

## New Isoflavone C-Glycosides from *Pueraria lobata*

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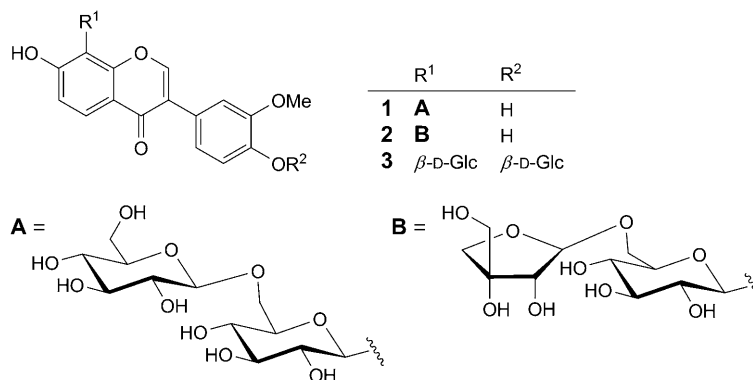
Three new isoflavone C-glycosides, along with two known isoflavone O-glycosides, were isolated from the roots of *Pueraria lobata* (WILLD.) OHWI. The structures of the new compounds were elucidated as 4',7-dihydroxy-3'-methoxyisoflavone 8-C-[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  6)]- $\beta$ -D-glucopyranoside (**1**), 4',7-dihydroxy-3'-methoxyisoflavone 8-C-[ $\beta$ -D-apiofuranosyl-(1  $\rightarrow$  6)]- $\beta$ -D-glucopyranoside (**2**), and 8-C- $\beta$ -D-glucopyranosyl-4',7-dihydroxy-3'-methoxyisoflavone 4'-O- $\beta$ -D-glucopyranoside (**3**) on the basis of spectroscopic methods, especially 2D-NMR and MS analyses. The known compounds isolated were identified by comparison of their physical and spectroscopic data with those reported in the literature.

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**Introduction.** – The root of *Pueraria lobata* (WILLD.) OHWI has been used as herbal medicine in China for centuries and used as dietary supplement in USA in recent years [1]. In Traditional Chinese Medicine, it is used in prescriptions for the treatment of fever, headache, diarrhea, and stiff neck with pain due to high blood pressure. It contains large amounts of isoflavonoids including puerarin, daidzin, daidzein. Puerarin was the predominant isoflavone (= 3-phenyl-4H-1-benzopyran-4-one) in the root, followed by 6''-O-xylosylpuerarin, mirificin, daidzin, and daidzein [2]. In persons at high risk of cardiovascular events, a greater isoflavone intake has been associated with better vascular endothelial function and lower carotid atherosclerotic burden [3]. As a part of systematic search for cardiovascular bioactive compounds from Chinese medicines [4], chemical investigation of the roots of *P. lobata* led to the isolation of three new isoflavonoids.

**Results and Discussion.** – The air-dried root of *P. lobata* (WILLD.) OHWI (20 kg) was extracted with 95% aqueous EtOH. The residue of the EtOH extract was suspended in H<sub>2</sub>O and then successively extracted with petroleum ether, AcOEt, and BuOH. Separation of the H<sub>2</sub>O-soluble portion over macroporous resin column, MPLC, and prep.-HPLC afforded three new isoflavone C-glycosides, **1–3** (Fig. 1), along with two known isoflavone O-glycosides, daidzein 4',7-diglucoside (**4**) and daidzin (**5**).

Compound **1** was obtained as grey amorphous powder. The HR-ESI mass spectrum of **1** displayed a quasi-molecular-ion peak at  $m/z$  631.1652 ( $[M + Na]^+$ ; calc. 631.1633) corresponding to the molecular formula C<sub>28</sub>H<sub>32</sub>O<sub>15</sub>. The IR spectrum of **1** revealed the presence of OH group (3388 cm<sup>-1</sup>), CO group (1630 cm<sup>-1</sup>), and aromatic ring (1595,

