Two Novel Ravenelones from the Edible Mushroom *Pulveroboletus ravenelii*

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Two novel butenolides, isoravenelone (1) and ravenelone (2), and a large amount of a known compound, vulpinic acid (3), were isolated from the methanolic extract of the Japanese fungus *Pulveroboletus ravenelii* (Boletales). These structures were elucidated by two dimensional (2D) NMR, MS, IR and UV spectra, and X-ray crystallographic analysis.

Key words *Pulveroboletus ravenelii*; butenolide; vulpinic acid

We have been studying the chemical constituents of Japanese and European inedible mushrooms and found that they produce various types of secondary metabolites, many of which show characteristic pharmacological activities such as superoxide anion radical release inhibition, anticancer, apoptosis and anti HVS-1 activity.[1—6] *Pulveroboletus ravenelii* (BECK. *et* CURT.) MURR. is grown to a limited extent in Japan, and also in east Asia and America.[7] Previously, vulpinic acid (0.12% of the fresh weight), atrometic acid, isoxerocomic acid, variegatic acid, xerocomorubin and variegatorubin were isolated from the fruit bodies of Australian *P. ravenelii*.[8] The same Japanese species was reported to contain vulpinic acid, permethyl ethers of methyl xerocomate, and methyl atrometate and its corresponding monochloro derivatives.[9] In continuation of our research of biologically active constituents of fungus belonging to Basidiomycetes, we have reinvestigated the fruit bodies of Japanese *P. ravenelii.* We report here the isolation and structures of two novel butenolides (**1**, **2**), along with a known compound, vulpinic acid (**3**).

The methanolic extract of the fruit bodies of *P. ravenelii* was partitioned between EtOAc and water. The EtOAc layer was evaporated and crystallized in MeOH to give compound **3** as a crystal. The mother liquid was not purified to a pure state by several separation procedures, thus it was methylated after the presence of one carbomethoxyl group (δ_H 3.62) was confirmed by ^{1}H-NMR spectrum. Then, the reaction mixture was purified by prep. HPLC to afford compounds **4** and **5**, which corresponded to methylated derivatives of compounds **1** and **2**, respectively. Compound **4** was obtained as a white powder, and its high resolution (HR)-FAB-MS showed $[M+H]^+$ at *m/z* 441.2625, suggesting the molecular formula to be $C_{27}H_{36}O_5$. The IR spectrum indicated the presence of a carbonyl group (1753 cm^{-1}) and a benzene ring (1610 cm^{-1}). The ^{1}H-NMR spectrum of **4** showed the presence of two methoxyl groups (δ_H 3.64, 3.81, each 3H, s), one olefinic proton (δ_H 6.69), three benzene rings, and two methine protons at δ_H 4.70 (1H, d, J=12.1 Hz) and 5.01 (1H, d, J=12.1 Hz), suggesting that they are *anti* configuration to each other (Table 1). The ^{13}C-NMR of **4** showed the presence of 27 carbons, including one ester carbonyl (δ_C 173.7) and one lactone carbon atom (δ_C 168.4). The ^{1}H–^{1}H correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), and heteronuclear multiple bond connectivity (HMBC) spectra suggested the presence of three aromatic rings which were located at C-6, C-7 and C-8, as shown in Fig. 1. The position of one methoxyl group (δ_C 60.5) was determined to be at C-3 due to the HMBC correlation with C-3. The other methoxyl group (δ_C 52.3) had a HMBC correlation with C-9, indicating the presence of a carbomethoxyl group in **4** (Fig. 1). Comparison of the ^{1}H-NMR data of **4** with those of compound **1** revealed that the methylation of compound **1** occurred at C-3. Furthermore, the 3-OMe had NOE correlations with H-2b, H-3b, H-5a and H-6a, and the absence of NOE between 3-OMe and H-6 indicated that the configuration of C_2–C_6 was *E*-form. Thus, compound **1** was determined to be as shown in Chart 1 and was named isoravenelone.

