Strychnochrysine, a New Bisindole Alkaloid from the Roots of *Strychnos nux-vomica*[1]

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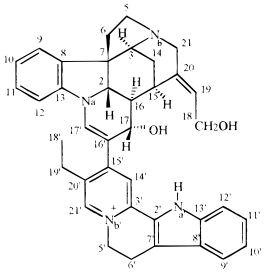
The reinvestigation of *Strychnos nux-vomica* resulted in the isolation of a colored monoquaternary bisindole alkaloid from the roots. The structure of this new orange substance, strychnochrysine **(1)**, was defined by detailed spectroscopic methods.

Strychnos nux-vomica L. (Loganiaceae), the most extensively studied *Strychnos* species because of its former importance in pharmacy, is a tree of up to 25 m in height, distributed in an area stretching from India and Sri Lanka to Indo-China. Almost all parts of *S. nux-vomica* seem to have been used for one medical purpose or another.[2,3] The root-bark contains numerous monomeric alkaloids in which strychnine and brucine are the main bases.[4−6] During the chemical screening of Asian *Strychnos* material, tertiary alkaloid extracts from the root-bark of *S. nux-vomica* were observed to include several minor components, which, on TLC, immediately colored blue when sprayed with ferric chloride−perchloric acid reagent.[4] One of these blue-coloring bases was later isolated and appeared to be identical with longicaudatine, a bisindole alkaloid initially found in *S. longicaudata*.[7] Moreover, the presence of yellow orange polar bases giving the same blue color with ferric chloride−perchloric acid reagent has been reported in root-bark of *S. nux-vomica*.[8] The UV spectrum of one of these bases (designated KBQ14)[8] was very similar to that of afrocurarine, a quaternary bisindole alkaloid isolated from *S. usambarensis*.[9,10] TLC comparison with an authentic material showed that they were not identical.[8] The structure of the compound KBQ14 has not yet been elucidated.

In a continuation of our search for potential active compounds from the *Strychnos* genus, we studied the alkaloidal extracts of an Indian sample of root of *S. nux-vomica*, which resulted in the isolation of several yellow and orange alkaloids. Among these minor components is an alkaloid that we named strychnochrysine, after the Greek word "chrysos" for gold or orange-yellow. In this paper, we report the isolation and structure elucidation of this new natural product (**1**).

Strychnochrysine (**1**) is an asymmetrical bisindole alkaloid that gave a blue color with the ferric chloride−perchloric acid reagent. Despite the presence of a quaternary nitrogen (corynanium part), **1** can be extracted by applying a continuous cyclic extraction process with methylene chloride. This pecularity is probably due to the presence of the tertiary amine function of the strychnan part counterbalancing the influence of the very polar quaternary corynanium portion of the molecule.



1 Strychnochrysine

The orange color of strychnochrysine can be explained by its UV spectrum showing maxima at 210, 252, 275, 311, and 428 nm. This highly conjugated chromophore is similar to that of afrocurarine and is not modified in alkali (differing from an anhydronium base). Its molecular weight, 583, corresponds to the elemental composition $C_{38}H_{39}N_4O_2$. The presence of a corynanium afrocurarine-like moiety as suggested by the UV spectrum is confirmed by mass fragments at *m/z* 115, 154, 167, 204, 219, 232, and 247.[10] However, the structure of **1** is mainly deduced from the analysis of $^1H-^1H$ COSY, HMQC, and HMBC spectral data (Table 1). In the aromatic region, two deshielded pyridinic protons at δ 8.58 and 7.83 due to H-14′ and H-21′ were observed, and therefore, the shifts of the C-14′ and the C-21′ can be established by HMQC at δ 122.0 and 140.8, respectively. In this aromatic region, the COSY spectrum showed that the eight other aromatic protons are found in two groups of four protons each, represented by two four-spin systems expected from two indole moieties. The protons at δ 7.70 (d), 7.46 (d), 7.32 (t), and 7.09 (t) belong to the dihydroflavopereirine (corynanium) skeleton, and those at δ 7.24 (d), 7.23 (t), 6.99 (t), and 6.77 (d) belong to the strychnan ring. The protonated aromatic carbons were assigned by their direct correlations observed in the HMQC spectrum. The HMBC spectrum confirms these attributions and allows the assignments of the quaternary carbons. The H-11′ correlated to C-13′, C-9′ (δ 140.1 and 119.4), while the

