

Indole Alkaloids from *Geissospermum reticulatum*

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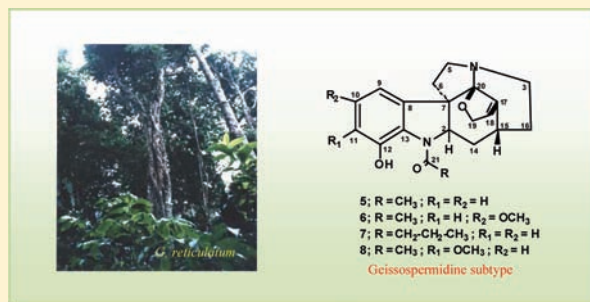
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Supporting Information

ABSTRACT: Ten indole alkaloids were isolated from *Geissospermum reticulatum*, seven (1–7) from the leaves and three (8–10) from the bark. Seven were aspidospermatan-type alkaloids (1–3, 5–9), including four (5–8) with a 1-oxa-3-cyclopentene group in their molecule, which we named geissospermidine subtype. Compounds 1–3, 5–8, and 10 had not been reported previously as natural products, while 4 and 9 were the known alkaloids *O*-demethylaspidospermine and flavopereirine. Their structures were determined by spectroscopic techniques including 1D and 2D NMR experiments (COSY, NOESY, HSQC, HMBC). Additionally, X-ray crystallographic analyses of 1, 2, and 6 were performed. Antiparasitic activities of the ethanolic and alkaloidal extracts and of the pure alkaloids were tested against *Trypanosoma cruzi* and *Leishmania infantum*. In general, the extracts exhibited selective action and were more active against *Leishmania* than against *Trypanosoma*. Alkaloid 4 was also very active against *L. infantum*.



Geissospermum reticulatum A. Gentry (Apocynaceae) is a tree commonly found throughout the Amazon rainforest of South America. Aqueous infusions of the bark of *Geissospermum* species are used by native communities for various medicinal purposes, exhibiting antimalarial, antitumoral, antioxidant, nociceptive, and antibacterial activities.¹ Of the 12 known species of this genus, four have been studied previously (*G. argenteum*, *G. laeve*, *G. sericeum*, and *G. vellosii*)^{2–6} and are sources of indole alkaloids of the strychnan (akuammicine subtype), corynanthean (normacusine B subtype), aspidospermatan (geissosvelline subtype), and flavopereirine β -carboline type.⁷

Indole alkaloids are known antiparasitic agents.⁸ Leishmaniasis and Chagas disease are parasitic diseases with high morbidity and mortality rates. Leishmaniasis is endemic in many tropical and subtropical countries, and Chagas disease is a public health menace in many Latin American countries (it is also spreading to the USA, Canada, and many parts of Europe and the Western Pacific as a result of migratory flows).⁹ In the case of both Leishmaniasis and Chagas disease, the drugs currently being used are far from satisfactory due to their side effects, and the search for new compounds to improve current treatments is of utmost importance.¹⁰

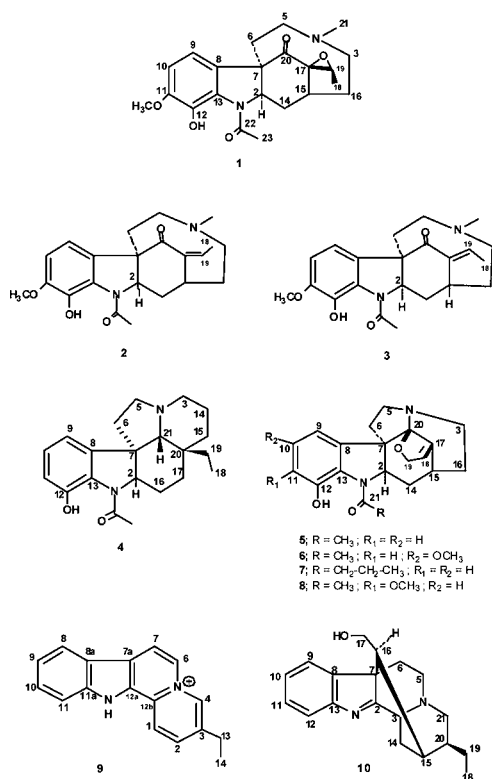
Here we report on the isolation of 10 indole alkaloids extracted from the leaves and bark of *G. reticulatum* as part of our ongoing search for plants from the Peruvian Amazonia for

use as antiparasitic agents.^{11,12} Compounds 1–3, 5–8, and 10 have not been reported previously as natural products, and aspidospermatan-type alkaloids 5–8 have a 1-oxa-3-cyclopentene group between C-17 and C-20, a structural subtype not previously described and which we have named geissospermidine. Alkaloids 4 and 9 are the known compounds *O*-demethylaspidospermine and the β -carboline alkaloid flavopereirine, respectively.^{13,14} The antiparasitic activities of the bark and leaf extracts and pure alkaloids against *Trypanosoma cruzi* and *Leishmania infantum* (the etiological agents of Chagas disease and leishmaniasis, respectively) and the cytotoxic effects on mammalian cells (CHO cell line) were tested.

RESULTS AND DISCUSSION

The *G. reticulatum* extracts exhibited antiparasitic action and were more active against *Leishmania* than *Trypanosoma* (Table S1, Supporting Information). An ethanolic extract of the bark (EB) exhibited a selective effect against *L. infantum*, while the alkaloidal extracts (acid and basic extracts from leaves and bark; AAL and BAL, AAB and BAB), were active against both parasites to varying degrees. Only the AAB extract was equally

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active against both parasites, and it was also moderately toxic to CHO mammalian cells at the lowest concentration tested. This indicated that *T. cruzi* is sensitive to *G. reticulatum* alkaloids, while *L. infantum* was affected by both alkaloidal and nonalkaloidal fractions. On the basis of these results, the alkaloidal extracts were selected for further chemical analysis.

