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Isolation of the Active Component Having the Uremia-Preventive Effect from *Salviae Miltiorrhizae Radix* Extract

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An attempt was made to isolate the active component which exhibits the improving effect on uremic symptoms from *Salviae miltiorrhizae Radix*. Systematic isolation from the aqueous extract of *Salviae miltiorrhizae Radix* was carried out and the effects of the fractions were assessed in terms of an index of decrease in blood urea nitrogen. It was found that one of the major blood urea nitrogen-decreasing components was compound I. Compound I, which was considered to be a tetramer of (dihydro) caffeic acid, showed improving effects on uremic symptoms, causing significant decreases in blood urea nitrogen, creatinine, methylguanidine, and guanidinosuccinic acid.

Keywords—*Salviae miltiorrhizae Radix*; chronic renal failure; blood urea nitrogen; serum creatinine; methylguanidine; guanidinosuccinic acid; (dihydro)caffeic acid tetramer; uremic rat

In a series of investigations of the effects of *Salviae miltiorrhizae Radix* (Chinese crude drug named "dan shen") in uremic rats, it has been found that the administration of aqueous extract from *Salviae miltiorrhizae Radix* resulted in significant decreases of urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid and inorganic phosphate, and a marked increase of guanidinoacetic acid in the serum.¹⁻³⁾ An increase in renal tissue blood flow with a decrease of blood pressure on oral administration of the extract to uremic rats suggested that the extract might alleviate the uremia by enhancing renal function.⁴⁾ Indeed, *Salviae miltiorrhizae Radix* extract apparently induced partial repair of renal dysfunction, markedly accelerating the excretion of urinary urea and creatinine.⁵⁾

In the present paper, an attempt was made to identify the active component of *Salviae miltiorrhizae Radix* extract that caused the improvement of the uremic state. Thus, systematic isolation and purification of the active components from *Salviae miltiorrhizae Radix* were carried out and the effects of the fractions were assessed in terms of an index of decrease in blood urea nitrogen. This paper deals with the isolation of the active components and also describes the effects of the major principle, tentatively named compound I, on blood urea nitrogen, creatinine, and guanidino compound levels in rats with chronic renal failure.

Materials and Methods

Extraction and Separation of Active Components from *Salviae Miltiorrhizae Radix*—*Salviae miltiorrhizae Radix* (*Salvia miltiorrhiza* BUNGE) produced in China was purchased from Tochimoto Tenkaido Co., Ltd., Osaka, Japan. The radix was finely powdered and successively extracted with H₂O (fr. A) and acetone (fr. B) according to Chart 1. Fraction A was chromatographed over an MCI-GEL CHP-20P (7.5 × 35 cm) column and elution was done using H₂O (fr. I), 50% aqueous MeOH (fr. II), and a mixture of MeOH-acetone (fr. III) successively as eluents.

