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A NEW BUTENOLIDE-TYPE FUNGAL PIGMENT FROM THE MUSHROOM *PULVEROBOLETUS RAVENELII*

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Abstract – A new butenolide-type fungal pigment, pulverolide (**1**) was isolated from the fresh fruiting bodies of *Pulveroboletus ravenelii*, together with a related known compound pulveraven A (**2**). The structure of pulverolide (**1**) was elucidated on the basis of spectroscopic studies including extensive 2D NMR experiments.

INTRODUCTION

Pulveroboletus ravenelii is a species in the Boletaceae, whose lemon yellow color is very conspicuous. It is a mushroom which is beautiful apart from taste. Previous chemical investigation of this mushroom led to the isolation of several butenolides, such as pulveraven A, pulveraven B, ravenelone and isoravenelone.[1,2] As a part of our work on the natural products from higher fungi of Yunnan Province, China,[3-6] the chemical constituents of *P. ravenelii* were investigated and a new butenolide-type fungal pigment pulverolide (**1**) was isolated, together with a related known compound pulveraven A (**2**). This paper describes the isolation and structural elucidation of the new compound, in addition its possible biogenetic pathway originated from vulpinic acid. It is worth while to note that this type of fungal pigment with the *2H*-furo[3,2-*b*]benzopyran-2-one skeleton is reported for the first time from the family Boletaceae.

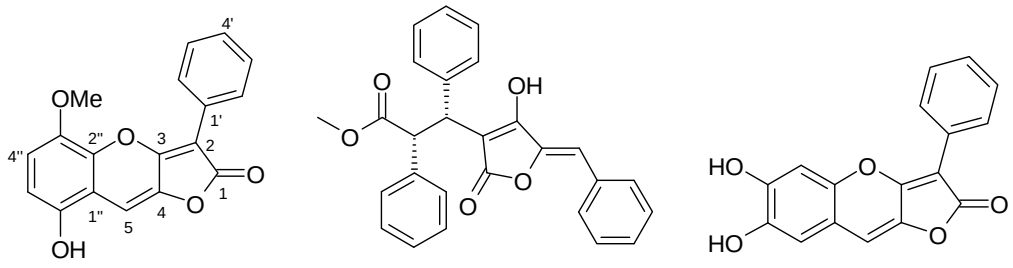
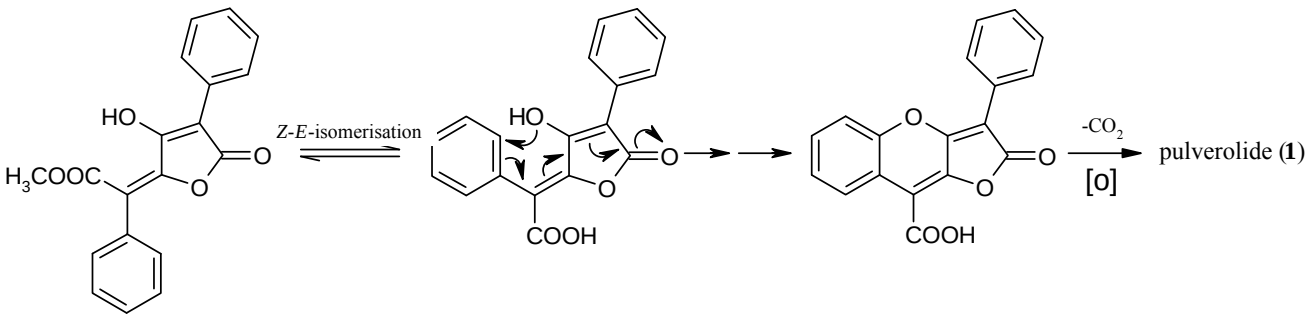


