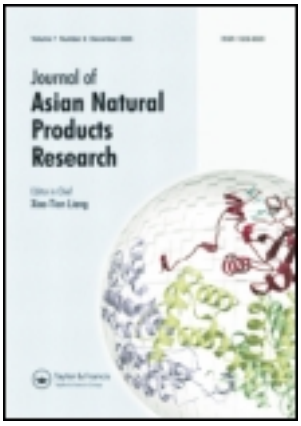

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Acetylated anthraquinone glycosides from *Cassia obtusifolia*

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Three new acetylated anthraquinone glycosides (**1**–**3**) were isolated from the seed of *Cassia obtusifolia,* together with one parent anthraquinone glycoside (**1a**). Their structures were determined on the basis of spectroscopic methods and physicochemical properties as obtusifoline-2-O-β-D-2, 6-di-*O*-acetylglucopyranoside (**1**), obtusifoline-2-O-β-D-glucopyranoside (**1a**), obtusifoline-2-O-β-D-3, 6-di-*O*-acetylglucopyranoside (**2**), and obtusifoline-2-O-β-D-4, 6-di-*O*-acetylglucopyranoside (**3**).

Keywords: Leguminosae; *Cassia obtusifolia*; acetylated anthraquinone glycosides; spectroscopic methods

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, the 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 treatment of purgation, red and tearing eyes, dizziness, headache, etc. [3]. In previous investigations of this plant, a number of compounds were isolated, including flavonoids, triterpenoids, anthrones, and anthraquinones [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 anthraquinone glycosides (**1**–**3**) were isolated and determined on the basis of spectroscopic methods and physicochemical properties, along with the parent anthraquinone glycoside **1a** (Figure 1). In this paper, the isolation and the structural elucidation of the three new compounds (**1**–**3**) are described.

2. Results and discussion

Compound **1** was obtained as yellow needles with the molecular formula $C_{26}H_{26}O_{12}$, determined on the basis of NMR and HR-ESI-MS data (*m/z* 553.1328 $[M + Na]^+$). The ^{13}C NMR spectrum exhibited 12 aromatic carbons (δ 152.0, 155.9, 140.1, 125.1, 118.4, 136.4, 124.0, 161.0, 124.5, 116.8, 129.7, 132.4) and two

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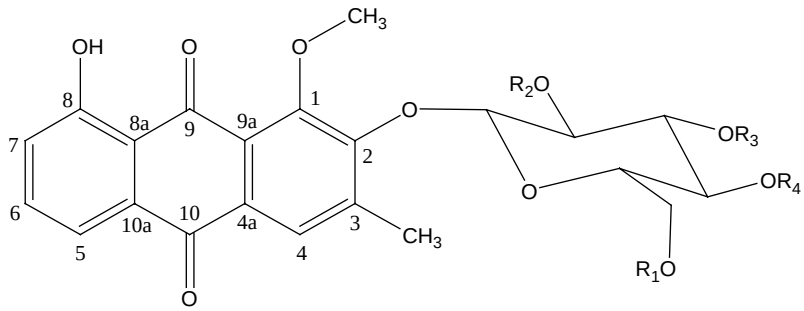

	R_1	R_2	R_3	R_4
1	**Ac**	**Ac**	**H**	**H**
1a	**H**	**H**	**H**	**H**
2	**Ac**	**H**	**Ac**	**H**
3	**Ac**	**H**	**H**	**Ac**

Figure 1. Structure of compound **1**–**3** and **1a**.

carbonyl carbons (δ 187.8, 181.3) suggestive of a typical anthraquinone structure. In the above 12 aromatic carbons, two aromatic carbons at δ 155.9 (C-2) and 161.0 (C-8) were substituted by hydroxy, one aromatic carbon at δ 152.0 (C-1) by a methoxyl (δ 61.4), and one aromatic carbon at δ 140.1 (C-3) by a methyl

