Indole Alkaloids from *Aspidosperma rigidum* and *A. schultesii* and their Antiparasitic Effects

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Five oxindole alkaloids, three plumerane-type alkaloids, subtype haplophitine, and one aspidospermatane-type alkaloid, subtype tubotaiwine, were isolated from the medicinal plants *Aspidosperma rigidum* and *A. schultesii*. One compound was identified as the transoid conformer of 18-oxo-*O*-methylaspidoalbine which was not previously described. The antiparasitic activity of all compounds against *Trypanosoma cruzi* and *Leishmania infantum* and their non-specific cytotoxicity against mammalian cells were also determined.

Key words: Aspidosperma rigidum, *Aspidosperma schultesii*, Indole Alkaloids

Introduction

The crisis of re-emerging infectious diseases and the resistance of many pathogens to currently used drugs are widely recognized as being of serious and immediate concern. The different forms of leishmaniasis require expensive treatments, and the medicines used today, pentavalent antimonial and/or pentamidine salts, exhibit toxicity along with numerous side effects. Nifurtimox and benznidazole, used to treat the acute stages of Chagas' disease, are poorly tolerated. However, higher plants are a potential source of new antiprotozoal drugs (Phillipson and Wright, 1991). Furthermore, alkaloids have been found to be more effective antileishmanial agents than other natural products (Mishra *et al*., 2009).

Medicinal plants are a very important component of the biodiversity and traditional medicine of the Peruvian Amazonian region (Rojas *et al*., 2003). Various trees of the genus *Aspidosperma* (Apocynaceae) are used in northwest Amazonia to prepare remedies against fever and rheumatism and as a source of timber (Schultes and Raffauf, 1990; Oliveira *et al*., 2009). Previous studies reported that the bark of *A. ramiflorum* exhibits antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, and that the aerial parts show antiviral activity (Tanaka *et al*., 2006; Verpoorte *et al*., 1983; Roming *et al*., 1992). Species belonging to the *Aspidosperma* genus were extensively reported as being useful in the treatment of malaria. Additionally, *Aspidosperma* extracts showed very good antiprotozoal activity *in vitro*, including leishmanicidal and trypanocidal activities (Weniger *et al*., 2001). This genus is characterized by the occurrence of indole alkaloids (Cordell, 1979; Mitaine *et al*., 1996; Pereira *et al*., 2007).

As part of our ongoing work on antiparasitic compounds from Peruvian Amazonian plants (Ruiz-Mesía *et al*., 2005), we have studied the South American species *A. rigidum* and *A. schultesii* (Arndt *et al*., 1967; Gould *et al*., 2002) which are used as a popular remedy in Peru (Kvist *et al*., 2006; Sanz-Biset *et al*., 2009). Here we describe the isolation and purification of nine alkaloids whose structures were established through a comprehensive NMR study and by comparison with published data of similar compounds. Additionally, their antiparasitic activity against *Trypanosoma cruzi* and *Leishmania infantum* and their non-specific cytotoxicity on cell cultures were evaluated.

