Sinensiols H–J, three new lignan derivatives from *Selaginella sinensis* (Desv.) Spring

Qinfeng Zhu¹, Beibei Gao², Qian Chen¹, Tiantian Luo¹, Guobo Xu¹ and Shanggao Liao*¹

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**Full Research Paper**

**Address:**

¹School of Pharmacy, Guizhou Medical University, No. 2 Dongqing Road, Guiyang, 550025, P. R. China and ²State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, No.132 Lanhei Road, Kunming, 650203, P. R. China

**Email:** Shanggao Liao* - lshangg@163.com

* Corresponding author

**Keywords:**
lignan derivatives; nitric oxide production inhibition; norlignans; *Selaginella sinensis*

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**Abstract**

One new lignan sinensiol H (1) and two new bisnorlignans, sinensiols I and J (2 and 3), along with three known compounds were isolated from the whole plants of *Selaginella sinensis*. Their structures were elucidated on the basis of 1D and 2D NMR spectroscopy as well as high-resolution mass spectrometry. The absolute configuration of 1 was established by ECD calculation. Compounds 2 and 3 represent rare examples of naturally occurring 9,9’-bisnorlignans. All the isolated compounds were assayed for their inhibitory effects on LPS-induced nitric oxide production in RAW 264.7 macrophages.

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**Introduction**

*Selaginella* is the only genus of Selaginellaceae. As a representative of the earliest and still-surviving vascular plant lineage that had arisen about 400 million years ago, it is important for studying the evolution of land plants [1,2]. This genus includes approximately 750 species worldwide, some of which are used in traditional medicines for the treatment of various diseases including diabetes, gastritis, hepatitis, skin diseases and urinary tract infections [3,4]. In fact, *S. tamariscina* and *S. pulvinata* are officially listed in the Chinese Pharmacopoeia for the treatment of amenorrhoea, dysmenorrhoea and traumatic injury [5]. *Selaginella sinensis*, an endemic species in China, is used as a folk medicine for the treatment of cholecystitis, hepatitis, nephritis, eczema and bleeding [6]. Previous phytochemical studies showed the presence of flavonoids, lignans, glucosides and pigments in the plant [7,8] while pharmacological evaluations showed that some of the compounds possessed antioxidant and antiviral activities [9-11]. However, chemical constituents responsible for its efficacy in treating various inflammatory diseases are still not clear. As part of our continuing research on the bioactive compounds from this genus
the chemical constituents of the whole plant of *S. sinensis* were investigated. As a result, three new lignan derivatives 1–3 together with three known lignan glycosides 4–6 (Figure 1) were isolated. Their isolation, structural elucidation and inhibitory effects on LPS-induced nitric oxide production are reported.

**Results and Discussion**

Sinensiol H (1) was isolated as a pale yellow amorphous powder. The negative HRESIMS [M − H]− at *m/z* 371.1133 (calcd for 371.1136) suggested its molecular formula to be C_{20}H_{20}O_{7}, corresponding to 11 degrees of unsaturation. The IR spectrum showed absorption bands characteristic of hydroxy group (3450 cm\(^{-1}\)), carbonyl (1765 cm\(^{-1}\)), and aromatic system (1608, 1516, 1490 cm\(^{-1}\)). Analysis of its \(^1\)H NMR (DMSO-\(d_6\)) data (Table 1) revealed the presence of two ABX benzene rings [\(\delta_H 6.92\) (d, \(J = 1.2\) Hz, 1H, H-2), 6.83 (d, \(J = 7.9\) Hz, 1H, H-5) and 6.79 (dd, \(J = 7.9, 1.2\) Hz, 1H, H-6); 6.59 (d, \(J = 1.5\) Hz, 1H, H-2′), 6.62 (d, \(J = 8.0\) Hz, 1H, H-5′), and 6.47 (dd, \(J = 8.0, 1.5\) Hz, 1H, H-6′)]. The \(^13\)C NMR (Table 1) and HSQC data showed signals due to twelve aromatic carbons, three methyl-enedioxy group (\(\delta_C 100.7\)), one methoxy group (\(\delta_C 55.4\)), and one methine. The chemical shift values of the 1D NMR of 1 were similar to those of the known compound 8′β-hydroxyhinokinin [14], the major difference being the absence of signals for a methylenedioxy (\(\delta_H 5.93, \delta_C 101.2\)) and the presence of signals for a methoxy group (\(\delta_H 3.67, \delta_C 55.4\)) in 1. The HMBC correlations (Figure 2) from 3′-OCH3 (\(\delta_H 3.67, s, 3H\)) to C-3′ indicated the methoxy group was located at C-3′. In the ROESY spectrum, the correlations of 8′-OH/H-2-7 and H-8/H-2-7 (Figure 3a) suggested a trans orientation of H-8 and 8′-OH. The experimental ECD spectrum of 1 (Figure S16 in Supporting Information File 1) showed two positive Cotton effects (CEs) at 204 and 231 nm, which matched well with those in the calculated ECD curve for the (8S,8′R)-stereoisomer (Figure 3b). Consequently, the structure of 1 was determined as shown in Figure 1, and named sinensiol H.

