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Three new acetylated benzyl-beta-resorcylate glycosides from *Cassia obtusifolia*

Xiaohui Wu [a,b,c], Jinlan Ruan [c], V.C. Yang [a,d], Zhiyuan Wu [b], Jianshi Lou [a], Hongquan Duan [a], Jun Zhang [a], Yanwen Zhang [a,*], Dean Guo [b,*]

[a] Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, College of Pharmacy, Tianjin Medical University, Tianjin 300070, China
[b] National Engineering Laboratory for TCM Standardization Technology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
[c] Key Laboratory of Natural Medicinal Chemistry and Resources Evaluation of Hubei Province, College of Pharmacy, Tongji Medical Center, Huazhong University of Science, Wuhan 430030, China
[d] Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA

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ABSTRACT

Three new acetylated benzyl-beta-resorcylate glycosides (**1**–**3**) were isolated from seeds of *Cassia obtusifolia*. Their structures were determined on the basis of the spectroscopic methods and physicochemical properties as 2-benzyl-4,6-dihydroxy benzoic acid-6-O-[2,6-O-diacetyl]-D-glucopyranoside (**1**), 2-benzyl-4,6-dihydroxy benzoic acid-6-O-[3,6-O-diacetyl]-D-glucopyranoside (**2**) and 2-benzyl-4, 6-dihydroxy benzoic acid-6-O-[4,6-O-diacetyl]-D-glucopyranoside (**3**), respectively.

1. Introduction

Cassia obtusifolia Linn, a member of the genus *Cassia* (Leguminosae), is a widely used traditional Chinese medicinal plant and widely distributed in China, Japan, Philippines and South Korea. It belongs to the economically and medically important family Leguminosae (Syn. Cesalpiniaceae), subfamily Ceasalpinioideae [1,2]. The seeds of the plant have been widely used for the treatments of purgation, red and tearing eyes, dizziness, and headache, etc. [3]. A number of compounds including flavonoids, triterpenoids, anthrones and anthraquinones were reported in previous literatures [4–8]. Anthraquinones were confirmed to exert the main effect on purgation [9]. We have previously reported three new compounds A–C from the seeds of *C. obtusifolia* [8]. As part of the continuous chemical constituents investigation of this plant, three new acetylated benzyl-beta-resorcylate glycosides (**1**–**3**) were isolated and determined on the basis of spectroscopic methods and physicochemical properties. In this paper, the isolation and the structural elucidation of the three new compounds (**1**–**3**) are described.

2. Experimental methods

2.1. General

IR spectra were recorded with a Perkin-Elmer 577 spectrometer as KBr pellet. NMR spectra were recorded with a Bruker AM-400 spectrometer with TMS as an internal standard. Diaion HP-20 (Mitsubishi Chem. Ind., Tokyo, Japan). All solvents were of analytical grade (Shanghai Chemical Plant). HRESIMS were obtained on a Marine instrument. Silica gel (200–300 mesh) C_{18}, reversed-phase silica gel (150–200 mesh, Merck) etc. were used for column chromatography,

* Corresponding authors. Tel.: +86 21 50271516; fax: +86 21 50272789.
E-mail addresses: zhangyanwen@tijmu.edu.cn (Y. Zhang), gda5958@163.com (D. Guo).

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and pre-coated silica gel GF_{254} plate (QingDao Marine Chemical Plant) was used for TLC.

2.2. Plant material

The seeds of *C. obtusifolia* were purchased from Shanghai Derentang Pharmaceutical Co. Ltd. The plants were authenticated by Prof. De-An Guo, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China. A voucher specimen (SC 0222009) was deposited in the Shanghai Research Center for Modernization of TCM, Shanghai Institute of Materia Medica.

2.3. Extraction and isolation

The seeds of *C. obtusifolia* (4.0 kg) were ground and extracted with 95% ethanol (10×4 L) for 10 times at room temperature. The ethanol extract was concentrated under vacuum to yield a crude extract (380.0 g), which was suspended in H_2O (4 L) and extracted with petroleum ether (3×4 L), $CHCl_3$ (3×4 L), EtOAc (3×4 L) and *n*-BuOH (3×4 L), sequentially. The EtOAc extract (26.0 g) was separated by column chromatography on silica gel (200–300 mesh, 300 g) eluting with a $CHCl_3$–MeOH gradient (30:1, 20:1, 10:1, 5:1, 2:1, 1:1) to yield fractions I–IIIV. Fraction V (1.2 g) was then repeatedly chromatographed on silica gel (200–300 mesh) column using $CHCl_3$–MeOH (3:1). The final purification was carried out by ODS, with eluting solvent MeOH–H_2O (1.2:1). This yielded new compound **1** (21 mg, t_R 9.0 min Fig. 1), compound **2** (19 mg, t_R 10.2 min Fig. 1) and compound **3** (27 mg, t_R 9.6 min Fig. 1).

2.3.1. 2-Benzyl-4,6-dihydroxy benzoic acid-6-O-[2,6-O-diacetyl]-D-glucopyranoside (**1**)

White amorphous powder (MeOH); IR bands (KBr): 3550, 3421, 1609, 1500, 1423, 1411, 1351, 1200, 1082, 840, 699 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (positive-ion mode) *m/z* 513.1367 $[M+Na]^+$ (calcd for $C_{24}H_{26}O_{11}Na$ 513.1373).

