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# Chrysopentamine, an Antiplasmodial Anhydronium Base from *Strychnos usambarensis* Leaves

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## Abstract

A new derivative of strychnopentamine was isolated from the leaves of *Strychnos usambarensis*. This compound, named chrysopentamine, was identified by detailed spectroscopic methods (UV, IR, HR-ESI-MS, 1D and 2D NMR). Chrysopentamine presented an original hydroxy substitution on C-14 and an aromatization of the ring D of strychnopentamine leading to anhydronium base properties and exhibited strong antiplasmodial properties (IC<sub>50</sub> less than 1  $\mu$ M).

*Strychnos usambarensis* Gilg (Loganiaceae) is a tree which has been used traditionally in the preparation of a curarizing arrow poison in Rwanda [1]. In addition to curarizing quaternary alkaloids, tertiary alkaloids showing antiplasmodial and cytotoxic activities were also found in the leaves and root bark [2], [3], [4], [5] and, recently, we have showed that isostrychnopentamine (**2**) induces apoptosis in colon cancer cells by an original p53-independent pathway [6]. In the course of the development of an effective procedure for the extraction of strychnopentamine (**1**) and isostrychnopentamine (**2**) from the leaves of *S. usambarensis*, we isolated a new anhydronium base showing potent antiplasmodial properties. This new compound was named chrysopentamine (**3**), based on the Greek word "chrysos" for gold or orange-yellow and on the analogy with strychnopentamine (five nitrogen atoms) (Fig. 1).

The UV spectrum of **3** suggested a highly conjugated  $\beta$ -carboline chromophore. A bathochromic shift was observed in alkaline solutions, suggesting an anhydronium base moiety or/and a

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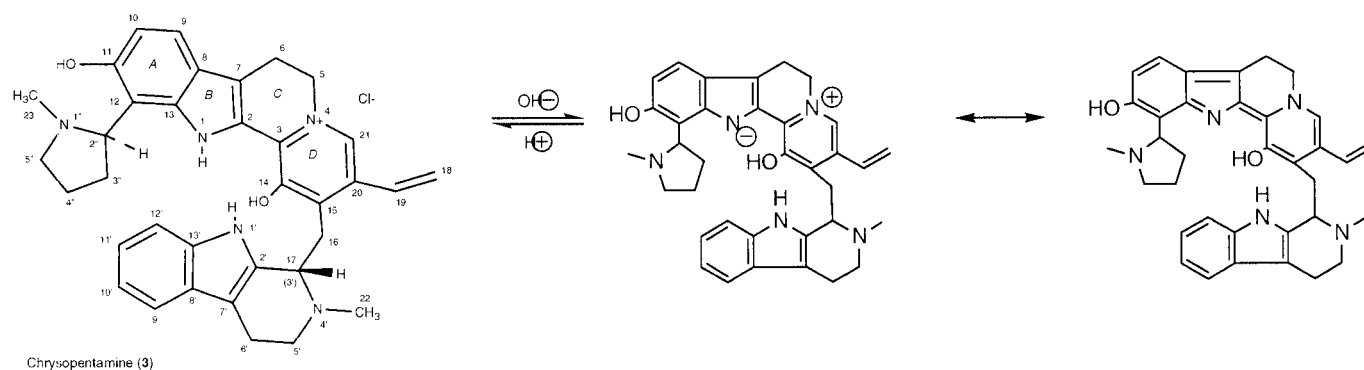
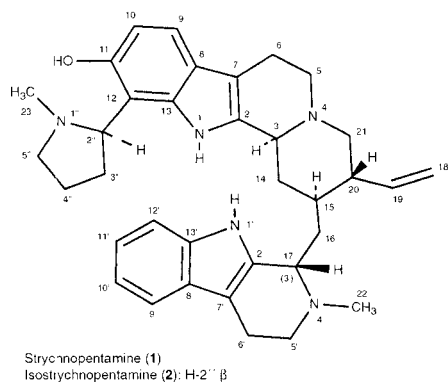


Fig. 1 Chemical structures of strychnopentamine (1), isostrychnopentamine (2), and chrysopentamine (3).

phenolic group. Despite the presence of a quaternary nitrogen in the corynanium part of the alkaloid, **3** was easily extractable by organic solvents under alkaline conditions (pH 8–9), indicating the presence of the zwitterionic form of an anhydronium base, as in dihydroflavopereirine (Fig. 1) [7]. The FT-IR spectrum showed NH and/or OH vibrations, vinyl vibrations, *ortho*-disubstituted and *ortho*-tetrasubstituted benzene vibrations, but no CO vibrations were detectable. Based on HR-ESI-MS,  $C_{35}H_{38}N_5O_2$  was determined as the molecular formula, with an  $[M + H]^+$  at  $m/z = 560.3044$ . This was indicative of the presence of five nitrogen atoms, one more than what was expected for a bisindole alkaloid.

