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CONODUSARINE, A NEW BIOLOGICALLY ACTIVE BISINDOLE ALKALOID FROM *TABERNAEMONTANA DIVARICATA*

Toh-Seok Kam* and Huey-Shen Pang

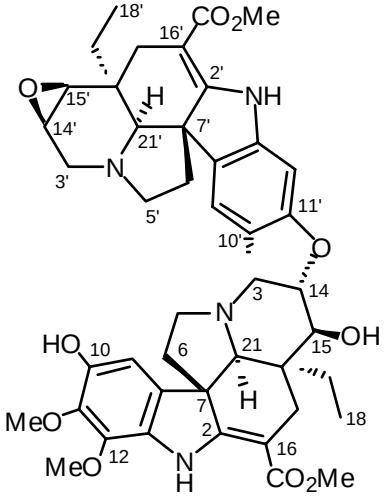
Department of Chemistry, University of Malaya
50603 Kuala Lumpur, Malaysia

Abstract – A new bisindole alkaloid of the vobasine-iboga type, conodusarine, was isolated from the stem-bark extract of *Tabernaemontana divaricata* and the structure established by spectroscopic analysis.

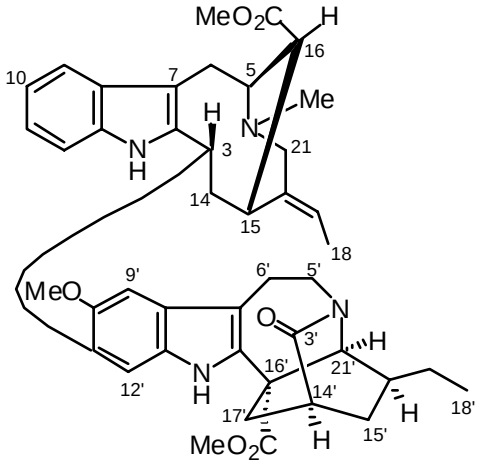
Plants of the genus *Tabernaemontana* (Apocynaceae) have a widespread distribution and are known to provide alkaloids of intriguing molecular structure as well as novel biological activity.[1-3] We have reported many examples of new alkaloids which are distinguished by their structural novelty, as well as useful bioactivity, from various Malaysian representatives of this genus.[4-19] For instance we have previously reported the structure of the novel tetracyclic indole voaharine[16,18] and taberhanine,[19] as well as the new bisindoles, conophylline, conophyllidine, conofoline, and conophyllinine from the leaf extract of *T. divaricata* (L.) R.Br. ex Roem. & Schult.[16-19] The bisindole conophylline (**1**) which is found in both the single-flower as well as the double-flower variety of *T. divaricata* has been recently found to exhibit important biological activity.[20-24] It was shown to be a potent inhibitor of *ras* functions,[20] and very recently, it has also been found to induce morphological change as well as insulin production in pancreatic acinar carcinoma AR42J cells.[24] We have isolated another new bisindole from the stem-bark extract of the double-flower variety of this plant, for which we give the trivial name conodusarine.

Conodusarine (**2**) was obtained as a light yellowish oil, with $[\alpha]_D$ +19^o (*c* 0.26, $CHCl_3$). The UV spectrum (λ_{max} 223, 286, 295, 312 nm) is characteristic of an indole chromophore while the IR spectrum showed absorptions due to NH (3388, 3254 cm^{-1}), ester (1727 cm^{-1}) and lactam carbonyl (1664 cm^{-1}) functions. The ESIMS spectrum showed an MH^+ at *m/z* 719, and HREIMS measurements gave the molecular formula $C_{43}H_{50}N_4O_6$. The presence of the fragment ions at *m/z* 194, 182, 180, 149, and 122 in the EIMS spectrum are characteristic of vobasine-iboga bisindoles.[25,26] The ^{13}C NMR spectrum showed a total of 42 carbon resonances indicating coincidence of two carbon resonances which was shown by subsequent 2-D experiments to be that of C(7) and C(12'). Examination of the ^1H and ^{13}C NMR spectral data with the aid of COSY, HMQC, and HMBC, confirmed the presence of vobasinyl and ibogan units in **2**. Thus the ^1H NMR spectrum of **2** (Table 1) showed the presence of two indole NH, an unsubstituted indole ring (vobasinyl), another indole ring substituted at C(10') and C(11') (iboga), one aromatic methoxy group (iboga), two ester carbomethoxy groups, one N-Me (vobasinyl), an ethylidene (vobasinyl), and an ethyl side chain (iboga). The

ester methyl associated with the vobasinyl 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 vobasinyl unit was observed as a broad one proton doublet at δ 5.11 (*J* 10.5 Hz), the presence of only one hydrogen indicating branching of the bisindole from C(3) of the vobasinyl moiety. The aromatic hydrogens of the iboga unit were observed as two singlets at δ 6.69 and 6.89, indicating substitution of the aromatic moiety at positions 10' and 11'.



