NOTE

Solalyratins A and B, new anti-inflammatory metabolites from *Solanum lyratum*

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Abstract A new coumestan (solalyratin A, **1**) and a novel cyclic eight-membered α,β-unsaturated ketone (solalyratin B, **3**), together with three known compounds, puerariafuran (**2**), coumestrol (**4**) and 9-hydroxy-2′,2′-dimethylpyrano[5′,6′:2,3]-coumestan (**5**), were isolated from the whole plant of *Solanum lyratum*. Their structures were elucidated on the basis of spectroscopic analyses. In vitro, compounds **1–5** showed anti-inflammatory activities, with IC_{50} values in the range 6.3–9.1 μM.

Keywords *Solanum lyratum* Thunb. · Solanaceae · Solalyratins A, B · Anti-inflammatory activity

Introduction

The Solanaceae family is a rich source of active secondary metabolites. In particular, *Solanum* is one of the largest genera in this family, including about 1400 species widely distributed throughout the world [1]. The plants in this genus always draw the attention of researchers due to continuing discoveries over many years of interesting biologically active constituents, including mainly steroids, steroidal alkaloids and their glycosides. *Solanum lyratum* Thunb. (Solanaceae) is a perennial herb which is native to China, Japan and Korea. This plant is commonly known as "Bai-

Ying" in traditional Chinese medicine and "Back-Mo-Deung" in traditional Korea medicine, and has been used as an antitumor, anti-inflammatory, immunomodulatory, anti-anaphylactic, and antioxidant agent [2–7]. In previous phytochemical studies on *S. lyratum*, we reported the isolation of a series of sesquiterpenoids and isoflavonoids [8–10]. As part of our ongoing search for new bioactive substances, a bioguided separation of extract from *Solanum lyratum* was performed, and led to the isolation of two new compounds, named solalyratins A and B (**1** and **3**), along with three known compounds, puerariafuran (**2**), coumestrol (**4**) and 9-hydroxy-2′,2′-dimethylpyrano[5′,6′:2,3]-coumestan (**5**). Their structures were elucidated by detailed spectral data. In addition, the anti-inflammatory activities of compounds **1–5** were investigated using the inhibition of β-glucuronidase release from polymorphonuclear leukocytes of rats induced by platelet activating factor (PAF), with IC_{50} values in the range 6.3–9.1 μM. Herein we report the isolation and structure elucidation, as well as the evaluation of anti-inflammatory activities, of these compounds.

Results and discussion

Compound **1** was isolated as yellow powder. The molecular formula was determined to be $C_{20}H_{16}O_5$ by HR-ESI mass spectrum, which showed a quasi-molecular ion peak at *m/z* 337.1072 [M + H]$^+$. The IR spectrum displayed absorption bands at 3339, 1661, 1617, 1593 and 1457 cm^{-1}, which were in agreement with hydroxyl, conjugated carbonyl and aromatic moieties. The UV spectrum of **1** exhibited absorption maxima at 250, 309 and 351 nm, which was characteristic of the coumestan skeleton [11, 12]. The ^{1}H-NMR spectrum displayed the presence of one set of ABX-type aromatic proton signals at δ_H 7.68

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Table 1 ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of compounds **1** and **3**