The NMR spectral data of **3** are listed in Table 1. The broad band decoupled  $^{13}C$ -NMR spectrum of **3** showed 35 carbon signals which were sorted by HSQC, HMBC and APT as two  $CH_3$ , nine  $CH_2$ , ten CH and 14 quaternary carbons. In the aromatic region, the COSY, TOCSY and HSQC spectra showed seven aromatic protons (represented by one four-spin system, one two-spin system and a singlet at  $\delta = 7.77$ ) and three protons from a vinylic side chain. The  $^1H$ - and  $^{13}C$ -NMR spectra of **3** were essentially similar to the spectra of **1** and **2** [8]. The principal differences with the **1** and **2** spectra were, in the aliphatic part of the  $^1H$  spectrum of **3**, the absence of H-3, H-14ab, H-15, H-20, H-21 and the deshielding of H-5, H-6, H-16 and H-17 and, in the aromatic part of the spectra, the appearance of a singlet at  $\delta = 7.77$ . This signal was correlated in the HMBC spectrum to C-5, C-20, C-19, C-3, C-2, C-15 and C-14, in the ROESY spectrum to H-5 and H-18, in the COSY spectrum to H-5 and H-19 and then attributed to H-21 (Table 1). The deshielded  $^1H$  and  $^{13}C$  shifts of CH-21 were consistent with

those of strychnochrysine or afrocurarine, possessing a pyridinium ring D in their corynane moiety [9], [10]. The aromatization of ring D of strychnopentamine could explain the major other modifications compared to the spectra of **1** and **2**. The HMBC spectrum allowed the assignment of the quaternary carbons of the pyridinium ring. The C-20 is correlated to H-21, H-18 and H-16b, while the C-15 is correlated to H-21 and H-16ab, C-3 is coupled to H-5 and H-21 and finally the C-14 is attributed to the quaternary signal at  $\delta = 160.9$ , which is correlated to H-16b and weakly with H-16a and H-21 (Table 1). This much deshielded value of C-14 could be explained by the presence of a supplementary hydroxy substituent at this position. The presence of a supplementary hydroxy substituent was expected from the HR-ESI-MS which has shown the presence of two oxygen atoms. This phenol at C-14 could also participate in the bathochromic shifts observed in the UV spectrum of **3** in alkaline conditions. The phenol at C-11 did not participate in the bathochromic shift, as it has been demonstrated that **1** and **2** possessed a cryptophenolic function and presented therefore no bathochromic shift [8]. Compound **3** exhibited a stable brown-greenish coloration after detection with Fast Blue B reagent (phenol reagent) on TLC plates, while **1** and **2** presented only a transient purple coloration (consequence of the cryptophenolic function), which suggested the presence of a supplementary phenolic function for **3**. This phenolic function could not be placed on the two indole moieties, because H-9, H-10, H-9', H-10', H-11' and H-12' were clearly identified by comparison with **1** and **2**. The only possibility left was then the C-14 pyridinium position. To confirm the presence of two hydroxy substitutions,  $^1H$ -NMR spectra of **1**, **2** and **3** were recorded in  $CDCl_3$ . The spectra

Table 1 <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data of chrysopentamine (**3**) (recorded at 500/125 MHz in MeOD)

