

# Isoflavanones and their *O*-glycosides from *Desmodium styracifolium*

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## Abstract

Two isoflavanones (5,7-dihydroxy-2',3',4'-trimethoxy-isoflavanone and 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxy-isoflavanone), four isoflavanone *O*-glycosides (5,7-dihydroxy-2',3',4'-trimethoxy-isoflavanone 7-*O*- $\beta$ -glucopyranoside, 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxy-isoflavanone 7-*O*- $\beta$ -glucopyranoside, 5,7-dihydroxy-2',4'-dimethoxy-isoflavanone 7-*O*- $\beta$ -glucopyranoside, and 5,7,4'-trihydroxy-2',3'-dimethoxy-isoflavanone 7-*O*- $\beta$ -glucopyranoside), and a coumaronochromone (3,5,7,4'-tetrahydroxy-coumaronochromone), along with 25 known compounds, were isolated from the aerial parts of *Desmodium styracifolium*. This is for the first time isoflavanone *O*-glycosides were isolated from a natural source.

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**Keywords:** *Desmodium styracifolium*; Fabaceae; Isoflavanones; Isoflavanone *O*-glycosides; Coumaronochromone

## 1. Introduction

The genus *Desmodium* (Fabaceae) is distributed in tropical and subtropical regions, with 27 species and 5 varieties growing in China (Li, 1992). The presence of isoflavones, isoflavanones, flavone *C*-glycosides, pterocarpanoids, and coumaronochromones in this genus have been reported (Ingham and Dewick, 1984; Mizuno et al., 1992; Su et al., 1993; Botta et al., 2003; Tsanuo et al., 2003).

*Desmodium styracifolium* (Osb.) Merr. is a Chinese medicine renowned for its heat-clearing and diuretic properties (The State Pharmacopoeia Commission of P.R. China, 2005). It is an important medicinal herb for treatment of renal stones (Chen et al., 2006; Hu, 2006) and its effect on calcium oxalate has been attributed to polysaccharides (Li et al., 1988), triterpenoids (Hirayama et al., 1993), and flavonoids (Kubo et al., 1989a; Lu et al., 2003). Cardio-cerebrovascular effects have also been reported in the literature (Xu et al., 1980; Ho et al., 1989). *D. styracifolium* contains flavonoid compounds such as vicenins and isovitexin (Yasukawa

et al., 1986; Su et al., 1993; Yang et al., 2004a); and a quantitative method for the detection of total flavonoids in this herb has been reported (Yang et al., 2004b). Triterpene glycosides (Kubo et al., 1989b; Aoshima et al., 2005) and an alkaloid (Yang et al., 1993) have also been reported from *D. styracifolium*. The volatile oil contents were also determined by a GC–MS method (Chen et al., 2005).

From the aerial parts of *D. styracifolium*, we have now isolated two new isoflavanones (1–2), four new isoflavanone 7-*O*-glycosides (3–6), and a new coumaronochromone derivative (7), together with 25 known compounds: lupeol (8) (Deng et al., 2000), lupeone (9) (Yan et al., 2001), sophoradiol (10) (Fish and Johnson, 1994; Yu and Yang, 1999), soyasapogenol B (11) (Kubo et al., 1989; Yu and Yang, 1999), (23*Z*)-9, 19-cycloart-23-ene-3 $\beta$ , 25-diol (12) (Della Greca et al., 1994), homoferreirin (13) (Linuma et al., 1994; Agrawal, 1989), isoferreirin (14) (Adesanya et al., 1985), hydnocarpin-D (15) (Yu and Yang, 1999; Guz and Stermitz, 2000), 3,9-dihydroxypterocarpan (16) (Chacha et al., 2005), cimicifugic acid (17) (Zhao et al., 2002), secundiflorol H (18) (Tanaka et al., 1998), dalbergiodin (19) (Osawa et al., 1992), genistein (20) (Kinjo et al., 1987), formononetin (21) (Huang and

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Tu, 2004), kaemferol (**22**) (Luo et al., 2003), 7,4'-dihydroxy-3'-methoxy-isoflavone (**23**) (Hirakura et al., 1997), aromadendrin (**24**) (Agrawal, 1989; Feng et al., 2003), 2'-hydroxygenistein (**25**) (Agrawal, 1989), desmoxiphyllin A (**26**) (Mizuno et al., 1992), chrysoeriol (**27**) (Yang et al., 2002), 5,7,4'-trihydroxy-coumaronochromone (**28**) (Hassanean, 1998), quercetin (**29**) (Huang and Tu, 2004), luteolin (**30**) (Zhao et al., 2003), 2,3-*trans*-3,5,7,2',4'-pentahydroxyflavanone (**31**) (El-Sohly et al., 1999), and orobol (**32**) (Agrawal, 1989). The known compounds were identified by comparing their spectroscopic data with those reported in the literature and by interpretation of the 1D and 2D NMR spectra. New structures were elucidated by means of various NMR experiments including COSY, DEPT, HMQC, HMBC, as well as X-ray crystallographic analysis.

## 2. Results and discussion

Compound **1** was obtained in racemic form. High-resolution EI MS displayed a molecular ion at  $m/z$  346.1043  $[M]^+$  (calcd 346.1047) establishing a molecular formula of  $C_{18}H_{18}O_7$ . The presence of an isoflavanone skeleton was deduced from the  $^1H$  ( $\delta$  4.46, *dd*,  $J = 11.2$  Hz, H-2a; 4.34, *dd*,  $J = 5.6$ , 10.8 Hz, H-2b; 4.17, *dd*,  $J = 5.6$ , 11.6 Hz, H-3) and  $^{13}C$  [ $\delta$  48.97 (C-3); 71.77 (C-2); 198.80 (C-4)] NMR spectroscopic data (Tables 1 and 2) (Yenesew et al., 2000; Tanaka et al., 2003). The  $^1H$  NMR spectrum further indicates the presence of a pair of *ortho*-coupled

