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Chloropestolide A, an Antitumor Metabolite with an Unprecedented Spiroketal Skeleton from *Pestalotiopsis fici*

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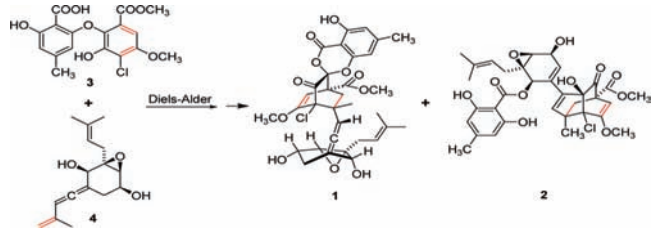
ABSTRACT



Chloropestolide A (1), a highly functionalized spiroketal with an unprecedented skeleton derived from a chlorinated bicyclo-[2.2.2]-oct-2-en-5-one ring and a 2,6-dihydroxy-4-methylbenzoic acid unit, has been isolated from the scale-up fermentation extract of *Pestalotiopsis fici*. The structure of 1 was elucidated by NMR spectroscopy and X-ray crystallography. 1 could be derived from the same Diels−Alder precursors as 2 and showed significant inhibitory effects on growth of two human cancer cell lines, HeLa and HT29, with GI_{50} values of 0.7 and 4.2 μM, respectively.

Chemical investigations of the plant endophytic fungi *Pestalotiopsis* spp. have led to discovery of a variety of bioactive natural products.[1−9] Our prior work on *Pestalotiopsis fici* (AS 3.9138 = W106-1) grown in different solid-substrate fermentation cultures afforded structurally diverse and biologically active secondary metabolites,[10−12] such as chloropupukeananin (**2**), the first pupukeanane chloride with anti-HIV-1 activity, and its biosynthetic Diels−Alder precursors (**3** and **4**).[10] To identify other minor active components

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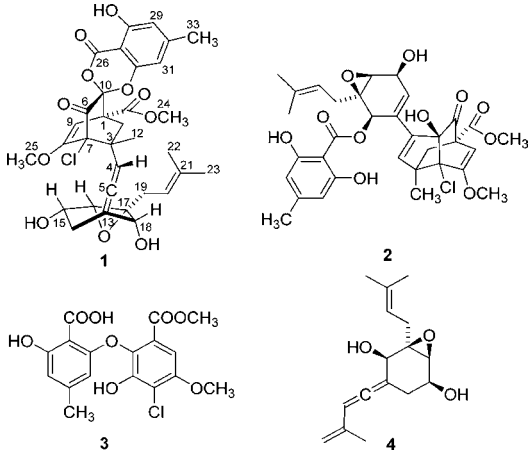
and/or Diels−Alder analogues of **2**, the fungus was refermented in a larger scale on rice to afford a crude extract showing inhibitory effects on growth of the human cancer cell lines, HeLa and HT29, and replication of the HIV-1 virus in C8166 cells. Its HPLC fingerprint revealed the presence of metabolites which were different from those isolated previously. Bioassay-directed fractionation of the extract led to the isolation of a highly functionalized spiroketal chloride with a novel skeleton named chloropestolide A (**1**) and metabolites **2**−**4** which were also identified in our previous work.[10] Details of the isolation, structure assignment, biogenesis, and biological activity of **1** are reported herein.



1 was assigned the molecular formula $C_{33}H_{35}ClO_{11}$ on the basis of its HRESIMS (*m*/*z* 665.1753 $[M + Na]^+$) and NMR data (Table 1). Analysis of its ^1H, ^{13}C, and HMQC NMR spectroscopic data revealed the presence of three exchangeable protons, six methyls (two methoxys), three methylenes, three oxymethines, 13 sp^2 carbons (five protonated), five sp^3 quaternary carbons including three heteroatom-bonded, and three carbonyl carbons. These data accounted for all the NMR resonances except for one chlorine atom. The ^1H and ^{13}C NMR spectra of **1** showed the signals for a 2,6-dihydroxy-4-methylbenzoate moiety nearly identical to those found in the spectra of **2** and **3** and a subunit similar to **4**. However, the resonances for the remaining portion of **1** differed significantly from those of **2**, indicating that **1** was related to **2** but with different structural features.

