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Indolomonoterpenic alkaloids from *Strychnos icaja* roots

Geneviève Philippe[a], Patrick De Mol[b], Monique Zèches-Hanrot[c], Jean-Marc Nuzillard[c], Monique-Hanrot Tits[a], Luc Angenot[a], Michel Frédérich[a,b,*]

[a]*University of Liège, Natural and Synthetic Drugs Research Center, Laboratoire de Pharmacognosie, Avenue de l'Hôpital 1, B36, B-4000 Liège, Belgium*
[b]*University of Liège, Laboratoire de Microbiologie Médicale, Liège, Belgium*
[c]*Laboratoire de Pharmacognosie, UMR 6013 CNRS, Bâtiment 18, Moulin de la Housse, F-51687 Reims cedex 2331, France*

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Abstract

In the course of our search for new antiplasmodial alkaloids from *Strychnos icaja*, we have isolated five alkaloids: three monomers, protostrychnine and genostrychnine, previously described in *Strychnos nux-vomica*, pseudostrychnine, already found in the leaves of the plant, a new bisindolic alkaloid, named strychnogucine C, and the first naturally occurring trimeric indolomonoterpenic alkaloid: strychnohexamine. This latter trimeric alkaloid presented an antiplasmodial activity against the FCA *Plasmodium falciparum* line near 1 μM.

Keywords: Loganiaceae; *Strychnos icaja*; Indolomonoterpenic alkaloid; Strychnogucine C; Protostrychnine; Strychnohexamine; *Plasmodium falciparum*

1. Introduction

Malaria is the major parasitic infection in many tropical and subtropical regions, leading to more than one million deaths (principally young African children) out of 400 million cases each year (Greenwood and Mutabingwa, 2002). More than half of the world's population live in areas where they remain at risk of malaria infection. During last years, the situation has worsened in many ways, mainly due to malarial parasites becoming increasingly resistant to several antimalarial drugs. Furthermore, the control of malaria is more and more complicated by the parallel spread of resistance of the mosquito vector to currently available insecticides. Discovering new drugs in this field is therefore a health priority. In this context, the search for new antimalarial compounds from tropical plants could be an economically affordable solution.

Strychnos icaja Baill. (Loganiaceae) is a tropical shrub common in tropical forest of central Africa (Congo, Rwanda, Cameroon, ...). This *Strychnos* species is mainly used by local populations as an arrow or ordeal poison, but has also been used by Pygmies tribes from Cameroon to treat persistent malaria (Neuwinger, 1996). *S. icaja* is well known for its toxicity, but the alkaloids responsible for this convulsant effect are mainly strychnine and 12-hydroxystrychnine (Sandberg and Kristianson, 1970). In previous works, some dimeric alkaloids derivated from sungucine (Kambu et al., 1980) and possessing potent and selective (*Plasmodium* towards human cells) antiplasmodial properties have been isolated from *S. icaja* (Frédérich et al., 2000, 2001).

We report herein the isolation and characterization of strychnogucine C (**3**), a new bisindolomonoterpenic alkaloid, and of protostrychnine (**1**), genostrychnine and pseudostrychnine (**2**). In the course of this work we have also isolated strychnohexamine (**4**), an original trimeric indolomonoterpenoid alkaloid. Antiplasmodial activities of these alkaloids have then been investigated here for the first time.

* Corresponding author. Tel.: +32-4-366-43-38; fax: +32-4-366-43-32.
E-mail address: m.frederich@ulg.ac.be (M. Frédérich).