Position	<sup>1</sup> H <sup>a</sup>	COSY H/H correlations	TOCSY H/H correlations	ROESY H/H correlations	<sup>13</sup> C <sup>b</sup>	HMBC <sup>c</sup> C-H correlations
2					126.69 (q)	6, 21
3					133.78 (q)	5, 21
5	4.67 (t, 7.4)	21, 6	6	6, 21	57.14 (CH <sub>2</sub> )	21, 6
6	3.28 (m)	5	5	5, 9	24.03 (CH <sub>2</sub> )	5
7					114.79 (q)	5, 6, 9, 10
8					120.28 (q)	10, 9, 6
9	7.39 (d, 8.6)	10	10	6, 10	120.02 (CH)	–
10	6.72 (d, 8.6)	9	9	9	113.62 (CH)	–
11					155.92 (q)	2'', 10, 9
12					108.40 (q)	2'', 10, 9
13					138.74 (q)	2'', 9, 10
14					162.46 (q)	16ab, 21
15					139.47 (q)	21, 16ab
16a	3.46 (m)	16b, 17	16b, 17	16b, 17	32.04 (CH <sub>2</sub> )	–
16b	3.63 (m)	16a, 17	16a, 17	16a, 17		
17 (3')	4.63 (m)	16ab	16ab	22, 16a, 16b	61.73 (CH)	16b, 5'b, 22, 5'a
18a	5.45 (d, 17.2)	18b, 19	18b, 19	18b	120.17 (CH <sub>2</sub> )	–
18b	5.66 (d, 10.9)	18a, 19	18a, 19	18a, 21, 19		
19	6.95 (dd, 11, 18)	21, 18ab	18ab	19b,	132.42 (CH)	21, 18b
20					135.38 (q)	21, 18ab, 16b
21	7.77 (s)	19, 5	–	5, 18b	127.28 (CH)	5
N4' Me (22)	2.95 (s)	–	–	17, 5'ab	41.39 (CH <sub>3</sub> )	5'b
2'					131.34 (q)	6a
5'a	3.32 (m)	5'b, 6'ab	5'b, 6'ab	5'b, 6', 22	49.19 (CH <sub>2</sub> )	22, 6
5'b	3.74 (m)	5'a, 6'ab	5'a, 6'ab	5'a, 22		
6'a	2.95	5'ab	5'ab	5'ab	18.62 (CH <sub>2</sub> )	5'a
6'b	3.02 (m)	5'ab	5'ab			
7'					107.66 (q)	5'ab, 6', 9'
8'					127.41 (q)	9', 10', 12'
9'	7.44 (d, 7.9)	10', 11'	10', 11', 12'	10'	119.06 (CH)	11', 10', 12'
10'	7.02 (t, 7.3)	9', 11', 12'	9', 11', 12'	11', 9'	120.28 (CH)	12'
11'	7.11 (t, 7.3)	10', 12', 9'	9', 10', 12'	10', 12'	123.07 (CH)	9', 12', 10'
12'	7.30 (d, 8.1)	11', 10'	9', 10', 11'	11'	112.16 (CH)	10', 9', 11'
13'					138.28 (q)	9', 11'
N1'' Me (23)	2.46 (s)	–	–	5''ab, 2''	40.73 (CH <sub>3</sub> )	2'', 5''a
2''	4.10 (t, 8.8)	3''ab	5''ab, 4'', 3''ab	3''b, 5''a, 23, 11'	66.67 (CH)	5''b, 23
3''a	1.96 (m)	3''b, 2'', 4''	3''b, 4'', 5''ab, 2''	3''b	32.87 (CH <sub>2</sub> )	2'', 5''b,
3''b	2.51 (m)	3''a, 2'', 4''	3''a, 4'', 5''ab, 2''	3''a, 4''		
4''a	2.10 (m)	5''ab, 3''ab	3''ab, 2'', 5''ab	5''ab, 3''b	24.03 (CH <sub>2</sub> )	5''b, 5''a, 3''b
4''b	2.11 (m)					
5''a	2.61 (m)	5''b, 4''	5''b, 4'', 3''ab, 2''	5''b, 4'', 23	57.14 (CH <sub>2</sub> )	23, 3''b,
5''b	3.49 (m)	5''a, 4''	5''a, 4'', 3''ab, 2''	5''a, 4'', 23		

<sup>a</sup> Chemical shifts (δ) in ppm from TMS. Multiplicities and coupling constants in Hz are in parentheses.<sup>b</sup> Carbon multiplicities in parentheses were deduced from APT spectrum.<sup>c</sup> Correlations from C to the indicated hydrogens.

of **1** and **2** showed two extra NH peaks and one extra phenol peak at δ = 11.80, while the spectrum of **3** exhibited two additional NH peaks and two additional phenol peaks at δ = 10.60 (OH-14) and δ = 11.44 (OH-11). An OH substitution on C-14 is not very frequent for a corynane type alkaloid, but a 14-hydroxyrauniticine has been previously described [11]. Finally, the linkage C-15/C-16/C-17 between the two portions of the molecule was confirmed by correlations in the HMBC spectrum (C-15/H-16b; C-14/H-16b; C-20/H-16b; C-17/H-16b; C-17/H-5ab) and in the COSY spectrum. The identity of the pyrrolidine ring was confirmed by correlations in the TOCSY spectrum (H-2''-H-3''-H-4''-H-5'').

