CHEMICAL REVIEWS

Review

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Chemical Insights into Ginseng as a Resource for Natural Antioxidants

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CONTENTS

1. INTRODUCTION

Ginseng is the root of *Panax ginseng C. A. Meyer* mainly produced in China, Korea, and America. The first book recording ginseng is *Shennongbencaojing* about 2000 years ago.[1] The storage conditions and the amount of dietary intake of ginseng are recored in the US pharmacopoeia.[2] Recent studies reveal that ginseng has some novel pharmacological effects on hypodynamia, anorexia, shortness of breath, palpitations, insomnia, impotence, hemorrhage, and diabetes.[3] The total extract from ginseng modulates calcium channels in pain and opioid-induced antinociception[4] and reduces brain polyamine levels in experimental animals.[5] Ginseng predominantly enhances cognitive performance and memory by modulating cerebroelectrical activity.[6] The active components in ginseng consist of ginsenosides, polyphenols, amino acids, and polysaccharides. The chemistry, biosynthesis, analysis, and tonic effects of ginsenosides were reviewed recently, and anticarcinogenic, immunomodulatory, anti-inflammatory, anti-allergic, antiatherosclerotic, antihypertensive, antidiabetic, anti-stress, and anticancer activities of ginseng are due to the action of ginsenosides.[7]

Environmental pollution deteriorates the living surroundings and changes the redox status of healthy bodies,[8] leading to aging and vital diseases consequently.[9] Maintaining health by dietary intake is more important than treating diseases by drugs. The supplementation of natural antioxidants is regarded as a prophylactic strategy toward diseases caused by oxidative stress.[10,11] Much work focuses on the phytochemistry of natural antioxidants such as polyphenolics,[12] phenylpropanoids,[13] and other active components,[14,15] in which phenolics attract much research attention because the phenolic hydroxyl group is able to suppress reactive oxygen species (ROS) and reactive nitrogen species (RNS). On the other hand, as the major active component in ginseng, ginsenoside is a dammarane- or oleanane-type tetracyclic triterpenoid sapogenin linked by sugar moieties. The antioxidant action of ginseng is an attractive research field that provides much information for dietary supplementation and pharmacological usage of ginseng products.

The pharmacological action of ginseng is related to the abilities of ginsenosides to regulate enzyme expression;[16] thus, many more individual ginsenosides are needed for comparing pharmacological activity and for exploring the structure–activity relationships. Chemical techniques play an important role in isolating and synthesizing various ginsenosides. As shown in Figure 1, the aim of this review is to summarize chemical aspects of ginsenosides and to introduce some *in vitro* results on the antioxidant actions of ginsenosides. The first aspect is that appropriate cultivation can enrich the amount of ginsenosides during the growth of ginseng. Hence, the influence of the cultivation conditions and additives on the accumulation of ginsenosides should be taken into consideration. The second aspect is how to extract ginsenosides from ginseng. Innovations in separation techniques are beneficial for obtaining much more of a single ginsenoside and can be employed in the isolation of ginsenoside-like compounds from other plants. The third aspect is to identify the structure of ginsenoside by mass spectra (MS) and nuclear magnetic resonance (NMR) spectra. MS can be used to confirm the kind of sugar moiety, while NMR gives information on the linkage position of the sugar moiety. The fourth aspect is to synthesize

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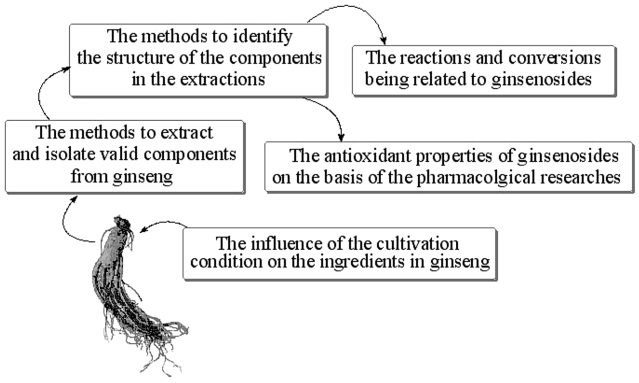

Figure 1. The organization of this review.

ginsenosides via enzyme-promoted reactions or organic reactions. Especially, the sugar moiety in a ginsenoside can change its position in the presence of a special enzyme; thus, one kind of ginsenoside converts into another one via enzyme-catalyzed reaction. Finally, although a large number of research proves that ginsenoside exhibits many pharmacological activities, the mechanism is still not very clear because only a few single ginsenosides are used in the same experimental system, and it is difficult to clarify the influence of the sugar moiety at different positions on pharmacological actions. Therefore, in the research on pharmacological mechanisms of ginsenosides, it seems not necessary to develop much more experimental systems, but enough types of single ginsenosides should be screened in the same experimental system. As a result, much more information on the structure−activity relationship can be obtained. In this review, some *in vitro* experimental systems are introduced because these experimental systems can be set up conveniently in an organic laboratory. Thus, the activity of the obtained antioxidants can be evaluated by using biological species. On the other hand, the activities of some single ginsenosides have been evaluated in these experimental systems. If many more single ginsenosides are used in these experimental systems, some general principles of ginsenoside activity against oxidative damage may be clarified.

2. INFLUENCES OF THE CULTIVATION CONDITIONS ON THE VALID COMPONENTS IN GINSENG

2.1. Structures of Representative Ginsenosides

Ginsenosides are various saponins with (20*S*)-protopanaxadiol (PD) or (20*S*)-protopanaxatriol (PT) being sapogenins. Table 1 collects some typical structures of PD- and PT-type ginsenosides. The sugar moieties attach to the 6- and 20-positions in PT-type ginsenosides and the 3- and 20-positions in PD-type ginsenosides. The differences of the position and kind of sugar moieties lead to various bioactivities of ginsenosides.[17]

2.2. Comparison of Ginsenoside Contents under Different Growth Conditions

The wild ginsengs are generally harvested after 8 or more years. The pharmacological activity of wild ginseng is higher than that of the cultivated ones because a large amount of ginsenosides is accumulated during the long-term growth period. However, short growth periods and growing location significantly affect the ginsenoside contents. For example, the contents of ginsenoside in American ginseng (*Panax quinquefolium*) are Rb1 > Rg1 > Re when wild and two-year-old ginsengs are transplanted from population surrounding to forest garden. The population surrounding affects the content of Re markedly, followed by Rb1, Rc, and Rb2. Both population surrounding and growing location attenuate the contents of Rg1 and Rd.[18] Furthermore, the light levels in the understory of a broadleaf forest also affect the contents of Rg1, Re, Rb1, Rc, Rb2, and Rd in one- and two-year-old American ginseng. The red and infrared lights increase Rd, Rc, and Rg1 40% in two-year-old ginseng.[19] The geographic location affects the contents of ginsenosides as well. The investigation of *Panax notoginseng* cultivated in Yunnan Province, China, indicates that the contents of R1, Rg1, Rb1, and Rd in *Panax notoginseng* produced in the southwest of the Province and harvested in autumn are higher than those from plants produced in other areas and harvested in other seasons.[20] The genetic sequences of *Panax notoginseng* and ratios of Rd/Rg1, Re/Rg1, and Rb1/Rg1 are quite different even though *Panax notoginseng* are harvested from the same farm in different years. The cultivation conditions markedly influence the quality of ginseng.[21]

2.3. Influence of CO_2 and Jasmonic Acid on Ginsenoside Contents

Some chemicals, such as CO_2 and jasmonic acid (structure shown in Figure 2), can increase the contents of ginsenosides and other antioxidants. The addition of jasmonic acid markedly increases ginsenoside content in a flask-type bioreactor and does not influence the weight and growth rate of ginsengs.[22] The treatment of *Panax notoginseng* with 2-hydroxyethyl jasmonate in a bioreactor increases the activities of protopanaxdiol 6-hydroxylase and Rd glucosyltransferase and changes the ratios of Rb/Rg and Rb1/Rd. Hence, jasmonic acid is able to increase ginsenoside contents.[23]

The treatment of *Panax ginseng* with different concentrations of CO_2 in a bioreactor increases the contents of phenolics, flavonoids, and proteins and the activity of the enzyme for the biosynthesis of phenolics. This is because CO_2 activates the pentose phosphate pathway and the shikimate/phenylpropanoid pathway to enrich phenolics and ginsenosides in *Panax ginseng*.[24] Moreover, CO_2 can induce the generation of ascorbate peroxidase, monodehydroascorbate reductase, glutathione reductase, catalase (CAT), guaiacol peroxidase, and superoxide dismutase (SOD) and can enhance the activities of glutathione-*S*-transferase and glutathione peroxidase in ginseng.[25] Therefore, the cultivation conditions and the application of chemicals directly affect the contents and kinds of the active components in ginseng.

3. ISOLATION OF THE ANTIOXIDANT COMPONENTS FROM GINSENG

The activities of total extracts from ginseng roots, flowers, stems, and leaves are compared in order to find which part contains a large amount of active components. For example, the activities of antioxidants and quinone reductase of American, Asian, and Siberian ginseng are compared, and American and Asian ginsengs are found to reduce HOCl efficiently, while Siberian ginseng can quench $ONOO^-$ rapidly.[26] However, it is difficult to confirm which component mostly contributes to the bioactivity in the case of total extracts employed. Therefore, it is necessary to apply suitable solvents and novel techniques for isolating various individual ingredients from ginseng. Although the root of *Panax ginseng C. A. Meyer* is defined as ginseng, the method of isolating ginsenosides from ginseng is also available

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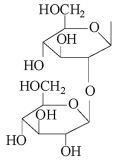
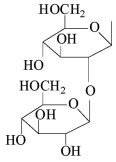
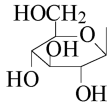
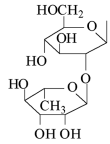
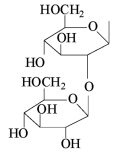
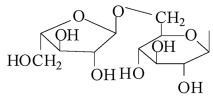
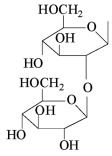
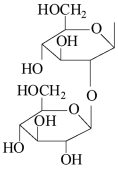
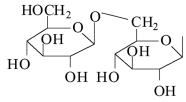
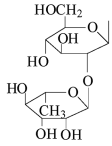
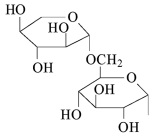
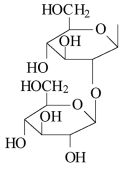
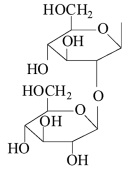
Table 1. Structures of Some Typical Ginsenosides

PD group	G_1	G_3	PT group	G_2	G_3
20(S)-protopanaxadiol (PD, G_1=G_3=H)			20(S)-protopanaxatriol (PT, G_2=G_3=H)		
Rh2		H	**Rh1**		H
Rg3		H	**Rg1**		
Rd			**Rg2**		H
Rc			**R1**		
Rb1			**Re**		
Rb3			**Rf**		H

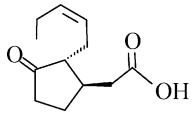


Figure 2. The structure of jasmonic acid.

for separating ginsenoside-related saponins from *Panax notoginseng*, *Panax quinquefolius*, and *Panax japonicus*. Hence, the following methods correlate with the extraction of dammarane- and oleanane-type tetracyclic triterpenoid saponins from ginseng-related plants.

3.1. Solvents

Ginsenosides are fixed in ginseng cells that can be destroyed in refluxing methanol within a few hours or in cool methanol for a long period. After methanol is evaporated under vacuum, the crude extract is dissolved in water, and the water phase is washed by nonpolar organic solvents to remove fatty acid. Then, water-saturated *n*-butanol and the mixture of chloroform

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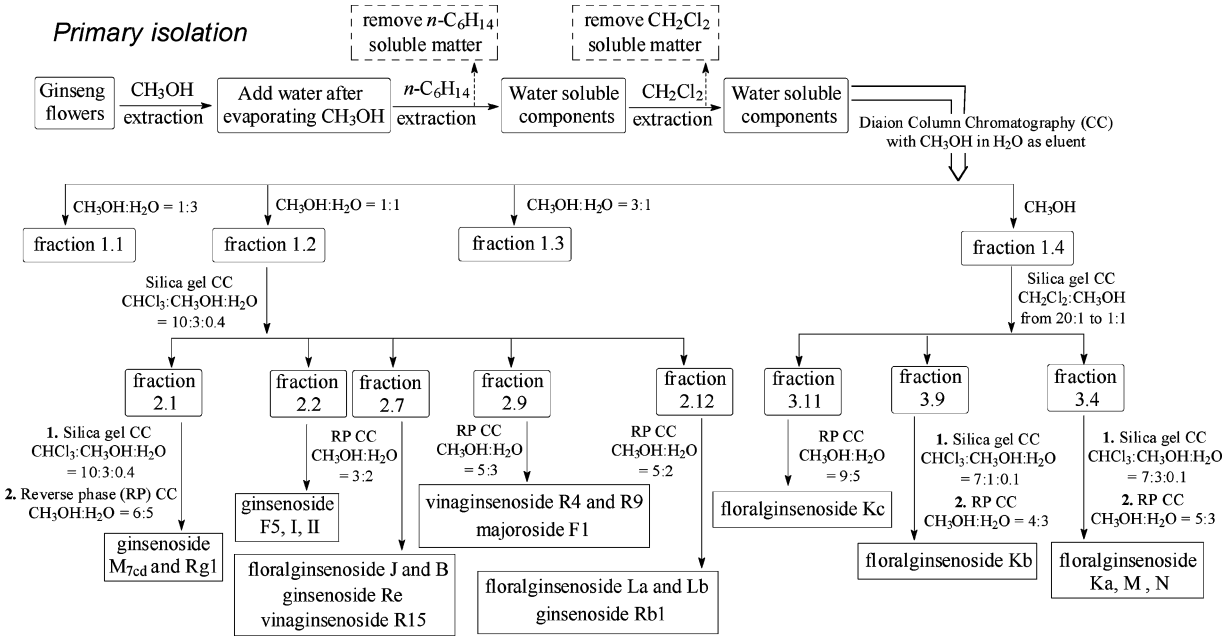

Figure 3. A typical process to isolate ginsenosides from flowers of *Panax notoginseng*.

