



## Bisbenzylisoquinoline alkaloids from *Guatteria boliviana* (Annonaceae)

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### Abstract

Together with known alkaloids, five new bisbenzylisoquinoline derivatives were isolated from the stem bark of *Guatteria boliviana* (Annonaceae), puertogalines-A **1** and -B **2**, (+)-guatteboline **3**, philogaline **4** and (–)-antioquine **5**. Their structures were elucidated by spectrometric methods and their antiparasitic activity was evaluated in vitro on *Leishmania* sp., *Trypanosoma cruzi* and *Plasmodium falciparum*. Their cytotoxic activity was also measured in KB cell line. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Guatteria boliviana*; Annonaceae; Bisbenzylisoquinoline; Two-dimensional NMR; Antiparasitic activity; Cytotoxicity; *Leishmania* sp; *Trypanosoma cruzi*; *Plasmodium falciparum*

### 1. Introduction

Previous chemical and pharmacological investigations have indicated that bisbenzylisoquinolines are important bioactive components existing in plants of the Annonaceae family. Bisbenzylisoquinolines are known to have various pharmacological activities including antiparasitic activity, in particular against *Leishmania* sp. (Fournet et al., 1993; Munshi et al., 1972) *Trypanosoma cruzi* (Rojas de Arias et al., 1994) and *Plasmodium* sp. (Likhitwitayawuid et al., 1993; Valentin et al., 1997; Angerhofer et al., 1999).

So, in a search for novel antiparasitic natural products, in collaboration with I.R.D. (Institut de Recherche pour le Développement), we have studied the stem bark of *Guatteria boliviana* (Fries, 1939). This

Annonaceous plant is traditionally used in Bolivia as febrifuge and vermifuge. As shown by screening tests, the ethanolic extract possessed an in vitro activity (100 µg/ml) against *Leishmania braziliensis* responsible for the cutaneous leishmaniasis of New World.

The present paper deals with the chemical and biological investigation of the stem bark alkaloidal extract of *Guatteria boliviana*. Five new bisbenzylisoquinoline derivatives more or less oxidized were isolated, puertogalines-A (**1**) and -B (**2**), (+)-guatteboline (**3**), philogaline (**4**) and (–)-antioquine (**5**), together with known alkaloids, an oxoaporphin, lanuginosine (Talapatra et al., 1969), and four bisbenzylisoquinoline derivatives, tiliageine (Tackie et al., 1974), funiferine (Tackie et al., 1973), sepeperine (Grundon and McGarvey, 1960) and pangkorimine (Lavault et al., 1987).

### 2. Results and discussion

The extraction was performed from stem bark's by

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usual procedure as described in the experimental and was repeatedly chromatographed to isolate 10 bisbenzylisoquinoline derivatives.

The molecular formula of puertogaline A (**1**),  $C_{34}H_{30}N_2O_6$ , was deduced from the HREIMS. The weak intensity of the molecular ion and the lack of the benzylic cleavage fragmentation peak suggested a conjugated compound (Guinaudeau et al., 1976). The UV absorptions were indicative of a bisbenzylisoquinoline structure (Guha et al., 1979) and showed a bathochromic shift upon addition of a base and an acid, indicating the presence of a phenolic and an imine function on the molecule. The  $^{13}C$  NMR spectrum (Table 2) showed two signals at  $\delta$  165.8 and 168.7, indicating the presence of two imine functions, confirmed by IR spectroscopy. The  $^1H$  NMR spectrum (Table 1) indicated the presence of two isolated AB systems corresponding to H- $\alpha$  and H- $\alpha'$ , corroborating the presence of the two imine functions. Signals corresponding to 10 protons were found in the aromatic region: three singlets, one AMX system and four double doublets suggesting that **1** was a tail-to-tail bisbenzylisoquinoline containing two diaryl ether bridges (Guinaudeau et al., 1976).

Moreover, this compound bears two aromatic methoxy groups as shown by two singlets at  $\delta$  3.85 and 3.95. The complete structural characterization of **1** was then accomplished by examination of the 2D homo and heteronuclear correlated NMR spectra (Table 3). On a 11–12' binding bisbenzylisoquinoline, the H-10 signal, on the C ring, was very up-fielded (*br s* at  $\delta$  5.61). This signal was coupled on the HMBC spectrum with C- $\alpha$  and the methylene protons correlated to the imine carbon C-1 ( $\delta$  168.7). This carbon was then  $^3J$  correlated with H-8, coupled with the MeO-bearing carbon at  $\delta$  142.0 (C-6). The 6 position of this MeO group was confirmed by NOE enhancement with H-5. The second MeO group resonating at  $\delta$  3.85 was located at C-6',  $^2J$  correlated with H-5' ( $\delta$  6.54) on the HMBC spectrum. This was corroborated by the NOE observed between 6'-OMe and H-5'. The presence of H-8 distinguished by a NOE effect with H-10 and H- $\alpha$ , and the chemical shift of C-7 and C-8' suggested that **1** bears a 7–8' diaryl ether linkage. Further analysis of COSY and HMBC spectra led to the complete assignment of all remaining protons and the unambiguous structure of **1** (Fig. 1).

