Naucleamides A—E, New Monoterpene Indole Alkaloids from *Nauclea latifolia*

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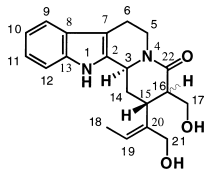
Five new monoterpene indole alkaloids, naucleamides A—E (1—5), were isolated from the bark and wood of *Nauclea latifolia*, and the structures and relative stereochemistry were elucidated from the spectroscopic data. Naucleamide E (5) is a unique monoterpene indole alkaloid possessing a pentacyclic ring system with an amino acetal bridge.

Key words monoterpene indole alkaloid; naucleamide; *Nauclea latifolia*; spectroscopic data; amino acetal bridge

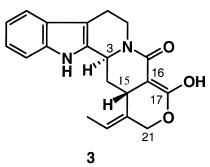
A number of monoterpene indole alkaloids such as nauclefine[1] and naulafine[2] have been isolated from plants of *Nauclea* species (Rubiaceae). Recently, several monoterpene indole alkaloids have been isolated from *Nauclea orientalis* which have interesting biological activity.[3,4] In our search for structurally unique constituents from higher plants, five new monoterpene indole alkaloids, naucleamides A—E (**1**—**5**), have been isolated from the bark and wood of *Nauclea latifolia*.[1,2] Nauleamide E (**5**) is a monoterpene indole alkaloid possessing a pentacyclic ring system with an amino acetal bridge. In this paper we describe the isolation and structural elucidation of **1**—**5**.

The bark and wood of *Nauclea latifolia* (Rubiaceae) were extracted with MeOH. The MeOH extracts were partitioned between hexane and 90% aqueous MeOH, and then the MeOH layer was partitioned with *n*-BuOH and H_2O. The *n*-BuOH-soluble portions were subjected to a silica gel column ($CHCl_3$–MeOH, 8 : 1) and then a C_{18} column (MeOH–H_2O, 1 : 1) to afford an alkaloidal fraction, which was purified by C_{18} HPLC (MeOH–H_2O, 7 : 3) to give naucleamides A (**1**, 0.0016%), B (**2**, 0.0016%), C (**3**, 0.0012%), D (**4**, 0.0012%), and E (**5**, 0.0008%) together with known related monoterpene indole alkaloids, strictosamide[5] (**6**, 0.15%), 10-hydroxystrictosamide[6] (**7**, 0.008%), and tetrahydrodesoxycordifoline[7] (**8**, 0.004%).

The molecular formula $C_{20}H_{24}N_2O_3$ of naucleamide A (**1**) was established by high resolution (HR)-FAB-MS [*m/z* 363.1687 $(M+Na)^+$, Δ −0.2 mmu]. The IR spectrum implied the presence of hydroxy (3427 cm^{-1}) and amide carbonyl (1611 cm^{-1}) groups. The UV spectrum of **1** was characteristic of an indole chromophore. The gross structure of **1** was deduced from detailed analysis of the ^1H- and ^{13}C-NMR data (Table 1) aided by two-dimensional (2D) NMR experiments (^1H–^1H correlation spectroscopy (COSY), ^1H-detected heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC)). The ^{13}C-NMR data indicated that the molecule possessed one lactam carbonyl, eight aromatic carbons, one trisubstituted olefin, two oxymethylenes, three methines, three methylenes, and one methyl group. The ^1H–^1H COSY spectrum revealed connectivities (Fig. 1) of C-5 to C-6, C-9 to C-12, C-3 to C-14, C-14 to C-17, and C-18 to C-19. HMBC correlations (Fig. 1) of H-3 to C-2 (δ_C 135.6), H-6 to C-7 (δ_C 111.2), H-9 to C-7, H-10 to C-8 (δ_C 129.3), and H-11 to C-13 (δ_C 138.8) indicated the presence of a tetrahydro-β-carboline ring (rings A, B, and C). The presence of a δ-lactam ring (ring D) with an oxymethylene (CH_2-17) at C-16 (δ_C 48.8) was elucidated by HMBC correlations of H_2-5 and H_2-17 to C-22 (δ_C 175.0). HMBC correlations of H-16, H_3-18, and H_2-21 to C-20 (δ_C 141.1) revealed the connectivity between C-15 (δ_C 33.0) and C-20 and the presence of a trisubstituted olefin with an oxymethylene at C-20 and a methyl group at C-19 (δ_C 126.9). Nuclear Overhauser effect spectroscopy (NOESY) correlations (Fig. 2) of H_3-18 to H-15 indicated that the geometry of the trisubstituted olefine was *E*. Thus the structure of naucleamide A was elucidated to be **1**. NOESY correlations of H-14a to H-16 and H-14b to H-15 and ^1H–^1H coupling constants ($J_{3,14a}$=5.9 Hz, $J_{3,14b}$=3.7 Hz, $J_{14a,15}$=11.1 Hz, and $J_{15,16}$=10.6 Hz) indicated a *trans* relationship between H-



