Strychnogucines A and B, Two New Antiplasmodial Bisindole Alkaloids from *Strychnos icaja*

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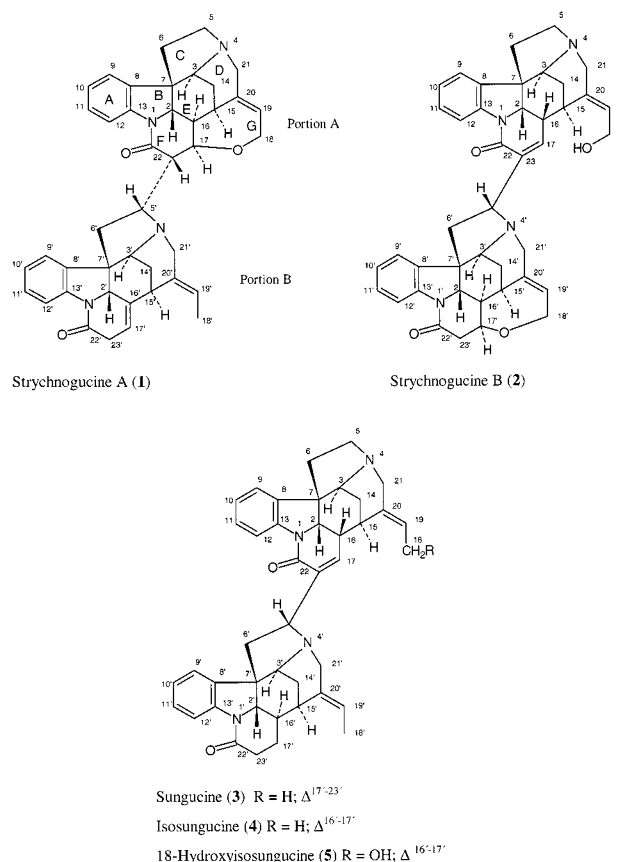
A reinvestigation of *Strychnos icaja* roots has resulted in the isolation of two tertiary quasi-symmetric bisindole alkaloids named strychnogucines A (**1**) and B (**2**). Their structures were identified by means of spectroscopic data interpretation. Compound **2** was highly active in vitro and compound **1** moderately active against four strains of *Plasmodium falciparum*. Strychnogucine B (**2**) was more active against a chloroquine-resistant strain than against a chloroquine-sensitive one (best CI_{50}, 80 nM against the W2 strain). In addition, this compound showed a selective antiplasmodial activity with 25−180 times greater toxicity toward *P. falciparum*, relative to cultured human cancer cells (KB) or human fibroblasts (WI38).

Malaria is the major parasitic infection in many tropical and subtropical regions, leading to approximately one million deaths out of 400 million cases each year. Increased resistance of *Plasmodium falciparum* to conventional treatments and the emergence of multidrug-resistant strains is a worldwide problem, and few alternative drugs are under development. Urgent efforts are therefore necessary to identify new classes of antimalarial drugs.

Strychnos icaja Baillon (Loganiaceae), a 20−100-m long liana distributed throughout central Africa, is the only African *Strychnos* species containing strychnine. Its roots were once used for the preparation of arrow and ordeal poisons.[1] This toxicity was ascribed to the monoindole alkaloids strychnine and hydroxystrychnine, isolated from the roots by Sandberg et al.[2] However, some tribes of pygmies from Cameroon have treated malaria with *S. icaja* roots.[1] The roots were studied chemically by Kambu et al.,[3] who isolated methylstrychnine, three tertiary monomers (icajine; 19,20-α-epoxynovacine; and 19,20-α-epoxy-15-hydroxynovacine), and two tertiary dimers (bisnordihydrotoxiferine and a new compound, sungucine). Recently, as part of our search for new potential antiplasmodial compounds[4,5] from African *Strychnos* species, we isolated three new sungucine (**3**) derivatives,[6] named isosungucine (**4**), 18-hydroxysungucine, and 18-hydroxyisosungucine (**5**) from *S. icaja*. These compounds, particularly 18-hydroxyisosungucine, were moderately active against *P. falciparum*.[6] In a continuation of our work on *S. icaja*, we have investigated further fractions that show interesting antiplasmodial activities and have isolated two new alkaloids, strychnogucines A (**1**) and B (**2**). Alkaloids **1** and **2** show moderate to high activity when evaluated against four strains of *P. falciparum*.