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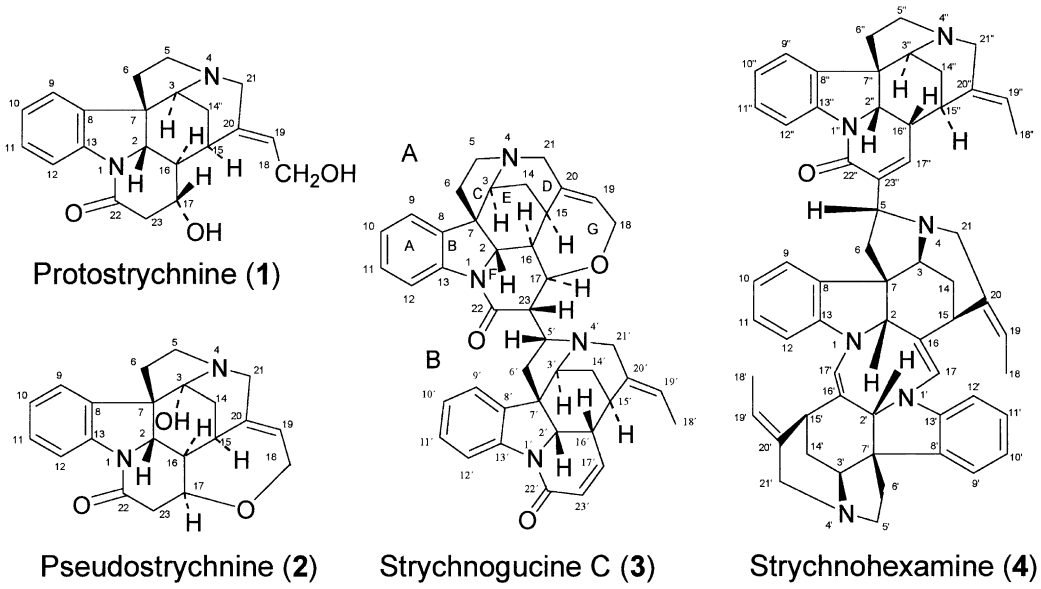

2. Results and discussion

Starting from an ethyl acetate extract of *S. icaja* root bark, sequential liquid–liquid extraction and chromatographic separations led to the isolation of the five indolomonoterpenic alkaloids. Alkaloids **1** and **2** were identified as protostrychnine (**1**) and pseudostrychnine (3-hydroxystrychnine, **2**), based on comparison of their ESI–MS, UV and ^{13}C NMR spectral data with literature (Baser et al., 1979; Bisset et al., 1973; Verpoorte et al., 1977; Baser and Bisset, 1982) and by a TLC comparison with an authentic sample for **2**. An other alkaloid has been identified as genostrychnine (strychnine-*N*-oxide) after UV, IR, ESI–MS, and chromatographic comparison with a reference sample and literature (Bisset, 1976). These three compounds (genostrychnine, **1** and **2**) were already known to be present in different species of *Strychnos,* such as *S. nux-vomica* and *S. ignatii* (Baser et al., 1979; Bisset, 1976; Datta and Bisset, 1990). Pseudostrychnine (**2**) has also been previously described in the leaves of *S. icaja* (Bisset et al., 1973). The identity of **1** and **2** has also been confirmed by ^{1}H NMR and COSY studies of these alkaloids. These data, which were not completely available in the literature, were listed in Table 1.

The discovery of protostrychnine in *S. icaja* is very important from a biosynthetic point of view. Effectively, protostrychnine is a key intermediate in the strychnine biosynthetic pathway hypothesis proposed by Heimberger and Scott (1973), being the last intermediate before the formation of strychnine and isostrychnine. Up to then, protostrychnine (and isostrychnine) had only been described in *S. nux-vomica* and *S. ignatii*. The discovery of protostrychnine and, recently, of isostrychnine (Frédérich et al., 2000) in *S. icaja*, the African strychnine-containing *Strychnos* species, confirms definitely this biosynthetic hypothesis.

Alkaloid **4**, named strychnohexamine, exhibiting a strong and fleeting purple coloration with cerium (IV) sulphate reagent, was the first naturally occurring trisindolic monoterpenoid alkaloid which has been isolated to date. Its structure has been described recently (Philippe et al., 2002). Although another trimeric indolomonoterpenic alkaloid has been previously obtained by biotechnology methods, strychnohexamine is the first one isolated directly from a plant source. The former trimeric compound was a derivative of tabersonine and was formed in micro traces (less than 1 mg) after incubation of tabersonine with an enzyme mixture obtained from leaves of mature *Catharantus roseus* plants (Schübel et al., 1989).

Compound **3** gave a blue fluorescence on TLC plate after pulverisation with cerium (IV) sulfate reagent. The UV spectrum was very close to that of strychnine and strychnogucine A (Frédérich et al., 2001), showing an absorption maximum at 254 nm. Based on HR–ESI–MS, $C_{42}H_{42}N_4O_3$ was determined as its molecular formula, with a [MH^+] at m/z 651,3210. This molecular formula was identical to that of strychnogucine A, and as for strychnogucine C, the ESI–MS fragmentation (daughters peaks) displayed two ions at m/z 335 and 317, corresponding to the masses of strychnine and deoxyisostrychnine, respectively. Compared by TLC with other alkaloids possessing this molecular weight, including strychnogucine A, compound **3** did not present any similarities.