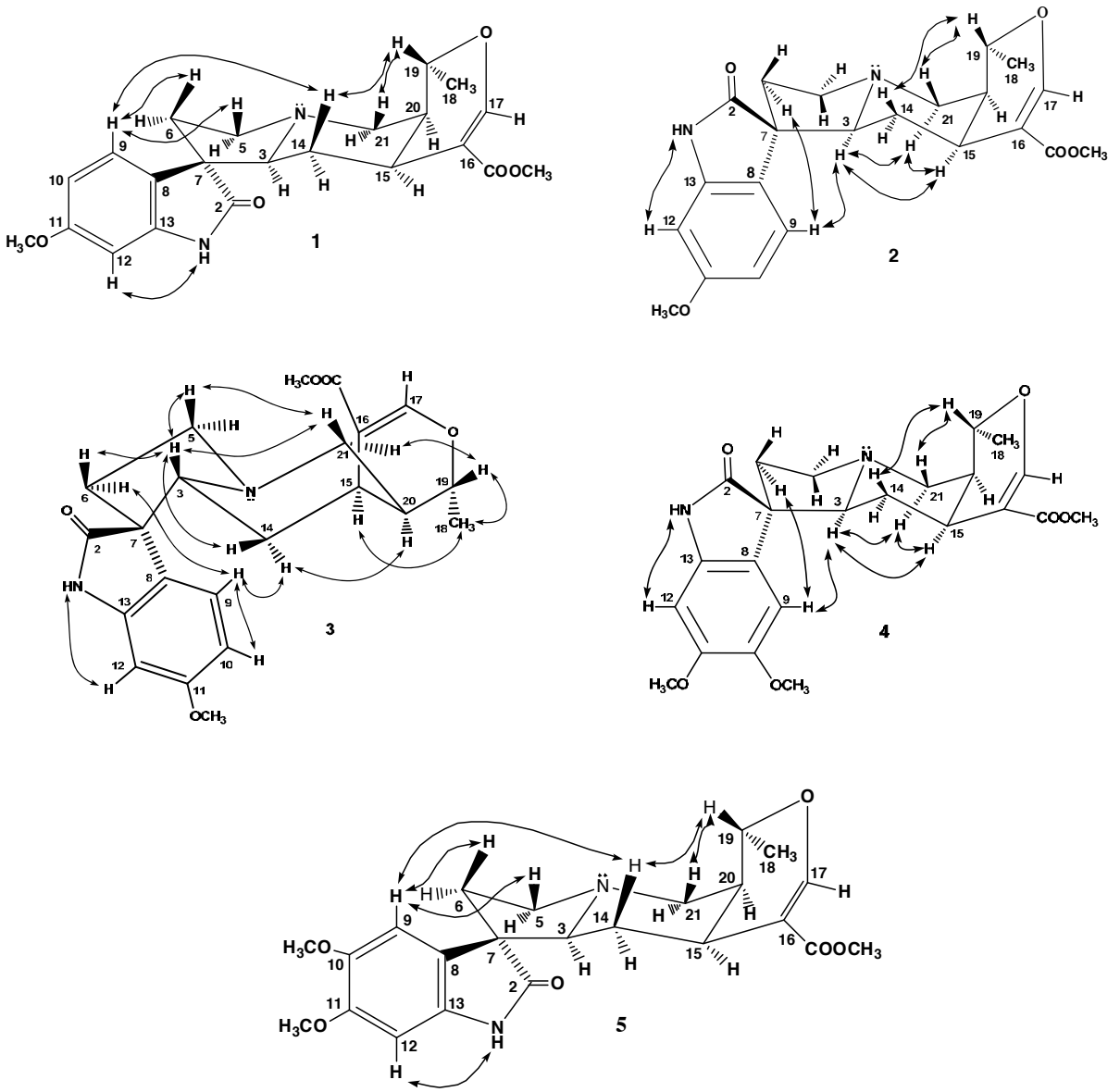

Fig. 1. NOESY of compounds **1**–**5**.

Results and Discussion

The study of the basic leaf, bark, and root extracts of *A. rigidum* and the basic bark extract of *A. schultesii* afforded nine alkaloids. Compounds **1**–**5** were found to be oxindole alkaloids, **6**–**8** to be plumerane-type alkaloids, subtype haplophitine, and **9** to be an aspidospermatane-type alkaloid, subtype tubotaiwine. All of these alkaloids have previously been isolated except for compound **8**, which is the transoid conformer of 18-oxo-*O*-methylaspidoalbine.

The spectroscopic data of alkaloids **1**–**5** displayed characteristic absorption bands for oxindole alkaloids (Titeux *et al.*, 1975), while the mass spectra of alkaloids **1**–**3** showed a molecular ion

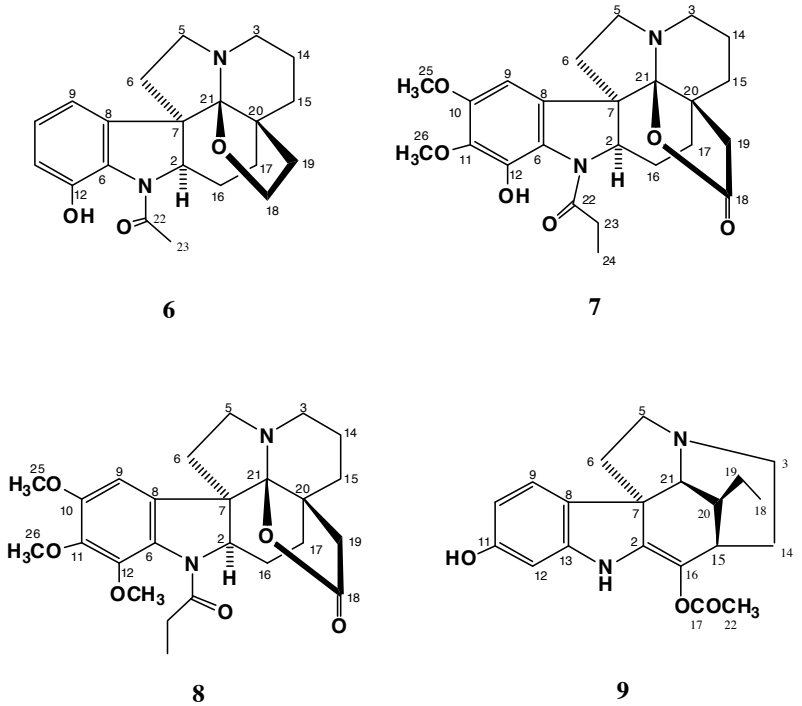

Fig. 2. Chemical structures of compounds **6**–**9**.

peak at *m/z* 398.18 corresponding to the molecular formula $C_{22}H_{26}N_2O_5$ and a fragmentation pattern characteristic of pentacyclic oxindole alkaloids (Gilbert *et al*., 1963). However, the molecular ion and fragment ion peaks of alkaloids **4** and **5** were 30 units (OMe) greater than those found for alkaloids **1**–**3**, indicating the presence of an additional methoxy group in the aromatic moiety. Their mass spectra were very similar to that reported for carapanaubine (Gilbert *et al*., 1963). A complete unambiguous assignment of the proton and carbon signals of alkaloids **1**–**5** (Fig. 1) was performed by ^{1}H-^{1}H COSY, ^{13}C DEPT, HSQC, and NOESY experiments (Tables I and II) and by comparison with published data for similar alkaloids (Pousset *et al*., 1967; Ripperger, 1977; Lounasmaa and Kan, 1980; Seki *et al*., 1993). Alkaloids **1**–**3** proved to be caboxine A, caboxine B, and isocaboxine •, and **4** and **5** were found to be carapanaubine and isocarapanaubine, previously isolated from *Cabucala fasciculata* (Titeux *et al*., 1975), *Aspidosperma carapanauba* (Giebert *et al*., 1963), and *Rauwolfia vomitoria* (Amer and Court, 1980), respectively.

Alkaloid **6** was obtained as •resin or solid?• with a molecular ion peak at *m/z* 354.1874, corresponding to the molecular formula $C_{21}H_{26}N_2O_3$, a fragmentation pattern similar to that of an aspidospermine-like skeleton with the presence of a tetrahydrofuran ring, and a base peak at *m/z* 138 (Djerassi *et al*., 1962). The spectroscopic data (IR, UV, ^{1}H and ^{13}C NMR) were essentially identical with those of haplocidine, previously isolated from different *Aspidosperma* species (Cava *et al*., 1963; Robert *et al*., 1983; Mitaine *et al*., 1996).

Alkaloids **7** and **8** were isolated as amorphous solids. Their mass spectra gave a molecular ion at *m/z* 442 and 456, respectively, and both exhibited an unusual fragmentation with *m/z* 160 as the base peak. Their UV spectra were nearly identical to that of the indole alkaloid *O*-methylaspidoalbine (Brown *et al*., 1966). The ^{1}H and ^{13}C NMR spectra of compounds **7** and **8** (1D and 2D NMR experiments, Table III) were closely related except for one peak of an additional methoxy group in the spectrum of alkaloid **8**. Additionally, the signal at δ_H 4.04 ppm (1H, dd, J = 11.2, 4.9 Hz) correlated (NOESY experiment) with signals at

Table I. ^{1}H and HSQC NMR data of alkaloids **1**–**5**.

