Conversion of Ginsenoside Rb1 into Six Types of Highly Bioactive Ginsenoside Rg3 and Its Derivatives by FeCl₃ Catalysis

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Ginsenoside Rb1 is an important saponin of ginseng(s); however, Rb1, with 3- O- and 20- O-sugar moieties, has low bioavailability. Here, we report the derivatization of ginsenoside Rb1 to completely generate six types of highly bioactive minor ginsenosides Rg3 and its derivatives by FeCl₃ catalysis, the reaction conditions are similar to enzymatic reaction conditions. In FeCl₃ catalysis, the only 20- O-sugar moiety of ginsenoside Rb1 was decomposed into the minor ginsenosides Rk1 and Rg5 with newly produced C-20 ethylene bands; but also hydrolyzed into 20(S)-Rg3 and 20(R)-Rg3; subsequently the C-24(25) ethylene bands of 20(S)-Rg3 and 20(R)-Rg3 were hydrated to 20(S)-25-OH-Rg3 and 20(R)-25-OH-Rg3. After separation of reaction mixture from 34 g ginsenoside-Rb1 by silica-gel-column, the 3.3 g sample I of TLC top-band consisting of Rg5 and Rk1, 8.7 g sample II of TLC middle-band consisting of 20(S)-Rg3 and 20(R)-Rg3, 3.5 g sample III of TLC bottom-band consisting of unknown product-I and -II including 20(S)-25-OH-Rg3, were obtained. The sample III consisting of unknown product-I and -II was purified by crystallization, and identified to 20(S)-25-OH-Rg3 and 20(R)-25-OH-Rg3 by HPLC-Evaporative Light Scattering Detector (ELSD) and NMR. Therefore, six types of minor-ginsenosides Rk1, Rg5, 20(S)-Rg3, 20(R)-Rg3, 20(S)-25-OH-Rg3 and 20(R)-25-OH-Rg3 were successfully prepared from ginsenoside Rb1 by FeCl₃ catalysis. FeCl₃ has low toxicity and is inexpensive, and the reaction conditions are similar to enzymatic reaction conditions; thus, this method is applicable to the development of ginseng-based drugs.

Key words ginsenosides Rb1; ginsenoside conversion; FeCl₃ catalysis; ginsenoside Rg3; Rg3 derivative

Ginseng, a well-known traditional medicinal herb, comprises 14 species within the genus Panax (Araliaceae family); however, the most widely used Panax species are Panax ginseng (Korean or Asian ginseng), P. quinquefolium (American ginseng), and P. notoginseng (Notoginseng or Sanchi ginseng).¹⁻⁴) The ginsenosides, which are believed to be the main active components in ginseng, are a special group of triterpenoid saponins that can be classified into dammarane- and oleanane-type ginsenosides. To date, over 150 ginsenosides have been identified from ginseng plants; however, 80–90% of ginsenosides are dammarane-type ginsenosides (Rb1, Rb2, Re, Rd, Rg1, Re, and Rf in Korea ginseng; Rb1, Re, Rb2, Rc, Rd, and Rg1 in American ginseng and; Rg1, Rb1, R1, Rd, and Re in Notoginseng), which shows that Rb1 is a representative ginsenoside of ginseng.⁵) Ginsenoside Rb1 cannot be directly absorbed by the human body due to the four glycosides of 3-O-Glc-Glc- and 20-O-Glc-Glc-substituted on the Rb1 molecular core, so Rb1 has low bioavailability.⁶⁻⁸) After oral intake of ginseng, the major natural ginsenosides are hydrolyzed in the human intestinal tract, and the major ginsenosides are converted into more active forms of the minor ginsenosides, which are subsequently absorbed and can then exhibit their physiological activity; however, the rates of conversion are very low.⁹⁻¹²) The minor ginsenosides Rg3, Rg5, Rk1 and other derivatives have various important pharmacological activities such as antitumor, antidiabetes, antithrombotic and anti-Alzheimer’s disease effects.¹³⁻¹⁶) Therefore, the production of highly bioactive and highly bioavailable minor ginsenosides (Rg3 and its derivatives) from high-abundance ginsenosides with low bioavailability is key to the development of natural saponins and ginseng-based medicines. The highly active minor ginsenoside Rg3 is obtained from the less active ginsenoside Rb1 using acidic or basic conversion methods, but this methodology usually results in the formation of many by-products and severe environmental pollution.¹⁷) Moreover, enzymatic or microbiological methods have been widely used in the conversion of ginsenoside into Rg3, Rg5, Rk1 and 25-OH-Rg3 as these methods possess many advantages such as mild conditions, fewer by-product, and increased environmental friendliness; however, the enzymatic method has many shortcomings such as high cost and ease of inactivation.¹⁸,²²) Metals or ionic liquids can be used in the production of low-molecular-weight organic compounds such as in the conversion of sugar into hydroxymethyl furfural, and applications of metal catalysis in the conversion of biomass and in the iron-catalyzed C–C bond-forming functionalization of heterocycles have revolutionized contemporary chemistry.²³,²⁴) Nevertheless, reports of metal catalysis for the production of high-molecular-weight natural products such as saponins are rare. Herein, we report the inexpensive conversion of low bioavailability ginsenoside Rb1 into highly bioactive minor ginsenoside Rg3 and its derivatives by FeCl₃.