Ten alkaloids (1–10) were isolated by successive purification by CC and PTLC. Compounds 1–3 had similar ¹H and ¹³C NMR spectra, indicating that they were closely related. The molecular formula of 1 was determined to be C₂₂H₂₈N₂O₅ by HREIMS, and elemental analysis was 16 amu higher than that obtained for compounds 2 and 3 (C₂₂H₂₈N₂O₄). IR and UV spectra of the three compounds showed a strong amide carbonyl band between 1622 and 1631 cm⁻¹ and absorptions characteristic of an *N*-acyldihydroindoline chromophore. The ¹H and ¹³C NMR spectra of compounds 1–3 showed identical shifts for two aromatic protons between δ_{H} 6.79–7.09 (d, *J* = 8.5 Hz) and 6.66–6.70 (d, *J* = 8.5 Hz), coupled with each other (COSY), a singlet at δ_{H} 10.6 (s, OH), one OCH₃ at δ_{H} 3.81–3.85 (s), and the *N*-COCH₃ group at δ_{H} 2.41–2.45 (s) and δ_{C} 169.3–169.4 (s, C=O). These signals were correlated with their carbon by HSQC and long-range correlations to neighboring carbon atom (HMBC) experiments (Table 1). These spectroscopic data were very similar to those published for geissovelline,¹⁵ a dihydroindole alkaloid, except that 1–3 had one less OCH₃. Moreover, compound 1 had a trisubstituted oxirane group [δ_{H} 2.75 (1H, q, *J* = 5.7 Hz), δ_{C} 63.9 (d)] coupled with a methyl signal at δ_{H} 1.14 (3H, d, *J* = 5.4 Hz); δ_{C} 13.4 (q) (COSY, HSQC) and δ_{C} 65.1 (s). The relative configuration of alkaloid 1 was obtained by a NOESY experiment (Figure 1) where the following spatial correlations were observed: the signal at δ_{H} 4.77 (H-2) with those at δ_{H} 1.88 (dd, H-6), 2.75 (m, H-5), 2.89 (ddd, H-3), the signal at δ_{H} 2.75 (q, H-19) with the proton at δ_{H} 1.80 (m, H-15), and a significant NOE between the methyl signal at δ_{H} 1.14 (d, H-18) and the aromatic proton at δ_{H} 6.79 (d, H-9).

Table 1. NMR Data for Compounds 1–3^a

position	1		2		3	
	δ_{H} mult (<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C}
2	4.77, dd (7, 11)	65.7	4.74, dd (6, 11)	65.0	4.78, dd (6, 11)	64.6
3	2.89, ddd (4, 13, 13)	58.9	2.77, m	56.9	2.83, m	56.7
3 β	3.18, dq (3, 14)		2.77, m		2.66, m	
5	2.75, m	54.3	2.77, m	51.9	2.80, m	51.9
5 β	2.60, dd (8, 14)		2.55, dd (8, 14)		2.57, dd (8, 14)	
6	3.06, m	43.8	2.97, m	43.8	2.92, m	44.4
6 β	1.88, dd (7, 13)		1.74, m		1.82, m	
7		58.4		56.7		55.7
8		124.9		127.8		127.8
9	6.79, d (8)	115.6	7.08, d (8)	115.8	7.09, d (8)	116.0
10	6.66, d (8)	109.6	6.70, d (8)	110.1	6.69, d (8)	110.0
11		150.4		150.1		150.1
12		137.0		136.9		136.8
13		127.7		127.4		127.4
14	2.46, ddd (3, 7, 14)	32.6	2.26, dq (3, 14)	33.7	2.29, dq (3, 14)	32.6
14 β	2.15, m		1.92, m		1.82, m	
15	1.80, m	38.2	2.77, m	39.6	3.23, m	29.8
16	1.56, dq (3, 15)	27.4	1.92, m	32.3	1.92, m	31.1
16 β	2.18, m		1.74, m		1.77, m	
17		65.1		137.9		138.6
18	1.14, d (5)	13.4	2.03, d (7)	15.2	1.70, d (7)	13.0
19	2.75, q (5)	63.9	5.76, q (7)	133.2	6.49, q (7)	127.4
20		192.5		185.3		184.3
21	2.08, s	41.2	1.94, s	40.6	1.90, s	40.3
22		169.4		169.3		169.3
23	2.42, s	22.7	2.41, s	22.7	2.43, s	22.8
OMe	3.85, s	56.4	3.81, s	56.4	3.81, s	56.4
OH	10.60, s		10.60, s		10.60, s	

^a500 MHz for ¹H, 125 MHz for ¹³C; chemical shifts in δ , coupling constants in Hz, CDCl₃.

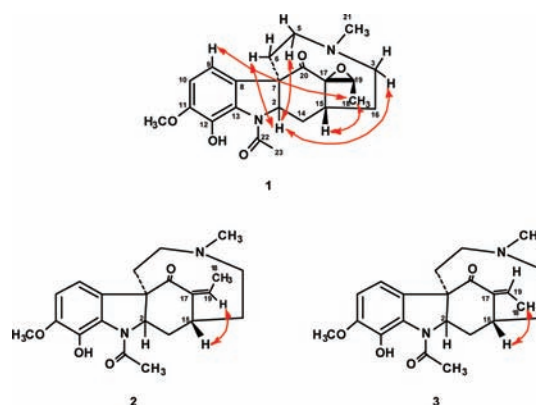


Figure 1. NOESY of compounds 1–3.

The NMR spectra of alkaloids 2 and 3 did not show the oxirane group chemical shifts but rather had signals corresponding to a methyl on a double bond and a methine

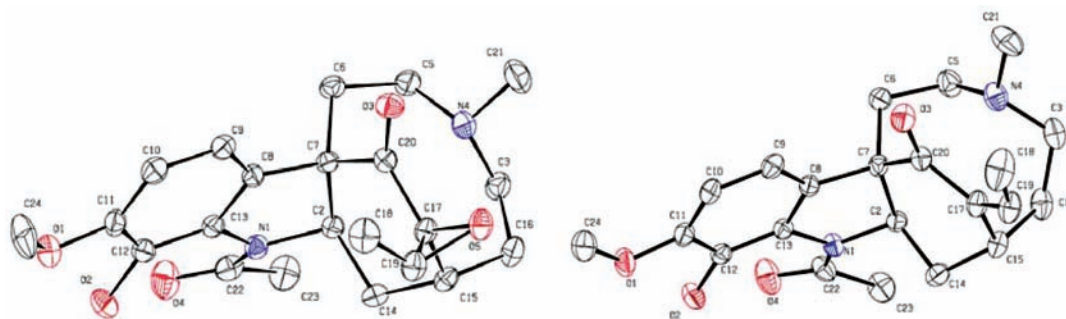


Figure 2. ORTEP drawings of compounds 1 and 2.

