Brevicompanine C, Cyclo-(D-Ile-L-Trp), and Cyclo-(D-Leu-L-Trp), Plant Growth Regulators from *Penicillium brevi-compactum*

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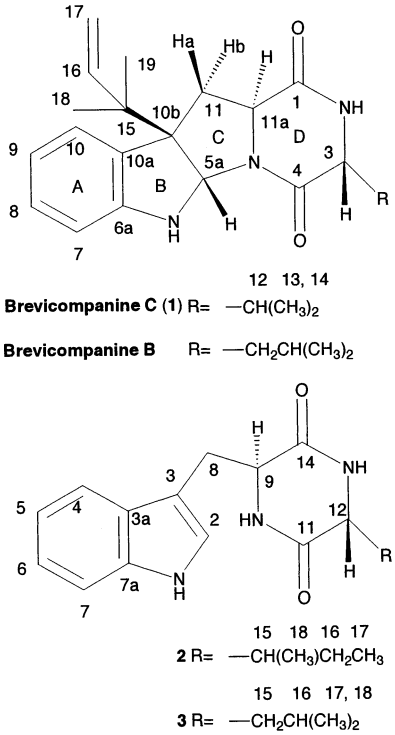
Received August 18, 2004

New plant growth regulators, named brevicompanine C (**1**), cyclo-(D-Ile-L-Trp) (**2**), and cyclo-(D-Leu-L-Trp) (**3**), have been isolated from *Penicillium brevi-compactum* Dierckx, and their structures have been established by spectroscopic methods including 2D NMR and chiral TLC analysis. Plant growth activities of **1**, **2**, and **3** have been examined using lettuce seedling bioassay methods. All compounds accelerated the root growth of the seedlings in proportion to their concentration from 1 to 100 mg/L.

Fungi have proven to be a valuable resource for the discovery of novel natural products, many of them potential targets for agrochemical and biomedical development. Diketopiperazines are a group of fungal metabolites that include plant growth regulators such as cyclo-(L-tryptophyl-L-phenylalanyl)[1] and fructigenine A[2] and mycotoxins such as aszonalenin[3] and okaramines A and B.[4−6] We have focused our attention on new plant growth regulators from fungi and previously isolated two diketopiperazines, brevicompanines A and B, from the filtrate of *Penicillium brevi-compactum* Dierckx (Moniliaceae).[7,8] Brevianamides A−F had been already isolated from the culture filtrate of this fungus besides brevicompanines A and B.[9,10] Further investigation for metabolites of this fungus has now led to the isolation of new metabolites designated brevicompanine C (**1**) from the mycelial mats and cyclo-(D-Ile-L-Trp) (**2**) and cyclo-(D-Leu-L-Trp) (**3**) from the culture filtrate. We report herein the isolation, structural determination, and biological activities of **1**−**3**.

The EtOAc-soluble neutral fraction (11.6 g) from the acetone extract of the mycelial mats of *P. brevi-compactum* was purified with silica gel column chromatography and preparative TLC to afford compound **1**.

Compound **1**, named brevicompanine C, was obtained as a colorless powder. The molecular formula of **1** was established as $C_{21}H_{27}N_3O_2$ by comparing its ^{1}H and ^{13}C NMR and EI mass data with those of brevicompanine B.[7] The UV, IR, and ^{1}H and ^{13}C NMR spectra of **1** revealed a close relationship to brevicompanine B. The IR absorption bands at 3380 and 1659 cm^{-1} and two signals at δ 165.6 and 169.7 in the ^{13}C NMR spectrum indicated that **1** possessed a diketopiperazine unit.[7] The positive reaction to the Ehrlich reagent and eight signals at δ 61.1, 78.4, 108.8, 118.8, 125.1, 128.7, 128.9, and 150.1 in the ^{13}C NMR spectrum indicated the presence of an indoline moiety.[2] A peak at *m/z* 284 ($M^+ - 69$) in the MS and five signals at δ 22.4, 22.9, 40.9, 114.5, and 143.5 in the ^{13}C NMR spectrum indicated the presence of a 1,1-dimethyl-2-propenyl group in **1**.[2,11] The ^{1}H and ^{13}C NMR spectra showed the presence of four methyl carbons, one aliphatic methylene carbon, four aliphatic and four aromatic methine carbons, one vinyl group, two amides, and two aliphatic and two aromatic quarternary carbons. In the ^{1}H NMR spectrum of **1**, the resonance due to the protons of the methylene was absent, indicating that **1** differed from brevicompanine B only by the presence of the isopropyl group on C-3 instead of the isobutyl group. These data suggested that the valine unit in **1** is replaced with a leucine moiety in brevicompanine B.[7]



