J. Antibiot. 59(10): 669–672, 2006

NOTE

THE JOURNAL OF ANTIBIOTICS

Marasmane Sesquiterpenes Isolated from *Russula foetens*

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Received: June 1, 2006 / Accepted: October 10, 2006

Abstract One lactone marasmane sesquiterpene named 8α,13-dihydroxy-marasm-5-oic acid γ-lactone (**1**) and one unsaturated marasmane sesquiterpene named 13-hydroxy-marasm-7(8)-en-5-methoxy γ-acetal (**2**) together with a known compound: 7α,8α,13-trihydroxy-marasm-5-oic acid γ-lactone (**3**) were isolated from the fruiting bodies of *Russula foetens*. Their structures were established on the basis of spectral methods (MS, IR, 1D and 2D NMR experiments).

Keywords *Russula foetens*, marasmane sesquiterpenes, structure determination

Members of the genus *Russula* are important symbionts, forming mycorrhiza with higher plants which explains in some cases their preference for growing among certain kinds of trees. The genus is one of the largest in Agaricales and is distributed worldwide; more than 100 species are reported to grow in China where mixed forests are their typical habitat. While secondary metabolites occurring in the fruiting bodies of *Lactarius* species have been well investigated, the *Russula* mushrooms have received less attention, notwithstanding the large number of existing species [1]. The fungal subdivision Basidiomycotina produces many toxic sesquiterpenes derived from the protoilludane skeleton. This skeleton is transformed and rearranged to a multitude of compounds. Fungal sesquiterpenes formed *via* the humulane-protoilludane biosynthetic pathway are characteristic for the subdivision Basidiomycotina [2]. Sesquiterpenes possessing the marasmane skeleton have been known for more than 50 years [3]. The marasmane sesquiterpenes often have antibiotic activities [4~8].

From the higher fungus *Russula foetens* we had isolated two marasmane sesquiterpenes: lactapiperanol A [9] and lactapiperanol E [10]. In this paper, we will report the isolation of another three compounds (**1**~**3**), of which **1** and **2** are new.

The fruiting bodies of *R. foetens* were collected at Ailao Mountain of Yunnan Province, China, in July, 2004 and identified by Prof. Mu Zang, Kunming Institute of Botany, the Chinese Academy of Sciences. The voucher specimen was deposited at the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences. The air-dried fruiting bodies of *R. foetens* (1 kg) were crushed and extracted with $CHCl_3$/MeOH (1/1, v/v) three times at room temperature. The combined extracts were concentrated *in vacuo* to give a syrup (70 g) which was chromatographed on a silica gel (1500 g) column, using a gradient elution with $CHCl_3$/MeOH (100 : 0 to 0 : 100, v/v) to afford six fractions. Fr. 2 ($CHCl_3$/MeOH, 95 : 5) was subjected to silica gel chromatography using a gradient elution with $CHCl_3$/acetone (100 : 0 to 50 : 50) and four fractions were obtained. Further separation of Fr. 2.1 ($CHCl_3$/acetone, 98 : 2) over silica gel with petroleum ether/acetone (100 : 1) afforded compound **2**. Fr. 2.4 ($CHCl_3$/acetone, 50 : 50) was purified over silica gel with $CHCl_3$/acetone (50 : 50) repeatedly to give compound **3**. Fr. 3 ($CHCl_3$/MeOH, 90 : 10) was subjected to Sephadex LH-20 to get three fractions. Fraction 3.2 was separated by preparative thin

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Table 1 The ^{1}H, ^{13}C-NMR assignments, HMBC and NOESY correlations of compound **1**

