Original Article

Chemical constituents from *Cornus officinalis* and their biological activity[1]

Abstract

Objective: To study the chemical constituents from *Cornus officinalis Sieb.*, Et Zucc, and their peroxisome proliferator-activated receptors (PPARs) agonist activity. **Materials and Methods:** The leaves of *C. officinalis* were extracted three times with 90% EtOH at room temperature. The ethanol extracts were combined and concentrated under reduced pressure to yield residue, which was isolated and purified by silica gel and reverse-phase C_{18} column chromatography. The structures were elucidated on the basis of spectroscopic evidence and their physiochemical characteristics. Cell-based luciferase reporter gene assays were used to evaluate PPARα/γ agonistic activities. **Results:** Five compounds were isolated and elucidated as 10-hydroxyhastatoside (1), β-dihydrocornin (2), isoquercitrin (3), loganin (4) and oleanolic acid (5). **Conclusion:** Compounds 1 and 2 were obtained from *C. officinalis* for the first time. Compound 3 exhibited moderate agonistic activities for PPARα, with EC_{50} values of 29.5 μM.

Key words:
Cornus officinalis, iridoid glycosides, peroxisome proliferator-activated receptors

Introduction

The genus *Cornus* belongs to the family Cornaceae and consists of approximately four species distributed throughout the northern hemisphere including Asia, Europe, and North America. Of the four species, two of them are endemic to China.[1]

Cornus officinalis Sieb., Et Zucc, is a tree mainly distributed in Shanxi, Gansu, and Shaanxi provinces in China. It has been considered one of the 25 plant-based drugs most frequently used in China, Japan, and Korea. This species has attracted considerable interest of medicinal chemists for decades. Its extracts have been used in Chinese herbal medicine as a tonic formula and considered to possess actions including strengthening the liver and kidney, preservation of essence, and antidiabetic activity.[2] Moreover, it has potential antioxidant,[3] antineoplastic,[4] anti-inflammatory, and analgesic activities.[5] Previous phytochemical investigations have revealed that the total iridoid glycosides are the major chemical constituents of this plant, such as morroniside and loganin, and a few polyphenols, monomeric, and trimeric hydrolyzable tannins.[6,7] Taking into consideration the biological activity of this plant, we investigated the leaves of this species, collected from Taibai mountain of China. As a result, five known compounds (1–5) were obtained [Figure 1] from the ethyl acetate fraction. We present herein the isolation and structural elucidation of these compounds, as well as their proliferator-activated receptor (PPAR) agonistic activities.

Materials and Methods

Materials

Optical rotations were measured on a JASCO DIP-370 digital polarimeter equipped with a halogen lamp (589 nm) and a 10 mm microcell. IR spectra were recorded on a Nicolet Magna-

[1]Dedicated to the late Dr. Fransworth, University of Illinois at Chicago for his pioneer work on bio-active natural products"

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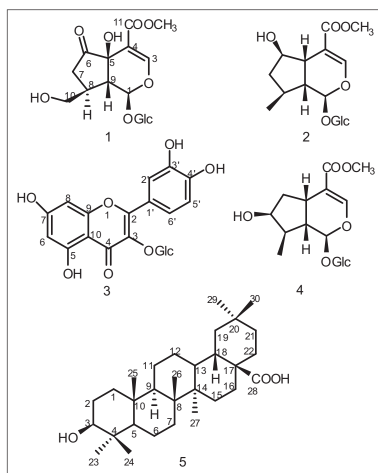

Figure 1: Compounds 1–5 isolated from ethyl acetate fraction of *Cornus officinalis*

IR 750 spectrophotometer. NMR spectra were acquired with a BrukerAvance 400 (400 MHz) for proton and BrukerAvance 400 (100 MHz) for carbon spectrometers using solvent signals (Methanol-d_4; δ_H4.78/δ_C 49.15) as references, and the chemical shift in ppm. The ESI-MS data were obtained using an Agilent 1100 series TOF MS with electrospray ionization. Preparative HPLC was carried out using Shimadzu with Shim-park RP-C18 column (20 × 200 mm) and SPD-M 20 A Multiple Wavelength detector and ELSD-LT ∏ detector.