Strychnogucine A (**1**)   Strychnogucine B (**2**)

Sungucine (**3**) R = H; $\Delta^{17'-23'}$

Isosungucine (**4**) R = H; $\Delta^{16'-17'}$

18-Hydroxyisosungucine (**5**) R = OH; $\Delta^{16'-17'}$

Results and Discussion

After further extraction and purification of *S. icaja* roots, we obtained two new alkaloids, strychnogucines A (**1**) and B (**2**), which were obtained as sungucine (**3**) derivatives possessing a strychnine substructure. Compound **1** was found in the same fractions as strychnine, while **2** occurred in the same fractions as the hydroxysungucines.[6]

The UV spectrum of **1** exhibited maxima at 214, 255, 281, and 289 nm, with values very similar to those of strychnine[7,8] or isostrychnine,[9] except for the maximum at 255 nm, which is more prominent in strychnine. The IR spectrum showed an amide absorption at 1666 cm^{-1}. This spectrum is very close to those of sungucine (**3**) and

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Table 1. ^1H (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data for Strychnogucine A (**1**) in $CDCl_3$

position	δ_H[a]	COSY H−H correlations	δ_C	HMBC[b] C−H correlations	DEPT
2	3.96 (d, 9.9)	16	60.34[c]	17, 3, 6, 16	CH
3	3.92 (m)	14a, 14b	60.12[c]	21a, 14a	CH
5a	2.91 (m)	5b, 6	50.33	21a, 21b, 6a	CH_2
5b	3.17 (m)	5a, 6			
6ab	1.87 (m)	5a, 5b	43.14	3, 5a	CH2
7			51.62	3, 6, 9	Q
8			132.99	10, 12, 3	Q
9	7.19 (m)	10	122.64	11, 12	CH
10	7.04 (m)	9, 11	124.36	12	CH
11	7.17 (m)	10,12	128.41[d]	9, 2	CH
12	8.02 (d, 7.02)	11	116.45	10, 11	CH
13			142.42	3, 11, 12	Q
14a	1.38 (t, 13.6)	14b, 3	27.00	21b	CH2
14b	2.19 (dt, 13.6, 3.9)	14a, 3, 15			
15	3.15 (m)	14b, 16	32.05	3, 21a, 14a, 16, 19	CH
16	1.15 (dd, 9.9, 3.2)	2, 15, 17	48.97	3, 14a	CH
17	4.14 (dd 3.2, 3.1)	16, 23	79.83	2, 3, 5′, 18, 23	CH
18a	4.07 (dd, 6.7, 13.1)	19	64.95	19	CH_2
18b	4.18 (dd, 6.4, 13.1)				
19	5.93 (m)	18ab	127.79	21a, 21b, 18, 2	CH
20			140.25	18, 21a, 21b, 14a, 16, 2	Q
21a	2.70 (d, 14.7)	21a	52.53	5a, 19	CH_2
21b	3.69 (d, 14.7)	21b			
22			170.99	23	Q
23	2.84 (3.1, 6.1)	5′, 17	52.18	5′, 6′a, 6′b, 17	CH
2′	4.38 (s)	17′, 23′	65.86	6′a, 6′b, 3′, 15′	CH
3′	3.84 (m)	14′b	61.96	6′b, 15′, 21′a	CH
5′	3.80 (ddd, 12.05, 6.1, 5.1)	6′a, 6′b, 23	61.59	17, 21′a, 21′b, 23, 6′a, 6′b	CH
6′a	1.75 (t, 12.05)	6′b, 5′	46.43	2′, 5′, 23	CH_2
6′b	2.24 (dd, 5.1, 12.05)	6′a, 5′			
7′			52.92	2′, 3′, 6′a, 6′b, 9′	Q
8′			133.95	3′, 10′, 12′	Q
9′	7.14 (m)	10′	122.35	11′, 12′	CH
10′	7.02 (m)	9′, 11′	123.75	12′	CH
11′	7.25 (m)	10′, 12′	128.63[d]	9′	CH
12′	8.13 (d, 7.56)	11′	114.80	10′, 11′	CH
13′			142.06	3′, 11′, 12′	Q
14′a	1.46 (m)	14′b, 15′	25.06	21′a	CH_2
14′b	2.34 (m)	14′a, 15′, 3′			
15′	3.54 (m)	14′a, 14′b	34.48	3′, 21′a, 14′a, 19′	CH
16′			141.64	2′, 15′, 23′	Q
17′	5.85 (m)	2′, 23′	120.32	2′, 15′, 23′	CH
18′	1.64 (d, 6.8)	19′, 21′b	12.6	19′	CH_3
19′	5.45 (q, 6.21)	18′	122.81	18′, 21′a, 21′b	CH
20′			135.94	18′, 21′a, 21′b, 14′a, 15′	Q
21′a	2.94 (d, 14.9)	21′b	51.92	3′, 5′, 19′	CH_2
21′b	3.72 (d, 14.9)	18′, 21′a			
22′			169.06	23′	Q
23′	3.10 (m)	2′, 17′	36.91		CH_2

