Enzymatic Preparation of Ginsenosides Rg_2, Rh_1, and F_1[1)]

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During investigation of the hydrolysis of a protopanaxatriol-type saponin mixture by various glycoside hydrolases, crude preparations of β-galactosidase from *Aspergillus oryzae* and lactase from *Penicillium* sp. were found to produce two minor saponins, ginsenoside Rg_2 [6-*O*-(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)-20(*S*)-protopanaxatriol] and ginsenoside Rh_1 (6-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol), respectively, in high yields. Moreover, a naringinase preparation from *Penicillium decumbens* readily gave an intestinal bacterial metabolite, ginsenoside F_1 (20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol), as the main product, with a small amount of 20(*S*)-protopanaxatriol from a protopanaxatriol-type saponin mixture. Also, a hesperidinase from *Penicillium* sp. selectively hydrolyzed ginsenoside Re into ginsenoside Rg_1. This is the first report on the enzymatic preparation of minor saponins, ginsenosides Rg_2 and Rh_1, and of an intestinal bacterial metabolite, ginsenoside F_1, with high efficiency from a protopanaxatriol-type saponin mixture.

Key words enzymatic preparation; ginsenoside Rg_2; ginsenoside Rh_1; ginsenoside F_1; *Aspergillus oryzae* β-galactosidase; *Penicillium* sp. lactase

Ginseng (the root of *Panax ginseng* C. A. Meyer, Araliaceae) is one of the most commonly used traditional medicines in China, Korea, Japan, and other Asian countries for the treatment of various diseases. Ginseng saponins (ginsenosides) have been regarded as the principal components responsible for the pharmacological activities of ginseng, and a large number of ginsenoside (G) derivatives have been identified in *Panax ginseng* and other *Panax* spp.[2)] Recently, cytotoxic effects of minor saponins, such as G-Rh_1 and G-Rh_2, on the growth of various cancer cells,[3—7)] and also inhibitory effects of human intestinal bacterial saponin metabolites, such as compound K and protopanaxatriol (Ppt), on the growth, invasion, and migration of tumor cells, have been reported.[8—10)] Conventional chemical methods, such as chemical synthesis, mild acid hydrolysis, or alkaline cleavage for the preparation of minor saponins and saponin metabolites, inevitably produced side reactions, such as epimerization, hydration and hydroxylation.[11—13)] In the previous paper,[14)] we first reported that a minor saponin, G-Rh_1, was produced in high yields (more than 90%) from major saponins such as G-Re and G-Rg_1 by a crude lactase preparation from *Penicillium* sp. In this paper, we examined the enzymatic preparation of G-Rh_1, G-Rg_2 (another minor saponin), and G-F_1 [a bacterial metabolite of G-Rg_1 in rat large intestine[15)]], with high efficiency from a Ppt-type saponin mixture that was readily obtained from ginseng extract.

Crude preparations of various glycoside hydrolases such as ten cellulases, a hemicellulase, a β-glucosidase, three hesperidinases, two naringinases, three β-galactosidases, seven lactases, four pectinases, five α-amylases, two maltogenic α-amylases, two β-amylases, a glucoamylase, three pullulanases, a dextranase, and an isomaltase were screened for their ability to produce minor saponins and an intestinal bacterial metabolite from a Ppt-type saponin mixture. HPLC results showed that the remarkable production of new peaks (tentatively named compounds I, II, III, IV, and V) from a Ppt-type saponin mixture occurred with crude preparations of four glycosidases, that is, a hesperidinase from *Penicillium* sp. (Enz. H), a β-galactosidase from *Aspergillus oryzae* (Enz. G), a lactase from *Penicillium* sp. (Enz. L), and a naringinase from *P. decumbens* (Enz. N), respectively (Fig. 1). These new peaks were not formed in the reactions with boiled enzyme preparations. Enz. H formed a large quantity of compound I having a retention time similar to that of G-Rg_1, together with compound III being similar in retention time to G-Rh_1, from a Ppt-type saponin mixture. Enz. G gave compound II (a main product), showing a retention time consistent with G-Rg_2, together with compound III and a small amount of compound IV having a retention time similar to that of G-F_1. The almost complete disappearance of the Ppt-type saponin mixture and the marked accumulation of compound III were observed in the incubated reaction mixture with Enz. L. Enz. N converted the initial saponin mixture into compound IV as a main product, and into compound V showing a much shorter retention time than that of compound IV, together with a small amount of compound III. The yields of compounds I, II, III, and IV measured by HPLC analysis were 60, 34, 68, and 50%, respectively, based on total ginsenosides in a Ppt-type saponin mixture. These compounds were isolated in a crystalline form or as a white powder from the 48 h-incubated reaction mixture on a semipreparative scale by extraction with *n*-butanol and column chromatography. They were identified by spectroscopy as follows: compound I, G-Rg_1; compound II, G-Rg_2; compound III, G-Rh_1, compound IV, G-F_1, and compound V, Ppt (Fig. 2). The ^{13}C-NMR spectral data of these compounds are shown in Table 1.

