ORGANIC LETTERS

2011
Vol. 13, No. 10
2670–2673

Virgatolides A−C, Benzannulated Spiroketals from the Plant Endophytic Fungus *Pestalotiopsis virgatula*

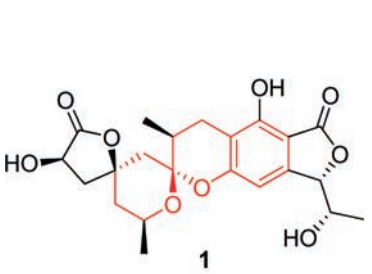
Jian Li,[†,‡] Li Li,[§] Yikang Si,[§] Xuejun Jiang,[†] Liangdong Guo,[†] and Yongsheng Che*[,†,∥]

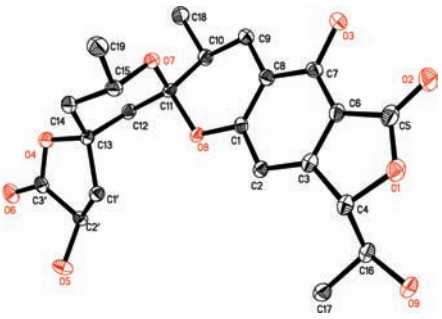
Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, People's Republic of China, Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China, and Beijing Institute of Pharmacology & Toxicology, Beijing 100850, People's Republic of China

cheys@im.ac.cn

Received March 23, 2011

ABSTRACT





Virgatolides A−C (1−3), unique metabolites with a 3′,4′,5′,6′-tetrahydrospiro[chroman-2,2′-pyran] core, were isolated from cultures of the plant endophytic fungus *Pestalotiopsis virgatula*. Compounds 1−3 possess two previously undescribed skeletons originating from a benzannulated 6,6-spiroketal and one (2 and 3) and two (1) γ-lactone units, respectively. The structure of 1 was secured by X-ray crystallography.

Natural products incorporating a benzannulated spiroketal unit have been reported from various sources as the bioactive principles.[1] A notable feature of this class of compounds is the presence of a benzannulated 5,5-,[2,3] or 6,5-,[4,5] or 6,6-spiroketal[6−8] moiety as their core skeletons. Naturally occurring benzannulated 6,6-spiroketals are rare. The only precedents include citreoviranol and its demethyl analogue isolated from the fungus *Penicillium citreoviride* B (IFO 4692),[6] chaetoquadrins A−C from the Ascomycete *Chaetominum quadrangulatum* strain 71-NG-22,[7] and the dimeric cynandiones from the rhizome of a Taiwanese folk medicine, *Cynanchum taiwanianum*.[8]

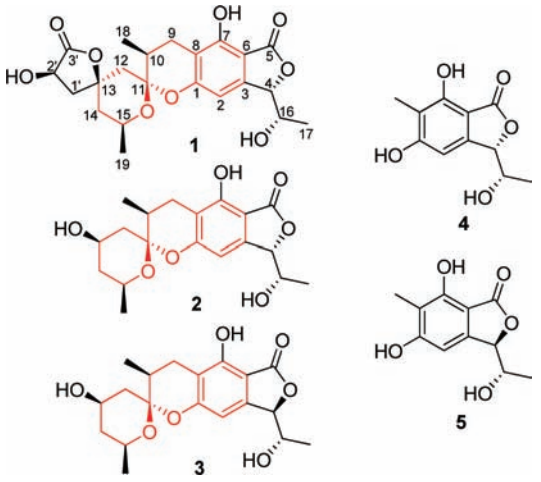
Endophytic fungi inhabiting normal tissues of the host plants are well-known producers of bioactive

[†] Chinese Academy of Sciences.
[‡] Graduate School of Chinese Academy of Sciences.
[§] Chinese Academy of Medical Sciences and Peking Union Medical College.
[∥] Beijing Institute of Pharmacology & Toxicology.

