Studies on Absorption, Distribution, Excretion and Metabolism of Ginseng Saponins. V.[1] The Decomposition Products of Ginsenoside Rb_2 in the Large Intestine of Rats

Masami KARIKURA,*[,a] Toshio MIYASE,[a] Hisayuki TANIZAWA,[a] Yoshio TAKINO,[a] Toshio TANIYAMA,[b] and Teruaki HAYASHI[b]

School of Pharmaceutical Sciences, University of Shizuoka,[a] 395, Yada, Shizuoka 422, Japan and Koshiro Co., Ltd.,[b] 2–5–8, Dosho-machi, Chuo-ku, Osaka 541, Japan. *Received April 4, 1990*

The decomposition of ginsenoside Rb_2 (Rb_2) in the rat large intestine after oral administration was investigated in detail. A part of Rb_2 was decomposed and six decomposition products (I—VI) were observed on thin-layer chromatogram. Among them, five products (I—V) were isolated, and identification of these compounds was done by carbon-13 nuclear magnetic resonance (^{13}C-NMR). On the basis of ^{13}C-NMR analysis, these compounds were identified as ginsenoside Rd (I), 3-*O*-β-D-glucopyranosyl-20-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol (II), ginsenoside F_2 (III), 20-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol (IV), and compound K (V), respectively.

Keywords ginsenoside Rb_2; metabolism; rat large intestine; prosapogenin; ^{13}C-NMR; TLC; HPLC; ginseng saponin

The root of *Panax ginseng* C. A. MEYER (Araliaceae) 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.[2] Almost all of the pharmacological studies involved were *in vitro* or *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 done 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 absorption of ginseng saponins, ginsenoside Rg_1 (Rg_1, 20(*S*)-protopanaxatriol saponin) and ginsenoside Rb_1 (Rb_1, 20(*S*)-protopanaxadiol saponin), in the rat gastrointestinal tract,[1] resulting in a further clarification of their pharmacodynamics. In these studies, we found an obvious difference in the decomposition modes of Rg_1 and Rb_1 in the rat large intestine. That is, Rg_1 was decomposed to ginsenoside Rh_1 and ginsenoside F_1 by enteric bacterias, whereas Rb_1 was decomposed to ginsenoside Rd (Rd) by an enteric enzyme. However, it remained to be clarified whether the mode of decomposition of Rg_1 and Rb_1 could be applied simply to other ginseng saponins. In the present study, we used ginsenoside Rb_2 (Rb_2), which is one of the main components of Ginseng Radix and posseses an improving action on arteriosclerosis,[3] and investigated in detail its decomposition products in the rat large intestine after oral administration.

Experimental

Materials Rb_2 was isolated from red ginseng supplied by the Japan–Korea Red Ginseng Co., Ltd., by high-performance liquid chromatography (HPLC). The experimental animals used were male Wistar rats, 8 weeks old and weighing 190—200 g, obtained from Shizuoka Laboratory Animal Center, Hamamatsu, Japan. The rats were deprived of food but given free acess to water 18 h prior to the experiments. SEP-PAK® C_{18} cartridges (SEP-PAK) were purchased from Japan Waters Co., Ltd., Tokyo, Japan. Other chemicals used were similar to those employed in our previous study.[1]

Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) ^{13}C-NMR spectra were measured with a JEOL model FX-90Q spectrometer with tetramethyl silane as an internal standard.

Thin-Layer Chromatography (TLC) TLC was performed on Merck precoated silica gel 60 F_{254} plates (0.25 mm thick). As a developing solvent for TLC, a $CHCl_3$–MeOH–H_2O (65 : 35 : 10, v/v, lower phase) mixture was used. The detection of spots on the TLC plates was done by spraying 1% of $Ce(SO_4)_2$–10% H_2SO_4 solution followed by heating (150 °C, 3—4 min).

Column Chromatography Silica gel 60 (230—400 mesh, Merck) and Bondapak C_{18} (Waters) were used for column chromatography.

