Chaetoglobosin U, a Cytochalasan Alkaloid from Endophytic *Chaetomium globosum* IFB-E019

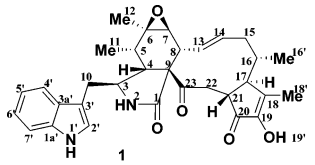
Gang Ding, Yong C. Song, Jing R. Chen, Chen Xu, Hui M. Ge, Xiao T. Wang, and Ren X. Tan*

Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

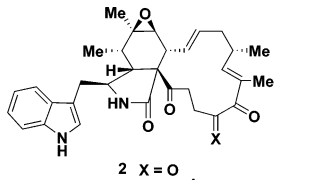
Received December 8, 2005

A new cytotoxic cytochalasan-based alkaloid named chaetoglobosin U (**1**), along with four known analogues, chaetoglobosins C (**2**), F (**3**), and E (**5**) and penochalasin A (**4**), has been characterized from the EtOAc extract of a solid culture of *Chaetomium globosum* IFB-E019, an endophytic fungus residing inside the stem of healthy *Imperata cylindrica*. The structure of chaetoglobosin U was determined through correlative analyses of its UV, IR, CD, MS, and 1D (^{1}H and^{13}C NMR and DEPT) and 2D NMR (COSY, NOESY, HMQC, and HMBC) data. Chaetoglobosin U (**1**) exhibited cytotoxic activity against the human nasopharyngeal epidermoid tumor KB cell line with an IC_{50} value of 16.0 μM, comparable to that (14.0 μM) of 5-fluorouracil co-assayed as a positive reference. The known analogues **2**−**5** were moderately active to the cell line, with IC_{50} values of 34.0, 52.0, 48.0, and 40.0 μM, respectively.

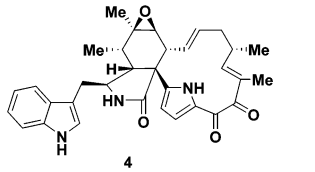
Endophytes, a rich source of bioactive metabolites, are considered to be one of the hot topics in the field of natural product chemistry.[1,2] In a continuation of our characterization of biologically active and/or structurally novel endophytic metabolites,[3,4] an endophyte, *Chaetomium globosum* IFB-E019, harbored inside the normal stem of *Imperata cylindrica* was shown to produce cytotoxic substances. With the follow-up fractionation procedure co-guided by cytotoxicity bioassay and ^{1}H NMR acquisition, the EtOAc solvent partition fraction of the $CHCl_3$−MeOH (1:1) extract from the biomass of its stationary fermentation was repeatedly chromatographed over silica gel and Sephadex LH-20 to give a new cytochalasan-based alkaloid (**1**) and four known analogues identified as chaetoglobosins C (**2**), E (**5**), and F (**3**)[5,6] and penochalasin A (**4**).[7] The results are discussed in this paper.