NMR spectral data of compound **3** were listed in Table 2. The broad band decoupled ^{13}C NMR spectrum showed 42 carbon signals which were sorted by HSQC and HMBC techniques as one methyl, eight methylenes, 23 methines and 10 quaternary carbons, including two carbons from a carbonyl group. Among the 23 methines,

Table 1
^{1}H NMR spectral data of compounds **1** and **2** (recorded respectively at 500 and 400 MHz) in $CDCl_3$

Position	Protostrychnine (**1**)		Pseudostrychnine (**2**)	
	^{1}H chemical shifts[a]	COSY H/H correlations	^{1}H chemical shifts[a]	COSY H/H correlations
2	3.82 (*d*, 11.2)	16	3.82 *(m)*	16
3	3.70 (*m*)	14a, 14b, 15, 21b		
5a	2.90 (*m*)	5b, 6a, 6b	2.85 *(m)*	5b, 6a, 6b
5b	3.18 (*m*)	5a, 6a, 6b	3.83 *(m)*	5a
6a	1.96 (*m*)	5a, 5b, 6b	1.80 *(m)*	5a, 6b
6b	2.53 (*m*)	5a, 5b, 6a	2.26 *(m)*	5a, 6a
9	7.38 (*d*, 7.5)	10, 11	7.89 *(d,* 7.8*)*	10
10	7.18 (*dt*, 7.5 and 0.6)	9, 11, 12	7.05 *(m)*	9, 11
11	7.29 (*td*, 1.0 and 8.0)	9, 10, 12	7.23 *(m)*	10, 12
12	7.97 (*d*, 8.0)	10, 11	8.11 *(m)*	11
14a	1.73 (*dd*, 2.5 and 11.3)	3, 14b, 15	1.80 *(d,* 13.2)	14b, 15
14b	2.18 (*dt*, 3.3 and 13.9)	3, 14a, 15	2.26 *(m)*	14a, 15
15	3.09 (*d*, 3.0)	3, 14a, 14b, 16, 21b	3.28 *(m)*	14a, 14b, 16
16	1.40 (*dt*, 3.4 and 10.6)	2, 15, 17	1.37 *(d*, 10.2)	2, 15, 17
17	3.97 (*t*, 9.1)	16, 23a, 23b	4.27 *(m)*	16, 23a, 23b
18a	4.13 (*ddd*, 1.6; 6.2 and 13.0)	18b, 19, 21a	4.05 *(m)*	19
18b	4.26 (*dd*, 7.2 and 13.0)	18a, 19, 21a	4.13 *(m)*	19
19	5.79 (*t*, 6.4)	18a, 18b, 21a, 21b	5.91 *(t,* 7.2)	18a, 18b
21a	3.19 (*m*)	18a, 18b, 21b	2.94 (*d,* 14.9)	21b
21b	3.60 (*d*, 17.5)	3, 15, 19, 21a	3.73 *(d,* 15.2)	21a
23a	2.39 (*d*, 16.5)	17, 23b	2.66 (*dd,* 3.4 and 17.4)	17, 23b
23b	3.25 (*dd*, 8.5 and 16.5)	17, 23a	3.12 (*m*)	17, 23a

[a] Multiplicities and coupling constants in Hz are in parentheses.

12 were in the aromatic region: the COSY spectrum showed eight aromatic protons, as expected from two indole moieties, and four methines, two of which belonging to two ethylidenic side chains. As in strychnogucine A, ^{1}H and ^{13}C chemical shifts of the portion A of the molecule were quasi-identical to those of strychnine. Among characteristic signals of strychnine, we had a highly shielded shift for H-16, due to the configuration H-16α of strychnine, and a deshielded shift for H-12, due to the influence of the carbonyl C-22. The only differences with strychnine were observed at the 23-position: the C-23 chemical shift was notably shielded (51.9 ppm instead of 42.3 ppm) and only one proton corresponded to H-23, indicating the implication of C-23 in a supplementary C–C bond. These data were very close to these of strychnogucine A. Nevertheless, in the portion B of the molecule, some differences were observed in the ^{1}H and ^{13}C NMR spectra between the two compounds. In the portion B of the alkaloid, H-17′ was correlated to a multiplet at δ 2.77 and to a doublet at δ 5.98, assigned respectively to H-16′ and H-23′. The presence of H-16 and the deshielded shift value for H-23′ (methine proton) indicated the presence of a 17′-23′ double bond instead of a 16′-17′ double bond as in strychnogucine A.

