Brunneins A–C, β-Carboline Alkaloids from *Cortinarius brunneus*

Axel Teichert, Jürgen Schmidt, Andrea Porzel, Norbert Arnold,* and Ludger Wessjohann

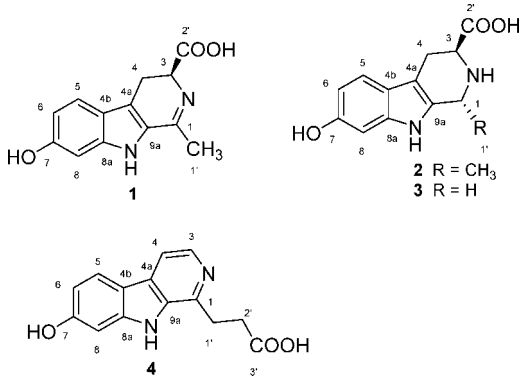
Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany

Received June 4, 2007

Four β-carboline alkaloids, brunneins A–C (**1**–**3**) and 3-(7-hydroxy-9*H*-β-carboline-1-yl)propanoic acid (**4**), were isolated from fruiting bodies of the agaricoid fungus *Cortinarius brunneus*. The structures of **1**–**3** were determined by analysis of NMR and MS data, and the structure of compound **4** was determined by comparison with published data. Brunnein A (**1**) exhibited very low cholinesterase inhibitory effects and no cytotoxicity.

The basidiomycetous genus *Cortinarius* is the most widely distributed genus in Agaricales and comprises about 4200 epithets.[1] The genus *Cortinarius* is divided into several subgenera based mainly on morphologic and microscopic properties. Species in *Cortinarius* form obligate ectomycorrhiza with conifers and deciduous trees. The genus *Cortinarius* is subdivided into the subgenera *Cortinarius*, *Leprocybe*, *Sericeocybe*, *Phlegmacium*, *Myxacium*, and *Telamonia* in Europe.[2,3] *Cortinarius brunneus* (Pers.) Fr. belongs to the subgenus *Telamonia*, wherein species with mostly brownish-colored fruiting bodies with hygrophan caps are combined. However, the ongoing circumscription of the genus as well as their subgenera based on molecular biological data suggested that the infrageneric delimitations should be rearranged.[4,5] Fruiting bodies of *C. brunneus* are usually umber to dark brown colored with a 3–8 cm diameter cap and a 6–10 cm stem. The species forms ectomycorrhiza usually with *Picea*. Chemical investigations of *C. brunneus* are few. The isolation of L-4-hydroxy-3-methoxyphenylalanine from *C. brunneus* is the only structural chemistry report so far.[6] Furthermore, two unknown greenish fluorescent compounds were used as chemotaxonomic markers for the infrageneric delimitation of *C. brunneus.*[7]

This study describes the isolation and structure elucidation of the new β-carboline alkaloids (−)-(3*S*)-7-hydroxy-1-methyl-4,9-dihydro-3*H*-β-carboline-3-carboxylic acid (**1**, brunnein A), (−)-(1*R*,3*S*)-7-hydroxy-1-methyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid (**2**, brunnein B), (−)-(3*S*)-7-hydroxy-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid (**3**, brunnein C), and 3-(7-hydroxy-9*H*-β-carboline-1-yl)propanoic acid (**4**), of which only the latter is known from the water moss *Fontinalis squamosa* Hedw. and roots of the shrub *Eurycoma harmandiana* Pierre distributed in Southeast Asia.[8,9]



The β-carboline alkaloids (**1**–**4**) were obtained as yellowish solids by a fluorescence-guided isolation (λ_{exc} = 366 nm) from the MeOH extract of *C. brunneus* after repeated column chromatography on Diaion HP 20, silica gel RP-2, Sephadex LH 20, and RP-18 HPLC.

Compound **1** exhibited a strong greenish fluorescence on TLC plates under UV light (λ = 366 nm). The UV spectrum displayed absorption bands at λ_{max} = 260 nm and λ_{max} = 375 nm. The negative ion electrospray (ESI) mass spectrum showed characteristic ions at *m/z* 243 ($[M - H]^-$) and *m/z* 199 ($[M - H - CO_2]^-$). The molecular formula $C_{13}H_{12}O_3N_2$ was deduced from the positive ion ESI-FTICR-MS (*m/z* 245.0919, $[M + H]^+$). LC-ESIMS/MS analyses in the positive ion mode showed three main fragments at *m/z* 199 ($[M - H_2CO_2]^+$), *m/z* 171 ($[M - H_2CO_2 - CO]^+$), and *m/z* 131, representing the hydroxyindolyl key ion. This fragmentation pattern revealed a dihydro-β-carboline skeleton substituted with a methyl and a carboxyl group at the dihydropyridine ring and a hydroxyl group located at the indole moiety.