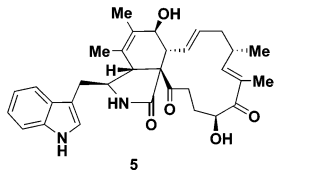
1



2 X = O
3 X = H, ¦Â-OH



4



5

Compound **1** was afforded as a light yellow powder. Its broadened IR absorption bands at 3056−3600 cm^{-1} were indicative of NH and/or OH, and those at 1694, 1677, 1637, 1549, and 1421 cm^{-1} of carbonyl and phenyl groups. The molecular formula of **1** was assigned as $C_{32}H_{36}O_5N_2$ by a set of quasimolecular ions at *m*/*z* 529 ($[M + H]^+$) and *m*/*z* 551 ($[M + Na]^+$) in its (+)-ESI mass spectrum, the latter having an exact mass of 551.2509 (calcd for $C_{32}H_{36}O_5N_2Na$: 551.2516), disclosed by subsequent high-resolution ESIMS measurement. This molecular formula assignment was reinforced by its ^{1}H and^{13}C NMR spectra acquired in DMSO-d_6, which exhibited a total of 32 carbon resonance lines and a sum of 36 hydrogens. Scrutiny of its ^{1}H and ^{13}C NMR spectra, in correlation with DEPT experiments, indicated that the 32 carbon signals arose from four methyl (two secondary, an allylic, and a tertiary), three sp^3-hybridized methylene, eight sp^3- and seven sp^2-hybridized methine, two sp^3- and five sp^2-hybridized quaternary, two ketone (δ 212.3 and 202.3), and an amide (δ 174.4) carbon. This carbon combination, along with the two nitrogen atoms in the molecular formula, indicated that compound **1** was most likely a cytochalasan-based alkaloid.[8] This postulation was subsequently confirmed by a correlative interpretation of its COSY, NOESY, HMQC, and HMBC spectra that allowed the unequivocal assignment of all ^{1}H and^{13}C NMR signals. Moreover, most of the ^{1}H and ^{13}C NMR signals of compound **1** resembled those of chaetoglobosin C (**2**), possessing as well a molecular formula of $C_{32}H_{36}O_5N_2$. However, the ^{13}C NMR of compound **1** failed to give the 19-ketone signal around δ 196.7 present in that of **2**. Comparison of the ^{1}H NMR spectra of **2** and **1** showed that the 17-methine signal exhibited around δ 6.0 in the ^{1}H NMR spectrum of **2** was shifted upfield to δ 2.14 in that of **1**, and the 21-methylene multiplet at δ 2.25 replaced a broadened methine doublet of **1** at δ 2.08. Moreover, the ^{1}H NMR spectrum of **1** exhibited a hydroxyl singlet at δ 9.00, which was indiscernible in that of **2**. These deviations in the NMR spectra between the two compounds indicated the presence of the 4,5-disubstituted 2-hydroxy-3-methylcyclopentone motif formed by the direct bonding of C-17 and C-21. This was confirmed by the cross-peak between H-17 and H-21 in its H−H-COSY spectrum as well as by HMBC correlations of C-18 with H-21 and of C-20 with H-17.

The stereochemistry of compound **1** was determined by comparing its ^{13}C NMR and NOESY spectra with those of its reported congeners, whose absolute configurations were established by X-ray diffraction analyses.[9,10] In the ^{13}C NMR spectrum of **1**, the chemical shifts of C-1 through C-16 and of C-2′ through C-7′ were nearly identical to those of chaetoglobosin A[5] and **2**, demonstrating that this substructure of **1** shared the absolute configurations with those of the two related compounds. This was further substantiated by the full detection of the anticipated NOESY correlations. Further evidence came from its NOESY spectrum displaying the cross-peaks of H-21 with H-4 and H-5 and the chemical shifts of the

* To whom correspondence should be addressed. Tel: +86-25-8359 2945. Fax: +86-25-8330 2728. E-mail: rxtan@nju.edu.cn.

10.1021/np050515+ CCC: $33.50
Published on Web 02/08/2006

C-17 and C-21 signals resonating at δ 52.9 and 50.8, close to those of chaetoglobosin S, possessing the 17*R*,21*R*-configuration.[11] To confirm this assumption, the CD spectrum of compound **1** was acquired. The positive Cotton effect of **1** (Δ_{319} +5.52), dependent on the chirality of the α,β-unsaturated cyclopentenone chromophore, was nearly identical to that of Δ^{14}-cholesten-3-ol-16-one benzoate, indicating that **1** also has 17*R*-stereochemistry.[12] The 21*R*-configuration was also evidenced from the absence of any NOE correlation of H-21 with H-17. We named compound **1** chaetoglobosin U.

Among compounds **1**−**5** tested against the human nasopharyngeal epidermoid tumor KB cell line, chaetoglobosin U (**1**) was the most cytotoxic, with an IC_{50} value of 16.0 μM, comparable to that (14.0 μM) of 5-fluorouracil co-assayed as a positive reference. The four known congeners **2**−**5** were moderately inhibitory to the cell line with IC_{50} values of 34.0, 52.0, 48.0, and 40.0 μM, respectively.

Around 30 chaetoglobosins, some being remarkably bioactive, have been previously characterized from cultures of fungi belonging to the genera *Calonectria*,[13] *Chaetomium*,[8] *Cylindrocladium*,[15] *Discosia*,[15] *Iplodia*,[16] *Penicillium*,[17] and *Phomopsis*.[18] These secondary metabolites, generated through multiple biochemical steps, have been suggested as biomarkers that could be allocated to differentiate *Penicillium* species.[19] However, most of the chaetoglobosins were characterized as metabolites of *Chaetomium* species. Interestingly, *Ch. globosum* was also isolated as an endophyte from Mormon tea genera and species, which failed to produce any isolatable amounts of chaetoglobosins during liquid fermentation.[20] However, the title endophytic strain we obtained from the normal stem of *I. cylindrica* did produce chaetoglobosin metabolites in liquid culture as described herein.

