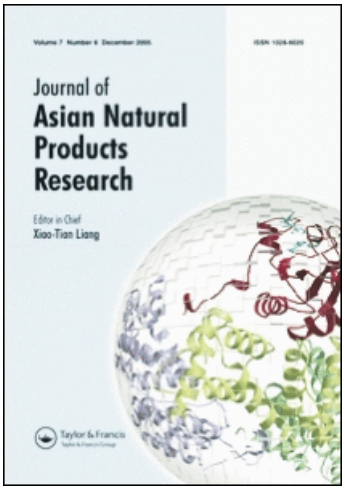

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A new lignan and anti-inflammatory flavonoids from *Kerria japonica*

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A new lignan and anti-inflammatory flavonoids from *Kerria japonica*

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A new lignan named kerinol (**1**) as well as 12 known compounds were isolated from the twigs of *Kerria japonica* (L.) DC (Rosaceae). Their structures were elucidated on the basis of spectroscopic analysis. Linariin (**3**) and isolinariin B (**4**), two major flavonoids of *K. japonica*, were found to exhibit significant anti-inflammatory activities in mice *in vivo* with an inhibitory rate of 25.5% ($p < 0.001$) and 13.1% ($p < 0.05$), respectively.

Keywords: *Kerria japonica* (L.) DC; Rosaceae; lignan; flavonoids; anti-inflammatory activity

1. Introduction

Kerria japonica (L.) DC (Rosaceae) is a plant distributed widely in the south of China. The flowers and twigs of *K. japonica* have been used in Chinese folk medicine with antitussive, antiarthritic, eliminating edema, and curing fever-toxic tumefaction effects.[1] Previous phytochemical studies on this plant have led to the isolation of di-*O*-palmitoyl-all-*trans*-xanthophyll and sutherlandin.[2] This paper describes the isolation and structure elucidation of a new lignan named kerinol (**1**) and 12 known compounds (Figure 1). The known compounds were identified as (−)-carinol (**2**),[3,4] linariin (**3**),[5] isolinariin B (**4**),[5] (−)-olivil,[6] sutherlandin-5-*trans*-*p*-coumarate,[7] (−)-masoniresinol,[6] (+)-pinoresinol,[8] prinsepiol,[9] 8-hydroxypinoresinol,[10] (+)-pinoresinol-4′-*O*-β-D-glucopyranoside,[8] (+)-1-hydroxy-syringaresinol,[8] and syringaresinol-4′-*O*-β-D-glucopyranoside[11] by comparing its spectroscopic data with reported values. All compounds were reported for the first time from the genus *Kerria*. In a preliminary pharmacological test, compounds **3** and **4** were found to exhibit significant anti-inflammatory activities in mice *in vivo* with an inhibitory rate of 25.5% ($p < 0.001$) and 13.1% ($p < 0.05$), respectively.

2. Results and discussion

Compound **1** was obtained as an amorphous powder with the molecular formula $C_{20}H_{26}O_8$ deduced from HR-ESI-MS and NMR analyses. The ^{1}H NMR spectrum of **1** showed proton signals due to ABX system at δ_H 7.00 (2H, d, $J = 1.5$ Hz), 6.72 (2H, d, $J = 8.1$ Hz), and 6.80 (2H, dd, $J = 8.1$, 1.5 Hz), two methoxy signals at δ_H 3.82 (6H, s), four benzylic proton signals at δ_H 3.10 (2H, d, $J = 13.6$ Hz) and 3.00 (2H, d, $J = 13.6$ Hz), and four oxygenated methylene signals at δ_H 3.60 (2H, d, $J = 10.6$ Hz) and 3.52 (2H, br d, $J = 10.6$ Hz). The ^{13}C NMR spectrum of **1** displayed 10 carbon resonances, separated by DEPT experiment into one methyl, two methylenes, three methines, and four quaternary carbons. A combined analysis of NMR data and molecular weight revealed that

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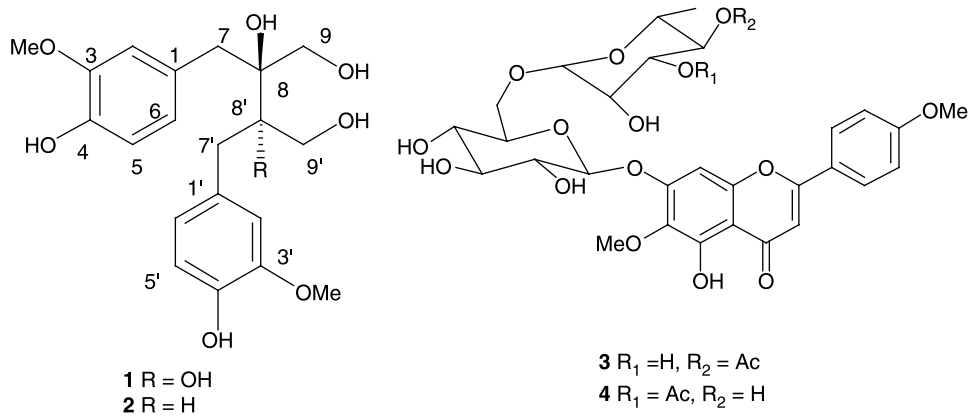