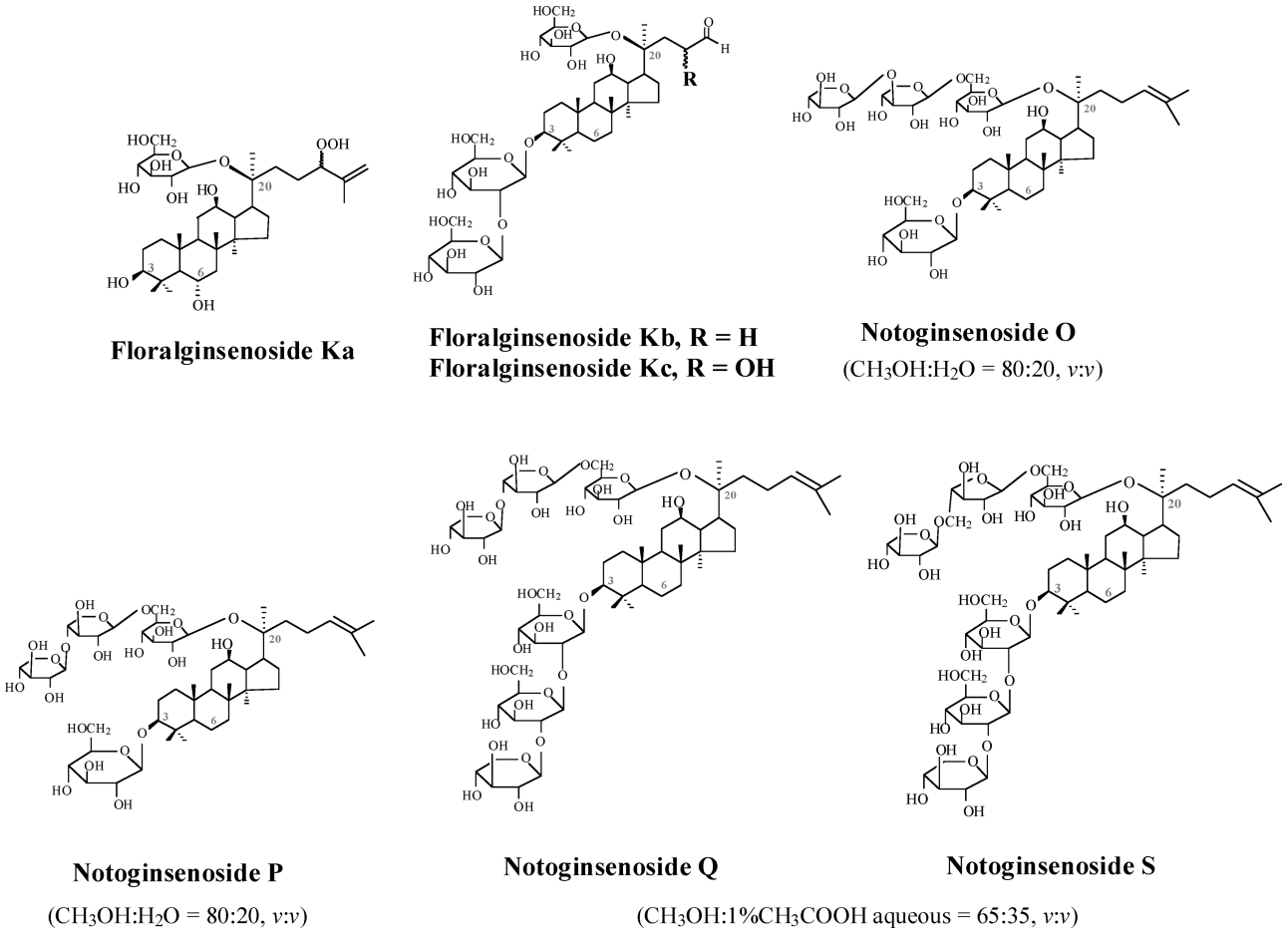

Figure 4. Structures of saponins with polysaccharide moieties.

and methanol are used to isolate individual ginsenosides. Figure 3 outlines a typical process to separate saponins from flower buds of *Panax notoginseng*. A reverse phase (RP) chromatography column (CC) and a mixture of methanol and water are applied to isolate saponins with polysaccharide moieties as shown in Figure 4.[27,28]

Water, methanol, and ethanol are applied to extract freeze-dried leaves of wild ginseng. The obtained mixture can trap 2,2-

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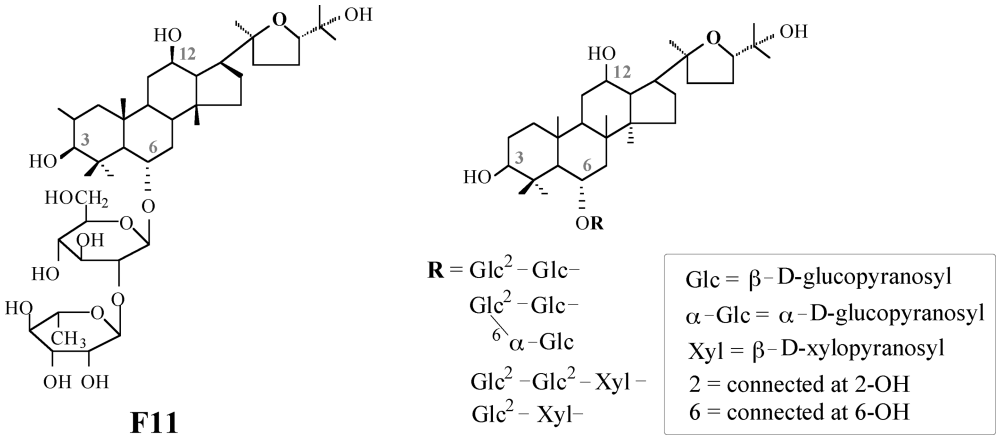

Figure 5. Structure of pseudoginsenoside and some representative compounds isolated from *Panax japonicus*.

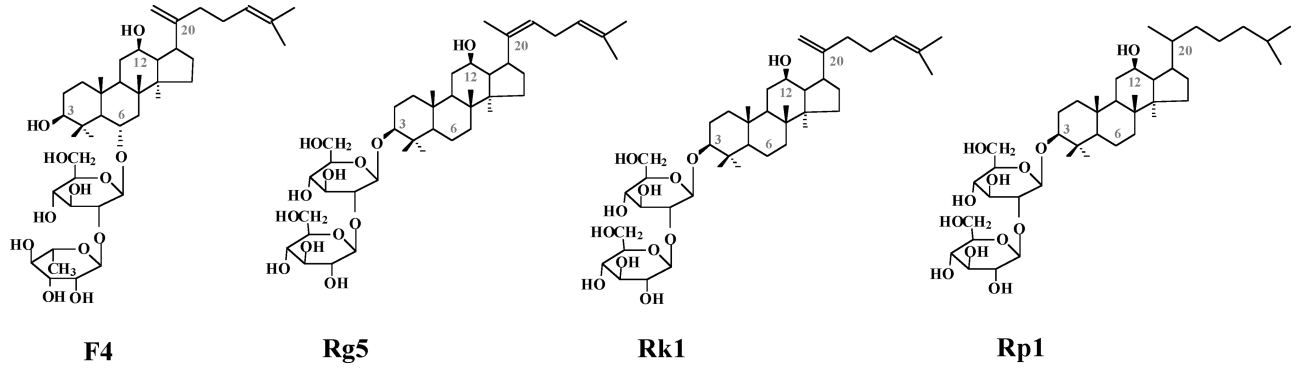

Figure 6. Structures of F4, Rg5, Rk1, and Rp1.

diphenyl-1-picrylhydrazyl radical (DPPH) and hydroxyl radical ($^{\bullet}$OH) and can chelate ferrous ion. The ethanol extract shows the highest radical-scavenging and Fe^{2+}-chelating activities, while aqueous extract has the highest activity to trap superoxide radical. Flavonoid, quercetin, and kaempferol are generated when the extracts are deglycosylated under acidic conditions.[29] Therefore, a solvent with high polarity is appropriate for extracting phenolics from ginseng. Although 65% glycerin aqueous solution can also extract saponins from *Panax quinquefolius*, the amount of the obtained saponins is obviously lower than when 50% ethanol aqueous solution is the solvent because ethanol can release ginsenosides effectively by destroying ginseng cells, while glycerin cannot destroy ginseng cells very well.[30] Water-saturated *n*-butanol is able to isolate pseudoginsenosides as shown in Figure 5.[31] Compound **F11**, a representative pseudoginsenoside isolated from American ginseng, includes a tetrahydrofuran ring at the 20-position of the sapogenin instead of a carbon chain.

Steam distillation is another useful way to destroy ginseng cells. The contents of ginsenosides are measured when American ginseng is steamed at 100−120 °C for 1 h or at 120 °C for 0.5−4 h. Consequently, the contents of Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd decrease, while the contents of Rh1, Rg2, Rg3, and Rh2 increase after steam distillation. In particular, the amount of Rg3 reaches a maximum value when ginseng is steamed at 120 °C for 2 h.[32] A large amount of Rg3 and Rh2 is detected in the extract from boiling water rather than from 80% ethanol aqueous solution at room temperature. So, Rg3 and Rh2 are regarded as the heating products in the process of the extraction.[33] Heating may also lead to the dehydrolyzation of hydroxyl group at 20-position, producing F4, Rg5, Rk1, and Rp1 (structures shown in Figure 6).

The abundant contents of Rg3, Rg5, and F4 enhance abilities of the corresponding ginseng extract to relax endothelium and to trap 2,2-diphenyl-1-picrylhydrazyl radical (DPPH),[34] while the content of arginine, the major amino acid in ginseng, decreases from 10.4 to 1.38 mg/g after steam distillation. On the other hand, steam distillation removes 92.9% of *β*-*N*-oxalyl-L-*α*,*β*-diaminopropionic acid that can cause crippling neurolathyrism.[35] Glutamine and arginine, two major free proteinogenic amino acids in ginseng, can react with carbonyl groups in sugar moieties to form Maillard reaction products (MRPs) with antioxidant activities.[36] The investigation on bioactivities of ginsenosides is important for the clinic usage of ginseng-related herbs[37] and therefore requires much more advanced techniques to be used in the separation of natural-occurring saponins from ginseng.

3.2. Microwave Irradiation

Using microwave irradiation shortens the heating period in the extraction of ginsenosides. In a microwave oven, ginseng powders mixed with 10-fold volume of 80% methanol aqueous solution are irradiated by 300 W microwave four times, each time being less than 30 s. The same amount of ginseng powder extracted in boiling methanol for 12 h provides a similar amount of the obtained ginsenosides to that from microwave irradiation. So, the microwave irradiation cannot cause the decomposition of ginsenosides.[38] With a condenser equipped to avoid the evaporation of the solvent, the extraction period may increase to 5 min each time, and the power of microwave irradiation increases to 700 W. Figure 7 illustrates the structures

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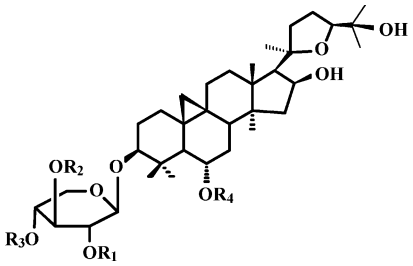

R_1	R_2	R_3	R_4
Ac	Ac	H	Glc
Ac	H	H	Glc
Glc	H	H	H
H	H	H	Glc

Ac = CH_3CO-

Figure 7. Structures of acetyl-astragalosides extracted from *Radix astragali* under microwave irradiation.

of astragalosides extracted from *Radix astragali* under microwave irradiation.[39] The microwave irradiation is still a thermal extraction method and is thereby not suitable for the extraction of heat-sensitive ginsenosides.

3.3. Sonication

Using ultrasonic vibration to extract ginsenosides avoids high temperature, and the efficiency is three times higher than the refluxing method.[40] The solvents and sonication conditions are screened in the extraction of Rb1, Rb2, Rc, Rd, Re, and Rg1 from American ginseng. The amount of the obtained ginsenosides in 70% methanol aqueous solution under sonication is higher than that in methanol at room temperature. Especially, it is found that Rd is the most sensitive to heating, followed by Rc and Rb1, while Rb2, Rg1, and Re are inert to heating.[41] Therefore, the application of ultrasonic vibration avoids the decomposition of thermal-sensitive ginsenosides in the process of the extraction.

3.4. Ultrahigh Pressure and Supercritical CO_2

An apparatus with ultrahigh pressure is applied to extract thermal-sensitive ginsenosides from American ginseng at room temperature by using water, ethanol, methanol, and *n*-butanol as solvents. It is found that 0.861% Rc can be extracted by ethanol within 2 min when the pressure ranges from 100 to 600 MPa.[42] As shown in Figure 8, ultrahigh pressure can be obtained by mechanical press[43] or by boiling solvent in a sealed autoclave.[44]

CO_2 can be liquified under 31 MPa at 35−60 °C, and this supercritical state of CO_2 is usually used to extract natural compounds because liquid CO_2 possesses high diffusivity and low viscosity and surface tension. The liquid CO_2 can extract 73 and 108 mg of ginsenosides at 35 and 60 °C within 4 h. If the ginseng roots are immersed in ethanol for 6 h in advance and then extracted by liquid CO_2, the amount of the obtained ginsenosides increases to 800 and 1141 mg.[45] But the polarity of CO_2 is not high enough to dissolve all ginsenosides, ethanol is thereby applied to increase the polarity of the supercritical CO_2, and as a result, much more ginsenosides (2028 mg) can be obtained. In addition, methanol and dimethyl sulfoxide (DMSO) are usually used to modify the polarity of the liquid CO_2 with the pressure ranging from 20.7 to 48.3 MPa. More than 90% of ginsenosides can be extracted at 110 °C in the case of four-times weight of organic solvent employed. However, high temperature and organic solvent may change the structure of the ginsenosides. For example, mono-*O*-acetyl Rb1 is detected when DMSO is used to modify liquid CO_2. High temperature and pressure may decompose DMSO to form an acetyl source and subsequently lead to the acetylation product of Rb1.[46] Therefore, some efficient biological methods are developed to isolate single ginsenosides. For example, in immunoaffinity column chromatography, the use of an antiginsenoside Rb1 monoclonal antibody can readily isolate Rb1 from total extract of ginseng roots[47] and from methanolic extract of *Araliaceous* species.[48] Thus, biological methods are powerful ways to isolate a certain ginsenoside.

4. IDENTIFICATION OF GINSENOSIDES

NMR is generally used to confirm the linkage position of sugar moieties on the sapogenin, and MS is applied to identify the kind of sugar moieties. High-performance liquid chromatography (HPLC) is used to measure the amount of ginsenoside.

4.1. NMR

^{1}H NMR signals of ginsenoside range from 3 to 6 ppm, in which hydrogen atoms of sapogenin and sugar moieties appear as overlapping and splitting peaks. So, it is difficult to assign which peak is derived from the hydrogen atom of sapogenin and which from that of the sugar moiety. Acetylation of hydroxyl groups in sugar moieties eliminates the corresponding ^{1}H signals, leading to assignment of the ^{1}H NMR signals of sapogenin conveniently.[49] The one-dimensional ^{1}H NMR spectrum cannot be applied to confirm the ginsenoside structure directly, but some softwares can be employed to treat complicated ^{1}H NMR spectra. For example, Chenomx

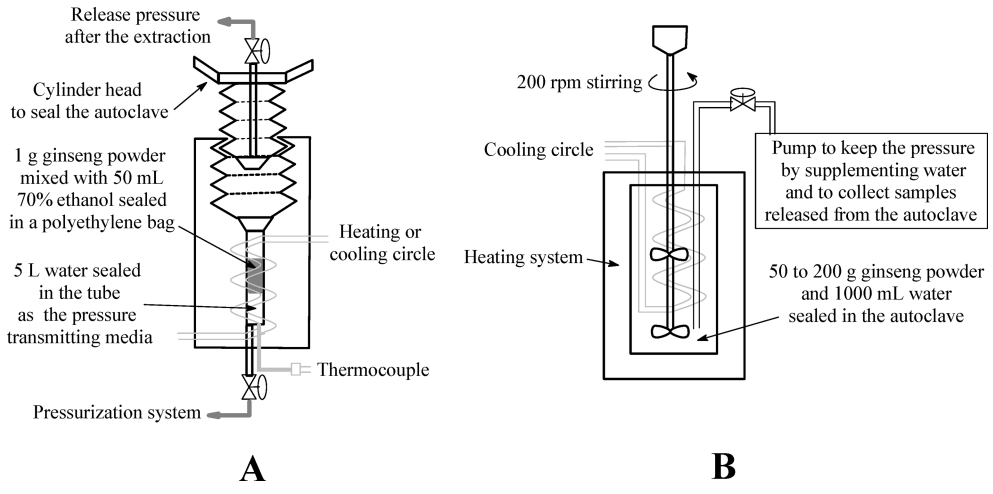

Figure 8. Mechanical (A) and continuous (B) ultrahigh pressure extraction apparatus.

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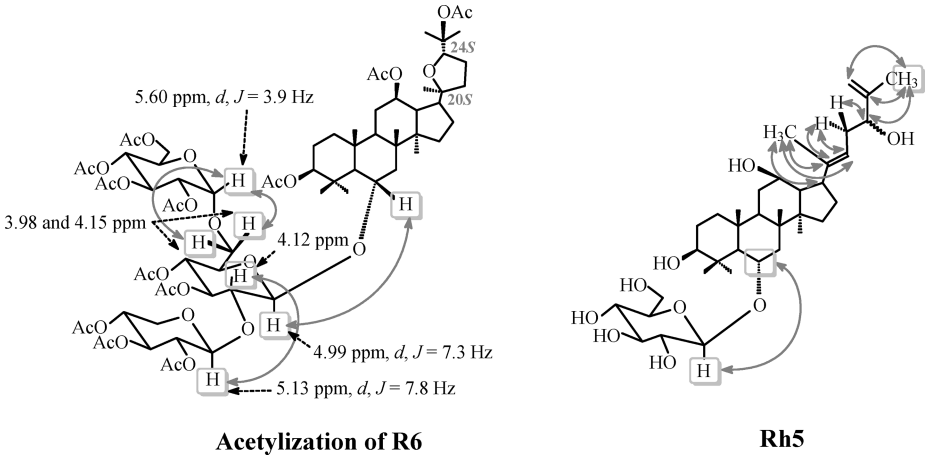

Figure 9. Long-range correlations among hydrogen atoms (acetylization of R6) and between carbon and hydrogen atoms (Rh5).

