

NEW TABERNAMINE DERIVATIVES FROM *TABERNAEMONTANA*

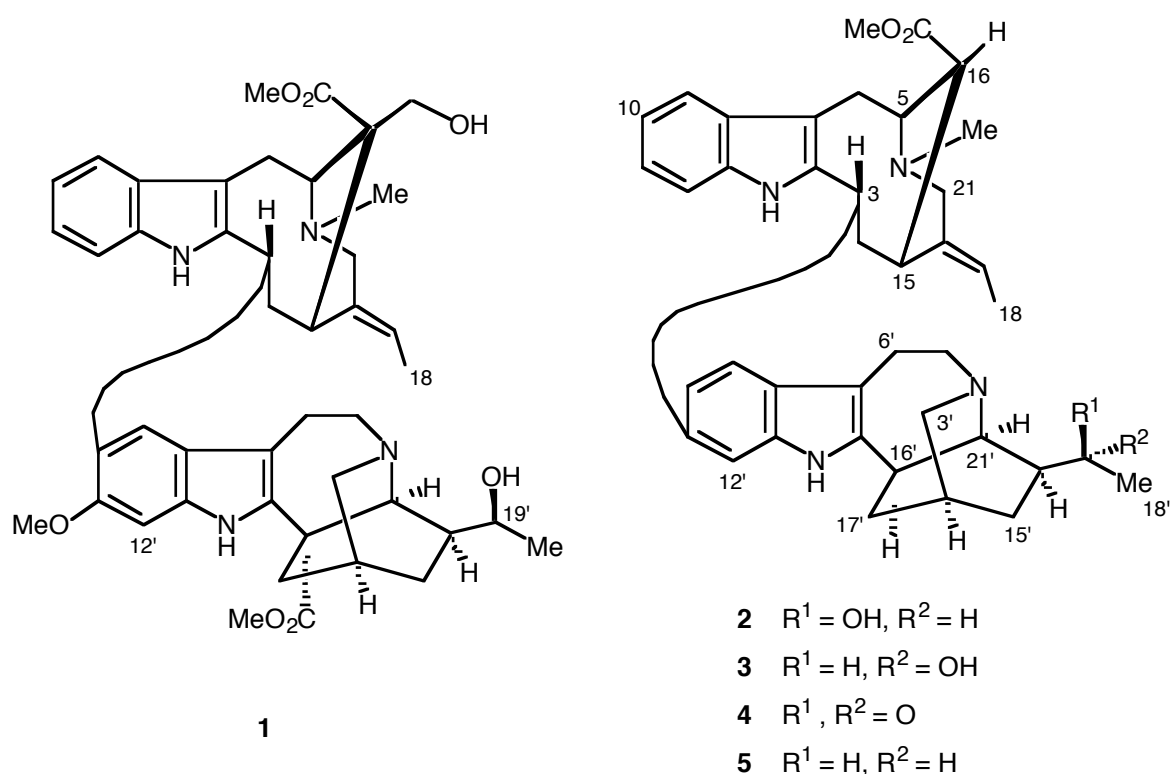
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Abstract – Three new bisindole alkaloids of the vobasine-iboga type, which are derivatives of tabernamine, viz., 19'(*S*)-hydroxytabernamine, 19'(*R*)-hydroxytabernamine, and 19'-oxotabernamine, were isolated from the stem-bark extract of *Tabernaemontana corymbosa* and the structures were established by spectroscopic analysis.

Plants of the genus *Tabernaemontana* (Apocynaceae) have a widespread distribution¹ and are rich sources of indole alkaloids.²⁻⁴ We have previously reported the structures of several alkaloids possessing new carbon skeletons from the Malayan species, *T. corymbosa* Roxb. Ex Wall.⁵⁻⁸ We have also reported the structures of two new bisindoles, vobasonidine and vobatricine,⁹ as well as the bioactivity of conodiparine A (**1**), a new vobasine-iboga bisindole obtained from the leaf extract of same plant, which was found to reverse multidrug-resistance in vincristine resistant KB cells.¹⁰ We now wish to report the structures of three new bisindoles related to tabernamine (**5**), from the stem-bark extract of this plant.

