STUDIES ON THE METABOLISM AND/OR DECOMPOTION AND DISTRIBUTION OF GINSENOSIDE Rb_2 IN RATS

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INTRODUCTION

The root of *Panax ginseng* C.A. Meyer(Araliceae) has been used in folk medicine for thousands of years in Korea, China and Japan.

Ginseng saponins, isolated from the root of Panax ginseng, have been regarded as the principal components responsible for the pharmacological activities of Ginseng Radix, such as tonic, hematopoietic, tranquilizing and sedative actions. There are many reports on the pharmacological, chemical and biochemical studies of ginseng saponins[1]. Almost all of the pharmacological studies involved were in vitro and in vivo experiments, not by oral administration. However, Ginseng Radix has been used for a long time as a crude drug taken orally. Therefore, evaluation of ginseng saponins should be down following oral administration. Our attention was focused on the absorption, distribution, excretion and metabolism of ginseng saponins after oral administration, since several pharmacological actions of ginseng saponins reported previously might be understood more clearly if these aspects could be clarified. From this view point, we studied and reported on the pharmacodynamics of ginsenoside Rg_1 (Rg_1, 20 (S) - protopanaxatiol saponin) and ginsenoside Rb_1 (Rb_1, 20 (S) - protopanaxadiol saponin) in rats[2]. In these studies, we found obvious differences in their pharmacodynamics of Rg_1 and Rb_1. That is, Rg_1 was easily hydrated in both rat stomach and in 0.1 N HCl, but Rb_1 was little decomposed in rat stomach. Rg_1 was decomposed to ginsenoside Rh_1 (Rh_1) and ginsenoside F_1(F_1) by enteric bacterias, whereas Rb_1 was decomposed to ginsenoside Rd(Rd) by an enteric enzyme. The amount of absorbed Rg_1 and Rb_1 were 1.9% and 0.1% of the dose, respectively. Rg_1 was excreted into rat urine and bile in a 2 : 5 ratio, while Rb_1 was mainly and gradually excreted into rat urine. However, it remained to be clarified whether the pharmacodynamics of Rg_1 and Rb_1 could be applied simply to other ginseng saponins. For that purpose, we need to undergo more studies about other ginseng saponins.

In this symposium, we would like to report the pharmacodynamics of ginsenoside Rb_2 (Rb_2), which is one of the main components of Ginseng Radix and posseses an improving action on arteriosclerosis[3], in rats after oral administration in detail.

EXPERIMENTAL

Material Rb_2 was isolated from red ginseng supplied by the korea Tabacco & Ginseng Public Corporation and the Japan - Korea Red Ginseng Co., Ltd., by high - performance liquid chromatography(HPLC). The experimental animals used were male Wister rats, 6 - 8 weeks old, obtained from Shizuoka Laboratory Animal Center, Hamamatu, Japan. The rats were deprived of food but given free access to water 18 hr prior to the experiments. Other chemicals used were similar to those employed in our previous study[2].

^{1}H - and ^{13}C - Nuclear Magnetic Resonance (^{1}H - and ^{13}C - NMR) ^{1}H - NMR spectra were measured with a JEOL model GSX - 500 (500 MHz) spectrometer and ^{13}C - NMR spectra were measured with a JEOL model FX - 90Q (22.5 MHz) and GSX - 270 (67.8 MHz) spectrometers.

Fast Atom Bombardment Mass Spectrometry(FAB - MS) FAB - MS measured with a JEOL JMS - SX102 mass spectrometer.

Thin - Layer Chromatography(TLC) Normal - phase TLC was performed on Merck precoted Silica gel 60 F_{254} plates (0.25 mm thick). Reverse - phase TLC was performed on Merck precoted RP - 8 F_{254}plates (0.25 mm thick). Developing solvents for normal - and reverse phase TLC were a $CHCl_3$ - MeOH - H_2O (65 : 35 : 10, v/v, lower phase) mixture and 35% aqueous CH_3CN, respectively. TLC spots were detected by spraying with 1% Ce $(SO_4)_2$ - 10% H_2SO_4 solution followed by heating(150C, 3 - 4 min).

Column chromatography Silica gel 60(230 - 240 mesh, Merck) and Bondapak C_{18} (Waters) were used for column chromatography.

HPLC HPLC was performed using a LC - 6A liquid chromatography(carried out with a YMC - packed column AQ - 312 (ODS, 5 μm, 6×150 mm, YMC) under the following conditions : 35%, 40% or 45% CH_3CN aqueous solution as the mobile phase, flow rate 1.0 ml/min, detection wavelength 202 nm. Isolation by HPLC was carried out with a YMC - packed column SH - 343 - 5 (ODS, 5μm, 20×250 mm, YMC) under the conditions : 60%, 70% or 85% CH_3CN aqueous solution as the mobile phase, flow rate 5.0 ml/min, detection wavelength 205 nm.

Chemical and Biological Decomposition of Rb_2 Rb_2 (100 mg/kg, 2% saqueous solution) was administered orally to rats. After exsanguination from the abdominal artery under ether anesthesia at a definite time after administration, the stomach and large intestine were removed. The gut contents were flushed with MeOH and treated according to the procedure in Chart 1.