No.	**1**[a]		**3**[b]	
	δ_H	δ_C	δ_H	δ_c
1	7.68 (1H, d, 8.5)	120.5 CH		182.6 C
2	6.95 (1H, dd, 8.5, 1.7)	113.9 CH	5.70 (1H, s)	113.1 CH
3		156.9 C		172.3 C
4	7.20 (1H, d, 1.7)	98.8 CH		86.9 C
4a		155.9 C		
5			2.46 (1H, m)	45.8 CH$_2$
			1.78 (1H, m)	
6		159.7 C	4.34 (1H, m)	67.0 CH
6a		102.0 C		
6b		103.3 C		
7		132.4 C	1.98 (1H, m)	47.5 CH$_2$
			1.54 (1H, m)	
8	7.70 (1H, d, 1.8)	119.3 CH		36.1 C
9		159.6 C	1.47 (3H, s)	26.7 CH$_3$
10	6.94 (1H, d, 1.8)	103.6 CH	1.28 (3H, s)	30.8 CH$_3$
10a		152.9 C		
11			1.79 (3H, s)	27.2 CH$_3$
11a		157.6 C		
11b		114.7 C		
1′		40.1 C		197.1 C
2′	6.28 (1H, dd, 17.5, 10.7)	146.8 CH	5.96 (1H, br s)	128.0 CH
3′	5.04 (1H, d, 10.7)	111.0 CH$_2$		160.5 C
	5.02 (1H, d, 17.5)			
4′	1.51 (3H, s)	26.5 CH$_3$		79.5 C
5′	1.51 (3H, s)	26.5 CH$_3$		41.6 C
6′			2.51 (1H, d, 17.6)	49.8 CH$_2$
			2.34 (1H, d, 17.6)	
7′			6.84 (1H, d, 15.7)	145.1 CH
8′			6.47 (1H, d, 15.7)	130.6 CH
9′				197.5 C
10′			2.31 (3H, s)	28.6 CH$_3$
11′			1.89 (3H, d, 1.3)	18.9 CH$_3$
12′			1.11 (3H, s)	23.1 CH$_3$
13′			1.03 (3H, s)	24.5 CH$_3$
–OH	10.77 (1H, s)			
–OH	10.02 (1H, s)			

Chemical shift values are in ppm and J values (in *parentheses*) are in Hz, and the assignments are based on HMQC, HMBC, and ^{1}H–^{1}H COSY experiments

[a] NMR data in DMSO-d_6

[b] NMR data in CDCl$_3$

(1H, d, J = 8.5 Hz, H-1), 7.20 (1H, d, J = 1.7 Hz, H-4) and 6.95 (1H, dd, J = 8.5, 1.7 Hz, H-2) assignable to the coumestan moiety; a pair of *meta*-coupled aromatic protons at δ_H 7.70 (1H, d, J = 1.8 Hz, H-8) and 6.94 (1H, d, J = 1.8 Hz, H-10) assignable to one tetrasubstituted benzene ring; one 1,1-dimethylallyl unit with resonances at δ_H 6.28 (1H, dd, J = 17.5, 10.7 Hz, H-2′), 5.04 (1H, d, J = 10.7 Hz, H$_a$-3′), 5.02 (1H, d, J = 17.5 Hz, H$_b$-3′), 1.51 (3H × 2, s, –CH$_3$); as well as two phenolic hydroxyl groups at δ_H 10.77 (1H, s) and 10.02 (1H, s). The ^{13}C-NMR (Table 1) and DEPT spectra exhibited signals

for 17 sp^2 carbons, including one methylene, six methines and ten quaternary carbons. These findings further confirmed that **1** had a coumestan skeleton. The location of the 1,1-dimethylallyl unit was established by HMBC correlation between H-2′ (δ_H 6.28) and C-7 (δ_C 132.4). On the basis of detailed analyses of ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC spectra, all proton and carbon signals were assigned. Therefore, the structure of **1** was characterized as shown in Fig. 1, and named solalyratin A.

Compound **3** was isolated and purified as white powder, and HR-ESI mass spectrum gave a quasi-molecular ion

