Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 413–417
Bioorganic & Medicinal Chemistry

Complanadines C and D, new dimeric alkaloids from *Lycopodium complanatum*

Kan'ichiro Ishiuchi,[a] Takaaki Kubota,[a] Yuzuru Mikami,[b] Yutaro Obara,[c] Norimichi Nakahata[c] and Jun'ichi Kobayashi[a,*]

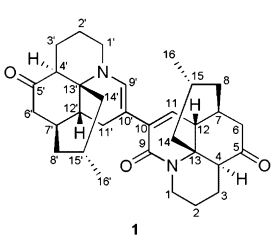
[a]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*
[b]*Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan*
[c]*Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan*

Received 1 September 2006; revised 20 September 2006; accepted 21 September 2006
Available online 12 October 2006

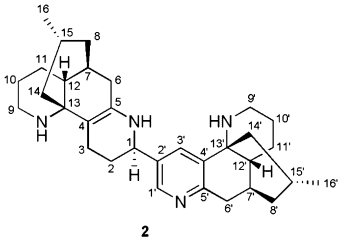
Abstract—Two new dimeric *Lycopodium* alkaloids, complanadines C (**1**) and D (**2**), have been isolated from the club moss *Lycopodium complanatum*, and the structures and relative stereochemistry of **1** and **2** were elucidated on the basis of the spectral data. Complanadine D (**2**) enhanced mRNA expression for NGF.

1. Introduction

Club moss (Lycopodiaceae) are known to be a rich source of *Lycopodium* alkaloids possessing unique heterocyclic ring systems such as $C_{16}N$, $C_{16}N_2$, and $C_{27}N_3$, which have attracted great interest from biogenetic, synthetic, and biological points of view.[1] In our continuing efforts to find new *Lycopodium* alkaloids,[2] two new dimeric *Lycopodium* alkaloids, complanadines C (**1**) and D (**2**), were isolated from the club moss *Lycopodium complanatum*. In this paper, we describe the isolation and structure elucidation of **1** and **2**.



1



2

2. Results and discussion

The club moss of *L. complanatum* collected at Nayoro in Hokkaido were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 10 with sat. Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 1:0 to 1:1, and then $CHCl_3$/MeOH, 1:0 to 0:1). The fraction eluted with hexane/EtOAc (10:1 to 1:1) was purified by a silica gel column (hexane/EtOAc, 50:1 and then $CHCl_3$/MeOH, 1:1) to give complanadine C (**1**, 0.00003% yield). The fraction eluted with $CHCl_3$/MeOH (1:0 to 50:1) in the amino silica gel column was purified by a silica gel column ($CHCl_3$/MeOH, 1:0 and then $CHCl_3$/MeOH/H_2O/TFA, 6:4:1:0.01) followed by C_{18} HPLC (MeCN/H_2O/TFA, 18.5:81.5:0.1) to yield complanadine D (**2**, 0.00007% yield).

Complanadine C (**1**) showed the pseudomolecular ion peak at *m/z* 503 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{32}H_{42}N_2O_3$, was established by HRESIMS [*m/z* 503.3270, $(M+H)^+$, Δ −0.4 mmu]. IR absorptions implied the presence of carbonyl (1704 and 1638 cm^{-1}) functionalities. ^{13}C NMR data (Table 1) revealed three carbonyl carbons, two sp^2 quaternary carbons, two sp^2 methines, two sp^3 quaternary carbons, eight sp^3 methines, 13 sp^3 methylenes, and two methyl groups. Among them,

Keywords: *Lycopodium complanatum*; *Lycopodium* alkaloids; Complanadines C and D.
* Corresponding author. Tel.: +81 11 706 3239; fax: +81 11 706 4989; e-mail: jkobay@pharm.hokudai.ac.jp

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.09.043

two methylenes (δ_C 47.3; δ_H 3.37 and 2.92, and δ_C 37.7; δ_H 4.42 and 3.08) were ascribed to those attached to a nitrogen.