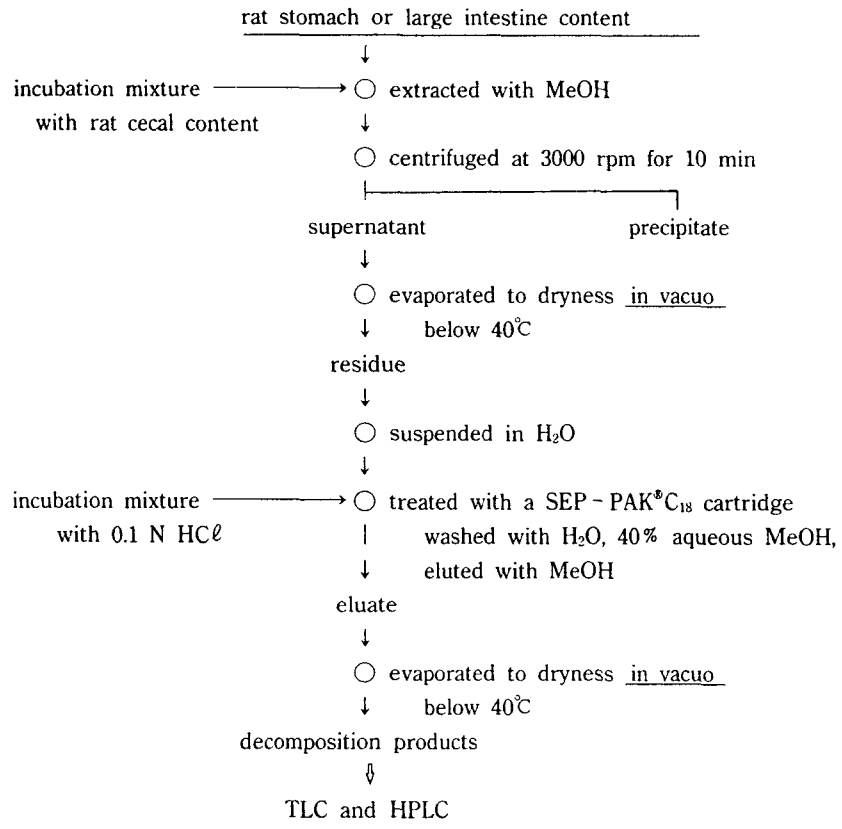


Chart 1. Procedure for Obtaining Decomposition Products of Rb_2

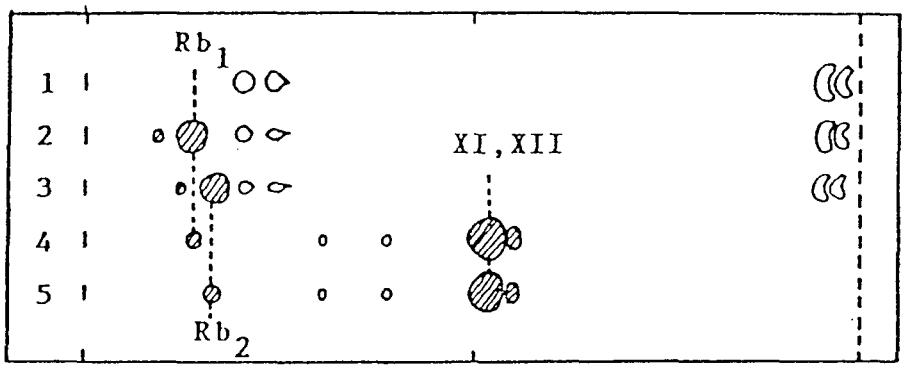


Fig. 1. TLC of Decomposition Products of Rb_1 and Rb_2 in Rat Stomach or in 0.1 N HCℓ
Developing solvent, $CHCℓ_3$—MeOH—H_2O (65 : 35 : 10, v/v, lower phase) ; plate, precoated silica gel 60 F_{254} (Merck) ; detecting reagent, 1% of $Ce(SO_4)_2$—10% H_2SO_4 solution, with heated at 150℃ for 4 min. 1, normal rat ; 2, Rb_1 (100 mg/kg, p.o.) - administered rat (1 h after treatment) ; 3, Rb_2 (100 mg/kg, p.o.) - administered rat (1.5 h after treatment) ; 4, Rb_1 in 0.1 N HCℓ (37℃, 24 h) ; 5, Rb_2 in 0.1 N HCℓ (37㎠, 24h).

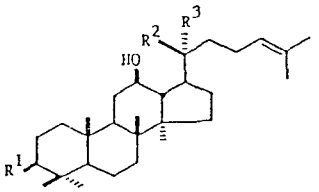
The Decompotion Products of Rb_2 in the Stomach of Rats[4, 6]

As shown in Fig. 1, Rb_2 was little decomposed in rat stomach after oral administration. A small quantity of a decompotion product whose Rf value is lower than that of Rb_2 was observed on normal phase TLC. On the other hand, 0.1 N HCl treatment, the acidity of which is similar to that of gastric juice, resulted in TLC decompotion products with higher Rf values than that of Rb_2 or the rat stomach decompotion product.

