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AN INDOLINIC CRYPTOALKALOID FROM *STRYCHNOS MATTOGROSSENSIS*

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Key Word Index—*Strychnos mattogrossensis*; Strychnaceae; indolinic alkaloids; strychnobrasiline; 12-hydroxy-11-methoxystrychnobrasiline; mattogrossine; 2D NMR spectra.

Abstract—A new indolinic cryptoalkaloid, mattogrossine, has been isolated from the roots and branches of *Strychnos mattogrossensis* collected near Manaus. Elucidation of its structure is based mainly on 2D NMR studies. Two other indolinic alkaloids were also obtained: strychnobrasiline and 12-hydroxy-11-methoxystrychnobrasiline, and their ^{13}C NMR data are provided.

INTRODUCTION

In the course of our investigations on *Strychnos* alkaloids, we have studied chemically roots and branches of *S. mattogrossensis* S. Moore from Amazonia, which is a South American species growing in the tropical forest of the basin of the Amazon (Colombia, Venezuela, Peru and Brazil). The name of the species is derived from 'mato grosso' (Portuguese = great forest). It is a woody vine provided with both spines and tendrils, and it can attain huge dimensions when old. It is found in various habitats but is especially common in inundated forest with muddy water rivers. *S. mattogrossensis* belongs to the section *Breviflorae* and is essentially like *S. nigricans* Prog. in Mart., apart from details of the flower and the fruit [1].

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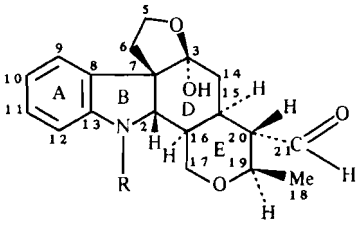
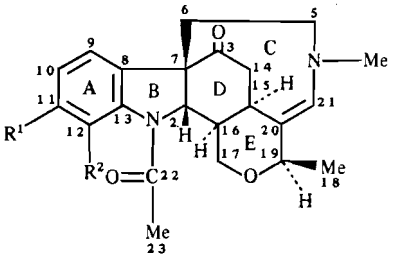
RESULTS AND DISCUSSION

Extraction of branches and roots yielded *ca* 0.1% of a mixture of tertiary bases from which three indolinic alkaloids have been isolated and their structures determined. No quaternary bases were detected. Both samples (roots and branches) yielded the same alkaloids as shown by TLC on silica gel. Strychnobrasiline (**3**) and 12-hydroxy-11-methoxystrychnobrasiline (**4**) were identified by comparison with literature data (UV, IR, MS and ^{1}H NMR) [2, 3]. Their ^{13}C NMR and CD data are presented here for the first time.

The present communication mainly reports the structure determination of a new product (**1**) to which we have given the trivial name mattogrossine. This does not afford a positive response with Dragendorff reagent, but is visualized on silica TLC plates by short-wave UV light (254 nm). Indeed, its UV spectrum exhibits the chromophore suggestive of a *N*-acetylindoline derivative. The elemental composition, $C_{21}H_{25}NO_5$, is established by



1 R = Ac
2 R = H



3 R^1 = H, R^2 = H
4 R^1 = OMe, R^2 = OH

high resolution FAB mass measurements of the [M + 1] peak at *m/z* 372, corresponding to $C_{21}H_{26}NO_5$ (calculated value 372.18055; observed value 372.17985).

The IR spectrum shows a band at 1720 cm^{-1} attributable to a saturated carbonyl (e.g. aldehyde grouping), and a broad band at 1642 cm^{-1} attributable to a *N*-acetylindoline. The NMR spectra of mattogrossine show a remarkable complexity due to the overlapping of the cisoid and transoid conformations of the *N*-acetyl group (Fig. 1).

A better understanding of the structure of the molecule is gained by examination of the ^1H and ^{13}C NMR spectra of deacetylmattogrossine (**2**) where these conformations are abolished. The ^1H NMR spectrum of **2**—characteristic of an unsubstituted indoline moiety—shows some similarities to, but also striking differences from that of **3**, indicating modification of the ring C [2, 3].

