The plasmas of dogs administered sulthiame orally were analyzed by the present method. The results are shown in Fig. 2. The plasma sulthiame level increased immediately after the administration, reached a maximum in 2 hr, and then decreased in a first order manner. The biological half-life was about 7.3 hr.

The bioequivalence of sulthiame-containing tablets (Ospolot Tablets) of two formulas, which were the same in content of sulthiame but different in the kind and contents of inactive ingredients, was demonstrated in dogs. Details will be reported elsewhere.

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Effect of Extract from Paeoniae Radix on Urea-nitrogen Concentration in Rat Serum. I[1)]

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Intraperitoneal administration of the extract from Paeoniae Radix decreased the urea-nitrogen (BUN) concentration in rat serum. An attempt was made to extract and purify the active components, monitoring the BUN-decreasing activity. The acetone fraction from Paeoniae Radix decreased BUN concentration. After partition between ethyl acetate (EtOAc) and water, BUN-decreasing activity was detected in the EtOAc fraction. Further purification was carried out by Sephadex LH-20 column chromatography, and 1,2,3,4,6-penta-O-galloyl glucose, (+)-catechin, and gallic acid were purified from the EtOAc fraction. (+)-Catechin and gallic acid showed no activity. It became clear that one of the BUN-decreasing components from Paeoniae Radix was 1,2,3,4,6-penta-O-galloyl glucose, which is a typical tannin of the gallotannins.

Keywords——paeoniae radix; blood urea nitrogen; gallotannin; 1,2,3,4,6-penta-O-galloyl glucose; (+)-catechin; gallic acid

It was previously reported that intraperitoneal administration of each of 6 Kanpo prescriptions from among 65 varieties resulted in decreases of urea-nitrogen (BUN) concentrations in rat serum.[2)] Furthermore, administration of each of 7 crude drugs (Rhei Rhizoma, Coptidis

Rhizoma, Ephedra Herba, Paeoniae Radix, Bupleuri Radix, Glycyrrhizae Radix, and Scutellariae Radix) from among the crude drugs which constitute the 6 Kanpo prescriptions reduced BUN concentration.[1)]

Urea nitrogen concentrations were decreased in the liver at 8 hr and in the kidney at 4—8 hr after treatment with extract from Rhei Rhizoma. The concentrations of seven amino acids, Gln, Ala, Gly, Ser, Glu, Met, and Arg, in rat plasma and the concentrations of three amino acids, Glu, Gln, and Asp, in the liver were reduced at 2 hr after the treatment. In addition, with respect to the balance of plasma amino acids across various organs at 2 hr after the administration, release of Gln from the liver was decreased and the uptake of Gln by non-hepatic visceral organs was also decreased. These results suggested that BUN-decreasing activity was caused by a decrease of urea synthesis due to a reduction of the concentrations of amino acids in plasma and the liver.[3)]

Furthermore, it has been found that the BUN-decreasing constituent from Rhei Rhizoma was in a tannin fraction.[4)] Thus, it was of interest to determine whether the same component from crude drugs was responsible for BUN-decreasing activity.

In this work, an attempt was made to extract and purify the active substance from Paeoniae Radix by monitoring the BUN-decreasing activity.

Materials and Methods

Animals——Male Wistar rats weighing *ca.* 140 g were used throughout the experiments. The animals were maintained in an air-conditioned room with lighting from 6 a.m. to 6 p.m. The room was kept at 25±1° and 60% relative humidity. Laboratory pellet chow (CLEA Japan Inc., Tokyo) and tap water were given freely.

Extraction and Fractionation——Paeoniae Radix purchased from Nakai Koshin-Do (Kobe) was extracted, fractionated, and purified according to Chart 1. The recovery of each fraction is indicated in Chart 1.

