Isariotins A–D, Alkaloids from the Insect Pathogenic Fungus *Isaria tenuipes* BCC 7831

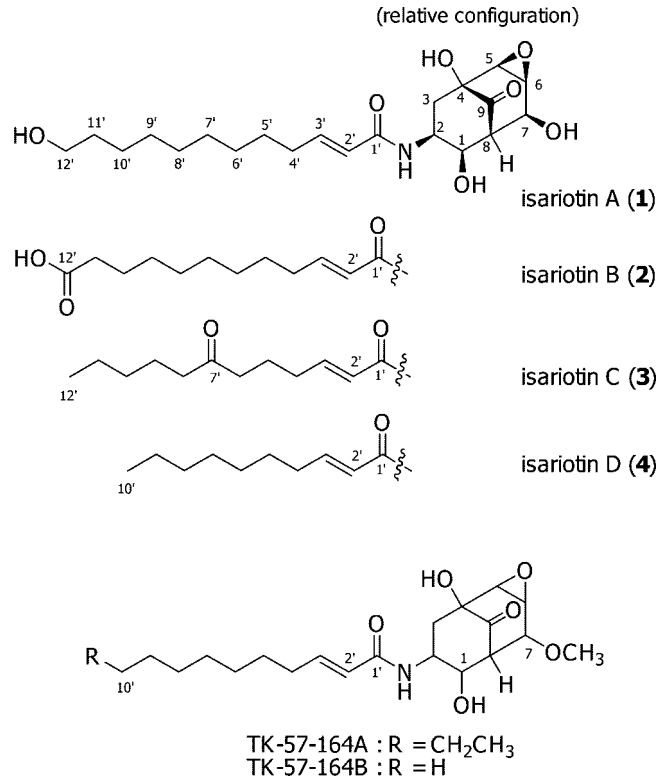
Rachada Haritakun, Prasert Srikitikulchai, Punsa Khoyaiklang, and Masahiko Isaka*

National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, 113 Phaholyothin Road, Klong Luang, Pathumthani 12120, Thailand

Received June 18, 2007

Isariotins A–D (**1**–**4**), alkaloids possessing a unique bicyclo[3.3.1]nonane ring, were isolated from the insect pathogenic fungus *Isaria tenuipes* BCC 7831. The structures of these compounds were elucidated primarily by NMR and mass spectroscopic analyses.

Fungi belonging to the genus *Isaria* are insect pathogens including Homoptera, Lepidoptera, and Coleoptera. This group of fungi are sources of a wide range of bioactive compounds[1] such as the potent immunosuppressant sphingoid ISP-I (from *I. sinclairii* ATCC 24400),[2] the antioxidative pseudo-dipeptide hanasanagin (from fruiting bodies of *I. japonica*),[3] and three types of insecticidal cyclodepsipeptides, isariins (from *I. cretacea*[4] and *I. felina*[5]), isarolides (from an *Isaria* sp.),[6] and isafelins/isaridins (from *I. felina*[7] and an *Isaria* sp.[8]). As part of our research program on bioactive secondary metabolites from fungi of Thai isolates, we have been continuously investigating the constituents of insect pathogenic fungi within the family Clavicipitaceae.[9] In this paper we describe the isolation and structure elucidation of four new alkaloids, isariotins A–D (**1**–**4**), from *Isaria tenuipes* BCC 7831. Currently, the taxonomy of *Isaria* species has been fluid due to their close relation with entomogenous species within the mega genus *Paecilomyces*.[10] On the basis of phylogenetic relationships analyzed using β-tuburin gene and ITS rDNA, it was recently proposed by our collaborating mycologists (at BIOTEC) that 10 of the entomogenous *Paecilomyces* species should be moved to the genus *Isaria*.[11] This report includes the reassignment of one of the most popular entomopathogenic fungi, *Paecilomyces tenuipes* (or *Isaria japonica*, commonly in Japan), to be named *Isaria tenuipes*.[11] A series of trichothecanes have been isolated from cultured fruiting bodies of *P. tenuipes*,[12] whereas we previously reported production of beauvericins in cell cultures of *P. tenuipes* (*I. tenuipes*) BCC 1614.[13]



