Humulane-Type Sesquiterpenoids from the Mushroom *Lactarius mitissimus*

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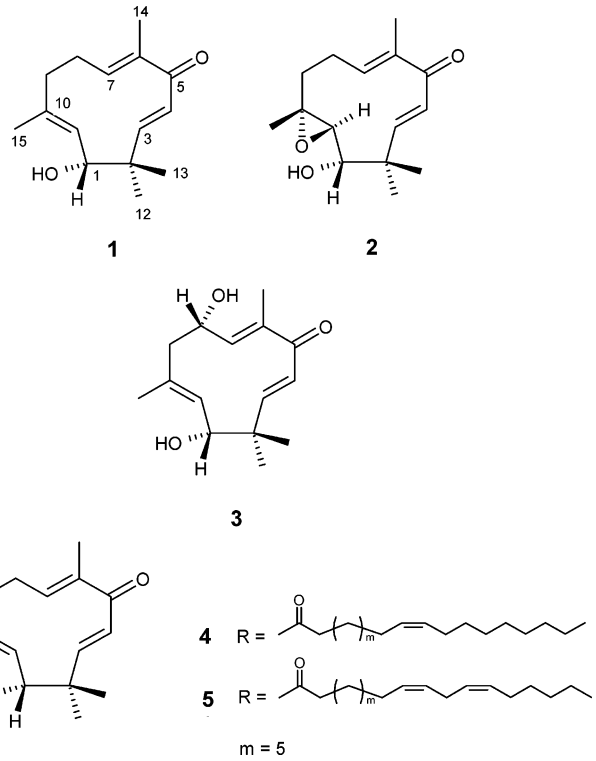
Five new humulane-type sesquiterpenes, mitissimols A (**1**), B (**2**), and C (**3**), and a mixture of mitissimol A oleate (**4**) and mitissimol A linoleate (**5**), were isolated from the fruiting bodies of *Lactarius mitissimus*. Their structures were elucidated on the basis of comprehensive spectroscopic techniques and necessary chemical methods. The relative stereochemistry of **1** was determined by single-crystal X-ray diffraction analysis.

Mushrooms that belong to the genus *Lactarius* (family Russulaceae, Basidiomycotina) contain a milky juice, which can be observed when the fruiting bodies are injured. Sesquiterpenes play an important biological role in the great majority of *Lactarius* species, being responsible for the pungency and bitterness of the milky juice and the change in color of the latex on exposure to air[1] and constituting a chemical defense system against various predators such as bacteria, fungi, animals, and insects.[2,3] Most *Lactarius* sesquiterpenes are lactaranes, secolactaranes, marasmanes, isolactaranes, norlactaranes, or caryophyllanes and are believed to be biosynthesized from humulene.[4−6] Fungi of the genus *Lactarius* have been shown to be a good source of bioactive secondary metabolites. Many sesquiterpenes have been isolated from *Lactarius* species.[7−10] However, the presence of humulanes in higher fungi has rarely been reported. One example is 2β-epoxy-6*Z*,9*Z*-humulene-8α-ol isolated from *L. hirtipes*.[11] As a continuing investigation of bioactive metabolites of *Lactarius* and *Russula* sp. in Yunnan Province of China,[12−16] the chemical constituents of the fruiting bodies of *Lactarius mitissimus* were investigated. This report deals with the isolation and structure elucidation of five new humulene sesquiterpenes (**1**−**5**) from the fruiting bodies of *L. mitissimus*.