aromatic protons ( $\delta$  6.84, *d*,  $J = 8.8$  Hz, and 6.70, *d*,  $J = 8.8$  Hz), a pair of *meta*-coupled aromatic protons ( $\delta$  5.88, *d*,  $J = 2.0$  Hz; and 5.86, *d*,  $J = 2.0$  Hz), and three methoxyl groups ( $\delta$  3.81, 3.80, and 3.78). In the EI MS, the appearance of a fragment ion at  $m/z$  194 resulting from *retro*-Diels–Alder (RDA) cleavage of the C-ring was in agreement with placement of three methoxyl groups in the B-ring and hence two hydroxyl groups were assigned to the A-ring. In the HMBC spectrum, the C-3 displayed a long-range correlation with an aromatic proton at  $\delta$  6.84 which was *ortho*-coupled with an aromatic proton at  $\delta$  6.70 (as shown in  $^1H$ – $^1H$  COSY spectrum). Thus, the *ortho*-coupled aromatic protons were assigned to H-6' ( $\delta$  6.84) and H-5' ( $\delta$  6.70), and the three methoxyl groups were located at C-2', C-3' and C-4', respectively. The chemical shift value of C-4 ( $\delta$  198.80) indicated the presence of a 5-OH functionality. Two *meta*-coupled aromatic protons were therefore assigned to H-6 ( $\delta$  5.88) and H-8 ( $\delta$  5.86), respectively, based on the HMBC results. Thus, compound **1** was elucidated to be 5,7-dihydroxy-2',3',4'-trimethoxyisoflavanone (Fig. 1), whose structure was unambiguously established by X-ray crystallographic analysis (Fig. 2).

Compound **2** was also obtained in racemic form. Its molecular formula was determined as  $C_{17}H_{14}O_7$  from the high-resolution EI MS data showing a  $[M]^+$  ion at  $m/z$  330.0728 (calcd. 330.0734). This compound was established to be an isoflavanone on the basis of its characteristic spectroscopic data: a set of three aliphatic proton signals ( $\delta$  4.51, *dd*,  $J = 11.0$  Hz, H-2a; 4.36, *dd*,  $J = 5.6$ , 10.8 Hz,

Table 1  
 $^1H$  NMR spectroscopic data for **1**–**7**<sup>a</sup>

Position	<b>1</b> <sup>b</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>c</sup>	<b>4</b> <sup>c</sup>	<b>5</b> <sup>c</sup>	<b>6</b> <sup>d</sup>	<b>7</b> <sup>b</sup>
2	4.46, <i>dd</i> (11.2, 11.2) 4.34, <i>dd</i> (5.6, 10.8)	4.51, <i>dd</i> (11.0, 11.0) 4.36, <i>dd</i> (5.6, 10.8)	4.50, <i>m</i>	4.59, <i>m</i> 4.47, <i>m</i>	4.52, <i>dd</i> (5.2, 10.8) 4.45, <i>dd</i> (5.6, 10.8)	4.54, <i>dd</i> (11.2, 11.2) 4.46, <i>m</i>	6.12, <i>s</i>
3	4.17, <i>dd</i> (5.6, 11.6)	4.19, <i>dd</i> (5.6, 11.2)	4.37, <i>m</i>	4.39, <i>m</i>	4.38, <i>dd</i> (5.2, 10.8) 4.38, <i>dd</i> (5.6, 11.2)	4.27, <i>dd</i> (5.6, 11.2)	
6	5.88, <i>d</i> (2.0)	5.90, <i>d</i> (2.0)	6.18, <i>s</i>	6.18, <i>d</i> (2.0)	6.16, <i>s</i>	6.18, <i>s</i>	5.92, <i>d</i> (2.0)
8	5.86, <i>d</i> (2.0)	5.88, <i>d</i> (2.0)	6.18, <i>s</i>	6.17, <i>d</i> (2.0)	6.16, <i>s</i>	6.18, <i>s</i>	5.91, <i>d</i> (2.0)
3'					6.61, <i>d</i> (1.6)		6.37, <i>d</i> (2.4)
5'	6.70, <i>d</i> (8.8)	6.52, <i>d</i> (8.0)	6.78, <i>d</i> (8.8)	6.63, <i>d</i> (8.0)	6.51, <i>dd</i> (1.6, 8.4)	6.62, <i>d</i> (8.4)	6.43, <i>dd</i> (2.4, 8.4)
6'	6.84, <i>d</i> (8.8)	6.66, <i>d</i> (8.0)	6.92, <i>d</i> (8.8)	6.73, <i>d</i> (8.0) 6.72, <i>d</i> (8.0)	7.07, <i>d</i> (8.4)	6.78, <i>d</i> (8.4) 6.79, <i>d</i> (8.4)	7.21, <i>d</i> (8.4)
2'-OCH <sub>3</sub>	3.80, <i>s</i>	3.89, <i>s</i>	3.74 <sup>e</sup> , <i>s</i>	3.81, <i>s</i>	3.73, <i>s</i>	3.80 <sup>f</sup> , <i>s</i>	
3'-OCH <sub>3</sub>	3.78, <i>s</i>		3.75 <sup>e</sup> , <i>s</i>			3.79 <sup>f</sup> , <i>s</i>	
4'-OCH <sub>3</sub>	3.81, <i>s</i>		3.79, <i>s</i>		3.76, <i>s</i>		
OCH <sub>2</sub> O		5.95, <i>s</i>		6.09, <i>d</i> (3.2)			
1''			4.99, <i>d</i> (6.8)	4.98, <i>d</i> (6.8) 4.99, <i>d</i> (6.8)	4.99, <i>d</i> (6.8)	5.11, <i>d</i> (7.6) 5.10, <i>d</i> (7.6)	
2''			3.23, <i>m</i>	3.23, <i>m</i>	3.25, <i>m</i>	3.53, <i>m</i>	
3''			3.26, <i>m</i>	3.29, <i>m</i>	3.28, <i>m</i>	3.61, <i>m</i>	
4''			3.14, <i>m</i>	3.15, <i>m</i>	3.14, <i>m</i>	3.51, <i>m</i>	
5''			3.40, <i>m</i>	3.33, <i>m</i>	3.41, <i>m</i>	3.61, <i>m</i>	
6''			3.69	3.69	3.69	3.91	
			3.45	3.74	3.47	3.74	

<sup>a</sup> Chemical shifts are expressed in  $\delta$  values.  $J$  values (Hz) are in parentheses.