The linkage C-23/C-5′ between the two portions of **3** was confirmed from the HMBC spectrum (H-5′ and the two H-6′ were correlated to C-23, while H-17 was correlated to C-5′), from the COSY spectrum (correlation between H-5′ and H-23), and from the correlations between H-23 and H-5′, H-17 and H-6′a, H-17 and H-3′ in the ROESY spectrum (Table 2 and Fig. 1).

The stereochemistry of **3** was then considered. The configurations H-15α, H-3α, H-2β, 7*R*, H-15′α, H-3′α, H-2′β and 7′*R* were those commonly accepted from biogenetic considerations (Klyne and Buckingham, 1974). This configuration is confirmed by the presence, in the CD spectra of a positive Cotton effect near 240 nm. This positive effect is indicative of a 2β,7β configuration in alkaloids with an indoline moiety (Klyne et al., 1965, 1968) and is observed, i.e., in the CD spectra of the monomers protostrychnine, pseudostrychnine (see Experimental) and isostrychnine (Frédérich et al., 2000), but also in the CD spectra of the dimers strychnogucine A, B (Frédérich et al., 2001) and sungucine (Frédérich et al., 2000), whose absolute configuration has been determined by X-ray analysis. In addition, this 2β,7β configuration is always biogenetically linked with a 3α,15α configuration (Klyne and Buckingham, 1974).

The H-16α (16*R*) and H-16′β (16′*S*) configurations were proposed after comparison of chemical shifts of C-2, C-6, C-14, C-7, C-3, C-16, C-21 and C-2′, C-6′, C-14′, C-7′, C-3′, C-16′, C-21′ with the published values for retuline, isoretuline (Massiot et al., 1988), strychnine (Verpoorte, 1980), and sungucine (Frédérich et al., 2000), and after observation of the coupling constants between H-2 and H-16 (10.5 Hz, antiperiplanar) and between H-2′ and H-16′ (6.8 Hz, periplanar). The deshielded value of H-16 (1.23 ppm) and the more

Table 2
^{1}H and ^{13}C NMR spectral data of compound **3** (recorded at 500/125 MHz) in $CDCl_3$