(1) Sperry, J.; Wilson, Z. E.; Rathwell, D. C. K.; Brimble, M. A. *Nat. Prod. Rep*. **2010**, *27*, 1117–1137.
(2) Zhang, P. C.; Xu, S. X. *Phytochemistry* **2001**, *57*, 1249–1253.
(3) Asari, F.; Kusumi, T.; Zheng, G. Z.; Cen, Y. Z.; Kakisawa, H. *Chem. Lett*. **1990**, 1885–1888.
(4) Stierle, A. A.; Stierle, D. B.; Kelly, K. *J. Org. Chem*. **2006**, *71*, 5357–5360.
(5) Bringmann, G.; Kraus, J.; Schmitt, U.; Puder, C.; Zeeck, A. *Eur. J. Org. Chem*. **2000**, *15*, 2729–2734.
(6) Shizuri, Y.; Shigemori, H.; Sato, R.; Yamamura, S.; Kawai, K.; Furukawa, H. *Chem. Lett*. **1988**, 1419–1422.
(7) Fujimoto, H.; Nozawa, M.; Okuyama, E.; Ishibashi, M. *Chem. Pharm. Bull*. **2002**, *50*, 330–336.
(8) Huang, P. L.; Lu, C. M.; Yen, M. H.; Wu, R. R.; Lin, C. N. *Phytochemistry* **1995**, *40*, 537–541.
(9) (a) Schulz, B.; Boyle, C.; Draeger, S.; Rommert, A. K.; Krohn, K. *Mycol. Res*. **2002**, *106*, 996–1004. (b) Strobel, G. *Microbes Infect*. **2003**, *5*, 535–544. (c) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *J. Nat. Prod*. **2004**, *67*, 257–268. (d) Zhang, H. W.; Song, Y. C.; Tan, R. X. *Nat. Prod. Rep*. **2006**, *23*, 753–771. (e) Liu, L.; Gao, H.; Chen, X.; Cai, X.; Yang, L.; Guo, L.; Yao, X.; Che, Y. *Eur. J. Org. Chem*. **2010**, *17*, 3302–3306.

10.1021/ol200770k
Published on Web 04/15/2011

secondary metabolites.[9] Chemical studies of the *Pestalotiopsis* genus have attracted much attention due to frequent discovery of structurally diverse and biologically active natural products.[10,11] During an ongoing search for new bioactive metabolites from the species of this genus, a strain of *Pestalotiopsis virgatula* (L147) isolated from the leaves of the trational Chinese medicinal plant *Dracontomelon duperreanum* Pierre was subjected to chemical study. An EtOAc extract prepared from cultures of solid-substrate fermentation showed cytotoxicity against HeLa (cervical epithelium) cells. Fractionation of this extract afforded virgatolides A−C (**1**−**3**), three benzannulated spiroketals possessing previously undescribed ring systems, together with their biosynthetically related known compounds, pestaphthalides A (**4**) and B (**5**).[12] Details of the structure elucidation, cytotoxicity, and hypothetical biogenesis of **1**−**3** are reported herein.