<sup>b</sup> In  $CD_3OD$ .

<sup>c</sup> In  $DMSO-d_6$ .

<sup>d</sup> In  $Acetone-d_6$ .

<sup>e,f</sup> Assignments in same vertical column may be interchangeable.

Table 2  
<sup>13</sup>C NMR spectroscopic data for 1–7

Position	1 <sup>a</sup>	2 <sup>a</sup>	3 <sup>b</sup>	4 <sup>b</sup>	5 <sup>b</sup>	6 <sup>c</sup>	7 <sup>a</sup>
2	71.77 <i>t</i>	71.54 <i>t</i>	70.11 <i>t</i>	69.86 <i>t</i>	69.48 <i>t</i>	71.33 <i>t</i> 71.29 <i>t</i>	111.97 <i>d</i>
3	48.97 <i>d</i>	48.08 <i>d</i>	46.79 <i>d</i> 46.70 <i>d</i>	47.32 <i>d</i> 47.20 <i>d</i>	46.29 <i>d</i> 46.18 <i>d</i>	48.04 <i>d</i> 47.87 <i>d</i>	79.09 <i>s</i>
4	198.80 <i>s</i>	198.71 <i>s</i>	197.57 <i>s</i> 197.53 <i>s</i>	197.50 <i>s</i> 197.47 <i>s</i>	197.43 <i>s</i> 197.40 <i>s</i>	198.56 <i>s</i> 198.51 <i>s</i>	193.09 <i>s</i>
4a	103.56 <i>s</i>	103.90 <i>s</i>	103.23 <i>s</i>	103.13 <i>s</i>	103.31 <i>s</i> 103.29 <i>s</i>	104.43 <i>s</i> 104.41 <i>s</i>	100.67 <i>s</i>
5	165.88 <i>s</i>	165.85 <i>s</i>	163.23 <i>s</i> 163.21 <i>s</i>	162.77 <i>s</i> 162.76 <i>s</i>	163.09 <i>s</i>	164.66 <i>s</i> 164.63 <i>s</i>	165.63 <i>s</i>
6	97.18 <i>d</i>	97.17 <i>d</i>	96.58 <i>d</i> 96.54 <i>d</i>	96.63 <i>d</i> 96.57 <i>d</i>	96.49 <i>d</i> 96.44 <i>d</i>	97.49 <i>d</i>	98.00 <i>d</i>
7	168.24 <i>s</i>	168.20 <i>s</i>	164.98 <i>s</i> 164.92 <i>s</i>	165.06 <i>s</i> 165.00 <i>s</i>	164.82 <i>s</i> 164.76 <i>s</i>	165.95 <i>s</i> 165.88 <i>s</i>	169.14 <i>s</i>
8	96.05 <i>d</i>	96.04 <i>d</i>	95.39 <i>d</i> 95.37 <i>d</i>	95.45 <i>d</i> 95.38 <i>d</i>	95.30 <i>d</i> 95.25 <i>d</i>	96.21 <i>d</i> 96.18 <i>d</i>	96.80 <i>d</i>
8a	165.13 <i>s</i>	165.12 <i>s</i>	162.68 <i>s</i>	163.32 <i>s</i>	162.60 <i>s</i> 162.58 <i>s</i>	163.78 <i>s</i>	160.89 <i>s</i>
1'	122.37 <i>s</i>	121.75 <i>s</i>	120.43 <i>s</i> 120.37 <i>s</i>	120.23 <i>s</i> 120.17 <i>s</i>	114.90 <i>s</i> 114.83 <i>s</i>	119.96 <i>s</i> 119.88 <i>s</i>	118.13 <i>s</i>
2'	153.18 <i>s</i>	143.04 <i>s</i>	151.23 <i>s</i>	141.19 <i>s</i>	157.86 <i>s</i>	152.32 <i>s</i> 157.85 <i>s</i>	162.07 <i>s</i>
3'	143.55 <i>s</i>	138.44 <i>s</i>	141.54 <i>s</i>	136.78 <i>s</i>	98.86 <i>d</i>	141.31 <i>s</i>	99.00 <i>d</i>
4'	155.19 <i>s</i>	150.90 <i>s</i>	153.21 <i>s</i>	148.88 <i>s</i>	160.06 <i>s</i>	151.60 <i>s</i>	162.04 <i>s</i>
5'	108.73 <i>d</i>	103.47 <i>d</i>	107.72 <i>d</i>	102.94 <i>d</i>	104.99 <i>d</i>	111.91 <i>d</i>	110.78 <i>d</i>
6'	126.14 <i>d</i>	124.73 <i>d</i>	124.71 <i>d</i> 124.66 <i>d</i>	123.59 <i>d</i> 123.52 <i>d</i>	130.88 <i>d</i> 130.79 <i>d</i>	125.61 <i>d</i> 125.52 <i>d</i>	126.67 <i>d</i>
2'-OCH <sub>3</sub>	61.24 <i>q</i>	59.80 <i>q</i>	60.21 <sup>d</sup> <i>q</i>	59.12 <i>q</i>	56.66 <i>q</i>	60.51 <sup>e</sup> <i>q</i> 60.49 <sup>e</sup> <i>q</i>	
3'-OCH <sub>3</sub>	61.02 <i>q</i>		60.50 <sup>d</sup> <i>q</i>			60.44 <sup>e</sup> <i>q</i>	
4'-OCH <sub>3</sub>	56.51 <i>q</i>		55.87 <i>q</i>		55.23 <i>q</i>		
OCH <sub>2</sub> O		102.60 <i>t</i>		101.25 <i>t</i>			
1''			99.53 <i>d</i> 99.42 <i>d</i>	99.54 <i>d</i> 99.45 <i>d</i>	99.47 <i>d</i> 99.38 <i>d</i>	100.63 <i>d</i> 100.54 <i>d</i>	
2''			72.98 <i>d</i> 72.97 <i>d</i>	72.97 <i>d</i>	72.95 <i>d</i>	74.15 <i>d</i>	
3''			76.30 <i>d</i>	76.29 <i>d</i>	76.29 <i>d</i>	77.41 <i>d</i>	
4''			69.50 <i>d</i>	69.50 <i>d</i>	69.73 <i>d</i>	70.75 <i>d</i>	
5''			77.11 <i>d</i>	77.11 <i>d</i>	77.07 <i>d</i>	77.59 <i>d</i>	
6''			60.57 <i>t</i>	60.56 <i>t</i>	60.55 <i>t</i>	62.21 <i>t</i>	

<sup>a</sup> In CD<sub>3</sub>OD.

