A New Marasmane Sesquiterpene from the Basidiomycete *Russula foetens*

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A new marasmane sesquiterpene, named lactapiperanol E (**1**), was isolated from the fruiting bodies of *Russula foetens* together with a known sesquiterpene lactapiperanol A (**2**). Their structures were established on the basis of spectral methods (MS, IR, 1D and 2D NMR experiments).

Key words: Russula foetens, Lactapiperanol E, Marasmane Sesquiterpene

Introduction

Large fungi of the genus *Russula* belong to subdivision Basidiomycotina, order Agaricales, family Russulaceae. They 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 produce 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 are known for more than 50 years [3]. Marasmic acid was found as an antibacterial substance in *Marasmius conigenus* [4], and its 9-hydroxy derivative, detected in another basidiomycete, displayed antifungal, cytotoxic and phytotoxic activity [5]. Velutinal [6] and its fatty acid esters [7] represent interesting examples of prodrugs. In most fungi only the esters are present which are cleaved to velutinal in case of injuries at the fruiting bodies [8]. The genus *Lactarius* contains marasmane sesquiterpenes [2]. Pilatin is an antibiotically active marasmane derivative from the culture of *Flagelloscypha pilatii*. It is a higher oxidized derivative of marasmic acid, causes frameshift mutations in *Salmonella typhimurium*, inhibits the growth of bacteria and fungi and is highly cytotoxic [9].

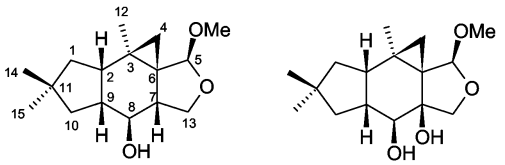
As part of our continuing research of bioactive metabolites from *Lactarius* and *Russula* sp. in Yunnan Province [10 – 16], the chemical constituents of *R. foetens* (Pers.) Pers. were investigated. This report describes the structure elucidation of two marasmane sesquiterpenes including the new compound lactapiperanol E (**1**) from this mushroom.

Results and Discussion

Lactapiperanol E (**1**) was obtained as an oil, $[\alpha]_D = +24.5$ (*c* 0.03, $CHCl_3$). Based on HRESI-MS ([M-OMe]$^+$ *m/z* 235.1724, calcd. for 235.1693) and NMR spectra, the molecular formula was determined to be $C_{16}H_{26}O_3$, which suggested a sesquiterpene skeleton with 4 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl groups at 3419 cm^{-1}. The ^{1}H and ^{13}C NMR spectra (Table 1) of **1** showed signals due to an acetal group [$\delta_H = 4.57$ (s, H-5); $\delta_C = 105.9$ (s, C-5)], an oxygenated methine [$\delta_H = 3.12$ (dd, 11.7, 7.9, H-8); $\delta_C = 72.5$ (d, C-8)], an oxygenated methylene [$\delta_H = 4.21$ (dd, 9.0, 7.8, H-13a), 3.99 (dd, 9.0, 1.9, H-13b); $\delta_C = 69.9$ (t, C-13)], a methoxy group [$\delta_H = 3.31$ (s); $\delta_C = 54.6$ (q)], three tertiary methyl groups [$\delta_H = 1.02$ (s, H-12), 0.99 (s, H-14), 1.08 (s, H-15); $\delta_C = 21.6$ (q, C-12), 31.5 (q, C-14), 32.1 (q, C-15)], a cyclopropane ring [$\delta_H = 0.66$ (d, 4.7, H-4a), 0.64 (d, 4.7, H-4b); $\delta_C = 21.9$ (t, C-4)], two methylenes [$\delta_H = 1.56$ (dd, 12.9, 6.7, H-1a), 1.42 (t, 12.9, H-1b),

Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) data of **1** and **2**.

Pos.	**1**		**2**	
1	1.56 (dd, 12.9, 6.7) 1.42 (t, 12.9)	45.0 (t)	1.58 (dd, 13.0, 6.7) 1.44 (t, 13.0)	45.1 (t)
2	2.54 (ddd, 13.3, 6.7, 6.6)	44.7 (d)	2.54 (ddd, 13.4, 6.7, 6.6)	45.2 (d)
3	–	20.6 (s)	–	22.3 (s)
4	0.66 (d, 4.7) 0.64 (d, 4.7)	21.9 (t)	0.85 (d, 5.0) 0.55 (d, 5.0)	17.7 (t)
5	4.57 (s)	105.9 (d)	4.63 (s)	106.4 (d)
6	–	36.3 (s)	–	41.9 (s)
7	1.91 (brt, 7.9)	49.2 (d)	–	77.6 (s)
8	3.12 (dd, 11.7, 7.9)	72.5 (d)	3.18 (d, 11.7)	73.2 (d)
9	1.51 (m)	44.1 (d)	1.77 (m)	38.7 (d)
10	1.66 (brd, 11.9) 1.52 (m)	42.2 (t)	1.74 (brd, 13.7) 1.54 (dd, 13.7, 8.2)	42.2 (t)
11	–	37.0 (s)	–	37.0 (s)
12	1.02 (s)	21.6 (q)	1.06 (s)	21.2 (q)
13	4.21 (dd, 9.0, 7.8) 3.99 (dd, 9.0, 1.9)	69.9 (t)	4.26 (d, 9.5) 3.94 (d, 9.5)	77.6 (t)
14	0.99 (s)	31.5 (q)	1.01 (s)	31.8 (q)
15	1.08 (s)	32.1 (q)	1.11 (s)	32.2 (q)
OCH_3	3.31 (s)	54.4 (q)	3.35 (s)	54.6 (q)