Table 1

$^1H$  NMR spectral data for compounds **1–5** ( $CDCl_3$ ,  $\delta$ , J Hz)

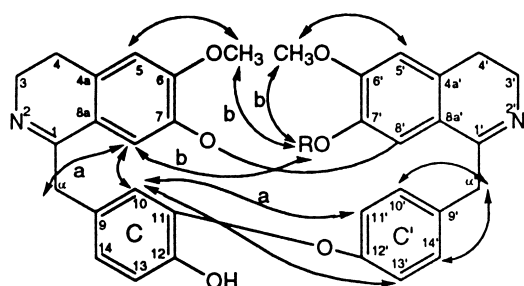
H	1	2	3	4	5
1			4.14 <i>br s</i>		4.00 <i>d</i> (8)
3 ax.	3.69–3.72 <i>m</i>	3.24 <i>m</i>	2.79 <i>m</i>	3.19 <i>m</i>	2.93 <i>m</i>
3 eq.		3.86 <i>m</i>	3.07 <i>m</i>	3.89 <i>m</i>	3.48 <i>m</i>
4 ax.	2.47–2.51 <i>m</i>	2.53 <i>m</i>	2.35 <i>br d</i> (16)	2.48 <i>m</i>	2.47 <i>m</i>
3 eq.			2.43 <i>m</i>		2.93 <i>m</i>
5	6.73 <i>s</i>	6.70 <i>s</i>	6.49 <i>s</i>	6.56 <i>s</i>	6.34 <i>s</i>
8	6.61 <i>s</i>	6.76 <i>s</i>	6.26 <i>s</i>		
$\alpha$	3.72 <i>m</i>	3.48 <i>m</i>	2.76 <i>m</i>	3.87 <i>m</i>	3.05 <i>m</i>
	3.25 <i>m</i>	3.80 <i>m</i>	2.94 <i>dd</i> (14 and 3)	4.58/4.59 <i>2d</i> (12) <sup>a</sup>	2.78 <i>m</i>
10	5.61 <i>br s</i>	5.85 <i>d</i> (2)	5.13 <i>br s</i>	6.60 <i>d</i> (2)	7.12 <i>br s</i>
13	6.71 <i>br s</i>	6.79 <i>d</i> (10)	6.75 <i>d</i> (8)	6.87 <i>d</i> (8)	6.85 <i>d</i> (8)
14	6.71 <i>br s</i>	6.86 <i>dd</i> (10 and 2)	6.64 <i>br d</i> (8)	7.47 <i>dd</i> (8 and 2)	7.27 <i>dd</i> (8 and 2)
1'					3.71 <i>t</i> (3)
3' ax.	3.50–3.60 <i>m</i>	3.56 <i>m</i>	3.63 <i>m</i>	3.19 <i>m</i>	3.12 <i>m</i>
3' eq.		3.88 <i>m</i>	3.88 <i>m</i>	3.89 <i>m</i>	
4'	2.65–2.75 <i>m</i>	2.71 <i>m</i>	2.71 <i>m</i>	2.48 <i>m</i>	2.71 <i>m</i>
5'	6.54 <i>s</i>	6.51 <i>s</i>	6.54 <i>s</i>	6.74 <i>s</i>	6.48 <i>s</i>
8'				7.52 <i>s</i>	6.94 <i>s</i>
$\alpha'$	4.67 <i>d</i> (15)	4.01 <i>d</i> (14)	4.02 <i>d</i> (14)	3.72 <i>m</i>	3.05 <i>m</i>
	3.92 <i>d</i> (15)	4.42 <i>d</i> (14)	4.50 <i>d</i> (14)	4.04/4.05 <i>2d</i> (13) <sup>a</sup>	
10'	6.72 <i>m</i>	6.75 <i>dd</i> (8 and 2)	7.19 <i>br d</i> (8)	7.15 <i>d</i> (2)	6.63 <i>br s</i>
11'	6.33 <i>dd</i> (8 and 2)	6.35 <i>dd</i> (8 and 2)	6.39 <i>dd</i> (8 and 2)		
13'	6.86 <i>dd</i> (8 and 2)	6.96 <i>dd</i> (8 and 2)	6.78 <i>dd</i> (8 and 2)	6.80 <i>d</i> (8)	6.86 <i>d</i> (8)
14'	7.12 <i>dd</i> (8 and 2)	7.33 <i>dd</i> (8 and 2)	7.42 <i>br d</i> (8)	7.28 <i>dd</i> (8 and 2)	7.20 <i>dd</i> (8 and 2)
NCH <sub>3</sub> -2					2.35 <i>s</i>
NCH <sub>3</sub> -2'					2.64 <i>s</i>
OCH <sub>3</sub> -6	3.95 <i>s</i>	3.92 <i>s</i>	3.90 <i>s</i>	3.92 <i>s</i>	3.82 <i>s</i>
OCH <sub>3</sub> -12					3.87 <i>s</i>
OCH <sub>3</sub> -6'	3.85 <i>s</i>	3.85 <i>s</i>	3.86 <i>s</i>	3.90 <i>s</i>	3.49 <i>s</i>
OCH <sub>3</sub> -7'		3.19 <i>s</i>	3.33 <i>s</i>		
OCH <sub>3</sub> -12'				3.85 <i>s</i>	

<sup>a</sup> Two conformers in equal amount.