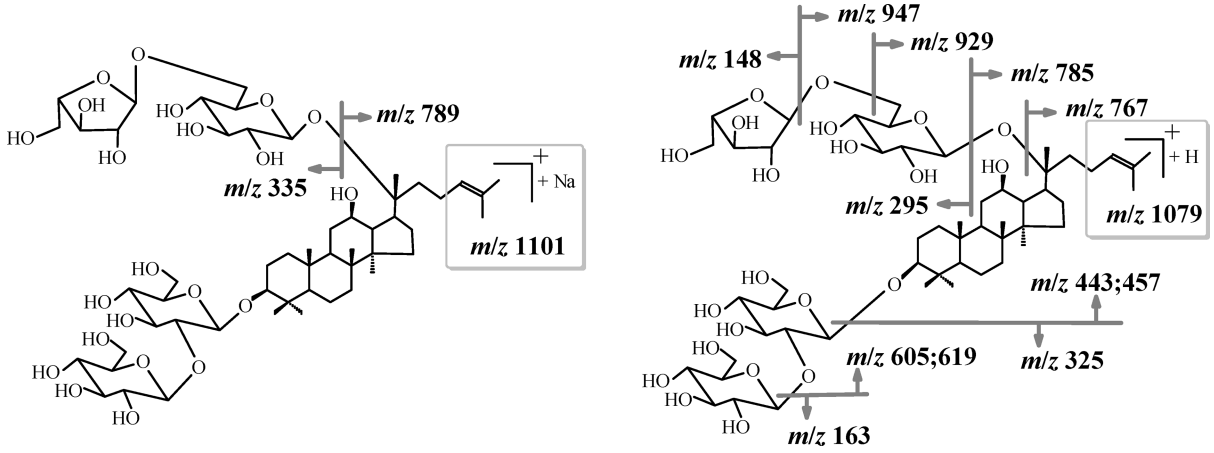

Figure 10. The fragmentation mode of Rc in the ion spray MS.

NMR Suite software (version 4.6, Chenomx Inc., Edmonton, Canada) contains baseline correction and automatic phasing algorithms, and SIMCA-P software (Umetrics, Kinnelon, NJ) provides unsupervised principal component analysis (PCA) and supervised partial least-squares discriminate analysis (PLS-DA) in analyzing ^{1}H NMR spectra. By use of the aforementioned softwares, some metabolites including coumarate, fumarate, glucose, and several amino acids are found. PCA and PLS-DA are reliable analytical methods to confirm the quality of commercial ginsengs.[50] On the other hand, ^{13}C NMR signals of the sapogenin skeleton range from 15 to 70 ppm, while those of sugar moieties range from 60 to 110 ppm. ^{13}C NMR signals of the C═C in the sapogenin range from 120 to 130 ppm.[51] So, it is easy to tell the ^{13}C NMR signals of sapogenin from those of sugar moieties. Futhermore, some novel NMR techniques such as ^{1}H,^{1}H-COSY, ROESY, heteronuclear multiple bond spectroscopy (HMBC), total correlation spectroscopy (^{1}H,^{13}C-TOCSY), and heteronuclear single quantum coherence spectroscopy (^{1}H,^{13}C-HSQC) reveal the long-range correlations among hydrogen atoms or between hydrogen and carbon atom from sapogenin and sugar moieties. As shown in Figure 9, two-dimensional NMR spectra are applied to confirm the linkage position of sapogenin and sugar moieties.[52]

4.2. MS

The complicated *m*/*z* detected by MS cannot be readily assigned to a concrete fragment deriving from ginsenoside. Advanced MS techniques, including electron impact MS, field desorption ionization MS, liquid secondary ionization MS, and liquid chromatography MS (LC/MS), are applied to identify the structure of ginsenoside. An ion spray (IS, nebulizer gas-assisted electrospray) technique in MS gives protonated or deprotonated molecular anions of Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1. The further fragment ions of these molecular cations or anions are analyzed by LC/MS/MS, and the kind of sugar moiety can be confirmed by *m*/*z* of the fragment ion. Figure 10 outlines a fragmentation mode of Rc in the ion spray MS.[53]

Gas chromatography (GC) equipped with electron impact MS (EI/MS) and HPLC equipped with electrospray ionization MS (ESI/MS) are applied to measure the molecular weights of ginsenosides ionized by H^+, Li^+, Na^+, NH_4^+, CH_3COO^-, Co^{2+}, Ni^{2+}, and Zn^{2+},[54−56] followed by the analysis of the kind of sugar moieties on the basis of *m*/*z* generated from the further fragmentation. Moreover, the electrospray ionization multistage tandem MS gives further fragment ions from the cleavage of sugar moieties[57] and the sapogenin skeleton.[58] An energy gradient neutral loss scan mode in a triple-quadrupole MS/MS is better than a fixed-energy neutral loss scan mode, and thus, glucuronides and sapogenins can be simultaneously detected in the total extracts from medicinal herbs.[59]

4.3. HPLC

An ODS C_{18} liquid chromatographic column and an ultraviolet detector with the wavelength at 203 nm are general way to

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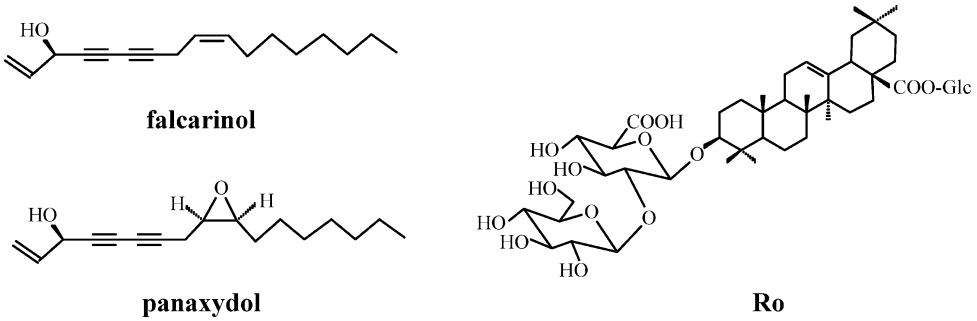

Figure 11. Structures of falcarinol, panaxydol, and ginsenoside Ro.

analyze ginsenosides. Acetonitrile aqueous solution acts as the eluent with the volume ratio of acetonitrile increasing from 18% to 55% or from 21% to 42% by a gradient program. R1 has the shortest retention time, followed by Rg1, Re, Rf, Rb1, Rc, Rb2, Rb3, and Rd in this case.[60] So, the majority of ginsenosides can be isolated and detected under this condition.[61] Recently, studies on the analysis of ginsenosides by HPLC mainly focus on (1) the application of statistical methods to treat the chromatographic peaks, (2) the selection of eluents to isolate ginsenoside epimers, (3) the variation of the wavelength to simultaneously detect more compounds in ginseng extracts, (4) the application of MS as the detector for HPLC, and (5) the development of biochemical methods to enrich a certain ginsenoside before HPLC analysis.

The principal component analysis (PCA) reveals the covariance and the correlation of ginsenoside distributions.[62] The partial least-squares (PLS) analysis predicts the antioxidant activity of medicinal herbs from HPLC fingerprint peaks.[63] Meanwhile, the ratio of acetonitrile and water gradually increases from 20:80 to 95:5 and then decreases to 20:80, resulting in a complete isolation of Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, (20*R*)-Rg2, Rg3, Rh1, and Rh2 in an ODS C_{18} column.[64] An appropriate component in the eluent can even isolate epimers of a single ginsenoside. For example, an eluent containing methanol and 4% aqueous solution of H_3PO_4 (65:35, v/v, pH = 5.1) is capable of isolating Rg2 into (20*R*)-Rg2 and (20*S*)-Rg2.[65] Furthermore, a photodiode array (PDA) UV detector simultaneously emits light with wavelength ranging from 200 to 600 nm. The mixture of CH_3OH and H_2O ranging from 40:60 to 75:25 and 100:0 and then to 40:60 affords complete isolation of Rb1, Rb2, Rc, Rd, Re, Rg1, Ro, malonyl-Rb1, malonyl-Rc, and malonyl-Rd as well as falcarinol and panaxydol. As shown in Figure 11, the conjugative system in falcarinol and panaxydol needs long wavelength of UV in the detection, and PDA is capable of detecting these compounds simultaneously[66] and obtaining three-dimensional UV spectra. HPLC equipped with a fluorometric detector can carry out simultaneous detection of amino acids and saponins including baicalin, wogonin-7-*O*-glucuronide, liquiritin apioside, glycyrrhizin, saikosaponin b1 and b2, Rg1 and Rb1, 6-gingerol, 6-shogaol, and arginine in medicinal herbs.[67] HPLC equipped with a pulsed amperometric detector can detect nonpolar ginsenosides. The potential in the amperometric detector ranges from −0.2 to +0.22 V or from −2.0 to +0.6 V along with the variation of the concentration of acetonitrile in aqueous solution, leading to the isolation of Rg5, Rk1 (structures in Figure 6), and astragalosides (structures in Figure 7).[68]

MS is the most sensitive detector of HPLC. In addition to the electron impact MS and the electrospray ionization MS, atmospheric pressure chemical ionization (APCI), triple quadrupole, ion trap (IT), Fourier transform ion cyclotron resonance (FTICR), time-of-flight (TOF), and multistage MS are able to control the fragmentation modes and, thus, are widely employed to detect pharmacological[69] and proteomic components in ginseng,[70] the metabolites of ginsenosides,[71] and the amounts and kinds of ginsenosides in drugs[72] and beverages.[73] Also, gas chromatography equipped with MS (GC/MS) can be used to determine phenolics in ginseng after the extracts are treated with trimethylsilyl chloride to form evaporative derivatives.[74]

Ginsenosides can be detected by other methods. Immunoaffinity using an antiginsenoside Rb1 monoclonal antibody can identify Rb1 specifically.[75] On a polyethersulfone membrane, the chromatographic immunostaining method is employed to determine Re with $CH_3OH/H_2O/CH_3COOH$ (45:55:1, volume ratio) as the eluent.[76] The enzyme-linked immunosorbent assay (ELISA) and Western blotting method are beneficial for the qualitative and quantitative measurement of ginsenosides at trace level. The ELISA method decreases the detectable limitation of PT from 50 pg/mL to 20 ng/mL.[77] Finally, the aspects of analytical chemistry in the research of ginseng are summed up as shown in Figure 12.

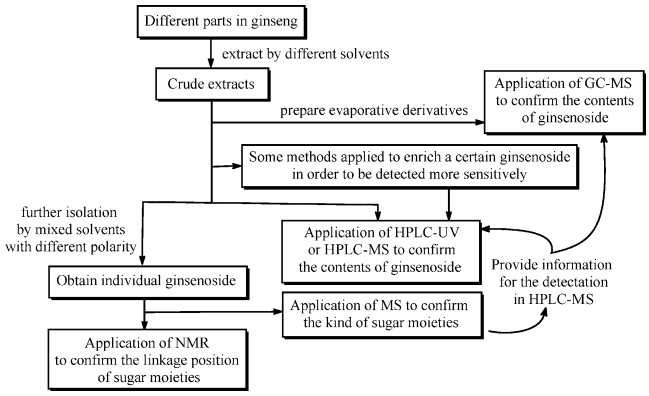

Figure 12. A schematic summarization of the aspects of analytical chemistry in the research of ginseng.

5. GINSENOSIDE-RELATED REACTIONS

The biological activities of saponins in medicinal plants have been reviewed recently,[78] and the *in vitro* culture technology is a useful way to enrich active components artificially.[79] Meanwhile, organic synthesis still plays an important role in the preparation and conversion of ginsenosides. In particular,

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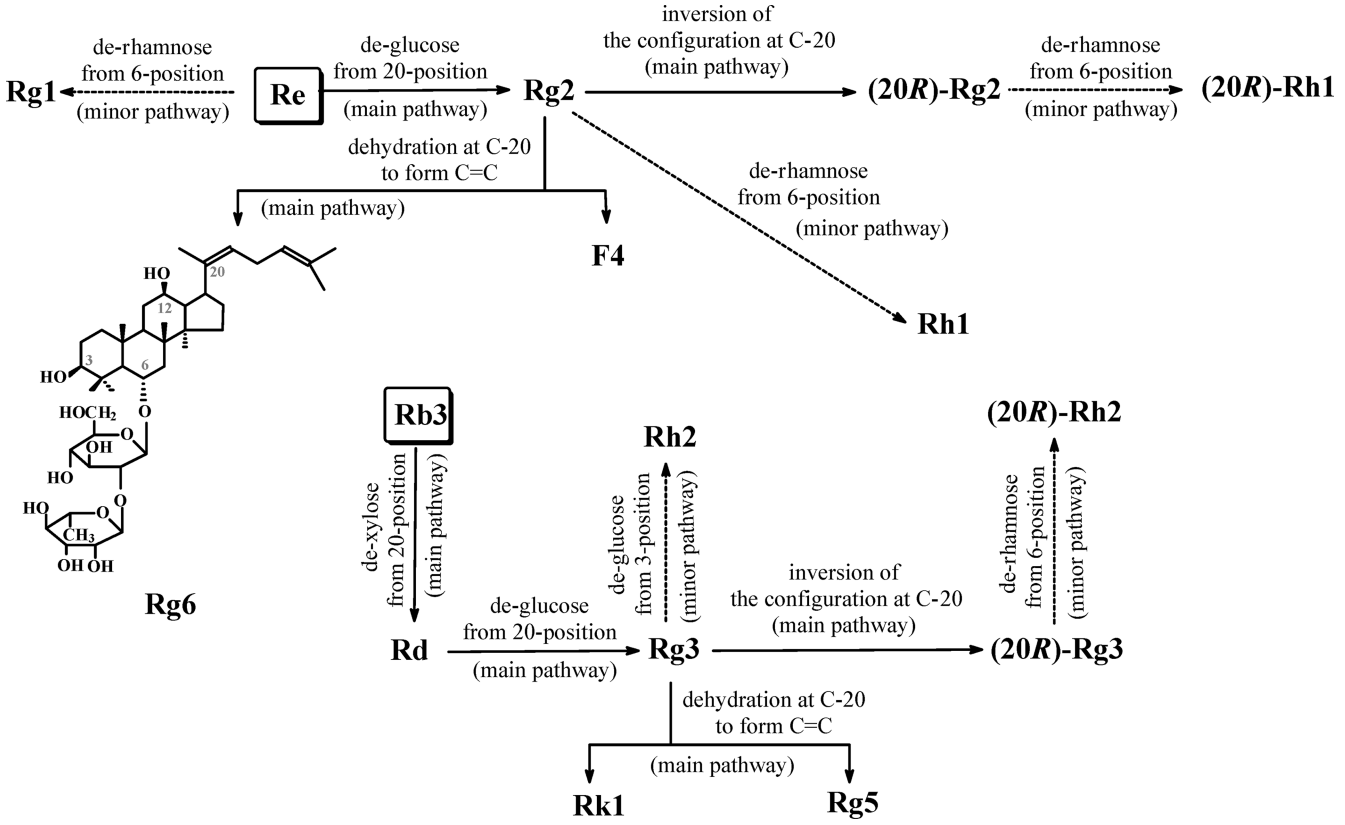

Figure 13. Proposed conversions of Re and Rb3 under acidic conditions.