Experimental Section

General Experimental Procedures. Melting points were determined on a Boetius micro melting point apparatus. Optical rotations were taken on a Perkin-Elmer 341 digital polarimeter. The UV spectrum was recorded on a Hitachi U-3000 spectrophotometer. CD spectra were obtained on a JASCO J-725 spectrometer, and the IR spectrum was measured on a Nexus 870 FT-IR spectrometer. ESIMS spectra were recorded on a Mariner System 5304 mass spectrometer. NMR data were acquired in DMSO-d_6 on a Bruker DRX500 NMR spectrometer with ^{1}H and ^{13}C NMR observed at 500 and 125 MHz with tetramethylsilane (TMS) and solvent signals as internal references. Silica gel (200−300 mesh) for column chromatography was produced by Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was purchased from Pharmacia Biotech, Sweden. All other chemicals used in this study were of analytical grade.

Fungal Identification. *Chaetomiun globosum* IFB-E019 was isolated from the fresh plant *Imperata cylindrica* collected in November 2001 from the seashore of Yancheng, Jiangsu Province, China. The specimen of *I. cylindrica*, authenticated by Prof. X. J. Tian, was preserved in the Herbarium of Nanjing University.

The isolated endophytic fungus IFB-E019 was identified according to the following morphological characteristics. Colonies of IFB-E019 on PDA grew slowly at 22 °C, attaining 50−53 mm in diameter in 9 days. The initially appearing white surface of the colony gradually turned dust-colored, and the back of the lawn looked black-brown with many granules in the middle of the colony on PDA plates. The surface of the colony had concentric rings. The shell of the vesicles was ovate and had a taupe coloration, adhering to the culture plate, with interascicular pseudoparenchyma. Vesicles were claviform and transparent, with eight spores. These morphological characteristics suggested an identification of the endophytic fungus as *Ch. globosum*,[21] which was reinforced by the sequence of its 18S rRNA that gave a 99% sequence similarity to those accessible by BLAST analyses of available *Ch. globosum* sequences.

Fermentation and Isolation. After growing on PDA medium at 28 °C for 5 days, the title endophyte, *Ch. globosum* IFB-E019, was inoculated into Erlenmeyer flasks (1000 mL) containing 300 mL of PDA medium. After incubation for 4 days at 28 ± 1 °C on a rotary shaker at 150 rpm, 20 mL of culture liquid was transferred as the seed into 250 mL flasks, each preloaded with the evenly mingled medium composed of 7.5 g of grain, 7.5 g of bran, 0.5 g of yeast extract, 0.1 g of sodium tartrate, 0.01 g of $FeSO_4{-}7H_2O$, 0.1 g of sodium glutamate, 0.1 mL of pure corn oil, and 30 mL of H_2O, and grown for 40 days at 28 ± 1 °C with the relative humidity in the range 60−70%. The harvested solid culture was dehydrated to a residue (3.5 kg, not completely dried), which was extracted at room temperature with a MeOH−$CHCl_3$ (1:1, v/v) mixture (5 L × 5). Evaporation of the solvent under reduced pressure gave a brown oil (300 g), to which 1000 mL of H_2O was added, and the combination was thoroughly mixed to yield a suspension. This was extracted successively with petroleum ether (1000 mL × 3), EtOAc (1000 mL × 3), and *n*-butanol (1000 mL × 3). The EtOAc fraction, shown to be cytotoxic, was concentrated in vacuo to give a residue (30.5 g), which was chromatographed on a silica gel column (60 × 10 cm) eluted with a $CHCl_3$−MeOH gradient ($CHCl_3$−MeOH = 1:0; 100:1; 100:2; 100:4; 100:8; 100:16; 0:1, each 1.5 L) to afford seven fractions (Fr-1: oil, Fr-2: 10.0 g, Fr-3: 2.0 g, Fr-4: 2.8 g, Fr-5: 3.1 g, Fr-6: 2.2 g, Fr-7: 4.6 g). The bioactive fraction Fr-2 was separated first on a silica gel column (60 × 5 cm) eluted with $CHCl_3$−MeOH mixtures (100:0, 200:1, 200:3, 200:4, 200:6, and 200:10) and then on a Sephadex LH-20 column (40 × 2.8 cm) with $CHCl_3$−MeOH (1:1, 80 mL) to give **1** (5 mg), **2** (300 mg), **3** (18 mg), and **4** (15 mg). Gel filtration of Fr-3 (moderately cytotoxic) over a Sephadex LH-20 column with $CHCl_3$−MeOH (1:1, 100 mL) gave **5** (20 mg).

