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PUBESCENONE, A NEW MARASMANE SESQUITERPENOID FROM THE MUSHROOM *LACTARIUS PUBESCENS*

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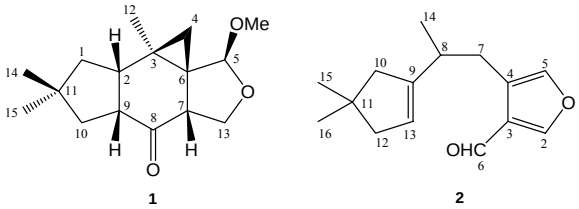
Abstract – A new marasmane sesquiterpenoid, named pubescenone (**1**), was isolated from the fruiting bodies of the basidiomycete *Lactarius pubescens* together with a known sesquiterpene aldehyde, lactaral (**2**). The structure of pubescenone (**1**) was elucidated on the basis of extensive spectral methods (MS, IR, 1D and 2D NMR experiments).

INTRODUCTION

Lactarius (Russulaceae, Basidiomycotina) is an important ecological and economic genus because of its ectomycorrhizal habit and edible in many regions of the world. The genus is also attractive because all species exude a clear or milky liquid (latex) of sesquiterpene origin when injured, possible functions for this exudate range from a storage medium of labile components to a role in a chemical defence system against parasites.[1,2] The sesquiterpenes found in *Lactarius* species are varying widely in both chemical structure and biological activity.[3] In pursuing our research on the biologically active novel metabolites of *Lactarius sp* in Yunnan Province of China,[4-8] we have studied the constituents of the fruiting bodies of *L. pubescens* which is an inedible mushroom growing in late summer in watered areas. Herein we reported the isolation and characterization of a new marasmane sesquiterpenoid, pubescenone (**1**), together with a known sesquiterpene aldehyde, lactaral (**2**) (Figure 1).



1 2

Figure 1: Structures of compounds **1** and **2**.

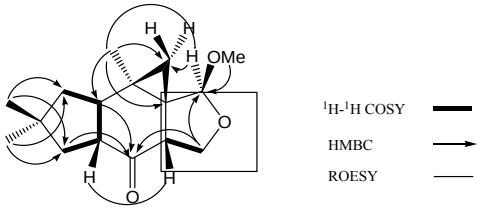


Figure 2: ^{1}H-^{1}H COSY, selected HMBC and ROESY correlations for **1**.

RESULTS AND DISCUSSION

Pubescenone (**1**) was isolated as a colorless oil, $[\alpha]_D^{26.3}$ + 108. The molecular formula was determined to be $C_{16}H_{24}O_3$ by a combination of HRESIMS and ^{13}C NMR data. IR spectral absorption at 1712 cm^{-1} indicated the presence of carbonyl functionality. The ^{1}H NMR spectrum (Table 1) displayed signals due

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

Position	δ_C	δ_H
C-1	45.5 (t)	1.57, 1H, dd, *J* = 12.4, 5.6, H-β; 0.95, 1H, dd, *J* = 13.2, 12.4, H-α
C-2	48.7 (d)	2.88, 1H, ddd, *J* = 13.2, 7.2, 5.6
C-3	20.9 (s)	-
C-4	20.7 (t)	1.16, 1H, d, *J* = 5.3, H-β; 0.89, 1H, d, *J* = 5.3, H-α
C-5	106.2 (d)	4.62, 1H, s
C-6	36.3 (s)	-
C-7	49.9 (d)	3.00, 1H, dd, *J* = 9.1, 5.0
C-8	209.7 (s)	-
C-9	49.1 (d)	2.55, 1H, dd, *J* = 7.6, 7.2
C-10	37.7 (t)	2.31, 1H, d, *J* = 13.5, H-α; 1.24, 1H, dd, *J* = 13.5, 7.6, H-β
C-11	37.5 (s)	-
C-12	21.0 (q)	1.11, 3H, s
C-13	66.4 (t)	4.36, 1H, dd, *J* = 8.9, 5.0, H-α; 4.15, 1H, dd, *J* = 9.1, 8.9, H-β
C-14	30.9 (q)	1.06, 3H, s
C-15	31.9 (q)	1.00, 3H, s
OMe	54.4 (q)	3.34, 3H, s

