

New Anti-inflammatory 4-Hydroxyisoflavans from *Solanum lyratum*

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Three new 4-hydroxyisoflavans, named lyratin A (1), lyratin B (2) and lyratin C (3), along with a known compound, 4,7,2'-trihydroxy-4'-methoxyisoflavan (4), were isolated from the whole plant of *Solanum lyratum*. Their structures were established by means of detailed physical data analyses. *In vitro*, four compounds showed anti-inflammatory activities with inhibitory ratios of release of β -glucuronidase from polymorphonuclear leukocytes of rats in the range of 30.3—38.6% at 10 μ M.

Key words *Solanum lyratum*; Solanaceae; 4-hydroxyisoflavan; lyratin; anti-inflammatory activity

The whole plant of *Solanum lyratum* THUNB (Solanaceae) is a renowned traditional Chinese medicine that has been used as anti-inflammatory, antitumor, antibacterial, and antioxidant agent.^{1–4} In our previous phytochemical studies, we reported isolation of several sesquiterpenoids, flavonoids, and amides, some of which showed significant cytotoxic activities.^{5–8} As part of our ongoing search for new natural compounds with biological activities, the ethanolic extract of *S. lyratum* was further investigated, which led to the isolation of three new 4-hydroxyisoflavans, named lyratin A (1), lyratin B (2), and lyratin C (3), together with a known 4-hydroxyisoflavan, 4,7,2'-trihydroxy-4'-methoxyisoflavan (4). On the basis of extensive spectroscopic analyses, the structures of the new compounds were elucidated. In addition, the isolated four 4-hydroxyisoflavans were screened for their anti-inflammatory activities with inhibitory rates of release of β -glucuronidase from polymorphonuclear leukocytes of rats in the range of 30.3—38.6% at 10 μ M. Herein we report the isolation, structure elucidation and anti-inflammatory activities of these four 4-hydroxyisoflavans.

Results and Discussion

Compound 1 was isolated as a yellow gum. The molecular formula was determined C₂₀H₂₂O₅ by HR-electrospray ionization (ESI)-MS, which indicated a quasi-molecular ion peak at m/z 343.1541 [M+H]⁺. The IR spectrum showed absorption bands at 3410, 1605, 1558, and 1459 cm⁻¹, which were in agreement with hydroxy and aromatic groups. The ¹H-NMR spectrum showed the presence of a pair of doublets at δ_H 3.57 (1H, dd, $J=11.0, 11.1$ Hz) and 4.19 (1H, dd, $J=5.0, 11.0$ Hz), a multiplet at δ_H 3.52 (1H, m), and a doublet at δ_H 5.45 (1H, d, $J=6.6$ 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 heteronuclear multiple quantum coherence (HMQC) experiment as a methylene carbon at δ_C 65.8 (C-2) and two methine carbon atoms at δ_C 39.3 (C-3) and 77.4 (C-4). In the ¹H-NMR spectrum, further signals were observed that showed the presence of 7-substituted ring A [δ_H 7.25 (1H, d, $J=8.4$ Hz, H-5), 6.48 (1H, dd, $J=2.0, 8.4$ Hz, H-6), and 6.25 (1H, d, $J=2.0$ Hz, H-8)], *ortho*-coupled aromatic doublets [δ_H 6.32 (1H, d, $J=8.0$ Hz, H-5') and 6.92 (1H, d, $J=8.0$ Hz, H-6')], a prenyl unit [δ_H 3.12 (2H, d, $J=6.9$ Hz, H-1''), 5.15 (1H, t, $J=6.9$ Hz, H-2''), 1.67 (3H, s, H-4''), and 1.59 (3H, s, H-5'')], and three hydroxyl units [δ_H 9.59 (1H, s), 9.21 (1H,