No.	**1**		2		3	
	δ_H[a]	HSQC[b]	δ_H	HSQC	δ_H	HSQC
2	-	181.8 s	-	181.1 s	-	181.5 s
3*α*	2.48 m	71.2 d	2.28 m	74.4 d	-	67.8 d
3*β*	-		-		2.29 dd (11.6, 2.3)	
4	-	-	-	-	-	-
5*α*	2.39 m	54.0 t	2.31 m	55.0 t	4.5 dt (8.7, 2.3)	54.3 t
5b	3.18 ddd (8.0, 8.0, 2.3)		3.26 ddd (10.8, 8.7, 2.9)		2.43 q (9.0)	
6*α*	2.35 m	34.9 t	1.94 m	34.5 t	2.35 m	35.4 t
6*β*	1.95 ddd (11.6, 7.4, 7.4)		2.40 m		2.04 m	
7	-	56.5 s	-	55.6 s	-	56.3 s
8	-	125.7 s	-	125.2 s	-	126.0 s
9	7.14 d (8.2)	125.5 d	7.08 d (8.2)	123.7 d	7.24 d (8.2)	125.9 d
10	6.55 dd (8.2, 2.3)	107.3 d	6.56 dd (8.2, 2.3)	107.4 d	6.53 dd (8.2, 2.3)	107.1 d
11	-	159.7 s	-	159.2 s	-	160.0 s
12	6.46 d (2.3)	96.8 d	6.40 d (2.3)	96.7 d	6.42 d (2.3)	97.0 d
13	-	141.2 s	-	141.5 s	-	141.5 s
14*α*	1.59 m	30.2 t	1.70 m	29.5 t	1.04 ddd (13.1, 11.9, 5.0)	27.4 t
14*β*	0.88 q (11.8)		1.44 q (12.4)		2.14 m	
15*α*	2.51 m	30.4 d	2.41 m	30.9 d	2.70 m	25.4 d
16	-	109.9 s	-	109.2 s	-	105.4 s
17	7.41 s	154.9 d	7.47 s	155.2 d	7.40 d (2.0)	154.1 d
18	1.40 d (6.2)	18.6 q	1.40 d (6.1)	19.0 q	1.21 d (6.6)	18.9 q
19	4.34 dq (10.4, 6.2)	72.1 d	4.54 dq (10.6, 6.1)	72.2 d	4.16 q (6.6)	75.0 d
20*α*	1.59 m	37.9 d	1.59 br s	37.9 d	1.91 m	37.3 d
21*α*	2.40 m	53.5 t	2.30 m	53.7 t	2.97 dd (11.1, 4.5)	53.8 t
21*β*	3.26 dd (11.9, 1.8)		3.29 dd (11.9, 1.7)		2.14 m	
N-H	8.17 br s		7.41 br s		7.66 s	
OMe-10	-	-	-	-	-	-
OMe-11	3.79 s	55.5 q	3.79 s	55.6 q	3.81 s	55.6 q
$CO_2\underline{C}H_3$	3.60 s	50.9 q	3.61 s	50.9 q	3.60 s	51.2 q
$\underline{C}O_2CH_3$	-	167.6 s	-	167.7 s	-	167.7 s

2.40 and 2.53 ppm (1H each, m, H-23A and H-23B) assigned to H-2*α* of alkaloid **7**. However, H-2*α* of **8** showed a shift of 0.40 ppm to lower field due to the influence of the carbonyl group without spatial correlation with H-23 (NOESY). This spectroscopic evidence suggested that the -$NCOCH_2CH_3$ group is oriented towards C-2*α*H in alkaloid **7**, being identified as 18-oxo-aspidoalbine, previously isolated from *A. exalatum* (Medina and Hurtado, 1977), while alkaloid **8** is the transoid conformer of alkaloid **7**. The spectroscopic data of **8** differed from that published for 18-oxo-*O*-methylaspidoalbine, and its optical activity had the same value with an opposite sign. This compound has not been described as a natural product (Fig. 2).

Alkaloid **9** was identified as 11-hydroxytubotaiwine based on its ^{1}H and ^{13}C NMR spectral data and by comparison with published data for similar alkaloids (Aimi *et al.*, 1994). The 2D NMR experiments confirmed the chemical shifts of the remaining protons of alkaloids **6**–**9**.

The results of the antiparasitic and cytotoxic activities assays of alkaloids **1**, **2**, **4**, and **6**–**8** are summarized in Table IV. Compound **1** had significant antiparasitic effects at a dose of 100 *μ*g/ml (only one dose could be tested due to scarce compound availability), and was more toxic against *L. infantum* than against *T. cruzi*; **2** was active against *T. cruzi* with an ED_{50} value within the upper range of the positive control nifurtimox, while **4**, **6**, **7**, and **8** were inactive. None of these compounds were toxic against mammalian CHO cells.