1 H-16 α
2 H-16 β



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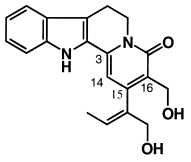

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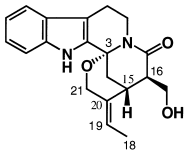

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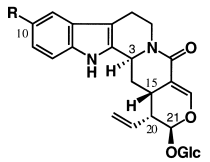
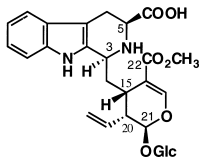


6 R = H
7 R = OH



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15 and H-16, α-orientations of H-3 and H-16, and a β-orientation of H-15. Therefore the relative stereochemistry of naucleamide A was assigned as **1**.

HR-EI-MS analysis of naucleamide B (**2**) revealed the molecular formula to be $C_{20}H_{24}N_2O_3$ [*m/z* 340.1782 (M^+), Δ −0.5 mmu], which was the same as that of naucleamide A (**1**). The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**, except for the signals around C-16. The ^{1}H–^{1}H COSY spectrum revealed connectivities of C-5 to C-6, C-9 to C-12, C-3 to C-14, C-14 to C-17, and C-18 to C-19. HMBC correlations of H_2-17 to C-22 (δ_C 174.1), H-16, H_3-18, and H_2-21 to C-20 (δ_C 140.1) indicated the presence of an oxymethylene (CH_2-17) at C-16 (δ_C 49.3) and a trisubstituted olefin connected to C-15 (δ_C 34.3) like **1**. The geometry of the trisubstituted olefine was estimated to be *E* by the NOESY correlation between H-15 and H_3-18. Thus the gross structure of **2** was elucidated to be the same as that of **1**. NOESY correlations of H-15 to H-14b and H-16 and ^{1}H–^{1}H coupling constants ($J_{3,14a}$=6.2 Hz, $J_{3,14b}$=4.1 Hz, $J_{14a,15}$=10.3 Hz, and $J_{15,16}$=5.4 Hz) indicated a *cis* relationship between H-15 and H-16, an α-orientation of H-3, and β-orientations of H-15 and H-16. Therefore naucleamide B (**2**) was assigned to be the 16-epimer of naucleamide A (**1**).

HR-EI-MS analysis of nucleamide C (**3**) revealed the molecular formula to be $C_{20}H_{20}N_2O_3$ [*m/z* 336.1482 (M^+), Δ +0.8 mmu]. The ^{1}H–^{1}H COSY spectrum revealed connectivities of C-5 to C-6, C-9 to C-12, C-14 to C-3 and C-15, and C-18 to C-19. HMBC correlations of H-3, H_2-5, and H-15 to C-22 (δ_C 166.6) indicated the presence of a δ-lactam ring (ring D), while HMBC correlations of H-15 to C-16 (δ_C 121.6) and C-17 (δ_C 172.5) revealed that **3** possessed an enol moiety at C-16. HMBC correlations of H-15 to C-21 (δ_C 74.4), H_3-18 to C-20 (δ_C 133.3), and H_2-21 to C-17 and C-20 indicated that ring D was fused to a dihydropyran ring (ring E) with a trisubstituted olefin (C-19 and C-20).[8] The geometry of the trisubstituted olefine was *E* by NOESY correlations of H-14b to H_3-18 and H-19 to H-21b. The ^{1}H–^{1}H coupling constants ($J_{3,14a}$=5.5 Hz, $J_{3,14b}$=3.7 Hz, $J_{14a,15}$=8.8 Hz, and $J_{14b,15}$=6.6 Hz) indicated an α-orientation of H-3 and a β-orientation of H-15. Therefore the structure and the relative stereochemistry of naucleamide C were assigned to be **3**.