Position	^{1}H NMR[a]	COSY H/H correlations	Position	^{13}C NMR	HMBC[b] C→H correlations
2	3.98 (*d*, 10.5)	16	2	59.7	6ab
3	3.90 (*m*)	14ab	3	60	6ab, 14b, 21a
5a	2.89 (*m*)	5b, 6ab	5	50	6a, 21ab
5b	3.17 (*m*)	5a, 6ab			
6a	1.85 (*m*)	6b, 5ab	6	42.9	2
6b	1.87 (*m*)	6a, 5ab			
7			7	51.7	2, 6ab, 9, 14b
8			8	132.6	2, 12, 13
9	7.17* (*m*)	10	9	122.2	11
10	7.06 (*m*)	9, 11	10	124.4	12
11	7.20 (*m*)	10, 12	11	128.5	9
12	8.02 (*d*, 8.0)	11	12	116.5	10
13			13	142.1	2, 9, 10, 11, 12
14a	1.52 (*d*, 14.0)	3, 14b, 15	14	26.7	
14b	2.40 (*dt*, 14.0 and 4.6)	3, 14a, 15			
15	3.22 (*m*)	14ab, 16, 19	15	31.9	14b, 21a
16	1.23 (*m*)	2, 15, 17	16	49.5	2, 14b
17	4.24 (*m*)	16,23	17	79.1	2, 18ab, 23, 5′
18a	4.12 (*dd*, 12.5 and 5.7)	18b, 19	18	65.1	
18b	4.16 (*dd*, 12.5 and 6.9)	18a, 19			
19	5.95 (*m*)	15, 18ab, 21b	19	128.4	18ab, 21ab
20			20	139.3	14a, 18ab, 21ab
21a	2.72 (*d*, 14.5)	21b	21	52.5	
21b	3.69 (*d*, 14.5)	19, 21a			
22			22	171	23
23	2.88 (*m*)	17, 5′	23	51.9	
2′	4.30 (*d*, 6.8)	16′	2′	64.9	3′, 6′ab, 17′
3′	3.52 (*d*, 4.3)	14′ab	3′	65.1	2′, 6′ab, 21′a
5′	4.04 (*m*)	23, 6′ab	5′	61.7	6′a, 21′a, 23
6′a	1.95 (*t*, 12)	5′, 6′b	6′	39.5	2′, 5′, 23
6′b	2.21 (*dd*, 12 and 5)	5′, 6′a			
7′			7′	51.4	2′, 6′ab, 9′, 14′ab
8′			8′	134.2	3′, 6′a, 12′, 13′
9′	7.18* (*m*)	10′	9′	122.2	11′
10′	7.08 (*m*)	9′, 11′	10′	124.1	12′
11′	7.24 (*m*)	10′, 12′	11′	128.5	9′
12′	8.20 (*d*, 8.1)	11′	12′	116.2	10′
13′			13′	141.5	5′, 9′, 10′, 11′, 12′
14′a	1.75 (*m*)	14′b, 15′	14′	22.5	
14′b	1.78 (*m*)	14′a, 15′			
15′	2.68 (*m*)	14′ab, 16′	15′	31	2′, 3′, 14′a, 19′, 21′a
16′	2.77 (*m*)	2′, 15′, 17′, 23′	16′	40.1	17′, 23′
17′	6.87 (*dd*, 6.5 and 9.8)	16′, 23′	17′	144	
18′	1.65 (*d*, 6.0)	19′, 21′b	18′	12.9	19′
19′	5.28 (*d*, 6.0)	15′, 18′, 21′b	19′	119.3	18′, 21′a
20′			20′	142.6	
21′a	3.13 (*d*, 16.8)	21′b	21′	49.7	5′, 19′
21′b	3.62 (*d*, 16.8)	19′, 21′a			
22′			22′	162	
23′	5.99 (*d*, 9.8)	16′, 17′	23′	122.8	

*These values could be interchanged.
[a] Multiplicities and coupling constants in Hz are in parentheses.
[b] Correlations from C to the indicated hydrogens.

shielded value of H-16′ (2.72 ppm) were in agreement with these configurations.

The configuration of H-5′ was suggested as β (H-5′*R*) after comparison of the H-6′ab multiplicities and the C-5′, C-6′, H-5′, and H-6′ chemical shifts with values for sungucine and isosungucine (Frédérich et al., 2000). This configuration was corroborated by the ROESY coupling between H-23 and H-5′ (Fig. 1) when no coupling was observed between H-5′ (β) and H-3′ (α).

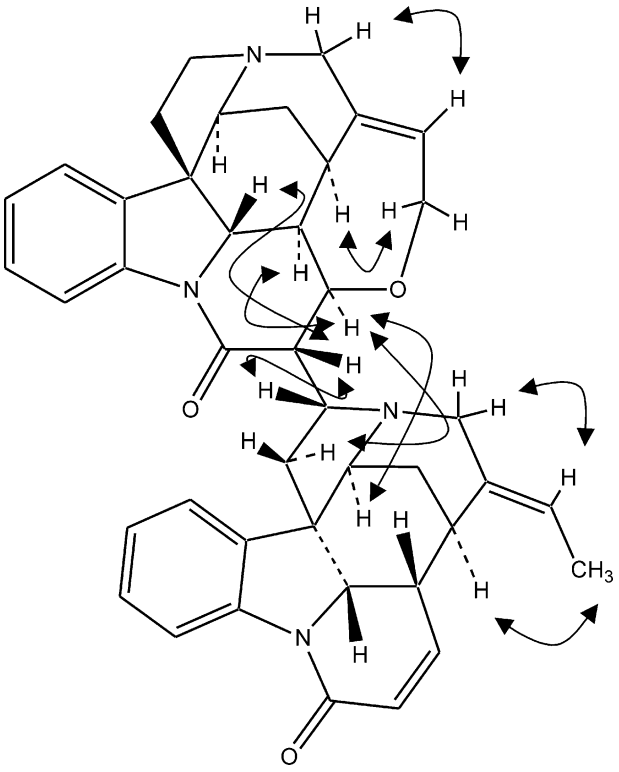

Fig. 1. Significant ROESY correlations for compound **4**.