Virgatolide A (**1**) was assigned a molecular formula of $C_{22}H_{26}O_9$ (10 degrees of unsaturation) by HRESIMS (*m*/*z* 457.1466 [M + Na]$^+$). Its ^{1}H and ^{13}C NMR spectra showed resonances for three exchangeable protons, three methyl groups, four methylenes, five methines (four of which are oxymethines), six sp^2 carbons (one protonated), two oxygenated sp^3 quaternary carbons including one of double oxygenation (δ_C 100.8), and two carboxylic carbons (δ_C 169.1 and 176.1, respectively). These data accounted for all the NMR resonances, suggesting that **1** was a pentacyclic compound. Analysis of the ^{1}H and ^{13}C NMR spectroscopic data of **1** (Table 1) revealed the same isobenzofuranone moiety with a hydroxyethyl group attached to C-4 as found in the coisolated known compound **4**.[12] Interpretation of the ^{1}H−^{1}H COSY NMR data of **1** established three isolated spin-systems, which were C-9−C-10−C-18, C-14−C-15−C19, and C-1′−C-2′ (including OH-2). HMBC correlations from H_2-9 to the sp^2 carbons C-1, C-7, and C-8 led to the connection of C-8 to C-9. While those of H_2-12 and H_3-18 with the C-11 oxygetated sp^3 carbon located C-11 between C-10 and C-12. In turn, cross peaks from H_2-1′ and H-2′ to the carboxylic carbon (C-3′) indicated that C-2′ is adjacent to C-3′. Additionl correlations from H_2-1′, H-2′, H_2-12, and H_2-14 to the oxygenated quaternary carbon (C-13) connected C-13 to C-12, C-14, and C-1′. HMBC cross peaks from the exchangeable proton at 6.05 ppm to C-1′, C-2′, and C-3′ located a free hydroxy group at C-2′. Considering the doubly oxygenated nature of C-11, and the chemical shifts for C-1 (δ_C 153.6) and C-15 (δ_C 64.3), the two C-11 bonded oxygen atoms were individually attached to C-1 and C-15, respectively, to complete the substructure for a 1,7-dioxaspiro[5.5]undecane moiety. In this circumstance, the C-3′ carboxylic carbon is required to acylate the C-13 oxygen to form the second γ-lactone ring to satisfy the unsaturation requirement of **1**, even though no additional evidence for this linkage was provided by the HMBC data. Therefore, the planar structure of virgatolide A was tentatively assigned as shown in **1**.

Table 1. NMR Spectroscopic Data for **1** in DMSO-d_6

position	$\delta_H{}^a$ (*J* in Hz)	$\delta_C{}^b$	HMBC (H → C#)
1		153.6	
2	6.56, s	102.5	1, 4, 6, 7, 8
3		147.7	
4	5.25, d (3.2)	82.5	3, 5, 17
5		169.1	
6		105.6	
7		156.9	
8		110.9	
9a	2.26, dd (16.8, 12.4)	23.8	1, 7, 8, 10, 18
9b	2.65, dd (16.8, 6.0)		1, 7, 8, 10, 11
10	1.90, m	33.3	7, 9, 11, 18
11		100.8	
12a	1.95, d (12.4)	39.6	10, 11, 13, 1′
12b	2.11, d (12.4)		11, 13, 14, 1′
13		81.0	
14a	1.58, t (12.4)	44.2	13, 15, 19, 1′
14b	1.99, t (12.4)		13
15	3.83, qt (12.4, 6.5)	64.3	19
16	4.06, m	66.2	3
17	1.06, d (6.5)	18.5	4, 16
18	1.04, d (6.5)	15.8	9, 10, 11
19	1.04, d (6.5)	21.0	14, 15
1′a	2.13, dd (13.2, 8.0)	40.9	12, 13, 14, 2′, 3′
1′b	3.16, dd (13.2, 8.0)		12, 13, 14, 2′, 3′
2′	4,58, ddd (13.2, 8.0, 6.0)	67.0	13, 1′, 3′
3′		176.1	
OH-7	9.63, s		1, 6, 8
OH-16	4.94, d (5.2)		4, 16, 17
OH-2′	6.05, d (6.0)		1′, 2′, 3′

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

Fortunately, the proposed structure for virgatolide A (**1**) was confirmed by single-crystal X-ray crystallographic analysis, and a perspective ORTEP plot is shown in

(10) (a) Pulici, M. S. F.; Koshino, H.; Uzawa, J.; Yoshida, S.; Lobkovsky, E.; Clardy, J. *J. Org. Chem.* **1996**, *61*, 2122–2124. (b) Gunatilaka, A. A. *J. Nat. Prod.* **2006**, *69*, 509–526. (c) Deyrup, S. T. S.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2006**, *69*, 608–611. (d) Xu, J.; Ker, J.; Sendker, J.; Wray, V.; Guan, H.; Edrada, R.; Lin, W.; Wu, J.; Proksch, K. *J. Nat. Prod.* **2009**, *72*, 662–665. (e) Xu, J.; Ebada, S. S.; Proksch, P. *Fungal Diversity* **2010**, *44*, 15–31.