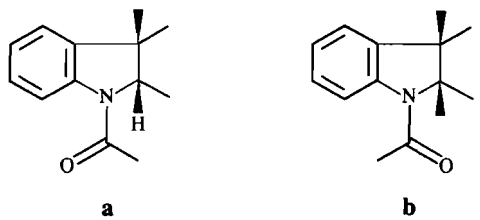
Conspicuous signals are an aldehydic proton at δ9.8, coupled with a methinic proton; four aromatic signals from a *N*-acetylindoline group; and a methyl doublet at δ1.3. In the ^{13}C NMR spectrum of **2**, the chemical shift of a quaternary carbon atom at δ104.9 indicates a hemiketal function such as the one found in venecurine [4]. We again note important differences with strychnobrasiline data (see Table 2). In fact, the structure **2** was established by combining biosynthetic considerations and data of two-dimensional NMR spectroscopy, firstly the 2D-correlation ^{13}C–^1H, and then the autocorrelated proton 2D NMR spectrum (COSY).

The results of the COSY spectrum of the aliphatic region of **2** are shown in Table 3. An isolated spin system can be established from the resonance (at δ4.20 and 4.08) of the H-5_A and H-5_B protons, which, as a consequence of being an oxymethylene system, resonate furthest downfield of the aliphatic region. This pair of protons is linked to the H-6_A and H-6_B protons resonating at δ2.7 and 2.4, respectively. (The strong deshielding of the H-5 protons is in accordance with the binding to the hemiketalic function located on C-3). A larger and useful structural fragment can be assembled from the remaining aliphatic protons. A convenient entry point in this system is afforded either by the H-19 oxymethine proton resonating at δ3.5 or by the H-20 methine proton (δ3.0). The vicinal and geminal connectivities provide the means of assembling completely the multispin substructure related with the hydrogens bonded to $C_{18}-C_{19}-C_{20}(-C_{21})-C_{15}(-C_{14})-C_{16}(-C_2)-C_{17}$. The proposed assemblage is confirmed from Relayed Coherence Transfer (RCT) and Long Range (LR) COSY experiments (Table 3). On the basis of this work, structures **1** and **2** are proposed for mattogrossine and its deacetylated derivative respectively.

The stereochemistry remains to be considered. The CD curve of mattogrossine shows a marked positive Cotton effect in the 250 nm region—as for strychnozairine—which establishes its configuration as 2β–7β (2*S*, 7*S*) [5]. The stereochemistry of C-19 and C-20 has been deduced from the 2D NOESY spectrum of **2** in which NOE connectivities were observed between Me-18 and H-21; thus, C-19 and C-20 respectively have *R* and *S* configurations, considering that ring E has the thermodynamically more stable chair conformation. This hypothesis is in close agreement with the comparison of the observed values of the coupling constants ($^3J_{19-20}$: 10.8 Hz; $^3J_{15-20}$: 11.2 Hz) and those calculated on the Dreiding model for a *trans*-diaxial position of H-19 and H-20; hence, the Me-18 and the aldehyde group are each in an equatorial position.

The observation of a 10.8 Hz vicinal coupling constant between H-16 and H-2 suggests the 16α-H (16*R*) configuration as in isoretuline [5]. The 3*S* and 15*R* configurations are strictly dependent on the configuration of C-7 and are in agreement with the biogenetic hypothesis [6].

As observed in strychnobrasiline [1, 2] and retuline [7], the restricted rotation around the N_1-acetyl group gives rise to two rotamers: rotamer **a** (acyl oxygen oriented towards the aromatic ring *ca* 70%) and rotamer **b** (*ca* 30%) shown in Fig. 1. The two rotamers of mattogrossine are easily discriminated because the acetamide group has a strong magnetic anisotropy.



a **b**

Fig. 1. Cisoid and transoid conformations of acylindoline derivatives.