Fig. 1. Compounds **1–3** from *Pueraria lobata*

1511  $\text{cm}^{-1}$ ). The UV absorption maxima at 224 and 248 nm, as well as the H-atom *singlet* at  $\delta(\text{H})$  8.36 (H–C(2)) in the  $^1\text{H-NMR}$  spectrum suggested **1** to be an isoflavone. Furthermore, the  $^1\text{H-NMR}$  spectrum showed signals for an aromatic *AX* system ( $\delta(\text{H})$  7.91 (*d*,  $J=8.8$ , 1 H), 6.96 (*d*,  $J=8.8$ , 1 H)), an *AMX* system ( $\delta(\text{H})$  7.16 (*d*,  $J=2.0$ , 1 H), 6.80 (*d*,  $J=8.2$ , 1 H), 7.02 (*dd*,  $J=8.2, 2.0$ , 1 H)), a MeO group ( $\delta(\text{H})$  3.79 (*s*, 3 H)), and signals of two anomeric H-atoms ( $\delta(\text{H})$  4.78 (*d*,  $J=9.9$ , 1 H), 4.13 (*d*,  $J=7.9$ , 1 H)). The  $^{13}\text{C-NMR}$  spectrum of **1** exhibited 15 aromatic C-atom signals due to a C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> system and twelve C-atom signals for two hexosyl moieties. Therefore, it could be concluded that **1** was an isoflavone glycoside. Comparison of the NMR data of **1** (Table) with those of the known compound 3'-methoxypuerarin [4] indicated that they were similar except for the appearance of the signals for an additional sugar residue in **1**. Acid hydrolysis of **1** afforded 3'-methoxypuerarin and D-glucose. The  $^1\text{H}$ -,  $^{13}\text{C}$ -, and 2D-NMR (COSY, HMBC, ROESY, and TOCSY) data also suggested that the two sugar units were  $\beta$ -D-glucopyranoses. The linkage of the two glucopyranosyl moieties at C(6'') and that of the inner glucopyranosyl moiety at C(8) of the aglycone were determined by the HMBs between  $\delta(\text{H})$  4.13 (H–C(1''') of Glc') and  $\delta(\text{C})$  69.0 (C(6'') of Glc), as well as between  $\delta(\text{H})$  4.78 (H–C(1'') of Glc) and  $\delta(\text{C})$  112.4 (C(8)), 156.2 (C(9)) and 161.9 (C(7)) (Fig. 2). Thus, the structure of **1** was established as 4',7-dihydroxy-3'-methoxyisoflavone 8-C-[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  6)]- $\beta$ -D-glucopyranoside<sup>1)</sup>.

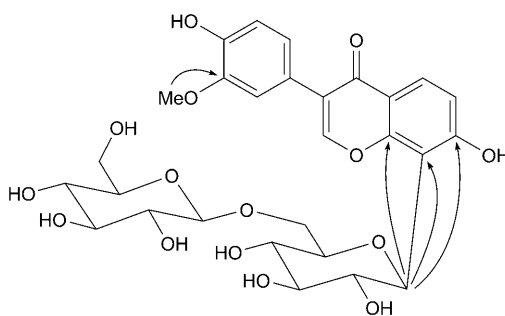
Compound **2** was obtained as grey amorphous powder. The molecular formula of **2** was determined as C<sub>27</sub>H<sub>30</sub>O<sub>14</sub> on the basis of a *quasi*-molecular-ion peak at  $m/z$  601.1544 ( $[M + \text{Na}]^+$ ; calc. 601.1528) in its HR-ESI mass spectrum. The UV and IR spectra of **2** were similar to those of **1**, suggesting that **2** was also an isoflavone glycoside. Detailed examination of 1D- and 2D-NMR spectra of **2**, and comparison with those of **1** revealed that they possessed the same aglycone moiety but differed in the saccharide portion. The  $^{13}\text{C-NMR}$  spectrum of **2** exhibited eleven C-atom signals, which were in good agreement with the published data for the sugar moiety of dadzein 8-C-[ $\beta$ -D-

<sup>1)</sup> Trivial numbering. For systematic names, see *Exper. Part*

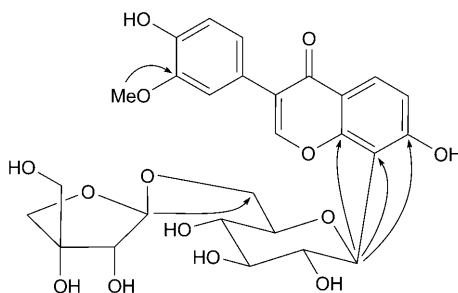
Table.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data of Compounds **1–3** ( $J$  in Hz). Isoflavone numbering.

