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Strychnohexamine from *Strychnos icaja*, a naturally occurring trimeric indolomonoterpenic alkaloid

Geneviève Philippe,[a] Elise Prost,[b] Jean-Marc Nuzillard,[b] Monique Zèches-Hanrot,[b] Monique Tits,[a] Luc Angenot[a] and Michel Frédérich[a,*]

[a]*University of Liège, Natural and Synthetic Drug Research Center, Laboratory of Pharmacognosy, B36, Av. de l'Hôpital 1, 4000 Liège, Belgique*

[b]*Laboratoire de Pharmacognosie, UMR 6013, CPCBAI, BP 1039, 51097 Reims, France*

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Abstract—A reinvestigation of *Strychnos icaja* roots has resulted in the isolation, from the EtOAC extract, of one tertiary trisindole alkaloid, named strychnohexamine. Its structure has been investigated by means of spectroscopic data interpretation (UV, IR, HRESIMS, 1D and 2D NMR). This is the first time that a natural trimeric indolomonoterpenic alkaloid has been isolated directly from a plant species. This compound is one of the largest alkaloids ever discovered.

Strychnos icaja Baill. (Loganiaceae) is a medium sized liana which is found in various vegetation zones of central Africa (rainforest, secondary forest, swamp and gallery forests). *S. icaja* is mainly used as an ordeal poison, but is also occasionally used in traditional medicine, notably for the treatment of chronic and persistent malaria.[1,2] Malaria is the major parasitic infection in many tropical countries, leading to approximately 1.1 million deaths each year. In the continuation of our search for new antimalarial agents, we described the isolation of six dimeric asymmetric alkaloids belonging to the strychnan group.[3,4] These alkaloids possess an atypical 5′-23 linkage between the two parts of the substance. This type of linkage is totally original and at this time exclusively described for these alkaloids from *S. icaja*. This kind of liaison could be reproduced in series and could allow the formation of 'polymeric' alkaloids. In this paper, we describe the isolation and the structural determination of strychnohexamine, a trimeric monoterpenoid indole alkaloid.

An EtOAc extract of *S. icaja* roots was subjected to sequential partitions by MPLC and preparative TLC. These fractionations led to the isolation of various alkaloids among which was strychnohexamine (**1**).[5]

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. The roots of *S. icaja* (500 g) were extracted as previously described[3,4] to give 28 g of alkaloidic extract. The pH 8 extract was fractionated first by medium pressure liquid chromatography (MPLC) on 180 g Merck LichroPrep Si 60 (40–63 μm, Merck 9336) with CH_2Cl_2/MeOH mixtures, to give fractions I–XXVI, detected by TLC (EtOAC/2-PrOH/NH_4OH, 80:15:5). Strychnohexamine (**1**) was present in weak amounts in fractions XVIII to XXII. The purification of **1** (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.

The UV and CD spectra of **1** were very similar to those of bisnordihydrotoxiferine **2**.[6] The only difference in the CD spectra is a maximum at 240 nm, as in sungucine **3**.[3] Furthermore, the compound showed, after being spread with $Ce(SO_4)_2/H_2SO_4$ on a TLC plate, a fleeting violet coloration, as for bisnordihydrotoxiferine **2**. The FT-IR spectrum indicated the presence of a carbonyl group (1664 cm^{-1}). The molecular formula, $C_{59}H_{60}N_6O_1$, was established by means of HRESIMS. It proved, for the first time, the existence of an indolomonoterpenic alkaloid containing six nitrogen atoms. NMR spectral data of **1** are listed in Table 1. In

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* Corresponding author. Tel.: +32-4-366-4338; fax: +32-4-366-4332; e-mail: m.frederich@ulg.ac.be

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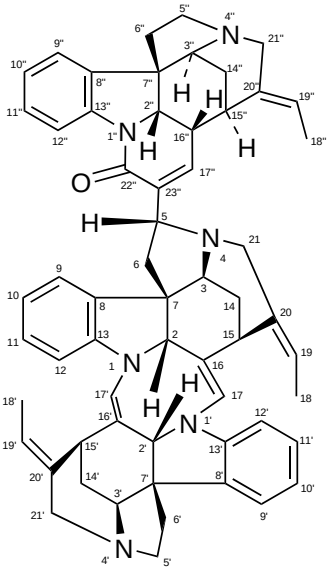
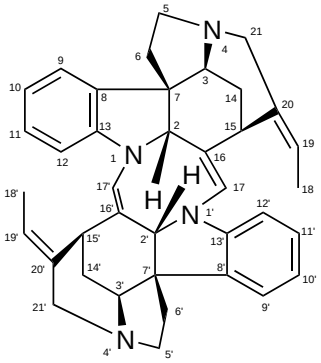
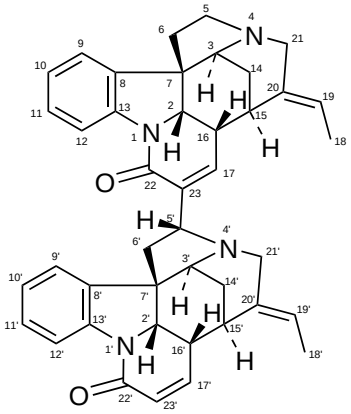