Fig. 1 Structures of compounds **1**–**5** isolated from *Solanum lyratum*
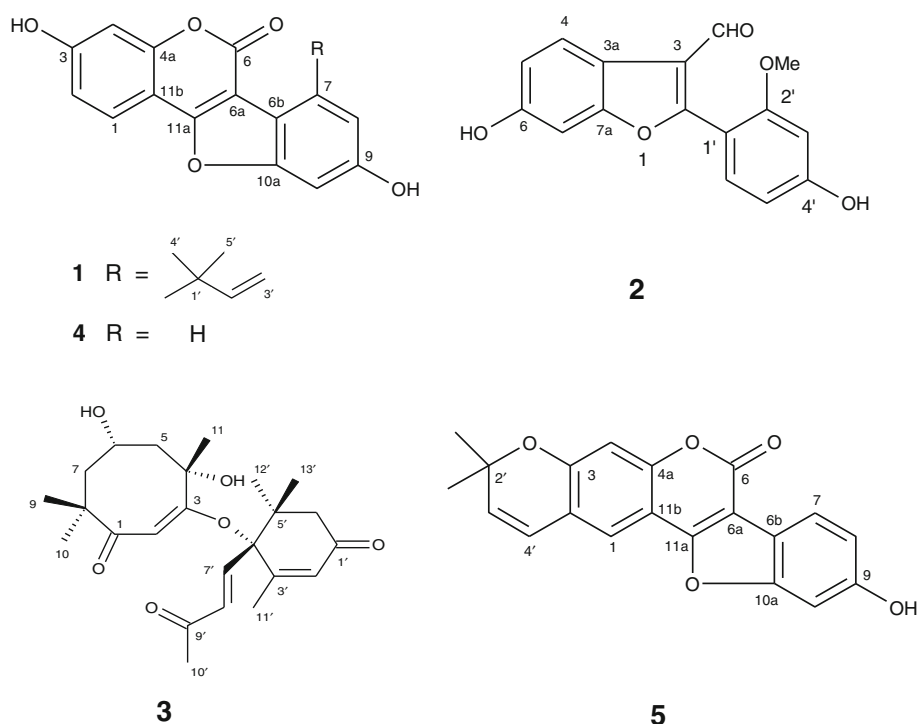
peak at *m/z* 419.2429 [M + H]⁺, corresponding to a molecular formula of $C_{24}H_{34}O_6$ with eight degrees of unsaturation. The IR spectrum showed absorption bands at 3437, 1713, 1665, 1424, 1370 and 1258 cm⁻¹, which were assignable to hydroxyl, conjugated carbonyl and double bond groups. The ¹H-NMR spectrum displayed the signals of seven tertiary methyl units at δ_H 2.31 (3H, s, H-10′), 1.89 (3H, d, *J* = 1.3 Hz, H-11′), 1.79 (3H, s, H-11), 1.47 (3H, s, H-9), 1.28 (3H, s, H-10), 1.11 (3H, s, H-12′) and 1.03 (3H, s, H-13′), a double bond with *E* configuration at δ_H 6.84 (1H, d, *J* = 15.7 Hz, H-7′) and 6.47 (1H, d, *J* = 15.7 Hz, H-8′), two trisubstituted olefinic protons at δ_H 5.96 (1H, br s, H-2′) and 5.70 (1H, s, H-2), and an oxygen-bearing proton at δ_H 4.34 (1H, m, H-6). The ¹³C-NMR (Table 1) and DEPT spectra exhibited 24 carbon resonances, including seven methyl, three methylene, five methine (one oxygenated, four olefinic) and nine quaternary (three carbonyl, two olefinic, two oxygenated) carbons. Furthermore, the ¹H–¹H COSY spectrum showed correlations in a sequence of two methylenes at δ_H 2.46 (1H, m, H$_a$-5), 1.78 (1H, m, H$_b$-5), 1.98 (1H, m, H$_a$-7) and 1.54 (1H, m, H$_b$-7) and a methine at δ_H 4.34 (1H, m, H-6), suggesting that H-6 coupled with the signals of H$_2$-5 and H$_2$-7. The latter, together with the crucial ¹H–¹³C long-range correlations from H-2 (δ_C 5.70) to C-1 (δ_C 182.6), C-3 (δ_C 172.3), C-4 (δ_C 86.9) and C-8 (δ_C 36.1) and from H-6 (δ_H 4.34) to C-4 (δ_C 86.9), C-5 (δ_C 45.8), C-7 (δ_C 47.5) and C-8, which were observed in the HMBC spectrum of **3**
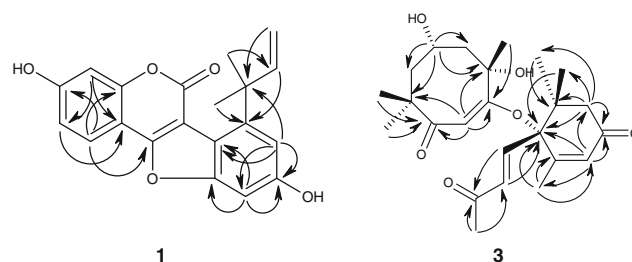