	<b>1<sup>a</sup></b>		<b>2<sup>a</sup></b>		<b>3<sup>a</sup></b>		<b>3<sup>b</sup></b>	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
H–C(2)	8.36 (s)	152.7	8.33 (s)	152.5	8.45 (s)	153.3	8.25 (s)	154.9
C(3)		123.0		123.1		122.7		125.1
C(4)		174.8		174.8		174.8		178.0
H–C(5)	7.91 ( <i>d</i> , $J=8.8$ )	126.1	7.89 ( <i>d</i> , $J=8.8$ )	126.0	7.94 ( <i>d</i> , $J=8.9$ )	126.2	8.04 ( <i>d</i> , $J=8.8$ )	128.1
H–C(6)	6.96 ( <i>d</i> , $J=8.8$ )	116.2	6.95 ( <i>d</i> , $J=8.8$ )	115.9	6.99 ( <i>d</i> , $J=8.9$ )	115.1	6.98 ( <i>d</i> , $J=8.8$ )	116.7
C(7)		161.9		161.0		161.8		163.1
C(8)		112.4		112.2		112.7		113.2
C(9)		156.2		156.4		156.3		157.8
C(10)		115.2		115.2		116.6		118.3
C(1')		123.1		122.9		125.9		128.1
H–C(2')	7.16 ( <i>d</i> , $J=2.0$ )	113.1	7.17 ( <i>d</i> , $J=1.9$ )	113.1	7.23 (br. s)	113.3	7.25 ( <i>d</i> , $J=1.9$ )	114.9
C(3')		147.2		147.2		148.6		150.6
C(4')		146.4		146.4		146.2		148.0
H–C(5')	6.80 ( <i>d</i> , $J=8.2$ )	115.2	6.81 ( <i>d</i> , $J=8.1$ )	115.2	7.12 (br. s)	115.3	7.21 ( <i>d</i> , $J=8.3$ )	118.0
H–C(6')	7.02 ( <i>dd</i> , $J=8.2, 2.0$ )	121.5	7.03 ( <i>dd</i> , $J=8.2, 1.9$ )	121.5	7.12 (br. s)	121.2	7.07 ( <i>dd</i> , $J=8.2, 1.9$ )	122.8
MeO	3.79 (s, 3 H)	55.6	3.79 (s, 3 H)	55.6	3.79 (s, 3 H)	55.7	3.89 (s, 3 H)	56.8
	Glc:		Glc:		Glc:		Glc:	
H–C(1'')	4.78 ( <i>d</i> , $J=9.9$ )	73.5	4.81 ( <i>d</i> , $J=9.6$ )	73.5	4.82 ( <i>d</i> , $J=10.0$ )	73.3	5.09 ( <i>d</i> , $J=9.7$ )	75.7
H–C(2'')	4.05 <sup>c</sup>	70.6	4.04 <sup>c</sup>	70.6	4.02 (br. <i>t</i> , $J=9.5, 1\text{ H}$ )	70.8	4.08–4.13 ( <i>m</i> , 1 H)	73.0
H–C(3'')	3.27 <sup>c</sup>	78.7	3.27 <sup>c</sup>	78.7	3.28 <sup>c</sup>	78.8	3.43 <sup>c</sup>	78.2
H–C(4'')	3.26 <sup>c</sup>	70.4	3.22 <sup>c</sup>	70.7	3.23 <sup>c</sup>	70.6	3.53 <sup>c</sup>	71.7
H–C(5'')	3.43 <sup>c</sup>	80.0	3.42 <sup>c</sup>	80.1	3.26 <sup>c</sup>	81.9	3.48 <sup>c</sup>	82.7
CH <sub>2</sub> (6'')	3.48–3.55 ( <i>m</i> , 1 H), 4.10 (1 H) <sup>c</sup>	69.0	3.37 (1 H) <sup>c</sup> , 3.91–3.93 ( <i>m</i> , 1 H)	68.3	3.43–3.47 ( <i>m</i> , 1 H), 3.67–3.73 ( <i>m</i> , 1 H)	61.5	3.86 (1 H) <sup>c</sup> , 3.70–3.74 ( <i>m</i> , 1 H)	62.8
	Glc:		Glc:		Glc:		Glc:	
H–C(1''')	4.13 ( <i>d</i> , $J=7.9$ )	103.1	4.78 ( <i>d</i> , $J=3.1$ )	109.0	4.95 ( <i>d</i> , $J=7.8$ )	100.1	4.93 ( <i>d</i> , $J=7.2$ )	102.8
H–C(2''')	2.93 ( <i>t</i> , $J=7.8$ )	73.3	3.73 ( <i>d</i> , $J=3.0$ )	75.7	3.30 <sup>c</sup>	73.5	3.51 <sup>c</sup>	74.9
H–C(3''')	3.05 <sup>c</sup>	76.7	–	78.7	3.31 <sup>c</sup>	77.2	3.49 <sup>c</sup>	77.9
CH <sub>2</sub> (4''')	3.05 <sup>c</sup>	70.0	3.84 ( <i>d</i> , $J=9.6$ ), 3.56 ( <i>d</i> , $J=9.7$ )	73.2	3.18 <sup>c</sup>	69.7	3.42 <sup>c</sup>	71.4
	Glc:		Glc:		Glc:		Glc:	
H–C(5''')	3.03 <sup>c</sup>	76.9	3.32 <sup>c</sup>	63.1	3.32 <sup>c</sup>	77.1	3.53 <sup>c</sup>	80.1
CH <sub>2</sub> (6''')	3.44 (1 H) <sup>c</sup> , 3.64 (br. <i>d</i> , $J=11.6, 1\text{ H}$ )	61.0	3.43–3.47 ( <i>m</i> , 1 H), 3.67–3.73 ( <i>m</i> , 1 H)	60.7	3.43–3.47 ( <i>m</i> , 1 H), 3.67–3.73 ( <i>m</i> , 1 H)	60.7	3.86 (1 H) <sup>c</sup> , 3.73–3.78 ( <i>m</i> , 1 H)	62.5

