A New Trihydroxy Fatty Acid from the Ascomycete, Chinese Truffle *Tuber indicum*

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ABSTRACT: From the chloroform/methanol extract of the fruiting bodies of the ascomycete Chinese truffle *Tuber indicum* Cooke et Massee, a new trihydroxylated monounsaturated fatty acid (**1**) has been isolated. The structure of this new linoleic acid–derived metabolite was established as 9,10,11-trihydroxy-(12*Z*)-12-octadecenoic acid by means of spectroscopic and chemical methods. The fatty acid composition of the chloroform-soluble fraction of this fungus was analyzed by gas chromatography–mass spectrometry. The content of the predominant unsaturated fatty acids (oleic and linoleic acids) is as high as 68%. The use of dimethyl disulfide adduct was effective in the determination of the position of the double bond, and the glycol oxidation fission reaction with sodium metaperiodate supported on silica gel was helpful in establishing the location of the trihydroxylic groups in the new fatty acid.

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Truffles, also known as "black diamonds," are subterranean edible fungi of the family Tuberaceae (order Tuberales) that grow in symbiosis with certain trees. There are more than 60 different kinds of truffles around the world (1), most of which grow in various parts of Europe, particularly in France. They are thought to be a "miracle of nature" and have been since ancient times the ultimate in gastronomy because of their superior nutritional attributes. In addition to their use as a costly food, truffles have been used in making liqueurs, for scenting tobacco, and in certain perfumes.

Recent studies have proven that some truffles contain steroids as major components (2,3) as well as volatile organic compounds for mushroom aroma (4–6). The white truffle (*Tuber magnatum* Pico) and the black truffle (*T. melanosporum* Vitt) are highly appreciated for their unique aroma, which is characteristically sulfurous. The predominant sulfur compounds in white truffle aroma are dimethyl sulfide and bis(methylthio)-methane and dimethyl sulfide in black truffle aroma (4). Interestingly, the ability of pigs to detect truffles underground has been linked to the presence of trace amounts of odorous steroidal pheromone in both black and white truffles (5).

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Abbreviations: CC, column chromatography; DMDS, dimethyl disulfide; EI–MS, electron impact–mass spectrometry; FAB–MS, fast atom bombardment–mass spectrometry; GC–MS, gas chromatography–mass spectrometry; HPODE, hydroperoxyoctadecadienoic acid; IR, infrared; LA, linoleic acid; LOX, lipoxygenase; MS, mass spectrometry; NMR, nuclear magnetic resonance; OA, oleic acid.

The lipid content of mushrooms has been the object of many investigations in relation to studies in subjects as varied as metabolism, nutrition, and medicine. In the past decades, it has been proven that fatty acids with 16 and 18 carbons in higher fungi are most abundant and that linoleic and/or oleic acids as principal unsaturated fatty acids (6–8) occur in complex bonding forms. Several higher mushrooms are also found to contain polyhydroxylated C_{18} fatty acids in the free acid forms (9–11).

About 25 species of the genus *Tuber* are found in China. Chinese truffles, *T. indicum* Cooke et Massee, are distributed mainly in the provinces of Yunnan and Sichuan. This truffle strongly resembles the black truffle. As part of our search for naturally occurring bioactive secondary metabolites of higher fungi in the Yunnan Province, we reported a rare polyhydroxylated ergosterol glycoside in the preceding paper (2). In continuation of our investigation on chemical constituents of *T. indicum*, a new fatty acid (**1**) was isolated from the fruiting bodies of this fungus, along with known compounds adenosine, uracil, 5-hydroxypyrrolidin-2-one, D-allitol, ergosterol, and nonanedioic acid (azelaic acid). The present report deals with the structural elucidation of this new metabolite, and the fatty acid composition of its chloroform-soluble fraction is briefly described.

