Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1693–1696

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

CONODIPARINES A-D, NEW BISINDOLES FROM *TABERNAEMONTANA*. REVERSAL OF VINCRISTINE-RESISTANCE WITH CULTURED CELLS

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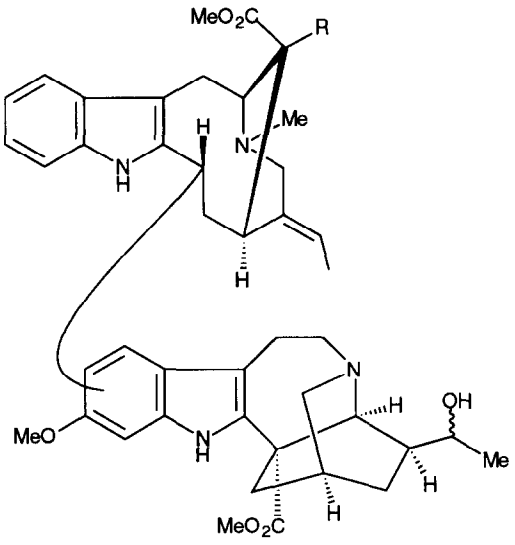
Received 20 April 1998; accepted 28 May 1998

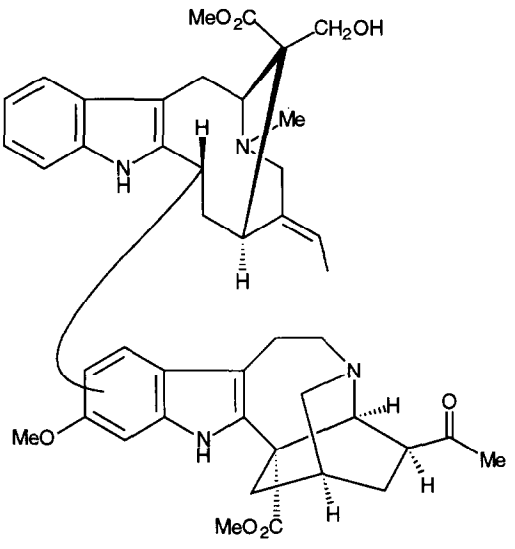
Abstract: Four new bisindoles of the vobasine-iboga type, conodiparines A-D were obtained from *Tabernaemontana corymbosa* which showed appreciable activity in reversing resistance in vincristine-resistant KB cells.

In continuation of our ongoing studies on bioactive principles from Malaysian plants,[1-4] we investigated the alkaloidal composition of *Tabernaemontana corymbosa* and wish to report the isolation of four new bisindoles, conodiparines A-D, which showed potential for reversal of multidrug resistance in vincristine-resistant KB cells.

The four new bisindoles (**1-4**) were obtained from the leaves. All four compounds showed UV spectra which are characteristic of indole chromophores (*e.g.*, 227, 286, 296 nm for **1**). The mass-spectra of all four compounds showed fragments at *m/z* 180, 136, 124 and 122 which are characteristic of vobasine-iboga bisindoles[5,6] and in addition, the fragment at *m/z* 367 due to the intact vobasinyl fragment was common in the mass-spectra of **1-4**, suggesting that they share a common vobasinyl monomeric moiety. Conodiparine A **1**, was obtained as an amorphous powder, $[\alpha]_D$ -34° ($CHCl_3$, *c* 0.05). The EIMS of **1** showed a molecular ion at *m/z* 750 and HRMS measurements gave the exact mass of the M^+ ion as 750.3991 corresponding to the molecular formula $C_{44}H_{55}N_4O_7$ (calcd M^+, 750.3993). The IR spectrum showed bands due to NH/OH (3388 cm^{-1}) and ester (1722 cm^{-1}) functions. The ^{13}C NMR spectrum showed a total of 44 separate resonances, in agreement with the formula derived from the HRMS. Examination of the ^1H and ^{13}C NMR spectra with the aid of COSY, HMQC and HMBC confirmed the presence of vobasinyl and iboga units. Thus the ^1H NMR spectrum of **1** (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

0960-894X/98/$19.00
PII: S0960-894X(98)00294-7





1 R = CH_2OH, 19'(*S*); C(3) - C(10') bond
2 R = CH_2OH, 19'(*S*); C(3) - C(12') bond
5 R = H, 19'(*R*); C(3) - C(10') bond
6 R = H, 19'(*R*); C(3) - C(12') bond

3 C(3) - C(10') bond
4 C(3) - C(12') bond

(vobasinyl), an ethylidene (vobasinyl), a hydroxymethyl (vobasinyl), and a hydroxyethyl group (iboga). The ester methyl associated with the vobasinyl unit is unusually shielded (δ 2.38) 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 5.14 (*J* 13 Hz) and the attachment of this carbon (3) to the aromatic C(10') is confirmed by the observed two and three bond correlations from C(10') to H(3) and C(3) to H(9') respectively in the HMBC spectrum. The placement of the methoxy substituent at C(11') of the iboga unit is supported by comparison of the aromatic carbon resonances with that of those in related vobasine-iboga bisindoles with similar substitution and branching such as conoduramine. Further proof of this is provided by the observed correlation from C(11') to H(9') in HMBC. The NMR spectral data in fact resemble that of the bisindole, 19'(*R*)-hydroxyconoduramine **5**,[7] recently isolated from *Tabernaemontana subglobosa* which has a similar mode of branching of the monomeric entities [C(3) to C(10') connection], except for the presence of a hydroxymethyl function at C(16) of the vobasinyl unit in **1**. The configuration of 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 allkaloid, 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.[7,8] Conodiparine B **2**, is readily shown to be constituted from the same monomeric units as in **1** from the spectral data. The difference from **1** is in the mode of branching of the monomeric units, which in **2** is from C(3) of the vobasinyl unit to C(12') of the iboga unit, as in the related compound 19'(*R*)-hydroxy-