<sup>a</sup>) Recorded in (D<sub>2</sub>O)DMSO at 300 MHz ( $^1\text{H}$ ) and 75 MHz ( $^{13}\text{C}$ ). <sup>b</sup>) Recorded in CD<sub>3</sub>OD at 400 MHz ( $^1\text{H}$ ) and 100 MHz ( $^{13}\text{C}$ ). <sup>c</sup>) Overlapped signals.

Fig. 2. Key HMBCs of compound **1**

apiofuranosyl-(1 → 6)]-β-D-glucopyranoside and genistein 8-C-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside [5]. Acid hydrolysis of **2** revealed the presence of 3'-methoxypuerarin and D-apiose. Thus, the sugar moiety of **2** was deduced to be β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside. In the HMBC spectrum of **2**, correlations between δ(H) 4.78 (H-C(1''') of Api) and δ(C) 68.3 (C(6'') of Glc), as well as between δ(H) 4.81 (H-C(1'') of Glc), and δ(C) 112.2 (C(8)), 156.4 (C(9)), and 161.0 (C(7)) were observed (Fig. 3), indicating that the sugar chain was attached to C(8) of the aglycone by C-glycosidic bond. The assignments of the <sup>1</sup>H- and <sup>13</sup>C-NMR chemical shift (Table) of **2** were completed with the aid of <sup>1</sup>H,<sup>1</sup>H-COSY, HSQC, HMBC, ROESY, and TOCSY data. Consequently, the structure of **2** was characterized as 4',7-dihydroxy-3'-methoxyisoflavone 8-C-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside.

