A New Flavonoid from *Selaginella tamariscina* (Beauv.) Spring

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A new flavonoid, 6-(2-hydroxy-5-carboxyphenyl)-apigenin (1), together with two new natural products, 3-(4-hydroxyphenyl)-6,7-dihydroxy coumarin (2), 1-methoxy-3-methylanthraquinone (3) and four known compounds, were isolated from *Selaginella tamariscina* (Beauv.) Spring. The structures of the new isolated compounds were elucidated on the basis of 1D and 2D NMR as well as ESI-HR-MS spectroscopic analysis.

Key words *Selaginella tamariscina*; Selaginellaceae; flavonoid; coumarin; anthraquinone

Selaginella tamariscina (Beauv.) Spring, one species from genus *Selaginella*, has been introduced in Chinese Pharmacopeia for the effectiveness of promoting blood circulation. Studies on the chemical constituents of *Selaginella* led to the discovery of many compounds including biflavonoids, phenylpropanoids, anthraquinones, steroids, alkaloids and organic acids. Recently, some unusual natural pigments with novel carbon framework named selaginellin, selaginellin A and selaginellin B were isolated from *S. sinensis*[1] and *S. tamariscina*.[2] Previously, we have isolated selaginellin C, the fourth novel unusual natural pigments from *S. pulvinata* Maxim. (Hook *et* Grev.)[3] and a new flavonoid from *S. tamariscina*.[4] As a continuation of our work, another new flavonoid, 6-(2-hydroxy-5-carboxyphenyl)-apigenin (**1**) was isolated from the 75% (v/v) ethanol extract of *S. tamariscina* together with two new natural products, 3-(4-hydroxyphenyl)-6,7-dihydroxy coumarin (**2**),[5] 1-methoxy-3-methylanthraquinone[6] (**3**) and four known compounds heveaflavone (**4**), amentoflavone (**5**), heptadecanoic acid (**6**) and β-sitosterol (**7**).

Results and Discussion

Compound **1** was obtained as amorphous yellow powder. The molecular formula, $C_{22}H_{14}O_8$, was determined on the basis of positive electron spray ionization mass spectrometry (ESI-MS) (*m*/*z* 407.0 $[M+H]^+$) and HR-ESI-MS data [*m*/*z*: 407.07851 $[M+H]^+$ (Calcd for $C_{22}H_{15}O_8$, 407.07669)]. Its UV spectrum showed the maximum absorption at 339, 253 and 223 nm. IR spectrum showed the presence of hydroxyl (3432.0 cm^{-1}), conjugated carbonyl (1654.4 cm^{-1}), and aromatic ring (1607.4, 1579.0, 1510.1 cm^{-1}). Examination of the ^{1}H- and ^{13}C-NMR of compound **1** and comparison to the corresponding signals in 6-(2-hydroxy-5-acetylphenyl)-apigenin reported previously[4] indicated that compound **1** had similar structure with 6-(2-hydroxy-5-acetylphenyl)-apigenin. As evident from the ^{1}H-NMR spectrum, five hydroxyl groups were exhibited at δ 13.09 (1H, s, 5-OH), δ 12.49 (1H, br s, 5″-COOH), δ 10.74 (1H, br s), δ 10.32 (1H, s) and δ 10.21 (1H, br s). An AA′XX′ coupling system signals at δ 7.55 (2H, d, *J*=8.8 Hz, H-2′, 6′) and δ 6.76 (2H, d, *J*=8.8 Hz, H-3′, 5′) indicated the *para*-substitution of ring B. In heteronuclear multiple bond coherence (HMBC) spectrum, the correlation peaks between δ 6.80 (H-3) and δ 163.9 (C-2), δ 182.4 (C-4), δ 121.4 (C-1′) suggested that C-3 was not be substituted (Fig. 2). Due to the ^{1}H–^{1}H correlation spectroscopy (COSY) and HMBC spectrum, three aromatic protons δ 7.87 (1H, dd, *J*=8.4, 2.0 Hz, H-4″), δ 7.79 (1H, d, *J*=2.0 Hz, H-6″) and δ 7.06 (1H, d, *J*=8.4 Hz, H-3″) should belong to ring D. The HMBC correlations between δ 167.5 (5″-COOH) and δ 7.87 (H-4″), δ 7.79 (H-6″) indicated that the carboxy group was located at C-5″. The HMBC correlations between δ 160.2 (C-2″) and δ 7.87 (H-4″), δ 7.79 (H-6″) indicated a hydroxyl group was located at C-2″. HMBC correlations between δ 103.9 (C-6) and δ 7.79 (H-6″) concluded the linkage of the carboxyphenyl was located at the C-6 position of ring A. On the basis of above evidences, compound **1** was determined to be 3-(2-(4-hydroxyphenyl)-5,7-dihydroxy-chromen-6-yl)-4-hydroxy-benzoic acid, named 6-(2-hydroxy-5-carboxyphenyl)-apigenin.

