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A New Unusual Natural Pigment from *Selaginella sinensis* and Its Noticeable Physicochemical Properties

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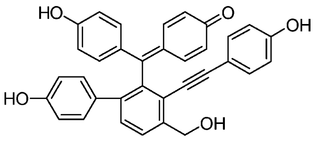

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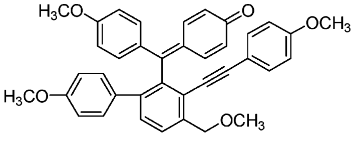

1a

A new unusual pigment with a novel carbon framework named selaginellin (**1**) was isolated from the acetone extract of *Selaginella sinensis*, and its methoxy derivative (**1a**) was synthesized. Both selaginellin **1** and **1a** are racemic compounds. The structure of selaginellin **1** was established as (*R*,*S*)-4-[(4′-hydroxy-4-(hydroxymethyl)-3-((4-hydroxyphenyl)ethynyl)biphenyl-2-yl)(4-hydroxyphenyl)methylene]-2,5-cyclohexadien-1-one and **1a** as (*R*,*S*)-4-[(4′-methoxy-4-(methoxymethyl)-3-((4-methoxyphenyl)ethynyl)biphenyl-2-yl)(4-methoxyphenyl)methylene]-2,5-cyclohexadien-1-one by the analysis of one- and two-dimensional NMR data, HR-ESIMS, EI-MS, IR, UV, CD, and single-crystal X-ray experiments, and the mechanism of their color change according to different pH values and fluorescent properties was studied.

Many *Selaginella* species have been used in traditional medicine to treat a variety of diseases in some countries[1−4] and have attracted considerable attention. *Selaginella sinensis*, rich in active biflavonoids,[5−8] was used extensively in traditional Chinese medicine.[2,3,5] From the whole of *Selaginella sinensis*, several known biflavones, such as amentoflavone and hinokiflavone,[5,8] were also isolated by us. At the same time, a new unusual pigment was isolated for which we proposed the name selaginellin (**1**, Figure 1), and its methoxy derivative (**1a**, Figure 1) was synthesized, as well. Both selaginellin **1** and **1a** are racemic compounds, and their structures are established by the analysis of ^{1}H NMR, ^{13}C NMR, and two-dimensional NMR, including HHCOSY, NOESY, HMQC, and HMBC, HR-ESIMS, EI-MS, IR, UV, CD, and single-crystal X-ray experiments. The mechanism of their color change according to different pH values and fluorescent properties was studied, as well.

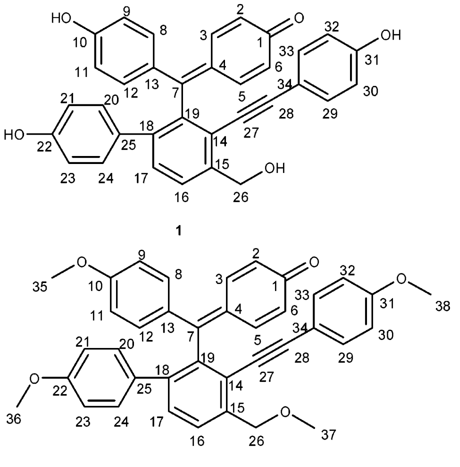

FIGURE 1. Structures of compounds **1** and **1a**.

The air-dried whole plant of *Selaginella sinensis* (5.5 kg), collected in the Guangxi Autonomous Region of China in August 1999, was powdered and extracted with acetone three times at room temperature, each extraction lasting 5 days. The acetone solution was filtered and evaporated under reduced pressure to give an acetone extract (28 g) which was subjected to column chromatography on silica gel eluting with a gradient of petroleum/acetone (from 50:1 to 1:1) to give eight fractions (monitored by TLC analysis) from which the seventh fraction, petroleum/acetone (2:1), yielded pure selaginellin **1** (108 mg).