The H-17α (17*R*) and H-23β (23*S*) configurations were proposed after comparison of chemical shifts of C-15 through C-23 and C-19 through C-21 with analogous data for strychnine (Sandberg et al., 1969) and strychnogucine A (Frédérich et al., 2001). These hypotheses were corroborated by the ROESY couplings observed between H-17 (α) and H-16 (α), H-17 (α) and H-3′ (α), H-23 (β) and H-2 (β).

Consequently, alkaloid **3** must have the following stereochemistry: H-15α (15*R*), H-3α (3*S*), H-2β (2*S*), 7*R*, H-16α (16*R*), H-17α (17*R*), H-23β (23*S*), H-5′β (5′*R*), H-15′α (15′*S*), H-16′β (16′*S*), H-3′α (3′*S*), H-2′β (2′*S*), and 7′*R*.

The in vitro antiplasmodial activities of isolated compounds have then been determined against the FCA chloroquine-sensitive strain of *Plasmodium falciparum* in comparison to chloroquine, quinine and other dimeric alkaloids from *S. icaja* (see Table 3). Strychnogucine C presented an IC_{50} between 10 and 20 μM, which was notably less active than other sungucine type alkaloids. This is a confirmation that the presence of a cyclisation of the ring G in the upper part of the molecule (portion A), as in strychnogucine A, or the presence of a 17′-23′ double bond, as in sungucine, has a negative impact on antiplasmodial activity. The most active compound from this class of alkaloids (quasi-symmetric dimers possessing a 5′-23 link) remains strychnogucine B, possessing a lower strychnine moiety and an upper isostrychnine II moiety (Frédérich et al., 2001). If the antiplasmodial selectivity of these sungucine-type alkaloids towards human cells has been previously demonstrated (Frédérich et al., 2001), it will be nevertheless important to check, in the future, if these alkaloids are really devoid of any convulsant activity. The monomers have also been tested against *Plasmodium* but were devoid of any antiplasmodial activity, as expected and previously observed for other monomers (Frédérich et al., 1999). On the other hand, strychnohexamine (**4**) presented an interesting antiplasmodial activity with an IC_{50} near 1 μM, which was about two times more potent than bisnordihydrotoxiferine.

Table 3
In vitro antiplasmodial activity of compounds **3** and **4**, quinine, and chloroquine on FCA line of *Plasmodium falciparum*

Compound	IC_{50} (μM)	IC_{90} (μM)	n[a]
Sungucine	7.816±1.137	26.256	3
Strychnogucine A	2.310±0.304	6.980	2
Strychnogucine B	0.617±0.067	3.785	2
Strychnogucine C (**3**)	16.057±0.764	33.535	2
Strychnohexamine (**4**)	1.097±0.099	4.296	3
Bisnordihydrotoxiferine	2.796±1.078	16.409	3
Chloroquine	0.02±0.002	0.119	9
Quinine	0.269±0.006	1.913	3

[a] n = Number of experiments.

3. Experimental

3.1. General

UV spectra were recorded on a Kontron Uvikon spectrophotometer, and the IR spectra were recorded on a Perkin-Elmer 1750 FTIR spectrometer. NMR spectra were recorded in $CDCl_3$ on a Bruker 500 MHz NMR spectrometer, with TMS as an internal reference. CD curves were determined on a Jobin Yvon CD6 dichrograph. ESIMS were obtained with a VG Autospec-Q (VG Analytical, Manchester, Liquid s/ms, Cs^+, 20 keV, resolution >5000) apparatus. Analytical TLC was performed on precoated Si gel F_{254} (Merck, 1.05735) plates. After development, the dried plates were examined under short-wave (254 nm) or long-wave (366 nm) UV light and sprayed with one of the following reagents: (a) Dragendorff's reagent, (b) 1% ceric sulfate in 10% sulfuric acid. LiChroprep Si 60 (15–25 μm, Merck 9336) was used for column chromatography. Si gel 60 PF 254 (Art.1.07747, Merck) was used for purification of alkaloids by preparative TLC (1.25 mm thick, 20×40 cm Si gel plates). All solvents used were analytical grade (Merck). The authentic sample of pseudostrychnine has been obtained from the late Professor Bisset, Chelsea College, University of London. The original sample of genostrychnine came from the collections of the laboratory, and had been

obtained from strychnine by hemi-synthesis (H_2O_2 treatment).

