Conjugated Ketonic Fatty Acids from *Pleurocybella porrigens*

Yoshiaki AMAKURA,*[,a,1)] Kazunari KONDO,[a] Hiroshi AKIYAMA,[a] Hideyuki ITO,[b] Tsutomu HATANO,[b] Takashi YOSHIDA,[c] and Tamio MAITANI[a]

[a] *National Institute of Health Sciences; 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan:* [b] *Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; 1–1–1 Tsushima-naka, Okayama 700–8530, Japan: and* [c] *Faculty of Pharmaceutical Sciences, Matsuyama University; 4–2 Bunkyo-cho, Matsuyama, Ehime 790–8578, Japan.*
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Three novel conjugated long-chain fatty acids (1—3) were obtained from aqueous methanol extracts of *Pleurocybella porrigens* together with nine known constituents including (8*E*,10*E*)-7,12-dioxo-8,10-octadecadienoic acid (ostopanic acid) (4). The structures of the new fatty acids were characterized as (14*RS*)-(10*E*,12*E*)-14-hydroxy-9-oxo-10,12-octadecadienoic acid (1), (12*RS*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid (2), and (10*E*,12*E*)-9,14-dioxo-10,12-octadecadienoic acid (3) by spectroscopic methods.

Key words *Pleurocybella porrigens*; Sugihiratake; Tricholomataceae; conjugated ketonic fatty acid

The fungus *Pleurocybella porrigens* (Japanese name: Sugihiratake), a basidiomycete of the Tricholomataceae family, is a small, flat, white mushroom. This edible mushroom grows during the late summer and autumn and is native to all districts of Japan including the Tohoku and Hokuriku areas. In the autumn of 2004, several perplexing acute encephalopathy cases were diagnosed in the Tohoku and Hokuriku areas, and the intake of this mushroom was identified as one of the common factors in these cases.[2—6)] As a first step to clarify the relationship between the acute encephalopathy cases and this mushroom, we conducted an investigation to determine the chemical constituents of *P. porrigens*.[7)] In the course of this work, we isolated and characterized three novel conjugated long-chain fatty acids (compounds **1**—**3**) together with nine known compounds [(8*E*,10*E*)-7,12-dioxo-8,10-octadecadienoic acid (ostopanic acid) (**4**),[8,9)] tryptophan, adenine, *trans*-cinnamic acid, ergosterol,[10)] triolein,[11)] α- and β-eleostearic acids,[12,13)] and methyl α-eleostearate[14)]] from aqueous methanol extract of *P. porrigens*. This paper deals with the structural elucidation of these new compounds based on spectroscopic methods.

Results and Discussion

A 50% aqueous methanol extract of *P. porrigens* was fractionated by chromatography on Diaion HP-20 with aqueous MeOH, then each fraction showing similar patterns on HPLC or TLC was combined. Further purification was performed by a combination of chromatography over MCI-gel CHP 20P with a step-wise gradient elution using aqueous MeOH, preparative HPLC, and preparative TLC to afford three novel compounds (**1**—**3**) along with nine known constituents.

