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RESEARCH NOTE

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Metabolite Analysis of *Panax ginseng* C. A. Meyer by HPLC According to Root Age

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Abstract In order to investigate the major metabolite patterns of aged *Panax ginseng* C.A. Meyer roots, the ginsenoside contents for white ginseng roots of various ages were compared. The 1-year to 6-year old roots were extracted with methanol, and then the methanol-soluble metabolites were analyzed by high performance liquid chromatography (HPLC). The metabolite contents of the 1-year and 2-year roots, including the ginsenosides and minor components, were not different, but the Rg_1, Re, and Rc ginsenoside contents between the 2-year and 3-year roots showed significant differences. Rg_1 and Rc increased significantly in the 1-year to 2-year roots, and Re increased significantly from the 3-year root age. Rd increased slightly until the 2-year age and decreased from the 3-year age. Based on the ginsenoside distributions and contents at various root ages, we have suggested 2 biogenesis schemes using the ginsenosides that have been isolated from the roots of *P. ginseng* so far.

Keywords: root age, high performance liquid chromatography (HPLC), ginsenoside, *Panax ginseng* C.A. Meyer, biogenesis

Introduction

Ginseng, which is the root of *Panax ginseng* C.A. Meyer, is one of the most widely used herbal medicines in the Orient, including Korea, China, and Japan. Ginsenosides are the major components having pharmacological and biological activities, including immune, cardiovascular, central nervous system, endocrine, anti-diabetic, anti-tumor, and antioxidant activities (1-7). So far, 26 ginsenosides have been isolated and identified from *P. ginseng* root. The major ginsenosides are glycosides that contain an aglycon with a dammarane skeleton, and an oleanane skeleton. The 2 major groups of ginsenosides are the protopanaxadiol (PPD) and protopanaxatriol (PPT) groups. The PPD group includes the ginsenosides Ra_1, Ra_2, Ra_3, Rb_1, Rb_2, Rb_3, Rc, Rd, Rg_3, quinquenoside (Q) R_1, malonyl-ginsenoside (G)-Rb_1, malonyl-G-Rb_2, malonyl-G-Rc, malonyl-G-Rd, koryoginsenoside R_2, and majoroside F_4. The PPT group includes the ginsenosides Re, Rf, Rg_1, Rg_2, Rh_1, 20-gluco-G Rf, notoginsenoside (N) R_1, and koryoginsenoside R_1. In addition, the oleanane type ginsenosides include Ro, polyacetylene G Ro, and Ro methylester (Fig. 1).

For the analysis of these ginsenosides, researchers have examined various methods using high performance liquid chromatography (HPLC) (8, 9). Currently, these ginsenosides are being used as standard compounds to evaluate the quality of *P. ginseng* (white ginseng). In this study, we describe the metabolite changes for various root ages of *P. ginseng*, as well as biogenesis of the ginsenosides.

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Materials and Methods

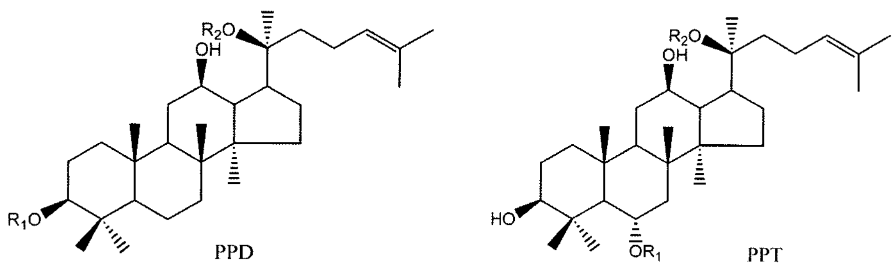
Materials The 1-year to 6-year old roots of *Panax ginseng* C.A. Meyer (*Cheonpung*) were cultured at Suwon, and obtained from KT&G Co., Ltd. (Suwon, Korea). The roots, which consisted of two 1-year old roots and ten roots per each-year old following, were lyophilized and powdered by a pulverizer.