Compound **1** was obtained as colorless crystals with the molecular formula $C_{15}H_{22}O_2$, as determined from the pseudomolecular ion at *m*/*z* 257.1516 $[M + Na]^+$ displayed in its HRESIMS. Its ^{13}C NMR spectrum included signals for a carbonyl (C═CCOC═C) (δ 203.7), one quaternary carbon (δ 42.0), two olefinic quaternary carbons (δ 138.6 and 137.8), four olefinic methyne carbons (δ 157.0, 148.1, 128.0, and 127.2), one methyne carbon (δ 75.6), two methylene carbons (δ 39.5 and 24.6), and four methyl carbons (δ 26.6, 17.1, 16.0, and 11.7). Its IR spectrum also showed bands at 3481 (OH), 1638 (C═CCOC═C), 1388, and 1359 (*gem*-dimethyl group) cm^{-1}. Comparisons of the ^1H and ^{13}C NMR spectra of **1** (Table 1) with those reported in the literature for zerumbone indicated closely related structures for these compounds, both displaying three double bonds, a hydroxyl group, and a conjugated carbonyl group.[17] The location of the hydroxyl group was determined to be at C-1, based on δ_H 4.22 (1H, d, J = 10.6, H-1) and δ_C 75.6 (d, C-1) assigned by the COSY, HMQC, and HMBC spectra. The geometry of the 3, 4 double bond was determined as *E* from the proton coupling constant ($J_{3,4}$ = 16.4 Hz). The 6*E*, 10*E* configuration was suggested from the carbon resonances of C-10 (δ 138.6), C-15 (δ 16.0), C-6 (δ 137.8), and C-14 (δ 11.7), which are characteristic for *E*, *E* configurations in related compounds.[18]



This was supported by a ROESY experiment, which showed significant correlations between H-1 and H-3, H-12, H-15; between H-11 and H-7, H-13; and between H-4 and H-13. Thus, compound **1** was elucidated as 3*E*,6*E*,10*E*-humulatrien-1α-ol-5-one, named mitissimol A, which was confirmed by single-crystal X-ray diffraction (Figure 1).

Compound **2** was obtained as a white solid. The molecular formula of **2** was determined to be $C_{15}H_{22}O_3$ on the basis of HRESIMS $[M + Na]^+$ *m*/*z* 273.1474 (calcd for $C_{15}H_{22}O_3Na$ 273.1466). The ^1H and ^{13}C NMR spectra of **2** (Table 1) were similar to those of **1**, which suggested that this compound possessed the same humulane skeleton. The key differences were that δ_C for carbons 10 and 11 in the spectrum of **2** (δ_C 65.8 and 63.5, respectively) were shifted upfield compared to those of **1** (δ_C 128.0 and 138.6, respectively). This characteristic difference was caused by the double bond in **1** being displaced by an epoxide ring in **2**. The HMBC spectra of **2** demonstrated the expected key correlations. The ROESY experiment showed correlations between H-1 and H-3, H-12, H-15; H-11 and H-7, H-9α; and H-4 and H-13. The geometry of the 3, 4 double bond was further determined to be *E* by considering the coupling constant ($J_{3,4}$ = 16.4 Hz) displayed in its

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Table 1. ^{1}H NMR and ^{13}C NMR Data for Mitissimols A (**1**), B (**2**), and C (**3**)[a]

	1		2		3	
no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	75.6 d	4.22 (d, 10.6)	76.1d	3.30 (d, 9.7)	76.0 d	4.19 (d, 10.7)
2	42.0 s		40.4 s		43.8 s	
3	157.0 d	5.69 (d, 16.4)	155.9 d	5.93 (d, 16.4)	161.4 d	5.84 (d, 16.5)
4	127.2 d	6.06 (d, 16.4)	129.2 d	6.25 (d, 16.4)	128.0 d	6.07 (d, 16.5)
5	203.7 s		202.5 s		206.5 s	
6	137.8 s		139.2 s		140.7 s	
7	148.1 d	5.90 (br d, 10.6)	147.0 d	6.05 (br d, 8.7)	147.8 d	5.73 (br d, 10.0)
8	24.6 t	2.47 (m); 2.28 (m)	24.5 t	2.41 (m)	65.6 d	4.61 (ddd, 10.4, 10.0, 5.5)
9	39.5 t	2.37 (m); 2.23 (m)	38.0 t	2.28 (ddd, 13.4, 3.9, 2.8); 1.38 (t d, 13.4, 7.1)	50.2 t	2.70 (dd, 11.4, 5.5); 2.12 (dd, 11.4, 10.4)
10	138.6 s		155.9 d	5.93 (d, 16.4)	135.8 s	
11	128.0 d	5.25 (br d, 10.6)	65.8 d	2.75 (d, 9.7)	130.9 d	5.30 (brd, 10.7)
12	26.6 q	1.14 (s)	26.8 q	1.16 (s)	26.9 q	1.11 (s)
13	17.1 q	1.14 (s)	17.1 q	1.24 (s)	17.6 q	1.13 (s)
14	11.7 q	1.79 (br s)	12.0 q	1.85 (br s)	12.1 q	1.88 (br s)
15	16.0 q	1.62 (br s)	16.7 q	1.29 (s)	17.3 q	1.65 (br s)