Strychnohexamine **1**



Bisnordihydrotoxiferine **2**



Sungucine **3**

the aromatic region, the COSY and HSQC spectra showed twelve aromatic protons (represented by three four-spin systems as expected from three indole moieties) and six methine protons (three of whom arise from three ethylidene side chains). In fact, the ^{1}H and ^{13}C chemical shifts of a first portion of the alkaloid were very close to those of bisnordihydrotoxiferine **2**.[6] The remaining signals were completely similar to those of the upper isostrychnine II part of **3**.[3] The linkage C-23″–C-5 between the two moieties was confirmed by correlations in the HMBC spectrum (C-5–H-17″), in the TOCSY spectrum (H-3″-14″-15″-16″ (-2″)-17″-5-6ab) and in the ROESY spectrum (H-17″-H5; H-17″-H3;…). All the ^{13}C chemical shifts were interpolated from the HMQC and HSQC spectra. Unfortunately, we obtained no correlations for C-23″ and we could not measure its chemical shift, expected to be close to 135 ppm (as in sungucine).

The stereochemistry was then considered. The configurations 15″*R*,3″*S*,7″*R*,2″*S*; 15*S*,3*S*,7*R*,2*S*; 15′*S*,3′*S*,7′*R*,2′*S* (H-2β, H-3α, H-15α) were those commonly accepted from biogenetic considerations.[7,8] Comparison of the chemical shifts of C-3″, C-6″, C-7″, C-14″ and C-16″ with the values for the same carbons in retuline and isoretuline[9] allowed the strychnan part of **1** to be placed in the retuline series with a H-16β (16*S*) functionality. This configuration was confirmed by the observation of the coupling constant between H-16″ and H-2″ (6.6 Hz, periplanar) and by the presence of a H-2″–H-16″ coupling in the ROESY spectrum. The H-5β (5*R*) configuration was attributed by comparison of the H-6b multiplicities and the C-5, C-6, H-5 and H-6 chemical shifts with values for sungucine. Furthermore, this stereochemistry is corroborated by the presence, in the ROESY spectrum, of correlations between H-3 and H-17″, H-5 and H-17″ and H-6b and H-17″, as observed in the dreiding model. The orientations of the ethylidene side chains are proposed to be *E*, because of the presence of correlations in the ROESY spectrum between, respectively, H-18″ and H-17″, H-18″ and H-15″, H-18 and H-17, H-18 and H-15, H-18′ and H-17′, H-18′ and H-15′, H-19″ and H-21″a, H-19 and H-21a, H-19′ and H-21′b.

This alkaloid **1** was then tested for antiplasmodial activity against *Plasmodium falciparum*, and was found to be moderately active, slightly more than bisnordihydrotoxiferine **2** (IC_{50} in the micromolar range).

In conclusion, strychnohexamine represents to our knowledge the only naturally occurring (directly isolated from a higher plant) trimeric indolomonoterpenic alkaloid described to date. A trimeric derivative of tabersonine has been previously isolated in traces (less than 1 mg), among with the dimers voafrine A and voafrine B, after incubation of tabersonine with an enzyme mixture obtained from leaves of mature *Catharantus roseus* plants.[10] The structure 3-hydroxy-14′-(3α″-tabersonyl)voafrine B has been proposed from only MS and UV data for this compound.[10]

The presence of strychnohexamine in *Strychnos icaja* opens the way to find higher 'polymeric' alkaloids possessing several C-5–C-23 linkages. The biosynthetic pathway leading to this kind of alkaloid from the monomers present in the plant (strychnine, isostrychnine I and II) must probably implicate radical chemistry and a specific enzyme, which seems to be highly active in *S. icaja*, since seven alkaloids possessing this linkage have been identified in this plant[3,4] *Strychnos nux-vomica*, the other *Strychnos* synthesizing strychnine and isostrychnine do not seem to possess this pathway, which would then be specific to *S. icaja*.