Figure 1. The X-ray data also allowed assignment of its relative configuration.

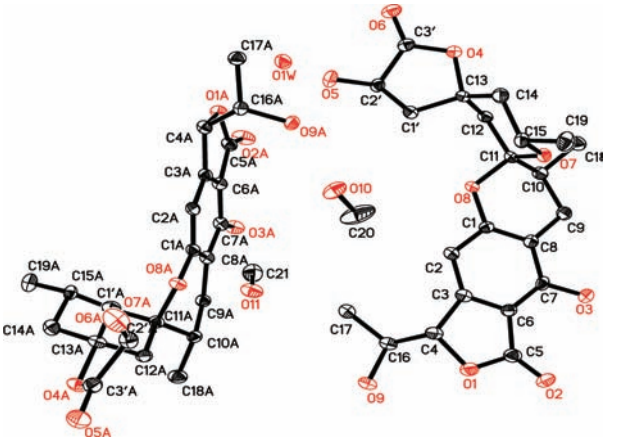


Figure 1. Thermal ellipsoid representation of **1**.

The absolute configuration of the C-4 stereogenic center was assigned by comparison of the CD spectrum of **1** (Figure 2) with that of pestaphthalide A (**4**).[12] The CD spectrum of **1** displayed a positive Cotton effect at around 215 ($\Delta\varepsilon$ +2.1) nm, and showed the same chirality as that of **4**,[12] indicating that C-4 is *S*-configured. Considering the relative configuration dertermined by X-ray data, the absolute configuration of 4*S*, 10*S*, 11*R*, 13*R*, 15*S*, 16*S*, and 2′*R* was assigned for **1**.

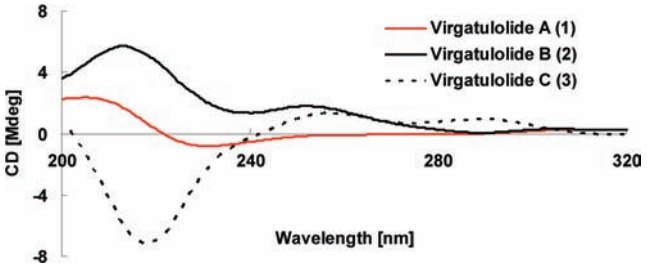


Figure 2. CD spectra of **1**–**3**.

Compound **2** gave a pseudomolecular ion $[M + Na]^+$ peak at *m*/*z* 387.1421 by HRESIMS, corresponding to a molecular formula of $C_{19}H_{24}O_7$ (eight degrees of unsaturation). Analysis of its NMR spectroscopic data (Table S1, Supporting Information) revealed structural features similar to those presented in **1**, except that the γ-lactone moiety spirally jointed to the 1,7-dioxaspiro[5.5] undecane unit at C-13 was replaced by an exchangeable proton (δ_H 4.87) in **2**, which was attached to C-13 on the basis of its HMBC correlations with C-12, C-13, and C-14, thereby completing the planar structure of **2** as shown. The relative and absolute configurations of **2** were deduced by analysis of its ^{1}H NMR *J*-values, NOESY data, and by analogy to **1**.

