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Evaluation of Corticosterone Secretion-inducing Activities of Ginsenosides and Their Prosapogenins and Sapogenins

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Ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 (GRb_1, GRb_2 and so on) isolated from the roots of *Panax ginseng* C.A. MEYER, and their 20*S*- and 20*R*-prosapogenins (*S*-PS and *R*-PS), 20*S*- and 20*R*-protopanaxadiols (*S*-PPD and *R*-PPD), and a mixture of 20*S*- and 20*R*-panaxadiols (*SR*-PD) at various doses were administered to rats intraperitoneally. Plasma corticosterone and glucose levels at 30 min after the administration were determined, and ED_{50} values for corticosterone level were estimated. The ED_{50} values of GRd, GRb_2, GRc and GRb_1 were 7.1, 20, 44 and 112 μmol/kg, respectively. ED_{50} of *S*-PS, the genuine prosapogenin of GRb_1, GRb_2, GRc and GRd, which has no sugar residue attached to the C-20 position, was 28 μmol/kg, and the artifact prosapogenin *R*-PS also showed nearly the same activity, 26 μmol/kg. The ED_{50} values of genuine and artifact sapogenins *S*- and *R*-PPD were 42 and 32 μmol/kg, respectively, but an epimeric mixture of two artifact sapogenins *SR*-PD showed no activity at a dose of 80 μmol/kg. The ED_{50} values of GRe and GRg_1, glycosides of 20*S*-protopanaxatriol, were 107 and >160 μmol/kg, respectively. Most of these 11 compounds did not significantly affect the plasma glucose level at 30 min after the treatment.

Keywords——*Panax ginseng*; ginsenoside; ginseng prosapogenin; ginseng sapogenin; corticosterone secretion-inducing activity; ED_{50} in μmol/kg

Studies on the isolation of saponins from the roots of *Panax ginseng* C.A. MEYER, ginsenosides Ro, Rb_1, Rb_2, Rb_3, Rc, Rd, Re, 20-gluco-Rf, Rf, Rg_1 and Rg_2 (GRo, GRb_1, GRb_2 and so on), and on the determination of their structures have been carried out by Shibata *et al.* and Sanada *et al.*[1-4] Kaku and Kawashima[5] isolated 20*S*-, 20*R*- and Δ^{20}-prosapogenins from the hydrolysate of GRb_1, GRb_2 and GRc. Pharmacological and biochemical studies on ginseng extract and saponin-containing fractions have been done, and they have revealed that ginseng roots show a wide variety of activities. Previously we conclusively showed that the active principle stimulating serum protein biosynthesis in the extract was saponin.[6] Pharmacological properties of pure ginsenosides, genuine sapogenin and prosapogenins were reported by Kaku, Saito and Kita and their groups.[7-10] Biochemical actions of pure ginsenosides were also reported by us and by Yamamoto, Higashi and Okuda and their groups.[6,11-19] Most of these reports showed that changes in the sugar or sapogenin moiety of ginseng saponins caused changes in the intensities of the action, when one or two fixed weights of various ginsenosides were given.

In the previous report[20-22] we showed that ginseng saponin increased plasma adrenocorticotropin (ACTH), corticosterone, and adrenocortical cyclic adenosine monophosphate (AMP) without significant change in plasma glucose in intact rats, but it did not increase plasma corticosterone in dexamethasone-treated rats, or adrenal cyclic AMP in hypophysectomized rats. We also found that escin, occurring in the seeds of the horse chestnut tree,[23] saikosaponins and their genuine sapogenins from Bupreuri radix[24,25] stimulated the pituitary adrenocortical system, accompaied with a transient increase of plasma glucose. In the present study, in order to clarify the relationship between chemical structure and biological activity, the cortico-

sterone secretion-inducing activities of 2 pure ginseng sapogenins, an epimeric mixture of two artifact sapogenins, 2 pure prosapogenins and 6 pure saponins were determined. The effects of these compounds on plasma glucose level were also determined.