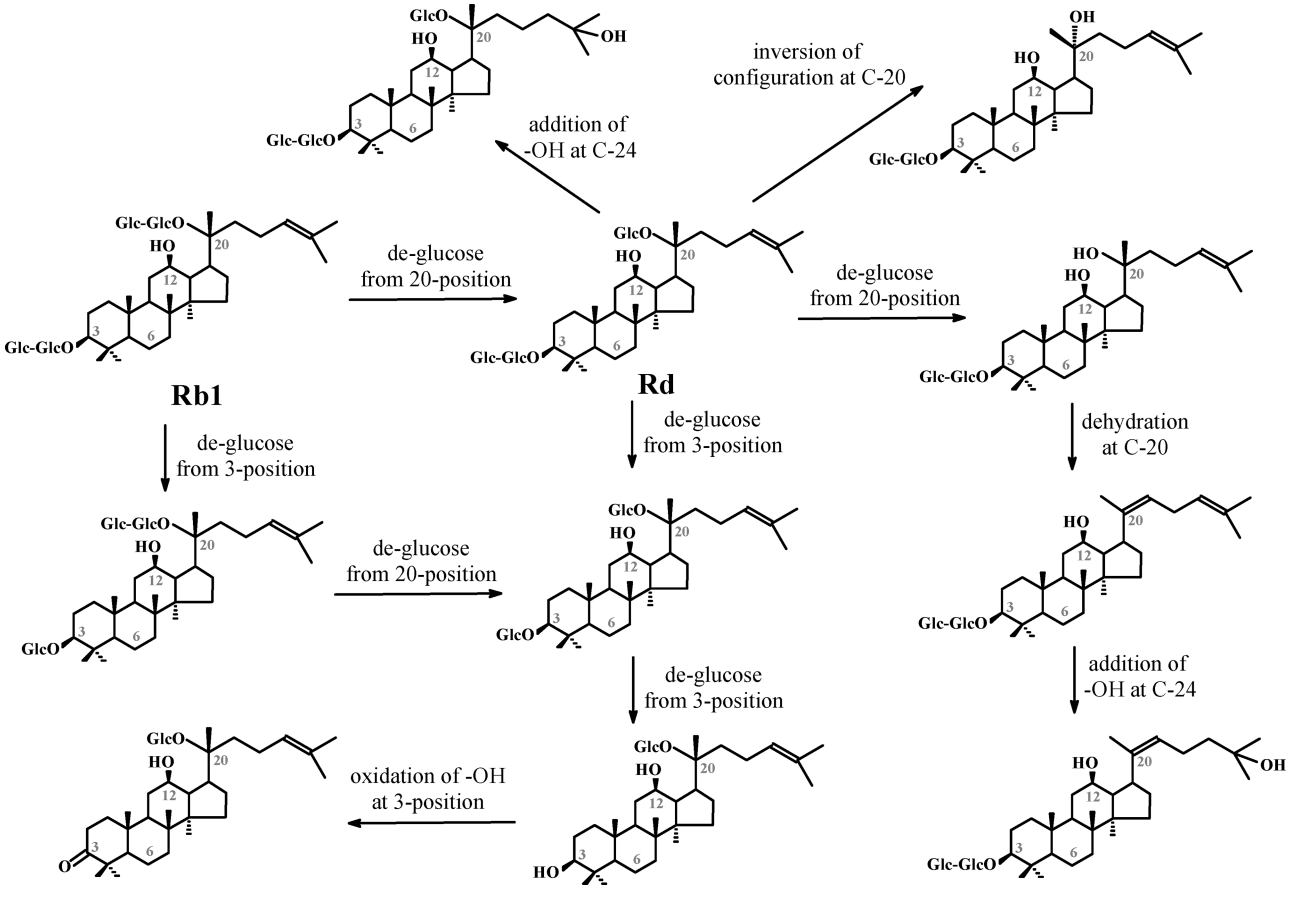

Figure 14. Proposed conversions of Rb1 in rat metabolism.

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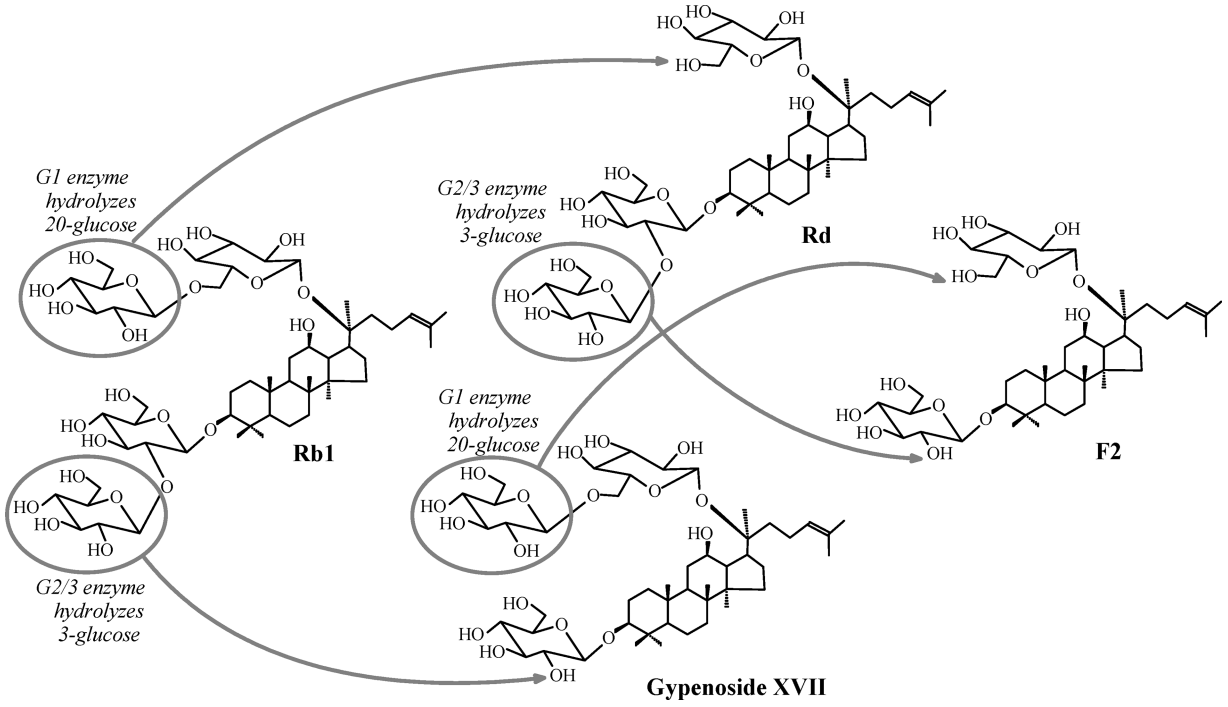

Figure 15. Different ways to deglycosylate Rb1 to form F2 catalyzed by G1 and G2/3 enzymes.

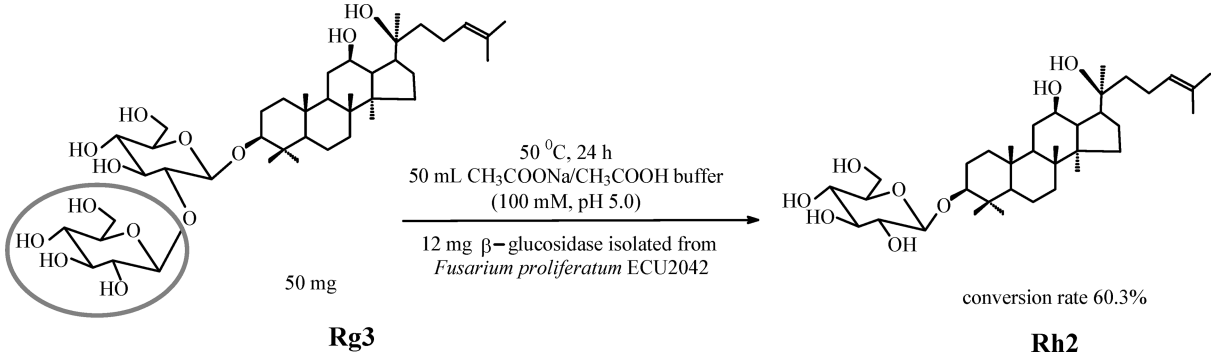

Figure 16. *β*-Glucosidase isolated from *Fusarium proliferatum* ECU2042 to convert Rg3 into Rh2.

enzyme-promoted reaction is an efficient way to synthesize ginsenoside and helps us to understand the metabolism process of ginsenosides. The reactions on ginsenosides include deglycosylation, glycosylation, sugar moiety-related reactions, and the synthesis of sapogenin.

5.1. Deglycosylation

An *in vitro* gastro-intestinal tract model is used to investigate the metabolites of ginsenosides. As shown in Figure 13, the bioconversion of ginsenosides involves a series of deglycosylations.[80] The determination of metabolites of Rb1 formed by microbe and rat reveals that the *in vivo* conversion of Rb1 may follow the process as shown in Figure 14.[81] The process shown in Figures 13 and 14 indicates that the sugar moieties at the 20-position are deglycosylated more readily than those at other positions, and the hydroxyl group at the 20-position is also readily dehydrolyzed to form C═C. The hydroxyl group at the 3-position can be oxidized to form carbonyl group, whereas, the hydroxyl group at the 12-position is inert to oxidation and dehydrolyzation.

Some efforts are contributed to find a special enzyme for the deglycosylation at a certain position of ginsenoside. A *β*-glucosidase (G-II) isolated from the phytopathogenic fungus *Cladosporium fulvum* (*syn. Fulvia fulva*) can specifically cleave the glycoside linkage between two *β*-glucose moieties at the 20-position in Rb1 to form Rd (see the conversion from Rb1 to Rd in Figure 14) but cannot deglycosylate other *β*-D-glucosidic linkages in PD-type ginsenosides.[82] Ginsenosidase type II isolated from *Aspergillus* sp. g48p strain exhibits high selectivity to cleave the linkage between polysaccharide at the 20-position in PD-type ginsenosides. This enzyme can only deglycosylate 20-*O*-*β*-glucoside in Rb1, 20-*O*-*β*-xyloside in Rb3, and 20-*O*-*α*-arabinoside in Rb2 and Rc, forming Rd and a little of Rg3 eventually. However, this enzyme is not active toward other sugar moieties in Rb1, Rb2, Rb3, and Rc and cannot hydrolyze the sugar moieties at the 20-position of PT-type ginsenosides such as Re, Rf, and Rg1. Hence, ginsenosidase type II is a specific enzyme for removing the terminal sugar moiety at the 20-position of PD-type ginsenosides.[83] The deglycosylation routines of ginsenosides largely depend upon the kind of enzyme even though the structure of the enzyme is not clear. In general, enzymes are characterized by the isolation procedure and molecular weight. For example, three enzymes named G1, G2, and G3 are isolated from ginseng pathogen *Pythium*

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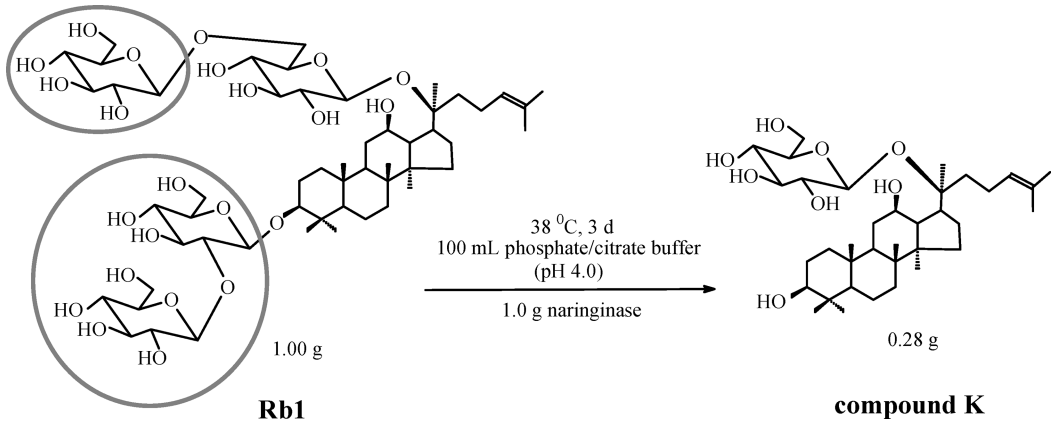

Figure 17. Naringinase deglycosylates disaccharides at the 3-position and terminal monosaccharide at the 20-position in Rb1.

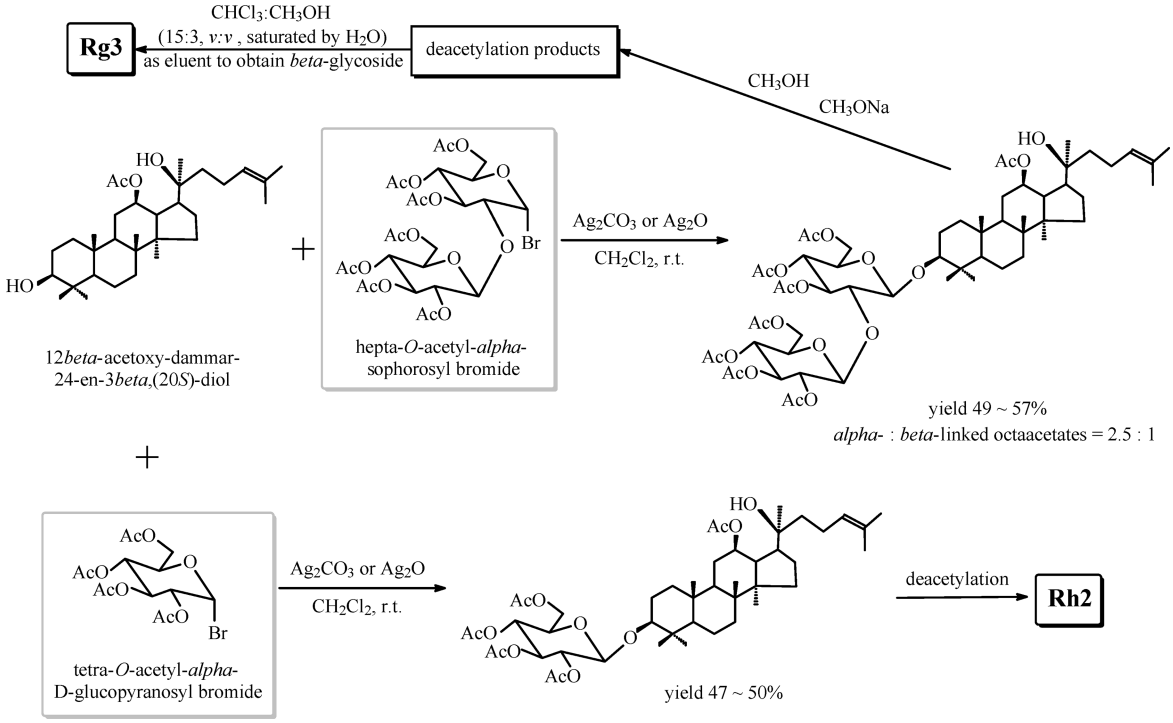

Figure 18. Glycosylation of 12β-acetoxy-dammar-24-en-3β,20*S*-diol to prepare Rg3 and Rh2.

irregulare by the precipitation in acetone, the enrichment on polybuffer exchanger, the filtration on Sephacryl S-200 HR gel column, and the anion exchange on Q Sepharose chromatography column in turn. The analysis of molecular weight indicates that G1 is likely a homodimer of 78 kDa subunits, while G2 and G3 are two monomeric enzymes of 61 and 57 kDa, respectively. These enzymes can deglycosylate the terminal monosaccharide from disaccharides at the 3- or 20-position of Rb1, Rc, Rb2, and Rd to yield ginsenoside F2 as the final product.[84] Figure 15 outlines the pathways of Rb1 to form F2 catalyzed by G1 and G2/3 or the inverse order.[85] The G1 enzyme specifically deglycosylates the terminal sugar moiety from the 20-position, while the G2/3 enzymes just deglycosylate the terminal sugar moiety from the 3-position of PD-type ginsenoside.

A microbial strain GS514 isolated from soil around ginseng roots can convert Rb1 into Rg3 by deglycosylating disaccharide at the 20-position in Rb1 with Rd being the intermediate.[86] As shown in Figure 16, β-glucosidase isolated from *Fusarium proliferatum* ECU2042 can deglycosylate the terminal glucose moiety at the 3-position in Rg3 to form Rh2.[87]

As shown in Figure 17, naringinase deglycosylates disaccharides at the 3-position and the terminal glucose moiety at the 20-position of Rb1 to form compound K.[88] A study on the cleavage of the glycosidic bond in Rg1, Rb1, and Ro indicates that glucoside at both 20- and 6-positions can be thoroughly deglycosylated by NaOH or CH_3ONa in *n*-butanol under oxygen or air, but the deglycosylation does not occur when oxygen or air is replaced by nitrogen. Thus, the antioxidant effectiveness of ginsenosides may be ascribed to the cleavage of sugar moieties in the presence of oxygen.[89] In the presence of the intestinal bacteria, the deglycosylation of sugar moieties at the 3-, 6-, or 20-position is the first step in the metabolism process of ginsenoside.