The gross structure of **1** was elucidated by analyses of 2D NMR data including the ^1H–^1H COSY, HOHAHA, HMQC, and HMBC spectra in CD_3OD (Fig. 1). Most of ^1H and ^{13}C NMR signals (Table 1) appeared to be due to each half moiety (units A and B) of a dimeric compound. In unit A (C-1 ∼ C-16), ^1H–^1H COSY and HOHAHA spectra revealed connectivities of C-1 ∼ C-4, C-7 ∼ C-8, C-7 ∼ C-12, C-8 ∼ C-15, C-11 ∼ C-12, and C-14 ∼ C-16. HMBC correlations of H-4 (δ_H 2.70) to C-5 (δ_C 209.9) and C-6 (δ_C 43.3), H-6b (δ_H 2.29) to C-5, and H-8b (δ_H 1.38) and H-12 (δ_H 2.73) to C-6 suggested connections of C-4 to C-6 through C-5 and C-6 to C-8 through C-7. Connections of C-4 to C-12, C-4 to C-14, and C-12 to C-14 through C-13 were deduced from HMBC cross-peaks of H-12 to C-13 (δ_C 61.7), H-14b (δ_H 1.16) to C-4 (δ_C 50.0) and C-13. HMBC cross-peaks of H-1b (δ_H 3.08) to C-9 (δ_C 166.5), H-11 (δ_H 5.96) to C-9, and H-12 (δ_H 2.73) to C-10 (δ_C 135.1) indicated connections of C-1 to C-9 through a nitrogen atom and C-9 to C-11 through C-10. In unit B (C-1′ ∼ C-16′), the ^1H–^1H COSY and HOHAHA spectra of **1** revealed three partial structures C-1′ ∼ C-4′, C-8′ ∼ C-15′ and C-14′ ∼ C-16′, and C-11′ ∼ C-12′. HMBC correlations of H-4′ (δ_H 2.62) and H-6′b (δ_H 2.23) to C-5′ (δ_C 211.7) suggested the connection of C-4′ to C-6′ through C-5′. Connections of C-6′ to C-12′, C-6′ to C-8′, and C-8′ to C-12′ through C-7′ were deduced from HMBC cross-peaks of H-6′b to C-12′ (δ_C 40.5), and H-8′b (δ_H 1.30) to C-6′ (δ_C 42.4) and C-12′. HMBC cross-peaks of H-4′ to C-13′ (δ_C 57.3) and H-14′a (δ_H 2.56) to C-12′ and C-13′ indicated connections of C-4′ to C-12′, C-4′ to C-14′, and C-12′ to C-14′ through C-13′. Connections of C-1′ to C-9′, C-1′ to C-13′, and C-9′ to C-13′ through a nitrogen atom were suggested from HMBC cross-peaks of H-1′b (δ_H 2.92) to C-9′ (δ_C 137.2) and C-13′, and H-9′ (δ_H 7.22) to C-13′. HMBC cross-peaks of H-9′ to C-11′ (δ_C 25.9) and H-11′a (δ_H 2.55) to C-10′ (δ_C 103.4) revealed the connection of C-9′ to C-11′ through C-10′. The connection of units A and B was provided from HMBC correlations of H-11 to C-10′ and H-9′ to C-10. Thus, the gross structure of complanadine C was elucidated to be **1**.

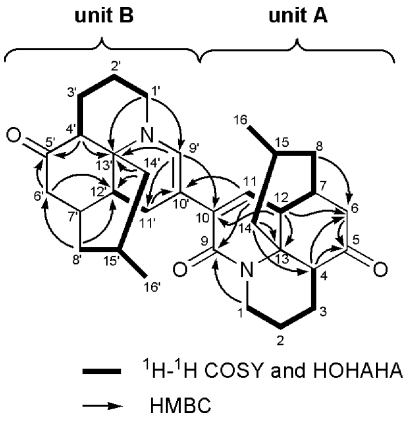


Figure 1. Selected 2D NMR correlations for complanadine C (**1**).

