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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.<sup>1,2</sup> The sesquiterpenes found in *Lactarius* species are varying widely in both chemical structure and biological activity.<sup>3</sup> In pursuing our research on the biologically active novel metabolites of *Lactarius sp* in Yunnan Province of China,<sup>4-8</sup> 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).

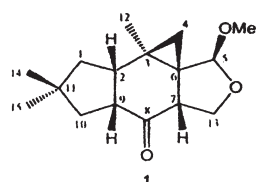
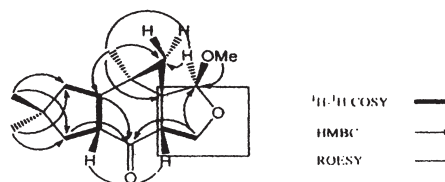
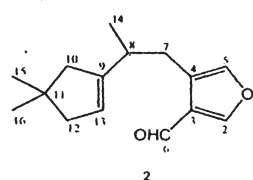


Figure 1: Structures of compounds 1 and 2.

Figure 2:  $^1\text{H}$ - $^1\text{H}$  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  $\text{C}_{16}\text{H}_{24}\text{O}_3$  by a combination of HRESIMS and  $^{13}\text{C}$  NMR data. IR spectral absorption at  $1712\text{ cm}^{-1}$  indicated the presence of carbonyl functionality. The  $^1\text{H}$  NMR spectrum (Table 1) displayed signals due

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR data ( $\text{CDCl}_3$ ) of pubescenone (**1**)

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

## EXPERIMENTAL

### 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  $\text{CDCl}_3$  solvent,  $\delta$  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%  $\text{H}_2\text{SO}_4$  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  $\text{CHCl}_3/\text{MeOH}$  (1:1, v/v, 2000 mL  $\times$  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 ( $\text{CHCl}_3/\text{MeOH}$ , 1:1 v/v) and column chromatography (silica gel) (petroleum ether).

**Pubescenone (1)**: Colorless oil.  $[\alpha]_D^{26.3} + 108$  (c 0.54,  $\text{CHCl}_3$ ). IR (KBr): 2952, 2926, 2866, 1712, 1453, 1635, 1309, 1192, 1161, 1039, 979, 939, 738  $\text{cm}^{-1}$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR data see Table 1. HRESIMS:  $[\text{M}+\text{Na}]^+$  287.1617 (calcd. for  $\text{C}_{16}\text{H}_{24}\text{O}_3\text{Na}$  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).

### ACKNOWLEDGMENTS

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### REFERENCES

1. S. Camazine and A. T. Lupo Jr, *Mycologia*, 1984, **76**, 355.
2. O. Sterner, R. Bergman, C. Franzén, and B. Wickberg, *Tetrahedron. Lett.*, 1985, **26**, 3163.
3. G. Vidari and P. Vita-Finzi, 'Chemistry and activity of sesquiterpenes from the genus *Lactarius*: Studies in natural product chemistry: structure and chemistry (Part D)', Vol. 17, ed. by A. Rahman, Elsevier, Amsterdam, 1995, pp. 153-195.
4. J. K. Liu, *Heterocycles*, 2002, **57**, 157.
5. L. Hu and J. K. Liu, *Z. Naturforsch.*, 2002, **57c**, 571.
6. D. Q. Luo, F. Wang, X. Y. Bian, and J. K. Liu, *J. Antibiot.*, 2005, **58**, 456.
7. X. L. Yang, D. Q. Luo, Z. J. Dong, and J. K. Liu, *Helv. Chim. Acta*, 2006, **89**, 988.
8. L. Z. Fang, H. J. Shao, W. Q. Yang, and J. K. Liu, *Helv. Chim. Acta*, 2006, **89**, 1463.
9. Y. Yaoita, K. Machida, and M. Kikuchi, *Chem. Pharm. Bull.*, 1999, **47**, 894; W. M. Daniewski, M. Gumulka, K. Ptaszynska, P. Skibicki, J. Krajewski, and P. Gluzinski, *Phytochemistry*, 1992, **31**, 913.

10. X. N. Wang, F. Wang, J. C. Du, H. M. Ge, R. X. Tan, and J. K. Liu, *Z. Naturforsch.*, 2005, **60b**, 1065.
11. G. Magnusson and S. Thorién, *Tetrahedron*, 1974, **30**, 1431.