Preparation of extracts The powdered samples of 250 mg/root were put into 50 mL Falcon tubes and extracted with 25 mL of 99.5% MeOH at room temp for 24 hr, 3 times. After shaking each tube, 5 mL from each tube were filtered by Acrodisc LC PVDF before injection in HPLC.

HPLC analysis HPLC analysis was used with an Agilent HPLC 1100 series and Zorbax-SB C_{18} column (4.6×250 mm, 5 μm, Agilent, Santa Clara, CA, USA). The analysis conditions were: injection volume of 20 μL, run time of 50 min, gradient (ACN, H_2O, 10 to 100, flow: 0.8 mL/min), column temp of 40°C, and UV detection at 205 nm.

Results and Discussion

The major metabolites of the aged roots of *P. ginseng* were compared using HPLC. The contents and distributions of the ginsenosides according to root age are shown in Table 1 and Fig. 2, respectively. The ginsenoside contents of the various root ages showed significant differences for the PPT and PPD ginsenoside types. For the PPT ginsenosides, the content of Rg_1 increased in the 1-year and 2-year roots, but decreased in the 3-year to 6-year old roots. On the contrary, the content of Re was low in the 1-year and 2-year old roots, but increased in the 3-year to 6-year old roots. These results suggest that Rg_1 was required for the formation of Re, which has a rhamnopyranose at the C-2′



Protopanaxadiol (PPD) type	R_1[1)]	R_2[1)]
Ginsenoside Ra$_1$	-glc(2→1)glc	-glc(6→1)arap(4→1)xyl
Ginsenoside Ra$_2$	-glc(2→1)glc	-glc(6→1)araf(2→1)xyl
Ginsenoside Ra$_3$	-glc(2→1)glc	-glc(6→1)glc(3→1)xyl
Ginsenoside Rb$_1$	-glc(2→1)glc	-glc(6→1)glc
Ginsenoside Rb$_2$	-glc(2→1)glc	-glc(6→1)arap
Ginsenoside Rb$_3$	-glc(2→1)glc	-glc(6→1)xyl
Ginsenoside Rc	-glc(2→1)glc	-glc(6→1)araf
Ginsenoside Rd	-glc(2→1)glc	-glc
20(*S*)-Ginsenoside Rg$_3$	-glc(2→1)glc	-H
Quinquenoside R$_1$	-glc(2→1)glc(6)Ac	-glc(6→1)glc
Koryoginsenoside R$_2$	-glc(2→1)glc	-glc(6→1)glc-22-ene
Malonyl-ginsenoside Rb$_1$	-glc(2→1)glc(6)Ma	-glc(6→1)glc
Malonyl-ginsenoside Rb$_2$	-glc(2→1)glc(6)Ma	-glc(6→1)arap
Malonyl-ginsenoside Rc	-glc(2→1)glc(6)Ma	-glc(6→1)araf
Malonyl-ginsenoside Rd	-glc(2→1)glc(6)Ma	-glc
Majoroside F$_4$	-glc	-glc
Protopanaxatriol (PPT) type		
Ginsenoside Re	-glc(2→1)rha	-glc
Ginsenoside Rf	-glc(2→1)glc	-H
20-Gluco-ginsenoside Rf	-glc(2→1)glc	-glc
Ginsenoside Rg$_1$	-glc	-glc
Ginsenoside Rg$_2$	-glc(2→1)rha	-H
Ginsenoside Rh$_1$	-glc	-H
Notoginsenoside R$_1$	-glc(2→1)xyl	-glc
Koryoginsenoside R$_1$	-glc(6-Ac)	-glc

[1)]glc, β-D-glucopyranosyl; arap, α-L-arabinopyranosyl; xyl, β-D-xylopyranosyl; araf, α-L-arabinofuranosyl; Ac, acetyl; Ma, malonyl; rha, α-L-rhamnopyranosyl.

Fig. 1. Currently known ginsenosides isolated from the roots of *P. ginseng*.

of glucose, rather than the formation of the other PPT and PPD ginsenosides in the 3-year old roots. The content of Rg$_1$ was highest in the 2-year old roots and Re and Rf were highest in the 3-year old roots. For the PPD ginsenosides, contents increased up until the 2-year old root age, but then slightly decreased at 3-years. The content of Rc increased in the 1 year- to 2-year old roots, but then decreased in the 3-year to 6-year roots. The content of Rb$_1$ was highest in the 5-year old roots, and Rg$_2$, Rc, Rb$_2$, and Rd were highest in the 2-year old roots.