Table 2. NMR Data for Compounds 5–8^a

position	5		6		7		8	
	H mult (J in Hz)	C	H mult (J in Hz)	C	H mult (J in Hz)	H mult (J in Hz)	C	
2	4.18, dd (6, 11)	63.3	4.15, dd (6, 11)	63.3	4.24, dd (6, 11)	62.1	4.18, dd (6, 11)	63.3
3	2.64, ddd, (2, 5, 14)	44.7	2.62, ddd (2, 4, 14)	44.6	2.65, ddd (2, 5, 14)	44.7	2.64, ddd, (2, 5, 14)	44.7
3 β	3.07, m		3.06, m		3.09, m		3.07, m	
5	2.92, ddd (6, 11, 11)	48.7	2.90, ddd (6, 11, 11)	48.6	2.91, ddd (6, 11, 11)	48.6	2.92, ddd (6, 11, 11)	48.7
5 β	3.45, dd (8, 10)		3.40, dd (8, 10)		3.45, dd (8, 10)		3.45, dd (8, 10)	
6	2.28, ddd (8, 11, 11)	36.5	2.26, ddd (8, 11, 11)	36.3	2.28, ddd (8, 11, 11)	36.3	2.28, ddd (8, 11, 11)	36.5
6 β	1.96, dd (7, 13)		1.95, dd (6, 13)		1.96, dd (7, 13)		1.96, dd (7, 13)	
7		60.2		60.1		60.2		59.6
8		135.2		136.3		135.7		126.9
9	7.30, dd (1, 8)	128.2	6.91, d (3)	103.4	7.30, dd (1, 8)	116.0	7.21, d (8)	115.9
10	7.07, t (8)	109.6		160.0	7.07, t (8)	128.8	6.71, d (8)	110.4
11	6.84, dd (1, 8)	118.4	6.38, d (3)	102.9	6.85, dd (1, 8)	118.5		150.1
12		146.7		147.4		146.9		137.1
13		128.3		121.9		128.5		129.0
14	2.19, ddd (4, 7, 13)	36.4	2.17, ddd (4, 7, 13)	36.5	2.18, ddd (4, 7, 13)	32.6	2.20, ddd (4, 6, 13)	36.7
14 β	1.57, td (3, 11)		1.56, td (3, 11)		1.57, td (3, 11)		1.57, td (3,11)	
15	3.09, m	28.6	3.06, m	28.7	3.09, m	28.7	3.08, m	28.8
16, β	1.83, m	25.6	1.83, m	25.7	1.83, m	25.7	1.82, m	26.6
17		135.6		135.7		135.2		135.8
18	5.62, s	118.8	5.62, s	118.8	5.62, s	118.8	5.63, s	118.8
19	4.73, dd (1, 13)	73.3	4.73, dd (1, 13)	73.3	4.74, dd (1, 13)	73.3	4.74, dd (1,13)	73.2
19 β	4.50, dd (1, 13)		4.51, dd (1, 13)		4.51, dd (1, 13)		4.50, dd (1,13)	
20		106.3		106.2		106.3		106.3
21		168.9		168.0		171.7		169.0
22A		23.0		22.4	2.57, quint (7)	36.6	2.36, s	22.7
22B					2.47, quint (7)			
23					1.82, m	19.1		
24					1.06, t (7)	13.9		
OMe			3.74, s	55.6			3.86, s	56.4
OH	10.40, s			10.70, s	10.40, s		10.44, s	

^a500 MHz for ¹H, 125 MHz for ¹³C; chemical shifts in δ , coupling constants in Hz, CDCl₃.

proton [δ_{H} 2.03 (3H, d, $J = 7.2$ Hz); δ_{C} 15.3 (q); 5.76 (1H, q, $J = 7.6$ Hz); δ_{C} 133.2 (d)] for 2 and [δ_{H} 1.70 (3H, d, $J = 7.2$ Hz); δ_{C} 13.0 (q); 6.49 (1H, q, $J = 7.0$ Hz); δ_{C} 127.4 (d)] for compound 3. These data and the NOEs (NOESY experiment) between the proton at δ_{H} 2.77 (m, H-15) and the proton at δ_{H} 5.76 (q, $J = 7.6$ Hz, H-19) for compound 2 and the proton signal at δ_{H} 3.23 (m, H-15) with the methyl signal at 1.70 (d, $J = 7.2$ Hz, H-18) for compound 3 allowed us to establish the *Z* and *E* configurations for alkaloids 2 and 3, respectively (Figure 1). Complete analyses of the ¹H NMR, ¹³C NMR, COSY, HSQC, and HMBC experiments permitted assignment of the remaining protons and carbons for 1–3 (Table 1). The molecular structures of 1 and 2 were confirmed by a single-

crystal X-ray diffraction study on crystals obtained from an *n*-hexane/EtAcO solvent mixture (Figure 2). Therefore, the structures of alkaloids 1–3 were established as 10-demethoxy-12-hydroxy-17,19-epoxygeissovelline, (*Z*)-10-demethoxy-12-hydroxygeissovelline, and (*E*)-10-demethoxy-12-hydroxygeissovelline, respectively.

The mass spectrum of 4 gave a molecular ion at m/z 340.2150 (C₂₁H₂₈N₂O₂ by HREIMS), as well as peaks at m/z 312 and 124 typical of the aspidospermine skeleton.¹⁶ Its IR and UV spectra showed absorption bands characteristic of a 12-hydroxy-*N*-acetyldihydroindole group.¹⁷ The ¹H and ¹³C NMR (COSY, HSQC, HMBC) spectra of 4 coincided with those of

an aspidospermine skeleton, supporting identification as *O*-demethylaspidospermine.^{13,18}

The structures of alkaloids 5–8 were established by analysis of 1D and 2D NMR data (COSY, HSQC, HMBC, NOESY) (Table 2). The similarity of these data indicated that these compounds were closely related. Alkaloids 5–8 were dihydroindole derivatives with the same extended fused ring system, which is a new skeleton. HREIMS of 5–8 provided molecular formulas of C₂₀H₂₂N₂O₃ (M⁺ 338.1610), C₂₁H₂₄N₂O₄ (M⁺ 368.1736), C₂₂H₂₆N₂O₃ (M⁺ 366.1977), and C₂₁H₂₄N₂O₄ (M⁺ 368.1720), respectively. The aromatic proton and carbon resonances (HSQC and HMBC) indicated that 5 was a 12-hydroxy-*N*-acyldihydroindole derivative, 6 was a 12-hydroxy-10-methoxy-*N*-acyldihydroindole derivative, 7 was a 12-hydroxy-*N*-butanoyldihydroindole derivative, and 8 was a 12-hydroxy-11-methoxy-*N*-acyldihydroindole derivative (Table 2). The chemical shift between ¹H 4.15–4.24 (1H, dd, *J* = 6.4, 11.2 Hz); ¹³C 62.1–63.3 (d) in the ¹H NMR spectra of 5–8 (Table 2) is attributed to a methine proton attached to a C-2 carbon (HSQC) bonded to a nitrogen. In alkaloid 6 this proton showed long-range correlations to the carbon resonances at ¹³C 36.5 (t, C-14), 60.1 (s, C-7), 121.9 (s, C-13), 136.3 (s, C-8), 168.0 (s, N-CO-CH₃), and 106.2 (s, C-20) (HMBC, Figure 3),

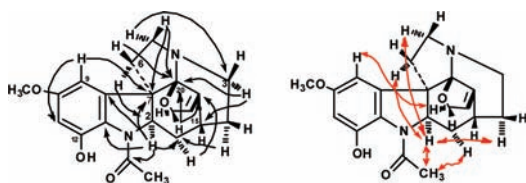


Figure 3. HMBC (→) and NOESY (↔) of compound 6.