HPLC HPLC was performed using a LC-6A liquid chromatograph (Shimadzu) with a SPD-6A ultraviolet detector (Shimadzu). HPLC was 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, 5 μm, 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.

Biological Decomposition of Rb_2 1) Decomposition of Rb_2 in Rat Large Intestine: Rb_2 (100 mg/kg, 2% aqueous solution) was administered orally to the rats. After exsanguination from the abdominal artery under anesthesia with ether at 6 h after administration, the large intestine was isolated and treated according to the procedure in Chart 1, and the decomposition products for TLC and HPLC were obtained.

2) Decomposition of Rb_2 by Rat Cecum Content (*in Vitro*): The whole

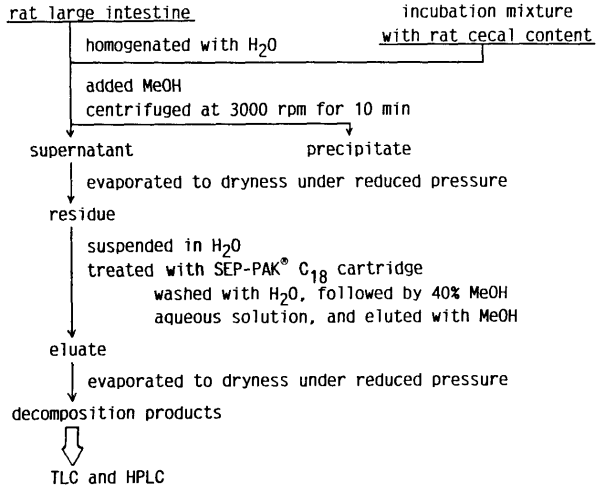


Chart 1. Assay Procedure for Obtaining Biological Decomposition Products of Rb_2

cecal content of a normal rat was suspended in 50 ml of 0.9% saline. Rb_2 (20 mg) was added to the suspension and the solution was incubated at 37 °C for 6 h. The decomposition products of Rb_2 were investigated by the procedure in Chart 1.

3) Isolation of Decomposition Products of Rb_2 (Chart 2): The whole cecal content of 3 normal rats was suspended in 100 ml of 0.9% saline solution containing 300 mg of Rb_2, and was incubated at 37 °C for 8 h. To this, 400 ml of MeOH was added, and the supernatant was obtained by centrifugation (3000 rpm, 10 min). The supernatant was evaporated to dryness below 40 °C under reduced pressure, and the residue obtained was suspended in 50 ml of water. The suspension was injected into a Bondapak C_{18} column (Waters, 25 × 200 mm) pre-equilibrated with water. After washing the column with water (1 l) and then with 40% MeOH aqueous solution (2 l), the eluate with 80% MeOH aqueous solution yielded three fractions after removal of the solvent under reduced pressure; fraction 1 (130 mg), fraction 2 (50 mg) and fraction 3 (90 mg). Fraction 1 was purified by column chromatography [silica gel 40 g, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, v/v, lower phase)] followed by HPLC (60% CH_3CN aqueous solution) to afford Rb_2 (50 mg) and I (30 mg). Fraction 2 was purified by column chromatography [silica gel 20 g, $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2 : 4 : 1, v/v, lower phase)] followed by HPLC (70% CH_3CN aqueous solution) to afford II (30 mg) and III (5 mg). Fraction 3 was purified by column chromatography [silica gel 40 g, $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2 : 4 : 1, v/v, lower phase)] followed by HPLC (85% CH_3CN aqueous solution) to afford IV (40 mg) and V (15 mg). These compounds were identified as ginsenoside Rd (I, Rd), 3-*O*-β-D-glucopyranosyl-20-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol (II), ginsenoside F_2 (III, F_2), 20-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol (IV), and compound K (V), by comparison of ^{13}C-NMR spectral data with the reported values.[4] Their chemical structures and ^{13}C-NMR spectral data are shown in Chart 3 and Table I, respectively.

