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NOTE

Russulfoen, a new cytotoxic marasmane sesquiterpene from *Russula foetens*

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The genus *Russula* is one of the largest in *Agaricales* and contains more than 100 species in forests worldwide. The family *Russulaceae* (subdivision *Basidiomycotina*) are important symbionts, forming mycorrhiza with higher plants and trees. The fungal subdivision *Basidiomycotina* produces many toxic sesquiterpenes derived from the protoilludane skeleton, which is transformed and rearranged to various sesquiterpenes, including marasmane sesquiterpenes,[1] which were discovered more than 50 years ago[2] and have antibacterial, antifungal, cytotoxic and phytotoxic activities.[3,4] Although a number of *Lactarius* species have been studied with regard to their secondary metabolites, marasmane sesquiterpenes, particularly,[1] the *Russula* mushrooms have been rarely investigated, despite the large number of species. Therefore, as a part of a systematic study of Korean poisonous mushrooms,[5,6] we investigated the constituents of the fruiting bodies of the mushroom *R. foetens*, widely distributed throughout Korea and other East Asian countries. This is a poisonous mushroom that contains gastrointestinal irritants and several marasmane sesquiterpenes.[7,8] Column chromatographic separation of its MeOH extract resulted in the isolation of a new marasmane sesquiterpene lactone named russulfoen (**1**), together with two known marasmane sesquiterpene lactones, 7α,8α,13-trihydroxy-marasm-5-oic acid γ-lactone (**2**)[9] and 8α,13-dihydroxy-marasm-5-oic acid γ-lactone (**3**),[8] one known ergosterol, (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**4**),[10] as well as (1*R*,2*R*)-1-phenylpropane-1, 2-diol (**5**)[11] that was isolated from the natural source for the first time (Figure 1). Their structures were elucidated on the basis of spectroscopic studies, including 2D-NMR experiments. In this study, we describe the isolation and structural elucidation of **1** and the cytotoxic activities of compounds **1**–**5**.

The air-dried and powdered fruiting bodies of *R. foetens* (7.2 g) were extracted with 80% MeOH two times at room temperature and filtered. The filtrate was evaporated under vacuum to afford a methanolic extract (1.3 g), which was suspended in H_2O, and then partitioned with *n*-hexane, $CHCl_3$ and *n*-BuOH, successively, yielding *n*-hexane (90 mg), $CHCl_3$ (120 mg) and *n*-BuOH fractions (150 mg). Each fraction was evaluated for cytotoxicity against A549, SK-OV-3, SK-MEL-2 and HCT-15 cell lines using the sulforhodamine B assay. It was found that the *n*-hexane and $CHCl_3$ soluble fractions were of moderate cytotoxicity against the tumor cell lines. The *n*-hexane soluble fraction (90 mg) was separated on a Sephadex LH-20 (Pharmacia, Uppsala, Sweden) column with CH_2Cl_2-MeOH (1:1) as the eluent to give three fractions (H1–H3). Fraction H2 (12 mg) was further purified by normal-phase preparative HPLC using a solvent of *n*-hexane-EtOAc (1:1) at a flow rate of 2.0 ml min^{-1} (Apollo Silica 5 μm column (Alltech, Nicholasville, KY, USA), 250×10 mm) to give compound **4** (5 mg). The $CHCl_3$ soluble fraction (120 mg) was separated on a Sephadex LH-20 column with CH_2Cl_2-MeOH (1:1) as the eluent to give two fractions (C1–C2). Fraction C2 (25 mg) was further purified by RP-C_{18} preparative HPLC (Econosil RP-18 10 μ column (Alltech), 250×10 mm; 60% MeOH) to afford compounds **2** (3 mg) and **5** (3 mg). The *n*-BuOH soluble fraction (150 mg) showing weak cytotoxicity was also separated on a Sephadex LH-20 column with CH_2Cl_2-MeOH (1:1) as the eluent to give four fractions (B1–B4). Fraction B3 (30 mg) was further purified by RP-C_{18} preparative HPLC (Econosil RP-18 10 μ column, 250×10 mm; 60% MeOH) to give compounds **1** (4 mg) and **3** (3 mg).

Compound **1** was obtained as a colorless oil. Its molecular formula was determined to be $C_{15}H_{22}O_4$ from the $[M+H]^+$ peak at *m/z* 267.1590 (calcd. for $C_{15}H_{23}O_4$, 267.1596) in the positive-ion high-resolution FAB-MS spectrum. The IR spectrum of **1** showed a broad hydroxyl band at 3388 cm^{-1} and a γ-lactone carbonyl absorption at 1751 cm^{-1}. Its UV spectrum revealed end absorption λ_{max} 217 nm with low extinction. The physico-chemical properties of **1** are summarized in Supplementary Information. The ^{1}H- and ^{13}C-NMR spectral data of **1** are shown in Table 1.