Fig. 3. Key HMBCs of compound **2**

Compound **3** was also isolated as grey amorphous powder. The molecular formula was determined as C<sub>28</sub>H<sub>32</sub>O<sub>15</sub> by HR-ESI-MS (*m/z* 631.1647 [*M* + Na]<sup>+</sup>). The IR and UV spectra of **3** were similar to those of **1** and **2**. In the <sup>1</sup>H-NMR spectrum of **3**, characteristic signals for H-C(2) of isoflavone (δ(H) 8.45 (*s*)) and two anomeric H-atoms (δ(H) 4.82 (*d*, *J* = 10.0), 4.95 (*d*, *J* = 7.8)) were observed, suggesting that **3** was also an isoflavone glycoside. Comparison of the NMR data of **3** (Table) with those of **1** and **2** revealed they possess the same aglycone, 4',7-dihydroxy-3'-methoxyisoflavone. Acid hydrolysis of **3** afforded 3'-methoxypuerarin and D-glucose. Analysis of <sup>1</sup>H,<sup>1</sup>H-COSY, HSQC, HMBC, ROESY, and TOCSY data confirmed the presence of two

glucopyranoses. The linkage positions of the sugar units could be deduced by an HMBC experiment in which correlations between H–C(1'') ( $\delta(\text{H})$  4.82) of glucose, and C(7) ( $\delta(\text{C})$  161.8), C(8) ( $\delta(\text{C})$  112.7), C(9) ( $\delta(\text{C})$  156.3) of aglycone, as well as between H–C(1''') ( $\delta(\text{H})$  4.95) of the other glucose, and C(4') ( $\delta(\text{C})$  146.2) of aglycone were observed, indicating a glucose was attached to C(8) by C-glycosidic bond, and the other glucose was connected to C(4') via O-glycosidic bond. Thus, the structure of **3** was deduced as 8-C- $\beta$ -D-glucopyranosyl-4',7-dihydroxy-3'-methoxyisoflavone 4'-O- $\beta$ -D-glucopyranoside.

The two known compounds obtained were identified as daidzein 4',7-diglucoside (**4**) [6] and daidzin (**5**) [7] by comparison of their physical and spectroscopic data with those reported in the literature.

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### Experimental Part

*General.* HPLC-Grade MeOH was from *Merck* (*Merck*, Germany), and D-Glucose and D-apiose were from *Sigma*. 3'-Methoxypuerarin (=4',7-dihydroxy-3'-methoxyisoflavone 8-C- $\beta$ -D-glucopyranoside) was previously isolated from *P. lobata* [4]. The deionized H<sub>2</sub>O used for HPLC was purified by a *Milli-Q* purification system (*Millipore*, USA). Hydrolysis was performed on a *Syncore* polyvap reactor (*Büchi*, Switzerland). Column chromatography (CC): macroporous resin (16–60 mesh; *Tianjin Haiguang Chemical Co. Ltd.*, Tianjin, P. R. China). Medium-pressure liquid chromatography (MPLC): *Büchi* MPLC apparatus (pump, *C-605*; pump manager, *C-615*; detector, UV photometer *C-635*; fraction collector, *C-660*; *Büchi*, Switzerland). HPLC and prep. HPLC: *Agilent 1100* series HPLC and prep. HPLC apparatus, resp; the prep. separation was run with a flow rate at 10 ml/min and monitored with a MWD detector at 256 nm. GC: *Jinghe GC-900* apparatus (*Jinghe Analytical Equipment Inc.*, Shanghai, P. R. China). Optical rotations: *Jasco P-1020* digital polarimeter. UV Spectra: *Jasco V-550* UV/VIS spectrophotometer (*Jasco*, Japan). IR Spectra: *Jasco FT/IR-480* plus spectrometer (*Jasco*, Japan). The <sup>1</sup>H-, <sup>13</sup>C-, and 2D-NMR spectra: *Bruker AV-300* or *AV-400* NMR spectrometers (*Bruker*, Germany), with (D<sub>6</sub>)DMSO as solvent; chemical shifts in ppm relative to TMS ( $\delta$  0.00) as an internal standard (<sup>1</sup>H) and  $\delta$  40.0 ppm from (D<sub>6</sub>)DMSO as a standard (<sup>13</sup>C). HR-ESI-MS: *Agilent 6210* ESI/TOF mass spectrometer (*Agilent*, USA).

*Plant Material.* The root of *Pueraria lobata* (WILLD.) OHWI was purchased in Anhui province, P. R. China in September 2007, and authenticated by Dr. *Xian-Tao Zhang* (Guangdong Research Institute of Chinese Traditional Medicine). A voucher specimen (No. ICMS200709011) was deposited with the Institute of Chinese Medical Sciences, University of Macau, Macau.

