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Studies on the Absorption, Distribution, Excretion and Metabolism of Ginseng Saponins. I.[1] Quantitative Analysis of Ginsenoside Rg_1 in Rats

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Quantitative analysis of ginsenoside Rg_1, isolated from red ginseng (*Panax ginseng* C.A. Meyer) and one of the main saponins, was experimented in rats for the studies of the absorption, distribution, excretion and metabolism of ginseng saponins.

The analysis procedure was developed as follows. Ginsenoside Rg_1 added to biological samples of rats was adsorbed on a Servachrom XAD-2 resin column after deproteinization with methanol. The adsorbed ginsenoside Rg_1 was eluted with 60% methanol aqueous solution and further subjected to thin-layer chromatography with chloroform–methanol–water (65: 35: 10, lower phase) as the developing solvent and 8% vanillin methanol solution/72% H_2SO_4 (1: 5) as the detecting reagent.

As reasonable recoveries and standard deviations were found in this procedure, the concentrations of ginsenoside Rg_1 in samples from rats treated with ginsenoside Rg_1 (100 mg/kg, *p.o.*) were determined by applying the described method.

Keywords——ginsenoside Rg_1; quantitative analysis; distribution in rat; dual-wavelength TLC scanner; Servachrom XAD-2 resin

The root of *Panax ginseng* C.A. Meyer (Araliaceae) is an important component in various prescriptions in Chinese traditional medicine. It has also been used for thousands of years as an important folk drug in Korea, China and Japan.

Ginseng saponins, isolated from the root of *Panax ginseng*, have been regarded as principal components manifesting the pharmacological activities of the drug. We also reported in a previous paper[2] that crude total ginseng saponins had an inhibitory effect on side effects induced by cortisone acetate. There are many reports of pharmacological[3] and chemical[4] studies on ginseng saponins. However, little is known about the absorption, distribution, excretion and metabolism of ginseng saponins.

In the present paper, we describe a method for the quantitative determination of ginsenoside Rg_1, one of the main ginseng saponins, in biological samples of rats.

Experimental

Materials——Experimental animals used were male Sprague-Dawley (JCL: SD, SPF) rats weighing 180—200 g. The rats were deprived of food but given free access to water for 18 h prior to the experiments.

Ginsenoside Rg_1 was isolated from red ginseng supplied by Japan Korea Red Ginseng Co., Ltd., by high-performance liquid chromatography (HPLC). Servachrom XAD-2 resin was purchased from Rohm and Haas Co., Ltd. Other chemicals used were *n*-butanol (*n*-BuOH), methanol (MeOH), *o*-vanillin and H_2SO_4 (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) and they were of reagent grade or were purified by distillation.

Thin-Layer Chromatography (TLC)——TLC was done on Merck precoated kieselgel 60 plates (0.25 mm thick). As a developing solvent for TLC, the following mixture was used: $CHCl_3$–MeOH–H_2O (65: 35: 10, v/v, lower phase). The detection of spots on TLC plates was done by spraying 8% vanillin in MeOH–72% H_2SO_4 (1: 5, v/v) or 10% H_2SO_4 followed by heating (140°C, 3—4 min). TLC densitograms were obtained on a Shimadzu CS-910 chromatogram scanner equipped with a dual wavelength spectrophotometer under the following conditions.

detecting wavelength: 530 nm

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reference wavelength: 780 nm
slit width: 1.25 × 1.25 mm
scanning mode: zig-zag

The peak areas were calculated by using an equipped integrator.

Biological Samples——Rat blood was taken from a polyethylene tube canulated into the right carotid artery of a rat under anesthesia with sodium pentobarbital (25 mg/kg, *i.p.*). After removal of the blood, tissue samples such as liver, kidney, heart, lung, spleen, stomach and intestines (digestive organ samples included their contents) were obtained from the rat. Serum was obtained by centrifugation at 3000 rpm for 15 min after clotting. Urine and feces samples were collected separately for 24 h by the use of a metabolic cage (KN-646, Natsume, Tokyo, Japan).