Table 1. Contents of major ginsenosides according to the root age of *P. ginseng* (ng/μL)

	1-year	2-year	3-year	4-year	5-year	6-year
Rg_1	70.792	102.900	60.078	48.726	63.026	46.056
Re	6.071	24.178	79.027	78.596	68.099	78.605
Rf	4.201	12.101	23.449	20.534	20.718	18.356
Rb_1	29.661	69.570	48.629	53.560	71.843	59.120
Rg_2	6.036	9.122	3.386	2.900	3.844	2.415
Rc	35.871	80.269	48.410	37.958	50.689	38.927
Rb_2	14.943	31.263	24.609	21.274	21.725	21.361
Rd	14.121	25.154	7.099	4.654	4.589	4.232

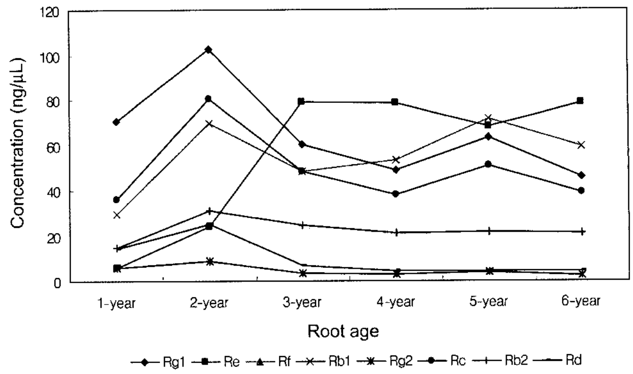


Fig. 2. Contents of major ginsenosides according to the root age of *P. ginseng*.

From these results, we suggest that Rg_1 and Re of the PPT ginsenosides, and Rc of the PPD type, could be used as indicator chemicals to distinguish between the 2-year and 3-year old roots.

The Rg_1 and Rb_1 ginsenosides have inhibitory effects on the central nervous system (CNS) (10, 11); Rg_1 and Rc have vasorelaxation effects, and Re enhances the immune system (12). Based on the increase of these ginsenosides in the 1-year and 2-year old roots and decrease in the 3-year to 6-year old roots, the ginsenoside distributions of *P. ginseng* by root age could be used as a quality control