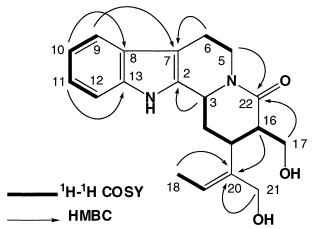

Fig. 1. ^{1}H–^{1}H COSY and Selected HMBC Correlations of Naucleamide A (**1**)

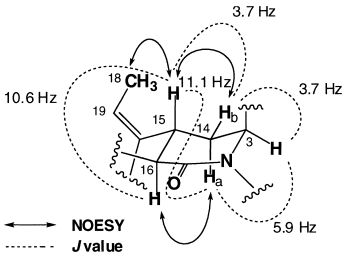

Fig. 2. Relative Stereochemistry of the δ-Lactam Ring of Naucleamide A (**1**)

Table 1. ^{1}H- and ^{13}C-NMR Data of Naucleamides A (**1**), B (**2**), and C (**3**) in CD_3OD

Position	**1**		**2**		**3**	
	^{1}H[a)]	^{13}C[a)]	^{1}H[a)]	^{13}C[a)]	^{1}H[a)]	^{13}C[a)]
1						
2		135.6		135.8		134.1
3	5.07 (m)	56.0	5.13 (dd, 6.2, 4.1)	56.1	5.05 (m)	54.8
4						
5(a)	4.98 (dd, 12.2, 5.0)	44.7	5.04 (dd, 12.8, 5.3)	44.4	5.04 (m)	43.4
(b)	3.10 (dt, 12.2, 4.2)		3.07 (ddd, 12.8, 12.4, 4.0)		3.08 (ddd, 12.6, 12.1, 4.3)	
6(a)	3.02 (m)	22.9	2.98 (m)	22.8	2.94 (m)	22.5
(b)	2.75 (m)		2.77 (m)		2.75 (dd, 15.4, 4.3)	
7		111.2		110.8		110.6
8		129.3		129.2		128.8
9	7.44 (d, 7.9)	119.5	7.45 (d, 7.9)	119.5	7.47 (d, 7.9)	119.3
10	7.03 (dd, 7.9, 7.2)	120.8	7.03 (dd, 7.9, 7.2)	120.6	7.05 (dd, 7.9, 7.8)	120.7
11	7.11 (dd, 8.1, 7.2)	123.2	7.11 (dd, 8.1, 7.2)	123.3	7.13 (dd, 8.1, 7.8)	123.3
12	7.34 (d, 8.1)	112.9	7.34 (d, 8.1)	113.0	7.36 (d, 8.1)	112.7
13		138.8		138.8		138.4
14(a)	2.60 (ddd, 14.1, 11.1, 5.9)	32.5	2.91 (ddd, 13.8, 10.3, 6.2)	29.9	2.50 (ddd, 14.3, 8.8, 5.5)	32.0
(b)	2.42 (dt, 14.1, 3.7)		2.42 (dt, 13.8, 4.1)		2.41 (ddd, 14.3, 6.6, 3.7)	
15	3.03 (m)	33.0	3.23 (m)	34.3	3.35 (m)	32.2
16	2.81 (ddd, 10.6, 5.2, 3.8)	48.8	2.61 (ddd, 5.4, 4.6, 3.8)	49.3		121.6
17(a)	3.91 (dd, 11.0, 3.8)	62.9	3.98 (dd, 11.3, 4.6)	62.9		172.5
(b)	3.59 (dd, 11.0, 5.2)		3.87 (dd, 11.3, 3.6)			
18	1.60 (d, 6.9)	14.0	1.60 (d, 6.9)	14.3	1.86 (d, 6.9)	14.6
19	5.75 (q, 6.9)	126.9	5.82 (q, 6.9)	127.6	5.87 (q, 6.9)	127.5
20		141.1		140.1		133.3
21(a)	4.15 (s)[b)]	64.8	4.24 (d, 12.7)	66.6	4.81 (d, 13.0)	74.4
(b)			4.12 (d, 12.7)		4.73 (d, 13.0)	
22		175.0		174.1		166.6

a) δ in ppm; *b*) 2H.

