Isolation of Bis-Indole Alkaloids with Antileishmanial and Antibacterial Activities from *Peschiera van heurkii* (Syn. *Tabernaemontana van heurkii)*

V. Muñoz[1], *C. Moretti*[1,2,3,4], *M. Sauvain*[1,2], *C. Caron*[3], *A. Porzel*[3], *G. Massiot*[3], *B. Richard*[3], *and L. Le Men-Olivier*[3]

[1] Insituto Boliviano de Biologia de Altura (IBBA), CP 717, La Paz, Bolivia
[2] Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), Département Santé, 213 rue La Fayette, F-75480 Paris Cedex 10, France
[3] Laboratoire de Pharmacognosie, associé au CNRS - URA 492, Faculté de Pharmacie, 51 rue Cognacq Jay, F-51096 – Reims Cedex, France
[4] Address for correspondence

Received: September 27, 1993; Revision accepted: January 29, 1994

Abstract

Extracts from leaves and stem bark of *Peschiera van heurkii* (Muell. Arg.) L. Allorge (syn. *Tabernaemontana van heurkii* Muell. Arg., Apocynaceae) have been assayed for antileishmanial and antibacterial activities. The activities were concentrated in the alkaloid fractions which yielded 20 indole and bis-indole alkaloids. The strongest leishmanicidal and antibacterial activities were observed with the dimeric alkaloids conodurine (**1**), *N*-demethylconodurine (= gabunine) (**2**), and conoduramine (**3**). Weak toxicity towards macrophage host cells and strong activity against the intracellular amastigote form of *Leishmania* were observed for compounds **1** and **2**. *In vivo*, **1** was less active than glucantime (= *N*-methylglucamine antimonate), the drug of reference, while **2** was devoid of activity at 100 mg/kg.

Key words

Peschiera van heurkii, Tabernaemontana, Apocynaceae, bis-indole alkaloids, conodurine, gabunine, conoduramine, leishmanicidal, antibacterial activities, *Leishmania amazonensis, Leishmania braziliensis.*

Introduction

An antileishmanian screening of medicinal plants used in Bolivia (1) showed that aqueous alcoholic extracts from *Peschiera van heurkii* (Muell. Arg.) L. Allorge gave *in vitro* activity against *Leishmania* spp. The antiparasitic activity was concentrated in the alkaloid fractions. Since to the best of our knowledge, no previous chemical work has been published on the species, we report here on the isolation and structural elucidation of the alkaloids and on their leishmanicidal and antibacterial activities.

Planta Med. 60 (1994) 455–459

Materials and Methods

Determination of leishmanicidal activity

Culture and maintenance of the *Leishmania*

Parasites: Leishmania amazonensis strain MHOM/GF/84/CAY H-142 was originally isolated in the French Guyana Institut Pasteur. *Leishmania braziliensis* strain MHOM/BR/75/M 2903 and *Leishmania donovani chagasi* strain MHOM/BR/74/PP75 were obtained from IBBA, a WHO reference laboratory; identifications were controlled by isoenzyme analysis.

In vitro test procedure on promastigotes culture of *Leishmania* spp.

Compounds were aseptically dissolved in liquid medium and DMSO (final concentration of DMSO less than 0.1 %) and placed in microcells Titertek 96 (Flow Laboratories) to obtain final concentrations of 100, 50, 25, and 12.5 μg/ml. All assays were done in triplicate. Each cell was cultured with 50 000 parasites at 27 °C. The activity of the compounds was evaluated after 72 h by optical observation on a drop of culture with an inverted phase microscope, by comparison with control cells (without extracts), and with pentamidine-containing cells (2).