s) and 8.31 (1H, s)]. In the heteronuclear multiple bond correlation (HMBC) spectrum, the cross peaks from the proton at δ_H 9.59 to C-7, from the proton at δ_H 9.21 to C-4', and from the proton at δ_H 8.31 to C-2' confirmed that three hydroxyl moieties were attached to C-7, C-4', and C-2', respectively. The long-range correlations from H-4 to C-2, C-3, C-5, C-9, and C-1', from H-3 to C-2, C-10, C-1', C-2', and C-6', as well as from H-2 to C-3, C-4, and C-1' indicated the presence of the C-ring. The position of the prenyl group was confirmed by HMBC experiment, which showed correlation between a vinylic proton H-2'' of the prenyl substituent and C-3', furthermore, the methylene protons H-1'' of the prenyl substituent showed correlations with C-2', C-3', and C-4'. The proton H-6' showed correlations with C-2', C-3', C-4', and C-3, while proton H-5' showed correlations with C-1', C-3', and C-4'. Thus the substitution in the B-ring was determined to be 2',4'-dihydroxy-3'- γ,γ -dimethylallyl. The relative configuration of 1 was determined from rotating frame Overhauser enhancement spectroscopy (ROESY) data. In the ROESY spectrum, cross peaks were observed from H-3 to H-2_b and from H-4 to H-2_a, confirming that the relative configuration of H-3 and H-4 was *trans*. Furthermore, the absolute configurations of C-3 and C-4 of 1 were established from circular dichroism (CD) spectrum. In a previous paper,⁹ it was reported that (3*S*,4*R*)-4-hydroxyisoflavan exhibits a negative Cotton effect at 220—250 nm and a positive Cotton effect at 250—300 nm, while (3*R*,4*S*)-4-hydroxyisoflavan showed positive and negative Cotton effects in the regions of 220—250 and 250—300 nm, respectively. Since the CD spectrum of 1 showed a negative Cotton effect at 239 nm and a positive Cotton effect at 285 nm, this suggests the absolute configurations of C-3 and C-4 as 3*S*, and 4*R*, respectively. On the basis of the above data and comprehensive 2D NMR experiments (HMQC and HMBC), the structure of compound 1 was identified as shown in Fig. 1, named lyratin A.

Compound 2 was obtained as a brown paste and gave a HR-ESI-MS ion peak at m/z 341.1385 [M+H]⁺, corresponding to a molecular formula of C₂₀H₂₀O₅. The IR spectrum displayed absorption bands at 3431, 1610, 1568, and 1457 cm⁻¹, which were assignable to hydroxy and aromatic moieties. Comparison of its ¹H-NMR spectrum with that of 1 showed that 2 had many features in common with 1. In contrast to compound 1, the ¹H-NMR spectrum of 2 displayed signals of one 2,2-dimethylpyran moiety [δ_H 5.68 (1H, d,

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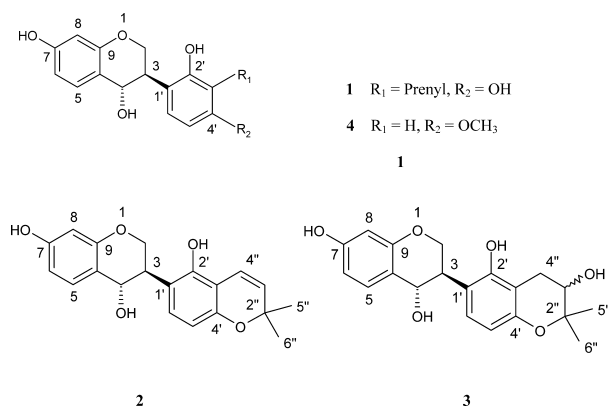