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

isosungucine (**4**),[6] with the principal difference being the disappearance of the enone absorption at 1630 cm^{-1}. The molecular formula, $C_{42}H_{42}N_4O_3$, was established by means of HRESIMS, showing an additional oxygen atom in comparison to **3** ($C_{42}H_{42}N_4O_2$). The ESIMS fragmentation (daughter peaks) displayed two ions at *m/z* 335 and 317, corresponding to the masses of strychnine and deoxyisostrychnine, respectively.

NMR spectral data of **1** are listed in Table 1. In the aromatic region, the COSY spectrum showed eight aromatic protons (represented by two four-spin systems as expected from two indole moieties) and three methine protons (C-17′ and two protons from two ethylidene side chains). Protonated aromatic carbons were assigned from their direct correlations observed in the HMQC spectrum. The HMBC spectrum (Table 1) confirmed these assignments and also allowed the assignment of quaternary aromatic carbons. Among the aromatic protons, H-12 and H-12′ were deshielded, as in strychnine,[10] isostrychnine,[6] and sunguicine (**3**),[6] due to the influence of the carbonyl at C-22.

Chemical shifts and HMBC and COSY correlations of portion A of **1** were very close to those of strychnine.[10,11] Notably, in the COSY spectrum, an "oxy" ethylidene side chain (H-19, triplet and H-18ab, two doublets of doublets), and a doublet (H-2) correlated to a multiplet (H-16), correlated, in turn, with H-15 and H-17, were observed. As in strychnine, the chemical shift of H-16 (δ 1.15) was very shielded. The presence of a ring F was deduced from the chemical shift of H-17 (δ 4.14, corresponding to the expected value for a H−C−O) and from the correlation between C-17 and H-18 in the HMBC spectrum (Table 1).

In portion B of **1**, H-17′ was correlated to a singlet at δ 4.38 and to a multiplet at δ 3.10, assigned, respectively, to H-2′ and H-23′ (aliphatic). The DEPT spectrum (Table 1) indicated that the signal for C-23′ is a methylene. These observations are in accordance with the presence of a C-16′/C-17′ double bond. Using the HMBC spectrum, the whole skeleton of portion B of alkaloid **1** was established, in every respect identical to the lower part of **4**. The linkage C-23−C-5′ between the two parts of **1** was confirmed from the HMBC spectrum (H-5′ was correlated to C-17 and C-23, while H-17 was correlated to C-5′) and from the COSY spectrum (correlation between H-5′and H-23).

The stereochemistry of **1** was then considered. The configurations H-15α, H-3α, H-2β, 7*R*, H-15′α, H-3′α, H-2′β,

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data for Strychnogucine B (**2**) in $CDCl_3$