In vitro test procedure on the amastigote forms

Mouse peritoneal macrophages were obtained according to the procedure already described by Sauvain et al. (3). One million non-inflammatory macrophages were collected from each BALB/c mouse. The adherent cells were cultured at 37 °C under 5 % CO_2 during two hours, then the plates were washed with RPMI + buffer (MOPS-Sigma, USA), without FCS to eliminate non-adherent cells. The supernatant was replaced by 0.5 ml/well of fresh medium RPMI + glutamine + FCS + antibiotics before infection by *L. amazonensis* amastigotes at a ratio of infecting organism to host cell of 5 : 1. Infection took place at 34 °C during a minimum of 2 hours, and the compounds were added to the culture maintained at 37 °C under 5 % CO_2 for 24 hours. The medium was then renewed and the cells left to incubate for another 24 hours before fixation. Plates were fixed with methanol and stained with 10 % Giemsa's stain (Specia, France). They were set up with Eukitt Resin (CML, France). Macrophages with and without parasites were counted under × 40 magnification. For each triplicate assay, the survival index (SI) of amastigotes was calculated relative to the control.

In vivo test procedure

Eight-weeks-old female mice BALB/c, 18–20 g, n = 8 for each random group, (Charles Rivers Ltd, Margate, UK) were infected with 2.5×10^5 amastigotes of *L. amazonensis* (LV79 strains) in the posterior feet (4). Treatment started 2 weeks after infection by a single intralesional injection. Growth of cutaneous lesions of the mice was calculated for 8 consecutive weeks, by measuring the diameter of the infected feet with a vernier caliper (Kroelin 10DI00T6) at weekly intervals. Results were compared with those obtained with glucantime.

Determination of antibacterial activity by means of the agar diffusion technique (5)

Microorganisms

The following strains were used as test organisms: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Mycobacterium smegmatis* CNCM 7326, *Escherichia coli* CIP 54127, and *Pseudomonas aeruginosa*.

Culture of microorganisms

S. aureus, E. coli, and *P. aeruginosa* were subcultured in beef extracts, peptone, and NaCl broth at 37 °C for 18 hours. *B. subtilis* and *M. smegmatis* were subcultured on agar test plates with serum at 37 °C for 48 hours.

Test plates

Each alkaloid was dissolved in a citrate-phosphate buffer (pH 7.0) which was used as control. Portions of 5 ml of medium warmed at 45 °C, were inoculated with the test organisms (final density 0.4 at 260 nm) and transferred into Petri dishes. (∅ = 9 cm) containing 12 ml of medium. In all test plates 5 holes (∅ = 1 cm) were made, into each of which 0.2, 0.15, 0.10, or 0.05 ml (corresponding to 80 μg, 60 μg, 40 μg, 20 μg) of the test solution was pipetted. Plates were then incubated at 37 °C. Diameters of inhibition were measured and each product was tested against each microorganism in triplicate.

Streptomycine (10 μg) was used as a control of the growth and as a reference for antibiotic activity. The inhibition zones given in Table **4** are the averages of the results of these three experiments.

Plant material

Plant material was collected in Bolivia by one of us (CM), in the tropical rain forest of Chapare. Voucher specimens (Moretti 1455) are deposited in the National Herbarium of La Paz, Bolivia. The botanical collections were identified by L. Allorge (Museum National d'Histoire Naturelle, Paris).

Extraction and isolation

From the powdered defatted leaves (980 g) and stem bark (480 g), alkaloids were displaced from their salt by means of a 10% (v/v) ammonia solution (588 and 288 ml, respectively) and extracted with ethyl acetate (20 and 25 l, respectively). They were then separated from neutral compounds by extraction with 2% aqueous sulfuric acid (five times, 6 and 9 l). Neutralization of the aqueous phase and extraction with chloroform yielded crude dried (with Na_2SO_4) alkaloid mixtures: 12.2 g/kg (leaves), 36.4 g/kg (stem bark).

The mixtures, 8 g (leaves) and 18 g (stem bark), were fractionated respectively by silica gel (70–230 mesh) column chromatography over 240 g (∅ column 40 mm, 50 ml fractions) or 540 g (∅ column 50 mm, 100 ml fractions), eluted with $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity followed by pure MeOH.