Table 1. ^1H and ^{13}C NMR data of complanadine C (**1**) in CD_3OD

	δ_H	δ_C
1a	4.42 (1H, dd, 13.6, 5.3)	37.7
1b	3.08 (1H, ddd, 13.2, 13.2, 2.4)	
2a	1.65 (1H, m)	24.3[α]
2b	1.49 (1H, m)	
3a	2.04 (1H, m)	18.6
3b	1.68 (1H, m)	
4	2.70 (1H, m)	50.0
5		209.9
6a	2.59 (1H, m)	43.3
6b	2.29 (1H, m)	
7	2.50 (1H, m)	36.8
8a	1.78 (1H, m)	41.0
8b	1.38 (1H, ddd, 12.6, 12.6, 4.2)	
9		166.5
10		135.1
11	5.96 (1H, d, 2.3)	127.9
12	2.73 (1H, m)	42.6
13		61.7
14a	2.59 (1H, m)	42.2[β]
14b	1.16 (1H, t, 12.6)	
15	1.55 (1H, m)	25.2
16	0.91 (3H, d, 6.2)	22.7
1′a	3.37 (1H, ddd, 13.8, 13.8, 3.0)	47.3
1′b	2.92 (1H, dd, 13.8, 4.8)	
2′	1.52 (2H, m)	24.9[α]
3′a	2.04 (1H, m)	19.2
3′b	1.62 (1H, m)	
4′	2.62 (1H, m)	48.0
5′		211.7
6′a	2.75 (1H, m)	42.4[β]
6′b	2.23 (1H, m)	
7′	2.31 (1H, m)	35.7
8′a	1.75 (1H, m)	42.5[β]
8′b	1.30 (1H, ddd, 12.6, 12.6, 3.6)	
9′	7.22 (1H, s)	137.2
10′		103.4
11′a	2.55 (1H, m)	25.9
11′b	2.19 (1H, m)	
12′	1.99 (1H, m)	40.5
13′		57.3
14′a	2.56 (1H, m)	41.1
14′b	0.91 (1H, m)	
15′	1.50 (1H, m)	24.9
16′	0.87 (3H, d, 6.2)	22.5

[α,β]These signals may be interchangeable.

The NOESY spectrum of **1** showed cross-peaks as shown in computer-generated 3D drawing (Fig. 2). In unit A, a chair-like conformation of a piperidine ring (N-1, C-1 ∼ C-4, and C-13) was suggested from NOESY correlations of H-14a to H-1b and H-3b. NOESY cross-peaks and $^3J_{H\text{-}14/H\text{-}15}$ (12.6 Hz) indicated a chair conformation of a cyclohexane ring (C-7 ∼ C-8, and C-12 ∼ C-15). In unit B, NOESY correlations of H-14′a to H-1′ and H-3′b suggested a chair-like conformation of a piperidine ring (N-1′, C-1′ ∼ C-4′, and C-13′). A chair conformation of a cyclohexane ring (C-7′ ∼ C-8′, and C-12′ ∼ C-15′) was deduced from NOESY correlations of H-8′ to H-16′, and H-12′ to H-8′b and H-14′b. Thus, the partial relative stereochemistry of complanadine C (**1**) was elucidated as shown in Figure 2.

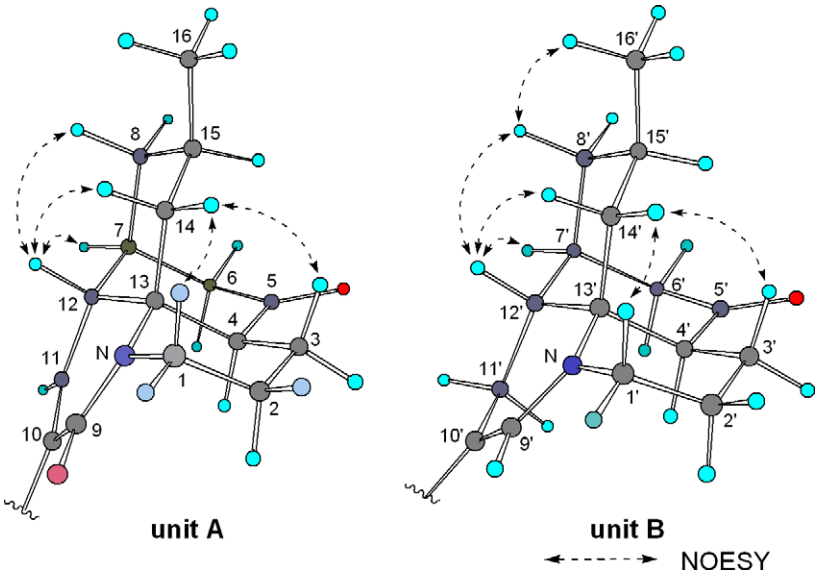


Figure 2. Selected NOESY correlations and relative stereochemistry for complanadine C (**1**).