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (100 and 125 MHz) data of strychnohexamine (recorded in $CDCl_3$). Chemical shifts (δ) in ppm from TMS. Multiplicities and coupling constants in Hz are in parentheses

No	^{1}H	COSY H/H correlations	^{13}C[a]	HMBC[b] C/H correlations
2/**2′**	5.25 (s)/5.23 (s)		70.8/72.0	3, 17, 17′, 6b/17, 17′, 6′a
3/**3′**	3.61/ND[c]	14b	63.6/65.4	2, 6b/
5/**5′a**	4.03/3.01	6a, 6b/5′b, 6′a, 6′b,	63.5/54.1	21a, 17″/
5′b	3.28	5′a, 6′a		
6a/6′a	2.28/2.28	6b, 5/6′b, 5′a, 5′b	48.8/42.3	/2
6b/**6′b**	2.65 (dd, 6.1, 12.9)/2.51	6a, 5/6′a, 5′a,		
7/**7′**	–		55.3/54.2	2, 6b, 9/2′, 9′
8/**8′**	–		134.2/134.3	10, 12/10′, 12′
9/**9′**	7.09/7.15	10/10′	128.2/128.1	11/11′, 6′b
10/**10′**	6.77/6.81	9, 11/9′, 11′	118.8/119.2	12/12′
11/**11′**	7.11/7.14	10, 12/10′, 12′	122.7/122.5	9/9′
12/**12′**	6.45/6.41	11/11′	107.6/107.0	10/10′
13/**13′**	–		146.8/146.7	11, 17′/11′, 17
14a/**14′a**	1.66/1.74	14b, 15/14′b, 15′	23.6/24.3	
14b/**14′b**	1.88/1.92	14a, 15, 3/14′a, 15′		
15/**15′**	3.70/3.71	14ab/14′ab	29.9/30.5	17, 19/17′
16/**16′**	–		119.6/117.9	17/17′
17/**17′**	6.44 (s)/6.31 (s)		130.5/130.1	19/19′
18/**18′**	1.81 (d, 5.2)/1.85 (d, 6.9)	19, 21a, 21b/19′	13.9/13.0	19/19′
19/**19′**	5.29 (m)/5.48 (q, 6.5)	18/18′	116.9/119.6	18/
20/**20′**	–		140.2/141.8	18/18′
21a/**21a′**	3.13/3.25	21b, 18/21′b	51.3	
21b/**21b′**	3.70/3.35	21a, 18/21′a	52.6	
2″	4.30 (d, 6.9)	16″	64.6	6″a, 6″b, 17″
3″	3.32	14″a	65.3	2″, 5″
5a″	3.09	5″b, 6″a, 6″b	53.6	6″b, 21″a
5b″	3.28	5″a, 6″a, 6″b		
6a″	2.16	6″b, 5″a, 5″b	37.0	2″
6b″	2.59 (dt, 13.2, 7.8)	6″a, 5″, 5″b		
7″	–		52.3	2″, 9″, 6″ab
8″	–		135.8	6″b, 10″, 12″, 6″b
9″	7.28	10″	122.4	11″
10″	7.11	9″, 11″	124.3	9″, 12″
11″	7.24	12″, 10″	128.2	9″
12″	8.22 (d, 7.9)	11″	116.4	10″
13″	–		141.6	9″, 11″, 12″
14a″	1.82	14″b, 15″, 3″	23.1	
14b″	1.93	14″a, 15″		
15″	2.74	14″a, 14″b	31.4	2″, 21″a, 14″b, 19″
16″	2.70	2″, 17″	37.8	17″
17″	7.01 (d, 6.7)	16″	137.0	
18″	1.73 (d, 6.8)	19″, 21″b	13.1	19″
19″	5.40 (m)	18″	119.6	18″
20″	–		140.4	16″, 18″, 21″a
21a″	3.33		52.4	19″, 5″ab
21b″	3.70	18″		
22″	–		162.3	17″
23″	–		ND[c]	

[a] These values are extrapolated from the HSQC and HMBC spectra.
[b] Correlations from C to the indicated hydrogens.
[c] ND = Not determined.

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5. **1**: White-yellowish 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, $C_{59}H_{60}N_6O_1$ requires 868.4828; FT-IR (KBr) cm^{-1}: 3429, 2926, 1664 (C=O), 1597 (C=C), 1486, 1418, 1383, 1261, 1095, 801, 754, 617; UV (MeOH) λ_{max} nm (log ε): 210 (3.55), 293 (3.25), 320 (2.99); CD (MeOH) $\Delta\varepsilon$ (nm): +2 (240), −16.6 (274), +16.6 (294), −33.3 (318).
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