SHORT REPORT

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Lyratin D, A New 4-Hydroxyisoflavan from the Whole Plant of *Solanum lyratum* Thunb

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Abstract: A new 4-hydroxyisoflavan, named lyratin D (**1**), was isolated from the whole plant of *Solanum lyratum* Thunb. Its structure and absolute configuration were elucidated through a comprehensive analysis of its HR-ESI-MS, 1D and 2D NMR, and circular dichroism spectroscopy data. Compound **1** was then assessed for its cytotoxic effects on RAW264.7 murine macrophage cells, and its antioxidant activity was evaluated through DPPH assay.

Keywords: *Solanum lyratum*; 4-hydroxyisoflavan; antioxidant.

1. Plant Source

The whole plant of *Solanum lyratum* Thunb. was collected from Sapa, Lao Cai, a province situated in the mountainous northwest region of Vietnam, in July 2019. The plant material was identified by Dr. Nguyen Viet Phong, one of the authors. A voucher specimen (accession code IMBC-SL1907) was deposited in the Department of Marine Medicinal Material, Institute of Marine Biochemistry, Hanoi, Vietnam.

2. Previous Studies

S. lyratum, belonging to the Solanaceae family, is a species of flowering plant known for its wide distribution across East Asia, including China, Korea, Japan, and Vietnam [1]. In traditional Chinese medicine, *S. lyratum* is recognized for its therapeutic properties such as heat-clearing, detoxification, wind-dispelling, and dampness-reducing effects [2]. Consequently, this plant has been

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extensively used across Asian countries to treat various conditions such as jaundice, edema, gonorrhea, cholecystitis, inflammation, and rheumatoid arthritis [2]. Previous studies have shown that *S. lyratum* contains diverse active compounds, including alkaloids, steroidal saponins, terpenoids, lignans, and flavonoids [3,4]. Notably, ethanol extracts from *S. lyratum* have demonstrated promising anticancer activities against human hepatocellular carcinoma SMMC-7721 cells and human osteosarcoma U-2 OS cells via the mitochondrial pathway [5,6]. Additionally, studies have isolated steroidal saponins and steroidal alkaloids from *S. lyratum*, revealing their cytotoxic effects by inhibiting tumor angiogenesis, promoting tumor cell apoptosis, enhancing immunity, and reducing inflammation while providing antioxidative properties [4,7,8].

Infertility, particularly male infertility, has become increasingly prevalent, accounting for approximately one-third of infertility cases [9]. The integrity of sperm cells is crucial for successful fertilization but is susceptible to damage from oxidative stress [10]. Despite inherent defense mechanisms, sperm cells may experience an imbalance between reactive oxygen species and antioxidant capacity, profoundly impacting male fertility [11]. Therefore, understanding the antioxidant potential of compounds from *S. lyratum* could provide insights into mitigating oxidative stress-related damage to sperm cells and improving male fertility results.

3. Present Study

General Experimental Procedures: The experimental procedures used in this study were similar to those reported in our previous research [12]. Optical rotation was obtained using a Jasco P-2000 digital polarimeter (Tokyo, Japan). NMR spectra were recorded on an Avance III-HD 500 NMR spectrometer (Bruker, Germany). High-resolution electrospray ionisation mass spectra (HR-ESI-MS) were obtained using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Circular dichroism (CD) spectrum was obtained using a Chirascan spectrometer (Applied Photophysics, Surrey, UK). Thin-layer chromatography was performed on Kieselgel 60 F_{254} or RP-18 F_{254S} plates (Merck, Darmstadt, Germany). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 40–63 μm) and Sephadex LH-20 resin (25–100 μm) (Merck).

Extraction and Isolation: The air-dried whole plant of *S. lyratum* (2.9 kg) was powdered and extracted three times with MeOH (1 h × 3) under ultrasonic conditions at room temperature (22–25°C), resulting in a MeOH residue (320 g) after the removal of the solvent. The extract was then suspended in water and partitioned with CH_2Cl_2 and EtOAc to obtain a CH_2Cl_2 extract (30 g), an EtOAc extract (26 g), and a water layer. The CH_2Cl_2 extract was subjected to silica gel CC and eluted with *n*-hexane:acetone (gradient 100:0–0:100, *v/v*) to obtain seven fractions (D1–D7). Fraction D6 (1.7 g) was separated using silica gel CC and eluting with CH_2Cl_2:EtOAc (4:1, *v/v*), followed by purification using a Sephadex LH-20 column and eluting with MeOH:H_2O (1:1, *v/v*), to isolate compound **1** (6.4 mg) (Figure 1).

Lyratin D ***(1)****:* A yellow gum; $[\alpha]_D^{20}$ = −98.6 (*c* 0.3, $CHCl_3$); CD (*c* 0.1 mM, MeOH) $\Delta\varepsilon_{285\ nm}$ +2.89, $\Delta\varepsilon_{238\ nm}$ −3.08; ^{1}H (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) data, see Table 1; HR-ESI-MS *m/z* 355.1527 $[M-H_2O+H]^+$ (calcd. for $C_{21}H_{23}O_5$, 355.1545).