Determination of Ginsenoside Rg_1 in Biological Samples——Serum (3 ml) was treated with MeOH (12 ml) according to the procedure in Chart 1, and it was prepared as a final 20% MeOH aqueous solution (5 ml). Tissue samples (whole organs except for liver), 2 g of liver sample and 0.5—5 g of feces samples were each homogenized with 5—9 ml of distilled water in a glass homogenizer. After homogenization, MeOH (30 ml) was added to extract ginsenoside Rg_1. The MeOH extracts of these samples were obtained by evaporation of the MeOH under reduced pressure, and then they were prepared as 20% MeOH aqueous solution (5 ml). Urine (5 ml) was also treated with MeOH (20 ml) and prepared as a final 20% MeOH aqueous solution (5 ml). The 20% MeOH aqueous solution was applied to an XAD-2 resin column (1.0 cm in diameter, 9.0 cm in height) pre-equilibrated with 20% MeOH aqueous solution. The eluate obtained with 60% MeOH aqueous solution was evaporated to dryness under reduced pressure. The residue was redissolved in MeOH and 5 μl of it was used as a sample for TLC. The *n*-BuOH extraction of serum is dealt with in Chart 1.

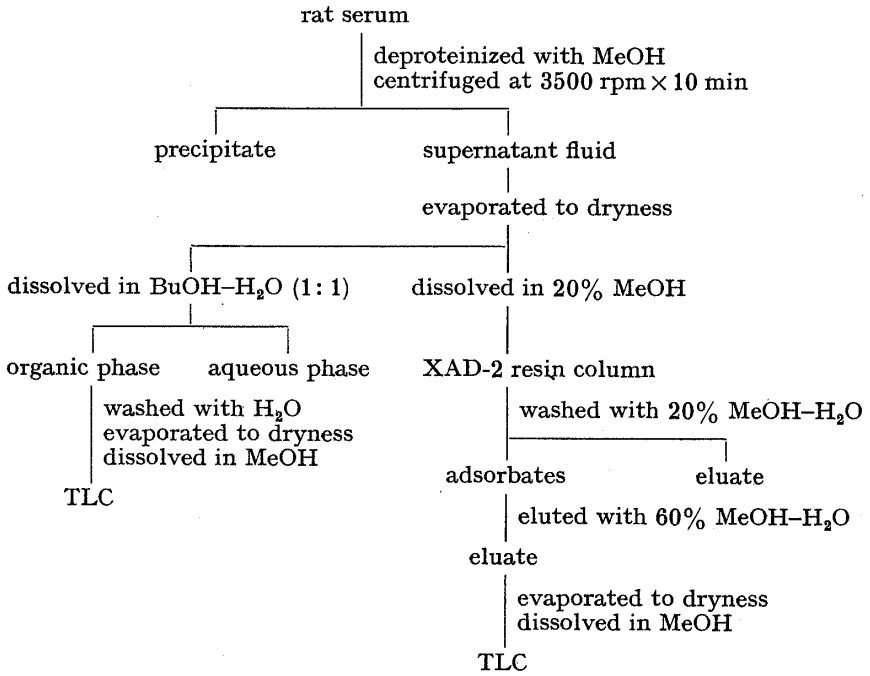

Chart 1. Assay Procedure for Ginsenoside Rg_1 in Serum

Recovery of Ginsenoside Rg_1——A definite volume of ginsenoside Rg_1 aqueous solution (1 mg/ml) was added to homogenates or solutions of biological samples of normal rats. Then ginsenoside Rg_1 in these biological samples was determined by using the method mentioned above.

Ginsenoside Rg_1 Administration——Ginsenoside Rg_1 was dissolved in distilled water to 20 mg/ml, and administered orally at a dose of 100 mg/kg to rats. One hundred and fifty min after the administration of ginsenoside Rg_1, the rats were killed under anesthesia and were dissected as described above. Then ginsenoside Rg_1 was determined by using the method described above.

