Two Further *Bis*-Indole Alkaloids from *Tabernaemontana bovina*

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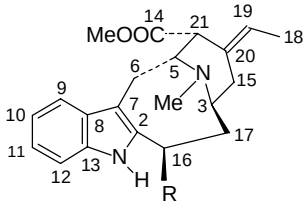
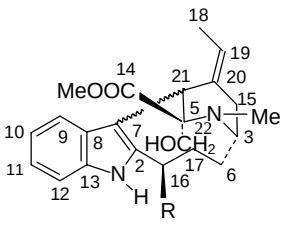
Abstract. The new *bis*-indole alkaloids tabernaemontabovine (**1**) and tabernaemontavine (**2**) have been isolated from aerial parts of *Tabernaemontana bovina*. Their structures have been assigned on the basis of APT, ^{1}H-^{1}H DQF COSY, gradient-selected HSQC and gradient-selected HMBC-spectra.

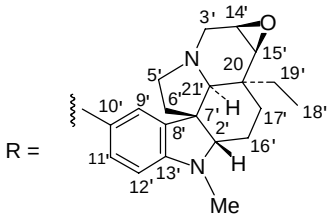
Species of the genus *Tabernaemontana*, belonging to the family of Apocynaceae, are a rich source for various types of indole alkaloids [1–4] and are widely used in the folk medicine [4]. The species *Tabernaemontana bovina* Lour., a ca. 1 m high shrub growing in Cochinchina [5], is used in the traditional medicine of Vietnam. Especially the roots are applied for the treatment of fever and jaundice [6]. Recently, we reported the structural elucidation of the novel indole alkaloids 3-oxomehranine and 14α,15β-dihydroxy-*N*-methylaspidospermine [7] as well as the *bis*-indole alkaloids tabernaebovine and methylenebismehranine [8], isolated besides a series of already known members from aerial parts of *T. bovina*. In the present study the identification of two further new *bis*-indole alkaloids, named tabernaemontabovine (**1**) and tabernaemontavine (**2**) from the same plant source is described.



1 (relative configuration)



2 (relative configuration)



(absolute configuration)

Results and Discussion

The elemental composition of tabernaemontabovine (**1**) and tabernaemontavine (**2**) were shown to be $C_{41}H_{50}N_4O_3$ and $C_{42}H_{52}N_4O_4$, respectively, by high-resolution mass spectrometry.

The ^{1}H and ^{13}C NMR signals of **1** and **2** (Table 1) and the structures of the alkaloids were assigned on the basis of APT, ^{1}H-^{1}H DQF COSY, gradient-selected HSQC and gradient-selected HMBC spectra.

Chemical shifts and coupling constants $J_{H,H}$ of **1** for the molecule half containing C-2' to C-21' were practically identical with those of analogous atoms of tabernaebovine and methylenebismehranine [8] indicating identical substructures. Relevant couplings for **1** were detected between H-3/H-15A, H-3/H-17α, H-3/H-17β, H-5/H-6A, H-5/H-6B, H-5/H-21, H-9/H-10, H-16/H-17α, H-16/H-17β, H_3-18/H-15B, H_3-18/H-19, H-5/C-7, H-5/C-14, H-6A/C-2, H-6A/C-7, H-6B/C-2, H-6B/C-7, H-16/C-2, H-16/C-7, H-21/C-14 and H-21/C-20. The stereochemistry was deduced by means of the NOESY spectrum. A NOE H-15B/H_3-18 (ca. 3.5 Å, Dreiding model) indicated the stereochemistry of the ethylidene moiety. A NOE H-15B/H-21 (ca. 3.7 Å) (H-15A = H-15α, H-15B = H–15β for **1**) is in agreement with the assumption of 1,3-*cis* quasi-diaxial arrangement of these hydrogen atoms. From the coupling constant $J_{H\text{-}5,H\text{-}21}$ = 2.4–3.4 Hz (Table 1) an equatorial conformation of H-5 can be derived assuming a chair conformation of the piperidine ring. In agreement with this conclusion no NOEs between H-21 and the 6-hydrogens were observed. A NOE H-3/H-6B (ca. 0.9 Å) (H-6A = H-6β, H-6B = H-6α for **1**) suggested that H-3 and H-6B possessed *cis* quasi-axial conformation with regard to the 8-membered ring. No NOE H-3/H-6A was detected. According to the coupling pattern (Table 1) H-16 had an axial conformation. All these observations

  1436-9966/99/3410X-506 $ 17.50 + .50/0

Table 1 ^{1}H and ^{13}C NMR data of compounds **1** and **2** [499.8/75.5 MHz, 2D: 499.8/125.7 MHz, $CDCl_3$, δ values, *J* (Hz) in parentheses, ^{1}H signals without multiplet specification taken from the 2D spectra]

