Two New Furanoditerpenes from *Saururus chinenesis* and Their Effects on the Activation of Peroxisome Proliferator-Activated Receptor γ

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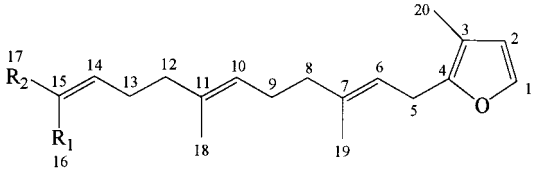
Two new acyclic furanoditerpene compounds, saurufuran A (**1**) and B (**2**), were obtained from the root of *Saururus chinensis*, and their structures were elucidated by means of 1D and 2D NMR spectroscopic analyses. Saurufuran A (**1**) is effective on the activation of peroxisome proliferator-activated receptor γ (PPARγ) with an EC_{50} value of 16.7 μM; however, saurufuran B (**2**), with an EC_{50} value of >100 μM, weakly activated the PPARγ.

As part of a search for peroxisome proliferator-activated receptor γ (PPARγ) agonist from plants, two new furanoditerpenes were isolated from the root of *Saururus chinensis*. *S. chinensis* (Saururaceae) is a perennial herb distributed mainly in southern Korea and China and used in folk medicine for the treatment of inflammation, tumors, jaundice, and gonorrhea.[1] Lignans, neolignans, aristolactams, and flavonoids have been isolated from *Saururus* species.[2−5]

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors, a family that includes the receptors for steroid hormones, retinoids, thyroid hormone, and vitamin D. Three mammalian PPARs have been identified to date, termed PPARα, PPARγ, and PPARδ. PPARs function as regulators of lipid and lipoprotein metabolism, glucose homeostasis, and cellular differentiation and also appear to control the inflammatory response.[6,7] PPARγ agonists have therapeutic potential in the treatment of type 2 diabetes, inflammatory disease, and certain cancers.[8,9] Here we describe the isolation and structure elucidation of two new acyclic furanoditerpene compounds, named saurufuran A (**1**) and B (**2**), and their effect on the activation of PPARγ.



Saurufuran A (**1**) was obtained as a pale yellow oil, and the molecular formula was established as $C_{20}H_{28}O_3$ by HRFABMS (*m/z* 317.2113 $[M + H]^+$, calcd for $C_{20}H_{29}O_3$ 317.2117) and by ^{13}C NMR. The IR spectrum of **1** showed absorptions for a carbonyl group at 1690 cm^{-1} and for a hydroxy group at 3400−2600 cm^{-1}. The ^1H NMR spectra showed signals at δ 7.20 (1H, d, J = 1.8 Hz) and 6.15 (1H, d, J = 1.8 Hz), which are characteristic of two vicinal protons in 2,3-disubstituted furan. The signal at δ 6.07 (tq, J = 7.4, 1.2 Hz), which coupled with vinyl methyl at δ 1.90 (d, J = 1.2 Hz) and the methylene at δ 2.63 (dt, J = 7.4, 7.4 Hz), was ascribed to the olefinic H-14 on the β-carbon of a α,β-unsaturated carbonyl system. The ^1H NMR also included signals for methyl on a furan ring at δ 1.96 and methylene at δ 3.28 (d, J = 7.3 Hz, H-5) coupled with the olefinic proton at δ 5.26 (dq, J = 7.3, 1.2 Hz, H-6), which was long-range coupled with the vinyl methyl at δ 1.71. Another set of vinyl methyl and allylically coupled olefinic protons were shown at δ 1.58 and 5.12, respectively. Complex patterns of methylene proton signals at δ 2.00−2.10 were also observed. The ^{13}C and DEPT NMR spectra of **1** showed 20 signals consisting of one acid carbonyl group, five sp^2 methines, five methylenes, five sp^2 quarternary carbons, and four methyl groups. The HMBC correlations (Figure 1) confirmed all the expected connectivities for the proposed acyclic furanoditerpene skeleton. In particular, major HMBC connectivities between C-3 and H-1, H-2, and H-20 and between C-4 and H-1, H-2, H-20, H-5, and H-6 were very important to locate the methyl and acyclic isoprenoid moiety to C-3 and C-4 of the furan ring, respectively. The chemical shift values of H-6 and H-10 indicated an *E* configuration for both C-6 and -10 double bonds (Table 1). However chemical shifts of H-14 methine (δ 6.07), H-13 methylene (δ 2.63), and H-17 (δ 1.90) are in good agreement with those for demeroperatic acid[10] and asiaticusin B,[11] indicating that compound **1** is the *Z* configuration at the C-15 position.