Table 1. ^{1}H NMR spectra of compounds 1–4 ($CDCl_3$, 400 MHz, values in ppm. TMS = 0)

H	1 (rot. a)	2	3 (rot. a)	4
2	4.1	3.54	4.63	4.62
5	A 4.2 B:4.08	A:4.17[a] B:4.01	A–B:2.5→2.7	AB 2.67
6	A 2.7 B:2.4	A:2.5 B:2.2	A–B:2.2→2.6	A:2.53 B:2.27
9	7.7	7.6	7.5	6.96
10	7.1	6.7	7.0–7.2	6.7
11	7.2	7.07	7.0–7.2	—
12	7.9	6.6	7.9	—
14	A 2.1 B:2.0	A 2.0 B:1.9	A 3.0 B:1.7	A:3.0 B:1.75
15	2.3	2.2	2.68	2.67
16	1.7	1.5	1.8	1.9
17	A 4.05 B:3.5	A:4.0[a] B:3.6	A 4.0 B:3.7	A:4.02 B:3.8
18	1.3	1.3	1.37	1.34
19	3.5	3.45	4.0	3.9
20	3.0	3.0	—	—
21	9.8	9.8	6.0	6.01
N–COMe	2.25	—	2.35	2.4
N–Me	—	—	2.2	2.2
O–Me	—	—	—	3.85
OH	—	—	—	10.08

[a]Interchangeable.

Mattogrossine (1) might biogenetically arise from 3 by oxidative fission of ring C, loss of N_4-Me and further cyclization *via* a hemiketal function correlating C-5 with C-3. The basicity of the only remaining nitrogen in the structure of mattogrossine is reduced by the acetyl group, and explains why this substance is not detected using Dragendorff reagent and can be considered as a cryptoalkaloid.

Table 2. ^{13}C NMR spectra of compounds 1–4 ($CDCl_3$, 100.8 MHz, values in ppm, TMS = 0)

C	1	2	3	4
2	64.2	61.3	63.4	65.4
3	104.25	104.9	193.4	192.9
5	64	64.8	53.6	53.5
6	37.1	36.9	40.7[a]	40.3[a]
7	57.5	58.5	57	57.01
8	133.7	130.5	133.2	136.9
9	125.5	126.7	125	115.9
10	125.2	120.25	125.3	110.1
11	128.2	128.7	128.3	150.6
12	119.5	110.8	119.3	127
13	142.1	150.6	141.5	129.5
14	34.6	35.6	41.5[a]	40.4[a]
15	33.9	34.3	41.8[b]	41.1[b]
16	36.9	37.7	41.2[b]	41.02[b]
17	68.1	69.5	68.6	68.4
18	20.7	21.07	16.98	16.9
19	74.6	75.01	77.3	77.26
20	55.1	55.6	136.8	137
21	204	205.2	134.3	134.4
N–CO Me	169.3	—	169.7	172.5
N–COMe	23.3	—	23.2	22.7
N–Me	—	—	42.5	42.5
O–Me	—	—	—	56.6

[a,b]Interchangeable.

EXPERIMENTAL

Plant material. Herbarium specimens, roots and branches of *Strychnos mattogrossensis* were collected in 1988 near Manaus (Municipio do Careira, Igarapé Grande do Lago do Rei), and voucher specimens (COELHO, D. Herb. no. 142800) are kept in the herbarium of INPA at Manaus, as well as in the Department of Plant Taxonomy, Agricultural University, Wageningen (The Netherlands) and in the Pharmaceutical Institute, University of Liège.

