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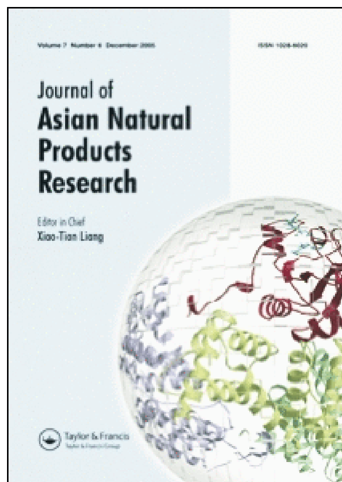

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Kai Xiao[a]; Qing-Hong Song[a]; Shu-Wei Zhang[a]; Li-Jiang Xuan[a]
[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

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Water-soluble constituents of the root barks of *Fraxinus rhynchophylla* (Chinese drug Qinpi)

KAI XIAO†‡, QING-HONG SONG†, SHU-WEI ZHANG† and LI-JIANG XUAN†*

†State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China
‡Laboratory of Toxin and Pharmarmacology, Faculty of Naval Medicine, Second Military Medical University, Shanghai 200433, China

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Chemical studies on the roots of *Fraxinus rhynchophylla* led to the isolation of fraxisecoside (**1**), a novel coumarin–secoiridoid hybrid glycoside, namely, fraxetin-8-*O*-[11′-methyl-oleosidyl-(7′ → 6″)]-β-D-glucopyranoside and 14 known compounds. Their structures were elucidated based on chemical evidence and spectroscopic analysis, including extensive 2D NMR methods. Compound **2** was first isolated as a pure compound. Compound **1** exhibited moderate PTP1B inhibition activity. Compounds **1** and **2** showed inhibition activity against B- and T-cell proliferation, without cytotoxicity.

Keywords: *Fraxinus rhynchophylla*; Oleaceae; Fraxisecoside; PTP1B inhibition activity

1. Introduction

Fraxinus rhynchophylla (Oleaceae) is one of the sources of the traditional Chinese herbal drug, *Cortex fraxini* (*Qinpi*). It has been used as an anti-inflammation agent for the treatment of enteritis, tracheitis, acute conjunctivitis, dysentery as well as psoriasis. There have been many chemical studies on other sources of *Cortex fraxini* (*Qinpi*); however, chemical studies on *Fraxinus rhynchophylla* are rare. To our knowledge, there is only one report about the isolation and nitric oxide synthase (iNOS) inhibitory activity of ferulaldehyde and scopoletin together with fraxidin [1].

In our investigation of the bark of *F. rhynchophylla*, a novel compound consisting of a coumarin glucoside unit and a secoiridoid glucoside unit linked *via* an ester function, named fraxisecoside (**1**), together with 14 known compounds were isolated. Compounds **2**–**15** were identified by comparison of their spectroscopic data with those reported in the literature as hydroxyframoside A (**2**) [2], jasmultiside (**3**) [3], esculin (**4**) [4], fraxin (**5**) [5], fraxetin (**6**) [5], magnolioside (**7**) [6], esculetin (**8**) [5], (–)-pinoresinol (**9**) [7], osmanthuside H (**10**) [8],

*Corresponding author. Email: ljxuan@mail.shcnc.ac.cn

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3,4-dihydroxyphenthyl alcohol (**11**) [3], naringenin (**12**) [9], ligstroside (**13**) [10], asperuloside (**14**) [11], and salidroside (**15**) [12]. Compound **2** used to be reported as a mixture and here it was isolated as a pure compound for the first time. The structure of fraxisecoside (**1**) was elucidated by chemical properties and spectroscopic analysis, including 1D and extensive 2D NMR ($^1H-^1H$ COSY, HMBC, HMQC, and ROESY) methods.

2. Results and discussion

The aqueous acetone extract of the bark of *F. rhynchophylla* was subjected to Sephadex LH-20 column chromatography to give several fractions. The fractions were further purified through a combination of column chromatography on Sephadex LH-20, MCI gel CHP20P, ODS and Toyopearl HW-40F to give compounds **1**–**15**.

