Vobasonidine and Vobatricine, Novel Bisindole Alkaloids from a Malayan *Tabernaemontana*

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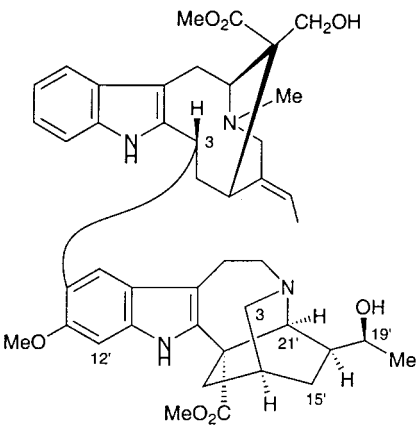
Two novel bisindole alkaloids of the vobasine-vobasine and vobasine-strychnan type, *viz*., vobasonidine (**2**) and vobatricine (**3**) were obtained from the leaf and stembark extracts, respectively, of the Malayan species *Tabernaemontana corymbosa*, and their structures were established by spectroscopic analysis.

1. Introduction. – Plants belonging to the genus *Tabernaemontana* (Apocynaceae) are notable for producing a wide variety of indole and bisindole alkaloids, including many with intriguing C-skeletons as well as novel biological activity [1][2]. Several Malayan members of the genus have been previously investigated and have provided many new indole as well as bisindole derivatives [3]. We have previously reported the structures of several new indole alkaloids possessing novel C-skeletons from the Malayan species *T. corymbosa* [4–7], and we now wish to report the structures of two novel bisindole derivatives from the same plant. The bisindole alkaloids documented thus far from *Tabernaemontana* include those of the ibogan-corynanthean (*e.g.* ervahanine A), ibogan-plumeran (*e.g.* ervafoline), corynanthean-aspidospermatan (*e.g.* vobparicine), ibogan-canthinone (*e.g.* isobona-fousine), and plumeran-macroline (*e.g.* pandicine) types [1][2]. We recently reported the structures and biological activity of several vobasine-ibogan bisindoles (*e.g.* conodiparine A **1**) obtained from the Malayan species *Tabernaemontana corymbosa*. These compounds (conodiparines A–D) were found to reverse multidrug-resistance (MDR) in vincristine-resistant KB cells [8]. We now wish to report the isolation of a novel bisindole of the vobasine-vobasine type, *viz*., vobasonidine (**2**), as well as the first example of a bisindole of the vobasine-strychnan type, *viz*., vobatricine (**3**), from the same plant.

Results and Discussion. – Vobasonidine (**2**) was obtained from the leaf extract as an optically active light yellowish oil. The UV spectrum showed absorption maxima at 229, 286, and 295 nm, characteristic of an indole chromophore, while the IR spectrum exhibited bands due to NH/OH (3382 cm^{-1}) and ester (1716 cm^{-1}) functions. The FAB-MS of **2** showed an $[M+H]^+$ peak at *m*/*z* 751, and HR-FAB-MS measurements established the molecular formula as $C_{44}H_{55}N_4O_7$ (see *Exper. Part*). The fragment ion observed at *m*/*z* 733 in the FAB-MS can be attributed to loss of H_2O, indicating the presence of an OH group. In addition, the characteristic peaks observed at *m*/*z* 180 and 122 are typical of vobasine-type compounds [9], while the fragment ion *m*/*z* 367 was also previously noted in the MS of conodiparine A (**1**), suggesting the presence of a similar vobasine moiety in vobasonidine [8]. Examination of the ^1H- and ^{13}C-NMR



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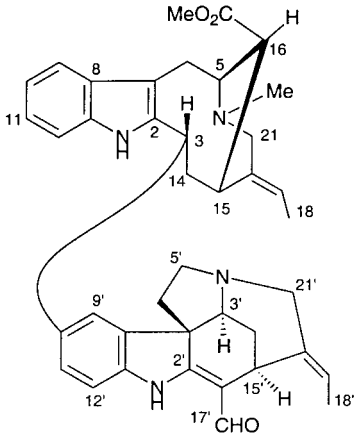