Compound 2 was obtained as a white amorphous powder. Its molecular formula was determined to be C_{20}H_{24}O_{6} by the HRESIMS peak at *m/z* 359.1497 [M − H]− (calcd for 359.1500). The IR spectrum of 2 showed the presence of hydroxy (3417 cm\(^{-1}\)) and aromatic (1593, 1509 cm\(^{-1}\)) groups. The \(^1\)H NMR spectrum recorded in MeOH-\(d_4\) (Table 1) of compound 2 displayed signals for two aromatic protons at \(\delta_H 6.35\) (d, \(J = 1.8\) Hz, H-6) and \(\delta_H 6.34\) (d, \(J = 1.8\) Hz, H-2), one
**Table 1**: $^1$H NMR and $^{13}$C NMR data of compounds 1–3 ($\delta$ in ppm and $J$ in Hz).

| No. | $^1$H | $^1$C | $^2$H | $^2$C | $^3$H | $^3$C |
|-----|-------|-------|-------|-------|-------|-------|
|     | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1   | 6.85 (d, 1.5, 1H) | 132.9 | 6.92 (d, 1.2, 1H) | 133.8 | 6.34 (d, 1.8, 1H) | 138.2 | 140.6 |
| 2   | 109.8 | 140.6 | 109.6 | 154.4 | 105.2 | 149.1 |
| 3   | 148.0 | 154.4 | 147.1 | 135.9 | 154.4 | 149.1 |
| 4   | 146.4 | 135.9 | 145.5 | 148.1 |
| 5   | 108.5 | 148.1 | 108.0 | 148.1 |
| 6   | 108.5 | 148.1 | 108.0 | 148.1 |
| 7   | 6.80 (dd, 7.9, 1.5, 1H) | 122.4 | 6.79 (dd, 7.9, 1.2, 1H) | 122.1 | 6.35 (d, 1.8, 1H) | 110.2 | 120.5 |
| 8   | 2.70 (dd, 8.9, 5.0, 1H) | 50.3 | 2.83–2.78 (m, 1H) | 49.9 | 5.67–5.57 (m, 1H) | 131.6 | 74.9 |
| 9   | 177.4 | 178.0 | 127.1 | 138.2 | 139.8 |
| 10  | 6.48 (d, 1.9, 1H) | 112.2 | 6.59 (d, 1.5, 1H) | 114.0 | 6.34 (d, 1.8, 1H) | 105.2 | 105.1 |
| 11  | 146.9 | 154.4 | 147.1 | 154.3 |
| 12  | 145.3 | 135.9 | 145.1 | 135.8 |
| 13  | 115.0 | 115.1 | 115.1 | 151.2 |
| 14  | 6.53 (d, 8.1, 1H) | 122.7 | 6.86 (d, 8.0, 1H) | 122.4 | 6.35 (d, 1.8, 1H) | 110.2 | 110.1 |
| 15  | 4.31 | 3.23–3.25 (m, 2H) | 41.7 | 3.23–3.25 (m, 2H) | 38.9 | 36.7 |
| 16  | 78.4 | 78.0 | 5.67–5.57 (m, 1H) | 131.6 | 28.7 |
| 17  | 4.18 (d, 10.0, 1H) | 77.0 | 4.14 (d, 9.4, 1H) | 3.81 (d, 9.4, 1H) | 75.5 |
| 18  | 3.91 (s, 3H) | 3.81 (s, 3H) | 56.4 | 56.4 | 61.0 |
| 19  | 3.67 (s, 3H) | 3.76 (s, 3H) | 61.0 | 61.0 |
| 20  | 3.84 (s, 3H) | 3.76 (s, 3H) | 61.0 | 61.0 |
| 21  | 3.94 (s, 2H) | 5.96 (s, 2H) | 100.7 | 5.93 (s, 2H) | 102.2 |
| 22  | 5.38 (s, 1H) | 8.78 (s, 1H) | 74.9 |