Table 2. ^{1}H- and ^{13}C-NMR Data of Naucleamides D (**4**) and E (**5**) in CD_3OD

Position	**4** ^{1}H[a)]	**4** ^{13}C[a)]	**5** ^{1}H[a)]	**5** ^{13}C[a)]
1				
2		127.4		135.0
3		139.4		84.2
4				
5(a)	4.50 (m)[b)]	43.0	4.99 (ddd, 12.8, 5.1, 1.4)	37.8
(b)			3.14 (ddd, 12.8, 12.2, 4.0)	
6(a)	3.19 (m)[b)]	21.2	2.86 (ddd, 15.5, 4.0, 1.4)	22.7
(b)			2.77 (ddd, 15.5, 12.2, 5.1)	
7		116.0		111.1
8		127.7		127.7
9	7.62 (d, 7.9)	121.2	7.51 (d, 7.7)	120.2
10	7.13 (dd, 7.9, 7.2)	121.6	7.05 (dd, 7.7, 7.3)	120.7
11	7.27 (dd, 8.1, 7.2)	126.2	7.16 (dd, 8.1, 7.3)	124.0
12	7.43 (d, 8.1)	113.6	7.37 (d, 8.1)	113.0
13		140.9		138.6
14(a)	6.56 (s)	104.2	2.63 (dd, 13.3, 4.7)	34.9
(b)			2.30 (dd, 13.3, 3.2)	
15		152.2	3.58 (dd, 4.7, 3.2)	30.5
16		127.4	2.58 (dd, 8.7, 4.3)	52.5
17(a)	4.65 (d, 12.0)	59.7	3.98 (dd, 11.0, 4.3)	64.3
(b)	4.41 (d, 12.0)		3.84 (dd, 11.0, 8.7)	
18	1.60 (d, 6.9)	15.4	1.77 (d, 6.8)	12.7
19	5.96 (q, 6.9)	125.7	5.54 (q, 6.8)	120.7
20		141.1		137.1
21(a)	4.30 (s)[b)]	67.8	4.30 (d, 13.7)	67.6
(b)			4.16 (d, 13.7)	
22		165.2		173.1

a) δ in ppm; *b*) 2H.

The molecular formula $C_{20}H_{20}N_2O_3$ of naucleamide D (**4**) was established by HR-EI-MS [*m/z* 336.1488 (M^+), Δ +1.4 mmu]. ^{13}C-NMR data (δ_C 104.2, 127.4, 139.4, 152.2 and 165.2) (Table 2) indicated the presence of a pyridone ring (ring D). HMBC correlations of H-14 to C-2 (δ_C 127.4), C-3 (δ_C 139.4), and C-16 (δ_C 127.4) revealed that ring C was fused to ring D, which was connected to an oxymethylene (C-17) at C-16 and a trisubstituted olefin at C-15 (δ_C 152.2) by HMBC correlations of H_2-17 to C-15 and C-22 (δ_C 165.2) and H-19 to C-15 and C-21 (δ_C 67.8) and H_2-21 to C-20 (δ_C 141.1). Therefore naucleamide D (**4**) was assigned to be 3,14,15,16-dehydronaucleamide A.