The different activity patterns of **1** and **2** could be attributable to the difference in the stereochemistry of the B-ring of these compounds. An additional methoxy group at C-10 in **4** resulted in a loss of trypanocidal activity compared with

4		5	
δ_H	HSQC	δ_H	HSQC
-	181.3 s	-	181.1 s
2.28 m	74.3 d	2.52 *m*	71.1 d
-		-	
-	-	-	-
3.34 m 3.30 dt (9.0, 2.7)	55.1 t	2.39 m 3.21 ddd (16.2, 9.4, 2.4)	53.9 t
1.97 m 2.37 m	34.3 t	2.36 m 1.95 m	34.9 t
-	56.4 s	-	57.2 s
-	123.7 s	-	124.4 s
6.74 s	108.5 d	6.89 s	109.3 d
-	145.3 s	-	144.9 s
-	149.5 s	-	149.1 s
6.48 s	95.5 d	6.51 s	95.3 d
-	134.2 s	-	133.5 s
1.71 m 1.51 q (12.4)	29.6 t	1.60 m 0.84 q (11.9)	29.6 t
2.42 dt (11.7, 4.5)	31.0 d	2.39 m	30.3 d
-	109.2 s	-	109.9 s
7.48 s	155.2 d	7.41 s	154.9 d
1.40 d (6.0)	19.0 q	1.40 d (6.3)	18.3 q
4.54 dq (10.5, 6.1)	72.2 d	4.33 dq (10.3, 6.1)	72.1 d
1.60 m	37.9 d	1.59 m	38.8 d
2.31 m 3.31 dd (11.9, 1.8)	53.7 t	2.40 m 3.76 dd (11.9, 1.9)	53.3 t
7.66 s		7.82 *s*	
3.89 s	56.4 q	3.81 s	56.7 q
3.87 s	57.1 q	3.83 s	56.2 q
3.61 s	50.9 q	3.61 s	50.9 q
-	167.8 s	-	167.5 s

[a] Coupling constants (Hz) are shown in parentheses.
[b] Multiplicities were determined by DEPT data.

2. This is the first report on the antileishmanial and antitrypanocidal effects of the *A. rigidum* alkaloids caboxine A (**1**) and caboxine B (**2**). However, the antiplasmodial and antileishmanial effects of *Aspidosperma* spp. have been linked to the presence of alkaloids. Three alkaloids (fendlerine, aspidoalbine, and aspidolimidine), isolated from the stem bark of *A. megalocarpon*, exhibited strong antimalarial activity *in vitro* (Mitaine *et al.*, 1998), and several *Aspidosperma* alkaloids were modest antiplasmodial agents (Mitaine-Offer *et al.*, 2002). Previous studies showed that an alkaloid extract of *A. ramiflorum* was effective against *Leishmania amazonensis* (Ferreira *et al.*, 2004). Ramiflorines A and B purified from *A. ramiflorum* showed significant activity against *L. amazonensis* with potency values similar to those of compound **2** [LD_{50} values of (16.3 ± 1.6) μg/ml and (4.9 ± 0.9) μg/ml, respectively] (Tanaka *et al.*, 2007). However, little is known about the antitrypanocidal components of *Aspidosperma* spp.

The mode of action of alkaloids **1** and **2** is not known. The main targets of antileishmanial compounds are mitochondria and ergosterol synthesis. The mechanism of action • involves mitochondrial functions (Sun and Zhang, 2008). Paromomycin inhibits protein synthesis by binding to 16S rRNA (Vicens and Westhof, 2001). Similarly to other polyene antifungals, amphotericin B interferes with ergosterol, the main component of fungal cell membranes (Baginski and Czub, 2009). Delorenzi *et al.* (2001) reported that a monomeric indole alkaloid, coronaridine, causes pronounced ultrastructural alterations in the mitochondria of promastigotes and amastigotes, as assessed by transmission electron microscopy; compounds **1** and **2** could have a similar mode of action.

Table II. Proposed stereochemistry for alkaloids **1**–**5** based on a NOESY experiment.

Alkaloid	Proton	NOESY	C-7 configuration	D/E function
1	H-9 H-14β	H-6β, H-19β H-9	*S*	*cis*
2	H-3α H-14β	H-9 H-19β	*R*	*cis*
3	H-9 H-3β H-18α H-19β	H-14α, H-6α, H-10 H-21β, H-5β H-15α, H-19β H-21α, H-18α	*R*	*cis*
4	H-3α H-14β	H-9 H-19β	*R*	*cis*
5	H-19β	H-18α, H-14β, H-21β, H-9	*S*	*cis*

Table III. ^{1}H, HSQC, HMBC, and NOESY NMR data of alkaloid **8**.

Proton	δ_H[a]	HSQC[b]	HMBC	NOESY
2α	4.40 m	68.1 d	-	H-6α, H-16α
3α	2.72 dd (4.0, 11.1)	43.4 t	C-5, C-14, C-15, C-21	H-5α
3β	2.81 td (2.0,11.6)		C-5, C-14, C-15, C-21	H-5β
5α	3.08 td (5.6, 8.8)	48.5 t	C-6, C-7, C-21	H-3α
5β	2.98 m		C-6, C-7	H-3β
6α	1.99 m	33.4 t	C-2, C-5, C-7, C-21	H-2α, H-17α
6β	1.85 m		C-2, C-5, C-7, C-21	-
7	-	59.4 s	-	-
8	-	133.8 s	-	-
9	6.90 s	103.8 d	C-7, C-10, C-11, C-13	OCH_3-10
10	-	151.5 s	-	-
11	-	141.6 s	-	-
12	-	144.3 s	-	-
13	-	126.9 s	-	-
14α	1.76 m	20.2 t	C-20	-
14β	1.51 m		C-3, C-15	H-19β, H-17β
15	1.51 m	33.9 t	C-3, C-17, C-21	H-17β
16α	1.91 m	24.1 t	C-20, C-21	-
16β	1.50 m		-	H-19β
17α	1.91 m	25.0 t	-	H-2α, H-6α
17β	1.51 dd (4.3, 13.6)		C-2, C-19, C-20, C-21	H-15β, H-19β
18	-	176.1 s	-	-
19α	2.30 d (16.3)	42.5 t	C-15, C-17, C-18, C-20	-
19β	1.88 d (16.3)		C-15, C-18, C-20, C-21	H-14β, H-16β, H-17β
20	-	40.3 s	-	-
21	-	107.7 s	-	-
22	-	174.5 s	-	-
23A	2.61 q (8.1)	27.4 t	C-22, C-24	-
23B	2.27 q (8.1)		C-22, C-24	-
24	1.08 t (7.4)	9.7 q	C-22, C-23	H-23A, H-23B
25	3.75 s	56.1 q	C-10	-
26	3.81 s	61.1 q	C-11	-
27	3.73 s	60.1 q	C-12	-

[a] Coupling constants (Hz) are shown in parentheses.
[b] Multiplicities were established by DEPT data.

