STUDIES ON THE ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM OF GINSENOSIDE Rg_1 AND Rb_1 IN RATS

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INTRODUCTION

The pharmacological activities of crude drugs used in oriental medicine have been gradually confirmed through many clinical and pharmacological studies during last decade. However, the pharmacokinetics, such as absorption, distribution, excretion and metabolism, of the main active components in crude drugs have been little studied. The pharmacokinetic study is very important for more understanding the effects of the drug.

The root of *Panax ginseng* C.A. Meyer (Araliaceae) is an important crude drug in various prescription 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 this crude drug. We[1] also reported that crude total ginseng saponins had an inhibitory effect on side effects induced by cortisone acetate. There are many reports of pharmacological[2] and chemical[3] studies on ginseng saponins. However, little is known about the absorption, distribution, excretion and metabolism of ginseng saponins. Although, Han et al.[4] and Chen et al.[5] reported on the pharmacokinetics of ginseng saponins isolated from *Panax* species, the pharmacokinetics of them are still not known in any details. Because, their reports involve several problems such as the analytical methods employed and the experimental animal used. Therefore, further experiments are necessary for understanding the pharmacokinetics of ginseng saponins.

For the studies of absorption, distribution, excretion and metabolism of ginseng saponins, we selected ginsenoside Rg_1 (Rg_1) and ginsenoside Rb_1 (Rb_1) as a representative of triol group saponins and diol group saponins, respectively, since they are contained at a relatively high concentrations in the ginsengs and have been most extensively examined for their pharmacological actions. We also chosen rats as the experimental animal since they have been widely used in pharmacological studies of Rg_1 and Rb_1. And we employed a combination method of thin layer chromatography (TLC) and spectrometry using a dual-wavelength TLC scanner which was developed by Sanada et al.,[6] because this method is simple and suitable for the determination of small amount of saponins when adequate separation methods for saponins from biological components of rats are employed.

We describe the results of studies on the absorption, distribution, excretion and metabolism of Rg_1 and Rb_1 in rats by using our new analytical assay methods.

ABSORPTION, DISTRIBUTION AND EXCRETION OF Rg_1 [7),8)]

1. Experimental Methods

1-1. Administration of Rg_1

Male Sprague-Dawley rats (JCL: SD, SPF) weighing 180-200g were kept away from food but not water for 18 hours prior to experiments. Rg_1 was dissolved in distilled water to 0.2%, and administered orally at a dose of 100mg/kg to rats by stomach tube. In addition, for intravenous experiments, 0.2% injection of Rg_1 dissolved in 0.9% saline was injected via the femoral vein at a dose of 5mg/kg to non-fasted rats.

1-2 Preparation of biological Samples

Blood was collected through a polyethylene tube inserted into the right carotid artery of a rat under anesthesia with sodium pentobarbital (25mg/kg, i.p.), coagulated and centrifuged at 3000 rpm for 15 minutes to isolate serum. Such tissues as the liver, kidney, heart, lung, spleen, brain and also the stomach, small intestine and the large intestine (digestive tract samples included their contents) were taken out as soon as possible after depleting blood. Urine and feces were collected separately with a metabolic cage. On the other hand, bile was collected periodically through a polyethylene tube cannulated into the bile duct of a rat fixed to Ballman's cage. Besides, obtaining the urine in the bladder in intravenous administration experiments, the rat bladder was cannulated with polyethylene tubing under anesthesia with sodium pentobarbital (25mg/kg, i.p.) Each rat was held in a Ballman's cage and the urine was collected periodically.

1-3 Determination of Rg_1 in Biological Samples

The biological samples containing Rg_1 were treated according to the procedure in Chart 1 and subjected to TLC. The procedure is briefly outlined below. Tissue samples (whole organs except for liver: 2g of the liver) and feces were homogenized with 5-9ml of distilled water. Serum (3ml) and each homogenate were treated with 12-30ml of methanol (MeOH) for extracting Rg_1. In the case of the brain, the homogenate was defatted with 20ml of benzene, then MeOH (20ml) was added to water layer separated by centrifugation at 3000 rpm for 10 minutes. The MeOH solution of the samples were evaporated to dryness under reduced pressure, and they were prepared as 20% MeOH aqueous solution. In the cases of urine and bile, MeOH was directly added to 20%. Each 20% MeOH aqueous solution was applied to Servachrome XAD-2 resin column (1 x 9cm) 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 5μl of it was used as a sample for TLC.

1-4 TLC

TLC was done on Merck precoated kieselgel 60 plates (0.25mm thick). As a developing solvent, $CHCl_3$-MeOH-H_2O (65 ; 35 : 10) mixture was used. The detection of spots on TLC plates was done by spraying 8% vanillin in MeOH-72% H_2SO_4 (1 : 5, v/v) followed by heating (140°C, 3-4 minutes). TLC densitograms were obtained on a Shimadzu CS-910 chromatogram scanner equipped with a dual-wavelength spectrophotometer under the following conditions,: detecting wavelength; 530nm, reference wavelength; 780nm, slit width; 1.25 x 1.25mm, scanning mode; zig-zag. The peak areas were calculated by using an equipped integrator.

2. Experimental Results

2-1 Oral Administration

2-1-1 Serum Concentration of Rg_1

As shown in Fig. 1, Rg_1 appeared in the serum as early as 15 minutes after oral administration. The serum concentration reached a peak of 0.9μg/ml 30 minutes after, then decreased to 0.2μg/ml at 2.5 hours and was practically undetectable 6 hours after.

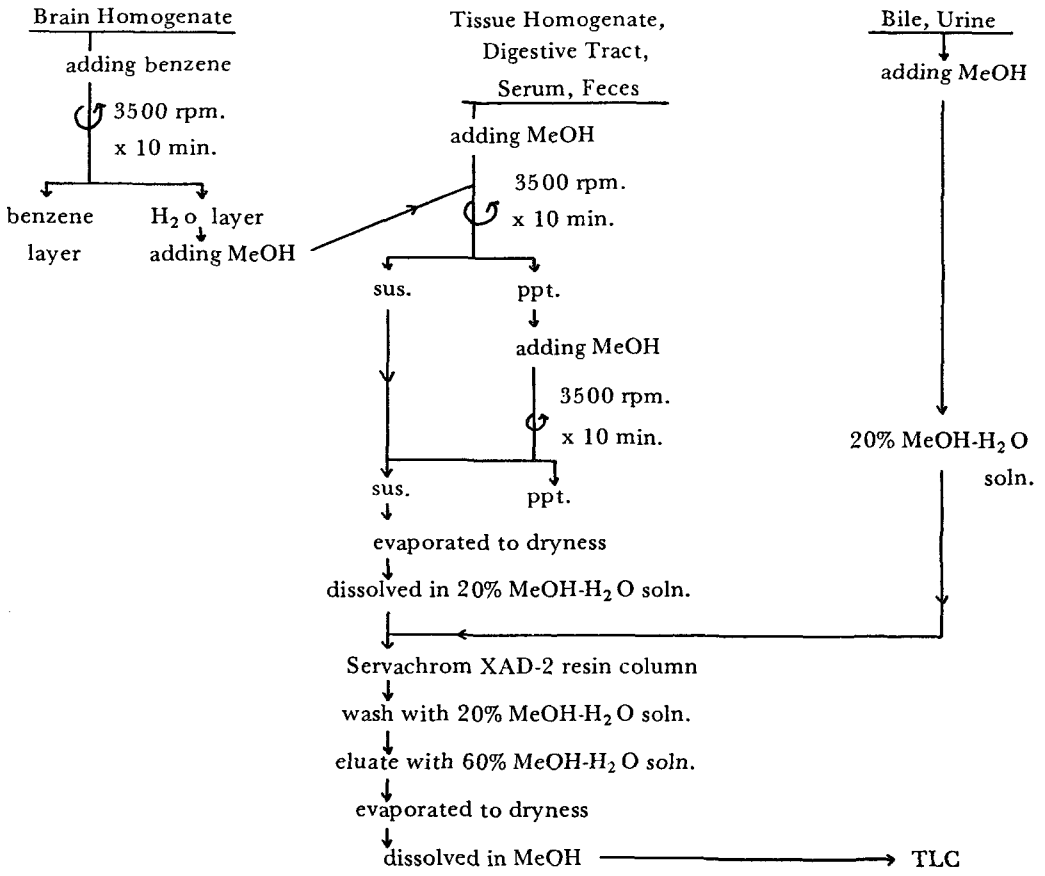


Chart 1. Assay Procedure for Ginsenoside Rg_1 in Biological Samples of Rats

2-1-2 Tissue Concentrations of Rg_1

Table 1 shows the tissue concentrations of Rg_1 after oral administration. Rg_1 concentrations in the liver and the kidney were slightly higher than those in other tissues and reached peaks of 3.5 ± 2.0 and 2.6 ± 1.5μg/g, respectively 1.5 hours after, but were undetectable 6 hours after. On the other hand, Rg_1 concentrations in the heart, lung and the spleen at all times after administration were below 1.5μg/g. Moreover, Rg_1 in the brain was also undetectable at any time after administration.

