



3',4',5',6' TETRADEHYDROLONGICAUDATINE Y, AN ANHYDRONIUM BASE FROM *STRYCHNOS USAMBARENSIS**

MICHEL FRÉDÉRICH,† JOELLE QUÉTIN-LECLERCQ,‡ ROSE GADI BIALA, VIVIANE BRANDT, JACQUES PENELLE, MONIQUE TITS and LUC ANGENOT

Université de Liège, Institut de Pharmacie, Service de Pharmacognosie, CHU, Tour 4, Avenue de L'Hôpital 1, B4000 Liège, Belgique

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Key Word Index—*Strychnos usambarensis*; Loganiaceae; stem bark; 2D-NMR; bis-indole alkaloid; anhydronium base.

Abstract—A new bisindole alkaloid, tetrahydrolongicaudatine Y, has been isolated from the stem bark of *Strychnos usambarensis* collected from the Ivory Coast. Elucidation of its structure is based mainly on spectroscopic studies and places it among the relatively small collection of zwitterionic indoloquinolizine alkaloids. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of our investigations on the alkaloids of *Strychnos* species, we further analysed the stem bark content of *S. usambarensis* collected from the Ivory Coast (liana form) [1]. The arborescent form of this species, whose roots are the main ingredient of an African arrow poison, contains several indole alkaloids [2, 3], among which are strychnopentamine and usambarensine-derivatives. These alkaloids also possess interesting anticancer and antiprotozoal properties [3–5]. The present paper describes the isolation and the structural elucidation of a new alkaloid, named 3',4',5',6' tetrahydrolongicaudatine Y (1).

RESULTS AND DISCUSSION

Tetrahydrolongicaudatine Y (1) is present not only in organic fractions containing tertiary alkaloids but also in the precipitate observed during extractions at the interface between organic and aqueous layers. It gives a blue colouration with cerium sulphate or iron chloride reagents, as do bis-indole compounds of the longicaudatine or toxiferine types [6]. The asymmetrical bis-indole structure was confirmed by the FAB mass spectrum which showed a $[M]^+$ at m/z

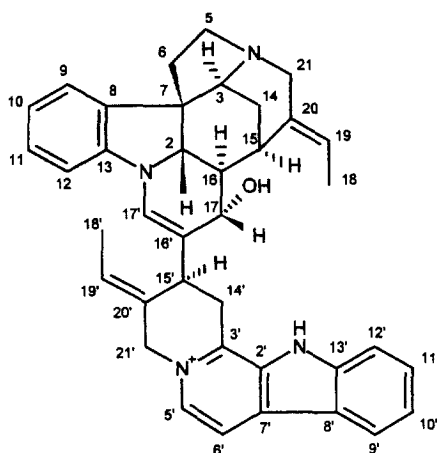
567, corresponding with the elemental composition $C_{38}H_{39}N_4O$. Besides the indole peaks at m/z 130 and 144, fragmentations were observed at m/z 551 $[M-16]^+$ and 550 $[M-17]^+$, indicative of an hydroxyl group, m/z 247, 233, 219, 206 and 115, suggesting the presence of a corynanium-type part [7], and m/z 122, consistent with an ethylidenic piperidine ring arising from a strychnan part [8]. The UV spectra, showing maxima at 204, 255, 308 and 367 nm in acidic or neutral solutions, suggested a β -carbolinium chromophore. A bathochromic shift, corresponding to the zwitterionic form (pH-dependent) anhydronium base [9], was observed in alkaline solutions, as in melinonine F, for example [7]. The IR spectrum showed NH and/or OH vibrations at 3405 cm^{-1} , but no CO absorptions were detectable.

The ^1H NMR spectrum showed the presence in the aromatic region, of two ethylidene side-chains (δ 5.7 q and 6.05 q , one proton each, δ 1.78 d and 1.61, d three protons each), two coupled pyridinic protons (δ 8.46 d and 8.39 d , $J = 6.5\text{ Hz}$), eight benzenic protons, corresponding to two unsubstituted indole nuclei, and a one-proton singlet at δ 6.05, in accordance with the enamincic H-17' [6] shift of a longicaudatine-like skeleton. In the aliphatic region, beside the two methyl doublets of the ethylidene side-chains at δ 1.78 and 1.61, we noted the presence of two deshielded interacting doublets at δ 5.30 and 5.24, which were assigned as H-21'A and H-21'B, because they were connected, in the COSY spectrum, with the protons of one of the ethylidene side-chains (δ 6.05 and 1.61, assigned as H-19' and H₃-18', respectively). All these protons are also connected (COSY spectrum) with a one-proton peak at δ 4.10, assigned as H-15', which was related