position	δ_H[a]	COSY H−H correlations	δ_C	HMBC[b] C−H correlations
2	4.35 (d, 5.9)	16	64.4	6a, 6b, 17
3	3.45 (m)	14a, 14b	64.9	2, 21a
5a	3.13 (m)	5b, 6ab	53.7	6b, 21a, 21b
5b	3.40 (m)	5a, 6ab		
6a	2.18 (m)	6b, 5ab	37.2	2
6b	2.67 (m)	6a, 5ab		
7			52.5	2, 6a, 6b, 9
8			135.8	6b, 10, 12
9	7.24 (m)[d]	10	122.4[c]	10, 11
10	7.11 (m)	9, 11	124.8	12
11	7.28 (m)[c]	10, 12	128.6[d]	9, 10
12	8.22 (d, 6.4)	11	116.6	9, 10
13			141.8	11, 12
14a	1.78 (m)	14b, 15, 3	22.8	
14b	1.79 (m)	14a, 15, 3		
15	2.77 (m)	14a, 14b	31.8	2, 3, 16, 19
16	2.79 (m)	2, 17	31.3	17
17	7.20 (d, 5.23)	16	137.6	16
18	4.37 (m)	19	57.7	19
19	5.63 (t)	18	64.7	18, 21a, 21b
20			142.7	18, 21a, 21b, 16
21a	3.37 (m)	21b	52.3	19
21b	3.74 (m)	21a		
22			162.3	17
23			134.9	6′a, 16, 17
2′	4.13 (d, 10)	16′	60.9	6′a, 6′b, 16′, 17′
3′	4.33 (m)	14′a, 14′b	59.1	5′, 21′a, 21′b
5′	4.19 (dd, 5, 12)	6′a, 6′b	60.7	6′a, 6′b, 17, 21′a, 21′b
6′a	1.86 (t, 12)	6′b, 5′	50.1	2′
6′b	2.56 (dd, 5, 12)	6′a, 5′		
7′			52.7	2′, 6′a, 6′b
8′			134.1	10′, 12′, 2′, 6′a
9′	7.26 (m)[d]	10′	122.6[c]	10′, 11′
10′	7.09 (m)	9′, 11′	124.5	12′
11′	7.30 (m)[c]	10′, 12′	128.8[d]	9′, 10′
12′	8.09 (d, 6.5)	11′	116.4	9′, 10′
13′			142.2	2′, 11′, 12′
14′a	1.78 (m)	14′b, 15′, 3′	28.7	16′
14′b	1.79 (m)	14′a, 15′, 3′		
15′	3.21 (m)	14′a, 14′b	31.3	3′, 14′a, 16′, 19′
16′	1.37 (m)	14′a, 15′, 2′, 17′	48.2	2′, 14′a, 15′
17′	4.35 (m)	16′, 23′a, 23′b	77.6	2′, 15′, 18′, 23′a, 23′b
18′a	4.05 (dd, 5.6, 13)	18′b, 19′	64.7	19′
18′b	4.11	18′a, 19′		
19′	6.01 (m)	18′a, 18′b	128.2	18′, 21′a, 21′b
20′			140.1	18′, 21′b, 16′, 14′a
21′a	2.81 (m)	21′b	51.7	18′, 19′
21′b	3.70 (m)	21′a		
22′			169.0	23′a, 23′b
23′a	2.70 (dd,	23′b, 17′	42.6	
23′b	3.21 (m)	23′a, 17′		

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

and 7′*R* were those commonly accepted from biogenetic considerations.[12,13] Comparison of the chemical shifts of C-3, C-6, C-7, C-14, C-16 with the values for the same carbons in retuline and isoretuline[14] allowed **1** to be placed in the isoretuline series with a H-16α functionality. The deshielded value of H-16 and the relatively high coupling constant between H-2 and H-16 (9.9 Hz) were in agreement with this configuration. A H-5′β configuration was suggested after comparison of the H-6′ab multiplicities and the C-5′, C-6′, H-5′, and H-6′ chemical shifts with values for **3** and **4**. A H-17α configuration was proposed from the coupling constant of 3.2 Hz between H-16 and H-17, which was compatible with a periplanar configuration (angle near 50° on a molecular model) and comparable with the value exhibited by strychnine.[10] On the other hand, a H-17β configuration would lead to different tensions in ring G. Nevertheless, the chemical shifts of C-15 through C-17 and C-19 through C-21 were identical to analogous data for strychnine.[7] The H-23β configuration was proposed after observation of coupling constants between H-23 and H-17 (identical to those observed in strychnine between H-17 and H-23β) and between H-23 and H-5′ (6 Hz, compatible with a periplanar configuration between H-5′β and H-23β). Consequently, alkaloid **1** 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-3′α (3′*S*), H-2′β (2′*S*), and 7′*R*.

The UV spectrum of strychnogucine B (**2**) exhibited maxima at 222, 252, 283, 292, and 304 nm, in a manner similar to **4**. The IR spectrum showed an amide absorption at 1666 cm^{-1} and an enone absorption at 1630 cm^{-1}, as in **3** and **4**. A molecular weight of 667.3295 was established by means of HRESIMS and corresponded to the molecular formula $C_{42}H_{42}N_4O_4$, showing two more oxygen atoms than **3**.[6] NMR spectral data of **2** are listed in Table 2.