Fig. 2 Key HMBC correlations of compounds **1** and **3**

(Fig. 2), revealed the presence of an eight-membered ring, which is an unusual structural moiety in natural products. In addition, the HMBC correlations from H-2′ (δ_H 5.96) to C-4′ (δ_C 79.5), C-6′ (δ_C 49.8) and C-11′ (δ_C 18.9), from H$_2$-6′ (δ_H 2.51 and 2.34) to C-1′ (δ_C 197.1), C-4′ (δ_C 79.5), C-5′ (δ_C 41.6), C-12′ (δ_C 23.1) and C-13′ (δ_C 24.5) and from H$_3$-11′ (δ_H 1.89) to C-2′ (δ_C 127.9), C-3′ (δ_C 160.5) and C-4′ indicated a cyclic six-membered α,β-unsaturated ketone moiety. In the ¹³C- and ¹H-NMR spectra (in CDCl₃, at 25°C) of **3**, the C-2 at δ_C 113.1 and C-3 at δ_C 172.3 of the eight-membered ring moiety which presented as an enol form, as well as H-2 at δ_H 5.70 attached to C-2, all displayed sharp singlets. When measured at 55°C and −65°C, the signal shapes of these proton and carbons had almost no change. In accordance with this behavior, it was concluded that the keto-enol interconversion was blocked

Fig. 3 Key ROESY correlations of compound **3**
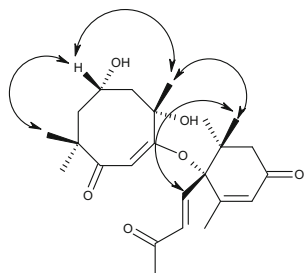
and gave rise to the NMR equivalence of the proton and carbons in positions 2 and 3. Taken together, these data confirmed that the cyclic six-membered α,β-unsaturated ketone moiety was connected to the eight-membered ring at C-3. Based on the above data and comprehensive 2D NMR experiments (^{1}H–^{1}H COSY, HSQC, HMBC), the structure of **3** was established as shown in Fig. 1. The relative configuration of **3** was determined from ROESY data. In the ROESY spectrum (Fig. 3), cross-peaks from H-6 to H$_3$-11 and H$_3$-9, as well as from H$_3$-13′ to H$_3$-11 and H-7′, indicated that H-6, H$_3$-9, H$_3$-11, H-7′ and H$_3$-13′ were co-facial and β-oriented, while H$_3$-12′ and H$_3$-10 were on the opposite side of the molecular plane and thus α-oriented. In this case, the molecular modeling indicated that the conjugated octanone ring was in a twisted boat conformation state and could possess the lowest energy.

Phytochemical studies of this plant also resulted in the isolation of three known constituents: puerariafuran (**2**), coumestrol (**4**) and 9-hydroxy-2′,2′-dimethylpyrano[5′,6′: 2,3]-coumestan (**5**) [13–15]. The three known compounds were identified by comparison of their physical and spectra data with reported values.

Compounds **1–5** and ginkgolide B were evaluated for anti-inflammatory activities using established methods [16, 17]; these compounds showed inhibitory effects on the release of β-glucuronidase from polymorphonuclear leukocytes (PMNs) of rats, and the IC$_{50}$ values were 7.8 μM (**1**), 8.6 μM (**2**), 9.1 μM (**3**), 7.4 μM (**4**), 6.3 μM (**5**) and 5.6 μM (ginkgolide B), respectively. Based on the bioassay results, it is concluded that compounds **1–5** possess anti-inflammatory activities similar to that of the positive control (ginkgolide B).

