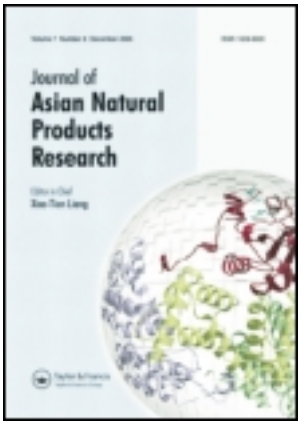

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Irisdichotins A–C, three new flavonoid glycosides from the rhizomes of *Iris dichotoma* Pall.

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Three new compounds including one flavonol glycoside, irisdichotin A (**1**), and two flavanonol glycosides, irisdichotins B (**2**) and C (**3**), were isolated from the rhizomes of *Iris dichotoma* Pall. Their structures were elucidated on the basis of spectroscopic methods.

Keywords: *Iris dichotoma*; irisdichotin A; irisdichotin B; irisdichotin C

1. Introduction

Iris dichotoma Pall. (Iridaceae) is a perennial herb that is native to China. Its rhizomes are commonly used in Chinese folk medicine for clearing heat and detoxifying, eliminating phlegm, swelling, and pain in the throat [1]. Several flavonoids had been previously reported from the rhizomes of *I. dichotoma* [2]. To find potential drug leads, the chemical constituents of *I. dichotoma* were further investigated. This paper deals with the isolation and structural elucidation of three new flavonoid glycosides, irisdichotins A–C (**1**–**3**; Figure 1).

2. Results and discussion

Irisdichotin A (**1**), yellow powder, had molecular formula $C_{23}H_{24}O_{12}$ on the basis of HR-ESI-MS data (*m/z* 493.1352, $[M + H]^+$). The IR spectrum indicated the presence of hydroxyl (3350 cm^{-1}) and carbonyl (1665 cm^{-1}). The ^1H NMR spectrum (Table 1) showed two MeO groups at δ_H 3.90 (s, 3H) and 3.86 (s, 3H), five aromatic protons at δ_H 6.86 (d, 1H, $J = 2.4$ Hz), 6.95 (d, 1H, $J = 8.4$ Hz), 7.04 (d, 1H, $J = 2.4$ Hz), 7.75 (dd, 1H, $J = 8.4$, 2.4 Hz), and 7.79 (d, 1H, $J = 2.4$ Hz), and two OH groups at δ_H 9.09 and 9.67 (s, 1H each) on aromatic rings, which were very similar to those of 3,5,3′-trihydroxy-7,4′-dimethoxyflavone [3]. In addition, six proton signals were found in the range of δ_H 3.0–4.0, together with the signal at δ_H 4.86 (d, 1H, $J = 7.8$ Hz) assigned as the anomeric proton, which suggested the existence of a sugar moiety. Comparison of the NMR spectra of **1** with those of 3,5,3′-trihydroxy-7,4′-dimethoxyflavone showed that the OH group at C-5 in 3,5,3′-trihydroxy-7,4′-dimethoxyflavone was replaced by an *O*-β-D-Glu group in **1**, as confirmed by typical ^{13}C NMR signals at δ_C 103.5, 73.6, 75.9, 69.9, 77.6, and 60.9 [4]. The HMBC correlations (Figure 2) of H-1″ at δ_H 4.86 (d, 1H, $J = 7.8$ Hz) with C-5 at δ_C 157.9 also revealed that the OGlu group was located at C-5. Acid hydrolysis of **1** gave glucose, which was identified by

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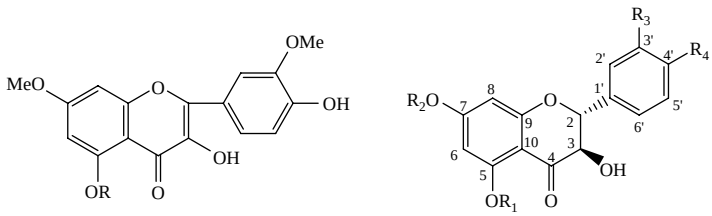

	R_1	R_2	R_3	R_4
2	D-Glc	Me	OMe	OH
3	Me	D-Glc	H	OH

Figure 1. Structures of compounds **1**–**3**.

comparing with authentic sample. The assignment of OH group of B ring was deduced from HMBC correlations between OH-4′ at δ_H 9.67 and C-5′ at δ_C 115.5, C-3′ at 148.5 and C-4′ at 147.4. On the other hand, the correlation between the methoxyl at δ_H 3.86 and C-3′ at δ_C 148.5 suggested that the MeO group was attached at C-3′ (Figure 2). Thus, the structure of **1** was elucidated as 3,4′-dihydroxy-7,3′-dimethoxyflavonol-5-*O*-β-D-glucopyranoside (Figure 1).