The ^{1}H NMR spectrum showed a three-spin coupling system with signals at δ 6.69 (1H, dd, H-6), 6.77 (1H, d, H-8), and 7.52 (1H, d, H-5), indicating a 3-fold-substituted aromatic ring. A second spin system consisted of H-3 (1H, dd, 4.08), H-4A (1H, dd, 3.14), and H-4B (1H, dd, 3.21). The position of the phenolic OH group at C-7 was deduced from comparison with literature ^{13}C NMR data of hyrtioerectine B and harmalol, naturally occurring analogue β-carboline alkaloids, possessing the hydroxyl function at C-6 and C-7, respectively.[10,11] Furthermore, the high-field ^{13}C chemical shift of C-8 at δ 96.3 is caused by the two electron-donating *ortho* substituents at C-7 and C-8a. The ^{1}H NMR methyl group singlet signal at δ 2.56 showed only two HMBC correlations, with C-1 (δ 160.9) and C-9a (δ 126.2), indicating the methyl group at C-1. The carboxyl group was located at C-3 which displayed a low-field ^{13}C chemical shift of δ 58.0. Analysis of the heteronuclear 2D NMR spectra resulted in an unambiguous assignment of all ^{1}H and ^{13}C NMR signals (Table 1). The absolute configuration of brunnein A was determined using a degradation method described in the literature.[12] Therefore, the structure of **1** was assigned as (−)-(3*S*)-7-hydroxy-1-methyl-4,9-dihydro-3*H*-β-carboline-3-carboxylic acid.

The spectroscopic data of compounds **2**–**4** resembled that of carboline **1**. The positive ion ESI-FTICR-MS afforded the molecular formula $C_{13}H_{14}O_3N_2$ (*m/z* 247.1078, $[M + H]^+$) for compound **2** and $C_{12}H_{12}O_3N_2$ (*m/z* 233.0925, $[M + H]^+$) for compound **3**, both suggesting eight degrees of unsaturation. The characteristic loss of 73 amu (HN═CH–CO_2H) observed in the LC-ESIMS/MS spectra of **2** and **3** being typical for a retro-Diels–Alder fragmentation suggested a tetrahydro-β-carboline skeleton for both compounds.[13,14]

The ^{1}H NMR spectrum of **2** (Table 2) differed from that of **1** only in the signal for the methyl group at δ 1.52 giving a doublet coupling with H-1. The corresponding carbon C-1 showed a ^{13}C high-field shift at δ 46.8 deduced from the HMBC correlation peak. The ^{1}H NMR spectrum of compound **3** (Table 2) differed from that of compound **2** by a loss of the methyl signal, this being replaced by two doublets at δ 4.44 (1H, d, H-1A) and δ 4.49 (1H, d, H-1B), indicating a methylene group (C-1).

* Corresponding author. Tel: +49-345-5582-1310. Fax: +49-345-5582-1319. E-mail: Norbert.Arnold@ipb-halle.de.

10.1021/np070259w CCC: $37.00
Published on Web 09/14/2007

Table 1. ^{1}H and ^{13}C NMR Data of Brunnein A (**1**) (in DMSO-d_6)

position	δ_C, mult.[a]	δ_H (J in Hz)	HMBC[b]
1	160.9, qC		
3	58.0, CH	4.08, dd (11.8, 8.6)	
4	22.9, CH_2	3.14, dd (17.5, 11.8)	3, 4a, 9a
		3.21, dd (17.5, 8.6)	
4a	121.9, qC		
4b	118.4, qC		
5	122.4, CH	7.52, d (8.7)	7, 8a
6	113.1, CH	6.69, dd (8.7, 1.4)	4b
7	157.9, qC		
7-*OH*		8.53, s	
8	96.3, CH	6.77, d (1.4)	4b, 6, 7
8a	141.8, qC		
9a	126.2, qC		
1′	18.9, CH_3	2.56, s	1, 9a
2′	169.5, qC	11.53, br, s	

[a] Chemical shifts of HSQC and HMBC correlation peaks. [b] Carbons correlating with the corresponding proton.