Results and Discussion

Isariotin A (**1**) was obtained in low quantity (1.8 mg) from the extract of a 7 L liquid culture (potato dextrose broth, PDB) grown in a 10 L bioreactor. On the other hand, the culture grown in shake flasks using the same liquid medium (40 × 250 mL, 10 L) contained a trace of isariotin B (**2**, 2.0 mg) but lacked **1**. When the flask fermentation was conducted using the same medium (PDB) under static conditions (80 × 250 mL, 20 L), four compounds, **1**–**4**, were isolated (11.3, 1.8, 14.0, and 9.9 mg, respectively). In all cultures, the major secondary metabolite constituent of the extracts was beauvericin. The spectroscopic data of beauvericin isolated from the fungus BCC 7831 were identical to those of the standard compound that we previously isolated from *I. tenuipes* (*P. tenuipes*) BCC 1614.[13]

The molecular formula of isariotin A (**1**) was established to be $C_{21}H_{33}NO_7$ using HRESIMS in combination with the ^{13}C NMR data. The IR spectrum showed intense broad absorption bands of amide and ketone carbonyls at ν_{max} 1674 and 1737 cm^{-1}, respectively, as well as a broad absorption band at 3400–3350 cm^{-1} assignable to hydroxy and NH groups. Inspection of ^{13}C, DEPT, and HMQC NMR data indicated that metabolite **1** contained an aliphatic ketone (δ_C 207.8), an amide carbonyl (δ_C 164.6), two olefinic methines, an oxygenated tertiary carbon (δ_C 75.7), six methines, an oxymethylene (δ_C 61.6, hydroxymethyl), and nine methylenes. The connection of C-3–C-2–C-1–C-8–C-7–C-6–C-5 was deduced from COSY and HMBC correlations. HMBC correlations from the tertiary alcohol proton (4-OH; δ_H 4.43, s) to C-3, C-4, and C-5 indicated the connection of C-3–C-4–C-5. The bicyclo[3.3.1]nonane ring system with a ketone bridge was suggested by the presence of HMBC correlations from H-3a, 4-OH, H-5, and H-8 to this carbonyl (C-10). The epoxide bridge between C-5 and C-6 was strongly suggested by the chemical shifts of these oxymethine carbons at δ_C 64.0 (C-5) and 57.6 or 57.5 (C-6) and by the absence of corresponding hydroxy proton resonances. The amide proton at δ_H 6.99 (br d, J = 8.1 Hz, exchangeable with D_2O) showed COSY correlation with H-2 (δ_H 4.82, m), whereas this NH proton exhibited an HMBC correlation to the δ_C 164.4 carbonyl carbon (C-1′). The amide carbonyl (C-1′) was connected with a *trans* olefin (J = 15.2 Hz), as revealed by the HMBC correlations from both olefinic protons (H-2′ and H-3′) to the carbonyl. C-3′ of the *trans* olefin was attached to a nine-carbon methylene chain, which was terminated by a hydroxymethyl group (C-12′); therefore, the planar structure of isariotin A (**1**) was proposed.

* Corresponding author. Tel: +66-25646700, ext. 3554. Fax: +66-25646707. E-mail: isaka@biotec.or.th.

10.1021/np070291q CCC: $37.00
Published on Web 09/07/2007

Table 1. ^{13}C NMR (125 MHz) Data for Isariotins A–D (**1**–**4**)

position	**1**[a]	**2**[b]	**3**[a]	**4**[b]
1	71.7	71.3	71.7	71.4
2	46.1	46.1	46.1	46.0
3	37.9	37.7	37.9	37.8
4	75.7	75.8	75.7	75.7
5	64.0	64.1	64.0	64.0
6	57.5[c]	57.5[e]	57.5[g]	57.5[h]
7	68.3	68.1	68.3	68.2
8	57.6[c]	57.8[e]	57.6[g]	57.6[h]
9	207.8	208.5	207.8	208.1
1′	164.6	165.9	164.4	165.1
2′	124.4	124.0	124.8	124.1
3′	143.3	144.2	142.5	143.9
4′	31.5	31.4	30.9	31.60[i]
5′	28.2	27.7	22.2[j]	28.2
6′	29.0[d]	29.3[f]	41.2	28.9
7′	29.0[d]	29.1[f]	208.8	29.7
8′	28.9[d]	29.0[f]	42.1	31.58[i]
9′	28.7[d]	28.4[f]	23.2	22.4
10′	25.7	25.3	31.2	13.4
11′	32.9	35.4	22.2[j]	
12′	61.6	179.7	13.3	

[a] Acquired in acetone-d_6. [b] Acquired in acetone-d_6/D_2O (2 drops). [c–i] Assignments of carbons with the same index can be interchanged. [j] The ^{13}C NMR resonances are superimposed.

