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INDOLE ALKALOIDS FROM *TABERNAEMONTANA BOVINA*

TRINH PHUONG LIEN, HELMUT RIPPERGER,* ANDREA PORZEL,* KURT MERZWEILER,† TRAN VAN SUNG, and GÜNTER ADAM*‡

Institute of Chemistry, National Centre for Natural Science and Technology of Vietnam, Nghia Do, Tu Liem, Hanoi, Vietnam; *Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany; †Institute of Inorganic Chemistry, Martin Luther University, Kurt-Mothes-Straße 2, D-06120 Halle/Saale, Germany

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Key Word Index—*Tabernaemontana bovina*; Apocynaceae; indole alkaloids; 3-oxomehranine; 14α,15β-dihydroxy-*N*-methylaspidospermine

Abstract—In addition to (-)-mehranine, hecubine, ibogaine, ibogaline, 19*R-epi* voacristine, 20-hydroxyconopharyngine and pedunculine the novel indole alkaloids 3-oxomehranine and 14α,15β-dihydroxy-*N*-methylaspidospermine have been isolated from the leaves and stems of *Tabernaemontana bovina*. The structure of 3-oxomehranine has been assigned by NMR investigations, the structure of 14α,15β-dihydroxy-*N*-methylaspidospermine by X-ray analysis and their absolute configurations by circular dichroism.

INTRODUCTION

Tabernaemontana bovina (Apocynaceae) is a *ca* 1 m high bush [1] growing in Nam-bo (Cochinchina), the constituents of which have not yet been studied. From leaves and stems besides the known indole alkaloids (-)-mehranine [2] (yield 0.0066%), hecubine [3] (yield 0.0007%), ibogaine [4] (yield 0.0017%), ibogaline [5] (yield 0.0010%), 19*R*-epi-voacristine [6] (yield 0.0003%), 20-hydroxyconopharyngine [7] (yield 0.0007%) and pedunculine [8] (yield 0.060%) the new alkaloids 3-oxomehranine (**1**) (yield 0.0005%) and 14α,15β-dihydroxy-*N*-methylaspidospermidine (**3**) (yield 0.0005%) have been isolated.

RESULTS AND DISCUSSION

The elemental composition of 3-oxomehranine (**1**) and 14α,15β-dihydroxy-*N*-methylaspidospermidine (**3**) were shown to be $C_{20}H_{24}N_2O_2$ and $C_{20}H_{28}N_2O_2$, respectively, by high-resolution mass spectrometry.

The ^1H and ^{13}C NMR signals of **1** (Table 1) were assigned on the basis of APT, $^1H-^1H$ DQF COSY, gradient-selected HSQC and gradient-selected HMBC spectra. The stereochemistry of **1** was investigated by NOESY spectroscopy (Figure 1). An important NOE H-9/H-21 (Dreiding model: *ca* 2.2 Å) revealed neighbourhood. The α/β assignment of the 5-, 6-, 16- and 17-protons were detected from the NOEs H-2/H-6β (*ca* 1.8 Å), H-5β/H-6β (ca 2.3 Å), H-2/H-16β (*ca* 2.4 Å) and H-17α/H_3-18. For the latter effect the observation of correlations H-19A/H-21 and H-19B/H-21 was important. These indicated that H-21 and the ethyl chain were located at the same surface of the molecule. On the other hand, NOEs H-2/H-6β and H-2/H-16β indicated location of the corresponding hydrogen atoms at the other side. The configuration of the epoxide ring was revealed by NOEs H-15/H_3-18 and H-15/H-19.

The constitution and relative stereochemistry of **3** were determined by X-ray analysis. Figure 2 shows the molecular structure obtained. Due to the lack of any heavy atoms, the absolute configuration could not be determined. The structural determination revealed that the two hydroxyl groups occupy equatorial positions. The torsion angle O-1, C-14, C-15, O-2 is 62.5°. In the crystal structure of **2**, the hydroxyl protons form weak hydrogen bonds to the nitrogen atoms of two adjacent molecules (O-N = 288 − 294 pm). Whereas one hydrogen (H-2) is bonded to the indole nitrogen N-1, the other (H-1) is bonded to N-4. This molecular arrangement leads to infinite double chains which are orientated parallel to the crystallographic axis (Figure 3).