Compound **5** was obtained as a white powder; its molecular formula was deduced to be $C_{27}H_{24}O_5$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectral data of **5** (Table 1) were very similar to those of compound **4**, except for differences in the NOE spectrum. There were NOE correlations between 3-OMe/H-6, H-2b, and H-3b, but no NOE correlation was observed between 3-OMe and H-5a and H-6a (Fig. 2), indicating that the configuration of C_2–C_6 was *Z*-form. Thus, compound **5** was a geometrical isomer of compound **4** at C-6. Compound **2** was reported as a ravenelone,[8] but only in structure. So, we wish to report here the full characterization of compound **2**.

The relative configurations of **4** and **5** were elucidated as shown in Fig. 3, by a coupling constant and CD spectrum that showed no typical Cotton effect because the dihedral

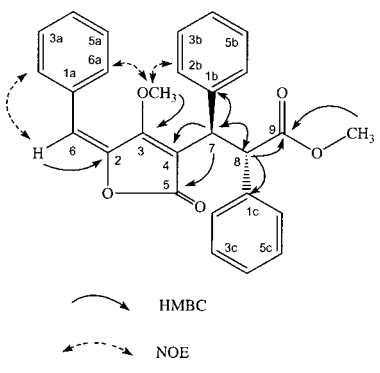

Fig. 1. The Important HMBC and NOE Correlations of **4**

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **4** and **5**

Position	Compound **4**		Compound **5**	
	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)
2		143.2		142.4
3		163.2		163.8
4		139.2		139.5
5		168.4		169.4
6	6.69 s	114.9	6.19 s	107.9
7	4.70 d (12.1)	44.7	4.83 d (11.8)	44.8
8	5.01 d (12.1)	53.0	5.09 d (11.8)	53.0
9		173.7		174.0
3-OMe	3.81 s	60.5	4.42 s	60.4
9-OMe	3.64 s	52.3	3.63 s	52.3
1a		136.7		136.8
2a, 6a	7.44 dd (0.8, 7.4)	129.9	7.72 d (7.4)	130.4
3a, 5a	7.35 t (7.1)	128.2	7.37 t (7.4)	128.7
4a	7.33 t (7.1)	128.7	7.30 t (7.4)	128.8
1b		114.7		106.5
2b, 6b	7.21 d (7.1)	128.6	7.16 d (7.7)	128.4
3b, 5b	7.11 t (7.1)	128.4	7.11 d (7.7)	128.4
4b	7.07 t (7.1)	126.9	7.14 d (7.7)	127.4
1c		114.7		106.5
2c, 6c	7.22 d (7.1)	128.5	7.22 d (7.1)	128.6
3c, 5c	7.15 t (7.1)	128.4	7.11 t (7.1)	128.4
4c	7.10 t (7.1)	127.4	7.07 d (7.1)	126.9

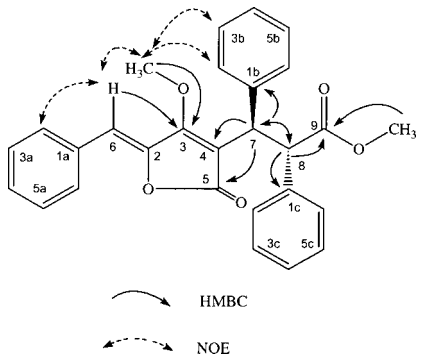


Fig. 2. The Important HMBC and NOE Correlations of **5**

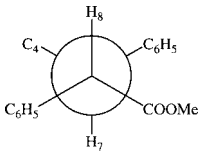
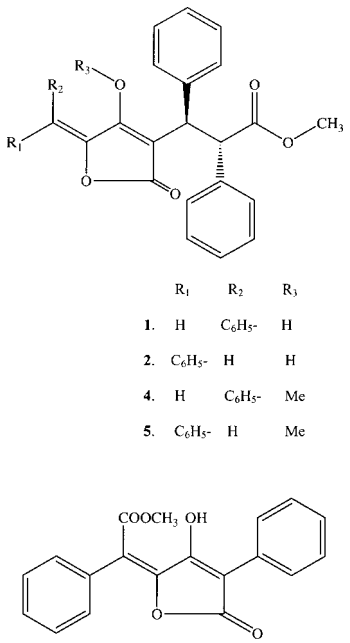


Fig. 3. The Conformations of Compounds **4** and **5**

angle of the two benzene rings was 180°. However, their absolute configuration remains to be clarified.

