NEW BISINDOLE ALKALOIDS FROM PETCHIA CEYLANICA

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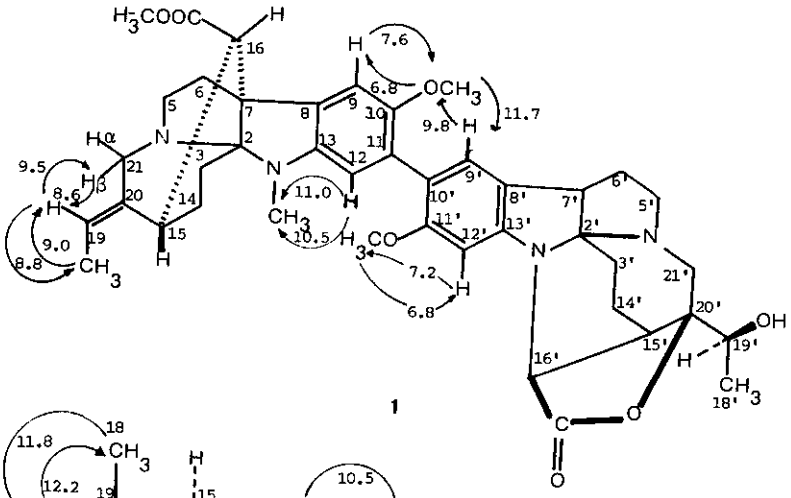
Abstract - Two new bisindole alkaloids, ceylanine (1) and ceylanicine (2) have been isolated from Petchia ceylanica and their structures have been established on the basis of spectroscopic techniques.

Petchia ceylanica (Apocynaceae) is a plant found in the lowlands of Sri Lanka. A number of bisindole alkaloids having vincorine-type moieties linked together have been previously reported from the plant[1,2]. As a result of isolation and structural studies, two new bisindole alkaloids, ceylanine (1) and ceylanicine (2) have been isolated from the leaves and the stem of the plant in 0.022% and 0.0258% yields (based on dried plant material) respectively. Ceylanine represents the first bisindole alkaloid having a vincorine-type unit linked to a plelocarpamine skeleton through a bond between the benzene rings of the two moieties.

Ceylanine (1) $[\alpha]_D^{26}$ (MeOH) = -124^o was isolated as a pale-coloured pink amorphous solid, susceptible to light. It showed λ_{max} (MeOH) 265, 300 and 330 nm in its uv spectrum. The ir spectrum (KBr) showed absorptions at 3400 cm^{-1} (OH), 1768 (C=O) and 1735 cm^{-1} (C=O). The mass spectrum exhibited a strong M^+ at m/z 722.3592 corresponding to the formula $C_{42}H_{50}N_4O_7$ (calcd. 722.3584, confirmed by FAB mass spectroscopy[3]). A prominent peak at m/z 694 ($C_{40}H_{46}N_4O_7$) corresponded to the loss of ethylene by a retro-Diels Alder cleavage of ring D[2], probably from the vincorine moiety. The remainder of the mass spectrum was uncharacteristic. The ^{1}H-nmr spectrum (300 MHz) of (1) showed a 3H singlet for the N-methyl group at δ 2.72, a 3H singlet for the ester methyl protons at δ 3.64, two 3H singlets for the six methoxy protons at δ 3.72 and 3.82, and four 1H singlets for the aromatic protons at δ 7.55, 6.90, 6.19 and 6.14 which were assigned to C-9', C-12, C-9 and C-12' protons respectively,



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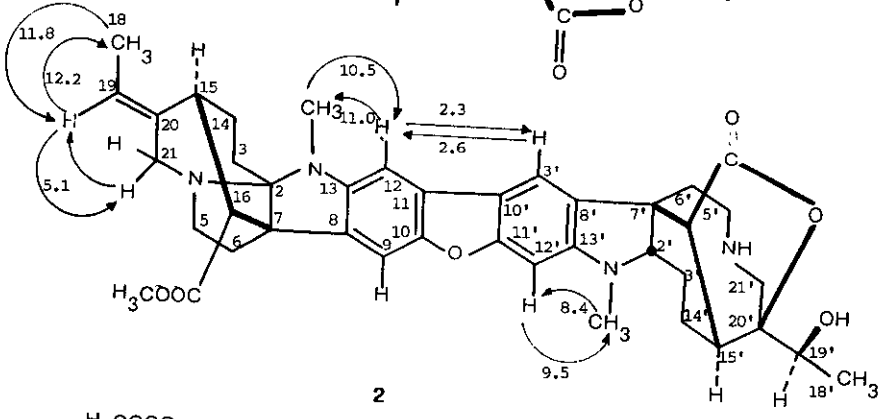

