by Prof. Dr. A. C. Webber, a plant taxonomist at the Departamento de Biologia of the Universidade Federal do Amazonas (UFAM). A voucher specimen (no. 7707) was deposited at the Herbarium of the UFAM.

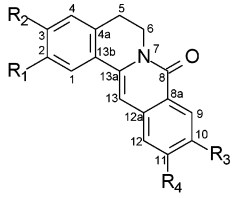
Extraction and Isolation. Dried and powdered bark (800 g) of *G. hispida* was successively extracted with *n*-hexane followed by MeOH, to yield hexane (14.0 g) and MeOH (45.7 g) extracts. TLC investigations indicated a high concentration of alkaloids in the MeOH extract. Therefore, an aliquot of the MeOH extract (44.0 g) was initially subjected to an acid−base extraction[10] to give the CH_2Cl_2 alkaloid

Table 2. NMR Data (400 MHz) for Compounds **3** and **4**

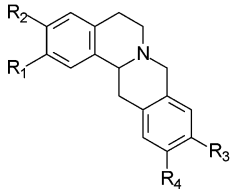
	3			**4**		
position	δ_C mult.[a]	δ_H mult.[a]	$^1H-^{13}C$ HMBC[a,b]	δ_C[a]	δ_H mult.[a]	$^1H-^{13}C$ HMBC[a,b]
1	107.2, CH	7.24 s	2, 3, 4a, 13a, 13b	107.9	7.26 s	2, 3, 4a, 13a, 13b
2	145.9, qC			148.3		
3	146.8, qC			149.9		
4	113.7, CH	6.82 s	2, 3, 5, 13b	110.6	6.75 s	2, 3, 5, 13b
4a	129.2, qC			128.3		
5	27.9, CH_2	2.91 m	4, 4a, 6, 13b	28.1	2.95 m	4, 4a, 6, 13b
6	39.8, CH_2	4.35 m	4a, 5, 8, 13a	39.8	4.38 m	4a, 5, 13a
8	161.3, qC			161.5		
8a	118.2, qC			118.4		
9	108.0, CH	7.82 s	8, 8a, 11, 12a	108.0	7.84 s	8, 8a, 10, 11, 12a
10	148.9, qC			148.9		
11	153.4, qC			153.3		
12	105.8, CH	6.94 s	8, 8a, 10, 11, 13	105.7	6.95 s	8, 8a, 10, 11, 13
12a	132.0, qC			132.1		
13	101.2, CH	6.82 s	8a, 12, 13a, 13b	101.1	6.86 s	8a, 12, 13a, 13b
13a	136.3, qC			136.1		
13b	122.2, qC			122.4		
2-OCH_3	56.1	4.00 s	2	56.3	3.99 s	2
3-OCH_3 (OH)				56.2	3.95 s	3
10-OCH_3	56.4	4.02 s	10	56.2	4.03 s	10
11-OCH_3	56.3	4.01 s	11	56.1	4.02 s	11

[a] The experiments were obtained in $CDCl_3$ at 293 K with TMS as internal reference (0.00 ppm). [b] Long-range $^1H-^{13}C$ HMBC correlations, optimized for 8 Hz, are from hydrogen stated to the indicated carbon.

Chart 2



	R_1	R_2	R_3	R_4
3	OCH_3	OH	OCH_3	OCH_3
4	OCH_3	OCH_3	OCH_3	OCH_3



	R_1	R_2	R_3	R_4
12	OH	OCH_3	OCH_3	OH
13	OCH_3	OH	OCH_3	OH

Table 3. Antioxidant Capacity of Alkaloids **2**, **5**, **6**, **7**, **9**, **12**, and **13**

alkaloid/controls	ORAC assay[a]
2	1.62 (2.86)
5	0.50 (2.67)
6	0.86 (0.95)
7	0.30 (1.38)
9	0.27 (0.66)
12	1.40 (4.15)
13	1.67 (0.62)
quercetin[b]	5.62 (0.90)
isoquercitrin[b]	5.25 (1.80)
caffeic acid[b]	2.95 (2.05)

[a] ORAC data expressed as relative Trolox equivalents for pure compound. [b] Positive controls. The results are the mean of a triplicate assay plus the corresponding relative standard deviation.

