

The First Humulene Type Sesquiterpene from *Lactarius hirtipes*

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Lactarius hirtipes, Humulene, 2 β , α -Epoxy-6Z, 9Z-Humuladien-8 α -ol

A new sesquiterpene of humulene type, named 2 β , α -epoxy-6Z, 9Z-humuladien-8 α -ol (**1**) together with a known compound lactarinic acid was isolated from the fruiting bodies of *Lactarius hirtipes*. Their structures were established on the basis of spectral evidence (MS, IR, NMR, HMBC, HMQC, ^1H - ^1H COSY, ROSEY experiments). Compound **1** was found as the first humulene-type sesquiterpene in higher fungi.

Introduction

The mushrooms belonging to the genus *Lactarius* (family Russulaceae, Basidiomycotina) form a milky juice when the fruiting bodies are injured. In the great majority of *Lactarius* species, different kinds of sesquiterpenes play an important biological role, being responsible for the pungency and bitterness of the milky juice, the change in the air of the color of the latex (De Bernardi *et al.*, 1993), and constituting a chemical defence system against various predators such as bacteria, fungi, animals, insects (Sterner *et al.*, 1985). Most of *Lactarius* sesquiterpenes belonging to the classes of lactaranes, secolactaranes, marasmanes, isolactaranes, norlactaranes, and caryophyllanes were believed to be biosynthesized from humulene (Vidari *et al.*, 1995; Bosetti *et al.*, 1989; Daniewski *et al.*, 1981; Ayer *et al.*, 1981.). For the subdivision Basidiomycotina, fungal sesquiterpenes formed via the humulane-protoilludane biosynthetic pathway are also characteristic (Abraham, 2001). However, no representative of humulene type of sesquiterpenes has ever been isolated so far from higher fungi. Only there are reports that α -humulene has been identified as volatile constituent of mushrooms (Raprior *et al.*, 1996). Continuing research on bioactive naturally occurring bioactive compounds of higher fungi in Yunnan Province, China (Liu, 2002), we have isolated lactarinic acid and 2 β , α -epoxy-6Z, 9Z-humuladien-8 α -ol (**1**), the first purified sesquiterpene with a humulene skeleton in higher fungi, from the entire fresh fruiting bodies of *Lactarius hirtipes* J. Z. Ying (Ying, 1991). The detailed struc-

ture and relative stereostructure of **1** were elucidated by spectroscopic means.

Results and Discussion

Compound **1** was obtained as white needles. M.p. 189–190.5 °C. $[\alpha]_{\text{D}} -21.3^\circ$. Based on high resolution EIMS ($[\text{M}]^+$: 236.1757, calc. 236.1776) and NMR data (Table I, II), its molecular formula was determined to be $\text{C}_{15}\text{H}_{24}\text{O}_2$, which indicated a sesquiterpene skeleton containing 4 degrees of unsaturation. The IR spectrum showed the absorption bands of hydroxyl group near 3296 cm^{-1} , gem-dimethyl group at 1387 , 1360 cm^{-1} , and double bonds at 1640 , 1610 cm^{-1} . The ^1H and ^{13}C NMR showed signals due to four tertiary methyl groups [δ_{H} , 1.02 (s, H₃-12), 1.16 (s, H₃-13, 15), 1.70 (s, H₃-14); δ_{C} , 30.5 (C-12), 16.1 (C-13), 23.0 (C-15), 12.9 (C-14)], one trisubstituted double bond [δ_{H} , 5.44 (m, H-6); δ_{C} , 124.9 (C-6), 143.1 (C-7)] and a disubstituted double bond [δ_{H} , 5.72 (dd, $J = 16.1$, 6.9 Hz, H-9), 5.37 (d, $J = 16.1$ Hz, H-10); δ_{C} , 132.1 (C-9), 139.3 (C-10)], an epoxide ring [δ_{H} , 2.56 (d, H-2); δ_{C} , 63.3 (C-2), 61.4 (C-3)], and no additional sp^2 or sp carbons. The structure was suggested to be bicyclic sesquiterpene by taking the degrees of unsaturation into account. With the aid of the DEPT spectrum (Table I), the remaining signals in the ^{13}C NMR spectrum of **1** could be attributed to three methylenes [δ_{H} , 1.59 (d, H-1 α , $J = 14.3$ Hz), 1.34 (dd, H-1 β , $J = 14.3$, 9.8 Hz), 2.02 (m, H-4 α), 1.16 (m, H-4 β), 2.46 (m, H-5 α), 2.08 (m, H-5 β); δ_{C} , 40.4 (C-1), 37.9 (C-4), 22.7 (C-5)], one methine bearing a hydroxyl group [δ_{H} , 4.69

Table I. ^{13}C NMR (125 MHz) Data (δ in ppm, for **1**) in CDCl_3 .