Figure 3 shows the hydrolysis of G-Re, G-Rf, G-Rg_1, and G-Rg_2 by crude preparations of four glycosidases. Enz. H readily hydrolyzed G-Re into G-Rg_1, and both G-Rf and G-Rg_2 into G-Rh_1, but had no activity against G-Rg_1. Enz. G hydrolyzed G-Re into G-Rg_2, G-Rf into G-Rh_1, and also G-Rg_1 into G-Rh_1 as a main product and a small amount of G-F_1, but had no activity against G-Rg_2. Enz. L hydrolyzed all of G-Re, G-Rf, G-Rg_1, and G-Rg_2 into G-Rh_1. It was also already observed with Enz. L that G-Re was hydrolyzed into G-Rh_1 *via* G-Rg_1, not *via* G-Rg_2, during investigation of the time-course of G-Rh_1 production from G-Re.[14)] Enz. N completely hydrolyzed both G-Re and G-Rg_1 into G-F_1, and also

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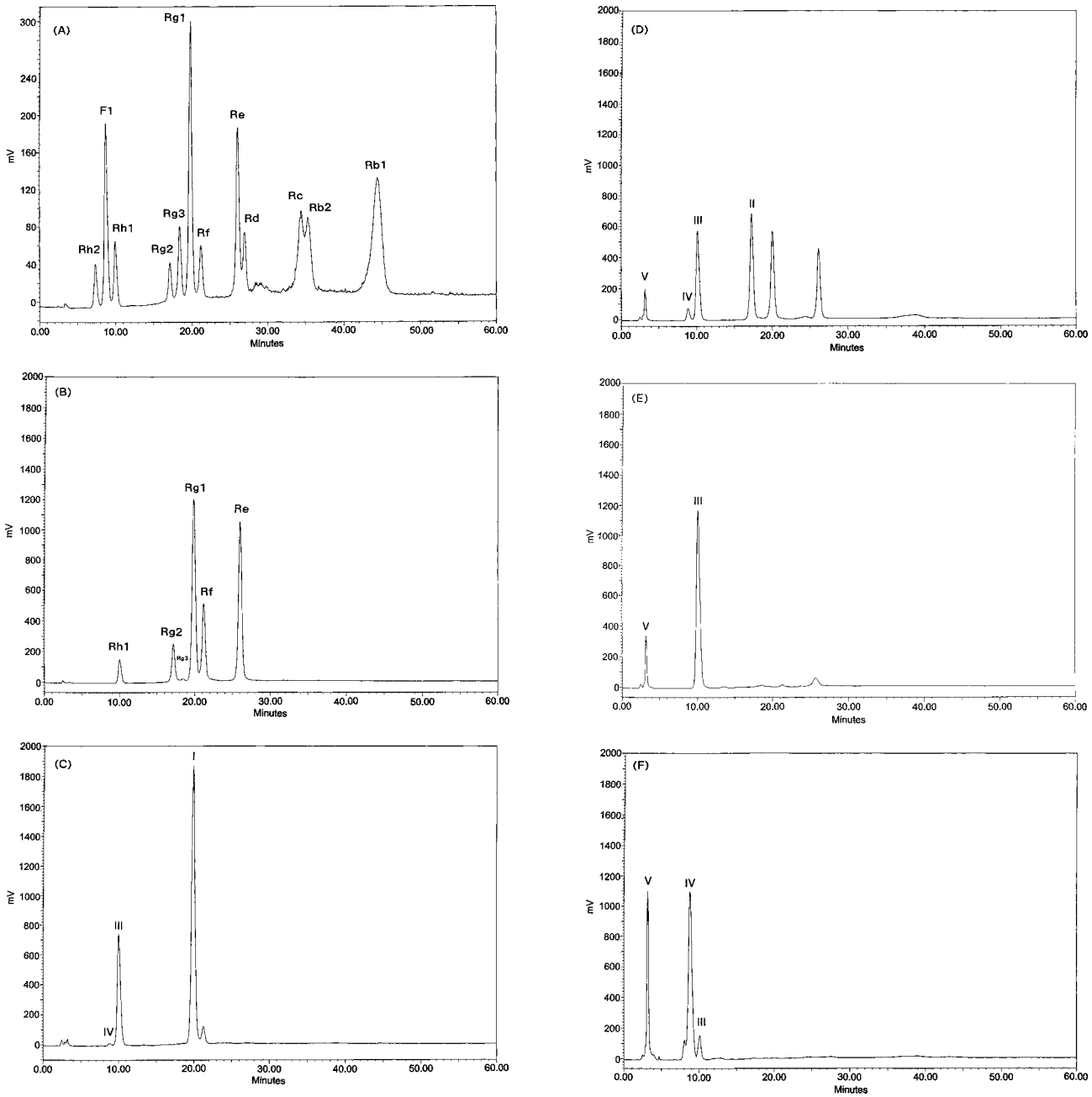