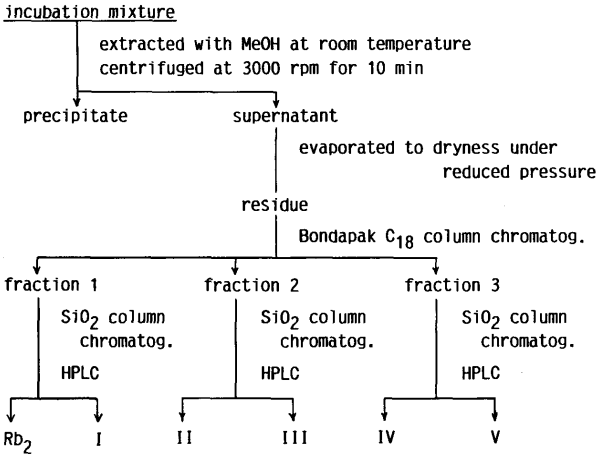
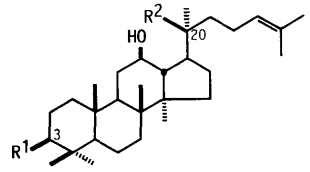


Chart 2. Isolation Procedure for Decomposition Products of Rb_2 by Rat Cecal Contents

Results and Discussion

As shown in Fig. 1, six decomposition products (I—VI) were observed by the TLC method in a sample of rat large intestine taken 6 h after oral administration of Rb_2



	R^1	R^2
Rb_2 (ginsenoside Rb_2)	: -O-glc$^{2\text{-}1}$glc	-O-glc$^{6\text{-}1}$arap
I (ginsenoside Rd)	: -O-glc$^{2\text{-}1}$glc	-O-glc
II	: -O-glc	-O-glc$^{6\text{-}1}$arap
III (ginsenoside F_2)	: -O-glc	-O-glc
IV	: -OH	-O-glc$^{6\text{-}1}$arap
V (compound K)	: -OH	-O-glc

Chart 3. Chemical Structures of Rb_2 and Its Biological Decomposition Products in Rat

glc; β-D-glucopyranosyl, arap; α-L-arabinopyranosyl.

TABLE I. ^{13}C-NMR Data of Rb_2, I, II, III, IV, and V[a]

		Rb_2	I	II	III	IV	V
Aglycone moiety	C-2	26.9	26.8	26.8	26.8	28.3	28.3
	C-3	89.1	89.1	89.0	89.0	78.2	78.1
	C-20	83.7	83.5	83.6	83.4	83.6	83.4
3-*O*-β-D-Glucopyranosyl moiety	C-1′	105.1	105.0	106.8	106.9		
	C-2′	83.7	83.5	75.8	75.8		
	C-3′	78.2[b]	78.0[b]	78.8[b]	78.8[b]		
	C-4′	71.8	71.8	72.1	72.1		
	C-5′	78.0[b]	78.0[b]	78.2[b]	78.2[b]		
	C-6′	62.8	63.0	63.3	63.2		
2′-*O*-β-D-Glucopyranosyl moiety	C-1″	106.1	105.9				
	C-2″	77.2	76.9				
	C-3″	79.2[b]	78.4[b]				
	C-4″	71.8	71.8				
	C-5″	78.2[b]	78.0[b]				
	C-6″	62.8	63.0				
20-*O*-β-D-Glucopyranosyl moiety	C-1‴	98.2	98.3	98.2	98.3	98.2	98.3
	C-2‴	75.0	75.1	75.0	75.2	74.9	75.2
	C-3‴	79.2[b]	79.1[b]	79.1[b]	79.2[b]	79.2	79.2[b]
	C-4‴	71.8	71.8	72.1	71.7	71.8	71.7
	C-5‴	76.8	78.0[b]	76.6	78.2[b]	76.7	78.1[b]
	C-6‴	69.4	63.0	69.3	63.0	69.2	63.0
6‴-*O*-α-L-Arabinopyranosyl moiety	C-1⁗	104.6		104.4		104.6	
	C-2⁗	72.2		72.1		72.2	
	C-3⁗	74.2		74.1		74.1	
	C-4⁗	68.6		68.4		68.5	
	C-5⁗	65.6		65.3		65.6	

a) Measured at 22.5 MHz in pyridine-d_5. Chemical shifts are in δ_c. *b*) Assignments may be interchangeable within the same column.

