Selaginellin A and B, Two Novel Natural Pigments Isolated from *Selaginella tamariscina*

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Two new unusual natural pigments were first isolated from the whole herbs of *Selaginella tamariscina*. The structure of selaginellin A (1) was established as (*R*,*S*)-4-[(4′-hydroxy-3-((4-hydroxyphenyl)ethynyl)biphenyl-2-yl)(4-hydroxyphenyl)methylene]-2,5-cyclohexadien-1-one and selaginellin B (2) as (*R*,*S*)-4-[(4′-methoxy-4-(methyl)-3-((4-methoxyphenyl)ethynyl)biphenyl-2-yl)(4-methoxyphenyl)methylene]-2,5-cyclohexadien-1-one, along with four known biflavonoids, amentoflavone (3), hinokiflavone (4), heveaflavone (5), and 7″-*O*-methylamentoflavone (6). Their chemical structures were elucidated by spectral analysis of electrospray ionization mass spectroscopy (ESI-MS), one-dimensional nuclear magnetic resonance spectroscopy (1D-NMR) and two-dimensional-nuclear magnetic resonance spectroscopy (2D-NMR) including ^{1}H-NMR, ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple bond coherence (HMBC), and single-crystal X-ray diffraction techniques.

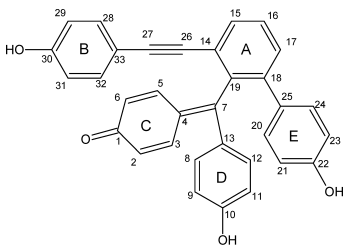
Key words *Selaginella tamariscina*; selaginellin A; selaginellin B

Selaginella tamariscina (BEAUV.) SPRING is a Chinese herbal medicine, that is widely distributed in China. There is a long history of using the whole herbs of *S. tamariscina* in traditional Chinese medicines, and there has been much pharmacology research on *Selaginella* species.[1—3] Previous phytochemical studies on the constituents of genus *Selaginella* led to the discovery of many compounds, including biflavonoids,[4—8] lignan,[8] lignanoside,[9] alkaloids,[10,11] *etc.* A natural pigment named selaginellin was isolated from *Selaginella sinensis* recently.[12] In the course of our studies on *S. tamariscina*, we examined the constituents of the ethanol extract of whole herbs of this species. We have isolated and elucidated two new natural pigments, selaginellin A (**1**) and selaginellin B (**2**), along with four known biflavonoids, amentoflavone (**3**), hinokiflavone (**4**), heveaflavone (**5**), and 7″-*O*-methylamentoflavone (**6**). The structures of these compounds were assayed by extensive spectral analysis including 2D-NMR and HR-MS spectra. We report herein the isolation and structure elucidation of two new unusual natural pigments, selaginellin A (**1**) and seleginellin B (**2**).

Results and Discussion

The EtOH extract of the whole herbs of *S. tamariscina* was concentrated and partitioned with chloroform and ethyl acetate. The ethyl acetate fraction was separated by a combination of chromatographies such as silica gel column chromatography, Sephadex LH-20 column chromatography, preparative thin-layer chromatography, and preparative high-performance liquid chromatography to yield two new compounds: selaginellin A (**1**) and selaginellin B (**2**), and four known biflavonoids. They were identified as amentoflavone (**3**),[13] hinokiflavone (**4**),[13] heveaflavone (**5**),[8] and 7″-*O*-methyl amentoflavone (**6**)[14] based on comparison of their physical and spectral data with literature values. The structures of compounds **1**—**6** are shown in Fig. 1.

Selaginellin A (**1**) was obtained as red needle crystal. The molecular formula of **1** was determined to be $C_{33}H_{23}O_4$, by HR-ESI-MS at *m*/*z* 483.15957, with the degree of unsaturation being 23. The melting point was 189—190 °C. UV λ_{max}^{MeOH} nm (log ε): 297 (3.15), 430 (3.27). IR ν^{KBr}, cm^{-1}: 1595, 1511, 1456, indicated the presence of a benzene ring. The ^{1}H-NMR (Table 1) spectrum showed that all hydrogen is aromatic protons. Three proton spin systems, including four *para*-benzene moieties [H-29, 31 (δ_H 6.56, 2H, d, J=8.5 Hz) and H-28, 32 (δ_H 6.91, 2H, d, J=8.5 Hz), H-8, 12 (δ_H 7.08, 2H, d, J=9.0 Hz) and H-9, 11 (δ_H 6.47, 2H, d, J=9.0 Hz), H-20, 24 (δ_H 6.72, 2H, d, J=8.5 Hz) and H-21, 23 (δ_H 6.48, 2H, d, J=8.5 Hz)], were identified by ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), and one hemi-benzene moiety