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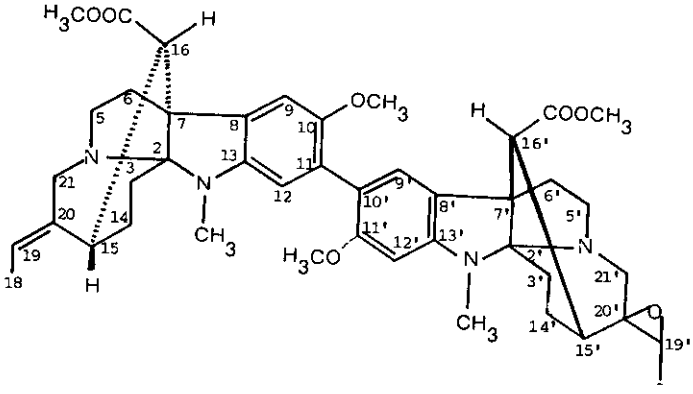

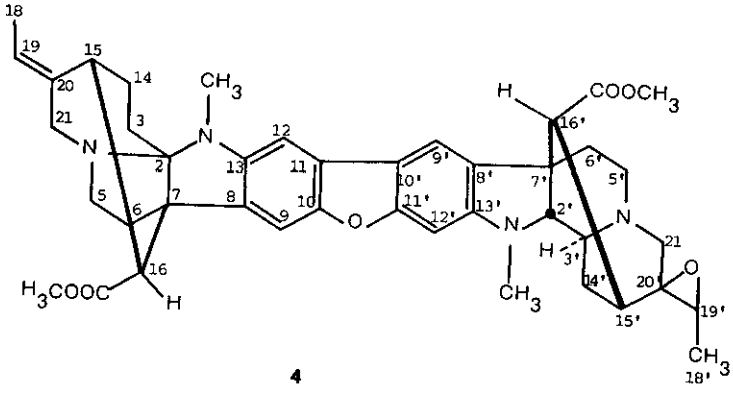

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the lack of splitting and the chemical shifts indicating that substituents were present at 10,10', 11 and 11' positions. A 3H double doublet at δ 1.59 (vinylic methyl at C-18) showed geminal coupling with the olefinic proton at C-19 ($J_{18,19}$= 6.8 Hz) and allylic coupling with the C-21 proton ($J_{18,21\beta}$= 1.0 Hz). A 3H split doublet at δ1.18 ($J_{18,19'}$= 6.30 Hz) was assigned to the 18'-methyl protons. The two doublets at δ 1.59 and 1.18 showed spin-spin coupling in the COSY-45 spectrum with quartets at δ 5.28 ($J_{19,18}$= 6.80 Hz) and 3.99 ($J_{19',18'}$= 6.30 Hz) respectively. The latter were assigned to 19 and 19' protons respectively. A double doublet at δ 3.75 assigned to the C-21β proton ($J_{21\beta,21\alpha}$= 14.5 Hz, $J_{21\beta,18}$ = 1.0 Hz) was coupled with a doublet at δ 2.98 (21αH, $J_{21\alpha,21\beta}$= 14.5 Hz), corresponding to the C-21α proton. The C-21'β and C-21'α protons resonated as doublets at δ 3.23 and 2.96 respectively ($J_{21'\alpha,21'\beta}$ = 15.5 Hz). A one-proton split doublet at δ 4.68 ($J_{16',15'}$= 6.0 Hz) was characteristic for the C-16'H. The upfield chemical shift for C-16'H established it to be β-oriented (appeared at 5.41 when α)[4-7]. The ^{13}C-nmr spectrum of (1) exhibited eleven methine, ten methylene and six methyl carbons (DEPT)[8]. Comparison of the ^{13}C-nmr spectrum of ceylanine (1) with that of peceylanine[1] (3) strongly indicated the occurrence of a common vincorine[9] moiety in it from the correspondence of chemical shifts and identity of signal multiplicities in both compounds (Table-1). The remaining signals for the second moiety represented five methylene, six methine and two methyl carbons (DEPT) but lacked an N-methyl group. The chemical shifts and multiplicities of these signals suggested that the second moiety of ceylanine possessed a pleiocarpamine-type skeleton[7]. The downfield chemical shift of C-16' at δ 59.7 (also supported by the ^{1}H-nmr spectrum : doublet at δ4.68 for C-16'H) suggested that C-16' is linked with N_a, as in pleiocarpamine and related compounds[4-7]. A quaternary carbon signal at δ 96.6 was characteristic for C-2' which is bonded to two nitrogen atoms[9], its low field position indicating the presence of isodihydropleiocarpamine-type unit[11]. The downfield chemical values of C-19', C-15' and C-20' at δ 69.44, 45.75 and 90.5 (in comparison to corresponding carbons in peceylanine 63.1, 38.9 and 63.8 respectively) indicated the formation of a γ-lactone ring system. This unit can be formed by linkage of C-16' with N_a in ceylanine (1) (instead of linkage with C-7' in peceylanine) followed by hydrolytic cleavage of the oxiran ring and lactone formation with the ester at C-16'. The carbonyl carbon of the lactone resonated at δ177.40. The presence of a γ-lactone was also supported by its ir spectrum (KBr) which showed absorption at δ1768 cm^{-1}. The stereochemistry of ethylidene side chain was established by NOE measurements. Irradiation of H-19 at δ 5.28 caused enhancement of H-21β at δ 3.75. Similarly irradiation of 18-H at δ 1.59 enhanced the olefinic proton at δ19-H at δ 5.28, suggesting E-stereochemistry at C-19. The R-configuration of the hydroxyl group at C-19' was established by Horeau's method[1]. On the basis of these data