Results

Calibration Curves

Calibration curves obtained with two detecting reagents are shown in Fig. 1. There was a linear relationship between the amount (0.5—3.0 μg) of ginsenoside Rg_1 applied and the

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integration value. However, the sensitivity of the vanillin–H_2SO_4 detecting reagent was superior to that of the 10% H_2SO_4 reagent. Thus, vanillin–H_2SO_4 detecting reagent was used routinely.

The slope of the calibration curve differed somewhat with each TLC plate as pointed out by Sanada *et al.*[5] Therefore, one μg of ginsenoside Rg_1 was applied to every TLC plate as a standard.

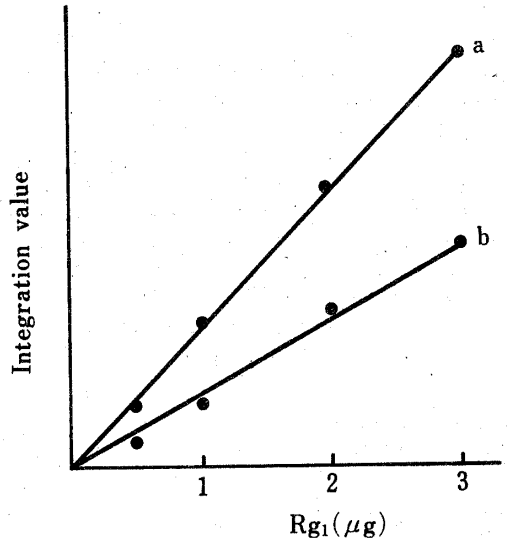

Fig. 1. Comparison of Detecting Reagents for Ginsenoside Rg_1 on a TLC

Plate: Merck Precoated Kieslgel 60.
Heating: 140°C for 4 min.
a) 8 % vanillin MeOH soln./72% H_2SO_4 (1: 5).
b) 10 % H_2SO_4.

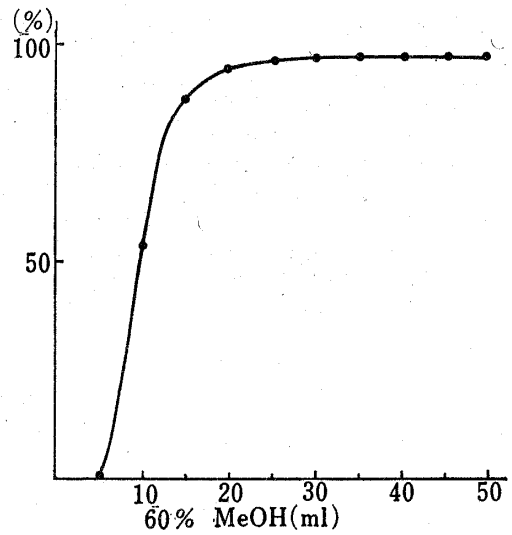

Fig. 2. Cumulative Recovery of Ginsenoside Rg_1 from an XAD-2 Resin Column

Adsorbed ginsenoside Rg_1: 20 mg.
Eluent: 60 % MeOH aqueous solution.

Elution of Ginsenoside Rg_1 from the XAD-2 Resin Column

Elution of ginsenoside Rg_1 (20 mg) from an XAD-2 resin column was performed by using 20%, 60% and 100% MeOH aqueous solutions as eluents.

Ginsenoside Rg_1 was not eluted by 20% MeOH aqueous solution. Nearly complete elution of ginsenoside Rg_1 was obtained by 60% and 100% MeOH aqueous solutions. The elution curve in the case of 60% MeOH aqueous solution is shown in Fig. 2. The recovery of ginsenoside Rg_1 was 94%.