The position of the hydroxyl group at C-7 in compounds **2** and **3** was determined as in case of **1**. The position and relative configuration of the carboxyl and the methyl group in **2** were established by 2D ROESY experiments, which showed correlations between Me-1′$_{ax}$ and H-3$_{ax}$.

The absolute configurations of compounds **2** and **3** were determined by comparison of their spectroscopic data with those from compound **1** and with literature data from analogue compounds, e.g. (−)-(3*S*)-6-hydroxy-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid.[15] As a consequence, the structure of compound **2** was assigned as (−)-(1*R*,3*S*)-7-hydroxy-1-methyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid, and compound **3** as (−)-(3*S*)-7-hydroxy-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid. Compound **4** was assigned as 3-(7-hydroxy-9*H*-β-carboline-1-yl)-propanoic acid, previously reported from *Fontinalis squamosa* and *Eurycoma harmandiana*.[8,9]

Fluorescent β-carboline derivatives are widespread in plants, but very rare in Basidiomycetes.[16] (−)-1-Methyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid has been reported from *Amanita muscaria* (L. ex Fr.) Hooker.[17] Harmane, a frequent plant alkaloid, is so far only known in Basidiomycetes from the culture broth of *Coriolus maximus*.[18] The β-carboline-1-propionic acid derivatives infractin, 6-hydroxyinfractin, and the pentacyclic infractopicrin were reported from *Cortinarius infractus*.[19] Infractopicrin is responsible for the bitter taste of this toadstool. Flazin, a β-carboline derivative substituted with a hydroxymethylfuran moiety, reported from Japanese soy sauce, has been detected in the fungus *Suillus granulatus*.[20,21] Infractin, *N*-methyl-β-carbolinium-1-propanoate, and a unique set of thiomethylated canthine-6-one derivatives were isolated from *Boletus curtisii*.[22]

Previous reports described β-carboline alkaloids as potential cholinesterase inhibitors. Tertiary aromatic β-carbolines showed only weak activity against acetylcholinesterase (AChE), while quaternary ones were found to be about one-sixth as potent as physostigmine in a frog rectus abdominis muscle assay.[23] These results were confirmed by recent reports about quaternary β-carbolines as AChE and butyrylcholinesterase (BChE) inhibitors using the Ellman assay.[24,25] Brunnein A (**1**) was tested exemplarily and exhibited less than 50% inhibitory activity at 10^{-4} M against both AChE and BChE. In conclusion, an additional carboxyl group at C-3 does not enhance the low activity of dihydro-β-carbolines against the cholinesterases. Tertiary β-carbolines were discussed as target-specific prodrugs activated by a β-carboline-2-*N*-methyltransferase, supported by the previous isolation of 2-*N*-methylnorharmane from human brain.[25−27] Additionally, an antiproliferative assay of brunnein A (**1**) against human PC3 prostate cancer cells indicated no cytotoxic effect up to 10^{-4} M.

Experimental Section

General Experimental Procedures. 1D (^{1}H) and 2D (HSQC, HMBC, COSY, ROESY) NMR spectra were recorded on a Varian Inova 500 NMR spectrometer operating at 499.81 MHz using a 3 mm microsample inverse detection probe. Chemical shifts were referenced to internal TMS ($\delta = 0$ ppm, ^{1}H) or DMSO-d_6 ($\delta = 39.5$ ppm, ^{13}C). For D_2O the chemical shifts were recorded with respect to the 0 ppm point determined by the manufacturer's indirect referencing method. Preparative HPLC was performed on a Varian ProStar 218 system with a PrepStar 330 photodiode array detector using a C_{18} column (ODS 5 μm, 150 × 20 mm i.di, YMC). The ESI and collision-induced dissociation (CID) mass spectra were obtained from a TSQ Quantum Ultra AM system (Thermofinnigan) equipped with a hot ESI source (HESI, electrospray voltage: 3.0 kV, sheath gas: nitrogen; vaporizer temperature: 50 °C; capillary temperature: 250 °C; collision gas: argon; collision pressure: 1.5 mTorr). The MS system was coupled with a Surveyor Plus micro-HPLC (Thermo Electron) and equipped with an Ultrasep ES RP18E-column (5 μm, 1 × 100 mm, SepServ). For the HPLC a gradient system was used starting from H_2O/CH_3CN (90:10) (each of them containing 0.2% HOAc) to 10:90 within 15 min; flow rate: 50 μL min^{-1}. The high-resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonics) equipped with an Infinity cell, a 7.0 T superconducting magnet (Bruker), a RF-only hexapole ion guide, and an external electrospray ion source (Agilent, off-axis spray). The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 μL h^{-1}. IR spectra were measured on a Thermo Nicolet 5700 FT-IR on an ATR crystal (diamond), CD spectra on a Jasco J-815 CD spectrometer, and UV spectra on a Jasco V-560 UV/vis spectrometer. Optical rotations were