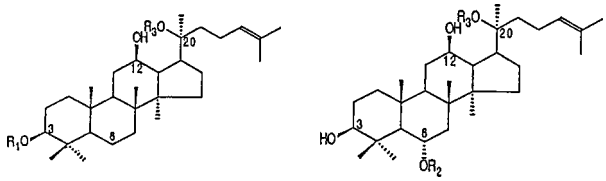
Fig. 1. HPLC Profiles of the Hydrolyzates of a Ppt-Type Saponin Mixture by Crude Preparations of Four Glycosidases

The reaction mixture containing 5 mg of a Ppt-type saponin mixture in 0.25 ml of methanol, 1.25 ml of 0.2 M sodium acetate buffer, 5 mg of enzyme preparation, and distilled water to make a final volume of 2.5 ml was incubated at 37 °C in the dark with gentle stirring. After a 48 h incubation, each reaction mixture was extracted twice with 1.0 ml of *n*-butanol saturated with water, then centrifuged. The *n*-butanol layer was washed twice with 0.3 ml of water, concentrated to dryness *in vacuo*, and the residue was dissolved in 0.75 ml of methanol. The methanol solution (20 μl) was applied to HPLC. Enzyme preparations and the pH of the buffer used in the reaction mixture were as follows. Enz. H: 4.0; Enz. G: 5.0; Enz. L: 4.5; and Enz. N: 4.0.

(A) A mixture of standard ginsenosides containing Rh2 (ginsenoside Rh_2), F1 (ginsenoside F_1, 322 ppm), Rh1 (ginsenoside Rh_1, 130 ppm), Rg2 (ginsenoside Rg_2, 120 ppm), Rg3 (ginsenoside Rg_3), Rg1 (ginsenoside Rg_1, 448 ppm), Rf (ginsenoside Rf, 116 ppm), Re (ginsenoside Re, 304 ppm), Rd (ginsenoside Rd), Rc (ginsenoside Rc), Rb2 (ginsenoside Rb_2), and Rb1 (ginsenoside Rb_1); (B) a Ppt-type saponin mixture which contained six peaks corresponding to Rh1 (279 ppm), Rg2 (825 ppm), Rg3 (27 ppm), Rg1 (2017 ppm), Rf (1108 ppm), and Re (1807 ppm); (C) a Ppt-type saponin mixture+Enz. H; (D) a Ppt-type saponin mixture+Enz. G; (E) a Ppt-type saponin mixture+Enz. L; and (F) a Ppt-type saponin mixture+Enz. N. Peaks I, II, III, IV, and V: compounds I, II, III, IV, and V.