EXPERIMENTAL PROCEDURES

Chromatographic and instrumental methods. Melting points were obtained on an XRC-1 apparatus (Sichuan University, Sichuan, People's Republic of China). Optical rotations were taken on a Horiba SEPA-300 automatic polarimeter (Horiba, Tokyo, Japan). The nuclear magnetic resonance (NMR) one- and two-dimensional NMR spectra were acquired on Bruker AM-400 and Bruker DRX-500 instruments (Karlsruhe, Germany); tetramethylsilane was used as an internal standard and coupling constants were represented in hertz. Mass spectrometry (MS) spectra were measured with a VG Autospec-3000 mass spectrometer (VG, Manchester, England). Infrared (IR) spectra were obtained in KBr pellets on a Bio-Rad (Richmond, CA) FTS-135 infrared spectrophotometer. Gas chromatography (GC)–MS was performed with a Finnigan 4510 GC–MS spectrometer (San Jose, CA) employing the electron impact (EI) mode (ionizing potential 70eV) and a capillary column (30 m × 0.25 mm) packed with 5% phenyl/95% methylsilicone on HP-5 (Hewlett-Packard, Palo Alto, CA). Hydrogen was

used as carrier gas and other conditions were as follows: hydrogen gas flow (30 mL/min), air flow rate (300 mL/min), hydrogen gas carrier flow rate (1.0 mL/min), and column temperature (160–240°C, rate of temperature increase: 5°C/min).

Materials. Column chromatography (CC) was performed over silica gel (200–300 mesh; Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China). Reversed-phase chromatography was carried out on LiChroprep^R RP-8 (40–63 μm) (Merck, Darmstadt, Germany). Thin-layer chromatographic analysis was carried out on plates precoated with silica gel F_{254} (Qingdao Marine Chemical Ltd.), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. All solvents were distilled before use.

Fungal samples. The dried fruiting bodies of *T. indicum* were purchased in Yunnan Province in April 2000 and identified by Profs. P.G. Liu and X.H. Wang (Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, People's Republic of China). A voucher specimen was deposited at the Herbarium of Kunming Institute of Botany.

Extraction and isolation. The dried and powdered fruiting bodies (4.7 kg) were extracted successively three times with $CHCl_3$ and four times with $CHCl_3$/MeOH (1:1, vol/vol) at room temperature. These extracts were concentrated to dryness *in vacuo*, respectively, to give both $CHCl_3$ (154 g) and $CHCl_3$/MeOH extracts (122 g). The $CHCl_3$/MeOH (1:1, vol/vol) extracts were chromatographed over silica gel using $CHCl_3$ by using increasing concentrations of MeOH in $CHCl_3$ as eluent. The fractions (3.5 g) eluted with $CHCl_3$/MeOH (95:5, vol/vol) were subjected to silica gel CC using $CHCl_3$/MeOH (9:1, vol/vol) to provide a residue (139 mg), which was rechromatographed on silica gel with cyclohexane/acetone (7:3, vol/vol) to furnish pure compound **1** (18.7 mg).

Fatty acid analysis. Two milliliters Et_2O/*n*-hexane (2:1, vol/vol), 2 mL MeOH, 2 mL 0.8 mol/L KOH-MeOH, and 19 mL H_2O were added to the 110 mg $CHCl_3$ extract containing fatty acids in a 25-mL volumetric flask; after shaking for 5 min, the supernatant obtained was subjected to GC–MS.