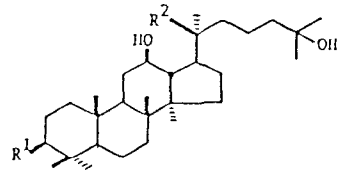
Initially, we investigated the major TLC spot (Rf value, 0.54) of the decompotion products obtained by 0.1 N HCl treatment. This spot was separated into two peaks by HPLC (ODS, 45% aqueous CH_3CN). The main products (XI and XII) were isolated

and purified by preparative HPLC, and identified by ^{13}C-NMR. Based on ^{13}C-NMR data, XI and XII were considered identical to 20 (S)-ginsenoside Rg_3- (20(S)-Rg_3) and Rg_3, respectively. The chemical structures of XI and XII are shown in Chart 2.

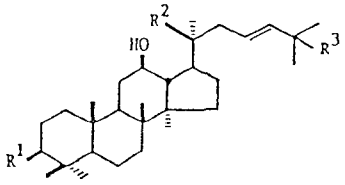
Next, we investigated an unidentified product in rat stomach after oral administration of Rb_2 (100 mg/kg), which was not found



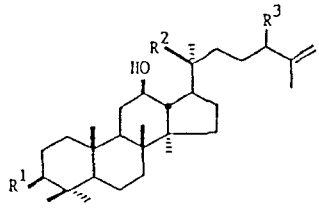
	R^1	R^2	R^3
Rb_2	-O-glc^2—glc	-O-glc^6—arap	$-CH_3$
VI	-O-glc^2—glc	-O-glc	$-CH_3$
VII	-O-glc	-O-glc^6—arap	$-CH_3$
VIII	-O-glc	-O-glc	$-CH_3$
IX	-OH	-O-glc^6—arap	$-CH_3$
X	-OH	-O-glc	$-CH_3$
XI	-O-glc^2—glc	-OH	$-CH_3$
XII	-O-glc^2—glc	$-CH_3$	-OH



	R^1	R^2
V	-O-glc^2—glc	-O-glc^6—arap



	R^1	R^2	R^3
I	-O-glc^2—glc	-O-glc^6—arap	-OH
III	-O-glc^2—glc	-O-glc^6—arap	-OOH



	R^1	R^2	R^3
II	-O-glc^2—glc	-O-glc^6—arap	-OH
IV	-O-glc^2—glc	-O-glc^6—arap	-OOH

Chart 2. Chemical Structures
glc, β-D-glucopyranosyl ; arap, α-L-arabinopyranosyl.

in 0.1 N HCl treated sample. From its TLC behavior, it was unlikely to be a hydrolysis product formed by elimination of O-glycosyl moieties from Rb_2. In our previous study[2d], C-25, 26 hydrated Rh_1 was found as a metabolite of Rg_1 in rat stomach. Therefore, we assumed the existence of a C-25, 26 hydrated derivative of Rb_2 (V) and synthesized it separately. Comparison of the unidentified product in rat stomach with V was performed only by TLC method, and Rf value of the unidentified product was the same as that of V. However, further comparisons could not be done by either HPLC with a ultraviolet detector or ^{13}C-NMR, since V had no side chain double bound and the unidentified product was too low yield. Therefore, we attempted to incubate Rb_2 with rat cecal contents to yield the unidentified product in large quantities, since we have found a similar TLC spot metabolites of Rb_2 in rat large intestine (see part 4). We isolated the fraction whose Rf value was identical to that of the unidentified product by silica gel column chromatography, and subjected it to ^{13}C-NMR. Its ^{13}C-NMR data indicated that it was not yet pure and the double honds existed in it. Therefore, it has become apparent that the unidentified product in rat stomach was not V. Based on these results, this fraction was subjected to HPLC, and we found that it was separated into 4 peaks by HPLC (ODS, 27% aqueous CH_3CN). Therefore, this fraction was further isolated by preparative HPLC to yield four compounds (I-IV), and identified by FAB-MS and ^{13}C-NMR. Based on their ^{13}C-NMR and FAB-MS data, I and II were identified as 20-O-[α-L-arabinopyranosyl (1→6)-β-D-glucopyranosyl]-12β, 25-dihydroxy-3β, 20(S)-dammar-23-ene-3-yl β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-12β, 24(S)-dihydroxy-3β, 20(S)-dammar-25-ene-3-yl β-D-glucopyranosyl (1→2)-β-D-glucopyranoside, respectively. By referring to the reported papers[5], it was reasonable to consider that the ^{13}C-NMR signals of III and IV (at δ 81.3 and 90.1 ppm, respectively) are assignable to that of the carbon attached to a hydroperoxyl group. In order to verify this presumption, Rb_2 was subjected to photosensitized oxygenation, and the products were identical to III and IV.

Consequently, III and IV were determined to be 20-O-[α-L-arabinopyranosyl (1→6)-β-D-glucopyranosyl]-12β-hydroxy-25-hydroperoxy-3β, 20(S)-dammar-23-ene-3-yl β-D-glucopyranosyl (→2)-β-D-glucopyranoside and 20-O-[α-L-arabinopyranosyl (1→6)-β-D-glucopyranosyl]-12β-hydroxy-24(S)-hydroperoxy-3β, 20(S)-dammar-25-ene-3-yl β-D-glucopyranosyl (1→2)-β-D-glucopyranoside, respectively. The chemical structures of I-IV are shown in Chart 2.