[a] Compounds **1** and **2** were measured at 400 MHz in $CDCl_3$, **3** in MeOD. Coupling constants are given in Hz.

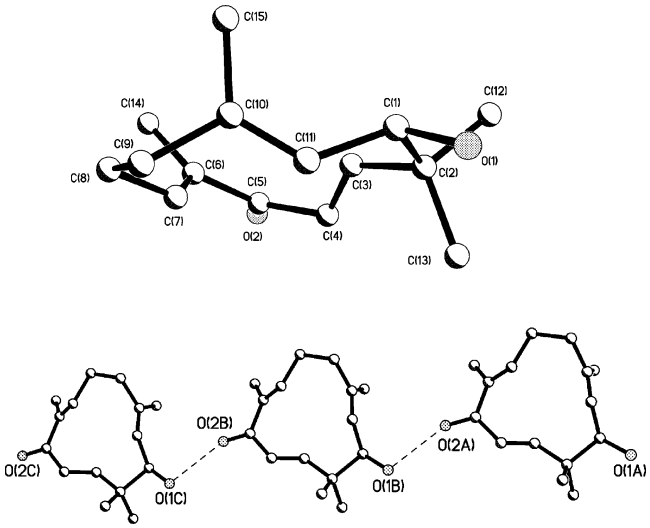

Figure 1. X-ray crystal structure of compound **1**.

^{1}H NMR spectrum. From these data, compound **2** was determined to be 10,11-epoxy-3*E*,6*E*-humuladien-1α-ol-5-one, named mitissimol B.

Compound **3** was obtained as a colorless crystal. The molecular formula of **3** was determined to be $C_{15}H_{22}O_3$ on the basis of HRESIMS $[M + Na]^+$ *m*/*z* 273.1467 (calcd for $C_{15}H_{22}O_3Na$ 273.1466). The ^{1}H and ^{13}C NMR spectra (Table 1) of **3** were similar to those of **1**, which suggested that this compound also possessed the same humulane skeleton. Key differences were that δ_C for carbons 8 and 9 in the spectrum of **3** (δ_C 65.6 and 50.2) were shifted downfield compared to those of **1** (δ_C 24.6 and 39.5, respectively). This characteristic difference was caused by a proton at position 8 of **1** being displaced by an OH group in **3**. The location of the OH group was further determined to be at C-8 by COSY, HMQC, and HMBC experiments. The relative stereostructure of **3** was determined by the ROESY experiment, which showed significant correlations between H-1 and H-3, H-15; H-11 and H-7, H-9α; H-8 and H-14, H-15; and H-3 and H-1, H-12, H-15. Thus, compound **3** was determined to be 3*E*,6*E*,10*E*-humulatrien-1α,8α-diol-5-one, named mitissimol C.