1 **2**

Fig. 1. Structures of **1** and **2**.

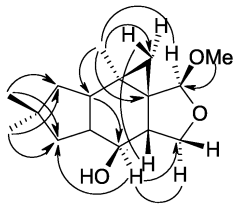


Fig. 2. Key HMBC and NOE correlations for **1**.

1.66 (brd, 11.9, H-10a), 1.52 (m, H-10b); $\delta_C = 45.0$ (t, C-1), 42.2 (t, C-10)], and three methines [$\delta_H = 2.54$ (ddd, 13.3, 6.7, 6.6, H-2), 1.91 (brt, 7.9, H-7), 1.51 (m, H-9); $\delta_C = 44.7$ (d, C-2), 49.2 (d, C-7), 44.1 (d, C-9)]. For there were no double bonds, the structure of a tetracyclic sesquiterpene can be suggested by taking four degrees of unsaturation into account. The NMR data were similar to those of the known compound **2** [17], which indicated that compound **1** was a marasmane derivative. The distinct difference between **1** and **2** is that: the quaternary carbon at C-7 of **2** [$\delta_C = 77.6$ (s, C-7)] is replaced by a methine carbon in **1** [$\delta_H = 1.91$ (brt, 7.9, H-7); $\delta_C = 49.2$ (d, C-7)]. In the light of the evidence mentioned above and key HMBC and NOE correlations (Fig. 2), the structure of **1** was therefore established as shown in Fig. 1 and the compound named lactapiperanol E.

Experimental Section

General

Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectrum was recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained with a Nexus 870 FT-IR with KBr pellets. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometers in $CDCl_3$ with TMS as an internal standard. EI-MS spectra were recorded with a VG Autospec-3000 spectrometer. HRESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer.

Silica gel (200 – 300 mesh, Qingdao Marine Chemical Inc., China) was 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 fruiting bodies of *R. foetens* were collected at the Ailao Mountains 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.

Extraction and isolation

The air-dried fruiting bodies of *R. foetens* (1 kg) were crushed and extracted with $CHCl_3$/MeOH (1/1, v/v) at room temperature. The combined extracts were concentrated *in vacuo* to give a syrup (70 g), which was then subjected to column chromatography on silica gel (1500 g, 200 – 300 mesh). Elution with $CHCl_3$/MeOH (100:0 – 0:100) afforded six fractions. Fr. 2 ($CHCl_3$/MeOH, 95:5) was subjected to silica gel chromatography with $CHCl_3$/acetone (100:0 – 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 (100:0 – 70:30) afforded compound **1**. Fr. 3 ($CHCl_3$/MeOH, 90:10) was subjected to silica gel chromatography with $CHCl_3$/MeOH (100:0 – 80:20), and four fractions were obtained. Further separation of Fr. 3.1 ($CHCl_3$/MeOH, 98:1) over silica gel with petroleum ether/acetone (100:0 – 70:30) gave compound **2**.

Lactapiperanol E (**1**): oil. – $[\alpha]_D$ +24.5 (c 0.03, $CHCl_3$). – IR (KBr): ν = 3419, 2950, 2929, 2868, 1740, 1453, 1381, 1365, 1192, 1134, 1089, 1044, 1012, 945, 756 cm^{-1}. – ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 1. – MS (EI, 70 eV): *m/z* (%) = 266 (3) $[M]^+$, 248 (5) $[M\text{-}H_2O]^+$, 235 (100) $[M\text{-}OCH_3]^+$, 217 (43), 199 (9), 189 (24), 173 (20).

Lactapiperanol A (**2**): colorless solid. – ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 1. – MS (EI, 70 eV): *m/z* (%) = 281 (3) $[M\text{-}1]^+$, 264 $[M\text{-}H_2O]^+$ (6), 251(77) $[M\text{-}OCH_3]^+$, 232 (100), 217 (46), 204 (50), 189 (67), 175 (39), 161 (52), 135 (88), 123 (84).

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