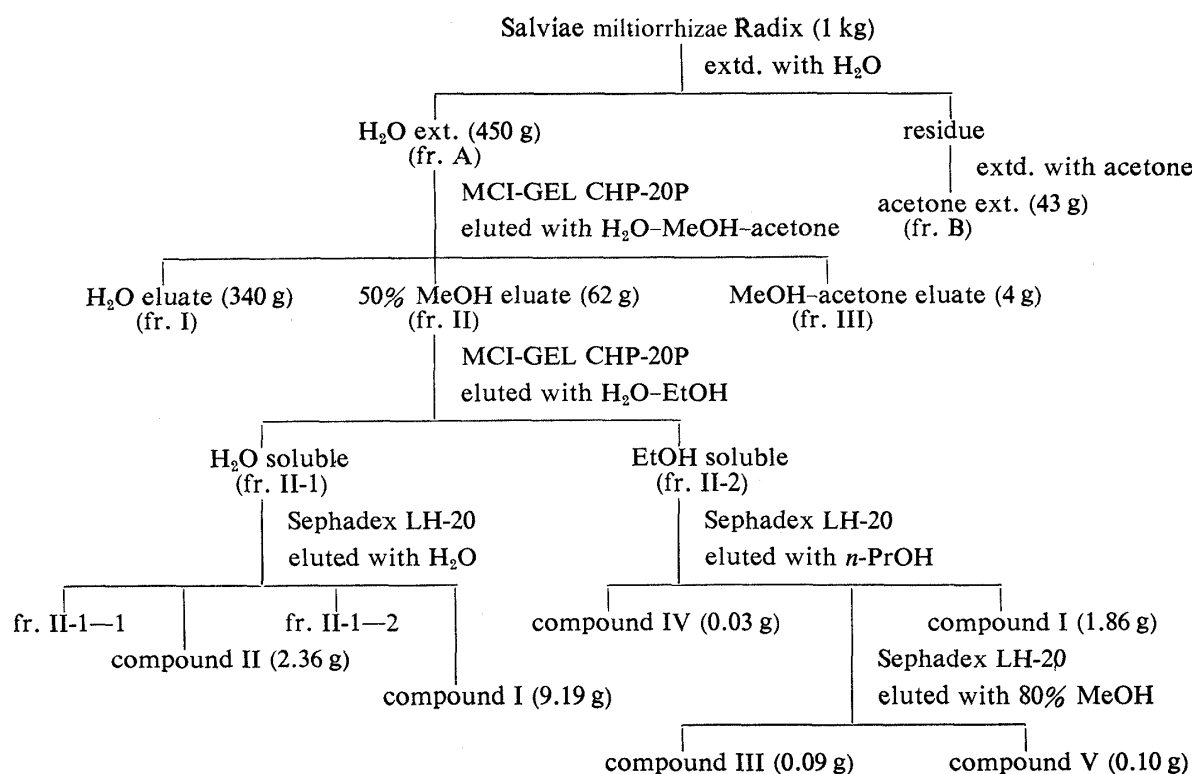


Chart 1. Extraction and Separation of Active Components from *Salviae Miltiorrhizae Radix*

Fraction II was subjected to repeated chromatography over MCI-GEL CHP-20P using an H₂O-EtOH mixture as the eluent to afford fractions II-1 and II-2. Fraction II-1 was further subjected to Sephadex LH-20 chromatography with H₂O to afford compounds I and II. Fraction II-2 was column-chromatographed on Sephadex LH-20, and elution with *n*-PrOH and then 80% MeOH gave compounds I, III, IV, and V.

Animals and Treatment—Male rats of the JCL: Wistar strain, with a body weight of 110–120 g, were kept in an animal room with an ambient temperature of 22 ± 1 °C and with lights on from 6 a.m. to 6 p.m. They were allowed to acclimate for several days and fed on a commercial feed (CLEA Japan Inc., Tokyo, type CE-2) during the adaptation period. Then they were fed *ad libitum* on an 18% casein diet containing 0.75% adenine, which produced rats with experimental renal failure.^{6–10} The 18% casein diet contained the following components (in 100 g): casein 18 g, α -cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture¹¹ 4 g, vitamin mixture¹¹ 1 g, cellulose powder 2 g, and choline chloride 0.1 g. To this diet, adenine was added and mixed at the level of 0.75 g/100 g of diet. During the adenine feeding period, the fractions obtained from *Salviae miltiorrhizae Radix* were administered orally for 21 d to rats as drinking water, while control rats received tap water. Throughout the experimental period, there were no statistically significant difference between the control and each group of fraction-treated rats with regard to change of body weight. Food intake of each rat was essentially proportional to weight change. On the 21st day of the feeding period, rats were sacrificed by means of a blow on the head and exsanguinated. Blood was collected in a conical centrifuge tube and the serum was separated by centrifugation immediately.