The relative configuration of the bicyclo[3.3.1]nonane ring moiety was deduced from ^{1}H–^{1}H *J*-values and NOESY correlation data. The coupling constant of 12.7 Hz observed between H-2 (δ_H 4.82, m) and one of the diastereotopic H-3 methylene protons at δ_H 1.99 (H-3b, t, J = 12.7 Hz) and the relatively weak NOESY cross-peak between these protons indicated the *trans*-diaxial relation. The methine H-1 resonated as a narrow multiplet, excluding any large *J*-value couplings. The presence of *W*-type long-range coupling (J = 1.8 Hz) for H-1 and H-3a (δ_H 2.31, ddd, J = 12.7, 5.0, 1.8 Hz) strongly supported the equatorial assignment of these protons. The NOESY correlations from H-1 and H-2 to H-7 established the configuration of C-7. According to the intense NOESY cross-peak of H-3a and H-5, the epoxide oxygen was placed on the β-face in the structure **1**.

The molecular formula of isariotin B (**2**), $C_{21}H_{31}NO_8$, was determined by HRESIMS. Since the resolution of ^{1}H NMR spectra recorded in DMSO-d_6 or acetone-d_6 was not clear for structural elucidation, all NMR data for **2** were acquired in acetone-d_6 with 2 drops of D_2O. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**. The significant difference was the absence of the hydroxymethyl group in **2** and the presence of a broad carbonyl carbon resonating at δ_C 179.7. The HMBC correlation from the methylene protons of the side chain (H-11′, δ_H 2.17, m) confirmed the terminal carboxylic acid functionality. Detailed analysis of the NMR data, in a similar fashion to that described above for **1**, revealed that the structure of the bicyclo[3.3.1]nonane core of **2** is identical to that of **1**. Isariotin B (**2**) was therefore defined to be the carboxylic acid analogue of isariotin A (**1**).

The IR spectrum of isariotin C (**3**), $C_{21}H_{31}NO_7$ (HRESIMS), exhibited an additional intense carbonyl absorption band at ν_{max} 1708 cm^{-1}, when compared with **1**. The ^{13}C NMR spectrum showed that one of the methylene carbons was replaced by an aliphatic ketone resonating at δ_C 208.8, and the ^{1}H NMR spectrum exhibited the resonance of a terminal methyl at δ_H 0.87 (3H, t, J = 7.1 Hz, H-12′). The COSY and HMQC data demonstrated the side chain structure, C-2′ to C-6′, and C-8′ to the terminal methyl group (C-12′). HMBC correlations from H-5′, H-6′, H-8′, and H-9′ to the ketone carbonyl carbon permitted the attachment of this ketone to C-7′. Again, the bicyclo[3.3.1]nonane core was proved to be identical to that of isariotins A and B by interpretation of the 2D NMR data.

Isariotin D (**4**) was slightly less polar when compared with other isariotins. Its molecular formula was determined by HRESIMS and ^{13}C NMR to be $C_{19}H_{29}NO_6$. The ^{1}H and ^{13}C NMR data for the bicyclo[3.3.1]nonane core were similar to those of **1**–**3**; hence, isariotin D (**4**) possesses a C_{10} carboxyamide group. The COSY and HMBC data revealed the structures of the head part of the α,β-unsaturated carboxyamide, C-1′–C-5′ position, and the tail end of the linear chain, C-8′–C-10′. The rest of the resonances, two methylenes, were assigned to C-6′ and C-7′. To determine the absolute configuration, we attempted to apply the modified Mosher method. However, we were unable to prepare the (*S*)- and (*R*)-MTPA esters of isariotin D (**4**) employing usual reaction conditions (excess MTPA-Cl, pyridine, rt), presumably because of the steric hindrance of the secondary alcohol moieties.