Table IV. Antiparasitic and cytotoxic effects of alkaloids **1**–**8** against *L. infantum*, *T. cruzi*, and mammalian CHO cells. Data is represented as average % mortality or % viability ± SE. Effective dose values are given in μg/ml (EC_{50} and 95% confidence limits).

Test	L. infantum		T. cruzi		CHO	
	% Mortality (100 μg/ml)	EC_{50}	% Mortality	EC_{50}	% Viability	EC_{50}
rigidum						
1	82.13 ± 1.8	nc	69.92 ± 4.2a	nc	65.07 ± 0.1	nc
2	20.68 ± 11.45	>100	68.92 ± 1.46	10.59 (7.96, 14.11)	89.67 ± 0.0	>100
4	28.34 ± 2.68	>100	35.87 ± 0.66	>100	99.38 ± 0.0	>100
6	0.00 ± 0.00	>100	35.58 ± 0.62	>100	nc	-
schultesii						
7	13.88 ± 5.95	>100	0.00	>100	94.30 ± 7.74	>100
8	6.06 ± 9.79	>100	0.00	>100	92.34 ± 6.72	>100
Amphotericin B	-	0.04 (0.01, 0.12)	-	-	-	10.25 (5.36, 19.61)
Nifurtimox	-	-	-	3.39 (1.40, 8.19)	-	13.91 (9.09, 21.30)

nc, not calculated. Some EC_{50} values (**1** on parasites and CHO cells, **2** and **4** on CHO cells) could not be calculated due to lack of product.

Experimental

General experimental procedures

Optical rotations were determined in $CHCl_3$ at room temperature using a Perkin-Elmer (•town•, MA, USA) 137 polarimeter. IR spectra were taken on a Perkin-Elmer (Barcelona, Spain) 1600 FT spectrometer. UV spectra were measured on a Hewlett-Packard (•town•, MN, USA) HP-8254-A instrument. NMR spectra were measured on a Bruker (Rheinstetten, Germany) AMX2 500 MHz spectrometer with pulsed field gradient using the solvent as internal standard ($CDCl_3$, at δ_H 7.26 ppm and δ_C 77.0 ppm). The programs used in two-dimensional (2D) NMR experiments (HMBC, HSQC, COSY, and NOESY) were those furnished with the manufacturer's software. EIMS and exact mass measurements were recorded on a Micromass Autospec (Manchester, UK) instrument at 70 eV. Alumina (Merck, Darmstadt, Germany, art. 1.01077) and silica gel 60 F_{254} (Merck, art. 105715) were used for column chromatography and preparative TLC, respectively. Alkaloids were visualized on TLC plates with Dragendorff's reagent.

Plant material

Leaves, bark, and roots of *A. rigidum* Standley were collected from adult flowering trees in March 2001 near the Pañacocha community (12 km from Iquitos, Peru, 120 m above sea level), and bark of *A. schultesii* was collected from the Allpahuayo Mishana biological station located at km 27 of the Iquitos-Nauta road (San Juan Bautista District, Maynas Province in the Departamento of Loreto, Peru), in June 2003. The materials were identified by Ing. J. Ruiz Macedo. Voucher specimens (No. 034316 and No. 035169) were deposited in the herbarium of the Universidad Nacional de la Amazonia Peruana, Iquitos, Peru.

Trypanocidal activity and cytotoxicity assays

Antitrypanocidal activity and non-specific toxicity were evaluated against epimastigote forms of *Trypanosoma cruzi* (Y strain) and CHO cells (mammalian Chinese hamster ovary cells), respectively, as described by (González-Coloma *et al.*, 2002).

Leishmanicidal activity

Leishmanicidal activity was evaluated against promastigote forms of *Leishmania infantum* (PB75 strain), cultured at 28 °C in RPMI medium supplemented with 10% fetal calf serum. Parasites in the logarithmic growth phase were distributed in 96-well flat-bottom plates. The compounds were dissolved in DMSO (<0.2%) and added to the cultures at several concentrations for 72 h. Amphotericin B was used as the reference drug,

and parasite viability was analysed by means of the modified •write out• (MTT) colorimetric assay (González-Coloma *et al.*, 2002). The activity was calculated as % mortality, and ED_{50} values (effective dose to obtain 50% culture growth) were determined from linear regression analysis.

The compounds were tested at concentrations of 100, 50, 25, 10, 5, and 1 μg/ml in order to determine the ED_{50} values, except **1** (on parasites and CHO cells), **2** and **4** (on CHO cells), which were tested at only one dose due to lack of product.

The non-specific toxicity of the extracts, evaluated on CHO cells, was calculated as % viability.

Extraction and isolation

Air-dried and powdered leaves and bark of *A. rigidum* (0.6 and 1.76 kg, respectively) and bark of *A. schultesii* (2.56 kg) were extracted repeatedly with ethanol (4 l). After removing the solvent under reduced pressure, the EtOH extracts of *A. rigidum* (72.3 and 16.9 g) and *A. schultesii* (165.9 g), respectively, were treated with 1.0 M H_2SO_4, filtered, and extracted with CH_2Cl_2 to obtain an acidic residue at pH 2 from *A. rigidum* (1.07 and 2.2 g) and *A. schultesii* (14.1 g). The acidic aqueous layers were then adjusted to pH 10 with concentrated NaOH and extracted with $CHCl_3$ to give a basic residue. The basic residue of *A. rigidum* (753.7 g) was subjected to column chromatography over alumina, eluted with *n*-hexane (100%), *n*-hexane/EtOAc, and EtOAc (100%), to afford 51 fractions of 250 ml each. Fractions 26–29 were combined and concentrated to produce a yellow foam (106.5 mg). Chromatography indicated that this was a mixture of three compounds, which were separated by preparative TLC on silica gel 60 F_{254} (art. 1.05715) eluted with *n*-hexane/EtOAc (40:60) to afford caboxine A (**1**) (57.7 mg), caboxine B (**2**) (10.4 mg), and isocaboxine B (**3**) (2.6 mg). The second basic residue (490.0 mg) was subjected to column chromatography over alumina and eluted under the same chromatographic conditions as described above producing two alkaloids, carapanaubine (**4**) (29.5 mg) and isocarapanaubine (**5**) (9.6 mg). Moreover, 1.5 kg of finely powdered roots of *A. rigidum* were extracted with EtOH and treated as described above to afford a basic residue (803.1 mg). Further purification of this residue by column chromatography over alumina under the same chromatographic conditions as described above afforded the alkaloid haplocidine (**6**) (12.0 mg). The basic extract (1.72 g) from *A. schultesii* was chromatographed on a Sephadex LH-20 column eluted with *n*-hexane/CH_2Cl_2/MeOH (3:1:1) to afford 54 fractions. Combining similar fractions allowed us to group them into the major fractions A (187.3 mg), B (147.7 mg), and C (275.5 mg). Alkaloid **7** (73.0 mg) was isolated from fraction A, alkaloid **8** (70.0 mg) from fraction B, and alkaloid **9** (9.0 mg) from fraction C.

