Free Radical Scavengers, Antioxidants and Aldose Reductase Inhibitors from *Camptosorus sibiricus* Rupr.

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Flavonoids and organic acids were recommended in the literature as the main active constituents of *Camptosorus sibiricus* Rupr. Assay-guided fractionation led to the isolation of 9 flavonoids and 8 phenolic acids. All compounds were tested for DPPH scavenging activity, SOD-like and aldose reductase inhibition. Among them, compounds **1**, **2**, **3**, **5**, **6**, **7**, **8**, **9**, **11**, **15** showed activities. The most active free radical scavenger and antioxidant was compound **8**, while compound **1** exhibited strong inhibiting activity of aldose reductase. The structure-activity relation was dicussed briefly.

Key words: *Camptosorus sibiricus* Rupr., DPPH Scavengers, Antioxidants, Aldose Reductase Inhibitors

Introduction

Camptosorus sibiricus is a famous herbal folk medicine widely distributed in North China, which has good therapy effects on vascular inflammation, diabetic complication and traumatism (41st Session of Graduate of Shenyang College of Pharmacy, 1977; Xu *et al*., 1989; Zhang *et al*., 1979). The total flavonoids and organic acids of *C. sibiricus* were recommended as the main active constituents against vascular inflammation (41st Session of Graduate of Shenyang College of Pharmacy, 1977). Some flavonoids of the herb with dilatation activity of blood vessels were reported in the literature (Zhang *et al*., 1979). In our intended research, assay-guided fractionation led to the isolation of 9 flavonoids and 8 phenolic acids. All the compounds were tested for DPPH scavenging activity, SOD-like and aldose reductase inhibition.

Materials and Methods

Plant material

The plant material was collected in Beining City, Liaoning Province, China, in July 2002, and identified by Prof. Qishi Sun (Pharmacognosy Laboratory, Shenyang Pharmaceutical University). A voucher specimen (No. 20020701) is deposited at the herbarium of Research Department of Natural Medicine, Shenyang Pharmaceutical University, Shenyang, China.

Extraction and isolation

The air-dried whole herb of *C. sibiricus* (4.2 kg) was extracted with 70% ethanol and successively partitioned three times with petroleum ether, EtOAc and *n*-BuOH to give 254.0 g, 40.0 g and 138.0 g residues, respectively. The EtOAc extract was chromatographed over a silica gel column eluted with a $CHCl_3$/MeOH gradient yielding compounds **1**, **2**, **3**, **10**–**17**. The *n*-BuOH extract was subjected to silica gel column chromatography gradiently eluted with $CHCl_3$/MeOH to give fraction a [$CHCl_3$/MeOH (100:3–100:13 v/v) gradient, combined eluate 8.5 g], fraction b [$CHCl_3$/MeOH (100:14–100:20 v/v) gradient, combined eluate 25.2 g] and fraction c [$CHCl_3$/MeOH (100:21–100:50 v/v) gradient, combined eluate 32.1 g]. Compounds **4**–**9** were isolated from fraction b by repeated preparative HPLC (ODS column, 216 × 25 mm; flow rate 5 ml/min). The structures were identified on the basis of 1D and 2D NMR data as reported previously (Li *et al*., 2004, 2006a, b).

DPPH decoloration assay

The free radical scavenging activity of the samples was assessed using the DPPH radical, according to the method reported by Matsuda *et al*. (2003). An ethanol solution of DPPH (100 μM, 1.0 ml) was mixed with different concentrations of

each test compound (0–100 μM, 0.5 ml) and 0.1 M acetate buffer (pH 5.5, 1.0 m), and the absorbance change at 517 nm was measured 30 min later. A solution without DPPH was used as a blank. Measurements were performed for three times, and the concentration required for 50% reduction (50% scavenging concentration, SC_{50}) of a 40 μM DPPH radical solution was determined graphically. α-Tocopherol was used as reference compound.

SOD-like activity assay

SOD assay was conducted with the XO/WST system reported by Ukeda *et al.* (1999). Into 2.5 ml of a 50 mM sodium carbonate buffer (pH 10.2), 0.1 ml of 3 mM xanthine, 3 mM EDTA, a WST (water-soluble tetrazolium, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate sodium salt) solution and the sample solution containing SOD or water were added. The reaction was initiated by adding an XO (xanthine oxidase) solution (0.1 ml). The reaction solution was incubated for 20 min at 37 °C, and then 0.1 ml HCl was added. The absorbance change was monitored with a Beckman DU-530 spectrophotometer at 450 nm.