Isolation of Ginsenoside Rg_1 in Biological Samples

XAD-2 resin column chromatography was compared with *n*-BuOH extraction for the pretreatment of biological samples containing ginsenoside Rg_1. In the XAD-2 resin column chromatography (elution with 100% MeOH) and the *n*-BuOH extraction method, ginsenoside Rg_1 on the TLC plate was incompletely separated from biological components. On the other hand, ginsenoside Rg_1 was completely separated from biological components on the TLC plate by using the fraction eluted with 60% MeOH aqueous solution from the XAD-2 resin column. The *Rf* value of ginsenoside Rg_1 was 0.24. Figure 3 shows the TLC chromatogram of a serum sample. XAD-2 resin column chromatography (elution with 60% MeOH aqueous solution) was thus adopted as a standard procedure.

Recoveries of Ginsenoside Rg_1 added to Biological Samples

Recoveries of ginsenoside Rg_1 (3—1000 μg) added to homogenates or solutions of isolated organs (liver, kidney, heart, lung, spleen, stomach, small intestine and large intestine), serum,

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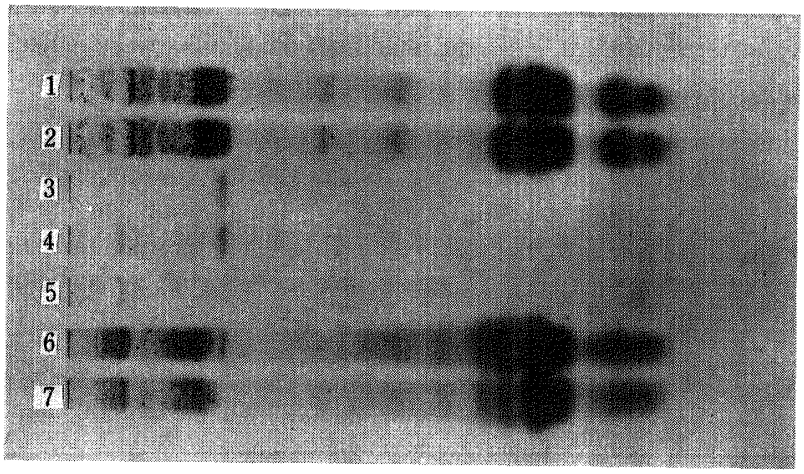

Fig. 3. Thin-Layer Chromatograms of Ginsenoside Rg_1 in Rat Serum

Developing solvent: $CHCl_3$–MeOH–H_2O (65: 35: 10, lower phase).
Plate: Merck precoated Kieselgel 60.
Detecting reagent: 8 % vanillin MeOH soln./72 % H_2SO_4 (1: 5), heating at 140°C for 3 min.
Sample size: 5 μl per spot.
1: BuOH extraction.
2: BuOH extraction + Rg_1.
3: Rg_1.
4: 60 % MeOH aq. eluate of XAD-2 resin column + Rg_1.
5: 60 % MeOH aq. eluate of XAD-2 resin column.
6: 100 % MeOH eluate of XAD-2 resin column + Rg_1.
7: 100 % MeOH eluate of XAD-2 resin column.

TABLE I. Recovery of Ginsenoside Rg_1 added to Rat Tissues, Fluids and Feces

Tissues or fluids[a]	Ginsenoside Rg_1 added (μg)	Recovery[b] (%)
Liver	30	96.3±1.4
	100	94.8±4.2
	1000	96.6±2.4
Kidney	30	91.3±4.9
	100	90.8±2.8
	1000	92.4±4.9
Heart	1000	96.9±1.8
Lung	1000	93.4±4.2
Spleen	1000	94.4±2.0
Stomach	1000	96.9±3.3
Small intestine	1000	98.2±3.2
Large intestine	1000	90.4±2.9
Serum	3	96.7±2.3
	30	96.0±3.0
	300	94.4±5.4
Urine	30	95.8±3.6
	100	95.1±3.9
	1000	98.4±1.1
Feces	1000	95.4±2.6

a) The employed amounts or volumes are as follows: liver (2 g), other tissues (whole organs), serum (3 ml), urine (5 ml) and feces (5 g).
b) Each value represents the mean ± S.D. of 3 experiments.