Cytotoxicity Assay. The in vitro cytotoxic activity was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colometric method.[22] KB cells were grown in RPMI medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (50 μg/mL). Cells were harvested at the log phase of growth and seeded into 96-well plates (100 μL/well at a density of 2 × 10^5 cells/mL). After 24 h incubation at 37 °C, 5% CO_2 to allow cell attachment, cultures were exposed to various concentrations of the isolated compounds for 48 h. Finally, MTT solution (2.5 mg/mL in PBS) was added (40 μL/well). Plates were further incubated for 4 h at 37 °C, and the formazan crystals formed were dissolved by adding 150 μL/well of DMSO. Absorption at 570 nm was measured with an ELISA plate reader, and the IC_{50} value was defined as the concentration at which 50% survival of cells was discerned.

Chaetoglobosin U (1): light yellow powder; mp 167−168 °C; $[\alpha]^{20}_D$ −17 (*c* 0.18, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 222 (0.479), 281 (0.089); CD (EtOH) $\Delta\epsilon_{319}$ +5.52 (*c* 4 × 10^{-4} M); IR (KBr) ν_{max} 3600, 3361, 1694, 1677, 1637, 1549, 1493; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.87 (1H, s, H-1′), 9.00 (1H, s, 19′-O*H*), 8.42 (1H, s, *H*NCO), 7.43 (1H, d, *J* = 7.7, H-4′), 7.30 (1H, d, *J* = 8.0, H-7′), 7.07 (1H, s, H-2′), 7.02 (1H, t, *J* = 7.2, H-5′), 6.96 (1H, t, *J* = 7.5, H-6′), 6.16 (1H, dd, *J* = 9.8, 15.2, H-13), 5.10 (1H, dt, *J* = 11.5, 23.0, H-14), 3.64 (1H, br s, H-3), 3.10 (1H, dd, *J* = 6.6, 15.2, H-22a), 2.86 (1H, d, *J* = 5.6, H-7), 2.71 (1H, dd, *J* = 4.9, 14.0, H-10a), 2.57 (1H, d, *J* = 6.0, H-4), 2.46 (1H, m, H-10b), 2.20 (1H, br d, *J* = 12.3, H-15a), 2.14 (2H, br d, *J* = 6.8, H-8, H-17), 2.08 (1H, br d, *J* = 4.3, H-21), 1.92 (3H, s, H-18′), 1.86 (1H, m, H-15b), 1.57 (1H, m, H-5), 1.49 (2H, br d, *J* = 13.3, H-16, H-22b), 1.12 (3H, s, H-11), 0.94 (3H, d, *J* = 6.7, H-16′), 0.70 (3H, d, *J* = 7.1, H-11); ^{13}C NMR (DMSO, 125 MHz) δ 212.2 (C, C-23), 203.3 (C, C-20), 174.4 (C, N*C*O), 149.0 (C, C-19), 147.2 (C, C-18), 136.6 (C, C-3a′), 133.6 (CH, C-14), 129.5 (CH, C-13), 127.9 (C, C-1a′), 124.7 (CH, C-2′), 121.3 (CH, C-5′), 118.9 (CH, C-6′), 118.5 (CH, C-4′), 112.0 (CH, C-7'), 109.4 (C, C-3′), 67.7 (C, C-9), 60.9 (CH, C-7), 57.5, (C, C-6), 52.9 (CH, C-17), 52.4 (CH, C-3), 50.8 (CH, C-21), 50.3 (CH, C-8), 48.9 (CH, C-4), 43.8 (CH_2, C-15), 42.5 (CH_2, C-22), 42.0 (CH, C-16), 36.8 (CH, C-5), 33.9 (CH_2, C-10), 21.5 (CH_3, C-16′), 19.6 (CH_3, C-12), 17.1 (CH_3, C-18′), 12.6 (CH_3, C-11); ESIMS *m*/*z* 529 $[M + H]^+$, 551 $[M + Na]^+$; HRESIMS *m*/*z* 551.2509 $[M + Na]^+$, calcd for $C_{32}H_{36}O_5N_2Na$ 551.2516.

