Food Chemistry 131 (2012) 1097–1103

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Glycolipids from the aerial parts of *Orostachys japonicus* with fatty acid synthase inhibitory and cytotoxic activities

HaiYan Zhang [a], Joonseok Oh [b], Tae-Su Jang [c], Byung Sun Min [d], MinKyun Na [a,*]

[a] College of Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Republic of Korea
[b] Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA
[c] College of Medicine, CHA University, Seoul 135-080, Republic of Korea
[d] College of Pharmacy, Catholic University of Daegu, Gyeongsan, Gyeongbuk 712-702, Republic of Korea

ARTICLE INFO

Article history:
Received 11 July 2011
Received in revised form 7 September 2011
Accepted 15 September 2011
Available online 21 September 2011

Keywords:
Orostachys japonicus
Crassulaceae
Orostachysterebroside A
Glycolipids
Fatty acid synthase
Cytotoxicity

ABSTRACT

Inhibition of fatty acid synthase (FAS) has been proposed as a new therapeutic target for the treatment of cancer. In our preliminary study, a MeOH extract of the aerial parts of *Orostachys japonicus* A. Berger (Crassulaceae) inhibited FAS activity (>95% inhibition at 62.5 μg/mL). Through bioassay-guided fractionation, we isolated three cerebrosides (**1**–**3**), including one new compound (**1**), and seven glyceroglycolipids (**4**–**10**) from a FAS inhibitory hexane-soluble fraction of the MeOH extract of *O. japonicus*. On the basis of physicochemical and spectroscopic analyses including 1D and 2D NMR, HRFABMS, and ESIMS/MS, the structure of the new compound **1** was 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*,6*Z*,10*E*)-2-[(2′*R*,6′*Z*,9′*Z*)-2-hydroxyeicosa-6′,9′-dienoylamino)]-1,3,4-trihydroxytetracosa-6,10-diene, and it was named orostachyscerebroside A. Compounds **1**–**10** were tested for their *in vitro* FAS inhibitory activity, and compounds **1**, **4**, **5**, **6**, **7**, and **8** inhibited the enzyme with micro-molar IC_{50} values both in the incorporation of [^{3}H] acetyl CoA (from 52.2 to 108.0 μM) and in the oxidation of NADPH (from 19.5 to 30.3 μM). Interestingly, compounds **1**, **4**, **5**, **6**, **7**, and **8** with FAS inhibitory activity showed cytotoxic activity against HL-60 (human leukemia) and A549 (human lung adenocarcinoma) cancer cells, while none of them displayed cytotoxicity against MCF-7 (human breast adenocarcinoma) cells. This is the first report on the inhibition of FAS by glycolipids from *O. japonicus* and their cytotoxic activity and may provide a scientific basis for the folk remedy using the plant to treat cancer.

1. Introduction

Fatty acid synthase (FAS) is a sophisticated multifunctional enzyme that is responsible for the reductive *de novo* synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and nicotinamide adenine dinucleotide phosphate (NADPH) (Wakil, 1989). Recently, inhibition of this enzyme was suggested to hold promise as a strategy for cancer chemoprevention because increased expression of FAS has emerged as a common phenotype of most human carcinomas (Kuhajda, 2000). Indeed, the expression and activity of FAS is extremely low in nearly all nonmalignant adult tissues, whereas FAS is significantly up-regulated or highly activated in various cancer cell types (Kuhajda, 2006). Therefore, FAS inhibitors could be developed into ideal anti-cancer agents that are selectively cytotoxic to human cancer cells. The pioneering observations that inhibition of FAS by cerulenin or C75 selectively kills cancer cells via apoptosis induction and tumor growth inhibition in a number of *in vitro* and *in vivo* studies have validated the reliance of cancer cell survival on FAS activity (Pizer et al., 2000; Wang et al., 2005; Zhou et al., 2003). Other reported FAS inhibitors such as the green tea polyphenol epigallocatechin-3-gallate (EGCG) and flavonoids (i.e., luteolin, quercetin, and kaempferol) inhibited cancer cell growth by inducing apoptotic cell death, also suggesting that those FAS inhibitors could be developed into a lead drug for anti-neoplastic properties (Lupu & Menendez, 2006). However, these reported FAS inhibitor candidates have limitations in practical use. Cerulenin was reported to be chemically unstable and C75 (Swinnen, Brusselmans, & Verhoeven, 2006), a more chemically stable derivative of cerulenin, induced significant weight loss in an *in vivo* assay (Pizer et al., 2000). Therefore, more potent and clinically suitable leads for FAS inhibitors should be explored from natural product libraries given the fact that 77.8% of approved anti-neoplastic agents during last two decades were either natural products themselves or structurally based on them, or were their semi-synthetic analogs (Newman & Cragg, 2007). The discovery of cerulenin and plantensimycin in a natural product pool as possible FAS inhibitors (Hata et al., 1960; Wright & Reynolds, 2007) confirms that such pools are a huge source of potential templates for FAS inhibitors. Hundreds of plant extracts were screened

* Corresponding author. Tel.: +82 53 810 2820; fax: +82 53 810 4654.
E-mail address: mkna@ynu.ac.kr (M. Na).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.09.058

for FAS inhibition, and a MeOH extract of the aerial parts of *Orostachys japonicus* (Crassulaceae) exhibited potent FAS inhibitory activity (>95% inhibition at 62.5 μg/mL).