The leaves of *C. officinalis* were collected from Taibaimountain, Shaaxi, China, in September 2009, and Voucher specimens (2009D028) were deposited at the Department of Chemistry, Xi'an University of Technology.

Extraction and isolation

The dried leaves of *C. officinalis* (10.0 kg) were extracted with 90% methanol and dried *in vacuo* to provide a crude extract. The crude extract (310 g) was sequentially separated on silica gel eluted by a step gradient of hexanes-ethyl acetate (100:0, 80:20, 50:50, and 0:100) and ethyl acetate-methanol mixtures (80:20, 50:50, and 0:100) to afford seven fractions (A–G). Fractions D, E, and F exhibited moderate PPARα/γ agonistic activities. Fr. D (5 g) was chromatographed on silica gel eluting successively with a gradient of ethyl chloroform/methanol (1:0 to 0:1) to give five subfractions (Fr. D_1-D_5). Fr. D_2 was further chromatographed on a silica gel eluted with chloroform/methanol in gradient (1:0 to 1:1) to obtain 2 (4 mg), 3 (6 mg), and 4 (5 mg). Fr. D_3 was separated by reversed-phase HPLC (Shim-park RP-C18 column; 5 μm; 20 × 250 mm; step gradient from 20% methanol in H_2O to 45% methanol in H_2O for 40 min and followed by 45–100% methanol in H_2O for 20 min, 8 mL/min) to give 1 (2 mg) and 5 (5 mg).

Determination of PPARα/γ agonistic activities

Cell-based luciferase reporter gene assays were used to evaluate PPARα/γ agonistic activities of compounds 2–4 as described previously.[8,9] Human hepatoma (HepG2) cells were cultured in DMEM/Ham's F12 medium supplemented with FBS (10%) and antibiotics (penicillin G sodium 100 U/mL and streptomycin 100 μg/mL) at 37 °C in an atmosphere of 95% humidity and 5% CO_2. At about 75% confluence, cells were harvested by trypsinization and transfected with firefly luciferase reporter gene constructs containing PPARα and γ in HepG2 cells. Briefly, 25 μg of DNA plasmids was added to 500 μL cell suspension (5 × 10^{6} cells) and incubated for 5 min at room temperature in BTX disposable cuvettes (4 mm gap). The cells were electroporated at 150 V (HepG2 cells) and a single 70 ms pulse in a BTX Electro Square Porator T 820 (BTX I, San Diego, CA). Transfected cells were plated in 96-well plate at a density of 5 × 10^{4} cells/well and grown for 24 h. The cells were treated with different concentrations of test compounds for 24 h followed by addition of 40 μL 1:1 mixture of Luc-Lite reagent and PBS containing 1 mmol calcium and magnesium. Luciferase activity was determined in terms of light output measured on a TopCountmicroplate reader (Packard Instrument Co., Meriden, CT) in a single photon counting mode. The pSV-β-galactosidase control plasmid (Promega) was used to normalize the transfection efficiencies. The ciprofibrate and ciglitazone were used as positive control for PPARα and PPARγ, respectively.

Results and discussion

The compounds were identified by comparison of their[1]H and[13] C NMR, MS, and IR spectral data with those reported in literature[11-13], respectively.

