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A Botryane Metabolite with a New Hexacyclic Skeleton from an Entomogenous Fungus *Hypocrea* sp.

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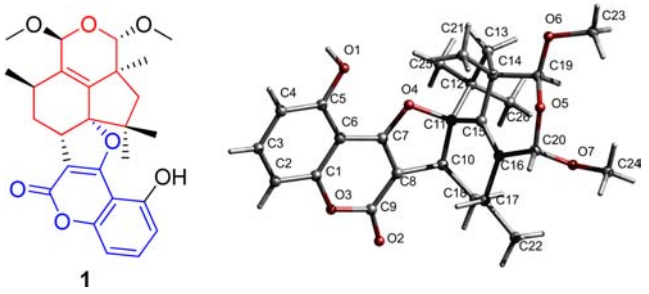
ABSTRACT



Hypocrolide A (1), a botryane metabolite with a new hexacyclic skeleton, was isolated from cultures of the entomogenous fungus *Hypocrea* sp. The proposed structure was confirmed by X-ray crystallography using Cu Kα radiation. The mixed-biogenetic skeleton could be derived from the hypothetical precursors related to coumarin and dihydrobotrydiol, and the latter may be derived from the coisolated 10-oxodehydrodihydrobotrydial (2) or a similar analogue.

Botryanes are sesquiterpenoids with the core skeleton of a bicyclic, nonisoprenoid system.[1] This class of natural products have been encountered only from a limited number of fungal species including the necrotrophic plant pathogen *Botrytis cinerea*,[1−7] the basidiomycete *Boletus edulis*,[8] the mitosporic fungus *Geniculosporium* sp. associated with the red alga *Polysiphonia* sp.,[9] and the ascomycete *Daldinia concentrica*.[10] As an important group of sesquiterpene metabolites, the botryanes showed a variety of biological activities. Notable representatives include phytotoxin botrydial, a hypersensitive response (HR) inducer produced by *B. cinerea*;[11] boledulins A−C, cytotoxic metabolites from *B. edulis*;[8] and several antimicrobial botryanes from *Geniculosporium* sp.[9] A recent structure−activity relationship (SAR) study of botryanes demonstrated that the presence of 1,5-dialdehyde functionality was critical for the cytotoxic effects of this class of compounds.[12] Biogenetically, the botryanes were generated from the key intermediate presilphiperfolan-8β-ol

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(PSP), which was derived from cyclization of the universal precursor farnesyl diphosphate (FPP).[13−15] The biosynthesis was catalyzed by the genes encoding synthases, in which the recombinant *BcBOT2* protein is responsible for the conversion of FPP to PSP (Figure 1).[14]

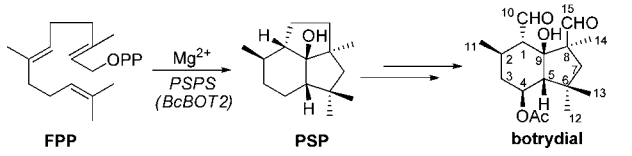
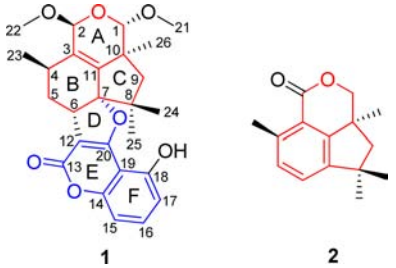


Figure 1. Biosynthesis of botrydial.

Serrataspis sp. is a Diaspididae insect feeding on the phloem or parenchyma of woody plants and grasses.[16] The parasitic insect shows putative mutualistic associations with fungal phytopathogen of the genus *Septobasidium*.[17] The insects obtain nutrition from the host plants, causing great loss in agriculture.[18] In the current work, the fungus *Hypocrea* sp. was isolated from a *Septobasidium*-infected insect, *Serrataspis* sp. collected at Cang Mountain, Dali, Yunnan Province, People's Republic of China. An EtOAc extract prepared from solid-substrate fermentation cultures displayed cytotoxicity against HeLa (cervical epithelium), A549 (lung carcinoma epithelial), and MCF-7 (breast cancer) cells. Fractionation of the extract afforded hypocrolide A (**1**), a mixed-biogenetic botryane derivative possessing a previously undescribed hexacycle, and the known compound, 10-oxodehydrodihydrobotrydial (**2**).[7] Details of the structure elucidation, cytotoxicity, and plausible biogenesis of **1** are reported herein.