both G-Rf and G-Rg_2 into G-Rh_1 and Ppt, showing a higher *Rf* value than that of G-F_1 on a thin layer chromatogram. These results showed that Enz. H, L, and N had α-rhamnosidase activity against -*O*-β-D-glucose (2←1)-α-L-rhamnose linkage attached to the C-6 hydroxyl group of aglycone in G-Re and G-Rg_2. Enz. G had selective β-glucosidase activity toward the C-20-*O*-β-D-glucosidic linkage in G-Re, but had no α-rhamnosidase activity, resulting in a remarkable accumulation of G-Rg_2 from G-Re. Enz. L had a higher activity of β-glucosidase which regioselectively hydrolyzed the C-20-*O*-β-D-glucosidic linkage in only G-Rg_1, together with high α-rhamnosidase activity against G-Re and G-Rg_2, to afford G-Rh_1 in a large quantity from a Ppt-type saponin mixture. In addition to α-rhamnosidase activity, Enz. N had higher β-glucosidase activity toward C-6-*O*-β-D-glucosidic linkage in G-Rg_1, to afford G-F_1 in a high yield. As shown in Fig. 1-(F),

	R_1	R_2	R_3
G-Re		-glc (2–1) rha	-glc
G-Rf		-glc (2–1) glc	-H
G-F_1		-H	-glc
G-Rg_1		-glc	-glc
G-Rg_2		-glc (2–1) rha	-H
G-Rh_1		-glc	-H
G-Rh_2	-glc		-H
C-K	-H		-glc
Ppd	-H		-H
Ppt		-H	-H



20(*S*)-protopanaxadiol 20(*S*)-protopanaxatriol

Fig. 2. Chemical Structures of Ginsenosides and Their Related Compounds

G-: ginsenoside; C-: compound; glc: β-D-glucopyranosyl; rha: α-L-rhamnopyranosyl; Ppd: 20(*S*)-protopanaxadiol; Ppt: 20(*S*)-protopanaxatriol.

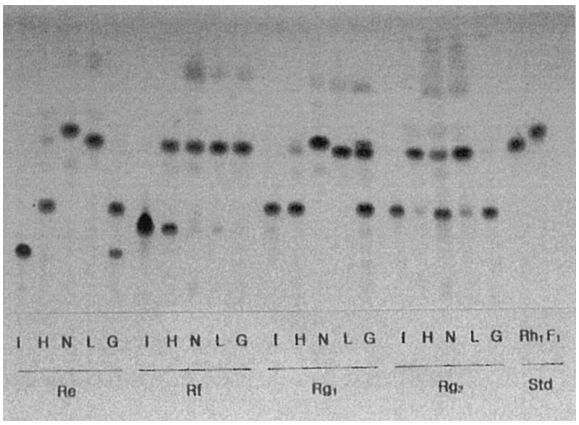


Fig. 3. A Thin-Layer Chromatogram of Hydrolyzates of Ginsenosides Re, Rf, Rg_1, and Rg_2 by the Preparation of Four Glycosidases

The reaction mixture containing 10 mg of ginsenoside Re (or Rf, Rg_1, Rg_2) in 0.25 ml of methanol, 10 mg of enzyme preparation, 1.25 ml of 0.2 M sodium acetate buffer and distilled water to make a final volume of 2.5 ml was incubated for 48 h at 37 °C. After incubation, the reaction mixture was extracted with 0.5 ml of *n*-butanol saturated with water and centrifuged. An aliquot (5 μl) of the *n*-butanol layer was applied on TLC. Enzyme preparations and the pH of the buffer used in the reaction mixture were the same as described in Fig. 1. I (Re, Rf, Rg_1, Rg_2), and Rh_1, F_1: standard ginsenosides Re, Rf, Rg_1, Rg_2, Rh_1, and F_1. Std: standard.

Enz. N also had β-glucosidase activity toward the C-6-*O*-β-glucosidic linkage in G-Rh_1, resulting in the formation of Ppt. From these results, the hydrolytic pathways of Ppt-type saponins by crude preparations of four glycosidases are shown in Fig. 4. Also, they reveal that Enz. G, L, and N are very useful in the selective production of minor saponins, G-Rg_2 and G-Rh_1, and an intestinal bacterial metabolite, G-F_1, respectively, from a Ppt-type saponin mixture.