The leaves afforded coronaridine (solvent $CHCl_3$/MeOH, 99:1), apodine and coronaridine hydroxyindolenine ($CHCl_3$/MeOH, 98:2), and hedrantherine ($CHCl_3$/MeOH, 95:5). The stem bark, afforded coronaridine ($CHCl_3$/MeOH, 99:1); elution with ($CHCl_3$/MeOH, 98:2) gave $N_{(a)}$-methylpericyclivine, conodurine (**1**), vobasine, conoduramine (**3**), perivine, gabunine (**2**) and 16-epiaffinine. More polar fractions with $CHCl_3$/MeOH, 80:20 to 50:50 gave accedinisine (**4**), normacusine B, and *N'*-demethylaccedinisine (**5**). Centrifugal TLC was carried out on a Chromatotron apparatus (Harrison Research) on silica gel PF 254 (4 mm thickness, elution with $CHCl_3$ and $CHCl_3$/MeOH).

Final purification of the alkaloids was performed by TLC on silica gel plates (Whatmann K6F, 0.5 mm thick) using $CHCl_3$/MeOH, 98:2, $CHCl_3$/MeOH, 90:10, or $CHCl_3$/MeOH/NH_4OH, 85:15:1, as eluent.

Characterisation of the alkaloids

Fifteen indole and bis-indole alkaloids were identified by comparison of their physical and spectral data with literature data, and by direct comparison with authentic samples. They are listed in the Table **1**. Four other compounds were separated in small quantities to elucidate their chemical structures. The structure of compound **5**, *N'*-demethylaccedinisine, was established by means of chemical correlation with **4**.

The novel isolation of **1**, **2**, **3**, and **4** has provided the opportunity to complete the literature data, by means of 2D-NMR experiments; ^{1}H- and ^{13}C-NMR were recorded in $CDCl_3$ at 300 and 75 MHz, respectively.

Conodurine (**1**) (8). ^{1}H-NMR: $\delta = 7.23$ (d, J = 8 Hz, H-9), 7.10 (m, H-10', H-11', H-12'), 6.82 (d, J = 8 Hz, H-10), 5.31 (m, H-3', H-19'), 3.97 (s, OMe), 3.70 (s, COOMe), 2.64 (s, NMe'), 2.51 (s, COOMe'), 1.67 (d, J = 7 Hz, H-18'), 0.84 (t, H-18). ^{13}C-NMR, see Table **5**.

Gabunine = N'-demethylconodurine (**2**) (8). ^{1}H-NMR: $\delta = 7.25$ (d, J = 8.4 Hz, H-9), 7.10 (m, H-10', H-11', H-12'), 6.82 (d, J = 8.4 Hz, H-10), 5.40 (q, H-19'), 5.30 (dd, J = 11 Hz, J' = 2 Hz, H-3'), 3.98 (s, OMe), 3.71 (s, COOMe), 2.56 (s, COOMe'), 1.68 (d, J = 7.0 Hz, H-18'), 0.81 (t, H-18). ^{13}C-NMR, see Table **5**.

Conoduramine (**3**) (8). ^{1}H-NMR: $\delta = 7.54$ (m, H-9'), 7.11–7.02 (m, H-10', H-11', H-12'), 6.90 (S, H-9), 6.80 (s, H-12), 5.35 (q, J = 7 Hz, H-19'), 5.13 (bd, J = 10 Hz, H-3'), 3.95 (s, OMe), 3.65 (s, COOMe), 2.62 (s, NMe'), 2.45 (s, COOMe'), 1.64 (d, J = 7 Hz, H-18'), 1.50 (m, H-19), 0.87 (t, J = 7 Hz, H-18). ^{13}C-NMR, see Table **5**.

Accedinisine (**4**) (14). ^{1}H-NMR: $\delta = 7.54$ (m, H-9'), 7.35 (d, J = 8 Hz, H-9), 7.20–7.00 (m, H-10', H-11', H-12, H-12'), 6.95 (d, J = 8 Hz, H-11), 5.40–5.35 (m, H-19, H-19'), 4.68 (d, J = 10 Hz, H-3'), 4.15 (d, J = 8 Hz, H-3), 4.05 (m, H-5'), 3.57 (s, N_1-Me), 3.30 (m, H-6'), 2.60 (N_4'-Me), 2.50 (s, COOMe'), 1.70 (m, H-18, H-18'). ^{13}C-NMR, see Table **5**.