1

2

A molecular formula of $C_{26}H_{30}O_7$ (12 degrees of unsaturation) was assigned to hypocrolide A (**1**) on the basis of HRESIMS (*m*/*z* 455.2064 $[M + H]^+$). Analysis of its ^1H, ^{13}C, and HMBC NMR data (Table 1) revealed the presence of one exchangeable proton (δ_H 7.20), six methyl groups including two methoxys, two methylenes, four methines with two doubly oxygenated, three sp^3 quaternary carbons, 10 olefinic/aromatic carbons (three of which were protonated), and one carboxylic carbon (δ_C 164.7). These data accounted for all the NMR resonances of **1** and six of the 12 unsaturations, suggesting that **1** was a hexacyclic compound. Interpretation of the $^1H-^1H$ COSY NMR data showed the presence of two isolated spin-systems, which were C-4−C-6 (including C-23) and C-15−C-17. In the HMBC spectrum of **1**, correlations from H-2 to C-3 and C-11 and from H-5 to C-3 indicated that C-2 and C-4 are both connected to C-3 of the C-3/C-11 olefin (Figure 2). HMBC cross peaks from H_3-26 to C-1, C-9, C-10, and C-11 revealed the connections of C-10 to C-1, C-9, and C-11. Mutual HMBC correlations observed between the C-1 and C-2 oxymethines indicated that both carbons are attached to the same oxygen atom, establishing a highly substituted 3,6-dihydro-2*H*-pyran unit (ring A). While correlations of H_3-24 and H_3-25 with C-7, C-8, and C-9 connected C-8 to both C-7 and C-9. In turn, cross peaks from H-6 to C-8 and C-11 enabled junction of the cyclohexene (ring B) and cyclopentane (ring C) at C-7/C-11, completing the 3,3a,4,5,5a,6,7,8-octahydro-1*H*-cyclopenta[*de*]isochromene unit. HMBC

Table 1. NMR Spectroscopic Data for **1** in $CDCl_3$

pos.	$\delta_H{}^a$ (*J* in Hz)	$\delta_C{}^b$	HMBC (H → C#)
1	4.50, s	101.9	2, 9, 10, 11, 21, 26
2	5.07, s	100.4	1, 3, 4, 10, 11, 22
3		137.7	
4	2.38, m	28.4	2, 3, 5, 6, 23
5	2.00, m[c]	35.4	3, 4, 6, 7/12, 23
	1.89, m[c]		
6	3.45, dd (9.42, 4.98)	33.4	4, 5, 8, 11, 7/12, 13, 20
7		106.1	
8		45.3	
9	1.81, d (14.2)	47.7	1, 7, 8, 10, 11, 24, 25, 26
	1.74, d (14.2)		
10		41.7	
11		138.8	
12		105.7	
13		164.7	
14		155.5	
15	6.92, dd (8.34, 0.78)	108.9	13, 14, 17, 19
16	7.42, t (8.34)	133.5	14, 15, 17, 18, 19
17	6.76, dd (8.34, 0.78)	110.9	15, 18, 19
18		153.4	
19		101.3	
20		160.0	
21	3.56, s	57.0	1
22	3.54, s	55.9	2
23	1.17, d (6.48)	17.9	3, 4, 5
24	0.96, s	27.1	7, 8, 9, 25
25	1.04, s	22.9	7, 8, 9, 24
26	1.29, s	21.3	1, 9, 10, 11
OH-18	7.20, s		17, 18, 19

[a] Recorded at 600 MHz. [b] Recorded at 150 MHz. [c] Mutiplicity due to signal overlapping.