Compound **1** (figure 1) was obtained as a crystal-like solid, $[\alpha]_D^{25}$ −78.8 (*c* 0.19, MeOH), with a molecular formula of $C_{33}H_{40}O_{20}$ which was deduced by ESI-MS and elemental

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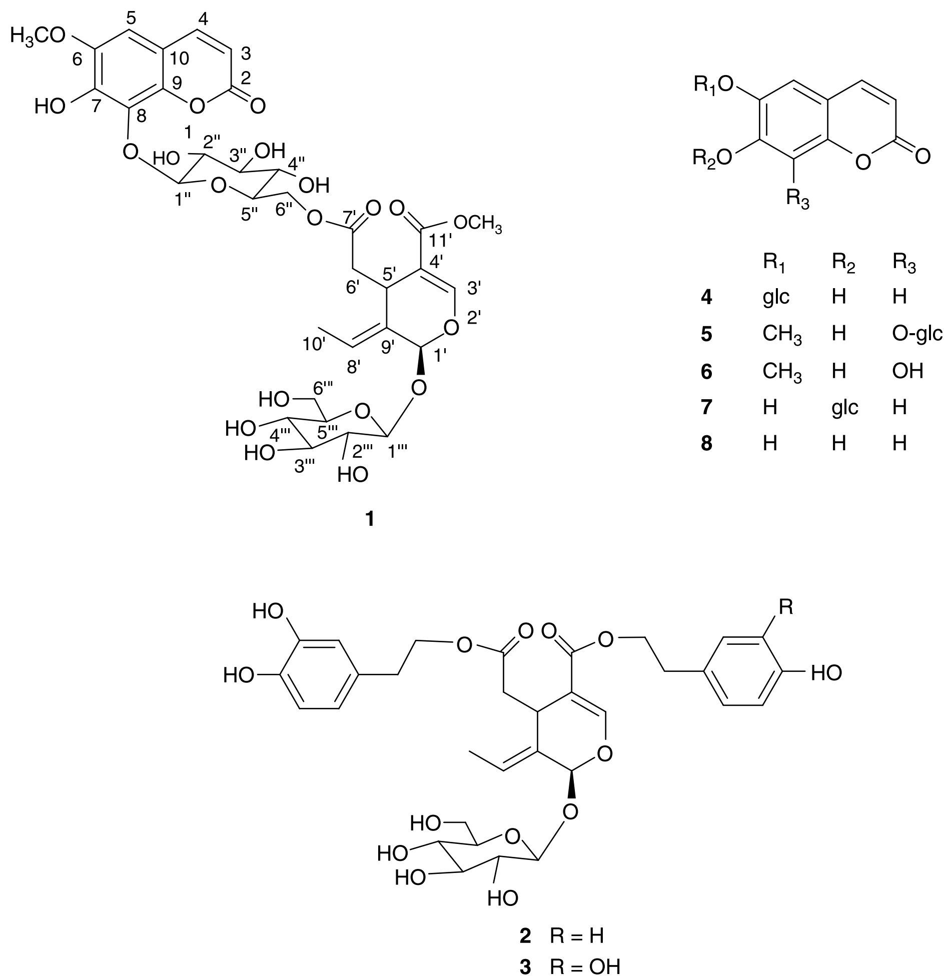

	R1	R2	R3
4	glc	H	H
5	CH_3	H	O-glc
6	CH_3	H	OH
7	H	glc	H
8	H	H	H

2 R = H
3 R = OH

Figure 1. Structures of fraxisecoside (**1**) and compounds **2**–**8**.

analysis. Positive result of Molish reaction suggested that **1** was a glycoside. The UV maxima at 232 and 349 nm (MeOH) and strong absorption bands of IR (1708, 1631, 1076 and 594 cm^{-1}) suggested the presence of a secoiridoid skeleton [2,13,14]. The IR spectrum exhibited strong bands for hydroxy (3411 cm^{-1}) and aromatic ring (1617 and 1504 cm^{-1}). ESI-MS gave quasimolecular ions $[M + Na]^+$ at *m/z* 779 and $[M-H]^-$ at *m/z* 755. In the ^1H NMR spectrum of **1**, the two protons resonating at δ 6.42 (1H, $J = 9.4$ Hz, H-3) and 8.05 (1H, $J = 9.4$ Hz, H-4) indicated a coumarin partial structure. In the ^1H NMR and ^{13}C NMR spectra of **1**, the signals of coumarin moiety were similar to those of the aglycon in **5**. In the NOESY spectrum, the signal at δ 7.14 showed correlation with H-4, so it is assigned to H-5. And also, the correlation of H-5 with protons at δ 4.10 suggested that the OCH_3 was attached to C-6. Hence, the coumarin moiety was assigned. While the signals at δ 7.64 (1H, s, H-3′), 6.24 (1H, q, $J = 7.0$ Hz, H-8′), 6.08 (1H, s, H-1′) and 1.83 (3H, d, $J = 7.0$ Hz, H-10′) and the corresponding carbon signals at δ 155.3, 125.2, 95.7, 13.9, together with the two carboxylic groups at δ 173.2 and 169.0 suggested the presence of a secoiridoid skeleton. The signals of the secoiridoid moiety were similar to those of the reported ones as framoside [2], insuloside [13] and escuside [14] isolated from the same genus. The rest signals could be assigned to two glucose moieties [anomeric protons δ 5.24 (1H, d, $J = 7.8$ Hz, H-1″) and 4.99 (1H, d, $J = 7.8$ Hz, H-1‴)] according to the results of acidic hydrolysis and the subsequent GC analysis [15]. The connection of the partial structures was established by HMBC and NOESY experiments (figure 2). In the HMBC experiment, the correlations between H-1‴ and C-1′, between H-1′ and C-1‴ (δ 101.3) indicated the glucose moiety was linked to C-1′ of the secoiridoid moiety. The NOESY correlation between H-1′ and H-1‴ further confirmed the linkage. The other glucose moiety was linked to C-8 as strongly evidenced by the HMBC correlation between the anomeric proton and C-8. But the carbon signal at δ 65.1 (C-6″, corresponding proton signals at δ 4.61 and 4.31 according to the HMQC experiment) indicated the hydroxyl group at C-6″ was esterified. In the HMBC experiment, H-6″ showed long-range correlations with C-7′ (δ 173.2), which indicated the carbonyl group was linked to C-6″. So from the evidence described above, the structure of compound **1** was elucidated as