This study is the first report in which the presence of Rb_2 hydroperoxides is confirmed in rat stomach and cecum. It appears that production of Rb_2 hydroperoxides is due to the lipoxygenases, which are widely distributed in vivo. Han et al[6] have reported that ginsenoside (Rg_1, Re and Rb_1) were hydrated easily in the side chain under mild acidic conditions (with 0.1 N HCl, at 37℃). In our result so far, C-25, 26 hydrated derivatives of Rg_1 were detected in both rat stomach and 0.1 N HCl,

but those of Rb_1 and Rb_2 were produced in neither media. This means that O - glycosyl moieties of Rb_1 and Rb_2 are fairly stable and hydration dose not occur in rat stomach. These facts led us that the decomposition modes are different between 20(S) - protopanaxartiol saponins and 20 (S) - protopanaxadiol saponins, and that 20(S) - protopanaxadiol saponins undergo oxygenation rather than hydration in the side chain. The decomposition pathway of Rb_2 is summarized in Chart 3.

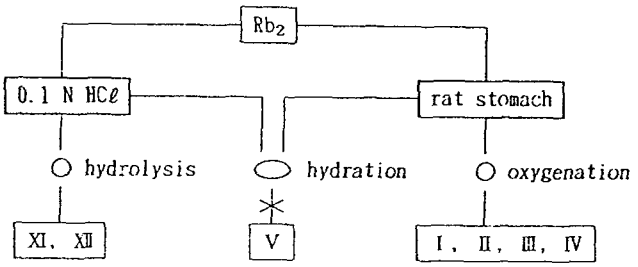


Chart 3. Decomposition Pathway of Rb_2 in Rat Stomach or in 0.1 N HCℓ

The Decomposition Products of Rb_2 in the Large Intestine of Rats[1a]

As shown in Fig. 2, six spots (containing 9 decomposition products, I - IV, and VI⇨V) of which Rf values on TLC were higher than that of Rb_2 were isolated and purified by normal - and reverse - phase column chromatography, and identification of these compound was done by ^{13}C - NMR. In the ^{13}C - NMR data for these compounds, all of the carbon signals due to the aglycone moiety appeared at almost the same positions as those of Rb_2. Therefore, these compounds were assumed to be prosapogenins of Rb_2. Based on these data, VI was considered identical to Rd. Compound VII was idetified to be 20 - O - [α - L - arabinopyranosyl (1→6) - β - D - glucopyranosyl] - 3 - 20 (S) - protopanaxadiol β - D - glucopyranoside. Then, VIII, IX and X were determined to be ginsenoside $F_2(F_2)$, 20 - O - [α - L - arabinopyranosyl (1→6) - β - D - glucopyranosyl] - 3 - 20(S) - protopanaxadiol and compound K, respectively. The formation of these prosapogenins of Rb_2 revealed that decomposition began with cleavage of the terminal glucose of sophorosyl group at the C - 3 hydroxyl group or the terminal arabinose of an oligosaccharide at the C - 20 hydroxyl group, and that the reaction proceeded stepwise via cleavage of sugar moieties at the C - 3 or C - 20 hydroxyl group, finally forming compound K. Moreover, the order of yield of the decomposition products was IX > X > VIII. This suggested the presence of β - glucosidase, which decomposes the glycoside chain predominantly at the C - 3 hydroxyl group, in rat large intestine. Consequently, the decomposition pathway of Rb_2 in rat large intestine can be assumed as Chart 4.

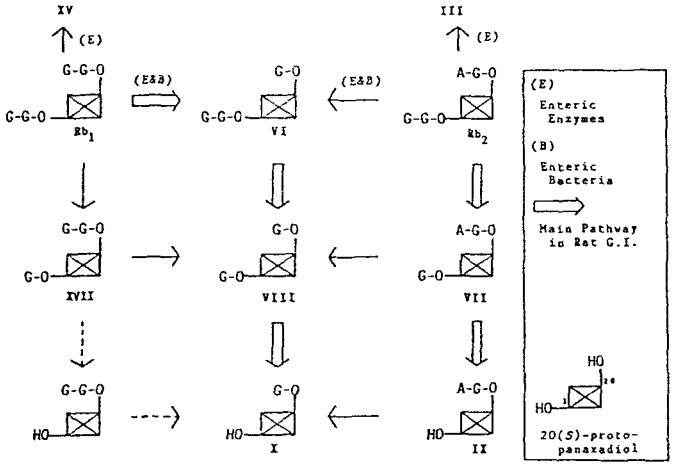


Chart 4. Decomposition Pathway of Rb_1 and Rb_2 in Rat Large Intestine or by Crude Hesperidinase

Comparison of the Decomposition Modes of Rb_1 and Rb_2 in the Digestive Tract of Rats[4c]

In order to clarify some similarities and differences of decomposition modes between 20 (S) - protopanaxadiol (20(S) - ppd) saponins, represented by Rb_1 and Rb_2, the decompositions of Rb_1 and Rb_2 in the rat gastrointestinal tract and 0.1 N HCl were investigated in detail. As the experimental methods which were used in this chapter were the same as those in above chapters, summarized results were herinafter described.

