TRINERVINE, A NEW INDOLE ALKALOID FROM *STRYCHNOS TRINERVIS*

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Abstract – A new indole alkaloid, trinervine (**1**), together with three known bases, bisnordihydrotoxiferine, longicaudatine, and normacusine B, was isolated from the roots of *Strychnos trinervis* (Vell.) Mart. Their structures were established by spectroscopic methods.

In the course of investigating the curarizing alkaloids of various *Strychnos* species of Brazil, Marini-Bettolo and his co-workers[1] reported in 1953 the results of their preliminary studies on the constituents of *Strychnos trinervis* (Vell.) Mart., a plant endemic to the northeastern region of Brazil. From extensive chromatographic studies, the presence of 23 alkaloids was indicated among which seven bisindole quaternary bases were identified.[2] In the ensuing three decades, no further details on the chemical constituents of this plant have appeared in the literature. We therefore became interested to investigate the roots of this plant.

The mixture of bases obtained from the chloroform soluble fraction of an ethanolic extract concentrate of the roots of *S. trinervis* was subjected to chromatographic separation over neutral Brockmann grade II–III alumina to yield four alkaloids. Of these, three were identified as known compounds – *bisnordihydrotoxiferine*,[3,4] *normacusine B*,[5] and longicaudatine[6] – by comparison of their spectroscopic properties (in particular, ^{1}H- and ^{13}C-nmr data) with those reported in the literature. The fourth alkaloid, named trinervine, appeared to be new. The evidence for the structure of this latter compound is summarized below.

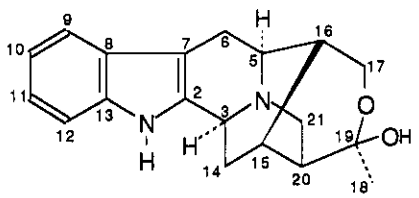
Trinervine was obtained as colourless needles from MeOH–EtOAc, mp 219–220°C, $[\alpha]_D^{22°}$ –7° (*c* 0.373, $CHCl_3$). The ir spectrum showed broad NH/OH absorption band around 3220 cm^{-1}. The uv absorption bands at λ_{max}^{EtOH} (log ε) 229 (4.33), 280 (3.62) and 289 (3.59) nm were indicative of the usual 2,3-disubstituted indole chromophore. Its electron impact mass spectrum indicated the molecular formula $C_{19}H_{22}N_2O_2$ ($M^{+\cdot}$ at *m/z* 310.1675; calcd.

Table 1. ^{1}H and ^{13}C Nmr spectral data of (**1**) [400 and 100.61 MHz respectively, $CDCl_3$–CD_3OH (3:1), δ values]

Position	^{1}H(*J* in Hz)	^{13}C
2		136.39 *s*
3	4.01 *dd* (10.30, 1.95)	47.96 *d*
5	3.39 *t* (5.80)	52.49 *d*
6	2.60 *d* (15.60)	26.60 *t*
	3.12 *dd* (15.60, 5.80)	
7		101.98 *s*
8		127.03 *s*
9	7.33 *d* (7.80)	117.45 *d*
10	7.10 *t* (7.80)	118.49 *d*
11	7.03 *t* (7.80)	120.72 *d*
12	7.43 *d* (7.80)	110.75 *d*
13		138.00 *s*
14	1.64 *m*	32.78 *t*
	1.89 *m*	
15	2.28 *m*	22.39 *d*
16	1.38 *m*	38.39 *d*
17	3.47 *dd* (10.70, 1.00)	64.34 *t*
	4.06 *d* (10.70)	
18	1.40 *s*	25.33 *q*
19		96.45 *s*
20	1.68 *m*	35.22 *d*
21	2.96 *dd* (13.70, 3.50)	50.08 *t*
	3.08 *dd* (13.70, 10.30)	