Urea Nitrogen Assay—Estimation of urea nitrogen concentration in serum was carried out according to the urease-indophenol method.¹²

Creatinine Assay—Serum creatinine was determined according to the Folin-Wu method.¹³

Guanidino Compound Assay—Trichloroacetic acid was added to serum to give 10% final concentration for deproteinization. The mixture was centrifuged at $1000 \times g$ for 10 min. The supernatant was applied to a Shimadzu LC-5A liquid chromatograph using a step gradient. Eluates were monitored (395 nm excitation, 500 nm emission) by using a fluorescence spectrometer, model RF-540 (Shimadzu Co., Kyoto, Japan).

Statistics—All data were treated statistically by the use of Student's *t*-test.

Results

Effect of the Fractions from *Salviae Miltiorrhizae Radix* on Blood Urea Nitrogen

Table I shows the effect on blood urea nitrogen of each fraction separated according to

TABLE I. Effect of Each Fraction on Urea Nitrogen Level in the Serum

Exp. No.	Material	Dose (mg/rat/d)	Urea nitrogen (mg/dl)
1	Control	—	110.2 ± 3.6 (100)
	Fraction A	15	82.8 ± 9.0 ^{a)} (75)
	Fraction B	15	84.8 ± 6.1 ^{a)} (77)
2	Control	—	112.1 ± 14.5 (100)
	Fraction I	15	103.4 ± 8.7 (92)
	Fraction II	15	72.8 ± 5.0 ^{a)} (65)
	Fraction III	15	78.4 ± 5.2 ^{a)} (70)

Values are means ± S.E. of 6 rats. Figures in parentheses are percentages of the control value. a) Significantly different from the control value, $p < 0.05$.

TABLE II. Effect of Compound I on Urea Nitrogen and Creatinine Levels in the Serum

Material	Dose (mg/rat/d)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)
Control	—	121.7 ± 4.1 (100)	3.67 ± 0.13 (100)
Compound I	2	97.9 ± 6.7 ^{a)} (80)	3.40 ± 0.24 (93)
Compound I	4	84.9 ± 3.6 ^{b)} (70)	2.62 ± 0.18 ^{b)} (71)
Compound I	8	87.0 ± 3.5 ^{b)} (71)	2.96 ± 0.05 ^{b)} (81)

Values are means ± S.E. of 6 rats. Figures in parentheses are percentages of the control value. a) Significantly different from the control value, $p < 0.01$, b) $p < 0.001$.

TABLE III. Effect of Compound I on Guanidino Compound Levels in the Serum

Material	Dose (mg/rat/d)	MG (μg/dl)	GSA (μg/dl)	GAA (μg/dl)
Control	—	10.3 ± 0.5 (100)	175.1 ± 5.6 (100)	132.1 ± 17.5 (100)
Compound I	2	9.7 ± 0.6 (94)	132.6 ± 4.3 ^{c)} (76)	139.1 ± 10.3 (105)
Compound I	4	8.4 ± 0.6 ^{a)} (82)	102.2 ± 11.1 ^{c)} (58)	114.7 ± 5.0 (87)
Compound I	8	7.6 ± 0.2 ^{b)} (74)	112.7 ± 10.2 ^{c)} (64)	129.7 ± 9.7 (98)

MG, methylguanidine; GSA, guanidinosuccinic acid; GAA, guanidinoacetic acid. Values are means ± S.E. of 6 rats. Figures in parentheses are percentages of the control value. a) Significantly different from the control value, $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$.

Chart 1. Fractions A and B from *Salviae miltiorrhizae Radix* both caused a significant decrease of urea nitrogen concentration in serum. The blood urea nitrogen-decreasing activities of fractions II and III were 35% and 30%, respectively, compared with that of the control, while fraction I had no effect.

Effect of Compound I on Urea Nitrogen and Creatinine Levels in the Serum

As shown in Table II, the blood urea nitrogen-decreasing activity of compound I was 20% or 30%, compared with the control, at the dose level of 2 or 4 mg/rat/d. The oral administration of 8 mg/rat/d also caused a significant reduction. Compound I decreased the

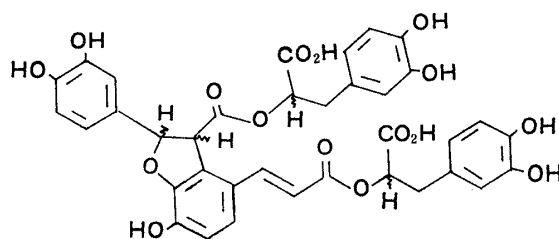


Fig. 1

level of creatinine in serum at doses of 4 and 8 mg/rat/d.