2-2-3 Amount of Rg_1 in the Digestive Tract

The time course of Rg_1 contents in the digestive tract after oral administration is shown in Fig. 2. The amounts of Rg_1 in the stomach and the small intestine 15 minutes after administration were 42.3 ± 1.6 and 35.6 ± 4.3% of the dose, respectively. At 60 minutes after, when most of the Rg_1 in the stomach transfered into the small intestine, the amount of Rg_1 in the stomach decreased to only 9.9 ± 2.3% of the dose and that in the small intestine increased largely to 70.8 ± 1.6% of the dose. Then, that in the large intestine 4 hours after was as high as 56.7 ± 8.5% of the dose. At 6 hours after, that in the small intestine was near the limit of detection and, on the contrary, 52.2 ± 2.7% of the dose was found in the large intestine. In addition, decomposition products of Rg_1 were detected in the stomach and the large intestine (as will be mentioned later).

2-1-4 Urinary, Fecal and Biliary Excretions of Rg_1

The cumulative excretions of Rg_1 into urine and feces are shown in Fig. 3 and that in bile is also in Fig. 4.

Table 1. Tissue levels of ginsenoside Rg_1 after oral administration of ginsenoside Rg_1 (100mg/kg) to rats

Tissue	Concentration (μg/g) Time after administration (h)					
	0.5	1.0	1.5	2.5	4.0	6.0
Brain	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Heart	N.D.	1.1±1.1	1.5±0.8	N.D.	N.D.	N.D.
Lung	N.D.	1.2±0.9	1.5±1.1	N.D.	N.D.	N.D.
Liver	1.1±0.7	2.3±1.4	3.5±2.0	2.2±1.5	1.4±0.9	N.D.
Kidney	0.9±0.7	1.8±1.0	2.6±1.5	1.9±1.1	1.5±0.9	N.D.
Spleen	N.D.	0.8±0.5	1.1±0.7	N.D.	N.D.	N.D.

Each value represents the mean ± S.E. of 4 animals.
N.D.: not detectable.

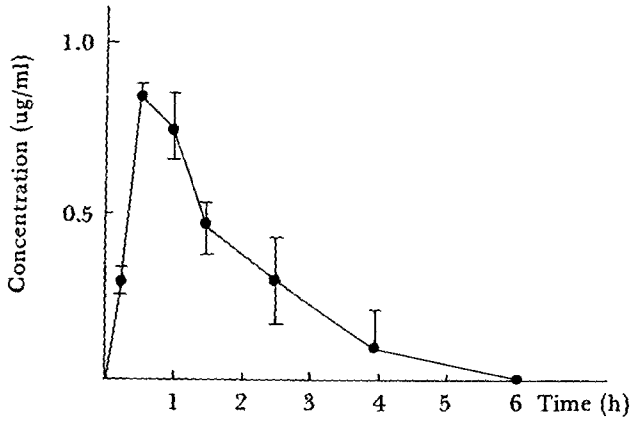


Fig. 1. Serum concentration of ginsenoside Rg_1 after oral administration of ginsenoside Rg_1 (100mg/kg) to Rats each point represents the mean ± S.E. of 3 animals.

The cumulative urinary and fecal excretions of Rg_1 within 24 hours were 0.40 ± 0.04 and 41.2 ± 2.6% of the dose, respectively. During 6-12 hours after administration, 57.2% of the total urinary excretion and 70.9% of the total fecal excretion were excreted. On the other hand, the amount of Rg_1 excreted into bile within 24 hours was 1.1 ± 0.1% of the dose and 34.1% of the total biliary excretion was excreted during 2-4 hours after administration. Rg_1 was still excreted into bile during 12-24 hours after administration.

2-2 Intravenous Administration

2-2-1 Serum Concentration of Rg_1

The serum concentration of Rg_1 was 8.9 ± 1.0μg/ml 2 minutes after injection and then decreased quite rapidly, as seen in Fig. 5. The half-life of disapearance of Rg_1 from the serum was 6.3 minutes.

2-2-2 Concentrations of Rg_1 in the Liver and the Kidney

Fig. 6 shows the time course of Rg_1 concentrations in the liver and the kidney after injection.

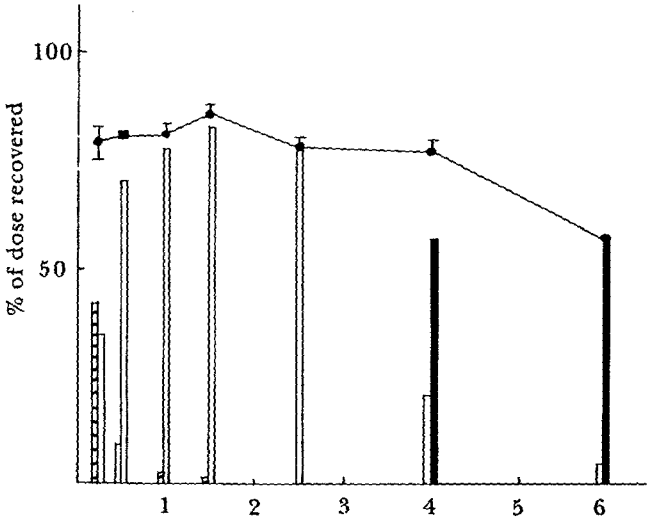


Fig. 2. Amounts of ginsenoside Rg_1 in the digestive tract of Rats after oral administration of ginsenoside Rg_1 (100mg/kg)
▨, stomach; □, small intestine: ■ large intestine —●— total.
Each point represents the mean ± S.E. of 3 animals.

The Rg_1 concentrations in both organs decreased biexponentially, rapidly in the α-phase and slowly in the β-phase. In the liver, the half-lives of Rg_1 were 5.3 minutes for α-phase and 34.7 minutes for β-phase, while those in the kidney were 5.7 and 36.1 minutes, respectively.

2-2-3 Urinary and Biliary Excretions of Rg_1

Cumulative excretions of Rg_1 into urine and bile are shown in Fig. 7 and 8, respectively.

The urinary excretion of Rg_1 was almost completed within 4 hours after injection and the amount of Rg_1 excreted into urine during that period was 21.6± 1.4% of the dose. Urinary excretion continued after that and the cumulative excretion within 12 hours was 23.5 ± 10.9% of the dose. On the other hand, more than 50% of

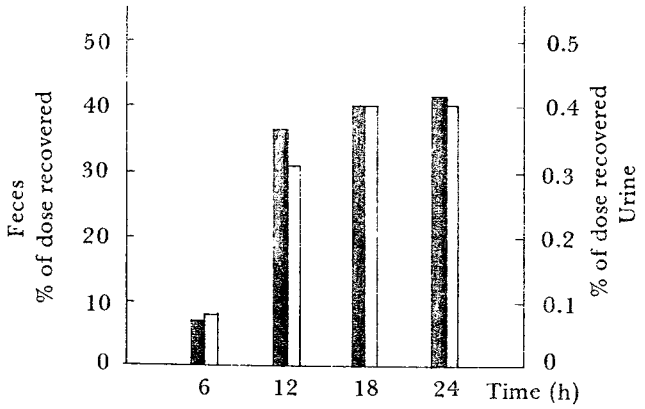


Fig. 3. Cumulative excretions of ginsenoside Rg_1 into urine and feces after oral administration of ginsenoside Rg_1 (100mg/kg) to rats
feces; urine.
Each point represents the mean of 3 animals.