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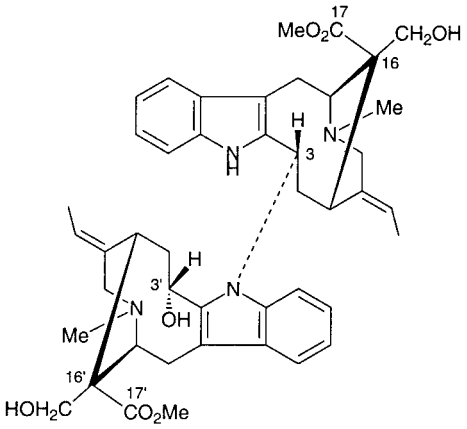

2

spectral data (*Tables 1* and *2*) with the aid of COSY, HMQC, and HMBC, indicated that vobasonidine is constituted from the union of two vobasine units. Thus, the ^{1}H-NMR spectrum of **2** showed the presence of two unsubstituted indole moieties, two methoxycarbonyl groups (δ 2.38, 2.35), two MeN groups (δ 2.52, 2.62), two ethylidene side chains (δ 1.76, 5.50; 1.73, 5.43), and two hydroxymethyl groups (δ(H) *ca.* 3.68; δ(C) 69.9, 70.3). The unusual shielding of the ester Me groups associated with both the vobasine units is in agreement with the configuration at C(16), which places both ester functions in the shielding zone of the aromatic rings. One vobasine unit corresponds to the same vobasine unit present in conodiparine A (**1**), as deduced from the similarity of the NMR data as well as the observation of the common *m*/*z* 367 fragment in the MS [8]. Since only one H–C(3) is present (δ 6.33), the dimer must be branched from C(3) of this unit. The NMR spectral data also indicated that the other vobasine unit, except

Table 1. *^{1}H-NMR Spectral Data for Compounds* **2** *and* **3** (400 MHz, $CDCl_3$)[a])

2				**3**			
H−C(3)	6.33 (*dd*, *J* = 13, 3)	H−C(3′)	5.62 (*dd*, *J* = 12, 3)	H−C(3)	4.54 (*dd*, *J* = 13, 3)	H−C(3′)	4.06 (*m*)
H−C(5)	3.92 (br. *t*, *J* = 9)	H−C(5′)	3.80 (*t*, *J* = 9)	H−C(5)	4.06 (*m*)	CH_2(5′)	3.10 (br. *dd*, *J* = 12, 6); 3.36 (*td*, *J* = 12, 5)
CH_2(6)	3.42 (*dd*, *J* = 14.8, 9); 3.73 (*m*)	CH_2(6′)	3.20 (*dd*, *J* = 14.8, 9); 3.50 (*dd*, *J* = 14.8, 9)	CH_2(6)	3.24 (*dd*, *J* = 14.7, 8); 3.44 (*dd*, *J* = 14.7, 11)	CH_2(6′)	1.88 (br. *dd*, *J* = 12, 5); 2.39 (*td*, *J* = 12, 6)
H−C(9)	7.54 (*dd*, *J* = 7.5, 2)	H−C(9′)	7.48 (*dd*, *J* = 7.5, 2)	H−C(9)	7.55 (*dd*, *J* = 8, 1.5)	H−C(9′)	7.18 (*d*, *J* = 1.5)
H−C(10)	7.03 (*m*)	H−C(10′)	6.95 (*m*)	H−C(10)	7.08 (*m*)	–	
H−C(11)	7.03 (*m*)	H−C(11′)	6.95 (*m*)	H−C(11)	7.08 (*m*)	H−C(11′)	6.97 (*dd*, *J* = 8, 1.5)
H−C(12)	7.03 (*m*)	H−C(12′)	7.19 (*dd*, *J* = 7.5, 2)	H−C(12)	7.08 (*m*)	H−C(12′)	6.79 (*d*, *J* = 8)[f])
CH_2(14)	2.39 (*m*); 3.35 (*q*, *J* = 13)	CH_2(14′)	2.18 (*m*); 2.93 (*q*, *J* = 12)	CH_2(14)	1.87 (*ddd*, *J* = 15, 7, 3); 2.62 (*m*)	CH_2(14′)	1.25 (*ddd*, *J* = 14, 4, 2); 2.52 (*ddd*, *J* = 14, 4, 2)
H−C(15)	3.66 (*m*)	H−C(15′)	3.49 (*m*)	H−C(15)	3.75 (*m*)	H−C(15′)	3.70 (*m*)
H−C(16)	–	H−C(16′)	–	H−C(16)	2.72 (*t*, *J* = 3)	–	
CH_2−C(16)	*ca.* 3.68; *ca.* 3.68	CH_2−C(16′)	*ca.* 3.68; *ca.* 3.68	–		H−C(17′)	9.32 (*s*)
Me(18)	1.76 (*dd*, *J* = 7, 1.6)[b])	Me(18′)	1.73 (*dd*, *J* = 7, 1)[b])	Me(18)	1.68 (*dd*, *J* = 6.7, 1.2)	Me(18′)	1.60 (*dt*, *J* = 7, 1.5)
H−C(19)	5.50 (*q*, *J* = 7)[c])	H−C(19′)	5.43 (*q*, *J* = 7)[c])	H−C(19)	5.36 (*q*, *J* = 6.7)	H−C(19′)	5.43 (*q*, *J* = 7)
CH_2(21)	3.01 (*d*, *J* = 13.8); 3.67 (*d*, *J* = 13.8)	CH_2(21′)	3.01 (*d*, *J* = 13.8); 3.67 (*d*, *J* = 13.8)	CH_2(21)	2.94 (*d*, *J* = 13.7); 3.73 (br. *d*, *J* = 13.7)	CH_2(21′)	2.96 (*d*, *J* = 15.7); 4.00 (br. *d*, *J* = 15.7)
NH	8.80 (br. *s*)	–		NH	7.52 (br. *s*)	NH′	10.26 (br. *s*)[f])
MeN	2.52 (*s*)[d])	Me′N	2.62 (*s*)[d])	MeN	2.60 (*s*)	–	
MeO	2.38 (*s*)[e])	Me′O	2.35 (*s*)[e])	MeO	2.43 (*s*)	–	