ceylanine was assigned structure (**1**).

Ceylanicine (**2**) was isolated as a light yellow-coloured amorphous solid, $[\alpha]_D^{26}$ (MeOH) = -98° from the extracts of the stem of Petchia ceylanica. It showed λ_{max} (MeOH) 245, 276, 355 nm in its uv spectrum, suggesting a dihydroindole skeleton with N-C-N linkage[1,3,9,12]. The ir spectrum (KBr) showed absorption at 3400 cm^{-1} (OH), 1720 cm^{-1} (ester carbonyl) and 1765 cm^{-1} (γ-lactone). Its molecular ion peak (M^+) occurred at m/z 692.3499 (calcd. for $C_{41}H_{48}N_4O_6$, 692.3492) which was confirmed by FAB mass spectroscopy[3]. Other major peak appeared at m/z 664, 524, 496 and 495. The mass fragmentation pattern was found to be almost identical to that of desmethylpeceyline, reported earlier[2]. Such pseudoakuammiline-type alkaloids e.g. corymine[13] exhibit a prominent retro Diels-Alder cleavage by opening of ring D. Its ^{1}H-nmr spectrum (300 MHz, $CDCl_3$) exhibited four 1H singlets at δ 7.78, 7.74, 6.71 and 6.65, assigned to the aromatic protons at C-9', C-12, C-9 and C-12' respectively. This indicated a similar substitution pattern in ceylanicine as in other dimers isolated from Petchia ceylanica[1,2]. The C-18 ethylidene methyl protons appeared as a split double doublet at δ 1.65 ($J_{18,19}$= 6.8 Hz, $J_{18,21\beta}$= 1.0 Hz), which showed vicinal coupling with the quartet at δ 5.41 for C-19H ($J_{19,18}$= 6.8 Hz).

The E-stereochemistry of ethylidene side chain was established by carrying out NOE difference measurements on H-19, H-18 and H-21β. Irradiation of the H-19 proton at δ 5.41 enhanced H-18 and H-21β protons whereas when H-18 at δ 1.65 was irradiated it enhanced H-19 at δ 5.41. The C-18' methyl protons resonated as a doublet at δ 1.29 ($J_{18',19'}$= 6.36 Hz), being coupled with the C-19' protons which resonated as a quartet at δ 3.69 ($J_{19',18'}$= 6.36 Hz). The R-configuration of the hydroxyl group at C-19' was established by Horeau's method[2]. Two N-methyl singlets appeared at δ 2.73 and 2.61, while a 3H singlet at δ 3.78 was assigned to the carbomethoxy methyl protons.