Position	**1**		**2**	
	H	C	H	C
2	–	137.8	–	137.4 [a])
3	3.74	52.4	3.91 *t* (9.0)	59.9
5	4.02 *td* (9.2, 2.4)	59.7	–	51.9
6A	3.24 *dd* (14.6, 7.9)	19.3	3.27 *dd* (15.0, 8.6)	17.1
6B	3.45 *dd* (14.3, 10.7)		3.50 *t* (10.4)	
7	–	110.4	–	110.4
8	–	129.8	–	130.0
9	7.54 *dd* (5.5, 2.4)	117.5	7.54 *dd* (6.6, 2.1)	117.6
10	7.06	121.6	7.05	121.8
11	7.06	118.8	7.05	118.9
12	7.06	109.7	7.05	109.8
13	–	136.0	–	136.2
NH	7.44 *s*	–	7.43 *s*	–
14	–	171.8	–	174.2
15A	2.91 *d* (14.0)	52.4	2.98 *d* (13.7)	52.1
15B	3.74		3.62 *d* (13.4)	
16	4.48 *dd* (12.8, 2.9)	44.7	4.48 *d* (11.9)	44.5
17α	2.58	39.1	2.60 *d* (15.0)	39.0
17β	1.84 *ddd* (11.9, 6.9, 3.2)		1.87 *ddd* (12.8, 4.7, 2.4)	
18	1.66 *dd* (6.7, 1.5)	12.2	1.65 *d* (5.8)	12.1
19	5.34 *q* (6.7)	118.6	5.40 *q* (6.4)	119.9
20	–	137.4 [a])	–	136.2
21	2.71 *d* (3.4)	47.0	3.47	35.8
22	–	–	3.71 *d* (3.4)	70.5
OMe	2.45 *s*	49.9	2.39 *s*	50.2
*N*Me	2.59 *s*	42.4	2.57 *s*	42.0
2'	3.34 *dd* (10.7, 5.2)	73.2	3.34 *dd* (10.7, 5.2)	73.2
3α'	2.36 *d* (12.8)	53.1	2.35 *d* (12.8)	53.1
3β'	3.54 *dd* (11.9, 1.0)		3.55 *d* (13.1)	
5α'	2.22	53.6	2.22	53.6
5β'	3.19 *td* (7.9, 2.4)		3.20 *td* (7.9, 1.5)	
6α'	1.62	40.6	1.62	40.6
6β'	2.27		2.28	
7'	–	51.3	–	51.3
8'	–	137.2 [a])	–	137.3 [a])
9'	6.86 *d* (1.2)	121.1	6.84 *s*	121.2
10'	–	134.8	–	134.8
11'	6.81 *dd* (7.6, 1.4)	126.8	6.77 *d* (7.9)	126.9
12'	6.24 *d* (7.6)	106.4	6.23 *d* (7.6)	106.4
13'	–	149.1	–	149.2
14'	3.30 *d* (3.7)	53.1	3.31 *d* (3.4)	53.1
15'	2.84 *d* (4.0)	57.7	2.85 *d* (4.0)	57.6
16α'	1.07	20.0	1.08	20.0
16β'	1.72		1.73	
17α'	1.34 *dt* (14.0, 4.0)	24.5	1.36 *d* (15.6)	24.3
17β'	1.76		1.78 *dd* (14.0, 2.0)	
18'	0.53 *t* (7.5)	7.2	0.55 *t* (7.3)	7.3
19'	1.03	27.8	1.06	27.8
20'	–	34.6	–	34.6
21'	2.21	66.3	2.20	66.5
*N*Me'	2.70 *s*	31.7	2.70 *s*	31.7

[a]) May be exchanged.

were in accord with the stereochemistry given in formula **1**.

Also for **2** chemical shifts and coupling constants $J_{H,H}$ for the molecule half containing C-2' to C-21' were practically identical with those of analogous atoms of tabernaebovine and methylenebismehranine [8] indicating identical substructures. Relevant couplings for **2** were detected between H-3/H-6A, H-3/H-6B, H-6A/H-17β, H-6B/H-17α, H-6B/H-17β, H-16/H-17α, H-16/H-17β, H_3-18/H-19, H-3/C-15, H-6B/C-3, H-15A/C-19, H-15A/C-20, H-16/C-2, H-17α/C-3, H_3-18/C-19, H_3-18/C-20, H-19/C-15, H-19/C-21, H-21/C-5, H-21/C-7, H-21/C-14, H-21/C-19, H-21/C-20, H-22/C-14, H-22/C-21 and *N*Me/C-5. The stereochemistry was studied by

means of the NOESY spectrum. NOEs H_3-18/H-21 (ca. 1.7 Å) and H-15A/H-19 (ca. 3.0 Å) indicated the stereochemistry of the ethylidene moiety. A NOE H-6A/H-22 (ca. 2.5 Å) suggested a quasi-diaxial *cis*-relation of the 22- and the 6-methylene groups and the configurations at C-3 and C-5 given in formula **2**. All these results were in accord with the stereochemistry of formula **2**. A NOE OMe/H-2' revealed a position of the CO_2Me group near the aromatic ring system of the other half of the molecule in agreement with the ^1H chemical shift of OMe at high field.