5.2. Glycosylation and Sugar Moiety-Related Reactions

The reaction between the hydroxyl group at the 3-position of dammarane-type sapogenin with acetylized glycosyl bromide

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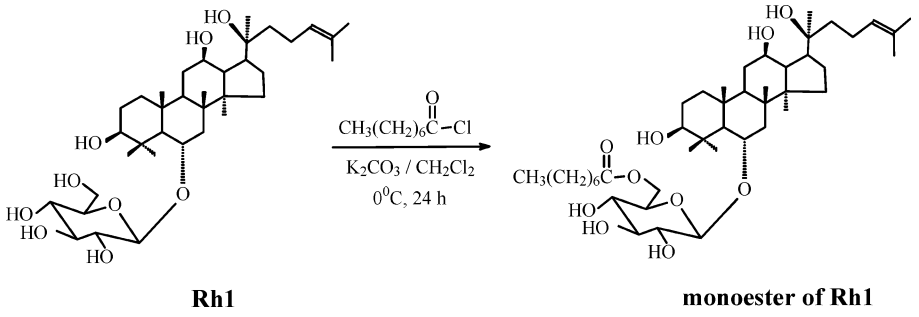

Figure 19. Chemical method to modify Rh1.

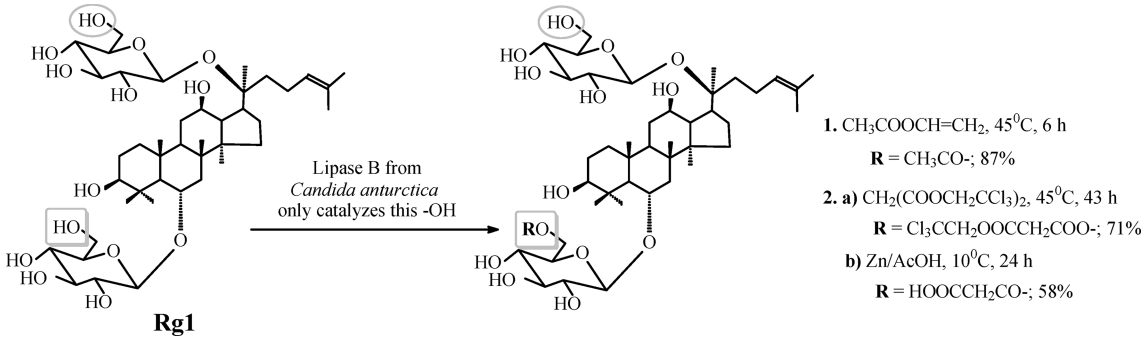

Figure 20. Lipase B from *Candida antarctica* shows higher regioselective activity.

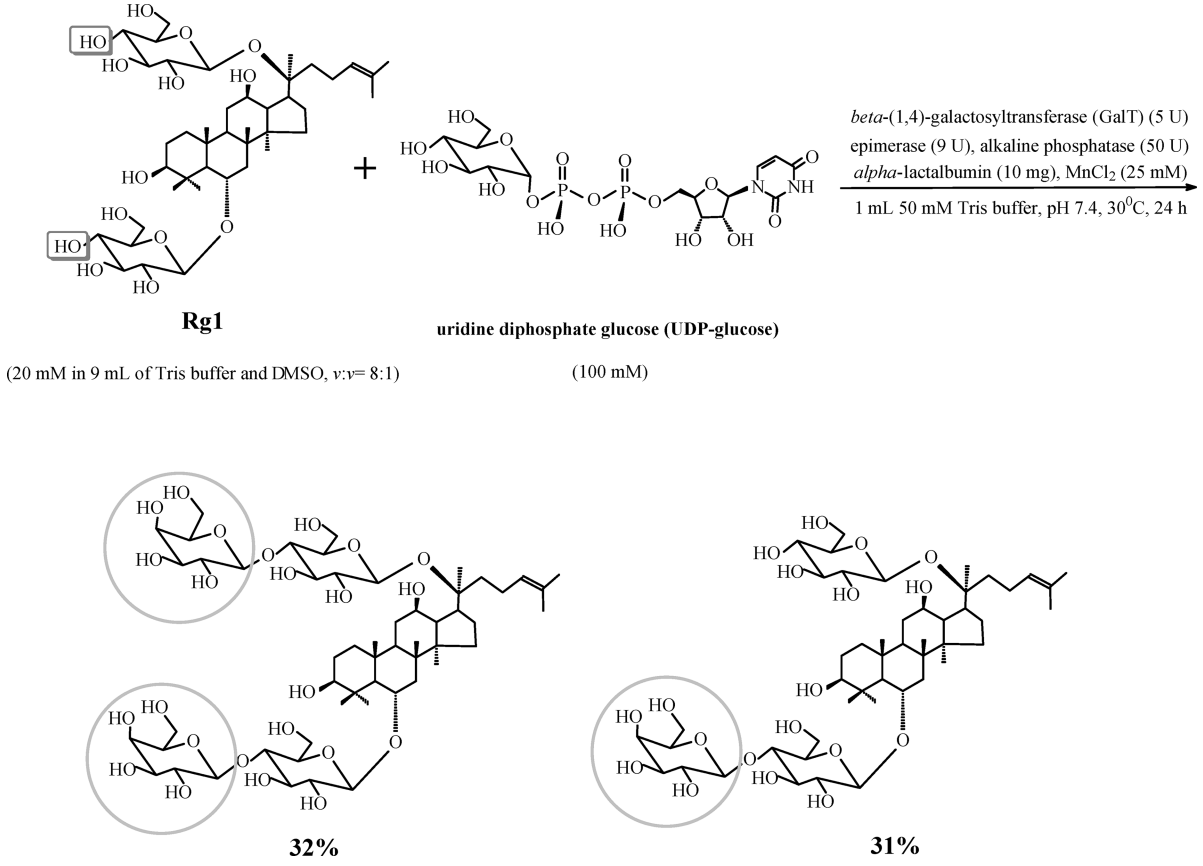

Figure 21. UDP-glucose as the glucose donor in the glycosylation of Rg1.

affords Rg3[90] and Rh2[91] as shown in Figure 18. The hydroxyl group at the 12-position in sapogenin is acetylized to form 12β-acetoxy-dammar-24-en-3β,20*S*-diol, which can react with hepta-*O*-acetyl-α-sophorosyl bromide or tetra-*O*-acetyl-α-D-glucopyranosyl bromide to produce the precursor of Rg3 or Rh2. Then, Rg3 and Rh2 are generated after deacetylization. The reactivities of hepta-*O*-acetyl-α-sophorosyl bromide and tetra-

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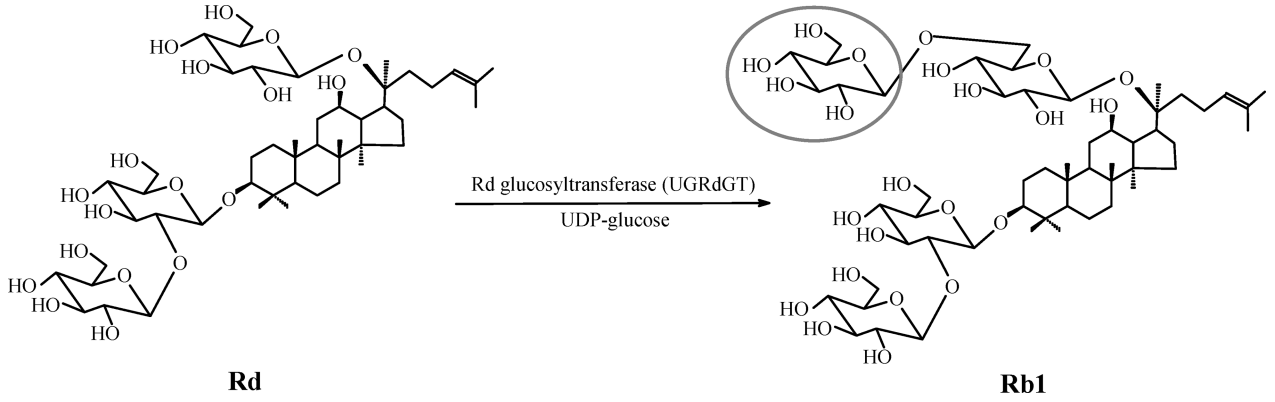

Figure 22. UGRdGT as an active enzyme in the biotransformation of Rd to Rb1.

O-acetyl-α-D-glucopyranosyl bromide toward the hydroxyl group at the 3-position are higher than that at the 20-position.

As shown in Figure 19, octanoyl chloride can react with $-CH_2OH$ in glucose of Rh1 to form an ester because the reactivity of the primary alcoholic group is higher than that of other types of hydroxyl groups. The monoester of Rh1 inhibits murine H22 hepatoma cells more efficiently than Rh1 itself because the long carbon chain is beneficial for monoester of Rh1 to transport into the cell membrane.[92] The esterification by fatty acid increases the lipophilicity of ginsenoside, resulting in good uptake *in vivo*.[93]

In novozyme 435 lipase-catalyzed esterification of Rb1, an acyl reagent with a short carbon chain gives higher yield than one with a long carbon chain in *tert*-amyl alcohol. But low yield is obtained when the esterification takes place in the mixture of *t*-butanol and pyridine (1:1, v/v). This enzyme cannot catalyze regioselective esterification of a specific hydroxyl group in Rb1, but the yield reaches 61% when vinyl decanoate reacts with Rb1 at 53 °C for 40 h in *tert*-amyl alcohol. Actually, the aforementioned synthesis condition is obtained by response surface methodology (RSM) analysis and, then, is proven by the experimental operation.[94] Although Rg1 has two $-CH_2OH$ in glucose moieties, lipase B isolated from *Candida antarctica* shows high regioselective activity and only catalyzes the transesterification between $-CH_2OH$ in the glucose moiety at the 6-position and vinyl acetate in *tert*-amyl alcohol to produce monoester of Rg1. As shown in Figure 20, Rg1 reacts with bis(2,2,2-trichloroethyl) malonate in the presence of lipase B, followed by the reduction via Zn/CH_3COOH to form 6′-*O*-carboxyacetyl Rg1.[95]

As shown in Figure 21, β-(1,4)-galactosyltransferase (GalT) isolated from bovine colostrums can catalyze the glycosylation of 4-OH in the glucose moiety at the 6- and 20-positions in Rg1 when uridine diphosphate glucose (UDP-glucose) acts as the glucose donor. The 4-OH in the glucose moiety at the 6-position in F1, Rh1, and Re can also be glycosylated under the same experimental conditions.[96] Furthermore, as shown in Figure 22, Rd glucosyltransferase (UGRdGT) catalyzes Rd to produce Rb1 when UDP-glucose acts as the glucose donor.[97] Therefore, the regioselection in the glycosylation of ginsenoside largely depends upon the kind of enzyme.

As shown in Figure 23, the glycosylation of oleanolic acid by using 4,6-di-*O*-acetyl-2-*O*-(2-*O*-2-(azidomethyl)benzoyl)-3-*O*-benzoyl-D-glucopyranosyl trifluoroactetimidate (AABBG) produces Ro at the total yield of 28%.[98] This method may be employed in the glycosylation of other sapogenins.

As shown in Figure 24, the immunostaining technique enhances the sensitivity and specificity in the analysis of ginsenoside by thin layer chromatography (TLC).[100] The TLC plate is made of polyvinylidene difluoride (PVDF) with bovine serum albumin (BSA) adhereing on the surface. The oxidation of $NaIO_4$ breaks the C−C bond in glucose moiety to form aldehyde groups that can condense with $-NH_2$ in BSA. As a result, the ginsenoside is fixed on the surface of PVDF. Then, the sapogenin part in the ginsenosides is immunostained by antiginsenoside Rb1 or antiginsenoside Rg1 monoclonal antibodies (MAbs).[99] The cleavage of C−C in sugar moieties under $NaIO_4$ is the key step although it cannot be confirmed which C−C bond is broken in the glucose moieties.

5.3. Sapogenin-Related Reactions

As shown in Figure 25, hydroxylation occurs in PD to form eight products in the presence of fungus *Mucor spinosus* AS 3.3450, which can also specifically oxidize the hydroxyl group at the 12-position in PD to form a carbonyl group.[101]

As shown in Figure 26, the skeleton of dammarane can be synthesized via the cyclization of 2,3-oxidosqualene catalyzed by various specific enzymes. Cycloartenol synthase, dammarenediol-II synthase, and β-amyrin synthase are able to catalyze the cyclization of squalene to form the sapogenin of ginsenoside.[102] Squalene derivatives provide all the carbon atoms in the sapogenin skeleton, and some specific enzymes drive a successive transfer of π-electrons to form the triterpenoid skeleton.[103]

6. ACTIVE COMPONENTS IN GINSENG

Figure 27 illustrates the essential role of *in vivo* oxidation in aging and some fatal diseases.[104] *In vivo* oxidation provides the energy for life and, meanwhile, generates many reactive oxygen and nitrogen species that cause oxidative stress. Oxidative stress changes the chemical components of lipid, membrane, DNA, and protein, inactivates enzymes, and degrades the central nervous system (CNS).[105] Since antioxidant therapy has become a popularly acceptable concept, nutritional and medicinal plants are applied to inhibit harmful oxidations.[106] In particular, the effects of ginseng extracts on tumors and diabetes have been widely investigated because these two diseases correlate directly with the oxidative damage. Presented here are the antioxidant properties of ginsenosides including apoptosis of tumor cells, inhibition of diabetes and neuron degradation, and reaction with free radicals.

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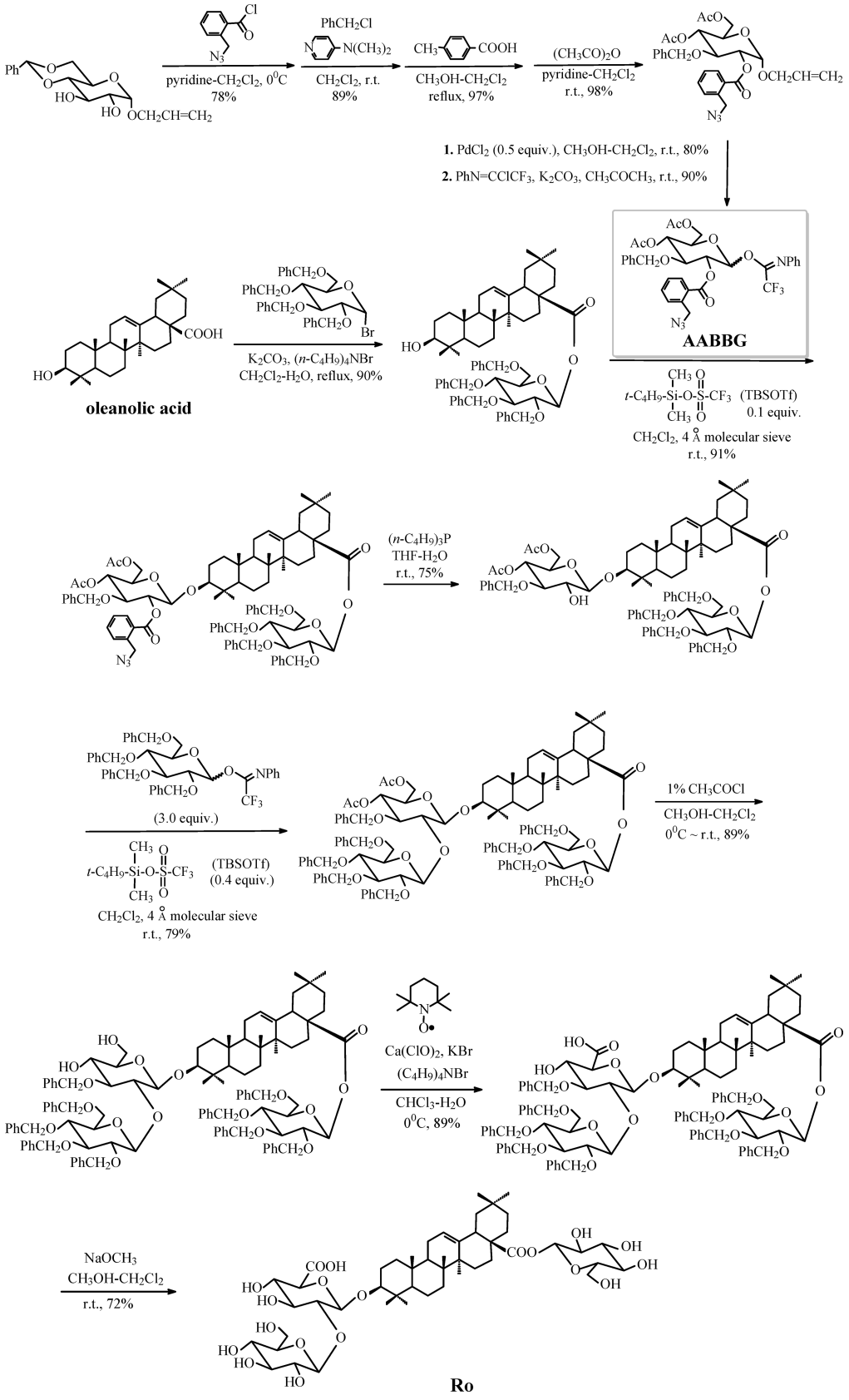

Figure 23. Synthetic routines of Ro.