Results and Discussion

Minor Ginsenoside Products from Ginsenoside Rb1 by FeCl₃ Ginnsenoside Rb1 has two sugar-moieties, 3-O-Glc-
Glc- and 20-O-Glc-Glc-. We accidentally found that only the 20-O-sugar moiety of ginsenoside Rb1 can be completely reacted into highly bioactive 20(S)-Rg3 (1), 20(R)-Rg3 (2), Rg5 (3), Rk1 (4), unknown product-I [20(S)-25-OH-Rg3] (5) and unknown product-II [20(R)-25-OH-Rg3] (6) by FeCl₃ catalysis using conditions similar to the enzymatic reaction conditions at a much lower cost than can be achieved with the enzymatic method. Minor ginsenoside products 20(S)-Rg3, 20(R)-Rg3, Rg5 and Rk1 were identified by HPLC using standards ginsenosides 20(S)-Rg3, 20(R)-Rg3, Rg5 and Rk1. Unknown minor ginsenoside product-I [20(S)-25-OH-Rg3] and product-II [20(R)-25-OH-Rg3] were identified by HPLC-Evaporative Light Scattering Detector (ELSD) and NMR methods.

A 2% solution of ginsenoside Rb1 in 40% ethanol was mixed with the same volume of 1.3 mol FeCl₃ solution, and the mixture was reacted at 40°C for 12 h (the final Rb1 concentration was 1%, and the FeCl₃ concentration was 0.65 mol). After the reaction, the reaction mixture was diluted with water, extracted with water-saturated n-butanol, washed with water, and dried by vacuum distillation to obtain the derivatized samples. Each sample (4 mg) was dissolved in 1 mL methanol and analyzed by TLC and HPLC as shown in Figs. 1A, B.

Figure 1A, B shows that the products from the reaction of ginsenoside Rb1 with FeCl₃ appear as three bands in the TLC (Fig. 1A) and six peaks in the HPLC chromatogram (Fig. 1B). Comparing the TLC and HPLC results, the product corresponding to the top band of the TLC was the mixture of the isomers Rg5 (3) and Rk1 (4), the product corresponding to the middle band of the TLC was the mixture of the isomers 20(S)-Rg3 (1) and 20(R)-Rg3 (2), and the product corresponding to the bottom band of the TLC was the mixture of unknown product-I (5) and product-II (6) including 20(S)-25-OH-Rg3 (5), which requires further structural clarification.

The effects of the concentration of substrate Rb1, the ethanol concentration in the reaction solvent, the temperature, and the reaction time on the Rb1 conversion were examined. The good condition of ginsenoside Rb1 conversion by FeCl₃ was that 1% Rb1 in 35 to 40% ethanol solvent was reacted at 40°C by 0.65 mol FeCl₃. To understand the effect of reaction time, a 1% solution of ginsenoside Rb1 in 35% ethanol was reacted with 0.65 mol FeCl₃ at 40°C for 10, 30 min, 1, 5, 9, 10, 12, 24 and 48 h. After reacting for 10 min, minor ginsenosides Rg5, Rk1, 20(S)-Rg3 and 20(R)-Rg3 began to be produced from ginsenoside Rb1; after reacting for 12 h, the ginsenoside Rb1 substrate was completely converted into Rb1, Rg5, 20(S)-Rg3, 20(R)-Rg3, unknown product-I and product-II. After reacting for 48 h, the yields of product-I and product-II had increased, and the yields of Rk1, Rg5, 20(S)-Rg3 and 20(R)-Rg3 had decreased (Fig. 1 Right). Therefore, the best conditions for producing the six types of minor ginsenosides from ginsenoside Rb1 were a 1% solution of ginsenoside Rb1 in 35–40% ethanol solvent reacting with 0.65 mol FeCl₃ at 40°C.

