

Article

Absorbable Phenylpropenoyl Sucroses from *Polygala tenuifolia*

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Abstract: Three phenylpropenoyl sucroses – sibiricose A5 (**1**), A6 (**2**) and 3',6-disinapoyl sucrose (**3**) – were isolated from the 30% EtOH extract of *Polygala tenuifolia*, which displayed antidepressant-like action. HPLC analysis indicated that the three phenylpropenoyl sucroses could be absorbed into serum. From the serum pharmacochimistry point of view, these three phenylpropenoyl sucroses might prevent or relieve depression.

Keywords: absorbable phenylpropenoyl sucrose; sibiricose A5; sibiricose A6; 3',6-disinapoyl sucrose; *Polygala tenuifolia*

1. Introduction

Polygala tenuifolia (local name: Yuan zhi) is widely distributed in China, and its root is an important herb used in Traditional Chinese Medicine to treat depression. A number of investigations had been carried out to identify the compounds responsible of the biological activity of Yuan zhi [1-4]. Some phenylpropenoyl sucroses from Yuan zhi became hot research topics because of their bioactivity of preventing memory disorders [5-8]. The understanding and diagnosis of many diseases mainly relies on the metabolic profiling of biological fluids such as urine and plasma, as their compositions reflect the biochemical status of a living organism and imply information about the biological processes associated with pathological conditions. The application of high-performance liquid chromatography (HPLC) for untargeted plasma metabolic profiling with direct comparison with authentic samples in

metabonomic research has attracted much attention in recent years [9,10]. In order to research the untargeted plasma metabolite profiling of phenylpropenoyl sucroses, we examined the metabolites in rat serum using HPLC by direct comparison with authentic samples after the rats are administered orally with Yuan zhi aqueous extracts. As a result, sibiricose A₅ (**1**), A₆ (**2**) and 3',6-disinapoyl sucrose (**3**) were absorbed into serum, as we report here for the first time.

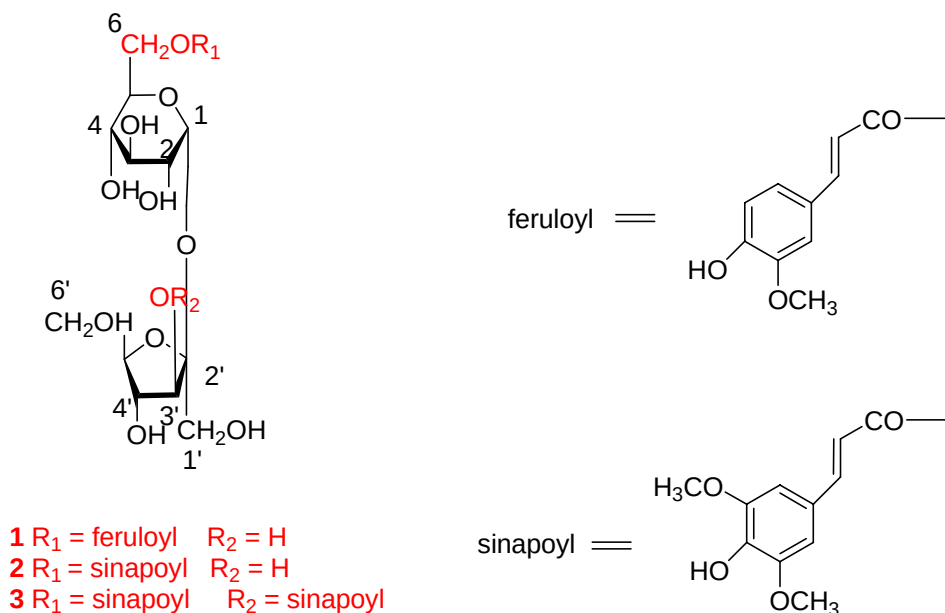
2. Results and Discussion

2.1. Identification of Compounds 1 to 3

The 30% EtOH extract of Yuan zhi exhibited more notable activity in the antidepressive assay than the 50%, 70% and 90% EtOH extracts [11]. The proportion of phenylpropenoyl sucroses in the 30% EtOH fraction of Yuan zhi is 44.88%, which is calculated by their peak area ratio in HPLC-DAD. Thus, we focus on the phenylpropenoyl sucroses-enriched fraction in this study.

Repeated column chromatography (CC) over Sephadex LH-20 and silica gel column led to the isolation of three phenylpropenoyl sucroses **1-3** (Figure 1) from this fraction. These compounds were determined to be sibiricose A₅ (**1**) [12], A₆ (**2**) [12] and 3',6-disinapoyl sucrose (**3**) [13], respectively, by comparison of their spectroscopic data with reported literature values and authentic samples. The full NMR assignments of **1**, **2** and **3** (Table 1) were achieved by the detailed 2D NMR analysis.

Figure 1. Structures of compounds **1-3**.