310.1681). Besides the molecular ion peak, the mass spectrum exhibited fragment ions at *m/z* 309 ($M^{+}\cdot$–$H\cdot$), 182, 169 and 168, which are diagnostic of an aromatic unsubstituted sarpagine-like skeleton,[7,8] thereby revealing a similar structural framework for trinervine. The peaks at *m/z* 292 ($M^{+}\cdot$–H_2O) and 291 (309–H_2O) attested to the presence of a hydroxyl group in the alkaloid. The absence of a carbonyl band in the ir spectrum suggested that the oxygen atoms might be present either as hydroxyl group or in an ether linkage. The ^{1}H nmr spectrum [400 MHz in $CDCl_3$–CD_3OD (3:1)] of trinervine showed signals corresponding to four aromatic protons (δ 7.00–7.50) confirming the occurrence of an aromatic unsubstituted

indole moiety. A three-proton signal at δ 1.40 indicated the presence of a methyl group attached to a quaternary carbon atom. From the chemical shift of this methyl group together with the absence of an ethylidene chain in the molecule, it was suspected that this quaternary centre, which should be carbon 19 of a sarpagine-like skeleton, is linked to carbon 17 through an oxygen atom possibly forming a hemiketal ring system as depicted in structure (**1**). Indeed, this became clearly evident from an examination[9] of its ^{13}C nmr spectrum which displayed 19 distinctly separate carbon signals as would have been expected from the molecular formula of the alkaloid. All the chemical shift values (Table 1) as well as the single frequency off-resonance multiplicities were in favour of structure (**1**) for trinervine. In particular, a signal due to a quaternary carbon at δ 96.45 revealed that this carbon should be attached to two heteroatoms and must therefore be the C-19 of a sarpagine–like skeleton. The existence of a strong nOe between C-18 methyl protons and the equatorial H-21 indicated the depicted stereochemistry at C-19.



(**1**)

On the basis of these considerations, structure (**1**) is suggested for trinervine. All the chemical shifts and couplings (Table 1) in the ^{1}H and ^{13}C and the 2D COSY spectra fully support this structure. Trinervine is thus structurally related to the recently published quaternary indole alkaloid venecurine[10] of which it should be the tertiary base. A direct correlation could not be carried out due to paucity of material.

EXPERIMENTAL

All melting points are uncorrected. Optical rotation was determined on a Perkin-Elmer model 141 polarimeter. Spectra were recorded with the following instruments: uv, Perkin-Elmer Lambda 5; ir, Nicolet 205 FT-IR spectrometer; ms, AEI MS50; nmr, ^{1}H (400 MHz) and ^{13}C (100.61 MHz) on a Bruker WM 400; chemical shifts are given in ppm relative to TMS (δ=0); abbreviations *s*, *d*, *t*, *q* and *m* in Table 1 refer to singlet, doublet, triplet, quartet and multiplet, respectively.

Plant materials

The roots of *Strychnos trinervis* (Vell.) Mart. (Loganiaceae) were collected in Recife, Brazil, in December 1984, by Professor Alda de A. Chiappeta. Voucher specimens were deposited at LTF, UFPB.

Isolation and purification of alkaloids. Extraction of the total alkaloid mixture (300 g dark syrup) from *S. trinervis* roots (1.5 kg) was carried out as previously described.[3] After separation of bisnordihydrotoxiferine (330 mg, 0.02%) from the nonpolar fraction, the aqueous part was brought to pH 9.5–10.0 by dropwise addition of conc. NH_4OH at 0–5°C.

The liberated bases were extracted with $CHCl_3$ and, after usual work up, the concentrated $CHCl_3$ extract (2.5 g) was chromatographed over Brockmann alumina, grade II–III, using solvents of increasing polarity starting from C_6H_6 to $CHCl_3$–MeOH mixtures (up to 4:1) as eluents. The combined $CHCl_3$ fractions were subjected to further chromatography over grade II–III alumina with Et_2O and Et_2O–MeOH mixtures (up to 3:1) yielding first a pale yellow solid (19.5 mg, 0.0013%) crystallizing from MeOH to give colourless needles, mp 275–276°C and identified as normacusine B. A second alkaloid, isolated as a slightly orange solid (195 mg, 0.013%), crystallized from MeOH–Et_2O in colourless needles, mp above 340°C and was identified as longicaudatine. The fractions eluted with $CHCl_3$–MeOH (19:1) were combined and re-chromatographed over grade II–III alumina with EtOAc–MeOH (9:1) to give trinervine (30 mg, 0.002%), which crystallized from MeOH–EtOAc in colourless needles, mp 219–220°C. The latter fractions eluted with $CHCl_3$–MeOH (9:1 and 4:1), giving a mixture of poor yielding more polar alkaloids, were grouped together and put aside for further investigation.

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