Position	δ_{C}	DEPT	HMBC (selected)
1	40.4	CH_2	H-2 α , 10, H ₃ -12 β , 13 α
2	63.3	CH	H-1 (α , β), 4 (α , β), H ₃ -15 β
3	61.4	C	H-1 (α , β), 2 α , 4 (α , β), 5 (α , β), H ₃ -15
4	37.9	CH_2	H-2 α , 5 (α , β), 6
5	22.7	CH_2	H-4 (α , β), 6
6	124.9	CH	H-4 (α , β), 5 (α , β), 8 β , H ₃ -14
7	143.1	C	H-8 β , 5 (α , β), H ₃ -14
8	78.1	CH	H-6, 9, 10, H ₃ -14
9	132.1	CH	H-8 β , 10
10	139.3	CH	H-1 (α , β), 8 β , H ₃ -12 β , 13 α
11	35.1	C	H-1 (α , β), 2 α , 9, 10, H ₃ -12, 13
12	30.5	CH_3	H-1 (α , β), 10, 13 α
13	16.1	CH_3	H-1 (α , β), 10, 12 β
14	12.9	CH_3	H-6, 8 β
15	23.0	CH_3	H-1 (α , β), 4 (α , β)

(d, H-8 β , $J = 6.9$ Hz), δ_{C} , 78.1 (C-8)] and a quaternary carbon [δ_{C} , 35.1 (C-11)]. The ^1H - ^1H COSY data, clearly indicated the presence of three partial structures by showing correlations between H-1 and H-2; H-4 and H-5, H-5 and H-6; H-8 and H-9, H-9 and H-10. This was confirmed by HMQC experiments. The correlations of H-1, 2, 4, 5, H₃-15 with C-3; H-8, 5, H₃-14 with C-7, H-1, 2, 9, 10, H₃-12, H₃-13 with C-11 in the HMBC spectrum of compound **1** (Table I), established the key linkage of the partial structures. Thus, **1** was suggested to be a humulene type sesquiterpene containing an 11-membered and an epoxide ring. The pronounced absorption at 972 cm^{-1} of IR spectrum, which was considered as a useful pointer to humulene based sesquiterpenoids, is a further evidence

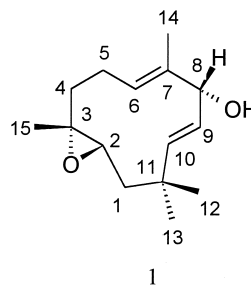


Fig. 1

for this proposed structure (Damodaran and Dev, 1968). Depend on the above spectroscopic analysis, the structure of this new compound was determined to be **1** as shown in Fig. 1.

To establish the relative stereostructure of **1**, the ROSEY experiment was carried out. Observed correlations between H-2 α and H-1 α , 4 α , 5 α ; H₃-15 and H-1 β , 5 β indicated that 2, 3-epoxide ring was *trans* form. This was confirmed by direct comparison of the chemical shift of C-2, C-3 with those of *trans* 2, 3-epoxy humulene epoxides. The signal (δ_{C} , 63.3 ppm, C-2) shifted down-field was assigned to the carbon bearing epoxide bond of β configuration, while up-field one (δ_{C} , 61.4 ppm, C-3) to the carbon bearing epoxide bond of α configuration (Heymann *et al.*, 1994). Detailed analysis of the ROSEY spectrum found that the correlations between H-8 β and H-10, H-1 β and H-10, which suggested the hydroxyl group at C-8 chiral center to be α configuration. The stereochemistry of 9, 10 double bond was determined to be *Z* by considering the coupling constant ($J_{9-10} =$

