Phenolic Metabolites from the Stems and Leaves of *Sophora flavescens*

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Two new compounds, (−)-(6a*R*,11a*R*)-4-methoxy-8,9-(methylenedioxy)pterocarpan 3-*O*-*β*-D-glucopyranoside (**1**) and 5-hydroxy-7-methoxyisoflavone 4′-*O*-*β*-D-xylopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**2**), were isolated, together with 30 known compounds from the stems and leaves of *Sophora flavescens* AITION. Their structures were elucidated by extensive spectroscopic analysis, including HR-ESI-MS data. A preliminary comparison of phenolic metabolite profiles, based on the qualitative HPLC analysis, indicated that the composition of the roots and the aerial parts were significantly different.

Introduction. – *Sophora flavescens* AITION, belonging to the Fabaceae family, is a perennial herb distributed in China, Russia, Japan, India, and Korea [1]. Its dried root is recorded in current Chinese Pharmacopoeia as *Sophorae Radix* (*SR*; *Kushen* in Chinese) and widely used as a traditional Chinese medicine for the treatment of jaundice, leucorrhea, carbuncles, pyogenic infections of the skin, scabies, as well as dysentery [2]. 293 Dose-forms of the preparations containing *SR* and its main alkaloids, including matrine, oxymatrine, sophoridine, and oxysophocarpine, or combinations with other herbal ingredients, were authorized to the Chinese market, such as anti-HBV *Kushensu* injection and as an antineoplastic compound *Kushen* injection [3]. As an important raw herbal material in the fields of medicines, cosmetics, biopesticides, and veterinary drugs, the demand for the root of *S. flavescens* has been growing with more extensive applications, and accordingly, *SR* inevitably faces the threat of resource exhaustion. To overcome the plant shortage, one strategy is the standardized cultivation according to *Good Agricultural Practice*. In addition, comprehensive utilization of medicinal and non-medicinal parts may also be an approach.

Chemical investigations of the root of *S. flavescens* led to the isolation and identification of numerous structurally diverse compounds such as alkaloids, flavonoids, chromones, saponins, *etc.* Quinolizidine alkaloids and prenylated flavonoids are the best-studied constituents, and up to now, 41 alkaloids and 108 flavonoids have been isolated from the root of *S. flavescens* [4]. Some studies have been reported on the composition and content of the alkaloids in the different organs of *S. flavescens*, such as roots, stems, leaves, flowers, and seeds [5–8]. However, no systematic and comprehensive investigation on the phenolic compounds of the aerial parts was conducted to date, with the exception of the quantification of trifolirhizin [9] and isokurarinone [10].

As part of our continuing interest in this plant, we have undertaken a detailed phytochemical investigation of the EtOH extract of the stems and leaves of *S. flavescens*. Our study resulted in the isolation of 32 compounds **1**–**32**, including a pterocarpan flavonoid, **1**, an isoflavone glycoside, **2**, and 17 compounds, **5**, **7**, **9**–**11**, **13**, **14**, **16**–**21**, and **29**–**32**, reported for the first time from this plant.

Results and Discussion. – The 80% EtOH extract of the stems and leaves of *S. flavescens* was subjected to repeated CC on macroporous resin *HPD-100*, silica gel (SiO_2), *Sephadex LH-20*, and *ODS*, as well as semi-preparative HPLC, to give new compounds **1** and **2**, along with known compounds maackiain (**3**) [11], trifolirhizin (**4**) [11], (+)-syringaresinol (**5**) [12], calycosin (**6**) [13], 7,3′-dihydroxy-5′-methoxyisoflavone (**7**) [14], genistein (**8**) [15], 5′-methoxydehydrodiconiferyl alcohol (**9**) [16], dehydrodiconiferyl alcohol (**10**) [17], 2′-hydroxygenistein (**11**) [18], daidzein (**12**) [19], orobol (**13**) [20], 7,3′,4′-trihydroxyisoflavone (**14**) [21], ononin (**15**) [22], 3′-*O*-methylorobol 7-*O*-*β*-D-glucopyranoside (**16**) [23], pratensenin 7-*O*-*β*-D-glucopyranoside (**17**) [24], genistin (**18**) [25], sophororicoside (**19**) [18], chrysoeriol 4′-*O*-*β*-D-glucopyranoside (**20**) [26], 5,7,3′-trihydroxyflavone 4′-*O*-*β*-D-glucopyranoside (**21**) [27], pseudobatigenin 7-*O*-*β*-D-xylopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**22**) [28], kushenol O (**23**) [29], 4′-methoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (**24**) [30], kakkanin (**25**) [31], lanceolarin (**26**) [32], liriodendrin (**27**) [12], protocatechic acid (**28**) [33], tachioside (**29**) [34], isotachioside (**30**) [35], koaburaside (**31**) [35], and leonuriside A (**32**) [36] (*Fig. 1*).