Caboxine A (**1**): Amorphous solid. – $[\alpha]_D^{25}$ –66.1° (*c*, 0.304, $CHCl_3$) [lit. $[\alpha]_D^{25}$ –68° $CHCl_3$ (Titeux *et al.*, 1975)]. – UV (EtOH): λ_{max} (log ε) = 294 (4.20), 286 (4.30), 260 (4.54), 213 (4.49) nm. – IR (NaCl): υ_{max} = 3246, 2950, 1707, 1686, 1629, 1505, 1458, 1210, 1154, 1085, 757 cm^{-1}. – ^1H NMR: see Table I. – EIMS: *m*/*z* = 398 $[M]^+$ (100), 383 (3), 381 (4), 367 (6), 223 (57), 208 (20), 189 (16), 180 (7), 175 (14), 69 (39). – HREIMS: *m*/*z* = 398.1806 $[M]^+$, calcd. for $C_{22}H_{26}N_2O_5$ 398.1841.

Caboxine B (**2**): Amorphous solid. – $[\alpha]_D^{25}$ –77.5° (*c*, 0.040, $CHCl_3$) [lit. –107.9°, $CHCl_3$ (Titeux *et al.*, 1975)]. – UV: λ_{max} (log ε) = 299 (4.3), 289 (4.4), 265 (4.5), 215 (4.4) nm. – IR (NaCl): υ_{max} = 3248, 1704, 1630, 1192 cm^{-1}. – ^1H NMR: see Table I. – EIMS: *m*/*z* = 398 $[M]^+$ (100), 383 (3), 381 (4), 367 (7), 223 (71), 208 (21), 189 (15), 180 (17), 175 (11), 153 (22), 69 (39). – HREIMS: *m*/*z* = 398.1826 $[M]^+$, calcd. for $C_{22}H_{26}N_2O_5$ 398.1841.

Isocaboxine • (**3**): Amorphous solid. – $[\alpha]_D^{25}$ +64.3° (*c*, 0.028, $CHCl_3$) [lit. $[\alpha]_D^{25}$ +53°, $CHCl_3$ (Titeux *et al.*, 1975)]. – UV: λ_{max} (log ε) = 299 (4.3), 289 (4.4), 265 (4.5), 215 (4.4) nm. – IR (NaCl): υ_{max} = 3238, 1703, 1623, 1193 cm^{-1}. – ^1H NMR: see Table I. – EIMS: *m*/*z* = 398 $[M]^+$ (100), 383 (3), 381 (4), 367 (7), 223 (76), 208 (23), 189 (16), 180 (7), 175 (14), 173 (13), 153 (3), 69 (39). – HREIMS: *m*/*z* = 398.1824 $[M]^+$, calcd. for $C_{22}H_{26}N_2O_5$, 398.1641.

Carapanaubine (**4**): Amorphous solid. – $[\alpha]_D^{25}$ –43.2° (*c*, 0.044, $CHCl_3$) [lit. $[\alpha]_D^{25}$ –101°, $CHCl_3$ (Gilbert *et al.*, 1963)]. – UV: λ_{max} (log ε) = 298 (4.2), 287 (4.4), 265 (4.4), 215 (4.5) nm. – IR (NaCl): υ_{max} = 3300, 1707, 1684, 1108 cm^{-1}. – ^1H NMR: see Table I. – EIMS: *m*/*z* = 428 $[M]^+$ (100), 413 (3), 411 (4), 397 (3), 223 (60), 208 (23), 205 (30), 190 (37), 180 (7), 69 (62). – HREIMS: *m*/*z* = 428.1965 $[M]^+$, calcd. for $C_{23}H_{28}N_2O_6$ 428.1947.

Isocarapanaubine (**5**): Amorphous solid. – $[\alpha]_D^{25}$ –63.3° (*c*, 0.120, $CHCl_3$) [lit. $[\alpha]_D^{25}$ –68° $CHCl_3$ (Pousset *et al.*, 1967)]. – UV: λ_{max} (log ε) = 299 (4.2), 288 (4.5), 265 (4.4), 214 (4.4) nm. – IR (NaCl): υ_{max} =

3298, 1701, 1627, 1190 cm^{-1}. – ^1H NMR: see Table I. – EIMS: m/z = 428 $[M]^+$ (100), 413 (3), 411 (3), 397 (4), 223 (38), 208 (15), 205 (10), 190 (7), 180 (5), 69 (25). – HREIMS: m/z = 428.1946 $[M]^+$, calcd. for $C_{23}H_{28}N_2O_6$ 428.1947.

