

## Revision of the Structure of Strychnochromine

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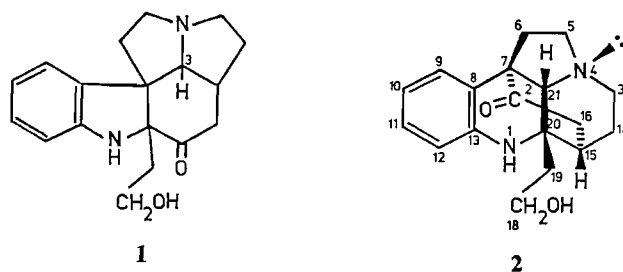
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**Key Words:** quinoline alkaloids; *Strychnos gossweileri*; Strychnaceae; 2D-NMR

**Abstract:** The structure of strychnochromine, an unusual alkaloid isolated from the root bark of *Strychnos gossweileri*, has been revised. Strychnochromine 2 possesses a new pentacyclic skeleton with a tetrahydroquinoline rather than indole or indoline moiety.

Chemical investigations of the constituents of the root bark of *Strychnos gossweileri* Exell have resulted in the isolation of a number of alkaloids among which is the new and peculiar strychnochromine<sup>1-5</sup>. From spectroscopic data (UV, IR, MS, one-dimensional <sup>1</sup>H and <sup>13</sup>C NMR spectra), structure **1**, based on an indoline moiety, was proposed for this alkaloid. Unfortunately, the lack of material and of reference compounds did not allow us to confirm this hypothetical structure and determine its stereochemistry<sup>6</sup>.

Recently, we had the opportunity to analyse a new sample of the root bark of *S. gossweileri* collected in the same area. Beside known alkaloids, we isolated a new anhydronium base<sup>7</sup> and further amounts of strychnochromine. In order to confirm its unusual structure and determine its stereochemistry, extensive 2D NMR experiments were carried out (COSY, X-H Corr, NOESY, COLOC). The COSY and X-H Corr experiments mostly confirmed the previous observations but the COLOC maps ( $J = 5, 7, 8, 10$  or  $15$  Hz) did not fit the proposed structure. In particular, the connectivities observed between C-3 and H-3 and the neighbouring H and C cannot be explained by structure **1**. Furthermore, it was not possible to find a structure based on an indoline skeleton that would agree with all the data.

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data in  $\text{CD}_3\text{OD}$ 

H	$\delta$ (ppm)	Correlations* (carbons)	C	$\delta$ (ppm)	Correlations* (protons)
3 A	2.82	3, 15	2	208.9	21, 15, 16 A-B
3 B	2.33	3, 5, 15, 14	3	44.7	3, 21
5 A	3.12	5, 21, 6	5	55.3	5 A-B, 6 B, 3 B
5 B	2.97	5, 21	6	28.3	6 A-B, 21, 5 A
6 A	3.25	6, 7	7	58.9	21, 6 A-B, 9, 16 A-B
6 B	1.94	6, 7, 5, 8	8	119.7	1, 21, 6 B, 10, 12
9	6.97	9, 7, 11, 13	9	127.0	9, 11
10	6.64	10, 8, 12	10	117.3	10, 12
11	7.04	11, 9, 13	11	128.4	11, 9
12	6.55	12, 8, 10	12	112.4	12, 10
14 A	2.12	14, 16	13	144.2	9, 11
14 B	1.64	14, 20, 15	14	27.8	14 A-B, 15, 16 A-B, 3 B
15	2.41	15, 16	15	40.8	15, 21, 14 B, 16 A-B, 3 A-B
16 A	2.93	16, 2, 7, 20, 15, 14	16	42.3	16 A-B, 15, 14 A
16 B	2.37	16, 2, 7, 20, 15, 14	18	58.7	18 A-B, 19 A-B
18 A	3.95	18, 20	19	39.0	19 A-B
18 B	3.95	18, 20	20	54.1	21, 15, 14 B, 16 A-B, 18 A-B, 19 A-B
19 A	2.29	19, 20, 18	21	67.1	21, 1, 5 A-B, 15, 19 B
19 B	2.18	19, 21, 20, 18			
21	2.88	21, 2, 7, 6, 20, 8, 15, 3			

\*Correlations observed by means of X-H CORR and COLOC experiments

The problem was solved by single-crystal X-ray analysis of the *p*-bromobenzoyl ester of strychnochromine.

The structure found, **2**, fully explains the 2D-NMR data obtained with strychnochromine (Table 1). The proposed numbering is based on the biogenetic hypothesis, but has to be proved by further studies involving, for example, the use of labelled precursors.