Position	δ_H	HMBC	NOESY	δ_C
1a	1.68 (dd, *J*=14.0, 6.8)			41.8 (t)
1b	1.47 (dd, *J*=14.0, 13.4)			
2	2.63(ddd, *J*=13.4, 6.8, 6.7)	C-8	H_3-14	45.2 (d)
3	/			28.4 (s)
4a	1.36 (d, *J*=4.2)			29.0 (t)
4b	0.96 (d, *J*=4.2)		H-7	
5	/			177.9 (s)
6	/			29.5 (s)
7	2.40 (ddd, *J*= 9.3, 9.2, 9.2)		H-4b	43.8 (d)
8	3.27 (dd, *J*=9.9, 10.2)	C-13, C-10	H_3-15, H-13a	73.6 (d)
9	1.59 (m)			44.8 (d)
10a	1.66 (dd, *J*=14.3, 1.0)			44.7 (t)
10b	1.58 (dd, *J*=14.3, 7.3)			
11	/			37.0 (s)
12	1.29 (s)	C-2, C-4, C-6		17.4 (q)
13a	4.18 (dd, *J*=7.3, 9.3)		H-8	71.5 (t)
13b	4.71 (t, *J*=9.3)	C-6		
14	1.26 (s)	C-1, C-10	H-2	31.8 (q)
15	1.05 (s)	C-1, C-10	H-8	32.4 (q)

layer chromatography with $CHCl_3$/MeOH (100 : 5) to afford compound **1** with strong fluorescence at 365 nm.

Compound **1** was isolated as oil. The molecular formula of compound **1** was determined to be $C_{15}H_{22}O_3$ on the basis of HR-EI-MS [*m*/*z* 250.1574 (Calcd. 250.1569)]. The IR spectrum of **1** showed a broad hydroxyl band at 3423 cm^{-1} and a lactonic carbonyl absorption at 1758 cm^{-1}. These features were confirmed by ^{13}C-NMR which exhibited signals for a C atom substituted with hydroxyl oxygen atom (δ 73.6, C-8, d) and a quaternary carbon (δ 177.9, C-5, s). Three methyl groups (δ 1.05, 1.26 and 1.29 respectively, each 3H, s) were shown in the ^{1}H-NMR spectrum of **1**. The molecular formula $C_{15}H_{22}O_3$ suggested **1** had five degrees of unsaturation. Each of the two H-4 protons showed a doublet in the ^{1}H-NMR spectrum with a characteristic coupling constant of *J*= about 5 Hz (δ 1.36, H-4a, d, *J*=4.2 Hz; δ 0.96, H-4b, d, *J*=4.2 Hz) allowed us to assign the marasmane skeleton, but not lactarane skeleton, to compound **1**. The ^{1}H-^{1}H COSY spectrum of **1** exhibited the connections between H-8 and H-9, H-9 and H-2, H-8 and H-7, H-7 and H-13_{ab} clearly. The HMBC spectrum shown in Table 1 gave the structure of **1**.

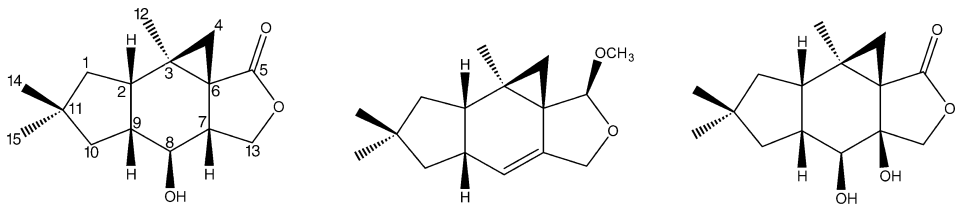
The NOESY cross peaks were observed between H-8 and H_3-15, H-8 and H-13a, H-7 and H-4b, H-2 and H_3-14. The coupling constant (d, *J*=10.2 Hz) for H-8 and H-9 suggested the hydroxyl group at C-8 had *trans* configuration. According to all the information, we could draw the stereo structure of compound **1**.