Selaginellin **1** is a red oil. The HR-ESIMS gives a pseudomolecular ion at *m*/*z* 513.1698 $[M + H]^+$ (calcd *m*/*z* 513.1697) corresponding to a molecular formula of $C_{34}H_{24}O_5$. The IR spectrum shows the absorption at 3374 cm^{-1} (−OH), 2201 cm^{-1} (C≡C), and 1593 cm^{-1} (benzene). UV (H_2O) λ_{max}: 296 nm (ϵ = 10 800), 447 nm (ϵ = 5300), 573 nm (ϵ = 4400). The CD spectrum of compound **1** shows that it has no Cotton effect. ^{1}H and ^{13}C NMR data are shown in Table 1, but it is difficult to determine the structure of **1** from these spectra data and consequently its methoxy derivative **1a** was synthesized. Compound **1a** is a yellow lumpy crystalline material (mp 168−169 °C), its molecular formula, $C_{38}H_{32}O_5$, was determined by HR-ESI at *m*/*z* 569.2311 $[M + H]^+$ (calcd *m*/*z* 569.2323), and the richly detailed proton and carbon NMR spectra are shown in Table 1. The IR spectrum shows the absorption at 2201 cm^{-1} (C≡C), 1630 cm^{-1} (C═O), and 1630 cm^{-1} (benzene). UV (CH_3OH) λ_{max}: 300 nm (ϵ = 10 200), 419 nm (ϵ = 6075). The CD

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(1) (a) Darias, V.; Bravo, L.; Rabanal, R.; Sanchez Mateo, C.; Gonzalez Luis, R. M.; Hernandez Perez, A. M. *J. Ethnopharmacol.* **1989**, *25*, 77−92. (b) MacFoy, C. A.; Sama, A. M. *J. Ethnopharmacol.* **1983**, *8*, 215−223. (c) Winkelman, M. *J. Ethnopharmacol.* **1986**, *18*, 109−131.

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TABLE 1. **NMR Spectral Data for Compound 1a ($CDCl_3$, 400 MHz) and 1 (CD_3COCD_3, 300 MHz)**

	1		**1a**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	199.1		187.0	
2	116.2	6.72 dd (8.1, 1.8)	113.9	6.76 m
3	137.3	6.82 dd (8.1, 1.8)	138.8	7.10 dd (8.4, 2.0)
4	130.5		131.3	
5	137.3	6.82 dd (8.1, 1.8)	139.8	7.10 dd (8.4, 2.0)
6	116.2	6.72 dd (8.1, 1.8)	113.2	6.76 m
7	154.9		158.7	
8	133.8	7.12 dd (8.1, 1.8)	132.6	6.83 m
9	115.4	6.52 dd (8.1, 1.8)	113.2	6.65 m
10	159.1		158.8	
11	115.4	6.52 dd (8.1, 1.8)	113.1	6.65 m
12	133.8	7.12 dd (8.1, 1.8)	132.7	6.83 m
13	143.2		141.1	
14	122.3		122.5	
15	141.5		141.5	
16	127.9	7.76 d (8.1)	127.8	7.64 d (8.0)
17	132.2	7.35 d (8.1)	129.8	7.31 d (8.0)
18	137.3		138.2	
19	141.9		141.5	
20	130.5	7.12 dd (8.1, 1.8)	131.2	6.79 m
21	115.4	6.52 dd (8.1, 1.8)	114.5	6.65 m
22	157.5		159.8	
23	115.4	6.52 dd (8.1, 1.8)	114.1	6.65 m
24	130.5	7.12 dd (8.1, 1.8)	131.3	6.79 m
25	131.0		132.9	
26	62.7	4.94 s	72.7	4.81 d (4.4)
27	84.4		84.2	
28	99.8		98.6	
29	133.8	7.02 dd (8.1, 1.8)	132.9	7.39 dd (8.4, 2.0)
30	116.2	6.62 dd (8.1, 1.8)	113.9	6.47 dd (8.4, 2.0)
31	161.3		160.5	
32	116.2	6.62 dd (8.1, 1.8)	113.2	6.47 dd (8.4, 2.0)
33	133.8	7.02 dd (8.1, 1.8)	132.7	7.47 dd (8.4, 2.0)
34	113.9		114.5	
35			55.2	3.75 s
36			55.2	3.75 s
37			58.8	3.58 s
38			55.2	3.76 s

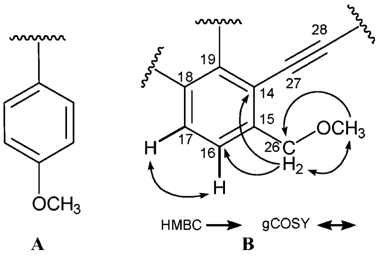

FIGURE 2. Substructure A and B of compound **1a**; HMBC and gCOSY correlations of B.