Compound **2** was also obtained as an amorphous yellow powder. The molecular formula, $C_{15}H_{10}O_5$, was determined

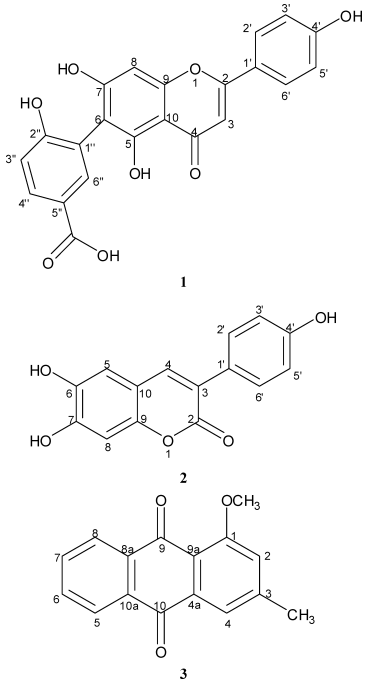

Fig. 1. Structure of Compounds **1**—**3**

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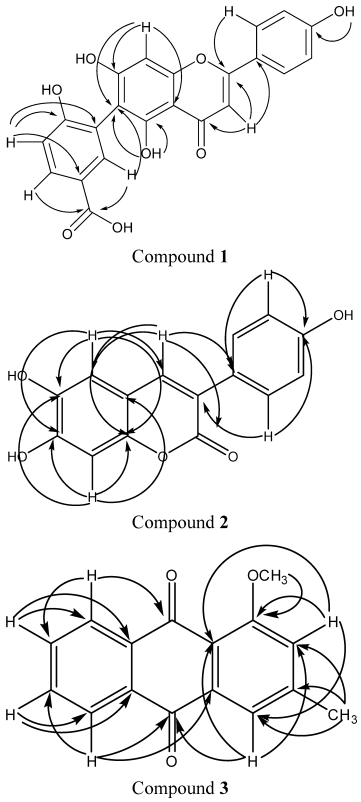

Fig. 2. Key HMBC Correlations of **1**—**3**

on the basis of positive ESI-MS (*m/z*: 271.3 $[M+H]^+$) and HR-ESI-MS data [*m/z*: 271.0596 $[M+H]^+$ (Calcd for $C_{15}H_{11}O_5$, 271.0606)]. Its UV spectrum showed the maximum absorption at 274 and 220 nm. Its IR spectrum showed typical absorptions of hydroxyl (3433.8 cm^{-1}), carbonyl (1716.5 cm^{-1}), and aromatic ring (1584.9, 1509.3 cm^{-1}). The structure of **2** was established from analysis of the ^1H- and ^{13}C-NMR spectra, as shown in Table 1. ^{13}C-NMR displayed 15 carbon signals, nine being typical for an esculetin (6,7-dihydroxy-coumarin),[7] the other 6 signals were ascribable to a *para*-substitution of phenyl moiety, which was verified by an AA′XX′ coupling system signals at δ 7.53 (2H, d, J=8.4 Hz, H-2′, 6′) and δ 6.80 (2H, d, J=8.4 Hz, H-3′, 5′) exhibited in ^1H-NMR. The signal at δ 157.5 showed that there was a hydroxyl located at C-4′. The HMBC correlations between δ 121.8 (C-3) and δ 7.53 (H-2′, 6′), δ 126.4 (C-1′) and δ 7.93 (H-4) (Fig. 2) concluded the linkage of the *p*-hydroxyphenyl was located at the C-3 position of esculetin.

