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Four new sesqui-lignans isolated from *Acanthopanax senticosus* and their diacylglycerol acyltransferase (DGAT) inhibitory activity

Jia-Lin Li [a,1], Na Li [a,1], Hyun-Sun Lee [b], Shan-Shan Xing [a], Shi-Zhou Qi [a], Zhen-Dong Tuo [a], Le Zhang [a], Ban-Ban Li [a], Jian-Guang Chen [a], Long Cui [a,*]

[a] College of Pharmacy, Beihua University, Jilin Province 132013, People's Republic of China
[b] Chemical Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Chungbuk 363-883, Republic of Korea

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ABSTRACT

Four new sesqui-lignans, (7*R*, 7′*R*, 7″*S*, 8*S*, 8′*S*, 8″*S*)-4′,5″-dihydroxy-3,5,3′,4″-tetramethoxy-7,9′:7′,9-diepoxy-4,8″-oxy-8,8′-sesquineo-lignan-7″,9″-diol (**1**), (7*R*, 7′*R*, 7″*S*, 8*S*, 8′*S*, 8″*S*)-4′,3″-dihydroxy-3,5,3′,5′,4″-pentamethoxy-7,9′:7′,9-diepoxy-4,8″-oxy-8,8′-sesquineo-lignan-7″,9″-diol (**2**), (7*R*, 7′*R*, 7″*S*, 8*S*, 8′*S*, 8″*S*)-3′,4″-dihydroxy-3,5,4′,5″-tetramethoxy-7,9′:7′,9-diepoxy-4,8″-oxy-8,8′-sesquineo-lignan-7″,9″-diol (**3**) and acanthopanax A (**7**) together with three known compounds (**4**–**6**) were isolated from the EtOAc-soluble extract of *Acanthopanax senticosus*. Their structures were elucidated on the basis of spectroscopic and physicochemical analyses. All the isolates were evaluated for *in vitro* inhibitory activity against DGAT1 and DGAT2. Among them, compounds **1**–**6** were found to exhibit selective inhibitory activity on DGAT1 with IC_{50} values ranging from 61.1 ± 1.3 to 97.7 ± 1.1 μM and compound **7** showed selective inhibition of DGAT2 with IC_{50} value 93.2 ± 1.2.

1. Introduction

Triglycerides (TG), the most leading storage form of energy in eukaryotic cells and essential for normal physiology. However, excess accumulation of TG in tissue can lead to a variety of disorders, such as obesity, insulin resistance and hepatic steatosis [1]. For these reasons, inhibition of TG synthesis has been considered as an efficient strategy for treatment of obesity and type II diabetes [2]. Diacylglycerol acyltransferase (DGAT) catalyzes the acyl residue transfer from acyl-CoA to diacylglycerol (DAG), which the exclusive key enzyme for the final step in TG synthesis [3]. Molecular studies showed: DGAT enzymes are encoded by two non-homology genes: DGAT1 and DGAT2, and both genes are ubiquitously expressed [4]. Meanwhile, DGAT1 as a member of the acyl CoA: cholesterol acyltransferase (ACAT) gene family is more homologous with ACAT1 and ACAT2 than with DGAT2, which is more closely related to the monoacylglycerol acyl transferase (MGAT) enzymes [5]. Recent studies have shown that: mice knockout DGAT1 has provided an understanding of the relationship between TG synthesis and metabolic syndrome like obesity and type II diabetes. These DGAT1 deficient mice were resistant to weight gain when fed a high-fat diet through mechanisms that involved improve energy expenditure and increased sensitivity in insulin and leptin [6]. Based on these aspects, searching for novel, selective, and orally bio-available DGAT1 inhibitors for the treatment of obesity and type II diabetes have been intensified.

Acanthopanax senticosus (Rupr. & Maxim.) Harms, a nontoxic herb belongs to the family of Araliaceae, which found in Northeast Asia. *A. senticosus* traditionally used as a tonic to treat rheumatism, diabetes, and hepatitis [7]. Previous phytochemical and biological investigations found its roots and stem barks include diterpenoids, triterpenoids, lignans, polyacetylenes, phenylpropanoids, flavonoids [8] and diphenyl ethers [9]. During our efforts on identifying new DGAT1 inhibitors from nature resource, a MeOH extract of the stem of *A. senticosus* exhibited DGAT inhibitory activity which led us to investigate this plant. In this study, we isolated four new sesqui-lignans along with three known compounds (Fig. 1), and tested their DGAT inhibitory activity.