In the circular dichroism spectra of **1** and **2** 8 Cotton effects were observed. They corresponded with each other concerning the signs and reflected therefore the same configurations at C-16. Basing on the X-ray analysis of (–)-mehranine hydrobromide [9] the absolute configurations of (–)-mehranine, 3-oxomehranine, and 14α,15β-dihydroxy-*N*-methylaspidospermine have been assigned [7]. Biogenetic considerations suggest that also the novel *bis*-indole alkaloids **1** and **2**, isolated from the same plant species, have identical steric structures with regard to the molecule halves containing the atoms C-2' to C-21'.

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Experimental

Isolation of compounds

Leaves and stems of *Tabernaemontana 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 was deposited in the Herbarium of the Institute of Pharmacy, Hanoi. The plant material was dried at room temperature, ground and extracted (3.0 kg) with 95% MeOH at room temperature. MeOH was evaporated *in vacuo*, and the aqueous solution was extracted with *n*-hexane, followed by EtOAc and *n*-BuOH. The solvents were evaporated *in vacuo*. The united residues of the EtOAc and *n*-BuOH extracts were partitioned between 0.2M HCl and toluene-Et_2O (1:1). After addition of $KHCO_3$ to the aqueous layer, the latter was extracted with $CHCl_3$-EtOH (2:1). Evaporation of the solvents *in vacuo* gave a mixture of alkaloids, which was chromatographed over silica gel with EtOAc-*n*-hexane (4:1) increasing the ratio of EtOAc to 100%, followed by EtOAc with increasing amounts of MeOH (maximum 30%). Raw tabernaemontabovine (**1**) and tabernaemontavine (**2**) were isolated.

Tabernaemontabovine **1**

The alkaloid was purified by column chromatography [silica gel, $CHCl_3$-MeOH (9:1)] and preparative TLC [silica gel, cyclohexane-Me_2CO-$NHEt_2$ (40:10:3)]. Yield 0.0017%, oil. $[\alpha]_D^{25}$ –74.2° (MeOH, *c* 0.50). CD (MeOH): $\Delta\varepsilon_{303}$ = –16.1, $\Delta\varepsilon_{296}$ = +27.8, $\Delta\varepsilon_{288}$ = +15.6, $\Delta\varepsilon_{275}$ = –28.9, $\Delta\varepsilon_{261}$ = +17.8, $\Delta\varepsilon_{239}$ = –36.7, $\Delta\varepsilon_{224}$ = +39.8, $\Delta\varepsilon_{209}$ = –124.0. R_f = 0.49 [silica gel, cyclohexane-$CHCl_3$–$NHEt_2$ (6:3:1)]. EIMS (70 eV) *m/z* (rel. int.): 646.3882 $[M]^+$ ($C_{41}H_{50}N_4O_3$; calcd. 646.3882) (43), 181.1093 [*N*-methylpiperidine ring + MeCH + CO_2Me + $H]^+$ ($C_{10}H_{15}NO_2$; calcd. 181.1103) (100), 122.0969 [181 – $CO_2Me]^+$ ($C_8H_{12}N$; calcd. 122.0970) (96).

Tabernaemontavine **2**

The alkaloid was purified by column chromatography [silica gel, *n*-hexane-Me_2CO–$NHEt_2$ (12:8:1) and silica gel, cyclohexane–$CHCl_3$–$NHEt_2$ (20:10:1)]. Yield 0.0008%, oil. $[\alpha]_D^{25}$ –56.8° (MeOH, *c* 0.50). CD (MeOH): $\Delta\varepsilon_{304}$ = –5.5, $\Delta\varepsilon_{296}$ = +10.6, $\Delta\varepsilon_{289}$ = +6.8, $\Delta\varepsilon_{275}$ = –7.4, $\Delta\varepsilon_{260}$ = +14.6, $\Delta\varepsilon_{239}$ = –6.6, $\Delta\varepsilon_{225}$ = +10.4, $\Delta\varepsilon_{207}$ = –77.6. R_f = 0.40 [silica gel, cyclohexane–$CHCl_3$–$NHEt_2$ (6:3:1)]. EIMS (70 eV) *m/z* (rel. int.): 676.4083 $[M]^+$ ($C_{42}H_{52}N_4O_4$; calcd. 676.3988) (45), 211 [*N*-methylpiperidine ring + MeCH + CO_2Me + CH_2OH + $H]^+$ (67), 180 [211 – $CH_2OH]^+$ (100).

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