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

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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. 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-spiroketal⁶⁻⁸ 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

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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 ¹H and ¹³C NMR spectra showed resonances for three exchangeable protons, three methyl groups, four methylenes, five methines (four of which are oxymethines), six sp² carbons (one protonated), two oxygenated sp³ quaternary carbons including one of double oxygenation ($\delta_{\rm C}$ 100.8), and two carboxylic carbons ($\delta_{\rm C}$ 169.1 and 176.1, respectively). These data accounted for all the NMR resonances, suggesting that 1 was a pentacyclic compound. Analysis of the ¹H and ¹³C 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 1H-1H 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 H₂-9 to the sp² carbons C-1, C-7, and C-8 led to the connection of C-8 to C-9. While those of H₂-12 and H₃-18 with the C-11 oxygetated sp3 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'. Addition correlations from H₂-1', H-2', H₂-12, and H₂-14 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-d₆

position	$\delta_{ ext{H}}{}^a (J \text{ in Hz})$	${\delta_{ m C}}^b$	$\mathrm{HMBC}(\mathrm{H} \to \mathrm{C} \#)$
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

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

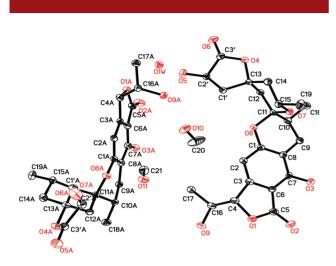


Figure 1. Thermal ellipsoid representation of 1.

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). 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**, indicating that C-4 is S-configured. Considering the relative configuration determined by X-ray data, the absolute configuration of 4S, 10S, 11R, 13R, 15S, 16S, and 2'R was assigned for **1**.

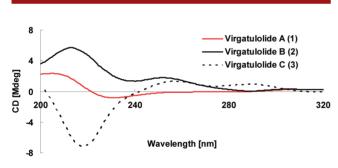


Figure 2. CD spectra of 1-3.

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 ($\delta_{\rm H}$ 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 ¹H 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 ¹H and ¹³C 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 (CD₃OD; δ_H/δ_C 5.29/ 82.7 and 4.13/66.4) were different from those in 3 (CD₃OD; $\delta_{\rm H}/\delta_{\rm C}$ 5.23/83.4 and 3.98/67.7), as well as the ¹H-¹H 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 ¹H 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 4R and 16S absolute configuration. Although the 1,7dioxaspiro[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 10S, 11R, 13R, and 15S configuration on the basis of its biosynthetic relevance to 1 and 2.

Compounds 1–3 showed modest cytotoxicity against HeLa cells, with IC₅₀ values of 19.0, 22.5, and 20.6 μ M, respectively (the positive control 5-fluorouracil showed an IC₅₀ value of 10.0 μ M).

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 unque 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(8H)-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

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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).

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Supporting Information Available. Experimental procedures, characterization data, NMR data of **2** and **3**, ¹H and ¹³C 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.

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