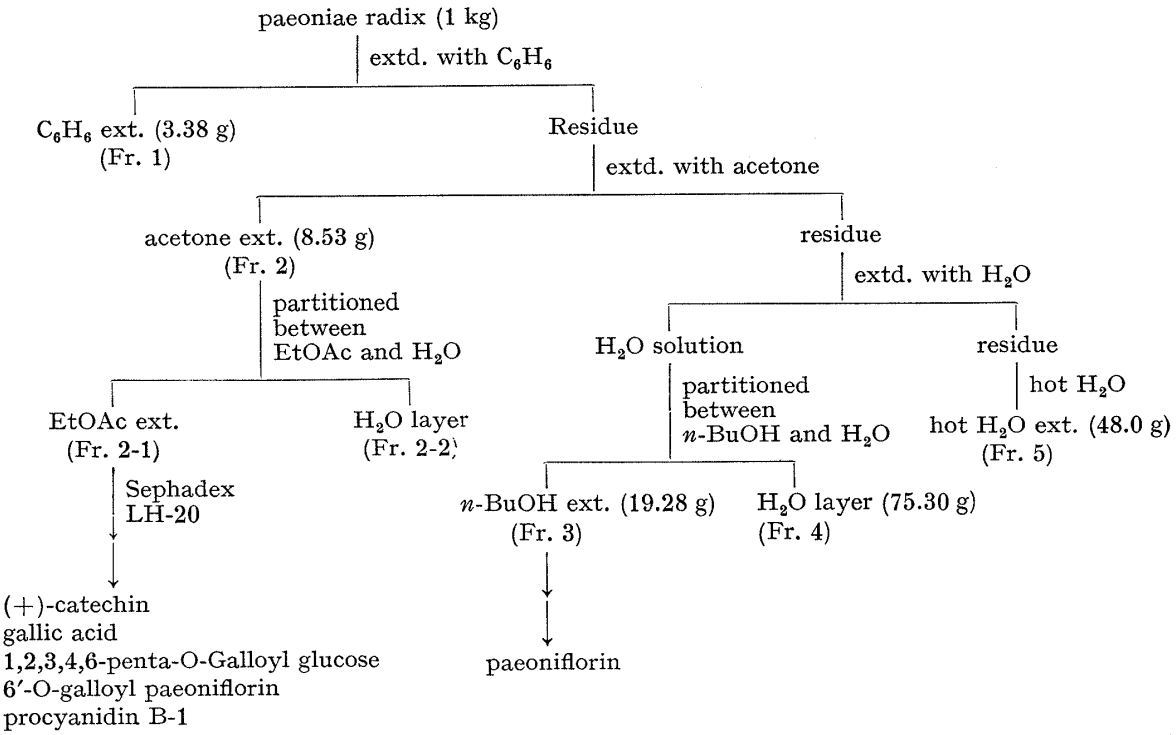

Chart 1. Extraction and Fractionation

Preparation of Serum——Each fraction made soluble in saline or 5% ethanol/saline was administered intraperitoneally to rats at 10 a.m. while control rats were treated with an equal volume of saline. Blood samples were collected by decapitation at 6 p.m., 8 hr after the treatment. The blood was allowed to stand for several hours in a cold room at 4°, and then serum was separated by centrifugation (1000×*g*, 10 min., 4°).

During the experiment, laboratory pellet chow was removed from the rat cage at 9 a.m. but water was given freely.

BUN Assay——Estimation of BUN concentration in serum was carried out with the commercial reagent, BUN "EIKEN" (Eiken Chemical Co., Tokyo), using a DSA-560 discrete sample analyzer (Beckman Instrument Inc., U.S.A.) according to the previous paper.[4] Determination of BUN was based on the urease-indophenol method.[5]

Results

Effect of Each Fraction from Paeoniae Radix on BUN Concentration

Table I shows the effect on BUN concentration of each fraction separated according to Chart 1 at 8 hr after intraperitoneal administration. It became clear that Fr. 2 (acetone ext.) caused a decrease of BUN concentration, while Frs. 3, 4 and 5 had no effect.

Table I. Effect of Each Fraction on BUN[a] Concentration at 8 Hr after Intraperitoneal Administration

Material	Dose mg/rat	No. of rats	BUN mg/100 ml[b]	(%)
Control (saline)		7	17.0±3.6	100
Fr. 2	25	6	11.0±2.1[c]	65
Fr. 3	25	6	15.2±2.0	89
Fr. 4	25	6	16.6±1.2	98
Fr. 5	25	6	16.7±1.6	98

a) Blood urea nitrogen.
b) Data are expressed as means±S.D.
c) $p<0.001$, student's t test.

Effect of Each Fraction from Fr. 2 on BUN Concentration

As shown in Chart 1, Fr. 2 was partitioned between ethyl acetate (EtOAc) (Fr. 2-1) and the aqueous phase (Fr 2-2). Each fraction was examined for BUN-decreasing activity after administration. As shown in Table II, the effect of graded doses of Fr. 2-1 on BUN concentration was approximately dose-dependent. The BUN concentration was decreased by 25—30% as compared with the control value upon administration of 5—12.5 mg/rat of Fr. 2-1, while Fr. 2-2 showed no effect (data not shown).

Table II. Effect of Fr. 2-1 on BUN Concentration at 8 hr after Intraperitoneal Administration

Dose mg/rat	No. of rats	BUN mg/100 ml[a]	(%)
Control (saline)	7	15.8±1.5	100
2.5	5	13.3±2.3[b]	84
5	6	11.8±2.0[c]	75
10	6	11.9±1.7[c]	75
12.5	6	11.1±1.1[d]	70

a) Data are expressed as means±S.D.
b) $p<0.05$, student's t test.
c) $p<0.01$.
d) $p<0.001$.