2. Experimental

2.1. General

Optical rotations were determined on a JASCO P-1020 polarimeter using a 100-mm glass microcell (JASCO, Tokyo, Japan). UV spectra were recorded in MeOH using a Shimadzu spectrometer (Shimadzu, Tokyo, Japan). IR spectra were recorded with a JASCO FT-IR 620 spectrophotometer (JASCO Corporation, Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were obtained from a Varian Unity Inova 500 MHz spectrometer (Varian Unity Inova, Phoenix, USA) using TMS as the internal standard. Mass spectra were obtained on a QTOF2 high resolution

* Corresponding author.
E-mail address: cuilong71@beihua.edu.cn (L. Cui).
[1] These authors contributed equally to this work.

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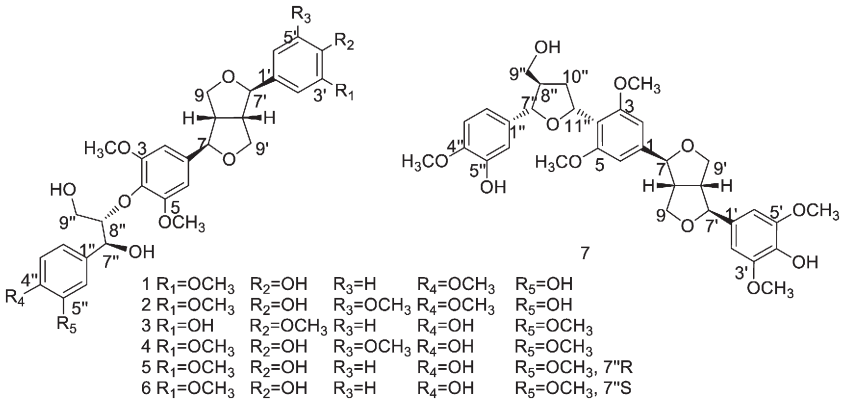

Fig. 1. Structures of compounds **1**–**7**.

mass spectrometer (Micromass, Wythenshawe, UK). Column chromatography was conducted using silica gel 60 (200 μm particle size, Yantai Xinde Chemical Co., Ltd., Yantai, China) and RP-18 (150–63 μm particle size, Merck, Darmstadt, Germany). Thin-layer chromatography precoated TLC silica gel 60 F_{254} plates from Merck were used. HPLC were carried out using a Shimadzu System LC-10AD pump equipped with a model SPD-10Avp UV detector (Shimadzu, Tokyo, Japan), and an Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size, Shiseido Fine Chemicals, Tokyo, Japan).

Bovine serum albumin and *sn*-1, 2-dioleoylglycerol was obtained from Sigma Chemical Co. (St. Louis, MO, USA). [1-^{14}C] oleoyl — CoA (250 μCi) was purchased from Amersham Pharmacia Biotech. Inc. (Little Chalfont, Buckinghamshire, England). [1-^{14}C] acetic acid (250 μCi) and [U-^{14}C] glycerol (50 μCi) were purchased from Amersham Biosciences (Buckinghamshire, UK).

2.2. Plant material

The stem of *A. senticosus* was collected in Jilin, Jilin province, People's Republic of China, and authenticated by Professor Gao Li (College of Pharmacy, Yanbian University). A voucher specimen of the plant (No. 20141121) was deposited at the College of Pharmacy, Beihua University, Jilin, People's Republic of China.

