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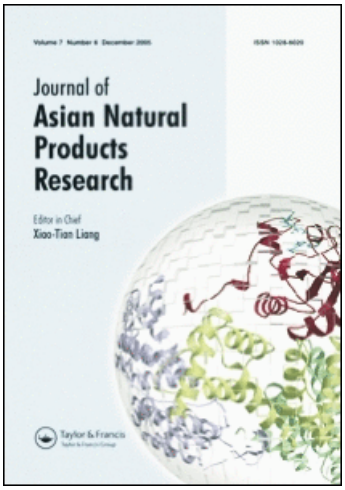

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Note

PTP1B inhibitors from *Saussrurea Lappa*

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A new lignan glycoside, named 1,5-dihydroxypinoresinol-4′-*O*-β-D-glucopyranoside (**1**), has been isolated from the EtOH extract of the roots of *Saussurea lappa*, together with twenty known compounds: (+)-1-hydroxypinoresinol-1-*O*-β-D-glucopyranoside (**2**), fraxiresinol-4′-*O*-β-D-glucopyranoside (**3**), (−)-olivil-4″-*O*-β-D-glucopyranoside (**4**), 4-allyl-2,6-dimethoxybenzene-1-*O*-β-D-glucopyranoside, syringin, costunolide-15-*O*-β-D-glucopyranoside, chlorogenic acid, aloe-emodin-8-*O*-β-D-glucopyranoside (**5**), rhein-8-*O*-β-D-glucopyranoside (**6**), chrysophanol (**7**), emodin, dehydrocostus lactone, costunolide, β-costic acid, reynosin, arbusculin A, α-cyclocostunolide, β-cyclocostunolide, santamarine and magnolialide. Three anthraquinones (**5**–**7**) showed moderate bioactivity against human Protein Tyrosine Phosphatase 1B (hPTP1B) *in vitro*.

Keywords: *Saussurea lappa*; PTP1B inhibitors; 1,5-Dihydroxypinoresinol-4′-*O*-β-D-glucopyranoside

1. Introduction

Saussureae Radix, the roots of *Saussurea lappa* Clarke (Compositae), is a Chinese traditional herbal medicine used as an aromatic stomachic and pertumery. Several sesquiterpenes, such as costunolide and dehydrocostus lactone, have been isolated from Indian *S. lappa* [1–3]. There have also been some reports describing the pharmacological activities of extracts or principal constituents of *S. lappa*, such as anti-ulcer [4,5], anti-carcinogenesis in rats [6,7], vasorelaxant effect [8], inhibitory effects on killing activity of cytotoxic T lymphocytes [9], and inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages [10]. Our studies on natural constituents with hPTP1B inhibitory activity [11–13] revealed that the EtOH extract of Chinese *S. lappa* has a potent inhibitory effect on PTP1B ($IC_{50} = 3.5\ \mu g\ mL^{-1}$). Chemical investigation on the water-soluble fraction yielded a new lignan glycoside, 1,5-dihydroxypinoresinol-4′-*O*-β-D-glucopyranoside (**1**), and nine known compounds: (+)-1-hydroxypinoresinol-1-*O*-β-D-glucopyranoside (**2**) [14], fraxiresinol-4′-*O*-β-D-glucopyranoside (**3**) [15], (−)-olivil-4″-*O*-β-D-glucopyranoside (**4**)

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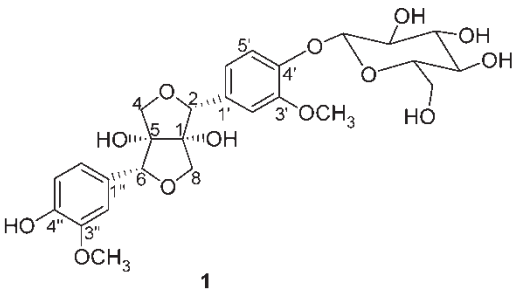
[16], 4-allyl-2,6-dimethoxybenzene-1-*O*-β-D-glucopyranoside [17], syringin [18], costunolide-15-*O*-β-D-glucopyranoside [19], chlorogenic acid [20], aloe-emodin-8-*O*-β-D-glucopyranoside (**5**) [21] and rhein-8-*O*-β-D-glucopyranoside (**6**) [22]. Chemical investigation on the EtOAc-soluble fraction yielded eleven known compounds: chrysophanol (**7**), emodin [21], dehydrocostus lactone, costunolide, β-costic acid, reynosin, arbusculin A, α-cyclocostunolide, β-cyclocostunolide, santamarine and magnolialide [19].



