

Strychnochry sine, a New Bisindole Alkaloid from the Roots of *Strychnos nux-vomica*¹

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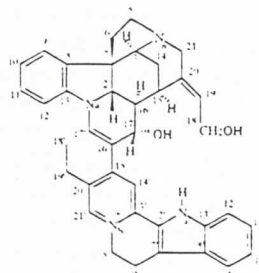
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The reinvestigation of *Strychnos nux-vomica* resulted in the isolation of a colored monoquaternary bisindole alkaloid from the roots. The structure of this new orange substance, strychnochry sine (1), was defined by detailed spectroscopic methods.

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 strychnochry sine, after the Greek word "chry sos" for gold or orange-yellow. In this paper, we report the isolation and structure elucidation of this new natural product (1).

Strychnochry sine (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 peculiarity is



1 Strychnochry sine

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.

The orange color of strychnochry sine 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 C₃₈H₃₉N₄O₂. 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. ^1H - and ^{13}C -NMR Data of Strychnochrysin (recorded in 400 MHz in CDCl_3)

position	$^1\text{H}^a$	COSY H/H correlations	HMBC ^b H/C correlations	$^{13}\text{C}^c$
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 (b) 2.90	5b, 6a, 6b 5a, 6a		53.7
6	(a) 2.60 (b) 1.95	6b, 5a, 5b 6a, 5a, 5b		42.8
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) (b) 1.80 (13.7)	14b, 3, 15 14a, 3, 15		29.6
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) (b) 4.10 (12.6, 6.4)	18b, 19 18a, 19		56.5
19	6.14 (t, 6.4)	18a, 18b		128.5
20				137.6
21	(a) 3.75 (16.0) (b) 3.25 (16.0)	21b 21a	C-3, C-19	57.3
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 C-16', C-15'	133.7
18'	1.40 (t)	19'a, 19'b	C-19', C-20'	14.1
19'	(a) 2.90 (b) 2.80	19'b 19'a	C-18' C-18'	24.5
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 H₂-5' and H₂-6' and a long-range correlation between H₂-5' 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 H₂-6' methylene protons showed correlations with C-7' and C-5' (δ 115.5, 55.3). The third methylene unit H₂-19' was clearly coupled to the methyl protons H₃-18' (δ

1.40). The HMBC spectrum showed H₃-18' 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-hydroxyisoretuline and 18-hydroxydesacetylisoeretuline.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-desacetylisoeretuline or Wieland-Gumlich alcohol¹²) 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 moieties 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-2 β (2*S*), 7 β (7*R*), H-3 α (3*S*), H-15 α (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 isoeretuline 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 desacetylisoeretuline).^{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 strychnochrysin belonged to the isoeretuline 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-16 α (16*R*) configuration, which was also encountered in longicaudatine⁷ 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-17 β (17*R*) configuration is similar to that of guianensine¹⁸ 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 Platform Micromass single quadrupole (30 eV) and with a VG Micromass 7070F (70 eV) apparatus. NMR spectral analyses were carried out with a Bruker DRX 400 Avance spectrometer at 400.13 MHz (^1H) 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 pre-coated Si gel F₂₅₄, 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 pH 8 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- $\text{Me}_2\text{CO-EtOH-NH}_4\text{OH}$ (25%) (45:45:7:3) and (b) $\text{MeOH-0.2 M NH}_4\text{NO}_3$ (3:2). The first system (65 plates) was useful for separating known tertiary alkaloids (brucine, strychnine and derivatives, longicaudatine) from strychnochrysin 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 strychnochrysin 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.

Strychnochrysin (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) ν_{max} 3419, 2924, 2853, 1637, 1598, 1556, 1480, 1384, 1330, 1280, 1245, 1098, 1048, 920, and 747 cm^{-1} ; ESIMS m/z 583.5 $[\text{M}]^+$ corresponding to $\text{C}_{38}\text{H}_{39}\text{N}_4\text{O}_2$; EIMS m/z $[\text{M}]^+$ 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); ^1H - and ^{13}C -NMR data, see Table 1. CD_{MeOH} ($\Delta\epsilon_{\text{nm}}$) $\Delta\epsilon_{277} + 0.425$; $\Delta\epsilon_{319} + 2.581$; $\Delta\epsilon_{386} + 0.636$; $\Delta\epsilon_{457} - 0.347$.

Acetylation of strychnochrysin. 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 $[\text{M}]^+$ (ESIMS).

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