

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 *S. usambarensis*. This compound, named chrysopentamine, was identified by detailed spectroscopic methods (UV, IR, HRESIMS, 1D and 2D NMR). Chrysopentamine presented an original hydroxyl substitution on C-14 and an aromatization of the ring D of strychnopentamine leading to anhydronium base properties and exhibited strong antiplasmodial properties (IC₅₀ inferior to 1 μM).

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 [2-5] 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 β -carbolinium chromophore. A bathochromic shift was observed in alkaline solutions, suggesting an anhydronium base moiety or/and a phenolic group. Despite the presence of a quaternary nitrogen in the corynanium part of the alkaloid, **3** was easily extractible 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 HRESI-MS, $C_{35}H_{38}N_5O_2$ was determined as the molecular formula, with a $[M+H]^+$ 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.

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 DEPT 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 δ 7.77) and three protons from a vinylic side chain. The 1H and ^{13}C 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 δ 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 strychnochrysin 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 δ 160.9, which is correlated to H-16b and weakly with H-16a and H-21 (Table **1** and Fig. **3**). This much deshielded value of C-14 could be explained by the presence of a hydroxyl substituent at this position. The presence of a supplementary hydroxyl substituent was expected from the HRESI-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 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 hydroxyl substitutions, $^1\text{H-NMR}$ spectra of **1**, **2** and **3** were recorded in CDCl_3 . The spectrum 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-hydroxy-rauniticine 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-5'ab) (Fig. **3**) 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-17 β (C-17*S*) 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-17*S* 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 H-2'' - H-11' correlation as strychnopentamine (**1**), while isostrychnopentamine (**2**) exhibited an H-23 - H-11' correlation, but no H-2'' - H-11' correlation. These differences in ROESY couplings were consistent with strychnopentamine (**1**) and isostrychnopentamine (**2**) molecular modeling [8, 14].

The *in vitro* antiplasmodial 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 IC₅₀ 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 tetrahydrolongicaudatine Y or guianensine [5], compound **3** exhibits the strongest antiplasmodial activity of this class of compounds.

Chrysopentamine (**3**), which exhibits strong antiplasmodial properties, is a novel indolomonoterpenoid alkaloid, which has five nitrogen atoms, an unusual phenolic substitution on C-14, and a pyridinium ring which put 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 HRESI-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 CH₂Cl₂ and then repeatedly extracted with CH₂Cl₂ at pH 8. The combined CH₂Cl₂ 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 heptane sulfonate (1g in 420 ml, fitted to pH 2 with H₃PO₄)

(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), 1g of strychnopentamine (**1**) (3200-3400 ml), 300 mg of 11-hydroxyusambarine (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-Me₂CO-H₂O (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/NH₄OH, 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+CH₃COOH 1%) λ_{\max} (log ϵ) = 231 (3.37), 290 (2.92), 320 (2.67), 457 (3.12); UV (MeOH) λ_{\max} (log ϵ) = 231 (3.37), 290 (2.92), 325 (2.67), 469 (3.12); UV (MeOH+NH₄OH 1%) λ_{\max} (log ϵ) = 231 (3.37), 290 (2.92), 325 (2.67), 469 (3.12); UV (MeOH+KOH 0.1 M 1%) λ_{\max} (log ϵ) = 231 (3.37), 292 (2.92), 360 (2.67), 510 (3.12); IR ν_{\max} (KBr) 3391, 2925, 2852, 1724 (w), 1622, 1589, 1554, 1450, 1308, 1255, 1232, 1157, 1119, 1039, 922, 804, 743, 599 cm⁻¹; CD (MeOH) nm ($\Delta\epsilon$) 218 (-6.25), 230 (+3.13), 244 (-0.52), 274 (+1.06), 293 (-0.25); ¹H (400 MHz) and ¹³C (100 MHz) NMR data are given in Table 1; ESIMS m/z 560 [MH⁺] (90), 519 (10), 376 (100), 185 (10); HRESIMS m/z [MH⁺] 560.3044 (calcd for C₃₅H₃₈N₅O₂, 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 [15-19]. Chloroquine diphosphate (Sigma C6628), and quinine base (Aldrich 14590-4) were used as antimalarial references.