<sup>b</sup> In DMSO-*d*<sub>6</sub>.

<sup>c</sup> In Acetone-*d*<sub>6</sub>.

<sup>d,e</sup> Assignments in same vertical column may be interchangeable.

H-2b; 4.19, *dd*, *J* = 5.6, 11.2 Hz, H-3) and three carbon resonances [ $\delta$  48.08 (C-3); 71.54 (C-2); and 198.71 (C-4)] (Tables 1 and 2). The <sup>1</sup>H NMR spectrum further revealed the presence of a pair of *ortho*-coupled aromatic protons ( $\delta$  6.52, *d*, *J* = 8.0 Hz; and 6.66, *d*, *J* = 8.0 Hz), a pair of *meta*-coupled aromatic protons ( $\delta$  5.88, *d*, *J* = 2.0 Hz; and 5.90, *d*, *J* = 2.0 Hz), a methoxyl group ( $\delta$  3.89, *s*) and a methylenedioxy group ( $\delta$  5.95, 2H, *s*). The fragments characteristic of a *retro*-Diels-Alder (RDA) fragmentation in the EI MS (*m/z* 330, 178) suggested that the methoxyl group and methylenedioxy groups were located on the B-ring, while two hydroxyl groups were on the A-ring. The HMBC spectrum displayed a long-range correlation between C-3 and an aromatic proton at  $\delta$  6.66. Thus, the *ortho*-coupled aromatic protons were assigned to H-5' ( $\delta$  6.52) and H-6' ( $\delta$

6.66). Since the carbon at  $\delta$  143.04 correlated with H-3 and the methoxyl group ( $\delta$  3.89) in the HMBC spectrum, it was assigned to C-2' bearing a methoxyl group. Consequently, the methylenedioxy group was assigned to C-3' and C-4'. The chemical shift value of C-4 ( $\delta$  198.71) was in agreement with the presence of a 5-OH group. The two *meta*-coupled aromatic protons were assignable to H-6 ( $\delta$  5.90) and H-8 ( $\delta$  5.88) based on analysis of HMBC data. On the basis of available evidence, compound 2 was characterized as 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxy-isoflavanone (Fig. 1), whose structure was also confirmed by X-ray crystallographic analysis (Fig. 3).

Since isoflavanones can readily racemize during extraction and purification procedures (Harborne and Mabry, 1982), it is not possible to exclude the potential of

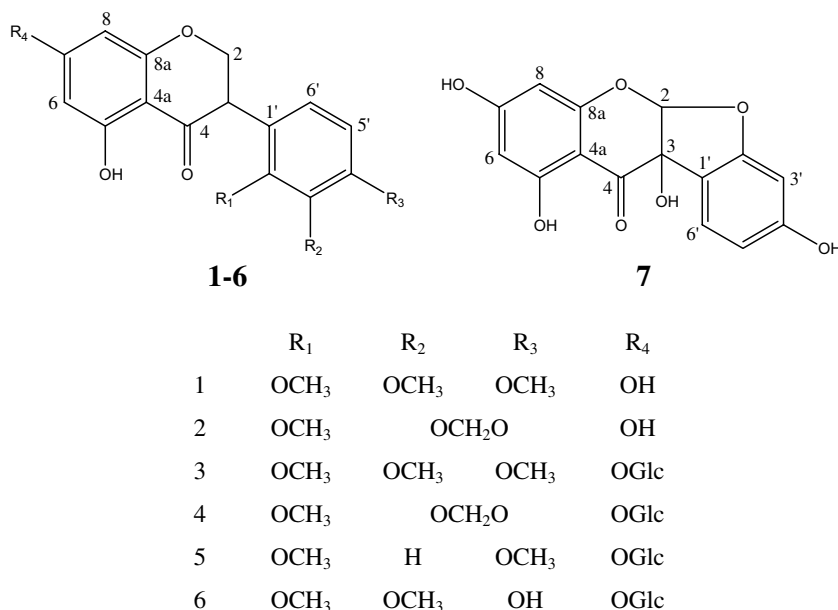
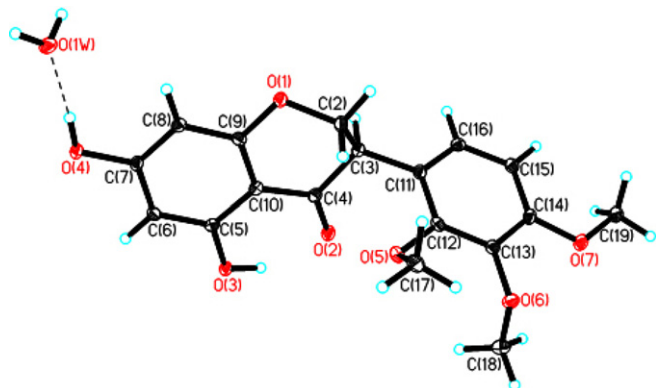
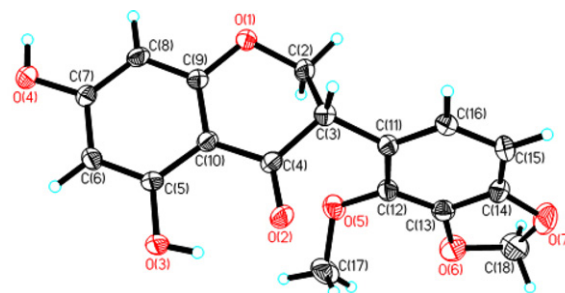


Fig. 1. Chemical structures of 1–7.

compounds **1** and **2** having undergone racemization during the experimental procedures.