Compound **3** was assigned the same molecular formula $C_{19}H_{24}O_7$ as **2** by HRESIMS (*m*/*z* 387.1420 $[M + Na]^+$). Its ^{1}H and ^{13}C NMR spectra showed resonances nearly identical with those of **2**, except that the chemical shifts for the C-4 and C-16 oxymethines in **2** (CD_3OD; δ_H/δ_C 5.29/82.7 and 4.13/66.4) were different from those in **3** (CD_3OD; δ_H/δ_C 5.23/83.4 and 3.98/67.7), as well as the ^{1}H–^{1}H coupling constant observed between H-4 and H-16 (3.0 Hz in **2**; 5.0 Hz in **3**). These data implied that **3** was a stereoisomer of **2**. Interpretation of its 2D NMR data established the same planar structure as **2**. Analysis of the ^{1}H NMR *J*-values and NOED data (Table S2, Supporting Information) indicated that the relative configuration of the 6,6-spiroketal moiety (1,7-dioxaspiro[5.5]undecane) remains the same as in **2**, whereas the C-4 hydroxyethyl attached isobenzofuranone unit possesses the same relative configuration as the other coisolated known compound, pestaphthalide B (**5**).[12] In addition, the CD spectrum of **3** (Figure 2) exhibited the same chirality as **5**, suggesting the 4*R* and 16*S* absolute configuration. Although the 1,7-dioxaspiro[5.5]undecane and isobenzofuranone units could not be directly correlated by spectroscopic evidence, the 6,6-spiroketal portion of **3** was presumably to have the 10*S*, 11*R*, 13*R*, and 15*S* configuration on the basis of its biosynthetic relevance to **1** and **2**.

Compounds **1**–**3** showed modest cytotoxicity against HeLa cells, with IC_{50} values of 19.0, 22.5, and 20.6 μM, respectively (the positive control 5-fluorouracil showed an IC_{50} value of 10.0 μM).

Virgatolides A–C (**1**–**3**) are new members of the rare benzannulated 6,6-spiroketal class of natural products with the characteristic 3′,4′,5′,6′-tetrahydrospiro[chroman-2,2′-pyran] core. They possess unqiue structural features by virture of the presence of two previously undescribed skeletons. Specifically, the benzannulated 6,6-spiroketal fused to the hydroxyethyl attached γ-lactone moiety at C-3/C-4 to form a 3,3′,4,4′,5′,6′-hexahydrospiro[furo[3,4-*g*]chromene-2,2′-pyran]-6(8*H*)-one new ring system in **2** and **3**, which further spirally joined the second γ-lactone unit at C-13 to form the new skeleton presented in **1**. To our knowledge, this is the first occurrence of the γ-lactone unit(s) in the benzannulated 6,6-spiroketals.

From a biosynthetic aspect, compounds **1**–**3** could be generated from a putative triacetic lactone, 3,6-dimethyl-4-hydroxy-2-pyrone (**6**),[13,14] and pestaphthalide (**4** and **5**) of

(11) (a) Liu, L.; Liu, S.; Jiang, L.; Chen, X.; Guo, L.; Che, Y. *Org. Lett*. **2008**, *10*, 1397–1400. (b) Liu, L.; Tian, R.; Liu, S.; Chen, X.; Guo, L.; Che, Y. *Bioorg. Med. Chem*. **2008**, *16*, 6021–6026. (c) Li, E.; Jiang, L.; Guo, L.; Zhang, H.; Che, Y. *Bioorg. Med. Chem*. **2008**, *16*, 7894–7899. (d) Li, E.; Tian, R.; Liu, S.; Chen, X.; Guo, L.; Che, Y. *J. Nat. Prod*. **2008**, *71*, 664–668. (e) Liu, L.; Li, Y.; Liu, S.; Zheng, Z.; Chen, X.; Guo, L.; Che, Y. *Org. Lett*. **2009**, *11*, 2836–2839. (f) Liu, L.; Liu, S.; Chen, X.; Guo, L.; Che, Y. *Bioorg. Med. Chem*. 2009, *17*, 606–613. (g) Liu, L.; Niu, S.; Lu, X.; Chen, X.; Zhang, H.; Guo, L.; Che, Y. *Chem. Commun*. **2010**, *46*, 460–462. (h) Liu, L.; Bruhn, T.; Guo, L.; Götz, D. G.; Brun, R.; Stich, A.; Che, Y.; Bringmann, G. *Chem.—Eur. J*. **2011**, *17*, 2604–2613.