Irisdichotin B (**2**), yellow powder, had the molecular formula $C_{23}H_{26}O_{12}$ according to the HR-ESI-MS data (*m/z* 517.1349 $[M + Na]^+$). The UV, IR, and NMR spectral data of **2** were similar to those of **1**. Comparison of the ^{13}C NMR and DEPT spectra of **2** with those of **1** revealed that the tetrasubstituted double bond at C-2,3 in **1** was replaced by two methine carbons in **2**, as confirmed by ^1H NMR signals at δ_H 5.04 (d, 1H, $J = 12.0$ Hz, H-2) and 4.47 (dd, 1H, $J = 12.0$, 4.8 Hz, H-3). The circular dichroism (CD) spectrum of **2**, which showed a positive Cotton effect in the region of 334 nm and a negative effect at 300 nm, established the *R* configuration at

Table 1. ^1H NMR spectral data of **1**–**3** (600 MHz, in DMSO at 27°, δ in ppm, J in Hz).

Position	**1**	**2**	**3**
H-2		5.04 (d, $J = 12.0$)	5.04 (d, $J = 12.0$)
H-3		4.47 (dd, $J = 12.0$, 4.8)	4.41 (dd, $J = 12.0$, 4.8)
H-6	6.86 (d, $J = 2.4$)	6.28 (d, $J = 2.4$)	6.48 (d, $J = 2.4$)
H-8	7.04 (d, $J = 2.4$)	6.48 (d, $J = 2.4$)	6.27 (d, $J = 2.4$)
H-2′	7.79 (d, $J = 2.4$)	7.10 (d, $J = 1.8$)	7.32 (d, $J = 9.0$)
H-3′			6.79 (d, $J = 9.0$)
H-5′	6.95 (d, $J = 8.4$)	6.79 (d, $J = 8.4$)	6.79 (d, $J = 9.0$)
H-6′	7.75 (dd, $J = 8.4$, 2.4)	6.92 (dd, $J = 8.4$, 1.8)	7.32 (d, $J = 9.0$)
MeO-5			3.78 (s)
MeO-7	3.90 (s)	3.79 (s)	
MeO-3′	3.86 (s)	3.78 (s)	
HO-4′	9.67 (s)	9.08 (s)	9.53 (s)
HO-3	9.09 (s)	5.41 (d, $J = 4.8$)	5.42 (d, $J = 4.8$)
Glucose			
H-1″	4.86 (d, $J = 7.8$)	4.87 (d, $J = 7.2$)	4.87 (d, $J = 7.8$)
H-2″	3.42 (m)	3.41 (m)	3.43 (m)
H-3″	3.33 (m)	3.37 (m)	3.35 (m)
H-4″	3.19 (m)	3.22 (m)	3.17 (m)
H-5″	3.51 (m)	3.46 (m)	3.48 (m)
H-6″	3.77 (m)	3.77 (m)	3.75 (m)

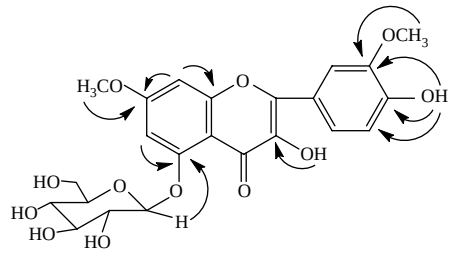

Figure 2. Key HMBC correlations of **1**.

the C-2 position [5]. The coupling constant between H-2 and H-3 ($J = 12.0$ Hz) also indicated the stereochemistry of C-3 to be *R* [6]. Finally, the structure of **2** was elucidated as 3β,4′-dihydroxy-7,3′-dimethoxyflavonone-5-*O*-β-D-glucopyranoside.