Table 4. Antimicrobial Activity of Alkaloids **5**, **6**, and **7**

	MIC[a] (μg mL^{-1})			
microorganism	**5**	**6**	**7**	**Controls**[b]
Kocuria rhizophila (ATCC 9341)[c]	–[e]	–	–	50.0
Staphylococcus aureus (ATCC14458)[c]	–	–	–	25.0
S. aureus penicilinase + (7+)[d]	–	–	–	25.0
S. aureus penicilinase − (8−)[d]	–	–	–	25.0
S. epidermidis (6ep)[d]	25.0	50.0	50.0	50.0
Escherichia coli (ATCC 10538)[c]	–	–	–	50.0
Pseudomonas aeruginosa (ATCC 27853)[d]	–	–	–	850
Candida albicans (ATCC 10231)[c]	–	–	–	12.5
C. tropicalis (CT)[d]	–	–	–	12.5
C. glabrata (ATCC 30070)[c]	–	–	–	12.5
C. parapsilosis (ATCC 22019)[c]	–	–	–	12.5
C. dubliniensis (ATCC 777)[c]	12.5	100.0	50.0	12.5
C. dubliniensis (ATCC 778157)[c]	25.0	100.0	100.0	12.5

[a] MIC (minimum inhibitory concentration) in μg mL^{-1}. [b] Positive controls: chloramphenicol for bacteria strains and ketoconazole for yeast strains. [c] Standard strain. [d] Field strain. [e] (−) no inhibition of development.

fraction (0.82 g) and the CH_2Cl_2 neutral fraction (7.79 g). The alkaloid fraction (0.80 g) was subjected to a 10% $NaHCO_3$-treated silica gel column chromatography[10] eluted with the following gradient systems: petroleum ether−CH_2Cl_2 from 100:0 to 10:90 followed by CH_2Cl_2−EtOAc from 100:0 to 10:90, and EtOAc−MeOH from 100:0 to 50:50. The eluted fractions were evaluated and pooled according to TLC analysis to afford 15 fractions. Fraction 8 (36.1 mg) was purified by preparative TLC eluted with CH_2Cl_2−MeOH (95:05) to give **4** (1.7 mg). Fraction 9 (32.0 mg) was fractionated by preparative TLC eluted with CH_2Cl_2−MeOH (95:05, 2×) to yield **1** (2.6 mg), **5** (2.1 mg), **9** (1.7 mg), **10** (0.7 mg), and **11** (0.8 mg). Fraction 10 (80.0 mg) was separated by preparative TLC eluted with petroleum ether−acetone (60:40, 3×), affording **6** (6.5 mg), **7** (5.2 mg), and a mixture of **7** and **8** (1.5 mg). Fraction 12 (60.9 mg) was separated by preparative TLC eluted with CH_2Cl_2−MeOH (90:10, 2×), yielding, after washing with $CHCl_3$ and recrystallization from $CHCl_3$−MeOH (2:1) **3** (1.3 mg), **12** (6.4 mg), and **13** (3.0 mg). Fraction 14 (85.5 mg) was also purified by preparative TLC eluted with CH_2Cl_2−MeOH (90:10, 3×), affording **2** (27.0 mg).

9-Methoxy-*O*-methylmoschatoline (1): orange, amorphous powder; UV λ_{max} (MeOH) (log ε) 208 (4.40), 238sh (4.26), 276 (4.39), 332sh (3.42), 367sh (3.07), 458 (3.49) nm; IR ν_{max} (film, $CHCl_3$) 2924, 2850, 1662, 1605, 1579, 1491, 1461, 1388, 1330, 1297, 1257, 1205, 1160, 1121, 1093, 1057, 1031, 1013, 991, 953, 862, 836, 764 cm^{-1}; ^1H and

^{13}C NMR data, Table 1; HR-ESIMS *m*/*z* 352.1322 (calcd for $C_{20}H_{17}NO_5 + H^+$, 352.1185).