respectively. The chemical shift at ¹³C 106.2 suggested that this signal could be attributed to a carbon of a carbinolamine group (N–C–O). Moreover, the methine proton (H-2) exhibited scalar coupling with protons at ¹H 2.17 and 1.56 (1H each, ddd, H-14) (COSY). The previous signal at higher field (1.56) was correlated with the carbon atoms at ¹³C 63.3 (d, C-2), 60.1 (s, C-7) and with the carbon at ¹³C 135.7 (s, C-17) (HMBC). This signal was connected with the chemical shift of a singlet at 5.62 (1H), ¹³C 118.8 (d) and two AB system protons at ¹H 4.73 (1H, dd, *J* = 1.1 and 12.6 Hz) and 4.51 (1H, br d, *J* = 12.5 Hz); ¹³C 73.3 (t), and the last signals correlated with a carbinolamine carbon at ¹³C 106.2 (s, C-20) in an HMBC experiment (Figure 3), confirming the presence of a 1-oxa-cyclopentene group in 5–8. The position of the 1-oxa-cyclopentene ring could be explained only by the structure proposed for alkaloids 5–8, where the signal at 4.73 (1H, dd) showed a NOE with H-9. The configuration at C-2 was established as C-2 H because it presented NOEs with H-5, H-6, H-16, and N-CO-CH₃ in a NOESY experiment (Figure 3). The remaining proton and carbon signals were assigned on the basis of spectroscopic data obtained from 1D and 2D NMR and were consistent with the proposed structures. Compounds 5–8 were classified as indole alkaloids of the aspidospermatan type with a 1-oxa-3-cyclopentene group between C-17 and C-20, thus comprising a new subtype. The molecular structure of 6 was confirmed by a single-crystal X-ray diffraction study on crystals obtained from an *n*-hexane/EtAcO solvent mixture (Figure 4) and, therefore, facilitated confirmation of the structures of alkaloids 5, 7, and 8 respectively.

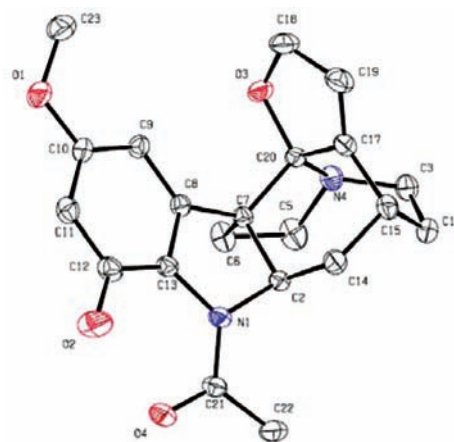


Figure 4. ORTEP drawing of compound 6.

The molecular formula of alkaloid 9 (C₁₇H₁₄N₂) was determined by HREIMS, and the ¹H and ¹³C NMR data (including 1D and 2D experiments in CD₃OD) were in agreement with values published previously for the known β -carboline alkaloid flavopereirine.¹⁴ This compound has been reported in the bark of *G. laeve*, *Strychnos longicaudata*, and *S. melinoniana*.¹⁹

The mass spectrum of alkaloid 10 showed a molecular ion at *m/z* 296, calculated for C₁₉H₂₄N₂O by HREIMS. The ¹H and ¹³C NMR spectra showed downfield signals at ¹H 7.33 (1H, d, *J* = 7.2 Hz), 7.21 (1H, t, *J* = 7.4 Hz), 7.32 (1H, t, *J* = 7.4 Hz), and 7.52 (1H, d, *J* = 7.4 Hz), which were correlated with their respective carbons at ¹³C 121.4 (d, C-9), 126.0 (d, C-10), 128.1 (d, C-11), and 120.4 (d, C-12) (HSQC). In addition, signals were detected from four quaternary carbons, three with downfield signals at ¹³C 193.4 (s, C-2), 144.7 (s, C-8), and 154.0 (s, C-13) and a fourth at ¹³C 66.4 (s, C-7). The marked downfield chemical shift of C-2 confirmed the presence of an unsubstituted indolenine.²⁰ The ¹H NMR spectrum also showed the presence of an ethyl side chain (methyl group at ¹H 0.97, 3H, t, *J* = 7.5 Hz and methylene protons at ¹H 1.35, 2H, m) and a pair of AB doublets due to the C-17 oxymethylene hydrogens at ¹H 3.86 and 3.93 (1H each, dd, *J* = 7.4, 11.2 Hz). The ¹H and ¹³C NMR data of alkaloid 10 were similar to those reported for akuammiline-type alkaloids.²⁰ 1D and 2D NMR experiments (HSQC and HMBC) allowed assignments of the remaining proton and carbon resonances (Table 3). The configuration at C-16 was established as C-16 H (¹H 2.86, m) because it presented NOEs with H-6 β , H-19, and H-21 β in a NOESY experiment (Figure 5). On the basis of these spectroscopic data, compound 10 was identified as a corynanthean indole alkaloid of the akuammiline type, which we named geissosreticulatine.

Assays against *L. infantum* and *T. cruzi* showed that 4 and 7 were the only active compounds. The alkaloids from the bark (8–10) could not be tested due to the low amounts available. The tested compounds all showed a GI₅₀ > 100 g/mL. Compound 4 (*O*-demethylaspidospermine) was very active against *L. infantum* (GI₅₀ = 7.7) with a lesser effect on *T. cruzi* (GI₅₀ = 41.7). The toxicity of 4 on CHO cells (GI₅₀ = 16.7) was lower than that of the reference drugs (amphotericin B and nifurtimox; GI₅₀ = 10.3 and 13.9, respectively). Compound 7 was active only against *L. infantum*, with a moderate effect (GI₅₀ = 52.2). The activity of 4 could explain that of the leaf alkaloidal extract. Compound 4 is structurally related to aspidospermine

Table 3. NMR Data for Compound 10^a

position	¹ H mult (J in Hz)	position
2		193.4
3	3.75, br s	70.2
5	3.24, m	57.8
5β	3.15, m	
6	1.91, m	33.8
6β	2.75, m	
7		66.4
8		144.7
9	7.33, d (7)	121.4
10	7.21, t (7)	126.0
11	7.32, t (7)	128.1
12	7.52, d (7)	120.4
13		154.0
14	1.63, dt (2, 14)	29.0
14β	1.07, dq (2, 14)	
15	1.95, br s	36.4
16	2.86, m	38.6
17A	3.86, dd (7, 11)	64.9
17B	3.93, dd (7, 11)	
18	0.97, t (7)	11.9
19A	1.35, m	25.0
19B	1.35, m	
20	1.65, m	41.9
21	3.16, m	51.8
21β	2.46, t (12)	

^a500 MHz for ¹H, 125 MHz for ¹³C; chemical shifts in δ , coupling constants in Hz, CDCl₃.