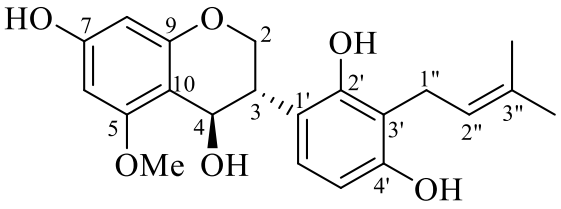

Figure 1. Structure of lyratin D (**1**) isolated from *S. lyratum*

Cell Culture and Cell Viability Assay: RAW264.7 murine macrophage cells (American Type Culture Collection - ATCC, MD, USA) were grown in Dulbecco's modified Eagle's medium (DMEM) high

glucose supplemented with 10% fetal bovine serum (FBS), penicillin G (100 units/mL), and streptomycin (100 μg/mL) in an incubator with 5% CO_2. Cells were seeded at the concentration of 5×10^3 cells/well in 96-well plates for 24 h before treating with compound **1** for an additional 24 h. Subsequently, cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) for 4 h. Dimethyl sulfoxide (DMSO) was used to dissolve formazan crystals before the cell viability was determined at the 540 nm absorbance using a BioTek Epoch 2 microplate reader (Agilent, CA, USA) [13].

DPPH Assay: The antioxidant activity of compound **1** was evaluated based on its radical scavenging effect on the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical [14]. Briefly, each well contained 20 μL of the sample, which was diluted in DMSO to final concentrations of 100, 50, 25, 12.5, and 6.25 μM. Then, 180 μL of DPPH, diluted to 150 μM in 80% ethanol, was mixed with the sample. The well contents were thoroughly mixed and incubated at room temperature (25 °C) for 30 min. When DPPH reacted with an antioxidant compound, the change in color from deep violet to light yellow was measured at 520 nm using a BioTek Epoch 2 microplate reader. Vitamin C (L-Ascorbic acid; Sigma-Aldrich, MO, USA) was used as a positive control. Radical-scavenging capacity was expressed as a percentage effect (%) and calculated using the following equation: Percentage effect (%) = [(*Acontrol* − *Asample*) / *Acontrol*] × 100%, where *Asample* is the absorbance of the sample and *Acontrol* is the absorbance of the control.

Compound **1** was isolated as yellow gum. Its molecular formula was determined as $C_{21}H_{24}O_6$ by the HR-ESI-MS with a dehydrated pseudo-molecular ion peak at *m/z* 355.1527 $[M-H_2O+H]^+$ (calcd. for $C_{21}H_{23}O_5$, 355.1545). The ^{1}H-NMR spectrum of compound **1** displayed the presence of a methylene group [δ_H 4.13 (1H, dd, *J* = 10.5, 4.7 Hz) and 3.46 (1H, d, *J* = 10.5 Hz)], and two methine groups at δ_H 3.37 (1H, dd, *J* = 6.8, 4.7 Hz) and 5.44 (1H, d, *J* = 6.8 Hz). These signals were assignable to two H-2 protons, H-3 and H-4 protons of a 4-hydroxyisoflavan skeleton. The corresponding carbons were identified by the HMQC spectrum as methylene carbon at δ_C 65.8 (C-2) and two methine carbon atoms at δ_C 38.7 (C-3) and 74.4 (C-4) (Table 1). In addition, in the ^{1}H-NMR spectrum, further signals were observed, indicating the presence of a 5,7-substituted ring [δ_H 6.08 (1H, d, *J* = 2.1 Hz, H-6) and 5.89 (1H, d, *J* = 2.1 Hz, H-8)], *ortho*-coupled aromatic doublets [δ_H 6.28 (1H, d, *J* = 8.0 Hz, H-5′) and 6.89 (1H, d, *J* = 8.0 Hz, H-6′)], a methoxy group [δ_H 3.77 (3H, s)], and a prenyl moiety [δ_H 3.13 (1H, dd, *J* = 13.7, 8.0 Hz, H-1″a), 3.05 (1H, dd, *J* = 13.7, 6.8 Hz, H-1″b), 5.18 (1H, t, *J* = 6.8 Hz, H-2″), 1.68 (3H, s, H-4″), and 1.59 (3H, s, H-5″)] (Table 1). The structure of compound **1** was further identified as a 4-hydroxyisoflavan by ^{1}H-^{1}H COSY and HMBC spectra (Figure 2). The ^{1}H-^{1}H COSY correlations of H_2-1/H_2-2/H-3/H-4 confirmed the linkage of methylene group C-2 and methine groups C-3 and C-4. The HMBC correlations of H-2 to C-3 and C-4, of H-3 to C-2, C-10, C-1′, C-2′, and C-6′, and of H-4 with C-2, C-3, C-5, C-9, and C-1′ indicated the position of the *ortho*-coupled aromatic. The position of the prenyl moiety was determined through HMBC correlations of the vinylic proton H-2″ with C-3′, and of the methylene protons H-1′ with C-2′, C-3′, and C-4′ (Figure 2). By analyzing the NMR data of compound **1**, it was found that its NMR signals were highly similar to those of lyratin A [15], except for the presence of an additional methoxy group in the structure of compound **1** compared to lyratin A (Table S1). The position of the methoxy group was determined by HMBC correlations of δ_H 3.77 (3H, s) with δ_H 160.8 (C-5) (Figure 2). Based on the above evidence, the planar structure of compound **1** was established.

