HETEROCYCLES, Vol. 79, 2009, pp. 765 - 771. © The Japan Institute of Heterocyclic Chemistry
Received, 29th September, 2008, Accepted, 27th October, 2008, Published online, 31st October, 2008.
DOI: 10.3987/COM-08-S(D)41

NAUCLEAMIDE F, A NEW MONOTERPENE INDOLE ALKALOID FROM *NAUCLEA LATIFOLIA*

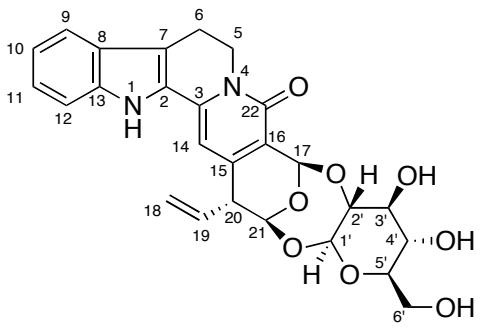
Yuka Kakuguchi, Haruaki Ishiyama, Takaaki Kubota, and Jun'ichi Kobayashi*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan. E-mail: jkobay@pharm.hokudai.ac.jp

Abstract - A new monoterpene indole alkaloid, naucleamide F (**1**), has been isolated from the bark and wood of *Nauclea latifolia*, and the structure and stereochemistry were elucidated on the basis of the spectral data. Naucleamide F (**1**) is a new monoterpene indole alkaloid consisting of a tetrahydro-β-carboline ring fused to a pyridone ring, and a 1,3,5-trioxepane ring fused to a dihydropyran ring and a glucose unit.

INTRODUCTION

A number of monoterpene indole alkaloids with biological activities have been isolated from *Nauclea* species (Rubiaceae).[1-4] In our search for bioactive metabolites from medicinal plants, we previously isolated new monoterpene indole alkaloids, naucleamides A~E[5], from the bark and wood of *Nauclea latifolia*. Further investigation of extracts from this plant resulted in the isolation of a new monoterpene indole alkaloid, naucleamide F (**1**), consisting of a heptacyclic ring system including a tetrahydro-β-carboline ring fused to a pyridone ring, and a 1,3,5-trioxepane ring fused to a dihydropyran ring and a glucose unit. Here we describe the isolation and structure elucidation of **1**.



1

RESULTS AND DISCUSSION

The bark and wood of *Nauclea latifolia* were extracted with MeOH. The MeOH extracts were partitioned between hexane and 90% aqueous MeOH, and then the MeOH layer was subsequently extracted with *n*-BuOH. The *n*-BuOH-soluble materials were purified by a silica gel column ($CHCl_3$-MeOH, 1:0 → 85:15) followed by a C_{18} column (MeOH-H_2O, 60:40) and C_{18} HPLC (CH_3CN-H_2O, 40:60) to afford naucleamide F (**1**, 0.0003%) together with known related monoterpene indole alkaloids, angustoline[6] (**2**, 0.0004%), compound **3**[7] (0.0004%), and compound **4**[7] (0.0003%).

Table 1. ^{1}H- and ^{13}C-NMR Data of Naucleamide F (**1**) in CD_3OD

Position	^{1}H[a]	^{13}C[a]
1	-	-
2	-	128.9
3	-	141.0
4	-	-
5a	4.42 (ddd, *J* = 6.0, 8.4, 14.1)	42.8
5b	4.65 (dt, *J* = 6.6, 14.1)	
6	3.17 (m) [b]	21.2
7	-	118.4
8	-	127.6
9	7.63 (d, 7.8)	121.3
10	7.13 (t, 7.2)	122.0
11	7.29 (t, 7.8)	126.5
12	7.44 (d, 8.4)	113.7
13	-	141.3
14	6.67 (s)	103.9
15	-	151.4
16	-	116.9
17	6.04 (s)	93.6
18a	5.32 (d, *J* = 10.8)	120.8
18b	5.38 (d, *J* = 17.4)	
19	5.86 (ddd, *J* = 7.8, 10.2, 17.4)	136.2
20	3.50 (d, *J* = 7.8)	48.8
21	5.51 (s)	96.6
22	-	178.8
1’	5.06 (d, *J* = 7.2)	99.8
2’	3.22 (d, *J* = 7.2)	82.2
3’	3.67 (m)	77.0
4’	3.30-3.40 (m)	71.4
5’	3.30-3.40 (m)	79.8
6’a	3.71 (dd, *J* = 4.2, 12.0)	63.3
6’b	3.88 (dd, *J* = 4.2, 12.0)	