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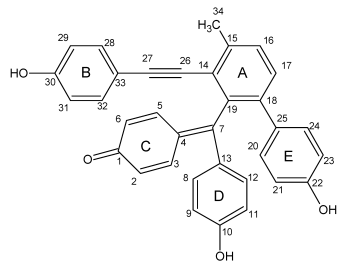

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Fig. 1. Structures of Isolated Compounds **1** and **2**

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Table 1. ^{1}H-, ^{13}C-NMR Data of Compound **1** at 500 MHz (for ^{1}H-NMR) and 125 MHz (for ^{13}C-NMR) and HMBC Data

	^{13}C-NMR	^{1}H-NMR	HMBC (H→C)
1	165.6		
2, 6	122.3	6.47 (2H, d, J=9.0 Hz)	C4
3, 5	138.9	7.08 (2H, d, J=9.0 Hz)	C1
4	131.4		
7	133.0		
8, 12	138.9	7.08 (2H, d, J=9.0 Hz)	C10
9, 11	122.3	6.47 (2H, d, J=9.0 Hz)	C13
10	165.6		
13	131.4		
14	126.4		
15	131.4	7.55 (1H, d, J=8.0 Hz)	C19, C17, C26
16	130.6	7.45 (1H, m)	C14, C18
17	131.0	7.26 (1H, d, J=8.0 Hz)	C19, C15
18	145.0		
19	142.3		
20, 24	131.0	6.72 (2H, d, J=8.5 Hz)	C22, C18, C17
21, 23	115.7	6.48 (2H, d, J=8.5 Hz)	C25, C22
22	158.0		
25	115.7		
26	87.2		
27	95.2		
28, 32	134.1	6.91 (2H, d, J=8.5 Hz)	C27, C30
29, 31	116.4	6.56 (2H, d, J=8.5 Hz)	C33, C30
30	159.4		
33	114.6		

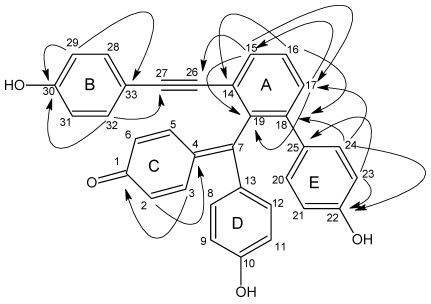

Fig. 2. Selected HMBC Correlation for Compound **1**

due to H-17 (δ_H 7.55) and H-16 (δ 7.45), H-15 (δ 7.26) were the consecutive protons in the same benzene ring. The chemical shifts and the coupling constants of these proton spin systems showed that **1** contained five benzene rings. The ^{13}C-NMR spectrum (Table 1) contained 19 signals, that could be classified using the DEPT data as nine methylenes and ten quaternary carbons, and revealed the presence of a symmetrical unit in the structure. δ_C 95.2 and δ_C 87.2 showed the presence of an acetylene bond. The total degree of unsaturation of five benzene rings and one acetylene was 22, which did not agree with the degree of unsaturation being 23, so there must be one double bond in the structure of compound **1**.

The proton–carbon connectivity of **1** was determined by HMQC experiments. The connectivity of the five benzene rings and one acetylene bond of compound **1** was determined by the heteronuclear multiple-bond correlation spectroscopy (HMBC) (Fig. 2). In the HMBC spectrum of compound **1**, correlation peaks were observed between H-28, 32 (δ_H 6.91) and C-27 (δ_C 95.2), C-30 (δ_C 159.4), also between H-15 (δ_H 7.55) and C-19 (δ_C 142.3), C-17 (δ_C 131.0), C-26 (δ_C 87.2),