Compound (**2**) was obtained as a light yellowish oil, with $[\alpha]_D -144^\circ$ (*c* 0.07, CHCl₃). The IR spectrum showed bands due to NH/OH (3600 cm⁻¹) and ester (1725 cm⁻¹) functions, while the UV spectrum (λ_{max} 230, 286, and 295 nm) is characteristic of an indole chromophore. The FABMS spectrum showed an MH⁺ at *m/z* 633 which analysed for C₄₀H₄₈N₄O₃ + H. The presence of the fragment ion at *m/z* 615 was due to loss of H₂O, suggesting the presence of a hydroxyl function, while the peaks at *m/z* 194, 182, 180, 136 and 122, which are also common in the MS spectra of compounds (**3-5**), are characteristic of vobasine-iboga bisindoles.¹¹ The ¹³C NMR spectrum showed a total of 40 carbon resonances in agreement with the molecular formula from the HRMS spectrum. The ¹H NMR spectrum of **2** shares many common features with that of tabernamine (**5**). Thus analysis of the ¹H NMR spectrum of **2** with the aid of COSY, HMQC



and HMBC revealed the presence of two indole *NH*, an unsubstituted indole ring (vobasiny), another indole ring substituted at C(11') (iboga), one ester carbomethoxy group (vobasiny), an *N*-methyl (vobasiny), an ethylidene (vobasiny), and a hydroxyethyl group (iboga). The ester methyl associated with the vobasiny unit is unusually shielded (δ 2.46) which is in agreement with the configuration of C(16), which places the ester function in the shielding zone of the aromatic ring. The H(3) resonance of the vobasiny unit was observed as a doublet of doublet at δ 4.64 with J 13 and 3 Hz, and since only one H(3) was present, the dimer must be branched from C(3). The attachment to the iboga unit is deduced to be at C(11) from the signals of the aromatic H and C-atoms and by comparison to tabernamine¹² and the ervahanines.¹³ This is further supported by the HMBC spectrum of **2** which showed long range correlations from H(10') and H(12') to C(3). The molecular formula of **2** is 16 mass units higher compared to that of tabernamine (**5**), suggesting replacement of H by a hydroxyl function. This is supported by comparison of the ¹H NMR spectrum of **2** with that of **5** which showed a general similarity, except that in **2**, signals due to a hydroxyethyl side chain at C(20') in place of an ethyl group, are observed (δ 4.14, *qd*, $J = 6.3, 1.5$ Hz; 1.10, *d*, $J = 6.3$ Hz). The corresponding carbon resonances for C(18') and C(19') were observed at δ 20.2 and 71.5 respectively, compared to δ 11.8 and 27.7 for **5**, confirming the presence of a hydroxyl function on C(19'). The configuration of the oxymethine C(19') is readily determined to be (*S*) from examination of the carbon shifts of C(15') and C(21') which correspond to that of the monomeric iboga alkaloid, heyneanine,

exemplifying the 19(*S*) series in iboga alkaloids with a hydroxyethyl side chain [*versus* that of 19-*epi*-heyneanine exemplifying the 19 (*R*) series]. The 19(*S*) compounds have the chemical shift of C(15) at *ca.* δ 23, which is shifted downfield by about 6.7 ppm, compared to those in the 19(*R*) compounds, for which the C(21) resonances are shifted upfield by about 5 ppm to *ca.* δ 54.7 compared to the 19(*S*) epimers.¹⁴ Compound (**2**) is therefore 19'(*S*)-hydroxytabernamine.