Materials and Methods

Ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 were isolated from the roots of *Panax ginseng* C.A. MEYER and purified[26] (Chart 1). Pure 20*S*- and 20*R*-prosapogenins (*S*-PS and *R*-PS) were isolated from the acidic hydrolysis products of GRb_1, GRb_2 and GRc[5] (Chart 1). 20*S*-Protopanaxadiol and 20*R*-protopanaxadiol (*S*-PPD and *R*-PPD), and a mixture of 20*S*- and 20*R*-panaxadiols (*SR*-PD) were prepared according to the procedures of Shibata *et al.*[27] (Chart 1). These saponins and sapogenins were dissolved in an appropriate volume of dimethylsulfoxide (DMSO), and then 19 volumes (9 volumes for *SR*-PD and *R*-PPD) of pyrogen-free saline were added to the solution to give a solution or suspension in 5% (10%) DMSO–saline. *SR*-PD, *S*-PPD and *R*-PPD in DMSO-saline yielded suspensions with floating fine particles, and *S*-PS and *R*-PS in DMSO-saline yielded suspensions with readily sedimentable fine particles. In DMSO-saline, GRd produced a slightly turbid solutions, and other saponins produced clear solutions. These solutions and suspensions were prepared just before use. GRe was soluble in 5% DMSO-saline, but about 1 h after the preparation GRe in the most concentrated solution partially precipitated as hydrates which were given to rats as a suspension. When 5 ml/kg of 5 or 10% DMSO-saline (control) was given to the experimental animals, it did not affect the basal levels of plasma corticosterone and glucose.

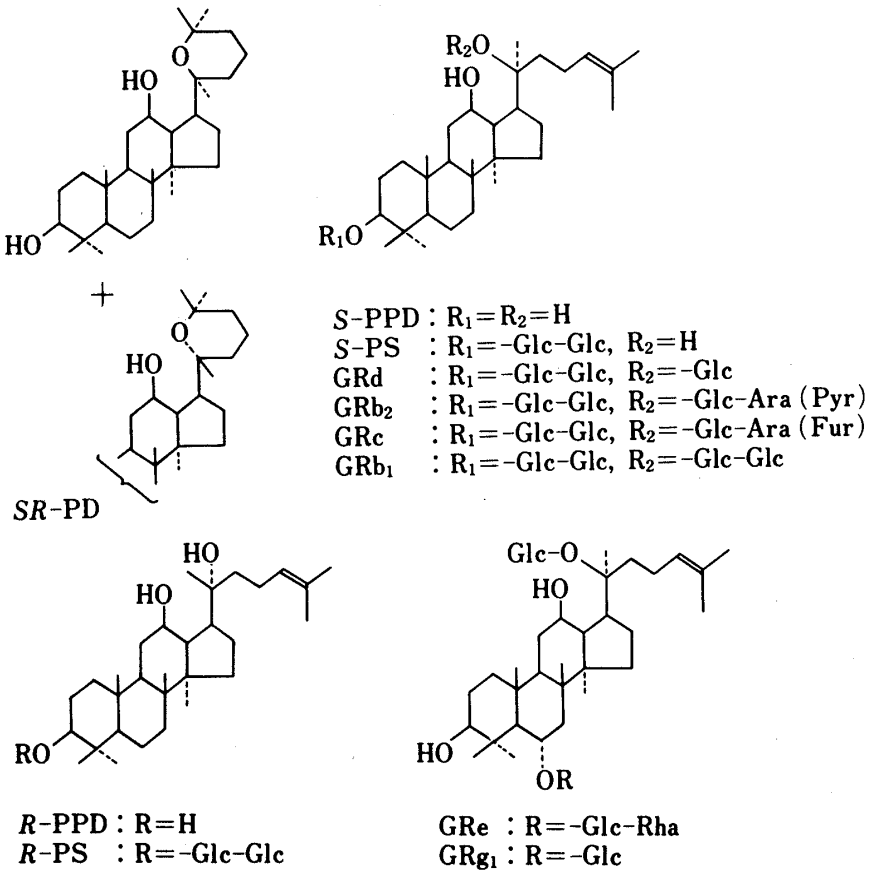

Chart 1. Structures of Ginseng Saponins, Prosapogenins and Sapogenins

Male Wistar rats of 130—150 g body weight were used. They were fed on laboratory chow (CE-2, CLEA Japan Inc., Tokyo) and tap water *ad libitum*, and maintained at 24°C, with 12 h of artificial light (light on: 0600—1800 h) for more than 6 d. To avoid stress-stimulated corticosterone release, they were "gentled" by daily handling for 4 consecutive days, twice a day in the morning and evening. Rats were decapitated with a guillotine at between 0900 and 1000 h, 30 min after the intraperitoneal injection of test drug or control solution. Trunk blood was collected in a chilled heparinized tube and centrifuged at 4°C, and the plasma was stored at −20°C.