Fig. 1. The Structures of Compounds 1–4 Isolated from *Solanum lyratum*

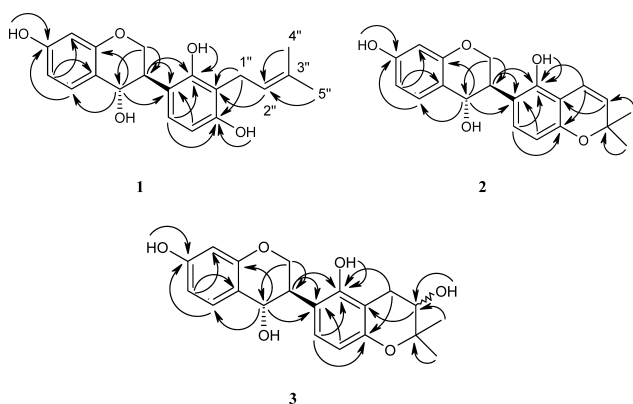


Fig. 2. Key HMBC Correlations of Compounds 1–3

$J=9.9$ Hz, H-3''), 6.37 (1H, d, $J=9.9$ Hz, H-4''), 1.35 (3H, s, H-5''), and 1.33 (3H, s, H-6''), which was cyclized with C-4' and C-2'' by an ether linkage, as supported by corresponding HMBC experiment. The relative configuration of the stereogenic centers of **2** was the same as those in **1**, as determined by the ROESY spectrum. Furthermore, based on the results of optical rotation and CD data, the absolute configurations of two chiral carbons of **2** were found in agreement with **1**. Consequently, the structure of compound **2** was established as shown in Fig. 1, named lyratin B.

Compound **3** was isolated and purified as a pale yellow gum, and the molecular formula was established as $\text{C}_{20}\text{H}_{22}\text{O}_6$ by HR-ESI-MS, which displayed a quasi-molecular ion at m/z 359.1490 $[\text{M}+\text{H}]^+$. The IR spectrum exhibited absorption bands at 3433, 1611, 1552, and 1455 cm^{-1} , which corresponded to hydroxy and aromatic groups. The ^1H - and ^{13}C -NMR spectra of **3** were similar to those of **2** except for the absence of the double bond across C-3''/C-4'' and the presence of an oxygenated methine proton [δ_{H} 3.58 (1H, dd, $J=5.3, 7.4$ Hz, H-3''); δ_{C} 67.4], a methylene moiety [δ_{H} 2.77 (1H, dd, $J=5.3, 16.8$ Hz, H_a-4'') and 2.33 (1H, dd, $J=7.4, 16.8$ Hz, H_b-4''); δ_{C} 25.9], and a hydroxyl group [δ_{H} 5.09 (1H, d, $J=4.7$ Hz)]. The long-range correlation between the proton at δ_{H} 5.09 and C-3'' indicated that the hydroxy group was attached to C-3''. With the aid of the ROESY data, it was readily confirmed that the relative configuration of the protons at C-3 and C-4 was also *trans*. Additionally, the absolute configurations of C-3 and C-4 were assigned as 3*S*, 4*R* on the

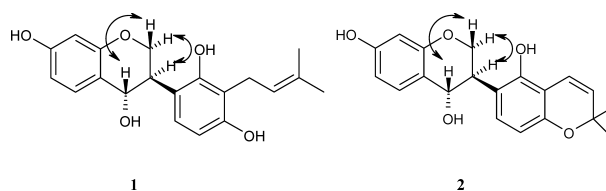


Fig. 3. Key ROESY Correlations of Compounds 1 and 2

basis of its CD spectrum, which displayed a negative Cotton effect at 239 nm and a positive Cotton effect at 285 nm.⁹⁾ In this way, except for C-3'', the absolute configurations of all chiral centers of **3** were established. Thus the structure of compound **3** was determined as shown in Fig. 1, named lyratin C.

The known compound was identified as 4,7,2'-trihydroxy-4'-methoxyisoflavan (**4**) by comparison of its physical and spectral data with those reported in the literature.¹⁰⁾

Compounds 1–4 and ginkgolide B were evaluated for anti-inflammatory activities using established methods,^{11,12)} and the inhibitory rates of release of β -glucuronidase from polymorphonuclear leukocytes (PMNs) of rats were 31.7%, 38.6%, 30.3%, 35.4% and 50.2% respectively at 10 μM . Based on the bioassay results, it may be concluded that these compounds have inhibitory activities on the release of β -glucuronidase from rat PMNs induced by platelet activating-factor (PAF).

Experimental

General Experimental Procedures Optical rotations were recorded by Perkin-Elmer 241 polarimeter. UV spectra were obtained by Shimadzu UV-160 spectrophotometer. IR spectra were determined by Perkin-Elmer 683 infrared spectrometer with KBr disks. CD spectra were recorded by JASCO-815 CD spectrometer. ESI-MS were measured by Bruker Esquire 3000 Plus spectrometer. HR-ESI-MS were recorded by Micromess Q-ToF Global mass spectrometer. NMR spectra were obtained by Varian Unity BRUKER 400 at 400 MHz (^1H) and 100 MHz (^{13}C) with tetramethylsilane (TMS) as internal standard. Silica gel (200–300 mesh) for column chromatography and silica gel GF254 for preparative TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, People's Republic of China.