Table 1. ¹H NMR spectral data for compounds (**2** - **5**) (400 MHz, CDCl₃)^a

H	2	3	4	5
3	4.64 dd (13, 3)	4.64 dd (13, 3)	4.62 dd (13, 3)	4.63 dd (13, 3)
5	4.04 ddd (10, 8, 3)	4.03 ddd (10, 8, 3)	4.04 ddd (10, 8, 3)	4.03 ddd (10, 8, 3)
6	3.35 m	3.24 m	3.27 m	3.25 dd (14.5, 8)
	3.50 dd (14.5, 10)	3.50 dd (14.5, 10)	3.50 dd (14.5, 10)	3.50 dd (14.5, 10)
9	7.56 dd (8, 1.5)	7.56 dd (8, 1.5)	7.56 dd (8, 1.5)	7.56 dd (7, 1)
10	7.04 m	7.05 m	7.06 m	7.05 m
11	7.04 m	7.05 m	7.06 m	7.05 m
12	7.04 m	7.05 m	7.06 m	7.05 m
14	1.97 m	1.93 m	1.93 m	1.97 m
	2.67 m	2.65 m	2.66 m	2.64 m
15	3.77 m	3.77 m	3.76 m	3.77 ddd (12, 7, 3)
16	2.72 t (3)	2.72 t (3)	2.72 t (3)	2.71 t (3)
18	1.65 dd (6.8, 1.5)	1.65 dd (6.7, 1.4)	1.64 dd (6.5, 1.5)	1.65 dd (6.7, 1.5)
19	5.32 q (6.8)	5.32 q (6.7)	5.32 q (6.5)	5.31 qd (6.7, 1)
21	2.90 d (14)	2.89 d (14)	2.91 d (14)	2.89 d (14)
	3.72 br d (14)	3.72 br d (14)	3.72 br d (14)	3.72 dd (14, 1.5)
NH	7.48 br s	7.52 br s	7.48 br s	7.51 br s
OMe	2.46 s	2.46 s	2.46 s	2.47 s
NMe	2.59 s	2.59 s	2.60 s	2.59 s
3'	3.01 m	3.01 m	3.11 m	2.93 dt (9, 3)
	3.01 m	3.01 m	3.11 m	3.01 dt (9, 2)
5'	3.35 m	3.12 m	3.27 m	3.09 m
	3.35 m	3.24 m	3.35 m	3.33 m
6'	2.61 m	2.69 m	2.63 m	2.64 m
	3.35 m	3.24 m	3.27 m	3.33 m
9'	7.36 d (8)	7.36 d (8.5)	7.35 d (8)	7.35 d (7)
10'	6.98 dd (8, 1.5)	6.99 dd (8.5, 1.5)	6.96 dd (8, 1.5)	6.96 dd (7, 1)
12'	7.00 br s	6.97 d (1.5)	7.01 br s	7.01 d (1)
14'	1.97 m	1.93 m	1.87 m	1.80 m
15'	1.59 m	1.81 m	1.75 ddt (14, 11, 3)	1.18 m
	1.97 m	1.87 m	2.06 m	1.76 m
16'	2.97 m	2.84 ddd (12, 3.5, 2)	2.80 br dd (11.5, 5)	2.85 ddd (11.8, 4, 1.5)
17'	1.59 m	1.56 m	1.54 ddd (13, 5, 3)	1.50 m
	2.04 m	2.00 m	1.98 m	1.97 m
18'	1.10 d (6.3)	1.25 d (6.4)	2.18 s	0.88 d (7)
19'	4.14 qd (6.3, 1.5)	3.86 qd (6.4, 2.5)	-	1.5 m
	-	-	-	1.5 m
20'	1.59 m	1.56 m	3.21 ddd (11, 6, 2.6)	1.5 m
21'	3.07 t (2)	3.31 t (2)	3.33 dd (2.6, 1.3)	2.79 t (1.5)
NH'	7.60 br s	7.57 br s	7.64 br s	7.47 br s

^a Assignments based on COSY, HMQC, and NOE.

Table 2. ^{13}C NMR spectral data for compounds (**2** - **5**) (100 MHz, CDCl_3)^a

C	2	3	4	5
2	137.4	137.4	137.4	137.4
3	45.3	45.2	45.3	45.1
5	59.8	59.7	59.7	59.6
6	19.4	19.4	19.4	19.2
7	110.3	110.3	110.2	110.1
8	129.7	129.7	129.8	129.6
9	117.6	117.5	117.6	117.4
10	119.0	119.0	119.0	118.9
11	121.7	121.7	121.7	121.6
12	109.9	109.9	109.8	109.8
13	136.0	136.0	136.0	135.9
14	38.9	38.9	38.9	38.9
15	33.6	33.6	33.6	33.5
16	46.9	47.0	47.0	47.0
18	12.3	12.3	12.3	12.2
19	118.9	118.8	118.9	118.6
20	137.5	137.6	137.5	137.6
21	52.3	52.4	52.3	52.3
OMe	50.0	49.9	50.0	49.8
CO	171.8	171.8	171.8	171.7
NMe	42.3	42.4	42.3	42.2
2'	141.0	141.1	141.3	142.0
3'	49.2	49.0	49.5	49.7
5'	52.8	52.7	54.5	54.0
6'	20.3	20.2	20.1	20.5
7'	108.5	108.5	109.3	108.8
8'	128.4	128.2	128.1	128.2
9'	118.2	118.2	118.0	117.9
10'	119.5	119.5	119.5	119.2
11'	139.3	139.3	139.1	138.6
12'	109.2	109.2	109.7	109.1
13'	135.0	134.9	134.4	134.6
14'	25.9	26.0	25.8	26.3
15'	23.0	29.0	24.7	31.9
16'	40.4	40.0	35.3	41.2
17'	34.3	34.1	34.3	34.0
18'	20.2	22.7	29.0	11.8
19'	71.5	71.5	209.3	27.7
20'	42.3	42.5	54.4	41.8
21'	60.8	54.6	55.3	57.5

^a Assignments based on HMQC and HMBC.