Compound **3** was obtained as an yellow crystal(CH_3Cl_3/MeOH), mp 183.7—185.3 °C, The molecular formula, $C_{16}H_{12}O_3$, was determined on the basis of positive EI-MS (*m/z*: 252 $[M]^+$), ESI-MS (*m/z*: 253.2 $[M+H]^+$) and HR-ESI-MS data [*m/z*: 253.0856 $[M+H]^+$ (Calcd for $C_{16}H_{13}O_3$, 253.0865)]. Its UV spectrum showed the maximum absorption at 386, 256 and 211 nm. Its IR spectrum showed the presence of carbonyl (1736.5 cm^{-1}) functionality. The structure of **3** was established from analysis of the ^1H- and ^{13}C-NMR spectra, as shown in Table 2. The extensive NMR spectral analyses indicated an 1-methoxy-9,10-anthraquinone skeleton.[8] Besides, the location of a methyl (δ 56.5) was identified by the correlations between δ 2.52 (–CH_3) and δ 118.5 (C-2), δ 2.52 (–CH_3) and δ 120.6 (C-4) in HMBC experiments (Fig. 2). The assignment of NMR data to compound **3** was further comfirmed by means of heteronuclear single quantum coherence (HSQC) and HMBC experiments.

Table 1. NMR Data for Compounds **1** and **2** in DMSO-d_6

Position	**1**		**2**	
	δ_C [a]	δ_H [b] (J in Hz)	δ_C [a]	δ_H [b] (J in Hz)
2	163.9	—	161.0	—
3	102.8	6.80 s	121.8	—
4	182.4	—	139.6	7.93 s
5	161.3	—	111.7	6.99 s
6	103.9	—	143.8	—
7	162.0	—	151.9	—
8	98.9	6.39 s	102.2	6.69 s
9	154.7	—	148.4	—
10	104.3	—	111.2	—
1′	121.4	—	126.4	—
2′	128.5	7.55 d (8.8)	129.7	7.53 d (8.4)
3′	116.1	6.76 d (8.8)	115.2	6.80 d (8.4)
4′	160.7	—	157.5	—
5′	116.1	6.76 d (8.8)	115.2	6.80 d (8.4)
6′	128.5	7.55 d (8.8)	129.7	7.53 d (8.4)
1″	121.6	—	—	—
2″	160.2	—	—	—
3″	115.6	7.06 d (8.4)	—	—
4″	131.0	7.87 dd (8.4, 2.0)	—	—
5″	119.2	—	—	—
6″	134.9	7.79 d (2.0)	—	—
5-OH	—	13.09 br s	—	—
5″-COOH	167.5	12.49 br s	—	—

[a] Recorded at 100 MHz. [b] Recorded at 400 MHz.

Table 2. NMR Data for Compound **3** in $CDCl_3$

Position	**3**	
	δ_C [a]	δ_H [b] (J in Hz)
1	160.6	—
2	118.5	7.15 br s
3	146.5	—
4	120.6	7.79 br s
4a	135.5	—
5	126.5	8.22 dd (7.5, 1.0)
6	134.2	7.77 dt (7.5, 1.0)
7	133.1	7.73 dt (7.5, 1.0)
8	127.2	8.28 dd (7.5, 1.0)
8a	132.5	—
9	182.3	—
9a	119.3	—
10	183.8	—
10a	135.1	—
1-OCH_3	56.5	4.05 s
3-CH_3	22.4	2.52 s

a) Recorded at 125 MHz. *b*) Recorded at 500 MHz.