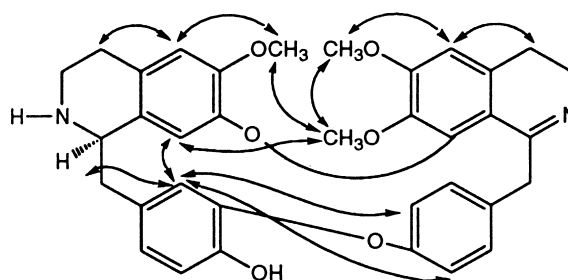
The second compound isolated from *Guatteria boliviana*, named puertogaline B (**2**), is another new bis-imine bisbenzylisoquinoline as shown by the UV and IR absorptions, and the  $^{13}\text{C}$  NMR spectrum showing two characteristic peaks at  $\delta$  168.0 and 165.2. From the HREIMS, the molecular formula was concluded to be  $\text{C}_{35}\text{H}_{32}\text{N}_2\text{O}_6$ , suggesting that compound **2** has one more methoxy group than **1**. Comparisons of the  $^1\text{H}$  and  $^{13}\text{C}$  spectral data with those of **1** indicated that one OH group was replaced by a MeO group ( $\delta$  3.19 on the  $^1\text{H}$  spectrum and 60.3 on the  $^{13}\text{C}$  spectrum) (Tables 1 and 2). A study of COSY, HMQC and HMBC correlations confirmed the similarity between **1** and **2** and led to the complete assignment of

all the remaining protons of **2** (Table 3). The heteronuclear  $^1\text{H}$ – $^{13}\text{C}$  correlations indicated particularly that the third MeO group was located at C-7'. The proton at C-5' was  $^2J$  or  $^3J$  coupled with two MeO-bearing carbon at  $\delta$  157.3 (C-6') and 137.5 (C-7'). The spatial proximity between these MeO groups was further supported by a NOE experiment. The structure of puertogaline B was thus established as the 7'-OMe derivative of **1**.

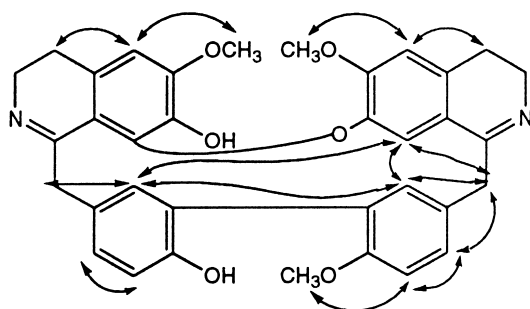
The molecular formula of (+)-guatteboline (**3**) was deduced as  $\text{C}_{35}\text{H}_{34}\text{N}_2\text{O}_6$  by HRMS ( $\text{M}^+$ ; 578.2428), suggesting the reduction of one of the imine functions of **2**. The UV spectrum showed characteristic absorptions for a bisbenzylisoquinoline structure and a bath-



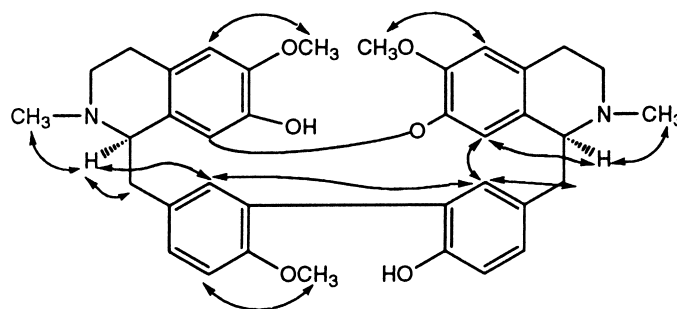
**1** Puertogaline A, R = H  
**2** Puertogaline B, R = CH<sub>3</sub>  
 a NOE only observed for **1**  
 b NOE only observed for **2**



**3** (+)-Guatteboline.



**4** Philogaline.



**5** (-)-Antioquine.

Fig. 1. Structures of the investigated BBIQ and their NOESY correlations.

ochromic shift upon addition of a base and an acid. The presence of one imine function in **3** was confirmed by the single  $^{13}\text{C}$  NMR resonance at  $\delta$  164.7 (Table 2). In comparison with **2**, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Tables 1 and 2) exhibited similar signals indicative of a tail-to-tail bisbenzylisoquinoline containing two diaryl ether bridges at 7–8' and 11–12'. The presence of one isolated AB system (at  $\delta$  4.02 and 4.50) showed the presence of only one imine function, the second one being replaced by an AMX system of H-1/H- $\alpha$  at 4.14, 2.76 and 2.94. As evident from the resonances at  $\delta$  3.33, 3.86 and 3.90, the structure of guatteboline includes three aromatic MeO groups. The fragmentation peak at  $m/z$  367 in the EI mass spectrum, corresponding to the benzylic cleavage of isoquinolines,