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urine and feces were investigated. As shown in Table I, mean recoveries of ginsenoside Rg_1 in all samples were more than 90% and standard deviations were generally below 5%.

Ginsenoside Rg_1 Concentration in Rats

Experiments were carried out on four rats. The spot (Rf=0.24) corresponding to ginsenoside Rg_1 was confirmed to be ginsenoside Rg_1 by TLC with the developing solvent BuOH–acetic acid–H_2O (4:1:5, upper phase, Rf=0.45). The metabolites[6] of ginsenoside Rg_1 were found in larger Rf values than ginsenoside Rg_1. The results are shown in Table II. The concentration of ginsenoside Rg_1 in rat tissues and serum were below 10 μg/g or ml. It was found that 77.3±3.9% of the dose remained in the digestive tract.

TABLE II. Ginsenoside Rg_1 Concentration in Rat Tissues after Oral Administration

Animal[a]		Concentration (μg/g or ml)								
No.	Weight (g)	Liver	Kidney	Heart	Lung	Spleen	Stomach	Small intestine	Large intestine	Serum
1	190	2.5	3.9	2.3	4.1	4.3	30.0	14890	80.0	0.4
2	195	1.3	4.3	2.2	2.1	3.6	150.0	14990	28.0	N.D.
3	200	3.5	8.3	5.6	3.2	9.5	20.0	14450	47.0	0.6
4	200	2.0	1.9	0.5>	2.9	2.4	20.0	16380	N.D.	0.2>

a) Ginsenoside Rg_1 was administered at a dose of 100 mg/kg. Rats were killed 150 min after administration. N.D.: not detected.

Discussion

Many studies have been reported on the quantitative analysis of ginseng saponins. The methods employed in those studies were gas liquid chromatography (GLC),[7] HPLC,[8] droplet counter current chromatography[9] and TLC.[5] However, those methods were not successful for the determination of ginseng saponins in animals. Chen[10] tried to determine ginseng saponins in rabbits by using GLC. He reported, however, that ginseng saponins could not be detected after oral administration. This indicates that the determination of ginseng saponins in animals treated with them is difficult.

We chose a combination method of TLC and spectrometry using a dual-wavelength TLC scanner which was developed by Sanada *et al.*,[5] because this method is simple and suitable for determination of a small amount of sample. We determined ginsenoside Rg_1, one of the main ginseng saponins, by using this method.

For the treatment of biological samples containing ginsenoside Rg_1, we used Servachrom XAD-2 resin which is an adsorption resin previously used for the separation of ginseng saponins.[11] In the separation of ginsenoside Rg_1 from biological components of rats, Servachrom XAD-2 resin column chromatography using 60% MeOH aqueous solution as an eluent was superior to the *n*-BuOH extraction which is commonly applied to crude drugs.

The vanillin–H_2SO_4 detecting reagent was more sensitive than the 10% H_2SO_4 one used by Sanada *et al.*[5] for the determination of ginsenoside Rg_1 on a TLC plate.

The mean recoveries of ginsenoside Rg_1 (3—1000 μg) added to rat tissues and fluids were more than 90% and the standard deviation was about 5% or less. The results indicate that our method for quantitative analysis of ginsenoside Rg_1 is applicable to rats treated with ginsenoside Rg_1. Therefore, we examined the effect of oral dosing of rats. The concentrations of ginsenoside Rg_1 in the tissues and serum of rats treated with it (100 mg/kg, *p.o.*) were all below 10 μg/(g or ml) at 150 min after the administration.

These data indicate that ginsenoside Rg_1 is absorbed from the digestive tract, in contrast to Chen's result. Further detailed studies are in progress.

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