Conodirinines A and B, Novel Vobasine-*Iboga* Bisindoles Incorporating an Additional Tetrahydro-1,3-oxazine Unit on the Vobasinyl Moiety

by **Toh-Seok Kam*** and **Kooi-Mow Sim**

Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia
(phone: 603-79674266; fax: 603-79674193; e-mail: tskam@um.edu.my)

Two novel bisindole alkaloids of the vobasine-*Iboga* type, *viz.*, conodirinines A and B, in which the vobasinyl unit has incorporated an additional tetrahydrooxazine ring, were obtained from the leaf extract of the Malayan species *Tabernaemontana corymbosa*, and their structures were established by spectroscopic analysis.

Introduction. – Plants belonging to the genus *Tabernaemontana* (Apocynaceae) are widely distributed and are rich sources of indole alkaloids [1]. These plants are notable for producing a wide variety of indole and bisindole alkaloids, including many with intriguing C-skeletons as well as novel biological activities [2][3]. Several Malayan members of the genus have been previously investigated, and have provided many new indole as well as bisindole derivatives [4]. We have previously reported the structures of several new indole and bisindole alkaloids that possess novel C-skeletons from the Malayan species, *T. corymbosa* ROXB. ex WALL [5–10], including conodiparine A (**1**), a new vobasine-*iboga* bisindole, which was found to reverse multidrug-resistance (MDR) in vincristine-resistant KB cells [9], and vobatricine (**2**), representing the first example of a bisindole of the vobasine-strychnan type [10]. We now wish to report the structures of two minor but novel bisindoles, constituted from vobasinyl and ibogan units, in which an additional tetrahydrooxazine ring has been incorporated into the vobasinyl moiety.

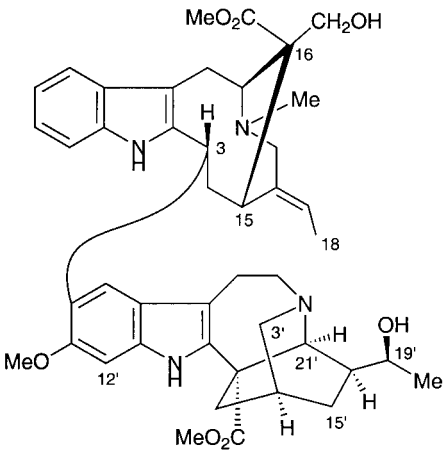
Results and Discussion. – Conodirinine A (**3**) was obtained in minute amounts from the leaf extract as a light yellowish oil with $[\alpha]_D = -88$ ($c = 0.03$, $CHCl_3$). The UV spectrum showed absorption maxima at 222, 287, and 294 nm, characteristic of an indole chromophore, while the IR spectrum showed bands due to NH/OH (3397 cm^{-1}) and ester (1728 cm^{-1}) functions. The FAB-mass spectrum of **3** showed an MH^+ peak at m/z 749, and HR-FAB-MS measurements established the molecular formula as $C_{44}H_{52}N_4O_7$ (see *Exper. Part*). The ^{1}H-NMR spectrum of conodirinine A (**3**) shares many common features with that of conodiparine A (**1**). Thus, analysis of the ^{1}H-NMR spectrum of **3** with the aid of COSY, HMQC, and HMBC revealed the presence of two indole NH, an unsubstituted indole ring (vobasinyl), another indole ring substituted at C(10′) and C(11′) (*Iboga*), one aromatic MeO group (*Iboga*), two ester MeOCO groups, an ethylidene (vobasinyl), a CH_2OH (vobasinyl), and a MeCHOH group (*Iboga*). The H–C(9′) and H–C(12′) *singlets* of the *Iboga* unit are coincidentally overlapped, while the unusually shielded ester Me group of the vobasine unit at δ 2.38 is consistent with the configuration at C(16), which places the ester function in the shielding zone of the aromatic ring. The characteristic upfield shift of the H–C(12′) and