Table 2  
 $^{13}\text{C}$  NMR spectral data for compounds **1–5** ( $\text{CDCl}_3$ )

H	1	2	3	4	5
1	168.7	168.0	55.1	167.7	62.9
3	45.0	44.5	42.2	45.7	44.4
4	25.7	26.1	29.7	27.9	22.4
4a	134.2	135.5	131.2	134.4	122.0
5	110.4	110.7	112.2	105.9	104.7
6	152.0	153.5	147.9	150.0	145.7
7	143.4	143.8	144.4	135.9	134.4
8	115.4	117.2	113.8	— <sup>a</sup>	141.7
8a	119.9	120.5	127.5	115.1	123.9
$\alpha$	40.1	44.8	38.5	43.7	39.8
9	127.7	128.3	122.8	130.2	137.8
10	116.1	116.5	116.4	134.6	135.3
11	143.9	147.8 <sup>b</sup>	148.6	125.2	125.5 <sup>c</sup>
12	148.3	144.2	143.7	152.4	152.9
13	115.8	115.8	114.7	118.0	110.7
14	121.9	123.1	123.4	130.1	129.4
1'	165.8	165.2	164.7	167.7	64.9
3'	45.7	45.9	46.5	45.7	47.3
4'	27.0	27.8	27.3	26.0	27.4
4'a	131.8	136.8	136.3	134.4	129.2
5'	105.1	105.6	106.0	111.1	112.6
6'	150.2	157.3	155.6	151.0	148.2
7'	134.7	137.5	138.3	142.2	142.6
8'	— <sup>a</sup>	144.2	130.9	115.4	119.1
8'a	114.5	115.7	116.0	119.2	129.8 <sup>d</sup>
a'	44.1	50.5	44.8	42.1	38.0
9'	134.5	135.7	135.8	129.1	130.3 <sup>d</sup>
10'	130.5	130.8	132.1	136.6	135.3 <sup>c</sup>
11'	121.9	121.9	121.7	126.6	128.0 <sup>c</sup>
12'	153.4	147.9 <sup>b</sup>	152.2	153.1	151.9
13'	121.9	121.9	122.2	111.1	116.8
14'	127.4	127.7	128.4	129.0	131.2
NCH3-2	—	—	—	—	42.3
NCH3-2'	—	—	—	—	43.6
OCH3-6	55.8	56.1	55.7	56.1	56.3
OCH3-12	—	—	—	—	56.4
OCH3-6'	55.8	56.1	56.0	56.1	55.8
OCH3-7'	—	60.3	60.2	—	—
OCH3-12'	—	—	—	56.1	—

<sup>a</sup> Not detected.

<sup>b–d</sup> Assignments may be interchanged.

indicated that **3** had three MeO groups in the upper part of the molecule. 2D homo and heteronuclear correlated NMR spectra clearly indicated the presence of the imine function at 1'–2' (Table 3). In fact, C-14' bearing the downfield proton at  $\delta$  7.42, was three-bond coupled on the HMBC spectrum with H- $\alpha'$ , two protons correlated with the imine carbon C-1'. Then, C-1' is  $^3J$  correlated with H-3', coupled with H-4'. The latter afforded to assign the signal at  $\delta$  6.54 to H-5' correlated with the two MeO groups at C-6' and C-7'. NOE experiment between H-5' and the methoxy group at C-6' and between the two MeO groups confirmed their location. The (1*R*) absolute configuration of (+)-guattebolin was deduced from the positive sign of the specific rotation and the circular dichroism (Battersby et al., 1965).

Compound **4**, named philogaline, is characterized by its unstability. The EIMS spectrum of **4** showed a molecular ion peak at  $m/z$  576 ( $\text{M}^+$ ), corresponding to the molecular formula  $\text{C}_{35}\text{H}_{32}\text{N}_2\text{O}_6$ . As previously observed, the UV and IR spectra indicated that **4** was a phenolic bisbenzylisoquinoline bearing one or two imine functions. The presence of an imine function was confirmed by one  $^{13}\text{C}$  NMR signal at  $\delta$  167.7. In the aromatic region of the  $^1\text{H}$  NMR spectrum, two AMX systems were found, corresponding to the rings C and C'. This  $^1\text{H}$  NMR spectral feature suggested that **4** was a tail-to-tail bisbenzylisoquinoline containing one 11–11' biphenyl bridge (Table 1). As evident from the resonances at  $\delta$  3.85, 3.90 and 3.92, the structure of **4** includes three MeO groups. The weak peak at  $m/z$  356 in the EI mass spectrum, corresponding to the benzylic cleavage, indicated that **4** had two MeO groups and one diaryl ether bridge on the upper part of the molecule. The full structural characterization of **4** was then accomplished by examination of the homonuclear and heteronuclear spectra showing the presence of two imine functions in the molecule (Table 3). The MeO group resonating at  $\delta$  3.90 was located at C-6', since this carbon was correlated with H-5' and H-8' appearing as singlets at  $\delta$  6.74 and 7.52, respectively. This latter proton was three-bond coupled with C-1' ( $\delta$  167.7) and showed a NOE enhancement with H-10' (ring C') and H- $\alpha'$  ( $\delta$  4.04–4.05). Moreover, the lack of H-8 allowed the location of the diarylether bridge at 8–7'. H-10' was also  $^3J$  coupled with the MeO-bearing carbon at  $\delta$  153.1 (C-12'). The 12' position of the MeO group ( $\delta$  3.85) was confirmed by NOE with H-13' ( $\delta$  6.80). The third MeO group ( $\delta$  3.92) should be located at C-6 since it displayed long-range coupling with C-6, which was  $^2J$  correlated with H-5. A NOE enhancement was also observed between MeO-6 and H-5 in the NOESY spectrum. Moreover, H-10 ( $\delta$  6.60) is correlated to C- $\alpha$  ( $\delta$  43.7) and one of the protons linked to C- $\alpha$  ( $\delta$  4.58/4.59) is  $^2J$  coupled with carbon C-1 ( $\delta$  167.7) of the second imine function resonating