Interpretation of the $^1H-^1H$ COSY NMR data of **1** identified three isolated proton spin systems, which were C-14−C-16 (including OH-15), C-18−OH-18, and C-19−C-20. Analysis of the HMBC data of **1** established the 2,3-epoxyvinylidenecyclohexan-1,4-diol moiety with an isoprenyl group attached to C-17, which is identical to that present in **4**. HMBC correlations from H_3-12 to C-2, C-3, C-4, and C-7, and from H_2-2 to C-3, C-4, C-7, and C-12 led to the connection of C-2, C-4, and C-12 to either C-3 or C-7. However, the downfield ^{13}C NMR chemical shift for C-7 (δ_C 82.3) and an HMBC cross-peak observed from H-9 to C-7 precluded C-7 from being directly joined to C-2, C-4, and C-12. Those correlations from H_2-2 and H-9 to the quaternary carbon C-1, the ketal carbon C-10, and the carboxyl carbon C-11 indicated that C-1 was connected to

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

pos.	$\delta_H{}^a$ (*J* in Hz)	$\delta_C{}^b$	HMBC (H → C#)
1		51.9	
2a	2.28, d (14)	40.3	1, 3, 4, 7, 9, 10, 11, 12
2b	2.66, dd (14, 3.0)		1, 3, 4, 7, 9, 10, 11, 12
3		44.1	
4	5.19, s	97.5	2, 5, 12, 13, 14, 18
5		202.8	
6		190.0	
7		82.3	
8		151.0	
9	5.63, s	97.8	1, 2, 7, 8, 10, 11
10		97.9	
11		169.4	
12	1.29, s	24.5	2, 3, 4, 7
13		105.8	
14a	2.07, dd (16, 5.5)	30.7	5, 13, 15, 16, 18
14b	2.31, ddd (16, 10, 3.5)		5, 13, 15, 16, 18
15	4.05, dd (10, 5.5)	68.5	
16	3.25, br s	63.4	14, 15, 17, 18, 19
17		65.9	
18	4.23, d (9.0)	68.2	5, 13, 14, 16, 19
19a	1.93, dd (16, 7.0)	33.8	16, 17, 18, 20, 21
19b	2.82, dd (16, 7.0)		16, 17, 18, 20, 21
20	5.10, t (7.0)	119.2	19, 22, 23
21		135.8	
22	1.65, s	18.1	20, 21, 23
23	1.71, s	26.0	20, 21, 22
24	3.79, s	53.4	11
25	3.77, s	57.2	8
26		163.8	
27		97.3	
28		161.4	
29	6.55, s	113.0	26, 27, 28, 31, 33
30		151.8	
31	6.45, s	108.2	26, 27, 29, 32, 33
32		154.4	
33	2.32, s	22.3	29, 30, 31
OH-15	4.30, br s		15
OH-18	3.92, d (9.0)		17, 18
OH-28	9.80, br s		

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

C-2, C-9, C-10, and C-11. In turn, correlations from the olefinic proton H-9 to C-7 and C-8 located C-7 at the allylic position of the C-8/C-9 olefin. Further HMBC correlations from H_3-24 to C-11 and from H_3-25 to C-8 indicated that C-8 and C-11 were each attached to a methoxy group. The exchangeable proton at δ_H 9.80 was deduced as the phenolic C-28−OH proton by comparison of the chemical shift values for C-28 and C-32 in **1** (δ_C 161.4 and 154.4, respectively) with those in **2** (δ_C 162.0 for both C-28 and C-32). An oxygenated sp^3 quaternary carbon signal (δ_C 97.9) was observed in the NMR spectrum of **1** instead of a ketone carbon (δ_C 196.4) in **2**, indicating that the C-10 ketone functionality was reduced to a ketal carbon. Considering the upfield chemical shifts for C-26 (δ_C 163.8 in **1** vs 170.9 in **2**) and C-32 (δ_C 154.4 in **1** vs 162.0 in **2**), these two carbons were connected to each of the C-10 oxygen atoms via an ether and an ester linkage, respectively. In addition, the

chemical shift value of C-7 (δ_C 82.3) and the hexacyclic nature of **1** required that the chlorine atom be directly attached to C-7 and the remaining ketone carbon C-6 be connected to both C-7 and C-10 by default to complete the bicyclo-[2.2.2]-oct-2-en-5-one ring. Collectively, these data permitted assignment of the gross structure of **1**.