Effect of Compound I on Levels of Guanidino Compounds in the Serum

The serum concentrations of various guanidino compounds after administration of compound I are shown in Table III. Methylguanidine (MG) in the serum was decreased significantly at the dosage level of 4 mg/rat/d of compound I. At the 8 mg/rat/d level, MG showed a decrease from 10.3 to 7.6 $\mu\text{g/dl}$ (a 26% change). Compound I resulted in a significant decrease in guanidinosuccinic acid (GSA). GSA-decreasing activities were 24%, 42%, and 36% at 2, 4, and 8 mg/rat/d, respectively, compared with the control value, while the decrease of guanidinoacetic acid (GAA) was not significant after the administration of the compound I in the dose range of 2 to 8 mg/rat/d.

Characterization of Compound I

Compound I is a pale yellowish brown amorphous powder showing a positive reaction to iron chloride. Its carbon-13 nuclear magnetic resonance (^{13}C -NMR) spectrum shows 4 carboxyl groups (δ 177.4, 176.6, 172.5, and 169.2), 1 methine (δ 56.7), and 2 methine groups linked to oxygen (δ 78.1 and 87.2). The proton nuclear magnetic resonance (^1H -NMR) spectrum showed *trans*-olefin hydrogens at 6.30 and 7.52 (each d, $J=16$ Hz) and mutually coupled methylene signals at the benzyl region of 4.49 and 5.62 (each d, $J=4$ Hz). Thus, compound I is considered to be the tetramer of (dihydro) caffeic acid (Fig. 1). Detailed structural analyses are in progress.

Discussion

It has been reported that *Salviae miltiorrhizae Radix* has the pharmacological actions of vasodilatation, hypotensive activity, antibacterial activity and lowering of blood lipid.¹⁴⁾ It has recently been used for the elimination of pains due to coronary insufficiency and hemostasis and for the acceleration of vasodilatation.¹⁵⁾ It was also reported that injection of *Salviae miltiorrhizae Radix* preparation was effective clinically for emergency treatment of myocardial ischemic findings.¹⁴⁾

On the other hand, Zhang *et al.* reported that an intravenous drip of *Salviae miltiorrhizae Radix* decreased blood levels of urea nitrogen and creatinine of uremic patients, while accelerating creatinine clearance.¹⁶⁾ Since oral administration to patients is widely used, the effect of oral treatment with *Salviae miltiorrhizae Radix* on uremic rats was investigated in our laboratory, and the extract was shown to improve the uremic state in our previous papers.^{2,3)}

In this work, we attempted to isolate the active components. Thus, the systematic isolation and purification of the active components from *Salviae miltiorrhizae Radix* were performed by monitoring the blood urea nitrogen-decreasing activity. Compound I (tetramer of (dihydro)caffeic acid) was found to be one of the active components. It is considered that compound I improves the adenine diet-induced uremic state in rats, since it decreased urea nitrogen, creatinine, MG, and GSA levels in the blood of these rats. However, a significant

increase in the level of GAA was not seen. Such observations are different from those following oral administration of *Salviae miltiorrhizae Radix* extract, as reported previously.^{2,3)}

We have reported that rhubarb has an improving effect on uremia^{17,18)} and its active component might be tannins of low molecular weight.¹⁹⁾ As shown in the present investigation, the active component of rhubarb for improving uremia is considerably different from that of *Salviae miltiorrhizae Radix*. Further detailed examinations are required to clarify the mechanisms involved. It is considered that *Salviae miltiorrhizae Radix* might act through several mechanisms, including suppressing the production of the uremia toxins, stimulating excretion of the uremic toxins, *etc.*

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