*9,10,11-Trihydroxy-(12Z)-12-octadecenoic acid (**1**).* White amorphous crystals ($CHCl_3$); m.p. 73–75°C; $[\alpha]_D^{22}$ = +10.0 (*c* 0.008, $CHCl_3$); IR (KBr) *v* 3368 (OH), 2928, 2852 (aliphatic C-H), 1713 (acidic C=O), 1464, 1399, 1242, 1075, 1017 (C-O), 940, and 723 $[(CH_2)_n]cm^{-1}$; EI–MS (70 eV) (relative intensity %) *m/z* 330 $[M]^+$ (0.5), 302 (1.0), 285 (1.5), 275 (1.0), 273 (1), 271 (1.2), 203 (7.0), 187 (4.5), 185 (41), 173 (7.5), 168 (50), 155 (29), 143 (2.5), 139 (12), 127 (19.5), 121 (5), 110 (14), 109 (35), 98 (24), 97 (29.5), 83 (68), 71 (29), 69 (58.2), 57 (77.5), and 55 (100); and ^1H and ^{13}C NMR data are given in Table 1. High-resolution fast atom bombardment (FAB)–MS (negative ion mode*s*) at *m/z* 329.2264 $[M-1]^-$, calcd. for $C_{18}H_{33}O_5$, 329.2328; FAB–MS (positive ion mode) *m/z* 331 $[M+1]^+$ (10), 313 $[M+1-H_2O]^+$ (23), 173 (100), 109 (25.5), 81(29.5).

*Acetylation of **1**.* Compound **1** (8.0 mg) was treated with Ac_2O/pyridine (1:1) for 36 h at room temperature to yield a crude product, to which 3 mL of water was added and then extracted with EtOAc (3 × 3 mL). The resulting residue was chromatographed on silica gel with *n*-hexane/EtOAc (10:1–8:2 vol/vol) to afford 8.9 mg of its triacetate derivative (**1a**) as a colorless oil. ^1H NMR 400 MHz ($CDCl_3$) δ ppm **1a**: 5.70 (1H, *dt*, *J* = 4.2, 6.7, 10.7 Hz), 5.64 (1H, *dd*, *J* = 11.0, 7.4 Hz), 5.29 (1H, *br d*, *J* = 9.7 Hz), 5.17 (1H, *t*, *J* = 5.0, 6.2 Hz), 4.95 (1H, *dd*, *J* = 2.9, 9.8 Hz), 2.20 (2H, *t*, *J* = 7.6 Hz), 1.98 (3H, *s*, $COCH_3$), 1.99 (3H, *s*, $COCH_3$), 2.04 (3H, *s*, $COCH_3$), 1.58 (4H, *m*), 1.22–1.27 ($[CH_2]_n$, *br s*), 0.86 (3H, *t*, *J* = 6.6 Hz, terminal methyl); EI–MS (70 eV) (relative intensity %) *m/z* 456 $[M]^+$ (1), 439 $[M-OH]^+$ (8), 411 (0.5), 410 (0.5), 399 (1), 397 $[M-AcO]^+$ (25), 336 $[M-2\times AcOH]^+$ (41), 294 $[M-2\times AcOH-Ac+1]^+$ (72), 287 (8), 277 (40), 276 $[M-3\times AcOH]^+$ (57), 248 (21), 241 (8), 233 (5), 227 (80), 219 (8.5), 215 (4), 182 (86), 169 (72), 168 (80), 155 (53), 143 (3), 140 (84), 139 (48), 127 (86), 122 (15), 110 (18), 109 (59),

TABLE 1
^1H (400 MHz) and ^{13}C (100 MHz) Nuclear Magnetic Resonance (NMR) Data of Compound 1 in Pyridine-d_5[a]

Atom. no.	^{13}C (multiplicity)		^1H (multiplicity, *J* in Hz)	^1H-^1H COSY
1	175.9	C		
2	34.9	CH_2	2.47 (*t, J* = 7.5 Hz)	H-3
3	25.7	CH_2	1.74 (*m*)	H-2, H-4
4–6	22.7–31.7	$3CH_2$	1.19–1.38 (*br m*)	
7	34.3	CH_2	2.25 (*m*)	H-6, H-8
8	34.3	CH_2	1.91 (*m*)	H-7, H-9
9	72.6	CH	4.26 (*m*)	H-8, H-10
10	78.6	CH	3.93 (*dd, J* = 6.7, 3.4 Hz)	H-9, H-11
11	67.8	CH	5.30 (*dd, J* = 9.1, 3.4 Hz)	H-10, H-12
12	132.6	CH	6.16 (*dd, J* = 11.0, 9.2 Hz)	H-11, H-13
13	131.5	CH	5.60 (*dt, J* = 11.0, 7.4 Hz)	H-12, H-14
14	28.2	CH_2	2.10 (*m*)	H-13
15–17	22.7–31.7	$3CH_2$	1.19–1.38 (*br m*)	
18	14.1	CH_3	0.80 (*t, J* = 7.0 Hz)	H-17

[a]Assignments were made by distortionless enhancement by polarization transfer and heteronuclear multiple quantum coherence analysis; COSY, correlation spectroscopy.