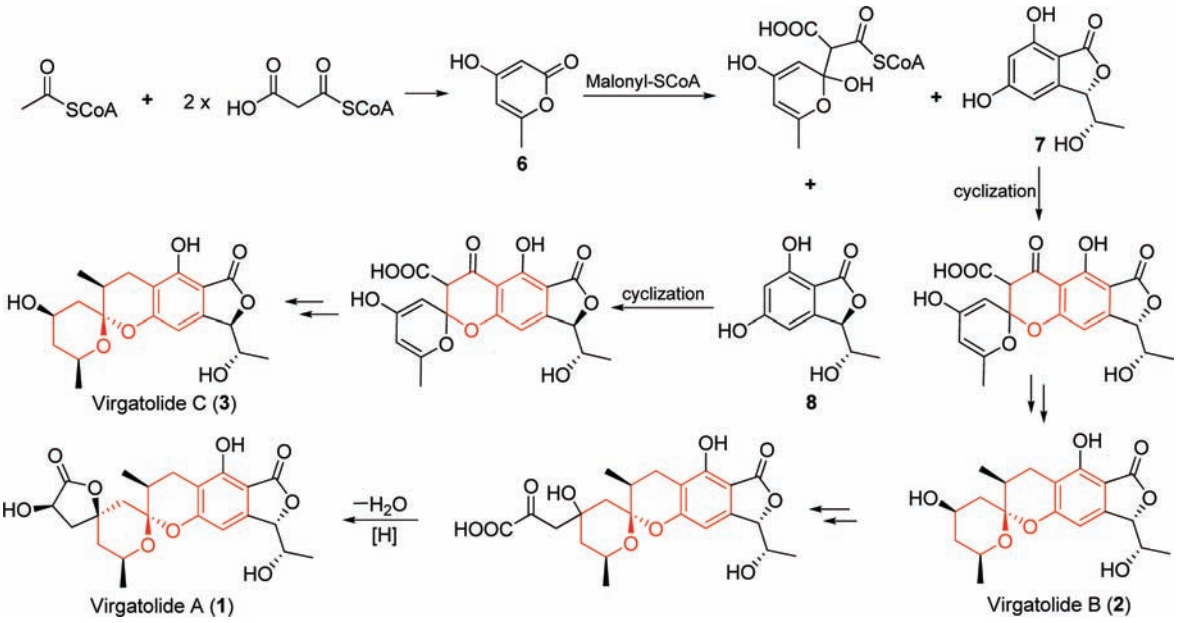
(12) Ding, G.; Liu, S.; Guo, L.; Zhou, Y.; Che, Y. *J. Nat. Prod*. **2008**, *71*, 615–618.

(13) Acker, T. E.; Brenneisen, P. E.; Tanenbaum, S. W. *J. Am. Chem. Soc*. **1966**, *88*, 834–837.

(14) Bentley, R.; Zwitkowits, P. M. *J. Am. Chem. Soc*. **1967**, *89*, 676–680.

(15) Uchida, K.; Watanabe, H.; Usui, T.; Osada, H.; Kitahara, T. *Heterocycles* **1998**, *48*, 2049–2060.

Scheme 1. Hypothetical Biosynthetic Pathways for **1**–**3**



intermediates, such as their demethyl analogues **7** and **8**,[15] via different reaction cascades as illustrated in the hypothetical biosynthetic pathways (Scheme 1).

Acknowledgment. Financial support from the National Natural Science Foundation of China (30925039), the Ministry of Science and Technolgy of China (2009CB522302 and 2009ZX09302-004), and the Chinese Academy of Sciences (KSCX2-EW-G-6) is gratefully acknowledged.

Supporting Information Available. Experimental procedures, characterization data, NMR data of **2** and **3**, ^{1}H and ^{13}C NMR spectra of **1**–**3**, and X-ray data of **1** (CIF file). This material is available free of charge via the Internet at http://pubs.acs.org.