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Original Article

A new coumarin glucoside ester from seeds oil leavings of *Xanthoceras sorbifolia*

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ABSTRACT

Objective: To study the coumarin from the seeds oil leavings of *Xanthoceras sorbifolia*.
Methods: The chemical constituents were isolated and purified by combination of silica gel, macroporous resin, Sephadex LH-20, and ODS column chromatography. Their structures were elucidated by spectral and chemical methods. The cytotoxicity of the new compound against 10 selected human cancer cell lines was assayed.
Results: Five comarins were isolated and identified as fraxetin-7-*O*-*β*-D-[6'-(3"–hydroxyl–3"- methylglutaryl)] glucopyranoside (**1**), fraxoside (**2**), fraxetin (**3**), scopoline (**4**), and esculetin (**5**), respectively.
Conclusion: Compound **1** is a new compound. Unfortunately, this compound exhibited no cytotoxicity with tested cell lines.

1. Introduction

Xanthoceras sorbifolia Bunge is a shrub distributed in northeast and north of China and has been used as a Chinese materia medica to treat arterial sclerosis, hyperlipemia, hyperpiesia, chronic hepatitis, and rheumatism (Li et al., 2007; Wang, 1998), in addition to their proliferating inhibitory activity against human cancer cell lines (Chan, 2007). Previous phytochemical studies on this plant revealed the presence of saponins, flavonoids, and coumarins from the fruit, seeds, barks, and husks (Chen, Takeda, & Ogihara, 1985; Fu et al., 2010; Li et al., 2006, 2007, 2008; Zhang and Bao, 2000; Ni and Zhang, 2009). And some of these compounds exhibited cytotoxicity toward various human cancer cell lines (Fu et al., 2010; Li et al., 2006, 2007, 2008). In our preceding paper (Yu et al., 2012), we reported some chemical constituents and their activity against 10 cell lines. The results showed that some compounds had cytotoxic activities. Here we reported the isolation and identification of a new coumarin glucoside (Fig. 1) together with four known coumarins, as well as inhibiting the proliferation activities of compound **1** against 10 human cancer cell lines.

2. Materials and methods

2.1. General

The NMR spectra were measured on Bruker ARX-300 and 600 spectrometers with TMS as the internal standard. HR-ESI-MS was obtained on an Agilent 6210 TOF mass spectrometer, Preparative HPLC system consisted of a LC-6AD intelligent preparative pump, SPD-20A intelligent UV/VIS detector, and YMC-Park ODS-A column (5 μm, ϕ 20 × 250 mm, YMC Co., Ltd.). Analytical HPLC system consisted of a Waters-600 controller pump, YMC column, waters-2400, and 996 photodiode array detectors. GC was carried out on an Agilent GC series system and performed with an HP-5 column (30 m × 0.25 mm × 0.25 μm, Agilent, Santa Clara, CA). Silica gel GF254 (1 – 40 μm) prepared for TLC and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Factory (Qingdao, China). ODS (40–70 μm) was purchased from Merck Chemical Company Ltd. (Darmstadt, Germany). Macroporous resin D101 was a product of Chemical Plant of Nankai University (Tianjin, China). Sephadex LH-20 was obtained from GE Healthcare (Little Chalfont, Buckinghamshire, UK). All the reagents were HPLC or analytical grade and purchased from Tianjin Damao Chemical Company (Tianjin, China).

2.2. Plant material and ten human cancer cell lines

The seeds of this plant were collected from Chifeng City, Inner Mongolia Autonomous Region of China, in October, 2015. The seeds

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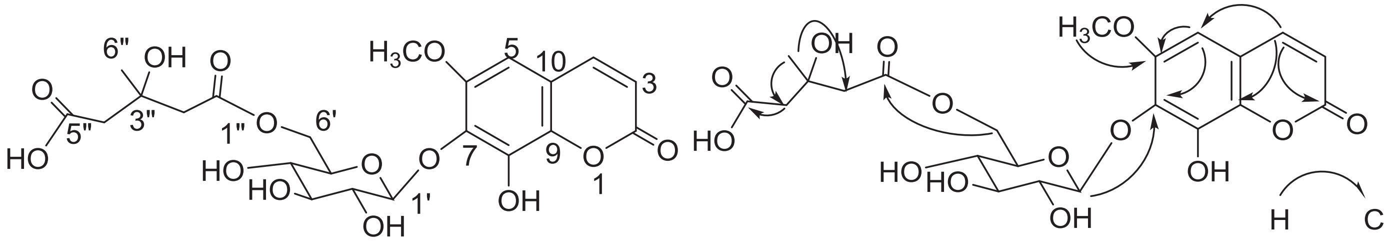

Fig. 1. Chemical structure and key HMBC correlations of compound **1**.

oil leavings were offered by Harbin Three-Happiness Bioengineering Pharmaceutical Co., Ltd. The voncher specimens (No.20151001) have been deposited in the College of Basic Science, Jinzhou Medical University.