Acknowledgment. This work was co-supported by a key project (20432030) and a grant (30300007) from the Natural Science Foundation of China.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Tan, R. X.; Zou, W. X. *Nat. Prod. Rep.* **2001**, *18*, 448−459.
(2) Strobel, G. A. *Microbes Infect.* **2003**, *5*, 535−544.

(3) Liu, J. Y.; Song, Y. C.; Zhang, Z.; Wang, L.; Guo, Z. J.; Zou, W. X.; Tan, R. X. *J. Biotechnol.* **2004**, *114*, 279−287.
(4) Liu, J. Y.; Liu, C. H.; Zou, W. X.; Tan, R. X. *Helv. Chim. Acta* **2003**, *86*, 657−660.
(5) Sekita, S.; Yoshihira, K.; Natori, S. *Chem. Pharm. Bull*. **1983**, *31*, 490−498.
(6) Springer, J. P.; Clardy, J.; Well, J. M.; Cole, R. J.; Kirksey, J. W.; Macfarlane, R. D.; Torgerson, D. F. *Tetrahedron Lett.* **1976**, *17*, 1355−1358.
(7) Numata, A.; Takahashi, C.; Ito, Y.; Minoura, K.; Yamada, T.; Matsuda, C.; Nomoto, K. *J. Chem. Soc., Perkin Trans*. **1995**, *1*, 239−245.
(8) Wenxu, J.; Feng, Y. J.; Blunt, J. W.; Cole, A. L.; Munro, M. H. J. *J. Nat. Prod*. **2004**, *67*, 1722−1725.
(9) Silverton, J, V.; Akiyama, T.; Kabuto, C.; Sekita, S.; Yoshihira, K.; Natori, S. *Tetrahedron Lett.* **1976**, *17*, 1349−1350.
(10) Silverton, J, V.; Kabuto, C. *Acta Crystallogr*. **1978**, *B34*, 588−593.
(11) Burlot, L.; Cherton, J.-C.; Convert, O.; Larpent, C.; Dennetier, B.; Dardoize, F. *Magn. Reson. Chem*. **1996**, *34*, 538−544.
(12) Djerassi, C.; Osiechi, J.; Herz, W. *J. Org. Chem.* **1957**, *22*, 1361−1366.
(13) Von, Wallbrunn. C.; Luftmann, H.; Bergander, K.; Meinhardt, F. *J. Gen. Appl. Microbiol.* **2001**, *47*, 33−38.
(14) Ichihara, A.; Katayama, K.; Teshima, H.; Oikawa, H.; Sakamura, S. *Biosci., Biotechnol. Biochem*. **1996**, *60*, 360−361.
(15) Donoso, R.; RiveraSagredo, A.; HuesoRodriguez, J. A.; Elson, S. W. *Nat. Prod. Lett*. **1997**, *10*, 49−54.
(16) Spondlin, C.; Tamm, C. *Helv. Chim. Acta* **1988**, *71*, 1881−1884.
(17) Andersen, B.; Smedsgaard, J.; Frisvad, J. C. *J. Agric. Food Chem.* **2004**, *52*, 2421−2428.
(18) Burlot, L.; Cherton, J. C.; Convert, O.; Correia, I.; Dennetiere, B. *Spectroscopy* **2003**, *17*, 725−734.
(19) Lund, F. *Lett. Appl. Microbiol*. **1995**, *20*, 228−231.
(20) Bashyal, B. P.; Wijeratne, E. M. K.; Faeth, S. H.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2005**, *68*, 724−728.
(21) Wei, J. C. *Handbook of Identification of Fungi*; Shanghai Science & Technology Press: Shanghai, 1979; pp 196−197.
(22) Mosmann, T. J. *Immunol. Methods* **1983**, *65*, 55−63.

NP050515+