Compound **3** was obtained as a white amorphous powder. Its positive ESI MS showed three quasi-molecular ion peaks at  $m/z$  509  $[M+H]^+$ , 531  $[M+Na]^+$ , and 547  $[M+K]^+$ , and the HRESI MS analysis of the quasi-molecular ion peak at  $m/z$  531.1479  $[M+Na]^+$  (calcd 531.1473) was consistent with a molecular formula of an  $C_{24}H_{28}O_{12}$ . The chemical shift values [ $\delta$  70.11 (C-2); 46.79 (C-3); and 197.57 (C-4)] were typical of an isoflavone structure (Table 2). The  $^1H$  NMR spectrum (Table 1) displayed a pair of *ortho*-coupled aromatic protons ( $\delta$  6.92,  $d$ ,  $J = 8.8$  Hz; and 6.78,  $d$ ,  $J = 8.8$  Hz), two chemically equivalent aromatic protons ( $\delta$  6.18,  $s$ ), three methoxyl groups ( $\delta$  3.79, 3.75, and 3.74), a chelated hydroxyl ( $\delta$  12.10, 5-OH), as well as protons belonging to a monosaccharide moiety. Fragment ion peaks in the EI MS at  $m/z$  346  $[508-163+H]^+$  and 194 indicated the presence of

a sugar moiety in the A-ring and three methoxyl groups in the B-ring. The observation of a HMBC correlation between C-3 ( $\delta$  46.79) and an aromatic proton at  $\delta$  6.92, which was *ortho*-coupled to an aromatic proton at  $\delta$  6.78, allowed the assignment of two *ortho*-coupled protons to H-5' ( $\delta$  6.78) and H-6' ( $\delta$  6.92), respectively. The three methoxyl groups were therefore located at C-1', C-2', and C-3' positions. The chemical shift value of C-4 ( $\delta$  197.57) indicated the presence of a chelated 5-OH group. Two chemically equivalent aromatic protons at  $\delta$  6.18 were assigned to H-6 and H-8 based on analysis of the HMBC data, which were also expected from biogenetic consideration. The sugar part was determined to be glucose from its NMR chemical shift values, and the glucosyl anomeric proton ( $\delta$  4.99) with a large coupling constant (6.8 Hz) suggested a  $\beta$  configuration. The linkage between the glucose and the aglycone was established on the basis of the HMBC correlation between the anomeric proton ( $\delta$  4.99, H-1'') and C-7 ( $\delta$  164.98). Compound **3** was therefore elucidated to be 5,7-dihydroxy-2',3',4'-trimethoxy-isoflavone 7-*O*- $\beta$ -glucopyranoside (Fig. 1).

Fig. 2. X-ray structure of **1** (a molecule of water was connected with C-5-OH by hydrogen bonding).Fig. 3. X-ray structure of **2**.

Compound **4** was obtained as a white amorphous powder. Its molecular formula was established to be  $C_{23}H_{24}O_{12}$ , based on the HRESI MS analysis of its quasi-molecular ion peak at  $m/z$  515.1161  $[M+Na]^+$  (calcd 515.1160). It was established to be an isoflavanone glycoside on the basis of analysis its NMR spectroscopic data (Tables 1 and 2). The  $^1H$  NMR spectrum showed the presence of a pair of *ortho*-coupled aromatic protons ( $\delta$  6.63, *d*,  $J = 8.0$  Hz; and 6.73, *d*,  $J = 8.0$  Hz), a pair of *meta*-coupled aromatic protons ( $\delta$  6.18, *d*,  $J = 2.0$  Hz; and 6.17, *d*,  $J = 2.0$  Hz), a methoxyl group ( $\delta$  3.81, *s*), a methylenedioxy group ( $\delta$  6.09), as well as protons belonging to a monosaccharide moiety. In the HMBC spectrum, C-3 ( $\delta$  47.32) correlated with an aromatic proton at  $\delta$  6.73. Thus, the *ortho*-coupled aromatic protons were assigned to 5'-H ( $\delta$  6.63) and 6'-H ( $\delta$  6.73), respectively. Long-range correlations were also observed between two quaternary carbons ( $\delta$  136.78 and 148.88) and the protons of a methylenedioxy group. In addition, the carbon at  $\delta$  136.78 exhibited long-range correlation with H-5', while the carbon at  $\delta$  148.88 had a long-range correlation with H-6'. These results led to the assignments of the quaternary carbons to C-3' ( $\delta$  136.78) and C-4' ( $\delta$  148.88); the methylenedioxy group was therefore located at C-3' and C-4'. The chemical shift value of C-4 ( $\delta$  197.50) was in agreement with the presence of a chelated 5-OH ( $\delta$  12.14). The *meta*-coupled aromatic protons were assigned to H-6 ( $\delta$  6.18) and 8-H ( $\delta$  6.17) based on the HMBC results. The sugar moiety was determined to be  $\beta$  glucose ( $\delta$  4.98, *d*,  $J = 6.8$  Hz, H-1'') from its NMR chemical shift values. In the HMBC spectrum, a long-range correlation between the anomeric proton of glucose ( $\delta$  4.98) and C-7 ( $\delta$  165.06) of the aglycone was also observed. All available evidence thus suggested that **4** was 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxy-isoflavanone 7-*O*- $\beta$ -glucopyranoside (Fig. 1).

Compound **5** was assigned a molecular formula of  $C_{23}H_{26}O_{11}$  from its positive HRESI MS:  $m/z$  501.1351  $[M+Na]^+$  (calcd. 501.1367). It was deduced to be an isoflavanone glycoside based on its NMR spectroscopic data (Tables 1 and 2). Its  $^1H$  NMR spectrum exhibited aromatic protons of an AMX system ( $\delta$  7.07, *d*,  $J = 8.4$  Hz; 6.51, *dd*,  $J = 1.6, 8.4$  Hz; 6.61, *d*,  $J = 1.6$  Hz), two chemical equivalent aromatic protons ( $\delta$  6.16, *s*), a chelated hydroxyl group ( $\delta$  12.17, 5-OH), two methoxyl groups ( $\delta$  3.76, 3.73) and protons corresponding to a monosaccharide. In the EI MS, the ion peaks at  $m/z$  316  $[478-163+H]^+$  and 164 arising from RDA cleavage of the C-ring indicated the presence of a sugar moiety on the A-ring and two methoxyl groups on B-ring. The placement of two methoxyl groups was confirmed from analysis of the HMBC data, establishing long-range correlations between 2'-OCH<sub>3</sub> ( $\delta$  3.73) and C-2' ( $\delta$  157.86), as well as between 4'-OCH<sub>3</sub> ( $\delta$  3.76) and C-4' ( $\delta$  160.06). Chemically equivalent aromatic protons at  $\delta$  6.16 were assigned to H-6 and H-8 based on the HMBC analysis. The sugar with its anomeric proton as a doublet at  $\delta$  4.99 (*d*,  $J = 6.8$  Hz, H-1'') was identified to be a  $\beta$  glucose. The