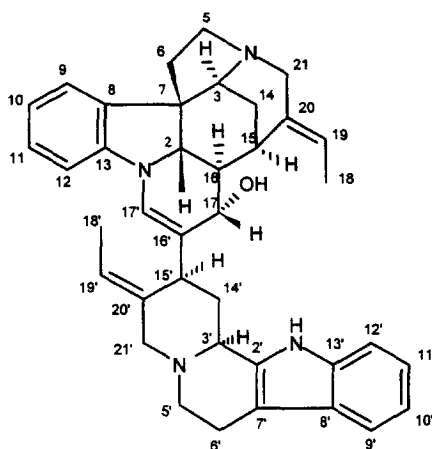
* This paper is dedicated to the memory of one of us (R.G.B) who died in a car accident on June 24th 1997.

† Author to whom correspondence should be addressed.

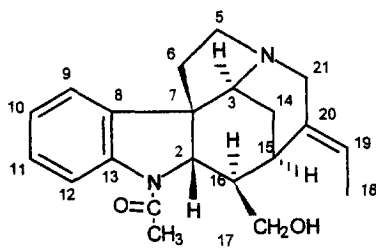
‡ Present address: Laboratoire de Pharmacognosie, Université Catholique de Louvain, Unité CHAM, UCL 7230, avenue E. Mounier, 72, B-1200 Bruxelles, Belgique.



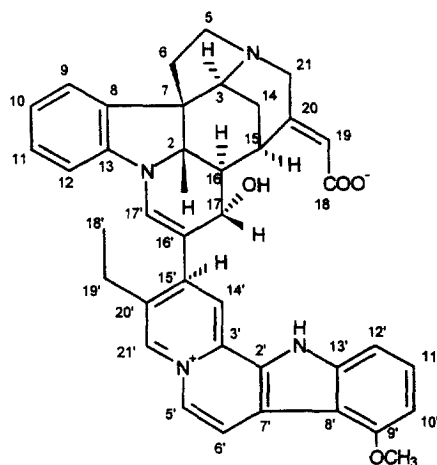
(1) 3',4',5',6', tetrahydrolongicaudatine Y.



(2) Longicaudatine Y



(3) Isoretuline



(4) Guianensine

to the two protons of a methylene group at δ 3.67 and 3.84, assigned as H-14' A and B. This H-15'-proton is also weakly connected with a one proton doublet at δ 4.02, a chemical shift in agreement with a linkage with an oxygen atom, assigned as H-17. Starting from this proton, the connectivities provide the means of assembling the multispin substructure related to the hydrogens bonded to C₁₇-C₁₆-(C₂)-C₁₅-C₁₄-C₃ of a strychnan-type skeleton. Another non-interacting spin system was observed in the COSY spectrum, constituting a CH₂-CH₂ unit associated with the protons at δ 1.60, 1.78, 2.60 and 3.12. This fragment probably arises from the tryptamine part of this moiety; the chemical shifts are in agreement with the fragment, N-

C(5)H₂-C(6)H₂-. The protons of the other ethylenic side-chain (δ 1.78 and 5.7, assigned as H₃-18 and H-19, respectively) were connected with the doublets located at δ 2.92 and 3.6 (H-21 A and B).

The ¹³C NMR spectra (total decoupling and DEPT) showed the presence, in the aliphatic region, of two methyl groups (ethylenic side-chains δ 13.5 and 15.0), six methylenes, six methines and one quaternary carbon atom, whose chemical shift was indicative of a C-7 from a strychnan skeleton (δ 51.3). In the aromatic region, we observed the presence of 13 methines and ten quaternary carbon atoms. Quaternary carbon atoms were identified by comparison between total decoupling and DEPT spectra.

All the chemical shifts of the protons and carbons are in agreement with the structure (**1**), as compared with the values described for alkaloids possessing similar sub-structures [6, 8, 10–13]. Nevertheless, we did not find any reference compounds to compare with the chemical shifts of the two H-21'. These deshielded values could be explained by the proximity of the quaternary ammonium and that of the ethylidene side-chain. The deshielded chemical shifts of the two H-14' are in agreement with the values attributed to H-14 of melinonine E [13].