The structures of isariotins are similar to those of substances TK-57-164A and TK-57-164B, recently reported in a patent as metabolites of an *Isaria* sp. TK-57 and was claimed to be active against plant pathogenic bacteria.[14] However, the patent literature

Table 2. ^{1}H NMR (500 MHz) Data for Isariotins A–D (**1**–**4**)

position	**1**[a]	**2**[b]	**3**[a]	**4**[b]
1	4.56 m	4.47 m	4.57 m	4.49 m
2	4.82 m	4.75 m	4.82 m	4.79 m
3	2.31 ddd (12.7, 5.0, 1.8)	2.30 m	2.32 ddd (12.7, 5.0, 1.7)	2.30 ddd (12.7, 5.0, 1.9)
	1.99 t (12.7)	2.02 t (12.7)	2.00 t (12.7)	2.01 t (12.7)
5	3.51[c] m	3.56 br d (3.7)	3.52 dd (3.7, 1.3)	3.54 dd (3.7, 1.3)
6	3.39 dd (3.7, 1.4)	3.42 br d (3.7)	3.40 dd (3.7, 1.3)	3.39 dd (3.7, 1.3)
7	4.35 dd (6.8, 4.8)	4.27 br d (7.6)	4.35 br dd (7.1, 4.6)	4.27 dd (6.7, 1.0)
8	2.94 ddd (7.7, 3.5, 1.4)	2.92 m	2.95 ddd (7.7, 3.4, 1.3)	2.93 m
1-O*H*	4.61 d (3.9)		4.65 d (3.7)	
4-O*H*	4.43 s		4.44 s	
7-O*H*	5.18 d (4.8)		5.17 (d, 4.7)	
N*H*	6.99 br d (8.1)		7.07 br d (8.1)	
2′	6.03 dt (15.2, 1.4)	6.06 br d (15.3)	6.04 dt (15.3, 1.5)	6.05 dt (15.3, 1.5)
3′	6.77 dt (15.2, 7.0)	6.78 dt (15.3, 6.9)	6.75 dt (15.3, 7.0)	6.79 dt (15.3, 7.0)
4′	2.16 m	2.15 br q (7.0)	2.16 dq (1.5, 7.0)	2.15 dq (1.5, 7.0)
5′	1.44 m	1.42 m	1.67 quintet (7.3)	1.42 m
6′	1.28–1.36[d] m	1.25–1.30[e] m	2.47 t (7.3)	1.24–1.32[f] m
7′	1.28–1.36[d] m	1.25–1.30[e] m		1.24–1.32[f] m
8′	1.28–1.36[d] m	1.25–1.30[e] m	2.42 t (7.4)	1.24–1.32[f] m
9′	1.28–1.36[d] m	1.25–1.30[e] m	1.52 quintet (7.4)	1.24–1.32[f] m
10′	1.28–1.36[d] m	1.52 m	1.26 m	0.86 t (7.0)
11′	1.48 m	2.17 m	1.29 m	
12′	3.51[c] m		0.87 t (7.1)	
12′-O*H*	3.43 t (5.2)			

[a] Acquired in acetone-d_6. [b] Acquired in acetone-d_6/D_2O (2 drops). [c–f] The ^{1}H NMR resonances with the same index are overlapped.

provided neither the relative configuration nor the NMR assignments of protons and carbons. By comparison of the ^{1}H and ^{13}C NMR spectroscopic data, it seems likely that TK-57-164s have the same relative configuration as isariotins. These *Isaria* metabolites are also partly related to gymnastatins, in particular gymnastatin G,[15] isolated from the sponge-derived fungus *Gymnascella dankaliensis* (OUPS-N134).[15,16] The plausible biosynthetic pathways of gymnastatins were proposed with L-tyrosine as the precursor of the bicyclo[3.3.1]nonane core.[15]

Isariotins did not show any activities in the assays against a malarial parasite (*Plasmodium falciparum* K1; up to a compound concentration of 20 μg/mL) and fungi (*Candida albicans*, *Magnaporthe grisea*; up to 50 μg/mL) nor cytotoxicity to three cancer cell lines (KB, BC, and NCI-H-187; up to 20 μg/mL) and Vero cells (up to 50 μg/mL). Compounds **1**, **3**, and **4** exhibited marginal activity against the mycobacterium *Mycobacterium tuberculosis* H_{37}Ra with the same MIC value of 200 μg/mL (486, 488, and 544 μM, respectively). The major metabolite of the fungus BCC 7831, beauvericin, has been known to exhibit significant antimalarial, antitubercular, and cytotoxic activities in the same assays.[13]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a Varian CARY 1E UV–visible spectrophotometer. FT-IR spectra were recorded on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker AV500D and DRX400 spectrometers. ESI-TOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