Experimental

General

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV-160 spectrophotometer. IR spectra were determined on a Perkin-Elmer 683 infrared spectrometer with KBr disks.

ESIMS were measured on a Bruker Esquire 3000 Plus spectrometer. HR-ESIMS were recorded on a Micromass Q-Tof Global mass spectrometer. NMR spectra were obtained on a Varian Unity BRUKER 400 at 400 MHz (^{1}H) and 100 MHz (^{13}C) with TMS as the 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 was collected in Linyi district, Shandong Province, People's Republic of China, in September 2009, and identified by Professor Yan–Yan Zhao, School of Pharmaceutical Science, Yantai University. The whole plant of *S. lyratu*m 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 × 3) with refluxing EtOH (80 L × 3). Evaporation of the solvent under reduced pressure provided the ethanolic extract (2.1 kg). The extract was dissolved and suspended in H$_2$O, and partitioned with CHCl$_3$, EtOAc and *n*-BuOH. The CHCl$_3$ fraction (217.1 g) was initially subjected to silica gel column (10 × 90 cm) chromatography (200–300 mesh, 2.0 kg) and eluted with cyclohexane–acetone at 95:5 (6.0 L), 90:10 (6.0 L), 85:15 (6.0 L), 80:20 (7.0 L), 75:25 (7.0 L), 70:30 (7.0 L), 60:40 (5.0 L), and 50:50 (3.0 L) to give eight fractions. Fraction 2 (2.9 g) was separated by CC over silica gel [eluted by cyclohexane–acetone (100:0–70:30)] to obtain compound **4** (157.3 mg). The fraction 6 (5.3 g) was separated by CC over silica gel [eluted by cyclohexane–acetone (95:5–55:45)], Sephadex LH-20 [100 g, eluted with EtOAc–EtOH, 50:50, v/v] and preparative TLC [CHCl$_3$–EtOAc, 5:1, v/v] to afford compounds **1** (56.6 mg), **2** (37.2 mg), **3** (15.7 mg) and **5** (91.4 mg).

Solalyratin A (1): Yellow powder. UV (CH$_3$OH) λ_{max}: 250, 309 and 351 nm. IR (KBr) ν_{max}: 3339, 1661, 1617, 1593, 1457 and 1085 cm^{-1}. ESIMS *m/z*: 337.2 [M + H]$^+$. HR-ESIMS *m/z*: 337.1072 [M + H]$^+$ (Calcd. for C$_{20}$H$_{16}$O$_5$, 337.1076). ^{1}H- and ^{13}C-NMR data, see Table 1.

Solalyratin B (3): White powder, $[\alpha]_D^{25}$ +60.1 (*c* 0.5, CH$_3$OH). UV (CHCl$_3$) λ_{max}: 219 nm. IR (KBr) ν_{max}: 3437, 2928, 1713, 1665, 1424, 1370, 1258, 1127 and 1025 cm^{-1}. ESIMS *m/z*: 419.2 [M + H]$^+$. HR-ESIMS *m/z*: 419.2429 [M + H]$^+$ (Calcd. for C$_{24}$H$_{34}$O$_6$, 419.2434). ^{1}H- and ^{13}C-NMR data, see Table 1.

Anti-inflammatory bioassays

The effects of compounds **1–5** and ginkgolide B on the release of β-glucuronidase in rat PMNs induced by PAF in vitro were evaluated. Rat PMNs were incubated with vehicle or various concentrations of test compounds at 37°C for 15 min. Cytochalasin B (10 μM) was then added. After 5 min, PAF (1 μM) was added prior to the termination of the reaction. The supernatants of the reaction were incubated with phenolphthalein glucuronic acid (0.4 mM) at 37°C for 18 h. The absorbance was read at 550 nm, and the IC$_{50}$ values were calculated. Ginkgolide B (Sigma, 98% pure) was used as positive control.

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