Ten human cancer cell lines are Hela (human cervical cancer cell 60 line), BGC-823 (human gastric adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), MDA-231 (human breast adenocarcinoma cell line), PC-3 (human prostatic carcinoma cell line), HepG-2 (human hepatocellular carcinoma cell line), A549 (human lung adenocarcinoma cell line), A375-S2 (human melanoma cell line), Hep2 (human laryngeal carcinoma cell line), and HT1080 (human sarcoma cell line).

2.3. Extraction and isolation

The seeds oil leavings (8 kg) were refluxed with 70% EtOH (30 L) for three times and each time for 2 h. The extracts were filtered and then concentrated under reduced pressure to yield a crude extract of ethanol (15 L), which was isolated on D-101 macroporous resins to give four fractions (water, 30%, 60%, and 95% EtOH).

The 30% EtOH fraction (24.5 g) was subjected to a silica gel column chromatography (CC) (200–300 mesh) eluted with a gradient of $CHCl_3$–CH_3OH (10:1, 8:2, 7:3, 6:4, 0:100), CH_3OH–CH_3COOH (1000:5), H_2O, and H_2O–CH_3COOH (1000:5) to afford 13 subfractions (SFr.1–13). SFr.2 was separated by Sephadex LH-20 in CH_3OH to afford five subfractions (SFr.21–25). SFr.23 was separated by PTLC with CH_2Cl_2-CH_3OH-H_2O (60:30:5) to obtain main color belt then purified by Prep-HPLC to afford compound **3** (4 mg). SFr.24 was separated by Prep-HPLC (CH_3OH-H_2O, 35:65) to afford **1** (26 mg). SFr.4 was chromatographed over silica gel CC eluted with CH_2Cl_2-CH_3COCH_3 (9:1**–**7:3) and CH_2Cl_2-CH_3OH (9:1–1:1) to obtain nine subfractions (SFr.41–49). SFr.44 was isolated by Prep-HPLC (CH_3OH-H_2O, 38:62) to afford compound **4** (3 mg). SFr.45 was subjected to a silica gel CC eluted with $CHCl_3$-CH_3OH (8:2) to obtain nine subfractions (SFr.45-1–SFr.45-9). SFr.45-5 was purified by Prep-HPLC (CH_3OH-H_2O, 25:75) to afford compound **2** (9 mg).

The 95% EtOH fraction was subjected to silica gel CC eluted with a gradient of CH_2Cl_2-CH_3OH (100:0–0:100) to afford 15 subfractions (SFr.1–15). SFr.4 was purified by Prep-TLC eluted with PE-CH_3COCH_3 (6:4) to obtain compound **5** (9 mg).

2.4. Sugar analysis

The absolute configuration of sugar was carried out according to the method we have already reported (Yu et al., 2012). The peaks of authentic samples of *D*-glucose and *L*-glucose, after treatment in the same manner were detected at 23.55 min and 24.27 min respectively. The sugar in compound **1** was identified as *D*-glucose according to its retention time 23.53 min.

2.5. Cytotoxic assay

The cytotoxicity assay was carried out according to the MTT (3-(4, 5-dimethylthiazole-2-yl)–2, 5-diphenyltetrazolium bromide) method (Yu et al., 2012). Compound **1** and 5-fluorouracil (positive control) were dissolved in DMSO to make stock solutions respectively, which were diluted to a working solution before use. Cells were cultured at 37°C and dispersed in replicates in 96-well plates with ten selected human cancer cell lines for 24 h. Compound **1** and 5-fluorouracil were then added to make various final concentrations (3.125 μmol/L, 6.25 μmol/L, 12.5 μmol/L, 25 μmol/L, and 50 μmol/L). Three replicate wells were used at each point in the experiment with three parallel control wells. The medium was removed and 100 μL DMSO was added to each well. The formazan crystals in the cells were solubilized with DMSO, and the level of MTT formazan was determined by measuring its absorbance at 490 nm with the Spectra Shell reader. The percentage of cell inhibition was calculated as follows:

$$\text{cell death } (\%) = [A_{490}(\text{control}) - A_{490}(\text{test})]/A_{490}(\text{control}) \times 100\%.$$

IC_{50} values were calculated with the LOGIT method.