Figure 1. Structures of compounds **1**–**4**.

compound **1** possesses a symmetrical structure and possibly with the dibenzylbutane lignan skeleton. The HMQC spectrum of **1** allowed the assignment of each proton signal to the relevant carbon resonance, and defined the three singlets at δ_H 7.32 (1H, s), 4.80 (1H, br s), and 3.90 (1H, s) to be hydroxy proton signals. The planar structural skeleton of **1** was further established on the basis of its HMBC spectrum in which $^1H-^{13}C$ long-range correlations were observed at H-2/C-4, C-6; H-5/C-3, C-1; H-6/C-2, C-4, C-7; H-7a and H-7b/C-2, C-6, C-9; H-9a and H-9b/C-8, C-7; 3-OMe/C-3; 4-OH/C-3, C-4, C-5; 8-OH/C-7, C-8, C-9 (Figure 2). The methoxyl substitution on the aryl ring was confirmed by the NOESY cross-peak between H-2 (δ_H 7.00) and 3-OCH_3 (δ_H 3.82) (Figure 2). Compound **1** was thus characterized as the new 1,2,3,4-butanetetrol,2,3-bis[(4-hydroxy-3-methoxyphenyl)methyl] and named kerinol. The specific optical rotation value $[\alpha]_D^{22}$ − 30.6 (*c* 0.32, MeOH) of compound **1** indicated that it is not a mesomer, which suggested the absolute configuration of C-8 and C-8′ to be *R*,*R* or *S*,*S*. According to literature,[12] the biosynthesis pathway of **1** may be related to (−)-masoniresinol or (−)-carinol (**2**) under the redox system.

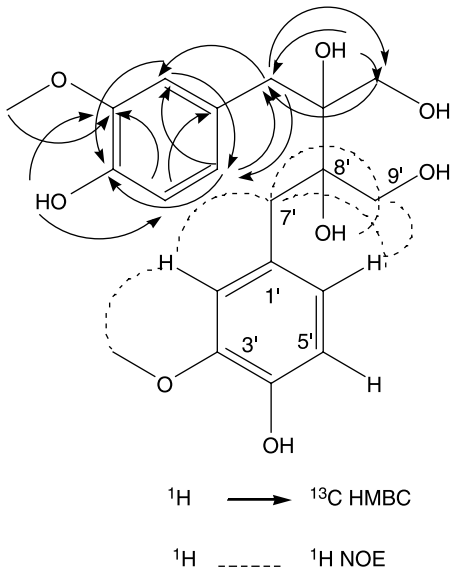

Figure 2. Key HMBC and NOE correlations for **1**.

According to our research results, compounds **3** and **4** were the main components of *K. japonica*, and their anti-inflammatory activities were evaluated in mice *in vivo*. As a result, the two flavanoid glycosides were found to exhibit a significant anti-inflammatory activity with inhibitory rates of 25.5 and 13.1% at a dose of 50 mg/kg/i.p., respectively. Therefore, compounds **3** and **4** were suggested to contribute in part to the antiarthritic and eliminating edema effect of *K. japonica*, although lignans from the same plant may also display an anti-inflammatory effect.[13] Additionally, sutherlandin-5-*trans*-*p*-coumarate, the cyano-containing compound, was supposed to contribute to the

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **1** (in acetone-d_6).

No.	δ_H (*J*, Hz)	δ_C	HMBC ($^{1}H \rightarrow {}^{13}C$)
1 (1′)		129.5 (s)	
2 (2′)	7.00 (d, 1.5)	115.2 (d)	C-4 (4′), C-6 (6′)
3 (3′)		147.3 (s)	
4 (4′)		145.4 (s)	
5 (5′)	6.72 (d, 8.1)	114.7 (d)	C-1 (1′), C-3 (3′)
6 (6′)	6.80 (dd, 8.1, 1.5)	124.1 (d)	C-2 (2′), C-4 (4′), C-7 (7′)
7a (7a′)	3.10 (d, 13.6)	37.6 (t)	C-2 (2′), C-6 (6′), C-9 (9′)
7b (7b′)	3.00 (d, 13.6)		C-2 (2′), C-6 (6′), C-9 (9′)
8 (8′)		77.7 (s)	
9a (9a′)	3.60 (d, 10.6)	64.0 (t)	C-7 (7′), C-8 (8′)
9b (9b′)	3.52 (br d, 10.6)		C-7 (7′), C-8 (8′)
3-OCH_3 (3′-OCH_3)	3.82 (s)	55.8 (q)	C-3 (3′)
4-OH (4′-OH)	7.32 (s)		C-3 (3′), C-4(4′), C-5(5′)
8-OH (8′-OH)	3.90 (s)		C-7(7′), C-8 (8′), C-9(9′)
9-OH (9′-OH)	4.80 (br s)		