Based on the above discussion, compounds **1** and **2** were determined to be 2-phenylmethylene-3-hydroxy-4-(2,3-diphenylpropionic acid methyl ester-3-yl)-5-oxo-2(2*H*)-furylidene, as shown in Chart 1.

Compound **3** was determined to be vulpinic acid by comparing its spectral data with literature values.[9] But the geometry of methoxyl carbonyl and phenyl groups has not been discussed, and remains to be clarified by NMR spectra. Thus, crystallographic analysis of vulpinic acid was carried out and its result was identical with that previously reported.[10]



	R_1	R_2	R_3
1.	H	C_6H_5-	H
2.	C_6H_5-	H	H
4.	H	C_6H_5-	Me
5.	C_6H_5-	H	Me

3

Chart 1

Experimental

UV spectra were obtained on a Shimadzu UV-1650PC in MeOH. IR spectra were recorded on a JASCO FT/IR-5300 spectrophotometer. Specific optical rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as a solvent. CD spectra were measured on a JASCO J-725 spectrometer in MeOH. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR), using $CDCl_3$ as a solvent. Chemical shifts were valued using tetramethyl silane (TMS) (δ 0.00) as an internal standard (^{1}H-NMR), and δ 77.03 (ppm) from $CDCl_3$ as a standard (^{13}C-NMR). Mass spectra, including high-resolution mass spectra, were recorded on a JEOL JMS AX-500 spectrometer. X-ray reflection data were measured on a DIP image diffractometer using Mo $K\alpha$ radiation (λ= 0.71073 Å). Preparative HPLC was performed on a Shimadzu liquid chro-

matograph LC-10AS with RID-6A and SPD-10A detectors using a 5 SL-II column. Column chromatography was carried out on silica gel 60 (0.2—0.5 mm, 0.04—0.063 mm, Merck).

Fungal Material *Pulveroboletus ravenelii* was collected in Okayama, Japan in September 2001 and identified by Makiko Nukada (Faculty of Food Culture, Kurashiki Sakuyo University, Kurashiki 710-0290, Japan). The voucher specimen (No. KSU01092621) was deposited at the Faculty of Food Culture, Kurashiki Sakuyo University, Kurashiki 710-0290, Japan.

Extraction and Isolation Dried fruit bodies of *P. ravenelii* (100 g) were extracted with MeOH. The filtrate was concentrated under reduced pressure to give a residue (34.4 g) which was partitioned between EtOAc and water. The EtOAc layer (5.8 g) was evaporated and crystallized from MeOH to give pulvinic acid (1.378 g), and the mother liquid (622 mg) was further subjected to SiO_2 column chromatography using $CHCl_3$: MeOH (9 : 1) to give a mixture (41.4 mg), followed by methylation with $(CH_3)_3SiCHN_2$ [1 ml (10% in hexane, commercially available Nacalai Tesque, Inc., Kyoto, Japan)] and MeOH (1 ml) for 3 h. The reaction mixture after evaporation of the solvent was purified by prep. HPLC using hexane : EtOAc (2 : 1) to afford compound **4** (2.07 mg) and compound **5** (3.98 mg).

Compound **4**: White powder, $[\alpha]_D^{20}$ −75.6° (c=0.45, $CHCl_3$). IR (KBr) cm^{-1}: 2927, 1753, 1610, 1495, 1454, 1167. UV λ_{max} (MeOH) nm (log ε): 227 (3.7), 321 (4.0). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see Table 1. HR-FAB-MS: m/z 441.2625 $[M+H]^+$ (Calcd for $C_{27}H_{37}O_5$: 441.2641).

Compound **5**: White powder, $[\alpha]_D^{20}$ −267.6° (c=0.78, $CHCl_3$). IR (KBr) cm^{-1}: 2951, 1754, 1613, 1495, 1454, 1168. UV λ_{max} (MeOH) (log ε): 220 (4.2), 324 (4.5). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see Table 1. HR-FAB-MS: m/z 441.1712 $[M+H]^+$ (Calcd for $C_{27}H_{37}O_5$: 441.2641).

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