observation of a HMBC correlation between the anomeric proton ( $\delta$  4.99) and C-7 ( $\delta$  164.82) of the aglycone suggested the position of linkage (C-7). Thus, compound **5** was concluded to be 5,7-dihydroxy-2',4'-dimethoxy-isoflavanone 7-*O*- $\beta$ -glucopyranoside (Fig. 1).

Compound **6** was also an isoflavanone glycoside. Its molecular formula ( $C_{23}H_{26}O_{12}$ ) was established from the analysis of positive HRESI MS:  $m/z$  517.1324  $[M+Na]^+$  (calcd 517.1316) and its NMR spectroscopic data (Tables 1 and 2). The  $^1H$  and  $^{13}C$  NMR spectra indicate the presence of a pair of *ortho*-coupled aromatic protons ( $\delta$  6.62, *d*,  $J = 8.4$  Hz; and 6.78, *d*,  $J = 8.4$  Hz), two chemical equivalent aromatic protons ( $\delta$  6.18), two methoxyl groups ( $\delta$  3.80 and 3.79), a chelated hydroxyl (5-OH), together with a  $\beta$  glucose moiety ( $\delta$  5.11, *d*,  $J = 7.6$  Hz). The two methoxyl groups were assigned to C-2' and C-3', respectively, based on analysis of the HMBC data. Since the proton at  $\delta$  6.78 correlated with C-3 ( $\delta$  48.04) in the HMBC spectrum, two *ortho*-coupled aromatic protons were then assigned to 5'-H ( $\delta$  6.62) and 6'-H ( $\delta$  6.78), respectively. The aromatic protons at  $\delta$  6.18 were assigned to 6-H and 8-H. The anomeric proton of glucose ( $\delta$  5.11) showed a long-range correlation with C-7 ( $\delta$  165.95) of the aglycone. Thus, compound **6** was identified to be 5,7,4'-trihydroxy-2',3'-dimethoxy-isoflavanone 7-*O*- $\beta$ -glucopyranoside (Fig. 1).

For compounds **3–6**, most NMR signals appeared as pairs (Tables 1 and 2). This feature could be attributed to epimerization at C-3. A similar observation has been reported in other studies (Botta et al., 2003; Tsanuo et al., 2003).

Compound **7** was identified as a coumaronochromone derivative based on a comparison of its NMR spectroscopic data with those reported in the literature (Wandji et al., 1995; Drewes et al., 2002). The molecular formula  $C_{15}H_{10}O_7$  was deduced from the HRESI MS quasi-molecular ion at  $m/z$  325.0321  $[M+Na]^+$  (calcd 325.0319) and its NMR spectroscopic data (Tables 1 and 2). The  $^{13}C$  NMR spectra displayed 15 carbons including six methines, and nine quaternary carbons. The  $^1H$  NMR spectrum exhibited an AMX system corresponding to three protons at  $\delta$  7.21 (*d*,  $J = 8.4$  Hz, H-6'), 6.43 (*dd*,  $J = 2.4, 8.4$  Hz, H-5'), and 6.37 (*d*,  $J = 2.4$  Hz, H-3'), two *meta*-coupled aromatic protons at  $\delta$  5.92 (*d*,  $J = 2.0$  Hz, H-6), and 5.91 (*d*,  $J = 2.0$  Hz, H-8), and a proton at  $\delta$  6.12 (*s*) assignable to H-2. Following detailed analysis of the DEPT, HMQC and HMBC spectra, compound **7** was elucidated to be 3,5,7,4'-tetrahydroxy-coumaronochromone (Fig. 1). In the HMBC spectrum, the long-range correlation between C-2' ( $\delta$  162.07) and H-2 ( $\delta$  6.12) further confirmed the presence of a furan ring. The presence of a 3-OH functionality in the structure might result from the addition of water to the C-2/C-3 double bond in ring C of the coumaronochromone skeleton (Wandji et al., 1995). A search in the literature indicated that the occurrence of coumaronochromones bearing 3-OH group is uncommon. Among the known structures, lupinols A–C and piscerythrol I were obtained



from *Piscidia erythrina*, *Lupinus albus*, and *Lupinus luteus* (Tahara et al., 1991), erysenegalensein J was isolated from *Erythrina sensegalensis* (Wandji et al., 1995), and kraussianones 4–5 were reported from *Eriosema kraussianum* (Dreves et al., 2002).

### 3. Concluding remarks

Plants of the Fabaceae family, especially the sub-family Papilionoideae, are a major source of isoflavanones. Several genera, such as *Erythrina*, *Glycyrrhiza*, *Desmodium*, *Sophora*, *Bolusanthus*, *Maackia*, *Swartzia*, *Platymiscium*, *Neorautanenia*, *Diphysa*, *Pachyrrhizus*, *Lespedeza*, and *Phaseolus*, have been reported to contain compounds of this type. As far as the genus *Desmodium* is concerned, nine isoflavanones were previously isolated, including six prenylated structures (desmodianones A–E and 6-methyltetrapterol A) from *D. canum* (Botta et al., 2003; Monache et al., 1996) and three geranylated isoflavanones (uncinane A–C) from *D. uncinatum* (Tsanuo et al., 2003). In the present study, compounds **1**–**6**, obtained from *D. styracifolium* are all simple isoflavanone structures. This is the first report of isoflavanone *O*-glycosides from a natural source. To the best of our knowledge, compounds of this type are hitherto unknown, although an isoflavanone C-glycoside was reported from *Pterocarpus macrocarpus* previously (Verma et al., 1986).