10-hydroxyhastatoside (1), colorless oil; ESI-MS m/z: 421 $[M+H]^+$;[1] H-NMR (CD_3OD, 400 MHz) δ_H: 7.71 (*br s*, 1H, H-3), 5.87 (*br s*, 1H, H-1), 4.57 (d, *J* = 8.0 Hz, 1H, H-1'), 3.86 (m, 2H, 2×H-10), 3.61 (3H, s, OMe), 3.68 (dd, *J* = 12.3, 2.0 Hz, 1H, H-6a'), 3.60 (overlapped, 1H, H-6b'),3.32 (m, 1H, H-5'), 3.27 (overlapped, 1H, H-3'), 3.23 (overlapped, 1H, H-4'), 3.12 (dd, *J* = 8.0, 6.0 Hz, 1H, H-2'), 2.71 (dd, *J* = 19.0, 10.6 Hz, 1H, H-7a), 2.64 (m, 1H, H-8), 2.24 (dd, *J* = 9.0, 2.4 Hz, 1H, H-9), 2.07 (dd, *J* = 19.0, 7.2 Hz, 1H, H-7b);^{13}C-NMR (CD_3OD, 100 MHz) δ_C: 95.2 (C-1), 157.3 (C-3), 105.6 (C-4), 74.0 (C-5), 214.0 (C-6), 35.3 (C-7), 33.0 (C-8), 47.0 (C-9), 64.0 (C-10), 168.2 (C-11), 100.0 (C-1'), 73.0 (C-2'), 76.1 (C-3'), 70.2 (C-4'), 77.0 (C-5'), 61.3 (C-6'),52.6 (11-OMe).[10]

β-dihydrocornin (2), colorless oil; ESI-MS m/z: 391 $[M+H]^+$;[1] H-NMR (CD_3OD, 400 MHz) δ_H: 7.36 (d, *J* = 1.2 Hz, 1H, H-3), 5.36 (d, *J* = 7.2 Hz, 1H, H-1), 4.58 (d, *J* = 8.0 Hz, 1H, H-1'), 4.08 (dd, *J* = 8.8, 5.6 Hz,1H, H-6a'),4.06 (m, 1H, H-6), 3.86 (dd, *J* = 10.4, 5.6 Hz,1H, H-6b'), 3.66 (s, 3H, OMe), 3.54-3.62 (overlapped with solvent signal, H-3' and H-4'), 3.32 (m, 1H, H-5'), 3.26 (dd, *J* = 9.0, 8.0 Hz,1H, H-2'), 2.79 (dd, *J* = 8.0, 2.8 Hz, 1H, H-5), 2.10 (m, 1H, H-8), 1.85 (m, 1H, H-9), 1.09 (d, *J* = 6.8 Hz, 3H, H-10);[13] C-NMR

(CD_3OD, 100 MHz) δ_C: 95.3 (C-1), 152.1 (C-3), 108.9 (C-4), 41.7 (C-5), 77.0 (C-6), 41.0 (C-7), 33.1 (C-8), 46.6 (C-9), 19.5 (C-10), 168.5 (C-11), 98.6 (C-1'), 73.3 (C-2'), 76.1 (C-3'), 70.1 (C-4'), 76.5 (C-5'), 61.3 (C-6'),50.5 (11-OMe).[10]

Isoquercitrin (3), colorless oil; ESI-MS m/z: 465 $[M+H]^+$;[1] H-NMR (pyridine-d5, 400 MHz) δ_H: 8.45 (d, J = 2.0 Hz, 1H, H-2'), 8.14 (dd, J = 8.4, 2.0 Hz, 1H, H-6'), 7.25 (d, J = 8.4 Hz, 1H, H-5'), 6.70 (d, J = 2.4 Hz,1H, H-8), 6.65 (d, J = 2.4 Hz,1H, H-6), 6.07 (d, 1H, J = 7.6 Hz,H-1''), 4.84 (dd, J = 9.6, 3.6 Hz, 1H, H-6a''), 4.61 (d, J = 9.6 Hz, 1H, H-6b''), 4.43 (m, 1H, H-3''), 4.33 (m, 1H, H-5''), 4.30 (m, 1H, H-4''), 4.20 (m, 1H, H-2'');[13] C-NMR (pyridine-d5, 100 MHz) δ_C: 62.4 (C-6''), 70.3 (C-4''), 73.9 (C-2''), 75.9 (C-3''), 78.21 (C-5''), 95.0 (C-8), 100.3 (C-6), 105.6 (C-l''), 106.0 (C-l0), 116.7 (C-2'), 118.3 (C-5'), 122.8 (C-l'), 123.62 (C-6'), 135.66 (C-3), 147.2 (C-3'), 150.2 (C-4'), 158.0 (C-9), 158.3 (C-2), 163.2 (C-5), 166.4 (C-7), 179.3 (C-4).[11]