reported antitussive effect of the plant.[14] All isolated compounds were reported for the first time from the genus *Kerria*.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured using Perkin–Elmer 341 polarimeter. IR spectra were recorded using a Perkin–Elmer 577 spectrometer. ESI-MS were measured using a Finnigan LCQ-DECA instrument, and HR-ESI-MS data were obtained on a Mariner spectrometer. The NMR experiments were run on a Bruker AM 400 spectrometer with TMS as the internal standard except when indicated otherwise. Preparative HPLC was carried out using a Varian SD-1 instrument equipped with a Merck NW25 C_{18} column (20 × 250 mm, 10 μm) and ProStar 320 UV–vis Detector. Column chromatographic separations were carried out using Si gel H60 (300–400 mesh), ZCX-II (100–200 mesh; Qingdao Mar. Chemical Group Corporation, Qingdao, China), and macroporous resin D101 (Huazhen Polymer Co., Ltd, Shanghai, China) as packing materials. Lobar column chromatography was carried out using Lichroprep Si60 (40–63 μm; Merck, Germany) equipped with a Lab Alliance Series I pump (LabAlliance Co., America). HSGF254 Si gel TLC plates (Yantai Chemical Industrial Institute, Yantai, China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC.

3.2 Plant material

The twigs of *K. japonica* were collected from the suburb of Chongqing in October 2006, and identified by Professor Jingui Shen of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (No. 061218SIMM) has been deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

Air-dried and powdered twigs (15 kg) of *K. japonica* were extracted with 95% EtOH four times, each for a week at room temperature. The extract was concentrated to dryness *in vacuo* and then suspended in 20% EtOH overnight. After filtration of the precipitated chlorophyll and removal of EtOH from the filtrate *in vacuo*, the aqueous residue (4 l) was partitioned with $CHCl_3$ (21 × 3) and *n*-butanol (21 × 3), successively, to yield the $CHCl_3$ extract (64 g) and *n*-butanol extract (165 g), respectively. The *n*-butanol extract (165 g) was subjected to a series of chromatographic techniques over macroporous resin D101, Si gel H60, Sephadex LH-20, JH-C_2H_5-C_6H_5, Lobar SiO_2 column, RP-18 preparative HPLC,

Table 2. Anti-inflammatory effects of linariin (**3**) and isolinariin B (**4**) in mice *in vivo*.

Treatment	Dose (mg/kg, i.p.)	Paw weight increase (mg)	Percentage of inhibition (%)	*p*
Control		93.8 ± 14.1		
Linariin (**3**)	50	69.8 ± 11.1	25.5	<0.001
Isolinariin B (**4**)	50	81.5 ± 9.96	13.1	<0.05

and SiO_2 PTLC to afford compounds **1** (18.5 mg), (−)-carinol **2** (11.6 mg), linariin **3** (2.3 g), isolinariin B **4** (1.6 g), (−)-olivil (30.8 mg), sutherlandin-5-*trans*-*p*-coumarate (40.8 mg), (−)-masoniresinol (12.2 mg), (+)-pinoresinol (24.2 mg), prinsepiol (10.5 mg), 8-hydroxypinoresinol (18.4 mg), (+)-pinoresinol-4′-*O*-β-D-glucopyranoside (5.7 mg), (+)-1-hydroxy-syringaresinol (7.6 mg), and syringaresinol-4′-*O*-β-D-glucopyranoside (36.4 mg).

*3.3.1 1,2,3,4-Butanetetrol,2,3-bis[(4-hydroxy-3-methoxyphenyl)methyl] (**1**)*

White amorphous powder; $[\alpha]_D^{22}$ − 30.6 (*c* 0.32, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3513–3249, 2937, 1600, 1517, 1430, 1363, 1276, 1279, 1124, 1016, 1031, 829, 561; ^1H and ^{13}C NMR spectral data, see Table 1; LRESIMS *m/z* 417.2 $[M + Na]^+$; HR-ESI-MS *m/z* 417.1534 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_8Na$, 417.1525).

3.4 Anti-inflammatory assay

Male Kunming mice (Shanghai Experiment Animal Center of Chinese Academy of Sciences, China) weighing 24 ± 1 g were used in this assay. The mice were randomly divided into three groups: control, compound **3** group, and compound **4** group ($n = 10$, each). These groups wereinjected with vehicle (0.9% NaCl), compound **3** (50 mg/kg), and compound **4** (50 mg/kg), respectively, in a volume of 1 ml/100 g body weight. Thirty minutes after injection, 20 μl of 0.2% carrageenin (Type II, Sigma Chemical Co., St. Louis, Mo., USA), prepared as a suspension in sterile 0.9% NaCl, was injected in the right hind paw of mice.[15] Four hours later, the mice were sacrificed and the increase in the paw weight was measured. The values were expressed as mean ± SD and the results were analyzed by Student's *t*-test. The results are listed in Table 2.

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