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Effect of Extract from Rhei Rhizoma on Urea-nitrogen Concentration in Rat Serum[1)]

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Intraperitoneal administration of the extract from Rhei Rhizoma decreased the urea-nitrogen (BUN) concentration in rat serum. An attempt was made to extract and partially purify the active substance(s), monitoring the BUN-decreasing activity. The activity was detected in the aqueous extract. After partition between butanol and water, biological activity was detected in both fractions. Further purification was carried out by dialysis and Sephadex LH-20 column chromatography, by which small molecular compounds, including anthraquinone glycosides, were eliminated. The BUN-decreasing activity was detected in fractions 3-2-1B, 3-2-2B, and 4-2-3-S1.

Keywords——Rhei Rhizoma; blood urea-nitrogen; extraction; fractionation; intraperitoneal administration

We previously reported that intraperitoneal administration of 5 varieties of crude drugs, Rhei Rhizoma (*Rheum officinale* Baillon), Coptidis Rhizoma (*Coptis japonica* Makino), Ephedrae Herba (*Ephedra distachya* Linne), Paeoniae Radix (*Paeonia albiflora* Pallas var.), and Bupleuri Radix (*Bupleurum falcatum* Linne), resulted in decreases of urea-nitrogen (BUN) concentration in rat serum (maximal at 6—8 hr after treatment) and that the extract from Rhei Rhizoma was the most effective.[1)] In addition, the concentrations of seven amino acids, Gln, Glu, Ala, Gly, Ser, Met, and Arg, in rat plasma and the concentrations of three amino acid, Glu, Gln, and Asp, in the liver were reduced at 2 hr after the treatment with extract from Rhei Rhizoma.[3)] Therefore, it was of interest to determine whether the same component was responsible for these effects.

In the present work, an attempt was made to extract and partially purify the active substance from Rhei Rhizoma by monitering the BUN-decreasing activity.

Materials and Methods

Animals——Male Wistar rats weighing *ca.* 140 g were used throughout the experiments. The animals were housed in air-conditioned quarters kept at 25° and 60% relative humidity. Rats were given laboratory pellet chow (CLEA Japan Inc., Tokyo) and tap water *ad libitum*.

Extraction and Fractionation——Powdered Rhei Rhizoma (雅黄; 350 g) was successively extracted with benzene (Fr. 1), acetone (Fr. 2), and water at room temperature and then with hot water (Fr. 5), giving Fr. 1 (1.05 g), Fr. 2 (53.6 g), and Fr. 5 (23.0 g). The water extract was partitioned between *n*-butanol and the aqueous phase to afford Fr. 3 (61.8 g) and Fr. 4 (55.8 g).

Fraction 3 (10.0 g) was redissolved in water and dialyzed for 7 days to obtain the dialyzed fraction (Fr. 3-1; 2.9 g) and the non-dialyzable fraction (Fr. 3-2). Concentrated Fr. 3-2 was separated into the water-soluble portion (Fr. 3-2-1; 2.36 g) and the methanol-soluble portion (Fr. 3-2-2; 3.09 g). Fraction 3-2-1

1) T. Nagasawa, S. Shibutani, and H. Oura, *Yakugaku Zasshi*, **99**, 71 (1979). This work was pressented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August, 1979.
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3) S. Shibutani, T. Nagasawa, T. Yokozawa, and H. Oura, *Yakugaku Zasshi*, **100**, 434 (1980).