The ^{1}H-NMR spectrum (Table 1) of **1** displayed signals for the presence of two methyl groups at δ_H 1.28 and 1.05 (each 3H, s), two oxygenated methylene groups at δ_H 4.45 (1H, t, J=10.0 Hz), 4.08 (1H, dd,

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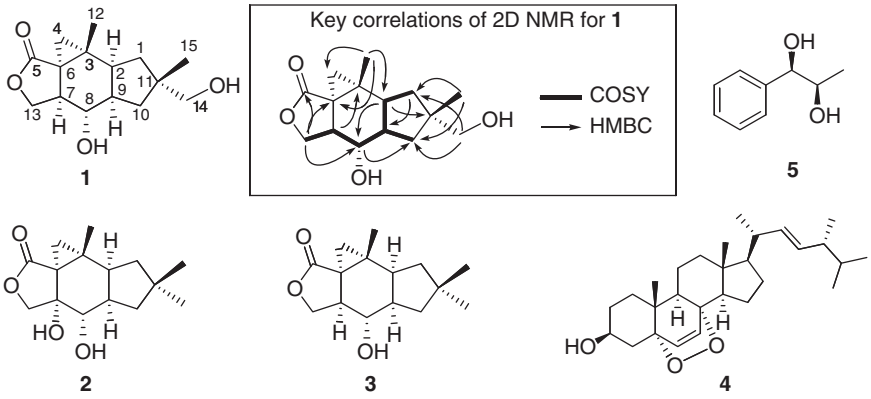

Figure 1 Structures of compounds **1**–**5**.

Table 1 ^{1}H- and ^{13}C-NMR data of 1 and 3

	1			*3*	
Position	δ_C	δ_H	*HMBC*	δ_C	δ_H
1α	37.5	1.62 (1H, dd, 14.0, 6.0)	C-3, 9, 10, 14, 15	41.8	1.68 (1H, dd, 14.0, 6.8)
1β		1.31 (1H, dd, 14.0, 12.0)			1.47 (1H, dd, 14.0, 13.4)
2	44.8	2.62 (1H, m)	C-4, 6, 8, 10, 11, 12	45.2	2.63 (1H, 13.4, 6.8, 6.7)
3	27.8			28.4	
4α	29.2	1.28 (1H, d, 4.5)	C-2, 5, 7, 12	29.0	1.36 (1H, d, 4.2)
4β		0.90 (1H, d, 4.5)			0.96 (1H, d, 4.2)
5	177.7			177.9	
6	29.5			29.5	
7	43.0	2.33 (1H, ddd, 10.0, 9.5, 7.0)	C-3, 4, 5, 9	43.8	2.40 (1H, ddd, 9.9, 9.3, 7.3)
8	73.3	3.25 (1H, dd, 11.0, 9.5)	C-2, 6, 10, 13	73.6	3.27 (1H, dd, 10.2, 9.9)
9	44.1	1.51 (1H, m)	C-1, 3, 7, 11	44.8	1.59 (1H, m)
10α	38.1	1.64 (1H, dd, 14.0, 2.0)	C-1, 2, 8, 14, 15	44.7	1.66 (1H, dd, 14.3, 1.0)
10β		1.55 (1H, dd, 14.0, 7.0)			1.58 (1H, dd, 14.3, 7.3)
11	40.2			37.0	
12	16.9	1.28 (3H, s)	C-2, 4, 6	17.4	1.29 (3H, s)
13α	71.5	4.45 (1H, t, 10.0)	C-5, 6, 8	71.5	4.71 (1H, t, 9.3)
13β		4.08 (1H, dd, 10.0, 7.0)			4.18 (1H, dd, 9.3, 7.3)
14α	70.8	3.46 (1H, d, 12.0)	C-1, 10, 15	31.8	1.26 (3H, s)
14β		3.43 (1H, d, 12.0)			
15	26.0	1.05 (3H, s)	C-1, 10, 14	32.4	1.05 (3H, s)

NMR data were obtained in 500 MHz for ^{1}H and 125 MHz for ^{13}C in CD_3OD, and values in parentheses are coupling constants in Hz.