[a] δ in ppm, [b] 2H

The molecular formula, $C_{26}H_{26}N_2O_8$, of naucleamide F (**1**) was established by HR-ESI-MS [*m/z* 517.1592 $(M+Na)^+$, Δ +0.5 mmu]. IR absorptions implied the presence of hydroxy (3443 cm^{-1}) and amide carbonyl (1645 cm^{-1}) functionalities. ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum suggested that **1** possessed one carbonyl, seven sp^2 quaternary carbons, six sp^2 methines, one sp^2 methylene, three sp^3 methylenes, one sp^3 methine, four sp^3 oxymethines, and three acetal methines. Among them, one oxymethylene carbon (δ_C 63.3), five oxymethine carbons (δ_C 82.2, 79.8, 77.0, 71.4, and 63.3), and one acetal methine carbon (δ_C 99.8) were ascribed to a glucopyranose unit (C-1'~C-6').[8] The ^1H-^1H COSY and TOCSY spectra of **1** revealed connectivities of four partial structures, C-5 to C-6, C-9 to C-12, C-18 to C-20, and C-1' to C-6'. HMBC cross-peaks of H-5 to C-7 and C-22, H-9 to C-7 and C-13, H-12 to C-8, H-14 to C-2 and C-3, and H-20 to C-14 and C-15 indicated the presence of a tetrahydro-β-carboline ring (N-1, C-2, C-3, N-4, and C-5~C-13) fused to a pyridone ring (C-3, N-4, C-14~C-16, and C-22) at C-3 and N-4, which was connected to an sp^3 methine (C-20). The presence of a 1,3,5-trioxepane ring (C-17, O-17, C-21, O-1', C-1', C-2', and O-2') fused to a dihydropyran ring (C-15~C-17, C-20, C-21, and O-17) at C-17 and C-21, and a glucose unit (C-1'~C-6' and O-5') at C-1' and C-2' was elucidated by HMBC correlations of H-17 to C-16 and C-2', H-21 to C-15 and C-17, and NOESY correlations for H-20 to H-21 and H-21 to H-1'.

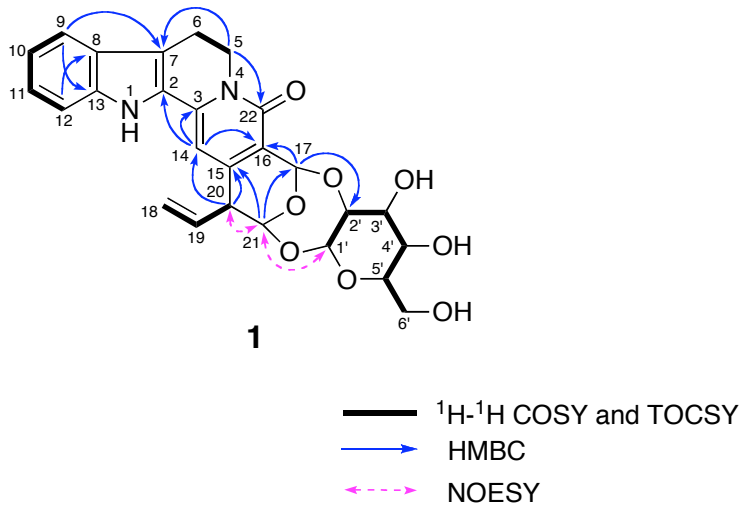


Figure 1. Selected 2D NMR correlations for naucleamide F (**1**).