C(12′) resonances indicated adjacent C(11′) oxygenation and allows placement of the aromatic MeO substituent at C(11′) of the *Iboga* unit [11]. This, in turn, suggests that the dimer is branched from C(3) of the vobasinyl unit (only 1 H–C(3) is observed) to C(10′) of the iboga unit, which is confirmed by the observed two- and three-bond correlations from C(10′) to H–C(3), and from C(3) to H–C(9′), respectively, in the HMBC spectrum of **3**. The signal of H–C(3) was observed as a *doublet* with $J = 13$ Hz, requiring H–C(3) and one of the H–C(14) to be *trans*-diaxial. This observation, coupled with the observed NOE interaction between H–C(3) and NH, confirms the α attachment of the iboga unit at C(3). The configuration at C(19′) is readily determined to be (*S*) from examination of the ^{13}C-NMR shifts of C(15′) and C(21′), which correspond to those of the monomeric *Iboga* alkaloid, heyneanine, exemplifying the (19*S*) series in *Iboga* alkaloids with a MeCHOH side chain [12]. Aside from these common features, there are also some distinct differences in the NMR spectra of these compounds. First, the *N*-Me resonance was conspicuously absent in the ^{1}H-NMR spectrum of **3**. Instead, a pair of low-field *AB doublets* corresponding to an CH_2O function (C(22)) was observed at δ 4.64 and 4.77. In the ^{13}C-NMR spectrum of **3**, two unusual low-field signals were observed at δ 76.7 and 88.0. The former is due to C(17)H_2O, while the latter is attributed to C(22)H_2O, which is α to both an O- and a N-atom. Since the molecular formula of **3** yielded a DBE value of 21, suggesting formation of an additional ring compared to conodiparine A (**1**), the above observations are consistent with formation of a tetrahydro-1,3-oxazine ring on the vobasinyl unit as shown in structure **3**. This is further supported by the observed long-range correlations from C(5), C(17), and C(21), to the H-atoms of the C(22)H_2O group in the HMBC spectrum of **3**.

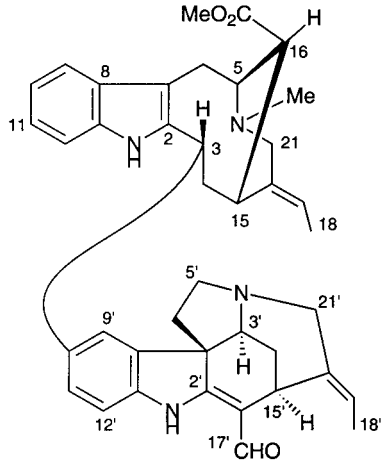
Conodirinine B (**4**) was obtained as a light yellowish oil, with $[\alpha]_D = -60$ ($c = 0.23$, $CHCl_3$). The MS data indicated that it is an isomer of **3** (MH^+ 749). The UV and IR spectra were also very similar to those of **3**. The NMR data indicated that, as in **3**, **4** has also incorporated an additional tetrahydro-1,3-oxazine ring as shown by the presence of a pair of low-field *AB doublets* at δ 4.64 and 4.75 in the ^{1}H-NMR spectrum, and the characteristic pair of downfield CH_2O signals at δ 76.4 and 88.2 in the ^{13}C-NMR spectrum. Unlike **3**, however, the dimer is now branched from C(3) of the vobasinyl unit to C(12′) of the *Iboga* unit. This is apparent from the coupling pattern of the aromatic H-atoms, where H–C(9′) and H–C(10′) are observed as a pair of *AB doublets* at δ 7.25 and 6.85 with $J = 8.8$ Hz. This is also confirmed by the observed C(12′) to H–C(3) correlation in the HMBC spectrum of **4**.

From a biogenetic viewpoint, conodirinines A and B, **3** and **4**, respectively, can be considered as arising from the appropriate tetracyclic precursors *via* *N*(4)-condensation with formaldehyde, followed by intramolecular cyclization. Such derivatives are known for the iboga compounds (*e.g.*, chippiine [13] and the dippinines [5]), and have been encountered only once in the vobasine series (pagicerine [14]). In any case, conodirinines A and B, **3** and **4**, respectively, represent the first examples of bisindoles that have incorporated an additional tetrahydro-1,3-oxazine ring on the vobasinyl moiety.