Irisdichotin C (**3**), white powder, had the molecular formula $C_{22}H_{24}O_{11}$ according to the HR-ESI-MS data (*m/z* 487.1224 $[M + Na]^+$). Comparison of the ^1H and ^{13}C NMR spectral data of **3** with those of **2** indicated that the differences between two compounds were the locations of methoxy and the sugar moiety at A-ring, and the substituent groups at B-ring. AA′BB′ spin system, which was exhibited at δ_H 7.32 (d, 2H, $J = 9.0$ Hz) and 6.79 (d, 2H, $J = 9.0$ Hz), and the HMBC correlation of OH at δ_H 9.53 with C-4′ at δ_C 157.7 suggested that the OH group was attached to C-4′. The OMe group was attached to C-5, based on HMBC correlations between OMe at δ_H 3.78 and C-5 at δ_C 165.6, as well as between H-6 at δ_H 6.48 and C-5 (Figure 3). The Glu group was attached to C-7, according to the HMBC correlations between H-1″ at δ_H 4.87 and C-7 at δ_C 159.6, and between H-8 at δ_H 6.27 and C-7. Finally, the structure was elucidated as 3β,4′-dihydroxy-5-methoxyflavonone-7-*O*-β-D-glucopyranoside.

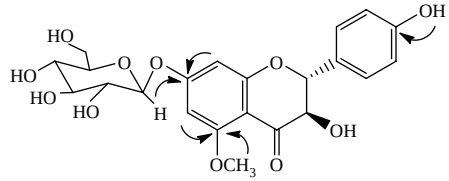

Figure 3. Key HMBC correlations of **3**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. UV spectra were recorded on a PGENRAL UV-T6 spectrophotometer. IR spectra were recorded on a SHIMADZU FTIR-8400s spectrometer. ^1H and ^{13}C NMR spectra (DMSO-d_6) were recorded on a Bruker Avance-600 instrument, and chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. CD spectra were recorded on a JASCO J-815 spectrophotometer. Mass spectra were obtained using a Thermo LTQ ORBITRAP XL mass spectrometer (for ESI and HR-ESI). Preparative HPLC was carried out with LabAlliance Series 1500 pump and LabAlliance Model 500 variable wavelength detector with a reversed-phase column (Alltech Alltima C18, 5 μm, 10 × 250 mm, at 3.5 l ml/min with detection at 260 nm).

3.2 Plant material

The rhizomes of *I. dichotoma* were collected at Jiufeng Mountain in Beijing, China, in April 2009, and identified by Prof. Pei-Gen Xiao, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (Bj090412) has been deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

3.3 Extraction and isolation

The air-dried rhizomes of *I. dichotoma* (5 kg) were refluxed with 90% EtOH (3 × 40 l, 2 h each). The extracts were concentrated under reduced pressure to syrup. The syrup was suspended in H_2O

(6l), followed by successive partitioning with petroleum ether, AcOEt, and BuOH. The BuOH extract (326.3 g) was subjected to column chromatography (CC; SiO_2; $CHCl_3$/MeOH/H_2O: 100:1:1 to 10:5:1) to afford 12 fractions (Frs. 1 ~ 12). Fr. 4 (21.1 g) was further subjected to repeated CC ($CHCl_3$/MeOH/H_2O: 50:3:1 to 10:3:1), and then with preparative HPLC (Alltima C18, 5 μm, 10 × 250 mm, MeOH/H_2O 47:53, flow rate 3.6 ml/min) to give compound **1** (7 mg) and compound **2** (5.6 mg). Fr. 11 (23.3 g) was further subjected to repeated CC ($CHCl_3$/MeOH/H_2O: 99:1 to 6:4:1) to afford 15 fractions (Fr. 11.1 ~ 11.15). Fr. 11.12 (9.8 g), eluted with $CHCl_3$/MeOH/H_2O (15:3:1), was subjected to repeated CC (SiO_2; $CHCl_3$/MeOH/H_2O 15:3:1), and then with prep. HPLC (MeOH/H_2O 50:50; flow rate 5 ml/min) to give compound **3** (5 mg).