The relative configuration of **1** was determined by differential NOE experiments. NOEs from H-18 and H_3-19 to H-5a and from H-5a to H-16 indicated that the B and C rings were connected with a *cis* junction. The coupling constant between H-11a and Ha-11 (J = 10.7 Hz) as well as NOEs from Ha-11 to H-5a and from H-3 to H-5a suggested that the vinyl allyl group on C-10b and methine protons of C-3, C-5a, and C-11a were of β, β, β, and α orientation, respectively.[7]

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10.1021/np040178p CCC: $30.25
Published on Web 01/21/2005

Acidic hydrolysis and chiral TLC analysis[12−14] of the resulting hydrolysate using D- and L-valine reference samples confirmed the absolute configuration of **1**. The absolute configuration of valine was determined to be D. Hence, the absolute configuration of **1** was established as 3*R*, 5a*R*, 10b*R*, and 11a*S*. The structure of **1** was therefore concluded to be (3*R*,5a*R*,10b*R*,11a*S*)-10*β*-(1,1-dimethyl-2-propenyl)-3-(methylethyl)-3,5α,6,10*β*,11,11α-hexahydro-2*H*-pyrazino[2′,1′-5,1]pyrrolo[2,3-*b*]indole-1,4-dione.[15,16]

The EtOAc-soluble neutral fraction (4.5 g) from the EtOAc extract of the culture filtrate was purified with silica gel column chromatography and preparative TLC to afford compounds **2** and **3**.

Compound **2** was obtained as a colorless powder. The molecular formula of **2** was established as $C_{17}H_{22}O_2N_3$ by HREIMS. The IR absorption bands at 3339 and 1676 cm^{-1} and two signals at δ 170.5 and 171.3 in the ^{13}C NMR spectrum indicated that **2** possessed a diketopiperazine unit.[7] The positive reaction to the Ehrlich reagent, nine signals at δ 38.9, 109.1, 112.1, 119.7, 120.0, 122.4, 126.0, 128.7, and 137.9 in the ^{13}C NMR spectrum, and a peak at *m/z* 130 in the MS indicated the presence of an indole-3-methylene moiety in **2**.[2,3] The ^1H and ^{13}C NMR spectra showed the presence of two methyl carbons, two aliphatic methylene carbons, three aliphatic and five aromatic methine carbons, three aromatic quarternary carbons, and two amides.

Hydrolysis of **2** with 6 M HCl at 110 °C for 8 h yielded L-tryptophan and D-isoleucine as degradation products. Thus, the structure of **2** was established as cyclo-(D-Ile-L-Trp).

Compound **3** was obtained as a colorless powder. Its molecular formula was established as $C_{17}H_{22}O_2N_3$ by HREIMS. The UV, IR, and ^1H and ^{13}C NMR spectra of **3** revealed a close relationship to **2**. The IR absorption bands at 3321 and 1676 cm^{-1} and two signals at δ 171.1 and 171.3 in the ^{13}C NMR spectrum indicated that **3** possessed a diketopiperazine unit.[7] The positive reaction to the Ehrlich reagent, nine signals at δ 41.9, 109.1, 112.1, 119.6, 120.0, 122.5, 125.9, 128.6, and 137.8 in the ^{13}C NMR spectrum, and a peak at *m/z* 130 in the MS indicated the presence of an indole-3-methylene moiety in **3**.[2,3] The ^1H and ^{13}C NMR spectra showed the presence of two methyl carbons, two aliphatic methylene carbons, three aliphatic and five aromatic methine carbons, three aromatic quarternary carbons, and two amides.

Hydrolysis of **3** with 6 M HCl at 110 °C for 8 h yielded L-tryptophan and D-leucine as degradation products. Thus, the structure of **3** was established as cyclo-(D-Leu-L-Trp).