A mixture of compounds **4** and **5** was obtained as a yellow oil, which demonstrated only one spot on silica gel plates developed with different solvent systems. The molecular formulas of **4** and **5** were determined to be $C_{33}H_{54}O_3$ at *m*/*z* 521.3980 $[M_4 + Na]^+$ (calcd for $C_{33}H_{54}O_3Na$ 521.3970) and $C_{33}H_{52}O_3$ at *m*/*z* 519.3811 $[M_5 + Na]^+$ (calcd for $C_{33}H_{52}O_3Na$ 519.3814) on the basis of HRESIMS, respectively. The IR spectrum indicated an ester carbonyl at 1736,

Table 2. ^{1}H NMR and ^{13}C NMR Data for Mitissimol A Oleate (**4**) and Mitissimol A Linoleate (**5**)[a]

	4		5	
no.	δ_C	δ_H	δ_C	δ_H
1	76.4 d	5.25 (d, 10.6)	76.4 d	5.25 (d, 10.6)
2	41.1 s		41.1 s	
3	155.6 d	5.68 (d, 16.4)	155.6 d	5.68 (d, 16.4)
4	127.8 d	6.06 (d, 16.4)	127.8 d	6.06 (d, 16.4)
5	203.3 s		203.3 s	
6	137.8 s		137.8 s	
7	148.3 d	5.89 (br d, 10.3)	148.3 d	5.89 (br d, 10.3)
8	24.6 t	2.14−2.50 (m)	24.6 t	2.14−2.50 (m)
9	39.4 t	2.14−2.50 (m)	39.4 t	2.14−2.50 (m)
10	140.9 s		140.9 s	
11	124.1 d	5.14 (br d, 10.6)	124.1 d	5.14 (br d, 10.6)
12	26.3 q	1.02 (s)	26.3 q	1.02 (s)
13	18.1 q	1.19 (s)	18.1 q	1.19 (s)
14	11.6 q	1.77 (br s)	11.6 q	1.77 (br s)
15	15.9 q	1.71 (br s)	15.9 q	1.71 (br s)
1′	173.2 s		173.2 s	
2′	34.4 t	2.30 (t, 7.6)	34.4 t	2.30 (t, 7.6)
3′	24.9 t	1.60 (m)	24.9 t	1.60 (m)
4′−7′	29.0−29.7 t	1.20−1.30 (m)	29.0−29.7 t	1.20−1.30 (m)
8′	27.1 t	1.95−2.05 (m)	27.1 t	1.95−2.05 (m)
9′	129.6 d	5.24−5.38 (m)	127.8 d	5.24−5.38 (m)
10′	129.9 d	5.24−5.38 (m)	128.0 d	5.24−5.38 (m)
11′	27.1 t	1.95−2.05 (m)	25.5 t	2.74 (t, 6.2)
12′	29.0−29.7 t	1.20−1.30 (m)	129.9 d	5.24−5.38 (m)
13′	29.0−29.7 t	1.20−1.30 (m)	130.1 d	5.24−5.38 (m)
14′	29.0−29.7 t	1.20−1.30 (m)	27.1 t	1.95−2.05 (m)
15′	29.0−29.7 t	1.20−1.30 (m)	29.0∼29.7 t	1.20−1.30 (m)
16′	31.8 t	1.20−1.30 (m)	31.4 t	1.20−1.30 (m)
17′	22.6 t	1.20−1.30 (m)	22.5 t	1.20−1.30 (m)
18′	14.0 q	0.85 (t, 6.9)	14.0 q	0.85 (t, 6.9)

[a] Compounds **4** and **5** were measured at 500 MHz in $CDCl_3$. Coupling constants are given in Hz.

