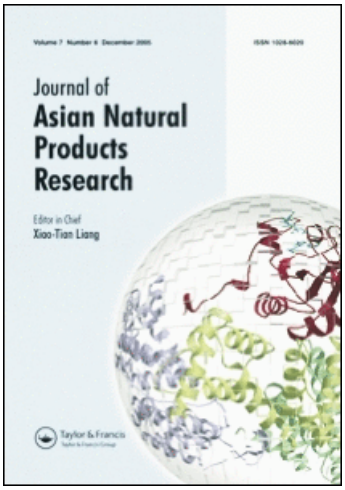

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Flavonoids from preparation of traditional Chinese medicines named Sini-Tang

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A new flavonoid, 7-hydroxyl-4′-*O*-β-D-(6″-*O*-α-hydroxylpropionyl)-glucopyranosyl dihydroflavone (**1**), together with 12 known flavonoids, has been isolated from the EtOAc fraction of the aqueous extract of Sini Tang. The structures of the compounds have been elucidated by spectral methods. The new compound comes from *Glycyrriza uralensis* Fisch., as determined by HPLC-ESI-MS.

Keywords: Sini Tang; Preparation; Flavonoids; *Glycyrriza uralensis* Fisch

1. Introduction

Sini Tang, a famous Chinese traditional decoction from the *Treatise on Febrile Disease*, consists of *Aconitum carmichaeli* Debx., *Zingiber officinale* Rosc. and *Glycyrriza uralensis* Fisch.. It is used to treat patients with symptoms of infirmity, being in a cold sweat, feeling cold in the limbs, lientery and faint pulse [1]. Our pharmacological research showed that the aqueous extract of Sini Tang has significant functions on cardiotonic, boosting blood pressure and antishock. It also inhibited the function of contracting vascular circle caused by KCl (60 mM) and phenephrine. To find the substantial foundation of therapy, we studied the chemical constituents of Sini Tang. Phytochemical analysis of it resulted in the isolation of a new compound (**1**), 7-hydroxyl-4′-*O*-β-D-(6″-*O*-α-hydroxylpropionyl) glucopyranosyl dihydroflavone, along with 12 known flavonoids. Antitumor tests of some isolated compounds (**1**–**5**) on NCI-H460, MCF-7 and SF-268 had been performed, but the activities are lower. This paper deals with the isolation, identification and structural elucidation of the new compound (**1**). The presence of **1** in *Glycyrriza uralensis* Fisch has been confirmed by the HPLC-ESI-MS method.