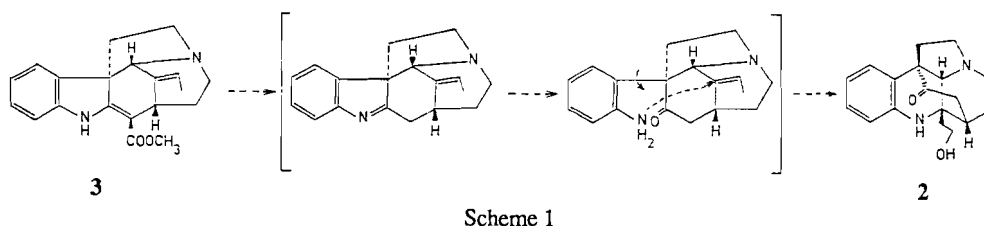
The strong absorption at 330 nm in the UV spectrum can now be explained by an interaction between two non-conjugated chromophores sterically arranged in such a way that there is an overlap of one  $\pi$  orbital of the aromatic ring with both the oxygen  $n$  orbital and the  $\pi^*$  orbital of the carbonyl group, allowing transfer of an electron from the aromatic ring to the antibonding orbital of the carbonyl group ( $\pi^*$ ). This produces intensification of the  $n \rightarrow \pi^*$  absorption, as already described<sup>8</sup>.

The positions of C-6 and H-6B [but not H-6A ] in the shielding zone of the carbonyl group explain their low  $\delta$  values in the NMR spectra and the large chemical shift difference between the two diastereotopic hydrogens H-6A and H-6B.

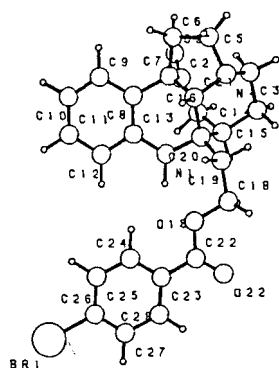
Two "W" patterns of long-range couplings are observed between H-21A and H-15 and between H-16A and H-14A. This explains the small coupling constants observed between those two pairs of protons, respectively 2.3 Hz and 1.5 Hz.

The structure of strychnochromine, with its tetrahydroquinoline ring, is very unusual in *Strychnos* species, but the formation of quinolines from indole alkaloids has already been mentioned in some other plants, for example: *Alstonia*, *Melodinus*, *Hunteria* or *Cinchona* species. Nevertheless, to our knowledge, the only other tetrahydroquinoline alkaloid in a *Strychnos* species is calebassinine, which has been found in a curare and in *S. solimoesana*<sup>9</sup>. However, the arrangement of the other rings in calebassinine is quite different. Strychnochromine appears to be the first alkaloid found in nature possessing this kind of pentacyclic skeleton.

From a biogenetic point of view, strychnochromine might derive from condylocarpine **3**, which has been found in other *Strychnos* species<sup>10-11</sup>, or from one of its derivatives. Scheme 1 represents a possible biogenetic pathway in which no C-C bond is broken. It has to be noted that alkaloids lacking C<sub>17</sub> have already been found in different *Apocynaceae*<sup>12</sup>.



The extraction and purification of strychnochromine followed the procedure recently described<sup>7</sup>. Strychnochromine was present in the lower chloroformic phase of fraction A and was purified by precipitation in hexane followed by fractionation through a medium-pressure Superformance<sup>®</sup> column filled with silica 60. The elution was carried out firstly with CHCl<sub>3</sub> and secondly with CHCl<sub>3</sub> : MeOH (9:1).



Crystal structure of strychnochromine *p*-bromobenzoate (Pluto view)

The *p*-bromobenzoyl ester was prepared as follows: 66 mg strychnochromine were dissolved in 20 ml dioxan containing 50 mg *p*-bromobenzoyl chloride and 18  $\mu$ l pyridine. The mixture was allowed to stand at room temperature for 16 hours. After removal of the solvents, the ester was purified by preparative TLC using the mixture EtOAc : isoPrOH : NH<sub>4</sub>OH 4.25% ( 60:25:15) as solvent system. Crystallization occurred in absolute ethanol at 4°C in the orthorhombic system, space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with *z*=4 molecules in a unit cell of dimensions: *a*= 7.475(3), *b*= 10.183(3), *c*= 27.906(9) Å, 1757 measured reflections (Siemens diffractometer: CuK $\alpha$   $\lambda$  = 1.5418 Å). The structure was determined by direct methods with SHELXS 86<sup>13</sup>; final R value = 0.070 for 1570 reflections with *I* > 0.0. The absolute configuration was based on anomalous scattering of Br and O and the Hamilton's test<sup>14</sup> (R = 0.075 for the other absolute configuration).

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