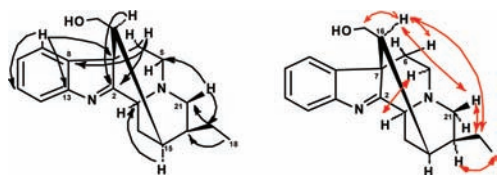


Figure 5. HMBC (→) and NOESY (↔) of compound 10.

and aspidopermidine, these alkaloids showing activity against strains of *Plasmodium falciparum* with resistance to chloroquine.²² However, this is the first report on their antiparasitic effects on *L. infantum*.

The activity of the bark extracts could be explained by the presence of alkaloids 8–10. Specifically, alkaloid 9 possesses antiplasmodial and cytotoxic activity and is able to interact selectively with cancer cells.^{4,21}

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Reichert Thermovar apparatus and are uncorrected. Optical rotations were determined in CHCl₃ at room temperature using a Perkin-Elmer 343 Plus polarimeter. IR spectra were taken on a Perkin-Elmer 1600 FT spectrometer. UV–vis spectra were recorded in EtOH on a Hewlett-Packard HP-8254 spectrophotometer. NMR spectra were measured on a Bruker AMX2 500 MHz spectrometer with pulsed field gradient and referenced to residual solvent signals (CDCl₃, at ¹H 7.26 and ¹³C 77.0). EIMS and HREIMS data were recorded on a VG-Micromass ZAB 2F instrument at 70 eV. Single-crystal X-ray diffraction analyses were conducted using an Oxford Diffraction Supernova Dual diffractometer equipped with an Atlas CCD using Cu K α radiation. Neutral alumina (Merck Art. 1097 and 5550) was used for column chromatography and TLC. Alkaloids were visualized on TLC with Dragendorff's reagent.

Plant Material. *G. reticulatum* was collected in CRI-IIAP at Puerto Maldonado (Departamento de Madre de Dios) at 280 m above sea level and identified by Ing. J. Ruiz Macedo. A voucher specimen (HIP no. 046008) has been deposited in the Herbarium of the Universidad Nacional de la Amazonia Peruana (UNAP), Iquitos, Perú.

Extraction and Isolation. The leaves (1.6 kg) and bark (3.2 kg) were dried and ground and then separately extracted with EtOH/H₂O (70/30) at room temperature, solvent being replenished every 72 h for 18 days. The extracts were filtered and concentrated under vacuum to give 40.2 g (2.5%) and 46.5 g (1.4%), respectively. These crude leaf and bark extracts were dissolved in a mixture of CH₂Cl₂ and 0.5 N H₂SO₄ (1:1) for 12 h at room temperature. The organic phase was filtered and dried over Na₂SO₄, and the solvent was removed to give two CH₂Cl₂ extracts (A, 14.2 g, 0.85%) and (B, 3.1 g, 0.19%), respectively. The pH of the remaining aqueous phase was adjusted to 10 with NaOH and repeatedly extracted with CH₂Cl₂. Evaporation of the solvent gave two crude alkaloidal extracts (C, 3.73 g, 0.23%; D, 3.60 g, 0.11%). Extracts A, B, C, and D were chromatographed on a neutral alumina column. Elution was carried out with hexane, hexane/EtAcO, and EtAcO/MeOH mixtures of increasing polarity to give the alkaloids 1 (73.0 mg, 0.4 \times 10⁻² %), 2 (105.0 mg, 0.6 \times 10⁻² %), 3 (58.3 mg, 0.3 \times 10⁻² %), 4 (24.2 mg, 0.15 \times 10⁻² %), 5 (40.0 mg, 0.25 \times 10⁻² %), 6 (22.1 mg, 0.13 \times 10⁻² %), and 7 (22.0 mg, 0.137 \times 10⁻² %) from extract A, 1 (15.0 mg, 0.4 \times 10⁻³ %), 8 (9.3 mg, 0.258 \times 10⁻³ %), and 9 (9.6 mg, 0.26 \times 10⁻³ %) from extract B, 1 (25.0 mg, 1.56 \times 10⁻² %) from extract C, and alkaloid 10 (7.9 mg, 2.4 \times 10⁻³ %) from extract D.

10-Demethoxy-12-hydroxy-17,19-epoxygeissosovelline (1): crystals, mp 316–318 °C; [α]_D²⁵ +34 (c 0.49, CHCl₃); UV (CHCl₃)_{max} (log ϵ) 211 (4.18), 232 (4.23), 293 (3.64), 327 (3.01) nm; IR (CHCl₃)_{max} 3448, 1700, 1631, 1454, 1249, 751 cm⁻¹; ¹H and ¹³C NMR see Table 1; EIMS *m/z* [M]⁺ 400 (100), 385 (11), 383 (9), 372 (8), 357 (17), 338 (28), 328 (18), 327 (79), 315 (19), 313 (15), 311 (17), 299 (9), 285 (15), 271 (12), 269 (37), 256 (8), 243 (13), 201 (10), 190 (12), 176 (18), 159 (33), 150 (12), 146 (16), 95 (11), 70 (32), 58 (58); HREIMS *m/z* 400.1976 [M]⁺ (calcd for C₂₂H₂₈N₂O₅, 400.1998); anal. C 65.8552, H 7.1247, N 7.2576, O 19.7625 calcd for C₂₂H₂₈N₂O₅.

(Z)-10-Demethoxy-12-hydroxygeissosovelline (2): crystals, mp 171–174 °C; [α]_D²⁵ +39.4 (c 0.104, CHCl₃); UV (EtOH)_{max} (log ϵ) 229 (4.33), 248 (4.09), 262 (4.14) nm; IR (CHCl₃)_{max} 1654, 1629, 1577, 1386, 1332, 1250, 1080, 753 cm⁻¹; ¹H and ¹³C NMR see Table 1; EIMS *m/z* [M]⁺ 384 (100), 369 (7), 356 (8), 341 (18), 325 (10), 314 (18), 313 (87), 299 (10), 272 (11), 271 (62), 270 (11), 257 (13), 256 (26), 202 (11), 190 (18), 189 (8), 176 (20), 161 (15), 138 (9), 124 (13), 122 (11), 81 (11), 72 (14), 70 (30), 67 (13), 59 (10), 58 (73), 57 (36); HREIMS *m/z* 384.2020 [M]⁺ (calcd for C₂₂H₂₈N₂O₄, 384.2049).