9-Methoxyisomoschatoline (2): red, amorphous powder; UV λ_{max} (MeOH) (log ε) 206 (4.03), 224sh (3.90), 284 (3.85), 303sh (3.71), 506 (2.98) nm; IR ν_{max} (KBr): 3388, 2955, 2922, 2851, 1660, 1602, 1575, 1554, 1493, 1408, 1370, 1319, 1257, 1212, 1172, 1125, 1089, 1059, 1039, 1015, 985, 944, 866, 829, 776, 708, 649, 623, 552, 467, 434 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HR-ESIMS *m*/*z* 338.1210 (calcd for $C_{19}H_{15}NO_5 + H^+$, 338.1028).

Isocerasonine (3): red, amorphous powder; UV λ_{max} (MeOH) (log ε) 204 (3.22), 209sh (3.18), 227 (3.18), 257 (3.08), 267sh (3.01), 334 (2.82), 348sh (2.76), 366sh (2.58) nm; IR ν_{max} (film, $CHCl_3$) 3380, 2919, 2849, 1638, 1611, 1584, 1509, 1464, 1262, 874 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; HR-ESIMS *m*/*z* 354.1270 (calcd for $C_{20}H_{19}NO_5 + H^+$, 354.1342).

8-Oxypseudopalmatine (4): light yellow, amorphous powder; UV λ_{max} (MeOH) (log ε) 203 (3.34), 207sh (3.31), 224 (3.25), 257 (3.11), 269sh (3.03), 331 (2.80), 348sh (2.72), 365sh (2.60) nm; IR ν_{max} (film, $CHCl_3$) 2918, 2849, 1640, 1605, 1584, 1513, 1463, 1261, 872 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; HR-ESIMS *m*/*z* 368.1414 (calcd for $C_{21}H_{21}NO_5 + H^+$, 368.1498).

Antioxidant Capacity by ORAC Assay. The antioxidant capacities of the MeOH extract, CH_2Cl_2 neutral and alkaloid fractions, and pure alkaloids **2**, **5**, **6**, **7**, **9**, **12**, and **13** were assessed through the ORAC assay. The ORAC assay measures scavenging activity against the peroxyl radical, using fluorescein as the fluorescent probe. The ORAC assays were carried out on a Synergy HT multidetection microplate reader system. The temperature of the incubator was set at 37 °C. The procedure was carried out according to the method established by Ou and co-workers[20] with modifications.[21] The data are expressed as μmol of Trolox equivalents (TE) per gram of extract or fraction on a dry basis (μmol of TE/g) and as the relative Trolox equivalent for pure compounds. In these tests, quercetin, isoquercitrin, and caffeic acid were used as positive controls. The analyses were performed in triplicate.

Antimicrobial Activity. Alkaloids **2**, **5**, **6**, **7**, **9**, **12**, and **13** were also evaluated for antimicrobial activity using the broth microdilution method (96-well microtiter plates), as previously described by Salvador et al.,[22] to give a concentration between 10 and 1000 μg mL^{-1}. The minimal inhibitory concentration (MIC) was calculated as the lowest concentration showing complete inhibition of a tested strain. In these tests, chloramphenicol and ketoconazole were used as positive controls, while the solution DMSO−sterile distilled water (5:95, v/v) served as the negative control. Each sensitivity test was performed in duplicate for each microorganism evaluated and was repeated three times. The strains of microorganisms utilized are shown in Table 4.