3.2. Plant material

The roots of *S. icaja* were collected near Kasongo-Lunda (Congo-Zaire). A voucher specimen of the plant (Duvigneaud H787) has been deposited in the herbarium of the Pharmaceutical Institute, at Liège and in the herbarium of the Belgian National Botanical Garden, at Meise.

3.3. Extraction and isolation

The roots of *S. icaja* (500 g) were macerated with 300 ml of EtOAc–ethanol–NH_4OH (96:3:1) and then percolated with EtOAc then with MeOH until complete extraction of alkaloids. The extract was concentrated under reduced pressure below 60 °C to yield 43 g of dry extract and then dissolved in EtOAc and extracted with 4% HOAc. The resulting acidic (pH 3) solution was extracted by CH_2Cl_2, then basified to pH 8 with Na_2CO_3 and repeatedly extracted with CH_2Cl_2. The same extraction was made at pH 10 (alkalinization with NH_4OH). The CH_2Cl_2 extracts obtained were dried over Na_2SO_4 and concentrated to yield crude alkaloid extracts (respectively 5, 28 and 1 g, at pH 3, 8 and 10). The pH 8 extract was fractionated by medium pressure liquid chromatography (MPLC) on 180 g Merck LichroPrep Si 60 (40–63 μm, Merck 9336) with a gradient of CH_2Cl_2/MeOH mixtures (0 to 10% MeOH), to give fractions I-XXVI, detected by TLC (EtOAC/2-PrOH/NH_4OH, 80:15:5), as previously described (Frédérich et al., 2001). Strychnohexamine (**4**) was present in weak amounts in fractions XVIII to XXII. The purification of **4** (10 mg) has been conducted by MPLC on Merck LiChroprep RP-8 (25–40 μm, 8 g) with MeOH–MeCN–H_2O (3:2:1) and finally on a Sephadex LH20 (20 g, Pharmacia Biotech) column with MeOH as mobile phase. Strychnogucine C (**3**) was present in fractions XVI and XVII (6570 to 8039 ml) along with strychnine, strychnogucine A and bisnordihydrotoxiferine. The four compounds were separated by MPLC on Merck LiChroprep RP8 (25–40 μm, 8 g) with MeOH–MeCN–H_2O (3:2:1); strychnine, 240 to 290 ml; strychnogucine A (18 mg) (1), 300 to 360 ml, strychnogucine C, 400 to 450 ml and bisnordihydrotoxiferine, 550 to 700 ml. Protostrychnine (**1**) and genostrychnine were isolated from the pH 10 extract. This extract has been fractionated by HSCCC. $CHCl_3$, MeOH and water were thoroughly equilibrated in suitable proportions (7:13:8) and the two phases separated. The lower organic phase was used as the stationary phase, and the upper aqueous phase as the mobile phase. We worked in the ascending mode. Compound **1** and genostrychnine were found alone in two successive fractions. These were concentrated under reduced pressure and then evaporated to dryness to yield, from the first fraction, 29 mg of protostrychnine (**1**) and, from the second, 35 mg of genostrychnine. Further purifications were not necessary. Pseudostrychnine (**2**) has been isolated from pH 3 extract. This extract has been fractionated by MPLC on Merck LiChroPrep Si 60 using the same mobile phase as for pH 8 extract. The first fraction obtained was pseudostrychnine (79 mg) and was followed by known alkaloids as vomicine, icajine, sungucine, strychnine.

3.4. Protostrychnine (*1*)

Amorphous buff colored solid. On TLC, gave a pale orange-pink coloration after spraying with cerium sulfate reagent and an orange-red coloration with ferric chloride reagent. The UV, IR, MS and ^{13}C NMR data were in agreement with those from the literature (Baser et al., 1979). CD_{MeOH}, $\Delta\varepsilon_{nm}$: $\Delta\varepsilon_{216}$ +6.0, $\Delta\varepsilon_{228}$ −0.2, $\Delta\varepsilon_{252}$ +2.6, $\Delta\varepsilon_{274}$ +1.1, $\Delta\varepsilon_{292}$ +1.5; ^1H NMR data are given in Table 2.