*3.3.1 3,4′-Dihydroxy-7,3′-dimethoxy-flavonol-5-O-β-D-glucopyranoside (**1**)*

3.3.1.1 Yellow powder. $[\alpha]_D^{25}$ −74.8 (*c* 0.05, MeOH). UV (MeOH) λ_{max} (log ε) 205 (4.68), 252 (4.33), 367 (4.36) nm; IR (KBr) ν_{max} cm^{-1}: 3320 (br), 2938, 2376, 1665, 1616, 1541, 1506, 1456, 1421, 1300, 1271, 1213, 1103, 1078, 993. ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2. HR-ESI-MS: *m/z* 493.1352 $[M + H]^+$ (calcd for $C_{23}H_{25}O_{12}$, 493.1346).

*3.3.2 3β,4′-Dihydroxy-7,3′-dimethoxy-flavonone-5-O-β-D-glucopyranoside (**2**)*

3.3.2.1 Yellow powder. $[\alpha]_D^{25}$ −56.4 (*c* 0.05, MeOH). UV (MeOH) λ_{max} (log ε) 202 (4.82), 227 (4.53), 283 (4.40) nm; CD (MeOH) $\Delta\varepsilon_{300\,nm}$ −2.295, $\Delta\varepsilon_{334\,nm}$ 1.252; IR (KBr) ν_{max} cm^{-1}: 3350 (br), 2952, 2922, 2902, 1681, 1612, 1573, 1440, 1257, 1163, 1103. ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2. HR-ESI-MS: *m/z* 517.1349 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_{12}Na$, 517.1322).

Table 2. ^{13}C NMR spectral data of **1**–**3** (150 MHz, in DMSO at 27°; δ in ppm).

Position	**1**	**2**	**3**
C-2	143.6 (s)	82.8 (d)	82.5 (d)
C-3	137.7 (s)	72.5 (d)	72.6 (d)
C-4	171.7 (s)	191.3 (s)	191.3 (s)
C-5	157.9 (s)	159.5 (s)	165.6 (s)
C-6	102.3 (d)	97.0 (d)	97.0 (d)
C-7	163.4 (s)	165.6 (s)	159.6 (s)
C-8	95.6 (d)	95.4 (d)	95.4 (d)
C-9	157.2 (s)	163.7 (s)	163.5 (s)
C-10	107.3 (s)	104.4 (s)	104.4 (s)
C-1′	121.4 (s)	128.0 (s)	127.5 (s)
C-2′	111.5 (d)	115.0 (d)	114.8 (d)
C-3′	148.5 (s)	147.3 (s)	129.3 (d)
C-4′	147.4 (s)	146.9 (s)	157.7 (s)
C-5′	115.5 (d)	112.2 (d)	129.3 (d)
C-6′	122.0 (s)	121.0 (s)	114.8 (s)
MeO-5			55.8 (q)
MeO-7	56.0 (q)	55.8 (q)	
MeO-3′	60.8 (q)	55.7 (q)	
C-1″	103.5 (d)	101.6 (d)	101.6 (d)
C-2″	73.6 (d)	73.3 (d)	73.3 (d)
C-3″	75.9 (d)	76.2 (d)	76.2 (d)
C-4″	69.9 (d)	69.7 (d)	69.8 (d)
C-5″	77.6 (d)	77.4 (d)	77.4 (d)
C-6″	60.9 (t)	60.7 (t)	60.8 (t)

*3.3.3 3β,4′-Dihydroxy-5-methoxy-flavonone-7-O-β-D-glucopyranoside (**3**)*

3.3.3.1 White powder. $[\alpha]_D^{25}$ −73.3 (*c* 0.12, MeOH). UV (MeOH) λ_{max} (log ε) 201 (4.75), 227 (4.76), 282 (4.55) nm; CD (MeOH) $\Delta\varepsilon_{300\,nm}$ −2.356, $\Delta\varepsilon_{334\,nm}$ 1.419; IR (KBr) ν_{max} cm^{-1}: 3350 (br), 2955, 2925, 2881, 1690, 1616, 1595, 1456, 1261, 1170, 1103. ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2. HR-ESI-MS: *m/z* 487.1224 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_{11}Na$, 487.1216).

3.4 Acid hydrolysis of compounds 1, 2, and 3

Each compound (3 mg) dissolved in 3 ml of HCl–H_2O–EtOH (2:1:2) was stirred at 80°C for 4 h. The hydrolysate was partitioned between EtOAc and H_2O. The aqueous layer was analyzed by silica gel TLC [$CHCl_3$–MeOH–H_2O (8:5:1)] and

identified by comparing with authentic samples, and the result showed that the sugar was glucose.

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