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Acetylenic acid analogues from the edible mushroom Chanterelle (*Cantharellus cibarius*) and their effects on the gene expression of peroxisome proliferator-activated receptor-gamma target genes

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ABSTRACT

A new acetylenic acid, (10*E*,14*Z*)-9-oxooctadeca-10,14-dien-12-ynoic acid (**1**), was isolated from the edible mushroom Chanterelle (*Cantharellus cibarius*), together with a known acetylenic acid, (10*E*,14*Z*)-9-hydroxyoctadeca-10,14-dien-12-ynoic acid (**2**) and their structures were determined through analysis of NMR and mass data. The new acetylenic acid (**1**) specifically activated peroxisome proliferator-activated receptor (PPAR)-γ with an EC_{50} value of 1.88 μM as measured by a reporter gene assay. Expression of PPAR-γ target genes were significantly altered as well, supporting the hypothesis that compound **1** is a PPAR-γ potential agonist that regulates transcription of the PPAR-γ target genes.

The peroxisome proliferator-activated receptors (PPARs) comprise an important subfamily of the nuclear hormone receptor superfamily that plays a critical role in the regulation of energy metabolism, cell proliferation, inflammation, and immunity.[1–3] Three subtypes of the PPAR nuclear fatty acid receptors have been identified: PPAR-α, PPAR-γ and PPAR-δ. PPAR-γ has been shown to be an important regulator of target genes involved in glucose and lipid metabolism. Therefore, PPAR-γ agonists are efficacious antidiabetic agents.[4–7] Moreover, these agonists have therapeutic potential in the treatment of other disease states such as atherosclerosis, inflammatory disease, and several cancers.[8–12]

In our search for biologically active agents derived from natural sources, a methanol extracts of *Cantharellus cibarius* Fr. (Chanterelle) exhibited significant activity in a reporter gene assay of PPAR-γ. The *C. cibarius* is an edible mushroom with a worldwide distribution, fruiting from fall to early spring. Its fruit body has been shown to protect against various diseases through its antioxidant activity. Several fatty acids, phenolic compounds, organic acid, carotenoids, and steroids from this mushroom have been shown to exhibit antioxidant and anti-inflammatory properties.[13–16] However, to the best of our knowledge, nothing has been reported about this species regarding the transcriptional activity on PPAR-γ. Bioassay-guided fractionation of the methanol extract of *C. cibarius* using a PPAR-γ reporter gene assay led to isolation of a new acetylenic acid, (10*E*,14*Z*)-9-oxooctadeca-10,14-dien-12-ynoic acid (**1**), as well as a known acetylenic acid, (10*E*,14*Z*)-9-hydroxyoctadeca-10,14-dien-12-ynoic acid (**2**) (Fig. 1).[13] We

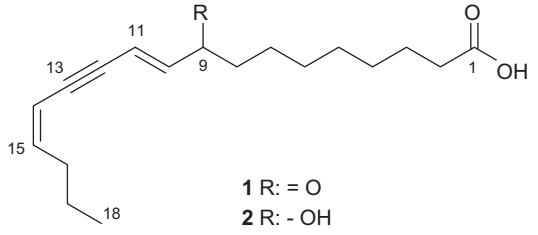

Figure 1. Structures of compounds **1** and **2**.

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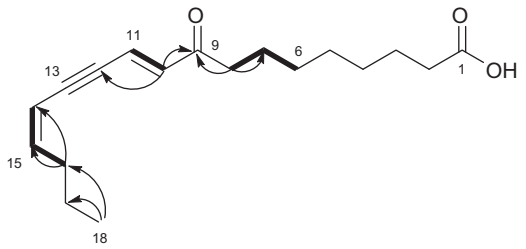

Figure 2. Selected HMBC (→) and COSY (—) correlations of compound **1**.

describe herein the isolation, structure elucidation, and biological evaluation of the compounds isolated.