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2. Results and discussion

Compound **1** was isolated as amorphous white powder, mp 230°C, $[\alpha]_D^{25}$ −6.6 (*c* 0.03, DMSO); its molecular formula was determined as $C_{24}H_{26}O_{11}$ from the positive HR-FABMS spectra ($[M + 1]^+$, *m/z* 491.1538, calcd. 491.1553). The ESI-MS (negative) spectrum of **1** shows ion peaks at *m/z* 489 $[M - H]^-$ and 255 $[(M - H)—C_3H_4O_2—C_6H_{10}O_5]^-$. The IR spectrum shows characteristic absorption bands for hydroxyl (3400 cm^{-1}), ester carbonyl (1720 cm^{-1}), α,β-unsaturated carbonyl (1650 cm^{-1}), aromatic ring (1580, 1510, 1470 cm^{-1}) and glycoside linkage (1130–1085 cm^{-1}). The UV absorption bands at λ_{max} (MeOH) (nm): 202 (sh), 210 (sh), 220, 270 and 302 (sh) are characteristic of dihydroflavone. The ^1H NMR spectrum of **1** exhibits signals of dihydroflavone: ABX-type aromatic proton signals at δ 7.66 (1H, d, $J = 8.6$ Hz, H-5), 6.52 (1H, dd, $J = 8.6$, 2.0 Hz, H-6) and 6.36 (1H, d, $J = 2.0$ Hz, H-8) due to the A ring; AA′BB′-type aromatic proton signals at δ 7.44 (2H, d, $J = 8.6$ Hz, H-2′,6′) and 7.06 (2H, d, $J = 8.6$ Hz, H-3′,5′) due to the B ring; the aliphatic proton signals at δ 5.54 (1H, dd, $J = 13.0$, 2.5 Hz, H-2), 3.12 (1H, dd, $J = 17.0$, 13.0 Hz, H-3_{trans}) and 2.67 (1H, dd, $J = 17.0$, 2.5 Hz, H-3_{cis}) are attributed to a CH—CH_2 system. ^{13}C NMR signals at δ 100.93, 73.96, 77.17, 70.73, 74.69, 64.51 and an anomeric proton at δ 4.94 (1H, d, $J = 7.5$ Hz) in the ^1H NMR suggest the presence of a β-glucopyranosyl moiety linked to C-4′ on comparison with the literature values for liquiritin (**12**) [2]. Furthermore, the typical downfield shift of C-6″ (+3.46 ppm) and the highfield shift of C-5″ (−2.72 ppm) of glycosyl in the ^{13}C NMR spectrum of compound **1** indicate that **1** is liquiritin (**12**) acylized at the C-6″. The ^{13}C NMR spectrum of **1** also reveals a methyl (δ 21.23), a methine (δ 66.77) with a hydroxyl group and an ester carbonyl (δ 175.28). Additionally, the ^1H NMR spectrum for **1** exhibits a doublet at δ 1.27(3H, d, $J = 7.0$ Hz) for a methyl group and a quartet at δ 4.14 (1H, q, $J = 7.0$ Hz) for a methine group. These data identify the acyl as an α-hydroxylpropionyl group. The HMBC spectrum shows the important correlation of the anomeric proton at δ 4.94 with the signal at δ 158.13, indicating that β-glucopyranosyl is linked to C-4′. The correlation between the proton at δ_H 4.05, 4.32 (H-6″) and the carbon signal at δ_c 175.28 confirms that the carbonyl of α-hydroxylpropionyl is linked to C-6″ of the β-glucopyranosyl. Consequently, **1** was identified as 7-hydroxyl-4′-*O*-β-D-(6″-*O*-α-hydroxylpropionyl)-glucopyranosyl dihydroflavone (figure 1). According to the biogenesis, this new compound should come from *Glycyrrhizin*. This conclusion was further confirmed by HPLC-ESI-MS: compound **1** could be detected at $t_R = 3.20$ min. with *m/z* 491.2 $[M + H]^+$ (figure 2).

In addition, the other isolated flavonoids from Sini Tang were identified as isoglycyrol (**2**) [3], formononetin (**3**) [4], neoglycyrol (**4**) [5], isoliquiritigenin (**5**) [6], liquiritigenin (**6**) [5],

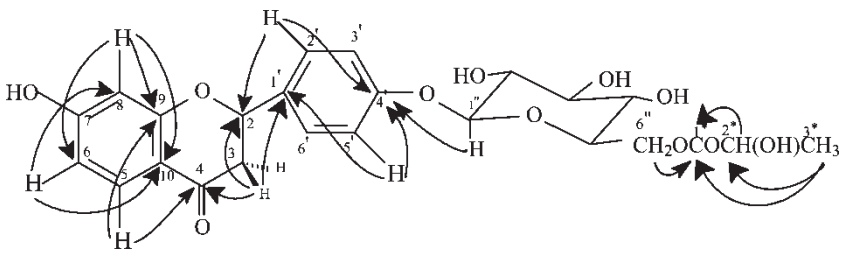

Figure 1. Structure and HMBC correlations for **1**.