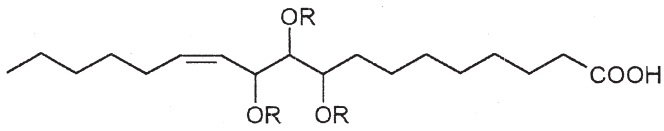
98 (43), 97 (39), 95 (55), 83 (75), 69 (60), 57 (75), 55 (100); high-resolution EI–MS *m/z* 456.2693 $[M]^+$ (calcd. for $C_{24}H_{40}O_8$, 456.2723).

*Sodium periodate oxidation of **1***. Sodium periodate (0.26 g in 1.2 mL water) was heated, with stirring, to 75°C over a period of 25 min. Silica (1.0 g) was added to the stirred solution. The mixture was then cooled and shaken vigorously for 20 min to give a coarse powder. CH_2Cl_2 (6 mL) was added and then, after stirring, a solution of **1** (6.4 mg) in 6 mL of CH_2Cl_2/MeOH (1:1, vol/vol). The reaction mixture was stirred at room temperature for 90 min, then filtered and washed with CH_2Cl_2. The combined supernatant was concentrated *in vacuo* to afford a mixture of aldehydes, which was subjected to EI–MS and ^{1}H NMR. EI–MS (70 eV) *m/z* 126 $[M]^+$ for 2-*cis*-octenal **2**, 172 $[M]^+$ for 9-oxo-nonanoic acid **3**; ^{1}H NMR 500 MHz ($CDCl_3$) δ ppm **2**: 9.51 (1H, *d*, *J* = 7.8 Hz, 1-H), 6.21 (1H, *dd*, *J* = 11.0, 7.8 Hz, 2-H), 6.85 (1H, *dd*, *J* = 11.0, 7.0 Hz, 3-H), 2.09 (2H, *dt*, *J* = 7.2, 7.8 Hz, 4-H), 0.88 (3H, *t*, *J* = 7.0 Hz, 8-H); and **3**: 9.76 (1H, *t*, *J* = 1.8 Hz, 9-H), 2.42 (2H, *t*, *J* = 7.3 Hz, 8-H), 2.34 (2H, *t*, *J* = 7.5 Hz, 2-H). ^{1}H NMR data were in agreement with those of authentic samples.

*Dimethyl disulfide (DMDS) derivative **4** of compound **1a***. To the solution of **1a** (3.1 mg) dissolved in DMDS (0.2 mL), DMDS (0.2 mL) and iodine (1 mg) were added. The mixture obtained was kept at 60°C for 44 h in a small-volume sealed vial. The reaction was quenched with aqueous $Na_2S_2O_3$ (5%), and the reaction mixture was extracted with *n*-hexane (0.6 mL). The extract was concentrated, and the residue was purified by silica gel CC using *n*-hexane/EtOAc (8:2, vol/vol) to give the DMDS adduct **4**. EI–MS (relative intensity %) (70 eV) *m/z* 550 $[M]^+$ (5), 533 (1), 506 (1.5), 452 (2), 419 (4), 404 (4), 390 (9), 355 (4), 340 (8), 323 (16), 309 (31), 239 (10), 226 (19), 183 (14), 167 (22), 154 (37), 131 (56), 111 (28), 97 (49), 83 (47.5), 69 (58), 57 (68.5), 55 (100); high resolution EI–MS *m/z* 131.0898 $[M]^+$ (calcd. for $C_7H_{15}S$, 131.0894).