conodurine **6**.[7] This is clearly shown by the aromatic-H resonances of the iboga unit which now appear as a pair of AB doublets at δ 7.25 and 6.83. Compounds **3** and **4** are C(19') oxo-analogues of **1** and **2** respectively. The IR spectra of **3** and **4** showed an additional carbonyl band at 1714 cm^{-1} due to the ketonic function and the mass spectra of these two compounds lack the (M - H_2O) fragment observed in **1** and **2**. The presence of the ketonic function at C(19') is also corroborated by the resonance at δ 208 in the ^{13}C NMR spectra of **3** and **4** in place of the oxymethine of **1** and **2**.

Table 1. ^1H and ^{13}C NMR Spectral Data for conodiparine A **1**[a]

Position	δ_C	δ_H	Position	δ_C	δ_H
2	137.8	-	2'	134.4	-
3	36.8	5.14 br d(13)	3'	51.1	2.73 br d (9); 2.91 m
5	60.1	3.92 br t (9)	5'	52.0	2.91 m; 3.28 m
6	17.3	3.28 m; 3.61 br t (11)	6'	21.4	2.80 m; 3.02 m
7	110.5	-	7'	109.8	-
8	130.0	-	8'	122.1	-
9	117.4	7.55 d (7)	9'	117.8	6.85 s
10	118.7	7.05 m	10'	127.6	-
11	121.5	7.05 m	11'	153.4	-
12	109.5	7.05 m	12'	92.7	6.81 s
13	136.0	-	13'	134.7	-
14	36.7	2.00 m; 2.63 m	14'	26.7	1.98 br s
15	35.8	3.52 m	15'	22.8	1.51 br t (12); 1.87 m
16	52.8	-	16'	53.9	-
17	70.5	*ca.* 3.70; *ca.* 3.70	17'	36.7	1.87 m; 2.52 m
18	12.0	1.65 d (6)	18'	20.2	1.06 d (6)
19	119.6	5.37 q (6)	19'	71.2	4.10 br q (6)
20	136.7	-	20'	39.4	1.40 m
21	51.9	2.96 d (14); 3.59 d (14)	21'	59.6	3.73 br s
CO_2Me	50.0	2.38 s	CO_2Me'	52.8	3.68 s
$\underline{C}O_2Me$	174.1	-	$\underline{C}O_2Me'$	174.9	-
*N*Me	41.9	2.56 s	11'-OMe	55.8	3.96 s
NH	-	7.59 br s	NH'	-	7.72 br s

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC, and HMBC.

Table 2. Cytotoxic activity of compounds **1-4**[a]

Compound	IC_{50}(μg/mL) (KB/S)	(KB/VJ300)	(KB/VJ300)*
1	19.2	13.5	1.45
2	20.8	15.0	2.45
3	21.4	17.0	5.60
4	18.6	13.6	4.60
Kopsoffinol	42	16.4	14.5

[a]KB/S and KB/VJ300 are vincristine-sensitive and -resistant human oral epidermoid carcinoma cell lines respectively;[9]

* with added vincristine 0.25 μg/mL, which did not affect the growth of the KB/VJ300 cells

The four dimeric compounds were screened for their potential in reversing multidrug resistance in vincristine-resistant KB cells and the results are presented in Table 2, which also shows the results for kopsoffinol, a bisindole of the eburnane-aspidofractinine type from *Kopsia* species[10] which we obtained from *Kopsia dasyrachis*.[11] The IC_{50} values of vincristine against sensitive (KB/S) and resistant (KB/VJ300) strains[9] are 0.014 and 1.05 μg/mL respectively in the present experiments. As shown in Table 2, compounds **1-4** are only weakly cytotoxic to both sensitive or resistant KB cells but show appreciable cytotoxicity when applied in the presence of vincristine. Among the four compounds, the highest level of activity in reversing vincristine resistance was shown by **1** while the bisindole kopsoffinol in contrast is completely inactive. This property of the vobasine-iboga type bisindole in reversing multidrug resistance is not unprecedented and has been very recently demonstrated in the known bisindole, conoduramine, which was observed to reverse resistance in vinblastine-resistant KB cells.[12] We shall report in a forthcoming full disclosure, structure elucidation of other bisindoles obtained from this plant, as well as comparison of their relative efficacy in reversing multidrug resistance.

Acknowledgement

We thank the University of Malaya and IRPA (Malaysia) and Grant-in-Aid (Ministry of Education, Science and Culture, Japan) for support of this work, Dr. J. K. MacLeod, Research School of Chemistry, Australian National University, for HRMS measurements, and Professor M. Kuwano, Department of Biochemistry, School of Medicine, Kyusyu University, for kind supply of the KB cell lines.

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