Haplocidine (**6**): •. – $[\alpha]_D^{25}$ +110.7° (*c*, 0.112, $CHCl_3$) [lit. $[\alpha]_D^{25}$ +231°, $CHCl_3$ (Cava *et al.*, 1963)]. – EIMS: m/z = 354 $[M]^+$ (30), 326 (39), 310 (63), 281 (3), 239 (4), 174 (2), 160 (18), 138 (100), 124 (3), 57 (15). – HREIMS: m/z = 354.1874 $[M]^+$, calcd. for $C_{21}H_{26}N_2O_3$ 354.1943. – ^1H and ^{13}C NMR: data identical to values published (Zeches *et al.*, 1995).

18-Oxo-aspidoalbine (**7**): Amorphous solid. – $[\alpha]_D^{25}$ +30.3° (*c*, 0.076, $CHCl_3$). – EIMS: m/z = 442 (65), 398 (36), 383 (11), 369 (17), 341 (10), 183 (11), 174 (17), 161 (50), 160 (100), 159 (17), 136 (14), 105 (13), 85 (11), 83 (16), 73 (13), 69 (12), 57 (18), 55 (19). – HREIMS: m/z = 442.2089 $[M]^+$, calcd. for $C_{24}H_{30}N_2O_6$ 442.2092. – ^1H and ^{13}C NMR: data identical to values published (Medina and Hurtado, 1977).

18-Oxo-O-methylaspidoalbine (**8**): Amorphous solid. – $[\alpha]_D^{25}$ –90.2° (*c*, 0.286, $CHCl_3$). – ^1H NMR: see Table I. – EIMS: m/z = 456 (70), 413 (9), 412 (33), 397 (14), 383 (36), 381 (17), 355 (17), 341 (5), 340 (10), 300 (15), 253 (15), 174 (19), 161 (65), 160 (100), 159 (17), 136 (15), 85 (8), 83 (13), 57 (19). – HREIMS: m/z = 456.2278 $[M]^+$, calcd. for $C_{25}H_{32}N_2O_6$ 456.2260.

11-Hydroxytubotaiwine (**9**): Isolated as resin. – $[\alpha]_D^{25}$ +589° (*c*, 0.023, $CHCl_3$). – EIMS: m/z = 340 (43), 283 (29), 281 (22), 246 (17), 245 (100), 198 (26), 197 (25), 196 (35), 184 (13), 183 (24), 170 (11), 167 (9), 160 (5), 126 (17), 124 (57), 110 (11), 96 (20), 95 (43), 82 (14), 84 (30), 71 (90). – HREIMS: m/z = 340.1791 $[M]^+$, calcd. for $C_{20}H_{24}N_2O_3$ 340.1782. – ^1H and ^{13}C NMR: data identical to values published (Aimi *et al.*, 1994).

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Aimi N., Uchida N., Oya N., Sakai S., Mendoza L. A., and Stockigt J. (1994), Isolation of two new nitrogenous metabolites from the cultured cells of *Aspidosperma quebracho-blanco*. Heterocycles **38**, 2411–2414.

Amer M. M. and Court W. E. (1980), Leaf alkaloids of *Rauwolfia vomitoria.* Phytochemistry **19**, 1833–1836.

Arndt R. R., Brown S. H., Ling N. C., Roller P., Djerassi C., Ferreira J. M., Gilbert F. B., Miranda E. C., Flores S. E., Duarte A. P., and Carrazzoni E. P. (1967), Alkaloid studies – LVIII. The alkaloids of six *Aspidosperma* species. Phytochemistry **6**, 1653–1658.

Baginski M. and Czub J. (2009), Amphotericin B and its new derivatives – mode of action. Curr. Drug Metab. **10**, 459–469.

Brown K. S., Sanchez W. E., Figueiredo A. A., and Ferreira-Filho J. M. (1966), Unusual mass spectral fragmentation of 21-oxoaspidoalbidine-type alkaloids. J. Am. Chem. Soc. **88**, 4984–4989.

Cava M. P., Talapatra S. K., Kamura K., Weisbach J. A., Douglas B., and Shoop E. C. (1963), Haplocine and haplocidine: New aspidospermine-type alkaloids from *Haplophyton cimicidum*. Chem. Ind. London, 1242–1243.

Cordell G. A. (1979), The alkaloids, chemistry and physiology. In: The *Aspidosperma* Alkaloids (Manske R. H. F. and Rodrigo R. G. A., eds.). Academic Press, New York, Vol. XVII, chap. 3, pp. 199.

Delorenzi J. C., Attias M., Gattass C. R., Andrade M., Rezende C., Pinto C. A. T., Henriques T. A., Bou-Habib D. C., and Saraiva E. M. B. (2001), Antileishmanial activity of an indole alkaloid from *Peschiera australis*. Antimicrob. Agents Chemother. **45**, 1349–1354.

Djerassi C., Antonaccio L. D., Budzikiewicz H., and Wilson J. M. (1962), Mass spectrometry in structural and stereochemical problems. The structure of the *Aspidosperma* alkaloid aspidoalbine. Tetrahedron Lett. **3**, 1001–1009.

Ferreira I. C. P., Lonardoni M. V. C., Machado G. M. C., Leon L. L., Filho L. G., Pinto L. H. B., and De Oliveira A. J. B. (2004), Anti-leishmanial activity of alkaloidal extract from *Aspidosperma ramiflorum*. Mem. I. Oswaldo Cruz **99**, 325–327.

Gilbert B., Aguayo B. J., Finch N., Taylor W. I., Budzikiewicz H., Wilson J. M., and Djerassi C. (1963), Mass spectrometry in structural and stereochemical problems. Carapanaubine, a new alkaloid from *Aspidosperma carapanauba* and some observations on mass spectra of oxindole alkaloids. J. Am. Chem. Soc. **85**, 1523–1528.

González-Coloma A., Guadaño A., de Ines C., Martinez-Díaz R., and Cortes D. Z. (2002), Selective action of acetogenin mitochondrial complex I inhibitors. Z. Naturforsch. **57c**, 1028–1034.

Gould K. A., Fredericksen T. S., Morales F., Kennard D., Putz F. E., Mortacedo B., and Toledo M. (2002), Post-fire tree regeneration in lowland Bolivia: implications for fire management. Forest Ecol. Manag. **165**, 225–234.