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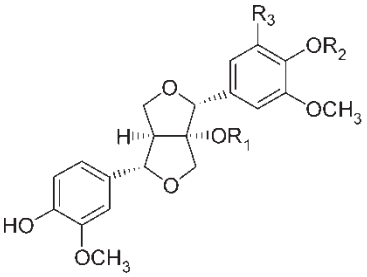
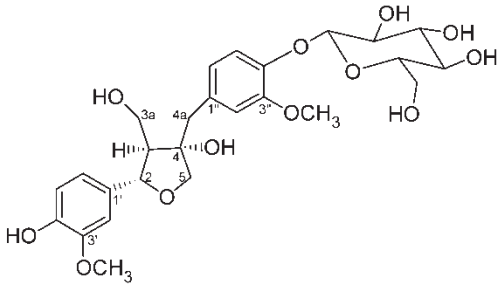


2 R_1=glc R_2=R_3=H
3 R_1=H R_2=glc R_3=OCH_3



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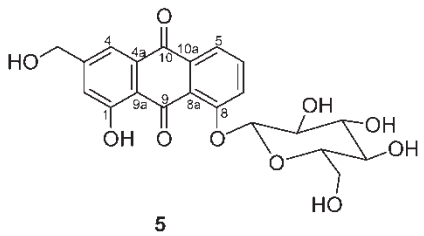

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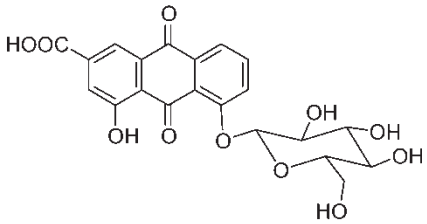

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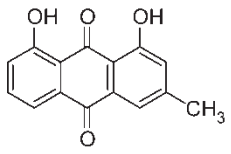

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2. Results and discussion

The molecular formula of **1** was established as $C_{26}H_{32}O_{13}$ on the basis of negative HR-ESIMS (*m/z* 551.1758 $[M - H]^-$) spectra. The UV spectrum showed maxima at 233 and 278 nm, which correspond to the furofuran-type lignan, *viz.* 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane [23]. The IR spectrum suggests the presence of hydroxyl group (3396 cm^{-1}) and benzene ring (1600, 1516, 1456 cm^{-1}). The ^1H NMR (table 1) signal pattern in the aromatic ring suggests the presence of two 1,2,4-trisubstituted aryl rings (δ_H: 7.10, d, $J = 1.6$ Hz; 7.14, d, $J = 8.3$ Hz; 6.94, dd, $J = 8.3, 1.6$ Hz; and 7.03, d, $J = 1.6$ Hz; 6.78, dd, $J = 8.1, 1.6$ Hz; 6.84, dd, $J = 8.1, 1.6$ Hz). It also exhibits signals of two methoxyl groups at δ 3.86 and 3.84, two oxygenated methylene groups (δ_H: 3.97–4.12, 4H, H_2-4 and H_2-8), two oxygenated methine groups (δ_H: 5.00, s, H-2; 4.96, s, H-6), and an anomeric proton doublet of D-glucopyranose at δ4.88 ($J = 7.2$ Hz).

^{13}C NMR and DEPT spectra indicate the presence of twenty-six carbon signals, including two 3-methoxy-4-hydroxyaryl groups (confirmed by HMBC spectrum), a glycopyranose group and six oxygenated carbons (two methylene, two methine and two quaternary carbons). Based on the NMR data of compound **1**, and comparison of the six-oxygenated carbons signals with those of kigeliol [24], the six oxygenated carbons of **1** formed a 3,7-dioxabicyclo[3.3.0]octane skeleton, which was supported by its HMBC cross peaks (figure 1).

The long-range relationships in the HMBC spectrum (figure 1) between the methoxy at δ 3.86 and the aromatic carbon at δ 113.8 (C-2′), and between methoxy at δ 3.84 and

Table 1. ^1H and ^{13}C NMR data of **1** (in CD_3OD, 400 MHz).