Loganin (4), colorless oil; ESI-MS m/z: 391 $[M+H]^+$;[1] H-NMR (CD_3OD, 400 MHz) δ_H: 7.40 (s, 1H, H-3), 5.28 (d, J = 4.4 Hz, 1H, H-1), 4.65 (d, J = 8.0 Hz, 1H, H-1'), 4.04 (t, J = 4.8 Hz, 1H, H-7), 3.83 (dd, J = 11.6, 1.6 Hz, 1H, H-6a'), 3.79 (dd, J = 1.6 Hz, 1H, H-6b'), 3.68 (s, 3H, OCH_3), 3.56 (m, 1H, H-3'), 3.26 (m, 1H, H-5'), 3.22-3.23 (m, 2H, H-4' and H-2'), 3.12 (m, 1H, H-5), 2.23 (m, 1H, H-9), 2.04 (m, 1H, H-6a), 1.88 (m, 1H, H-8), 1.62 (m, 1H, H-6b), 1.12 (d, J = 6.8 Hz, 3H, H-10);[13] C-NMR (100 MHz, CD_3OD) δ_C:170.1 (C-11), 152.7 (C-3), 114.6 (C-4), 100.6 (C-1'), 98.3 (C-1), 79.1 (C-5'), 78.6 (C-3'), 75.5 (C-7), 75.3 (C-2'), 72.1 (C-4'), 63.3 (C-6'), 52.2 (OCH3), 47.1 (C-9), 41.4 (C-6), 40.5 (C-8), 32.7 (C-5), 13.9 (C-10).[12]

Oleanolic acid (5), colorless oil; ESI-MS m/z: 456 $[M]^+$;[1] H-NMR (CD_3OD, 400 MHz) δ_H: 5.15 (t, J = 3.4 Hz, 1H, H-12), 3.21 (dd, J = 11.4, 4.2 Hz, 1H, H-3), 2.80 (dd, J = 14.0, 4.0 Hz, 1H, H-18), 1.04 (s, 3H, H-27), 0.96 (s, 3H, H-25), 0.93 (s, 3H, H-30), 0.85 (s, 3H, H-29), 0.84 (s, 3H, H-23), 0.80 (s, 3H, H-24), 0.75 (s, 3H, H-26).[13] C-NMR (CD_3OD, 100 MHz) δ_C: 183.2 (C-28), 143.5 (C-13), 122.4 (C-12), 79.0 (C-3), 55.4 (C-5), 47.6 (C-9), 46.6 (C-17), 45.9 (C-19), 41.7 (C-14), 41.0 (C-18), 39.4 (C-8), 38.2 (C-1), 37.8 (C-4), 37.1 (C-10), 33.9 (C-21), 33.2 (C-29), 32.6 (C-22), 32.6 (C-7), 30.8 (C-20), 28.2 (C-23), 27.8 (C-15), 25.1 (C-27), 23.7 (C-30), 23.6 (C-2), 23.5 (C-11), 23.0 (C-16), 18.3 (C-6), 17.2 (C-26), 15.6 (C-24), 15.4 (C-25).[13]

Compounds 2–5 were evaluated for agonistic activity for PPARα/γ. Unfortunately, only compound 3 exhibited moderate agonistic activities for PPARα, with EC_{50} values of 29.5 μM. The other compounds revealed no activity for PPARα/g.

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