The absolute configuration of (-)-mehranine monohydrobromide was assigned by X-ray analysis [9]. The absolute configurations of the new alkaloids **1** and **3** were recognized by comparison of their long wave length circular dichroisms with that of (-)-mehranine (**2**) (Table 2). The short wave length Cotton effects should not be compared, because the com-

‡ Author to whom correspondence should be addressed.

Table 1. ^{1}H and ^{13}C NMR data of alkaloids **1** and **3** (300/75 MHz, $CDCl_3$, δ values, J Hz in parentheses, ^{1}H signals without multiplet specification taken from the 2D spectra)

Position	1 H	C	H	C
2	3.22 *d* (5.2)	67.0		71.4
3	-	165.2		56.8
5α	3.82 *dd* (12.5, 9.5)	42.3		51.8
5β	3.32			
6α	1.55	36.2*		39.5*
6β	2.32 *dd* (13.0, 6.8)			
7	-	53.3	-	52.4
8	-	131.5	-	135.4
9	6.98 *d* (7.0)	121.7	7.03 *d* (7.4)	121.6
10	6.69 *t* (7.3)	117.6	6.64 *dt* (0.8, 7.4)	117.2
11	7.14 *dt* (0.9, 7.6)	128.6	7.08 *dt* (1.1, 7.7)	127.6
12	6.44 *d* (7.9)	107.0	6.38 *d* (7.7)	106.6
13	-	150.2	-	150.6
14	3.33 *d* (3.7)	49.4	3.88 *dt* (4.9, 9.8)	69.3
15	3.25 *d* (3.7)	59.4	3.40 *d* (9.9)	78.5
16α	1.20	20.0		21.1
16β	1.81			
17α	1.48	20.0		25.5
17β	1.20			
18	0.85 *t* (7.4)	7.3	0.83 *t* (7.4)	8.1
19A	1.25	27.0		23.1
19B	1.55			
20	-	36.5*	-	39.8*
21	4.13 *s*	64.8		66.5
*N*Me	2.78 *s*	31.3	2.74 *s*	31.5

* May be exchanged.

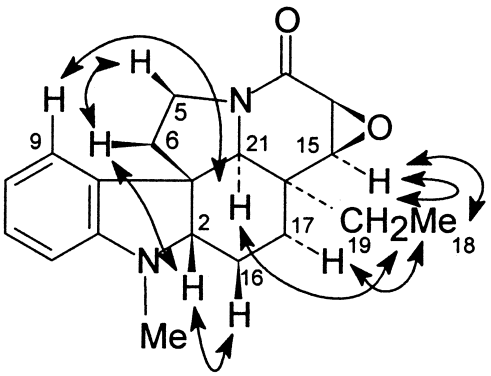


Fig. 1.

pounds possess additional chromophores (amide, epoxide and hydroxyl) absorbing in this region. Surprisingly, **2** displayed no effect at *ca* 270 nm, whereas compound **3** showed no effect at 297 nm.

The NMR signals of **3** Table 1 were assigned by comparison with the values of **1** and peduncularidine [8].

EXPERIMENTAL

Leaves and stems of *T. bovina* Lour. were collected in Tan Lac, Hoa Binh, Vietnam, in March 1997. The species was identified by Dr Nguyen Tap, Hanoi. A voucher specimen is deposited in the Herbarium of the Institute of Pharmacy, Hanoi. Plant material (3 kg) was dried at room temp., ground and extracted