In the aromatic part of the COSY spectrum of **2**, two indole moieties (eight aromatic protons), one hydroxyethylidene chain (δ 5.63 and 4.37), one oxyethylidene chain (ether oxyde, δ 6.01, 4.05 and 4.22), and one methine proton at δ 7.20, attributed to H-17, were all observed. All proton and carbon chemical shifts of strychnogucine B (**2**) corresponded to those, on one hand, of strychnine (portion B)

Table 3. Antiplasmodial Activity of Compounds **1**−**5** and of Three Reference Compounds

compound	FCA 20 Ghana IC$_{50}$ μM ± SD[a]	FCA 20 Ghana IC$_{90}$ μM	FCA 20 Ghana n[b]	W2 Indochina IC$_{50}$ μM ± SD[a]	W2 Indochina IC$_{90}$ μM	W2 Indochina n[b]
strychnogucine A (**1**)	2.310 ± 0.304 (1.50)	6.980	2	ND	-	
strychnogucine B (**2**)	0.617 ± 0.067 (0.41)	3.785	2	0.085 ± 0.01 (0.057)[c]	0.358	2
sungucine (**3**)	7.816 ± 1.137 (4.95)[d]	26.256	3	10.139 ± 3.08 (6.428)[d]	33.15	2
isosungucine (**4**)	1.315 ± 0.252 (0.835)[e]	7.059	3	0.265 ± 0.093 (0.168)[f]	1.716	2
18-hydroxyisosungucine (**5**)	0.847 ± 0.141 (0.551)[e]	4.348	4	0.140 ± 0.046 (0.091)[c,f]	1.352	2
chloroquine	0.020 ± 0.002 (0.006)	0.119	9	0.284 ± 0.017 (0.091)	1.745	6
quinine	0.269 ± 0.006 (0.087)	1.913	3	0.413 ± 0.011 (0.134)	1.718	2

compound	F 32 Tanzania IC$_{50}$ μM ± SD[a]	F 32 Tanzania IC$_{90}$ μM	F 32 Tanzania n[b]	PFB Brazil IC$_{50}$ μM ± SD[a]	PFB Brazil IC$_{90}$ μM	PFB Brazil n[b]
strychnogucine A (**1**)	4.813 ± 2.162	9.543	2	3.199 ± 0.144	9.403	2
strychnogucine B (**2**)	0.510 ± 0.260	3.228	2	0.202	1.150	1
18-hydroxyisosungucine (**5**)	1.263 ± 0.191	5.349	3	0.431 ± 0.203	1.644	2
chloroquine	0.014 ± 0.004	0.063	2	0.540 ± 0.330	1.544	5
mefloquine	0.023 ± 0.014	0.354	4	0.002 ± 0.001	0.006	2

[a] Values are expressed as mean ± standard deviation. All tests were realized in duplicate. [b] n = number of experiments. [c−f] Values bearing the same superscript in the same row or column were not significantly different ($p \leq 0.05$).

Table 4. Cytotoxic Activities on Human Cancer Cell Lines and Antiprotozoal Selectivity Index of Compounds **2**−**5**[a,b]

	KB IC$_{50}$	WI38 IC$_{50}$	KB/ FCA SI	KB/ W2 SI	WI38/ FCA SI	WI38/W2 SI
strychnogucine B (**2**)	>15	15.5	> 24.3	>176	25.12	182.4
sungucine (**3**)	6.2	6.0	0.8	0.6	0.8	0.6
isosungucine (**4**)	9	9.2	6.8	34.0	7.0	34.7
18-hydroxyisosungucine (**5**)	16.2	16.8	19.1	115.7	19.83	120
usambarensine[c]	9.7	4.62	14.8	36.6	3.1	7.8
emetine[c]	0.056	ND[d]	ND	ND	ND	ND

[a] IC$_{50}$ values are expressed in μM. [b] Selectivity index (SI) is defined as the ratio of cytotoxicity over antiplasmodial activity. [c] Reference compound. [d] ND = not determined.