N'-Demethylaccedinisine (**5**). Grey then yellow with Ceric-spray. TLC (silica gel), $CHCl_3/CH_3OH/NH_4OH$, 85:15:0.1, R_f 0.11. UV λ_{max}^{MeOH} nm (log ε): 230 (4.60), 287 (4.20), 294 (4.16). $[\alpha]_D$: $-72°$ (c 0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450–1725. ^{1}H-NMR: 7.54 (m, H-9'), 7.20–6.90 (m, six aromatics), 5.38–5.30 (m, H-19, H-19'), 4.67 (d, J = 9 Hz, H-3'), 4.18 (d, J = 8 Hz, H-3), 4.05 (m, H-5'), 3.57 (s, N_1-Me), 2.43 (s, COOMe'), 1.65 (m, H-18, H-18'). Methylation with formaldehyde/$NaBH_4$ (15) yielded accedinisine (**4**) (TLC, ^{1}H-NMR, and EIMS identical).

Results and Discussion

Fractionation of the alkaloid content led to the isolation of 20 alkaloids from the leaves and stem bark. Fifteen of them are known compounds. To the best of our knowledge, *N'*-demethylaccedinisine (**5**) was isolated for the first time from plants. The leishmanicidal activity of the alkaloids on the promastigote forms of *Leishmania amazonensis* (*L.a.*) and *L. braziliensis* (*L.b.*) are reported in Table **2**. The most potent compound was *N*-demethylconodurine (**2**) which remained active against *L.b.* at 10 μg/ml. Conodurine (**1**) and conoduramine (**3**) showed moderate activity. Apodine and accedinisine (**4**) were devoid of activity at 50 μg/ml.

Their activities on amastigote forms of *L.a.* are shown in Table **3**, and compared with their toxicity on normal macrophages. The alkaloids tested had no toxic effects on macrophage host cells. Conodurine (**1**) showed an SI of 47 % at 100 μg/ml. Gabunine (**2**) exhibited a strong activity with an SI of 3 % at 25 μg/ml. At this dose, glucantime was inactive.

The effect of glucantime and of conodurine (**1**) on the development of *L. amazonensis* lesions in BALB/c mice is presented in Fig. 1. Compound **1** had little effect on cutaneous lesions, compared with glucantime. Increasing the doses of conodurine (**1**) to 200 mg/kg gave rise to a strong cutaneous toxicity (mutilation). Gabunine (**2**) was devoid of activity at 100 mg/kg.

Table 1 Alkaloids isolated from leaves and stem bark of *Peschiera van heurkii* (Muell. Arg.) L. Allorge.

Alkaloids	Leaves*	Stem bark*	TLC**	UV	IR	MS	^{1}H-NMR	^{13}C-NMR	Ref.
affinisine		3 %	+	+		+	+		(6)
normacusine B		1 %	+	+	+	+	+		(6)
N(a)-methylpericyclivine		0.5 %	+	+		+	+		(7)
perivine		0.5 %	+	+		+	+		(8)
16-epiaffinine		2 %		+	+	+	+		(9)
vobasine		14 %	+	+		+	+		(8)
vobasinol		0.5 %	+	+	+	+	+		(10)
coronaridine	5 %	3 %	+	+		+	+		(8)
coronaridine hydroxyindolenine	1 %		+	+	+	+	+		(11)
apodine	2 %			+	+	+	+		(12)
hedrantherine	0.5 %			+	+	+	+		(13)
conodurine (1)		2 %	+	+		+	+	+	(8)
gabunine (2)		1 %	+	+		+	+	+	(8)
conoduramine (3)		6 %	+	+	+	+	+	+	(8)
accedinisine (4)		3 %		+	+	+	+	+	(14)
N'-demethylaccedinisine (5)		0.5 %		+	+	+	+		

* Percentage of pure compounds of the total alkaloidal content.
** Direct comparison with authentic sample.

