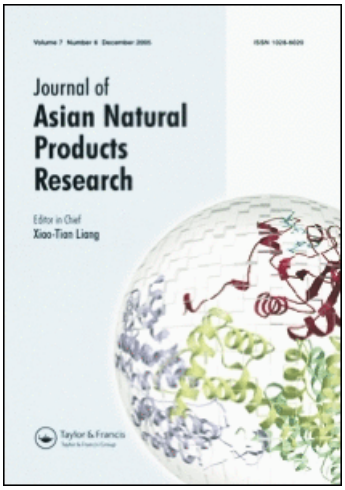

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ORIGINAL ARTICLE

Two new isoflavone glycosides from *Mucuna birdwoodiana*

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Two new isoflavone glycosides, mucodianins E (**1**) and F (**2**), have been isolated from the vine stems of *Mucuna birdwoodiana* Tutch. Their structures have been established as retusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**1**) and 8-*O*-methylretusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**2**) by means of spectroscopic analysis and chemical methods.

Keywords: *Mucuna birdwoodiana* Tutch.; Leguminosae; isoflavone glycosides; retusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside; 8-*O*-methylretusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside

1. Introduction

Mucuna birdwoodiana Tutch. (Leguminosae) is distributed in southern China. The vine stems of this plant are used to treat pain or numbness of the wrists, knees, or other joints, and irregular menstruation, and named as 'ji-xue-teng' in folk medicine [1]. Previous phytochemical investigation showed that the seeds of this plant contained L-DOPA [2] and the stems included phenolic [3] and triterpenoid [4] compounds. In our recent research on the vine stems of this plant, two new isoflavone glycosides, retusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**1**, mucodianin E) and 8-*O*-methylretusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**2**, mucodianin F), were isolated together with six known isoflavone glycosides. Their structures were determined by the spectroscopic analysis and chemical methods.

2. Results and discussion

Compound **1** was isolated as a white solid. The HR-ESI-MS of **1** exhibited a pseudomolecular ion at *m/z* 579.1718 $[M+H]^+$, consistent with a molecular formula of $C_{27}H_{30}O_{14}$. Positive ESI-MS/MS of **1** showed a quasimolecular ion $[M+Na]^+$ at *m/z* 601.2, and fragment ions at *m/z* 469.1 $[M+Na-132]^+$, 447.1 $[M-132]^+$, 307.1 $[M+Na-132-162]^+$, and 285.1 $[M-132-162]^+$, indicating losses of pentosyl and hexosyl moieties from the quasimolecular ion and the molecular weight of the aglycone was 284. The characteristic resonances for H-2 at δ 8.44 in the ^{1}H NMR spectrum and C-2 at δ 153.5 in the ^{13}C NMR spectrum suggested

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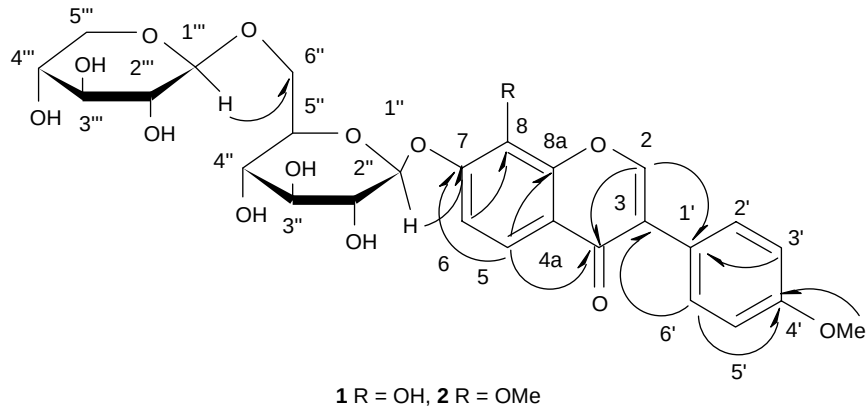

Figure 1. Chemical structures and key HMBC correlations of compounds **1** and **2**.

that **1** was an isoflavone glycoside [5]. In the ^{1}H NMR spectrum, two doublets at δ 7.52 (2H, d, $J = 9.0$ Hz, H-2′, 6′) and 6.99 (2H, d, $J = 9.0$ Hz, H-3′, 5′) appearing as an AA′BB′ type suggested the presence of a *para*-substituted ring B with a methoxyl group (δ 3.78) positioned at C-4′; this was confirmed by the HMBC experiment (Figure 1). In addition, a 7,8-disubstituted ring A was evident from two *ortho*-coupled protons at δ 7.55 (1H, d, $J = 9.0$ Hz) and 7.39 (1H, d, $J = 9.0$ Hz) and the HMBC correlations between H-5 at δ 7.55 and the carbonyl carbon (C-4) at δ 175.1. Moreover, according to the molecular weight of the aglycone moiety of **1**, the aglycone can be assigned as 7,8-dihydroxy-4′-methoxyisoflavone (retusin). Acid hydrolysis of **1** yielded glucose and xylose moieties detected by direct co-TLC comparison with authentic samples. The β-configuration of the two sugars was concluded from the signals at δ 4.84 (Glu H-1″, $J = 7.2$ Hz) and 4.18 (Xyl H-1‴, $J = 7.5$ Hz) in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of **1** exhibited 11 aliphatic carbon signals except for the methoxy, which were in good agreement with the published data for the sugar moiety of β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside [6,7]. Meanwhile, the correlations of Glu H-1″ at δ 4.84 with C-7 at δ 148.6 suggested that the *O*-glucose moiety was attached to C-7 (Figure 1). Therefore, compound **1** was elucidated as retusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside, named mucodianin E.