Compound **1** was obtained as white powder. The molecular formula was deduced as $C_{23}H_{24}O_{11}$ from a *quasi*-molecular-ion peak at *m*/*z* 499.1211 ($[M+Na]^+$) in the HR-ESI-MS. The ^{1}H-NMR spectrum (*Table 1*) indicated the presence of four aromatic H-atoms (*δ*(H) 7.19 (*d*, *J* = 8.7, 1 H), 6.93 (*d*, *J* = 8.7, 1 H), 6.84 (*s*, 1 H), and 6.39 (*s*, 1 H), of a OCH_2O group (*δ*(H) 5.87 (*d*, *J* = 15.0, 2 H)), of a MeO group (*δ*(H) 3.84 (*s*, 3 H)), and of an anomeric H-atom (*δ*(H) 4.94 (*d*, *J* = 7.3, 1 H)). OCH_2O H-atoms are usually non-equivalent in pterocarpans [11][37][38]. The multiplicity and the coupling constants observed for the OCH_2O group in **1** were in full agreement with the data reported for trifolirhizin (**4**) [11]. The ^{13}C-NMR spectrum (*Table 1*) exhibited signals of 23 C-atoms, of which 17 accounted for the aglycone moiety. The remaining signals were in good accordance with the presence of a glucose unit (*δ*(C) 101.3, 73.5, 76.6, 69.9, 76.8, and 61.1). The spectral data of compound **1**, except the presence of an additional MeO group, were similar to those of the co-occurring known compound trifolirhizin (**4**) [11]. The position of the MeO group was deduced to be C(4) of **1** from the HMBCs (*Table 1* and *Fig. 2*) between MeO (*δ*(H) 3.84) and C(4) (*δ*(C) 137.9). The other HMBCs from OCH_2O (*δ*(H) 5.87) to C(8) (*δ*(C) 141.9) and C(9) (*δ*(C) 148.2) revealed the position of OCH_2O moiety between C(8) and C(9). The key HMBC between H–C(1′) (*δ*(H) 4.94) and C(3) (*δ*(C) 151.1) indicated that the glucopyranosyl unit was linked to C(3) of the aglycone moiety. Acid hydrolysis, followed by GC analysis, revealed that the glucose was of the D-series. The *β*-configuration of sugar moiety was established on the basis of the coupling constant (*J* = 7.3) of the anomeric H-atom. A smaller coupling between H–C(6a) (*δ*(H) 3.48–3.53 (*m*, 1 H)) and H–C(11a) (*δ*(H) 5.51 (*d*, *J* = 7.0, 1 H)), as well as the correlations between H–C(6a) and H–C(11a) in the NOESY spectrum indicated that H–C(6a) and H–C(11a) were *cis*-oriented. The absolute