*Extraction and Isolation.* The air-dried and chipped root of *P. lobata* (20 kg) was extracted with 95% aq. EtOH under reflux for three times (3, 2, and 2 h, resp.). The extract was filtered and concentrated *in vacuo* to yield a brownish residue, which was suspended in 12 l of H<sub>2</sub>O and then successively extracted with petroleum ether (PE; 3  $\times$  6 l), AcOEt (3  $\times$  12 l), and BuOH (3  $\times$  12 l) to yield 98 g of a PE-soluble fraction, a 160 g of AcOEt-soluble fraction, a 1108 g of BuOH-soluble fraction, and a 935 g of H<sub>2</sub>O phase fraction, resp. Half of the H<sub>2</sub>O fraction was concentrated *in vacuo* to remove the org. solvent, diluted with H<sub>2</sub>O, and finally loaded to a *D101* macroporous resin column eluted with H<sub>2</sub>O, EtOH/H<sub>2</sub>O 4:6 (v/v), and EtOH. The EtOH/H<sub>2</sub>O 4:6 (v/v) fraction was collected and concentrated to give a residue (36.2 g), which was separated by MPLC over a *C18* column to afford fractions *B1–B5*. Compound **3** (20 mg) and mirificin-4'-O- $\beta$ -D-glucoside (reported in [4]) were separated from *Fr. B2* by prep. HPLC on a *Phenomenex Synergi 4 $\mu$  Polar-RP* column (250 mm  $\times$  22 mm I.D., 4  $\mu$ m) with MeOH/H<sub>2</sub>O 1:4 (v/v). *Fr. B3* was separated by prep. HPLC on an *Alltima C18* column (250 mm  $\times$  22 mm I.D., 10  $\mu$ m) with MeOH/H<sub>2</sub>O 25:75 (v/v) to provide six subfractions, *BA1–BA6*. Compounds **1** (4 mg) and **4** (7 mg) were

purified by prep. HPLC on the *Synergi 4 $\mu$  Polar-RP* column with MeOH/H<sub>2</sub>O 25:75 (v/v) from subfractions BA2 and BA4, resp. A prep. HPLC separation of *Fr. B5* over the *Alltima C18* column with MeOH/H<sub>2</sub>O 30:70 (v/v) provided two subfractions BB1 and BB2, and two pure compounds daidzein 8-C-*apiosyl*(1 $\rightarrow$ 6)glucoside and 6''-*O*-xylosylpuerarin (reported in [4]). Compounds **2** (4 mg) and **5** (5 mg) were purified from the subfractions *Fr. BB1* and *BB2* by prep. HPLC over the *Phenomenex Synergi 4 $\mu$  Polar-RP* column with MeOH/H<sub>2</sub>O 30:70 (v/v), resp.

*4',7-Dihydroxy-3'-methoxyisoflavone 8-C-[ $\beta$ -D-Glucopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside* (= *(1S)-1,5-Anhydro-6-O- $\beta$ -D-glucopyranosyl-1-[7-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-4-oxo-4H-chromen-8-yl]-D-glucitol*; **1**). Grey amorphous power (MeOH).  $[\alpha]_{\text{D}}^{25} = +32.2$  ( $c = 0.15$ , DMSO). UV (MeOH): 224 (4.43), 248 (4.50). IR (KBr): 3388, 2922, 1630, 1595, 1511, 1445, 1391, 1268, 1073. HR-ESI-MS: 631.1652 ( $[M + Na]^+$ , C<sub>28</sub>H<sub>32</sub>NaO<sub>15</sub>; calc. 631.1633).

*4',7-Dihydroxy-3'-methoxyisoflavone 8-C-[ $\beta$ -D-Apiofuranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside* (= *(1S)-1,5-Anhydro-6-O- $\beta$ -D-apiofuranosyl-1-[7-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-4-oxo-4H-chromen-8-yl]-D-glucitol*; **2**). Grey amorphous power.  $[\alpha]_{\text{D}}^{25} = +49.5$  ( $c = 0.12$ , DMSO). UV (MeOH): 222 (4.51), 246 (4.45). IR (KBr): 3359, 2933, 1630, 1595, 1518, 1445, 1383, 1273, 1082. HR-ESI-MS: 601.1544 ( $[M + Na]^+$ , C<sub>27</sub>H<sub>30</sub>NaO<sub>14</sub>; calc. 601.1528).