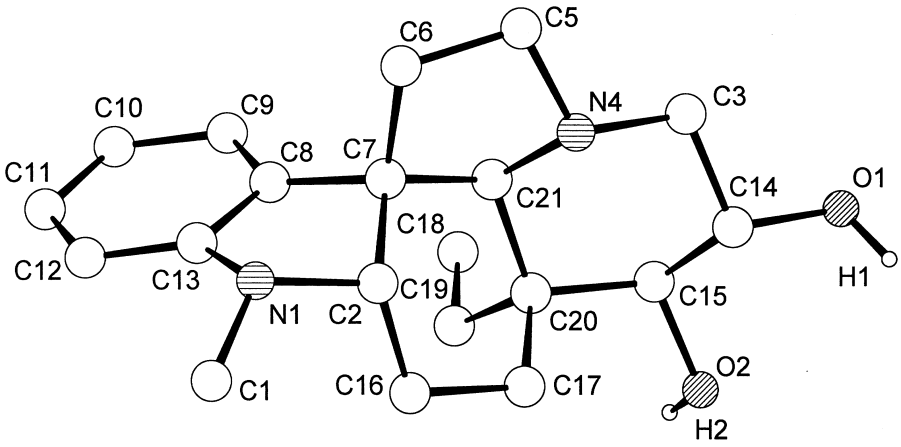


Fig. 2. Molecular structure of compound **3** (hydrogen atoms except OH are omitted)

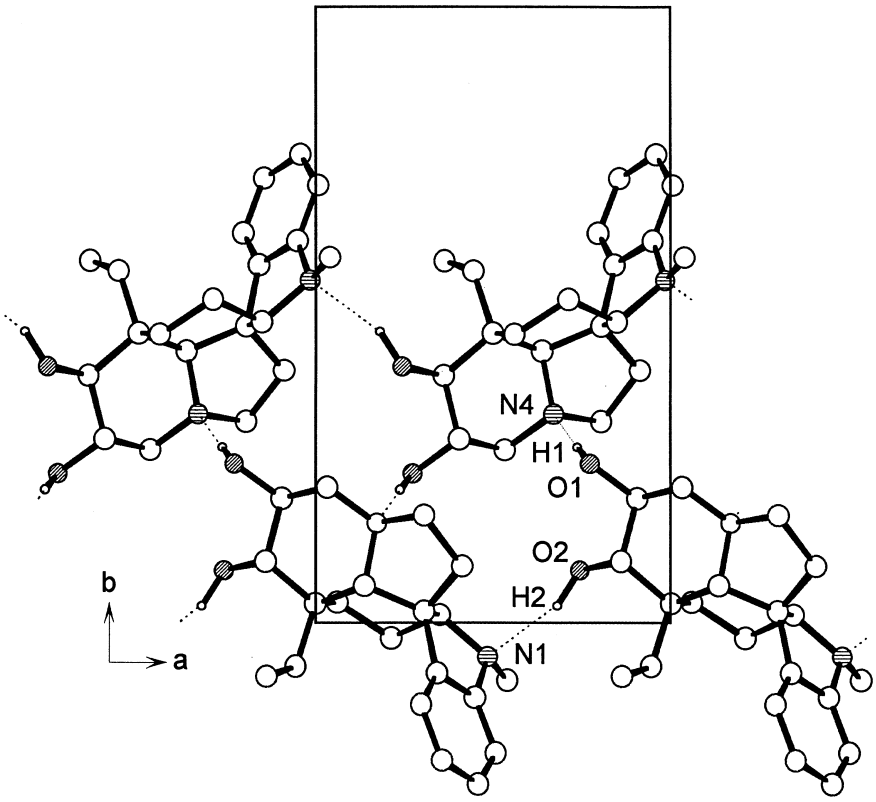
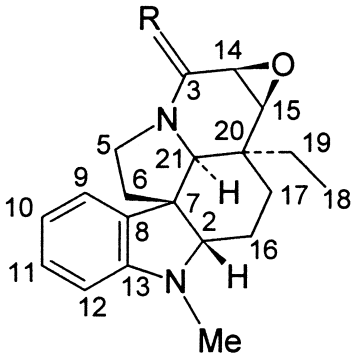


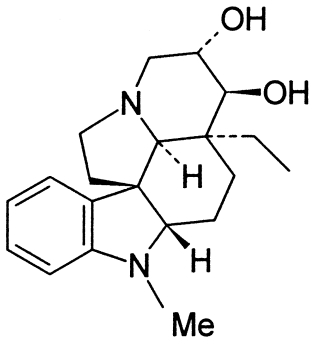
Fig. 3. Molecular arrangement of alkaloid **3** in the crystal (hydrogen bonds represented by dashed lines)

Table 2. Circular dichroism of alkaloids **1**–**3** (MeOH)