C═CCOC═C at 1662, and a geminate dimethyl at 1386, 1364 cm^{-1}. The FABMS (positive) gave the molecular related peaks $[M + 1]^+$ at *m*/*z* 498 ($C_{33}H_{54}O_3$+H) and 496 ($C_{33}H_{52}O_3$+H), and EIMS exhibited major fragments of $M^+ - 266$ or $M^+ - 264$ at *m*/*z* 234, which was consistent with the molecular fragment $C_{15}H_{22}O_2$. The ^{13}C NMR spectrum (Table 2) of **4** and **5** showed characteristic signals of esters composed of humulane sesquiterpenes and long chain fatty acids, which were assigned to a carbonyl carbon (C═CCOC═C) (δ_C 203.3), one quaternary carbon (δ_C 41.1), two olefinic quaternary carbons (δ_C 140.9 and 137.8), four olefinic methyne carbons (δ_C 155.6, 148.3, 124.1, and 128.0), one methyne carbon (δ_C 75.6), two methylene carbons (δ_C 39.4 and 24.6), and four methyl carbons (δ_C 26.3, 18.1, 15.9, and 11.6); 173.2 (CO), 34.4 (CH_2), 29.6−24.9 (CH_2), 130.1, 129.9, 129.6, 127.8 (double bond), 14.0 (CH_3), respectively. This was further confirmed by δ_H

5.25 (1H, d, 10.6, H-1) of **4** and **5** and δ_H 4.22 (1H, d, 10.6, H-1) of **1** in the ^{1}H NMR spectra (Table 2).

On methanolysis, the mixture of compounds of **4** and **5** yielded fatty acid methyl esters **4a** and **5a**. The EIMS of **4a** and **5a** displayed molecular ions at *m/z* 352 $[M_{4a}]^+$and 350 $[M_{5a}]^+$. Compounds **4a** and **5a** (8 mg) were treated in 0.5 mL of CS_2 by addition of 0.5 mL of DMDS and 3 mg of I_2. The reaction was carried out in a 10 mL flask closed with a Teflon-lined cap and kept 40 h at 60 °C. Samples were then treated with $Na_2S_2O_3$ solution (5% in distilled H_2O) and twice extracted with petroleum ether. The organic extract was subjected to EIMS analysis. The MS spectrum gave remarkable fragment-ion peaks at *m/z* 173, 131 for A_1 and A_2 and 185 for C_1 and C_2, respectively, due to cleavage of the bond between the carbons bearing a methylthio group. These data indicated that the double bond in **4a** is at C-9′, and the double bonds in **5a** are at C-9′ and C-12′, respectively.[19] The geometry of the C-9′/C-10′ and C-12′/C-13′ double bonds were determined to be *Z* on the basis of NMR data.[19,20] The two methyl esters were identified as methyl oleate and methyl linoleate.

Thus, the structures of **4** and **5** were determined to be the 9-octadecenoic ester of 3*E*,6*E*,10*E*-humulatrien-1α-ol-5-one and the 9,12-octadecadienoic ester of 3*E*,6*E*,10*E*-humulatrien-1α-ol-5-one, respectively, and were named mitissimol A oleate and mitissimol A linoleate, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained with a Tensor 27 using KBr pellets. NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 spectrometers in $CDCl_3$ with TMS as an internal standard. MS (EI, FAB) were recorded with a VG Autospec-3000 spectrometer. ESI and HRESIMS were recorded with an API QSTAR Pulsar 1 spectrometer. GC-MS analysis was performed with a HP 6890GC/5973 MS (Agilent Technologies) instrument equipped with a HP-5MS capillary column, 30 m long, 0.25 mm i.d., 0.25 μm film thickness; chromatographic conditions: injector 250 °C, detector 250 °C. The temperature of the column oven was increased from (40 °C) to 80 °C at 30 °C/min, and thereafter at 3 °C/min up to 250 °C; N_2 was used as carrier gas and the flow was 1 mL/min. Individual compounds were identified by comparison with standards of a mass spectrum database (WILEY7N.L and NIST98.L) and literature spectra. Silica gel (200−300 mesh, Qingdao Marine Chemical Inc., P. R. 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 ethanol.

Fungal Material. The fresh fruiting bodies of *Lactarius mitissimus* were collected at Ailao Mountain, Yunnan Province, China, in July 2003 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen (HKAS 37876) was deposited in the Herbarium of Kunming Institute of Botany, CAS.