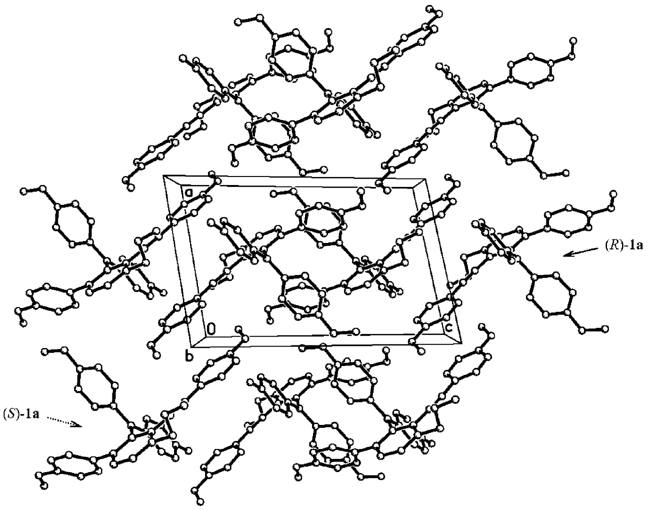

FIGURE 3. The packing diagram of compound **1a**; (*R*)-**1a** and (*S*)-**1a** are indicated by solid and dashed arrows, respectively.

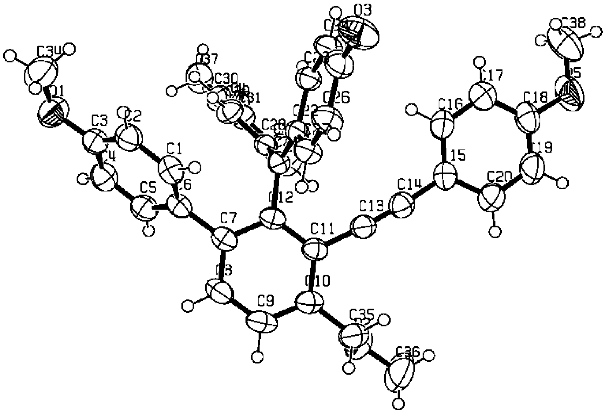

FIGURE 4. X-ray crystal structure of compound (*R*)-**1a**.

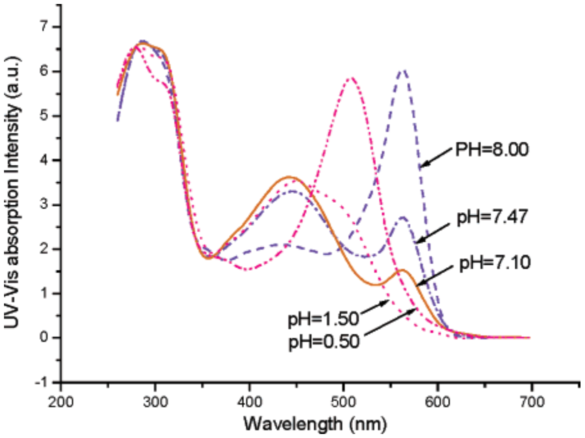

FIGURE 5. UV−vis absorption intensity for compound **1** in water at different pH values.

spectrum of compound **1a** shows that it has no Cotton effect. Both ^1H NMR and ^{13}C NMR spectra of **1a** show that it has four methoxy groups (δ_H 3.75, 3.75, 3.58, 3.76; δ_C 55.2, 55.2, 58.8, 55.2), one of which is connected to CH_2, and the others which are respectively connected to a benzene ring (δ_C 158.8 (C-10), 159.8 (C-22), 160.5 (C-31)) and lead to three substructures A (Figure 2). The other protons (CH) of **1a** amount to 18. Further analysis of the ^{13}C NMR and DEPT of compound **1a** reveals one C═O (δ_C 187.0), one C≡C (δ_C 98.6, 84.2), one CH_2 (δ_C 72.7), C-7 (δ_C 158.7),[9] and two other benzene rings. Extensive analysis of the gCOSY, gHMBC, NOESY, and NOE spectra leads to substructure B of compound **1a** (Figure 2), which was assembled on the basis of gCOSY (H-16/H-17 and H-26/H-37), HMBC (H-26/C-16, H-26/C-14, H-37/C-26, H-33/C-28, and H-29/C-28 have correlation peaks and C-27 has no correlation peaks; Figure 2), and NOE (H-26/H-37 and H-16/H-17). However, the structure of compound **1a** could not be