Corticosterone was determined in duplicate by the competitive protein binding method of Murphy.[28] Human serum was used as the corticoid binding globulin. Standard corticosterone and ^{3}H-corticosterone (51 Ci/mM) were from Merck and the Radiochemical Centre, respectively. Plasma glucose was determined in duplicate by the glucose oxidase method. A mixture of ginseng saponins did not affect the determinations of plasma corticosterone and glucose. The ED_{50} values for corticosterone secretion-inducing activity were evaluated from the log dose-response curve. Mean basal level of corticosterone was 4.4 μg/100 ml for 31 rats which were given DMSO-saline in 5 separate experiments. The maximum level of corticosterone was 40.0 μg/100 ml for 20 rats in 4 groups which were given GRd (20 or 40 μmol/kg) or GRb_2 (80 μmol/kg) in 3 separate experiments. Thus, ED_{50} was evaluated as dose at 22.2 μg/100 ml plasma corticosterone level.

Results

Graded doses (20, 40 and 80 μmol/kg) of sapogenins were administered to rats intraperitoneally, and the plasma corticosterone level was determined 30 min after the treatment (Fig. 1). Genuine sapogenin *S*-PPD (Chart 1) increased plasma corticosterone to the maximum level at a dose of 80 μmol/kg, and its ED_{50} was 42 μmol/kg (19 mg/kg) as estimated from the log dose-response curve in Fig. 1 (Table I). *R*-PPD an artifact epimer of *S*-PPD also had a similar order of activity, 32 μmol/kg (15 mg/kg). While both the genuine and artifact PPD were active, an epimeric mixture of other artifact sapogenins *SR*-PD was inactive at a dose of 80 μmol/kg. *SR*-PD and *R*-PPD were almost insoluble in DMSO, and *S*-PPD was hardly soluble in DMSO, so they were given to rats as suspensions in 10 or 5% DMSO-saline. They were found to be still present aggregate forms in the peritoneal cavity of some of the rats 30 min after the treatment.

The dose-response experiment on *S*-PS and *R*-PS, genuine and artifact prosapogenins of GRb_1, GRb_2, GRc and GRd (Chart 1), was done at doses of 10, 20, 40 and 80 μmol/kg (Fig. 1). ED_{50} of *S*-PS was 28 μmol/kg, and artifact prosapogenin *R*-PS also had a similar level of activity, 26 μmol/kg (Table I). These prosapogenins were more soluble than sapogenins, and the 20*S*-epimer was more soluble than the 20*R*-epimer. They were administered as suspensions in 5% DMSO-saline. Some aggregates of *R*-PS were found in the peritoneal cavity of a few animals, but no aggregates of *S*-PS were found at doses of 10 to 80 μmol/kg (7.8 to 63 mg/kg).

TABLE I. ED_{50} Values of Ginseng Saponins and Their Sapogenins and Prosapogenins for Corticosterone Secretion-inducing Activity

Comp.	Sugar residue[a] at C-3	C-6	C-20	ED_{50} (μmol/kg)	Ratio of ED_{50}	ED_{50} (mg/kg)	Hemoly.[b] (μg/ml)	LD_{50}[c] (mg/kg)
SR-PD	—	—	—	>80[d]	>11	> 37	—	—
S-PPD	—	—	—	42	5.9	19	—	—
R-PPD	—	—	—	32	4.5	15	—	—
S-PS	-GG	—	—	28	4.0	22	—	—
R-PS	-GG	—	—	26	3.6	20	—	—
GRd	-GG	—	-G	7.1	1.0	7.0	200	324
GRb_2	-GG	—	-GAp	20	2.9	23	400	305
GRc	-GG	—	-GAf	44	6.2	49	1600	410
GRe	—	-GR	-G	107	15	107	600	405
GRb_1	-GG	—	-GG	112[e]	16	128	2700	1110
GRg_1	—	-G	-G	>160[f]	>23	>131	2800	1250

a) G, glucose; Ap, arabinopyranose; Af, arabinofranose; R, rhamnose.
b) Hemolytic concentration, calculated from Kaku *et al.*[7]
c) Kaku *et al.*[7]
d) Not effective at 80 μmol/kg.
e) ED_{50} was estimated by extrapolation.
f) Not effective at 160 μmol/kg.