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Supporting Information Available: 1D and 2D NMR and MS spectra of **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Chatrou, L. W.; Rainer, H.; Maas, P. J. M. Annonaceae (Soursop Family). In *Flowering Plants of the Neotropics*; Smith, N.; Mori, S. A.; Henderson, A.; Stevenson, D. W.; Heald, S. V., Eds.; Princeton University Press: Princeton, NJ, 2004; pp 18−20.
(2) Corrêa, M. P. *Dicionário das Plantas úteis do Brasil e das Exóticas Cultivadas*; IBDF: Rio de Janeiro, 1984.
(3) Silva, D. B.; Tulli, E. C. O.; Militão, G. C. G.; Costa-Lotufo, L. V.; Pessoa, C.; Moraes, M. O.; Albuquerque, S.; Siqueira, J. M. *Phytomedicine* **2009**, *16*, 1059–1063.
(4) Hsieh, T.-J.; Chang, F.-R.; Chia, Y.-C.; Chen, C.-Y.; Chiu, H.-F.; Wu, Y.-C. *J. Nat. Prod.* **2001**, *64*, 616–619.
(5) Muhammad, I.; Dunbar, D. C.; Takamatsu, S.; Walker, L. A.; Clark, A. M. *J. Nat. Prod.* **2001**, *64*, 559–562.
(6) Costa, E. V.; Marques, F. A.; Pinheiro, M. L. B.; Vaz, N. P.; Duarte, M. C. T.; Delarmelina, C.; Braga, R. M.; Maia, B. H. L. N. S. *J. Nat. Prod.* **2009**, *72*, 1516–1519.
(7) Costa, E. V.; Pinheiro, M. L. B.; Silva, J. R. A.; Maia, B. H. L. N. S.; Duarte, M. C. T.; Amaral, A. C. F.; Machado, G. M. C.; Leon, L. L. *Quim. Nova* **2009**, *32*, 78–81.
(8) Costa, E. V.; Teixeira, S. D.; Marques, F. A.; Duarte, M. C. T.; Delarmelina, C.; Pinheiro, M. L. B.; Trigo, J. R.; Maia, B. H. L. N. S. *Phytochemistry* **2008**, *69*, 1895–1899.
(9) Zhang, Z.; ElSohly, H. N.; Jacob, M. R.; Pasco, D. S.; Walker, L. A.; Clark, A. M. *J. Nat. Prod.* **2002**, *65*, 856–859.
(10) Costa, E. V.; Pinheiro, M. L. B.; Xavier, C. M.; Silva, J. R. A.; Amaral, A. C. F.; Souza, A. D. L.; Barison, A.; Campos, F. R.; Ferreira, A. G.; Machado, G. M. C.; Leon, L. L. P. J. *J. Nat. Prod.* **2006**, *69*, 292–294.
(11) Boyom, F. F.; Ngouana, V.; Zollo, P. H. A.; Menut, C.; Bessiere, J. M.; Gut, J.; Rosenthal, P. J. *Phytochemistry* **2003**, *64*, 1269–1275.
(12) Waechter, A.-I.; Cavé, A.; Hocquemiller, R.; Bories, C.; Muñoz, V.; Fournet, A. *Phytother. Res.* **1999**, *13*, 175–177.
(13) Erkens, R. H. J.; Maas, P. J. M. *Rodriguésia* **2008**, *59*, 401–406.
(14) Ribeiro, J. E. L. S.; Maas, P. J. M.; Maas, H.; Miralha, J. M.; Webber, A. In *Annonaceae*; Ribeiro, J. E. L. S.; Hopkins, M. J. G.; Vicentini, A.; Sothers, C. A.; Costa, M. A. S.; Brito, J. M.; Souza, M. A. D.; Martins, L. H. P.; Lohmann, L. G.; Assunção, P. A. C. L.; Pereira, E. C.; Silva, C. F.; Mesquita, M. R.; Procópio, L. C., Eds.; INPA: Manaus, 1999; pp 121−135.
(15) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1983**, *46*, 761–835.
(16) González, M. C.; Zafra-Polo, M. C.; Blázquez, M. A.; Serrano, A.; Cortes, D. *J. Nat. Prod.* **1997**, *60*, 108–110.
(17) Harrigan, G. G.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Chan, G. W.; Johnson, R. K. *J. Nat. Prod.* **1994**, *57*, 68–73.
(18) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1979**, *42*, 325–360.
(19) Costa, E. V.; Pinheiro, M. L. B.; Marques, F. A.; Braga, R. M.; Maia, B. H. L. N. S. *Biochem. Syst. Ecol.* **2009**, *37*, 43–45.
(20) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.
(21) Salvador, M. J.; Ferreira, E. O.; Mertens-Talcott, S. U.; Castro, W. V.; Butterweck, V.; Derendorf, H.; Dias, D. A. *Z. Naturforsch.* **2006**, *61c*, 19–25.
(22) Salvador, M. J.; Ferreira, E. O.; Pral, E. M. F.; Alfieri, S. C.; Albuquerque, S.; Ito, I. Y.; Dias, D. A. *Phytomedicine* **2002**, *9*, 566–571.

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