The structure proposed explains the instability of the molecule, which easily loses two protons (probably H-15' and one H-14') and rearranges into a fully aromatized compound. This hypothesis is corroborated by the formation of an orange-coloured compound (ES/MS: m/z 565) after staying a few minutes on TLC.

The stereochemistry still has to be considered. The similarity between the chemical shifts of C-3, C-7 and C-15 in strychnine [6] and tetrahydrolongicaudatine Y, suggest the same configuration for these carbons in the two alkaloids. The proposed relative configurations of C-15 and C-15' are those commonly accepted from the biogenetical hypothesis: 15 α and 15' α [14, 15]. Examination of molecular stereomodels shows that the 2 β -, 7 β - and 3 α -configurations are strictly dependent on the configuration 15 α . In addition, the proposed stereochemistry is in agreement with the positive Cotton effect in the 250 nm region of the CD spectrum [15]. The large coupling constant observed between H-16 and H-2 (11.6 Hz) and the chemical shifts of C-6, C-14, C-16 and C-21 are consistent with the configuration 16 α , as in isoretuline (**3**) [10], but opposite of that proposed, on the basis of only ^1H NMR data, for afrocurarine [12]. The value of $^3J_{16-17}$ (10.3 Hz) could indicate an antiperiplanar configuration, so H-17 must be β , as in guianensine (**4**) [11]. The orientations of the side-chains are proposed to be *E*, because of the absence of connection in the NOESY spectrum between, respectively, H₃-18 and H-21A and H-21B, and H₃-18' and H₂-21'.

Treatment of (**1**) with NaBH₄ in MeOH for one hour at room temperature gave longicaudatine Y (**2**) [16]. The identity of the resulting compound was confirmed by the UV spectrum, as well as by diode-array LC and TLC comparisons with an authentic sample.

Tetrahydrolongicaudatine Y could be in an intermediate biosynthetic state between longicaudatine-type and guianensine-type products.

EXPERIMENTAL

Plant material

Bark of *S. usambarensis* Gilg. was collected from the Ivory Coast by Prof. F. Sandberg (Uppsala) and identified by Dr A. J. M. Leeuwenberg. Reference specimens (voucher No. 7916) have been deposited at Wageningen (The Netherlands).

Extraction and isolation

Powdered bark was macerated during 24 hr with MeOH-HOAc (99:1) and percolated with the same solvent. After concn of the extract under red. pres. and filtration, the soln was washed with CHCl₃. During this operation, a ppt. containing (**1**) was obtained. Na₂CO₃ was then added to pH 8 and the aq. soln extracted with CHCl₃. The remaining aq. soln was then acidified to pH 4 and quaternary alkaloids ppted by a satd soln of picric acid. (**1**) was also present in both CHCl₃ frs (in minor quantities) and in the picrate fr. It was purified from the ppt. obtained after extraction with CHCl₃. This ppt. was dispersed in H₂O-HOAc (99:1) and filtered. The aq. soln was basified to

Table 1. ^1H and ^{13}C NMR data of compound **1** (CD₃OD)