The relative stereochemistry of **1** was deduced from NOESY correlations of H-17 to H-19, H-20 to H-21, H-1' to H-21, H-3', and H-5', and a *J*-value for H-20/H-21 (~0 Hz) as shown in Figure 2.

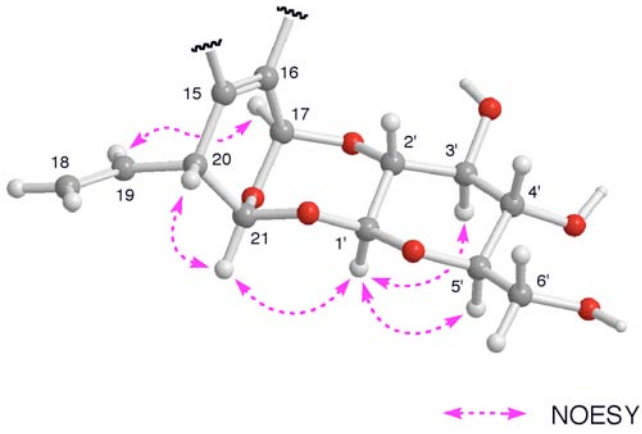


Figure 2. Selected NOESY correlations and relative stereochemistry for a part (C-15~C-21 and C-1'~C-6') of naucleamide F (**1**).

Since the sugar moiety was elucidated to be D-glucopyranose by chiral HPLC analysis of *O*-benzoyl derivatives of the methanolysis products of naucleamide F (**1**),[9] the absolute stereochemistry of naucleamide F (**1**) was assigned as shown in Figure 2.

The absolute stereochemistries of known related monoterpene indole alkaloids **2**~**4**, whose stereochemistries remains unsolved,[6,7] were elucidated as describe below. The absolute configurations at C-3 of **3** and **4** were assigned as both *R* on the basis of the negative Cotton effects at 279 nm (Δε -0.29) and 253 nm (Δε -0.72), respectively,[10] while the absolute configurations at C-19 of **2** ~ **4** were elucidated to be *S*, *S*, and *R* on the based of the Δδ values obtained for (*S*)- and (*R*)-MTPA esters of **2** ~ **4**, respectively [11] (Figure 3).

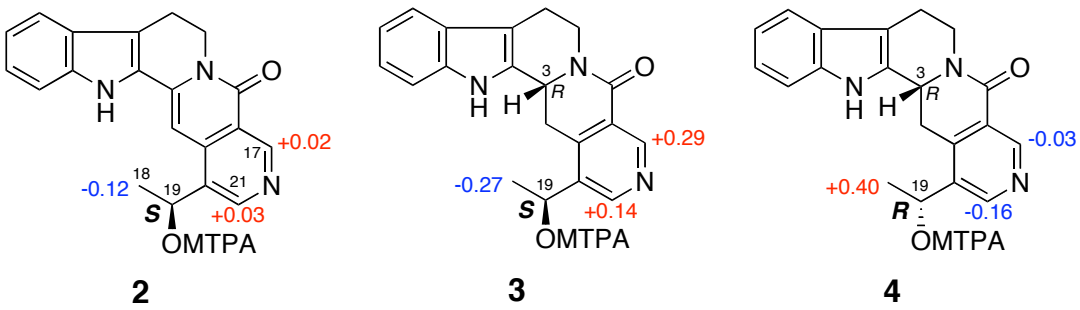


Figure 3. Δδ values [Δδ (in ppm) δ_S - δ_R] obtained for (*S*)- and (*R*)-MTPA esters of compounds **2** ~ **4**.