HR-EI-MS analysis of naucleamide E (**5**) revealed the molecular formula to be $C_{20}H_{22}N_2O_3$ [*m/z* 338.1631 (M^+), Δ +0.1 mmu]. The IR spectrum implied the presence of hydroxy (3433 cm^{-1}) and amide carbonyl (1633 cm^{-1}) groups. The UV spectrum of **5** was characteristic of an indole chromophore. The gross structure of **5** was deduced from detailed analysis of the ^{1}H- and ^{13}C-NMR data (Table 2) aided by 2D NMR experiments. The ^{13}C-NMR data indicated that the molecule possessed one lactam carbonyl, eight aromatic carbons, one trisubstituted olefin, one oxygenated quarternary carbon, two oxymethylenes, two methines, three methylenes, and one methyl group. The ^{1}H–^{1}H COSY spectrum revealed connectivities (Fig. 3) of C-5 to C-6, C-9 to C-12, C-14 to C-15, C-16 to C-17, and C-18 to C-19. HMBC correlations (Fig. 3) of H-5 and H_2-15 to C-3 (δ_C 84.2), H-15 and H_2-17 to C-22 (δ_C 173.1) revealed the presence of a δ-lactam ring (ring D) with an oxymethylene at C-16 (δ_C 52.5), while the presence of a trisubstituted olefin at C-19 and C-20 (δ_C 137.1) was indicated by HMBC correlations of H_3-18 to C-20, H-19 to C-15 (δ_C 30.5), and H_2-21 to C-19 (δ_C 120.7). The geometry of the trisubstituted olefin was estimated to be *E* by NOESY correlations (Fig. 4) of H-15 to H_3-18. The HMBC correlation of H_2-21 to C-3 indicated the presence of a tetrahydropyran ring (C-3, C-14, C-15, C-20, C-21, and O-3; ring E) fused to ring D. NOESY correlations of H-14b to H-17b, H-15 to H-17a, and H-16 to H-21a, and ^{1}H–^{1}H coupling constants ($J_{14a,15}$=4.7 Hz, $J_{14b,15}$=3.2 Hz, and $J_{15,16}$= <1.0 Hz) indicated both chair conformations of rings D and E, a *cis*-ring junction between rings D and E, an α-orientation of H-16, and a β-orientation of H-15. Therefore the structure and the relative stereochemistry of naucleamide E were assigned to be **5**.

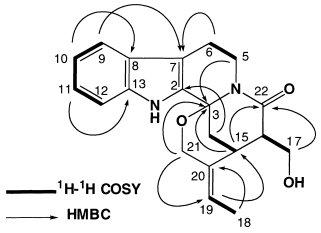

Fig. 3. ^{1}H–^{1}H COSY and Selected HMBC Correlations of Naucleamide E (**5**)

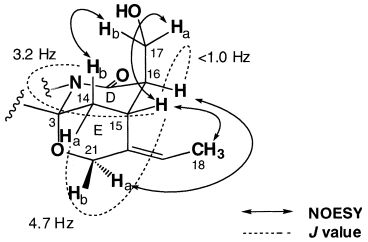

Fig. 4. Relative Stereochemistry of Rings D and E of Naucleamide E (**5**)

Naucleamides A—E (**1**—**5**) are new monoterpene indole alkaloids from *N. latifolia.* This type of monoterpene indole alkaloid is rare, although vallesiachotamine has been obtained from the Peruvian plant *Vallesia dichotoma*.[9] Nauleamide E (**5**) is a unique monoterpene indole alkaloid possessing a pentacyclic ring system with an amino acetal bridge. Biosynthetically, naucleamides A—E (**1**—**5**) may be derived from strictosamide (**6**) through reductive and/or oxidative cleavage of ring E.

Experimental

General Experimental Procedures Optical rotations were determined on a JASCO P-1030 polarimeter. UV and IR spectra were obtained on JASCO Ubest-35 and JASCO FT/IR-230 spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker ARX-500 and Bruker AMX-600 spectrometers. The 3.35 and 49.8 ppm resonances of residual CD_3OD were used as internal references for ^{1}H- and ^{13}C-NMR spectra, respectively. FAB mass spectra were measured on a JEOL HX-110 spectrometer using a glycerol matrix, and EI-MS was obtained on a FABmate spectrometer operating at 70 eV.

Plant Material The bark and wood of *N. latifolia* were collected at Calabar, Nigeria, in 2000. A voucher specimen has been deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University, Japan.