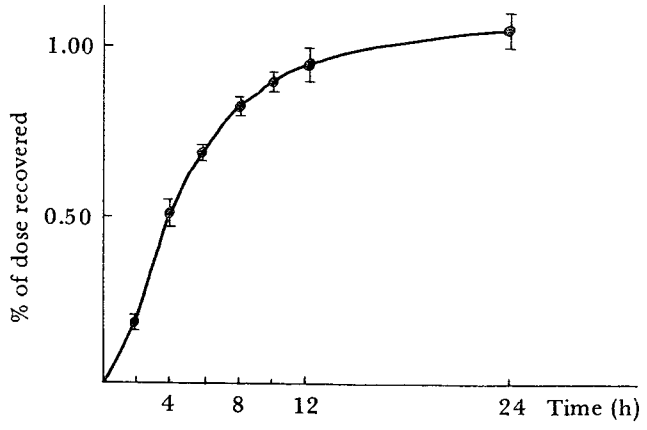


Fig. 4. Cumulative excretion of ginsenoside Rg_1 into bile after oral administration of ginsenoside Rg_1 (100mg/kg) to rats
Each point represents the mean±S.E. of 3 animals.

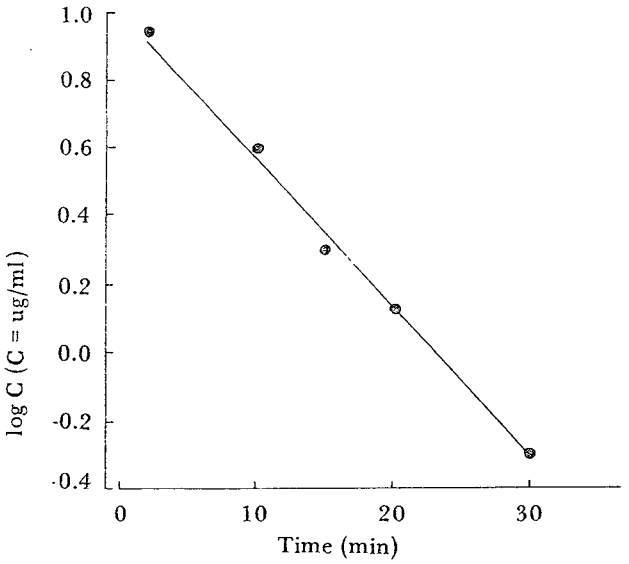


Fig. 5. Serum concentration of ginsenoside Rg_1 after intravenous administration of ginsenoside Rg_1 (5mg/kg) to Rats
Each point represents the mean of 3 animals.

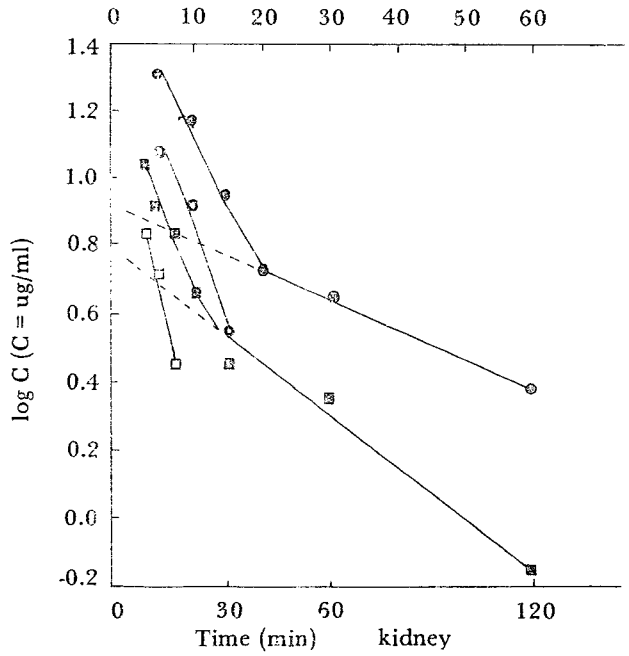


Fig. 6. Tissues levels of ginsenoside Rg_1 after intravenous administration of ginsenoside Rg_1 (5mg/kg) to rats
○, ●, liver; □, ▨, kidney.
Each point represents the mean of 3 animals.

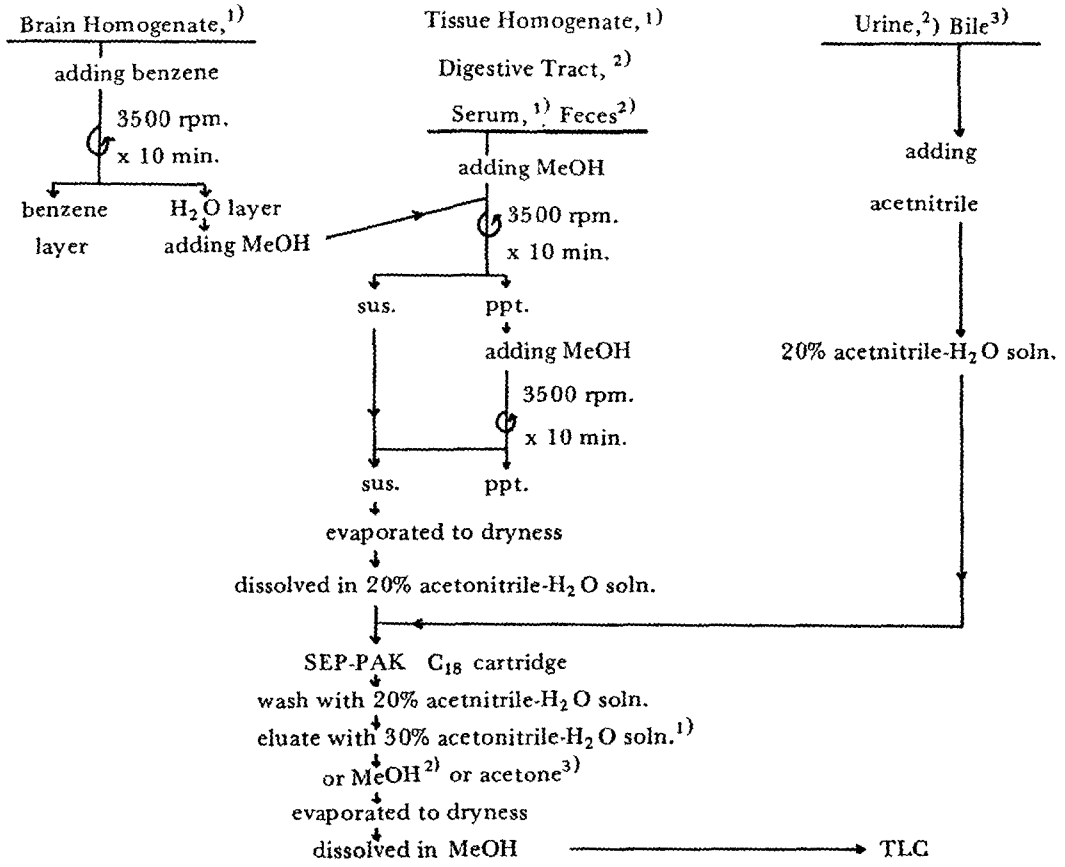


Chart 2. Assay Procedure for Ginsneoside Rb_1 in Biological Samples of Rats

The number of the biological sample means the treatment with the eluating solvent of the same number.

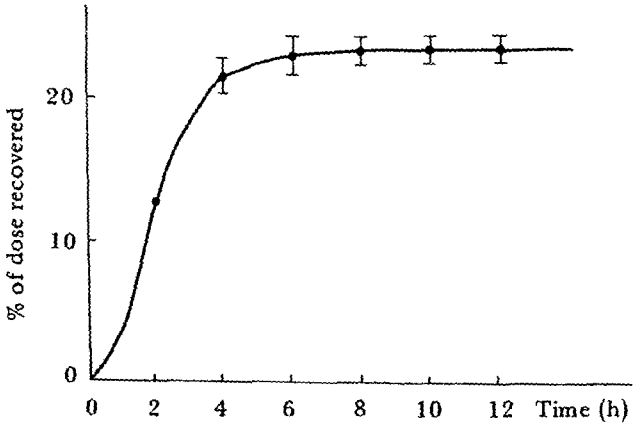


Fig. 7. Cumulative excretion of ginsenoside Rg_1 into urine after intravenous administration of ginsenoside Rg_1 (5mg/kg) to rats
Each point represents the mean ± S.E. of 3 animals.