As in the previous study[4c], we also noticed that Rb_1 was only a little decomposed in rat stomach, and that a small quantity of Rb_1 was changed into a substance with an Rf value lower than that of Rb_1 on normal - phase TLC (Fig. 1), and which was detected among the decomposition products of Rb_1 in the rat large intestine. This was similar to the case of Rb_2, suggesting that hydroperoxidation might occur in rat stomach. We succeeded in isolating four products (VIII - XVI) derived from Rb_1 by incubation with rat cecal contents. Based on their ^{13}C - NMR and FAB - MS and FAB - MS data, we determined XV, a main product, to be 20 - O - [β - D - glucopyranosyl (1→6) - β - D - glucopyranosyl] - 12β - hydroxy - 25 - hydroperoxy - 3β, 20 (S) - dammar - 23 - ene - 3 - yl β - D - glucopyranosyl (1→2) - β - D - glucopyranoside. Although XIII, XIV and XVI could not be determined by ^{13}C - NMR because of their small yield, we assumed that they were likely to be the 25 - hydroxy - 23 - en (XIII), 24 - hydroxy - 25 - en (XIV) and 24 - hydroperoxy - 25 - en (XVI) derivatives of Rb_1, as in the case of Rb_2. Next, we investigated the effect of treating Rb_1 with 0.1 N HCl. The result was same as that of Rb_2. The major products were 20(R, S) - Rg_3. The above data showed more clearly that 20(S) - ppd saponins (Rb_1 and Rb_2) undergo partial hydroperoxidation in rat stomach, whereas they are easily hydrolyzed by 0.1 N HCl, and that the decomposition mode of 20 (S) - ppd saponins is different from that of 20(S) - ppt saponin (Rg_1), which easily undergoes both hydrolysis and hydration in rat stomach and 0.1 N HCl.

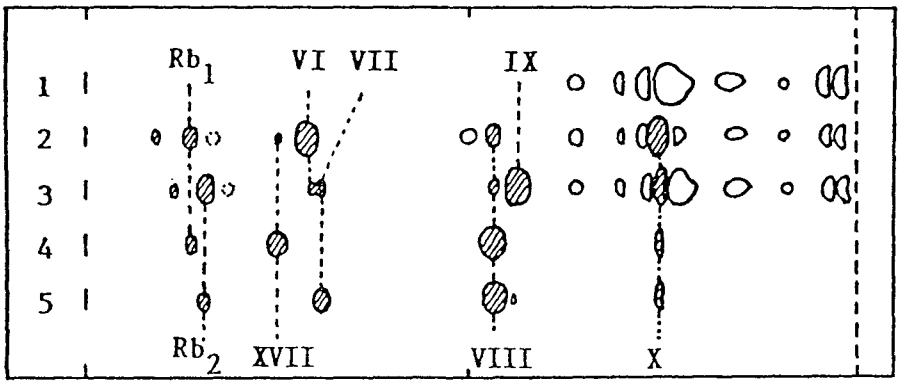


Fig. 2. TLC of Decomposition Products of Rb_1 and Rb_2 in Rat Large Intestine or by Crude Hesperidinase
TLC conditions were the same as those described in Fig. 5. 1, normal rat ; 2, Rb_1 (100 mg/kg, p.o.) - administered rat (3 h after treatment) ; 3, Rb_2 (100 mg/kg, p.o.) - administered rat (6 h after treatment) ; 4, Rb_1 by crude hesperidinase ; 5, Rb_2 by crude hesperidinase.

On the other hand, five decomposition products were observed by normal - phase TLC (Fig. 2) in the samples of rat large intestine after oral administration of Rb_1 (100 mg/kg) and incubation of Rb_1 with rat cecal contents. Those products with higher Rf values than Rb_1 were identified as gypenoside XVII (G-XVII, XVII), Rd (VI), F_2 (VIII) and compound K(X) by comparison of their TLC and HPLC behaviors with those of authentic samples, and the product with lower Rf value than Rb_1 was XV, described above. The formation of these prosapogenins of Rb_1 revealed that the decomposition pathways of Rb_1 and Rb_2 are similar, i.e. decomposition begins with cleavage of the terminal sugar moiety at the C - 3 or C - 20 hydroxy group, and the reaction proceeds via stepwise cleavage of sugar moieties, finally forming compound K (Chart 4). However, the rate of decomposition of Rb_1 seemed to differ from that of Rb_2. Then, the time courses of decomposition of Rb_1 and Rb_2 were tested by using the incubation system with rat cecal contents. Consequently, in the case of Rb_1, Rb_1 had already disappeared by 1 hr, and decomposition had proceeded to compound K after 3 hr, when Rd, the major intermediate product, was no longer detectable. On the other hand, hardly any of the Rb_2 had disappeared after 3 hr, and major intermediate product (IX) still remained. Therefore, we concluded that in rat large intestine there are obvious differences in the mode of decomposition between Rb_1 and Rb_2. These results led us to consider that because the sugar moieties of Rb_1 are all glucose and those of Rb_2 are glucose and arabinose, these might be responsible for the differnet rates of decomposition. Also it was speculated that decomposition of 20 (S) - ppd saponins began with clevage of the terminal sugar moiety at the C - 20 hydroxyl group, followed by the terminal sugar moiety at the C - 3 hydroxyl group by β - glucosidase present in rat large intestine. However, in Rb_2, one molecule of arabinose as the terminal sugar moiety at the C - 20 hydroxyl group resists attack by β - glucosidase. As a result, hydrolysis of the glycoside chain of Rb_2 probably occurs predominantly at the C - 3 hydroxyl group (chart 4). This assumption is strongly supported by the observation that the major intermediate product of Rb_1 was Rd, despite the fact that the sugar moieties of Rb_1 are all glucose, and also that in the case of Rb_2, the disappearance of Rd was fast.