Plant Material *Solanum lyratum* THUNB was collected in Linyi district, Shandong Province, People's Republic of China, in September 2006, and identified by Professor Yan-yan Zhao, School of Pharmaceutical Science, Yantai University. The whole plant of *S. lyratum* was harvested and air-dried at room temperature in the dark. A voucher specimen (YP06089) has been deposited at the herbarium of the School of Pharmaceutical Science, Yantai University.

Extraction and Isolation The air-dried whole plant of *S. lyratum* (20.0 kg) was finely cut and extracted three times (1 h \times 3) with refluxing EtOH. Evaporation of the solvent under reduced pressure provided the ethanolic extract. The extract was dissolved and suspended in H_2O , and partitioned with CHCl_3 , EtOAc, and *n*-BuOH. The CHCl_3 fraction (217.1 g) was initially subjected to silica gel column (10 \times 90 cm) chromatography (200–300 mesh, 2.0 kg) and eluted with cyclohexane–acetone at 95:5 (6.0), 90:10 (6.0), 85:15 (6.0), 80:20 (7.0), 75:25 (7.0), 70:30 (7.0), 60:40 (5.0), and 50:50 (3.0) to give ten fractions. Fraction 5 (3.6 g) was separated by CC over silica gel [eluted with cyclohexane–acetone, (100:0–70:30)], Sephadex LH-20 [100 g, eluted with EtOAc–EtOH, 50:50, v/v], and preparative TLC [CHCl_3 –EtOAc, 7:1, v/v] to afford compounds **2** (18.6 mg) and **4** (11.3 mg). Fraction 7 (4.2 g) was isolated by CC on silica gel [eluted by CHCl_3 – CH_2COCH_3 , (100:0–60:40)] and preparative TLC [CHCl_3 –EtOAc, 4:1, v/v] to give compounds **1** (25.4 mg) and **3** (39.1 mg).

Lyratin A (**1**): A yellow gum. $[\alpha]_{\text{D}}^{25} -113.5^\circ$ ($c=0.36, \text{CHCl}_3$). UV (CHCl_3) λ_{max} : 209 and 297 nm. IR (KBr) ν_{max} : 3410, 1632, 1605, 1558, 1459 and 1023 cm^{-1} . CD ($c=0.87\times 10^{-3}, \text{MeOH}$) $\Delta\epsilon$ (nm): -1.37 (239), $+1.40$ (285). ESI-MS m/z : 343.3 $[\text{M}+\text{H}]^+$. HR-ESI-MS m/z : 343.1541 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{20}\text{H}_{23}\text{O}_5$, 343.1545). ^1H - and ^{13}C -NMR data, see Table 1.

Table 1. NMR (400 MHz for ^1H and 100 MHz for ^{13}C , in $\text{DMSO}-d_6$) Data of Compounds **1**—**3**^{a,b)}