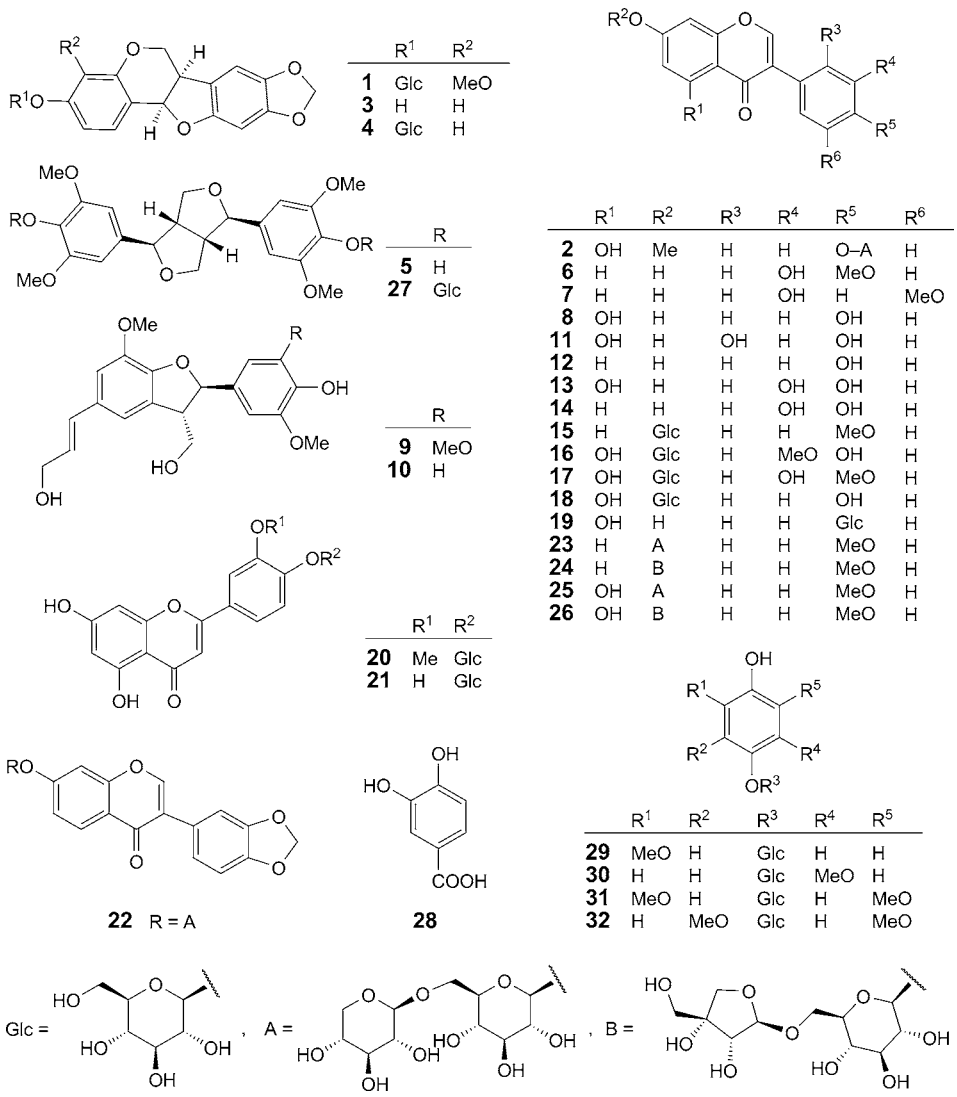

Fig. 1. *The structures of compounds* **1**–**32** *and reference substances* **I**–**IX**

configuration (6a*R*, 11a*R*) was determined from the negative optical rotation ($[\alpha]_D^{20} = -12$ ($c = 1.0$, MeOH)), the positive *Cotton* effect of 306 nm for the ^1L_b band, and the negative *Cotton* effect of 213 nm for the ^1L_a band [39]. Accordingly, the structure of **1** was determined as depicted in *Fig. 1*.

Compound **2** was isolated as white powder. The HR-ESI-MS revealed a *quasi*-molecular-ion peak at *m*/*z* 579.1708 ($[M+H]^+$), providing the a molecular formula $C_{27}H_{30}O_{14}$. The ^{1}H- and ^{13}C-NMR spectra (*Table 2*) indicated the presence of a 1,4-disubstituted aromatic ring (δ(H) 7.51 (*dd*, $J = 1.8$, 6.8, 2 H) and 7.14 (*dd*, $J = 1.8$, 6.8,

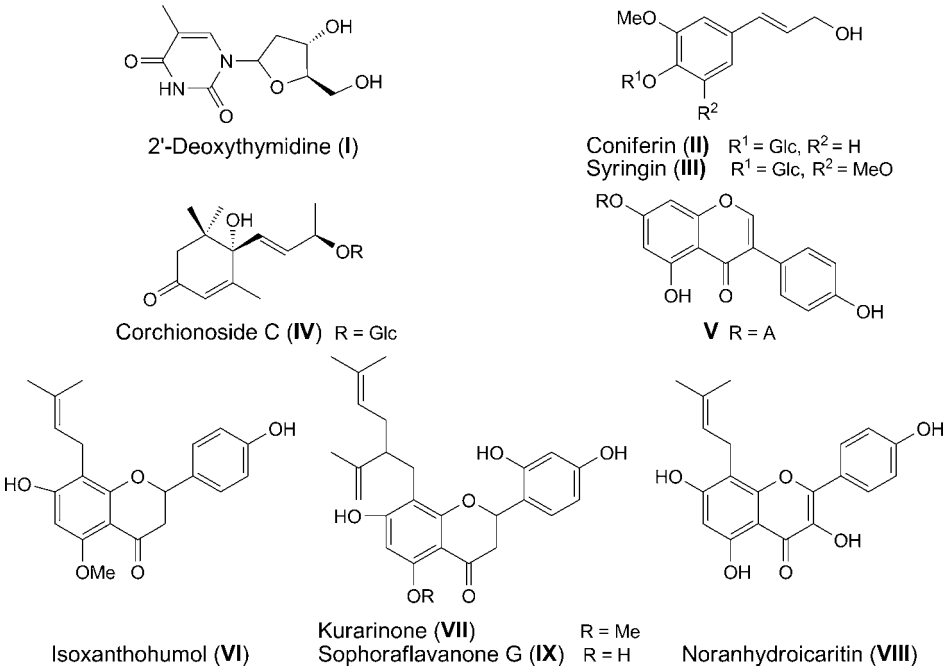

Fig. 1. (cont.)