Kohda *et al.* reported on the enzymatic hydrolysis of G-Rb_1, G-Rb_2, G-Rc, and G-Rg_1 with crude hesperidinase, naringinase, cellulase, pectinase, amylase, and emulsin.[16] Among them, crude hesperidinase, naringinase, and pectinase gave a prosapogenin, compound K, as a main product

Table 1. ^{13}C-NMR Chemical Shifts of Compounds I, II, III, IV and V

	I	II	III	IV	V
C-1	39.5	39.3	39.4	39.3	39.4
C-2	27.7	27.7	27.9	28.1	27.8
C-3	78.4	78.4	78.6	78.4	78.6
C-4	40.2	39.9	40.3	40.3	40.3
C-5	61.2	60.7	61.4	61.7	61.4
C-6	77.9	74.2	78.0	67.9	67.1
C-7	44.9	46.0	45.2	47.4	47.0
C-8	40.9	41.1	41.1	41.2	41.1
C-9	49.8	49.7	50.2	49.9	50.2
C-10	39.5	39.5	39.6	39.3	39.6
C-11	30.1	32.1	32.0	30.9	32.0
C-12	70.1	70.9	71.0	70.2	71.0
C-13	48.9	49.7	48.2	49.1	48.2
C-14	51.2	51.6	51.6	51.3	51.6
C-15	29.9	32.1	31.1	30.8	31.2
C-16	26.8	26.7	27.2	26.6	26.8
C-17	51.6	54.6	54.5	51.6	54.7
C-18	17.4	18.7	17.6	17.4	17.6
C-19	17.4	17.6	17.6	17.4	17.3
C-20	83.1	72.9	73.0	83.2	73.0
C-21	22.2	27.0	26.8	22.3	27.0
C-22	35.9	35.7	35.8	36.1	35.8
C-23	23.1	22.9	23.0	23.2	23.0
C-24	125.7	126.3	126.3	125.9	126.3
C-25	130.8	130.7	130.6	130.9	130.8
C-26	25.6	25.8	25.8	25.7	25.8
C-27	17.6	17.6	17.4	17.7	17.7
C-28	31.6	32.0	31.2	31.9	31.7
C-29	16.2	17.1	16.4	16.5	16.4
C-30	17.4	16.8	16.8	17.6	16.7
Sugar moieties					
6-Glc					
1	105.8	101.7	105.9		
2	75.3	79.4	75.4		
3	80.0	78.2	79.5		
4	71.6	72.5	71.3		
5	80.0	78.3	80.0		
6	62.9	63.0	63.1		
Rha					
1		101.9			
2		72.2			
3		72.4			
4		74.1			
5		69.4			
6		19.1			
20-Glc					
1	98.0			98.2	
2	74.9			75.1	
3	79.0			79.2	
4	71.3			71.6	
5	77.9			78.2	
6	62.9			62.8	

Glc: β-D-glucopyranosyl, Rha: α-L-rhamnopyranosyl.

from a mixture of G-Rb_1, G-Rb_2, and G-Rc. And also, crude hesperidinase (Tanabe Seiyaku Co. Osaka, Japan) hydrolyzed G-Rg_1 to give Ppt and glucose in almost quantitative yield. In this work, however, among all preparations of three hesperidinases, eleven cellulases, three naringinases, and four pectinases tested, only a naringinase (Enz. N) had high hydrolytic activity against G-Re, G-Rg_1, and a Ppt-type saponin mixture. A hesperidinase preparation from *A. niger* (Tanabe Seiyaku Co.) tested could not hydrolyze these saponins. These results suggest the hydrolysis took place with other glycosidases present in the crude preparations of hesperidinase that was used by Kohda *et al.*[16] Also, these results show

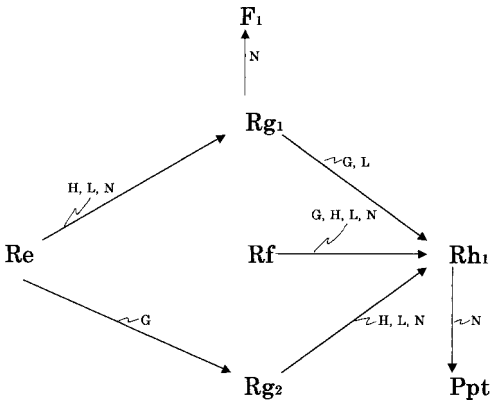


Fig. 4. Hydrolytic Pathways of Ppt-Type Saponins by Crude Preparations of Four Glycosidases

Re: ginsenoside Re, Rf: ginsenoside Rf, Rg_1: ginsenoside Rg_1, Rg_2: ginsenoside Rg_2, Rh_1: ginsenoside Rh_1, F_1: ginsenoside F_1, and Ppt: 20(*S*)-protopanaxatriol.
H: *Penicillium* sp. hesperidinase, N: *P. decumbens* naringinase, L: *Penicillium* sp. lactase, and G: *A. oryzae* β-galactosidase.

that the hydrolysis of glycosidic linkages, such as -*O*-β-D-glucose(2←1)-α-L-rhamnose in G-Re and G-Rg_2, -*O*-β-D-glucose(2←1)-β-D-glucose in G-Rf, C-6-*O*-β-D-glucose in G-Rg_1, and C-20-*O*-β-D-glucose in G-Re and G-Rg_1, might be due to other enzymes contaminated in crude preparations of four glycosidases. The isolation and characterization of these contaminated enzymes which specifically hydrolyze the above-mentioned glycosidic linkages are in a future study.