Alkaloid	CD				
1	$\Delta\epsilon 328 = +1.25$	$\Delta\epsilon 297 = -1.39$	$\Delta\epsilon 265 = +2.18$	$\Delta\epsilon 248 = -5.93$	$\Delta\epsilon 203 = -17.20$
2	$\Delta\epsilon 325 = +0.36$	$\Delta\epsilon 297 = -0.33$	–	$\Delta\epsilon 251 = -2.55$	$\Delta\epsilon 226 = +1.63$
3	$\Delta\epsilon 325 = +0.82$	–	$\Delta\epsilon 272 = +1.04$	$\Delta\epsilon 247 = -1.29$	$\Delta e 202 = -4.27$



1 R = O
2 R = H_2



3

with 95% MeOH at room temp. MeOH was evapd *in vacuo*, and the aq. soln extracted with *n*-hexane, followed by EtOAc and *n*-BuOH. Solvents were evapd *in vacuo*.

Isolation of compounds. The residue of the *n*-hexane extract was partitioned between 0.2 M HCl and toluene-Et_2O (1:1). After addition of $KHCO_3$ to the aq. layer, the latter was extracted with $CHCl_3$-EtOH (2:1). Evapn of solvents *in vacuo* gave a mixt. of alkaloids, which was chromatographed over silica gel with $CHCl_3$-EtOAc (19:1) increasing the amount of EtOAc to 40%. (-)-Mehranine, hecubine and 3-oxomehranine (**1**) were eluted. The combined residues of the EtOAc and BuOH extracts were treated, as described for the *n*-hexane extract and the "alkaloid" fr. chromatographed over silica gel with EtOAc-*n*-hexane (4:1) increasing the ratio of EtOAc to 100%, followed by chromatography over silica gel with EtOAc with increasing amounts of MeOH (maximum 30%). Pedunculine, 19*R*-*epi*-voacristine, ibogaine, ibogaline, 20-hydroxyconopharingine and 14α,15β-dihydroxy-*N*-methylaspidospermidine (**3**) were isolated.

3-*Oxomehranine* (**1**). Purified by silica gel prep. TLC EtOAc-*n*-hexane-$NHEt_2$ (15:10:1)]. Oil. $[\alpha]_D^{26}$ -19.1° ($CHCl_3$, $c = 0.40$). R*f* 0.49 [silica gel, cyclohexane-$CHCl_3$-$NHEt_2$ (6:3:1)]. EI-MS (70 eV) *m*/*z* (rel. int.): 324.1812 $[M]^+$ ($C_{20}H_{24}N_2O_2$, calcd 324.1838) (82), 157 (100).

14α,15β-*Dihydroxy*-N-*methylaspidospermidine* (**3**). Purified by silica gel CC *n*-hexane-Me_2CO-$NHEt_2$ (25:25:2)]. Mp 200–203° (from Me_2CO). $[\alpha]_D^{30}$ +15.3° (MeOH, *c* 0.06). R*f* 0.09 [silica gel, cyclohexane-$CHCl_3$-$NHEt_2$ (6:3:1)]. EI-MS (70 eV) *m*/*z* (rel. int.): 328.2119 $[M]^+$ ($C_{20}H_{28}N_2O_2$, calcd 328.2151) (20), 156 (100).

Crystal data of 14α,15β-*dihydroxy*-N-*methylaspidospermidine*. $C_{20}H_{28}N_2O_2$, M = 328.44, orthorhombic, space group P $2_12_12_1$, with a = 8.448(2), b = 14.162(3) and c = 14.372(3) Å, Z = 4, D_c = 1.269 g cm^{-3}, Mo-K_α radiation, 2 θmax. = 52°, 12986 collected reflections, 3360 independent reflections, 2386 reflections with $F > 4\sigma(F)$, 329 parameters. R1[$I > 2\sigma(I)$] = 0.0369, wR2 (all data) = 0.0788. The intensity data were collected on a STOE IPDS at room temperature. The structure was solved by direct methods (SHELXS-86) and refined on F^2 (SHELXL-93) [10]. All non-hydrogen atoms were refined anisotropically. The hydrogen positions were located from difference Fourier maps and refined isotropically. The structure plots were created with the program Diamond [11]. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this paper.

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