Extraction and Isolation. The fresh fruiting bodies of *L. mitissimus* (1.6 kg) were extracted with 95% aqueous EtOH (15 L) in maceration. The EtOH solution was evaporated in vacuo to give the extract (101 g), which was suspended in H_2O and extracted with EtOAC (4.5 L). The EtOAc extracts were evaporated under reduced pressure, giving 33.5 g of residue, which was subjected to silica gel column chromatography (CC) (column: 5.8 cm × 78 cm; silica gel: 850 g) eluting with $CHCl_3$/MeOH from 100:0 (v/v) to 50:50 (v/v) to give eight fractions. The fraction eluted with $CHCl_3$/MeOH (100:0, v/v) was subjected to chromatography eluting with petroleum ether/acetone (50:1, v/v) to give five fractions, then fraction 5 was purified by preparative TLC (petroleum ether/acetone, 6:1 (v/v)) and Sephadex LH-20 CC eluted by $CHCl_3$/MeOH (1:1, v/v) to afford yellow **4** and **5** (48 mg) (TLC: petroleum ether/acetone, 6:1 (v/v), R_f = 0.6). The fraction eluted by $CHCl_3$/MeOH (98:2, v/v) was further subjected to silica gel CC eluting with petroleum ether/acetone at 25:1, 15:1, 10:1, and 5:1 (v/v) to give fractions 1−4. Fraction 2 eluted with petroleum ether/acetone (15:1, v/v) was further purified by preparative TLC (silica gel, petroleum ether/acetone, 2:1 (v/v)) and Sephadex LH-20 CC eluted by $CHCl_3$/MeOH (1:1, v/v) to afford compound **1** (66 mg) (TLC: petroleum ether/acetone, 3:1 (v/v), R_f = 0.5). The fraction eluted by $CHCl_3$/MeOH (95:5, v/v) was further subjected to silica gel CC eluting with petroleum ether/acetone at 8:1, 6:1, 10:1, 4:1, 2:1, 1:1, and 1:5 (v/v) to give fractions 1−7. Fraction 2 eluted with petroleum ether/acetone (2:1, v/v) was further purified by preparative TLC (silica gel) with $CHCl_3$/isopropanol, 9:1 (v/v), and Sephadex LH-20 CC eluted by $CHCl_3$/MeOH (1:1, v/v) to afford compound **3** (26 mg) (Et_2O/MeOH, 10:1 (v/v), R_f = 0.5). Fraction 3 eluted with petroleum ether/acetone (10:1, v/v) was further purified by preparative TLC (silica gel, petroleum ether/acetone, 7:1 (v/v)) and Sephadex LH-20 CC eluted by $CHCl_3$/MeOH (1:1, v/v) to afford compound **2** (8 mg) (petroleum ether/acetone, 2:1 (v/v), R_f = 0.5).

Mitissimol A (1): colorless crystals ($CHCl_3$); mp 169−171 °C; $[\alpha]_D^{25.2}$ −42.0 (*c* 0.40, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 252 (4.30) nm; IR (KBr) ν_{max} 3481, 2988, 2959, 2925, 2881, 1638, 1445, 1388, 1297, 1273, 1045, 968, 900 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$, 400 MHz), see Table 1; FABMS (pos) *m/z* 235 $[M + 1]^+$, 469 $[2M + 1]^+$; HRESIMS (pos) *m/z* 257.1516 ($C_{15}H_{22}O_2Na$, calcd 257.1517).