3.5. Genostrychnine

Genostrychnine was identified by comparison with an authentic sample (TLC, UV, IR, ESI–MS).

3.6. Pseudostrychnine (*2*)

White needle-crystallized powder. On TLC, gave a fleeting blue coloration after spraying with cerium sulfate reagent and an orange coloration with ferric chloride reagent. CD_{MeOH}, $\Delta\varepsilon_{nm}$: $\Delta\varepsilon_{205}$ −17.48, $\Delta\varepsilon_{214}$ −9.42, $\Delta\varepsilon_{225}$ −20.22, $\Delta\varepsilon_{259}$ 3.72; $\Delta\varepsilon_{277}$ 0.97 FT–IR ν_{max} (C_2Cl_4) cm^{-1}: 3593 (OH), 2952, 1681 (C=O lactam), 1596, 1477, 1389, 1290. The UV, MS and ^{13}C NMR data (Baser et al., 1979) were in agreement with those from the literature (Verpoorte et al., 1977). ^1H NMR data are given in Table 2.

3.7. Strychnogucine C (*3*)

White-yellowish amorphous powder. On TLC, gave a blue fluorescence at 366 nm after spraying with cerium sulfate reagent; UV λ_{max} nm (log ε) (MeOH): 208 (4,63), 255 (4,14), 283 (3,93); FT–IR υ_{max} (KBr) cm^{-1}: 3437, 2926, 1665, 1594, 1482, 1461, 1385, 1287, 1095, 1051, 819, 758, 566, 425; CD_{MeOH}, $\Delta\varepsilon_{nm}$: $\Delta\varepsilon_{205}$ −15.6, $\Delta\varepsilon_{240}$ −0.39, $\Delta\varepsilon_{275}$ −1,56, $\Delta\varepsilon_{298}$ −0,39 ; ^1H and ^{13}C NMR data are given in Table 1; ESI–MS *m/z* 651 [MH^+] (90), 335 (50), 317 (100), 274 (5), 134 (20) (daughters); HRESIMS *m/z* [MH^+] 651.3213 (calcd for $C_{42}H_{43}N_4O_3$, 651.3335).

3.8. Strychnohexamine (*4*)

White-yellowish amorphous powder. On TLC gave a fleeting purple coloration after spraying with cerium

sulfate reagent; ESI–MS: m/z 869 (MH^+) (100), 855, 674, 629, 583, 546, 510, 438, 391, 345, 258, 247, 234, 231, 222, 208, 194, 144, 122; HRESIMS: m/z 868.48057, (calcd for $C_{59}H_{60}N_6O_1$, 868.4828); FT–IR υ_{max} (KBr) cm^{-1}: 3429, 2926, 1664 (C=O), 1597 (C=C), 1486, 1418, 1383, 1261, 1095, 801, 754, 617; UV λ_{max} nm (log ε) (MeOH): 210 (3.55), 293 (3.25), 320 (2.99); CD_{MeOH}, $\Delta\varepsilon_{nm}$: $\Delta\varepsilon_{240}$ +2, $\Delta\varepsilon_{274}$ −16.6, $\Delta\varepsilon_{294}$ +16.6, $\Delta\varepsilon_{318}$ −33.3. NMR data have been previously described (Philippe et al., 2002).

3.9. Antiplasmodial assays

Continuous in vitro cultures of asexual erythrocytic stages of the four *P. falciparum* strains were maintained following the procedure of Trager and Jensen (1976) and as described previously (Frédérich et al., 2000). Chloroquine diphosphate (Sigma C6628), mefloquine HCl (Roche) and quinine base (Aldrich 14590-4) were used as antimalarial references. Each test sample was applied in a series of eight four-fold dilutions (final concentrations ranging from 20 μg/ml to 0.0012 μg/ml) and was tested in duplicate. Parasite growth was estimated by determination of [^{3}H]hypoxanthine incorporation as described by Desjardins et al. (1979) and modified by Mirovsky et al. (1990). The Student t-test was used to test the significance of differences between results obtained for different samples. Statistical significance was set at $P \leqslant 0.05$.

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