The ^{13}C-nmr spectrum ($CDCl_3$, 75 MHz) indicated the presence of ten methine, ten methylene, five methyl and thirteen quaternary carbon atoms in addition to an ester carbonyl and a γ-lactone carbonyl group (Table 1). Correlation of the carbon chemical shifts of alkaloid (2) with the known bisindole alkaloid pelankine[1] (4) indicated the presence of a common vincorine[10] moiety in both alkaloids. The ^{1}H and ^{13}C-nmr data indicated the attachment of the two moieties to form the common unsymmetrically substituted dibenzofuran system found in both ceylanicine (**2**) and pelankine (**4**). The other moiety in ceylanicine showed four methine, five methylene and two methyl carbon signals (DEPT). A downfield methine at δ 78.69 for C-2' indicated β-confi-

guration of the proton at this centre (in α-configuration C-2' resonates at δ 70.6)[14]. The N-methyl carbon was deshielded by ca 6 ppm in comparison to other related alkaloids, a feature found when C-2' is tertiary[9]. As in ceylanine (1), the chemical shifts of C-19', C-15', C-20' in ceylanicine (2) appeared downfield at δ 68.72, 45.91, 87.1 respectively in comparison to pelankine (4). This is attributed to the formation of a γ-lactone ring in the molecule by opening of the oxiran ring between C-19' and C-20' and subsequent cyclization with the C-16' ester group. On the basis of these studies, structure 2 is proposed for ceylanicine. The akuammiline-type moiety in ceylanicine does not have a bond between N_b and C-3', the C-3' lacks any oxygen substituent and there is no unsaturation between 2',3' or between 3',14' positions. Ceylanicine is therefore unique in this respect. It may arise in nature by the reductive removal of an oxygenated functionality at C-3'.

TABLE-1

Carbon	(1)	(3)	(2)	(4)	Carbon	(1)	(3)	(2)	(4)
2	97.6[c]	97.2	97.8	97.5	2'	96.6[c]	96.3	78.7	96.3
3	40.5[c]	41.4	40.6	41.3	3'	40.3[c]	40.7	29.9	53.8
5	54.6[b]	54.8	53.9	54.9	5'	48.0[b]	54.8	50.2	49.9
6	20.4[a]	20.4	20.5	20.6	6'	20.3[b]	21.2	33.4	33.1
7	58.3[a]	57.2	57.0	57.1	7'	57.1[a]	56.0	41.0	42.5
8	135.5	135.6	136.9	135.1	8'	135.0	127.6	134.8	135.8
9	108.5	108.5	107.4	107.4	9'	127.3	126.2	111.6	112.5
10	148.9	149.2	149.7	149.3	10'	116.1	115.7	116.8	116.3
11	127.2	127.3	123.5	123.5	11'	155.3	156.8	156.6	156.7
12	111.25	110.4	95.0	94.1	12'	95.6	89.9	93.7	93.1
13	145.7	143.2	145.6	145.4	13'	148.6	149.2	152.6	152.5
14	26.0	26.1	26.9	26.3	14'	33.3	24.9	33.8	33.4
15	34.4	34.5	34.6	34.7	15'	45.7	38.9	45.9	39.3
16	50.7	50.7	50.2	51.1	16'	59.7	50.5	48.6	47.0
18	13.3	13.4	13.5	13.5	18'	16.8	14.4	17.2	15.8
19	122.6	121.7	122.0	122.0	19'	69.4	63.1	68.7	65.5
20	138.5	138.0	138.9	139.0	20'	81.1	63.8	81.7	65.8
21	60.0	58.2	58.2	58.3	21'	57.8	56.8	54.8	55.7
N-Me	27.7	27.7[e]	27.2	27.8[f]	N-Me	-	27.1	34.4	33.8[f]
OCH_3	51.4	51.3[e]	51.6	51.5[f]	$-OCH_3$	-	51.3[e]	-	51.4[f]
$Ar-OCH_3$	55.7	55.5	-	-	$Ar-OCH_3$	57.3	57.5[d]	-	-
C=O	173.6	173.5	173.4	173.5	C=O	177.4	173.3	177.5	173.1

a,b,c,d,e,f : These assignments may be reversed although given here are preferred.

EXPERIMENTAL

Mp was recorded in air bath-type melting point apparatus, spectra were recorded on Pye-Unicam sp-800 UV spectrometer, Jasco-IR-I spectrometer MAT 112 and 312 double focussing mass spectrometer connected to PDP 11/34 computer system and Bruker AM 300 NMR instruments.

Isolation of ceylanine

Ceylanine was obtained from the leaves of Petchia ceylanica collected from Kekutera district,

Sri Lanka. Initial extraction of ceylanine was done by the procedure described earlier[2,11]. Ceylanine was isolated from the fraction obtained by eluting with $CHCl_3$-MeOH (7:3). The bases present in this fraction were subjected to thin layer chromatography (silica gel, $CHCl_3$-MeOH 5:1) to yield ceylanine as major alkaloid.