2 H); δ(C) 130.7 and 116.7) and a 1,2,3,5-tetrasubstituted aromatic ring (δ(H) 6.68 (*d*, $J = 2.3$, 1 H) and 6.43 (*d*, $J = 2.3$, 1 H); δ(C) 98.6 and 93.0). A deshielded signal at δ(H) 8.45 (*s*, 1 H) was characteristic of H–C(2) of an isoflavone. The signal at δ(H) 12.90 (*s*, 1 H) revealed the presence of a chelated OH group at C(5). The signal of a MeO group at δ(H) 3.87 (*s*, 3 H) was also observed in the ^{1}H-NMR spectrum. The signals at δ(H) 4.86 (*d*, $J = 7.4$, 1 H) and 4.19 (*d*, $J = 7.6$, 1 H) were assigned to sugar anomeric H-atoms, based on the ^{1}H,^{1}H-COSY and HSQC experiments. In addition, the signals at δ(C) 100.8, 73.7, 76.5, 70.1, 77.0, and 68.6, as well as at δ(C) 104.3, 73.9, 76.9, 70.0, and 66.1 indicated that the sugar moiety consisted of glucopyranosyl and xylopyranosyl units. The D absolute configuration of both sugars was determined by acid hydrolysis, followed by GC analysis, and the β-configuration was established on the basis of the coupling constants of the anomeric H-atoms (δ(H) 4.86 ($J = 7.4$, H–C(1″)) and 4.19 ($J = 7.6$, H–C(1‴)) of glucose and xylose, respectively. In the HMBC spectrum (*Fig. 3*), the correlations from H–C(1″) (δ(H) 4.86) to C(4′) (δ(C) 157.8), and from H–C(1‴) (δ(H) 4.19) to C(6″) (δ(C) 68.6), as well as from H_α–C(6″) (δ(H) 3.58) and H_β–C(6″) (δ(H) 3.95) to C(1‴) (δ(C) 104.3) suggested that the xylopyranosyl was linked to C(6″) of glucopyranosyl, and glucopyranosyl was linked to C(4′) of the aglycone. The other HMBC from MeO (δ(H) 3.87) to C(7) (δ(C) 165.8) revealed that the MeO group was located at C(7) of *A* ring (*Fig. 3*). Actually, the spectral data of compound **2** were similar to those of the known compound of kakkanin (**25**) [31], and both compounds differ only in the respective linkage positions of the sugar moiety and the MeO group. Thus, the structure of compound **2** was determined as shown in *Fig. 3*.

Table 1. *^{1}H- and ^{13}C-NMR Data* (at 600 and 150 MHz, resp.; in CD_3OD) *of* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	^{1}H,^{1}H-COSY	HMBC
1	7.19 (*d*, *J* = 8.7)	125.7	H–C(2)	C(2), C(3), C(4), C(4a), C(11a)
2	6.93 (*d*, *J* = 8.7)	109.9	H–C(1)	C(3), C(4), C(4a), C(11b)
3		151.1		
4		137.9		
4a		149.5		
6	4.35 (*dd*, *J* = 4.6, 10.6, H_α), 3.68 (*d*, *J* = 10.6, H_β)	66.4	H_β–C(6), H–C(6a) H_α–C(6), H–C(6a)	C(4a), C(6a), C(6b), C(11a) C(4a), C(6a), C(6b), C(11a)
6a	3.48 – 3.53 (*m*)	40.2	H_α–C(6), H_β–C(6), H–C(11a)	C(6), C(6b), C(7), C(10a), C(11a), C(11b)
6b		118.0		
7	6.84 (*s*)	104.6		C(6a), C(6b), C(8), C(9), C(10), C(10a)
8		141.9		
9		148.2		
10	6.39 (*s*)	92.8		C(6b), C(8), C(9), C(10a)
10a		154.1		
11a	5.51 (*d*, *J* = 7.0)	78.2	H–C(6a)	C(1), C(4a), C(6), C(6a), C(6b), C(10a), C(11b)
11b		116.2		
1′	4.94 (*d*, *J* = 7.3)	101.3	H–C(2′)	C(3), C(3′)
2′	3.48 – 3.53 (*m*)	73.5	H–C(1′), H–C(3′)	C(4′)
3′	3.48 – 3.53 (*m*)	76.6	H–C(2′)	C(1′)
4′	3.41 – 3.47 (*m*)	69.9	H–C(3′), H–C(5′)	C(2′)
5′	3.41 – 3.47 (*m*)	76.8	H_α–C(6′), H_β–C(6′)	C(6′)
6′	3.72 (*dd*, *J* = 12.2, 7.2, H_α), 3.89 (*d*, *J* = 12.2, H_β)	61.1	H_β–C(6′), H–C(5′) H_α–C(6′), H–C(5′)	C(4′), C(5′) C(4′), C(5′)
OCH_2O	5.87 (*d*, *J* = 15.0)	101.2		C(8), C(9)
MeO	3.84 (*s*)	60.3		C(4)