Experimental

Chemical Reagents and Enzymes A Ppt-type saponin mixture containing G-Re, G-Rf, G-Rg_1, G-Rg_2, and G-Rh_1, and each of these ginsenosides were obtained from standardized ginseng extract containing 13—14% of total saponin (Korea Ginseng and Tobacco Research Institute) by the reported methods.[17,18] G-F_1 was isolated from the leaves of the same plant (*Panax ginseng* C. A. MEYER, Araliaceae) by the reported procedures.[19] G-Rh_1 was produced from G-Re by a lactase preparation from *Penicillium* sp. as described previously.[14] The purity of these compounds was assessed by TLC, HPLC, and ^{13}C-NMR spectroscopy. Crude preparations of four glycosidases, *i.e.*, a β-galactosidase from *A. oryzae* (Enz. G) (Kohjin Co., Ltd., Tokyo, Japan), a lactase from *Penicillium* sp. (Enz. L) (KI Chemical Ind., Co., Ltd., Iwata, Shizuoka-ken, Japan), a hesperidinase from *Penicillium* sp. (Enz. H), and a naringinase from *P. decumbens* (Enz. N) (both from Sigma Chemical Co., St. Louis, MO, U.S.A.) were purchased commercially.

Analyses TLC: A silica gel G-60 F_{254} TLC plate (E. Merck); developing solvent, chloroform–methanol–water (65 : 35 : 10, v/v/v, lower phase); detection, 20% sulfuric acid in ethanol and heating (105—110 °C, 10 min). HPLC: pump, Waters 510; auto sampler, Waters 717 plus; data system, Waters Millenium 32; column, Waters Carbohydrate (3.9×300 mm, 5 μm); mobile phase, gradient conditions with acetonitrile–water–2-propanol (80 : 5 : 15, v/v/v) (solvent A) and acetonitrile–water–2-propanol (80 : 20 : 15, v/v/v) (solvent B) were as follows: the ratios of solvent A/solvent B at running times of 0, 20, 50, and 60 min were 70/30, 0/100, 0/100, and 70/30, respectively; flow rate, 1 ml/min; detector, evaporative light scattering detector model ELSD 2000 (Alltech Co.), (temperature: 92 °C, nebulizing gas: nitrogen). FAB-mass: JEOL mass spectrometer model JMS-HX-110/110A; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): Burker spectrometer model AMX 400 in C_5D_5N with tetramethylsilane as an internal standard.

Isolation of Enzymatic Hydrolysis Products of a Ppt-Type Saponin Mixture For the semipreparative preparation of hydrolysis products (compounds I, II, III, IV and V) from a Ppt-type saponin mixture, a reaction mixture containing 0.5 g of a Ppt-type saponin mixture in 6.25 ml of methanol, 0.5 g of enzyme preparation, 31.25 ml of 0.2 M acetate buffer and water to make a final volume of 62.5 ml was incubated for 48 h at 37 °C. The pH of each buffer used was the same as described in Fig. 1. After incubation, two volumes of methanol were added to the reaction mixture, and the mixture was adjusted to pH 6.5, heated for 10 min in a boiling water bath, and centrifuged. The supernatant was concentrated, then extracted with *n*-butanol. The *n*-butanol layer was concentrated to dryness and dissolved in methanol. Each methanol solution obtained from the reaction mixture with Enz.H, G, L, or N was subjected to the following combined column chromatography: the methanol solution obtained with Enz. H was chromatographed on a silica gel 60 (230—400 mesh) column with chloroform–methanol–water (7 : 3 : 1, v/v/v), and then rechromatographed on an ODS C_{18} column using methanol–water (7 : 3, v/v) as a solvent to afford compound I (163 mg); the methanol solution obtained with Enz. G was purified by silica gel column chromatography eluted with chloroform–methanol–water (8 : 3 : 1, v/v/v), followed by rechromatography on a silica gel column with chloroform–methanol–ethyl acetate–water (2 : 2 : 4 : 1, v/v/v/v) to afford compound II (93 mg); the methanol solution obtained with Enz. L was chromatographed on a silica gel column with chloroform–methanol–water (7 : 3 : 1, v/v/v), and then rechromatographed on a silica gel column using chloroform–methanol–water (90 : 10 : 5, v/v/v, lower phase) as a solvent to afford compound III (182 mg); the one-half volume of methanol solution obtained with Enz. N was chromatographed on a silica gel column with chloroform–methanol (9 : 1, v/v), followed by rechromatography on a silica gel column using chloroform–methanol (12 : 1, v/v) as an eluent to afford compound IV (65 mg). The residual methanol solution was chromatographed on a silica gel column developed with chloroform–methanol–benzene (10 : 1 : 0.4, v/v/v), and then rechromatographed on a silica gel column using *n*-hexane–ethyl acetate (1 : 2, v/v) to afford compound V (31 mg). The identification of these compounds was made by comparison of MS, ^{1}H- and ^{13}C-NMR spectral data with those of authentic samples.