Reference samples of sibiricose A₅ (**1**), A₆ (**2**) and 3',6-disinapoyl sucrose (**3**) were prepared by repeated CC on Sephadex LH-20, ODS-A, silica gel and RP-HPLC of the three crude samples.

Table 1. ^1H -NMR (500 and 400 MHz) and ^{13}C -NMR (125 and 100 MHz) data of compounds **1–3** in CDCl_3 (δ in ppm, J in Hz).

Position	1		2		3	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}
Glycone moiety						
Glc-1	93.3	5.36 (d, 4)	93.3	5.37 (d, 4)	92.9	5.50 (d, 3.7)
2	73.1	3.53 (d, 10.0, 4.0)	73.1	3.51 (d, 10.0, 4.0)	73.1	3.50 (m)
3	74.9	3.69 (dd, 10.0, 9.0)	75.0	3.75 (dd, 10.0, 9.5)	75.0	3.69 (t, 9.3)
4	71.2	3.38 (dd, 9.0, 9.0)	71.2	3.34 (dd, 9.5, 9.0)	71.9	3.38 (m)
5	73.8	3.88 (m)	74.5	3.89 (m)	72.8	4.29 (m)
6	62.3	3.77 (dd, 12.0, 4.5)	62.4	4.31 (dd, 12.0, 4.5)	65.6	4.48 (br d, 10.0)
		3.81 (m)		4.52 (m)		4.06 (m)
Fru-1	65.4	3.60 (d, 12.0)	65.4	3.62 (d, 12.0)	65.4	3.60 (d, 12.0)
		3.69 (d, 12.0)		3.65 (d, 12.0)		3.63 (d, 12.0)
2	104.4		104.8		104.8	
3	79.7	5.40 (d, 8.0)	79.6	5.46 (d, 8.0)	79.6	5.58 (d, 7.5)
4	74.8	4.33 (dd, 8.0, 7.5)	74.5	4.07 (dd, 8.0, 7.5)	74.4	4.51 (t, 8.0)
5	84.1	3.88 (m)	84.2	3.88 (m)	84.2	4.10 (m)
6	62.9	3.84 (m)	62.9	3.89 (m)	63.7	3.99 (m)
		3.84 (m)		3.80 (m)		4.20 (m)
Aglycone moiety						
R ₁ -1	127.5		129.5		125.1	
2	112.1	6.74 (d, 2.0)	107.1	6.90 (s)	106.8	7.12 (s)
3	149.6		149.5		149.0	
4	149.0		130.7		140.5	
5	116.5	6.68 (d, 8.0)	149.5		149.0	
6	124.3	6.59 (dd, 8.0, 2.0)	107.1	6.90 (s)	106.8	7.12 (s)
7	147.7	7.71 (d, 15.6)	147.2	7.64 (d, 15.6)	146.0	8.09 (d, 16.0)
8	115.1	6.36 (d, 15.6)	115.8	6.38 (d, 15.6)	115.2	6.90 (d, 16.0)
9	168.3		169.1		166.8	
-OCH ₃	56.6	3.86 (s)	56.8	3.87 (s)	56.4	3.80 (s)
R ₂ -1					125.2	
2					106.9	7.12 (s)
3					149.2	
4					140.5	
5					149.2	
6					107.9	7.12 (s)
7					146.7	7.98 (d, 15.6)
8					115.6	6.67 (d, 15.6)
9					167.7	
-OCH ₃					56.9	3.80 (s)

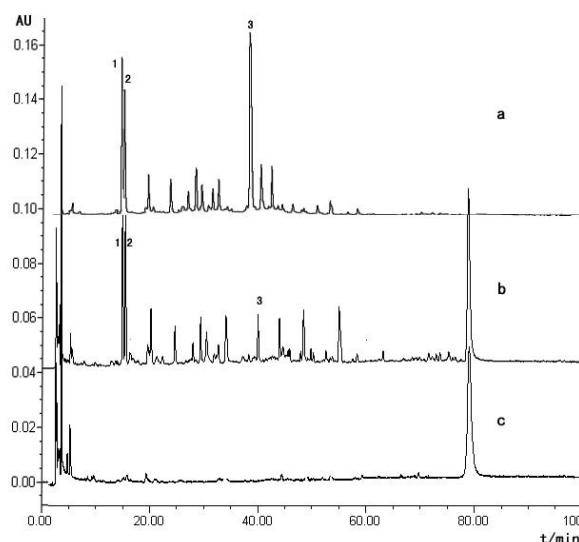
2.2. Untargeted Plasma Metabolite Profiling of Yuan zhi

The aqueous extract of *P. tenuifolia* (2.5 g crude drug/mL) was administered orally to male Sprague-Dawley (SD) rats at a dose of 1.56 g/kg. The orbital blood samples (3 mL) of the rats were

collected at 60 min post-dose. The serum containing the extracts of *P. tenuifolia* and blank control serum were analyzed by HPLC at 330 nm after a series of preconditioning (Figure 2). As shown in the Figure, HPLC analysis indicated that three phenylpropenoyl sucroses sibiricoside A5 (**1**), A6 (**2**) and 3',6-disinapoyl sucrose (**3**) of Yuanzhi, can be absorbed into serum. Our feasibility study showed the proportions of sibiricoside A5 (**1**), A6 (**2**) and 3',6-disinapoyl sucrose (**3**) in the fraction were 11.06%, 8.89%, 24.29%, respectively, by calculating their peak area ratio in HPLC-DAD. It was also reported that the fraction containing these three oligosaccharide polyesters as main chemical constituents had obvious antidepressant activity [11].

