Enzymatic Preparation of Ginsenosides Rg₂, Rh₁, and F₁¹⁾

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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 Rg₂ [6-*O*-(α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl)-20(*S*)-protopanaxatriol] and ginsenoside Rh₁ (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 F₁ (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 Rg₁. This is the first report on the enzymatic preparation of minor saponins, ginsenosides Rg₂ and Rh₁, and of an intestinal bacterial metabolite, ginsenoside F₁, with high efficiency from a protopanaxatriol-type saponin mixture.

Key words enzymatic preparation; ginsenoside Rg₂; ginsenoside Rh₁; ginsenoside F₁; *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-Rh₁ and G-Rh₂, 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-Rh₁, was produced in high yields (more than 90%) from major saponins such as G-Re and G-Rg₁ by a crude lactase preparation from *Penicillium* sp. In this paper, we examined the enzymatic preparation of G-Rh₁, G-Rg₂ (another minor saponin), and G-F₁ [a bacterial metabolite of G-Rg₁ in rat large intestine¹⁵⁾], 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-Rg₁, together with compound III being similar in retention time to G-Rh₁, from a Ppt-type saponin mixture. Enz. G gave compound II (a main product), showing a retention time consistent with G-Rg₂, together with compound III and a small amount of compound IV having a retention time similar to that of G-F₁. 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-Rg₁; compound II, G-Rg₂; compound III, G-Rh₁; compound IV, G-F₁, and compound V, Ppt (Fig. 2). The ¹³C-NMR spectral data of these compounds are shown in Table 1.

Figure 3 shows the hydrolysis of G-Re, G-Rf, G-Rg₁, and G-Rg₂ by crude preparations of four glycosidases. Enz. H readily hydrolyzed G-Re into G-Rg₁, and both G-Rf and G-Rg₂ into G-Rh₁, but had no activity against G-Rg₁. Enz. G hydrolyzed G-Re into G-Rg₂, G-Rf into G-Rh₁, and also G-Rg₁ into G-Rh₁ as a main product and a small amount of G-F₁, but had no activity against G-Rg₂. Enz. L hydrolyzed all of G-Re, G-Rf, G-Rg₁, and G-Rg₂ into G-Rh₁. It was also already observed with Enz. L that G-Re was hydrolyzed into G-Rh₁ via G-Rg₁, not via G-Rg₂, during investigation of the time-course of G-Rh₁ production from G-Re.¹⁴⁾ Enz. N completely hydrolyzed both G-Re and G-Rg₁ into G-F₁, 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 μ l) 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 Rh₂), F1 (ginsenoside F₁, 322 ppm), Rh1 (ginsenoside Rh₁, 130 ppm), Rg2 (ginsenoside Rg₂, 120 ppm), Rg3 (ginsenoside Rg₃), Rg1 (ginsenoside Rg₁, 448 ppm), Rf (ginsenoside Rf, 116 ppm), Re (ginsenoside Re, 304 ppm), Rd (ginsenoside Rd), Rc (ginsenoside Rc), Rb2 (ginsenoside Rb₂), and Rb1 (ginsenoside Rb₁); (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-Rg₂ into G-Rh₁ and Ppt, showing a higher *Rf* value than that of G-F₁ on a thin layer chromatogram. These results showed that Enz. H, L, and N had α -rhamnosidase activity against $-O\text{-}\beta\text{-D-glucose (2\leftarrow 1)-}\alpha\text{-L-rhamnose}$ linkage attached to the C-6 hydroxyl group of aglycone in G-Re and G-Rg₂. Enz. G had selective β -glucosidase activity toward the C-20- $O\text{-}\beta\text{-D-glucosidic}$ linkage in G-Re, but had no α -rhamnosidase activity, resulting in a remarkable accu-

mulation of G-Rg₂ from G-Re. Enz. L had a higher activity of β -glucosidase which regioselectively hydrolyzed the C-20- $O\text{-}\beta\text{-D-glucosidic}$ linkage in only G-Rg₁, together with high α -rhamnosidase activity against G-Re and G-Rg₂, to afford G-Rh₁ 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- $O\text{-}\beta\text{-D-glucosidic}$ linkage in G-Rg₁, to afford G-F₁ 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

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, Rg₁, and Rg₂ by the Preparation of Four Glycosidases

The reaction mixture containing 10 mg of ginsenoside Re (or Rf, Rg₁, Rg₂) 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 μ l) 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, Rg₁, Rg₂), and Rh₁, F₁: standard ginsenosides Re, Rf, Rg₁, Rg₂, Rh₁, and F₁. Std: standard.