(E)-10-Demethoxy-12-hydroxygeissosovelline (3): crystals, 194–197 °C; [α]_D²⁵ +13.9 (c 0.08, CHCl₃); UV (EtOH)_{max} (log ϵ) 233 (4.23), 248 (4.14), 259 (4.14) nm; IR (CHCl₃)_{max} 3012, 2928, 2853, 1661, 1622, 1576, 1456, 1250, 1080, 752 cm⁻¹; ¹H and ¹³C NMR see Table 1; EIMS *m/z* [M]⁺ 384 (100), 369 (10), 356 (21), 341 (32), 325 (11), 314 (20), 313 (93), 299 (12), 283 (9), 272 (15), 256 (35), 202 (11), 190 (23), 176 (26), 161 (17), 138 (11), 124 (13), 122 (14), 72 (11), 70 (28), 58 (62); HREIMS *m/z* 384.2071 [M]⁺ (calcd for C₂₂H₂₈N₂O₄, 384.2089).

O-Demethylaspidospermine (4): oil; [α]_D²⁵ +110.4 (c 0.268, CHCl₃); UV, IR, and ¹H NMR see refs 13 and 18; ¹³C NMR (CDCl₃, 100 MHz) 169.6 (NCOCH₃, C-22), 147.2 (C, C-12), 141.1 (C, C-8), 127.7 (CH, C-10), 126.9 (CH, C-13), 117.5 (CH, C-11), 113.5 (CH, C-9), 70.8 (CH, C-21), 69.7 (CH, C-2), 53.7 (CH₂, C-3), 53.2 (C, C-7), 52.9 (CH₂, C-5), 39.1 (CH, C-19), 35.3 (C, C-20), 34.0 (CH₂, C-15), 25.7 (CH₂, C-16), 23.3 (NCOCH₃, C-23), 22.9 (CH₂, C-6), 21.9 (CH₂, C-14), 21.5 (CH₂, C-17), 6.7 (CH₃, C-18); EIMS *m/z* [M]⁺ 340 (25), 339 (20), 312 (7), 297 (3), 160 (3), 146 (3), 125 (8), 124 (100), 91 (2), 69 (2), 55 (3); HREIMS *m/z* 340.2134 [M]⁺ (calcd for C₂₁H₂₈N₂O₂, 340.2150).

Geissospermidine (5): crystals; mp 281–285 °C; [α]_D²⁵ +107.9 (c 0.14, CHCl₃); UV (EtOH)_{max} (log ϵ) 219 (4.15), 258 (3.71), 292 (3.68) nm; IR (CHCl₃)_{max} 2934, 2855, 1629, 1601, 1576, 1474,

1380, 1361, 1254, 1041, 750 cm^{-1} ; ^1H and ^{13}C NMR see Table 2; EIMS m/z [M]⁺ 338 (100), 295 (19), 253 (6), 201 (8), 160 (71), 159 (99), 150 (17), 146 (82), 138 (24), 117 (19), 91 (17), 77 (34), 53 (15); HREIMS m/z 338.1610 [M]⁺ (calcd for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_3$, 338.1630).

10-Methoxygeissospermidine (6): crystals; mp 242–244 °C; [α]_D²⁵ +118.4 (c 0.294, CHCl_3); UV (EtOH) λ_{max} (log ϵ) 211 (4.15), 260 (3.71), 293 (3.68) nm; IR (CHCl_3) λ_{max} 3482, 2933, 2852, 1629, 1600, 1578, 1455, 1371, 1155, 1044, 750 cm^{-1} ; ^1H and ^{13}C NMR see Table 2; EIMS m/z [M]⁺ 368 (73), 353 (10), 326 (11), 3325 (15), 311 (5), 233 (11), 232 (12), 231 (35), 204 (7), 191 (9), 190 (58), 189 (100), 176 (44), 159 (12), 150 (51), 138 (22), 136 (10), 123 (13), 83 (10), 77 (14); HREIMS m/z 368.1737 [M]⁺ (calcd for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$, 368.1736).

N-Deacetyl-N-butanoylgeissospermidine (7): resin; [α]_D²⁵ +62.9 (c 0.054, CHCl_3); UV (EtOH) λ_{max} (log ϵ) 211 (4.15), 260 (3.71), 293 (3.68) nm; IR (CHCl_3) λ_{max} 3400, 2926, 2853, 1626, 1600, 1576, 1466, 1347, 1257, 752 cm^{-1} ; ^1H and ^{13}C NMR see Table 2; EIMS m/z [M]⁺ 366 (64), 351 (4), 296 (11), 295 (15), 281 (7), 229 (10), 159 (100), 150 (25), 146 (24), 138 (23), 71 (7); HREIMS m/z 366.1977 [M]⁺ (calcd for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_3$, 366.1943).

11-Methoxygeissospermidine (8): amorphous; [α]_D²⁵ +94.8 (c 0.058, CHCl_3); UV (EtOH) λ_{max} (log ϵ) 227 (4.21), 260 (3.77), 295 (3.43) nm; IR (CHCl_3) λ_{max} 2930, 1632, 1599, 1451, 1380, 1248, 1040, 749 cm^{-1} ; ^1H and ^{13}C NMR see Table 2; EIMS m/z [M]⁺ 368 (100), 358 (6), 325 (16), 232 (16), 231 (28), 190 (47), 182 (92), 176 (32), 174 (12), 161 (24), 150 (21), 138 (20), 84 (8); HREIMS m/z 368.1720 [M]⁺ (calcd for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$, 368.1736).

Flavopereirine (9): resin; [α]_D²⁵ −15.8 (c 0.038, CHCl_3); EIMS m/z [M]⁺ 264 (65), 232 (8), 231 (20), 203 (5), 169 (7), 157 (10), 115 (7), 105 (16), 91 (100), 77 (6), 65 (8); HREIMS m/z 246.1154 [M]⁺ (calcd for $\text{C}_{17}\text{H}_{14}\text{N}_2$, 246.1157); ^1H and ^{13}C NMR data identical to those published.¹⁴

Geissosreticulatine (10): amorphous, yellow; [α]_D²⁵ −144.3 (c 0.158, CHCl_3); IR (CHCl_3) λ_{max} 3354, 2931, 2872, 1607, 1454, 1061, 855, 749 cm^{-1} ; ^1H and ^{13}C NMR, Table 3; EIMS m/z [M]⁺ 296 (68), 278 (34), 265 (24), 236 (42), 222 (31), 194 (100), 180 (55), 167 (35), 135 (68), 123 (68), 94(62); HREIMS m/z 296.1875 [M]⁺ (calcd for $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}$, 296.1889).

Crystal Data for 1, 2, and 6. Intensity data were collected at room temperature on an Enraf-Nonius KappaCCD diffractometer with Mo K radiation ($\lambda = 0.71707 \text{ \AA}$). Cell refinement and data reduction were performed with COLLECT²³ and DENZO.²⁴ The structures were solved by direct methods using SIR97.²⁵ Refinements were performed with SHELXL-97²⁶ using full-matrix least-squares with anisotropic displacement parameters for all the non-hydrogen atoms. For 1, all the H-atoms were located in successive difference Fourier synthesis and isotropically refined. The H atoms for 2 and 6 were placed in calculated positions and refined using a riding model. Calculations were mostly made using the WinGX²⁷ set of programs. Molecular graphics were computed with PLATON.²⁸ The ellipsoids were drawn at the 30% probability level, and H atoms omitted for the sake of clarity. Figures 2 and 4 show computer-generated perspectives of the final X-ray models of alkaloids 1, 2, and 6. Crystallographic data (excluding structure factor tables) for the structures reported have been deposited at the Cambridge Crystallographic Data Center as supplementary publication no. 856234 for 1, 856233 for 2, and 856232 for 6. Copies of the data can be obtained free of charge upon request to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: int. + (1223) 336 033); e-mail: deposit@ccdc.cam.ac.uk.