H	δ	Correlations*	C	δ
2	2.81 (<i>d</i> : 11.6 Hz)	16	2	71.6
3	3.74 (<i>br. s</i>)	14AB, 15	3	62.9
5A	3.12 (<i>m</i>)	5B, 6AB	5	53.8
5B	2.60 (<i>m</i>)	5A, 6AB	6	41.2
6A	1.78 (<i>m</i>)	5AB, 6B	7	51.3
6B	1.60 (<i>m</i>)	5AB, 6A	8	132.3
9	7.03 (<i>d</i> : 7.3 Hz)	10, 11	9	123.8
10	6.68 (<i>t</i>)	9, 11, 12	10	121.4
11	6.99 (<i>t</i>)	10, 12	11	129.9
12	6.45 (<i>d</i> : 7.9 Hz)	10, 11	12	109.8
14A	2.00 (<i>dt</i>)	3, 14B, 15	13	147.4
14B	1.65 (<i>m</i>)	3, 14A, 15	14	29.2
15	3.12 (<i>m</i>)	14AB, 16, 19	15	27.5
16	1.67 (<i>m</i>)	2, 15, 17	16	48.9
17	4.02 (<i>d</i> : 10.3 Hz)	16	17	69.2
18	1.78 (<i>d</i>)	19, 21AB	18	13.5
19	5.70 (<i>q</i> : 6.7 Hz)	15, 18, 21B	19	126.6
21A	3.60 (<i>br. d</i>)	18, 21B	20	133.6 [†]
21B	2.92 (<i>br. d</i>)	18, 19, 21A	21	57.9
5'	8.46 (<i>d</i> : 6.5 Hz)	6'	2'	142.4
6'	8.39 (<i>d</i> : 6.5 Hz)	5'	3'	145.3
9'	8.28 (<i>d</i> : 7.9 Hz)	11', 10'	5'	133.1
10'	7.38 (<i>t</i>)	9', 11', 12'	6'	116.8
11'	7.70 (<i>d</i>)	10', 12', 9'	7'	131.7 [†]
12'	7.70 (<i>d</i>)	10', 11'	8'	123.7
14'A	3.84 (<i>m</i>)	14'B, 15'	9'	124.2
14'B	3.67 (<i>m</i>)	14'A, 15'	10'	123.2
15'	4.13 (<i>br. s</i>)	17, 17', 18', 19', 21' AB, 14'AB	11'	133.1
17'	6.08 (<i>s</i>)	15'	12'	114.0
18'	1.61 (<i>d</i> : 6.8 Hz)	15', 19', 21'AB	13'	135.9
19'	6.05 (<i>q</i> : 6.8 Hz)	15', 18', 21'A-B	14'	30.6
21'A	5.3 (<i>d</i> : 14.5 Hz)	15', 18'	15'	35.5
21'B	5.24 (<i>d</i> : 14.5 Hz)	15', 18'	16'	121.5
			17'	125.8
			18'	15.0
			19'	128.5
			20'	131.8 [†]
			21'	61.9

* Observed by means of 2D-COSY spectrum.

[†] These values may be interchanged.

pH8 (Na_2CO_3) and extracted with CHCl_3 . The organic layer was evapd and the residue fractionated firstly by High Speed Counter Current Chromatography (HSCCC) in a multilayer-coil separator-extractor fitted with 2.6 mm i.d. coiled tubing and CHCl_3 -MeOH- H_2O (9:15:9) as solvent. The lower CHCl_3 phase was used as stationary phase and the upper aq. phase was pumped from the tail of the column to the head. Frs containing (1) were then purified on a Fractogel TSK HW 40S column with EtOH and finally by prep. TLC on silica gel (2 mm) in EtOAc-*iso*PrOH- NH_4OH 4.25% (12:25:13).

Tetradehydroolongicaudatine Y

White powder when freshly isolated. Easily oxidizable to orange product. Blue colour with cerium sulphate and iron (III) chloride reagents on TLC. UV (MeOH) λ_{max} nm (log ϵ): 204 (4.56), 255 (4.27), 308 (4.15), 367 (3.5); (MeONa) λ_{max} nm (log ϵ): 212 (4.89), 283 (4.43), 318 (3.93); 422 (3.32). CD (MeOH) $\Delta\epsilon$ nm: $\Delta\epsilon_{200}$: -19.8; $\Delta\epsilon_{256}$: +5.67; $\Delta\epsilon_{273}$: -1.47; $\Delta\epsilon_{301}$: +4.2; $\Delta\epsilon_{312}$: -0.68; $\Delta\epsilon_{322}$: +1.6; $\Delta\epsilon_{338}$: -1.13, $\Delta\epsilon_{376}$: +0.28. FAB-MS m/z (rel. int.): 567 (100), 551 (2.4), 550 (1.9), 549 (3), 385 (7.5), 333 (5), 325 (5.3), 301 (6), 284 (7.7), 273 (14), 271 (15.2), 257 (15.3), 247 (85.6), 233 (39.2), 219 (31.4), 206 (25.6), 195 (17), 193 (16.8), 182 (60.2), 168 (24.6), 154 (21), 144 (35.3), 130 (25), 122 (17), 115 (29), 108 (13). IR (KBr) ν_{max} cm^{-1} : 3405, 2924, 1640, 1482, 1460, 1336, 1250, 1045, 758, 579. ^1H and ^{13}C NMR in Table 1. The NMR spectra were recorded on a Bruker 400 MHz spectrometer. Tetradehydroolongicaudatine Y must be stored in a cool place, protected from light and oxygen.

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