Crystal Data for 1. Crystals of **1** suitable for X-ray analysis were recrystallized from MeOH and were found to belong to the orthorhombic space group *P*2(1)2(1)2(1). Crystal data: $C_{15}H_{22}O_2$, M = 234.33, a = 9.1505(18) Å, b = 9.5977(19) Å, c = 16.152(3) Å, V = 1418.5(5) A^3, Z = 4, d = 1.097 Mg/m^3, Mo Kα radiation, linear absorption coefficient 0.071 mm^{-1}. A crystal of dimensions 0.49 × 0.28 × 0.22 mm was used for X-ray measurements on a MAC DIP-2030K diffractometer equipped with a graphite monochromator; a maximum 2θ value of 90° was set. The total number of independent reflections measured was 7305, 2517 of which were considered to be observed. The structure was solved by the direct method with SHELXS-97 and expanded using difference Fourier techniques, refined by the program NOMSCD[18] and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were R_f = 0.0423, R_w = 0.1049. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CDDC 601319). Copies of these data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retriving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (int) +44-1223/336-033; e-mail: deposit@ccdc.cam.ac.uk].

Mitissimol B (2): colorless needles ($CHCl_3$); mp 200−201 °C; $[\alpha]_D^{25.5}$ −1.0 (*c* 0.59, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 244 (3.43) nm; IR (KBr) ν_{max} 3507, 3011, 2995, 2962, 2924, 1651, 1641, 1466, 1386, 1294, 1271, 1061, 975, 911 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; ESIMS (pos) *m/z* 273 $[M + Na]^+$, 523 $[2M + Na]^+$; HRESIMS (pos) *m/z* 273.1474 ($C_{15}H_{22}O_3Na$, calcd 273.1466).

Mitissimol C (3): colorless crystals (MeOH); mp 193−196 °C; $[\alpha]_D^{25.1}$ +99.0 (*c* 0.64, MeOH); UV (MeOH) λ_{max} 252 (4.11) nm; IR (KBr) ν_{max} 3424 (OH), 3037, 2962, 2928, 1647 (C═CC═OC═C), 1477, 1386, 1099, 1026, 994, 901 cm^{-1}; ^{1}H NMR and ^{13}C NMR (400 MHz, MeOD), see Table 1; ESIMS (pos) $[M + Na]^+$ 273, $[2M + Na]^+$ 523; HRESIMS (pos) *m/z* 273.1467 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$ 273.1466).

A mixture of mitissimol A oleate (**4**) and mitissimol A linoleate (**5**) was obtained as a yellow oil: UV ($CHCl_3$) λ_{max} (log ϵ) 251 (4.24) nm; IR (neat) 3006, 2925, 2854, 1736, 1662, 1464, 1386, 1364, 1268, 1181, 1098, 978, 908 cm^{-1}; ^{1}H NMR and ^{13}C NMR (400 MHz, $CDCl_3$), see Table 1. According to the peak ratio in the ^{1}H NMR, the amount of mitissimol A oleate (**4**) and mitissimol A linoleate (**5**) in the mixture was almost 1:1. ESIMS (pos): (a) for **4**, *m/z* 521 $[M + Na]^+$; (b) for **5**, *m/z* 519 $[M + Na]^+$. HRESIMS (pos): (a) for **4**, *m/z* 521.3980 ($C_{33}H_{54}O_3Na$, calcd 521.3970); (b) for **5**, *m/z* 519.3811 ($C_{33}H_{52}O_3Na$, calcd 519.3814).

Methanolysis of 4 and 5. Compounds **4** and **5** (25 mg) in 10 mL of MeOH were treated with 0.2 M HCl (2 mL) for 12 h. The resultant mixture was extracted with Et_2O (30 mL) to obtain an organic layer, which was dried with Na_2SO_4. The residue from the Et_2O extract of **4a** and **5a** was analyzed by GC-MS directly. The result displayed the molecular ions at *m/z* 352 $[M_{4a}]^+$ and 350 $[M_{5a}]^+$. A sesquiterpene was isolated from the products of hydrolysis, which was identified as mitissimol A (**1**).