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

Position	¹ H ^a	COSY H/H correlations	TOCSY H/H correlations	ROESY H/H correlations	¹³ C ^b	HMBC ^c 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 ₂)	21, 6
6	3.28 (m)	5	5	5, 9	24.03 (CH ₂)	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 ₂)	-
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 ₂)	-
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 ₃)	5'b
2'					131.34 (q)	6'a
5'a	3.32 (m)	5'b, 6'ab	5'b, 6'ab	5'b, 6', 22	49.19 (CH ₂)	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 ₂)	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 ₃)	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 ₂)	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 ₂)	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 ₂)	23, 3''b,
5''b	3.49 (m)	5''a, 4''	5''a, 4'', 3''ab, 2''	5''a, 4'', 23		

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

Table 2

In vitro antiplasmodial 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 ₅₀ nM ± SD ^a	IC ₉₀ nM	n ^b	IC ₅₀ nM ± SD ^a	IC ₉₀ nM	n ^b	IC ₅₀ nM ± SD ^a	IC ₉₀ nM	n ^b
Strychnopentamine (1)	117 ± 33	443	4	N.D. ^c	N.D. ^c		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

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

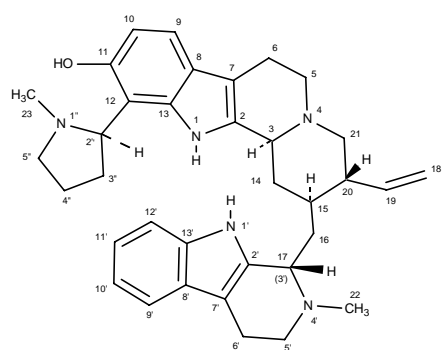
b. "n" = number of experiments.

c. N.D. = not determinated.

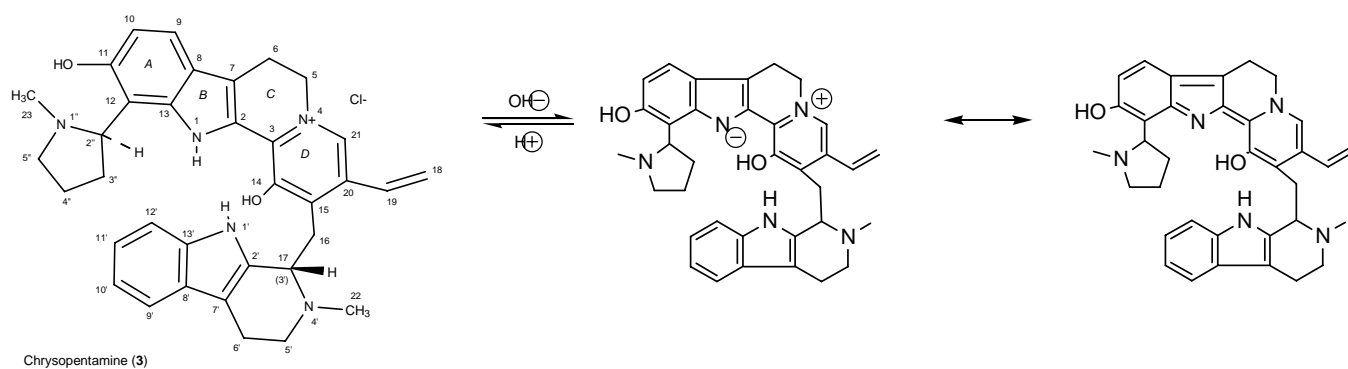
Figure legend:

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

Fig. 1.



Strychnopentamine (1)
Isostrychnopentamine (2): H-2'' β



Chrysopentamine (3)