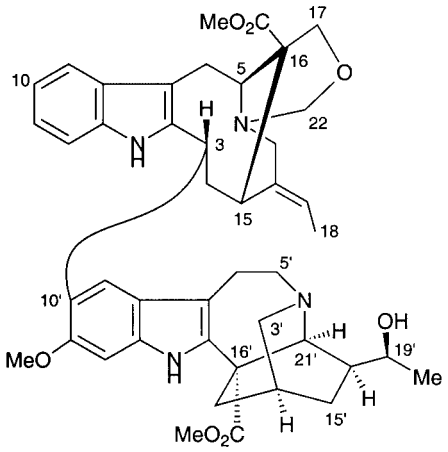
We would like to thank Dr. *A. J. M. Leeuwenberg*, Laboratory of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen, The Netherlands, for identification of plant material, the University of Malaya, and the *Ministry of Science, Technology, and Environment*, Malaysia (IRPA), for financial support.



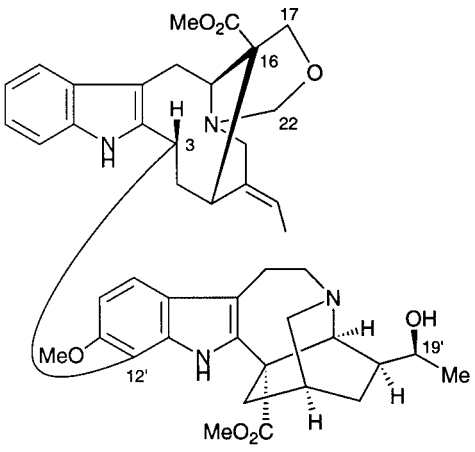
1



2



3



4

Experimental Part

General. Optical rotations: *JASCO DIP-370* digital polarimeter. UV Spectra: λ_{max} (log ε); *Shimadzu UV-3101PC* spectrophotometer. IR Spectra: ν [cm^{-1}]; *Perkin-Elmer 1600* Series FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: in $CDCl_3$ with TMS as internal standard on a *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 were obtained at The Research School of Chemistry, Australian National University, Canberra, Australia.

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. 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 material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as described in detail in [15]. The alkaloids were isolated by initial column chromatography on silica gel with $CHCl_3$ with increasing proportions of MeOH, followed by rechromatography of appropriate partially resolved fractions by centrifugal TLC. Initial chromatography of the basic fraction from the leaves provided essentially seven fractions. Rechromatography of *Fraction 6* with MeOH/$CHCl_3$, followed by centrifugal TLC ($CHCl_3$/MeOH 100:1; NH_3-saturated) gave compounds **3** and **4** (yield 0.0009 and 0.0069 g kg^{-1}, resp.).

Conodirinine A (**3**). Light yellowish oil. $[\alpha]_D = -88$ ($c = 0.03$, $CHCl_3$). UV (EtOH): 222 (4.56), 287 (4.00), 294 (4.01). IR (dry film): 3397, 1728. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 748 (17, M^+), 730 (35), 700 (11), 395 (40), 365 (17), 338 (65), 180 (55), 136 (55), 149 (100), 124 (38), 122 (55). FAB-MS: 749 ($[MH]^+$). HR-FAB-MS: 749.3935 (calc. for $C_{44}H_{52}N_4O_7 + H$, 749.3914).

Conodirinine B (**4**). Light yellowish oil. $[\alpha]_D = -60$ ($c = 0.23$, $CHCl_3$). UV (EtOH): 222 (4.62), 286 (4.05), 293 (4.03). IR (dry film): 3376, 3259, 1726. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 748 (40, M^+), 730 (100), 700 (15), 395 (39), 365 (18), 338 (70), 180 (36), 136 (20), 149 (52), 124 (15), 122 (25). FAB-MS: 749 ($[MH]^+$). HR-FAB-MS: 749.3941 (calc. for $C_{44}H_{52}N_4O_7 + H$, 749.3914).