Finally, we investigated the decompotion modes of Rb_1 and Rb_2 in the rat large intestine, paticularly noting whether enteric enzymes or enteric bacteria are predominantly responsible. The results are shown in Chart 4. Thus the respective prosapogenins of Rb_1 and Rb_2, except for Rd and the hydroperoxides of Rb_1 and Rb_2, are produced by tetracycline - resistant bacteria. Rd and the hydroperoxides (XV and III) of Rb_1 and Rb_2 are produced by enteric enzymes.

We therefore concluded that the decomposition of 20 (S) - ppd saponins (Rb_1 and Rb_2) in rat large intestine differed from that of 20 (S) - ppt saponin (Rg_1) in rat stomach, and that the decomposition modes of Rb_1 Rb_2 were different because of variations in the terminal sugar moiety at the C - 20 hydroxyl group.

The Absorption, Distribution and Excretion of Rb_2 in the Rats

1. Isotope Labeling of Rb_2[4 d]

In view of the many important findings on the pharmacological activities of ginsenosides, their low absorption rates, including our data, seem unconvincing and we, therefore, felt it necessary to determine whether or not these were correct. Tritium(^3H) labeling of a ginsenoside was seen as useful to resolve this, because the methods of determining ginsenosides and their metabolites in biological samples using HPLC or TLC - densitometry are not always perfect and may have inadvertently been missed. The existence of ^3H is easily found because of its high detection sensitivity. However, the synthesis of a radioisotopic ginseng saponin has been thought to be very difficult owing to its natural product. In fact, there is no report on labeling of a specific position in a ginseng saponin using ^{14}C or ^3H.

We attempted to obtain ^3H labeled Rb_2 as shown in Chart

5. Rb_2 was partially acetylated to XVIII by acetic anhydride and pyridine. that is, the C - 12 hydroxy group of Rb_2 was not acetylated. This selective acetylation was previously reported by Tanaka et al[7]. Then the C - 12 hydroxy group of XVIII was oxidized by chromic acid and pyridine to yield XIX. The formation of ^3H labeled Rb_2 was expected when Rb_2 was reproduced by reduction of XIX with ^3H labeled reductants.

As C - 12 epimerization occurs in this reduction, we investigated to select the best reduction condition for synthesis of ^3H labeled Rb_2. Thus we concluded that the most suitable reduction condition for XIX to obtain Rb_2 in good yield was 15 hr for refluxing, using 2 - PrOH as the solvent and 200 - fold $NaBH_4$ to XIX in mole ratio. The yield of [12 - ^3H] - Rb_2 and [12 - ^3H] - epi - Rb_2 which were isolated by HPLC (ODS, 35% aqueous CH_3CN) was 3.5% and 70.5%, respectively. The ratio of Rb_2 to 12 - epi - Rb_2 was 20.1. The specific activities of [12 - ^3H] - Rb_2 and [12 - ^3H] - epi - Rb_2 were 18.2 and 19.4 KBq/μmol, respectively. Total reduction percentage of XIX by [^3H] - $NaBH_4$ was the same as that by $NaBH_4$, but the ratio of Rb_2 to 12 - epi - Rb_2 was poorer than that of $NaBH_4$ (8.6). The cause of this is not clear, but may be due to "an isotopic effect". This method of specific position labeling of Rb_2 may also be applicable to other ginsenoside.