Table 1. ^{1}H 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				
4	7.86 (s)	7.92 (s)	7.90 (s)	7.89 (s)
4a				
5	7.65 (dd, 1.2, 8.4)	7.68 (dd, 1.2, 8.2)	7.68 (dd, 1.2, 8.3)	7.60 (dd, 1.3, 8.2)
6	7.74 (dd, 8.0, 8.4)	7.73 (dd, 8.0, 8.2)	7.79 (dd, 7.8, 8.3)	7.69 (dd, 7.8, 8.2)
7	7.33 (dd, 1.2, 8.0)	7.35 (dd, 1.2, 8.0)	7.30 (dd, 1.2, 7.8)	7.27 (dd, 1.3, 7.8)
8				
8a				
9				
9a				
10				
10a				
1-OCH_3	3.91 (s)	3.88 (s)	3.80 (s)	3.94 (s)
3-CH_3	2.41 (s)	2.40 (s)	2.38 (s)	2.45 (s)
Glc:1′	4.96 (d, 8.0)	5.00 (d, 8.0)	5.00 (d, 7.8)	5.02 (d, 8.0)
2′	4.67 (m)	3.30 (m)	3.20 (m)	3.22 (m)
3′	3.45 (m)	4.88 (m)	3.47 (m)	3.33 (m)
4′	3.29 (m)	3.39 (m)	4.66 (m)	3.30 (m)
5′	3.26 (m)	3.37 (m)	3.98 (m)	3.07 (m)
6′	4.22 (dd, 12.0, 2.0)	4.20 (dd, 12.0, 2.0)	3.82 (dd, 12.0, 5.0)	3.62 (m)
	3.94 (dd, 12.0, 5.7)	3.99 (dd, 12.0, 5.0)	3.77 (dd, 12.0, 2.0)	3.55 (m)
2′-$OCOCH_3$	2.10 (s)			
3′-$OCOCH_3$		2.08 (s)		
4′-$OCOCH_3$			2.11 (s)	
6′-$OCOCH_3$	1.93 (s)	1.91 (s)	1.88 (s)	

(δ 17.3). Examination of the ^{1}H and ^{13}C NMR spectroscopic data of **1** (Tables 1 and 2) indicated that the molecule consisted of an anthraquinone, one methyl, one methoxyl, one sugar, and two acetyl moieties.

The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of obtusifoline [10], apart from glycosidation chemical shifts signals of C-1 (δ 152.0), C-2 (δ 155.9), and C-3 (δ 140.1) and the presence of additional two acetyl groups. The sugar moiety was identificated as D-glucose ($[\alpha]_D^{20} + 39.6$, *c* 0.1, H_2O) by acid hydrolysis and by comparison with an authentic sample. The β-configuration of the glycosidic bond was deduced from the ^{1}H and ^{13}C NMR spectral data of the sugar moiety ($J = 8.0$ Hz). So the sugar moiety was determined as β-D-glucose, and it connected to the C-2 position of the anthraquinone aglycone by the HMBC correlation of H-1′/C-2. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of **1a** [11], except for the two additional acetyl groups in **1**. Alkaline hydrolysis of **1** with 1% KOH gave the same deacetylated glycoside **1a**, which was identified by TLC. In the HMBC spectrum, the correlations between H-2′/2′-$OCOCH_3$ and H-6′/6′-$OCOCH_3$ indicated that the acetyl moieties were connected to C-2′ and C-6′ (Figure 2). On the basis of the above observations, the structure of compound **1** was established as obtusifoline-2-O-β-D-2, 6-di-*O*-acetylglucopyranoside.

Table 2. ^{13}C NMR (100 MHz) data of **1**–**3** and **1a** (δ values, in DMSO-d_6).

Position	Compound **1**	Compound **2**	Compound **3**	Compound **1a**
1	152.0	152.2	152.0	154.3
2	155.9	155.7	156.0	153.5
3	140.1	139.9	140.3	142.4
4	125.1	124.8	125.0	125.1
4a	124.5	124.5	124.3	124.4
5	118.4	118.2	117.9	118.0
6	136.4	136.3	136.4	136.5
7	124.0	124.1	124.0	123.9
8	161.0	161.2	161.0	160.8
8a	116.8	116.9	116.6	116.6
9	187.8	187.9	188.0	187.8
9a	129.7	129.6	129.5	129.7
10	181.3	181.4	181.1	181.4
10a	132.4	132.3	132.2	132.2
1-OCH_3	61.4	61.2	61.3	61.0
3-CH_3	17.3	17.5	17.6	17.6
Glc:1′	104.5	106.8	107.0	106.7
2′	75.4	74.0	75.6	75.9
3′	76.2	78.7	75.7	78.1
4′	71.7	71.2	72.4	71.2
5′	75.4	75.5	73.5	77.9
6′	64.7	64.3	64.0	62.3
2′-$OCOCH_3$	173.1			
	21.7			
3′-$OCOCH_3$		173.1		
		21.3		
4′-$OCOCH_3$			172.6	
			21.4	
6′-$OCOCH_3$	173.5	173.9	173.1	
	21.1	21.0	21.1	

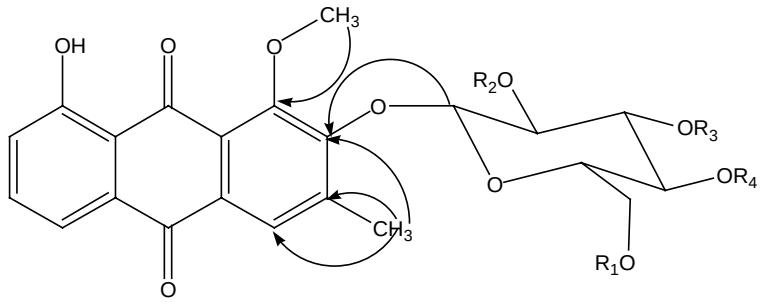

Figure 2. Selected HMBC correlations (H → C) for compounds **1**–**3** and **1a**.