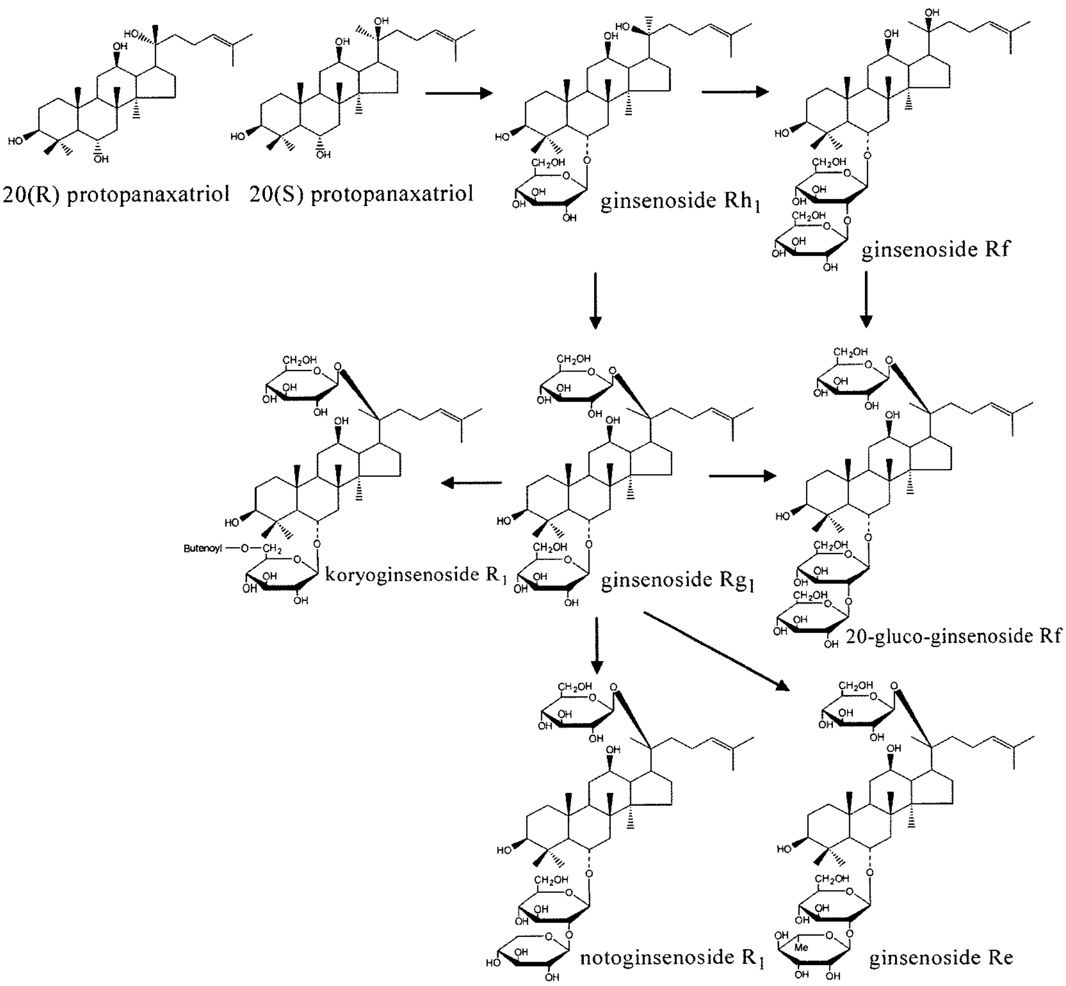


Fig. 3. Biogenetic relationships of the PPT-type ginsenosides in *P. ginseng*.

factor.

According to the changes in ginsenoside content by root age, and the types of ginsenosides that have been isolated so far from *P. ginseng* roots, we proposed 2 biogenesis schemes for the PPD and PPT ginsenoside types.

The biogenesis of the PPT ginsenosides is proposed in relation to the PPT ginsenosides that have been isolated so far from *P. ginseng* (white ginseng) roots. First, 20(*R*, *S*) protopanaxatriol is a precursor of all the PPT ginsenosides found in *P. ginseng*. 20(*R*, *S*) protopanaxatriol is β-D-glucopyranosylated at C-6 to yield ginsenoside Rh_1. The Rh_1 ginsenoside is β-D-glucopyranosylated at C-2′ → C-1″ to yield ginsenoside Rf, as well as at C-20 to yield ginsenoside Rg_1, a precursor of 4 other ginsenosides, respectively. First, ginsenoside Rg_1 is butenoylated at the C-6′ of glucose to yield koryoginsenoside R_1. Second, ginsenoside Rg_1 is xylopyranosylated at C-2′ → C-1″ to yield notoginsenoside R_1. Third, ginsenoside Rg_1 is rhamnopyranosylated at C-2′ → C-1″ to yield ginsenoside Re. Fourth, ginsenoside Rg_1 is β-D-pyranosylated at C-2′ → C-1″ to yield 20-gluco-ginsenoside Rf. Also, 20-gluco-ginsenoside Rf is yielded from β-D-pyranosylation at the C-20 of ginsenoside Rf. The biogenetic relationships of the PPT ginsenosides found in the roots of *P. ginseng* are summarized in Fig. 3.