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous crystals ($CHCl_3$), m.p. 73–75°C. The molecular formula of **1** was determined to be $C_{18}H_{34}O_5$ by high-resolution negative ion FAB–MS (*m/z* 329.2264 $[M - 1]^-$, calcd. 329.2328 for $C_{18}H_{33}O_5$) and ^{13}C NMR spectra. Upon treatment with Ac_2O/pyridine, **1** was acetylated to furnish its acetate **1a** (Scheme 1), which showed a molecular ion at *m/z* 456, corresponding to the molecular composition of $C_{24}H_{40}O_8$ as determined by high-resolution EI–MS at *m/z* 456.2693 (calcd. for $C_{24}H_{40}O_8$, 456.2723). The IR spectrum of **1** revealed the absorption bands of hydroxyls at 3368 and 1017 cm^{-1}, and a carboxylic carbonyl at 1713 cm^{-1}. Also, the bands appearing at 2928, 2852, 1464, 1420, and 723 cm^{-1} revealed its paraffinic nature (12). Compound **1** was considered to be a straight-chain compound due to a terminal methyl group at δ 14.1 ppm (13) in its ^{13}C NMR spectrum. The ^{1}H NMR spectrum of **1** showed the presence of one terminal methyl at δ

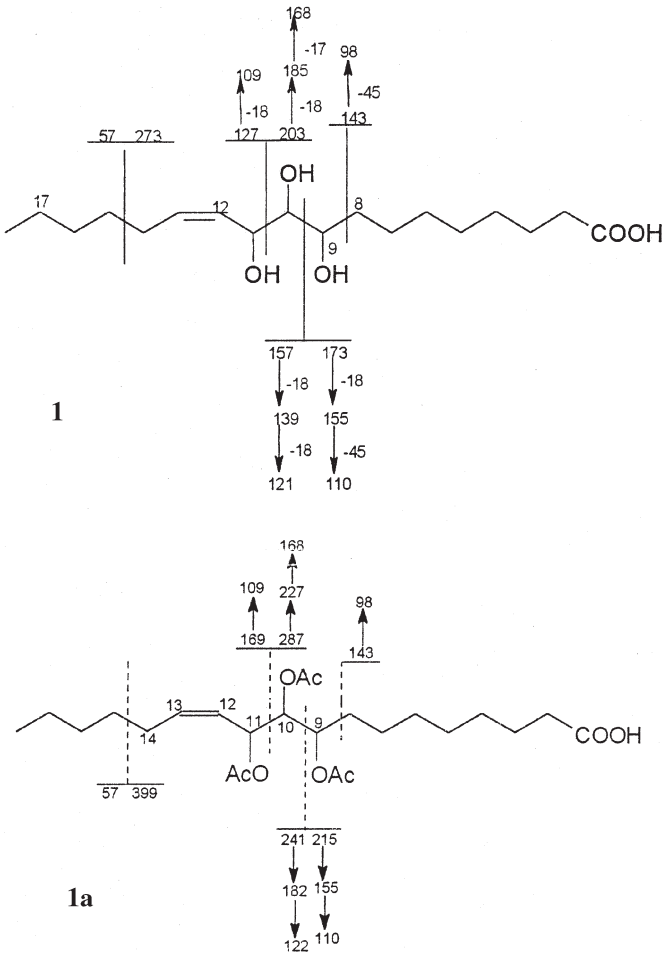


1 R = H
1a R = Ac

SCHEME 1

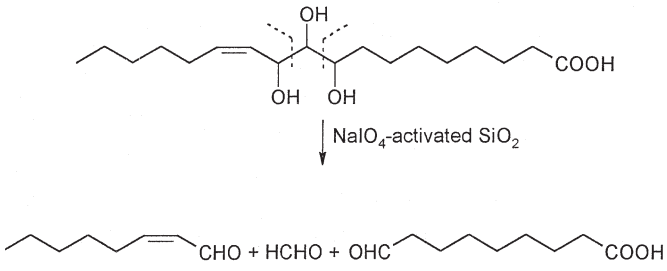
0.80 (3H, *t*), and methylenes at δ 1.19–1.38 (*br m*). The ^{13}C NMR (distortionless enhancement by polarization transfer) spectrum of **1** further furnished a quaternary carbon, 5 methines, 11 methylenes, and 1 methyl (Table 1), in which one carboxylic carbon (*C*OOH) at δ 175.9 was given. These data revealed that **1** was an unbranched fatty acid.