3. Results

Compound **1** was obtained as a yellow crystal. The ESI-TOF-MS data at m/z 537.1229 $[M+Na]^+$ revealed its molecular formula to be $C_{22}H_{26}O_{14}$. The ^1H NMR signals at δ_H 7.89 (1H, d, $J = 9.5$ Hz, H-4), 6.22 (1H, d, $J = 9.5$ Hz, H-3), and 7.05 (1H, s, H-5) were assigned to a 6, 7, 8-trisubstituted coumarin skeleton due to the HMBC correlations of H-5 [7.05(1H, s)]/C-4 (144.6), C-6 (145.4), and C-7 (131.2). The signals of one methoxy protons at δ_H 3.82 (3H, s) suggested the existence of a methoxy moiety in **6**, which was supported by the HMBC (Fig. 1) correlations of H-3.82/C-6. One anomeric protons at δ_H 4.97 (1H, d, $J = 7.6$ Hz) in conjunction with the carbon chemical shifts of one corresponding anomeric carbons at δ_C 103.5 (C-1′**)** indicated the presence of a glycosyl moieties. The β-anomeric configuration for the glucose was determined from its $^3J_{H1,H2}$ coupling signal 7.6 Hz. ^1H NMR δ_H 1.11 (3H, s), 2.52 (1H, d, $J = 11.1$ Hz), 2.41 (1H, d, $J = 11.1$ Hz), and 2.39 (2H, s) and connected with ^{13}C NMR δ_C 172.4, 170.3, 27.1, 45.2, 45.2, and 68.8 suggested that this compound including an 3"-methyl-3"-hydroxyglutaric acid moiety (Dubois, Wierer, & Wager, 1990). The NMR data indicated that **1** contained the same aglycone as fraxoside (**2**), which has been reported previously. The difference between them was an additional signal δ_C 172.4, 170.3, 27.1, 45.2, 45.2, and 68.8 assignable to one 3′′’-methyl-3′′’-hydroxyglutaric acid group in the NMR spectrum of **1**. The HMBC correlation of δ_H 4.21, 3.99/δ_C 170.3 verified that 3′′’-methyl-3′′’-hydroxyglutaric acid group was attached to C-6’. Correlation peaks was observed from the signal at δ_H 4.97 (H-1’) to δ_C 131.2 (C-7) which indicated that the sugar chains were located at C-7. The anometric of the glucose was determined as β-configured because of the large $^3J_{H\text{-}1,\,H\text{-}2}$ coupling constants (7.6 Hz). Acid hydrolysis of **1** afforded D-glucose as determined through GC analysis. The ^1H and ^{13}C NMR data were full assigned by the HSQC and HMBC spectra (Table 1). Therefore, compound **1** was characterized as fraxetin-7-*O*-β-*D*-[6’-(3′′’-hydroxyl-3′′’-methylglutaryl)] glucopyranoside.

Table 1
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for compound **1** in DMSO–d_6.

No.	^{13}C NMR	^{1}H NMR J (Hz)	No.	^{13}C NMR	^{1}H NMR J (Hz)
2	160.0		3′	76.0	3.27 (1H, m)
3	112.2	6.22 (1H, d, 9.5)	4′	70.0	3.18 (1H, m)
4	144.6	7.89 (1H, d, 9.5)	5′	74.1	3.38 (1H, m)
5	105.0	7.05 (1H, s)	6′	63.3	4.21 (1H, d, 10.4) 3.99 (1H, dd, 10.4, 6.8)
6	145.4		1″	170.3	
7	131.2		2″	45.2	2.52 (1H, d, 11.1) 2.42 (1H, d, 11.1)
8	143.7		3″	68.8	
9	142.8		4″	45.2	2.39 (2H, s)
10	110.0		5″	172.4	
1′	103.5	4.97 (1H, d, 7.6)	6″	27.1	1.11 (3H, s)
2′	73.8	3.36 (1H, m)	OCH$_3$	56.1	3.82 (3H, s)

Four known compounds, fraxoside (**2**) (Shi, Xu, & Kong, 2009), fraxetin (**3**) (Ma & Wang, 2010), scopoline (**4**) (Wang, Wang, Gan, Wu, & Liu, 2011), and esculerin (**5**) (Liu, Wang, Chen, Wu, & Wang, 2003) were also elucidated on the basis of their spectroscopic profiles and comparison to published data in the literatures. Compound **1** was tested for its cytotoxicity against Hela, BGC-832, MCF-7, MDA-231, PC-3, HepG-2, A594, A375-S2, Hep2, and HT1080 cell lines by MTT method. Unfortunately, it did not exhibite cytotoxicity.

Conflict of interest

The authors declare no conflict of interest.

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