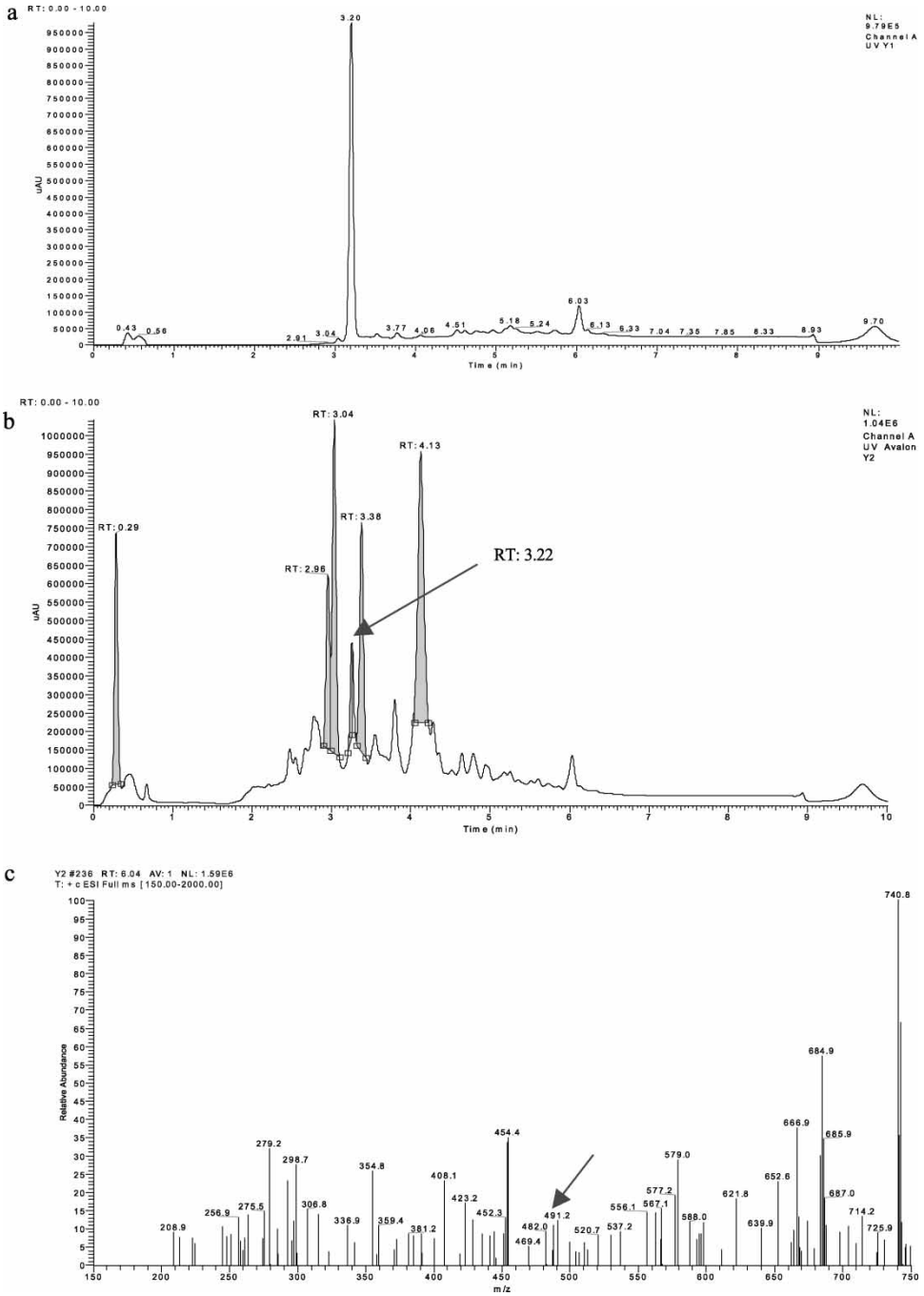

Figure 2. HPLC-ESI-MS spectrum of **1** in *Glycyrriza uralensis* Fisch sample. (a) HPLC chromatogram of **1**; (b) HPLC chromatogram of *Glycyrriza uralensis*; (c) mass spectrum of selective-ion current of **1**.

kumatakenin B (**7**) [7], medicarpin-*O*-β-D-glucoside (**8**) [8], 6″-*O*-acetylliquiritin (**9**) [2], isoononin (**10**) [7], isoliquiritin (**11**) [9], liquiritin (**12**) [2] and sophoraflavone B (**13**) [10].

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-4 micro-melting apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 983G infrared spectrometer. UV spectra were obtained on a Philips PYE Unicam Pu 8800 instruments. NMR spectra were run on a Varian INOVA-500 NMR spectrometer with TMS as internal standard. EIMS were obtained on a VG ZAB-2F mass spectrometer and ESIMS, FABMS, HR-FABMS were performed on a Autospec-Utima ETOF spec mass spectrometer. HPLC-MS detection was carried out by reversed-phase liquid chromatography (mobile phase of water and acetonitrile, a linear gradient from 5 to 95% of acetonitrile, eluted for 10 min) and mass spectrometry (Agilent series 1100) with a UV detector at $\lambda = 254$ nm (ESI ionization source).

3.2 Plant material

Aconitum carmichaeli Debx., *Zingiber officinale* Rosc. and *Glycyrriza uralensis* Fisch were purchased from the Medicinal Material company of China in December 2001 and identified by Professor Wen-Yan Lian. A voucher specimen (No. 1124) has been deposited in the Herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** and **12** in DMSO-d_6.