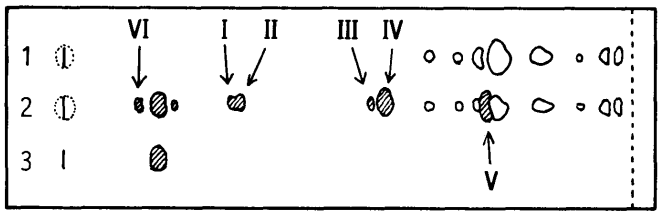


Fig. 1. Thin-Layer Chromatogram of Decomposition Products of Rb_2 in Rat Large Intestine

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 heating at 150 °C for 4 min. 1; normal rat, 2; Rb_2 (100 mg/kg, *p.o.*)-administered rat (6 h after treatment), 3; Rb_2.

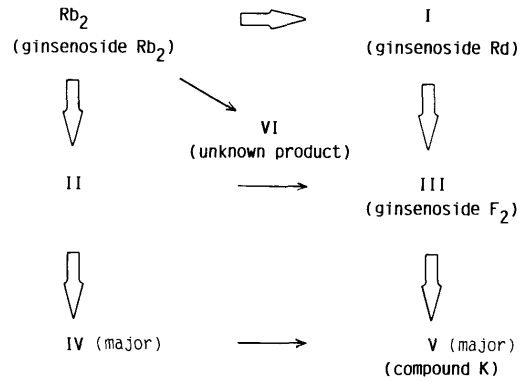


Chart 4. Decomposition Pathway of Rb_2 in Rat Large Intestine and by Rat Cecal Contents

⇒; main pathway.

(100 mg/kg) and after 6 h incubation of Rb_2 (20 mg) with rat cecal contents at 37 °C (*in vitro*).

The products (I—V), with *Rf* values on TLC higher than that of Rb_2, were isolated and purified by normal and reverse-phase column chromatography, and identification of these compounds 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. By comparison of the ^{13}C-NMR spectrum for product I with that for Rb_2, the number of sugar moieties decreased from four in Rb_2 to three in I, whose anomeric carbon signals appeared at δ 98.3, 105.0 and 105.9 ppm. Based on these results, I was considered identical to Rd, which is 20(*S*)-protopanaxadiol having two glucose molecules at the C-3 hydroxyl group in a sophorosyl-type structure and one glucose molecule at the C-20 hydroxyl group, by comparison of the ^{13}C-NMR data for I with the reported values.[4] Based on the ^{13}C-NMR spectrum, in which the anomeric carbon signals appeared at δ 98.2, 104.4 and 106.8 ppm, the structure of product II was suggested to be 3-*O*-β-D-glucopyranosyl-20-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol, and this was supported by comparison of the ^{13}C-NMR data for II with the reported values.[4] The ^{13}C-NMR data for product III, in which the anomeric carbon signals appeared at δ 98.3 and 106.9 ppm, led us to consider that III is the hydrolysis product of Rb_2, which has one glucose molecule at both the C-3 and C-20 hydroxyl groups. By comparison of the ^{13}C-NMR data for III with those already reported,[4] III was considered identical to ginsenoside F_2 (F_2). Product IV, whose anomeric carbon signals appeared at δ 98.2 and 104.6 ppm in the ^{13}C-NMR data, was suggested to have no sugar moiety at the C-3 hydroxyl group, and IV was identified as 20-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol by comparison of the ^{13}C-NMR data for IV with the values reported previously.[4] The ^{13}C-NMR data for product V, in which the anomeric carbon signal appeared at δ 98.3 ppm, led us to consider that V is 20(*S*)-protopanaxadiol, having one glucose molecule at the C-20 hydroxyl group. By comparison of the ^{13}C-NMR data for V with the values reported previously,[4] V was considered identical to compound K.