O. japonicus A. Berger (Crassulaceae) is a perennial herb that is distributed in Korea, China, and Japan. In Korea, it is widely utilized as a traditional remedy for hepatitis, boils, hemorrhoids (Kim, 1984), fever, gingivitis, and cancers (Lee, Seo, Shin, Lee, & Sung, 2008). In addition to the medicinal application, the plant is also used as a healthy food (Lee et al., 2011), and several kinds of health beverages and food supplements containing *O. japonicas* are commercialized in Korea. On the basis of these traditional clinical practices, a number of bioactivities of the medicinal plant have been verified, including the calpain inhibitory effect (Kim et al., 2009), anti-ulcerogenic effects (Jung, Choi, Nam, & Park, 2007), augmentation of hepatic alcohol dehydrogenase, anti-apoptotic activity in neuronal cells (Yoon et al., 2000), and antioxidant effect (Lee et al., 2011). Despite the traditional practices for alleviating cancer-related diseases, the number of reports investigating the possibility that *O. japonicus* could provide useful and novel compounds for the treatment of cancer diseases is relatively negligible. Furthermore, whether the medicinal plant could be a lucrative chemical pool to produce a lead template to develop into a FAS inhibitor has never been researched. Previous phytochemical studies only reported that *O. japonicus* produced secondary metabolites including epicatechin gallate (Kim et al., 2009), acylated sterol glucoside (Yoon, Min, Lee, Park, & Choi, 2005), and flavonoids (Sung, Jung, & Kim, 2002), which leaves a vast selection of unexplored secondary metabolites with the potential to produce cytotoxic lead compounds. Bioassay-guided fractionation was therefore performed using the MeOH extract of *O. japonicus*, which showed promising *in vitro* inhibitory activity on FAS in the preliminary screening. Three cerebrosides (**1**–**3**) including one new compound **1** named orostachyscerebroside A, and seven glyceroglycolipids (**4**–**10**) were purified from the hexane-soluble fraction of the MeOH extract. All the isolated compounds were evaluated for FAS inhibitory activity and cytotoxic activities against HL-60 (human leukemia), A549 (human lung adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cancer cells. This is the first report on the inhibition of FAS by glycolipids from *O. japonicus* and their cytotoxic activity.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were measured on a JASCO DIP-370 (Tokyo, Japan) automatic digital polarimeter. VLC was performed on Merck silica gel (70–230 mesh). MPLC was carried out with Biotage Isolera™. Reversed phase C_{18} SNAP Cartridge KP-C_{18}-HS (120 g, Biotage) and silica gel SNAP Cartridge HP-Sil (100 g, Biotage) were used for MPLC. HPLC separation was performed with a Gilson system with a UV detector and a Luna C_{18} column (250 × 21.2 mm, 10 μm). High and low resolution FABMS data were obtained on a JEOL JMS-700. The LCQ advantage trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) was equipped with an electrospray ionization (ESI) source. TLC was performed on glass plates precoated with silica gel F_{254} (20 × 20 cm, 200 μm, 60 Å, Merck). NMR spectra were recorded on JEOL ECA-500 (^{1}H, 500 MHz, ^{13}C, 125 MHz) and Varian VNS600 (^{1}H, 600 MHz, ^{13}C, 150 MHz) spectrometers. Conventional pulse sequences were used for COSY, HMQC and HMBC. All NMR experiments were performed at 294 K, using pyridine-d_5 (**1**–**3**) or CD_3OD (**4**–**10**) as the solvent. Chemical shifts were given on the δ scale and referenced by pyridine-d_5 as an internal standard (δ_H=7.19, δ_C=123.5) or CD_3OD as an internal standard (δ_H = 3.31, δ_C = 49.0). Coupling constants (J) are in Hz. Data processing was carried out with MestReNova v6.0.2 program. Optical density (OD) values in the cytotoxic activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were read on a TECAN ELISA Microplate Reader.

2.2. Plant material

The aerial parts of *O. japonicus* were purchased from "Gaya Wasong", a commercial farm in Gyeongnam, Korea, in July 2008, and identified by Prof. MinKyun Na of the College of Pharmacy, Yeungnam University. A voucher specimen (2008-0196A) was deposited in the herbarium of College of Pharmacy, Yeungnam University.