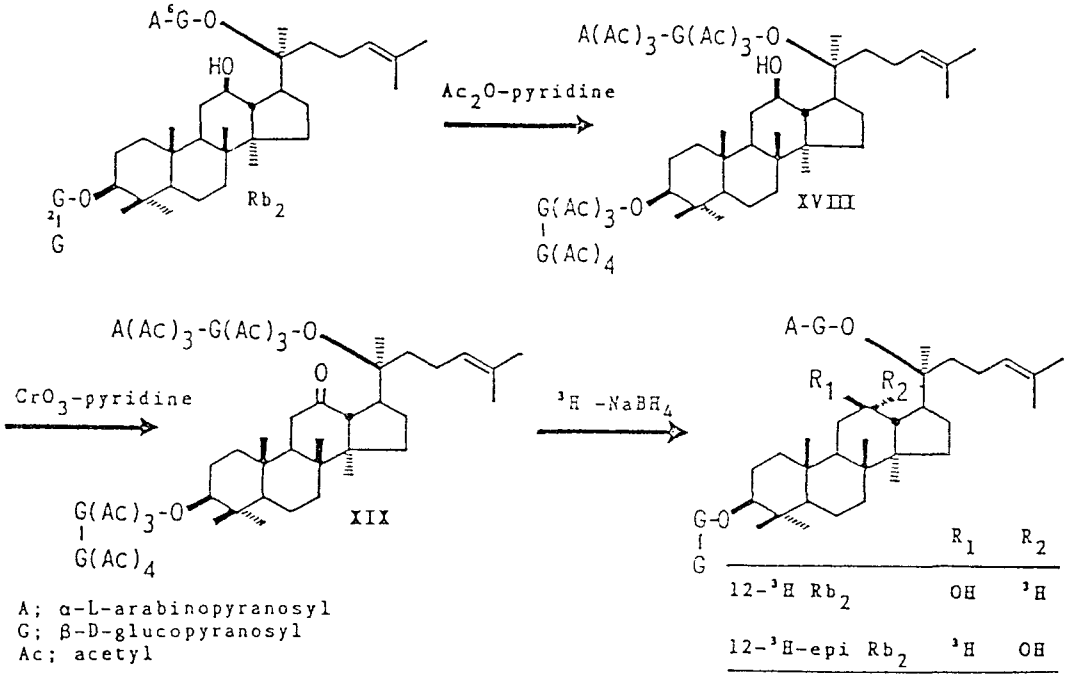


Chart 5. Synthetic Route of [12 - ^3H] Rb_2

2. The Absorption, Distribution and Excretion of ^3H Labeled Rb_2 in the Rat after Oral Administration

First, we examined the biological equivalency of [12 - ^3H] - Rb_2 and [12 - ^3H] - epi - Rb_2 in the distribution in rats, because the yield of [12 - ^3H] - epi - Rb_2 was superior to that of [12 - ^3H] - Rb_2 as mentioned above. [12 - ^3H] - Rb_2 or [12 - ^3H] - epi - Rb_2 was diluted with Rb_2 or 12 - epi - Rb_2 (final dose : 4.46 MBq/100 mg/kg) and administered orally to rats. The serum and tissue concentrations of Rb_2 which were converted from their radioactivities to the equivalent Rb_2 were evaluated at 6 hr. The concentrations of Rb_2 in the serum and tissues of the group I ([12 - ^3H] - epi - Rb_2 + Rb_2) were almost the same when compared with those of the group II ([12 - ^3H] - Rb_2 + - Rb_2). However, the concentrations of Rb_2 in them of the group III ([12 - ^3H] - epi - Rb_2 + 12 - epi - Rb_2) were about 30% lower than those of other two groups. Therefore, we decided to use [12 - ^3H] - epi - Rb_2 insted of [12 - ^3H] - Rb_2, and to dilute it with a large quantity of Rb_2.

The time course of serum concentration of radioactivitiy after oral administration of [12 - ^3H] - epi - Rb_2 + Rb_2 in rats is shown in Fig. 3, together with that of cold Rb_2 concentration which was determined by HPLC after oral administration of Rb_2 (100 mg/kg). The radioactivitiy and Rb_2 were recongnized at a early time and reached each maximum level, 3.55± 0.57 μg eq/ml at 12 hr and 1.16± 0.33 μg/ml, respectively. The radioactivitiy and Rb_2 were practically undetectable at 48 hr and 24 hr, respectively. These data mean that [12 - ^3H] - epi - Rb_2 and Rb_2 were absorbed rapidly from the upper part of the gastrointestinal tract and its degraded and/or metabolized compounds were also absorbed gradually from the lower part of rat digestive tract.

Next, the time courses of tissue radioactivity after oral administration of [12 - ^3H] - epi - Rb_2 + Rb_2 in rats were investiga-

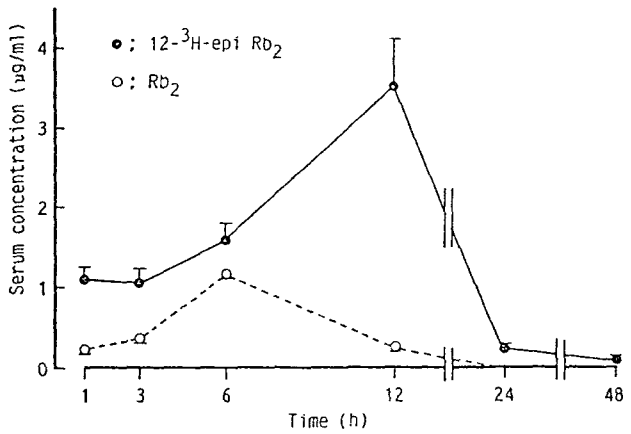


Fig. 3. Changes of Serum Concentration of [12-^{3}H]-epi-Rb$_2$ and Rb$_2$ in Rats[a]

a) [12-^{3}H]-epi-Rb$_2$ (4.46 MBq/100 mg/kg) or Rb$_2$ (100 mg/kg) was administered orally to rats. Each point is the mean± S.D. of 4 rats.

ted. As shown in Table 1, the profiles of the time variations of Rb$_2$ concentration in main tissues of rat were closely similar to that of serum, that is, the concentrations of main tissues reached their maximum at 12 hr after and then declined smoothly. In comparison of the tissue levels at 12 hr, the highest concentration was obtained in the liver (8.00± 0.54 μg eq/g, wet. tissue : 0.251% of the dose) and follwed by the kidney>the lung>the heart. And also the distributions of radioactivity in the brain and testis, which are closely related to the pharmacological activities of Ginseng Radix, were found, though the concentrations in the both organs were considerably lower than other tissues. In addition, Rb$_2$ is a first example to prove that a ginseng saponin distributes widely in the whole body.