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correlations from H_3-21 to C-1 and from H_3-22 to C-2 located the two *O*-methyls at C-1 and C-2, respectively. Further HMBC cross peaks from OH-18 to C-17, C-18, and C-19, and from H-15 to C-14 and C-19 established the trisubstituted aryl unit (ring F) with a hydroxy group attached to C-18. A weak but distinctive four-bond correlation[19] from H-15 to C-13 (Figure S6, Supporting Information) suggested that the C-13 carboxylic carbon acylated the C-14 (δ_C 155.5) oxygen to form an ester linkage. Additional correlations from H-6 to C-13 (δ_C 164.7) and C-20 (δ_C 160.0), plus ^{13}C NMR chemical shift consideration for C-12 (δ_C 105.7), indicated that the three carbons form an α,β-unsaturated ketone with C-12 connected to C-6. Considering the ^{13}C NMR chemical shifts of C-7 (δ_C 106.1), C-19 (δ_C 101.3), and C-20, and the unsaturation requirement of **1**, C-19 is attached to C-20 to form a coumarin unit, and C-7 and C-20 are attached to the only remaining oxygen atom by default to form ring D, even though no additional evidence for these linkages were provided by the HMBC data. Thereby, the gross structure of **1** was tentatively proposed as shown.

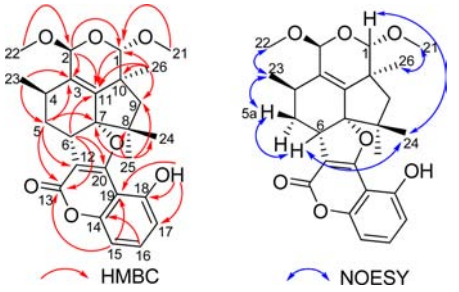

Figure 2. Selected key HMBC and NOESY correlations of **1**.

The relative configuration of **1** was deduced by analysis of NOESY data. NOESY correlations of H_3-24 with H-1 and H-6, H-6 with H-5a (δ_H 2.00), and of H_3-23 with H-5a and H_3-22 revealed their proximity in space, while that of H_3-21 with H_3-26 placed these protons on the opposite face of the ring system.

The absolute configuration of **1** was assigned using the CD exciton chirality method.[20] A strong UV absorption at λ_{max} 236 nm (Figure 3) was contributed by the coumarin moiety.[21] Corresponding to the UV maximum, **1** exhibited a split CD curve with the positive first Cotton effect (CE) at 244 nm ($\Delta\varepsilon$ +3.23) and negative second CE at 223 nm ($\Delta\varepsilon$ −0.54), which was caused by the transition reaction from the coumarin and the olefin chromophores with a dihedral angle of 119.2° (Figure 3). Therefore, the absolute configuration of 1*S*, 2*S*, 4*R*, 6*S*, 7*S*, and 10*S* was assigned for **1**.

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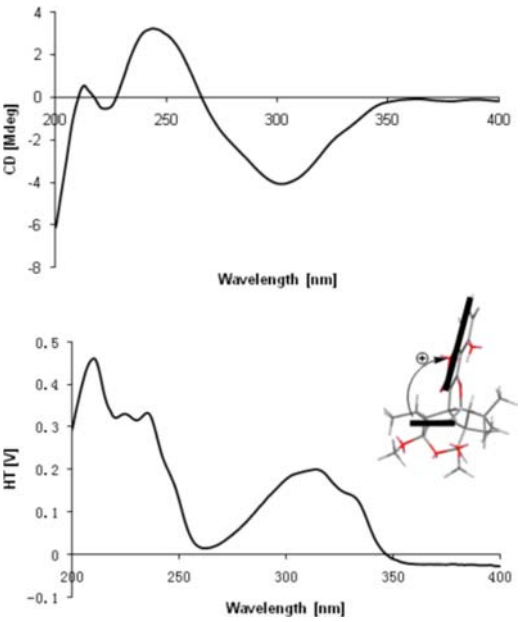

Figure 3. CD (top) and UV (bottom) spectra of **1** in MeOH; arrows denote the electric transition dipole of coumarin and C3/C11 olefin chromophores.