Table 2. ^{1}H and ^{13}C NMR Data of Brunnein B (**2**) (in DMSO-d_6) and Brunnein C (**3**) (in D_2O)

	brunnein B (**2**)			brunnein C (**3**)		
position	δ_C, mult.[a]	δ_H (J in Hz)	HMBC[b]	δ_C, mult.[a]	δ_H (J in Hz)	HMBC[b]
1	46.8, CH	4.53, q (6.7)	9a, 1′	40.3, CH_2	4.44, d (15.6)	
					4.49, d (15.6)	
3	53.4, CH	3.71, dd (7.9, 5.4)	4a, 2′	57.0, CH	4.08, dd (11.0, 5.3)	
4	23.4, CH_2	2.87, dd (16.1, 7.9)	3, 4a, 9a, 2′	22.2, CH_2	3.03, dd (16.4, 11.0)	4a, 5
		3.00, dd (16.1, 5.4)			3.39, dd (16.4, 5.3)	
4a	105.9, qC			105.9, qC		
4b	120.3, qC			124.4, qC		
5	118.6, CH	7.19, d (8.4)	4a, 7, 8a	119.0, CH	7.48, d (8.5)	7
6	109.5, CH	6.52, dd (8.4, 1.9)	8	109.4, CH	6.77, dd (8.5, 2.2)	5
7	153.7, qC			151.8, qC		
7-OH		8.86, br, s			n.d.	
8	97.1, CH	6.69, d (1.9)	4b, 7	97.2, CH	6.94, d (2.2)	5, 7
8a	137.9, qC			137.5, qC		
9a	131.1, qC			n.d.		
1′	19.0, CH_3	1.52, d (6.7)	1, 9a	173.9, qC	n.d.	
2′	170.2, qC	n.d.				

[a] Chemical shifts of HSQC and HMBC correlation peaks. [b] Carbons correlating with the corresponding proton. n.d. = not detected.

measured on a JASCO DIP-1000 polarimeter. Column chromatography was performed on Diaion HP 20 (Supelco), Sephadex LH20 (Pharmacia), and silica gel RP-2 (Merck). TLC was carried out on precoated aluminium TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm) using *n*-BuOH/HOAc/H_2O (4:1:1, v/v) as solvent system. For AChE and BChE inhibition and antiproliferative assays a Genios Pro (Tecan) microplate reader was used.

Fungal Material. *C. brunneus* (Pers.) Fr. was collected under *Pinus picea* in Paintner Forest, Kelheim, Germany (September 19, 2000, leg./det. N. Arnold, coll. 10/00 and 11/00). Voucher specimens are deposited at the Leibniz Institute of Plant Biochemistry Halle, Germany (IPB).

Extraction and Isolation. Lyophilized fruiting bodies of *C. brunneus* (125 g) were crushed in a mixer and extracted twice with 2 L of 80% aqueous MeOH in an ultrasound bath for 1 h at room temperature. The yellow-brown solution was concentrated to dryness *in vacuo* to give a dark brown residue (6.1 g). The crude extract was adsorbed on Diaion HP-20 resin and fractionated by sequential elution with H_2O and MeOH. The MeOH fractions containing the greenish fluorescent compounds **1**–**4** were further purified by repeated column chromatography on silica gel RP-2 using 40% aqueous MeOH as solvent and Sephadex LH 20 with MeOH. Preparative HPLC on a ODS C-18 column using H_2O (A) and CH_3CN (B) as eluents (linear gradient: 0–20 min, 3 %–25 % B, flow rate of 20 mL min^{-1}) afforded compounds **1** (1.5 mg), **2** (0.5 mg), **3** (0.5 mg), and **4** (0.6 mg).

Brunnein A (1): yellow-greenish solid; $[\alpha]^{26}_D$ −22 (*c* 0.02, MeOH); CD (MeOH) $[\theta]_{243}$ −1434, $[\theta]_{307}$ +1836, $[\theta]_{356}$ −3029, $[\theta]_{407}$ +1256; UV (MeOH) λ_{max} (log ε) 260 (3.3), 375 (3.6) nm; IR ν_{max} 3235 (br), 2929, 1558, 1374, 1262, 1083,1026, 806 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1; ESI-FTICR-MS *m/z* 245.0919 ($[M + H]^+$, calcd for $C_{13}H_{13}O_3N_2^+$, 245.0921); LC-ESI-CID-MS (positive ion mode, −20 eV) *m/z* (rel int. %) 245 (47), 199 (100), 171 (3), 162 (5).