Naucleamide F (**1**) is a new monoterpene indole alkaloid consisting of a tetrahydro-β-carboline ring fused to a pyridone ring, and a 1,3,5-trioxepane ring fused to a dihydropyran ring and a glucose unit. Naucleamide F (**1**) is a rare monoterpene indole alkaloid possessin a glucose unit connected to terpenoid

unit *via* two ether bonds, though an iridoid having a similar unit has been reported from the bark of *Eucommia ulmoides*.[8]

EXPERIMENTAL

General Experimental Procedures

Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on a JASCO FT/IR-5300 spectrophotometers and Shimadzu UV-1600PC, respectively. ^{1}H, ^{13}C and 2D NMR spectra were measured on a JEOL JMN-EX400, a JEOL ECA500, and a 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. ESI mass spectra were measured on a JEOL JMS-700TZ spectrometer.

Extraction and Isolation

The bark and wood (300 g) of *Nauclea latifolia* were extracted with MeOH (1.5 L), and the extracts were partitioned between hexane (200 mL x 3) and 90% aqueous MeOH (200 mL). The MeOH layer was partitioned between *n*-BuOH (200 mL x 3) and H_2O (200 mL). The *n*-BuOH-soluble portions (3.4 g) were subjected to a silica gel column chromatography ($CHCl_3$-MeOH, 1:0 $\rightarrow$ 85:15) to afford fraction **a** (583 mg). Fraction **a** was separated by a C_{18} column chromatography (MeOH-H_2O, 60:40) followed by C_{18} HPLC (Capcell Pak RP-18, Shiseido Co. Ltd, 10 x 250 mm; flow rate 2.5 mL/min; UV detection at 210 nm; eluent CH_3CN/H_2O, 40:60) to afford naucleamide F (**1**, 0.85 mg, t_R 17 min), angustoline (**2**, 1.3 mg, t_R 42 min), compound 3 (**3**, 1.2 mg, t_R 30 min), and compound 4 (**4**, 0.69 mg, t_R 28 min).

Naucleamide F (1): pale yellow amorphous solid; $[\alpha]_D^{25}$ +44 (*c* 0.21, MeOH); UV (MeOH) λ_{max} 210 nm (log ε 3.97), 261 (3.44), 289 (3.28), 301 (3.18), and 354 (3.57); IR (KBr) cm^{-1}: 3443, 2920, 1645; ^{1}H- and ^{13}C-NMR (Table 1); ESI-MS *m/z* 517 $(M+Na)^+$; HR-ESI-MS *m/z* 517.1592 $(M+Na)^+$ (calcd. for $C_{26}H_{26}N_2O_8Na$, 517.1587).

Stereochemical assignment of the sugar unit in naucleamide F (1).

Naucleamide F (**1**, 0.1 mg) was treated with 3% HCl/MeOH (300 μL) at 110 °C for 1h. After the solvent was removed by nitrogen stream, to the residue was added EtOAc (100 μL), and the EtOAc solution was extracted with H_2O (100 μL x 3). The aqueous fraction evaporated in vacuo was treated pyridine (100 μL), triethylamine (15 μL), and benzoyl chloride (15 μL), at rt for 21 h. After addition of MeOH (100 μL), the reaction mixture was extracted with *n*-hexane (100 μL x 3). The *n*-hexane-soluble fraction was evaporated in vacuo to afford 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivative of the sugar units of **1**.

Authentic D- and L-glucose were treated with benzoyl chloride as described above to afford 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivatives of D- and L-glucose, respectively. The 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivatives were subjected to chiral HPLC analyses using Chiralpak OD-R (Daicel Chemical Industry, Ltd., 4.6 x 250 mm; flow rate 0.5 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 95:5). The retention time of 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivative of methanolysis product of **1** was found to be 18.6 min, while the retention times of authentic 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose and 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl-α-L-glucopyranose were found to be 18.6 and 20.2 min, respectively.

Preparation of (*S*)- and (*R*)-MTPA esters of compounds 2~4.