The stereochemistry of **3** has still to be considered. The H-17β (C-17S) configuration was attributed by comparison of the CD spectra of **3** with those of **1** and **2** [2] and usambarine [12]. All these alkaloids gave a positive Cotton effect near 280 nm, which is indicative of a C-17S configuration [13]. This stereochemistry is corroborated by the presence, in the ROESY spectrum of the same correlations for H-17 for **1**, **2**, and **3** (Table 1). The H-2''α (C-2''R) was proposed after comparison of the ROESY correlations of **3** with those of **1** (H-2''α) and **2** (H-2''β). Compound **3** presented an H-2''-H-11' correlation as strychnopentamine (**1**), while isostrychnopentamine (**2**) exhibited an H-23-H-

**Table 2** *In vitro* antiparasmodial activity of chrysopentamine (3), isostrychnopentamine (1), strychnopentamine (2), quinine, and chloroquine against three *Plasmodium falciparum* cell lines

Compound	FCA 20/Ghana (chloroquine-sensitive line)			FCB1-R/Colombia (moderately chloroquine-resistant line)			W2/Indochina (Laos) (chloroquine-resistant line)		
	IC <sub>50</sub> nM ± SD <sup>a</sup>	IC <sub>90</sub> nM	n <sup>b</sup>	IC <sub>50</sub> nM ± SD <sup>a</sup>	IC <sub>90</sub> nM	n <sup>b</sup>	IC <sub>50</sub> nM ± SD <sup>a</sup>	IC <sub>90</sub> nM	n <sup>b</sup>
Strychnopentamine (1)	117 ± 33	443	4	N.D. <sup>c</sup>	N.D. <sup>c</sup>		145 ± 20	2982	4
Isostrychnopentamine (2)	120 ± 42	450	2	104 ± 36	386	3	152 ± 9	628	2
Chrysopentamine (3)	579 ± 376	1918	2	550 ± 149	1980	6	507 ± 227	1774	2
quinine	269 ± 6	1910	3	200 ± 33	2740	4	413 ± 11	1720	3
chloroquine	11 ± 5	71	6	32 ± 19	84	3	284 ± 17	1750	5

<sup>a</sup> Values are expressed as mean ± standard deviation. All tests were realized in duplicate.

<sup>b</sup> n = number of experiments.

<sup>c</sup> N.D. = not determined.

11' correlation, but no H-2''-H-11' correlation. These differences in ROESY couplings were consistent with strychnopentamine (1) and isostrychnopentamine (2) by molecular modeling [8], [14].

The *in vitro* antiparasmodial activity of chrysopentamine (3) was determined against three *Plasmodium falciparum* cell lines in comparison to chloroquine, quinine, strychnopentamine (1) and isostrychnopentamine (2) (see Table 2). Chrysopentamine presented an IC<sub>50</sub> around 500 nM against all tested *Plasmodium* lines. Compound 3 possessed a comparable activity against all chloroquine-resistant and -sensitive lines of *P. falciparum*, which is indicative of the absence of cross-resistance with chloroquine. Compared with other indolic anhydronium bases or quaternary alkaloids, such as tetradehydrolongicaudatine Y or guianensine [5], compound 3 exhibits the strongest antiparasmodial activity of this class of compounds.

Chrysopentamine (3), which exhibits strong antiparasmodial properties, is a novel indolomonoterpenoid alkaloid, which has five nitrogen atoms, an unusual phenolic substitution on C-14, and a pyridinium ring which puts the alkaloid in the relatively small family of indolic anhydronium bases.

## Materials and Methods

**General experimental procedures:** UV, IR, CD and NMR spectrometers and procedures have been described previously [15] (NMR spectra were measured with a Bruker Avance 500 MHz spectrometer). The HR-ESI-MS were recorded on a Q-TOF III micromass spectrometer (Waters-Micromass, Manchester, UK). All solvents and current chemicals used were of analytical grade (Merck chemicals, VWR international).