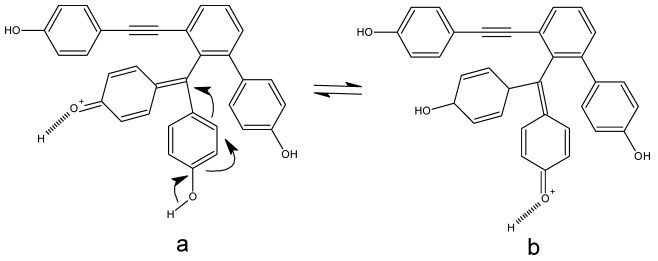

Fig. 3. The Tautomerism of Compound **1**

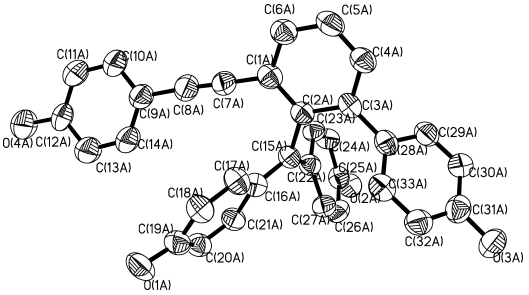

Fig. 4. Stereoview of Compound **1** from X-Ray Crystallographic Analysis

H-20, 24 (δ_H 6.72) and C-18 (δ_C 145.0), C-17 (δ_C 131.0), C-22 (δ_C 158.0). Moreover, H-17 (δ_H 7.26) and C-19 (δ_C 142.3), C-15 (δ_C 131.4), H-16 (δ_H 7.45) and C-14 (δ_C 126.4), C-18 (δ_C 145.0), H-29, 31 (δ_H 6.56) and C-33 (δ_C 114.6), C-30 (δ_C 159.4). Thus the structure of compound **1** was established to be selaginellin A, (*R*,*S*)-4-[(4′-hydroxy-3-((4-hydroxyphenyl)ethynyl)biphenyl-2-yl)(4-hydroxyphenyl)methylene]-2,5-cyclohexadien-1-one.

The chemical shift of ^{1}H-NMR and ^{13}C-NMR of C benzene ring and D benzene ring of **1** are similar which does not agree with the structure of selaginellin A, and the ^{1}H-NMR and ^{13}C-NMR of selaginellin A in CD_3OD is quite different from those of selaginellin in CD_3COCD_3 as previously published.[12] This observation may have been caused by the tautomerism of a and b with the contribution of active hydrogen (Fig. 3).[12]

The relative stereochemistry of compound **1** was deduced from X-ray crystallography. A view of the solid state conformation is provided in Fig. 4. Bond distances and bond angles are provided in Table 2.

Selaginellin B (**2**) was obtained as red needle crystal. The molecular formula of **2** was determined to be $C_{34}H_{25}O_4$, by HR-ESI-MS at *m/z* 497.17419, with the degree of unsaturation being 23. The melting point was 190—191 °C; UV λ_{max}^{MeOH} nm (log ε): 300 (3.18), 431 (3.21). IR ν^{KBr}, cm^{-1}: 1597, 1512, 1488, 1449, indicated the presence of a benzene ring. The IR spectrum of compound **2** agreed with that of compound **1**. The ^{1}H- and ^{13}C-NMR (Table 2) of compound **2** were closely similar to those of compound **1** except for the appearance of a methyl group proton [δ_H 2.54 (3H, s)] attached to the carbon (δ_C 21.2) in compound **2**. The HMBC spectrum revealed correlation peaks between H-33 of 15-CH_3 (δ_H 3.25) and C-26 (δ_C 86.0), C-19 (δ_C 139.9), C-14 (δ_C 125.8), C-16 (δ_C 130.6), which confirmed the substitution of methyl at C-15 position (Fig. 5). Thus, the structure of compound **2** was established to be selaginellin B, (*R*,*S*)-4-[(4′-methoxy-4-(methyl)-3-((4-methoxyphenyl)ethynyl)-

Table 2. ^{1}H-, ^{13}C-NMR Data of Compound **2** at 500 MHz (for ^{1}H-NMR) and 125 MHz (for ^{13}C-NMR) and HMBC Data

	^{13}C-NMR	^{1}H-NMR	HMBC (H→C)
1	166.5		
2, 6	122.2	6.46 (2H, d, J=9.0 Hz)	C4
3, 5	139.0	7.09 (2H, d, J=9.0 Hz)	C1
4	131.6		
7	133.1		
8, 12	139.0	7.09 (2H, d, J=9.0 Hz)	C10
9, 11	122.2	6.46 (2H, d, J=9.0 Hz)	C13
10	166.5		
13	131.6		
14	125.8		
15	131.3		
16	130.6	7.37 (1H, d, J=8.0 Hz)	C14, C18
17	131.6	7.15 (1H, d, J=8.0 Hz)	C19, C18, C15
18	142.2		
19	139.9		
20, 24	131.3	6.71 (2H, d, J=8.5 Hz)	C22, C18, C17
21, 23	115.7	6.48 (2H, d, J=8.5 Hz)	C25, C22
22	157.8		
25	115.7		
26	86.0		
27	100.0		
28, 32	133.9	6.91 (2H, d, J=8.5 Hz)	C27, C30
29, 31	116.4	6.53 (2H, d, J=8.5 Hz)	C33, C30
30	159.3		
33	114.8		
34 (15-CH_3)	21.2	2.54 (3H, s)	C26, C19, C14, C16