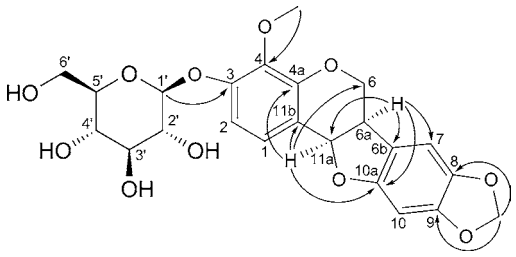

Fig. 2. *The key HMBCs* (H → C) *of compound* **1**

A number of diverse flavonoids have been isolated from the roots of *S. flavescens*, and most of them are prenylated derivatives with potent bioactivities, such as sophoraflavanone G and kurarinone. The present investigation provides a detailed metabolic profile of the aerial part. Isoflavonoids were the main group of compounds, moreover, no prenylated flavonoid was obtained from the aerial parts. A preliminary

Table 2. *^{1}H- and ^{13}C-NMR Data* (at 600 and 150 MHz, resp.; in CD_3OD) *of* **2**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	^{1}H,^{1}H-COSY	HMBC
2	8.45 (*s*)	155.3		C(3), C(4), C(9), C(1′)
3		122.7		
4		180.8		
5		162.2		
6	6.43 (*d*, *J* = 2.3)	98.6	H–C(8)	C(5), C(7), C(8), C(10)
7		165.8		
8	6.68 (*d*, *J* = 2.3)	93.0	H–C(6)	C(6), C(7), C(9), C(10)
9		158.0		
10		105.9		
1′		124.5		
2′/6′	7.51 (*dd*, *J* = 1.8, 6.8)	130.7	H–C(3′/5′)	C(3), C(4′)
3′/5′	7.14 (*dd*, *J* = 1.8, 6.8)	116.7	H–C(2′/6′)	C(1′), C(4′)
4′		157.8		
1″	4.86 (*d*, *J* = 7.4)	100.8	H–C(2″)	C(4′), C(3″)
2″	3.25 – 3.28 (*m*)	73.7		C(4″)
3″	3.54 (*t*, *J* = 9.0)	76.5		C(1″)
4″	3.17 (*t*, *J* = 9.0)	70.1		C(2″)
5″	3.26 – 3.31 (*m*)	77.0	H_α–C(6″), H_β–C(6″)	C(6″)
6″	3.58 (*dd*, *J* = 11.1, 6.6, H_α), 3.95 (*d*, *J* = 11.1, H_β)	68.6	H–C(5″)	C(1‴), C(4″), C(5″) C(4″), C(5″)
1‴	4.19 (*d*, *J* = 7.6)	104.3	H–C(2‴)	C(6″), C(3‴)
2‴	2.96 (*t*, *J* = 8.2)	73.9	H–C(1‴)	C(4‴)
3‴	3.05 – 3.08 (*m*)	76.9		C(1‴)
4‴	3.24 – 3.28 (*m*)	70.0		C(2‴)
5‴	3.66 (*dd*, *J* = 11.3, 5.3, H_α), 2.92 (*t*, *J* = 11.3, H_β)	66.1	H–C(4‴), H_β–C(5‴) H–C(4‴), H_α–C(5‴)	C(3‴), C(4‴) C(3‴), C(4‴)
MeO–C(7)	3.87 (*s*)	56.6		C(7)
OH	12.90			

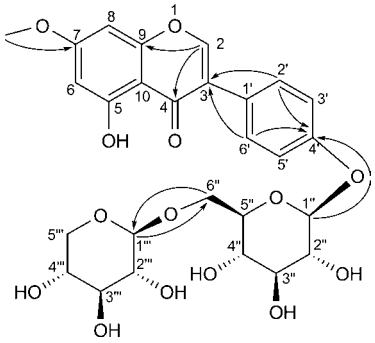

Fig. 3. *The key HMBCs* (H → C) *of compound* **2**

comparison of phenolic metabolite profiles indicated that the chemical compositions of the roots and the aerial parts were rather different. On the basis of qualitative HPLC chromatograms, recorded at 254 nm (*Figs. 4* and *5*), of roots and aerial parts collected