Effect of Each Purified Fraction from Fr. 2-1 on BUN Concentration

As shown in Chart 1, 1,2,3,4,6-penta-0-galloyl glucose,[6] (+)-catechin, and gallic acid were purified from Fr. 2-1 by means of column chromatography on Sephadex LH-20 with

Table III. Effect of Each Component on BUN Concentration at 8 hr after Intraperitoneal Administration

Material	Dose mg/rat	No. of rats	BUN mg/100 ml[a]	(%)
Exp. I. Control (5% ethanol/saline)		12	14.6±2.1	100
1,2,3,4,6-Penta-O-galloyl glucose	0.5	5	13.2±1.5	90
	1	10	11.9±1.8[b]	82
	2.5	9	10.9±1.3[c]	75
	5	5	10.9±1.0[a]	75
Exp. II. Control (saline)		17	14.3±2.1	100
(+)-Catechin	0.5	6	13.6±1.4	95
	1	4	13.1±1.8	92
	2.5	5	14.7±1.3	103
Paeoniflorin	0.5	6	15.0±2.0	105
	1	6	15.1±2.1	106
	2.5	6	13.8±1.3	97
Gallic acid	0.5	6	14.8±1.6	103
	1	5	13.9±1.0	97
	2.5	6	12.3±1.8	86
Glucose	0.5	5	13.5±1.6	94
	1	5	14.1±2.2	98
Gallic acid + Glucose	2.5 + 0.5	6	13.0±1.4	91

a) Data are expressed as means±S.D.
b) $p<0.01$, Student's t test.
c) $p<0.001$.

ethanol–acetone.[7] Paeoniflorin, which is a known component of Paeoniae Radix[8] and Moutan Cortex radicis (*Paeonia suffruticosa* Andr),[9] was purified from Fr. 3. Each purified component was examined for BUN-decreasing activity, and the results are shown in Table III.

1,2,3,4,6-Penta-0-galloyl glucose (2.5—5 mg/rat) reduced BUN concentration by 25% as compared with the control. (+)-Catechin and paeoniflorin did not affect the BUN concentration. Gallic acid and glucose, which are the structural components of galloyl glucose, showed no effect (Experiment II).

Discussion

An aqueous extract from Rhei Rhizoma reduced BUN concentration after intraperitoneal administration.[4] In the present paper, the procedure of extraction from Paeoniae Radix was carried out similarly to that of extraction from Rhei Rhizoma. Acetone fraction (Fr. 2) form Paeoniae Radix resulted in a decrease of BUN concentration (Table I), Therefore, an attempt was made to extract and purify the active substances from Paeoniae Radix, monitoring the BUN-decreasing activity. It was found that one of the BUN-decreasing components was 1,2,3,4,6-penta-o-galloyl glucose (Table III).

1,2,3,4,6-Penta-o-galloyl glucose is a typical tannin of the gallotannins (hydrolyzable tannins). Upon hydrolysis of the tannin, gallic acid and glucose are obtained. However, as shown in Table III, gallic acid and glucose did not show BUN-decreasing activity, so that the appearance of BUN-decreasing activity seemed to require the linkage of gallic acids to glucose by dehydration.

On the other hand, it was presumed that the BUN-decreasing components of Rhei Rhizoma were catechol tannins (condensed tannins),[4,7] which are structurally different from 1,2,3,4,6-penta-0-galloyl glucose purified from Paeoniae Radix. Therefore, it is of great interest to determine whether the BUN-decreasing activity is a common effect of tannins.

In the future, we expect to examine the effect on BUN concentration of 6′-o-galloyl paeniflorin and procyanidin B-1, which can be isolated from Fr. 2-1 (EtOAc ext.), but only in very small amounts.

Furthermore, we are examining the relationship between the structure and the activity of various tannins, and the mode of action of the BUN-decreasing activity.

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Chemical and Biochemical Studies on Carbohydrate Esters. X.[1] Plant Growth Inhibition by Pure Anomers of Synthetic 1-O-Lauroyl-D-glucopyranose[2]

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Anomerically pure, synthetic 1-O-lauroyl-α- and -β-D-glucopyranoses exhibited strong plant growth inhibiting activity in the Avena coleoptile straight growth test at a final concentration of 500/3 ppm. The growth inhibition ratios obtained with α- and β-anomers were 94.2% and 81.6%, respectively. Under the same conditions, 2-O- and 4-O-lauroyl-D-glucopyranoses and the mono-, di-, and poly-substituted products obtained by selective lauroylation of maltose were all found to be ineffecuive. In view of these results, the presence of a lauroyl function, either axial or equatorial, at the C-1 position of a monosaccharide unit appears to be important for this biological activity.

Keywords——plant growth inhibiting effect; Avena coleoptile straight growth test; 1-O-lauroyl-α-D-glucopyranose; 1-O-lauroyl-β-D-glucopyranose; 2-O-lauroyl-D-glucopyranose; 4-O-lauroyl-D-glucopyranose; maltose esters of lauric acid

In our previous study,[4] a variety of fatty acid esters of D-glucose, sucrose, and trehalose were examined for their plant growth regulating abilities. It was found that mixtures containing the α- and β-anomers of 1-O-lauroyl-D-glucopyranose in different ratios could produce strong plant growth inhibition, when their abscisic acid (ABA)-like activities were evaluated in Nitsch's Avena coleoptile straight growth assay.[5] Under similar test conditions, lauric acid in the free form, 1-O-acyl-D-glucopyranosyl esters of caprylic, myristic, and stearic acids,