Compound (**3**) was obtained as a light yellowish oil, with $[\alpha]_{\text{D}} -139^\circ$ (c 0.13, CHCl_3). The UV, IR and MS spectra of **3** were virtually identical to that of **2**. Examination of the ^1H and ^{13}C NMR spectral data of **3** (Tables 1 and 2) revealed that the spectra of **3** and **2** were very similar except for the chemical shifts of H(19') in the ^1H NMR spectrum, and C(15') and C(21') in the ^{13}C NMR spectrum, suggesting that **3** and **2** are C(19') epimers. The observed chemical shifts of C(15') and C(21') at δ 29.0 and 54.6 allowed

assignment of the C(19') configuration as *R* by analogy to 19-*epi*-heyneanine (*vide supra*).¹⁴ Compound (**3**) is therefore 19'(*R*)-hydroxytabernamine.

Compound (**4**) was obtained as a colourless oil, with $[\alpha]_D -158^\circ$ (*c* 0.08, CHCl₃). The UV spectrum was similar to that of tabernamine (**5**), showing absorption maxima typical of an indole chromophore. The FABMS spectrum showed an MH⁺ at *m/z* 631, and HRFABMS spectral measurements yielded the formula C₄₀H₄₆N₄O₃. The IR spectrum was similar to that of **5**, except for the presence of an additional carbonyl band at 1709 cm⁻¹ due to a ketonic function. The presence of the carbonyl function in **4** is corroborated by the carbon resonance at δ 209.3 in the ¹³C NMR spectrum. The ¹H NMR spectrum of **4** is similar to that of **5**, except for the signals of the C(20') ethyl side chain, which is replaced by a signal due to an acetyl group (δ 2.18). Based on these observations, compound (**4**) is 19'-oxotabernamine.

In addition to the three new compounds, the known bisindole tabernamine (**5**), was also obtained as the major alkaloid compared to the three new derivatives.¹⁵ In all the four compounds (**2-5**), the signal of H(3) was observed as a doublet of doublet with *J* = 13, and 3 Hz, requiring H(3) and one of the H(14) to be *trans*-diaxial. This observation, coupled with the observed NOE interaction between H(3) and NH, confirm the α attachment of the iboga unit at C(3).

EXPERIMENTAL

General. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ¹H- and ¹³C-NMR spectra were recorded in CDCl₃ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz respectively. EIMS, HREIMS, FABMS and HRFABMS spectra were obtained on a JEOL JMS-AX505H mass spectrometer, courtesy of Dr. K. Komiyama of the Kitasato Institute, Japan.

Plant Material. Plant material was collected in Perak, Malaysia (May, 1996) and were identified by Dr. A. J. M. Leeuwenberg, Laboratory of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen, The Netherlands.¹⁶

Extraction and Isolation. Extraction of the ground stem-bark material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere.^{17,18} The alkaloids were isolated by initial column chromatography on silica gel using CHCl₃ with increasing

proportions of MeOH followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were Et₂O, Et₂O-MeOH (25:1), and EtOAc/cyclohexane (1:1, NH₃-saturated). The yields (g kg⁻¹) of the alkaloids (**2-5**) were as follows: **2** (0.0013), **3** (0.0026), **4** (0.0015), **5** (0.0305).

19'(S)-Hydroxytabernamine (2): Light yellowish oil; $[\alpha]_D -144^\circ$ (c 0.07, CHCl₃). UV (EtOH) $\lambda_{\max}$ (log ϵ) 230 (4.64), 286 (4.09), 295 (4.04) nm. IR (dry film) $\nu_{\max}$ 3600, 1725 cm⁻¹. ¹H-NMR and ¹³C-NMR data, see Tables 1 and 2. FABMS m/z 633 [MH]⁺ (19), 615 (2), 337 (4), 307 (18), 194 (5), 182 (7), 181 (10), 180 (16), 136 (100), 122 (17). HRFABMS m/z 633.3802 (calcd for C₄₀H₄₈N₄O₃ + H, 633.3804).