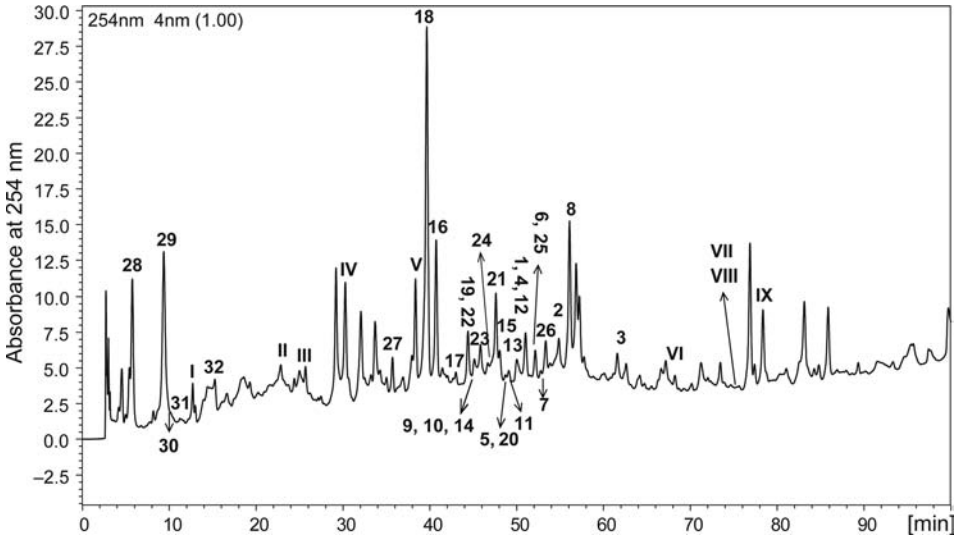

Fig. 4. *HPLC of the extract of the stems and leaves of* S. flavescens *and peak assignments.* Peak numbers correspond to compound numbers in *Fig. 1.*

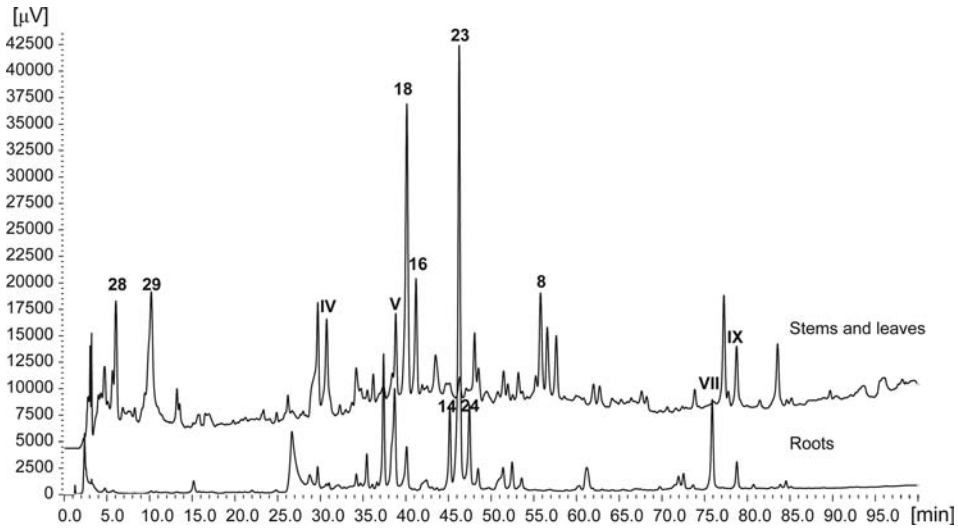

Fig. 5. *HPLC of the extract of the stems and leaves as well as roots of* S. flavescens

from the main growing area, Changzhi County, 6″-*O*-*β*-D-xylopyranosylgenistin (**V**), genistein (**8**), 3′-*O*-methylorobol 7-*O*-*β*-D-glucopyranoside (**16**), genistin (**18**), and sophoraflavanone G (**IX**) were the constituents of the aerial parts, whereas kushenol O (**23**), 7,3′,4′-trihydroxyisoflavone (**14**), 4′-methoxyisoflavone 7-*O*-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (**24**), and kurarinone (**VII**) were predominant in the roots. The aerial parts are also characterized by the presence of quite common H_2O-

soluble constituents, such as protocatechic acid (**28**), tachioside (**29**), isotachioside (**30**), koaburaside (**31**), leonuriside A (**32**), and 2′-deoxythymidine (**1**).

In summary, this work appears to be the first study on the phenolic metabolite profile of aerial parts of *S. flavescens*, which resulted in the isolation of 32 compounds, including a pterocarpan flavonoid, an isoflavone glycoside, and 17 compounds reported for the first time from this plant. Compared with roots, the aerial parts possess more isoflavonoids than prenylated flavanoids. In view of the obvious differences in phenolic compound compositions, it will probably be difficult to use the aerial parts as an alternative resource for roots to overcome the plant shortage. However, they may be of some interest in the cosmetics field, since the isoflavonoids in *S. flavescens* have been reported to possess significant antioxidant and tyrosinase-inhibition activity [40][41].