In addition, it is reported that 3',6-disinapoyl sucrose (**3**) can protect the SH-SY5Y human neuroblastoma cells from lesion, and sibiricoside A5 (**1**) can protect the PC12 cells from the damage induced by glutamic acid [11,14]. Therefore, from the point of view of serum pharmacochimistry, our results indicate that the three phenylpropenoyl sucroses might prevent or relieve depression.

Figure 2. Plasma metabolite HPLC profiles of *Yuan zhi* extract.



^a 30% EtOH extract; ^b Serum of rats after oral administration of *Yuan zhi*; ^c Blank control.
Peaks 1: sibiricoside A₅ (**1**); 2: sibiricoside A₆ (**2**); 3: 3',6-disinapoyl sucrose (**3**).

3. Experimental

3.1. General

Column chromatography (CC) was performed on Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.), SP825 (Mitsubishi Chemical Co.), Chromatorex ODS (Fuji Silysia Chemical Co., Ltd.) and silica gel (Qingdao Haiyang Chemical Co.). TLC was carried on silica gel G pre-coated plates (Qingdao Haiyang Chemical Co.) with CHCl₃-MeOH-H₂O (9:1:0.1 or 7:3:0.5). The spots were detected by spraying with 10% H₂SO₄ ethanol solution followed by heating. ¹H- and ¹³C-NMR, HSQC and HMBC spectra were recorded in DMSO-d₆ with Bruker AM-400 and DRX-500 spectrometers. The HPLC was performed with a Waters 2695 HPLC system on a Agela Venusil MP-C18 column (5 μm, 250 × 4.6 mm, Agela, China) and with column temperature set at 30 °C, using a diode-array detection (Shimadzu SPD-M10A).

3.2. Plant Material

The roots of *P. tenuifolia* were collected from Shanxi province, China, and identified by Yuting Chen (Beijing University of Chinese Medicine).

3.3. Extraction and Isolation

Dried plant material (300 g) of *P. tenuifolia* was refluxed with ethanol for 3 h (4 times). After removal of the organic solvent under reduced pressure, the aqueous fraction was concentrated to a small volume (100 mL) and subjected to a SP825 macroporous adsorptive resin column eluting with H₂O and 30% EtOH to afford two fractions. The concentrated 30% EtOH was applied to CC on silica gel (CHCl₃-MeOH-H₂O, 9:1:0.1-7:3:0.5), Sephadex LH-20 and ODS-A, eluting with H₂O-MeOH (1:0-0:1) to afford compounds **1** (14 mg), **2** (6 mg) and **3** (15 mg), respectively.

3.4. HPLC Analysis

The plasma sample (0.5 mL) was placed in a 5 mL polypropylene tube, and monopotassium phosphate (0.2 mL) and acetonitrile (1.5 mL) were added to the tube. The tube was vortexed-mixed for 90 s. The precipitated protein was removed by centrifugation at 4,000 rpm for 30 min. The organic layer was transferred to another tube, and evaporated to dryness by N₂ at 50 °C. The residue was added 0.2 mL distilled water to dissolve, and then filtered through a 0.45 µm Millipore filter film. Aliquots of 20 µL were injected into the HPLC system. The mobile phase consisted of A, CH₃CN; B, H₂O (0.1% phosphoric acid); gradient: 11-16% A linear in 10 min; 16-22% A linear in 10 min; 22-23% A linear in 5 min; 23-28% A linear in 9 min; 28% A linear in 11 min; 28-33% A linear in 10 min; 33-39% A linear in 15 min; 39-42% A linear in 15 min; 42-55% A linear in 10 min; 55-70% A linear in 5 min; isocratic on 70-11% A for 10 min. The mobile phase was degassed automatically using the electronic degasser system. The flow rate was 1.0 mL/min. The optimum wavelength was set at 330 nm [15].

3.5. Animal Experiments

Male SD rats (250 ± 20 g body weight) were provided by Vital River Experimental Animal Co., Ltd. (license number: SCXK2006-0009). Rats were kept in an environmentally controlled breeding room for three days before starting the experiment and were fed with standard laboratory food and water. SD rats had free access to water but no food for 12 h before the experiments. The extract of Yuan zhi was administered orally to rats at a dose of 1.56 g/kg, and the blood samples (3 mL) were collected at 60 min post-dose. The plasma samples were immediately separated by centrifugation at 4,000 rpm for 10 min.

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Sample Availability: Samples of the compounds are available from the authors.

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