Compound **2** was a white solid. The molecular formula, $C_{28}H_{32}O_{14}$, was inferred from the positive HR-ESI-MS at *m/z* 593.1877 $[M+H]^+$, suggesting that one hydroxyl group was substituted by a methoxyl group compared to **1**. The ^{1}H NMR spectrum of **2** was almost identical with that of **1** in the δ 2.93–5.01 region, indicating the same identity and pattern of sugar substitution. Inspection of the aromatic region of the NMR spectral data of compound **2** showed a close resemblance with **1**, only the *ortho*-coupled protons were downfield-shifted [H-5 at δ 7.83 (+0.28), H-6 at δ 7.43 (+0.04)], so the 8-hydroxyl group in **1** was substituted by a methoxyl group in **2**. The HMBC spectrum (Figure 1) confirmed the location of each substituent on the aglycone nucleus and the sequence of the oligosaccharide chain. Thus, the structure of compound **2** was determined to be 8-*O*-methylretusin 7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside and named mucodianin F.

The structures of the other six known isoflavone glycosides were identified as formononetin 7-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (**3**) [8], formononetin 7-*O*-β-D-glucopyranoside

(ononin, **4**) [9], 7-hydroxy-4′,8-dimethoxyisoflavone 7-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (**5**) [9], 7-hydroxy-4′,8-dimethoxyisoflavone 7-*O*-β-D-glucopyranoside (**6**) [10], genistein 7-*O*-β-D-glucopyranoside (genistin, **7**) [11], and retusin 7-*O*-β-D-glucopyranoside (**8**) [12] by comparison of the ^{1}H NMR, ^{13}C NMR, and MS spectral data with the reported values in the literature.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT digital melting-point apparatus with microscope and are uncorrected. UV spectra were obtained on a JASCO U-650 spectrophotometer. The optical rotations were measured on a JASCO P-2000 spectrophotometer. IR spectra were run on an IMPACT 400 spectrometer. HR-ESI-MS were performed on an Autospec Ultima-TOF mass spectrometer and ESI-MS on an Agilent 1100 LC/MSD Trap-SL mass spectrometer. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and HMBC spectra were run on an INOVA-500 spectrometer with TMS as the internal standard, and ^{1}H NMR (300 MHz) spectrum on a Mercury-300 spectrometer. Sephadex LH-20 (Pharmacia, Uppsala, Sweden), macroporous resin D101 (26–60 mesh; Tianjin, China), and polyamide resin (30–50 mesh; Zhejiang, China) were used for column chromatography (CC). HPLC separations were performed on a preparation YMC-Pack ODS-A column (10 μm, 250 × 20 mm i.d.; YMC, Kyoto, Japan) equipped with a Shimadzu SPD-6A UV spectrophotometric detector and a Thermo Constametric pumping system.

3.2 Plant material

The vine stem of *M. birdwoodiana* was collected in the County of Jin Xiu, Guangxi Province, China, in August 2006, and identified by Prof. Shou-Yang Liu, Guangxi Traditional Chinese Medical College. A voucher specimen (No. S2263) has been deposited in the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