Crystal data for 1: $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_5$, $M_w = 400.5$, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $a = 11.545(3) \text{ \AA}$, $b = 13.385(2) \text{ \AA}$, $c = 13.018(4) \text{ \AA}$, $V = 2011.7(9) \text{ \AA}^3$, ($\text{Mo K } \alpha$) = 0.09 mm^{-1} , $\rho_{\text{calc}} = 1.32 \text{ g cm}^{-3}$; $S = 1.07$, final R indices: $R_1 = 0.053$ and $R_w = 0.139$ for 2520 observed from 2822 independent and 8854 measured reflections ($\lambda_{\text{max}} = 28.6^\circ$, $I > 2 (I)$ criterion and 267 parameters); maximum and minimum residues are 0.23 and $-0.27 \text{ e } \text{\AA}^{-3}$, respectively.

Crystal data for 2: $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_4$, $M_w = 384.5$, orthorhombic, space group $P2_12_12_1$, $a = 9.235(5) \text{ \AA}$, $b = 14.029(6) \text{ \AA}$, $c = 15.432(7) \text{ \AA}$, $V = 1999.3(17) \text{ \AA}^3$, $Z = 4$, ($\text{Mo K } \alpha$) = 0.09 mm^{-1} , $\rho_{\text{calc}} = 1.28 \text{ g cm}^{-3}$, $S =$

1.13, final R indices: $R_1 = 0.059$ and $R_w = 0.167$ for 2559 observed from 2855 independent reflections ($\lambda_{\text{max}} = 28.6^\circ$, $I > 2 (I)$ criterion and parameters); maximum and minimum residues are 0.25 and $-0.25 \text{ e } \text{\AA}^{-3}$, respectively.

Crystal data for 6: $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$, $M_w = 368.4$, monoclinic, space group $P2_1$, $Z = 2$, $a = 7.110(4) \text{ \AA}$, $b = 8.783(5) \text{ \AA}$, $c = 14.748(6) \text{ \AA}$, $\beta = 98.91(4)^\circ$, $V = 909.9(8) \text{ \AA}^3$, ($\text{Mo K } \alpha$) = 0.09 mm^{-1} , $\rho_{\text{calc}} = 1.35 \text{ g.cm}^{-3}$; $S = 1.04$, final R indices: $R_1 = 0.033$ and $R_w = 0.087$ for 2102 observed from 2206 independent and 6688 measured reflections ($\lambda_{\text{max}} = 27.5^\circ$, $I > 2 (I)$ criterion and 250 parameters); maximum and minimum residues are 0.19 and $-0.14 \text{ e } \text{\AA}^{-3}$, respectively.

Leishmanicidal Activity. Leishmanicidal activity was assayed on promastigote forms of *L. infantum* PB75 strain, cultured at 28 °C in RPMI medium supplemented with 10% fetal calf serum. Parasites in logarithmic growth phase from an initial culture with 1×10^6 promastigotes/mL were distributed in 96-well flat-bottom plates. Each well was filled with 90 μL of culture after 2 days of incubation. Extracts and compounds were tested (extracts at 800, 400, and 200 $\mu\text{g/mL}$; compounds at 100, 10, and 1 $\mu\text{g/mL}$) for 48 h. Amphotericin B was used as the reference drug, and parasite viability was analyzed by a modified MTT colorimetric assay as described previously.²² When compounds showed activity, intermediate doses were assayed and GI_{50} values (concentration inhibiting 50% of parasite growth) were determined from linear regression analysis (Statgraphics Plus, version 5.1). All assays were carried out in triplicate.

Antitrypanosomal Activity. This activity was evaluated on epimastigote forms of *T. cruzi* Y strain cultured in LIT medium supplemented with 10% fetal calf serum. Parasites in the logarithmic growth phase from an initial culture with 2×10^6 epimastigotes/mL were distributed in 96-well flat-bottom plates. Each well was filled with 90 μL of culture after 2 days of incubation. Extracts and compounds were tested (extracts at 800, 400, and 200 $\mu\text{g/mL}$; compounds at 100, 10, and 1 $\mu\text{g/mL}$) for 72 h. Nifurtimox was used as the reference drug, and parasite viability was analyzed by a modified MTT colorimetric assay method as described above.²² The activity (%GI and GI_{50}) was calculated as described above for *Leishmania*. All assays were carried out in triplicate.

Cytotoxicity Assays. Cytotoxicity was assayed using mammalian Chinese hamster ovary (CHO) cells cultured in RPMI medium supplemented with 10% L-glutamine and 10% fetal calf serum at 37 °C under humidified atmospheric conditions of 5% $\text{CO}_2/95\%$ air. Cells were seeded in 96-well flat-bottom microplates with 100 μL of medium per well (initial densities of 10^4 cells per well) and incubated under the same conditions. After 24 h the medium was removed and fresh medium added containing the extracts or compounds. Cells were exposed for 48 h to the extracts and compounds (extracts at 800, 400, and 200 $\mu\text{g/mL}$; compounds at 100, 10, and 1 $\mu\text{g/mL}$). Toxicity of reference drugs (amphotericin B and nifurtimox) was also evaluated. Cell viability was analyzed by the MTT colorimetric assay method as described previously.²² Where compounds showed activity, intermediate doses were assayed and GI_{50} values (concentration inhibiting 50% growth of the cells) were determined from linear regression analysis (Statgraphics Plus, version 5.1). All assays were carried out in triplicate.