Brunnein B (2): yellow solid; $[\alpha]^{25}_D$ −35 (*c* 0.03, MeOH); CD (MeOH) $[\theta]_{231}$ −9266, $[\theta]_{266}$ −1053, $[\theta]_{283}$ +215, $[\theta]_{301}$ −557; UV (MeOH) λ_{max} (log ε) 219 (4.4), 299 (3.4) nm; IR ν_{max} 3329 (br), 2941, 2831, 1638, 1556, 1454, 1397, 1111,1021 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 2; ESI-FTICR-MS *m/z* 247.1078 ($[M + H]^+$, calcd for $C_{13}H_{15}O_3N_2^+$, 247.1077); LC-ESI-CID-MS (negative ion mode, +20 eV) *m/z* (rel int, %) 245 (6), 201 (100), 199 (15), 174 (6), 172 (35), 158 (14).

Brunnein C (3): yellowish solid; $[\alpha]^{22}_D$ −57 (*c* 0.02 MeOH); CD (MeOH) $[\theta]_{234}$ −585, $[\theta]_{266}$ −2537; UV (MeOH) λ_{max} (log ε) 222 (4.4), 267 (3.5), 296 (3.5) nm; IR ν_{max} 3384 (br), 2921, 2852, 1602, 1405, 1155, 1042, 806 cm^{-1}; ^1H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O), see Table 2; ESI-FTICR-MS *m/z* 233.0925 ($[M + H]^+$, calcd for $C_{12}H_{13}O_3N_2^+$, 233.0921); LC-ESI-CID-MS (positive ion mode, −15 eV) *m/z* (rel int, %) 233 (11), 160 (100).

3-(7-Hydroxy-9*H*-*β*-carboline-1-yl)propanoic acid (4): yellow-brownish solid; UV (MeOH) λ_{max} (log ε) 245 (4.2), 304 (3.7), 327 (3.72) nm, IR ν_{max} 3384 (br), 2921, 2852, 1602, 1405, 1155, 1042, 806 cm^{-1}; ^1H and ^{13}C NMR data agree with literature data;[8,9] ESI-FTICR-MS *m/z* 257.0922 ($[M + H]^+$, calcd for $C_{14}H_{13}O_3N_2^+$, 257.0921); LC-ESI-CID-MS (positive ion mode, − 20 eV), *m/z* (rel int, %) 257 (35), 239 (100), 211 (10), 197 (12), 174 (6).

Degradation Procedure for Stereochemistry Determination. The absolute configuration of brunnein A (**1**) was determined following a method described in the literature.[12]

Bioassays. The antiproliferative assay against PC3 cells was carried out in 96-well microtiter plates using a starting cell density of 4×10^4 mL^{-1} per well and 24 h incubation time at 37 °C under 5 % CO_2 with the test substances. The cells were visually examined using a phase-contrast microscope for signs of cytotoxicity and additionally checked with XTT reagent (Roche) by determination of the particular absorbance at $\lambda = 490$ nm. Vincristine sulfate was used as a positive control at two different concentrations (10^{-5} and 10^{-8} M) with cell growth inhibitons of 49% and 16%, respectively.

Acetyl- (AChE) and butyrylcholinesterase (BChE) inhibition assays were carried out in 96-well microtiter plates based on Ellman's method.[28,29] A mixture containing 25 μL of acetyl- or butyrylthiocholine iodide solution (15 mM), 125 μL of 5,5′-dithiobis-2-nitrobenzoic acid (3 mM), and 25 μL of sample solved in TRIS buffer (pH 8) was added to a microwell together with bovine acetylcholinesterase (0.22 U/mL) and equine butyrylcholinesterase (0.11 U/mL), respectively. The colorimetric measurement was carried out at $\lambda = 405$ nm. Physostigmine hydrochloride was used as a positive control (IC_{50} 63.3 μM for AChE and 3.5 μM for BChE inhibition assay). All chemicals and enzymes used for this assay were purchased from Sigma.

Acknowledgment. The authors are indebted to Mrs. C. Kuhnt for LC-MS/MS and Mrs. M. Süsse for CD, IR, and UV measurements as well as Mrs. C. Bobach for the performance of the antiproliferative assay.