Ceylanine was isolated as a pale-coloured pink amorphous material, mp 280°C (dec.), $[\alpha]_D^{26}$ (CH_3OH) = -124°, uv (MeOH) λ_{max} (log ε) nm, 265 (4.39), 300 (3.29) and 330 (3.09), ir (KBr) ν_{max} (cm^{-1}): 3400 (OH), 1768 (C=O) and 1735 (C=O), ms m/z (EI, FAB, rel.int. %), 722,3592 (100%) and 644(8%), ^{1}H-nmr ($CDCl_3$ + few drops of CD_3OD) δ: 1.18 (3H, d, $J_{18,19}$ = 6.30 Hz, H-18'), 1.59 (3H, dd, $J_{18,19}$= 6.8 Hz, $J_{18,21\beta}$ = 1.0 Hz, H-18), 2.72 (3H, s, N-Me), 2.96 (1H, d, $J_{21'\alpha,21'\beta}$= 15.5 Hz, H-21'α), 2.98 (1H, d, $J_{21\alpha,21\beta}$= 14.5 Hz, H-21α), 3.23 (1H, d, $J_{21'\beta,21'\alpha}$ = 15.5 Hz, H-21'β), 3.64 (3H, s, OCH_3), 3.72 (3H, s, Ar-OCH_3), 3.75 (1H, dd, $J_{21\beta,21\alpha}$ = 14.5 Hz, $J_{21\beta,18}$= 1.0 Hz, H-21β), 3.82 (3H, s, Ar-OCH_3), 3.99 (1H, q, $J_{19',18'}$ = 6.30 Hz, H-19'), 5.28 (1H, q, $J_{19,18}$= 6.8 Hz, H-19), 6.14 (1H, s, H-12'), 6.19 (1H, s, H-9), 6.90 (1H, s, H-12), 7.55 (1H, s, H-9'). ^{13}C-nmr ($CDCl_3$ + few drops of CD_3OD) : Table-1.

Isolation of ceylanicine

Ceylanicine was isolated from the ethanolic extract of the air dried stem of Petchia ceylanica. The ethanolic extract was evaporated to a gum (4.1 kg), and the total alkaloids obtained by extraction into 5% HCl (10 lit.) from $CHCl_3$. The aqueous layer was then basified to pH-9 with NH_3, extracted again with $CHCl_3$ (25 lit.) and dried over anhydrous Na_2SO_4. Evaporation of this concentrated $CHCl_3$ extract yielded crude alkaloidal fraction (100 g). The crude alkaloids were chromatographed on a silica gel column (2.0 kg). The column was successively eluted with increasing polarities of pet.ether, chloroform, acetone and methanol. The ceylanicine containing fraction was eluted with pet ether-chloroform (3:1) and ceylanicine was then purified by preparative TLC on silica gel using chloroform and pet.ether, 3:1, with a few drops of diethylamine.

Ceylanicine was isolated as light yellow-coloured amorphous solid, mp 250°C (dec.), $[\alpha]_D^{26}$ (CH_3OH) = -98°, uv (MeOH) λ_{max} (log ε) nm; 245 (435), 270 (4.03), 355 (3.39), ir KBr ν_{max} (cm^{-1}); 3400 (-OH), 1765 (C=O), 1720 (C=O). ms m/z (EI, FAB, rel.int. %): 692.3499 (18%), 6.64 (12%), 524 (6%), 496 (12%) and 495 (10%), ^{1}H-nmr ($CDCl_3$) δ: 1.29 (3H, d, $J_{18',19'}$= 6.36 Hz, H-18'), 1.65 (3H, dd, $J_{18,19}$= 6.8 Hz, $J_{18,21\beta}$ = 1.0 Hz, H-18), 2.61 (3H, s, N-Me), 2.73 (3H, s, N-Me), 3.69 (1H, q, $J_{19',18'}$= 6.36 Hz, H-19'), 3.78 (3H, s, OCH_3), 5.41 (1H, q, $J_{19,18}$= 6.8 Hz, H-19), 6.65 (1H, s, H-12'), 6.71 (1H, s, H-9), 7.74 (1H, s, H-12), 7.78 (1H, s, H-9'), ^{13}C-nmr ($CDCl_3$) : Table-1.

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