The ^{13}C NMR spectral signals at δ ppm 67.8 (CH), 72.6 (CH), and 78.6 (CH) and the ^{1}H NMR resonances at δ ppm 5.30 (1H, *dd*, *J* = 9.1, 3.4 Hz), 4.26 (1H, *dt*, *J* = 9.1, 2.6 Hz), and 3.93 (1H, *dd*, *J* = 6.7, 3.4 Hz) infer the existence of three hydroxy groups in the molecule. This conclusion was further confirmed by the presence of the nine-proton (3H, each) singlets of three ester methyl groups at δ 1.98, 1.99, and 2.04 in the ^{1}H NMR spectrum of **1a** as well as by a typical ion peak at *m/z* 276 due to the loss of three molecules of AcOH from the molecular ion of **1a**, respectively. A set of diagnostic fragment ions of *m/z* 173→155 and 157→139→121, 203→185→168, and 127→109 in the EI–MS of **1** (Scheme 2) showed these hydroxyls to be located at C-9, C-10, and C-11. This assignment was in turn supported by a series of characteristic mass spectral fragment ions at *m/z* 215→155 and 241→182→122, 287→227→168, and 169→109 in the EI–MS of **1a** (Scheme 2). To further prove the positions of trihydroxylic groups, two short-chain aldehydes, **2** and **3,** along with a formaldehyde (Scheme 3) were prepared by the $NaIO_4$-activated silica gel oxidation of **1**. The aldehydes thus obtained were analyzed by ^{1}H NMR and EI–MS without further separation. The conjugated aldehyde **2** with an adjacent double bond gave three typical low-field protons at δ ppm 9.51 (1H, *d*, *J* = 7.8 Hz, H-1), 6.21 (1H, *dd*, *J* = 11.0, 7.8 Hz, H-2), and 6.85 (1H, *dd*, *J* = 11.0, 7.0 Hz, H-3), which was in agreement with the spectrum of an authentic sample. The assignment of H-2 appearing at δ 6.12 ppm is based on the conjugated effect in **2**. Because the olefinic protons of **2** had the coupling constant of $J_{2,3}$ = 11.0 Hz, the double bond was deduced to be *cis*, and the position of this double bond turned out to be at C-2/C-3 due to the coupling between the olefinic proton (H-2) and the aldehydic proton (H-1) by $J_{1,2}$ = 7.8 Hz. Additionally, the aldehyde protons of **3** were recognized as a triplet at δ ppm 9.76 (*t*, *J* = 1.8 Hz, H-9). The absence of a methyl group and the presence of two methylenes as triplets at δ 2.34 and 2.42, respectively, indicate that the carboxyl group was present in **3**. These data show that the oxidation products had key structures **2** and **3**. The precursor of **3**, namely, 9-oxononanoic acid, was found to be 9-hydroperoxy-10,12-octadecadienoic acid (9-HPODE) (14).