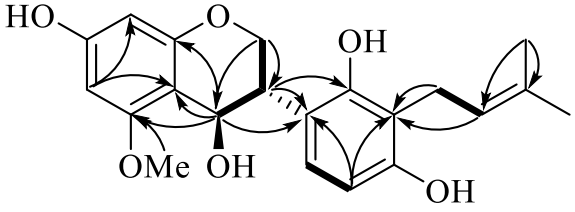

Figure 2. Key ^{1}H-^{1}H COSY (bold) and HMBC (→) correlations for lyratin D (**1**)

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data for lyratin D (**1**) in DMSO-d_6 (δ in ppm)

Position	**δ_C, type**	**δ_H (*J* in Hz)**
2	65.8, CH_2	4.13, dd (10.5, 4.7)
		3.46, d (10.5)
3	38.7, CH	3.37, dd (6.8, 4.7)
4	74.4, CH	5.44, d (6.8)
5	160.8, C	–
6	92.6, CH	6.08, d (2.1)
7	159.4, C	–
8	95.3, CH	5.89, d (2.1)
9	156.9, C	–
10	100.7, C	–
1′	117.2, C	–
2′	158.4, C	–
3′	110.3, C	–
4′	155.8, C	–
5′	106.8, CH	6.28, d (8.0)
6′	121.7, CH	6.89, d (8.0)
1″	22.5, CH_2	3.13, dd (13.7, 8.0)
		3.05, dd (13.7, 6.8)
2″	122.6, CH	5.18, t (6.8)
3″	130.3, C	–
4″	17.4, CH_3	1.68, s
5″	25.6, CH_3	1.59, s
5-OMe	55.5, CH_3	3.77, s
7-OH		9.65, br s
4′-OH		9.19, br s

The assignments were based on DEPT, HMQC and HMBC experiments.

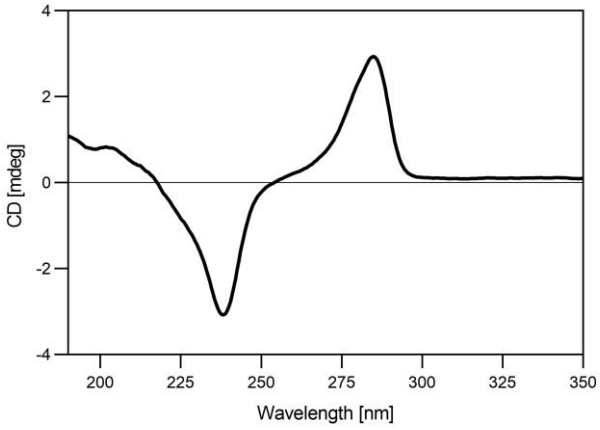

Figure 3. CD spectrum of lyratin D (**1**)

The relative configuration of compound **1** was determined from the NOESY spectrum. NOESY correlations were observed from H-3 to H-2b and from H-4 to H-2a, confirming that the relative configuration of H-3 and H-4 was *trans*. Additionally, the absolute configuration of C-3 and C-4 of compound **1** was identified by the CD spectrum (Figure 3). Previous studies indicated that (3*R*,4*S*)-4-hydroxyisoflavan exhibited positive and negative Cotton effects in the 220 to 250 nm and 250 to 300

nm regions, respectively [16-19]. Conversely, (3*S*,4*R*)-4-hydroxyisoflavan displayed the opposite Cotton effects, with a negative Cotton effect in the 220 to 250 nm region and a positive Cotton effect in the 250 to 300 nm region [16-19]. As shown in Figure 3, the CD spectrum of compound **1** showed a negative Cotton effect at 238 nm and a positive Cotton effect at 285 nm, confirming the absolute configurations of C-3 and C-4 as 3*S* and 4*R*. Thus, compound **1** was elucidated and named lyratin D.

Since the cytotoxicity of immune modulators is an undesirable property, the toxicity of compound **1** on RAW264.7 macrophage cells was studied in this study. The results indicated that compound **1**, at concentrations ranging from 3.125 to 25 μM, did not affect the viability of RAW264.7 cells. To evaluate the antioxidant activity, the DPPH assay was used to assess the free radical scavenging activity of compound **1**, with vitamin C used as the positive control. In comparison to the positive control, at concentrations of 25, 100, and 400 μM, vitamin C exhibited inhibitions of 18.24 ± 2.22%, 56.45 ± 2.00%, and 84.28 ± 0.77%, respectively. On the other hand, compound **1** demonstrated inhibitions of 9.90 ± 1.56%, 23.66 ± 0.11%, and 36.86 ± 0.10% at the same concentrations of 25, 100, and 400 μM, respectively.

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Supporting Information

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