2.3. Extraction and isolation

The dried aerial parts of *O. japonicus* (1.8 kg) were extracted with methanol (MeOH, 3 × 8 L) at room temperature for 7 days. The dried MeOH extract (395 g) was suspended in water and partitioned sequentially with *n*-hexane, CH_2Cl_2, and EtOAc. The *n*-hexane-soluble fraction (53 g) that showed FAS inhibitory activity was subjected to silica gel VLC (20 × 25 cm), and eluted with a stepwise gradient of *n*-hexane/EtOAc and EtOAc/MeOH [90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 100:0, 60:40, 0:100 (each 3 L)] to give 10 fractions (Fr. 1–10). Fr. 3–9 showed higher FAS inhibitory activity than the original fraction. Fr. 9 (2.8 g, eluted with 60:40) was further divided into five fractions (Fr. 9–1 to 9–5) using MPLC [silica gel SNAP Cartridge HP-Sil (100 g, Biotage) column; UV 254 and 210 nm] with a gradient of CH_2Cl_2/MeOH (from 95:5 to 100:0; v/v). Fr. 9–3 (1.18 g) was fractionated by C_{18} [C_{18} SNAP Cartridge KP-C_{18}-HS (120 g, Biotage) column; UV 254 and 210 nm] MPLC using a gradient of MeOH/H_2O (from 60:40 to 70:30; v/v) to yield five subfractions (Fr. 9-3-1 to 9-3-5). Purification of Fr. 9-3-2 (784.1 mg) using the reversed phase HPLC [Luna 10u C_8 column; 21.2 × 250 mm; 10 μm particle size; 6 mL/min; MeOH/H_2O (from 75:25 to 100:0; v/v); UV 210 and 203 nm] and reversed phase HPLC [Luna C_{18} column; 21.2 × 250 mm; 10 μm particle size; 6 mL/min; MeOH/H_2O (from 75:25 to 100:0; v/v); UV 210 and 203 nm] resulted in the isolation of compounds **1** (25.0 mg, t_R = 79 min), mixture of **2** and **3** (63.7 mg, t_R = 73 min), **4** (22.2 mg, t_R = 69 min), **5** (5.1 mg, t_R = 71 min), **6** (17.8 mg, t_R = 61 min), **7** (22.2 mg, t_R = 66 min), **8** (5.1 mg, t_R = 67 min), **9** (7.1 mg, t_R = 29 min), **10** (13.3 mg, t_R = 31 min).

Compound **1**: colorless amorphous powder; $[\alpha]_D^{25}$ + 6.8° (*c* = 0.1, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z*: 866 $[M + H]^+$, 704 $[(M + H) - 162]^+$; ESIMS/MS *m*/*z*: 866 $[M + H]^+$, 848 $[(M + H) - H_2O]^+$, 704 $[(M + H) - 162]^+$, 686 $[(M + H) - H_2O]^+$, 500 $[(M + H) - 366]^+$. HRFABMS *m*/*z*: 866.6724 $[M + H]^+$ (Calcd for $C_{50}H_{91}NO_{10}$: 866.6721).

Compounds **2** and **3**: white amorphous powder; $[\alpha]_D^{25}$ + 7.8° (*c* = 0.1, MeOH); ^{1}H and ^{13}C NMR data, see Supplementary data; FABMS *m*/*z*: 736 $[M + Na]^+$, 574 $[(M + H) - 162]^+$, 534 $[(M + H) - 162 - H_2O]^+$, 482 [fatty acid unit + Na]$^+$.

Compound **4**: colorless oil; $[\alpha]_D^{25}$ + 47.6° (*c* = 0.1, MeOH); ^{1}H and ^{13}C NMR data, see Supplementary data; FABMS *m*/*z*: 939 $[M + Na]^+$, 777 $[(M + Na) - 162]^+$, 405, 363, 347; ESIMS/MS *m*/*z*: 939 $[M + Na]^+$, 777 $[(M + Na) - 162]^+$, 659 $[(M + Na) - R_1COOH]^+$, 683 $[(M + Na) - R_2COOH]^+$, 497 $[(M + Na) - 162 - R_1COOH]^+$, 521 $[(M + Na) - 162 - R_2COOH]^+$, 405, 363, 347.

Compound **5**: colorless oil; $[\alpha]_D^{25}$ + 32.1° (*c* = 0.1, MeOH); ^{1}H and ^{13}C NMR data, see Supplementary data; FABMS *m*/*z*: 965 $[M + Na]^+$, 405, 363, 347; ESIMS/MS *m*/*z*: 965 $[M + Na]^+$, 803 $[(M + Na) - 162]^+$, 681 $[(M + Na) - R_1COOH]^+$, 687 $[(M + Na) - R_2COOH]^+$, 519 $[(M + Na) - 162 - R_1COOH]^+$, 405, 363, 347.

Compound **6**: colorless oil; $[\alpha]_D^{21}$ + 14.6° (*c* = 0.1, MeOH); ^{1}H and ^{13}C NMR data, see Supplementary data; FABMS *m*/*z*: 991 $[M + Na]^+$, 347; ESIMS/MS *m*/*z*: 991 $[M + Na]^+$, 829 $[(M + Na) - 162]^+$, 811 $[(M + Na) - 162 - H_2O]^+$, 681 $[(M + Na) - R_1COOH]^+$, 713