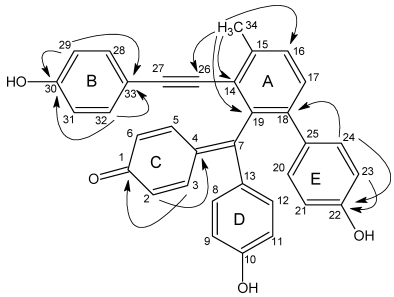

Fig. 5. Selected HMBC Correlation for Compound **2**

biphenyl-2-yl)(4-methoxyphenyl)methylene]-2,5-cyclohexadien-1-one.

Experimental

General Experimental Procedures All melting points were determined by Kofle instrument and were uncorrected. UV spectra were recorded by Agilent 8453 spectrophotometer. IR absorption spectra were obtained with Nicolet Impact 400 FT-IR instrument as a film on KBr disk. ^{1}H and ^{13}C spectra were obtained with INOVA-500 instrument. Chemical shifts were reported in parts per million on the δ scale with TMS as internal standard, and coupling constants were in Hertz. Single-crystal X-diffraction analysis was measured on a MAC DIP-2030K diffractometer; HR-ESI-MS was recorded on JMS-T100CS system. Column chromatography was performed with silica gel and Sephadex LH-20. TLC was performed on precoated Silica plates with $CHCl_3$–MeOH system and spots were detected by vis illumination. Waters Prep LC 4000 System was used for preparing compound **1** with 2487 detector.

Plant Material The whole herbs of *S. tamariscina* were collected in Beijing, P. R. China, in October 2005. A voucher specimen (ST-01-2005) was deposited at the Museum for Materia Medica, National Institute for the Control of Pharmaceutical and Biological Products, Beijing.

Extraction and Isolation Whole herbs of *S. tamariscina* (15 kg) were ground to a coarse powder and extracted with 95% ethanol (10 l×3). The ethanol extract was evaporated *in vacuo* to yield a dark residue (352 g). The ethanol extract was re-extracted with chloroform and ethyl acetate. The ethyl acetate extract (43 g) was chromatographed on silica gel column (10×70 cm) with chloroform–methanol gradient system to yield 4 fractions. Frs. 2 and 3 were chromatographed by Sephadex LH-20 and PTLC to afford amentoflavone (**3**, 100 mg), hinokiflavone (**4**, 21 mg), heveaflavone (**5**, 14 mg), and 7′-*O*-methylamentoflavone (**6**, 19 mg). Fr. 4 was chromatographed by Sephadex LH-20 and prepared HPLC to yield selaginellin A (**1**, 25 mg) and selaginellin B (**2**, 18 mg).

Selaginellin A (**1**): Red needle crystal, mp 191—192 °C. UV λ_{max}^{MeOH} nm (log ε): 297 (3.15), 430 (3.27); IR ν^{KBr}, cm^{-1}: 3150, 2799, 2669, 1595, 1511, 1456, 1377, 1336, 1241, 1162, 902, 836; HR-ESI-MS *m*/*z*: 483.15957 (Calcd for $C_{33}H_{23}O_4$: 483.15960); ^{1}H- and ^{13}C-NMR data of **1** are shown in Table 1. Crystal size: 0.40×1.00×3.00 mm; crystal system: triclinic system; space group: P-1; unit cell parameters: a=10.188 (1), b=17.987 (4), c=20.614 (4) Å, V=3381.7 (3) Å^3, Z=2, D_{calcd}=1.087 g/cm^3. The diffraction data were collected by MAC DIP-2030K Imaging Plate diffractometer with Mo$K\alpha$ Radiation, $2\theta_{max}$ 50.0°, 7747 independent reflections, observed 6361 were used for refinement. The structure was solved by direct method and refinement by the full matric least square methods, the final Rf=0.0748, Rw_w=0.1968(w=1/$\sigma|F|^2$). The Cambridge Crystallographic Data Centre (CCDC) deposition number is 679453.

Selaginellin B (**2**): Red needle crystal, mp 190—191 °C. UV λ_{max}^{MeOH} nm (log ε): 300 (3.18), 431 (3.21); IR ν^{KBr}, cm^{-1}: 3133, 2800, 2669, 1597, 1488, 1448, 1377, 1336, 1264, 1164, 911, 834; HR-ESI-MS *m*/*z*: 497.17419 (Calcd for $C_{34}H_{25}O_4$: 497.17528); ^{1}H- and ^{13}C-NMR data of **2** are shown in Table 2.

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