and, on the other hand, of isostrychnine II (portion A) (monomer of **3**). The linkage between the two parts of the alkaloid (C-5′ to C-23) was deduced from HMBC coupling between C-23 and H-6′a and between C-5′and H-17. The strychnine moiety (portion B) was seen in **2** to be the lower part of the dimer. The stereochemistry of **2** was then considered. The configurations H-15α (15*R*), H-3α (3*S*), H-2β (2*S*), 7*R*, H-15′α (15′*R*), H-3′α (3′*S*), H-2′β (2′*S*),7′*R*, and H-17′α (17′*S*) were deduced from biogenetic considerations.[12,13] The H-16β (16*R*) and H-16′α (16′*R*) configurations were proposed after comparison of chemical shifts of C-2, C-6, C-14, C-7, C-3, C-16, and C-21 with the published values for retuline, isoretuline,[14] strychnine,[15] and sungucine[6] and after observation of the coupling constants between H-2 and H-16 (5.9 Hz, periplanar) and between H-2′ and H-16′ (10 Hz, antiperiplanar). Chemical shifts of H-16 (δ 2.79) and H-16′ (δ 1.37) in **2** were also compatible with these configurations. The H-5′β configuration was attributed by comparison with **3** and the H-17′α configuration was proposed by comparison of ^{13}C NMR chemical shifts in ring G with strychnine.

In vitro antiplasmodial activity against four *P. falciparum* strains of compounds **1** and **2** in comparison to chloroquine, quinine, mefloquine, sungucine (**3**), isosungucine (**4**), and 18-hydroxyisosungucine (**5**) is shown in Table 3. When compared with other sungucine derivatives, only **2** possessed an interesting activity on *P. falciparum*. Cyclization in ring G in the lower portion of the molecule increased antiplasmodial activity, compared with hydroxyisosungucine.[6] Thus, strychnogucine B (**2**) is the most active compound of the series, with 12 to 120 times higher activity than **3** for the FCA and W2 plasmodial strains. Moreover, the activity of compound **2** was higher against the chloroquine-resistant strains W2 and PFB than against the chloroquine-sensitive strains FCA and F32 of *P. falciparum* (Table 3). Strychnogucine B (**2**) was cytotoxic against KB human cancer cell line and against WI38 human fibroblasts (Table 4). However, KB cells and WI38 fibroblasts were less sensitive to **2** than to **3** and **4**. Strychnogucine B (**2**) exhibited 25−180-fold higher activity against the *P. falciparum* strain than human cancer cell lines, thus indicating a good selectivity.

Experimental Section

General Experimental Procedures. UV and visible spectra were recorded on a Kontron Uvikon spectrophotometer, and the IR spectra were recorded on a Perkin-Elmer 1750 FTIR spectrometer. NMR spectra were measured with a Bruker DRX 400 Avance spectrometer at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C), at 25 °C. Chemical shifts were recorded in δ (ppm) based either on δ TMS = 0, and the coupling constants (*J*) are in hertz. ^{13}C NMR assignments were established partly through comparison of chemical shifts with published data for similar compounds and partly through interpretation of HMBC and HMQC spectra. All programs used in performing the 2D NMR experiments come from the Bruker library. 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 or (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).

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.