Enz. N also had β -glucosidase activity toward the C-6-*O*- β -glucosidic linkage in G-Rh₁, 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-Rg₂ and G-Rh₁, and an intestinal bacterial metabolite, G-F₁, respectively, from a Ppt-type saponin mixture.

Kohda *et al.* reported on the enzymatic hydrolysis of G-Rb₁, G-Rb₂, G-Rc, and G-Rg₁ 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. ¹³C-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-Rb₁, G-Rb₂, and G-Rc. And also, crude hesperidinase (Tanabe Seiyaku Co. Osaka, Japan) hydrolyzed G-Rg₁ 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-Rg₁, 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, Rg₁: ginsenoside Rg₁, Rg₂: ginsenoside Rg₂, Rh₁: ginsenoside Rh₁, F₁: ginsenoside F₁, 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 $\leftarrow$ 1)- α -L-rhamnose in G-Re and G-Rg₂, *-O*- β -D-glucose(2 $\leftarrow$ 1)- β -D-glucose in G-Rf, C-6-*O*- β -D-glucose in G-Rg₁, and C-20-*O*- β -D-glucose in G-Re and G-Rg₁, 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-Rg₁, G-Rg₂, and G-Rh₁, 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-F₁ was isolated from the leaves of the same plant (*Panax ginseng* C. A. MEYER, Araliaceae) by the reported procedures.¹⁹ G-Rh₁ 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 ¹³C-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 F₂₅₄ 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 Millennium 32; column, Waters Carbohydrate (3.9 $\times$ 300 mm, 5 μ m); 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; ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz): Burkert spectrometer model AMX 400 in C₅D₅N 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 C₁₈ 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, ¹H- and ¹³C-NMR spectral data with those of authentic samples.

Compound I: A white powder; FAB-MS, *m/z* 801 (M+1)⁺; ¹H-NMR (400 MHz, C₅D₅N), 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 ¹³C-NMR spectrum corresponded to that in the literature^{14,20} for G-Rg₁.

Compound II: Colorless needles from ethanol; mp 187–189 °C; FAB-MS, *m/z* 785 (M+1)⁺; ¹H-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, CH₃ 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 ¹³C-NMR spectrum corresponded to that in the literature²¹ for G-Rg₂.

Compound III: A white powder; FAB-MS, *m/z* 639 (M+1)⁺; ¹H-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 ¹³C-NMR spectrum corresponded to that in the literature^{14,20} for G-Rh₁.

Compound IV: A white powder from ethanol; FAB-MS, *m/z* 639 (M+1)⁺; ¹H-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 ¹³C-NMR spectrum corresponded to that in the literature²⁰ for G-F₁.

Compound V: Colorless needles from methanol–water; mp. 219–222 °C; FAB-MS, *m/z* 477 (M+1)⁺; ¹H-NMR (CDCl₃), 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 ¹³C-NMR spectrum corresponded to that in the literature²⁰ for Ppt. These compounds I, II, III, IV and V were identified as 6,20-di-*O*- β -D-glucopyranosyl-20(S)-protopanaxatriol (G-Rg₁), 6-*O*-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl]-20(S)-protopanaxatriol (G-Rg₂), 6-*O*- β -D-glucopyranosyl-20(S)-protopanaxatriol (G-Rh₁), 20-*O*- β -D-glucopyranosyl-20(S)-protopanaxatriol (G-F₁), 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 Rh₁, from Ginsenosides Re and Rg₁,” re-

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- 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).