[a]) Assignments based on COSY and HMQC. [b–e]) Assignments may be reversed. [f]) NOE observed between two H-atoms.

Table 2. *^{13}C-NMR Spectral Data for Compounds* **2** *and* **3** (100 MHz, $CDCl_3$)[a])

2				**3**			
C(2)	133.3	C(2′)	136.1	C(2)	137.4	C(2′)	168.9
C(3)	51.3	C(3′)	65.6	C(3)	44.8	C(3′)	61.6
C(5)	59.8	C(5′)	59.2	C(5)	59.7	C(5′)	56.5
C(6)	17.2[b])	C(6′)	17.3[b])	C(6)	19.2	C(6′)	46.4
C(7)	110.3	C(7′)	108.8	C(7)	110.4	C(7′)	58.3
C(8)	129.2[c])	C(8′)	129.1[c])	C(8)	129.7	C(8′)	136.4
C(9)	117.7	C(9′)	118.3	C(9)	117.6	C(9′)	120.3
C(10)	118.7	C(10′)	118.9	C(10)	119.0	C(10′)	140.0
C(11)	122.2	C(11′)	122.1	C(11)	121.7	C(11′)	127.1
C(12)	110.4	C(12′)	110.7	C(12)	109.7	C(12′)	110.4
C(13)	136.2	C(13′)	135.0	C(13)	135.9	C(13′)	141.4
C(14)	32.5	C(14′)	37.9	C(14)	38.6	C(14′)	30.8
C(15)	33.6	C(15′)	33.3	C(15)	33.4	C(15′)	31.2
C(16)	52.4	C(16′)	52.4	C(16)	47.0	C(16′)	111.2
CH_2OH	69.9[d])	CH_2OH	70.3[d])		–	C(17′)	188.6
C(18)	12.1[e])	C(18′)	12.2[e])	C(18)	12.3	C(18′)	12.9
C(19)	120.4[f])	C(19′)	120.8[f])	C(19)	118.9	C(19′)	120.9
C(20)	135.7[g])	C(20′)	135.9[g])	C(20)	137.6	C(20′)	139.2
C(21)	51.8[h])	C(21′)	51.9[h])	C(21)	52.3	C(21′)	56.6
MeN	41.9[i])	Me′N	42.1[i])	MeN	42.2		–
MeO	49.8[j])	Me′O	50.4[j])	MeO	49.8		–
CO	174.4[k])	CO′	175.0[k])	CO	171.5		–