Complanadine D (**2**) showed the pseudomolecular ion peak at *m/z* 487 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{32}H_{46}N_4$, was established by HRESIMS [*m/z* 487.3801, $(M+H)^+$, Δ +1.6 mmu]. Most of ^1H and ^{13}C NMR signals of **2** (Table 2) seemed to be due to each half moiety [units C (C-1 ~ C-16) and D (C-1′ ~ C-16′)] of a dimeric compound, of which the ^1H and ^{13}C NMR spectra were similar to those of complanadine A[3], except for lacking NMR signals for one of two trisubstituted pyridine rings in complanadine A. The ^1H–^1H COSY and HOHAHA spectra of **2** revealed the connection of C-1 ~ C-3 and HMBC correlations of H-1 (δ_H 4.44) and H_2-3 (δ_H 1.96 and 1.57) to C-5 (δ_C 140.0), and H_2-6 (δ_H 2.50 and 1.77) to C-4 (δ_C 98.6) and C-5, indicated the existence of a trisubstituted tetrahydropyridine ring in unit C. The connection between the tetrahydropyridine ring in unit C and a pyridine ring in unit D was provided by HMBC correlations of H_2-2 (δ_H 2.14 and 1.96) to C-2′ (δ_C 140.2), and H-1′ (δ_H 8.24) and H-3′ (δ_H 7.89) to C-1 (δ_C 52.9) (Fig. 3). Thus, the gross structure of complanadine D (**2**) was assigned as *N*-1,1,2,3-tetrahydro form of complanadine A.

The NOESY spectrum of **2** was similar to that of complanadine A, suggesting that the relative stereochemistry was the same as that of complanadine A except for the tetrahydropyridine ring in unit C. Analysis of the NOESY spectrum of **2** revealed a pseudochair form of the tetrahydropyridine ring (N-1, C-1 ~ C-5) and an α-configuration of H-1 (Fig. 4).

Complanadine C (**1**) is the first dimeric *Lycopodium* alkaloid containing a lycopodane-type $C_{16}N$ skeleton, while complanadine D (**2**) is *N*-1,1,2,3-tetrahydro form of complanadine A. Effects of complanadine D (**2**) on neurotrophic factor biosynthesis in 1321N1 human astrocytoma cells were examined by a semiquantitative RT-PCR method,[4,5] and it was found that the mRNA expressions for NGF were enhanced by **2**. Complanadine D (**2**) exhibited cytotoxicity against murine leukemia L1210 cells (IC_{50}, 7 μg/ml) in vitro, while **1** did not show such activity (IC_{50} > 10 μg/ml). Complanadines C (**1**) and D (**2**) showed antimicrobial activity against *Cryptococcus neoformans* (MIC, 0.52 and 0.26 μg/ml, respectively) and *Aspergillus niger* (MIC, 2.05 and 4.16 μg/ml, respectively).

3. Experimental

3.1. General

The IR spectrum was recorded on a JASCO FT/IR-230 and a Shimadzu UV-1600PC spectropolarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd). The 3.31 and 49.5 ppm resonances of residual CD_3OD were used as internal references for ^1H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

3.2. Plant material

The club moss *L. complanatum* was collected at Nayoro in Hokkaido in 2004. The botanical identification was made by Mr. N. Yoshida, Health Sciences University of Hokkaido. The voucher specimen has been deposited in the herbarium of Hokkaido University.