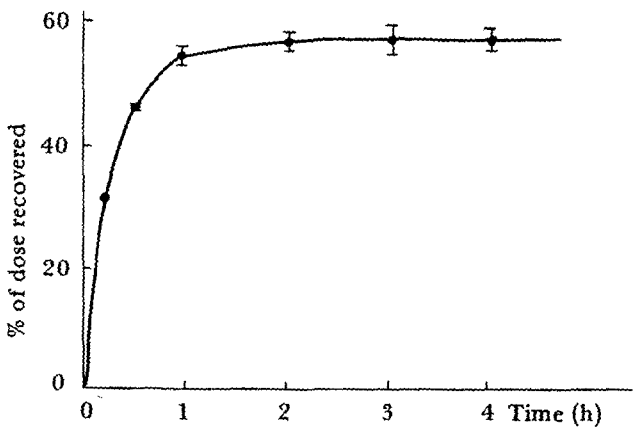


Fig. 8. Comulative excretion of ginsenoside Rg_1 into Bile after intravenous administration of ginsenoside Rg_1 (5mg/kg) to rats
Each point represents the mean±S.E. of 3 animals.

the total biliary excretion of Rg_1 was excreted within 15 minutes after injection and the cumulative excretion within 4 hours was 57.2 ± 1.7% of the dose.

ABSORPTION, DISTRIBUTION AND EXCRETION OF Rb_1 [9]

1. Experimental Methods

1-1 Administration of Rb_1

Rb_1 was administered in the same manner as Rg_1.

1-2 Preparation of Biological Samples

Biological samples were prepared in the same manner as metnioned for Rg_1.

1-3 Determination of Rb_1 in Biological Samples

The assay procedure for Rb_1 is summarized in Chart 2. The principal manner was the same as that of Rg_1. However, SEP-PAK$^{@}C_{18}$ cartridge was used in stead of Servachrom XAD-2 resin column. Acetonitrile and acetone in addition to MeOH were newly used as extractants and/or eluates.

1-4 TLC

TLC and densitometry were done in much the same way as in Rg_1 except for the detecting wavelength (535nm) and the developing solvents used for TLC. The developing solvents used for TLC were: solvent A [1-butanol/acetic acid/H_2O (4:1:5, upper phase)] for serum sample; solvent B [$CHCl_3$/1-butanol/MeOH/H_2O (4:8:3:4. lower phase)] for kidney and spleen samples; solvent C [$CHCl_3$/MeOH/H_2O (13:7:2, lower phase)] for samples other than the above.

2. Experimental Results

2-1 Oral Administration

2-1-1 Serum and Tissue Concentrations of Rb_1

The concentrations of Rb_1 in serum and tissues including the liver, kidney, heart, lung, spleen and the brain were determined at 15, 30, 60, 150, 240 and 360 minutes after administration. However, Rb_1 concentration in any of them was less than 0.2μg/(ml or g), the lower limit of detection, at any time.

2-1-2 Amount of Rb_1 in the Digestive Tract

As shown in Fig. 9, the amount of Rb_1 determined was 35.5 ± 8.5% of the dose in the stomach and 43.0 ± 13.6% of the dose in the small intestine 15 minutes after administration, and 17.2 ± 5.6 and 65.2 ± 13.9% of the dose, respectively, at 30 minutes after. Most of the Rb_1 administered was transfered from the stomach to the small intestine 60 minutes after administration and Rb_1 was detected mainly in the small intestine during 1-2.5 hours. At 4 hours after, 34.8 ± 7.3% of the Rb_1 dosed was found in the small intestine and 28.4 ± 10.7% was in the large intestine. However, Rb_1 was detected in very small amount (3.3%) in the small intestine but not in the large intestine 6 hours after administration.

Furthermore, decomposition products of Rb_1 were demonstrated in the stomach and the large intestine (as will be mentioned later).

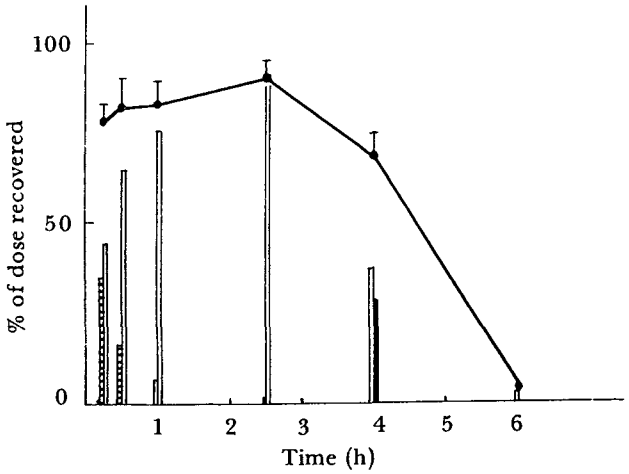


Fig. 9. Amounts of ginsenoside Rb_1 in the digestive tract after oral administration of ginsenoside Rb_1 (100mg/kg) to rats
▨, stomach; □, small intestine; ■, large intestine; —●— total.
Each point represents the mean ± S.E. of 3 animals.

2-1-3 Urinary, Fecal and Biliary Excretion of Rb_1

Cumulative urinary and fecal excretions of Rb_1 are shown in Fig. 10 and 11, respectively.

The maximum excretions of Rb_1 into urine and feces were observed during 12-18 and 6-12 hours after administration, respectively. The amount of Rb_1 excreted into urine for 48 hours after administration accounted for 0.05 ± 0.01% of the dose and cumulative fecal excretion within 24 hours was 10.8 ± 1.5% of the dose. On the other hand, Rb_1 in bile was detectable during 6-8 hours, but its concentration was below the limit of determination.

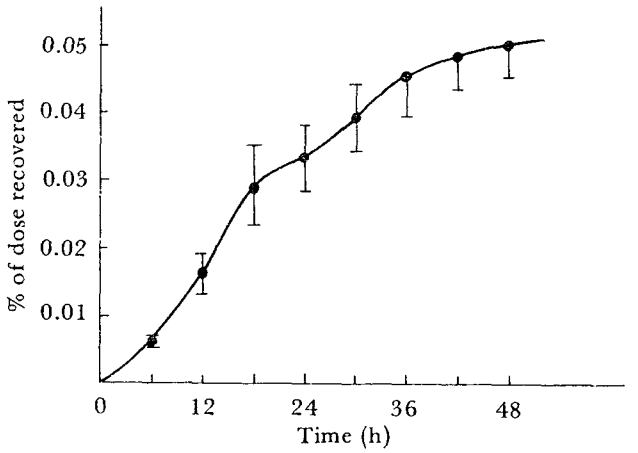


Fig. 10. Cumulative excretion of ginsenoside Rb_1 into urine after oral administration of ginsenoside Rb_1 (100mg/kg) to rats
Each point represents the mean ± S.E. of 3 animals.

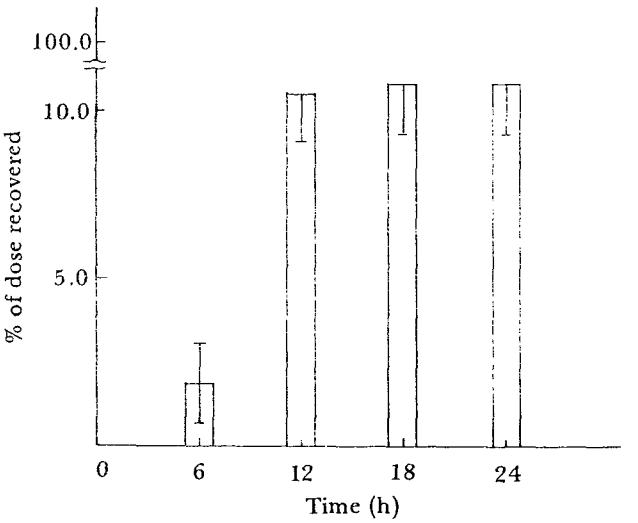


Fig. 11. Cumulative excretion of ginsenoside Rb_1 into feces after oral administration of ginsenoside Rb_1, (100mg/kg) to rats
Each point represents the mean ± S.E. of 3 animals.