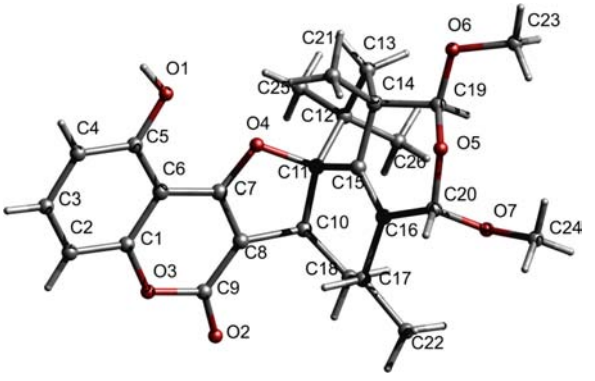

Figure 4. Thermal ellipsoid representation of **1**. (Note: A different numbering system is used for the structural data deposited with the CCDC.).

Finally, the proposed structure of **1** was confirmed by single-crystal X-ray crystallography. The perspective ORTEP plot is shown in Figure 4. In addition, the presence of a relatively high percentage of oxygen in **1** exhibited enough anomalous dispersion of Cu Kα radiation to allow assignment of the absolute configuration with the Flack parameter value close to 0.0.[19] Therefore, the absolute configuration of **1** was established based on the value of the Flack absolute structure parameter 0.05(13),[22] which is consistent with that deduced from the CD data.

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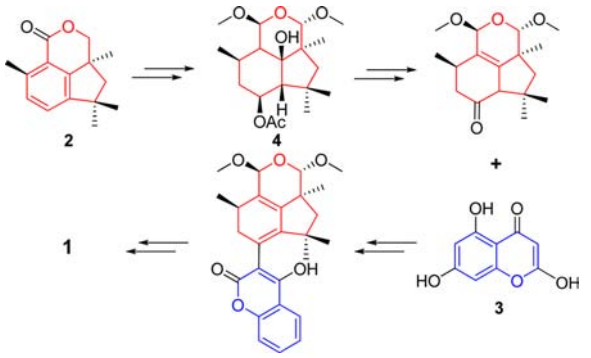
Scheme 1. Hypothetical Biosynthetic Pathways for **1**



To verify that **1** is an authentic natural product, a portion of the EtOAc extract was subjected to HPLC–MS analysis using HPLC-grade H_2O and CH_3CN as solvents. Compound **1** was identified on the HPLC–MS chromatogram of the crude extract by comparison of its retention time and ESIMS data with the pure compound (Figure S8, Supporting Information), indicating that **1** is indeed a naturally occurring metabolite.

Compound **2** was identified as the botryane δ-lactone, 10-oxodehydrodihydrobotrydial, which was previously isolated from the phytopathogenic fungus *B. cinerea*, by comparison of its NMR and MS data with literature values.[7]

Compound **1** was evaluated for cytotoxicity against human tumor cell lines, HeLa, A549, and MCF-7. It was cytotoxic to all the cell lines, showing IC_{50} values of 11.8, 22.0, and 20.4 μM, respectively (the positive control cisplatin showed IC_{50} values of 4.7, 7.8, and 4.9 μM against the three cell lines).

Hypocrolide A (**1**) is a botryane metabolite with the unique 5/6/6/5/6/6 ring system. Although several natural products and synthetic compounds with partial structural similarity to **1** have been reported, which incorporated either a decahydro-1*H*-cyclopenta[*de*]isochromene,[7] or a 6b,7,8,9,10,10a-hexahydro-6*H*-benzofuro[3,2-*c*]chromen-6-one[23,24] core, **1** possesses a previously undescribed hexacycle, which could be derived from the hypothetical precursors related to coumarin and dihydrobotrydiol (Scheme 1). The latter may be derived from the coisolated 10-oxodehydrodihydrobotrydial (**2**)[7] or a similar analogue, whereas the coumarin intermediate may be derived from 2,5,7-trihydroxychromone (**3**).[25] Although **3** was not isolated in the current work, several metabolites possessing the same 4*H*-chromen-4-one moiety as **3** were found in the same strain including 2,8-dihydroxy-3-methyl-9-oxoxanthene-1-carboxylic acid methyl ester,[26] and microsphaeropsones B and C.[27] The proposed precursors and the reaction cascades leading to the generation of **1** are illustrated in Scheme 1.

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Supporting Information Available. Experimental procedures, characterization data, ^1H, ^{13}C, and 2D NMR spectra, 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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The authors declare no competing financial interest.