Table 1. *^{1}H-NMR Data for Compounds* **3** *and* **4** (400 MHz, $CDCl_3$)[a])

3				**4**			
H–C(3)	5.15 (br. *d*, *J* = 13)	CH_2(3′)	2.78 (br. *d*, *J* = 9); 2.94 (*m*)	H–C(3)	5.31 (*dd*, *J* = 13, 3)	CH_2(3′)	2.46 (br. *d*, *J* = 9); 2.85 (*m*)
H–C(5)	4.18 (*m*)	CH_2(5′)	2.94 (*m*); 3.48 (*m*)	H–C(5)	4.18 (*m*)	CH_2(5′)	3.01 (*m*); 3.61 (*m*)
CH_2(6)	3.48 (*m*); 3.78 (*m*)	CH_2(6′)	2.94 (*m*); 3.09 (*m*)	CH_2(6)	3.61 (*m*); 3.61 (*m*)	CH_2(6′)	3.01 (*m*); 3.01 (*m*)
H–C(9)	7.63 (*d*, *J* = 6.9)	H–C(9′)	6.84 (*s*)	H–C(9)	7.75 (br. *d*, *J* = 7.4)	H–C(9′)	7.25 (*d*, *J* = 8.8)
H–C(10)	7.10 (*m*)	H–C(10′)	–	H–C(10)	7.17 (*td*, *J* = 7.4, 1)	H–C(10′)	6.85 (*d*, *J* = 8.8)
H–C(11)	7.10 (*m*)	H–C(11′)	–	H–C(11)	7.11 (*td*, *J* = 7.4, 1)	H–C(11′)	–
H–C(12)	7.05 (*d*, *J* = 6.9)	H–C(12′)	6.84 (*s*)	H–C(12)	7.04 (br. *d*, *J* = 7.4)	H–C(12′)	–
CH_2(14)	2.05 (*m*); 2.47 (*m*)	H–C(14′)	2.05 (*m*)	CH_2(14)	2.04 (*ddd*, *J* = 15, 7, 3); 2.57 (*m*)	H–C(14′)	1.58 (*m*)
H–C(15)	3.78 (*m*)	CH_2(15′)	1.52 (*m*); 1.95 (*m*)	H–C(15)	3.88 (*dd*, *J* = 11, 7)	CH_2(15′)	1.30 (*m*); 1.72 (*m*)
CH_2(17)	3.64 (*d*, *J* = 11); 3.67 (*d*, *J* = 11)	CH_2(17′)	1.95 (*m*); 2.54 (*m*)	CH_2(17)	3.66 (*d*, *J* = 10.8); 3.73 (*d*, *J* = 10.8)	CH_2(17′)	0.67 (br. *d*, *J* = 14); 1.72 (*m*)
Me(18)	1.66 (*d*, *J* = 6)	Me(18′)	1.13 (*d*, *J* = 6)	Me(18)	1.67 (*dd*, *J* = 6.8, 1.8)	Me(18′)	1.02 (*d*, *J* = 6)
H–C(19)	5.21 (*q*, *J* = 6)	H–C(19′)	4.18 (*m*)	H–C(19)	5.19 (*q*, *J* = 6.8)	H–C(19′)	4.08 (*m*)
H–C(20)	–	H–C(20′)	1.52 (*m*)	H–C(20)	–	H–C(20′)	1.30 (*m*)
CH_2(21)	3.29 (*d*, *J* = 16); 4.33 (*d*, *J* = 16)	H–C(21′)	3.65 (br. *s*)	CH_2(21)	3.25 (*d*, *J* = 16); 4.19 (*d*, *J* = 16)	H–C(21′)	3.72 (br. *s*)
CH_2(22)	4.64 (*d*, *J* = 10); 4.77 (*d*, *J* = 10)	11′-OMe	3.99 (*s*)	CH_2(22)	4.64 (*d*, *J* = 10); 4.75 (*d*, *J* = 10)	11′-OMe	3.99 (*s*)
NH	7.56 (br. *s*)	NH′	7.81 (br. *s*)	NH	7.73 (br. *s*)	NH′	7.48 (br. *s*)
MeO	2.44 (*s*)	OMe′	3.70 (*s*)	MeO	2.53 (*s*)	OMe′	3.69 (s)

[a]) Assignments based on COSY and HMQC.