Plant growth activities of **1**, **2**, **3**, IAA, and GA_3 were examined using bioassay with lettuce seedlings (Figure 1). Compound **1** inhibited the hypocotyl elongation by 46% of control at a concentration of 100 mg/L, but **2** and **3** showed no inhibitory effect on the hypocotyl elongation at the same concentration. IAA inhibited the hypocotyl elongation from 1 to 100 mg/L. In contrast, GA_3 accelerated the hypocotyl elongation by 433% of control at a concentration of 1 mg/L and showed the same effect at the concentrations of 10 and 100 mg/L. Compounds **1**, **2**, **3**, and GA_3 accelerated the root growth in proportion to their concentration from 1 to 100 mg/L. **1**, **2**, **3**, and GA_3 accelerated the growth by 212%, 167%, 164%, and 164% of control at a concentration of 100 mg/L, respectively. In contrast, IAA inhibited the root growth from 1 to 100 mg/L.

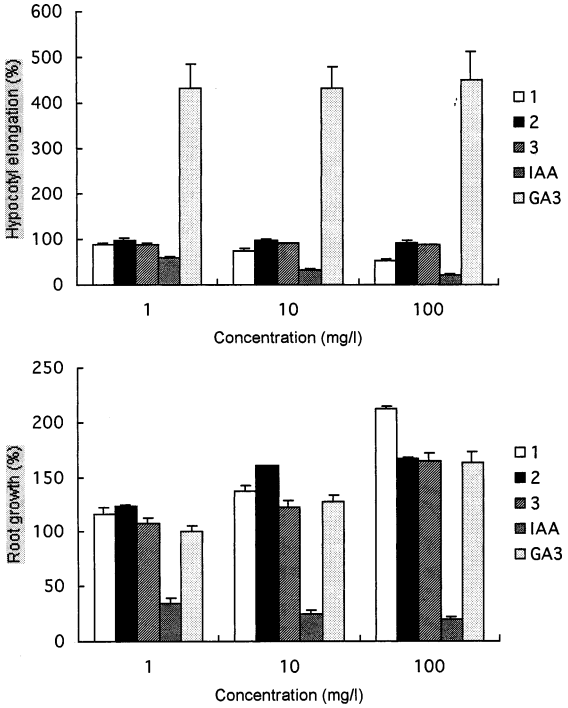

Figure 1. Effects of compounds **1**, **2**, **3**, IAA, and GA_3 on the growth of lettuce seedlings.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were determined on a Horiba SEPA-200 polarimeter. The UV spectra were recorded on a Shimazu UV-2200 spectrophotometer and the IR spectra on a Jasco FT IR-7000 spectrometer. The ^1H and ^{13}C NMR spectra were recorded with a JEOL JNM-GX 270 and a JEOL JNM-ESP 500 NMR spectrometer at 270 and 68 MHz and 500 and 125 MHz, respectively. Chemical shifts are expressed in δ values with solvents as internal standards. EIMS and HREIMS data were obtained with a Hitachi M80 and M-2000 mass spectrometers, respectively. Silica gel (Wako Pure Chemical Industries, Ltd., 75−150 μm) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.2 mm) were used for preparative TLC. Reversed-phase HPLC purification was performed on a Wakosil 5C18 column (Wako Pure Chemical Industries, Ltd., 7.5 × 250 mm) using a Shimadzu LC-3A pump with a flow rate of 2 mL min^{-1}. Chiralplate purchased from Macherey-Nagel was used for separation of enantiomers. Gibberelline (GA_3) and indole-3-acetic acid (IAA) were purchased from Wako Pure Chemical Industries, Ltd.

Fungal Material and Fermentation. *P. brevi-compactum* was collected from the soil at Tottori University in May 1997 and authenticated by Dr. R. A. Samson, Fungal Biodiversity Centre, Institute of the Royal Netherlands Academy of Arts and Sciences. A voucher specimen (No. 526) is deposited at the Laboratory of Bioorganic Chemistry in the Department of Biological and Environmental Chemistry, Faculty of Agriculture, Tottori University. Seventy-six 500 mL Erlenmeyer flasks, each containing 250 mL of Czapek-Dox medium supplemented with 3% polypeptone, were individually inoculated with one 1 cm^2 agar plug taken from a stock culture of the fungus maintained at 20 °C on potato dextrose agar. The fungus was statically grown at 25 °C for 21 days.