Compounds **2** and **3** were obtained as yellow needles having the same molecular formula $C_{26}H_{26}O_{12}$ as **1** on the basis of HR-ESI-MS and NMR analysis. And the ^{1}H and ^{13}C NMR spectra of **2** and **3** were similar to those of **1** apart from the chemical shifts of C-2′, C-3′, and C-4′ in compound **2** and the chemical shifts of C-3′, C-4′, and C-5′ in compound **3**, due to the different link site of the acetyl groups to β-D-glucose among **1**, **2**, and **3**. The acetyl groups of **2** were linked to C-3′ and C-6′ by the HMBC correlations of H-3′/3′-$OCOCH_3$ and H-6′/6′-$OCOCH_3$. The HMBC correlations between H-4′/4′-$OCOCH_3$ and H-6′/6′-$OCOCH_3$ indicated that the acetyl moieties were connected to C-4′ and C-6′ of **3**. Thus, **2** and **3** were characterized as obtusifoline-2-O-β-D-3, 6-di-*O*-acetylglucopyranoside and obtusifoline-2-*O*-β-D-4, 6-di-*O*-acetylglucopyranoside, respectively.

3. Experimental

3.1 General experimental procedures

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 Chemical Industries, Tokyo, Japan). All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, China). HRESIMS were obtained on a Marine instrument. Silica gel (200–300 mesh), C_{18} reversed-phase silica gel (150–200 mesh, Merck, Mumbai, India) etc. were used for column chromatography, and pre-coated silica gel GF_{254} plate (QingDao Marine Chemical Plant, QingDao, China) was used for TLC.

3.2 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, China. A voucher specimen (SC 0222009) has been deposited in the Shanghai Research Center for Modernization of TCM, Shanghai Institute of Materia Medica.

3.3 Extraction and isolation

The seeds of *C. obtusifolia* (4.0 kg) were ground and extracted with 95% ethanol (10 × 4 l) at room temperature. The ethanol extract was concentrated under vacuum to leave a residue that 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–III. Fraction II (0.9 g)

was then repeatedly chromatographed on a silica gel (200–300 mesh) column using $CHCl_3$:MeOH (5:1). The final purification was carried out by ODS, with eluting solvent MeOH:H_2O (1:1). This yielded new compounds **1** (28 mg, t_R 10.8 min), **2** (31 mg, t_R 9.2 min), and **3** (25 mg, t_R 11.6 min).

3.3.1 Obtusifoline-2-O-β-D-2, 6-di-O-acetylglucopyranoside (1)

Yellow needles; IR (KBr) ν_{max}: 3869, 3215, 2900, 1751, 1666, 1641, and 1550 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; HRESIMS (positive-ion mode): *m/z* 553.1328 $[M + Na]^+$ (calcd for $C_{26}H_{26}O_{12}Na$, 553.1322).

3.3.2. Obtusifoline-2-O-β-D-3, 6-di-O-acetylglucopyranoside (2)

Yellow needles; IR (KBr) ν_{max}: 3880, 3236, 2911, 1760, 1682, 1600, and 1541 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; HRESIMS (positive-ion mode): *m/z* 553.1325 $[M + Na]^+$ (calcd for $C_{26}H_{26}O_{12}Na$, 553.1322).

3.3.3. Obtusifoline-2-O-β-D-4, 6-di-O-acetylglucopyranoside (3)

Yellow needles; IR (KBr) ν_{max}: 3852, 3220, 2934, 1738, 1652, 1644, and 1558 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; HRESIMS (positive-ion mode): *m/z* 553.1326 $[M + Na]^+$ (calcd for $C_{26}H_{26}O_{12}Na$, 553.1322).

3.4 Alkaline hydrolysis of 1–3

Compound **1** (7 mg) was hydrolyzed with 1% KOH (0.5 ml) for 1 h at room temperature. After acidification with 1% HCl until pH 5, the reaction mixture was extracted with *n*-BuOH. The *n*-BuOH extract was purified on silica gel ($CHCl_3$:MeOH:H_2O, 3:1:0.1) 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, $R_f = 0.30$).

3.5 Acid hydrolysis of 1–3

Compounds **1**, **2**, and **3** (3 mg each) were refluxed in 10% HCl and 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 gel plate and by comparing with authentic sample, respectively. The R_f values were 0.15, 0.15, and 0.16 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. The optical rotation was $[\alpha]_D^{20} + 39.6$ (**1**), $[\alpha]_D^{20} + 35.9$ (**2**), $[\alpha]_D^{20} + 38.2$ (**3**). Thus, D-glucose was identified.

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