Compound **1** was obtained as a pale yellow solid. Its molecular formula $C_{18}H_{30}O_4$ was determined by high-resolution (HR)-electrospray ionization (ESI)-MS. ^{1}H-NMR and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra of **1** showed signals due to two sets of hydrogens on *trans*-olefinic carbons coupled with each other [δ 6.24 (1H, d, J=15 Hz, H-10), 6.29 (1H, dd, J=6.6, 15 Hz, H-13), 6.45 (1H, dd, J=10.2, 15 Hz, H-12), 7.30 (1H, dd, J=10.2, 15 Hz, H-11)], one methine [4.21 (1H, m, H-14)], ten methylene [1.33—1.41 (8H, m, H-4, 5, 6, 17), 1.58 (2H, m, H-15), 1.62—1.66 (6H, m, H-3, 7, 16), 2.23 (2H, t, J=7.2 Hz, H-2), 2.66 (2H, t, J=7.2 Hz, H-8)], and one methyl [0.97 (3H, t, J=7.2 Hz, H-18)] hydrogens. The ^{13}C-NMR spectrum showed carbon resonances due to a carboxylic acid at δ 180.0 (C-1) and a carbonyl carbon at δ 203.9 (C-9) along with four olefinic carbons [δ 128.7 (C-12), 130.4 (C-10), 144.3 (C-11), 148.4 (C-13)], one tertiary carbon possessing a hydroxyl group [δ 72.6 (C-14)], ten methylene carbons [δ 23.7 (C-17), 25.6 (C-7), 27.2 (C-3), 28.7 (C-16), 30.2, 30.3, 30.5 (C-4, 5, 6), 37.8 (C-15), 37.9 (C-2), 41.1 (C-8)], and a methyl carbon [δ 14.4 (C-18)]. These spectral features along with four unsaturation degrees in the molecular formula and UV data suggested **1** to be a C18 unsaturated oxo fatty acid with linear structure. The ^{1}H–^{13}C heteronuclear multiple-bond connectivity (HMBC) spectrum showed long-range correlations as follows: H-2 and C-1, 3, 4; H-8 and C-6, 7, 9; H-10 and C-9, 12; H-11 and C-9, 10, 13; H-12 and C-11, 14; H-13 and C-11, 14; H-14 and C-12; H-15 and C-13, 14, 16, 17; and H-18 and C-16, 17, indicating the locations of a carbonyl carbon at C-9 and a tertiary carbon at C-14. Although the proposed structure (**1**) has an asymmetric carbon at C-14, **1** was regarded as a racemate

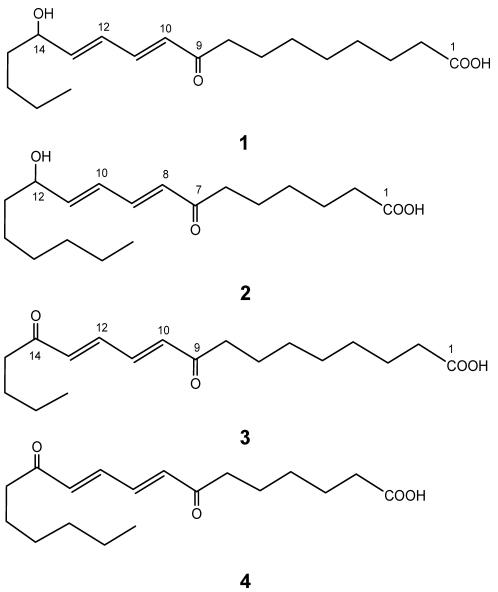

Fig. 1

* To whom correspondence should be addressed. e-mail: amakura@nihs.go.jp

because of its optical inactivity {$[\alpha]_D^{23}$ ±0° (EtOH)}. Thus compound **1** was characterized as (14*RS*)-(10*E*,12*E*)-14-hydroxy-9-oxo-10,12-octadecadienoic acid (**1**).

Compound **2** was isolated as a pale yellow solid, $[\alpha]_D^{23}$ ±0° (c=0.5, EtOH). The HR-ESI-MS of **2** had a molecular ion peak at m/z 333.2347 $[M+Na]^+$, consistent with the molecular formula $C_{18}H_{30}O_4$, and its UV spectrum was very similar to that of **1**. The patterns of ^{1}H- and ^{13}C-NMR spectra of **2** were also closely similar to those of **1**. The HMBC spectrum gave the following long-range correlations: H-2 and C-1, 3, 4; H-6 and C-4, 5, 7; H-8 and C-7, 10; H-9 and C-7, 11; H-11 and C-9, 12; H-12 and C-10, 11, 14; H-13 and C-11, 12; and H-18 and C-16, 17, indicating the locations of a carbonyl carbon at C-7 and a tertiary carbon bearing a hydroxyl group at C-12. In addition, its optical inactivity indicated that **2** was also in a racemic form, and therefore this compound was assigned as (12*RS*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid.