Fungal Material. The fungus used in this study was isolated on a Lepidoptera larva and identified by Dr. Nigel L. Hywel-Jones, BIOTEC. It was deposited in the BIOTEC Culture Collection (BCC) as BCC 7831 on July 15, 1998. This strain (BCC 7831) was originally described as *Paecilomyces tenuipes*, and it has recently been renamed as *Isaria tenuipes* by the depositor (N. L. Hywel-Jones).[11]

Fermentation and Isolation. *I. tenuipes* BCC 7831 was maintained on potato dextrose agar at 25 °C, and the agar was cut into plugs (1 × 1 cm) and inoculated into 8 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 6 days on a rotary shaker (200 rpm), each primary seed culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB), and incubated at 25 °C for 6 days on a rotary shaker (200 rpm). Each 25 mL portion of the secondary cultures (in 8 flasks) was transferred into 80 × 1 L Erlenmeyer flasks each containing 250 mL of PDB, and final fermentation was carried out at 25 °C for 29 days under static conditions. The cultures were harvested by filtration. The filtrate was extracted three times with EtOAc and concentrated under reduced pressure to leave a brown gum (1.80 g). This crude extract was passed through a Sephadex LH-20 column (4.0 × 60 cm) with MeOH as eluent to obtain 11 fractions, Fr-1–Fr-11. Fr-4 (387 mg) was mainly composed of beauvericin (^{1}H NMR in $CDCl_3$). Fr-5 (297 mg) was applied to Si gel column chromatography (MeOH/CH_2Cl_2, step gradient elution). The subfraction Fr-5-3 (114 mg) was beauvericin (^{1}H and ^{13}C NMR and MS data were identical to those of the authentic sample).[13] Fr-5-5 (24 mg) was subjected to preparative HPLC using a reversed-phase column (NovaPak HR C_{18}, 2.5 × 10.0 cm, 6 μm; mobile phase MeOH/H_2O = 40:60, flow rate 8 mL/min), followed by a short column on Si gel to furnish **3** (2.7 mg) and **4** (3.8 mg). The subfraction Fr-5-10 (41 mg) was subjected to preparative HPLC (MeOH/H_2O = 35:65) and a Si gel short column to obtain **2** (1.8 mg). The Sephadex LH-20 column fraction, Fr-6 (618 mg), was fractionated by Si gel column chromatography (MeOH/CH_2Cl_2, step gradient elution) to obtain 13 subfractions, Fr-6-1–Fr-6-13. Fr-6-5 (66 mg) was repeatedly subjected to Si gel column and preparative HPLC to furnish **3** (11.3 mg) and **4** (6.1 mg). Compound **1** (11.3 mg) was purified from Fr-6-9 (90 mg) by a combination of Sephadex LH-20 and Si gel column chromatography and preparative HPLC.

Isariotin A (1): colorless, amorphous solid; $[\alpha]^{27}_D$ −45 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.16) nm; IR (KBr) ν_{max} 3385, 2920, 1737, 1674, 1642, 1078 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI-TOF) *m/z* 434.2164 [M + Na]$^+$ (calcd for $C_{21}H_{33}NO_7Na$, 434.2149).

Isariotin B (2): colorless, amorphous solid; $[\alpha]^{26}_D$ −23 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.24) nm; IR (KBr) ν_{max} 3303, 2928, 1737, 1669, 1633, 1556, 1408, 1080 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI-TOF) *m/z* 448.1951 [M + Na]$^+$ (calcd for $C_{21}H_{31}NO_8Na$, 448.1942).

Isariotin C (3): colorless, amorphous solid; $[\alpha]^{27}_D$ −25 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.20) nm; IR (KBr) ν_{max} 3357, 2932, 1738, 1708, 1668, 1626, 1542, 1082, 757 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI-TOF) *m/z* 432.2016 [M + Na]$^+$ (calcd for $C_{21}H_{31}NO_7Na$, 432.1993).

Isariotin D (4): colorless, amorphous solid; $[\alpha]^{27}_D$ −37 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.03) nm; IR (KBr) ν_{max} 3329, 2927, 1739, 1668, 1630, 1543, 1083 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI-TOF) *m/z* 390.1885 [M + Na]$^+$ (calcd for $C_{19}H_{29}NO_6Na$, 390.1887).

Acknowledgment. Financial support from National Center for Genetic Engineering and Biotechnology is gratefully acknowledged.