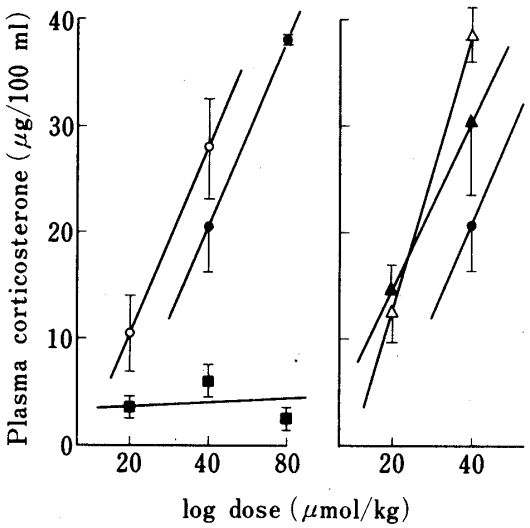

Fig. 1. Effect of Graded Doses of 20*S*- and 20*R*-Protopanaxadiols (*S*-PPD and *S*-PPD), a Mixture of 20*S*- and 20*R*-Panaxadiols (*SR*-PD), and 20*S*- and 20*R*-Prosapogenins (*S*-PS and *R*-PS) on Rat Plasma Corticosterone Level

Plasma samples were taken 30 min after intraperitoneal administration of 5.0 ml/kg of 5 or 10% DMSO-saline, or sapogenin or prosapogenin suspension. Each point represents the mean ±S.E. of 4 to 12 rats. ○, *R*-PPD; ●, *S*-PPD; ■, *SR*-PD; △, *R*-PS; ▲, *S*-PS.

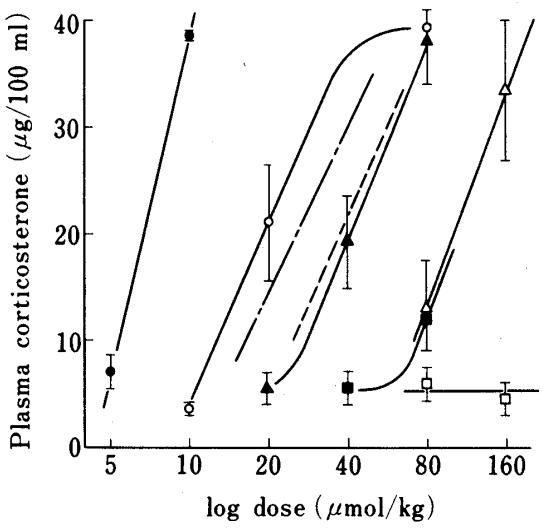

Fig. 2. Effect of Graded Doses of Ginsenosides Rd, Rb_2, Rc, Rb_1, Re and Rg_1 (GRd, GRb_2 and so on) on Rat Plasma Corticosterone Level

Plasma samples were taken 30 min after intraperitoneal administration of 5.0 ml/kg of 5% DMSO-saline or saponin suspension or solution. Each point represents the mean ±S.E. of 4 to 6 rats. ●, GRd; ○, GRb_2; ▲, GRc; △, GRe; ■, GRb_1; □, GRg_1. —·—·—, *S*-PS; ------, *S*-PPD.

The ED_{50} values of 6 purified ginsenosides, 4 glycosides of 20*S*-PPD (GRd, GRb_2, GRc and GRb_1) and 2 glycosides of 20*S*-protopanaxatriol (GRe and GRg_1) (Chart 1), on corticosterone secretion-inducing activity were also estimated in the same way as those of sapogenins and prosapogenins (Fig. 2 and Table I). ED_{50} of GRd was 7.1 μmol/kg (7.0 ml/kg), and GRd was the most effective compound among 11 samples on a molar basis. The ED_{50} values of GRb_2, GRc and GRe were 20, 44 and 107 μmol/kg, respectively. GRb_1 did not increase plasma corticosterone at a dose of 10, 20 or 40 μmol/kg, but at a dose of 80 μmol/kg it increased plasma corticosterone significantly ($p<0.05$: 11.9±3.0 μg/100 ml, $n=6$ *vs.* 3.7±1.0 μg/100 ml, $n=6$). ED_{50} of GRb_1 was estimated at 112 μmol/kg by extrapolation based on the assumption that the dose-response curve of GRb_1 was parallel to that of GRe. GRg_1 was inactive at doses of 40, 80 and 160 μmol/kg. The order of solubility of these 6 samples in 5% DMSO-saline was parallel to that of ED_{50}, that is GRd<GRb_2<GRc, GRb_1<GRe and GRg_1 on a molar basis, and none of these saponins, except GRe at a dose of 160 μmol/kg, yielded any aggregates in the peritoneal cavity of the rats at 30 min after the treatment.