	1		
Position	*^1H*	*^{13}C*	*HMBC*
1	–	89.4	
2	5.00 s	89.0	C-1, C-1′, C-2′, C-6′
4	eq 4.12 d (3.3) ax 3.99 br. s[a]	77.1[b]	C-2, C-5, C-6
5	–	89.5	
6	4.96 s	89.3	C-5, C-1″, C-2″, C-6″
8	eq 4.09 d (3.4) ax 3.97 br. sa	77.0[b]	C-1, C-2, C-6
1′	–	133.5	
2′	7.10 d (1.6)	113.8	C-1′, C-4′, C-6′
3′	–	150.7	
4′	–	147.9	
5′	7.14 d (8.3)	117.7	C-1′, C-3′, C-4′, C-6′
6′	6.94 dd (1.6; 8.3)	121.7	C-2′, C-4′, C-5′
1″	–	129.8	
2″	7.03 d (1.6)	113.2	C-1″, C-3″, C-4″, C-6″
3″	–	148.9	
4″	–	147.7	
5″	6.78 d (8.1)	115.9	C-1″, C-3″, C-4″
6″	6.84 dd (1.6; 8.1)	121.9	C-2″, C-4″
Glc-1	4.88 d (7.2)	103.1	C-4′
Glc-2	3.48 m	75.2	Glc-1, Glc-3, Glc-4
Glc-3	3.47 m	78.1	Glc-1, Glc-2, Glc-4, Glc-5
Glc-4	3.38 m	71.6	Glc-3, Glc-5, Glc-6
Glc-5	3.39 m	78.4	Glc-1, Glc-4, Glc-6
Glc-6	3.69 dd	62.8	Glc-4, Glc-5
OCH_3	3.86 s	57.1	C-3′, C-2′
OCH_3	3.84 s	56.7	C-3″, C-2″

Assignment based on DEPT, NOESY, HMQC and HMBC experiments. *J* values in parentheses in Hz.
[a,b] Tentative assignments, and may be interchanged.

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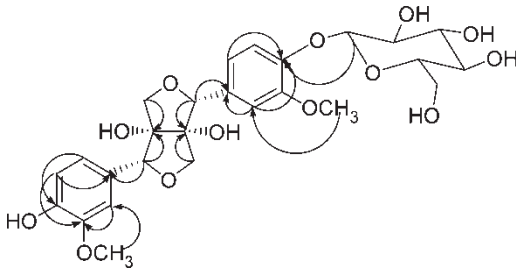

Figure 1. Key HMBC correlations for compound **1**.

the aromatic carbon at δ 113.2 (C-2″), support the idea that the two methoxy groups are at C-3′ and C-3″, respectively. HMBC cross peaks between the methine at δ 5.00 (H-2) and the aromatic carbon at δ 133.5 (C-1′), and between the methine at δ 4.96 (H-6) and the aromatic carbon at δ 129.8 (C-1″), reveal that the two 3-methoxy-4-hydroxyaryl groups are at C-2 and C-6, respectively. The D-glucopyranose at 4′-OH was deduced from the long-range relationship between the C-4′ (δ 147.9) and the H-Glc-1 (δ 4.88, d, $J = 7.2$ Hz).

^{1}H NMR is useful in establishing the stereochemistry of 2,6-diary-3,7-dioxabicyclo[3.3.0]octane lignan. In *epi* and *dia* series, an axial aryl group will cause an upfield shift of the axial proton in the opposite methylene ring to δ 3.25–3.45 [25]. The two methylene proton signals of **1** appeared between δ 3.97 and 4.12; therefore, it should possess diequatorial aryl groups. Thus, the structure of **1** was elucidated as 2*R*,6*R*-bis(3-hydroxy-4-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane-1*S*,5*S*-diol-4′-*O*-β-D-glucopyranoside.

The other compounds were identified by comparison of their physical and spectral data with those of reported values.

The 21 compounds were all tested for their inhibitory activity against hPTP1B *in vitro*. Anthraquinones and anthraquinone glycosides, except emodin, showed moderate inhibitory activity (results are summarized in table 2).