Compound I: A white powder; FAB-MS, *m/z* 801 $(M+1)^+$; ^{1}H-NMR (400 MHz, C_5D_5N), eight methyl signals at δ: 0.82, 1.04, 1.17, 1.59, 1.60, 1.60, 1.62, and 2.07 (all 3H, all s), two anomeric proton signals due to two β-glucosidic linkages at 5.03 (1H, d, *J*=7.8 Hz) and 5.18 (1H, d, *J*=7.7 Hz), 3.52 (1H, dd, H-3), 3.95 (1H, overlapped, H-12), 4.51 (1H, dd, H-6), and 5.25 (1H, t, H-24); the ^{13}C-NMR spectrum corresponded to that in the literature[14,20] for G-Rg_1.

Compound II: Colorless needles from ethanol; mp 187—189 °C; FAB-MS, *m/z* 785 $(M+1)^+$; ^{1}H-NMR, eight methyl signals at δ: 0.95, 0.97, 1.20, 1.36, 1.40, 1.64, 1.69, and 2.14 (all 3H, all s), 1.81 (3H, *J*=6.0 Hz, CH_3 in rhamnose), two anomeric proton signals due to one rhamnosidic linkage at 4.81 (1H, br s) and one β-glucosidic linkage at 5.28 (1H, d, *J*=6.8 Hz), 3.50 (1H, dd, H-3), 4.00 (1H, ddd-like, H-12), 4.69 (1H, dd, H-6), 5.35 (1H, t, H-24); the ^{13}C-NMR spectrum corresponded to that in the literature[21] for G-Rg_2.

Compound III: A white powder; FAB-MS, *m/z* 639 $(M+1)^+$; ^{1}H-NMR, eight methyl signals at δ: 0.83, 1.03, 1.19, 1.42, 1.61, 1.64, 1.67, and 2.08 (all 3H, all s), one anomeric proton signal due to β-glucosidic linkage at 5.04 (1H, d, *J*=7.8 Hz), 3.54 (1H, dd, H-3), 3.95 (1H, overlapped, H-12), 4.54 (1H, dd, H-6), and 5.34 (1H, t, H-24); the ^{13}C-NMR spectrum corresponded to that in the literature[14,20] for G-Rh_1.

Compound IV: A white powder from ethanol; FAB-MS, *m/z* 639 $(M+1)^+$; ^{1}H-NMR, eight methyl signals at δ: 0.98, 1.02, 1.09, 1.45, 1.59, 1.60, 1.62, and 1.99 (all 3H, all s), one anomeric proton signal due to β-glucosidic linkage at 5.19 (1H, d, *J*=7.6 Hz), 3.52 (1H, dd, H-3), 3.93 (1H, overlapped, H-12), 4.34 (1H, dd, H-6), and 5.24 (1H, t, H-24); the ^{13}C-NMR spectrum corresponded to that in the literature[20] for G-F_1.