References and Notes

(1) Isaka, M.; Kittakoop, P.; Thebtaranonth, Y. In *Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts*; White, J. F., Jr., Bacon, C. W., Hywel-Jones, N. L., Spatafora, J. W., Eds.; Marcel Dekker, Inc.: New York, 2003; pp 355–398.
(2) Fujita, T.; Inoue, K.; Yamamoto, S.; Ikumoto, T.; Sasaki, S.; Toyama, R.; Chiba, K.; Hoshino, Y.; Okumoto, T. *J. Antibiot.* **1994**, *47*, 208–215.
(3) Sakakura, A; Suzuki, K.; Katsuzaki, H.; Komiya, T.; Imamura, T.; Aizono, Y.; Imai, K. *Tetrahedron Lett.* **2005**, *46*, 9057–9059.
(4) (a) Vining, L. C.; Taber, W. A. *Can. J. Chem.* **1962**, *40*, 1579–1584. (b) Wolstenholme, W. A.; Vining, L. C. *Tetrahedron Lett.* **1972**, 2693–2696.
(5) (a) Baute, R.; Deffieux, G.; Merlet, D.; Baute, M.-A.; Neveu, A. *J. Antibiot.* **1981**, *34*, 1261–1265. (b) Deffieux, G.; Merlet, D.; Baute, R.; Bourgeois, G.; Baute, M.-A.; Neveu, A. *J. Antibiot.* **1981**, *34*, 1266–1271.
(6) Briggs, L. H.; Fergus, B. J.; Shannon, J. S. *Tetrahedron Suppl. 8* **1968**, *22*, 269–278.
(7) Guo, Y. X.; Liu, Q. H.; Ng, T. B.; Wang, H. X. *Peptides* **2005**, *26*, 2384–2391.
(8) (a) Sabareesh, V.; Ranganayaki, R. S.; Raghothama, S.; Bopanna, M. P.; Balaram, H.; Srinivasan, M. C.; Balaram, P. *J. Nat. Prod.* **2007**, *70*, 715–729. (b) Ravindra, G.; Ranganayaki, R. S.; Raghothama, S.; Srinivasan, M. C.; Gilardi, R. D.; Karle, I. L.; Balaram, P. *Chem. Biodiversity* **2004**, *1*, 489–504.
(9) Isaka, M.; Kittakoop, P.; Kirtikara, K.; Hywel-Jones, N. L.; Thebtaranonth, Y. *Acc. Chem. Res.* **2005**, *38*, 813–823.
(10) Luangsa-ard, J. J.; Hywel-Jones, N. L.; Samson, R. A. *Mycologia* **2004**, *96*, 773–780.
(11) Luangsa-ard, J. J.; Hywel-Jones, N. L.; Manoch, L.; Samson, R. A. *Mycol. Res.* **2005**, *109*, 581–589.
(12) (a) Kikuchi, H.; Miyagawa, Y.; Sahashi, Y.; Inatomi, S.; Haganuma, A.; Nakahata, N.; Oshima, Y. *Tetrahedron Lett.* **2004**, *45*, 6225–6228. (b) Kikuchi, H.; Miyagawa, Y.; Nakamura, K.; Sahashi, Y.; Inamori, S.; Oshima, Y. *Org. Lett.* **2004**, *6*, 4531–4533. (c) Kikuchi, H.; Miyagawa, Y.; Sahashi, Y.; Inatomi, S.; Haganuma, A.; Nakahata, N.; Oshima, Y. *J. Org. Chem.* **2004**, *69*, 353–356.
(13) (a) Nilanonta, C.; Isaka, M.; Kittakoop, P.; Palittapongarnpim, P.; Kamchonwongpaisan, S.; Pittayakhajonwut, D.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2000**, *66*, 756–758. (b) Nilanonta, C.; Isaka, M.; Kittakoop, P.; Trakulnaleamsai, S.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron* **2002**, *58*, 3355–3360.
(14) Nakagawa, A.; Nishikawa, N.; Takahashi, S.; Yamamoto, K. WO 2004-074269 A1.
(15) Amagata, T.; Minoura, K.; Numata, A. *J. Nat. Prod.* **2006**, *69*, 1384–1388.
(16) Amagata, T.; Doi, M.; Ohta, T.; Minoura, K.; Numata, A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3585–3599.

NP070291Q