Derivatization of 4a and 5a. The mixture of **4a** and **5a** (8 mg) was treated in 0.5 mL of CS_2 by addition of 0.5 mL of DMDS and 3 mg of I_2. The reaction was carried out in a 10 mL flask closed with a Teflon-

lined cap and kept 40 h at 60 °C. Samples were treated with $Na_2S_2O_3$ solution (5% in distilled H_2O) and twice extracted with petroleum ether. The organic extract was subjected to EIMS analysis, and the MS spectrum gave fragment-ion peaks at A_1 (*m/z* 173), A_2 (*m/z* 131), C_1 and C_2 (*m/z* 185), respectively.

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References and Notes

(1) De Bernardi, M.; Garlaschelli, L.; Toma, L.; Vidari, G.; Vita-Finzi, P. *Tetrahedron* **1993**, *49*, 1489−1504.
(2) Sterner, O.; Bergmann, R.; Kihlberg, J.; Wickberg, B. *J. Nat. Prod.* **1985**, *48*, 279−288.
(3) Abraham, W. R. *Cur. Med. Chem.* **2001**, *8*, 583−606.
(4) Vidari, G.; Vita-Finzi, P. *Metabolites of the genus Lactarius (Basidiomycetes): Chemistry and biological activities of the series studies in structure and chemistry (part D) of the series studies in natural product chemistry*; Atta-ur-rahman, Ed.; Elsevier: Amsterdam, 1995; pp 153−206.
(5) Daniewski, W. M.; Grieco, P. A.; Huffman J. C.; Rymikewicz, A.; Wawrzum, A. *Phytochemistry* **1981**, *20*, 2733−2734.
(6) Ayer, W.; A. Browne, L. M. *Tetrahedron* **1981**, *37*, 2199−2248.
(7) Garlaschelli, L.; Mellerio, G.; Vidari, G.; Vita-Finiz, P. *J. Nat. Prod.* **1994**, *57*, 905−910.
(8) Kopczacki, P.; Gumulka, M.; Masnyk, M.; Grabarczyk, H.; Nowak, G.; Daniewski, W. M. *Phytochemistry* **2001**, *58*, 775−787.
(9) Daniewski, W. M.; Gumulka, M.; Pankowska, E.; Ptaszyńska, K.; Bloszyk, E.; Jacobsson, U.; Norin, T. *Phytochemistry* **1993**, *32*, 1499−1502.
(10) Krawczyk, E.; Luczak, M.; Kobus, M.; Bańka, D.; Daniewski W. M. *Planta Med.* **2003**, *69*, 552−554.
(11) Hu, L.; Liu, J. K. *Z. Naturforsch.* **2002**, *57*c, 571-574.
(12) Tan, J. W.; Dong, Z. J.; Hu, L.; Liu, J. K. *Helv. Chim. Acta* **2003**, *86*, 307−309.
(13) Tan, J. W.; Xu, J. B.; Dong, Z. J.; Luo, D. Q.; Liu, J. K. *Helv. Chim. Acta* **2004**, *87*, 1025−1028.
(14) Luo, D. Q.; Wang, F.; Bian, X. Y.; Liu, J. K. *J. Antibiot.* **2005**, *7*, 456−459.
(15) Wang F. Luo D. Q., Liu J. K. *J. Antibiot.* **2005**, *6*, 412−415.
(16) Liu, J. K. *Chem. Rev.* **2005**, *105*, 2723−2744.
(17) Nakatani, N.; Jitoe, A.; Masuda, T.; Yonemori, S. *Agric. Biol. Chem.* **1991**, *55*, 455−460.
(18) Lu, Y.; Wu, B. M. *Chin. Chem. Lett.* **1992**, *3*, 637−640.
(19) Yamada, K.; Hara, E.; Miyamoto, T.; Higuchi, R.; Isobe, R.; Honda, S. *Eur. J. Org. Chem.* **1998**, 371−378.
(20) Fusetani, N.; Yasumoto, K.; Matsunaga, S.; Hirota, H. *Tetrahedron Lett.* **1989**, *30*, 6891−6894.

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