[a]) Assignments based on HMQC and HMBC. [b–k]) Assignments may be reversed.

for the presence of a hydroxymethyl substituent at C(16′), corresponds to vobasinol, as suggested by the observed C(3′) oxymethine resonance at δ 65.6. The ^{13}C-NMR spectrum of **2** showed a total of 44 peaks in agreement with the formula derived from mass measurements. Although all the C-signals of both vobasine units could be distinguished, it is clear that, except for the C(3)/C(3′) and C(14)/C(14′) signals, the signals occur in pairs, with only small differences (0.1 – 2 ppm) in the chemical shifts of the corresponding C-atoms of each pair. This has been previously observed, *e.g.* in the biskopsingine dimer nitaphylline [10]. The ^{1}H-NMR spectrum of **2** showed the presence of only one indole NH (δ 8.80), and since both the indole rings are unsubstituted, the dimer must be branched from the indole N(1′) of the vobasinol-like unit to C(3) of the other vobasine unit. The mode of connection of the monomeric units is supported by the observation of three-bond heteronuclear correlations from C(2′) to H–C(3) in the HMBC spectrum. Based on the foregoing discussion and further spectral data, the structure of vobasonidine is as shown by **2**. Only one other vobasine-vobasine bisindole with a similar C(3)–N(1′) linkage has been reported, *viz.*, hazuntamine, from *Hazunta modesta* (*T. modesta*) [11].

The signals due to H–C(3′) and C(3′) in the vobasinol-like unit were observed at δ 5.62 (*dd*, $J = 12$, 3 Hz) and 65.6, resp., while the signals due to H–C(3) and C(3) in the other vobasine unit appeared at δ 6.33 (*dd*, $J =$ 13.5, 3 Hz) and 51.3, resp. Irradiation of the H–C(3) signal at δ 6.33 resulted in an NOE enhancement of the NH signal at δ 8.80 and *vice versa*, requiring these two H-atoms to be in spatial proximity to each other. This observation, coupled with the J(3,14) and J(3′,14′) values of 13.5 and 12 Hz, resp., is in accord with the β-configuration of H–C(3) as well as of H–C(3′) [11].

Vobatricine (**3**), was obtained from the stem-bark extract as an optically active light yellowish oil. The UV spectrum showed absorption bands at 225, 287, 295, and 369 nm indicating the presence of an indole and a 2-methyleneindole chromophore [12], while the IR spectrum showed bands due to NH (3310 cm^{-1}), ester (1719 cm^{-1}), and conjugated carbonyl (1643 cm^{-1}) functions. In the EI-MS of **3**, a molecular ion appeared at *m*/*z* 628, which analyzed for $C_{40}H_{44}N_4O_3$. The observation of characteristic fragment ions at *m*/*z* 194, 182, 180, and 122 indicated the presence of a vobasine moiety, while the fragment ion at *m*/*z* 121 is typical of strychnan-type alkaloids [9]. The ^{13}C-NMR spectrum showed a total of 40 separate resonances, in agreement with the formula derived from the HR-MS. A notable feature of the ^{13}C-NMR spectrum was the presence of a downfield signal at δ 188.6 arising from the carbonyl of an α, β-unsaturated aldehyde moiety. This was supported by the observation of an aldehyde signal in the ^{1}H-NMR spectrum (*s* at δ 9.32). The latter showed a total of only seven aromatic signals, indicating branching of the bisindole from the aromatic site of one monomeric unit. Examination of the ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) with the aid of COSY, HMQC, and HMBC confirmed the presence of vobasine and strychnan units as indicated already by the MS. Thus the ^{1}H-NMR spectrum of **3** established the presence of two indole NH, an unsubstituted indole ring (vobasine), another indole ring substituted at C(10′) (strychnan), one methoxycarbonyl group (vobasine), one *N*-methyl (vobasine), and two ethylidene side chains. The strychnan unit was readily identified to be norfluorocurarine from the ^{13}C-NMR data, of which one set corresponded closely to those of an intact norfluorocurarine, with the exception of changes involving the aromatic C-signals. The vobasine unit on the other hand, except for the absence of the hydroxymethyl substituent at C(16), corresponded to that of conodiparine A (**1**) [8] as well as of vobasonidine (**2**) (*vide supra*), as evident from the similarity of the NMR data. As in **2**, the unusual shielding of the ester Me group associated with the vobasine unit (δ 2.43) is consistent with the configuration at C(16) in which the ester function is directed towards the shielding zone of the aromatic ring. The H–C(3) resonance of the vobasine unit suggests its β-configuration (*dd* at δ 4.54, $J = 13$ and 3 Hz). The attachment of C(3) is to the aromatic C(10′) of the strychnan unit, as established by the NMR signals of the aromatic H- and C-atoms and by comparison with the ervahanines [13]. This conclusion is also consistent with the observed three-bond correlations from C(3) to H–C(9′) and H–C(11′) in the HMBC spectrum. Based on the above, the structure of vobatricine is as shown by **3**. Vobatricine represents the first example of a vobasine-strychnan bisindole alkaloid.