The effect of these ginseng sapogenins and saponins on blood glucose level was determined with aliquots of all the plasma samples used for corticosterone determination. Plasma glucose levels of most groups of samples were nearly equal to the control level (145±3 mg/100 ml for 6 rats). The levels of a few groups of samples were higher than the control, but the difference were not significant statistically. When administered at the maximum dose, 40 μmol/kg for GRd, 160 μmol/kg for GRe and GRg_1, and 80 μmol/kg for the others, plasma glucose level was significantly higher than the control for *S*-PS ($p<0.01$, 167±5 mg/100 ml for 4 rats *vs.* 144±2 mg/100 ml for 5 rats) and for GRe ($p<0.02$, 200±17 mg/100 ml for 6 rats *vs.* 146±2 mg/100 ml for 5 rats). The reason for the higher glucose levels in these two cases is not clear at present.

Discussion

In the present study it was found that nine compounds among a congeneric series of 11 test compounds had activity to increase plasma corticosterone. Sapogenins *S*-PPD and *R*-PPD, and prosapogenins *S*-PS and *R*-PS had significant activity, and GRd was the most effective (ED_{50}: 7.1 μmol/kg), while an artifact sapogenin mixture (*SR*-PD) and GRg_1 did not increase plasma corticosterone at doses of 80 and 160 μmol/kg, respectively (Table I). When ginseng saponins and sapogenins increased plasma corticosterone, most of them did not affect plasma glucose at even the highest dose. In this respect, ginseng saponins and sapogenins are essentially different from escin[23] and saikosaponins,[24] which affect on plasma glucose.

On a molar basis, ED_{50} of genuine sapogenin *S*-PPD was 42 μmol/kg, and it was less effective than GRd, but more effective than GRb_1. However, *S*-PPD was administered as a suspension, so this ED_{50} value should be larger than the true ED_{50} value for completely solubilized *S*-PPD. This is also the case for *R*-PPD, *S*-PS, *R*-PS and *SR*-PD. When a fixed concentration (30 μM) of *S*-PPD or one of its derivatives was added to the organ culture medium of chicken embryonic dorsal root or sympathetic ganglia, GRb_1 and GRd showed a marked potentiating activity on nerve growth factor-mediated nerve fiber outgrowth, but *S*-PPD did not show such potentiating activity.[9] An artifact sapogenin *R*-PPD, which is an epimer of *S*-PPD, also surprisingly showed activity to increase plasma corticosterone, and it was less soluble but more effective than the genuine sapogenins. This suggests that *R*-PPD has rather higher potency than *S*-PPD. On the other hand, Saito and Lee[9] showed that hydroxylation of the side chain of GRd as in ginsenosides F6-a and F6-bc reduced the potentiating activity on nerve growth factor-mediated nerve fiber outgrowth. In the present study, an epimeric mixture of artifact sapogenins *SR*-PD formed by the ring closure of the open side chain of sapogenin did not show any activity at doses of 20, 40 and 80 μmol/kg, when it was given to rats as a suspension. Therefore these results suggest that a genuine sapogenin *S*-PPD has a corticosterone secretion-inducing activity, that the configuration of the OH group at C-20 of sapogenin does not seriously affect the activity, and that the open side chain of the sapogenin is essential for the activity.