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 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 solution was basified to pH 8 with Na_2CO_3 and repeatedly extracted with $CHCl_3$. The same extractions were made at pH 10 (alkalinization with NH_4OH) and pH 12 (alkalinization with NaOH). The $CHCl_3$ extracts obtained were dried over Na_2SO_4 and concentrated to yield crude alkaloid extracts (respectively 28, 1, and 0.5 g at pH 8, pH 10, and pH 12). Because they exhibited less potent activity against *P. falciparum* strains, pH 10 and 12 extracts were conserved for further investigation. The pH 8 extract was fractionated first by MPLC on 180 g Merck LichroPrep Si 60 (40−63 μm, Merck 9336) with CH_2Cl_2−MeOH mixtures (0 to 1 L: 100% CH_2Cl_2; 1 to 2.2 L: 0.5% MeOH; 2.2 to 3.1 L: 1% MeOH; 3.1 to 5 L: 2% MeOH; 5 to 7 l: 3% MeOH; 7 to 8.9 L: MeOH 4%; 8.9 to 9.6 L: MeOH 5%; 9.6 to 11 L: MeOH 7.5%; 11 to 12 L: 10% MeOH; 12 to 13 L: 50% MeOH; 100% MeOH), to give fractions I−XXVI, detected by TLC (EtOAc−2-PrOH−NH_4OH, 80:15:5). The purification of **3**−**5**, and hydroxysungucine has been described elsewhere.[6] Strychnogucine A (**1**) was present in fractions XVI and XVII (6570 to 8039 mL) along with strychnine and bisnordihydrotoxiferine. The three compounds were separated by MPLC on Merck LiChroprep RP_8 (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, and bisnordihydrotoxiferine, 550 to 700 mL. Strychnogucine B (**2**) was present in fractions XIX−XXII (8930−11 450 mL, 2 g) along with strychnine, isostrychnine, 18-hydroxysungucine, 18-hydroxyisosungucine (**5**), and other unidentified alkaloids. These fractions were purified by high-speed counter-current chromatography in a multilayer-coil separator−extractor fitted with 2.6-mm i.d. coiled tubing and hexane−EtOAc−EtOH−H_2O (1:3:1:4) as solvent. The lower aqueous phase was used as stationary phase, and the upper EtOAc phase was pumped from the tail of the column to the head. This separation gave eight fractions (1, 0−20 mL; 2, 20−50 mL; 3, 50−100 mL; 4−8, fractions of 100 mL). The fractions (2−5) containing strychnogucine B (15 mg) (**2**), hydroxysungucine, and hydroxyisosungucine (**5**) were purified by preparative TLC on Si gel in EtOAc−2-propanol−NH_4OH 25% (7:2:1) and finally on a Sephadex LH-20 (20 g, Pharmacia Biotech) column with MeOH as the mobile phase. Usambarensine was isolated in our laboratory as previously described.[5]

Strychnogucine A (1): white amorphous powder; on TLC, gave a blue fluorescence at 366 nm after spraying with cerium sulfate reagent; UV (MeOH) λ_{max} (log ε) 214 (3.22), 255 (2.98), 281 (2.64), 289 (2.58) nm; IR ν_{max} (KBr) 3435, 3042, 2929, 2859, 1666, 1596, 1482, 1461, 1395, 1327, 1287, 1149, 1108, 1052, 1028, 870, 818, 756 cm^{-1}; CD (MeOH) Δε (nm) −73.8 (210), −19.6 (218), −22.49 (222), +15.01 (243), −13.14 (266), +2.19 (273); ^1H (400 MHz) and ^{13}C (100 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 651 [MH$^+$] (30), 335 (32), 317 (100), 300 (10), 274 (80), 258 (8), 246 (9), 232 (20), 217 (13), 182 (3), 144 (4), 96 (4); HRESIMS *m/z* [MH$^+$] 651.3332 (calcd for $C_{42}H_{43}N_4O_3$, 651.3335).

Strychnogucine B (2): white amorphous powder; on TLC, gave a blue fluorescence at 366 nm after spraying with cerium sulfate reagent; UV (MeOH) λ_{max} (log ε) 222 (4.45), 252 (4.12), 283 (3.91), 292 (3.86), 304 (3.81) nm; IR ν_{max} (KBr) 3435, 2923, 2853, 1666, 1630, 1595, 1481, 1462, 1417, 1283, 1110, 1047, 757 cm^{-1}; CD (MeOH) Δε (nm) −4.78 (217), −6.8 (224), −3.26 (236), −6.52 (249), −6.2 (264), −0.18 (316) ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 667 [MH$^+$] (15), 531 (40), 359 (5), 335 (17), 317 (7), 272 (10), 264 (55), 234 (35), 220 (59), 194 (23), 184 (100), 168 (8), 156 (22), 144 (8), 122 (19); HRESIMS *m/z* [MH$^+$] 667.3295 (calcd for $C_{42}H_{43}N_4O_4$, 667.3284).

***Plasmodium falciparum* Strains.** Four *P. falciparum* strains were used in this study: the F32 chloroquine-sensitive and mefloquine-resistant strain from Tanzania, the PFB chloroquine-resistant strain from Brazil, the W2 chloroquine-resistant strain from Indochina, and the chloroquine-sensitive FCA 20 from Ghana. These strains were provided by Prof. P. Grellier (Laboratoire de Biologie Parasitaire et Chimiothérapie, Muséum d'Histoire Naturelle, Paris), Prof. J. Le Bras (Hôpital Bichat-Claude Bernard, Laboratoire de Parasitologie, Centre National de Référence de la Chimiosensibilité du Paludisme, Paris), and Prof. M. Wéry (Tropical Medicine Institute, Antwerpen, Belgium).