to three tertiary methyl groups (δ_H 1.11, s, H-12; 1.06, s, H-14; 1.00, s, H-15), a cyclopropane ring (δ_H 1.16, d, *J* = 5.3, H-4β; 0.89, d, *J* = 5.3, H-4α), two methylenes (δ_H 2.31, d, *J* = 13.5, H-10α; 1.24, dd, *J* = 13.5, 7.6, H-10β; 1.57, dd, *J* = 12.4, 5.6, H-1β; 0.95, dd, *J* = 13.2, 12.4 H-1α), three methines (δ_H 3.00, dd, *J* = 9.1, 5.0, H-7; 2.88, ddd, *J* = 13.2, 7.2, 5.6, H-2; 2.55, dd, *J* = 7.6, 7.2, H-9), a methoxy group (δ_H 3.34, s), an oxygenated methylene (δ_H 4.36, dd, *J* = 8.9, 5.0, H -13α; 4.15, dd, *J* = 9.1, 8.9 H -13β), and an acetal group (δ_H 4.62, s, H-5). The ^{13}C NMR spectrum analyzed together with the DEPT and HMQC NMR spectra revealed 16 carbon signals including four sp^3 methines (δ_C 106.2, C-5; 49.9, C-7; 49.1, C-9; 48.7, C-2), three quaternary sp^3 carbons (δ_C 37.5, C-11; 36.3, C-6; 20.9, C-3), four methylene carbons (δ_C 66.4, C-13; 45.5, C-1; 37.7, C-10; 20.7, C-4), three methyl carbons (δ_C 31.9, C-15; 30.9, C-14; 21.0, C-12), a methoxy carbon at δ_C 54.4, and a keto carbon at δ_C 209.7 (C-8). The above data suggested a marasmane sesquiterpenoid skeleton for **1**.[9] Comparison of its NMR spectral data with those of the known compound lactapiperanol E clearly indicated that **1** is a marasmane derivative,[10] the only notable difference between lactapiperanol E and **1** was that the sp^3 carbon at δ_C 72.5 in lactapiperanol E was replaced by the sp^2 carbon at δ_C 209.7 in compound **1**. The stereochemistry of H-2 in compound **1** was established as β on the basis of the significant correlations between H-2 and H-4β (δ_H 1.16, d, *J* = 5.3), and H-2 and H-9β (δ_H 2.55, dd, *J* = 7.6, 7.2) in ROESY experiments. Further, the structure of **1** was confirmed by combined ^{1}H-^{1}H COSY, key HMBC and ROESY correlations analysis (see Figure 2). Consequently, the structure of compound **1** was deduced to be as shown in Fig. 1, and named pubescenone.

Lactaral (**2**), a known sesquiterpene aldehyde, was also obtained from *L. pubescens*, and its physical and spectroscopic data are in good agreement with those reported in the literature.[11]

EXPERMENTAL

General Experimental Procedures

Optical rotation was measured on a Horiba SEPA-300 polarimeter. IR spectrum was obtained on a Bruker Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometers in $CDCl_3$ solvent, δ in ppm and *J* in Hz. EIMS was recorded with a VG Autospec-3000 spectrometer and HRESIMS was recorded with an 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 EtOH.

Mushroom Material

The fungus *L. pubescens* were collected at Ailao Mountains, Yunnan Province, China, in July, 2005 and identified by Prof. Mu Zang, Kunming Institute of Botany. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

Air-dried fruiting bodies of *L. pubescens* (1.5 kg) were exhaustively extracted with $CHCl_3$/MeOH (1:1, v/v, 2000 mL × 5) at rt. The combined extracts were filtered and concentrated in vacuo to afford a deep brown gum (105 g), which was suspended in water and extracted with EtOAc. The EtOAc extract (31 g) was then subjected on column chromatography (silica gel) using a petroleum ether/acetone gradient elution. Compound **1** (18 mg) was obtained from fraction 4 (petroleum ether/acetone, 90:10 v/v) by repeated column chromatography (silica gel) (petroleum ether/ EtOAc, 10:1 v/v). Compound **2** (24 mg) was obtained from fraction 1 (petroleum ether) by combined Sephadex LH-20 ($CHCl_3$/MeOH, 1:1 v/v) and column chromatography (silica gel) (petroleum ether).

Pubescenone (**1**): Colorless oil. $[\alpha]_D^{26.3}$ + 108 (c 0.54, $CHCl_3$). IR (KBr): 2952, 2926, 2866, 1712, 1453, 1635, 1309, 1192, 1161, 1039, 979, 939, 738 cm^{-1}. ^1H and ^{13}C NMR data see Table 1. HRESIMS: $[M+Na]^+$ 287.1617 (calcd. for $C_{16}H_{24}O_3Na$ 287.1623). EIMS: *m/z* 264 (7), 232 (84), 204 (82), 189 (54), 175 (29), 161 (36), 149 (47), 135 (33), 123 (62), 105 (64), 91 (66), 81 (100).

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