### 4. Experimental

#### 4.1. General

Optical rotations were performed using a Jasco P-1020 Polarimeter. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopic data were acquired with a JEOL JNM-EX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively, with TMS as internal standard. EI MS were determined on a Finnigan TSQ7000 triple quadrupole mass spectrometer, whereas APCI MS employed an Agilent HP 1100 series SL Trap MSD. X-ray crystallographic data were obtained using a Siemens P4-RA diffractometer. Column chromatographic (CC) was carried out on silica gel (100–200 mesh, 230–400 mesh), RP-18 silica gel (40–63 μm) and Sephadex LH-20 respectively, whereas TLC was conducted on silica gel 60 F<sub>254</sub> and RP-18 F<sub>254</sub> plates.

#### 4.2. Plant material

The aerial parts of *Desmodium styracifolium* were collected in Guangdong Province, P.R. China, in August 2003, and identified by Prof. DUAN Jin-Ao, Nanjing University of Traditional Chinese Medicine. A voucher specimen (No. SCM102003) was deposited in School of Chinese Medicine, Chinese University of Hong Kong.

#### 4.3. Extraction and isolation

The finely powdered aerial parts of *D. styracifolium* (25 kg) were refluxed with EtOH–H<sub>2</sub>O (9.5:0.5, 200 L × 2, 60 min each). The EtOH was removed under reduced pressure to give a residue, which was suspended in hot water duration and partitioned successively with *n*-hexane and EtOAc. The hexane-soluble portion was separated on a macroporous resin column eluted with MeOH this being followed by silica gel CC using a gradient of increasing polarity (petroleum ether–EtOAc, 9:1 → 8:2 → 7:3 → 6:4 → 5:5, 3 L each) to obtain fractions H-1, H-2, H-3, H-4, and H-5. Compounds **8** (30 mg) and **9** (25 mg) were crystallized from fraction H-2. Compounds **10** (40 mg), **11** (15 mg), and **12** (12 mg) were obtained from the fraction H-3 by repeated silica gel CC eluted with hexane–EtOAc (10:0 → 7:3) and hexane–acetone (10:0 → 8:2). Fraction H-4 was further separated on a macroporous resin column eluted with MeOH–H<sub>2</sub>O (9:1) followed by silica gel CC eluted with hexane–acetone (9:1 → 8:2) to obtain compounds **13** (25 mg) and **14** (15 mg). Fraction H-5 was further separated into 128 fractions by silica gel CC eluted with hexane–acetone (9:1 → 7:3); colorless crystals crystallized from fractions 88–95. These were dissolved in MeOH and purified on a Sephadex LH-20 column eluted with MeOH to afford compounds **1** (15 mg) and **2** (20 mg), respectively. The EtOAc-soluble fraction was separated on a macroporous resin column eluted with MeOH–H<sub>2</sub>O (9:1), followed by silica gel CC eluted with dichloromethane–acetone (9:1 → 5:5) to afford fractions E-1, E-2, E-3. Fraction E-1 was subjected to a macroporous resin column eluted with MeOH–H<sub>2</sub>O (8.5:1.5) and then purified by repeated CC on silica gel, RP-18 and Sephadex LH-20 to obtain compounds **15** (13 mg), **16** (10 mg), **17** (16 mg), **18** (28 mg), **19** (20 mg), **20** (30 mg), **21** (25 mg), **22** (15 mg), **23** (10 mg), **24** (15 mg), **25** (12 mg), **26** (15 mg), **27** (17 mg), and **28** (10 mg), respectively. Fraction E-2 was separated on a macroporous resin column eluted with MeOH–H<sub>2</sub>O (8.5:1.5) followed by Sephadex LH-20 CC and then silica gel CC to obtain five fractions: E-2-1 to E-2-5. Compounds **7** (10 mg), **29** (12 mg), **30** (15 mg), **31** (9 mg), and **32** (14 mg) were obtained from fractions E-2-3 and E-2-4 by repeated CC on RP-18 and Sephadex LH-20. Fraction E-3 was further applied to a macroporous resin column eluted with MeOH–H<sub>2</sub>O (8:2). After passing through a Sephadex LH-20 column eluted with methanol, the fraction rich in flavonoids was separated into 90 fractions by silica gel CC eluted with a CH<sub>2</sub>Cl<sub>2</sub>–MeOH mixture (8:2 → 6:4). Fractions 64–81 were combined and further purified on RP-18 eluted with MeOH–H<sub>2</sub>O (3:7 → 4:6) and Sephadex LH-20 columns eluted with MeOH to yield **3** (16 mg), **4** (12 mg), **5** (15 mg), and **6** (100 mg).

#### 4.4. (±)5,7-Dihydroxy-2',3',4'-trimethoxy-isoflavanone (**1**)

Colorless crystals, C<sub>18</sub>H<sub>18</sub>O<sub>7</sub>; m.p. 104–106 °C; UV λ<sub>max</sub><sup>MeOH</sup> nm: 243, 265, 330. [α]<sub>D</sub><sup>25</sup> ± 0° (MeOH, c 0.01). For

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, see Tables 1 and 2. EI MS  $m/z$ : 346, 194. HREI MS  $m/z$ : 346.1043  $[\text{M}]^+$  (calcd 346.1047).

4.5. ( $\pm$ )5,7-Dihydroxy-2'-methoxy-3',4'-methylenedioxy-isoflavanone (2)

Colorless crystals,  $\text{C}_{17}\text{H}_{14}\text{O}_7$ ; m.p. 108–110 °C; UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 243, 267, 320.  $[\alpha]_{\text{D}}^{25} \pm 0^\circ$  (MeOH,  $c$  0.01). For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, see Tables 1 and 2. EI MS  $m/z$ : 330, 178. HREI MS  $m/z$ : 330.0728  $[\text{M}]^+$  (calcd 330.0734).