The 2,3-epoxyvinylidenecyclohexan-1,4-diol unit in **1** was assigned the same relative configuration as **4** by comparison of its $^1H-^1H$ coupling constants and NOESY data with those of **4**. NOESY correlations (Figure 1) of H-2a with H-4 and

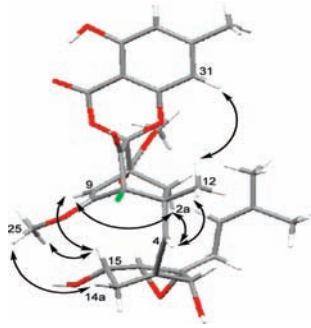


Figure 1. Key NOESY correlations for **1**.

H-9; H_3-12 with H-2b, H-4, and H-31; H-14a with H_3-25; and H-15 with H-9 and H_3-25 established the relative configurations of C-3 and C-10. Ultimately, the structure of **1** was further confirmed by X-ray crystallographic analysis (Figure 2).[13] The presence of a chlorine atom in **1** allowed

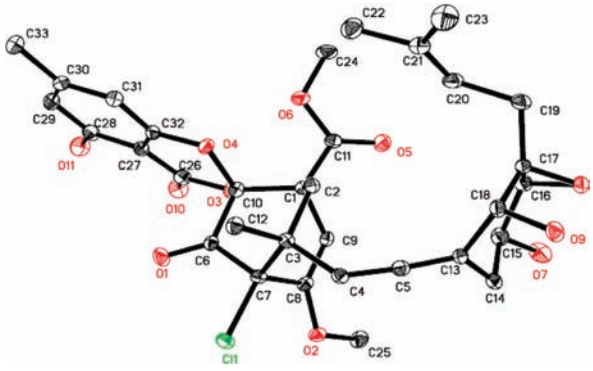


Figure 2. Thermal ellipsoid representation of **1**.

assignment of the absolute configurations of all the stereogenic centers (1*R*, 3*R*, 7*S*, 10*R*, 15*S*, 16*S*, 17*R*, and 18*R*).

Chloropestolide A (**1**) was tested for in vitro activity against HIV-1 replication in C8166 cells,[10] and it showed an inhibitory effect with an IC_{50} value of 64.9 μM (the CC_{50} value is greater than 100 μM; the positive control indinavir sulfate showed an IC_{50} value of 8.2 nM). Chloropupukeananin (**2**) has been demonstrated to be an anti-HIV-1 agent more potent than **1** in our previous study;[10] therefore, the anti-HIV-1 effect of the crude extract was mainly attributed to the presence of **2**. Compounds **1** and **2** were also evaluated against human tumor cell lines, HeLa and HT29, and both showed significant inhibitory effects on growth of the two cell lines (Table 2). **1** is about five to ten times the potency of the positive control 5-fluorouracil against the HeLa cells.

Table 2. Antitumor Effects of Compounds **1** and **2**

compound	HeLa (GI_{50}, μM)	HT29 (GI_{50}, μM)
1	0.7	4.2
2	1.4	6.7
5-fluorouracil	8.0	12.0

Natural products possessing the bicyclo-[2.2.2]-oct-2-en-5-one moiety have mainly been isolated from plants. Examples include 7-isopropyl-5-methyl-5-bicyclo-[2.2.2]-octen-2-one from the root oil of *Angelica archangelica* L.;[14] obtunone, chamaecypanone C, and obtunorlignan A from the heartwood of *Chamaecyparis obtusa* var. *formosana*;[15,16] heterotropanone from the plant *Heterotropa takaoi*;[17] helisorin and helisterculins A and B from the Indonesian medicinal plant *Helicteres isora*;[18] yunnaneic acids A−D from the roots of *Salvia yunnanensis*;[19] gnetin B from two *Gnelum* species;[20] and aquaticol from the medicinal plant *Veronica anagallis-aquatica*.[21] The only fungal metabolite incoporating the bicyclo-[2.2.2]-oct-2-en-5-one moiety was sorbiquinol from *Trichodertna longibrczchiatum*.[22] In addition, some compounds with a chlorinated bicyclo-[2.2.2]-oct-2-en-5-one core have been synthesized.[23,24] Chloropestolide A (**1**) differs significantly from the known precedents by having a previously undescribed chlorinated spiroketal skeleton derived from a chlorinated bicyclo-[2.2.2]-oct-2-en-5-one ring and a 2,6-dihydroxy-4-methylbenzoic acid moiety.