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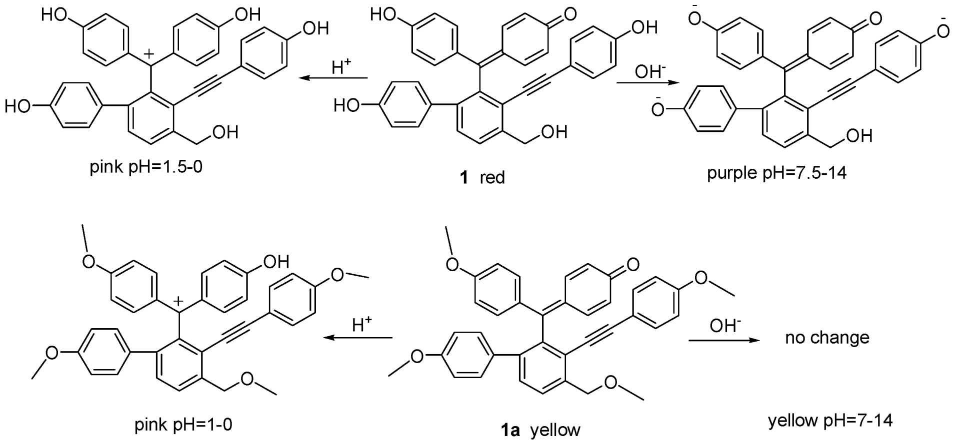
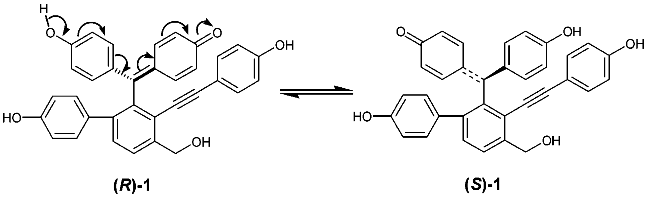
[9] Lycka, A.; Snobl, D.; Koutek, B.; Pavlickova, L.; Soucek, M. *Collect. Czech. Chem. Commun.* **1981**, *46*, 1775−1787.

SCHEME 1. Plausible Mechanisms of Color Change for Compound 1 and 1a with Different pH Values



SCHEME 2. The Tautomerism of Compound 1



established from the spectral data, and so a single crystal was prepared for X-ray crystallographic analysis.

The structure of compound **1a** is positively confirmed by X-ray crystallography. The packing diagram shows that compound **1a** is a racemate, and (*R*)-**1a** and (*S*)-**1a** are indicated in Figure 3, which shows that it has no optical activity. X-ray crystal structure of compound (*R*)-**1a** is shown in Figure 4. The absolute configuration comes from the bond of C7−C19 (Figure 1) which forms a chiral axis for steric hindrance. The structure of **1a** is established as (*R,S*)-4-[(4′-methoxy-4-(methoxymethyl)-3-((4-methoxyphenyl)ethynyl)biphenyl-2-yl)(4-methoxyphenyl)-methylene]-2,5-cyclohexadien-1-one. Compound **1** has no optical activity either, so it is a racemate too and is established as (*R,S*)-4-[(4′-hydroxy-4-(hydroxymethyl)-3-((4-hydroxyphenyl)-ethynyl)biphenyl-2-yl)(4-hydroxyphenyl)methylene]-2,5-cyclohexadien-1-one.

The physical and chemical properties of compound **1** and **1a** are noteworthy, and the color change of compounds **1** and **1a** according to different pH values was studied (Figure 5), and the plausible mechanism is shown in Scheme 1.[10,11] The pH ranges for the color change of compound **1** are 7.5−8.0 (red to purple) and 1.5−1 (red to pink). Noticeably, the former range (7.5−8.0) is not only very narrow but also very close to pH = 7.0 (neutral), which is very significant. Compound **1a** has only one pH range for its color change, which is 1.5−1 (yellow to pink), and so the two compounds, especially compound **1**, may be used not only as a natural pigment but also as an acid−base indicator.

The fluorescent properties of compounds **1** and **1a** were also studied. Compound **1** has no fluorescent property, which is probably due to fluorescence quenching caused by tautomerism of (*R*)-**1** and (*S*)-**1** (Scheme 2).[12] Compound **1a** has strong excellent bluish violet fluorescence (Figure 6), which may be possibly due to its well-conjugated structure and molecular rigidity. The strong bluish violet fluorescent property and chemical thermodynamic stability of compound **1a** make it potentially valuable as a luminescent material.

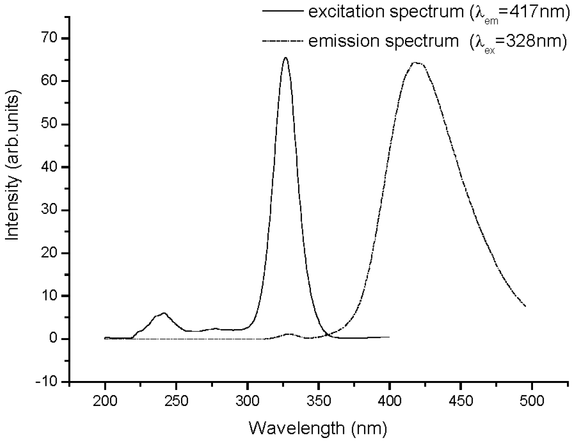


FIGURE 6. Fluorescence spectra for compound **1a** in methanol.