Extraction and Isolation. The mycelial mats obtained after filtration of the culture broth (19 L) were extracted three times with acetone. This acetone extract (15.3 g) was redissolved in EtOAc and partitioned twice with a saturated $NaHCO_3$ solution. The EtOAc-soluble neutral phases were combined and concentrated *in vacuo*. The resulting residue (11.6 g) was first fractionated by column chromatography on silica gel (*n*-hexane/acetone). The fraction (881 mg), obtained by elution with 40% acetone, was chromatographed on a silica gel column (*n*-hexane/EtOAc). The fraction (110 mg), obtained

by 70% EtOAc, was further purified by preparative TLC (toluene/acetone, 7:3, v/v) developed once to afford 54 mg of **1**.

The filtrate (19 L) was adjusted to pH 2.0 with 2 M HCl solution. The filtrate was successively extracted with EtOAc and concentrated *in vacuo*. This EtOAc extract (14.3 g) was redissolved in EtOAc and partitioned twice with a saturated $NaHCO_3$ solution. The EtOAc-soluble neutral phases were combined and concentrated *in vacuo*. The resulting residue (4.5 g) was first fractionated by column chromatography on silica gel (*n*-hexane/acetone). The fraction (32 mg), obtained by elution with 50% acetone, was further purified by preparative TLC ($CHCl_3$/MeOH,95:5, v/v). One solid was recrystallized from acetone to afford 5.6 mg of **2**, and another solid (15.9 mg) was fractionated via C_{18} reversed-phase HPLC. Preparative HPLC using a column with 40% acetonitrile as eluent gave **3** (7 mg).

Brevicompanine C (1): colorless crystals (EtOAc); mp 94−96 °C; $[\alpha]^{20}_D$ −321.7° (*c* 0.6, EtOH); UV (EtOH) λ_{max} (log ϵ) 207 (3.97), 245 (3.36), 302 (3.08) nm; IR (KBr) ν_{max} 3380 (NH), 2972 (alkane), 1659 (N−C═O), 1609 (C═C), 1468, 1444, 1143, 1081, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 7.15 (1H, d, J = 7.8 Hz, H-10), 7.08 (1H, dd, J = 7.8, 7.3 Hz, H-8), 6.95 (1H, br s, N*H*), 6.75 (1H, dd, J = 7.3, 7.3 Hz, H-9), 6.59 (1H, d, J = 7.8 Hz, H-7), 5.97 (1H, dd, J = 16.6, 10.0 Hz, H-16), 5.59 (1H, s, H-5a), 5.12 (1H, d, J = 10.0 Hz, H-17), 5.07 (1H, d, J = 16.6 Hz, H-17), 3.90 (1H, dd, J = 10.7, 5.9 Hz, H-11a), 3.76 (1H, m, H-3), 2.53 (1H, dd, J = 12.0, 5.9 Hz, H-11), 2.39 (1H, dd, J = 12.0, 10.7 Hz, H-11), 2.22 (1H, m, H-12), 1.12 (3H, s, H-18), 1.01 (3H, s, H-19), 0.96 (3H, d, J = 6.8 Hz, H-13), 0.82 (3H, d, J = 6.8 Hz, H-14); ^{13}C NMR ($CDCl_3$,68 MHz) δ 169.7 (C, C-1), 165.6 (C, C-4), 150.1 (C, C-6a), 143.5 (CH, C-16), 128.9 (CH, C-8), 128.7 (C, C-10a), 125.1 (CH, C-10), 118.8 (CH, C-9), 114.5 (CH_2, C-17), 108.8 (CH, C-7), 78.4 (CH, C-5a), 66.1 (CH, C-3), 61.1 (C, C-10b), 57.9 (CH, C-11a), 40.9 (C, C-15), 37.0 (CH_2, C-11), 33.4 (CH, C-12), 22.9 (CH_3, C-19), 22.4 (CH_3, C-18), 18.8 (CH_3, C-13), 16.9 (CH_3, C-14); EIMS *m/z* 353 $[M]^+$ (22), 284 (100), 256 (11), 185 (7), 157 (35), 130 (30), 72 (6).