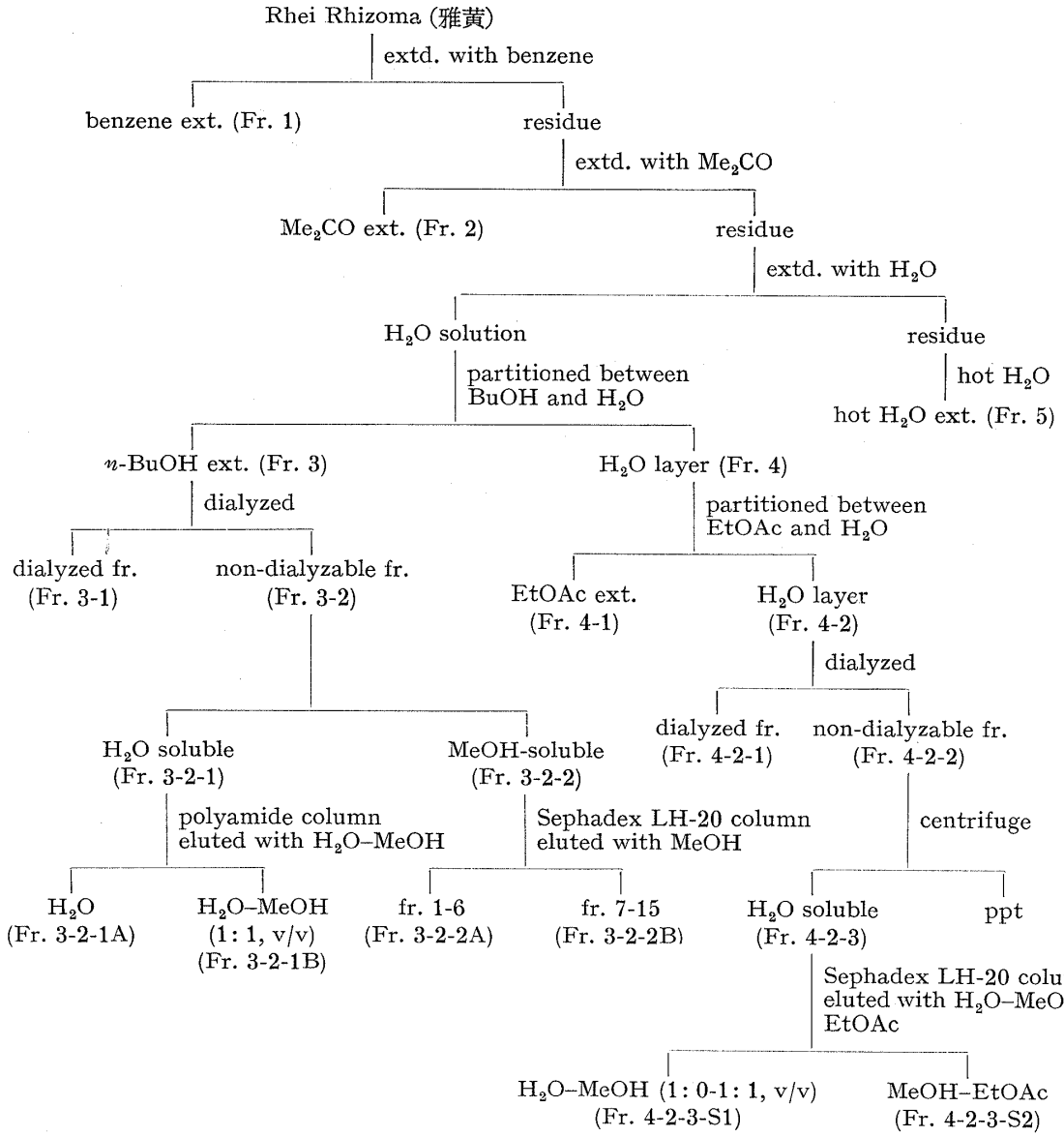

Chart 1. Extraction and Fractionation Procedures

(1.0 g) was then column-chromatographed on polyamide and eluted with water and water-methanol (1: 1, v/v) to give Fr. 3-2-1A (117 mg) and Fr. 3-2-1B (178 mg), respectively. Fraction 3-2-2 (1.0 g) from the water-insoluble portion was column-chromatographed on Sephadex LH-20 with methanol to give Fr. 3-2-2A (29 mg) and Fr. 3-2-2B (343 mg).

Fraction 4 (10 g) was partitioned between ethyl acetate and water. The ethyl acetate layer was evaporated to dryness (Fr. 4-1; 131 mg). The aqueous layer (Fr. 4-2) was dialyzed against water for 14 days to provide the dialyzed fraction (Fr. 4-2-1; 3.3 g) and the non-dialyzable fraction (Fr. 4-2-2; 4.87 g). Fraction 4-2-2 was centrifuged ($800 \times g$, 10 min), giving a precipitate and water-soluble portion (Fr. 4-2-3; 4.2 g). Fraction 4-2-3 (300 mg) was column-chromatographed on Shephadex LH-20, eluting with water, water methanol (1: 1, v/v), methanol, and ethyl acetate to give Fr. 4-2-3-S1 (142 mg) and Fr. 4-2-3-S2 (123 mg).

Preparation of Serum——Each fraction (0.5 ml of saline solution) was administered intraperitoneally to rats at 10 a. m., while control rats were treated with an equal volume of saline at the same time. Blood samples were collected by decapitation at 6 p. m., 8 hr after treatment.[1] The blood was allowed to stand for several hours in a cold room at 4°, and then sera were separated by centrifugation ($1000 \times g$, 10 min).

BUN Assay——Estimation of BUN concentration in serum was carried out with commercial reagent, BUN"EIKEN" (Eiken Chemical Co., Tokyo), using a DSA-560 discrete sample analyzer (Beckman Instrument, Inc., U.S.A). Determination of BUN was based on the urease-indophenol method. The reaction mixture was incubated at 37° for 18.75 min, allowed to stand at room temperature for 2.25 min, and then the BUN concentration was determined by colorimetry at 550 nm with urea solution as a standard.

Results and Discussion

Effects of the Fractions from Rhei Rhizoma on BUN Concentration in Rat Serum

It became clear that the hot water extract from Rhei Rhizoma caused a decrease of BUN concentration in rat serum after a single intraperitoneal administration.[1] As shown in Table I, the BUN concentration in rat serum decreased after intraperitoneal administration of 5 mg of Fr. 3 or Fr. 4. In addition, the BUN-decreasing effects of Fr. 3 and Fr. 4 were confirmed by treatment with graded dose of these fractions. Eight hours after administration, Fr. 3 (5 mg/rat) and Fr. 4 (5 mg/rat) reduced the BUN concentration in serum by 63% as compared with the control.