Compound **3**, a pale yellow solid, was assigned the molecular formula $C_{18}H_{28}O_4$ as deduced from its HR-ESI-MS. Although the ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **1** and **2**, **3** showed an additional carbonyl carbon signal instead of the signal due to a tertiary carbon bearing a hydroxyl group [C (H)-14 of **1** or C (H)-12 of **2**] in the ^{13}C-NMR. Additionally, ^{1}H-NMR and COSY spectra of **3** indicated the presence of four conjugated olefinic proton signals at around δ 6.6 and 7.3 (each 2H). These spectral features suggested that **3** is a straight-chain fatty acid with a conjugated diene–dione moiety, which was verified by spectral similarity between **3** and ostopanic acid, (8*E*,10*E*)-7,12-dioxo-8,10-octadecadienoic acid, (**4**) with a *E*,*E*-dienyl diketone structure. The structural difference between **3** and **4** was inferred from the HMBC spectrum of **3**, which showed the following correlations: H-2 and C-1, 4; H-3 and C-1; H-7, 16 and C-9, 14; H-8, 15 and C-7, 9, 14, 17; H-10, 13 and C-8, 9, 11, 12, 14, 15; H-11, 12 and C-9, 10, 13, 14; and H-18 and C-16, 17, indicating the locations of carbonyl carbons at C-9 and C-14. Based on these data, the structure containing a conjugated 10,12-diene-9,14-dione moiety was assigned to **3**. Thus compound **3** was characterized as (10*E*,12*E*)-9,14-dioxo-10,12-octadecadienoic acid.

Experimental

General Optical rotations were measured by Jasco P-1020 digital polarimeter. UV spectra were obtained by Shimadzu UVmini-1240 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded in methanol-d_4 (CD_3OD) on a Varian INOVA AS600 instrument (600 MHz for ^{1}H and 150 MHz for ^{13}C). The chemical shifts are given in δ (ppm) based on those of the solvent signals (δ_H 3.31; δ_C 49.0) in a tetramethylsilane (TMS) scale. HR-ESI-MS were recorded on JEOL JMS-T100LC mass spectrometer. HPLC using a photo-diode array detector was performed at 40 °C as follows: (Condition 1) column: L-column ODS (5 μm, 150×4.6 mm i.d., Chemicals Evaluation and Research Institute, Tokyo, Japan), mobile phase: acetonitrile–3% acetic acid in water at a flow rate of 1.0 ml/min, (Condition 2) column: L-column ODS (5 μm, 250×10 mm i.d., Chemicals Evaluation and Research Institute, Tokyo, Japan), mobile phase: acetonitrile–3% acetic acid in water at a flow rate of 3.0 ml/min, and detection at 280 nm. Column chromatography was performed with Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan) and MCI GEL CHP 20P (75—150 μm, Mitsubishi Chemical Corporation). TLC was performed on Silica gel 60 F_{254} plates (0.2, 1.0 mm layer thickness, Merck, Darmstadt, Germany), and the spots were detected by ultraviolet irradiation (254, 365 nm). All other chemicals were of analytical reagent grade.

Material *P. porrigens* samples were collected during the fall of 2004 from local health centers and the prefectural institutes of Public Health and Environment in Japan through the Ministry of Health, Labour and Welfare of Japan.