2-2 Intravenous Administration

2-2-1 Serum Concentration of Rb_1

The serum Rb_1 concentration was 83.8 ± 12.9 μg/ml at 5 minutes and decreased rapidly until one hour after injection, but subsequently it decreased very slowly to 1.1 ± 0.03μg/ml at 72 hours (Fig. 12). The half-lives of disappearance of Rb_1 from the blood was 11.6 minutes in the α-phase and 14.5 hours in the β-phase.

2-2-2 Concentrations of Rb_1 in Tissues

Time course of Rb_1 concentrations in the liver, kidney, heart and the lung are shown in Fig. 13. The concentrations of Rb_1 in the kidney, heart and the liver were 9.0 ± 1.6, 5.3 ± 0.9 and 2.9 ± 0.6μg/g, respectively, 5 minutes after injection and decreased nearly in parallel with the serum Rb_1 concentration after that. In the heart and the lung the Rb_1 concentration decreased to 2.1 ± 0.4 and 0.8 ± 0.2μg/ml, respectively, 24 hours after and the Rb_1 was undetectable 48 hours after. In the kidney the concentration of Rb_1 decreased to 0.77 ± 0.3μg 48 hours after and was undetectable 72 hours after. On the other hand, the Rb_1 concentration in the lung showed a maximum peak (5.0μg/g) 30 to 60 minutes after injection. Thereafter it decreased relatively slowly to 1.8 ± 0.04μg/g 24 hours after. In the brain and the spleen, Rb_1 was detectable, but its concentration was below the limit of determination at even 5 minutes after injection.

2-2-3 Urinary and Biliary Excretions of Rb_1

The cumulative urinary and biliary excretions of Rb_1 after injection are shown in Fig. 14 and 15, respectively.

Most of the urinary excretion of Rb_1 was completed within 48 hours after injection, but

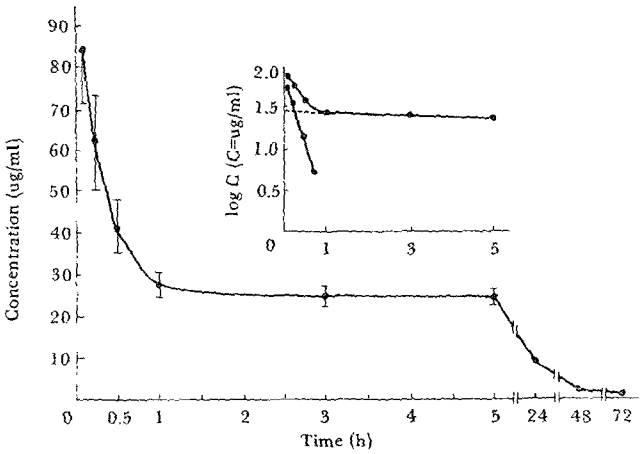


Fig. 12. Serum concentration of ginsenoside Rb_1 after intravenous administration of ginsenoside Rb_1 (5mg/kg) to rats
Each point represents the mean ± S.E. of 3 animals.

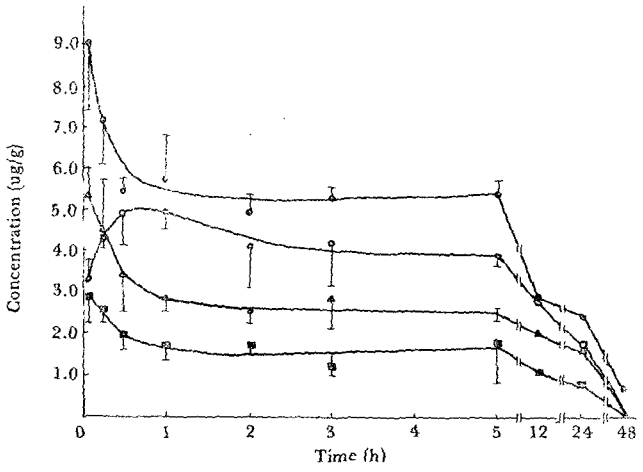


Fig. 13. Tissue levels of ginsenoside Rb_1 after intravenous administration of ginsenoside Rb_1 (5mg/kg) to rats
—●—, kidney; —▲—, heart; —○—, lung; —■—, liver.
Each point represents the mean ± S.E. of 3 animals.

still continued after that. The cumulative urinary excretion of Rb_1 was as much as 44.4 ± 2.6% of the dose within 120 hours after injection. On the other hand, its excretion into bile was almost completed within 12 hours after injection. The cumulative biliary excretion of Rb_1 was 0.83 ± 0.60% of the dose within 24 hours.

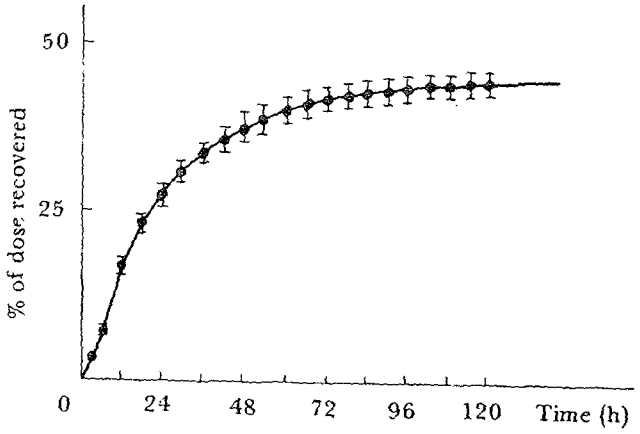


Fig. 14. Cumulative excretion of ginsenoside Rb_1 into urine after intravenous administration of ginsenoside Rb_1 to rats
Each point represents the mean ± S.E. of 3 animals.

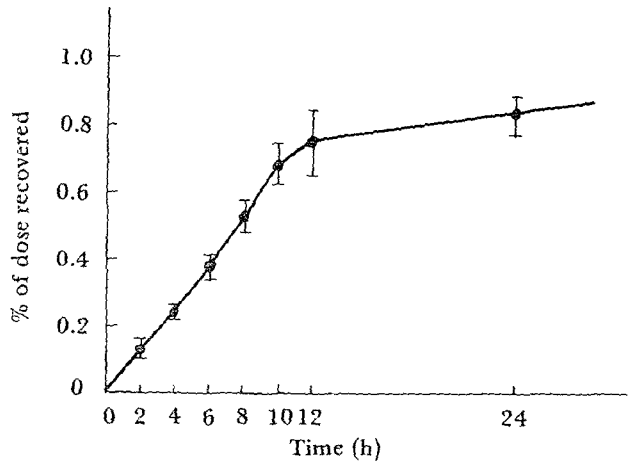


Fig. 15. Cumulative excretion of ginsenoside Rb_1 into bile after intravenous administration of ginsenoside Rb_1 to rats
Each point represents the mean ± S.E. of 3 animals.

DECOMPOSITION OF Rg_1 and Rb_1 IN THE DIGESTIVE TRACT[10]

1. Experimental Methods

1-1 Experiments on Decomposition

Male SD rats (180-200g in weight) were deprived of food but given free access to water for 18 hours prior to the experiments. After oral administration of Rg_1 and Rb_1 (100mg/kg, 2%

aqueous solution) separately to rats, the stomach and the large intestine were isolated and treated according to the detailed procedure described in our paper.[10] The decomposition products were compared with authentic samples by normal and reversed phase TLC. Some of decomposition products were isolated and analyzed for their chemical structures with ^{13}C-NMR. Moreover, *in vivo* and *in vitro* experiments were carried out under various conditions to clarify the process of decompositions of Rg_1 and Rb_1.