Figure 1. Structures of compounds (**1)**, **(2)** and (**3)**

RESULTS AND DISCUSSION

Compound (**1**) was obtained as yellow needles. The HRESIMS spectrum shows a quasi-molecular ion peak $[M–H]^-$ at m/z 307.0598 corresponding to the molecular formula $C_{18}H_{11}O_5$, requiring thirteen degrees of unsaturation. The IR spectrum indicates the presence of a hydroxyl group at 3399 cm^{-1}, a carbonyl group at 1745 cm^{-1} and a benzene ring at 1613, 1601, 1585 cm^{-1}. By careful comparison with the literature data, the ^{13}C NMR spectrum of **1** is very similar to those of aurantricholide A (**3**) which was recently isolated from *Tricholoma aurantium*.[7] Further comparison of the ^1H NMR spectra of **1** and **3** indicates this two compounds possess the same 2*H*-furo[3,2-*b*]benzopyran-2-one skeleton. The main differences in the ^1H NMR spectra are that: a) two *ortho*-coupled aromatic proton signals at δ 7.17 and 7.12 (each 1H, d, J = 9.0 Hz) are observed in **1**; b) the existence of one methoxyl group at δ 3.90 (3H, s) suggests that a hydroxyl group in **3** is methylated in **1**. The position of the methoxyl group and the hydroxyl group was determined to be attached at C-3′′ and C-6′′, respectively, on the basis of the HMBC spectrum (Table 1), by which the important correlation peaks between δ_H 7.26 (1H, s, H-5) and δ_C 157.9 (s, C-3), 141.8 (s, C-4), 143.7 (s, C-2′′) and 145.7 (s, C-6′′), and between δ_H 3.90 (3H, s, OCH_3) and δ_C 144.9 (s, C-3′′), 113.0 (d, C-4′′), are observed. In the light of the evidences mentioned above, the structure of **1** was therefore elucidated as shown in Figure 1, named pulverolide. Compound (**1**) with a rare 2*H*-furo[3,2-*b*]benzopyran-2-one skeleton may originate biogenetically from vulpinic acid (**4**) (Scheme 1).

Comparison of the physicochemical properties with literature data allowed to identify the known compound as pulveraven A (**2**).[1] No matter where the fruiting bodies of *P. ravenelii* were collected whether in America, Japan or China, pulveraven A (**2**) has been isolated without exception,[1,2] so we think this compound possesses important chemtaxonomic significance for this species.



vulpinic acid (**4**)

Scheme 1. Plausible biogenetic pathway of **1** from vulpinic acid (**4**)

Table 1. NMR data for pulverolide (**1**) in acetone-d_6

Position	^{13}C-NMR	^{1}H-NMR	HMBC
1	167.2 (s)		
2	96.5 (s)		
3	157.9 (s)		
4	141.8 (s)		
5	101.3 (d)	7.26 (s)	C-3, C-4, C-2″, C-6″
1'	130.3 (s)		
2', 6'	127.3 (2×d)	8.19 (d, 8.0)	C-2, C-4′
3', 5'	129.3 (2×d)	7.48 (dd, 8.0, 7.4)	C-1′
4'	128.2 (d)	7.33 (t, 7.4)	C-2′, C-6′
1"	110.8 (s)		
2"	143.7 (s)		
3"	144.9 (s)		
4"	113.0 (d)	7.17 (d, 9.0)	C-2″, C-6″
5"	107.5 (d)	7.12 (d, 9.0)	C-1″, C-3″
6"	145.7 (s)		
OCH_3	57.0 (q)	3.90 (s)	C-3″, C-4″
OH		8.84 (br s)	

EXPERIMENTAL

General Experimental Procedures

Melting point was obtained on XRC-1 apparatus and is uncorrected. IR spectrum was obtained on Bruker Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker DRX-500 spectrometer in aceton-d_6 solvent (δ_H 2.04 ppm, δ_C 29.8 ppm). ESIMS and HRESIMS were recorded with API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Fungal Material

The fresh fruiting bodies of *P. ravenelii* were purchased at market in Nanhua County of Yunnan Province, China, in August 2005 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, CAS.

Extraction and Isolation

The fresh fruiting bodies of *P. ravenelii* (100 g) were immersed in 80 % acetone (2.0 L) and left at rt. Then the acetone extraction was concentrated and partitioned between EtOAc and water. The EtOAc extract (1.5 g) was applied on a silica gel column and eluted stepwise with petroleum ether and acetone solvent system. Fraction 1 (175 mg) from petroleum ether/acetone (10:1, v/v) was subjected on a Sephadex LH-20 gel column eluting with $CHCl_3$/MeOH (1:1, v/v) to give a residue mainly containing **1**, which was further purified on a silica gel column eluting with petroleum ether/acetone (42:1, v/v) to yield pure compound (**1**) (4.0 mg) as yellow needles. Fraction 1 was evaporated to dryness, and pulveraven A (16 mg) was recovered from the residue by recrystallized from petroleum ether/acetone.

Pulverolide (1)

Yellow needles (acetone), mp 215-217°C, R_f = 0.52 ($CHCl_3$/MeOH=70/1). UV (MeOH) λ_{max} (ε): 231 (26,700), 255 (14,400), 372 (19,000) nm. IR (KBr): 3399, 3093, 2924, 2853, 1745, 1661, 1634, 1613, 1601, 1585, 1489, 1470, 1440, 1278, 1021 cm^{-1}. ESIMS (neg.): 307 $[M-1]^-$, 615 $[2M-1]^-$. HRESIMS (neg.): Calcd. for $C_{18}H_{11}O_5$ $[M-1]^-$: 307.0606. Found: 307.0598.

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