Table 2 *In vitro* activity of alkaloids on various strains of promastigote forms of *Leishmania* spp.

Drugs[a]	Strains[b]	Concentrations[c] μg/ml 50	25	12.5	10
conodurine	H-142	0	0	0	
	2903	++	0	0	
gabunine	H-142	++	0	0	
	2903	+++	+++	+	
conoduramine	H-142	+	0		
	2903	++	+		
accedinisine	H-142	0			
	2903	0			
apodine	H-142	0			
	2903	0			
stem bark (Alk. extr.)	H-142	+			
	2903	++			
stem bark (Alk. ct)	H-142	++			
	2903	+++			
leaves (Alk. ct)	H-142	++			
	2903	+++			
pentamidine	H-142				+++
	PP75				+++
	2903				+++

[a] (Alk. extr.): alcoholic extracts; (Alk. ct.): alkaloid content.
[b] H-142: *Leishmania amazonensis* strain; 2903: *Leishmania braziliensis* strain.
[c] 0: promastigotes identical to control; +: about 75 % promastigotes, with a few degenerative forms; ++: about 50 % promastigotes, with degenerative forms; +++: 0 % promastigotes.

Table 3 *In vitro* activity of alkaloids on *L. amazonensis* amastigotes compared with their toxicity towards macrophages.

Alkaloids	Concentrations μg/ml	Viability[a] of macrophages (%)	Survival[b] of amastigotes-SI (%)
conodurine	100	100	47
	50	100	59
	25	100	70
gabunine	25	100	3
	12.5	100	98
conoduramine	10	90	30
	5	100	77
	2.5	100	91
accedinisine	20	100	80
	10	100	100
	5	100	100
glucantime	800	100	3
	400	100	21
	200	100	53
	100	100	93
	50	100	98
	25	100	100
dimethyl sulfoxide	0.2 %	100	90
	0.1 %	100	100

[a] Viability = mean % of macrophages surviving.
[b] S.I. % = index of surviving intracellular amastigotes.

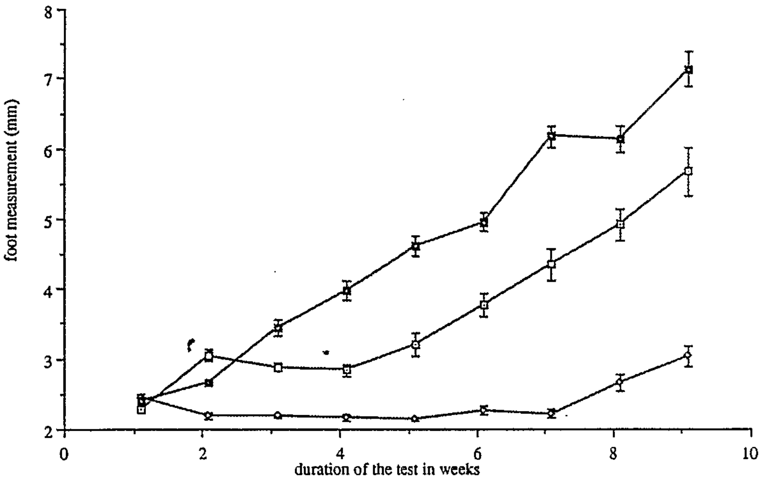
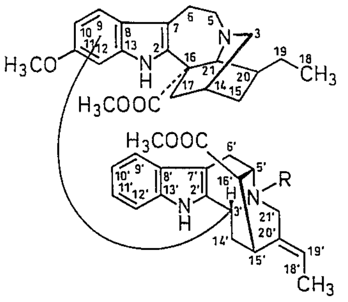


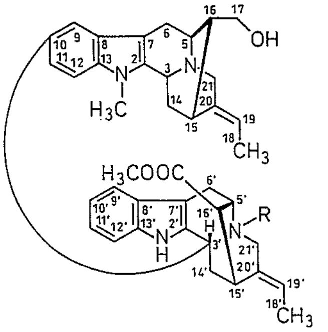
Fig. 1 *In vivo* activity of glucantime and conodurine base on *Leishmania amazonensis* infection in BALB/c mice (± SEM).