***In Vitro* Antiplasmodial Testing.** Continuous in vitro cultures of asexual erythrocytic stages of the four *P. falciparum* strains were maintained following the procedure of Trager and Jensen,[16] as described previously.[6] 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 4-fold dilutions (final concentrations ranging from 20 μg/mL to 0.0012 μg/mL) and was tested in duplicate. Parasite growth was estimated by the determination of [^{3}H]hypoxanthine incorporation as described by Desjardins et al.[17] and modified by Mirovsky et al.[18] The Student's *t*-test was used to evaluate the significance of differences between results obtained for different samples. Statistical significance was set at $p \leq 0.05$.

Evaluation of Cytotoxic Potential. The WI38, HeLa, and KB cell lines were cultured as described previously.[6] Compounds were tested on cells in 96-well microplates using the tetrazolium salt WST-1 (Boehringer) colorimetric assay.[6] Emetine (Sigma E2375) and usambarensine base[19] were used as reference compounds. Means ± standard errors were calculated. A Student's *t*-test was performed (statistical significance was set at $p \leq 0.007$).

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References and Notes

(1) Neuwinger, H. D. *African Ethnobotany: Poisons and Drugs, Chemistry, Pharmacology, Toxicology*; Chapman & Hall: London, 1996; p 569.
(2) Sandberg, F.; Roos, K.; Ryrberg, K. J.; Kristianson, K. *Acta Pharm. Suec.* **1969**, *6*, 103−108.
(3) Kambu, K.; Coune, C.; Angenot, L. *Planta Med.* **1980**, *37*, 161−164.
(4) Frederich, M.; Hayette, M. P.; Tits, M.; De Mol, P.; Angenot, L. *Antimicrob. Agents Chemother.* **1999**, *43*, 2328−2331.
(5) Frederich, M.; Tits, M.; Hayette, M. P.; Brandt, V.; Penelle, J.; DeMol, P.; Llabres, G.; Angenot, L. *J. Nat. Prod.* **1999**, *62*, 619−621.
(6) Frederich, M.; De Pauw-Gillet, M. C.; Llabres, G.; Tits, M.; Hayette, M. P.; Brandt, V.; Penelle, J.; De Mol, P.; Angenot, L. *Planta Med.* **2000**, *66*, 262−269.
(7) Sandberg, F.; Lunell, E.; Ryrberg, K. J. *Acta Pharm. Suec.* **1969**, *6*, 79−102.
(8) Sandberg, F.; Roos, K.; Ryrberg, K. J.; Kristiansson, K. *Tetrahedron Lett.* **1968**, *59*, 6717−6718.
(9) Galeffi, C.; Delle Monache, E. M.; Marini-Bettolo, G. B. *J. Chromatogr.* **1974**, *88*, 416−418.
(10) Lounasmaa, M.; Tolvanen, A. *Heterocycles* **1986**, *24*, 3229−3281.
(11) Verpoorte, R.; Hylands, P. J.; Bisset, N. G. *Org. Magn. Reson.* **1977**, *9*, 567−571.
(12) Klyne, W.; Buckingham, J. *Atlas of Stereochemistry: Absolute Configurations of Organic Molecules*; Chapman & Hall: London, 1974; p 151.
(13) Klyne, W.; Swan, R.; Bycroft, B.; Schmid, H. *Helv. Chim. Acta* **1969**, *49*, 833−841.
(14) Massiot, G.; Massoussa, B.; Jacquier, M. J.; Thépénier, P.; Le Men Olivier, L.; Delaude, C.; Verpoorte, R. *Phytochemistry* **1988**, *27*, 3293−3304.
(15) Verpoorte, R. *J. Pharm. Sci.* **1980**, *69*, 865−867.
(16) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673−675.
(17) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(18) Mirovsky, P.; Gay, F.; Bustos, D.; Mazier, D.; Gentilini, M. *Trans. R. Soc. Trop. Med. Hyg.* **1990**, *84*, 511−515.
(19) Bonjean, K. A.; Depauw-Gillet, M. C.; Quetin-Leclercq, J.; Angenot, L.; Bassleer, R. J. *Anticancer Res.* **1996**, *16*, 1129−1137.

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