$^a$Recorded at 600/150 MHz for $^1$H/$^{13}$C in CDCl$_3$. $^b$Recorded at 600/150 MHz for $^1$H/$^{13}$C in DMSO-$d_6$. $^c$Recorded at 600/150 MHz for $^1$H/$^{13}$C in MeOH-$d_4$.

methine at $\delta_H$ 5.67–5.57 (m, H-8), one methylene at $\delta_H$ 3.23–3.25 (2H, m, H-7) and two methyl groups at $\delta_H$ 3.78 (3H, s, 3-OCH$_3$) and $\delta_H$ 3.76 (3H, s, 4-OCH$_3$). The $^{13}$C NMR spectrum of 2 (Table 1) revealed 10 carbon signals for a benzene, one olefinic carbon, one methylene and two methoxy groups. The above mentioned 1D NMR data of 2 in combination with its molecular formula indicated that the compound must be a symmetrical dimeric benzene derivative. Further analysis of NMR data suggested that the structure of 2 was quite similar to that of (E)-5,5′-(but-2-ene-1,4-diyl)bis(3-methoxybenzene-1,2-diol) [15]. The main difference was that the hydroxy group at C-4 and C-4′ in (E)-5,5′-(but-2-ene-1,4-diyl)bis(3-methoxybenzene-1,2-diol) was substituted by a methoxy group in 2, which was confirmed by the HMBC correlation (Figure 2) from $\delta_H$ 3.76 (4-OCH$_3$, 4′-OCH$_3$) to $\delta_C$ 135.9 (C-4, C-4′). The absorption band near 999 cm$^{-1}$ in the IR spec-
trum (Figure S26 in Supporting Information File 1) indicated that the double bond has an \( E \) configuration [16-19]. Therefore, the structure of compound 2 was established as shown in Figure 1, and named as sinensiol I.

Sinensiol J (3) was isolated as a white amorphous powder. Its HRESIMS showed \([\text{M} + \text{HCOO}]^-\) at \( m/z \) 391.1394 (calcld for 391.1398), consistent with the molecular formula of \( \text{C}_{19}\text{H}_{22}\text{O}_{6} \). The \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR data (Table 1) of 3 were extremely similar to those of the \( \text{rac-1-[(benzo[d][1,3]dioxol-5-yl)-4-(3,4,5-trimethoxyphenyl)]butan-1-ol} \) [20], the significant difference being the absence of signals for a methoxy group in the \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR spectra. The flat ECD curve (Figure S38 in Supporting Information File 1) and nearly zero optical rotation of 3 (\( [\alpha]_{D}^{20.8} \) \( -1.34, \ c \ 0.28, \ \text{MeOH}) \) suggested that 3 was possibly a racemic mixture. Enantioseparation of 3 by HPLC using a chiral-pak IA column provided the enantiomers with a ratio about 3:2 (Figure S28, Supporting Information File 1) suggested its mixture feature. Unfortunately, the limited amount available of this compound did not allow the elucidation of its absolute configuration.

The remaining known compounds were identified as (\(+\))-piroresinol di-O-\( \beta \)-d-glucopyranoside (4) [21], dehydrodiconiferyl alcohol-4-O-\( \beta \)-d-glucopyranoside (5) [22], and lari-resinol-4-O-\( \beta \)-d-glucopyranoside (6) [23] (Figure 1) by comparing their physiochemical properties and spectral data with those reported in the literature.

**Biological activity**

The isolated compounds were screened for their inhibitory effects on the LPS-induced NO production in RAW 264.7 macrophages. \( \text{N}^\text{G}-\text{Monomethyl}-\text{L-arginine} \) monoacetate salt (\( \text{L-NMMA}, \ \text{Sigma} \)) was used as the positive control. As a result, compounds 1, 2, 4, and 5 showed mild inhibitory activities with inhibition rates in the range of 9.47–18.75\%, compound 3 showed moderate activity with an inhibition rate of 42.06 \( \pm \) 2.02\% at a concentration of 50 \( \mu \text{M} \) (\( \text{L-NMMA}, \ 59.31 \pm 2.19\% \), Table 2).