1 R = CH_3, C3'→C12 bond; conodurine

2 R = H, C3'→C12 bond; *N*-demethylconodurine (gabunine)

3 R = H, C3'→C10 bond; conoduramine



4 R = H, accedinisine

5 R = H, *N'*-demethylaccedinisine

Fig. 2 Chemical structures of alkaloids **1**, **2**, **3**, **4**, and **5**.

Table 4 Inhibition of growth of some Gram-positive and Gram-negative bacteria.

	Zone of inhibition, diameter in mm											
compounds	*S. aureus*			*E. coli*		*P. aeruginosa*	*M. smegmatis*			*B. subtilis*		
µg/ml	60	40	20	80	60	80	60	40	20	60	40	20
conodurine	24	22	18	13	12	12	37	32	28	24	21	18
conoduramine	22	21	18	14	13	11	30	28	27	22	20	19

Alkaloids N_a-methylpericyclivine, perivine, normacusine B, 16-epi-affinine, vobasinol and vobasine were inactive.

Table 5 ^{13}C-NMR (75 MHz, $CDCl_3$) spectral data of conodurine (**1**), gabunine (**2**), conoduramine (**3**), and accedinisine (**4**).

Carbons	conoduramine	conodurine	gabunine	accedinisine
2	135.4	137.6	136.9	138.9
3	51.7	52.9	52.9	49.6
5	53.1	51.1	51.2	54.5
6	22.1	21.9	21.9	27.2
7	110.2	109.9	109.0	103.8
8	122.7	124.4	124.4	126.4
9	119.4	117.0	117.2	117.6
10	127.2	104.9	104.8	118.7
11	152.5	151.9	151.9	121.6
12	93.0	114.3	114.1	107.7
13	138.0	135.0	134.9	137.6
14	27.4	26.9	27.0	33.2
15	32.0	31.8	31.7	27.8
16	55.0	54.5	54.4	44.5
17	36.5	33.7	33.7	65.2
18	11.5	11.5	11.5	12.7
19	26.7	26.5	26.5	117.6
20	39.0	38.8	38.7	136.2
21	57.4	57.5	57.4	56.5
16-$\underline{C}$OOMe	175.7	174.8	175.0	
16-COO$\underline{Me}$	52.3	52.2	52.6	
11-OMe	56.0	56.8	56.8	
1 (N-Me)				29.4
2′	135.9	135.9	135.9	136.2
3′	45.1	35.1	35.2	45.7
4′ (N-Me)	42.0	42.3		42.4
5′	60.0	59.5	60.0	59.9
6′	19.8	19.5	24.0	19.6
7′	110.0	108.9	109.0	109.0
8′	129.9	129.4	129.1	130.0
9′	117.3	117.9	118.2	117.7
10′	118.9	118.7	119.7	119.0
11′	121.4	122.0	123.3	121.6
12′	109.8	109.7	109.7	109.9
13′	134.9	135.9	134.9	136.2
14′	36.5	34.6	34.7	39.0
15′	33.5	33.4	33.6	33.9
16′	46.6	47.2	47.0	47.1
18′	12.2	12.2	12.3	12.3
19′	118.8	119.3	120.1	119.2
20′	137.2	136.7	136.1	136.5
21′	52.4	52.3	52.6	52.6
16′-COOMe	171.0	171.6	171.2	171.7
16′-$\bar{C}$OO$\underline{Me}$	49.9	49.9	50.3	50.1

Conodurine (**1**) and conoduramine (**3**) were the most active against Gram-positive and Gram-negative microorganisms (Table **4**). Gabunine (**2**) and accedinisine (**4**) were slightly active against *B. subtilis*.