3.3. Extraction and isolation

The club moss *L. complanatum* was crushed and extracted with MeOH. The MeOH extract was treated with 3% tartaric acid (pH 3) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 (aq) to pH 10 and extracted with $CHCl_3$ to give a crude alkaloidal fraction. A part of the alkaloidal fraction was purified by an amino silica gel column (hexane/EtOAc, 1:0 to 1:1, and then $CHCl_3$/MeOH, 1:0 to 0:1). The fraction eluted with hexane/EtOAc (10:1 to 1:1) was

Table 2. ^{1}H and ^{13}C NMR data of complanadine D (**2**) in CD_3OD

	δ_H	δ_C
1	4.44 (1H, dd, 4.3, 4.3)	52.9
2a	2.14 (1H, m)	30.3
2b	1.96 (1H, m)	
3a	1.96 (1H, m)	18.5
3b	1.57 (1H, m)	
4		98.6
5		140.0
6a	2.50 (1H, dd, 16.8, 5.8)	32.0
6b	1.77(1H, m)	
7	1.92 (1H, m)	34.8
8a	1.70 (1H, m)	44.3
8b	1.26 (1H, ddd, 12.6, 12.6, 3.8)	
9a	2.76 (1H, m)	42.6
9b	2.31 (1H, ddd, 11.5, 11.5, 2.8)	
10a	1.61 (1H, m)	26.3
10b	1.47 (1H, m)	
11a	1.66 (1H, m)	27.8
11b	1.55 (1H, m)	
12	1.55 (1H, m)	44.6
13		60.2
14a	1.70 (1H, m)	45.5
14b	0.96 (1H, dd, 11.5, 11.5)	
15	1.80 (1H, m)	28.0
16	0.94 (3H, d, 6.6)	22.4
1′	8.24 (1H, d, 2.2)	146.0
2′		140.2
3′	7.89 (1H, d, 2.2)	133.5
4′		137.3
5′		157.8
6′a	3.13 (1H, dd, 18.7, 7.1)	35.4
6′b	2.65 (1H, d, 18.2)	
7′	2.11 (1H, m)	34.6
8′a	1.80 (1H, m)	44.8
8′b	1.39 (1H, ddd, 12.6, 12.6, 3.8)	
9′a	2.74 (1H, m)	42.3
9′b	2.42 (1H, ddd, 12.6, 12.6, 2.7)	
10′	1.64 (2H, m)	25.6
11′a	1.56 (1H, m)	27.2
11′b	1.14 (1H, m)	
12′	1.70 (1H, m)	44.8
13′		57.7
14′a	1.47 (1H, m)	51.8
14′b	1.30 (1H, dd, 12.1, 12.1)	
15′	1.14 (1H, m)	27.1
16′	0.80 (3H, d, 6.6)	22.2

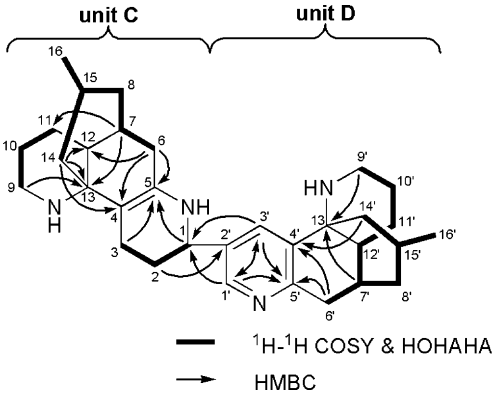


Figure 3. Selected 2D NMR correlations for complanadine D (**2**).

purified by a silica gel column (hexane/EtOAc, 50:1 and then $CHCl_3$/MeOH, 1:1) to give complanadine C (**1**, 0.00003% yield). The fraction eluted with $CHCl_3$/MeOH (1:0 to 50:1) in the amino silica gel column was purified by a silica gel column ($CHCl_3$/MeOH, 1:0 and then $CHCl_3$/MeOH/H_2O/TFA, 6:4:1:0.01) followed by C_{18} HPLC [eluent, MeCN/ H_2O/TFA (18.5:81.5:0.1); flow rate, 2 ml/min; UV detection at 210 nm] to yield complanadine D (2, 0.00007% yield, t_R = 14 min).

3.4. Complanadine C (1)

Colorless amorphous solid; $[\alpha]_D^{22}$ −9° (*c* 0.2, MeOH); IR (neat) ν_{max} 1704, 1638, and 1614 cm^{-1}; UV (MeOH) λ_{max} 223 (ε 4900) and 347 nm (1900); ^{1}H and ^{13}C NMR data (see Table 1); ESIMS *m/z* 503 $(M+H)^+$; HRESIMS *m/z* 503.3270 (M+H; calcd for $C_{32}H_{43}N_2O_3$, 503.3274).