	1		***12***	
C	δ_H	δ_C	δ_H	δ_C
2	5.54 (1H dd, $J = 13.0, 2.5$ Hz)	79.51	5.54 (1H dd, $J = 12.6, 1.9$ Hz)	79.04
3	3.12 (1H dd, $J = 17.0, 13.0$ Hz)		3.14 (1H dd, $J = 16.7, 12.6$ Hz)	
	2.67 (1H dd, $J = 17.0, 2.5$ Hz)	44.08	2.68 (1H dd, $J = 16.7, 1.9$ Hz)	43.55
4		190.75		190.31
5	7.66 (1H d, $J = 8.6$ Hz)	129.29	7.66 (1H d, $J = 8.6$ Hz)	128.79
6	6.52 (1H dd, $J = 8.6, 2.0$ Hz)	111.44	6.52 (1H dd, $J = 8.6,\ 1.5$ Hz)	110.95
7		165.51		165.02
8	6.36 (1H d, $J = 2.0$ Hz)	103.44	6.36 (1H d, $J = 1.5$ Hz)	102.97
9		163.91		163.43
10		114.42		113.94
1′		133.41		132.74
2′,6′	7.44 (2H d, $J = 8.6$ Hz)	128.84	7.45 (2H d, $J = 8.4$ Hz)	128.31
3′,5′	7.06 (2H d, $J = 8.6$ Hz)	117.04	7.07 (2H d, $J = 8.4$ Hz)	116.56
4′		158.13		157.84
1″	4.94 (1H d, $J = 7.5$ Hz)	100.93	4.91 (1H d, $J = 7.3$ Hz)	100.68
2″		73.96		73.57
3″		77.17		76.95
4″		70.73		70.07
5″		74.69		77.41
6″		64.51		61.05
1*		175.28		
2*	4.14 (1H q, $J = 7.0$ Hz)	66.77		
3*	1.27 (3H d, $J = 7.0$ Hz)	21.23		

3.3 Extraction and isolation

A mixture of *Aconitum carmichaeli* Debx. (4.8 kg), *Zingiber officinale* Rosc. (3.2 kg) and *Glycyrriza uralensis* Fisch. (4.8 kg) was added to 6–8 times that amount of water and extracted at 100°C for 1 h (3 ×). The extracted solution was added then to 95% EtOH to deposit 24 h. The EtOH solution was subsequently filtered and concentrated under reduced pressure. The aqueous residue was partitioned with light petroleum, $CHCl_3$, EtOAc and n-BuOH, respectively. The EtOAc-soluble portion was then chromatographed over silica gel, eluting with Et_2O, EtOAc, Me_2CO and MeOH, successively. The Et_2O fraction was then subjected to silica-gel column chromatography, eluting with a gradient of $CHCl_3$—Me_2CO solvent, and purified on Sephadex LH-20 to give **2** (30 mg), **3** (80 mg), **4** (30 mg) **5** (60 mg), and **6** (100 mg). The EtOAc fraction was chromatographed over silica gel, eluting with a gradient of $CHCl_3$—MeOH—H_2O. The combination of similar fractions on the basis of TLC analysis then afforded six fractions. Fraction 2 was subjected to silica-gel column chromatography, eluting with $CHCl_3$—MeOH, and purified on Sephadex LH-20 to give **7** (50 mg), **8** (30 mg), **9** (20 mg). Fraction 3 was isolated by the same method as above to afford **10** (50 mg) and **1** (50 mg). Fraction 4 was subjected to polyamide column chromatography, eluting with $CHCl_3$—MeOH, and purified on Sephadex LH-20 to give **11** (100 mg), **12** (220 mg) and **13** (200 mg).

Compound **1**: white powder; mp 230°C; $[\alpha]_D^{25}$ −6.6 (*c* 0.03, DMSO), UV (MeOH) λ_{max} (nm): 202 (sh), 210 (sh), 220, 270, 302 (sh); IR (KBr) ν_{max} (cm^{-1}): 3400 (br), 2900, 1720, 1650, 1610, 1580, 1510, 1470, 1285, 1240, 1130, 1085, 890, 830; ^1H and ^{13}C NMR data, see table 1; ESIMS $[M-1]^-$ *m/z*: 489; FABMS: *m/z* 491 ($[M+H]^+$, 10), 445 (23), 419 (10), 282(100), 256 (45), 225 (16), 159 (52), 130 (45); HR-FABMS $[M+H]^+$ *m/z* 491.1538 (calcd. for $C_{24}H_{26}O_{11}$, 491.1553).

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