1



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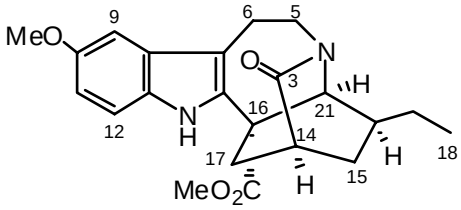

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Irradiation of the singlet at δ 6.69 (δ_C 110.5), resulted in NOE enhancement of NH', whereas similar irradiation of the signal at δ 6.89 (δ_C 98.9) resulted in enhancement of OMe' and H(6'). This allowed assigment of the former as H(12') and the latter as H(9') and as a result, places the aromatic methoxy substituent at position 10' and the point of branching of the bisindole at position 11'. This conclusion is also in accord with the observed upfield shift of the C(9') signal at δ 98.9, which is indicative of adjacent C(10') oxygenation.[14] Another notable feature of the NMR spectral data of the ibogan unit is the conspicuous absence of the signals due to both H(3'). Instead a lactam carbonyl signal is observed in the ^{13}C NMR spectrum at δ 175.7 which is assigned to C(3'). The assignment is supported by the HMBC data which showed three-bond correlations to this carbon from H(5') and H(21'). In addition, the presence of the lactam carbonyl function at position 3' has resulted in the downfield shifts of H(5β'), H(14'), and H(21') {compared to coronaridine}, as a result of proximity to the lactam carbonyl function.

Table 1. ^{1}H and ^{13}C NMR spectral data of conodusarine (**2**) (400 MHz, $CDCl_3$)[a]

Position	δ_C	δ_H	Position	δ_C	δ_H
2	137.8	-	2'	134.3	-
3	37.5	5.11 br d (10.5)	3'	175.7	-
5	59.7	4.01 ddd (11, 8, 2.5)	5'	42.5	3.09 m
6	19.5	3.21 dd (14, 8)			4.39 ddd (16, 14.5, 6.5)
		3.45 dd (14, 11)	6'	21.0	3.13 m
7	110.5	-			3.13 m
8	129.6	-	7'	108.5	-
9	117.2	7.53 br d (8)	8'	126.2	-
10	118.6	7.05 m	9'	98.9	6.89 s
11	121.3	7.05 m	10'	151.0	-
12	110.0	7.05 m	11'	130.6	-
13	135.8	-	12'	110.5	6.69 s
14	36.4	2.06 m	13'	130.4	-
		2.43 m	14'	37.8	2.43 m
15	33.5	3.76 m	15'	30.6	1.25 m
16	46.6	2.69 t (2.5)			1.85 ddd (13, 10, 3)
18	12.1	1.66 d (6.5)	16'	55.3	-
19	118.4	5.30 q (6.5)	17'	35.6	2.00 m
20	137.5	-			2.43 m
21	52.3	2.87 d (13.5)	18'	11.2	0.93 t (7)
		3.68 d (13.5)	19'	27.4	1.34 dq (14, 7)
*N*H		7.84 br s			1.46 dq (14, 7)
OMe	49.8	2.46 s	20'	35.2	1.64 m
C=O	171.4	-	21'	56.0	4.41 br s
*N*Me	42.2	2.56 s	*N*H'	-	7.96 br s
			OMe	52.7	3.61 s
			C=O	172.7	-
			10'-OMe	55.9	3.98 s

[a] Assignments based on COSY, HMQC, HMBC, and NOE.

At this stage it can be deduced that conodusarine is constituted from union of an unsubstituted vobasinyl and a 3'-oxo-10'-methoxyibogan unit {or 3-oxovoacangine (**3**)}, with a C(3)-C(11') bond linking the two monomeric moieties, which is in fact the 3'-oxo derivative of the known bisindole, voacamine. This is confirmed by a comparison of the NMR spectral data of conodusarine (**2**) with that of voacamine.[27] The stereochemistry at C(3) of the vobasinyl unit can be determined from a combination of two observations. Firstly, the signal for H(3) is a doublet with *J ca.* 11 Hz, requiring H(3) and one of the H(14) to be *trans*-diaxial. Furthermore, irradiation of H(3) results in NOE enhancement of NH and *vice versa*, requiring these two hydrogens to be in mutual proximity.[27] These two observations are only satisfied in the case where H(3) has β stereochemistry, since in the alternative arrangement in which the stereochemistry of H(3) is α, the conformation adopted by the central ten-membered ring, in order that one H(14) is *trans* to H(3), would result in H(3) pointing into the concave face of the middle ring, and therefore away from NH, in which case NOE between NH and H(3) would have been impossible.[14] The relative configuration of the iboga unit, as well as the stereochemistry at C(20'), are presumed to be similar to that of 3-oxovoacangine (**3**)[28] (see experimental section) and voacamine,[27] by comparison of the NMR spectral data of **2** with these compounds. Since all three compounds occur in the plant, such a conclusion would also be reasonable from a biogenetic