The dry material of *C. cibarius* (9 kg) was extracted three times with MeOH at room temperature. Removal of the solvent in vacuo yielded a MeOH extract (2 kg). The MeOH extract was suspended in distilled water and then partitioned with *n*-hexane and EtOAc (35 g), successively. A portion of the EtOAc-soluble layer (30 g) was subjected to silica gel CC (70–230 mesh; $\Phi = 7$ cm, $L = 25$ cm), and eluted with a stepwise gradient of $CHCl_3$–MeOH system (1:0, 80:1, 50:1, 30:1, 17:1, 10:1, 7:1, 3:1, v/v) to yield 10 fractions (CCE1 ~10). Fraction CCE5 (2.3 g) was subjected to RP-18 CC ($\Phi = 5$ cm, $L = 35$ cm) eluted with MeCN–H_2O (stepwise, 50:50 to 100:0, v/v) to give 13 fractions (CCE5-1 ~13). Among the above 13 fractions, compound **1** (10.4 mg)[17] and compound **2** (33.5 mg)[17] were isolated from fraction CCE5-7 (550 mg) by column chromatography ($\Phi = 3$ cm, $L = 15$ cm) on silica gel using mixture of $CHCl_3$–acetone (stepwise, 1:0, 80:1, 50:1, 30:1, 20:1, 10:1, 5:1, 3:1, 2:1, v/v) as an eluting solvent.

Compound **1** was isolated as a colorless oil, in which the molecular formula was established as $C_{18}H_{26}O_3$ by negative HRESIMS (*m/z* 289.1952 $[M-H]^-$; calcd for $C_{18}H_{25}O_3$, 289.1960). The ^{13}C NMR and HSQC spectra of the compound **1** revealed 9 saturated methylene carbons, one primary methyl group (δ_C 13.7), four olefinic carbons (δ_C 147.0, 136.4, 123.3, and 108.7), one acetylenic functionality (δ_C 96.4, and 91.0), one carboxyl group (δ_C 178.7), and one ketone group (δ_C 199.3). A carbonyl carbon signal at δ_C 178.7 in ^{13}C NMR spectrum, and the presence of a broad methylene protons signal at ca. δ_H 1.34 indicated that compound **1** was a fatty acid. The presence of an acetylenic function was evident from the presence of two quaternary carbon signals at δ_C 96.4 and 91.0. Connectivities from C-18 to C-14 were straight forward by interpretation of ^1H–^1H COSY and HMBC spectra (Fig. 2). The HMBC cross-peak between H-10 (δ_H 6.49) and C-12 (δ_C 91.0) together with the upfield shift of C-11 (δ_C 123.3) and C-14 (δ_C 108.7) indicated that one of the acetylene group could be placed between the two double bonds.[18,19] The location of the ketone group was assigned by the observed HMBC correlations from H-8 (δ_H 2.55) and H-10 (δ_H 6.49) to C-9 (δ_C 199.3). These correlations clearly indicated that a ketone group was located at C-9. Further, the ^1H–^1H COSY cross-peaks were observed for H-2/H-3, H-3/H-4, and H-7/H-8. We assigned *E*- and *Z*-geometry of two double bonds, C-10/C-11 and C-14/C-15, on the basis of their coupling constants of 16.0 and 11.0 Hz, respectively. Therefore, the structure of the new acetylenic acid (**1**) was determined as (10*E*,14*Z*)-9-oxooctadeca-10,14-dien-12-ynoic acid. Additionally, a known acetylenic acid was identified as (10*E*,14*Z*)-9-hydroxyoctadeca-10,14-dien-12-ynoic acid (**2**) by analysis of its NMR data and comparison of its physical and spectral data with those of literature values.[13]