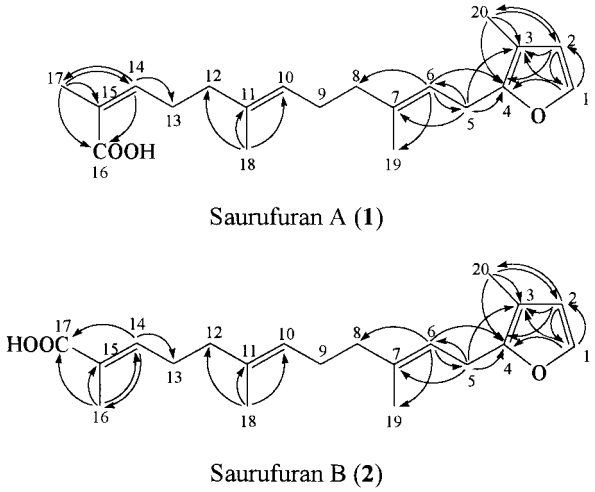


Figure 1. Long-range $^{13}C-^1H$ correlations (from HMBC) of **1** and **2**.

Saurufuran B (**2**) was given as a pale yellow oil. The ^1H NMR spectrum of **2** was similar to that for **1**, except the chemical shift values of the H-14 methine signal (δ 6.85), which was shifted downfield by 0.78 ppm, and of the H-13

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Table 1. ^{1}H and ^{13}C NMR Chemical Shifts for Saurufuran A (**1**) and B (**2**)[a]

	1		2	
no.	^{13}C	^{1}H	^{13}C	^{1}H
1	139.77	7.20 d (1.8)[b]	139.78	7.21 d (1.8)
2	112.84	6.15 d (1.8)	112.86	6.15 d (1.8)
3	113.39		113.40	
4	150.09		150.12	
5	25.17	3.28 d (7.3)	25.17	3.28 d (7.2)
6	120.04	5.26 dq (7.3, 1.2)	120.12	5.26 dq (7.2, 1.2)
7	136.42		136.31	
8	39.54	2.02 br t (7.0)	39.46	2.02 br t (6.9)
9	26.45	2.11 td (7.0, 6.9)	26.41	2.22 td (6.9, 6.6)
10	124.89	5.12 br t (6.9)	125.06	5.13 br t (6.6)
11	134.18		133.85	
12	39.02	2.07 t (7.4)	38.09	2.09 t (7.4)
13	28.12	2.63 td (7.4, 7.4)	27.51	2.27 tq (7.4, 7.3)
14	146.46	6.07 tq (7.4, 1.2)	144.66	6.85 dq (7.3, 1.2)
15	126.10		126.66	
16	173.49		12.14	1.83 d (1.2)
17	20.42	1.90 d (1.2)	172.00	
18	15.83	1.58 br s	15.97	1.60 br s
19	16.12	1.71 br s	16.11	1.70 br s
20	9.77	1.96 br s	9.78	1.96 br s

[a] Recorded in $CDCl_3$ at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). [b] *J* values (in Hz) in parentheses.

methylene signal (δ 2.27), which was shifted upfield by 0.36 ppm with respect to those of **1**. These chemical shift values are in good agreement with those for centipedic acid[12] asiaticusin A,[11] indicating that **2** is the *E* isomer at the C-15 position of compound **1**.

The effect on the PPARγ activation by saurufuran A (**1**) and B (**2**) was evaluated using a reporter gene assay.[13] Saurufuran A (**1**) activated the PPARγ dose-dependently, and the potency was comparable to that of ciglitazone.[14] The maximum fold activation by saurufuran A (100 μM) was 13.5-fold compared to the negative control, while that by ciglitazone (100 μM) was 15-fold. The EC_{50} values (concentration of compound required to induce 50% of the maximum luciferase activity) for activation of PPARγ by saurufuran A (**1**) and ciglitazone were 16.7 and 14.9 μM, respectively. However, saurufuran B (**2**) showed very weak activity with an EC_{50} value of >100 μM, indicating that the geometry of the terminal carboxyl group would be important for the binding to PPARγ. The activation of PPARγ by furanoditerpenoid is reported for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-370 polarimeter at 25 °C. IR and UV spectra were obtained on a JASCO IR Report-100 and a Milton Roy 3000 spectrometer, respectively. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), and HMBC spectra were obtained on a Varian Unity NMR spectrometer using $CDCl_3$ as a solvent. HRFABMS were measured on a JEOL HX 110 mass spectrometer. Kieselgel 60 (Merck Nos. 9385 and 7729) and Sephadex LH20 (Sigma Chemical Co., St. Louis, MO) were used for column chromatography. Fetal bovine serum, media, and supplement materials for cell culture were purchased from GIBCO-BRL (Gaithersberg, MD).