Extraction and Separation The leaves (100 g) were extracted with MeOH (500 ml×3), and the extracts were partitioned between hexane (100 ml×3) and 90% aqueous MeOH (100 ml). The MeOH layer was partitioned with *n*-BuOH (10 ml×3) and H_2O (100 ml). The *n*-BuOH-soluble portions (3.9 g) were subjected to silica gel column chromatography ($CHCl_3$–MeOH, 8 : 1) to afford fractions **a** (450 mg) and **b** (300 mg). Fraction **a** was separated

by C_{18} column chromatography (MeOH–H_2O, 1 : 1) followed by C_{18} HPLC (Develosil ODS-HG-5, Nomura Co. Ltd., 1.0×25 cm: flow rate 2.5 ml/min; UV detection at 210 nm; eluent MeOH–H_2O, 7 : 3) to afford naucleamides A (**1**, 1.6 mg, t_R 15 min), B (**2**, 1.6 mg, t_R 14 min), C (**3**, 1.2 mg, t_R 30 min), D (**4**, 1.2 mg, t_R 16 min), and E (**5**, 0.8 mg, t_R 22 min), while fraction **b** was purified by C_{18} column chromatography (MeOH–H_2O, 1 : 1) to give strictosamide (**6**, 150 mg), 10-hydroxystrictosamide (**7**, 8.0 mg), and tetrahydrodesoxycordifoline (**8**, 4.0 mg).

Naucleamide A (**1**): A colorless amorphous solid; $[\alpha]_D^{23}$ −113° (c=0.33, MeOH); UV λ_{max} (MeOH) nm (log ε): 225 (4.20), 282 (3.60); IR (KBr) cm^{-1}: 3427, 1611; ^{1}H- and ^{13}C-NMR (Table 1); FAB-MS *m/z* 363 $(M+Na)^+$; HR-FAB-MS *m/z* 363.1687 $(M+Na)^+$ (Calcd for $C_{20}H_{24}N_2O_3Na$, 363.1689).

Naucleamide B (**2**): A colorless amorphous solid; $[\alpha]_D^{23}$ −42° (c=0.27, MeOH); UV λ_{max} (MeOH) nm (log ε): 226 (4.26), 282 (3.60); IR (KBr) cm^{-1}: 3428, 1644; ^{1}H- and ^{13}C-NMR (Table 1); EI-MS *m/z* 340 (M^+); HR-EI-MS *m/z* 340.1782 (M^+) (Calcd for $C_{20}H_{24}N_2O_3$, 340.1787).

Naucleamide C (**3**): A colorless amorphous solid; $[\alpha]_D^{23}$ −47° (c=0.20, MeOH); UV λ_{max} (MeOH) nm (log ε): 224 (4.20), 281 (3.53); IR (KBr) ν_{max} cm^{-1}: 3430, 1632; ^{1}H- and ^{13}C-NMR (Table 1); EI-MS *m/z* 336 (M^+); HR-EI-MS *m/z* 336.1482 (M^+) (Calcd for $C_{20}H_{20}N_2O_3$, 336.1474).

Naucleamide D (**4**): A colorless amorphous solid; UV λ_{max} (MeOH) nm (log ε): 215 (4.18), 372 (4.08), 389 (4.00); IR (KBr) cm^{-1}: 3420, 1640; ^{1}H- and ^{13}C-NMR (Table 2); EI-MS *m/z* 336 (M^+); HR-EI-MS *m/z* 336.1488 (M^+) (Calcd for $C_{20}H_{20}N_2O_3$, 336.1474).

Naucleamide E (**5**): A colorless amorphous solid; $[\alpha]_D^{24}$ −40° (c=0.13, MeOH); UV λ_{max} (MeOH) nm (log ε): 225 (4.26), 275 (3.60) nm; IR (KBr) cm^{-1}: 3433, 1633; ^{1}H- and ^{13}C-NMR (Table 2); EI-MS *m/z* 338 (M^+); HR-EI-MS *m/z* 338.1631 (M^+) (Calcd for $C_{20}H_{22}N_2O_3$, 338.1630).

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