19'(R)-Hydroxytabernamine (3): Light yellowish oil; $[\alpha]_D -139^\circ$ (c 0.13, CHCl₃). UV (EtOH) $\lambda_{\max}$ (log ϵ) 231 (4.59), 286 (4.07), 295 (4.02) nm. IR (dry film) $\nu_{\max}$ 3391, 3260, 1721 cm⁻¹. ¹H-NMR and ¹³C-NMR data, see Tables 1 and 2. EIMS m/z 632 [M]⁺ (48), 614 (14), 337 (6), 307 (10), 194 (22), 182 (55), 181 (100), 180 (70), 136 (20), 122 (94). HREIMS m/z 632.3732 (calcd for C₄₀H₄₈N₄O₃, 632.3726).

19'-Oxo-tabernamine (4): Colourless oil; $[\alpha]_D -158^\circ$ (c 0.08, CHCl₃). UV (EtOH) $\lambda_{\max}$ (log ϵ) 231 (4.57), 284 (4.03), 296 (3.98) nm. IR (dry film) $\nu_{\max}$ 3390, 1722 cm⁻¹. ¹H-NMR and ¹³C-NMR data, see Tables 1 and 2. FABMS m/z 631 [MH]⁺ (48), 194 (10), 182 (13), 181 (25), 180 (35), 136 (100), 122 (35). HRFABMS m/z 631.3645 (calcd for C₄₀H₄₆N₄O₃ + H, 631.3648).

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REFERENCES AND NOTES

1. A. J. M. Leeuwenberg, *Tabernaemontana: The Old World Species*; Royal Botanic Gardens, Kew, 1991.
2. T. A. van Beek, R. Verpoorte, A. Baerheim Svendsen, A. J. M. Leeuwenberg, and N. G. Bisset, *J. Ethnopharmacol.*, 1984, **10**, 1.

3. B. Danieli and G. Palmisano, in 'The Alkaloids', ed. by A. Brossi, Academic Press, Orlando, 1986, Vol. 27, Chap. 1, pp. 1-130.
4. T. S. Kam, in 'Alkaloids: Chemical and Biological Perspectives', ed. by S. W. Pelletier, Pergamon, Amsterdam, 1999, Vol. 14, Chapter 2, pp. 285-435.
5. T. S. Kam, K. M. Sim, and T. M. Lim, *Tetrahedron Lett.*, 1999, **40**, 5409.
6. T. S. Kam, K. M. Sim, and T. M. Lim, *Tetrahedron Lett.*, 2000, **41**, 2733.
7. T. S. Kam, K. M. Sim, and T. M. Lim, *Tetrahedron Lett.*, 2001, **42**, 4721.
8. T. S. Kam and K. M. Sim, *Heterocycles*, 2001, **55**, 2405.
9. T. S. Kam and K. M. Sim, *Helv. Chim. Acta*, 2002, **85**, 1027.
10. T. S. Kam, K. M. Sim, T. Koyano, M. Toyoshima, M. Hayashi, and K. Komiyama, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1693.
11. H. Takayama, S. Suda, I. S. Chen, M. Kitajima, N. Aimi, and S. Sakai, *Chem. Pharm. Bull.*, 1994, **42**, 280.
12. P. Perera, F. Sandberg, T. A. van Beek, and R. Verpoorte, *Phytochemistry*, 1985, **24**, 2097.
13. X. Z. Feng, C. Kan, H. P. Husson, P. Potier, S. W. Kan, and M. Lounasmaa, *J. Nat. Prod.*, 1981, **44**, 670.
14. E. Wenkert, D. W. Cochran, H. E. Gottlieb, E. W. Hagaman, R. B. Filho, F. J. A. Matos, and M. I. L. M. Madruga, *Helv. Chim. Acta*, 1976, **59**, 2437.
15. The configuration of the double bond in the vobasynyl unit in **2-4** is assigned as *E* by chemical shift analogy of C(15) and C(21) with those of tabernamine (**5**).
16. Herbarium voucher specimens (GK 604) are deposited at the Herbarium of the Department of Chemistry, University of Malaya, Malaysia, and at Wageningen.
17. T. S. Kam and P. S. Tan, *Phytochemistry*, 1990, **29**, 2321.
18. T. S. Kam, K. Yoganathan, C. H. Chuah, and W. Chen, *Phytochemistry*, 1993, **32**, 1343.