Experimental Part

General. Optical rotations: *Jasco DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-3101PC* spectrophotometer; λ_{max}(log ε) in nm. IR Spectra: *Perkin-Elmer 1600*-FT-IR spectrophotometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: $CDCl_3$ solns. with $SiMe_4$ as internal standard; *Jeol JNM-LA-400* spectrometer at 400 and 100 MHz, resp. API-MS: *Perkin-Elmer API-100* instrument. EI-MS, HR-EI-MS, and FAB-MS: *Jeol JMS-AX505 H* mass spectrometer, by courtesy of Dr. *K. Komiyama* of the Kitasato Institute, Tokyo, Japan; *m*/*z* (rel. %).

Plant Material. Plant material was collected in Perak, Malaysia (May, 1996) and identified by Dr. *A. J. M. Leeuwenberg*, Laboratory of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen, The Netherlands. Herbarium voucher specimens (GK 604) are deposited at the Herbarium of the Department of Chemistry, University of Malaya, Malaysia, and at Wageningen.

Extraction and Isolation. Extraction of the ground leaf and 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 [14]. The alkaloids were isolated by initial column chromatography (silica gel, gradient MeOH/$CHCl_3$), followed by rechromatography of appropriate partially resolved fractions by centrifugal TLC. Initial chromatography of the basic fraction from the leaves provided essentially 7 fractions. Rechromatography of the last, *Fr. 7*, with MeOH/$CHCl_3$, followed by centrifugal TLC (Et_2O/MeOH 20:1) gave **2** (yield 0.0051 g kg^{-1}). Initial chromatography of the basic fraction from the stem-bark gave a total of 11 fractions. Rechromatography of *Fr. 11* with MeOH/$CHCl_3$, followed by centrifugal TLC (Et_2O/MeOH 5:1) gave **3** (yield 0.025 g kg^{-1}).

Vobasonidine (= *3-Hydroxy-16,16′-bis(hydroxymethyl)[1,3′-bivobasan]-17,17′-dioic Acid Dimethyl Ester*; **2**): Light yellowish oil. $[\alpha]_D = -80$ ($c = 0.31$, $CHCl_3$). UV (EtOH): 229 (4.20), 286 (3.66), 295 (3.64). IR (dry film): 3382, 1716. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 751 (24, $[M+H]^+$), 733 (5), 391 (14), 367 (45), 196 (8), 180 (100), 122 (9). HR-FAB-MS: 751.4075 ($[C_{44}H_{54}N_4O_7+H]^+$; calc. 751.4071).

Vobatricine (= *3-(2,16,19,20-Tetradehydro-17-oxocuran-10-yl)vobasan-17-oic Acid Methyl Ester* (**3**). Light yellowish oil. $[\alpha]_D = -575$ ($c = 0.13$, $CHCl_3$). UV (EtOH): 225 (4.62), 287 (4.16), 295 (4.15), 369 (4.36). IR (dry film): 3310, 1719, 1643. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 628 (20, M^+), 521 (5), 448 (12), 435 (8), 220 (4), 205 (10), 194 (7), 182 (12), 181 (40), 180 (26), 122 (34), 121 (6), 107 (100). HR-EI-MS: 628.3417 ($C_{40}H_{44}N_4O_3^+$; calc. 628.3413).

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