Cyclo-(D-Ile-L-Trp) (2): colorless crystals (acetone); mp 237 °C; $[\alpha]^{20}_D$ +82.0° (*c* 0.5, EtOH); UV (EtOH) λ_{max} (log ϵ) 205 (sh) (4.07), 220 (4.20), 272 (sh) (3.47), 280 (3.49), 289 (3.43) nm; IR (KBr) ν_{max} 3339 (NH), 2966 (alkane), 1676 (N−C═O), 1458, 1097, 808, 740 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 7.51 (1H, dd, J = 8.1, 1.0 Hz, H-7), 7.22 (1H, dd, J = 8.1, 1.0 Hz, H-4), 6.97 (1H, ddd, J = 8.1, 7.0, 1.0 Hz, H-6), 6.96 (1H, s, H-2), 6.89 (1H, ddd, J = 8.1, 7.0, 1.0 Hz, H-5), 4.13 (1H, m, H-9), 3.35 (1H, dd, J = 14.4, 4.2 Hz, H-8), 3.09 (1H, dd, J = 14.4, 4.1 Hz, H-8), 2.74 (1H, dd, J = 2.7, 0.8 Hz, H-12), 1.74 (1H, m, H-15), 1.05 (2H, m, H-16), 0.65 (3H, d, J = 6.5 Hz, H-18), 0.55 (3H, t, J = 7.3 Hz, H-17); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 171.3 (C, C-14), 170.5 (C, C-11), 137.9 (C, C-7a), 128.7 (C, C-3a), 126.0 (CH, C-2), 122.4 (CH, C-6), 120.0 (CH, C-5), 119.7 (CH, C-4), 112.1 (CH, C-7), 109.1 (C, C-3), 58.2 (CH, C-12), 57.1 (CH, C-9), 38.9 (CH_2, C-8), 31.1 (CH, C-15), 26.2 (CH_2, C-16), 14.2 (CH_3, C-18), 11.9 (CH_3, C-17); EIMS *m/z* 299 $[M]^+$ (13), 130 (100); HREIMS *m/z* 299.1634 (calcd for $C_{17}H_{22}O_2N_3$, 299.1634).

Cyclo-(D-Leu-L-Trp) (3): colorless crystals (acetone); mp 237 °C; $[\alpha]^{20}_D$ +53.2° (*c* 0.6, EtOH); UV (EtOH) λ_{max} (log ϵ) 207 (sh) (4.02), 220 (4.18), 272 (sh) (3.46), 280 (3.47), 289 (3.41) nm; IR (KBr) ν_{max} 3321 (NH), 2959 (alkane), 1676 (N−C═O), 1458, 1319, 839, 742 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 7.51 (1H, dd, J = 8.1, 1.0 Hz, H-7), 7.22 (1H, dd, J = 8.1, 1.0 Hz, H-4), 6.97 (1H, ddd, J = 8.1, 7.0, 1.0 Hz, H-6), 6.96 (1H, s, H-2), 6.89 (1H, ddd, J = 8.1, 7.0, 1.0 Hz, H-5), 4.12 (1H, dd, J = 4.1, 3.8 Hz, H-9), 3.35 (1H, dd, J = 14.6, 3.8 Hz, H-8), 3.05 (1H, dd, J = 14.6, 4.1 Hz, H-8), 1.44 (1H, m, H-12), 1.34 (2H, dd, J = 7.8, 4.6 Hz, H-15), 1.23 (1H, m, H-16), 0.64 (3H, d, J = 6.2 Hz, H-18), 0.52 (3H, d, J = 6.2 Hz, H-17); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 171.3 (C, C-14), 171.1 (C, C-11), 137.8 (C, C-7a), 128.6 (C, C-3a), 125.9 (CH, C-2), 122.5 (CH, C-6), 120.0 (CH, C-5), 119.6 (CH, C-4), 112.1 (CH, C-7), 109.1 (C, C-3), 57.5 (CH, C-12), 53.3 (CH, C-9), 41.9 (CH_2, C-8), 31.0 (CH, C-16), 25.1 (CH_2, C-15), 23.1 (CH_3, C-18), 22.0 (CH_3, C-17); EIMS *m/z* 299 $[M]^+$ (13), 130 (100); HREIMS *m/z* 299.1636 (calcd for $C_{17}H_{22}O_2N_3$, 299.1633).

Hydrolysis and Chiral TLC Analysis of 1−3. Compound **1** (2 mg) was suspended in 6 M HCl (0.5 mL) and heated at 110 °C for 8 h. The mixture was cooled to room temperature and evaporated to dryness before dissolving the residue in 50% aqueous MeOH (0.5 mL). TLC analysis was carried out using a slight modification of the Chiralplate method.[12−14] A sample (2 μL) of the reaction mixture and authentic samples (2 μL) of both D- and L-valine (1% w/v solutions) were applied to the Chiralplate and the plate was developed with MeOH/H_2O/MeCN (50:50:200, v/v/v). The plate was then allowed to dry and dipped in a 0.3% solution of ninhydrin in acetone. Gentle heating using a hair-dryer allowed visualization of the valine as a purple spot. D- and L-valine, R_f 0.52 and 0.60, respectively, could be confidently separated and assigned using this method. The valine component of the hydrolysis mixture of **1** comigrated with D-valine and was therefore assigned accordingly.