1-2 TLC

Normal phase TLC was performed on Merck precoated kieselgel 60 plates (0.25mm thick), using the following solvent systems as developing solvents: (A), 1-butanol/acetic acid/H_2O (4:1:5, upper phase); (B), $CHCl_3$/1-butanol/MeOH/H_2O (4:8:3:4, lower phase); (C), $CHCl_3$/MeOH/H_2O (13:7:2, lower phase). Reversed phase TLC was performed on Merck precoated kieselgel 60 silanized plates by using 60% MeOH aqueous solution as the developing solvent. Detection was achieved by spraying 8% vanillin in MeOH-72% H_2SO_4 (1:5, v/v) followed by heating (140°C, 3-4 minutes).

2. Experimental Results

2-2-1 Decomposition of Rg_1 in the Stomach

As shown in Fig. 16, three decomposition products (Rg_1-prosapogenins, I, II, III, named by Han et al.[11]) were observed by normal phase TLC. However, each of three products was further separated into two steric isomer (20-C(S) and 20-C(R) epimers), I_1 and I_2, II_1 (Rh_1) and II_2 and III_1 and III_2, respectively by reversed phase TLC.

2-2-2 Decomposition of Rg_1 in the Large Intestine

As thin layer chromatogram of decomposition products (IV, V) of Rg_1 in the large intestine 6 hours after oral administration of Rg_1 is shown in Fig. 17. The products IV and V were identified

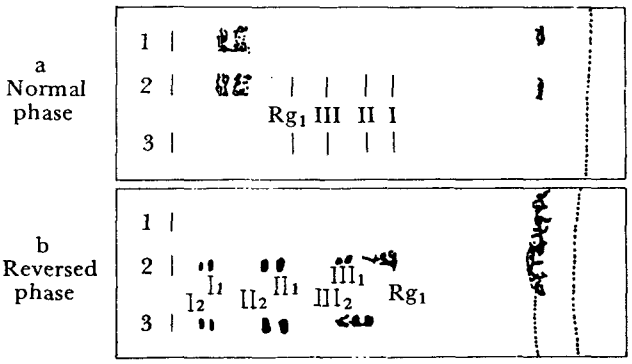


Fig. 16. Thin-Layer chromatograms of decomposition products of ginsenoside-Rg_1 in rat stomach or in 0.1N HCl solution
Developing solvents: a, $CHCl_3$-MeOH-H_2O (65:35:10, lower phase); b, 60% MeOH. plates: a, Merck precoated silica gel 60; b, Merck precoated silica gel 60 silanized. Detecting reagent: 8% vanillin-MeoH solution/72% H_2SO_4 (1:5), with heating at 140°C for 3 min. 1, normal rat; 2, Rg_1 (100mg/kg, p.o.)-administered rat (30min after treatment); 3, 0.1N HCl solution (37°C, 1h).

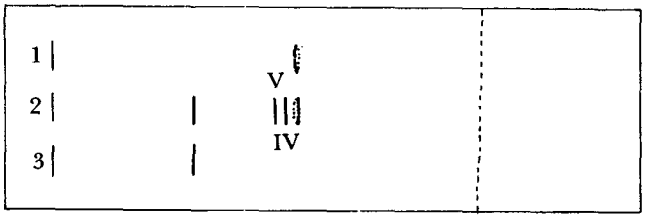


Fig. 17. Thin-layer chromatogram of decomposition Products of ginsenoside-Rg_1 in rat large intestine
Developing solvent: CHCL3-MeOH-H_2O (65:35:10, lower phase). 1, normal rat; 2, Rg_1 (100mg/kg, p.o.)-administered rat (6h after treatment); 3, standard Rg_1.

as F_1 and Rh_1, respectively, by normal phase TLC and reversed phase TLC.

2-2-3 Decomposition of Rb_1 in the Stomach

A thin layer chromatogram of decomposition product of Rb_1 in the stomach is shown in Fig. 18. Very small amount of decomposition product(VI was found.

2-2-4 Decomposition of Rb_1 in the Large Intestine

A thin layer chromatogram of decomposition products (VI, VII, VIII) of Rb_1 in the large intestine 4 hours after oral administration is

shown in Fig. 19. VII, a main decomposition product, was identified as Rd by TLC and by comparison of ^{13}C-NMR datum.[12]

2-2-5 Decomposition Patterns of Rg_1 and Rb_1 in the Digestive Tract

The decomposition patterns of Rg_1 and Rb_1 in rat stomach and large intestine which were estimated by the results of *in vivo* and *in vitro* experiments are summarized in Chart 3.

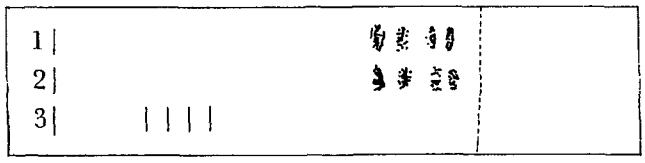


Fig. 18. Thin-layer chromatogram of decomposition products of ginsenoside-Rb_1 in rat stomach or in 0.1N HCl solution
Developing solvent: $CHCl_3$-MeOH-H_2O (65:35:10, lower phase). 1, normal rat; 2, Rb_1, (100mg/kg, p.o.)-administered rat (30min after treatment); 3, 0.1N HCl solution (37°C, 1h).

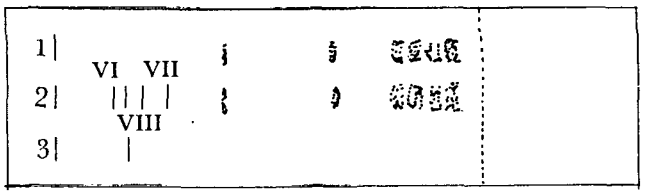


Fig. 19. Thin-layer chromatogram of decomposition products of ginsenoside-Rb_1 in rat large intestine
Developing solvent: $CHCl_3$-MeOH-H_2O (65:35:10, lower phase). 1, normal rat; 2, Rb_1 (100mg/kg, p.o.) administered rat (4h after treatment); 3, standard Rb_1.

The chemical structures of Rg_1, Rb_1 and their decomposition products which were identified by TLC and ^{13}C-NMR etc. are shown in Chart 4.

In addition, these decomposition products could not be found in rat tissues such as the liver and the kidney by our TLC methods.

DISCUSSION

The absorption, distribution, excretion and metabolism of ginseng saponins have still not been completely elucidated in spite of reports of Han et al.[4] and Chen et al..[5] Han et al. studied the absorption, distribution and excretion of Rg_1 in rabbits by means of TLC-colorimetry and the radioisotopic method. However, their assay methods used had several disadvantages, as pointed out by Chen et al..[5] On the other hand, the gas liquid chromatography developed by Chen et al.[5] had too low a sensitivity. Thus, they reported that no Rg_1 was found in the plasma and urine of rabbit after oral administration. Therefore, we first attempted to establish a new microdetermination methods for Rg_1 and Rb_1 in biological samples of rats.

For the pre-treatment of biological samples containing Rg_1 and Rb_1, we used Servachrom XAD-2 resin column (for Rg_1) and SEP-PAK@ C_{18} cartridge (for Rb_1). And a combination method of TLC and spectrometry using a dual-wave-length TLC scanner which was developed by Sanada et al.[6] was employed for the microdeterminations of Rg_1 and Rb_1. The lower limites of determinations in our methods were 0.2 ug/spot in both Rg_1 and Rb_1. As our methods for quantitative analysis of Rg_1 and Rb_1 were applicable to rats treated with Rg_1 and Rb_1, we investigated the absorption, distribution, excretion and metabolism of them in rats after oral and intravenous administration.

I. Rg_1

In general, the amount of absorbed the drug after oral administration can be estimated by using the following equation: $P(\%) = (UB_O/UB_V) \times 100$, where UB_O is the sum of urinary and biliary excretions (% of the dose) after oral administration and UB_V is the same sum after intravenous administration. In the case of Rg_1, P was calculated as 1.9% of the dose, and hence amount of absorbed Rg_1 seems to be more than 1.9% of the dose even if the minimum value is taken.

In addition, as Rg_1 was found in the serum as early as 15 minutes after oral administration, the absorption of Rg_1 was assumed to occur rapidly in the upper part of rat digestive tract.