3.3 Extraction and isolation

Crushed dry vine stem of *M. birdwoodiana* (10 kg) was extracted with 50% EtOH (3× 10 liters) under reflux to yield a crude extract (1600 g). The extract was passed over a D101 macroporous resin CC (8 kg) eluted with a gradient of aqueous EtOH (0, 30, 60, 75, 100%, v/v) to yield five fractions (1–5). Fraction 3 (145 g) was further fractionated on polyamide resin CC (2.5 kg) eluted with a gradient of aqueous EtOH (0, 15, 30, 50, 70, 100%, v/v) to give six fractions (3.1–3.6). Fraction 3.2 (4.3 g) was separated by reversed-phase silica gel (150 g) CC and eluted with 5–45% aqueous MeOH to provide six fractions (3.2.1–3.2.6). Fraction 3.2.6 (380 mg) was purified by Sephadex LH-20 CC and preparative HPLC (45% aqueous MeOH, 4 ml/min) to yield compound **2** (4.3 mg, t_R = 86.2 min). Fraction 3.3 (16.3 g) was subjected to reversed-phase silica gel (300 g) CC and eluted with gradient aqueous MeOH (20, 25, 30, 35, 40, 50, 100%, v/v) to give seven fractions (3.3.1–3.3.7). Fraction 3.3.3 (1.3 g) was purified by Sephadex LH-20 CC (eluted with MeOH) to yield compound **7** (8.5 mg). Fraction 3.3.4 (1.6 g) was also purified by Sephadex LH-20 CC (eluted with MeOH) to give compound **4** (10.5 mg) and preparative HPLC (45% aqueous MeOH, 4 ml/min) to afford compound **1** (5.2 mg, t_R = 79.2 min). Fraction 3.3.5 (1.8 g) was subjected to Sephadex LH-20 CC (eluted with MeOH) and preparative HPLC (50% aqueous MeOH, 4 ml/min) to yield compounds **3** (13.1 mg, t_R = 50.2 min) and **8** (2.1 mg, t_R = 42.8 min). Fraction 3.3.6 (980 mg) was performed on preparative HPLC (48% aqueous MeOH, 4 ml/min) to

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** and **2** in DMSO-d_6.

	1		**2**	
No.	^{1}H	^{13}C	^{1}H	^{13}C
2	8.44 s	153.5	8.47 s	153.6
3		122.8		123.1
4		175.1		174.8
4a		119.7		119.4
5	7.55 d (9.0)	115.2	7.83 d (9.0)	120.6
6	7.39 d (9.0)	114.0	7.43 d (9.0)	114.3
7		148.6		154.1
8		135.0		136.8
8a		145.7		149.9
1′		124.2		124.0
2′/6′	7.52 d (9.0)	130.1	7.52 d (9.0)	130.1
3′/5′	6.99 d (9.0)	113.6	6.99 d (9.0)	113.6
4′		159.0		159.0
4′-OMe	3.78	55.1	3.78	55.1
8-OMe			3.93	61.3
Glu-1″	4.84 d (7.2)	101.9	5.01 d (7.2)	100.7
2″	3.64 m	73.3	3.64 m	73.2
3″	3.40 m	76.5	3.40 m	76.5
4″	3.31 m	69.8	3.31 m	69.7
5″	3.69 m	75.5	3.70 m	76.0
6″	4.00 d (9.4), 3.15 m	68.4	3.98 d (10.2), 3.26 m	68.4
Xyl-1‴	4.18 d (7.5)	104.0	4.15 d (7.5)	104.0
2‴	2.94 m	73.4	2.93 m	73.4
3‴	3.04 m	76.1	3.04 m	76.4
4‴	3.09 m	69.6	3.17 m	69.6
5‴	3.57 m, 2.98 d (10.2)	65.6	3.57 m, 2.98 d (10.2)	65.6

give compounds **5** (5.1 mg, $t_R = 86.2$ min) and **6** (4.1 mg, $t_R = 75.8$ min).

3.3.1 *Mucodianin E (**1**)*

White solid (MeOH); mp 189.1–190.1°C; $[\alpha]_D^{25} = -62.4$ ($c = 0.06$, MeOH); UV (MeOH) λ_{max} (log ε) (nm): 258 (4.44), 210 (5.09), 203 (5.12); IR ν_{max} (cm^{-1}): 3499, 3378, 3256, 2954, 2929, 2887, 1624, 1598, 1513, 1389, 1291, 1260, 1079, 1055, 1025, 980; ^{1}H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS *m/z*: 579.1718 $[M+H]^+$ (calcd for $C_{27}H_{31}O_{14}$, 579.1708).

3.3.2 *Mucodianin F (**2**)*

White solid (MeOH); mp 214.5–216.0°C; $[\alpha]_D^{25} = -17.1$ ($c = 0.04$, MeOH); UV (MeOH) λ_{max} (log ε) (nm): 255 (4.54), 220 (5.61); IR ν_{max} (cm^{-1}): 3440, 3081, 2942, 1626, 1597, 1566, 1513, 1448, 1286, 1248, 1106, 1083, 1044, 990, 830, 668; ^{1}H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS *m/z*: 593.1877 $[M+H]^+$ (calcd for $C_{28}H_{33}O_{14}$, 593.1865).

3.3.3 *Sugar composition analysis of compounds **1** and **2***

Each compound (2 mg) was refluxed with 1 M HCl (dioxane–H_2O, 1:1, 2 ml) at 95°C for 2 h. After drying under a stream of nitrogen, the residue was suspended in H_2O and extracted with EtOAc. The aqueous layer was neutralized with $NaHCO_3$ and concentrated under reduced pressure to dryness to give a residue of the sugar fraction. Xylose and glucose were detected from the residue by co-TLC ($CHCl_3$:MeOH:H_2O:HOAc, 16:9:2:2; detection with a spray agent: 4% α-

naphthol–EtOH–5% H_2SO_4) with the authentic samples (R_f: 0.43 and 0.20).

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