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 100–200, 200–300, or 300–400 mesh; *Qingdao Haiyang, Co., Ltd.*, Qingdao, P. R. China), *ODS* (40–63 μm, *Merck*, DE-Darmstadt), *Sephadex LH-20* (*Pharmacia*, SE-Uppsala), and *HPD-100* macroporous resin (*Hebei Baoen Chemical Corporation*, Hebei, P. R. China). *RP-TLC* (*Merck*, USA): Precoated silica gel GF_{254} plates (*Qingdao Haiyang Co., Ltd.*, Qingdao, P. R. China). Semi-prep. HPLC: *Alltech* HPLC system equipped with a *Binary 426* solvent delivery pump; *UV-2000* detector; column, *Agilent* C_{18}, 5 μm, i.d. 9.6 × 250 mm. Anal. HPLC: *Prominence LC-20A* series instrument, *SPD-20A*/*20AV* detector; column, *Phenomenex Luna* C_{18}, 5 μm, i.d. 4.6 × 250 mm. GC: *Agilent 7890A* GC system. Optical rotation: *Rudolph* automatic polarimeter. Circular dichroism (CD): *JASCO J-815* spectropolarimeter. UV Spectra: *Shimadzu UV-2550* spectrophotometer. NMR Spectra: *Bruker AVIII-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS: *Agilent-1200-LC*/*MSD-Trap* (ESI) and *Agilent-6520 Q-TOf* (HR-ESI) spectrometers; in m/z.

Plant Materials. The dried stems and leaves, as well as roots of *S. flavescens* were collected from Changzhi City, Shanxi Province, P. R. China in November 2011, and authenticated by Mrs. *RuiZhen Wang*, *Shanxi Zhendong Pharmaceutical Co., Ltd.* Voucher specimens (No: KSY2011 and KS2011) were deposited with the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences.

Extraction and Isolation. The powdered stems and leaves of *S. flavescens* (10 kg, dry weight) were soaked 16 h with EtOH/H_2O 4:1 (200 l) and extracted 4 h under reflux to give 300 g of the extract. The extract was dispersed with H_2O (10 l) and subjected to CC (macroporous resin *HPD-100*, H_2O, EtOH/H_2O 3:7, 5:5, 7:3, and 95:5): *Frs. 1–5*. *Fr. 4* (1.3 g) was repeatedly subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 15:1:0.1): **3** (3.5 mg). *Fr. 3* (11.1 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 720:9:5, 360:9:5, 180:9:5, 90:9:5, 45:9:5, 18:9:5, 18:9:3): *Subfrs. 3A–3X*. *Fr. 3B* (270 mg) was purified by CC (SiO_2; $CHCl_3$/MeOH/H_2O 1080:9:5), followed by semi-prep. HPLC (MeOH/H_2O 45:55): **5** (12.3 mg). *Fr. 3C* (120 mg) was purified by CC (SiO_2; $CHCl_3$/MeOH/H_2O 360:9:5), followed by recrystallization from MeOH: **6** (7.2 mg). *Fr. 3D* (120 mg) was purified by semi-prep. HPLC (MeOH/H_2O 48:52): **7** (7.8 mg) and **8** (18.1 mg). *Fr. 3E* (230 mg) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 180:9:5) and purified by semi-prep. HPLC (MeOH/H_2O 35:65): **9** (4.8 mg) and **10** (4.2 mg). *Fr. 3F* (400 mg) was separated by CC (SiO_2; $CHCl_3$/MeOH/H_2O 180:9:5; and *Sephadex LH-20*; MeOH) and purified by semi-prep. HPLC (MeOH/H_2O 45:55): **11** (8.0 mg) and **12** (7.2 mg). *Fr. 3H* (390 mg) was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:2) and purified by semi-prep. HPLC (MeOH/H_2O 40:60): **1** (19.5 mg). *Fr. 3I* (1.01 g) afforded **4** (738.5 mg), **13** (4.3 mg), and **14** (5.4 mg) after purification by CC (*Sephadex LH-20*; MeOH/H_2O 4:1), followed by semi-prep. HPLC (MeOH/H_2O 35:65 for **14** or 40:60 for **13**). *Fr. 3J* (1.05 g) was subjected to CC (*Sephadex LH-20*; MeOH/H_2O 4:1) and semi-prep.