SCHEME 2

Furthermore, the ^{1}H NMR signals at δ 5.60 (1H, *dt*, *J* = 11.0, 7.4 Hz) and 6.16 (1H, *dd*, *J* = 11.0, 9.1 Hz) and ^{13}C NMR signals at δ 131.5 (CH) and 132.6 (CH) indicated the presence of a disubstituted double bond in **1**. The location and configuration of the double bond were determined as follows. The corresponding fragment ions at *m/z* 57, 127, and 203 due to the formation of $[C_4H_9]^+$, $[CH_3(CH_2)_4CH{=}CHCH(OH)]^+$, and $[CH(OH)CH(OH)(CH_2)_7COOH]^+$ species arising from allylic cleavages between C-14 and C-15 and between C-10 and C-11 supported the location of the olefinic linkage between C-12 and C-13 in the molecule, as evidenced from allylic cleavages of **1a** at *m/z* 57 $[C_4H_9]^+$,169 $[CH_3(CH_2)_4CH{=}CHCH(OAc)]^+$, and 287 $[CH(OAc)CH(OAc)(CH_2)_7COOH]^+$ fragment ions.



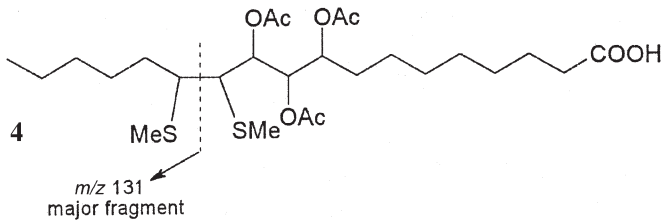
SCHEME 3

Moreover, the high-resolution EI–MS spectrum of the DMDS derivative **4** of **1a** showed a remarkable fragment-ion peak at *m/z* 131.0898, corresponding to the chemical composition of $C_7H_{15}S$ (calcd. 131.0894) due to cleavage of the bond between the carbons bearing a methylthio group (Scheme 4). This result also supports the location of the double bond between C-12 and C-13 in **1**.

It is known, on the other hand, that the geometry of the double bond in a long-chain alkene can be determined from the ^{13}C NMR chemical shift of the methylene carbon next to the olefinic carbon, namely, the carbon signal observed between δ 27–28 ppm in *cis* type and between δ 32–33 ppm in *trans* type (15). The *cis* stereochemistry of this double bond was deduced from the chemical shift of C-14 (δ = 28.2 ppm). This *cis* configuration was also supported by the large vicinal coupling constant ($J_{12,13}$ = 11.0 Hz) displayed between H-12 and H-13. From the ^{1}H-^{1}H correlation spectroscopy spectrum, the correlations between H-8 at δ 1.91 (*m*) and H-9 at δ 4.26 (*m*), H-9 and H-10 at δ 3.93 (*dd*), H-10 and H-11 at δ 5.30 (*dd*), H-11 and H-12 at δ 6.16 (*dd*), H-12 and H-13 at δ 5.60 (*dt*), and H-13 and H-14 at δ 2.10 (*m*) were observed in **1**. It strongly confirmed that **1** contained the partial structure -CH_2-HC=CH-CH(OH)-CH(OH)-CH(OH)-CH_2- (C-8 to C-14). Consequently, the preceding evidence led to the establishment of the structure of **1** as 9,10,11-trihydroxy-(12*Z*)-12-octadecenoic acid (Scheme 1).

The major fatty acids from a $CHCl_3$-soluble extract of *T. indicum* were linoleic acid (LA) (61.6%), stearic acid (16.0%), palmitic acid (13.7%), and oleic acid (OA) (6.66%). The high level of unsaturated fatty acids (68%) may contribute in part to explaining the nutritional quality of this mushroom.

Lipid peroxidation processes induced by lipoxygenase (LOX; EC 1.13.11.12), a nonheme iron-containing dioxygenase enzyme, are reported to occur in plant systems; they were observed, for instance, in *Rudbeckia fulgida* (16), in wheat flour suspensions (17), and in rice plants (18). The oxidation of the two unsaturated acids, LA and linolenic acid, is then catalyzed by LOX to lead, *via* an antarafacial process, to the hydroperoxide 9-HPODE or 13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE), and to mixtures of 9- and 13-HPODE depending on LOX type and appropriate conditions (14). Subsequently, HPODE are metabolized in the presence of various enzymes to mono-, epoxy-, and trihydroxy fatty acids and even a cascade of catabolic products. According to the pathway of metabolism, it is possible that the previously unknown metabolite reported in the present study is biosyn-