The strongest leishmanicidal and antibacterial activities were observed with the "dimeric" alkaloids composed of vobasine and isovoacangine-like units (**1** and **3**) or affinisine-vobasine units (**4**). Antiparasitic activities of other indole alkaloids have been reported: Vinblastine (VLB) and olivacine are active *in vitro* against *Trypanosoma cruzi* (16). VLB acts as a microtubule formation inhibitor and displays a strong antineoplastic effect (16); its cytotoxicities on mammalian cells reduce their antiparasitic values. Conodurine (**1**) and gabunine (**2**) were also shown to be cytotoxic (17). Interestingly, our results show that **1**, **2**, and conoduramine (**3**) displayed a weak toxicity towards macrophage host cells, associated with a strong activity against the intracellular parasite cells. Suppression of more than 90% of the amastigotes was achieved with gabunine (**2**) at the concentration of 3 μg/ml with no damage to macrophages. These alkaloids showed a good selective toxicity against parasites. However, the *in vivo* study showed that conodurine (**1**) was less active than the drug of reference. Toxic effects rapidly appeared with increasing doses. It has been shown that olivacine also failed to prevent infection of mice with *T. cruzi* trypomastigotes (16). It seems that inactivation of gabunine (**2**), which was very active *in vitro* on the intracellular amastigote form, might occur in the host.

Acknowledgements

This work was supported in part by a grant from the International Foundation of Science. It was performed in the framework of the "Medicinal Plants of Chapare Region" project undertaken by ORSTOM and the San Simon University of Cochabamba, Bolivia.

References

[1] Deharo, E., Moretti, C., Muñoz, V., Sauvain, M., Ruiz, E. (1992) S. T. P. Pharma. pratiques 2, 189–192.

[2] Fournet, A., Angelo, A., Muñoz, V., Roblot, F., Hocquemiller, R., Cavé, A. (1992) J. Ethnopharm. 37, 156–164.

[3] Sauvain, M., Dedet, J. P., Kunesch, N., Poisson, J., Gayral, P., Gantier, J. C., Kunesch, G. (1993) Phytother. Res. 7, 167–171.

[4] Trotter, E. R., Peters, W., Robinson, B. L. (1980) Ann. Trop. Med. Hyg. 74, 300–318.

[5] Caron, C., Hoizey, M. J., Le Men-Olivier, L., Massiot, G., Zèches, M., Choisy, C., Le Magrex, E., Verpoorte, R. (1988) Planta Med. 54, 409–412.

[6] Lounasmaa, M., Jokela, R., Tolvanen, A., Kwong Kan, S. (1985) Planta Med. 6, 519–521.

[7] Mafhoud, A. (1991) Diplôme d'Etude Approfondie, Faculté de Pharmacie, Reims – France, (pers. comm.).

[8] Kingston, D. G. I., Li, B. T., Ionescu, F. (1977) J. Pharm. Sci. 66, 1135–1138.

[9] Van Beek, T. A., Kuijlaars, F. L. C., Thomassen, P. H. A. M., Verpoorte, R., Baerheim Svendsen, A. (1984) Phytochemistry 23, 1771–1778.

[10] Clivio, P., Richard, B., Hadi, H. A., David, B., Sevenet, T., Zèches, M., Le Men, L. (1990) Phytochemistry 29, 3007–3011.

[11] Das, B. C., Fellion, E., Plat, M. (1967) C. R. Acad. Sci. Paris 264, 1765–1767.

[12] Iglesias, R., Diatta, L. (1975) Revista Cinic 6, 141–146.

[13] Naranjo, J., Hesse, M., Schmid, H. (1972) Helv. Chim. Acta 55, 1849–1866.

[14] Achenbach, H., Schaller, E. (1976) Chem. Ber. 109, 3527–3536.

[15] Sondengam, B. L., Hentchya Hémo, J., Charles, G. (1973) Tetrahedron Lett. 261–263.

[16] Wright, C. W., Phillipson, J. D. (1990) Phytother. Res. 4, 127–139.

[17] Kingston, D. G. I., Gerhart, B. B., Ionescu, F., Mangino, M. M., Sami, S. M. (1978) J. Pharm. Sci. 67, 249–251.