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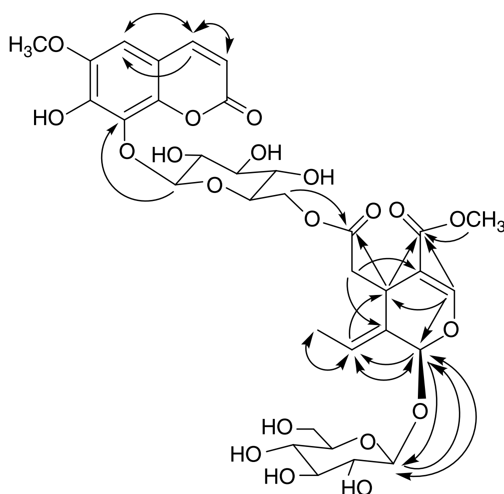

Figure 2. Significant HMBC (from H to C) and NOE correlations for fraxisecoside (**1**).

Table 1. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectral data of compound **1**.

No.	*Proton*	*Carbon*
2		163.6
3	6.42, d, $J = 9.4$ Hz	113.4
4	8.05, d, $J = 9.4$ Hz	146.6
5	7.14, s	106.3
6		147.7
7		144.8
8		133.0
9		146.1
10		112.4
6—OCH_3	4.10, s	57.3
1′	6.08, s	95.7
3′	7.64, s	155.3
4′		109.9
5′	3.94, overlap	31.9
6′	2.60, dd, $J = 15.0$, 11.0 Hz	41.9
	2.80, dd, $J = 15.0$, 3.5 Hz	
7′		173.2
8′	6.24, q, $J = 7.0$ Hz	125.2
9′		131.9
10′	1.83, d, $J = 7.0$ Hz	13.9
11′		169.0
11′—OCH_3	3.87, s	52.2
1″	5.24, d, $J = 7.8$ Hz	105.4
2″	3.78, m	75.6
3″	3.76, m	76.0
4″	3.64, m	71.9
5″	3.59, m	78.3
6″	4.61, m	65.1
	4.31, m	
1‴	4.99, d, $J = 7.8$ Hz	101.3
2‴	3.51, m	75.0
3‴	3.77, m	78.0
4‴	3.52, m	71.7
5‴	3.52, m	78.8
6‴	4.10, m	62.9
	3.85, m	

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fraxetin-8-*O*-[11′-methyl-oleosidyl-(7′ → 6″)]-β-D-glucopyranoside, which was named fraxisecoside and is shown in figure 1.

Compound **2** was obtained as yellowish amorphous powder, $[\alpha]_D^{25}$ −127.5 (*c* 0.19, MeOH). The UV spectrum showed a maximum at 223 nm. The IR absorption bands (1704, 1627, 1076, 561 cm^{-1}) suggested that compound **2** also possesses a secoiridoid skeleton. ESI-MS gave quasimolecular ions $[M-H]^-$ at *m*/*z* 645 and $[M + Na]^+$ at *m*/*z* 669.

Compounds **1**–**3** were tested *in vitro* to screen their bioactivities. Using the established protocols, compounds **1**–**3** showed no inhibition (MIC > 100 μg/ml) against the three microbes *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and a fungus (*Candida albicans*). Compounds **1**–**3** showed no inhibition against the matrix metalloproteinase collagenase-1 and stromelysin-1. In a PTP1B assay, compounds **1** and **2** exhibited moderate inhibition (IC_{50} 21 μM and 50 μM, respectively). Compounds **1**–**3** were found to inhibit the proliferation of the lymphocyte T and B cells (table 2); however, they showed no cytotoxicity against T and B cells in an MTT assay [16].