**Plant material:** The leaves of *Strychnos usambarensis* Gilg were collected by one of the authors (L.A.) in Akagera National Park, Rwanda and identified by Dr. A. J. M. Leeuwenberg. Voucher specimens of the plant were deposited in the herbarium of the National Botanical Garden of Belgium at Meise and in the herbarium of the Pharmaceutical Institute, at Liège (N° 1397).

**Extraction and isolation:** The leaves of *S. usambarensis* (767 g) were percolated with 20 L of EtOAc (pH 8) until complete extraction of

alkaloids. The extract was dissolved in 1% HOAc, washed with CH<sub>2</sub>Cl<sub>2</sub> and then repeatedly extracted with CH<sub>2</sub>Cl<sub>2</sub> at pH 8. The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were concentrated to give 6.55 g crude alkaloid extracts. Three g of extract were then fractionated by MPLC on 180 g of Merck Lichrospher 60 RP Select B (12 μm) with a gradient of acetonitrile in sodium heptanesulfonate (1 g in 420 mL, fitted to pH 2 with H<sub>3</sub>PO<sub>4</sub>) (0 to 800 mL: 10% acetonitrile; 800 mL to 1.5 L: 15% acetonitrile; 1.3 to 1.8 L: 20% acetonitrile; 1.8 to 5 L: 25% acetonitrile; 5 L to end: 30% acetonitrile), to give 80 mg of strychnophylline (200–400 mL), 30 mg of strychnofoline (500–600 mL), 20 mg of chrysopentamine (3) (2900–3000 mL), 1 g of strychnopentamine (1) (3200–3400 mL), 300 mg of 11-hydroxy-usambarine (3700–4400 mL) and 600 mg of isostrychnopentamine (2) (4600–4800 mL). The fractions were precipitated by Mayer's reagent and each precipitate was dissolved in MeOH-Me<sub>2</sub>CO-H<sub>2</sub>O (6:2:1) and the alkaloids converted to the chloride by passage through an Amberlite® IRA-420 column (VWR international). The fractions were detected by TLC (EtOAc/2-PrOH/NH<sub>4</sub>OH, 90:8:2). Compound 1 was finally purified on a Sephadex® LH20 (Amersham bioscience, Roosendaal, The Netherlands) column with methanol as mobile phase.

**Chrysopentamine (3):** Orange amorphous powder; coloration yellow, orange and red in solution, respectively at pH 3, 9 and 12; UV (MeOH + CH<sub>3</sub>COOH 1%): λ<sub>max</sub> (log ε) = 231 (3.37), 290 (2.92), 320 (2.67), 457 (3.12); UV (MeOH): λ<sub>max</sub> (log ε) = 231 (3.37), 290 (2.92), 325 (2.67), 469 (3.12); UV (MeOH + NH<sub>4</sub>OH 1%): λ<sub>max</sub> (log ε) = 231 (3.37), 290 (2.92), 325 (2.67), 469 (3.12); UV (MeOH + KOH 0.1 M 1%): λ<sub>max</sub> (log ε) = 231 (3.37), 292 (2.92), 360 (2.67), 510 (3.12); IR (KBr): ν<sub>max</sub> = 3391, 2925, 2852, 1724 (w), 1622, 1589, 1554, 1450, 1308, 1255, 1232, 1157, 1119, 1039, 922, 804, 743, 599 cm<sup>-1</sup>; CD (MeOH): (Δε) 218 (−6.25), 230 (+3.13), 244 (−0.52), 274 (+1.06), 293 (−0.25); <sup>1</sup>H- (400 MHz) and <sup>13</sup>C- (100 MHz) NMR data are given in Table 1; ESI-MS: m/z = 560 [MH<sup>+</sup>] (90), 519 (10), 376 (100), 185 (10); HR-ESI-MS: m/z [MH<sup>+</sup>] = 560.3044 (calcd. for C<sub>35</sub>H<sub>38</sub>N<sub>5</sub>O<sub>2</sub>: 560.3026).

**Plasmodium falciparum cell lines and assays:** Three *Plasmodium falciparum* cell lines were used in this study. These strains, the culture conditions and assay procedures were described previously [15], [16], [17], [18], [19]. Chloroquine diphosphate (Sigma C6628), and quinine base (Aldrich 14590–4) were used as antimalarial references.

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