

Two Further Bis-Indole Alkaloids from *Tabernaemontana bovina*

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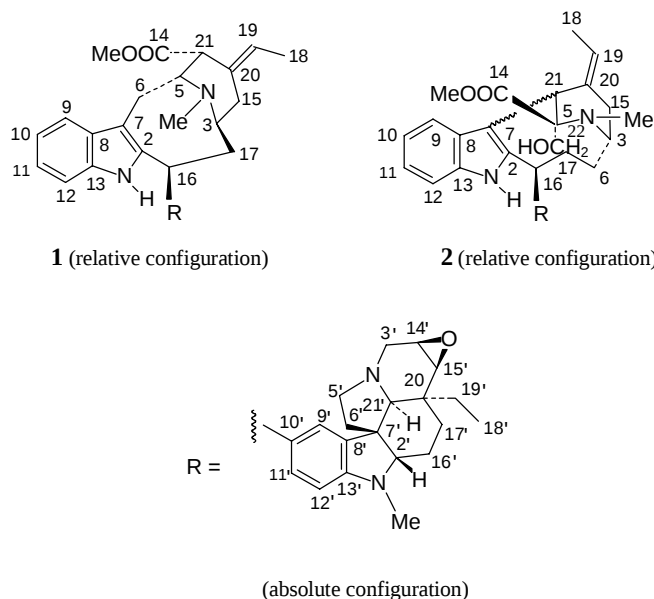
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Abstract. The new bis-indole alkaloids tabernaemontabovine (**1**) and tabernaemontavine (**2**) have been isolated from aerial parts of *Tabernaemontana bovina*. Their structures have been

assigned on the basis of APT, ^1H - ^1H 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 14 α ,15 β -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.



Results and Discussion

The elemental composition of tabernaemontabovine (**1**) and tabernaemontavine (**2**) were shown to be $\text{C}_{41}\text{H}_{50}\text{N}_4\text{O}_3$ and $\text{C}_{42}\text{H}_{52}\text{N}_4\text{O}_4$, respectively, by high-resolution mass spectrometry.

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

Chemical shifts and coupling constants $J_{\text{H,H}}$ 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-17 α , H-3/H-17 β , H-5/H-6A, H-5/H-6B, H-5/H-21, H-9/H-10, H-16/H-17 α , H-16/H-17 β , H₃-18/H-15B, H₃-18/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/H₃-18 (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-15 α , H-15B = H-15 β for **1**) is in agreement with the assumption of 1,3-*cis* quasi-diaxial arrangement of these hydrogen atoms. From the coupling constant $J_{\text{H-5,H-21}} = 2.4\text{--}3.4\text{ Hz}$ (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-6 β , H-6B = H-6 α 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

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

^{a)} May be exchanged.

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

Also for **2** chemical shifts and coupling constants $J_{\text{H,H}}$ 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-17 β , H-6B/H-17 α , H-6B/H-17 β , H-16/H-17 α , H-16/H-17 β , H₃-18/H-19, H-3/C-15, H-6B/C-3, H-15A/C-19, H-15A/C-20, H-16/C-2, H-17 α /C-3, H₃-18/C-19, H₃-18/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 NMe/C-5. The stereochemistry was studied by

means of the NOESY spectrum. NOEs H₃-18/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 CO₂Me 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 14 α ,15 β -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-Et₂O (1:1). After addition of KHCO₃ to the aqueous layer, the latter was extracted with CHCl₃-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, CHCl₃-MeOH (9:1)] and preparative TLC [silica gel, cyclohexane-Me₂CO-NHEt₂ (40:10:3)]. Yield 0.0017%, oil. [α]_D²⁵ –74.2° (MeOH, *c* 0.50). CD (MeOH): $\Delta\epsilon_{303} = -16.1$, $\Delta\epsilon_{296} = +27.8$, $\Delta\epsilon_{288} = +15.6$, $\Delta\epsilon_{275} = -28.9$, $\Delta\epsilon_{261} = +17.8$, $\Delta\epsilon_{239} = -36.7$, $\Delta\epsilon_{224} = +39.8$, $\Delta\epsilon_{209} = -124.0$. *R*_f = 0.49 [silica gel, cyclohexane-CHCl₃-NHEt₂ (6:3:1)]. EIMS (70 eV) *m/z* (rel. int.): 646.3882 [M]⁺ (C₄₁H₅₀N₄O₃; calcd. 646.3882) (43), 181.1093 [*N*-methylpiperidine ring + MeCH + CO₂Me + H]⁺ (C₁₀H₁₅NO₂; calcd. 181.1103) (100), 122.0969 [181 – CO₂Me]⁺ (C₈H₁₂N; calcd. 122.0970) (96).

Tabernaemontavine 2

The alkaloid was purified by column chromatography [silica gel, *n*-hexane-Me₂CO-NHEt₂ (12:8:1) and silica gel, cyclohexane-CHCl₃-NHEt₂ (20:10:1)]. Yield 0.0008%, oil. [α]_D²⁵ –56.8° (MeOH, *c* 0.50). CD (MeOH): $\Delta\epsilon_{304} = -5.5$, $\Delta\epsilon_{296} = +10.6$, $\Delta\epsilon_{289} = +6.8$, $\Delta\epsilon_{275} = -7.4$, $\Delta\epsilon_{260} = +14.6$, $\Delta\epsilon_{239} = -6.6$, $\Delta\epsilon_{225} = +10.4$, $\Delta\epsilon_{207} = -77.6$. *R*_f = 0.40 [silica gel, cyclohexane-CHCl₃-NHEt₂ (6:3:1)]. EIMS (70 eV) *m/z* (rel. int.): 676.4083 [M]⁺ (C₄₂H₅₂N₄O₄; calcd. 676.3988) (45), 211 [*N*-methylpiperidine ring + MeCH + CO₂Me + CH₂OH + H]⁺ (67), 180 [211 – CH₂OH]⁺ (100).

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