PPAR transactivation ability of both compounds **1** and **2** was investigated using a reporter gene assay in CHO cells cotransfected with a PPAR-γ expression vector and a PPRE-driven luciferase reporter-gene construct.[20] Compounds **1** and **2** did not activate PPAR-α or PPAR-δ at concentrations up to 20 μg/mL. However, the new acetylenic acid (**1**) did selectively activate PPAR-γ with an EC_{50} value of 1.88 μM, while compound **2** was inactive (EC_{50} value >20 μg/mL). Under the same experimental conditions, the EC_{50} value of troglitazone, a PPAR-γ agonist used as a positive control, was 0.44 μM. Next we further analyzed the effects of compound **1** on PPAR-γ target gene transcription in HepG2 hepatocytes using quantitative real-time PCR (Fig. 3).[21] PPAR-γ expression was upregulated dose-dependently up to 2.43-fold. Although compound **1** did not alter the expression of SCD-1, a gene regulating the rate-limiting step of monounsaturated fatty acid synthesis, compound **1** reduced the expression of two

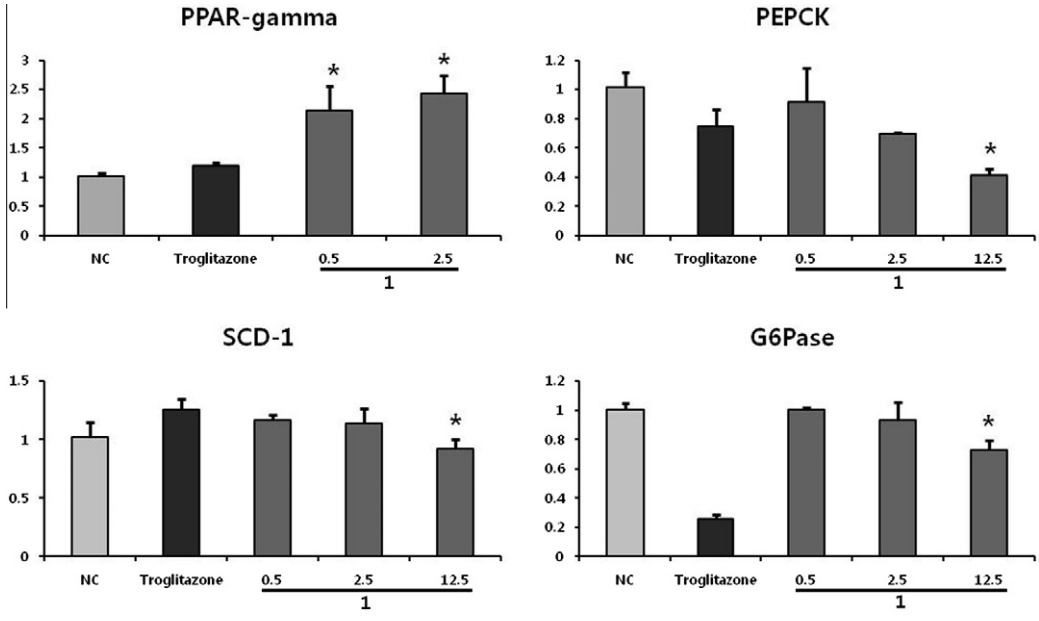

Figure 3. The expression of PPAR-γ target genes in the HepG2 hepatocytes. Cells were stimulated with either troglitazone (10 μM) or compound **1** at various concentrations (μM) and the expression of genes was assessed with real-time PCR. NC: nontreated control; PEPCK: phosphoenolpyruvate carboxykinase; SCD-1: stearyl CoA desaturase; G6Pase: glucose-6 phosphatase.*P <0.05 compared with NC.

Table 1
NMR data compounds **1** and **2** in $CDCl_3$[a]

No.	δ_C		δ_H, mult (x*J* in Hz)			
	1	**2**	**1**		**2**	
1	178.7	178.9				
2	33.6	33.7	2.35	t (7.5)	2.35	t (7.5)
3	24.6	24.8	1.63	m	1.64	m
4	28.8	28.9	1.34	m	1.33	m
5	29.0	29.0	1.34	m	1.33	m
6	29.0	29.2	1.34	m	1.33	m
7	24.0	25.2	1.64	m	1.64	m
8	41.0	37.2	2.55	t (7.5)	1.55	m
9	199.3	72.4			4.18	dd (12.5, 6.5)
10	136.4	144.7	6.49	d (16.0)	6.11	dd (15.5, 6.0)
11	123.3	114.3	6.80	dd (16.0, 2.5)	5.86	td (15.5, 1.5)
12	91.0	91.0				
13	96.4	88.0				
14	108.7	108.9	5.66	dd (11.0, 2.5)	5.58	dd (10.5, 1.5)
15	147.0	144.5	6.08	td (11.0, 7.5)	5.92	td (10.5, 7.5)
16	32.6	32.4	2.32	ddd (15.0, 6.0, 1.5)	2.30	ddd (15.0, 6.0, 1.5)
17	22.0	22.2	1.47	dd (15.0, 7.5)	1.45	dd (15.0, 7.5)
18	13.7	13.8	0.95	t (7.5)	0.95	t (7.5)