In conclusion, to the best of our knowledge, selaginellin **1** is a new, unusual racemic natural product with a novel carbon framework which has distinctive physical and chemical properties as does its derivative. Both selaginellin **1** and its derivative **1a** may be used as pigments, compound **1** as an acid−base indicator and compound **1a** in luminescent material fields.

Experimental Section

Plant Materials: The herb of *Selaginella sinensis* was collected in the Guangxi Autonomous Region of China in August 1999, and the plant material (200002822) was identified by Prof. Ru-neng Zhao, College of Pharmacy, Lanzhou University. Two voucher specimens are preserved in the herbarium of the College of Pharmacy and our laboratory.

Selaginellin 1: a red oil; $R_f = 0.25$ (petroleum/acetone 1:1); the IR spectrum shows the absorption at 3374 cm^{-1} (−OH), 2201 cm^{-1} (C≡C), 1593 cm^{-1} (C═C); UV (H_2O, λ_{max}) 296 nm (ϵ = 10 800), 447 nm (ϵ = 5300), 573 nm (ϵ = 4400). Its molecular formula, $C_{34}H_{24}O_5$, is determined by HR-ESIMS at *m/z* 513.1698 [M + H]$^+$

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[10] Orndorff, W. R.; Gibbs, R. C.; Mcnulty, S. *J. Am. Chem. Soc.* **1925**, *47*, 2767−2777.

[11] Lees, W. A.; Burawoy, A. *Tetrahedron* **1963**, *19*, 419−438.

[12] Kirste, B.; Harrer, W.; Kurreck, H.; Schubert, K.; Bauer, H.; Gierke, W. *J. Am. Chem. Soc.* **1981**, *103*, 6280−6286.

(calcd m/z 513.1697) and FABMS at m/z 513.4 $[M + H]^+$. The ^1H and ^{13}C NMR spectral data are shown in Table 1.

Compound 1a: a yellow lumpy crystalline material, mp 168−169 °C; $R_f = 0.3$ (petroleum/acetone 3:1); the molecular formula of **1a**, $C_{38}H_{32}O_5$, is determined by HR-ESIMS at m/z 569.2311 $[M + H]^+$ (calcd m/z 569.2323); EI-MS m/z 43, 55, 75, 121, 135, 151, 175, 247, 268, 376, 508, 525, 537, 568; IR (film) ν 2925, 2833, 2201, 1630, 1602, 1508, 1248, 1172 cm^{-1}; UV (CH_3OH, λ_{max}) 300 nm ($\epsilon = 10\,200$), 419 nm ($\epsilon = 6075$). These richly detailed proton and carbon NMR spectra are shown in Table 1.

Synthesis of 1a: The methylation reaction with diazomethane was carried out in the fume cupboard. About 90 mg of selaginellin (**1**) was added to excess diazomethane in anhydrous ether cooled in ice; after the reaction finished, the solvent was evaporated and the product was purified by column chromatography on silica gel and crystallization.

X-ray Crystallography. A single crystal of **1a** was obtained from the solvent (petroleum/CH_2Cl_2, 3:1, solvent evaporation); $C_{38}H_{32}O_5$, $M_r = 568.64$, triclinic, space group $P\bar{1}$, $a = 9.882(2)$ Å, $b = 11.409(2)$ Å, $c = 14.987(3)$ Å, $\alpha = 103.72(1)°$, $\beta = 95.62(2)°$, $\gamma = 108.65(1)°$, $V = 1527.46\ (59)$ Å^3, $Z = 2$, $D_{calcd} = 1.236$ g/cm^3. The final R value is 0.0369 ($R_w = 0.0885$) for 3514 reflections $[I > 2\sigma(I)]$. The packing diagram shows that the crystal of compound **1a** is a racemic conglomerate (R,S)-**1a**, and the richly detailed data are shown in the Supporting Information.

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Supporting Information Available: Isolation and general experimental procedures, HR-ESIMS and CD spectra of compound **1**, one-dimensional ^1H and ^{13}C NMR (DEPT), X-ray crystallographic data, CD, UV, together with HR-ESIMS, HHCOSY, and NOESY of compound **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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