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Table 1. ^{1}H- and ^{13}C-NMR Data of Strychnochrysine (recorded in 400 MHz in $CDCl_3$)

position	^{1}H[a]	COSY H/H correlations	HMBC[b] H/C correlations	^{13}C[c]
2	3.87 (d, 11.3)	16	C-17, C-13	71.2
3	3.70	14a,14b	C-2	62.2
5	(a) 3.25	5b, 6a, 6b		53.7
	(b) 2.90	5a, 6a		
6	(a) 2.60	6b, 5a, 5b		42.8
	(b) 1.95	6a, 5a, 5b		
7				50.8
8				134.8
9	7.24 (d)	10	C-13, C-11	123.3
10	6.99 (t)	9, 11	C-12, C-8	122.0
11	7.23 (t)	10, 12	C-9, C-13	128.7
12	6.77 (d)	11	C-10, C-8	108.8
13				144.3
14	(a) 2.30 (13.7)	14b, 3, 15		29.6
	(b) 1.80 (13.7)	14a, 3, 15		
15	3.24	14a, 14b		27.1
16	2.10 (11.3, 10.2)	2, 17		49.8
17	5.15 (d, 10.2)	16, 17′		68.0
18	(a) 4.25 (12.6, 6.4)	18b, 19		56.5
	(b) 4.10 (12.6, 6.4)	18a, 19		
19	6.14 (t, 6.4)	18a, 18b		128.5
20				137.6
21	(a) 3.75 (16.0)	21b	C-3, C-19	57.3
	(b) 3.25 (16.0)	21a		
2′				138.6
3′				141.0
5′	4.55	6′		55.3
6′	3.30	5′	C-7′, C-5′	22.7
7′				115.5
8′				124.8
9′	7.46 (d)	10′	C-11′,C-13′,C-7′	119.4
10′	7.09 (t)	9′, 11′	C-12′, C-8′	120.8
11′	7.32 (t)	10′, 12′	C-9′, C-13′	126.0
12′	7.70 (d)	11′	C-8′, C-10′	114.0
13′				140.1
14′	8.58 (s)	21′		122.0
15′				156.2
16′				119.0
17′	7.20 (s)	17	C-17, C-2	133.7
			C-16′, C-15′	
18′	1.40 (t)	19′a, 19′b	C-19′, C20′	14.1
19′	(a) 2.90	19′b	C-18′	24.5
	(b) 2.80	19′a	C-18′	
20′				135.5
21′	7.83 (s)	14′, 19′, 5′	C-5′, C-15′, C-3′	140.8

[a] Chemical shift (δ) in ppm from TMS, multiplicities, and coupling constants in Hz are in parentheses, overlapped signals *J* unresolved. [b] Correlations from H to the indicated carbons. [c] Chemical shift (δ) in ppm from TMS.

H-9′ is correlated to C-13′, C-11′, and C-7′ (δ 140.1, 126.0, and 115.5); the H-10′ is shown coupled to C-8′, C-12′ (δ 124.8, 114.0), as the H-12′ correlated also to C-8′ and C-10′ (δ 124.8 and 120.8). Similar correlations were observed for the indoline ring: the H-11, H-9, and H-2 correlated to C-13 (δ 144.3), while the H-10 and H-12 correlated to C-8 (δ 134.8).

Examination of NMR spectra reveals, for the corynanium part, three methylene groups at δ 55.3, 22.7, and 24.5, which were assigned to C-5′, C-6′, and C-19′, respectively. In the COSY spectrum, a strong correlation between H_2-5′ and H_2-6′ and a long-range correlation between H_2-5′ and H-21′ were observed. Similarly, connectivities were observed in the HMBC spectrum between H-21′ and C-5′ (δ 55.3), as well as with two quaternary carbons: C-15′ and C-3′ (δ 156.2, 141.0). The H_2-6′ methylene protons showed correlations with C-7′ and C-5′ (δ 115.5, 55.3). The third methylene unit H_2-19′ was clearly coupled to the methyl protons H_3-18′ (δ 1.40). The HMBC spectrum showed H_3-18′ correlated to C-19′ and to C-20′ (δ 24.5, 135.5).