at the same chemical shift as C-1'. We could observe that H- $\alpha$  (4.58) and H- $\alpha'$  (4.04) were resonating as double signals on the  $^1\text{H}$  NMR spectrum (Table 1). This original feature is probably caused by the strictness of the imine functions.

The molecular formula of (–)-antioquine (**5**),  $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$ , was determined by HRMS ( $m/z$  608.2866). The UV and IR spectra indicated the presence of hydroxy groups on a bisbenzylisoquinoline structure. As evident from the resonances at  $\delta$  2.35, 2.64, 3.49, 3.82 and 3.87 in the  $^1\text{H}$  NMR spectrum, the structure of **5** includes two N–Me and three MeO groups (Table 1). As precedently observed for **4**,  $^1\text{H}$  NMR spectral features in the aromatic region and the peaks on the EI mass spectrum at  $m/z$  382 and 191 suggested that **5** was another tail-to-tail (8–7', 11–11')-bisbenzylisoquinoline containing two methoxy groups on the upper part. This was confirmed by two-dimensional NMR spectra that allow to locate the three MeO groups at C-6, C-6' and C-12 (Table 3). In fact, in the HMBC spectrum, H-5 and H-5' were coupled with C-6 and C-6', two carbons  $^3J$  correlated with MeO groups resonating, respectively, at  $\delta$  3.82 and

3.49. This was corroborated by the NOE observed between 6-OMe and H-5, and 6'-OMe and H-5'. The third MeO group at  $\delta$  3.87 was correlated to C-12, a carbon also coupled with H-14. This proton H-14 was correlated on the COSY spectrum with H-10, a distinctive proton showing NOESY correlations with H-1 and H-10'. The 12 position of this MeO group was confirmed by the NOE observed with H-13. Compound **5** possessed two asymmetric centers. In this case, the absolute configuration is determined, as previously reported, by  $^1\text{H}$  NMR features and the sign of the specific rotation (Berthou et al., 1988). For **5**,  $^1\text{H}$  NMR features are typical of 'syn' configuration: aromatic protons in the range of 6.34–7.27 ppm, H-14 downfield compared to H-14', and H-10 appearing at  $\delta$  7.12. Next, from the specific rotation of  $-170^\circ$ , we could deduce the (1*R*, 1'*S*) configuration. This was confirmed by circular dichroism: one positive (222 nm) and two negative Cotton effects (248 and 285 nm). Compound **5** is a new alkaloid, (–)-antioquine, enantiomer of (+)-antioquine, previously isolated from various plants (Cortes et al., 1985).

The antiparasitic activity was investigated for pur-

Table 3  
HMBC correlations for compounds **1–5** ( $\text{CDCl}_3$ )