Compound **2** was isolated from the very small polar fraction. Its HR-EI-MS with the molecular ion peak at *m*/*z* 248.1769 (calcd. 248.1776) was consistent with the molecular formula $C_{16}H_{24}O_2$ which suggested a sesquiterpene skeleton with 5 degrees of unsaturation. The IR spectrum of **2** showed no hydroxyl group existed. From the ^{13}C-NMR and ^{1}H-NMR, we can see signals of one methoxy group (δ_C 54.5, δ_H 3.34, 3H), three methyl groups (δ_C 32.0, δ_H 0.99, 3H; δ_C 31.9, δ_H 1.01, 3H; δ_C 21.3, δ_H 1.20, 3H), one oxygenated methylene (δ_C 69.0, δ_H 4.55, 2H), one methylene with two doublets in ^{1}H-NMR (δ_C 26.1, δ_{Ha} 0.77 and δ_{Hb} 0.93, 2H), two methylenes (δ_C 44.2, δ_{Ha} 1.51 and δ_{Hb} 1.31, 2H; δ_C 48.2, δ_{Ha} 1.78 and δ_{Hb} 1.33, 2H), one acetal group (δ_C 109.0, δ_H 4.80, 1H), one unsaturated methine (δ_C 115.2, δ_H 4.84, 1H), two methines (δ_C 42.4, δ_H 2.40, 1H; δ_C 39.1, δ_H 2.45, 1H), one unsaturated quaternary carbon (δ_C 139.1). The above spectral data suggested compound **2** was also a marasmane sesquiterpenoid with a methoxy group. The C–H long-range correlations observed in HMBC spectrum (Table 2) confirmed the structure of compound **2**. Its stereo structure was determined by NOESY spectrum (Table 2).

The spectral data and physical properties of 7α,8α,13-trihydroxy-marasm-5-oic-acid γ-lactone (**3**) were identical

Table 2 ^{1}H, ^{13}C-NMR data, HMBC and NOESY correlations of compound **2**

Position	δ_H	HMBC	NOESY	δ_C
1a	1.51 (m)			44.2 (t)
1b	1.31 (dd, *J*=5.2, 12.1)			
2	2.40 (m)	C-8		42.4 (d)
3	/			34.5 (s)
4a	0.77 (d, *J*=3.7)			26.1 (t)
4b	0.93 (d, *J*=3.7)			
5	4.80 (s)		H_3-12	109.0 (d)
6	/			24.9 (s)
7	/			139.1 (s)
8	4.84 (m)	C-10, C-13		115.2 (d)
9	2.45 (m)			39.1 (d)
10a	1.78 (dd, *J*=13.3, 6.5)			48.2 (t)
10b	1.33 (dd, *J*=13.3, 10.3)			
11	/			37.4 (s)
12	1.20 (s)	C-2, C-4, C-6	H-5	21.3 (q)
13a	4.55 (m)			69.0 (t)
13b				
14	1.01 (s)	C-1, C-10		31.9 (q)
15	0.99 (s)	C-1, C-10		32.0 (q)
OCH_3	3.34 (s)	C-5		54.5 (q)



1 **2** **3**

Fig. 1 Structures of the three marasmane compounds isolated from *Russula foetens*.

with the data previously reported [11].

8α,13-dihydroxy-marasm-5-oic acid γ-lactone (**1**). $[\alpha]_D^{25}=+42.2$ (*c* 0.005, CH_2Cl_2). EI-MS *m/z* (rel. int.): 250 ($[M]^+$, 15), 232 ($[M-H_2O]^+$, 17), 217 $[M-H_2O-CH_3]^+$, (40), 135 (100). IR (KBr): ν=3423, 2925, 2867, 1758. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 M, $CDCl_3$): Table 1.

13-Hydroxy-marasm-7(8)-en-5-methoxy γ-acetal (**2**). $[\alpha]_D^{25}=+50.1$ (*c* 0.003, CH_2Cl_2). EI-MS *m/z* (rel. int.): 248 (15), 233 (41), 216 (100), 201 (18), 188 (17), 145 (20), 133 (22). ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 2.

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