viewpoint, on the assumption that **2** is derived either from oxidation of voacamine or from coupling of the appropriate vobasinyl precursor with 3-oxovoacangine. From the above considerations, the structure and relative configuration of the new bisindole is as shown in **2**.

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. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz respectively. ESIMS was obtained on a Perkin Elmer API 100 instrument. EIMS and HREIMS measurements were carried out at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania.

Plant Material. Details of collection, identification, and deposition of voucher specimens have been reported previously.[18]

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 HCl as has been described in detail elsewhere.[29] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Initial chromatography of the basic fraction from the stem-bark provided essentially 11 fractions. Rechromatography of fraction 8, using MeOH/$CHCl_3$, followed by successive centrifugal TLC (EtOAc-petroleum ether, 1:1, NH_3-saturated; EtOAc) gave compound **2** (yield 6.5 mg kg^{-1}).

Conodusarine (**2**): Light yellowish oil; $[\alpha]_D$ +19^o (*c* 0.26, $CHCl_3$). UV (EtOH) λ_{max} (log ε) 223 (4.77), 286 (4.31), 295 (4.30), 312 (4.01) nm. IR (dry film) υ_{max} 3388, 3254, 1727, 1664 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR data, see Table 1. EIMS *m/z* 718 $[M]^+$ (20), 523 (15), 194 (7), 182 (26), 180 (15), 149 (3), 122(12). HREIMS *m/z* 718.3747 (calcd for $C_{43}H_{50}N_4O_6$, 718.3730).

3-Oxovoacangine (**3**): Light yellowish oil; $[\alpha]_D$ -52^o (*c* 0.05, $CHCl_3$). UV (EtOH) λ_{max} (log ε) 222 (4.17), 282 (3.67), 299 (3.56), 311 (3.35) nm. IR (dry film) υ_{max} 3279, 1732, 1662 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$, Me_4Si) δ_H 0.98 (3H, t, *J* = 7.5 Hz, H-18), 1.39 (1H, ddt, *J* = 13, 8, and 2 Hz, H-15), 1.42 (1H, dq, *J* = 14 and 7.5 Hz, H-19), 1.53 (1H, dq, *J* = 14 and 7.5 Hz, H-19), 1.74 (1H, m, H-20), 1.99 (1H, ddd, *J* = 13, 10, and 3 Hz, H-15), 2.30 (1H, ddd, *J* = 13, 4, and 3 Hz, H-17), 2.61 (1H, m, H-14), 2.64 (1H, dd, *J* = 13 and 1.5 Hz, H-17), 3.17 (2H, m, 2 x H-6), 3.22 (1H, dt, *J* = 13 and 4.5 Hz, H-5), 3.72 (3H, s, CO_2Me), 3.84 (3H, s, 10-OMe), 4.49 (1H, ddd, *J* = 13, 4, and 2.5 Hz, H-5), 4.50 (1H, br s, H-21), 6.80 (1H, dd, *J* = 8.5 and 2.5 Hz, H-11), 6.91 (1H, d, *J* = 2.5 Hz, H-9), 7.13 (1H, d, *J* = 8.5 Hz, H-12), and 7.92 (1H, br s, NH). ^{13}C-NMR (100 MHz, $CDCl_3$, Me_4Si) δ_C 11.3 (C-18), 21.2 (C-6), 27.6 (C-19), 31.0 (C-15), 35.4 (C-20), 35.9 (C-17), 38.2 (C-14), 42.7 (C-5), 53.0 (CO_2Me), 55.6 (C-16), 56.0 (10-OMe), 56.1 (C-21), 100.5 (C-9), 109.1 (C-7), 111.3 (C-12), 112.5 (C-11), 128.2 (C-8), 130.9 (C-13), 134.7 (C-2), 154.2 (C-10), 173.0 (C-3), and 175.8 (CO_2Me). ESIMS *m/z* 383 $[MH]^+$ ($C_{22}H_{26}N_2O_4$ + H).

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