C	1	2	3	4	5
1	H-8, - $\alpha$	H-8, - $\alpha$ (3.48)	H-8	H- $\alpha$ (4.58/4.59)	H- $\alpha$ (2.78), NCH <sub>3</sub> -2
3	H-4				NCH <sub>3</sub> -2
4	H-5	H-5	H-5	H-5	H-5
4a	H-8	H-8	H-8	H-4	H-1, -5
5					
6	H-5, -8, OCH <sub>3</sub> -6	H-5, -8, OCH <sub>3</sub> -6	H-5, -8, OCH <sub>3</sub> -6	H-5, OCH <sub>3</sub> -6	H-5, OCH <sub>3</sub> -6
7	H-5, -8	H-8	H-5, -8	H-5	H-5
8					
8a	H-5	H-5	H-5	H-5	H-4 eq.
$\alpha$			H-10, -14	H-10	H-1
9	H- $\alpha$	H- $\alpha$ (3.80), -13	H-10	H-13	H-1, - $\alpha$ , -13
10	H- $\alpha$		H-14	H- $\alpha$ (4.58/4.59), -14	H- $\alpha$ , -10, -14
11	H-10	H-10, -13	H-10, -13	H-10', -13	
12	H-10	H-10, -14	H-10, -13, -14	H-10, -13, -14	H-14, OCH <sub>3</sub> -12
13					
14	H-10	H- $\alpha$ (3.80), -10		H- $\alpha$ , -10	H- $\alpha$
1'	H-3', - $\alpha'$	H-3', - $\alpha'$	H-3', $\alpha'$	H- $\alpha$ , - $\delta$	N-CH <sub>3</sub> -2
3'	H-4'	H-4'	H-4'		
4'	H-5'	H-3' ax., -5'	H-5'	H-5'	
4'a	H-4', -5'	H-3' eq., -4', -5'	H-3', -4'	H-4', -8'	H-5'
5'	H-4'		H-4'		
6'	H-5', OCH <sub>3</sub> -6'	H-5', OCH <sub>3</sub> -6'	H-5', OCH <sub>3</sub> -6'	H-5', -8', OCH <sub>3</sub> -6'	OCH <sub>3</sub> -6'
7'		OCH <sub>3</sub> -7'	H-5', OCH <sub>3</sub> -7'	H-5', -8'	H-5'
8'					
8'a	H-4', -5'	H-5'	H-4', -5'	H-5'	
$\alpha'$	H-10', -14'		H-10'	H-10'	
9'	H- $\alpha'$ , -11', -13'	H- $\alpha'$ , -11'	H- $\alpha'$ , -11', -13'	H-10'	
10'	H- $\alpha'$ (4.67), -10', -14'	H- $\alpha'$ (4.42)	H- $\alpha'$ (4.50), -14'	H- $\alpha'$ (3.72)	H-14'
11'	H-13'			H-10, -13'	
12'	H-10', -11', -13', -14'		H-10', -11', -13', -14'	H-10', -13', -14', OCH <sub>3</sub> -12'	H-14'
13'	H-11'				
14'	H- $\alpha'$ (4.67)	H- $\alpha'$ (4.42), -10'	H- $\alpha'$ , -10'		

Table 4

In vitro activity of *Guatteria boliviana* alkaloids on the trypomastigote forms of *Trypanosoma cruzi*, strain Y

Compounds	IC <sub>50</sub> (µg/ml)	IC <sub>90</sub> (µg/ml)
Lanuginosine	> 250	> 250
Pangkorimine	114.8	245.9
Funiferine	29.7	88.2
Tiliageine	175.1	370.7
Antioquine	47.4	87.9
Puertogaline A	136.3	260.3
Puertogaline B	43.9	163.1
Sepeerine	78.1	285.3
Guatteboline	57.9	96.5

ified compounds on *Leishmania* sp., *Trypanosoma cruzi* and *Plasmodium falciparum* and cytotoxic activity was evaluated. On *Leishmania* sp., only three compounds, puertogaline A and B and sepeerine showed moderate inhibition at 100 µg/ml, but this activity was equivalent to the value measured with the crude ethanolic extract in the screening tests (Hocquemiller et al., 1991).

The alkaloids were also tested on trypomastigote forms of *Trypanosoma cruzi* responsible for Chagas disease (Table 4) (Fournet, 1991). All the bisbenzylisoquinoline alkaloids were active at 250 µg/ml and three of them, funiferine, antioquine and guatteboline exhibited an IC<sub>90</sub> lower than 100 µg/ml. However, the substantial differences among the different structures did not allow for the elaboration of any structure–activity relationship.

As shown in Table 5, each of the isolates was accessed for cytotoxic and antimalarial potential. Antiplasmodial activity was performed on two strains of *Plasmodium falciparum*, chloroquine-sensitive, D6 and

chloroquine-resistant, W2 (Likhitwitayawuid et al., 1993). At the same time, cytotoxicity was measured on KB cell line and the selectivity index was calculated (ratio of LC<sub>50</sub> in KB cell line to the LC<sub>50</sub> in the parasites). Due to the small amount available, some of the bisbenzylisoquinoline alkaloids could not be evaluated. Regarding antimalarial activity, none of the isolates demonstrated strong activity and their selectivity index were in the range 9–92, whereas agents, such as quinine or artemisinin typically yield ratio > 1000. Nevertheless, we can notice that the presence of an imine functionality seemed to reduce the antimalarial activity. For example, on strains D6, funiferine, bearing one NH and one N–Me function, and sepeerine, di-N–Me derivative, showed both appreciable activity (SI = 92). A lower activity was observed for puertogaline B, a bis-imine alkaloid (SI = 15). In general, activity on strain D6 was higher than activity on strain W2, with one exception for guatteboline, a mono-imine bisbenzylisoquinoline.

### 3. Experimental

#### 3.1. General

UV spectra were recorded on a Philips PU 8700 spectrophotometer and IR spectra on a Perkin-Elmer 841 spectrometer. Circular dichroism curves were measured on an autodichrograph Mark V. Jobin Yvon. All <sup>1</sup>H and two-dimensional NMR spectra were recorded in CDCl<sub>3</sub> (δ ppm) on Bruker ARX 400 spectrometer operating at 400 MHz. <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> on Bruker AMX 200 spectrometer operating at 50 MHz. HREIMS spectra were obtained on a Kratos ms-80 spectrometer.