Table 2. Inhibition against B- and T-cell proliferation.

Compounds	*Concentrations (μg/ml)*	*Proliferation inhibition against B cells*	*Proliferation inhibition against T cells*
1	1	11%	1%
	10	18%	3%
	100	32%	8%
2	1	17%	5%
	10	14%	− 1%
	100	61%	55%
3	1	1%	− 1%
	10	16%	3%
	100	78%	56%

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3. Experimental

3.1 General experimental procedures

Optical rotations were recorded in CH_3OH using a Perkin–Elmer 241 automatic digital polarimeter. ^1H NMR, ^{13}C NMR, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker DRX-400 spectrometer (^1H 400 MHz and ^{13}C 100 MHz). The carbon multiplicities were obtained by DEPT experiment. ESI-MS and FAB-MS were obtained using a Finnigan MAT-90 instrument. UV was carried out on a Varian Cary 300 Bio instrument. IR was recorded on a Hitachi 275-50 IR spectrometer. Elemental analysis was carried out on an Elementar Vario EL instrument. Gas chromatography (GC) was run on a HP 1890 gas chromatography. Sephadex LH-20 (Pharmacia), Toyopearl HW40F (Tosoh), MCI-gel CHP20P (Mitsubishi), and Cosmosil ODS (40–60 μm, Nacalai Tesque Inc.) were used for column chromatography.

3.2 Plant material

The root bark of *F. rhynchophylla* was collected from Anhui province, China, in October 2000, and was identified by the author. A voucher specimen (No. FR001) is deposited at Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.

3.3 Extraction and isolation

The 70% aqueous acetone extract of the root bark of *F. rhynchophylla* was filtered. The solution was evaporated *in vacuo* to remove EtOH and was condensed to a suitable volume. Then it was subjected to chromatography on MCI gel CHP20P column eluted with H_2O and aqueous MeOH (10–80%) successively. The sugar fraction, eluted by water, was discarded and the MeOH eluates were subjected to Sephadex LH-20 chromatography, eluting with aqueous MeOH from water to 80% MeOH gradiently to give five fractions, which were subjected to a combination of column chromatography on Sephadex LH-20, MCI gel CHP20P, Cosmosil ODS and Toyopearl HW-40F to give **1** (16 mg), **2** (8 mg), **3** (6 mg), **4** (11 mg), **5** (10 mg), **6** (13 mg), **7** (8 mg), **8** (10 mg), **9** (300 mg), **10** (160 mg), **11** (20 mg), **12** (36 mg), **13** (17 mg), **14** (25 mg), and **15** (9 mg), respectively.

3.3.1 Fraxisecoside (1). Crystal-like solid, $[\alpha]_D^{25}$ −78.8 (*c* 0.19, MeOH); UV λ_{max} (MeOH, nm): 232, 349; IR ν_{max} (KBr, cm^{-1}): 3411, 1708, 1631, 1617, 1577, 1504, 1442, 1417, 1307, 1161, 1076, 594; ^1H NMR and ^{13}C NMR spectral data, see table 1; ESI-MS *m/z*: 617 [M-Glc + Na]$^+$, 779 M + Na]$^+$, 755 [M−H]$^-$; Elemental analysis (%) C 56.68, H 5.42 (calcd for $C_{33}H_{40}O_{20}\cdot H_2O$, C 56.74, H 5.48).

3.4 Acid hydrolysis of fraxisecoside (1)

A solution of fraxisecoside (**1**) (2 mg) in 7% HCl/EtOH (3:7) was refluxed for 4 h and then the mixture was diluted with H_2O and extracted with Et_2O. The aqueous layer was neutralised with 1 M NaOH and it was then subjected to TLC analysis on Kieselgel 60 F_{254} (Merck) [$CHCl_3$/MeOH/H_2O (30:12:4), 9 ml and HOAc, 1 ml] and paper chromatography [*n*-BuOH/HOAc/H_2O (4:1:5)] with standard sugars. The presence of glucose was established. The neutralised aqueous layer was then passed through an Amberlite IRA-60E column, the aqueous eluate was concentrated *in vacuo* and treated with thiazolidine as described previously [15]. Only the D-glucose derivative was detected by GC (GC conditions: column, Supelco SPB^{-1}, 0.25 mm × 27 m, column temperature 230°C; carrier gas, N_2; t_R, D-glucose derivative 17.9 min, L-glucose derivative 17.3 min).

3.5 Biological assays

The inhibition of T- and B-cell proliferation was assayed according to the literature procedure [16].

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