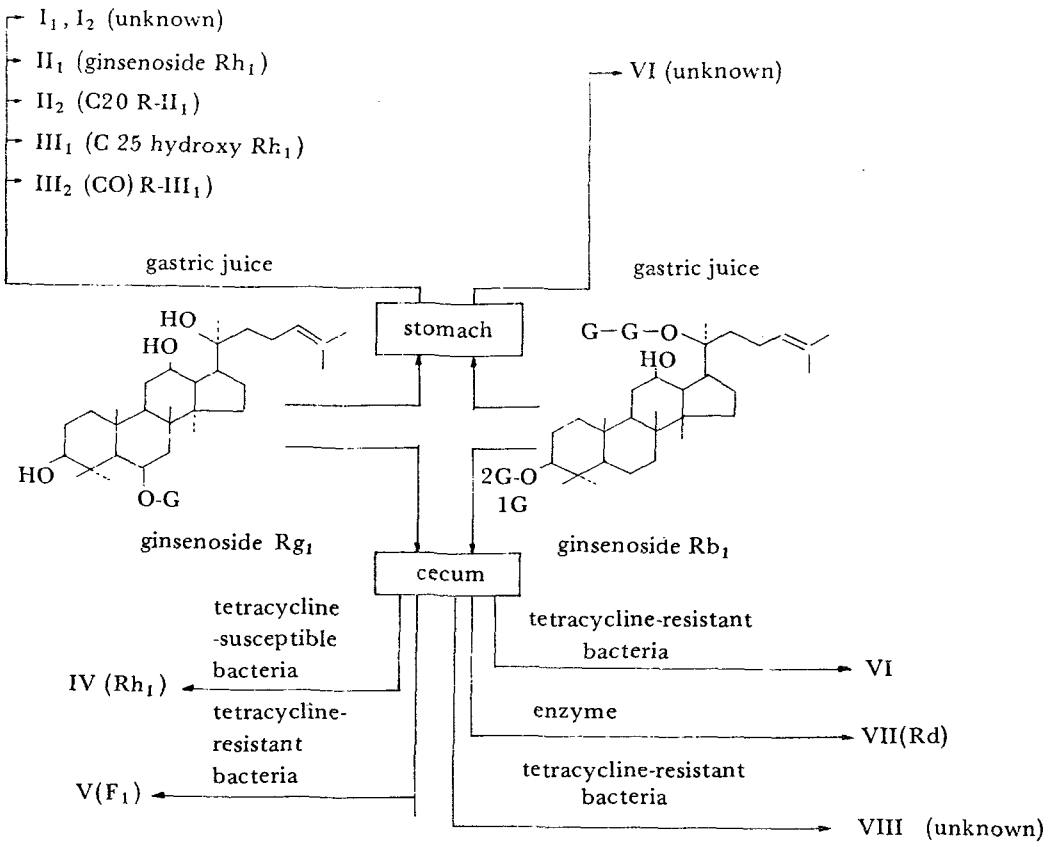


Chart 3. Decomposition of Ginsenoside-Rg_1 and -Rb_1 in the Stomach and Cecum of Rats

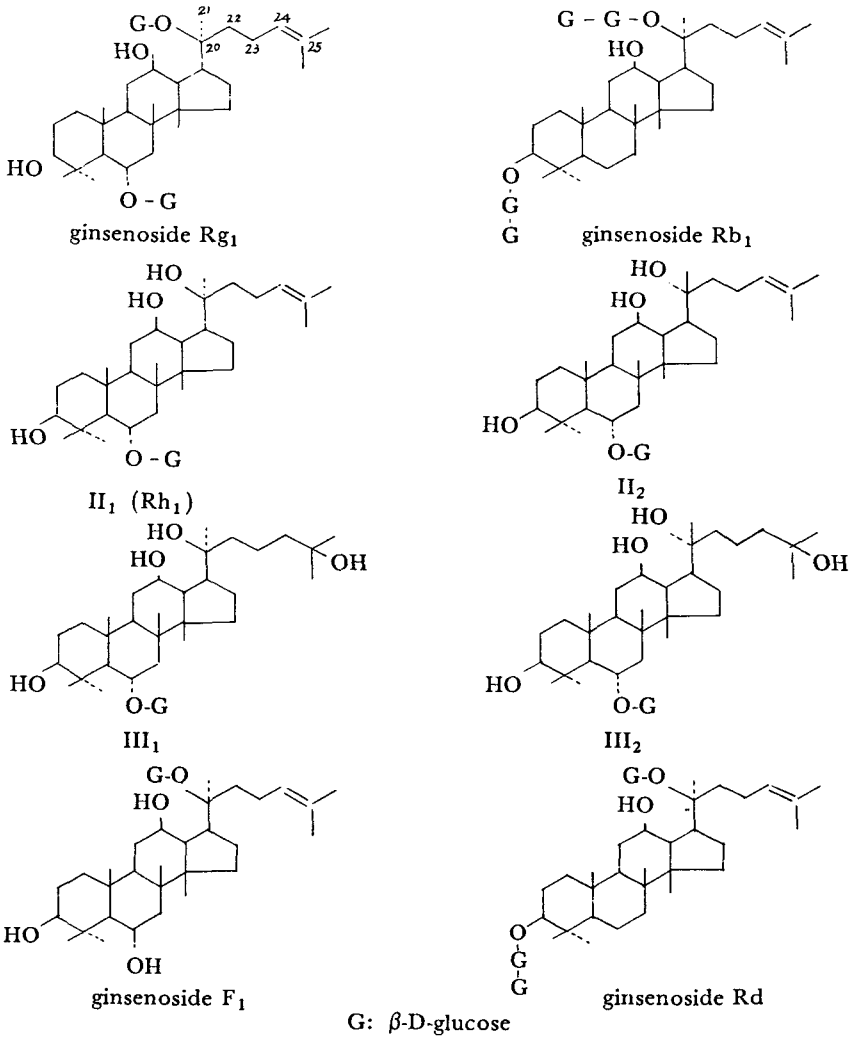
The serum concentration of Rg_1 reached its peak 30 minutes after oral administration and then declined rapidiy. The concentrations of tissues (except for the brain) reached a maximum around 1.5 hours after oral administration and declined smoothly. There were no tissues to which Rg_1 was distributed specifically. Therefore, Rg_1 administered orally was concluded to be absorbed rapidly and to be distributed widely in rat tissues except for the brain.

The urinary and biliary excretion of Rg_1 occurred in a 2:5 ratio in both oral and intravenous administration of Rg_1 to rats. This result is consistent with a general theory for biliary excretion, since the minimum threshold of molecular weight in rat biliary excretion is said to be 325 ± 50 and that of Rg_1 is 801.

As about 80% of the dose of Rg_1 was excreted in an unchanged form into urine and bile after intravenous injection to rats, it seems that Rg_1 is hardly metabolized in rat liver. On the othe hand, a part of Rg_1 was decomposed in the rat stomach after oral administration and six decomposition products were observed on a reversed phase TLC. In the large intestine, Rg_1 was decomposed to Rh_1 and F_1 by tetracycline-susceptable bacteria and tetracycline-resistant bacteria, respectively. However, approximately 40% of the oral administered Rg_1 was excreted into feces in an unchanged form. This point differed largely from that of Rb_1.

II. Rb_1

The concentration of Rb_1 in the serum and tissues after oral administration to rats was less than the lower limit of determination. This result suggested that the absorption of Rb_1 from the digestive tract is extremely slight. In fact, the amount of absorbed Rb_1 was calculated 0.11% of



G: β-D-glucose

Chart 4.

the dose. This value is quite low compared with that of Rg_1.

The serum concentration of Rb_1 after intravenous administration declined biexponentially and the half-life of the β-phase was extremely long (14.5 hours). This was in contrast to the exponential decline of Rg_1 with half-life of 6.3 minutes. As Chen et al.[5] reported that diol group saponins possess higher plasma protein binding activity than triol group saponins, so the longer half-life of Rb_1 might be related to this. In addition, the longer retention of Rb_1 in the rat body was supported by the facts that urinary Rb_1 excretion continued for longer time compared to that of Rg_1.