On the other hand, the excretions of radioactivity into the urine and bile were relatively smooth and the cumulative excretion were 3.0% of the dose (within 48 hr) and 0.7% of the dose (within 24 hr), respectively. The cumulative fecal excretion within 48 hr was 87.3% of the dose. Therefore, the amount of absorbed radioactivity after oral administration, which is calculated simply using the sum of urinary and biliary excretions, seems to be at least 3.7% of the dose. This value of Rb$_2$, obviously differed from that of Rb$_1$ (0.11% of the dose)[2c], is appreciably high compared with that of Rg$_1$ (1.9% of the dose)[2b]. This means that Rb$_2$, including its degraded and/or metabolized compounds, is absorbed relatively easily from the gastrointestinal tract of rats, compared with Rg$_1$ and Rb$_1$. And it also means that individual ginseng saponins have their own pharmacodynamics.

Table 1.Time Courses of [^{3}H] Radioactivity in Rat Tissues

Time(h)	Liver	Kidney	Lung	Heart	Spleen
1	0.313± 0.030	0.239± 0.015	0.258± 0.082	0.714± 0.033	0.099± 0.030
3	1.008± 0.260	0.346± 0.026	0.178± 0.030	0.119± 0.021	0.073± 0.008
6	6.621± 0.775	0.964± 0.131	0.801± 0.065	0.699± 0.029	0.548± 0.055
12	8.003± 0.540	2.642± 1.146	2.164± 0.736	1.838± 0.614	1.075± 0.516
24	0.726± 0.342	1.077± 0.719	0.092± 0.019	0.098± 0.031	0.101± 0.022
48	0.447± 0.158	0.292± 0.096	0.084± 0.044	0.086± 0.005	0.105± 0.025

Time(h)	Brain	Testis	Muscle	Fat
1	0.120± 0.058	0.051± 0.025	0.450± 0.302	0.050± 0.002
3	0.055± 0.006	0.105± 0.036	0.396± 0.184	0.152± 0.073
6	0.129± 0.066	0.222± 0.054	0.379± 0.113	0.502± 0.061
12	0.253± 0.132	0.489± 0.287	0.843± 0.441	1.101± 0.360
24	0.044± 0.011	0.077± 0.031	0.054± 0.009	0.308± 0.207
48	0.061± 0.008	0.058± 0.017	0.062± 0.010	0.130± 0.054

a) [12-^{3}H]-epi-Rb$_2$ (4.46 MBq/100 mg/kg) was administered to rats. Radioactivity was expressed as μg eq/g · wet · tissue. Each value is the mean± S.D. of 4 rats.

CONCLUSION

The pharmacodynamics of Rb$_2$ in rats after oral administration was investigated in detail. In the stomach, though Rb$_2$ was little decomposed, a small quantity of metabolites in which 25-hydroperoxy-23-ene derivative of Rb$_2$ was a main compound was found. This finding of the hydroperoxy derivative of Rb$_2$ was the first one in biological samples. Rb$_2$ was decomposed and/or metabolized to 9 compounds, including hydroperoxy derivatives of Rb$_2$, and Rd, F$_2$ and compound K, etc, in the large intestine. The decomposition modes of Rb$_1$ and Rb$_2$ in rat stomach were done in the same manner which was distinct from Rg$_1$, but those in the large intestine were different because of variations in the terminal sugar moiety at C-20 hydroxy group.

Next, a ^{3}H labeling of Rb$_2$ was examined and succeeded to label the C-12 position of Rb$_2$ with ^{3}H. The synthesized [12-^{3}H]-Rb$_2$ and [12-^{3}H]-epi-Rb$_2$ were administered orally to rats and the biological equivalency of [12-^{3}H]-epi-Rb$_2$

to [12-^{3}H]-Rb_2 was proved when it was diluted with a large quantity of Rb_2. The serum and tissue radioactivity indicated that Rb_2 was absorbed rapidly from the upper parts of digestive tracts and its metabolized and/or decomposed compounds of Rb_2 were also absorbed gradually. The radioactivity distributed widely in the whole body, including the brain and testis. The amount of absorbed radioactivity was seemed to be at least 3.7% of the dose, which figure was based on urinary excretion (3.0% of the dose, within 48 hr) and biliary excretion (0.7% of the dose, within 24 hr). This means that Rb_2, including metabolized and/or decomposed compounds of Rb_2 is absorbed more easily than Rg_1 and Rb_1, and also suggests that more studies on pharmacodynamics of another ginseng saponins are necessary.

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