Position	δ_{H}	J	^1H - ^1H COSY	ROSEY
1 α	1.59	d, 14.3	H-1 β	H-1 β , 2 α , 5 α
1 β	1.34	dd, 14.3, 9.8	H-1 α , 2 α	H-4 β , 10, H ₃ -12 β
2 α	2.56	d, 9.8	H-1 β	H-1 α , 4 α , 5 α , H-1 α , H ₃ -13 α
4 α	2.02	9.8	H-4 β , 5	H-2 α , 5 α , H ₃ -13 α
4 β	1.16	m	H-4 α , 5	H-1 β , 15 β
5 α	2.46	m	H-4, 5 β , 6	H-1 α , 4 α , 5 β
5 β	2.08	m	H-4, 5 α ,	H-4 β , 8 β
6	5.44	m	H-5 α , 5 β , 14	H-5 β , 8 β
8 β	4.69	d, 6.9	H-9	H-6, 10
9	5.72	dd, 16.1, 6.9	H-8 β , 10	H ₃ -13 α
10	5.37	d, 16.1	H-9	H-1 β , 8 β
H ₃ -12 β	1.02	s		H-1 β , H ₃ -13 α , 15 β
H ₃ -13 α	1.16	s		H-1 α , 2 α , 9, H ₃ -12 β
H ₃ -14	1.70	s		H-5 α
H ₃ -15 β	1.16	s		H-1 β , 5 β , H ₃ -12 β

Table II. ^1H NMR (500 MHz) Data (δ in ppm, J in Hz, for **1**) in CDCl_3 .

16.1 Hz) displayed in ^1H NMR spectrum and the ROSEY correlations between H-10 and H-1 β ; H-9 and H₃-13 α . Also based on observed correlations between H-6 and H-5 β ; H₃-14 and H-5 α , the stereochemistry of 6, 7 double bond was established as *Z* configuration. Therefore, compound **1** was determined to be 2 β , α -epoxy-6*Z*, 9*Z*-humuladien-8 α -ol.

Experimental

General

IR: *Perkin-Elmer 577* spectrometer, KBr pellets; in cm^{-1} . 1D, 2D NMR: *DRX-500* spectrometer; δ in ppm, *J* in Hz, TMS int. standard. MS: *VG Autospec-3000* spectrometer; *m/z* (rel. %). Optical rotation: *JASCO-20C* digital polarimeter at 20 °C.

Mushroom material

The fresh fruiting bodies of *Lactarius hirtipes* were collected in Lijiang, Yunnan Province, P. R. China. A voucher specimen (HKAS 38820) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

Fresh fruiting bodies of *L. hirtipes* (6 kg) were soaked into 95% ethanol without cutting to destroy the enzyme. After filtration, the fruiting bodies were air-dried and crushed into powder (598 g), which was exhaustively extracted with MeOH

(1000 ml x 5) at 45 °C. The combined filtrations were evaporated *in vacuo* to afford a red gum (62 g), which was suspended in water and extracted with EtOAc. The EtOAc extracts were evaporated. under red. press., giving 8.2 g of a residue which was purified on column chromatography (Si gel H-60, 5 x 40 cm) using a petroleum ether-acetone gradient elution. 23 mg **1** was obtained from fraction 2 (petroleum ether:acetone, 9:1 v/v) by repeated RP-C18 column chromatography and recrystallization. 4.1 g lactarinic acid was obtained from fraction 1 (petroleum ether:acetone, 20:1 v/v) and fraction 2 by recrystallization.

The physical and spectroscopic data of lactarinic acid in agreement with those reported in the literature (Tulloch *et al.*, 1977).

2 β , α -Epoxy-6*Z*,9*Z*-humuladien-8 α -ol (**1**), white needles. M.p. 189.0–190.5 °C. $[\alpha]_{\text{D}} -21.3^\circ$ (CHCl_3 , *c* 0.32). IR: 3296 (br. OH), 2954, 1640 (C=C), 1610, 1452, 1387, 1360, 1297, 972. HREIMS: 236.1757 ($\text{C}_{15}\text{H}_{24}\text{O}_2$, $[\text{M}]^+$: Calc. 236.1776). EIMS: 236 (91, $[\text{M}]^+$), 221 (4, $[\text{M}-\text{CH}_3]^+$), 203 (6, $[\text{M}-\text{H}_2\text{O}-\text{CH}_3]^+$), 175 (6), 162 (13), 152 (5), 135 (27), 121 (24), 109 (100), 95 (78), 83 (73), 69 (77), 55 (73). ^1H , ^{13}C NMR: see Tables I, II.

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