6.1. Effects on Cells and Diseases

Chemical carcinogens (structures in Figure 28), biological issues, and irradiation initiate the proliferation of tumor cells in an *in vitro* experimental system. The addition of antioxidants causes apoptosis of these tumor cells.[107] Table 2 lists some

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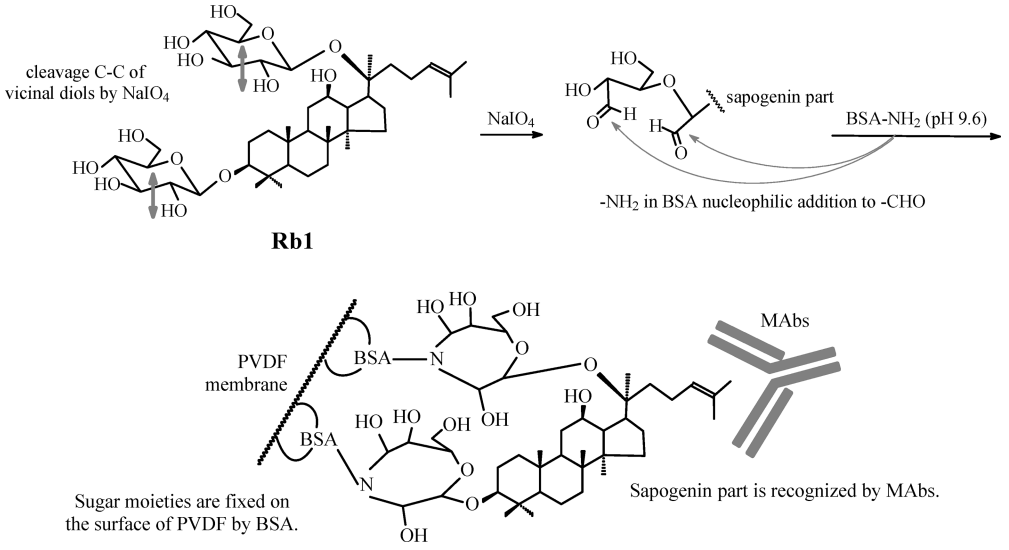

Figure 24. The process of immunostaining Rb1 on PVDF membrane.

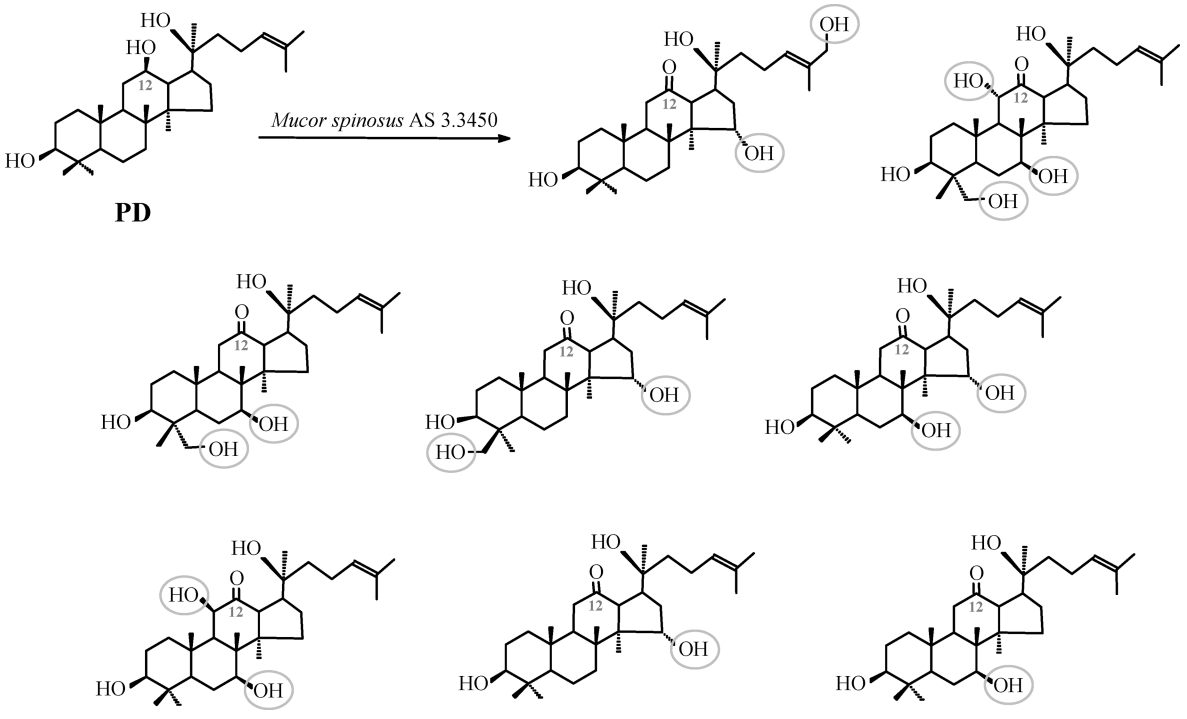

Figure 25. Fungus *Mucor spinosus* AS 3.3450-catalyzed hydroxylation of PD.

typical studies on the activities of total extracts, individual ginsenosides, and sapogenins.

As can be seen in Table 2, the aforementioned works consist of mainly initiators and cell lines in the research of carcinogenesis. The initiators involve chemical carcinogens, radicals, irradiations, and cytotoxins. The use of total extract just gives an overall effect of ginseng on tumor cells. It is difficult to identify which ingredient in ginseng inhibits the proliferation of tumor cells. Some researches emphasize the solvent applied to extract ginseng because a certain solvent is beneficial for dissolving a certain kind of component in ginseng. For example, methanolic extract of ginseng cannot stimulate the formation of tumor necrosis factor-α (TNF-α) in rat alveolar macrophages, but water extract exhibits high activity. Because methanol mainly extracts ginsenosides and water mainly extracts polysaccharide, the formation of TNF-α is mainly ascribed to the polysaccharide.[112] It is better to compare the bioactivity of individual ginsenosides in the same experimental system. For example, Rh1 and Rg1 were applied to hinder human breast cancer cells (MCF-7). Rh1 induces the responsive genes of estrogen,[141] and Rg1 increases the expression of insulin growth factor I receptor (IGF-IR), which can mediate the signaling pathways in MCF-7.[142] The individual ginsenosides are employed to suppress the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by acetylcholine (ACh). PT-type ginsenosides are more efficient than PD-type ones in this case.[131] Hemin can cause hemolysis of erythrocytes by accelerating the potassium leakage, dissociating skeletal proteins, and prohibiting some enzymes in erythrocyte membrane. As shown in Figure 29, the

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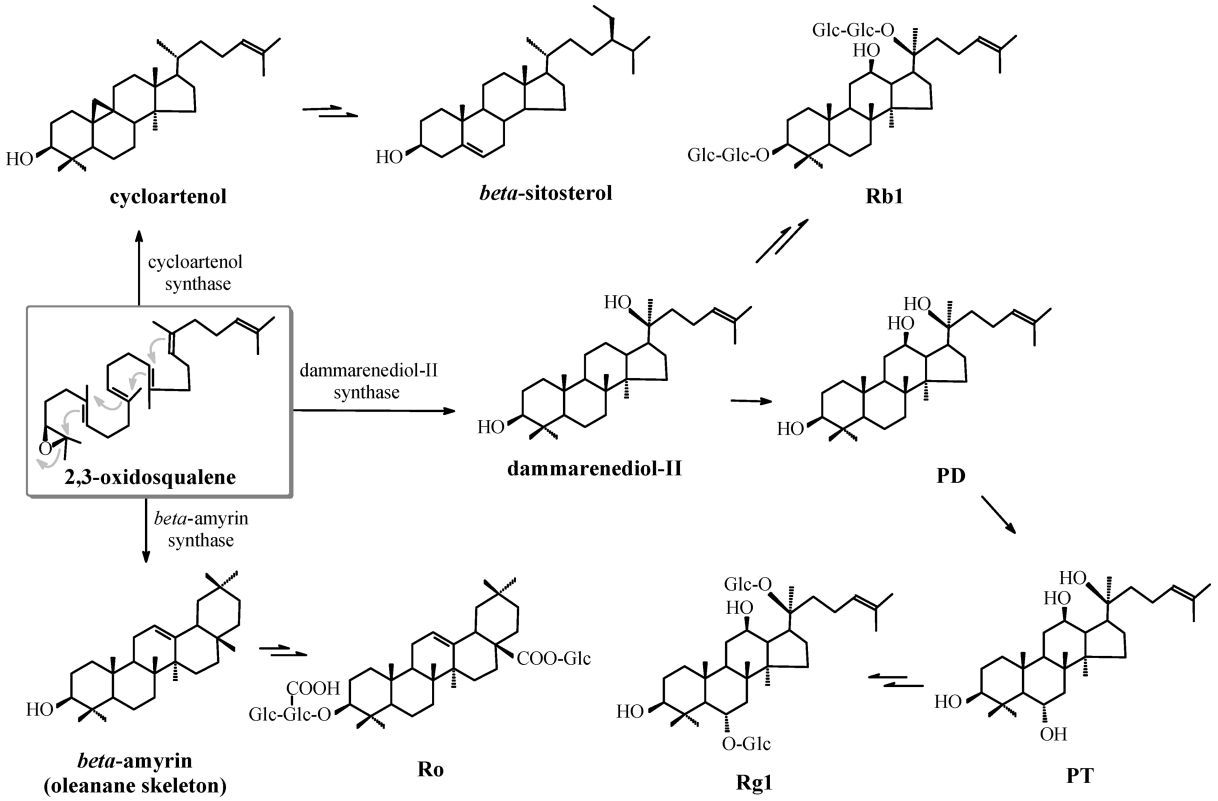

Figure 26. The enzyme-assisted cyclization of 2,3-oxidosqualene to form the dammarane-type skeleton.

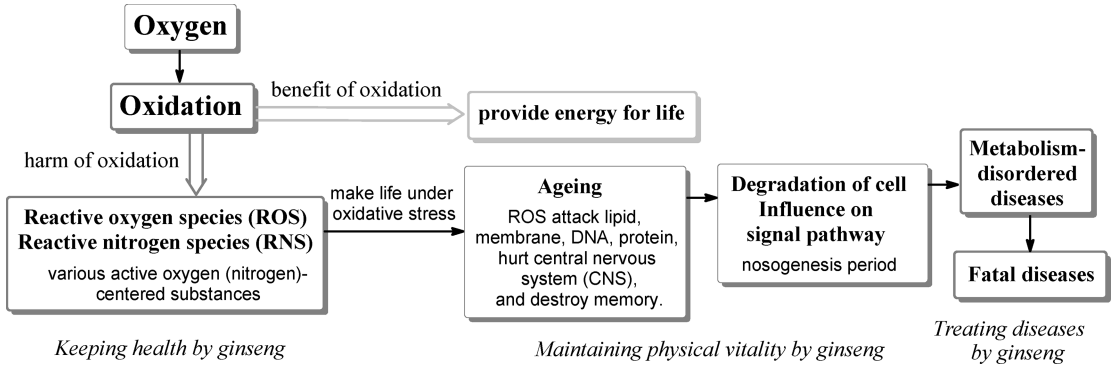

Figure 27. The functions of ginseng on different pathologic stages.

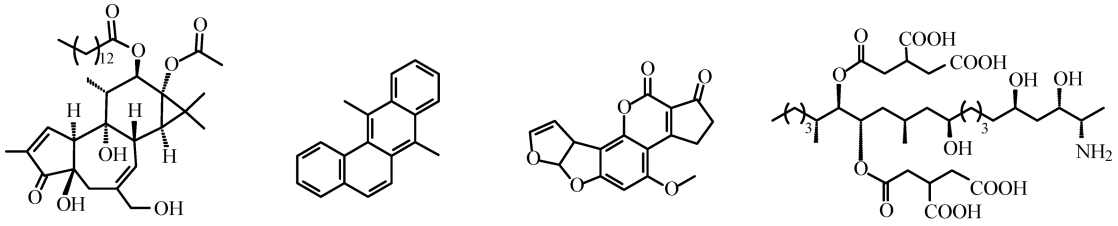

Figure 28. Structures of some chemical carcinogens.

protective activities of mixed ginsenosides (total), twelve individual ginsenosides, and PD and PT are compared in hemin-induced hemolysis of human erythrocytes.[132] PT-type ginsenosides together with PT itself possess similar anti-hemolysis activities (66−79%) as that of the mixed ginsenosides (74%). For PD-type ginsenosides, Rc has the highest activity (55%), followed by Rd, Rb1, and Rb3, but the corresponding sapogenin, PD, promotes hemolysis (106%). Meanwhile, Rh2 and Rg3 increase hemolysis percentages to 152% and 222%, respectively. Rg3 can initiate hemolysis (130%) even in the absence of hemin. By comparison of ginsenoside structures, the antihemolysis effects of Rc (55%),

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Table 2. *In Vitro* Experimental Models for Evaluating the Active Components in Ginseng

initiators	cell lines	active components	ref
xanthine-xanthine oxidase, 12-*O*-tetradecanoylphorbol-13-acetate, and photolysis of H_2O_2	human promyelocytic leukemia cells (HL-60) and strand scission in ϕX174 supercoiled DNA	methanol extract of heat-treated ginseng	108
12-*O*-tetradecanoylphorbol-13-acetate and 7,12-dimethyl benz[*a*]anthracene	HL-60 cells and papilloma	Rg3	109
aflatoxin B_1 and fumonisin	precancerous lesions in female Sprague−Dawley rats	water extract of Korean ginseng	110
lipopolysaccharide	liver injury in rats	methanol extract of heat-treated ginseng	111
lipopolysaccharide	rat alveolar macrophages	polysaccharide and Rb1	112
lipopolysaccharide	microcirculatory disturbance in rat mesentery	Rb1, Rg1, R1	113
$CdCl_2$	hepatic injury in Swiss albino mice	total extract of ginseng	114
7,12-dimethyl benz[*a*]anthracene	skin papillomagenesis in Swiss albino mice	Rp1	115
12-*O*-tetradecanoylphorbol-13-acetate and H_2O_2	rat liver epithelial cell (WB-F344)	Rb2 and epicatechin	116
H_2O_2	human umbilical cord vein endothelial cells	(20*S*)-Rg2, PT	117,118
tert-butyl hydroperoxide	liver injury (HepG2 cells)	compound K, Rb1, Rb2, Rc	119
D-galactosamine	primary cultured mouse hepatocytes	Rb1, Rb2, Rc, Rd, Re, Rg1, Rh1, Rh5, R25, R1, R2, RT4, R10, Rh4, PT oxide II	120
γ-ray radiation	human lung cancer cells (NCI-H460)	compound K	121
γ-ray radiation	apoptosis of rat intestinal epithelial cells	Rd	122
cyclophosphamide	apoptosis of mouse bone marrow cells and peripheral lymphocyte cells	total extract of ginseng	123
cytokine	apoptosis of pancreatic β-cells (MIN6N8)	total extracts of red and white ginseng	124
serum deprivation	apoptosis of human endothelial cells	(20*S*)-Rg3	125
thioacetamide	fibrosis of hepatic stellate cells	Rg1	126
transforming growth factor-β1	fibrosis of rat renal tubular epithelial cells (NRK-52E)	Rg1	127
gut ischemia/reperfusion	liver fibrosis and hepatic microvascular dysfunction in rats	water extract of ginseng	128
acetylcholine (ACh)	secretion of catecholamines from bovine adrenal chromaffin cells	Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1, Rh2, Ro, Rs1, PD, PT, and the metabolites of ginsenosides	129−131
hemin	hemolysis of erythrocytes	Rb1, Rb3, Rc, Rd, Re, Rg1, Rg2, Rg3, Rh1, Rh2, R1, F11, PD, and PT	132
collagen	platelet aggregation	total extracts of raw and steamed *Panax notoginseng*, *ginseng*, and *quinquefolium*	133
hypoxia/reoxygenation	injury of rat cardiomyocyte	Rg1	134
2,2′,5,5′-tetrachlorobiphenyl	human neuroblastoma (SK-N-MC) cells	water extracts of red ginseng	135
	human neuroblastoma (SK-N-SH) cells	Rb1, Rb3, R4, Fa	136
	adult and neonatal rat cardiomyocytes	total extracts of American, Indian, Siberian, and Asian ginseng	137
	guinea pig ventricular myocytes	total extract of ginseng, Re	138
	human colorectal cancer cells (HCT116 and SW480)	70% ethanol aqueous solution extract of steamed American ginseng	139
	human colon cancer cells (HCT116 parental and *p21*)	water extract of ginseng, polysaccharides	140
	human breast cancer cells (MCF-7)	Rh1, Rg1	141,142
	human carcinoma cells (KBV20C)	Rg3	143
	human colorectal cancer (SW-480) cells	Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, Rg3, Rh1, Rh2, (20*R*)-Rg2	144
	osteosarcoma cells (MG-63)	Rg1, cinnamic acid, and tanshinone IIA	145
	human leukemia cells (THP-1)	PD, PT, Rh1, Rh2, Rg3	146
	human glioma (SF188 and U87MG) cells	PD	147
	human pancreatic cancer cells	25-hydroxyl or 25-methoxy substituted PD	148
	human prostate cancer LNCaP and PC3 cells, and mouse PC3 xenograft tumor	25-hydroxyl substituted PD and PT	149
	human mammary gland carcinoma cells (MDA-MB-435)	sapogenin of F11 and its derivatives	150