**Conclusion**

In summary, three new lignan derivatives, sinensioils H–J (1–3) and three known compounds (4–6), were obtained from the
whole plants of *Selaginella sinensis*. The absolute configuration of compound 1 was established by comparison of calculated and experimental ECD spectra. Compounds 2 and 3, which possess a 1,4-diphenylbutane skeleton, are rare examples of naturally occurring 9,9′-bisnorlignans. In in vitro bioassays, compound 3 was found to show a moderate inhibitory effect on NO production in LPS-induced RAW 264.7 cells with an inhibitory rate of 42.06 ± 2.02% at 50 μM.

**Table 2: Inhibitory effects of compounds 1–6 on LPS-stimulated NO production.**

| sample | concentration (μM) | inhibition (%) |
|--------|-------------------|---------------|
| 1      | 50                | 18.75 ± 2.13  |
| 2      | 50 69.16 ± 0.81 (cytotoxicity) |               |
| 3      | 12.5              | 15.93 ± 1.37  |
| 4      | 50                | 42.06 ± 2.02  |
| 5      | 50                | 11.40 ± 0.81  |
| 6      | 50                | 9.47 ± 2.38   |
| L-NMMA | 50                | 3.36 ± 2.38   |

| sample | concentration (μM) | inhibition (%) |
|--------|-------------------|---------------|
| 1      | 50                | 59.31 ± 2.19  |

*Positive control.*

**Identification of new compounds**

**Compound 1**: pale yellow amorphous powder, \([\alpha]_{D}^{20.9} = -1.35 (c 0.24, MeOH); IR (KBr) ν max: 3433, 2937.2, 1593.0, 1241.6, 1035 cm⁻¹; ECD (c 2.2 × 10⁻⁴ M, MeOH) Δε (λ max) 204 (+4.36), 231 (+1.79) nm; H and ¹³C NMR data, see Table 1; HRESIMS (m/z): [M – H]^+ for C₂₃H₁₉O₇, 371.1136; found, 371.1133.

**Compound 2**: white amorphous powder, \([\alpha]_{D}^{20.8} = -1.34 (c 0.28, MeOH); IR (KBr) ν max: 3433, 2937.2, 1593.0, 1241.6, 814.9 cm⁻¹; H and ¹³C NMR data, see Table 1; HRESIMS (m/z): [M + COOH]^− for C₂₃H₂₃O₈, 391.1398; found, 391.1394.

**Compound 3**: white amorphous powder, \([\alpha]_{D}^{20.8} = -1.34 (c 0.28, MeOH); IR (KBr) ν max: 3433, 2937.2, 1593.0, 1241.6, 814.9 cm⁻¹; H and ¹³C NMR data, see Table 1; HRESIMS (m/z): [M + COOH]^− for C₂₃H₂₃O₈, 391.1398; found, 391.1394.

**Nitric oxide production inhibitory assay**

The inhibitory activity against the production of NO was evaluated using LPS induced RAW 264.7 cells as previously reported [24]. The cells were seeded in 96-well plates and co-incubated with the test compounds and positive control drug at a concentration of 50 μM or 12.5 μM, followed by stimulation with 1 μg/mL LPS for 18 h. The viability of RAW 264.7 cells was determined by an MTS assay to exclude the interference of the cytotoxicity of the test compounds before the nitric oxide (NO) production assay. NO production in each well was assessed by measuring the accumulation of nitrite in the culture medium. The results were expressed as a percentage of the positive control. Experiments were operated in triplicate. All
values are described as mean ± SD of three independent experiments.

Supporting Information

Supporting Information File 1

ECD calculation method of compound 1 and HPLC analysis of 3 and NMR, MS, and IR spectra of compounds 1–3.

[https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-18-146-S1.pdf]

Funding

This work was supported by the Project for Youth Science and Technology Talent of Guizhou Provincial Education Department (No. [2021]156), the Natural Science Foundation of Yunnan province (No. 202001AT070052), Doctoral Fund of Guizhou Medical University (No. [2020]005) and the New-Young Talent Project of Guizhou Medical University (No. 19NSP077).

ORCID® IDs

Qinfeng Zhu - https://orcid.org/0000-0001-6877-1838

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