Table 5

Antimalarial and cytotoxic activities of *Guatteria boliviana* alkaloids<sup>a</sup>

Products	KB cell line, LC <sub>50</sub> (ng/ml)	<i>Plasmodium falciparum</i> , D6 <sup>b</sup>		<i>Plasmodium falciparum</i> , W2 <sup>c</sup>	
		LC <sub>50</sub> (ng/ml)	SI	LC <sub>50</sub> (ng/ml)	SI
Antioquine	6700	118.7	56	132.7	50
Tiliageine	3200	48.9	65	107.7	30
Funiferine	10500	114.0	92	183.3	57
Sepeerine	6800	73.6	92	100.1	68
Pangkorimine	2600	134.7	19	284.5	9
Guatteboline	6000	207.5	29	72.5	83
Puertogaline B	4800	316.4	15	183.2	26
Chloroquine	–	1.8	≈ 5000	94.9	≈ 200
Quinine	> 20000	9.2	> 2174	59.8	> 334
Artemisinin	> 20000	2.6	> 7692	4.2	> 4762

<sup>a</sup> Ratio of LC<sub>50</sub> in KB cells to the LC<sub>50</sub> in the parasites (selectivity index).

<sup>b</sup> Chloroquine-sensitive strains of *Plasmodium falciparum*.

<sup>c</sup> Chloroquine-resistant strains of *Plasmodium falciparum*.

### 3.2. Plant material

Stem barks and leaves of *Guatteria boliviana* Winkl (Fries, 1939) were collected by C. Moretti in February 1989, in Puerto Aurora, Department of Chaparé, Bolivia. Voucher specimens were deposited in the National Herbarium of La Paz, Bolivia (no. SA 134).

### 3.3. Extraction and isolation

Extraction for preliminary biological studies: dried and powdered leaves and stem barks (10 g) were macerated with ethanol (50 ml) for two days. The extracts were filtered and evaporated to dryness, affording the ethanolic extract. Extraction method for alkaloids: powdered stem barks (384 g) were successively extracted in a Soxhlet apparatus with petroleum ether (bp 40–65°) (residue 2.2 g) and CH<sub>2</sub>Cl<sub>2</sub> after alkalification with 6% NH<sub>4</sub>OH. The methylene chloride layer was extracted with water acidified with 5% HCl (methylene chloride residue 1.5 g). The aqueous layer was further alkalified with 6% NH<sub>4</sub>OH and extracted with CH<sub>2</sub>Cl<sub>2</sub>, affording a CH<sub>2</sub>Cl<sub>2</sub> extract (12.3 g) containing the total alkaloids. Silica gel GF<sub>254</sub> was used for tlc. The CH<sub>2</sub>Cl<sub>2</sub> extract was fractionated by several successive chromatographies on Si gel (0.032–0.063 mm) and Si gel 60 N and 60 H.

### 3.4. Puertogaline A (1)

C<sub>34</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub>, amorphous, [ $\alpha$ ]<sub>D</sub><sup>20</sup> 0 (CHCl<sub>3</sub>/MeOH, 3/1, *c* 0.5); UV  $\lambda_{\max}$  (EtOH) nm (log  $\epsilon$ ): 204 (4.64), 235 sh (4.45), 278 (4.09); (OH<sup>−</sup>) 217 (4.29), 241 (4.42), 305 (3.85); (H<sup>+</sup>) 204 (4.64), 247 (4.28), 338 (4.10); IR  $\nu_{\max}^{\text{film}}$  cm<sup>−1</sup>: 3000, 1604, 1502, 1275, 1096, 862; HREIMS *m/z* (%): 562.2077 (M<sup>+</sup>, 17) [562.2103 calcd], 330 (10), 281 (7), 265 (7), 178 (12), 161 (14), 108 (67), 107 (97), 58 (100). For <sup>1</sup>H and <sup>13</sup>C NMR (400 and 50 MHz, CDCl<sub>3</sub>) data, see Tables 1 and 2.

### 3.5. Puertogaline B (2)

C<sub>35</sub>H<sub>32</sub>N<sub>2</sub>O<sub>6</sub>, amorphous, [ $\alpha$ ]<sub>D</sub><sup>20</sup> 0 (CHCl<sub>3</sub>, *c* 0.77); UV  $\lambda_{\max}$  (EtOH) nm (log  $\epsilon$ ): 205 (4.64), 235 (4.50), 278 (4.17); (OH<sup>−</sup>) 214 (4.36), 237 (4.51), 279 (4.20); (H<sup>+</sup>) 214 (4.45), 246 (4.32), 339 (4.20); IR  $\nu_{\max}^{\text{film}}$  cm<sup>−1</sup>: 3300, 2943, 1602, 1562, 1505, 1358, 1318, 1286, 1141, 1100, 1017, 842; HREIMS *m/z* (%): 576.2260 (M<sup>+</sup>, 100) [576.2260 calcd], 577 (52), 575 (20), 561 (13), 395 (1), 206 (13), 175 (8), 107 (8), 77 (8). For <sup>1</sup>H and <sup>13</sup>C NMR (400 and 50 MHz, CDCl<sub>3</sub>) data, see Tables 1 and 2.