Plant Material. The dried roots of *S. chinensis* were collected at Jeju Island, Korea, in October 1999 and identified by Dr. Kyong Soon Lee, a plant taxonomist at Chungbuk National University. A voucher specimen (No. 99101) was deposited in the Korea Research Institute of Bioscience and Biotechnology.

Extraction and Isolation. The dried roots of *S. chinensis* (3.2 kg) were extracted three times with MeOH (5 L) at room temperature. The MeOH extract (250 g) was partitioned between CH_2Cl_2 and water. The CH_2Cl_2-soluble fraction (95 g) was chromatographed on a Si gel column (6 × 40 cm) with an *n*-hexane−acetone step gradient system with increasing polarity. The fraction (1.5 g out of 13 g) eluted with *n*-hexane−acetone (10:1) showed 5.2-fold of luciferase activity at 20 μg/mL compared to the negative control. Further separation was made by Sephadex LH20 column chromatography using MeOH as an eluent to obtain the fraction of PPARγ agonist activity, which showed 9.8-fold of luciferase activity at 20 μg/mL compared to the negative control. The active fraction (450 mg) was finally purified by preparative HPLC (J'sphere ODS-H80, 10 × 150 mm, YMC, Japan) eluted with CH_3CN-H_2O (60:40) at a flow rate of 8 mL/min to give saurufuran A (**1**) (100 mg, t_R 48 min) and saurufuran B (**2**) (7.3 mg, t_R 42 min).

Saurufuran A (1): pale yellow oil; $[\alpha]^{25}_D$ −5.8° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207.4 (4.10), 280.7 (3.22) nm; IR (film) ν_{max} 3400 and 2600 (OH), 1690 (C=O), 1640 (C=C), 1501, 1450, 1380, 1260, 1160, 1090, 890, 760 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HRFABMS *m*/*z* 317.2113 [M + H]$^+$ (calcd for $C_{20}H_{29}O_3$, 317.2117).

Saurufuran B (2): pale yellow oil; $[\alpha]^{25}_D$ 0° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205.5 (4.09), 280.5 (3.34) nm; IR (film) ν_{max} 3450 and 2800 (OH), 1695 (C=O), 1650 (C=C), 1450, 1380, 1260, 1220, 1090, 890, 760 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HRFABMS *m*/*z* 317.2113 [M + H]$^+$ (calcd for $C_{20}H_{29}O_3$, 317.2117).

Bioassay for Activation of PPARγ. The effect on the activation of PPARγ by saurufuran A (**1**) and B (**2**) was evaluated using a reporter gene assay described by Forman, B. M., et al.[13] with some minor modifications. Briefly, a plasmid pFA-GAL4-PPAR chimera expression construct, containing the ligand binding domain of human PPAR-γ (amino acids 176−477), was prepared in the vector pFA-CMV (Stratagene, La Jolla, CA), while the pFR-Luc (Gal4-UAS-Luciferase) construct was from Stratagene (La Jolla, CA). NIH-3T3 cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin. NIH-3T3 cells, grown in 24-well culture plates, were transiently transfected with expression vectors (500 ng each) for pFA-GAL4-PPAR, pFR-Luc, and pSV-β-galctosidase (Promega, Madison, WI) using Lipofectamine plus reagent for 3 h according to the instructions of the manufacturer (Gibco/BRL, Gaithersburg, MD). Various concentrations of **1** and **2** (1−100 μM) were added 24 h after transfection, and whole cell lysates were prepared 16 h after treatment and assayed for luciferase and β-galactosidase activity using a luminometer (EG&G Berthold, Bad Wildbad, Germany) and spectrophotometer, respectively. Transfections were performed in triplicate, and activation was normalized to galactosidase activity.

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