On the other hand, the chemical structure of product VI, whose *Rf* value on TLC was lower than that of Rb_2, could not be determined because of its small yield. However, VI is unlikely to be a hydrolysis product formed by elimination of *O*-glycosyl moieties from Rb_2.

As described above, our investigation concerning the decomposition of Rb_2 in rat large intestine and by rat cecal contents successfully yielded intermediate hydrolysis products. The formation of these prosapogenins from Rb_2 revealed that decomposition began with cleavage of the terminal glucose of a 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 (Chart 4).

The order of yield of the decomposition products was IV > V > III. This suggested the presence of β-glucosidase, which decomposes the glycoside chain predominantly at the C-3 hydroxyl group, in rat large intestine. A study of decomposition by crude hesperidinase (a type of β-glucosidase), enteric enzymes and enteric bacteria is now under way.

Acknowledgements The authors are grateful to the Korea Ginseng and Tabako Research Institute and the Japan–Korea Red Ginseng Co., Ltd., for the supply of pure Rb_2. This work was supported in part by a grant from the Medical Society for Red Ginseng Research.

References

1) *a*) Y. Takino, T. Odani, H. Tanizawa, and T. Hayashi, *Chem. Pharm. Bull.*, **30**, 2196 (1982); *b*) T. Odani, H. Tanizawa, and Y. Takino, *ibid.*, **31**, 292 (1983); *c*) *Idem*, *ibid.*, **31**, 1059 (1983); *d*) *Idem*, *ibid.*, **31**, 3691 (1983).
2) *a*) O. Tanaka, "Metabolism and Disease," Vol. 10 (Special Issue for Wakan-yaku), Nakayama Shoten, Tokyo, 1973, pp. 548—555; *b*) J. Shoji, "Panax Ginseng," ed. by H. Oura, A. Kumagai, S. Shibata, and K. Takagi, Kyoritsu Shuppan, Tokyo, 1981, pp. 10—41; *c*) S. Shibata, *Gendai Tōyō Igaku*, **3**, No. 3, 62 (1982); *d*) O. Tanaka, *Yakugaku Zasshi*, **105**, 323 (1985); *e*) I. Kitagawa, T. Taniyama, M. Yoshikawa, Y. Ikenishi, and Y. Nakagawa, *Chem. Pharm. Bull.*, **37**, 2961 (1989).
3) *a*) T. Mitsuma, K. Izawa, T. Yokozawa, H. Oura, M. Yamamoto, and Y. Kawashima, *J. Med. Pharm. Soc. WAKAN-YAKU*, **1**, 15 (1984); *b*) T. Yokozawa, T. Kobayashi, H. Oura, and Y. Kawashima, *Chem. Pharm. Bull.*, **33**, 722 (1985).
4) *a*) S. Yahara, O. Tanaka, and I. Nishioka, *Chem. Pharm. Bull.*, **26**, 3010 (1985); *b*) H. Besso, R. Kasai, Y. Saruwatari, T. Fuwa, and O. Tanaka, *ibid.*, **30**, 2380 (1982); *c*) H. Koizumi, S. Sanada, Y. Ida, and J. Shoji, *ibid.*, **30**, 2393 (1982); *d*) H. Besso, R. Kasai, J. Wei, J. Wang, Y. Saruwatari, T. Fuwa, and O. Tanaka, *ibid.*, **30**, 4534 (1982); *e*) R. Kasai, H. Besso, O. Tanaka, Y. Saruwatari, and T. Fuwa, *ibid.*, **31**, 2120 (1983); *f*) T. Namba, K. Matsushige, T. Morita, and O. Tanaka, *ibid.*, **34**, 730 (1986).