Compound V: Colorless needles from methanol-water; mp. 219—222 °C; FAB-MS, *m/z* 477 $(M+1)^+$; ^{1}H-NMR ($CDCl_3$), eight methyl signals at δ: 0.90, 0.93, 0.98, 1.06, 1.13, 1.19, 1.63, and 1.69 (all 3H, all s), 3.18 (1H, dd, H-3), 3.57 (1H, ddd-like, H-12), 4.09 (1H, ddd, H-6), and 5.14 (1H, t, H-24); the ^{13}C-NMR spectrum corresponded to that in the literature[20] for Ppt. These compounds I, II, III, IV and V were identified as 6,20-di-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol (G-Rg_1), 6-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol (G-Rg_2), 6-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol (G-Rh_1), 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol (G-F_1), and 20(*S*)-protopanaxatriol (Ppt), respectively.

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References and Notes

1) The complete title of this paper is "Enzymatic Preparation of Minor Saponins and Intestinal Bacterial Saponin Metabolites of Ginseng. Part II." Part I is the "Enzymatic Preparation of Genuine Prosapogenin, 20(*S*)-Ginsenoside Rh_1, from Ginsenosides Re and Rg_1," re-

ported in *Biosci. Biotechnol. Biochem.*, **64**, 2739—2743 (2000).
2) Tanaka O., Kasai R., "Progress in the Chemistry of Organic Natural Products," ed. by Herz W., Grisebach H., Kirby G. W., Tamm C., Springer-Verlag, New York, 1984, pp. 1—76.
3) Odashima S., Ohta T., Kohno H., Matsuda T., Kitagawa I., Abe H., Arichi S., *Cancer Res.*, **45**, 2781—2784 (1985).
4) Ota T., Fujikawa-Yamamoto K., Zong Z., Yamazaki M., Odashima S., Kitagawa I., Abe H., Arichi S., *Cancer Res.*, **47**, 3863—3867 (1987).
5) Lee H. Y., Kim S. I., Lee S. K., Chung H. Y., Kim K. W., Proc. 6th Int. Ginseng Symp., Seoul, 1993, pp. 127—131.
6) Park M. T., Cha H. J., Jeong J. W., Lee H. Y., Kim S. I., Baek N. I., Kim O. H., Kim K. W., *J. Ginseng Res.*, **22**, 216—221 (1998).
7) Park J. A., Lee K. Y., Oh Y. J., Kim K. W., Lee S. K., *Cancer Lett.*, **121**, 73—81 (1997).
8) Wakabayashi C., Hasegawa H., Murata J., Saiki I., *Oncology Res.*, **9**, 411—417 (1997).
9) Wakabayashi C., Hasegawa H., Murata J., Saiki I., *J. Traditional Med.*, **14**, 180—185 (1997).
10) Wakabayashi C., Murakami K., Hasegawa H., Murata J., Saiki I., *Biochem. Biophys. Res. Commun.*, **246**, 725—730 (1998).
11) Han B. H., Park M. H., Han Y. N., Woo L. K., Sankawa U., Yahara S., Tanaka O., *Planta Med.*, **44**, 146—149 (1982).
12) Chen Y., Nose M., Ogihara Y., *Chem. Pharm. Bull.*, **35**, 1653—1655 (1987).
13) Elyakov G. B., Atopkina L. N., Uvarova N. I., Proc. 6th Int. Ginseng Symp., Seoul, 1993, pp. 74—83.
14) Ko S. R., Suzuki Y., Choi K. J., Kim Y. H., *Biosci. Biotechnol. Biochem.*, **64**, 2739—2743 (2000).
15) Odani T., Tanizawa H., Takino Y., *Chem. Pharm. Bull.*, **31**, 3691—3697 (1983).
16) Kohda H., Tanaka O., *Yakugaku Zasshi*, **95**, 246—249 (1975).
17) Sanada S., Kondo N., Shoji J., Tanaka O., Shibata S., *Chem. Pharm. Bull.*, **22**, 421—428 (1974).
18) Sanada S., Kondo N., Shoji J., Tanaka O., Shibata S., *Chem. Pharm. Bull.*, **22**, 2407—2412 (1974).
19) Yahara S., Tanaka O., Komori T., *Chem. Pharm. Bull.*, **24**, 2204—2208 (1976).
20) Yahara S., Kaji K., Tanaka O., *Chem. Pharm. Bull.*, **27**, 88—92 (1979).
21) Ko S. R., Suzuki Y., Kim Y. H., Choi K. J., *Biosci. Biotechnol. Biochem.*, **65**, 1223—1226 (2001).