3. Experimental

3.1 General experimental procedures

Melting points were determined using an XT-4 point apparatus and are uncorrected. Optical rotations were determined using a Perkin–Elmer Polarimeter 341. UV spectra were measured on a Shimadzu UV-250 spectrometer. IR spectra were obtained on a Perkin-Elmer 599B spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-Vx300M spectrometer, except for compounds **1** (Bruker AM-400 spectrometer). EIMS were measured on a Shimadzu GCMS-QP5050A spectrometer, and HR-ESIMS were measured on a Bruker Atex III spectrometer.

Table 2. hPTP1B inhibitory activity of **5**–**7** (IC_{50} μM).

	EtOH extract ($\mu g\ ml^{-1}$)	***5***	***6***	***7***	*$NaVO_3$* ($\mu g\ ml^{-1}$)
PTP1B	3.5	26.6	11.5	25.6	2.0

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3.2. Plant material

Roots of *Saussrurea lappa* were collected from Lijiang, Yunnan province, China, in July 2002, and identified by Professor Feng-chang Lou. A voucher specimen (2002011) has been deposited at the herbarium of Chinese National Center for Drug Screening, Shanghai, China.

3.3 Biological assays for the inhibition of hPTP1B

For human PTP1B enzyme (100 nM), *p*-nitrophenyl phosphate (p-NPP) (10 mM) was used as substrate. Na_3VO_4 was employed as a positive control ($IC_{50} = 2\,\mu M$). Three independent measurements were performed for IC_{50} determinations [26].

3.4 Extraction and isolation

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Dried, powdered plant material (5 kg) was extracted with EtOH (3 ×) at room temperature during 3 weeks. The extract was evaporated to dryness to obtain the EtOH extract. This extract was then dissolved in water and extracted with EtOAc (3 ×, each 1 L) to obtain a water solution and a EtOAc solution. The former was subjected to D-101 macroporous resin column, eluting with EtOH-water (10% → 20% → 30% → 40% → 50%), to yield five fractions. Fractions 3–5 were then subjected to silica gel (200–300 mesh) column chromatography, eluting with $CHCl_3$–CH_3OH–H_2O (from 20:1:0.1 to 4:1:0.1), then further separated by flash ODS (CH_3OH–H_2O) and Sephadex LH-20 (CH_3OH–H_2O, 1:1) columns to give ten pure compounds: 1,5-dihydroxy-pinoresinol-4′-*O*-β-D-glucopyranoside (**1**) (56 mg), (+)-1-hydroxypinoresinol-1-*O*-β-D-glucopyranoside (**2**) (35 mg), fraxiresinol-4′-*O*-β-D-glucopyranoside (**3**) (28 mg), (−)-olivil 4″-*O*-β-D-glucopyranoside (**4**) (66 mg), 4-allyl-2,6-dimethoxybenzene-1-*O*-β-D-glucopyranoside (28 mg), syringin (900 mg), costunolid-15-β-D-glucopyranoside (34 mg), chlorogenic acid (120 mg), aloe-emodin-8-*O*-β-D-glucopyranoside (**5**) (39 mg) and rhein-8-*O*-β-D-glucopyranoside (**6**) (24 mg).

The EtOAc extract was subjected to silica-gel column chromatography, eluting with light petroleum and EtOAc gradient, and further separated on flash ODS (CH_3OH–H_2O) to give eleven pure compounds: chrysophanol (**7**) (85 mg), emodin (18 mg), dehydrocostus lactone (2 g), costunolide (2.5 g), β-costic acid (55 mg), reynosin (45 mg), arbusculin A (22 mg), α-cyclocostunolide (78 mg), β-cyclocostunolide (17 mg), santamarine (65 mg) and magnolialide (29 mg).

1,5-Dihydroxypinoresinol-4′-*O*-β-D-glucopyranoside (**1**): amorphous powder from MeOH; $[\alpha]_D^{26}$: −41.2 (*c* 1.0, CH_3OH); UV λ_{max} (nm): 233 (3.86) and 278 (3.72); IR (KBr) ν_{max} (cm^{-1}): 3396 (OH), 2933, 2877 (aliphatic CH), 1600, 1516, 1456 (benzene C═C); negative HR-ESIMS: 551.1758 $[M - H]^-$ (calcd. for $C_{26}H_{31}O_{13}$, 551.1764); ^1H and ^{13}C NMR data see table 1.

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