The other half of the structure was also deduced from NMR data. The chemical shifts of the protons and the carbons (Table 1) support the structure of a strychnan-type skeleton with four methylene groups (C-5, C-6, C-14, and C-21), a hydroxyethylidene unit (C-20, C-19, C-18), and five methine groups (C-2, C-3, C-15, C-16, and C-17). The chemical shifts of C-19 and C-18 (δ 128.5 and 56.5) were similar to those of 18-hydroxy-isoretuline and 18-hydroxydesacetylisoretuline.[11] The assignments of H-17 at δ 5.15 and C-17 at δ 68.0 were consistent with a 17-hydroxy group. The presence of two hydroxyl functions was in agreement with the mass spectrum (base peak at *m*/*z* 182 as in the spectrum of the 18-hydroxy-desacetylisoretuline or Wieland−Gumlich alcohol[12]) and with NMR data (e,g., one proton triplet H-19 at δ 6.14). In support of this, a diacetate was formed on acetylation, the structure of which was confirmed by mass spectral analysis.

The linkage between the two moeities is deduced from the presence of a lowfield singlet at δ 7.20 (H-17′), which correlated not only to C-16′ and C-15′ (δ 119.0, 156.2) but also to C-2 and C-17 (δ 71.2, 68.0). The deshielding of the isolated enaminic H-17′ can be explained by the presence of a highly conjugated chromophore similar to that of afrocurarine.[10] As compared to the spectrum of longicaudatine,[7] the C-17 signal is upfield, a result of configurational changes due to the opening of the seven-membered ring and similar to that of protostrychnine.[13] The signal for C-16, which is two bonds away from the OH group, is (as would be expected) shifted downfield. The C-15 signal showed an upfield shift in agreement with the γ-effect.[14]

The stereochemistry still must be considered. The proposed relative configurations of C-2, C-7, C-3, and C-15 are those commonly accepted from a biogenetic hypothesis: H-2β (2*S*), 7β(7*R*), H-3α (3*S*), H-15α (15*R*).[15] The establishment of the configuration of C-16 of **1** is critical, because the influence of the tetrahydropyridinic linkage ring is difficult to determine. Nevertheless, in 1988, Massiot and colleagues established a ^{13}C-NMR database for the assignments of the spectra of the retuline and isoretuline series of alkaloids, which differ in the orientation of the hydroxymethyl group at C-16 and in the piperidinic ring conformation (boat in desacetylretuline, chair in desacetylisoretuline).[16,17] According to the values of chemical shifts of C-2, C-6, C-14, C-16, and C-21, it was deduced that strychnochrysine belonged to the isoretuline series. In support of this assumption, the coupling constant between H-2 and H-16 (J = 11.3 Hz) is in good agreement with a H-16α (16*R*) configuration, which was also encountered in longicaudatine[7] and guianensine.[18] The configuration of C-17 was finally deduced from the large coupling constant between H-17 and H-16 (J = 10.2 Hz), which indicated an antiperiplanar position of both protons. This H-17β (17*R*) configuration is similar to that of guianensine[18] and longicaudatine Y.[19]

Experimental Section

General Experimental Procedures. UV and visible spectra were recorded on a Kontron Uvikon 922 spectrophotometer, the IR spectrum was recorded as a

KBr pellet on a Perkin−Elmer 1750 FTIR spectrometer. ESIMS and EIMS were obtained, respectively, with a VG Plateform Micromass single quadrupole (30 eV) and with a VG Micromass 707OF (70 eV) apparatus. NMR spectral analyses were carried out with a Bruker DRX 400 Avance spectrometer at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C), at room temperature. The chemical shifts are recorded in δ (ppm) based either on δ TMS = 0 or δ residual $CHCl_3$ 7.25 and $CDCl_3$ 77 ppm, and the coupling constants (J) are in Hertz. The ^{13}C-NMR spectral assignments have been established partly through a comparison of the chemical shifts with the published data for similar compounds and partly through the appearance of signals in HMBC and HMQC spectra. CD spectrum was measured with a Jobin Yvon CD6 dichrograph. Analytical TLC were performed in precoated Si gel F_{254}, Art. 1.05735 (E. Merck) 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) 0.2 M ferric chloride in 35% perchloric acid; (b) 1% ceric sulfate in 10% sulfuric acid; (c) Dragendorff's reagent.