The concentrations of Rb_1 in tissues such as the kidney, heart and the liver after intravenous injection to rats were relatively high, and appeared to be correlated with the serum concentration. On the other hand, the Rb_1 concentrations in the brain and the spleen were very low.

Rb_1 was excreted more into urine than into bile. In general, it has been described[13] that large molecular-weight compounds which are strongly bound with serum alubumin are more easily excreted into bile than into urine. However, Rb_1 (molecular weight; (1109.3) which is assumed to be corresponding to this was mainly excreted into urine. Futher studies are desirable.

In the rat stomach, a small part of Rb_1 was decomposed after oral administration and unidentified decomposition product of Rb_1

was observed on the TLC plates. In the large intestine, Rb_1 was very quickly decomposed to Rd and two unidentified products by enteric enzyme and tetracycline-resistant bacteria, respectively. Therefore, approximately 10% of the oral administered Rb_1 was excreted into feces in an unchanged form. In addition, decomposition products of Rb_1 as well as Rg_1 were not found in rat tissues except for the digestive tract by our TLC methods. Therefore, these products are suggested to be poorly absorbed from the rat digestive tract and might play a major role in the pharmacological activities of Rg_1 and Rb_1.

Our results clarified that both Rg_1 and Rb_1 concentrations in tissues after oral administration were very low in rats. Therefore, these saponins amy act through hormons or directly affect the target tissues at the hormonal concentrations. Our experiments were all conducted with micrograms order of cold and unlabelled compounds. Our methods are excellent in simplicity and selectivity, but has some problems in sensitivity for these saponins in biological samples. Taking into account our results, a radioimmunoassay of Rg_1 developed by Sankawa et al[14] may be the most exceptive one when this method could be improved to be more simple and easy.

We earnestly hope that all components contained in the ginseng will be examined for their absorption, distribution, excretion and metabolism to clarify. Then, their mechanisms of pharmacological actions will be explained on the molecular basis from the pharmacokinetical point of view in future.

Fulder: I'd like to know about the difference between Rb_1 and Rg_1 in the way and in the length of half-life of saponin binding to protein in the serum.

Takino: I didn't estimate the half-life of Rb_1 and Rg_1-binding to the protein in the serum but I'm working on it.

Sandberg: In one of your slides, it showed that Rg_1 was not found in the brain, which, I think, is very important finding. You showed that Rg_1 was not detectable in the brain. What about Rb_1?

Tankino: Rb_1 also can't be found in the brain. A very small amount of Rb_1 and Rg_1 might be distributed in the brain and we were unable to detect them with our method.

F.J. Lee: In some experiments you used hundred milligrams of ginsenoside per Kilogram body weight. I think it is a rather extremely large amount as an individual ginsenoside. What is your opinion about that?

Takino: The sensitivity of detecting method of ginsenoside is not so good. Therefore, large amount of ginsenoside must be used for the detection of ginsenoside in the tissue or serum.

진세노사이드 Rg_1과 Rb_1의 흡수·분포·배설 및 대사에 관한 연구

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인삼의 주된 사포닌으로서 Rg_1과 Rb_1을 흰쥐에 투여하였을 경우에 이들 물질이 흰쥐의 장기에 흡수 또는 분포되는 상태와 배설에 대하여 연구하였다. 진세노사이드 Rg_1은 경구 투여량의 약 1.9%가 소화관의 상부에서 흡수되었으며, 투여한지 30분 후에 최고 혈중 농도에 이르렀고 조직에서는 1.5시간 걸렸다. 그러나 뇌에서는 확인되지 않았으며 뇨와 담즙에는 2 : 5의 비로 배설되었다.

Rb_1을 100㎎/㎏ 경구투여한 결과, 소화관에서는 거의 흡수가 되지 않았으며, 한편 정맥주사(5㎎/㎏)의 경우는 혈중 Rb_1의 농도가 지수적으로 감소하였으며, B-phase의 반감기는 14.5시간이었다. 정맥주사후 혈청과 조직에 장시간 잔존은 활성을 나타내는 혈청단백과의 결합과 관련이 있는 것으로 사료되며 시간에 따라 뇨로 배설되나 담즙에서는 확인되지 않았다.

Rg_1과 Rb_1을 경구투여한 후 TLC와 ^{13}C-NMR을 이용하여 위와 대장에서의 분해 상태를 연구한 결과 위에서 Rg_1의 일부가 분해, 6종류의 분해 산물이 reverse phase TLC상에서 관찰되었고 이들 분해 산

물은 약산성(0.1N HCl, 37℃) 조건하에서 Rg_1의 가수분해산물과 동일하였다.

한편, Rb_1경구투여후 위장에서 얻은 시료중에서 미확인 분해산물이 관찰되었으며, 이 분해산물은 약산성 조건하에서 Rb_1의 가수분해산물과는 상이하다는 사실을 확인하였다.

대장에서, Rg_1은 미생물 tetracycline-susceptible bacteria와 tetracycline-resist bacteria에 의해 Rh_1과 F_1으로 분해되었으며, Rb_1은 장내의 효소와 tetracycline-resistantant bacteria에 의해 Rd와 2 종류의 미확인 물질로 분해되었다.

REFERENCES

1. H. Tanizawa, H. Numano, T. Odani, Y. Takino, T. Hayashi, and S. Arichi, Yakugaku Zasshi, 101, 169 (1981).
2. H. Saito, Taisha, 10, 556 (1973); H. Oura and S. Hiai, Taisha, 10,564(1973); M. Yamamoto, Taisha, 10, 591 (1973); T. Yokozawa and H. Oura, Chem. Pharm. Bull., 24, 987 (1976); Y. Shibata, T. Nozaki, T. Higashi, S. Sanada, and J. Shoji, Chem. Pharm. Bull., 24, 2818 (1976); S. Odashima, Y. Nakayabu, N. Honjo, H. Abe, and S. Arichi, Eur. J. Cncer, 15, 885 (1979); T. Yokozawa, N. Kitahara, S. Okuda, and H. Oura, Chem. Pharm. Bull., 27, 419 (1979); S. Hiai, H. Yokoyama, H. Oura, and Yano, Endocrinol. Jpn., 26, 661 (1979); S. Hiai, H. Yokoyama, and H. Oura, Endocrinol. Jpn., 26, 737 (1979).
3. Y. Nagai, O. Tanaka, and S. Shibata, Tetrahedron, 27, 881 (1971); S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, Chem. Pharm. Bull., 22, 421 (1974); S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, Chem. Pharm. Bull., 22, 2407 (1974); S. Sanada and J. Shoji, Chem. Pharm. Bull., 26, 1694 (1978); S. Yahara, K. Kaji, and O. Tanaka, Chem. Pharm. Bull., 27, 88 (1979).
4. B. H. Han, E. B. Lee, W. C. Yun, and L. N. Woo, Korean Biochem. J., 9, 21 (1976); B. H. Han and I. M. Chang, Korean J. Ginseng Sci., 2, 17 (1977).
5. S. E. Chen, R. J. Sawchuk, and E. J. Staba, Eur. J. Drug Metabolism, 5, 161 (1980).
6. S. Sanada, J. Shoji, and S. Shibata, Yakugaku Zasshi, 98, 1048 (1978).
7. Y. Takino, T. Odani, H. Tanizawa, and T. Hayashi, Chem. Pharm. Bull., 30, 2196 (1982).
8. T. Odani, H. Tanizawa, and Y. Takino, Chem. Pharm. Bull., 31, 292 (1983).
9. T. Odani, H. Tanizawa, and Y. Takino, Chem. Pharm. Bull., 31, 1059 (1983).
10. T. Odani, H. Tanizawa, and Y. Takino, Chem. Pharm. Bull., 31, 3691 (1983).
11. B. H. Han, M. H. Park, Y.N. Han, L. K. Woo, U. Sankawa, S. Yahara, and O. Tanaka, Planta Medica, 44, 146 (1982).
12. O. Tanaka and S. Yahara, Phytochemistry, 17 1353 (1978).
13. R. W. Brauer, J. Am. Med. Ass., 169, 1462 1959).
14. U. Sankawa, C. K. Sung, B. H. Han, T. Takiyama, and K. Kawashima, Chem. Pharm. Bull., 30, 1907 (1982).