Rd (59%), and Re (66%) may be ascribed to sugar moieties at 3- (or 6-) and 20-position simultaneously. Furthermore, the absence of sugar moieties at the 20-position makes Rh2, Rg3, and Rh1 strongly induce apoptosis of human leukemia cells (THP-1).[146] The destroying effect of Rh2 on membrane is applied to hinder lipid rafts in the membrane of HeLa cells, causing apoptosis of these cells.[151] These works largely focus on the abundant components of individual ginsenoside such as the Rg and Rh series; the influence of the chiral carbon at the 20-position in Rg3 on the bioactivity is clarified clearly.[152]

Diabetes is another disease caused by oxidative stress and the lack of antioxidants and is an experimental model to evaluate

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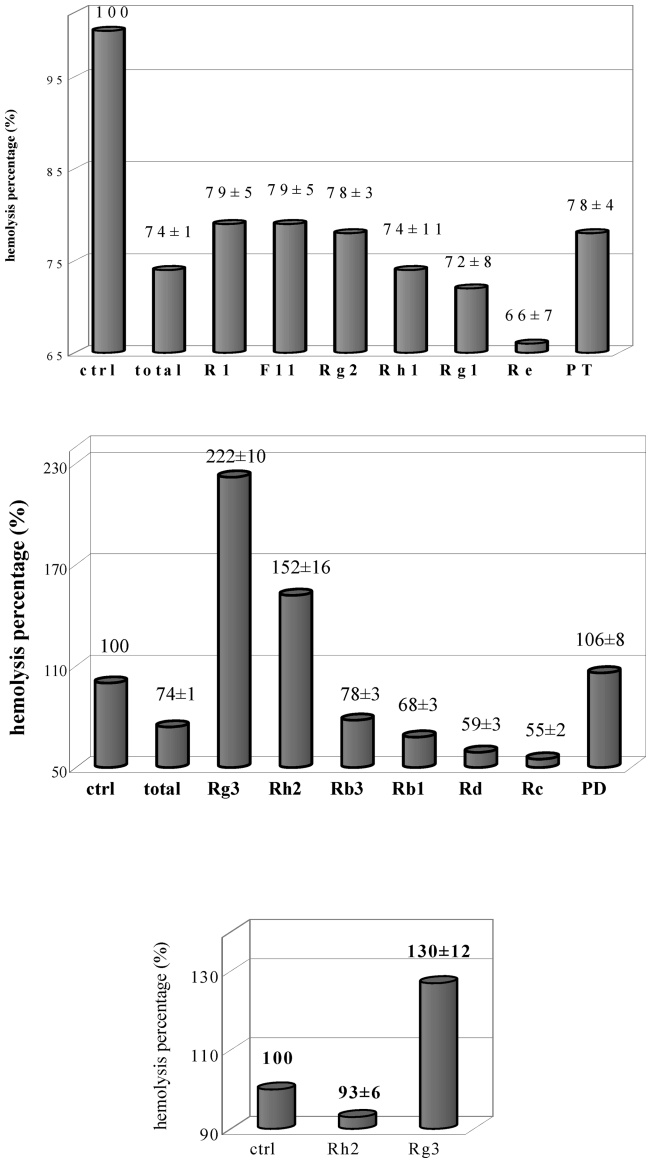

Figure 29. Hemin (4.0 μM)-induced hemolysis of human erythrocytes (1.0%, v/v in phosphate buffered saline (PBS)) is inhibited by 20.0 μM ginsenosides and PD and PT except Rh2 and Rg3. Rh2 and Rg3 (20.0 μM) cause hemolysis of erythrocytes (1.0%, v/v in PBS) as 4.0 μM hemin.[132]

the antioxidant activity. Table 3 collects some diabetes-related experimental models together with some other oxidative stress-induced diseases. The functions of ginseng components are evaluated by measuring various biochemical indexes.

As can be seen in Table 3, total extract of ginseng is usually employed in animal-related experiments. The complicated isolation process makes individual ginsenosides too expensive for the large consumption in the *in vivo* experiments. Although the amounts of various individual ginsenosides in total extract can be identified by HPLC, it cannot definitely point out which ginsenoside plays the major role in treating diseases. Thus, it is important to compare the activities of individual ginsenosides in the same experiment in order to reveal the *in vivo* mechanism of ginsenoside. For example, when the obese mice are fed various individual ginsenosides, it is found that Rb1, Rb2, Rc, and Rd inhibit pancreatic lipase efficiently, while Rg1 and Re cannot.[165] This finding implies that sugar moieties at both the 3- and 20-positions in PD-type ginsenoside are active to decrease pancreatic lipase, while sugar moieties at the 6- and 20-positions in PT-type ginsenoside do not exhibit this bioactivity.

6.2. Effects on Nervous System

Ginseng can prevent aging, disorders of the central nervous system (CNS), imbalance of nitric oxide (NO),[171] and neurotoxicity.[172] Table 4 lists some typical studies of ginsenosides in the CNS.

The experimental materials include bronchial and cerebral arterial strips and canine corpus cavernosum. Acetylcholine (ACh), acrylamide, prostaglandin, nicotine, electricity, and UV light cause the contraction of experimental materials.[173,174,187] The contents of NO, cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP) are measured in the presence of the total extract of ginseng and individual ginsenosides. Or, some chemical or biological factors are applied to cause oxidative stress of neurocytes and to attenuate neurological functions. The usage of the total extract of ginseng and individual ginsenosides can inhibit the aforementioned damages. For example, PC12 cells, primary astrocytes, mesencephalic or spinal cord neuron cultures, and hippocampal CA1 neurons are exposed to H_2O_2, dopamine, $^{\bullet}OH$, glutamate, and 1-methyl-4-phenylpyridinium ion or stimulated by epidermal growth factor and cerebral ischemic reperfusion factor. The application of ginsenosides can modify the biochemical indices of middle cerebral artery occlusion of male Wistar−Kyoto rats, Alzheimer of SAMP8 mice, and acrylamide-fed Sprague−Dawley rats. Dietary supplementation of total extract of ginseng or individual ginsenosides improves the biochemical indices of these experimental animals. The mechanism of ginsenosides in protecting neural systems can be summarized on the basis of experimental results from individual ginsenosides applied. For example, the antiamnestic and antiaging effects of Rg1 and Rb1 are due to increased expression of brain derived neurotrophic factor, BCL-2, and antioxidant enzyme, to form new synapses and to inhibit apoptosis and calcium overload.[190] Total extract of ginseng inhibits *N*-methyl-D-aspartate (NMDA)-induced increase of intracellular $[Ca^{2+}]$ in cultured hippocampal neurons.[191] Moreover, Rg3 inhibits NMDA receptor by increasing the concentration of glycine[192] and attenuates homocysteine-induced excitotoxicity.[193] The components of *β*-*N*-oxalyl-L-*α*,*β*-diaminopropionic acid, *γ*-aminobutyric acid, glutamine, and arginine are the neuroexcitatory compounds in old ginsengs.[194] If more individual ginsenosides are employed in the same experimental system and more neuroexcitatory compounds are found in ginseng, the mechanism of ginsenosides toward neural system will be further clarified.

6.3. Effects on Radicals

The radical-induced oxidations of lipids, membranes, DNA, and proteins are proven to be chemopathogenesis in aging[195] and some fatal diseases.[196,197] Much work focuses on the expression of oxidative damage,[198] the determination of antioxidant levels,[199] and the designation of antioxidants.[200] The high activity to trap radicals implies that the antioxidant may be a candidate as a chemopreventive drug.[201] The radical-scavenging properties of ginseng extracts and individual ginsenosides are collected in Table 5.

As can be seen in Table 5, water extract is usually used to determine the radical-scavenging properties of ginseng. Tissue-cultured adventitious roots of ginseng with poly(ethylene

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Table 3. *In Vivo* Experimental Models for Evaluating the Active Components in Ginseng

initiators and disease	measurement of biochemical indices	active components	ref
streptozotocin-induced diabetic rats	blood glucose level, thiobarbituric acid reactive substances (TBARS), glutathione peroxidase, catalase (CAT), superoxide dismutase (SOD)	water extract of wild *Panax ginseng* leaf	153
streptozotocin-induced diabetic rats	urinary protein, creatinine clearance level, serum glucose level, glycosylated protein, N^{ε}-(carboxymethyl)lysine and receptors for advanced glycation endproduct expressions	water extract of heat-treated American ginseng	154
streptozotocin-induced diabetic rats	blood glucose level, hepatic glycogen, total cholesterol level, intravenous glucose tolerance test	malonyl-ginsenosides, malonic acid, PD	155
streptozotocin-induced diabetic rats	blood glucose level, total cholesterol and triglyceride levels, glutathione and TBARS, C-reactive protein	Re	156,157
Zucker diabetic fatty rats	body and organ weight, food intake, blood glucose level, plasma cholesterol and triglyceride levels, peroxisome proliferator-activated receptor	Asian and American ginseng	158
C57BL/6J *ob/ob* mice	blood glucose level, intraperitoneal glucose tolerance test	ethanol extracts of ginseng root and berry, water extracts of American *ginseng* root and *Scutellaria baicalensis*	159,160
C2C12 myotubes	glucose uptake in insulin-dependent or insulin-independent signaling pathway	Rc	161
benzo[α]pyrene-induced hepatotoxicity	plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT), glutathione (GSH) and glutathione *S*-transferase (GST), TBARS, immunohistochemical analysis for cytochrome P450 1A1	water extract of *Panax ginseng* root	162
nicotineamideadenine dinucleotidephosphate (NADPH)/ CCl_4- or Fe^{2+}/ascorbic acid- induced lipid peroxidation of liver microsomes	TBARS	saponin mixture of *Astragalus corniculatus Bieb. (Fabaceae)*	163
apolipoprotein E-knockout (apoE-KO) mice	serum total cholesterol and triglyceride levels, oxidative low density lipoprotein (oxLDL), cell differentiation antigen 40, matrix metalloproteinase 9 expression	total extract of *Panax notoginsenosides*	164
high-fat diet-induced obesity in mice	pancreatic lipase activity, plasma triacylglycerol level, body and parametrial adipose tissue weights	water extract and Rg1, Re, Rb1, Rc, Rb2, Rd	165
2,3,7,8-tetrachlorodibenzo-*p*-dioxin-exposed guinea pig	NADPH-cytochrome P450 reductase activity, TBARS, 7-ethoxycoumarin-*O*-deethylase and benzphetamine-*N*-demethylase activities	water extract of *Panax ginseng*	166
glycation of hemoglobin	TBARS	ethanol extract of Korean red ginseng	167
ethanol-induced teratogenesis of female ICR mice	quantitative real-time PCR analysis, morphological scoring of embryos	total extract of black ginseng	168
acute cold-induced stress of male Sprague−Dawley rats	catecholamines, glucocorticoids, the tricarboxylic acid cycle, tryptophan (nicotinate), gut microbiota metabolites	total extract of ginseng	169
H3N2 influenza virus antigen in female ICR mice	specific IgG and IgG isotypes, hemagglutination inhibition assay, lymphocyte proliferation, IFN-γ and IL-5	Re	170

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Table 4. Nitric Oxide and Nervous System Employed To Evaluate the Active Components in Ginseng

experimental system	measurements of biochemical indices	active components	ref
acetylcholine (ACh)-induced contraction of human bronchial strips	contents of NO and cyclic guanosine monophosphate (cGMP)	total extract of ginseng	173
electric-stimulated or chemically induced contraction of monkey cerebral arterial strips	relaxation of cerebral arterial strips	water extract of *Panax ginseng*	174
H_2O_2-induced oxidative stress of primary astrocytes	cell viability, catalase (CAT), SOD, glutathione peroxidase and reductase	water extract of *Panax ginseng*	175
$^{\bullet}$NO production from sodium nitroprusside in phosphate-buffered saline	the amount of $^{\bullet}$NO measured based on Griess reaction	methanol extracts of white ginseng	176
acrylamide-induced neurotoxicity of Sprague−Dawley rats	SOD, lactate dehydrogenase, Na^+- and K^+-adenosine triphosphate enzyme, serum creatine kinase activity, 3,5,3′-triiodothyronine and L-thyroxine, TBARS in brain homogenate	total extract of *Panax ginseng* containing 3.6% Rg3	177
1-methyl-4-phenylpyridinium ion-induced neurotoxicity of PC12 cells	thioredoxin-1 expression, cell viability, content of lactate dehydrogenase released from damaged cells	PT-type ginsenoside with Rg1, R1 and Re as the main components	178
Alzheimer's disease-caused memory loss and impairment in aged SAMP8 mice	glutathione peroxidase, SOD, TBARS, postsynaptic density-95, phosphor-*N*-methyl-D-aspartate receptor 1, phospho-calcium-calmodulin dependent kinase II, phospho-protein kinase A, catalytic β subunit, protein kinase C γ subunit, brain derived neurotrophic factor	total extract containing Ra (2.9%), Rb1 (18.3%), Rb2 (9.1%), Rc (9.7%), Rd (8.2%), Re (23.4%), Rf (3.5%), Rg1 (7.6%), Rg2 (3.6%), Rg3 (4.7%), Ro (3.8%)	179
H_2O_2-induced oxidative stress of primary spinal cord neuron cultures in Sprague−Dawley rat embryos	surviving neurons and the length of neuritis, apoptotic spinal cord neurons	total extract of *Panax ginseng*, Rb1, Rg1	180
glutamate-induced oxidative stress of primary mesencephalic culture	lactate dehydrogenase release, propidium iodide uptake, *N*-methyl-D-aspartate receptor	Rb1, Rg1	181
epidermal growth factor-responsive neural stem cells differentiation	immunocytochemistry and confocal microscopy, fluorescence images of intracellular $[Ca^{2+}]$	Rg3, Rk1, Rg5	182
$^{\bullet}$OH-promoting ischemic damage of hippocampal CA1 neurons	intracerebroventricular infusion, hippocampal blood flow	Rb1	183
dopamine-induced apoptosis of PC12 cells	DNA fragmentation, nitrite content, 2,7-dichlorofluorescein fluorescent to monitor intracellular ROS, caspase-3 activity, cytochrome *c* release	Rg1	184
cerebral ischemia-reperfusion induced impairment of neurological responses, memory and caudate-putamen neuronal apoptosis in a vascular dementia rat model	expression of BCL-2, HSP70, BAX and tumor protein 53 (P53)	Rg2	185
glutamate-induced neurotoxicity in PC12 cells	TBARS, NO content, intracellular $[Ca^{2+}]$, protein expression levels of calpain II, caspase-3 and Aβ1−40	Rg2	186
acetylcholine (ACh)- or UV-induced contraction of canine corpus cavernosum	contents of NO, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP)	Rg3	187
Ca^{2+}- and H_2O_2-induced swelling of mitochondrial permeability in rat brain	mitochondrial swelling, ROS, mitochondrial energy metabolism	(20*S*)-Rg3	188
middle cerebral artery occlusion in male Wistar−Kyoto rats	regional cerebral blood flow, SOD, glutathione peroxidase, TBARS and ATP contents	(20*S*)-Rg3	189