*8-C- $\beta$ -D-Glucopyranosyl-4',7-dihydroxy-3'-methoxyisoflavone 4'-O- $\beta$ -D-glucopyranoside* (= *(1S)-1,5-Anhydro-1-[3-[4-( $\beta$ -D-glucopyranosyloxy)-3-methoxyphenyl]-7-hydroxy-4-oxo-4H-chromen-8-yl]-D-glucitol*; **3**). Grey amorphous power.  $[\alpha]_{\text{D}}^{25} = -23.1$  ( $c = 0.17$ , DMSO). UV (MeOH): 223 (4.41), 250 (4.57). IR (KBr): 3372, 2922, 1629, 1594, 1512, 1445, 1390, 1268, 1205, 1074. HR-ESI-MS: 631.1647 ( $[M + Na]^+$ , C<sub>28</sub>H<sub>32</sub>NaO<sub>15</sub>; calc. 631.1633).

*Acid Hydrolysis.* Each compound (2 mg) was hydrolyzed with 10 ml of 2M HCl for 2 h at 100°. The mixture was evaporated to dryness, and partitioned between BuOH and H<sub>2</sub>O. 3'-Methoxypuerarin was detected in the BuOH layer by HPLC over an *Alltech Alltima C18* column (250 mm  $\times$  4.6 mm, I.D., 5  $\mu$ m) at  $t_{\text{R}}$  12.4 min (mobile phase: MeOH/H<sub>2</sub>O 30:70; flow rate: 1 ml/min). The H<sub>2</sub>O layer was concentrated to dryness to yield sugar residue, which was dissolved in anhyd. pyridine (1 ml), reacted with L-cysteine methyl ester hydrochloride (2 mg) at 60° for 2 h, and concentrated to dryness. The mixture was then reacted with 1-(trimethylsilyl)-1H-imidazole (0.2 ml) at 60° for 1 h. The product was partitioned between cyclohexane and H<sub>2</sub>O, and the org. layer was analyzed by GC (column: *AT-SE-30* (0.5  $\mu$ m  $\times$  0.32 mm  $\times$  30 m); column temp.: 230°; detector temp.: 270°; injection temp.: 270°; and carrier gas: N<sub>2</sub>). The same reaction and analysis were applied for standard sugars. The derivatives of D-glucose and D-apiose showed peaks at  $t_{\text{R}}$  26.3 and 12.4 min, resp. As a result, D-glucose was detected from **1** and **3**, and D-apiose was detected from **2**.

#### REFERENCES

- [1] J. K. Prasain, A. Reppert, K. Jones, D. R. Moore II, S. Barnes, M. A. Lila, *Phytochem. Anal.* **2007**, *18*, 50.
- [2] G. Du, H. Y. Zhao, Q. W. Zhang, G. H. Li, F. Q. Yang, Y. Wang, Y. C. Li, Y. T. Wang, *J. Chromatogr. A* **2010**, *1217*, 705.
- [3] Y.-H. Chan, K.-K. Lau, K.-H. Yiu, S.-W. Li, H.-T. Chan, S. Tam, X.-O. Shu, C.-P. Lau, H.-F. Tse, *Am. J. Clin. Nutr.* **2007**, *86*, 938.
- [4] G.-H. Li, Q.-W. Zhang, W.-H. Hang, Y.-T. Wang, *Asian Chem. Lett.* **2009**, *13*, 35.
- [5] J. Kinjo, J. Furusawa, J. Baba, T. Takeshita, M. Yamasaki, T. Nohara, *Chem. Pharm. Bull.* **1987**, *35*, 4846.
- [6] Z. Li, R. B. Shi, B. Liu, *Chin. Tradit. Patent Med.* **2009**, *31*, 431.
- [7] K. Hirakura, M. Morita, K. Nakajima, K. Sugama, K. Takagi, K. Niitsu, Y. Ikeya, M. Maruno, M. Okada, *Phytochemistry* **1997**, *46*, 921.

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