2.3.2. 2-Benzyl-4,6-dihydroxy benzoic acid-6-O-[3,6-O-diacetyl]-D-glucopyranoside (**2**)

White amorphous powder (MeOH); IR bands (KBr): 3537, 3410, 1622, 1499, 1435, 1400, 1362, 1199, 1070, 856, 709 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (positive-ion mode) *m/z* 513.1371 $[M+Na]^+$ (calcd for $C_{24}H_{26}O_{11}Na$ 513.1373).

2.3.3. 2-Benzyl-4,6-dihydroxy benzoic acid-6-O-[2,6-O-diacetyl]-D-glucopyranoside (**3**)

White amorphous powder (MeOH); IR bands (KBr): 3543, 3410, 1618, 1509, 1435, 1412, 1358, 1194, 1065, 849, 695 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (positive-ion mode) *m/z* 513.1369 $[M+Na]^+$ (calcd for $C_{24}H_{26}O_{11}Na$ 513.1373).

2.4. Alkaline hydrolysis of **1**–**3**

Compound **1** (5 mg) was hydrolyzed with 1% KOH (0.5 mL) for 1 h at room temperature. Then acidification with 1% HCl until pH 5, and the reaction mixture was extracted with *n*-BuOH. The *n*-BuOH extract was purified on silica gel ($CHCl_3$–MeOH–H_2O, 4:1:0.1) to give **1a** (3 mg). Compounds **2** and **3** (5 mg) were treated in the same manner as **1** to afford **1a**, which was determined by co-TLC ($CHCl_3$–MeOH–H_2O, 3:1:0.1, R_f = 0.18).

Table 1
^1H NMR (400 MHz) data of **1–3** and **1a** (δ values, *J* in Hz, in DMSO-d_6).

Position	Compound **1**	Compound **2**	Compound **3**	Compound **1a**
1	–	–	–	–
2	–	–	–	–
3	6.52 (d, 2.4)	6.52 (d, 2.4)	6.50 (d, 2.4)	6.45 (d, 2.4)
4	–	–	–	–
5	6.32 (d, 2.4)	6.31 (d, 2.4)	6.31 (d, 2.4)	6.42 (d, 2.4)
6	–	–	–	–
2-COOH	–	–	–	–
1′	–	–	–	–
2′	7.04 (m)	7.01 (m)	7.01 (m)	7.05 (m)
3′	7.15 (m)	7.15 (m)	7.13 (m)	7.13 (m)
4′	7.08 (m)	7.06 (m)	7.05 (m)	7.08 (m)
5′	7.15 (m)	7.15 (m)	7.13 (m)	7.13 (m)
6′	7.04 (m)	7.01 (m)	7.01 (m)	7.05 (m)
7′	2.83 (2H, m)	2.83 (2H, m)	2.81 (2H, m)	2.78 (2H, m)
Glc:1″	4.99 (d, 8.0)	4.96 (d, 8.0)	4.94 (d, 8.3)	4.92 (d, 8.3)
2″	3.62 (m)	3.52 (m)	3.49 (m)	3.49 (m)
3″	3.49 (m)	3.61 (m)	3.50 (m)	3.46 (m)
4″	3.38 (m)	3.43 (m)	3.57 (m)	3.39 (m)
5″	3.70 (m)	3.72 (m)	3.80 (m)	3.63 (m)
6″	4.26(dd, 12.0, 2.0)	4.18(dd, 12.0, 2.0)	3.88(dd, 12.0, 5.0)	4.09 (d, 10.3)
	3.95(dd, 12.0, 5.7)	3.97(dd, 12.0, 5.0)	3.76(dd, 12.0, 2.0)	3.78 (m)
2″-$OCOCH_3$	2.09 (s)	–	–	–
3″-$OCOCH_3$	–	2.12 (s)	–	–
4″-$OCOCH_3$	–	–	2.08 (s)	–
6″-$OCOCH_3$	1.98 (s)	1.91 (s)	1.89 (s)	–

2.5. Acid hydrolysis of **1**–**3**

Compounds **1**, **2** and **3** (3 mg each) were refluxed with 10% HCl and were stirred at 90 °C for 3 h, respectively. The aglycone was extracted with EtOAc. The aqueous layers of the acid hydrolysis of **1**, **2** and **3** were neutralized with $NaHCO_3$ and then concentrated. D-Glucose was determined in each aqueous layer by TLC on a silica G plate comparison with authentic sample, respectively. The R_f values were 0.14, 0.14 and 0.12 with $CHCl_3$–MeOH–H_2O (3:1:0.1) and *n*-BuOH–HOAc–H_2O abbreviated to BAW (4:1:5, upper layer) as developing system, respectively. By measuring its optical rotation ($[\alpha]_D^{20}$ + 41.6 (**1**), $[\alpha]_D^{20}$ + 39.2 (**2**), $[\alpha]_D^{20}$ + 40.4 (**3**)),D-glucose was identified.

2.6. Cytotoxicity bioassay

Cytotoxic activity was tested against a human hepatoblastoma cell line (HepG 2) using the MTT method [10], according to a previous literature [11]. The OD value was read on a plate reader at wavelength of 570 nm. 5-Fluorouracil was used as positive control.

3. Results and discussion

Compound **1** was obtained as a white amorphous powder (MeOH) with the molecular formula $C_{24}H_{26}O_{11}$, determined on the basis of NMR and HRESIMS data (*m/z* 513.1367 [M+