(13) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 731103). Copies of the data can be obtained, free of charge, on application to the director, CCDC 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or email: deposit@ccdc.cam.ac.uk).

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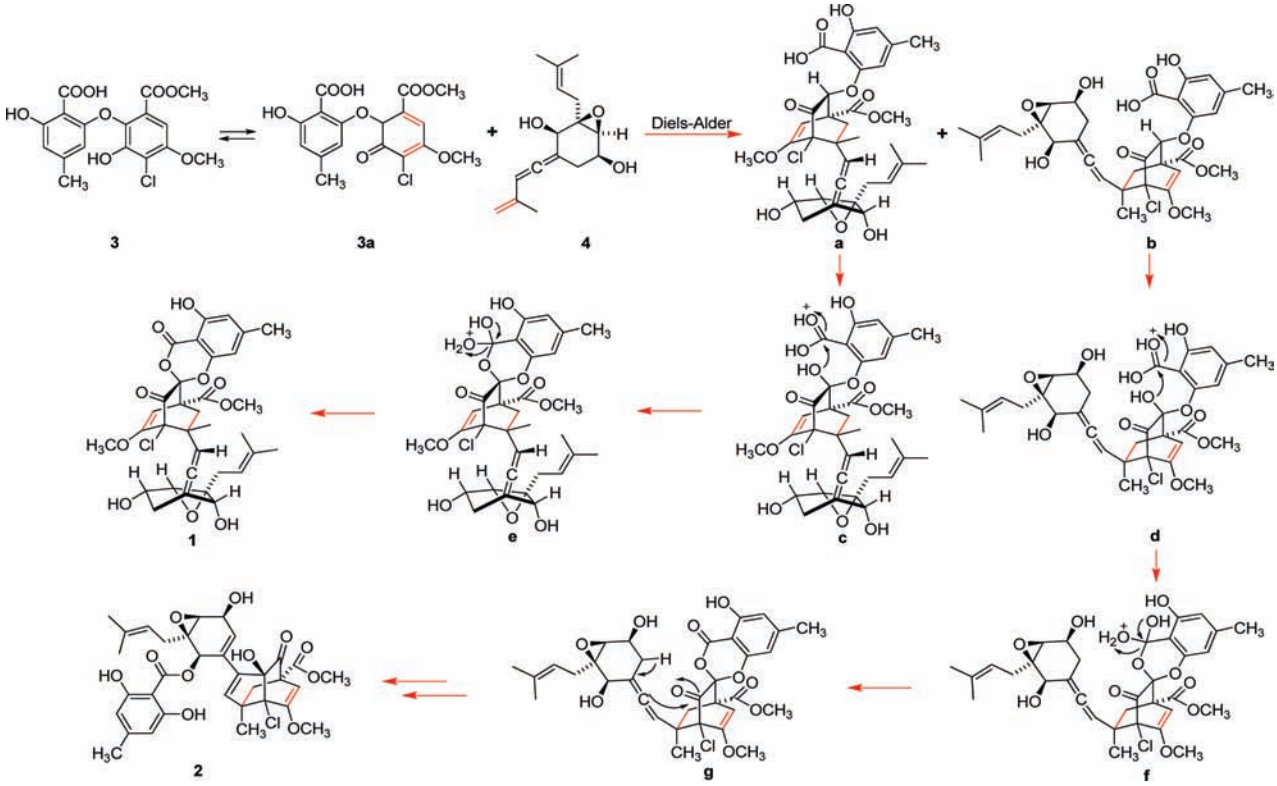
Scheme 1. Proposed Biosynthetic Pathway for **1**



Biogenetically, **3** and **4** could be the biosynthetic precursors for **1** and **2**,[10] first via an inverse-electron-demand Diels−Alder reaction[25,26] to form the key intermediates **a** and **b** (Scheme 1) and then followed by a series of reactions through different routes to form **1** and **2**. The discovery of **1** might imply that the biosynthetic pathway initially proposed for **2** in our previous work is more complex, possibly with more intermediates (such as **g**) being involved prior to the formation of the tricyclo-[4.3.1.$0^{3,7}$]-decane skeleton.[10] These results strongly suggest that further work is warranted to maximize the metabolic potential of *P. fici* to identify other "missing" building blocks for chloropupukeananin (**2**).

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Supporting Information Available: Experimental procedures, characterization data, and ^{1}H and ^{13}C NMR spectra of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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