Extraction and Isolation Fresh bodies of *P. porrigens* (*ca.* 1 kg) were homogenized in 50% aqueous MeOH (6 l) then filtered. The residue was further extracted with 50% aqueous MeOH (3 l) at room temperature. The combined filtrate was concentrated *in vacuo*. The concentrated solution was passed through a Diaion HP-20 column with a step-wise gradient of aqueous MeOH, then all fractions containing the same HPLC peak or TLC spot were combined. Further fractionation and purification were performed by chromatography over MCI-gel CHP 20P with aqueous MeOH in a step-wise gradient mode, preparative HPLC (Condition 2) with the following conditions: linear gradients of acetonitrile–3% acetic acid in water from 5 : 95 to 95 : 5 in 50 min], and preparative TLC [Silica gel 60 F_{254} plates (1.0 mm layer thickness), solvent: $CHCl_3$–MeOH–acetic acid (95 : 5 : 0.03), *n*-hexane–ethyl acetate (9 : 1)] to give three novel compounds [(**1**) (2.5 mg), (**2**) (1.7 mg), and (**3**) (1.6 mg)] along with (8*E*,10*E*)-7,12-dioxo-8,10-octadecadienoic acid (ostopanic acid) (**4**) (1.5 mg), tryptophan (8.0 mg), adenine (1.5 mg), *trans*-cinnamic acid (1.5 mg), ergosterol (6 mg), triolein (15 mg), α-eleostearic acid (11 mg), β-eleostearic acid (1.0 mg), and methyl α-eleostearate (3.0 mg).

Compound **1**: Pale yellow solid. $[\alpha]_D^{23}$ ±0° (c=1.0, EtOH). UV λ_{max} (EtOH) nm (log ε): 273 (4.37). ^{1}H-NMR δ: 0.97 (3H, t, J=7.2 Hz, H-18), 1.33—1.41 (8H, m, H-4, 5, 6, 17), 1.58 (2H, m, H-15), 1.62—1.66 (6H, m, H-3, 7, 16), 2.23 (2H, t, J=7.2 Hz, H-2), 2.66 (2H, t, J=7.2 Hz, H-8), 4.21 (1H, m, H-14), 6.24 (1H, d, J=15 Hz, H-10), 6.29 (1H, dd, J=6.6, 15 Hz, H-13), 6.45 (1H, dd, J=10.2, 15 Hz, H-12), 7.30 (1H, dd, J=10.2, 15 Hz, H-11). ^{13}C-NMR δ: 14.4 (C-18), 23.7 (C-17), 25.6 (C-7), 27.2 (C-3), 28.7 (C-16), 30.2, 30.3, 30.5 (C-4, 5, 6), 37.8 (C-15), 37.9 (C-2), 41.1 (C-8), 72.6 (C-14), 128.7 (C-12), 130.4 (C-10), 144.3 (C-11), 148.4 (C-13), 180.0 (C-1), 203.9 (C-9). HR-ESI-MS m/z: 333.2039 $[M+Na]^+$ (Calcd for $C_{18}H_{30}O_4$+Na, 333.2042).

Compound **2**: Pale yellow solid. $[\alpha]_D^{23}$ ±0° (c=0.5, EtOH). UV λ_{max} (EtOH) nm (log ε): 273 (4.33). ^{1}H-NMR δ: 0.95 (3H, t, J=7.2 Hz, H-18), 1.33—1.40 (8H, m, H-4, 15, 16, 17), 1.58 (2H, m, H-13), 1.62—1.66 (6H, m, H-3, 5, 14), 2.26 (2H, t, J=7.2 Hz, H-2), 2.66 (2H, t, J=7.2 Hz, H-6), 4.21 (1H, m, H-12), 6.23 (1H, d, J=15 Hz, H-8), 6.28 (1H, dd, J=6, 15 Hz, H-11), 6.45 (1H, dd, J=10.2, 15 Hz, H-10), 7.31 (1H, dd, J=10.2, 15 Hz, H-9). ^{13}C-NMR δ: 14.4 (C-18), 23.7 (C-17), 25.5 (C-14), 26.2 (C-5), 26.7 (C-3), 30.0, 30.3 (C-4, 15), 32.9 (C-16), 36.9 (C-2), 38.0 (C-13), 41.0 (C-6), 72.6 (C-12), 128.8 (C-10), 130.4 (C-8), 144.3 (C-9), 148.4 (C-11), 179.0 (C-1), 203.7 (C-7). HR-ESI-MS m/z: 333.2023 $[M+Na]^+$ (Calcd for $C_{18}H_{30}O_4$+Na, 333.2042).