Kvist L. P., Christensen S. B., Rasmussen H. B., Mejia K., and González A. (2006), Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. J. Ethnopharmacol. **106**, 390–402.

Lounasmaa M. and Kan S. K. (1980), A 400 MHz ^{1}H NMR study of the eight basic heteroyohimbine alkaloids. Tetrahedron **36**, 1607–1611.

Medina J. D. and Hurtado J. A. (1977), Alkaloids of the seeds of *Aspidosperma exalatum monachino*. Planta Med. **32**, 130–132.

Mishra B. B., Singh R. K., Srivastava A., Tripathi V. J., and Tiwari V. K. (2009), Fighting against leishmaniasis: Search of alkaloids as future true potential antileishmanial agents. Mini-Rev. Med. Chem. **9**, 107–123.

Mitaine A. C., Mesbah K., Richard B., Petermann C., Arrazola S., Moretti C., Zèches-Hanrot M., and Le Men-Olivier L. (1996), Alkaloids from *Aspidosperma* species from Bolivia. Planta Med. **62**, 458–461.

Mitaine A. C., Weniger B., Sauvain M., Lucumi E., Aragón R., and Zeches-Hanrot M. Z. (1998), Indole alkaloids from the trunk bark of *Aspidosperma megalocarpon*. Planta Med. **64**, 487.

Mitaine-Offer A. C., Sauvain M., Valentin A., Callapa J., Mallie M., and Zèches-Hanrot M. (2002), Antiplasmodial activity of *Aspidosperma* indole alkaloids. Phytomedicine **9**, 142–145.

Oliveira V. B., Freitas M. S. M., Mathias L., Braz-Filho R., and Vieira I. J. C. (2009), Biological activity and indole alkaloid of the genus *Aspidosperma* (Apocynaceae): a review. Rev. Bras. •. Med. **11**, 92–99.

Pereira M. M., Lisieux R., Jácome R. P., Alcântara A. F. C., Alves R. B., and Raslan D. S. (2007), Alcalóides indólicos isolados de espécies do gênero *Aspidosperma* (Apocynaceae). Quim. Nova **30**, 970–983.

Phillipson J. D. and Wright C. W. (1991), Medicinal plants against protozoal diseases. Trans. R. Soc. Trop. Med. Hyg. **85**, 155–165.

Pousset J. L., Poisson J., Shine R., and Shamma M. (1967), Determination de la stéréochimie des alcaloïdes oxindoliques. Bull. Soc. Chim. Fr. **8**, 2766–2779.

Ripperger H. (1977), Isolation of isopteropodin from *Hamelia patens*. Pharmazie **32**, 415–416.

Robert G. M. T., Ahond A., Poupat C., Potier P., Jolles C., Jousselin A., and Jacquemin H. (1983), *Aspidosperma* de Guyane: Alcaloides de *Aspidosperma marcgravianum*. J. Nat. Prod. **46**, 694–707.

Rojas R., Bustamante B., Bauer J., Fernández I., Albán J., and Lock O. (2003), Antimicrobial activity of selected Peruvian medicinal plants. J. Ethnopharmacol. **88**, 199–204.

Roming T. L., Weber N. D., Murray B. K., North J. A., Wood S. G., Hughes B. G., and Cates R. G. (1992), Antiviral activity of Panamanian plants extracts. Phytother. Res. **6**, 38–43.

Ruiz-Mesía L., Ruiz-Mesía W., Reina M., Martínez-Díaz R., De Inés C., Guadaño A., and González-Coloma A. (2005), Bioactive *Cinchona* alkaloids from *Remijia peruviana*. J. Agric. Food Chem. **53**, 1921–1926.

Sanz-Biset J., Campos-de-la-Cruz J., Epiquién-Rivera M. A., Cañigueral S. (2009), A first survey on the medicinal plants of the Chazuta valley (Peruvian Amazon). J. Ethnopharmacol. **122**, 333–362.

Schultes R. E. and Raffauf R. F. (1990), The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia. Dioscorides Press, Portland.

Seki H., Takayama H., Aimi N., Sakai S., and Ponglux D. (1993), A nuclear magnetic resonance study on the eleven stereoisomers of heteroyohimbine-type oxindole alkaloids. Chem. Pharm. Bull. **41**, 2077–2086.

Sun T. and Zhang Y. (2008), Pentamide binds to tRNA through non-specific hydrophobic interactions and inhibits aminocylation and translation. Nucleic Acids Res. **36**, 1654–1664.

Tanaka J. C. A., da Silva C. C., de Oliveira A. J. B., Nakamura C. V., and Dias Filho B.P. (2006), Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. Braz. J. Med. Biol. Res., **39**, 387–391.

Tanaka J. C. A., da Silva C. C., Ferreira I. C. P., Machado G. M. C., Leon L. L., and de Oliveira A. J. B. (2007), Antileishmanial activity of indole alkaloids from *Aspidosperma ramiflorum*. Phytomedicine, **14**, 377–380.

Titeux F., Le Men-Oliver L., and Le Men J. (1975), Structure des caboxines: alcaloides oxindoliques du *Cabucala fasciculata*. Phytochemistry **14**, 565–568.

Verpoorte R., Van Beek T. A., Thomassen P., Aandewiel J., and Baerheim-Svendsen A. (1983), Screening of antimicrobial activity of some plants belonging to the Apocynaceae and Loganiaceae. J. Ethnopharmacol. **8**, 287–302.

Vicens Q. and Westhof E. (2001), Crystal structure of paromomycin docked into the eubacterial ribosomal decoding A site. Structure **9**, 647–658.

Weniger B., Robledo S., Arango G. J., Deharo E., Aragón R., Muñoz V., Callapa J., Lobstein A., and Anton R. (2001), Antiprotozoal activities of Colombian plants. J. Ethnopharmacol. **78**, 193–200.

Zeches M., Mesbah K., Richard B., Moretti C., Nuzillard J. M., and Le Men-Olivier L. (1995), Alkaloids from leaves and stems of *Vallesia glabra*. Planta Med. **61**, 89–91.