No.	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2	3.57 (dd, 11.0, 11.1, H_a-2) 4.19 (dd, 5.0, 11.0, H_b-2)	65.8 CH_2	3.61 (dd, 10.9, 11.0, H_a-2) 4.23 (dd, 4.9, 10.9, H_b-2)	65.9 CH_2	3.62 (dd, 10.8, 11.0, H_a-2) 4.22 (dd, 4.9, 10.8, H_b-2)	66.0 CH_2
3	3.52 (m)	39.3 CH	3.56 (m)	39.0 CH	3.54 (m)	39.2 CH
4	5.45 (d, 6.6)	77.4 CH	5.58 (d, 6.2)	78.5 CH	5.52 (d, 6.5)	78.0 CH
5	7.25 (d, 8.4)	132.0 CH	7.29 (d, 8.4)	132.1 CH	7.29 (d, 8.4)	132.1 CH
6	6.48 (dd, 2.0, 8.4)	109.6 CH	6.48 (dd, 2.2, 8.4)	109.7 CH	6.49 (dd, 2.2, 8.4)	109.6 CH
7		158.6 C		158.8 C		158.7 C
8	6.25 (d, 2.0)	102.3 CH	6.27 (d, 2.2)	102.8 CH	6.27 (d, 2.2)	102.8 CH
9		156.2 C		156.4 C		156.3 C
10		111.6 C		111.1 C		111.3 C
1'		117.2 C		119.5 C		117.5 C
2'		158.2 C		154.9 C		157.7 C
3'		110.4 C		105.1 C		103.8 C
4'		155.8 C		152.9 C		153.5 C
5'	6.32 (d, 8.0)	107.0 CH	6.28 (d, 8.0)	107.9 CH	6.25 (d, 8.2)	108.2 CH
6'	6.92 (d, 8.0)	121.7 CH	7.06 (d, 8.0)	124.3 CH	7.02 (d, 8.2)	122.5 CH
1''	3.12 (d, 6.9, 2H)	22.4 CH_2				
2''	5.15 (t, 6.9)	122.6 CH		75.7 C		76.8 C
3''		130.0 C	5.68 (d, 9.9)	129.9 CH	3.58 (dd, 5.3, 7.4)	67.4 CH
4''	1.67 (s, 3H)	17.6 CH_3	6.37 (d, 9.9)	115.8 CH	2.77 (dd, 5.3, 16.8, H_a-4'') 2.33 (dd, 7.4, 16.8, H_b-4'')	25.9 CH_2
5''	1.59 (s, 3H)	25.4 CH_3	1.35 (s, 3H)	27.4 CH_3	1.24 (s, 3H)	20.5 CH_3
6''			1.33 (s, 3H)	27.4 CH_3	1.13 (s, 3H)	25.4 CH_3
7-OH	9.59 (s)		9.64 (s)		9.63 (s)	
2'-OH	8.31 (s)		8.30 (s)		8.33 (s)	
4'-OH	9.21 (s)					
3''-OH					5.09 (d, 4.7)	

a) Chemical shift values were in ppm and J values (in Hz) were presented in parentheses. b) The assignments were based on DEPT, HMQC and HMBC experiments.

Lyratin B (**2**): A brown paste. $[\alpha]_{\text{D}}^{25} -106.2^\circ$ ($c=0.32$, CHCl_3). UV (CHCl_3) λ_{max} : 211 and 298 nm. IR (KBr) ν_{max} : 3431, 1634, 1610, 1568, 1457 and 1010 cm^{-1} . CD ($c=0.59 \times 10^{-3}$, MeOH) $\Delta\epsilon$ (nm): -1.98 (220), -1.40 (235), $+0.79$ (285). ESI-MS m/z : 341.2 $[\text{M}+\text{H}]^+$. HR-ESI-MS m/z : 341.1385 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{20}\text{H}_{21}\text{O}_5$, 341.1389). ^1H - and ^{13}C -NMR data, see Table 1.

Lyratin C (**3**): A pale yellow gum. $[\alpha]_{\text{D}}^{25} -79.8^\circ$ ($c=0.33$, CHCl_3). UV (CHCl_3) λ_{max} : 206 and 295 nm. IR (KBr) ν_{max} : 3433, 1611, 1552, 1455 and 1033 cm^{-1} . CD ($c=0.84 \times 10^{-3}$, MeOH) $\Delta\epsilon$ (nm): -2.17 (239), $+1.23$ (285). ESI-MS m/z : 359.4 $[\text{M}+\text{H}]^+$. HR-ESI-MS m/z : 359.1490 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{20}\text{H}_{23}\text{O}_6$, 359.1495). ^1H - and ^{13}C -NMR data, see Table 1.

Anti-inflammatory Bioassays The effects of compounds **1**—**4** and ginkgolide B on the release of β -glucuronidase in rat PMNs induced by PAF *in vitro* were measured. Rat PMNs were incubated with vehicle or various concentrations of test compounds at 37°C for 15 min. Then, cytochalasin B ($10\ \mu\text{mol l}^{-1}$) was added. After 5 min, PAF ($1\ \mu\text{mol l}^{-1}$) was added prior to termination of the reaction. The supernatants of the reaction were incubated with phenolphthalein glucuronic acid ($0.4\ \text{mmol l}^{-1}$) at 37°C for 18 h. The absorbance was read at 550 nm then the inhibitory ratio was calculated. Ginkgolide B (Sigma; 98% pure) was used as positive control.

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