Effects of Further Purified Fractions from Fractions 3 on BUN Concentration in Rat Serum

Fraction 3 was further separated according to Chart 1. Table II shows the effect of each of the resulting partially purified fractions on BUN concentration in rat serum at 8 hr after

Table I. Effects of Fractions from Rhei Rhizoma on Blood Urea-nitrogen Concentration

Materials	Dose (mg/rat)	No. of rats	Blood urea-nitrogen (mg/100 ml)	Blood urea-nitrogen (%)
(Experiment I)				
Control (saline)		6	17.4±0.9	(100)
Fr. 2	5	6	15.2±2.3	(87)
Fr. 3	1	4	15.6±0.8	(90)
	2.5	6	14.1±2.2[a]	(81)
	5	6	10.9±1.6[b]	(63)
Fr. 4	1	6	15.7±1.5	(90)
	2.5	6	13.8±1.9[a]	(79)
	5	6	11.0±2.8[b]	(63)
Fr. 5	5	6	15.9±1.4	(91)

Data are expressed as means±S.D.
a) Significantly different from the control value, $p<0.01$.
b) Significantly different from the control value, $p<0.001$.

Table II. Effects of Partially Purified Fractions from Fraction 3 of Rhei Rhizoma on Blood Urea-nitrogen Concentration

Materials	Dose (mg/rat)	No. of rats	Blood urea-nitrogen (mg/100 ml)	Blood urea-nitrogen (%)
(Experiment II)				
Control (saline)		14	17.3±2.0	(100)
Fr. 3-1	5	12	15.2±3.8	(88)
Fr. 3-2-1	5	13	11.2±1.7[c]	(65)
Fr. 3-2-1A	5	7	16.5±3.2	(95)
(Experiment III)				
Control[a]		7	13.8±1.9	(100)
Saline		7	14.3±1.4	(103)
Fr. 3-2-1B[b]	5	7	10.7±1.4[d]	(78)
Fr. 3-2-2A[b]	5	6	12.9±2.4	(94)
Fr. 3-2-2B[b]	5	7	9.5±1.1[c]	(69)
Sennoside-A[b]	5	7	14.3±1.4	(104)

Data are expressed as means±S.D.
a) Control rats were treated with 4% acacia-saline solution.
b) Each material was suspended in 4% acacia-saline solution.
c) Significantly different from the control value, $p<0.001$.
d) Significantly different from the control value, $p<0.01$.

treatment. In experiment II, the fractions soluble in saline were examined. Administration of Fr. 3-2-1A had no effect. In experiment III, fraction insoluble in saline, suspended in 4% acacia-saline solution, were tested. The treatments with Fr. 3-2-2B and Fr. 3-2-1B resulted in decreases of BUN concentration in rat serum, the reductions being 69% and 78%, respectively, compared with the control values. The control (4% acacia-saline solution), Fr. 3-2-2A, and sennoside-A did not affect the BUN concentration in rat serum after intraperitoneal administration.

Effects of Further Purified Fractions from Fraction 4 on BUN Concentration in Rat Serum

Since Fr. 4 had a BUN-decreasing effect as shown in Table I, Fr. 4 was further separated by the procedures shown in Chart 1. The resulting partially purified fractions were examined for BUN-decreasing activity after intraperitoneal administration of 5 mg doses to rats, and the results are shown in Table III. BUN-decreasing activity was detected in Fr. 4-2-2, Fr. 4-2-3, and Fr. 4-2-3-Sl, the reductions being 67%, 62%, and 62%, respectively, compared with the control. In contrast, Fr. 4-1, Fr. 4-2-1, and Fr. 4-2-3-S2 had no effect. Accordingly, the BUN-decreasing activity detected in Fr. 4 may be due to Fr. 4-2-3-Sl. The dialysis and Sephadex LH-20 column chromatography procedures would have eliminated low molecular compounds, including anthraquinone glycosides, suggesting that the BUN-decreasing active compound(s) in Rhei Rhizoma may be of high molecular weight. Further studies are in progress to isolated the active compound(s) and to investigate the nature of the BUN-decreasing effect produced by treatment with Rhei Rhizoam extract.

Table III. Effects of Partially Purified Fractions from Fraction 4 of Rhei Rhizoma on Blood Urea-nitrogen Concentration

Materials	Dose (mg/rat)	No. of rats	Blood urea-nitrogen (mg/100 ml)	Blood urea-nitrogen (%)
(Experiment IV)				
Control (saline)		7	15.4±3.1	(100)
Fr. 4-1	5	7	13.4±1.9	(88)
Fr. 4-2-1	5	7	15.1±1.9	(98)
Fr. 4-2-2	5	7	10.3±1.9[a]	(67)
(Experiment V)				
Control (saline)		7	14.2±1.5	(100)
Fr. 4-2-3	5	7	8.9±1.5[b]	(62)
(Experiment VI)				
Control (saline)		7	17.0±3.6	(100)
Fr. 4-2-3-S1	5	7	10.6±1.5[b]	(62)
Fr. 4-2-3-S2	5	7	15.0±1.6	(88)

Data are expressed as means±S.D.
a) Significantly different from control value, $p<0.01$.
b) Significantly different from control value, $p<0.001$.