3.5. Complanadine D (2)

Colorless amorphous solid; $[\alpha]_D^{21}$ −32° (*c* 1.0, MeOH); IR (neat) ν_{max} 3276, 1669, and 1565 cm^{-1}; UV (MeOH) λ_{max} 209 (ε 15,100) and 272 nm (3900); ^{1}H and ^{13}C NMR data (see Table 2); ESIMS *m/z* 487 $(M+H)^+$; HRESIMS *m/z* 487.3817 (M+H; calcd for $C_{32}H_{47}N_4$, 487.3801).

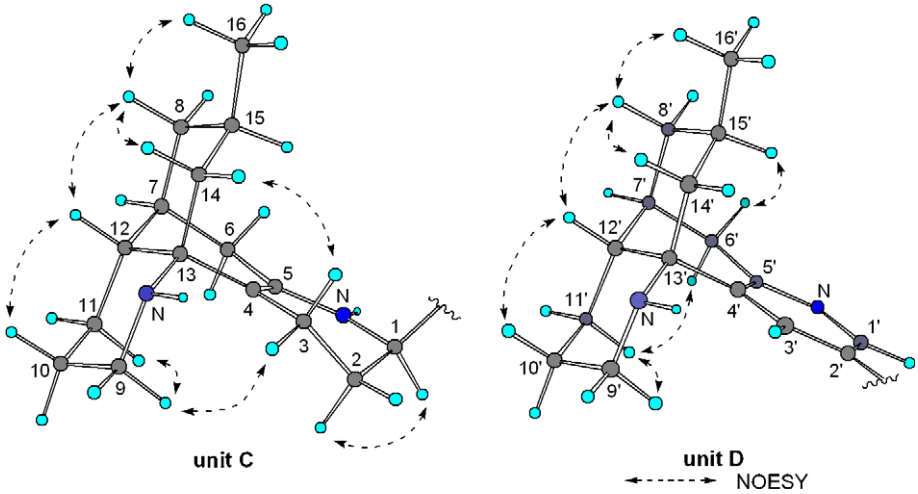


Figure 4. Selected NOESY correlations and relative stereochemistry for complanadine D (**2**).

Acknowledgments

The authors thank Prof. H. Morita, Hoshi University, for help with plant collection, Ms. S. Oka and Ms. M. Kiuchi, Center for Instrumental Analysis, Hokkaido University, for measurements of ESIMS, and Mr. N. Yoshida, Health Sciences University of Hokkaido, for botanical identifications of the plant. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References and notes

1. Reviews of *Lycopodium* alkaloids, see: (a) Kobayashi, J.; Morita, H.. In *The Alkaloids*; Cordell, G. A., Ed.; Academic: New York, 2005; Vol. 61, p 1; (b) Ayer, W. A.; Trifonov, L. S.. In *The Alkaloids*; Cordell, G. A., Brossi, A., Eds.; Academic: New York, 1994; Vol. 45, p 233; (c) Ayer, W. A. *Nat. Prod. Rep.* **1991**, *8*, 455; (d) MacLean, D. B.. In *The Alkaloids*; Brossi, A., Ed.; Academic: New York, 1985; Vol. 26, p 241; (e) MacLean, D. B.. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic: New York, 1973; Vol. 14, p 348; (f) MacLean, D. B.. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic: New York, 1968; Vol. 10, p 305.
2. Ishiuchi, K.; Kubota, T.; Hoshino, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Bioorg. Med. Chem.* **2006**, *14*, 5995–6000, and references cited therein.
3. Kobayashi, J.; Hirasawa, Y.; Yoshida, N.; Morita, H. *Tetrahedron Lett.* **2000**, *41*, 9069–9073.
4. Obara, Y.; Kobayashi, H.; Ohta, T.; Ohizumi, Y.; Nakahata, N. *Mol. Pharmacol.* **2001**, *59*, 1287–1297.
5. Morita, H.; Ishiuchi, K.; Haganuma, A.; Hoshino, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Tetrahedron* **2005**, *61*, 1955–1960.