To a solution of **2** (0.1 mg) in CH_2Cl_2 (100 μL) were added (*R*)-MTPACl (0.68 mg), triethylamine (2.0 μl), and *N*,*N*-demethyl-aminopyridine (4.1 mg). The mixture was allowed to stand at rt for 3 h. After evapolation of the solvent, the residue was applied to a silica gel column to give the (*S*)-MTPA ester of **1**. The (*R*)-MTPA ester of **2** and (*S*)- and (*R*)-MTPA esters of **3** and **4** were prepared according to the same procedure as described above.

(*S*)-MTPA ester of 2: ^{1}H NMR (CD_3OD) δ9.40 (H-17), 8.67 (H-21), 6.69 (H-19), 1.82 (H-18); ESIMS *m*/*z* 548 $(M+H)^+$.

(*R*)-MTPA ester of 2: ^{1}H NMR (CD_3OD) δ9.38 (H-17), 8.64 (H-21), 6.40 (H-19), 1.94 (H-18); ESIMS *m*/*z* 548 $(M+H)^+$.

(*S*)-MTPA ester of 3: ^{1}H NMR (CD_3OD) δ9.40 (H-17), 8.67 (H-21), 6.69 (H-19), 1.51 (H-18); ESIMS *m*/*z* 546 $(M+H)^+$.

(*R*)-MTPA ester of 3: ^{1}H NMR (CD_3OD) δ9.11 (H-17), 8.53 (H-21), 6.44 (H-19), 1.78 (H-18); ESIMS *m*/*z* 546 $(M+H)^+$.

(*S*)-MTPA ester of 4: ^{1}H NMR (CD_3OD) δ9.37 (H-17), 8.53 (H-21), 6.69 (H-19), 2.20 (H-18); ESIMS *m*/*z* 546 $(M+H)^+$.

(*R*)-MTPA ester of 4: ^{1}H NMR (CD_3OD) δ9.40 (H-17), 8.69 (H-21), 6.39 (H-19), 1.80 (H-18); ESIMS *m*/*z* 546 $(M+H)^+$.

ACKNOWLEDGMENTS

We thank Ms. S. Oka (Center for Instrumental Analysis, Hokkaido University) for measurements of ESIMS. This work was partly supported by a grant from a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. F. Hotellier, P. Delaveau, and J. L. Pousset, *Phytochemistry*, 1975, **14**, 1407.
2. F. Hotellier, P. Delaveau, and J. L. Pousset, *Planta Med*., 1979, **35**, 242.
3. Z. Zhang, H. N. ElSohly, M. R. Jacob, D. S. Pasco, L. A. Walker, and A. M. Clark, *J. Nat. Prod.*, 2001, **64**, 1001.
4. H. Takayama, O. Ohmori, M. Sakai, M. Funahashi, M. Kitajima, D. Santiarworn, B. Liawruangrath, and N. Aimi, *Heterocycles*, 1998, **49**, 40.
5. H. Shigemori, T. Kagata, H. Ishiyama, F. Morah, A. Ohsaki, and J. Kobayashi, *Chem. Pharm. Bull.*, 2003, **51**, 58.
6. F. Hotellier, P. Delaveau, and J. L. Pousset, *Phytochemistry*, 1975, **14**, 1407.
7. C. A. J. Erdelmeier, U. Regenass, T. Rali, and O. Sticher, *Planta Med.*, 1992, **58**, 43.
8. C. Takamura, T. Hirata, T. Ueda, M. Ono, H. Miyashita, T. Ikeda, and T. Nohara, *J. Nat. Prod.*, 2007, **70**, 1312.
9. J. Kobayashi, T. Kubota, M. Takahashi, M. Ishibashi, M. Tsuda, and H. Naoki, *J. Org. Chem.*, 1999, **64**, 1478.
10. C. M. Lee, W. F, Trager, and A. H. Beckett, *Tetrahedron*, 1967, **23**, 375.
11. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.