References and Notes

(1) http://www.indexfungorum.org/Names/Names.asp.
(2) Moser, M. In *Kleine Kryptogamenflora*; Gams, H., Ed.; Gustav Fischer Verlag: Stuttgart, New York, 1983; pp 347–415.
(3) Horak, E. *Röhrlinge und Blätterpilze in Europa*; Elsevier Spektrum Akademischer Verlag: München, 2005; pp 392–456.
(4) Garnica, S.; Weiβ, M.; Oertel, B.; Oberwinkler, F. *Can. J. Bot.* **2005**, *83*, 1457–1477.
(5) Peintner, U.; Moncalvo, J.-M.; Vilgalys, R. *Mycologia* **2004**, *96*, 1042–1058.
(6) Dardenne, G.; Marlier, M.; Welter, A. *Phytochemistry* **1977**, *16*, 1822–1824.
(7) Arnold, *N. Morphologisch-anatomische und chemische Untersuchung der Untergattung Telamonia (Cortinarius, Agaricales)*; IHW Verlag: Eching, 1993.
(8) Salm, R. F.; Zinsmeister, H. D.; Eicher, T. *Phytochemistry* **1998**, *49*, 887–892.
(9) Kanchanapoom, T.; Kasai, R.; Chumsri, P.; Hiraga, Y.; Yamasaki, K. *Phytochemistry* **2001**, *56*, 383–386.
(10) Youssef, D. T. A. *J Nat. Prod.* **2005**, *68*, 1416–1419.
(11) Coune, C. A.; Angenot, L. J. G.; Denoël, J. *Phytochemistry* **1980**, *19*, 2009–2011.
(12) Bringmann, G.; God, R.; Schäfer, M. *Phytochemistry* **1996**, *43*, 1393–1403.
(13) Kühne, H.; Hesse, M. *Mass Spectrom. Rev.* **1982**, *1*, 15–28.
(14) Gutsche, B.; Herderich, M. *Fresenius J. Anal. Chem.* **1998**, *360*, 836–839.
(15) Brossi, A.; Focella, A.; Teitel, S. *J. Med. Chem* **1973**, *16*, 418–420.
(16) Allen, J. R. F.; Holmstedt, B. R. *Phytochemistry* **1980**, *19*, 1573–1582.
(17) Matsumoto, T.; Trüb, W.; Gwinner, R.; Eugster, C. H. *Helv. Chim. Acta* **1969**, *52*, 716–720.
(18) Takeuchi, T.; Ogawa, K.; Hironobu, I.; Hiroyuki, S.; Katsuko, U.; Toshiharu, N.; Kato, M.; Hamao, U.; Osamu, T. *J. Antibiot.* **1973**, *26*, 162–167.
(19) Steglich, W.; Kopanski, L.; Wolf, M.; Moser, M.; Tegtmeyer, G. *Tetrahedron Lett.* **1984**, *25*, 2341–2344.
(20) Nakatsuka, S.; Feng, B.; Goto, T.; Kihara, K. *Tetrahedron Lett.* **1986**, *27*, 3399–3402.
(21) Liu, J.-K. *Chem. Rev.* **2005**, *105*, 2723–2744.
(22) Bröckelmann, M. G.; Dasenbrock, J.; Steffan, B.; Steglich, W.; Wang, Y.; Raabe, G.; Fleischhauer, J. *Eur. J. Org. Chem.* **2004**, *23*, 4856–4863.
(23) Ghosal, S.; Bhattacharya, S. K.; Mehta, R. *J. Pharm. Sci.* **1972**, *61*, 808–810.
(24) Becher, P. G.; Beuchat, J.; Gademann, K.; Jüttner, F. *J. Nat. Prod.* **2005**, *68*, 1793–1795.
(25) Schott, Y.; Decker, M.; Rommelspacher, H.; Lehmann, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5840–5843.
(26) Gearhart, D. A.; Neafsey, E. J.; Collins, M. C. *Neurochem. Int.* **2002**, *40*, 611–620.
(27) Matsubara, K.; Collins, M. A.; Akane, A.; Ikebuchi, J.; Neafsey, E. N.; Kagawa, M.; Shiono, H. *Brain Res.* **1993**, *610*, 90–96.
(28) Ellman, G. L.; Courtney, D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88–95.
(29) Rhee, I. K.; van de Meent, M.; Ingkaninan, K.; Verpoorte, R. *J. Chromatogr. A* **2001**, *915*, 217–223.

NP070259W