2.3. Extraction and isolation

The stem of *A. senticosus* (10.0 kg) was extracted with MeOH at room temperature for two weeks and the MeOH solution was concentrated to get a crude extract. This extract was suspended in H_2O, partitioned successively with *n*-hexane, EtOAc, and BuOH to afford *n*-hexane, EtOAc, and BuOH soluble fractions, respectively. A portion of the EtOAc-soluble fraction (81.0 g) was chromatographed over a silica gel column using a gradient of CH_2Cl_2/MeOH (from 50:1, 30:1 to 1:1), and was separated into 9 fractions (Fr.1–Fr.9). Fr.5 (CH_2Cl_2/MeOH 100:1, 21.0 g) was chromatographed over silica gel, eluted with a stepwise gradient of EtOAc/*n*-hexane (from 1:3, 1:2 to 2:1) to afford 8 fractions (Fr.5.1–Fr.5.8). Further purification of Fr. 5.7 (3.8 g) was subjected to silica gel column eluted with CH_2Cl_2/EtOAc (4:1, 3:1 to 1:5) to get 7 fractions, after which the Fr.5.7.4 (740.0 mg) was subjected to the RP-18 column (40.0 × 3.5 cm) and eluted with MeOH/H_2O (3:7, 4:6 to 7:3) to afford 6 fractions. Fr.5.7.4.5 (71.0 mg) was separated by HPLC, using an isocratic solvent system of 55% MeOH in H_2O over 60 min yielded compound **1** (2.0 mg, t_R = 35.4 min) and Fr.5.7.4.5.3 (30 mg). Further purification of Fr.5.7.4.5.3, using an isocratic solvent system of 40% CH_3CN in H_2O over 60 min yielded compound **7** (3.0 mg, t_R = 39.5 min). Fr.5.7.5 (840.0 mg) was subjected to the RP-18 column (40.0 × 3.5 cm) and eluted with MeOH/H_2O (3:7, 4:6 to 7:3) to afford 7 fractions. Fr.5.7.5.4 (260.0 mg) was separated by HPLC, using an isocratic solvent system of 50% MeOH in H_2O over 75 min yielded compounds **4** (16 mg, t_R = 61.8 min), **5** (37 mg, t_R = 52.9 min), **6** (38.7 mg, t_R = 68.3 min) and Fr.5.7.5.4.2 (22 mg). Fr.5.7.5.4.2 using an isocratic solvent system of 47% MeOH in H_2O over 85 min yielded compounds **2** (1.5 mg, t_R = 77.5 min) and **3** (2.0 mg, t_R = 84.2 min).

2.4. Spectroscopic data

2.4.1. (7R, 7′R, 7″S, 8S, 8′S, 8″S)-4′,5″-dihydroxy-3,5,3′,4″-tetramethoxy-7,9′:7′,9-diepoxy-4,8″-oxy-8,8′-sesquineo-lignan-7″,9″-diol (1)

Pale yellow powder; $[\alpha]_D^{25}$ −4.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.25), 236 (3.65), 278 (3.10) nm; IR (KBr) ν_{max} 3419, 2935, 1563, 1502, 1448, 1412, 1234, 1212, 1100, 1025, 806 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data are presented in Table 1; HREIMS *m/z*: 584.2262 $[M]^+$ (calcd. for $C_{31}H_{36}O_{11}$, 584.2258).

2.4.2. (7R, 7′R, 7″S, 8S, 8′S, 8″S)-4′,3″-dihydroxy-3,5,3′,5′,4″-pentamethoxy-7,9′:7′,9-diepoxy-4,8″-oxy-8,8′-sesquineo-lignan-7″,9″-diol (2)

Pale yellow powder; $[\alpha]_D^{25}$ −3.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.32), 233 (3.45), 276 (3.04) nm; IR (KBr) ν_{max} 3403, 2966, 1543, 1512, 1468, 1242, 1234, 1102, 1021, 802 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are presented in Table 1; HREIMS *m/z*: 614.2368 $[M]^+$ (calcd. for $C_{32}H_{38}O_{12}$, 614.2363).

2.4.3. (7R, 7′R, 7″S, 8S, 8′S, 8″S)-3′,4″-dihydroxy-3,5,4′,5″-tetramethoxy-7,9′:7′,9-diepoxy-4,8″-oxy-8,8′-sesquineo-lignan-7″,9″-diol (3)

White powder; $[\alpha]_D^{25}$ −4.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.21), 233 (3.61), 273 (3.08) nm; IR (KBr) ν_{max} 3412, 2955, 1541, 1510, 1441, 1408, 1229, 1207, 1121, 1022, 804 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are presented in Table 1; HREIMS *m/z*: 584.2260 $[M]^+$ (calcd. for $C_{31}H_{36}O_{11}$, 584.2258).

2.4.4. Acanthopanax A (7)

White powder; $[\alpha]_D^{25}$ −29.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.43), 247 (3.98), 289 (3.28) nm; IR (KBr) ν_{max} 3532, 2835, 1512, 1503, 1408, 1245, 1216, 1109, 1018, 798 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data are presented in Table 1; HREIMS *m/z*: 624.2568 $[M]^+$ (calcd. for $C_{34}H_{40}O_{11}$, 624.2571).

2.5. DGAT1 and DGAT2 assays

DGAT activity assays were carried out as described previously [10]. Briefly, DGAT activity in total membranes prepared from DGAT2- or DGAT1-overexpressing Sf-9 and HEK293 Tet-on cells was determined by measuring the formation of [^{14}C]-triacylglycerol from [^{14}C]-oleoyl CoA. The reaction mixture for the DGAT1 assay contained 175 mM Tris