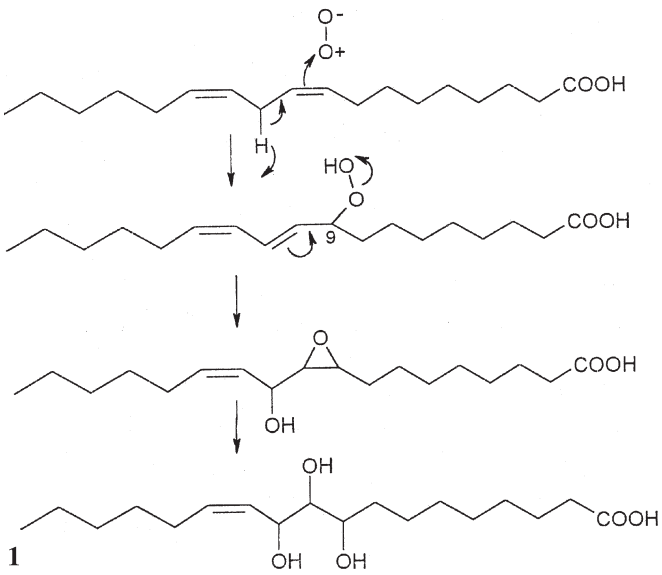


SCHEME 4

thetically derived from LA *via* the intermediate 9-HPODE (Scheme 5) in *T. indicum*. Hermann and Erwin (19) found that 9,10,11-trihydroxy-12-*trans*-octadecenoic acid from LA, a geometrical isomer of the title compound **1**, was present in the bee and may be considered one of the components responsible for stale bee flavors. There is no doubt that the first discovery of **1** could be required for an understanding of the role of *T. indicum* in mushroom metabolism.

The unsaturated fatty acids possess a broad spectrum of biological properties in both animals and plants. In particular, LA-derived hydroxylated unsaturated C-18 fatty acids have attracted considerable attention. Some of these compounds show biological activity, e.g., 9,10-dihydroxy-8-oxo-12*Z*-octadecaenoic acid exhibits cytotoxicity against HeLa cells and an inhibitory effect on tea pollen growth (9); 9*S*,12*S*,13*S*-trihydroxyoctadeca-10*E*-enoic acid is active against rice blast fungus (18); 9,10,13-trihydroxy-*trans*-11-octadecaenoic acid has prostaglandin E-like activity (20); and 13*S*-hydroxy-9,11-octadecadienoic acid (*S*-coriolic acid) shows nematocidal action (11). A fatty acid mixture consisting of linoleic, oleic, and palmitic acids as main components showed nematocidal activity, and the most active compound was LA with 50% inhibition lethal dose (LD_{50}) values between 5 and 10 μg/mL (11). LA and OA displayed antibacterial activity, the former inhibiting the growth of all the Gram-positive bacterial species with a minimum inhibitory concentration between 0.01 and 1.0 mg/mL, whereas the latter is active against three of the five Gram-positive bacteria at a minimum inhibitory concentration of 1.0 mg/mL. A synergistic effect between the two fatty acids was observed against *Staphylococcus aureus* and *Micrococcus kristinae* (21). The fungal metabolites that may be formed in the fruiting bodies of macrofungi constitute their chemical defense system against parasites as well as various predators such as bacteria, fungi, animals, and insects (22). This newly isolated fatty acid, in combination with LA and OA, might be presumed to act as phytoalexins existing in hypogenous fungi. Further investigation will be performed.

LA inhibits the activities of mammalian DNA polymerases; the addition of ergosterol peroxide to a polymerase reaction mixture led to selective enhancement of the inhibitory effect of LA on DNA polymerase β (23). Fortuitously, we have previously reported finding this sterol in this mushroom (2). The occurrence of LA and the sterol further offers the possibility of gaining insight into the biological functions of truffles.



SCHEME 5

ACKNOWLEDGMENTS

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