[a] The assignments were based on DEPT, HSQC, and HMBC experiments.

gluconeogenesis genes, G6Pase and PEPCK, comparable to the effect of troglitazone. These results suggest that the acetylenic acid analogue isolated from *C. cibarius* with PPAR-γ transcriptional activity, may have a benefit in the treatment of diabetes. However, further pharmacological investigation and in vivo physiological functions of this compound are needed.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.070.

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17. *Cantharellus cibarius* was purchased from Forest Wild Mushroom Business, Chengdu City, China on January 2007 and identified by one of the authors, H. Z. Jin. A voucher specimen (200701-CC) has been deposited at College of Life Sciences and Biotechnology, Korea University, Seoul, Korea.(10*E*,14*Z*)-9-oxooctadeca-10,14-dien-12-ynoic acid (**1**):Colorless oil; UV (MeOH) λ_{max} (log ε) 218 (3.95), 309 (4.21) nm; IR (KBr) ν_{max} 3420, 2927, 2856, 2360, 1735, 1245, 761 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS (negative mode) *m*/*z* 289.2 $[M-H]^-$, 579.3 $[2\,M-H]^-$; HRESIMS (negative mode) *m*/*z* 289.1952 $[M-H]^-$ (calcd for $C_{18}H_{25}O_3$, 289.1960). (10*E*,14*Z*)-9-hydroxyoctadeca-10,14-dien-12-ynoic acid (**2**): Colorless oil; $[\alpha]_D^{27}$ −3.5° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max}266 (4.13), 280 nm (4.02); ^1H and ^{13}C NMR data, see Table 1; ESI-MS (negative mode) *m*/*z* 291 $[M-H]^-$, 583 $[2\,M-H]^-$.
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20. After seeding CHO cells in 24-well plates, cells were co-transfected with the reporter vector pGL3-PPRE3-TK-luc, a human PPAR-expression vector, and an expression vector encoding β-galactosidase using Hilymax reagent (Dojindo Laboratories, Rockville, MD USA). The medium was removed after 4 h and replaced with high-glucose DMEM for 18 h. The cells were treated with the compounds (0, 5, 20, 40, 60, 80, or 100 μM) or troglitazone (10 μM) for 24 h and the luciferase activity was measured using a Firefly Luciferase Assay Kit (Biotium, Inc., Hayward, CA, USA) according to the manufacturer's protocol. Data were normalized relative to the β-galactosidase activity in the same lysate using β-Galactosidase Enzyme Assay System (Promega, Madison, WI, USA).
21. Total RNA was isolated from cells with an RNAiso Plus Kit (Takara Bio Inc., Otsu, Japan), then 2 μg of total RNA was reverse-transcribed with oligo(dT) and PrimerScript Reverse Transcriptase (Takara Bio Inc.) in a 20-μL of reaction volume. PCR was performed using these primers and iQ SYBR Green Supermix reagent (Bio-Rad Laboratories, Inc.) on an iQ5 iCycler system (Bio-Rad Laboratories, Inc.). The PCR conditions consisted of an initial denaturation step (95 °C for 3 min) followed by 60 cycles of 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s. To determine primer specificity, a 71-cycle melting curve was carried out beginning at 55 °C and increasing by 0.5 °C every 10 s. Gene expression levels were normalized to the corresponding value for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and analyzed using iQ5 System Software (version 2).