The biogenesis of the PPD ginsenosides is proposed in relation to the PPD ginsenosides that have been isolated thus far from the roots of *P. ginseng*. 20(*R*, *S*) protopanaxadiol is a precursor of all the PPD ginsenosides found in *P. ginseng*. 20(*R*, *S*) protopanaxadiol is β-D-glucopyranosylated at C-2′ → C-1″ of C-3 to yield ginsenoside Rg_3, and at C-3 and C-20 to yield majoroside F_4. Ginsenoside Rd, which is a precursor of 5 ginsenosides, is yielded from β-D-glucopyranosylation at C-2′ → C-1″ of majoroside F_4 as well as at the C-20 of ginsenoside Rg_3. First, ginsenoside Rd is malonylated at C-6″ to yield malonyl ginsenoside Rd. Second, ginsenoside Rd is xylopyranosylated at C-6″ ′ → C-1″″ and at C-20 to yield ginsenoside Rb_3. Ginsenoside Rb_3 is malonylated at C-6″ to yield malonyl ginsenoside Rb_3, successively. Third, ginsenoside Rd is glucopyranosylated at C-6‴ → C-1″″ to yield ginsenoside Rb_1. Ginsenoside Rb_1 is malonylated at C-6″ to yield malonyl ginsenoside Rb_1, glucopyranosylated at C-6″ ′ → C-1″″ to yield koryoginsenoside R_2, acetylated at C-6″ to yield quinquenoside R_1, and xylopyranosylated at C-3″″ → C-1″″ ′ to yield ginsenoside Ra_3. Fourth, ginsenoside