■ ASSOCIATED CONTENT

📄 Supporting Information

Table S1 and ^1H and ^{13}C NMR spectra of alkaloids 1–3, 5–8, and 10 are available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) (a) Milliken, W. *Econ. Bot.* **1997**, *51*, 212–237. (b) Vigneron, M.; Deparis, X.; Deharo, E.; Bourdy, G. *J. Ethnopharmacol.* **2005**, *98*, 351–360. (c) Bertani, S.; Bourdy, G.; Landau, I.; Robinson, J. C.; Esterre, Ph.; Dehero, E. *J. Ethnopharmacol.* **2005**, *98*, 45–54. (d) Werner, J. A. T.; Oliveira, S. M.; Martins, D. F.; Mazzardo, L.; Dias, J. F. G.; Lordello, A. L. L.; Miguel, O. G.; Royes, L. F.; Ferreira, J.; Santos, A. R. *S. J. Ethnopharmacol.* **2009**, *125*, 163–169. (e) Correia, A. F.; Segovia, J. F. O.; Goncalves, M. C. A.; De Oliveira, V. L.; Silveira, D.; Carvalho, J. C. T.; Kanzaki, L. I. *B. Eur. Rev. Med. Pharmacol.* **2008**, *12*, 369–380. (f) Munoz, V.; Sauvain, M.; Bourdy, G.; Callapa, J.; Bergeron, S.; Rojas, I.; Bravo, J.; Balderrama, L.; Ortiz, B.; Gimenez, A.; Deharo, E. *J. Ethnopharmacol.* **2000**, *69*, 127–137. (g) Mbeunkui, F.; Grace, M. H.; Lategan, C.; Smith, P. J.; Raskin, I.; Lila, M. A. *J. Ethnopharmacol.* **2012**, *139*, 471–477.
- (2) Paccioni, J. P.; Husson, H. P. *Phytochemistry* **1978**, *17*, 2146–2147.
- (3) Chiaroni, A.; Riche, C.; Paies, M.; Goutarel, R. *Tetrahedron Lett.* **1976**, *51*, 4729–4730.
- (4) Steele, J. C. P.; Nigel, C. V.; Kite, G. C.; Simmonds, M. S. J.; Warhurst, D. C. *J. Nat. Prod.* **2002**, *65*, 85–88.
- (5) Ishiyama, H.; Matsumoto, M.; Sekiguchi, M.; Shigemori, H.; Ohsaki, A.; Kobayashi, J. *Heterocycles* **2005**, *66*, 651–658.
- (6) Rapoport, H.; Moore, R. E. *J. Org. Chem.* **1962**, *27*, 2981–2985.
- (7) Volkan Kisakurek, M.; Leeuwenberg, A. J. M.; Hesse, M. In *Alkaloids Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; John Wiley & Sons: New York, 1983; Vol. 1, Chapter 5, pp 211–376.
- (8) (a) Fernández, L.; Syke, M.; Andrews, K.; Avery, V. M. *Int. J. Antimicrob. Agents* **2010**, *36*, 275–279. (b) Staerk, D.; Lemmich, E.; Christensen, J.; Kharasami, A.; Olsen, C. E.; Jaroszewski, J. W. *Planta Med.* **2000**, *66*, 531–536.
- (9) (a) Hotez, P. J.; Molyneux, D. H.; Fenwick, A.; Kumaresan, J.; Sachs, S. E.; Sachs, J. D.; Savioli, L. *N. Engl. J. Med.* **2007**, *357*, 1018–1027. (b) Rassi, A., Jr.; Rassi, A.; Marín-Neto, J. A. *Lancet* **2010**, *375*, 1388–1402.
- (10) (a) Correa, D. S.; Tempone, A. G.; Reimao, J. Q.; Taniwaki, N. N.; Romoff, P.; Fávero, O. A.; Sartorelli, P.; Mecchi, M. C.; Lago, J. H. *Parasitol. Res.* **2011**, *109*, 231–236. (b) Croft, S. L.; Barrett, M. P.; Urbina, J. A. *Trends Parasitol.* **2005**, *21*, 508–512. (c) Ghosh, S.; Debnath, S.; Hazra, S.; Hartung, A.; Thomale, K.; Schutheis, M.; Kapkova, P.; Schurigt, U.; Moll, H.; Holzgrabe, U.; Hazra, B. *Parasitol. Res.* **2001**, *108*, 861–871. (d) Murray, H. W.; Berman, J. D.; Davies, C. R.; Saravia, N. G. *Lancet* **2005**, *366*, 1561–1577.
- (11) Ruiz-Mesia, L.; Ruiz-Mesia, W.; Reina, M.; Martínez-Díaz, R.; de Inés, C.; Guadano, A.; González-Coloma, A. *J. Agric. Food Chem.* **2005**, *53*, 1921–1926.
- (12) Reina, M.; Ruiz-Mesia, W.; Ruiz-Mesia, L.; Martínez-Díaz, R.; González-Coloma, A. *Z. Naturforsch.* **2011**, *66c*, 225–234.
- (13) Verpoorte, R.; Koskuyck, E.; Tjin, A.; Tsoi, A.; Ruijgrok, C. L. M.; De Jong, G.; Baerheim, S. *Planta Med.* **1983**, *48*, 283–289.
- (14) Giri, V. S.; Maiti, B. C.; Pakrashi, S. C. *Heterocycles* **1984**, *22*, 233–236.
- (15) Moore, R. E.; Rapoport, H. *J. Org. Chem.* **1973**, *38*, 215–230.
- (16) Biemann, K.; Spitteller-Friedmann, M.; Spitteller, G. *J. Am. Chem. Soc.* **1963**, *85*, 631–638.
- (17) Ferreira, J. M.; Gilbert, B.; Owellen, R. J.; Djerassi, C. *Experientia* **1963**, *19*, 585.
- (18) (a) Laguna, A.; Dolejs, L.; Novotny, L. *Collect. Czech. Chem. Commun.* **1980**, *45*, 1419. (b) Thomas, D. W.; Schones, H. K.; Biemann, K. *Experientia* **1969**, *25*, 678–679.
- (19) Southon, I. W.; Buckingham, J. *Dictionary of Alkaloids*; Chapman and Hall: London, 1989; Vol. I.
- (20) Subramanian, G.; Hiraku, M.; Hayashi, H.; Koyano, T.; Komiyama, K.; Kam, T.-S. *J. Nat. Prod.* **2007**, *70*, 1783–1789.
- (21) Beljanski, M.; Beljanski, M. S. *Exp. Cell Biol.* **1982**, *50*, 79–87.
- (22) González-Coloma, A.; Reina, M.; Sáenz, C.; Lcret, R.; Ruiz-Mesia, L.; Arán, V. J.; Sanz, J.; Martínez-Díaz, R. A. *Parasitol. Res.* **2012**, *110*, 1381–1392.
- (23) Nonius, B. V. *KappaCCD Server Software*; Nonius, B. V.: Delft, The Netherlands, 2000.
- (24) Otinowski, Z.; Minor, W. *Methods in Enzymology*, Vol. 276, *Macromolecular Crystallography, Part A*, Carter, C. W., Jr.; Sweet, R. M., Eds.; Academic Press: New York, 1997; pp 307–326.
- (25) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Burla, M. C.; Polidori, G.; Camalli, M.; Spagna, R. *SIR97*; University of Bari: Italy, 1997.
- (26) Sheldrick, G. M. *SHELXL97*; University of Gottingen: Germany, 1997.
- (27) Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837–838.
- (28) Spek, A. L. *PLATON*; University of Utrecht: The Netherlands, 2003.