Si gel 60 PF (254 + 366) Art. 1.07747 (Merck) was used for purification of alkaloids in preparative TLC (1.25 mm thick, 20 × 40 cm Si gel plates). All solvents used were analytical grade (E. Merck).

Plant Material. *Strychnos nux-vomica* L. was collected in September 1982, at Midnapur, West Bengal, India, and identified by the late Prof. N. G. Bisset, King's College, University of London. A voucher sample (NGB 23289) has been deposited in the Pharmaceutical Institute, University of Liège, Belgium. The root-bark was not separated from the root wood prior to extraction.

Extraction and Isolation. The ground roots of *S. nux-vomica* L. (430 g) were macerated for 48 h with MeOH−HOAc (99:1) and then percolated with the same solvent mixture. The percolate was concentrated under reduced pressure below 60 °C, the resulting acidic solution was filtered, basified to pH8 with Na_2CO_3, and repeatedly extracted with $CHCl_3$ to remove the alkaloids. The yellow organic layers were pooled, dried over Na_2SO_4, and concentrated to give a crude alkaloid extract (13 g). Preparative TLC was carried out using two consecutive solvent systems: (a) toluene−Me_2CO−EtOH−NH_4OH (25%) (45:45:7:3) and (b) MeOH-0.2 M NH_4NO_3 (3:2). The first system (65 plates) was useful for separating known tertiary alkaloids (brucine, strychnine and derivatives, longicaudatine) from strychnochrysine and other polar compounds, which remain on the start line and give, after elution with $CHCl_3$, a residue amounting to 1.2 g. The second system (10 plates) was used for separating strychnochrysine from a mixture of several other colored compounds to be separated in the future. The plates were scanned under UV light. On each plate, the main top zone, which gives an orange fluorescence under 366 nm, was outlined, scraped off, and eluted with $CHCl_3$ to yield 65 mg of orange amorphous powder of **1**.

Strychnochrysine (1): orange-colored amorphous powder; on TLC (a) gives immediately a blue color with ferric chloride−perchloric acid reagent and a blue-green color with cerium sulfate reagent; UV (MeOH) λ_{max} (log ϵ) 210 (4.25), 252 (3.80), 275 (3.76), 311 (3.88), and 428 (3.75) nm; IR (KBr) υ_{max} 3419, 2924, 2853, 1637, 1598, 1556, 1480, 1384, 1330, 1280, 1245,1098, 1048, 920, and 747 cm^{-1}; ESIMS m/z 583.5 $[M]^+$ corresponding to $C_{38}H_{39}N_4O_2$; EIMS mlz $[M]^+$ undetected; 518 (0.4), 504 (0.8), 475 (1), 463 (1.5), 455 (3), 449 (3), 439 (5), 429 (6), 415 (9), 401 (11), 298 (8), 284 (10), 274 (18), 260 (27), 247 (23), 246 (29), 232 (27), 219 (28), 204 (22), 196 (27), 182 (100), 168 (28), 167 (22), 154 (26), 144 (32), 130 (50), 115 (23), 104 (18), 91 (34); ^{1}H- and ^{13}C-NMR data, see Table 1. CD $_{MEOH}$ ($\Delta\epsilon_{nm}$) $\Delta\epsilon_{277}$ + 0.425; $\Delta\epsilon_{319}$ +2.581; $\Delta\epsilon_{386}$ +0.636; $\Delta\epsilon_{457}$ − 0.347.

Acetylation of strychnochrysine. Compound **1** (1 mg) was acetylated (Ac_2O, 0.2 mL, pyridine, 1 mL, and stored for 14 h at room temperature). Reagents were removed by evaporation under vacuum to yield the diacetylated compound with a molecular ion at m/z 667 $[M]^+$ (ESIMS).

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