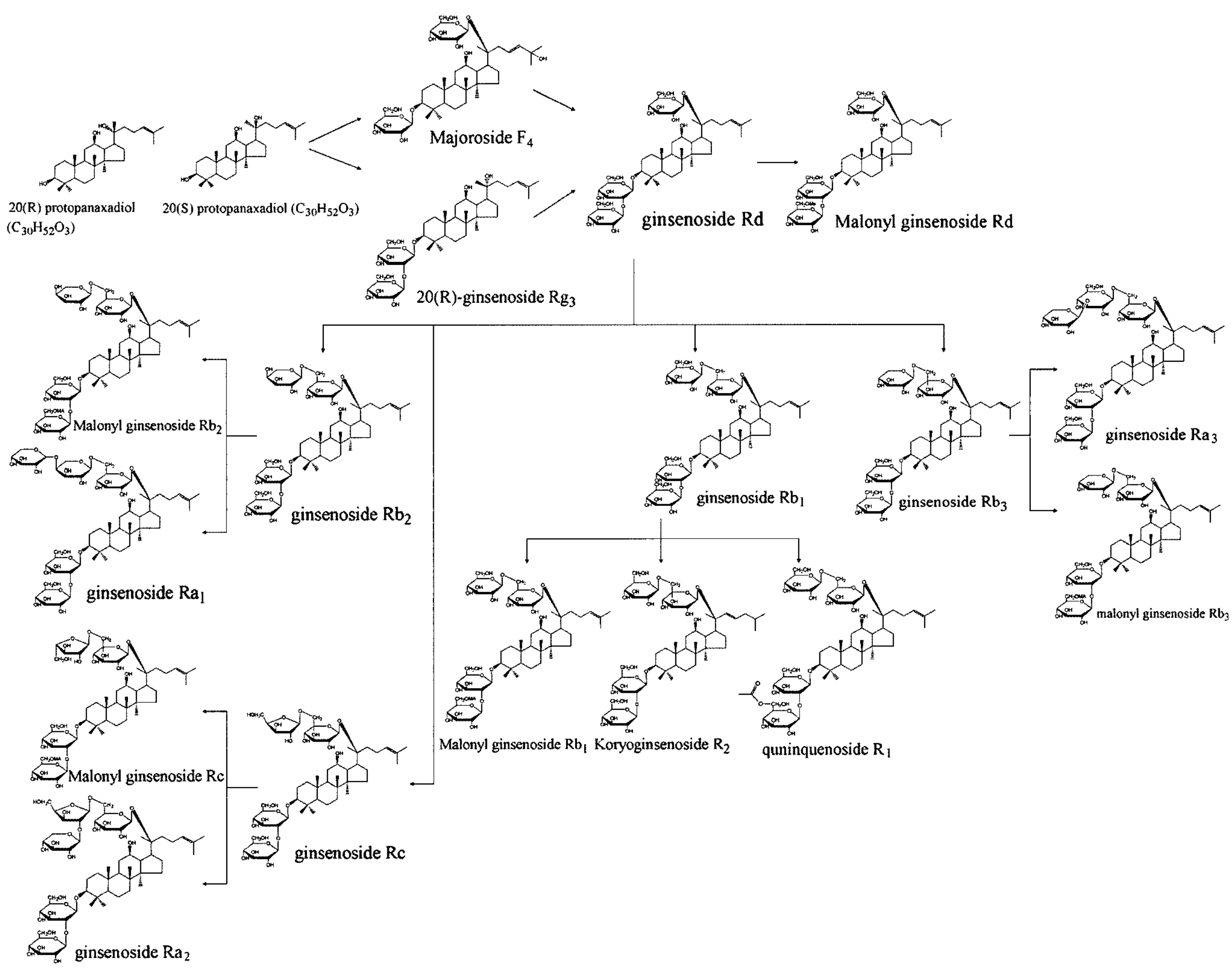


Fig. 4. Biogenetic relationships of the PPD-type ginsenosides in *P. ginseng*.

Rd is arabinofuranosylated at C-20 to yield ginsenoside Rc. Ginsenoside Rc is malonylated at C-6″ to yield malonyl ginsenoside Rc. Also, ginsenoside Rc is xylopyranosylated and arabinofuranosylated at C-6″ ′ to yield ginsenoside Ra_2, respectively. Fifth, ginsenoside Rd is arabinopyranosylated at C-6″ ′ to yield ginsenoside Rb_2. Ginsenoside Rb_2 is malonylated at C-6″ to yield malonyl ginsenoside Rb_2. Also, Rb_2 is xylopyranosylated and arabinopyranosylated at C-6″ ′ to yield ginsenoside Ra_1, respectively. The biogenetic relationships of the PPD ginsenosides found in the roots of *P. ginseng* are summarized in Fig. 4.

References

1. Nah SY, Park HJ, Mccleskey EW. A trace component of ginseng that inhibits Ca^{2+} channels through a pertussis toxin-sensitive G protein. P. Natl. Acad. Sci. USA 92: 8739-8743 (1995)
2. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: Multiple constituents and multiple actions. Biochem. Pharmacol. 11: 1685-1693 (1999)
3. Sato K, Mochizuki M, Saiki I, Yoo YC, Samukawa K, Azuma I. Inhibition of tumor angiogenesis and metastasis by a saponin of *Panax ginseng*, ginsenoside-Rb_2. Biol. Pharm. Bull. 17: 635-639 (1994)
4. Mochizuki M, Yoo CY, Matsuzawa K, Sato K, Saiki I, Tonooka S, Samukawa K, Azuma I. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb_2, 20 (*R*), and 20 (*S*)-ginsenoside-Rg_3, of Red ginseng. Biol. Pharm. Bull. 18: 1197-1202 (1995)
5. Kim SH, Kim JY, Park SW, Lee KW, Kim KH, Lee HJ. Isolation and purification of anticancer peptides from Korean ginseng. Food Sci. Biotechnol. 12: 79-82 (2003)
6. Seog HM, Jung CH, Kim YS, Park HS. Phenolic acids and antioxidant activities of wild ginseng (*Panax ginseng* C.A. Meyer) leaves. Food Sci. Biotechnol. 14: 371-374 (2005)
7. Kim IH, Choe EO. Effects of red ginseng extract added to dough on the lipid oxidation of frying oil and fried dough during frying and storage. Food Sci. Biotechnol. 12: 67-71 (2003)
8. Kwon SW, Han SB, Park IH, Kim JM, Park MK, Park JH. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. J. Chromatogr. A. 921: 335-399 (2001)
9. Samukawa K, Yamashita H, Matsuda H, Kubo M. Simultaneous analysis of saponins in ginseng radix by high performance liquid chromatography. Chem. Pharm. Bull. 43: 137-141 (1995)
10. Tsang D, Yeung HW, Tso WW, Pech H. ginseng saponins: Influence on neurotransmitter uptake in rat brain synaptosomes. Planta Med. 3: 221-224 (1985)
11. Benishin CG. Actions of ginsenoside Rb_1 on choline uptake in central cholinergin nerve endings. Neurochem. Int. 21: 1-5 (1992)
12. Nah SY. Ginseng: Recent advances and trends. Korean J. Ginseng Sci. 21: 1-12 (1997)