Compounds **2** (2 mg) and **3** (2 mg) were hydrolyzed according to the same procedure as described for **1**, respectively. D- and L-tryptophan, R_f 0.56 and 0.67, respectively, D- and L-isoleucine, R_f 0.55 and 0.60, respectively, and D- and L-leucine, R_f 0.51 and 0.56, respectively, could be confidently separated and assigned using this method. The tryptophan and isoleucine components of the hydrolysis mixture of **2** comigrated with L-tryptophan (R_f 0.66) and D-isoleucine (R_f 0.56) and were therefore assigned accordingly. The tryptophan and leucine components of the hydrolysis mixture of **3** comigrated with L-tryptophan (R_f 0.67) and D-leucine (R_f 0.51) and were therefore assigned accordingly.

Bioassay for the Growth of Lettuce Seedlings. Lettuce seedlings (*Lactuca sativa* cv. Kingcisco) were purchased from Takii Nursery and sown in a Petri dish (150 × 25 mm) lined with a filter paper containing deionized H_2O. After 1 day under continuous light (100 $\mu E/m^2$ s) at 24 °C, seedlings were selected for uniformity (radicles; 2 mm) and transferred into a mini-Petri dish (35 × 15 mm) lined with filter paper containing 1 mL of deionized H_2O and a defined amount of the test compound. The Petri dishes were kept at 24 °C for 4 days under continuous light (100 $\mu E/m^2$ s). The length of the hypocotyls and roots treated with the compounds was measured, and the mean value of the length was compared with an untreated control.[7] Triplicate experiments were conducted.

References and Notes

(1) Kimura, Y.; Tani, K.; Kojima, A.; Sotoma, G.; Okada, K.; Shimada, A. *Phytochemistry* **1996**, *41*, 665−669.
(2) Arai, K.; Kimura, K.; Mushiroda, T.; Yamamoto, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2937−2939.
(3) Kimura, Y.; Hamasaki, T.; Nakajima, H. *Tetrahedron Lett.* **1982**, *23*, 225−228.
(4) Yamazaki, M.; Fujimoto, H.; Kawasaki, T. *Chem. Pharm. Bull.* **1980**, *28*, 245−254.
(5) Murao, S.; Hayashi, H.; Takiuchi, K.; Arai, M. *Agric. Biol. Chem.* **1988**, *52*, 885−886.
(6) Hayashi, H.; Takiuchi, K.; Murao, S.; Arai, M. *Agric. Biol. Chem.* **1988**, *52*, 2131−2133.
(7) Kusano, M.; Sotoma, G.; Koshino, H.; Uzawa, J.; Chijimatsu, M.; Fujioka, S.; Kawano, T.; Kimura, Y. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2823−2826.
(8) Matsumura, K.; Kitahara, T. *Heterocycles* **2001**, *54*, 727−733.
(9) Birch, A. J.; Wright, J. J. *Tetrahedron* **1970**, *26*, 2329−2344.
(10) Birch, A. J.; Russell, R. A. *Tetrahedron* **1972**, *28*, 2999−3008.
(11) Marchelli, R.; Dossena, A.; Pochini, A.; Dradi, E. *J. Chem. Soc., Perkin Trans. 1* **1977**, 713−717.
(12) Brinkman, U. A. Th.; Kamminga, D. *J. Chromatogr.* **1985**, *330*, 375−378.
(13) Günther, K. *J. Chromatogr.* **1988**, *448*, 11−30.
(14) Jiang, Z.; Barret, M.; Boyd, K. G.; Adams, D. R.; Boyd, A. S. F.; Burgess, J. G. *Phytochemistry* **2002**, *60*, 33−38.
(15) Caballero, E.; Avendano, C.; Menendez, J. C. *Tetrahedron: Asymmetry* **1998**, 967−981.
(16) Caballero, E.; Avendano, C.; Menendez, J. C. *Tetrahedron: Asymmetry* **1998**, 3025−3038.

NP040178P