### 3.6. (+)-Guatteboline (3)

C<sub>35</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>, amorphous, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +138° (CHCl<sub>3</sub>, *c*

0.8); UV  $\lambda_{\max}$  (EtOH) nm (log  $\epsilon$ ): 205 (4.85), 231 sh (4.56), 283 (4.21); (H<sup>+</sup>) 225 (4.51), 286 (4.15); (OH<sup>−</sup>) 205 (4.85), 239 sh (4.42), 290 (3.98), 336 (4.13); IR  $\nu_{\max}^{\text{film}}$  cm<sup>−1</sup>: 3300, 2943, 1599, 1561, 1508, 1450, 1413, 1359, 1115, 1018, 970, 752; HREIMS *m/z* (%): 578.2428 (M<sup>+</sup>, 45) [578.2417 calcd], 367 (14), 214 (12), 175 (8), 107 (42), 58 (100); DC nm (EtOH):  $\Delta\epsilon_{219} + 6.2$ ,  $\Delta\epsilon_{261} - 0.1$ ,  $\Delta\epsilon_{294} + 0.1$ ,  $\Delta\epsilon_{320} - 0.1$ . For <sup>1</sup>H and <sup>13</sup>C NMR (400 and 50 MHz, CDCl<sub>3</sub>) data, see Tables 1 and 2.

### 3.7. Philogaline (4)

C<sub>35</sub>H<sub>32</sub>N<sub>2</sub>O<sub>6</sub>, amorphous, [ $\alpha$ ]<sub>D</sub><sup>20</sup> 0 (MeOH, *c* 0.23); UV  $\lambda_{\max}$  (EtOH) nm (log  $\epsilon$ ): 205 (4.38), 231 sh (4.26), 284 (3.89); (OH<sup>−</sup>) 232 (4.27), 302 (3.80); (H<sup>+</sup>) 205 (4.36), 246 (4.14), 329 (3.85); IR (film)  $\nu_{\max}^{\text{film}}$  cm<sup>−1</sup>: 3300, 2936, 1602, 1561, 1501, 1459, 1241, 1193, 1132; EIMS *m/z* (%): 576 (M<sup>+</sup>, 36), 545 (13), 515 (2), 430 (2), 356 (2), 175 (23), 28 (100). For <sup>1</sup>H and <sup>13</sup>C NMR (400 and 50 MHz, CDCl<sub>3</sub>) data, see Tables 1 and 2.

### 3.8. (−)-Antioquine (5)

C<sub>37</sub>H<sub>40</sub>N<sub>2</sub>O<sub>6</sub>, amorphous, [ $\alpha$ ]<sub>D</sub><sup>20</sup> −170° (CHCl<sub>3</sub>, *c* 0.2); UV  $\lambda_{\max}$  (EtOH) nm (log  $\epsilon$ ): 205 (4.72), 287 (3.90); (OH<sup>−</sup>) 228 (4.26), 294 (3.80); IR  $\nu_{\max}^{\text{film}}$  cm<sup>−1</sup>: 3400, 1615, 1585, 1504, 1451, 1279, 1118, 808, 732; HREIMS *m/z* (%): 608.2866 (M<sup>+</sup>, 89) [608.2886 calcd], 607 (71), 382 (31), 381 (74), 367 (29), 191 (100), 174 (32), 168 (23), 86 (27); DC nm (EtOH):  $\Delta\epsilon_{222} + 5.1$ ,  $\Delta\epsilon_{248} - 4.0$ ,  $\Delta\epsilon_{285} - 1.4$ . For <sup>1</sup>H and <sup>13</sup>C NMR (400 and 50 MHz, CDCl<sub>3</sub>) data, see Tables 1 and 2.

### 3.9. Bioassays

In vitro study on the promastigote forms of *Leishmania* (Hocquemiller et al., 1991): the extracts and the compounds were dissolved in DMSO and evaluated against the promastigote forms of *Leishmania amazonensis* (strain IFLA/BR/67/PH8), *L. donovani* (strain MHOM/IN/83/HS70) and *L. braziliensis* (strain MHOM/BR/M2903). Microorganisms are deposited in the “Instituto de Investigaciones en Ciencias de la Salud (IICS)”, Asunción, Paraguay. Each assay was performed in triplicate. The viability of the parasites was estimated by direct observation after 24 h incubation at 28°C, with an inverted microscope. The positive controls were Glucantime (Rhône-Poulenc, France) and Pentamidine (May and Baker, UK).

In vitro study on the trypomastigote forms of *Trypanosoma cruzi* (Fournet, 1991): the extracts and the compounds were dissolved in DMSO and evaluated against the trypomastigote forms of *Trypanosoma cruzi* (strain Y). Microorganism is deposited in the “Insti-

tuto de Investigaciones en Ciencias de la Salud (IICS)”, Asunción, Paraguay. Each assay was performed in triplicate. The parasites were counted after 24 h incubation at 4°C. The positive control was gentian violet.

Assays for cytotoxic and antimalarial potential: determination for cytotoxic and antimalarial activities were performed as described previously (Likhitwitayawuid et al., 1993).

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