Experimental

General Experimental Procedures Melting points were measured on Buchi-540 melting point apparatus (uncorrected); UV spectra were performed on a Shimadzu UV-2450 instrument (Japan); IR spectra were obtained on a Nicolet Avatar (U.S.A.) 360 FT-IR instrument as a film on KBr disk; NMR spectra were recorded on a Varian (U.S.A.) INOVA-400 spectrometers and Bruker AV-500 spectrometer with tetramethylsilane (TMS) as internal standard; MS spectra were measured on an LCQ-Advantage (U.S.A.) mass spectrometer and Q-Trap LC/MS/MS System Turboinospray source (Applied Biosystems/MDS Sciex U.S.A.); HR-ESI-MS spectra were recorded on a Micromass Zabspec (U.K.) HR-MS spectrometer and JMS-

T100 CS. Column was run using silica gel (200—300 mesh). The prep-HPLC was performed on LC-8A (Shimadzu, pre-ODS, 20×250 mm, 10 μm, UV detecter, MeOH–H_2O).

Plant Material Herbs of *S. tamariscina* were collected in Jiangxi Province, P. R. China, and were identified by Associate Prof. Jin-Ping Li (Central South University, Changsha, P. R. China). A voucher specimen was deposited in School of Pharmaceutical Sciences, Central South University (No. JB-003).

Extraction and Isolation The air-dried sample of *S. tamariscina* (14.0 kg) was soaked twice with cold 75% EtOH. After removal of the solvent under reduced pressure, the extract (1450 g) was chromatographed over D-101 macroporous resin column with EtOH/H_2O (v/v) gradient elution (0, 30, 60, 95%). The 60% EtOH portion was subject to a combination of chromatographies such as silica gel column chromatography, gel permeation chromatography and preparative HPLC to yield compound **1** (10.7 mg), **2** (7.8 mg), **3** (9.7 mg), **4** (107.3 mg), **5** (314.5 mg), **6** (52.6 mg), **7** (232.6 mg).

6-(2-Hydroxy-5-carboxyphenyl)-apigenin (**1**) $C_{22}H_{14}O_8$: Amorphous yellow powder; UV λ_{max} (MeOH): 339, 253, 223 nm; IR bands (KBr): 3432.0, 1654.4, 1607.4, 1579.0, 1510.1 cm^{-1}; ^{1}H- and ^{13}C-NMR see Table 1; ESI-MS *m*/*z*: 407.0 $[M+H]^+$; ESI-HR-MS *m*/*z*: 407.07851 $[M+H]^+$ (Calcd for $C_{22}H_{15}O_8$), 407.07669.

3-(4-Hydroxyphenyl)-6,7-dihydroxycoumarin (**2**) $C_{15}H_{10}O_5$: Yellow amorphous powder; ^{1}H- and ^{13}C-NMR see Table 1; UV λ_{max} (MeOH) nm: 274, 220; IR (KBr) cm^{-1}: 3433.8, 1716.5, 1584.9, 1509.3; EI-MS *m*/*z*: 288.3 $[M+H_2O]^+$, ESI-MS (*m*/*z*: 271.3 $[M+H]^+$) and HR-ESI-MS data [*m*/*z*: 271.0596 $[M+H]^+$ (Calcd for $C_{15}H_{11}O_5$, 271.0606)].

1-Methoxy-3-methylanthraquinone (**3**) $C_{16}H_{12}O_3$: Yellow amorphous powder; mp 183.7—185.3 °C; EI-MS *m*/*z*: 252 $[M]^+$, 237, 209, 206, 178; ESI-MS (*m*/*z*: 253.2 $[M+H]^+$) and HR-ESI-MS data [*m*/*z*: 253.0856 $[M+H]^+$ (Calcd for $C_{16}H_{13}O_3$, 253.0865)]; ^{1}H- and ^{13}C-NMR see Table 2; IR (KBr) cm^{-1}: 2953.4, 2849.5, 1736.6, 1457.3, 1397.7, 1384.9.

Identification of compounds **4**, **5** were performed by comparing NMR data with those reported in the literature.[9,10] Identification of compounds **6**, **7** were supported by TLC comparison with authentic compounds.

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