Compound **3**: Pale yellow solid. UV λ_{max} (EtOH) nm (log ε): 279 (4.14). ^{1}H-NMR δ: 0.98 (3H, t, J=7.2 Hz, H-18), 1.33—1.41 (8H, m, H-4, 5, 6, 17), 1.63—1.66 (6H, m, H-3, 7, 16), 2.26 (2H, t, J=7.2 Hz, H-2), 2.71 (4H, t, J=7.2 Hz, H-8, 15), 6.62, 6.64 (each 1H, m, H-10, 13), 7.33, 7.35 (each 1H, m, H-11, 12). ^{13}C-NMR δ: 14.2 (C-18), 23.3 (C-17), 25.1 (C-7), 26.7 (C-3), 27.3 (C-16), 30.2, 30.3, 30.4 (C-4, 5, 6), 36.8 (C-2), 41.4, 41.6 (C-8, 15), 137.3, 137.4 (C-10, 13), 140.8, 140.9 (C-11, 12), 178.5 (C-1), 202.7, 202.8 (C-9, 14). HR-ESI-MS m/z 331.1861 $[M+Na]^+$ (Calcd for $C_{18}H_{28}O_4$+Na, 331.1885).

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References and Notes

1) Present address: *Faculty of Pharmaceutical Sciences, Matsuyama University, 4–2 Bunkyo-cho, Matsuyama, Ehime 790–8578, Japan*.
2) Obara K., Okawa S., Kobayashi M., Takahashi S., Watanabe S., Toyoshima I., *Rinsho Shinkeigaku*, **45**, 253—256 (2005).
3) Kuwabara T., Arai A., Honma N., Nishizawa M., *Rinsho Shinkeigaku*, **45**, 239—245 (2005).
4) Kurokawa K., Sato H., Nakajima K., Kawanami T., Kato T., *Rinsho Shinkeigaku*, **45**, 111—116 (2005).
5) Kato T., Kawanami T., Shimizu H., Kurokawa K., Sato H., Nakajima K., Nomoto T., Seta T., Kamei T., Yoshino H., Sasagawa I., Ito M., Karasawa S., Kimura H., Suzuki Y., Degawa N., Tagawa A., Ataka K., Ando S., Omae T., Shikama Y., *No To Shinkei*, **56**, 999—1007 (2004).
6) Gejyo F., Honma N., Higuchi N., Ataka K., Teramura T., Alchi B., Suzuki Y., Nishi S., Narita I., *Kidney Int.*, **68**, 188—192 (2005).
7) Akiyama H., Toida T., Sakai S., Amakura Y., Kondo K., Sugita-Konishi Y., Maitani T., *J. Health Sci.*, **52**, 73—77 (2006).

8) Hamburger M., Handa S. S., Cordell G. A., Kinghorn A. D., Farnsworth N. R., *J. Nat. Prod.*, **50**, 281—283 (1987).
9) Sheu J.-H., Yen C.-F., Huang H.-C., Hong Y.-L. V., *J. Org. Chem.*, **54**, 5126—5128 (1989).
10) Yokokawa H., Mitsuhashi T., *Phytochemistry*, **20**, 1349—1351 (1981).
11) Meara M. L., *J. Chem. Soc.*, **1949**, 2154—2157 (1949).
12) Chisholm M. J., Hopkins C. Y., *J. Org. Chem.*, **27**, 3137—3139 (1962).
13) Dyer J. M., Chapital D. C., Kuan J. C., Mullen R. T., Turner C., McKeon T. A., Pepperman A. B., *Plant Physiol.*, **130**, 2027—2038 (2002).
14) Tolberg W. E., Paschke R. F., Wheeler D. H., *J. Am. Oil Chem. Soc.*, **38**, 102—104 (1961).