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Table 5. The Evaluation of the Active Components in Ginseng by Interaction with Radicals

experimental system	active components	ref
inhibition of Fe^{2+}/cysteine-induced lipid peroxidation of rat liver microsome measured by TBARS	water extract of ginseng	202
inhibition of H_2O_2 and ferric nitrilotriacetate-induced peroxidation of arachidonic acid measured by electron spin resonance (ESR) with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as spin label, and by TBARS	water extract of ginseng	203
interaction with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), $^{\bullet}OH$, ·NO, and $O_2^{-\bullet}$ measured by ESR with DMPO as spin label	water extract of Korean ginseng	204
chelation of Fe^{2+}, Cu^{2+}, and Fe^{3+}, inhibition of Fe^{2+}, Cu^{2+}, or Fe^{3+}-induced peroxidation of linoleic acid measured by ammonium thiocyanate, oxygen consumption in linoleic acid emulsion with hemoglobin as prooxidant, scavenging DPPH, inhibition of $^{\bullet}OH$-induced oxidations of deoxyribose and DNA, inhibition of autoxidation of mouse brain homogenate	total extract of American ginseng	205
inhibition of Cu^{2+}-induced oxidation of human low density lipoprotein (LDL), AAPH-induced oxidations of pBR322 plasmid DNA and soybean α-phosphatidylcholine bilayer lipid, cleavage of deoxyribose by $^{\bullet}OH$, and interaction with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\cdot}$)	methanol extract of ginseng	206
interaction with $^{\bullet}OH$ measured by 2′,7′-dichlorofluorescin diacetate, $O_2^{-\bullet}$ measured by dihydroethidium, inhibition of the oxidative injury of cardiomyocytes exposed to either H_2O_2 or antimycin A	75% ethanol extract of American ginseng berry, and Re	207−209
scavenging $O_2^{-\bullet}$, DPPH, $^{\bullet}OH$, and H_2O_2, chelating Fe^{2+}, and reducing Fe^{3+}	water extracts of *Salvia miltiorrhiza* and *Panax notoginseng*	210
measurement of Maillard reaction product (MRP), scavenging $O_2^{-\bullet}$, DPPH, $^{\bullet}OH$, $ONOO^-$, allophycocyanin assay	extracts of Korean and American heat-processed ginseng	211
AAPH-induced hemolysis of erythrocytes, Fe^{2+} and ascorbic acid-induced lipid peroxidation of brain homogenates of Sprague−Dawley rats	water or *n*-butanol extracts of *Panax quinquefolium*, *Panax notoginseng*, *Codonopsis pilosula*, *Pseudostellaria heterophylla*, and *Glehnia littoralis*	212
scavenging DPPH, inhibition of the peroxidation of linoleic acid, and prevention of the formation of lipid peroxide in Fe^{2+} and ascorbic acid-induced oxidation of rat liver homogenate	ethyl acetate extract of Korean ginseng leaves	213
scavenging DPPH in alginate biodegradable film incorporating white and red ginseng extracts	extract of white and red ginseng	214
inhibition of the oxidation of deoxyribose induced by ferrous ammonium sulfate, ascorbic acid, and H_2O_2, determination of TBARS and $O_2^{-\bullet}$, inhibition of self-oxidation of 1,2,3-phentriol	polysaccharides in boiling-water extract of ginseng	215
scavenging $^{\bullet}OH$ measured by ESR with DMPO as spin label	(20*S*)-Rg3, (20*R*)-Rg3, Rk1, Rg5, Rb1	216,217
AAPH-induced hemolysis of human erythrocytes	Rb1, Rb3, Rc, Rd, Re, Rg1, Rg2, Rg3, Rh1, Rh2, R1, PD, and PT	218,219

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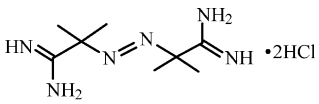
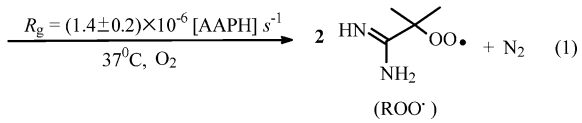
glycol)[220] and linoleic or α-linolenic acids[221] in a bioreactor exert high antioxidant capacity because much more ginsenosides and phenolics are enriched by the culture treatment. The tissue-culture treatment together with oxygen or Cu^{2+} employed can enhance the activities of glucose-6-phosphate dehydrogenase, shikimate dehydrogenase, cinnamyl alcohol dehydrogenase, and phenylalanine ammonia lyase, and increase the amount of phenolics, flavonoids, cysteine, and non-protein thiol in ginseng roots. As a result, the ability of ginseng extract to trap DPPH increases about 76%.[222,223] •OH usually acts as the radical resource in screening the radical-scavenging properties of ginseng extract. The extract from red ginseng exhibits higher ability to trap •OH than that from white ginseng when •OH is measured by ESR with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) being the spin labeling agent. The high ability of red ginseng is due to abundant contents of (20*S*)-Rg3, (20*R*)-Rg3, Rk1, and Rg5 produced by heating original ginseng to form red ginseng.[216] Moreover, Rb1 can convert into (20*S*)-Rg3, (20*R*)-Rg3, Rk1, and Rg5 in the heating process. (20*S*)-Rg3 and Rg5 play the main role in scavenging •OH, while (20*R*)-Rg3 and Rk1 only show weak activities toward •OH. In addition, Maillard reaction products (MRPs) formed between Rb1 and glycine in the heating process have high ability to scavenge •OH.[217] Re cannot trap DPPH because no hydrogen atom in Re can be abstracted by N-centered radical.[209] Also, the protective effect of water extract from ginseng on DNA against radiation-induced damage is better than that of individual ginsenosides,[224] and the radical-scavenging property of total extract from ginseng is ascribed to free or esterified phenolics and polysaccharides.[215] It is found that white ginseng contains a similar amount of phenolic acids (27.2 mg/100 g) to the red ginseng (26.8 mg/100 g).[225]

The antioxidant capacities of total extract of ginseng and 11 individual ginsenosides,[218] as well as corresponding sapogenins, PD, and PT,[219] have been compared in 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced hemolysis of human erythrocytes. Rb1, Rb3, Rc, Rd, Rg3, and Rh2 are typical PD-type ginsenosides, and Rh1, Rg1, Rg2, R1, and Re are typical PT-type ginsenosides. As shown in eq 1, AAPH



AAPH



(1)

provides peroxyl radical (ROO•) with a stable rate (R_g) via decomposition at 37 °C and is often used as peroxyl radical resource in the oxidations of LDL[226] and erythrocytes.[227]

Figure 30 outlines the effects of the mixtures of PD- or PT-type ginsenosides on AAPH-induced hemolysis of human erythrocytes. The mixture of PD-type ginsenosides protects erythrocytes against AAPH-induced hemolysis effectively with increasing concentration, and the mixture of PT-type ginsenosides exhibits a completely reverse result. However, both PD and PT promote hemolysis with increasing concentration, indicating that the sapogenins of PD- and PT-type ginsenosides play a prooxidant role in this case. Furthermore, the effects of various individual ginsenosides on AAPH-induced hemolysis

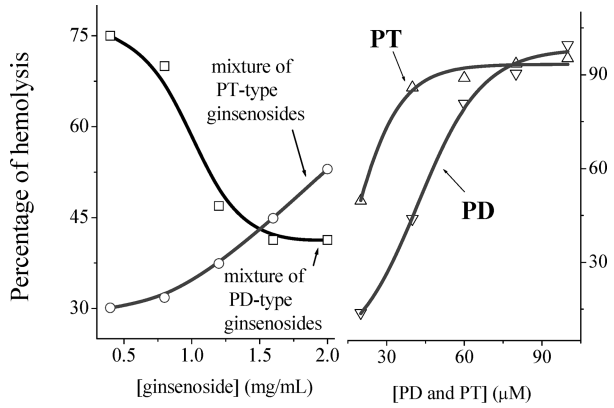


Figure 30. The effects of the mixtures of PD- or PT-type ginsenosides together with PD and PT on AAPH-induced hemolysis of human erythrocytes. Erythrocytes were suspended in PBS (3.0%, v/v), and 40 mM AAPH was added at 37 °C for 3 h.[219]

are illustrated in Figure 31.[219] In addition to Rh2 and Rg3, other individual ginsenosides prevent hemolysis concentration-dependently. The absence of sugar moieties at the 20-position makes Rh2 and Rg3 promote hemolysis with increasing concentration. In contrast, the absence of sugar moieties at the 20-position does not affect Rg2 and Rh1 to prevent hemolysis, demonstrating that sugar moieties at the 6- and 20-positions make PT-type ginsenosides inhibit AAPH-induced hemolysis.

Further research deals with the process of ginsenosides in inhibiting AAPH-induced hemolysis. Figure 32 outlines a typical hemolysis process in the presence of various concentrations of Rb1. The blank experiment shows that hemolysis does not take place immediately when AAPH is added to erythrocytes suspension. The endogenous antioxidant systems protect erythrocytes against the attack from radicals to generate an inhibition period (t_{inh}).[228] The t_{inh} increases with the concentration of Rb1, and the relationships between t_{inh} and concentrations of Rb1 along with other individual ginsenosides are illustrated in Figure 33. Rc and Rb1 inhibit AAPH-induced hemolysis in a concentration-dependent manner. Rb1 contains a sucrose moiety at the 3- and 20-positions; Rc contains a sucrose moiety at the 3-position and a xylosylglucose moiety at the 20-position. Hence, disaccharide moieties at the 3- and 20-positions enhance the ability of ginsenosides to protect erythrocytes. The protective effects of other ginsenosides on erythrocytes do not increase with the concentration. PD-type ginsenosides including Rb3, Rh2, Rd, and Rg3 and PT-type ginsenosides including Rg1 and Rh1 even improve the hemolysis with the increase of their concentrations, while Re is inert in this case.

7. CONCLUSION AND PERSPECTIVES

It is widely accepted that maintaining health is more important than treating diseases. Not only does botanical dietary intake supplement necessary nutrition, but the antioxidant components potentially reinforce the endogenous antioxidant systems. In the case of quality assurance and strict standardization control, ginseng may be a dietary supplementation in the future.[229] But the following aspects still need investigating.

(1) The conditions that affect the accumulation of antioxidant components in ginseng during the cultivation should be further clarified by analyzing ginsenosides,

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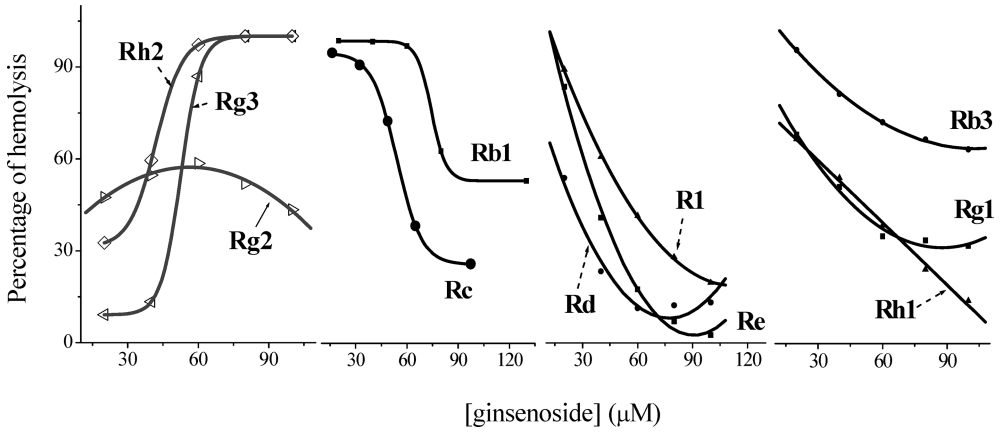

Figure 31. The effects of various individual ginsenosides on AAPH-induced hemolysis of human erythrocytes. Erythrocytes were suspended in PBS (3.0%, v/v), and 40 mM AAPH was added at 37 °C for 3 h.[219]

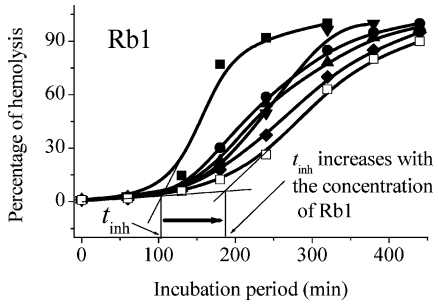

Figure 32. The relationship between the percentage of hemolysis and concentration of Rb1 in AAPH-induced hemolysis of human erythrocytes. The concentration of Rb1 is 0, 5, 10, 15, 20, and 25 μM (from the left line to the right one), respectively. The concentrations of AAPH and erythrocytes are 39 mM and 2.4% (v/v in PBS).[218]

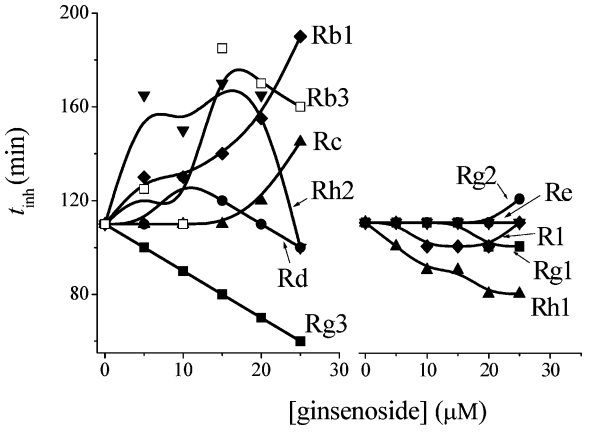

Figure 33. The relationship between the concentration of individul ginsenosides and inhibition time, t_{inh}, in AAPH-induced hemolysis of human erythrocytes. The concentrations of AAPH and erythrocytes are 39 mM and 2.4% (v/v in PBS), respectively.[218].

phenolics, and polysaccharides. Meanwhile, improvement of analysis and isolation technique helps us to obtain more ginsenosides and novel antioxidants in ginseng.

(2) Many more synthetic studies on the construction of sapogenin, glycosylation, and deglycosylation should be carried out chemically and biochemically because these synthetic works can provide a large amount of a certain ginsenoside for special pharmacological action.

(3) The systematic comparison of the activities of various individual ginsenosides in the same biological experimental system will clarify the influence of the position and the kind of sugar moiety on the bioactivity. The mutual effects among different components in ginseng should also be explored since the pharmacological actions of ginseng are not the simple sum of various components.

Based on the detailed results from all the investigation aspects, it is reasonable to believe that ginseng will be not only a medicinal herb to treat diseases but also a resource of natural antioxidants to maintain health.

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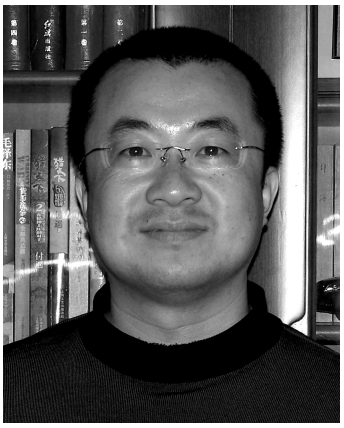
Biography



Zai-Qun Liu (born April 24, 1968, Changchun, China) studied at Jilin University (Changchun, China) from 1986 to 1996 and received his B.Sc. in 1990, M.Sc. in 1993 (major in organic synthesis under microwave irradiation), and Ph.D. in 1996 (major in the template effect of amine on the synthesis of aluminophosphate molecular sieves). He was a Postdoctorate Fellow at Lanzhou University (Lanzhou, China) from 1996 to 1998 and began with research work on antioxidants. He became Associate Professor in 1999 and Professor

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