J=10.0, 7.0 Hz), and 3.46 (1H, d, *J*=12.0 Hz), 3.43 (1H, d, *J*=12.0 Hz), and an oxygenated methine group at δ_H 3.25 (1H, dd, *J*=11.0, 9.5 Hz). Analysis of ^{1}H- and ^{13}C-NMR spectra together with HSQC indicated that 15 carbon signals were composed of a carbonyl carbon, two methyl carbons, five methylene carbons (including two oxygenated methylene carbons), four methine carbons (including one oxygenated methine carbon) and three quaternary carbons. The presence of the signals of H-4 at δ_H 1.28 (1H, d, *J*=4.5 Hz, H-4α) and 0.90 (1H, d, *J*=4.5 Hz, H-4β) together with 15 carbon signals of **1** allowed us to assign the marasmane skeleton to compound **1**, because the ^{1}H-NMR spectrum of marasmane sesquiterpene shows two H-4 protons as a doublet with a characteristic coupling constant of *J*=~5 Hz.[8,9] The ^{1}H- and ^{13}C-NMR resonances of **1** were similar to those of **3**,[8] except for the presence of additional signals (δ_H 3.46 and 3.43) assignable to the oxygenated methylene group (Table 1). The signals assignable to C-1 (δ_C 37.5), C-14 (δ_C 70.8) and C-15 (δ_C 26.0) were present in ^{13}C-NMR spectrum of **1** instead of the corresponding signals for C-1 (δ_C 41.8), C-14 (δ_C 31.8) and C-15 (δ_C 32.4) of **3**, which supported the presence of the oxygenated methylene group of C-14. The structure of **1** was confirmed by analysis of the ^{1}H-^{1}H COSY, HSQC and HMBC spectroscopic data (Table 1, Figure 1).

The coupling constant (*J*=11.0 Hz) for H-8 and H-9 suggested that they exist as *trans* form.[9] The absolute configurations of **1** were established on the basis of the convenient Mosher ester procedure in combination with NOESY experiment.[12] Esterification of **1** yielded diastereoisomeric α-methoxy-α-trifluoromethylphenylacetate (MTPA) diesters, bis[(*S*)-MTPA] ester (**1s**) and bis[(*R*)-MTPA] ester (**1r**). Diagnostic ^{1}H-NMR chemical shift differences between the MTPA esters of **1** [$\Delta\delta=\delta_S-\delta_R$] (Figure 2) revealed the absolute configuration at C-8 to be *S*. This suggested that H-8 has the β-orientation. The cross peaks between H-8 and H_3-12, between H-8 and H_3-15, between H-7 and H-9, between H-4 and H-7, between H-2 and H-9, between H-2 and

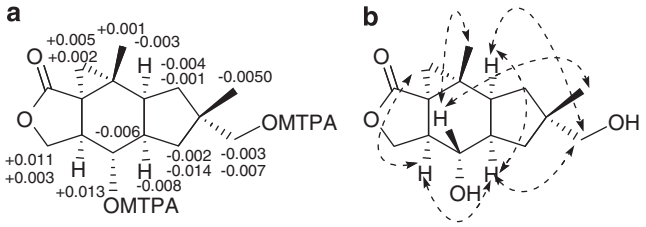

Figure 2 $\Delta\delta$ Values (δ_S–δ_R) in p.p.m. of the two α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters derived from **1** (**a**) and Key NOESY correlations of **1** (**b**).

Table 2 Cytotoxic activities of compounds (1–5) isolated from *R. foetens*

	IC_{50} (μM)			
Compound	*A549*	*SK-OV-3*	*SK-MEL-2*	*HCT-15*
1	23.0	28.2	15.4	>30.0
2	25.7	>30.0	>30.0	20.9
3	29.4	28.7	22.5	>30.0
4	19.5	18.4	17.8	19.9
5	>30.0	>30.0	23.5	>30.0
Doxorubicin	0.021	0.003	0.012	0.038

IC_{50} value of compounds against each cancer cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*.

H-14, and between H-9 and H-14 in the NOESY spectrum suggested that the cyclopropane ring at C-3/6 takes the α-orientation and the oxygenated methylene group of C-14 also takes the α-orientation (Figure 2). Thus, the structure of **1** was established as 8α,13,14-trihydroxymarasm-5-oic acid γ-lactone and the compound was named russulfoen.

Cytotoxic activities of the isolated compounds (**1–5**) were evaluated against the A549, SK-OV-3, SK-MEL-2 and HCT-15 human tumor cell lines *in vitro* using the sulforhodamine B assay.[13] The marasmane sesquiterpenes (**1–3**) exhibited moderate cytotoxicity against the A549, SK-OV-3, SK-MEL-2 and HCT-15 cell lines (Table 2). Compound **4** also exhibited moderate cytotoxicity against the four human tumor cell lines.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)