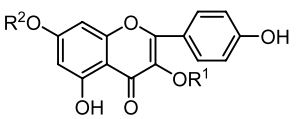
Aldose reductase (AR) assay

Aldose reductase activity was assayed by the method described by Matsuda *et al.* (2002). The supernatant fluid of a rat lens homogenate was used as the crude enzyme. The incubation mixture contained 135 mM Na,K-phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 μl of enzyme fraction, with or without 25 μl of sample solution, in a total volume of 0.5 ml. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μl 0.5 M HCl. Then, 0.5 ml 6 M NaOH containing 10 mM imidazole was added, and the solution heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a fluorophotometer (Luminescence Spectrometer LS50B, Perkin Elmer, England) at an excitation wavelength of 360 nm and an emission at 460 nm.

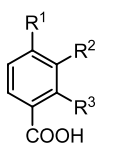
Results

From the ethanol extract of *C. sibiricus*, 9 flavonoids and 8 phenolic acids were isolated, among which compounds **7**, **8**, and **9** (Fig. 1) were characteristic constituents (Li *et al.*, 2006a), and assessed for free radical scavengers, antioxidants and AR inhibitors in selected assays (Table I). Most flavonoids, especially the three characteristic compounds, exhibited high activities in the assays.

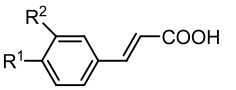
The SOD-like activity and activity towards the DPPH radical were both mainly due to the *ortho*-dihydroxy moiety of the phenolic acid and the caffeoyl group of the flavonoids. Compounds **11** and **15**, phenolic acids with an *ortho*-dihydroxy moiety, and compounds **7** and **8**, flavonoids caffeoylated at the saccharide chain, exhibited high DPPH decoloration and SOD-like activities. It can be con-



	R^1	R^2
1	H	H
2	H	Rha
3	Glu	H
4	Glu	Rha
5	$Glu_{2\text{-}1}Glu$	H
6	$Glu_{2\text{-}1}Glu$	Glu
7	$Glu_{2\text{-}1}Glu$ (6-caffeoyl)	H
8	$Glu_{2\text{-}1}Glu$ (6-caffeoyl)	Glu
9	$Glu_{2\text{-}1}Glu$ (6-coumaroyl)	Glu



	R^1	R^2	R^3
10	OH	H	H
11	OH	OH	H
12	OCH_3	OH	H
13	OH	H	OH



	R^1	R^2
14	H	H
15	OH	OH
16	OH	H
17	OGlu	H

Fig. 1. Structures of compounds **1**–**17** isolated from *Camptosorus sibiricus.*

Table I. Effects of compounds **1**–**17** on DPPH scavenging activity, SOD-like and aldose reductase inhibition (ARI) experiments[a].

Compound	DPPH SC_{50} [μM]	SOD-like IC_{50} [μM]	ARI IC_{50} [μM]
1	12.1 ± 0.8	23.5 ± 1.2	4.9 ± 0.7
2	24.0 ± 1.2	10.6 ± 1.1	13.0 ± 1.2
3	–	–	11.5 ± 1.1
4	–	–	–
5	–	38.4 ± 1.6	20.0 ± 1.3
6	18.0 ± 1.0	10.0 ± 1.1	29.7 ± 1.6
7*	6.7 ± 0.8	6.8 ± 0.6	12.3 ± 1.0
8*	2.6 ± 0.8	2.9 ± 0.5	22.0 ± 1.1
9*	–	–	46.1 ± 1.7
10	–	–	–
11	10.2 ± 1.0	4.1 ± 0.7	98.7 ± 2.1
12	–	–	–
13	–	–	–
14	–	–	–
15	10.0 ± 1.1	4.0 ± 0.5	46.8 ± 1.9
16	–	–	–
17	–	–	–

* Characteristic constituents of *Camptosorus sibiricus.*
[a] Data are means ± SD of 3 independent experiments.

cluded that the caffeoyl group increased the antioxidant activity of the flavonoids.

Most flavonoids exhibited strong AR inhibitory activities. But among the phenolic acids only protocatechuic acid (**11**) and caffeic acid (**15**) showed weak inhibitory activity. Comparison of their structures indicates that free hydroxy and caffeoyl groups on flavonoids enhance the inhibitory activities.

Discussion

The total flavonoids and organic acids from *C. sibiricus* were recommended as the main active constituents for vascular inflammation, and some flavonoids with activity of dilatation of blood vessels from the herb were reported (Zhang *et al.*, 1979). In our intended research, we isolated the constituents of the total flavonoid and phenolic acid part of *C. sibiricus* and tested their DPPH scavenging, SOD-like and aldose reductase inhibition activities to clarify the active composition against diabetic complication, liver cancer and traumatism.

The free radical scavenging and antioxidant activity found in *C. sibiricus* could be associated with the main phenolic acid and caffeoylated flavonoid compounds, giving support to the use of *C. sibiricus* as a medicinal plant to treat liver cancer and traumatism. The compounds with AR inhibitory activity were mainly flavonoids, comprising the characteristic constituents of *C. sibiricus.* The result indicated the total flavonoids play an important role when using *C. sibiricus* against diabetic complication.

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