A genuine prosapogenin *S*-PS, 3-*O*-β-D-glycopyranosyl(1→2)-β-D-glycopyranoside of *S*-PPD, was more soluble and showed higher corticosterone secretion-inducing activity than its aglycone. An artifact prosapogenin *R*-PS, the epimer of *S*-PS, also showed a significant activity, and it was more soluble and more effective than its aglycone. Thus, the sugar residue at C-3 apparently contributed to both the solubility and activity of *S*- and *R*-PS. However, increase in the solubility might increase absorption, and increase in the absorption might increase the activity without increase in potency. Moreover, in the present study the aglycone was found to be effective, but we have no information about absorption, distribution, metabolism and excretion of the aglycones and glycosides under *in vivo* conditions. The possibility cannot be excluded that the sugar moieties of *S*-PS and *R*-PS contributed indirectly to the activity due to an increase in the solubility. On the other hand, *R*-PS was less soluble but slightly more effective than its epimer. This suggests that *R*-PS and *R*-PPD tend to have higher potency than *S*-PS and *R*-PS, respectively. Some pharmacological activities of ginseng prosapogenins and saponins were studied by Kita *et al.*[10] and 20*S*-PS, GRb_1 and GRe were classified as being of biphasic type, while 20*R*- and Δ^{20}-prosapogenins were non-effective.

GRd or 20-*O*-β-D-glucopyranoside of *S*-PS was 4 times more effective than *S*-PS. The order of solubility of GRd, *S*-PS and *S*-PPD in 5% DMSO-saline was GRd>*S*-PS>*S*-PPD, and it was the same as the order of the activity. Therefore it seems likely that the sugar moiety at C-20 of GRd contributed to the solubility, like the sugar moiety at C-3 of *S*- and *R*-PS.

The ED_{50} values of GRb_2, GRc and GRb_1, which are monoglycosides of GRd at the end of the GRd molecule, were 20, 44 and 112 μmol/kg (or 23, 49 and 128 mg/kg), respectively. The order of their solubility was GRb_1 and GRc>GRb_2>GRd. Thus, the addition of

glycoside to GRd increased the solubility, but it markedly reduced the potency of GRd, in contrast to the sugar moieties of *S*-PS and GRd. GRe and GRg_1 could be assumed to be GRd derivatives with a free β-OH group at C-3 and glycoside moiety at the C-6α position. The ED_{50} values of GRe and GRg_1 were 107 and >160 μmol/kg, and they were more soluble than GRd and GRb_2. This suggests that either or both the free OH group and the additional sugar moiety markedly reduce the corticosterone secretion-inducing potency of GRd.

Kaku *et al.*, in a dose response experiment, determined the hemolytic concentration and LD_{50} of 7 pure ginseng saponins (Table I).[7] The order of hemolytic concentration was GRd, GRb_2, GRe, GRc, GRf (not tested in the present work), GRb_1 and GRg_1. No hemolyzed plasma was found in the present *in vivo* study, but the order was the same as that of the activity on corticosterone, except for GRe, and there was a close correlation between them (Table I). The order of LD_{50} in mice was GRb_2, GRd, GRe, GRc, GRb_1, GRg_1 and GRf. This order is also similar to that of the present study except for GRd and GRe, but there was little correlation between them. The ratio of LD_{50} to ED_{50} of GRd, GRb_2, GRc, GRe, GRb_1 and GRg_1 were 47, 13, 8.4, 3.8, 8.7 and <9.5, respectively.

Various biochemical actions *in vitro* or *in vivo* of pure ginseng saponins were studied by addition or injection of one or two fixed weights, but not molarities, of each ginsenoside. ACTH-induced lipolysis in fat cell suspension was inhibited by addition of several ginsenosides. GRb_2 was more effective, and GRh_1, GRg_1 and GRb_1 were less effective than the others (GRd was not tested).[19] Oura *et al.* reported that GRd among 6 ginsenosides was the most effective, and GRb_1 was not effective in serum protein biosynthesis in mice.[6] Yamamoto *et al.*[11] reported that several ginsenosides were effective on deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein and lipid synthesis and levels of cyclic AMP and guanosine monophosphate (GMP) in rat bone marrow, but GRb_1 was not. On the other hand, Higashi *et al.*[12,15,17] reported that GRb_1 among 3 or 5 main ginsenosides enhanced hepatic cholesterol biosynthesis, synthesis of the protein moiety of serum lipoproteins and hepatic RNA synthesis, and that GRg_1 was not effective or was less effective than the others in all three activities. Thus, the order of effectiveness of each ginsenoside in various kinds of biochemical activities *in vitro* or *in vivo* seems to be rather similar in most cases. These results and our present results suggest that *in vivo* active form(s) of ginsenosides are common.

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