HPLC (MeOH/H_2O 35:65): **15** (10.9 mg), **16** (19.5 mg), and **17** (3.0 mg). *Fr. 3K* (730 mg) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 72:9:5) and purified by semi-prep. HPLC (MeOH/H_2O 25:75 or 32:68): **18** (10.1 mg), **19** (13.9 mg), and **20** (2.0 mg). *Fr. 3M* (1.13 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 36:9:5, 27:9:5, 18:9:5; and *Sephadex LH-20*, MeOH/H_2O 4:1) and purified by semi-prep. HPLC (MeCN/H_2O with 0.1% HCOOH 20:80): **22** (6.0 mg), **23** (25.1 mg), **24** (10.1 mg), **25** (6.8 mg), **26** (3.2 mg), and **2** (2.4 mg), as well as **21** (3.5 mg, MeCN/H_2O with 0.1% HCOOH 22:78). *Fr. 2* (60 g) was submitted to CC (SiO_2; AcOEt/MeOH/$NH_3 \cdot H_2O$ 60:2:1, 45:2:1, 30:2:1, 15:2:1): *Frs. 2A–2N. Fr. 2B* (3.062 g) afforded **27** (60.0 mg), **28** (10.0 mg), **29** (17.6 mg), **30** (16.1 mg), **31** (4.7 mg), and **32** (11.9 mg) after purification by semi-prep. HPLC (MeOH/H_2O with 0.1% HCOOH 5:95 or 2:98) and recrystallization from MeOH.

Acid Hydrolysis and Identification of the Sugar Moieties of Compounds **1** *and* **2**, *and Determination of Their Absolute Configurations.* The absolute configurations of the sugar moieties were determined according to the method described in [42]. Each of **1** and **2** (2 mg) was hydrolyzed with 2.0M CF_3COOH (TFA; 2 ml) for 3 h at 110°. The mixture was extracted with AcOEt, and the H_2O layer was concentrated. The residue was dissolved in the anh. pyridine (100 µl), and L-cysteine methyl ester hydrochloride (0.06M, 100 µl) was added. The mixture was heated at 60° for 1 h, and then HMDS/TMCS (hexamethyldisilazane/trimethylchorosilane) 1:1 (150 µl) was added. The mixture was heated at 60° for 30 min, and then centrifuged. Two µl of the supernatant were analyzed by GC on a *HP-5* column (30 m, i.d. 0.320 mm; column temp., 230°, carrier gas, N_2). The derivative of **1** gave a peak at t_R 12.072 (D-glc), and that of **2** gave the peaks at t_R D-xylose (7.249 min) and D-glucose (12.053 min). Standard D-glucose and D-xylose were subjected to the same procedure: t_R 12.156 min (D-glc) and 7.253 min (D-xyl), resp.

*(6a*R*,12a*R*)-6a,12a-Dihydro-4-methoxy-6*H*-[1,3]dioxolo[5,6]benzofuro[3,2-*c*][1]benzopyran-3-yl β-*D*-Glucopyranoside* (**1**). White powder. $[\alpha]_D^{20} = -12$ ($c = 1.0$, MeOH). UV (MeOH): 309 (8635). CD ($c = 0.1$, MeOH): 213 (−61.26), 306 (+4.72). ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 1*. ESI-MS (pos.): 499.1 ($[M+Na]^+$), 515.1 ($[M+K]^+$). ESI-MS (neg.): 475.0 ($[M-H]^-$). HR-ESI-MS: 499.1211 ($[M+Na]^+$, $C_{23}H_{24}NaO_{11}^+$; calc. 499.1216).

*4-(5-Hydroxy-7-methoxy-4-oxo-4*H*-chromen-3-yl)phenyl 6-O-β-D-Xylopyranosyl-β-D-glucopyranoside* (**2**). White powder. $[\alpha]_D^{20} = -126$ ($c = 1.0$, MeOH). UV (MeOH): 261 (34541). ^{1}H- and ^{13}C-NMR ((D_6)DMSO): *Table 2*. ESI-MS (pos.): 579.0 ($[M+H]^+$), 601.0 ($[M+Na]^+$). ESI-MS (neg.): 577.0 ($[M-H]^-$). HR-ESI-MS: 579.1708 ($[M+H]^+$, $C_{27}H_{31}O_{14}^+$; calc. 579.1714).

Sample Preparation for HPLC Analyses. The powdered sample (1.0 g) was extracted with MeOH (50 ml) by ultrasonication at r.t. The extract was concentrated, and the residue was dissolved with MeOH (10 ml). The extract was filtered through a 0.22-mm micropore membrane (*Jinteng Corp.*, Tianjin, P. R. China) and used for HPLC analyses.

HPLC Analysis of the Root and Aerial Part of S. flavescens. The analyses were performed at 35° by HPLC on a *Phenomenex Luna* C_{18} column (4.6 × 250 mm, 5 µm). The mobile phase consisted of MeOH (*A*) and H_2O (*B*). A linear gradient of 5–100% *A* in 100 min was used. The flow rate was 1.0 ml/min. Detection was at 254 nm. The samples of root and aerial part, together with the isolated compounds, **1**–**32**, and reference substances, **I**–**IX**, from our compound collection, were analyzed under the same chromatographic conditions. Peaks were identified by comparison of the t_R values and on-line UV spectra.

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