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# Virgatolides A–C, Benzannulated Spiroketal from the Plant Endophytic Fungus *Pestalotiopsis virgatula*

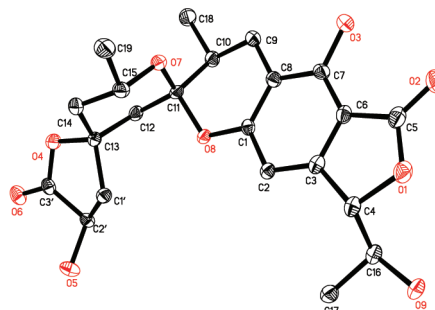
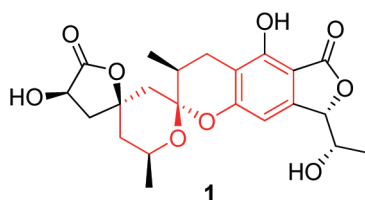
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## ABSTRACT



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)  $\gamma$ -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.<sup>1</sup> A notable feature of this class of compounds is the presence of a benzannulated 5,5-,<sup>2,3</sup> or

6,5-,<sup>4,5</sup> or 6,6-spiroketal<sup>6–8</sup> moiety as their core skeletons. Naturally occurring benzannulated 6,6-spiroketal are rare. The only precedents include citreoviranol and its demethyl analogue isolated from the fungus *Penicillium citreoviride* B (IFO 4692),<sup>6</sup> chaetoquadrins A–C from the Ascomycete *Chaetominum quadrangulatum* strain 71-NG-22,<sup>7</sup> and the dimeric cyanandiones from the rhizome of a Taiwanese folk medicine, *Cynanchum taiwanianum*.<sup>8</sup>

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

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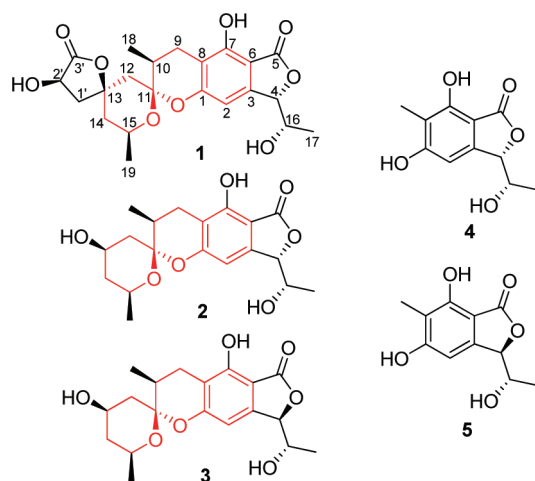
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secondary metabolites.<sup>9</sup> Chemical studies of the *Pestalotiopsis* genus have attracted much attention due to frequent discovery of structurally diverse and biologically active natural products.<sup>10,11</sup> During an ongoing search for new bioactive metabolites from the species of this genus, a strain of *Pestalotiopsis virgatula* (L147) isolated from the leaves of the traditional 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**).<sup>12</sup> 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 [ $M + Na$ ]<sup>+</sup>). Its  $^1H$  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 ( $\delta_C$  100.8), and two carboxylic carbons ( $\delta_C$  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  $^{13}C$  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**.<sup>12</sup> 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–C-19, 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 oxygenated  $sp^3$  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'. Additional 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 ( $\delta_C$  153.6) and C-15 ( $\delta_C$  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  $\gamma$ -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 $\rightarrow$ 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'

<sup>a</sup> Recorded at 400 MHz. <sup>b</sup> Recorded at 100 MHz.

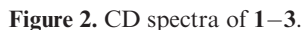
Fortunately, the proposed structure for virgatolide A (**1**) was confirmed by single-crystal X-ray crystallographic analysis, and a perspective ORTEP plot is shown in

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

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intermediates, such as their demethyl analogues **7** and **8**,<sup>15</sup> 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**,  $^1\text{H}$  and  $^{13}\text{C}$  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>.