Extraction and purification of alkaloids. Dry powder of the branches (or roots) was impregnated with NH_4OH and percolated with EtOAc. 2% H_2SO_4 was added to the soln and the

Table 3. Selected ^{1}H 2D NMR data for 2 ($CDCl_3$ 400 MHz)

COSY	COSY RCT	COSY LR	NOESY
H-2/H-16	H-14A/H-16	H-14A/H-17A	Me-18/H-20
H-5A/H-5B	H-14B/H-16	H-14A/H-17B	Me-18/H-21
H-5A/H-6A	H-14A/H-20	H-16/H-17A	
H-5A/H-6B	H-14B/H-20		
H-5B/H-6A	H-15/H-17A	+ Regular	
H-5B/H-6B	H-15/Me-18	couplings	
H-6A/H-6B	H-15/H-19		
H-14A/H-14B	H-15/H-21		
H-14A/H-15	H-16/H-17A		
H-14B/H-15	H-16/H-20		
H-15/H-16	Me-18/H-20		
H-15/H-20	H-19/H-21		
H-16/H-17B			
H-17A/H-17B	+ Regular		
Me-18/H-19	couplings		
H-19/H-20			
H-20/H-21			
+ Aromatic couplings			

mixture concd under red. pres.; the resulting aq. soln was filtered and extracted with Et_2O. The ethereal layer (extract A) was removed and partially evapd to give **1** that spontaneously crystallized. The aq. soln was then basified with Na_2CO_3 to pH 8 and re-extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4 and evapd to give extract B. This extract was fractionated by reversed-phase liquid chromatography on a Lobar column [LichroPrep RP 8 (40–63 μm)] with a mixture of MeOH and 0.02 M aq. NH_4OAc (3:2). The separation was monitored by TLC. Two alkaloids were detected, respectively, in fractions B_1 and B_2. Fraction B_1 was concd, basified and extracted with $CHCl_3$ to yield crude strychnobrasiline after evapn under red. pres.; **3** crystallized from EtOAc cooled to 6°. Fraction B_2 was purified by prep. TLC on silica gel with $CHCl_3$–MeOH (1:1) as solvent. After elution, **4** crystallized from MeOH cooled to 6°.

Mattogrossine (**1**). White powder: UV λ_{max}^{MeOH} (nm) (log ε): 213 (3.73) 252 (3.54) CD ($CHCl_3$): $\Delta\varepsilon_{249}$ +29.22. IR ν_{max}^{KBr} (cm^{-1}): 3400; 2900; 1720, 1642, 1625, 1586, 1485, 1410, 1395, 1340, 1280, 1220, 1135, 1080, 1050, 1010, 775. FABMS (rel. int.) 372 (35.7) 354 (2.4) 144 (100) 130 (26.7) 118 (5.9) 92 (12) 77 (9) 43 (71). ^{1}NMR spectrum: see Table 1. ^{13}C NMR spectrum: see Table 2.

Deacetylmattogrossine (**2**). *Preparation.* A soln of 50 mg of mattogrossine in 50 ml 1 M HCl was refluxed under N_2 for 4 hr. The soln was then basified with NaOH to pH 11 and extracted with $CHCl_3$. After working up, **2** was obtained. This derivative gave a strong positive response with Dragendorff reagent. ^{1}H NMR spectrum: see Table 1; ^{13}C NMR spectrum: see Table 2; 2D COSY, COSY RCT, COSY LR and NOESY data: see Table 3.

Strychnobrasiline (**3**). MS UV, IR, ^{1}H NMR (partim), see lit. data [2, 3]. CD (MeOH) Δε 260; +34.13. ^{1}H NMR. The spectrum is complicated by the presence of two rotamers (see Fig. 1) (*ca* 66% rotamer a, 33% rotamer b). Complete data for the main rotamer are given in Table 1. ^{13}C NMR: see Table 2. The assignments were made by comparison with data for similar compounds [8, 9]. CoTLC: with strychnobrasiline as internal standard confirms the identity of the compounds [10].

12-*hydroxy*-11-*methoxystrychnobrasiline* (**4**). MS, UV, IR, ^{1}H NMR (partim) see literature data [1, 2], CD (MeOH): Δε 267: +10.3. ^{1}H NMR. No rotamers because of the H-bonding linking the acetamide to the 12-hydroxyl group. Full data are given in Table 1. ^{13}C NMR: see Table 2. Heteronuclear chemical shift 2-D correlation has supported the assignments of the resonances mentioned in Tables 1 and 2.

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