Table 2. *^{13}C-NMR Data for compounds* **3** and **4** (100 MHz, $CDCl_3$)[a])

3				**4**			
C-Atom		C-Atom		C-Atom		C-Atom	
C(2)	136.1	C(2′)	135.0	C(2)	136.7	C(2′)	134.0
C(3)	36.8	C(3′)	50.5	C(3)	35.3	C(3′)	50.6
C(5)	61.0	C(5′)	47.0	C(5)	60.8	C(5′)	46.8
C(6)	25.0	C(6′)	25.9	C(6)	25.4	C(6′)	25.8
C(7)	109.2	C(7′)	108.2	C(7)	109.4	C(7′)	108.1
C(8)	129.6	C(8′)	122.2	C(8)	129.2	C(8′)	123.4
C(9)	117.7	C(9′)	117.9	C(9)	118.2	C(9′)	117.2
C(10)	119.2	C(10′)	128.1	C(10)	119.8	C(10′)	105.5
C(11)	122.1	C(11′)	153.9	C(11)	122.7	C(11′)	152.5
C(12)	109.9	C(12′)	92.8	C(12)	109.8	C(12′)	114.5
C(13)	136.1	C(13′)	135.0	C(13)	136.2	C(13′)	135.2
C(14)	36.8	C(14′)	29.7	C(14)	35.1	C(14′)	29.6
C(15)	39.3	C(15′)	22.7	C(15)	39.2	C(15′)	22.7
C(16)	52.5	C(16′)	53.5	C(16)	51.8	C(16′)	52.3
C(17)	76.7	C(17′)	36.3	C(17)	76.4	C(17′)	34.5
C(18)	11.7	C(18′)	20.3	C(18)	11.6	C(18′)	20.1
C(19)	115.2	C(19′)	70.2	C(19)	114.4	C(19′)	70.2
C(20)	137.2	C(20′)	38.0	C(20)	140.3	C(20′)	38.8
C(21)	49.8	C(21′)	59.0	C(21)	49.7	C(21′)	58.9
C(22)	88.0	MeO–C(11′)	55.9	C(22)	88.2	MeO–C(11′)	56.7
MeO	50.5	MeO′	53.3	MeO	50.6	MeO′	53.0
CO	173.3	CO′	172.4	CO	173.5	CO′	172.5

[a]) Assignments based on HMQC and HMBC.

REFERENCES

[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, N. G. Bisset, *J. Ethnopharmacol.* **1984**, *10*, 1.
[3] B. Danieli, G. Palmisano, in 'The Alkaloids', Ed. A. Brossi, Academic Press, Orlando, 1986, Vol. 27, Chapt. 1, pp. 1–130.
[4] T.-S. Kam, in 'Alkaloids: Chemical and Biological Perspectives', Ed. S. W. Pelletier, Pergamon, Amsterdam, 1999, Vol. 14, Chapt. 2, pp. 285–435.
[5] T.-S. Kam, K. M. Sim, *Heterocycles* **2001**, *55*, 2405.
[6] T.-S. Kam, K. M. Sim, T. M. Lim, *Tetrahedron Lett*. **1999**, *40*, 5409.
[7] T.-S. Kam, K. M. Sim, T. M. Lim, *Tetrahedron Lett*. **2000**, *41*, 2733.
[8] T.-S. Kam, K. M. Sim, T. M. Lim, *Tetrahedron Lett*. **2001**, *42*, 4721.
[9] T.-S. Kam, K. M. Sim, T. Koyano, M. Toyoshima, M. Hayashi, K. Komiyama, *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1693.
[10] T.-S. Kam, K. M. Sim, *Helv. Chim. Acta* **2002**, *85*, 1027.
[11] H. Takayama, S. Suda, I. S. Chen, M. Kitajima, N. Aimi, S. Sakai, *Chem. Pharm. Bull*. **1994**, *42*, 280.
[12] E. Wenkert, D. W. Cochran, H. E. Gottlieb, E. W. Hagaman, R. B. Filho, F. J. A. Matos, M. I. L. M. Madruga, *Helv. Chim. Acta* **1976**, *59*, 2437.
[13] T. A. Van Beek, R. Verpoorte, A. B. Svendsen, R. Fokkens, *J. Nat. Prod*. **1985**, *48*, 400.
[14] M. Bert, G. Baudouin, F. Tillequin, M. Koch, *Heterocycles* **1985**, *23*, 2505.
[15] T.-S. Kam, P. S. Tan, *Phytochemistry* **1990**, *29*, 2321.

Received July 11, 2002