4.6. 5,7-Dihydroxy-2',3',4'-trimethoxy-isoflavanone 7-O- $\beta$ -glucopyranoside (3)

White amorphous powder,  $\text{C}_{24}\text{H}_{28}\text{O}_{12}$ ; UV  $\lambda_{\text{max}}^{\text{DMSO}}$  nm: 240, 275, 305.  $[\alpha]_{\text{D}}^{25} - 27^\circ$  (DMSO,  $c$  0.003). For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, see Tables 1 and 2. EI MS  $m/z$ : 346, 194. ESI MS  $m/z$ : 509  $[\text{M}+\text{H}]^+$ , 531  $[\text{M}+\text{Na}]^+$ , 547  $[\text{M}+\text{K}]^+$ . HRESI MS  $m/z$ : 531.1479  $[\text{M}+\text{Na}]^+$  (calcd 531.1473).

4.7. 5,7-Dihydroxy-2'-methoxy-3',4'-methylenedioxy-isoflavanone 7-O- $\beta$ -glucopyranoside (4)

White powder,  $\text{C}_{23}\text{H}_{24}\text{O}_{12}$ ; UV  $\lambda_{\text{max}}^{\text{DMSO}}$  nm: 239, 278, 301.  $[\alpha]_{\text{D}}^{25} - 30^\circ$  (DMSO,  $c$  0.002). For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, see Tables 1 and 2. ESI MS  $m/z$ : 515  $[\text{M}+\text{Na}]^+$ , 531  $[\text{M}+\text{K}]^+$ . HRESI MS  $m/z$ : 515.1161  $[\text{M}+\text{Na}]^+$  (calcd 515.1160).

4.8. 5,7-Dihydroxy-2',4'-dimethoxy-isoflavanone 7-O- $\beta$ -glucopyranoside (5)

White amorphous powder,  $\text{C}_{23}\text{H}_{26}\text{O}_{11}$ ; UV  $\lambda_{\text{max}}^{\text{DMSO}}$  nm: 235, 290.  $[\alpha]_{\text{D}}^{25} - 35^\circ$  (DMSO,  $c$  0.002). For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, see Tables 1 and 2. EI MS  $m/z$ : 316, 164. ESI MS  $m/z$ : 479  $[\text{M}+\text{H}]^+$ , 501  $[\text{M}+\text{Na}]^+$ , 517  $[\text{M}+\text{K}]^+$ . HRESI MS  $m/z$ : 531.1351  $[\text{M}+\text{Na}]^+$  (calcd 531.1367).

4.9. 5,7,4'-Trihydroxy-2',3'-dimethoxy-isoflavanone 7-O- $\beta$ -glucopyranoside (6)

White amorphous powder,  $\text{C}_{23}\text{H}_{26}\text{O}_{12}$ ; UV  $\lambda_{\text{max}}^{\text{DMSO}}$  nm: 250, 360.  $[\alpha]_{\text{D}}^{25} - 25^\circ$  (acetone,  $c$  0.006). For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, see Tables 1 and 2. ESI MS  $m/z$ : 517  $[\text{M}+\text{Na}]^+$ . HRESI MS  $m/z$ : 517.1324  $[\text{M}+\text{Na}]^+$  (calcd 517.1316).

4.10. 3,5,7,4'-Tetrahydroxy-coumaronochromone (7)

White powder,  $\text{C}_{15}\text{H}_{10}\text{O}_7$ ; UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 285, 340.  $[\alpha]_{\text{D}}^{25} + 18^\circ$  (methanol,  $c$  0.0014). For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, see Tables 1 and 2. APCI MS  $m/z$ : 285  $[\text{M}-\text{H}_2\text{O}+\text{H}]^+$ ; ESI MS  $m/z$ : 325  $[\text{M}+\text{Na}]^+$ ; HRESI MS  $m/z$ : 325.0321 (calcd 325.0319).

4.11. X-ray data for compound 1 and 2

Compound 1 was recrystallized from MeOH– $\text{H}_2\text{O}$ .  $\text{C}_{18}\text{H}_{18}\text{O}_7 + \text{H}_2\text{O}$ ,  $M_r = 364.34$ ; Crystal size  $0.40 \times 0.30 \times 0.25 \text{ mm}^3$ ; monoclinic, space group  $\text{P2(1)/n}$ ,  $a = 10.7488(9) \text{ \AA}$ ,  $b = 7.6622(6) \text{ \AA}$ ,  $c = 21.2506(18) \text{ \AA}$ ,  $V = 1719.0(2) \text{ \AA}^3$ ,  $Z = 4$ ,  $D_c = 1.408 \text{ Mg/m}^3$ . The structures were refined by full-matrix least-squares on  $F^2$  using SHELEXL-97 (Sheldrick, 1997). Final discrepancy indices of  $R_1 = 0.0339$ ,  $wR_2 = 0.0822$  and  $\text{GOOF} = 1.033$  for observed data with  $I > 2\sigma(I)$ . The final difference electron density map contains maximum and minimum peak heights of 0.291 and  $-0.201 \text{ e \AA}^{-3}$ .

Compound 2 was recrystallized from MeOH– $\text{H}_2\text{O}$ .  $\text{C}_{17}\text{H}_{14}\text{O}_7$ ,  $M_r = 330.28$ ; Crystal size  $0.40 \times 0.35 \times 0.30 \text{ mm}^3$ ; monoclinic, space group  $\text{P2(1)/c}$ ,  $a = 8.6579(19) \text{ \AA}$ ,  $b = 7.4500(17) \text{ \AA}$ ,  $c = 23.049(5) \text{ \AA}$ ,  $V = 1467.3(6) \text{ \AA}^3$ ,  $Z = 4$ ,  $1.495 \text{ Mg/m}^3$ . The structures were refined by full-matrix least-squares on  $F^2$  using SHELEXL-97 (Sheldrick, 1997). Final discrepancy indices of  $R_1 = 0.0494$ ,  $wR_2 = 0.1158$  and  $\text{GOOF} = 1.038$  for observed data with  $I > 2\sigma(I)$ . The final difference electron density map contains maximum and minimum peak heights of 0.203 and  $-0.190 \text{ e \AA}^{-3}$ .

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center. CCDC 612572 (for compound 1) and 612573 (for compound 2) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via [www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html) (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; deposit@ccdc.cam.ac.uk).

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