According above results, when producing the six types of minor ginsenosides Rk1, Rg5, 20(S)-Rg3, 20(R)-Rg3, product-I and product-II from Rb1, the 1% ginsenoside Rb1 in 35 to 40% ethanol solvent was reacted at 40°C for 12 h by 0.65 mol FeCl₃.

Preparation and Separation of Products from Rb1 by FeCl₃. In the preparation of minor ginsenosides, the 22 g of ginsenoside Rb1 was dissolved in 1 L of 35% ethanol–water, and the solution was mixed with the same volume of 1.3 mol...
FeCl₃, in 35% ethanol–water, and then the mixture was reacted at 40°C for 12 h. After the reaction, water was added to the solution, and the mixture was tared with AB-8 macroporous-resin to remove sugars and other impurities and then subjected to a D-280 anion exchange resin column to decolorize the material; the products were concentrated and dried by vacuum distillation to afford 13 g of the product showing three components by TLC were pooled and dried by vacuum distillation to obtain three samples each showing one band in TLC; 3.3±0.8 g of sample I (top band in TLC), 8.7±0.9 g of sample II (middle band in TLC), and 3.5±0.8 g of sample III (bottom band in TLC) as shown in Fig. 2A. The experiment was repeated 3 times.

When separated samples I, II and III, each showing one band in TLC, from Rb₁ were analyzed by HPLC with a photodiode array detector; sample I was shown to contain the isomers Rg₅ and Rk₁; and sample II was the isomers 20(S)-Rg₃ and 20(R)-Rg₃ based on comparison to standards of Rg₅, Rk₁, 20(S)-Rg₃ and 20(R)-Rg₃ (Fig. 2B); however, separated sample III containing unknown product-I and -II did not show an HPLC-active peak. Sample III, containing unknown product-I and -II, was examined using HPLC-ELSD and showed two peaks as shown in Fig. 2C.

**Purification and Identification of Unknown Product-I and II from Sample III**

To further clarify the contents of sample III (one band in TLC), containing unknown product-I and -II, sample III was further separated and purified by crystallization to obtain pure compounds. A 3 g portion of sample III was dissolved in 60 mL of 60% methanol and stored at room temperature to obtain 0.25 g of a white precipitate (product-II) in 98% purity. After the precipitation of product-II, the remaining solution was dried by vacuum distillation, dissolved in hot methanol, and cooled in an ice bath to generate a precipitate. This procedure was repeated several times to generate 0.18 g of pure product-I; the HPLC-ELSD retention time of product-I (98% purity) was the same as that of the 20(S)-25-OH-Rg₃ standard, which proved that product-I was 20(S)-25-OH-Rg₃.

An NMR method was used to determine the structures of product-I and -II. Although product-I was identified by comparison to the 20(S)-25-OH-Rg₃ standard by HPLC-ELSD, to more carefully confirm their structures, the 13C-NMR spectra of product-I and -II were collected. The NMR data in Table 1 correspond with previous reports, which confirms the structure of product-I is 20(S)-25-OH-Rg₃: 12β,20(S),25-trihydroxy dammar-3-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl, and the structure of product-II is 20(R)-25-OH-Rg₃: 12β,20(R),25-trihydroxy dammar-3-β-D-glucopyranosyl-β-(1→2)-β-D-glucopyranosyl.

**Pathways of Ginsenoside Rb₁ Conversion by FeCl₃ into Rg₃ and Its Derivates**

It is shown from above results that a 1% solution of ginsenoside Rb₁ was completely converted into six types of minor ginsenosides Rg₃ and its derivatives by 0.65 mol FeCl₃ at 40°C in 12 h. According to Fig. 1 Right, the minor ginsenosides 20(S)-Rg₃ (1) and 20(R)-Rg₃ (2), Rg₅ (3) and Rk₁ (4) began to be produced from ginsenoside Rb₁ after the same reaction time; subsequently, the C-24(25) double bonds of 20(S)-Rg₃ and 20(R)-Rg₃ were hydrated to 20(S)-25-OH-Rg₃ (5) and 20(R)-25-OH-Rg₃ (6). However, the 3-O-sugar-moiety of ginsenoside Rb₁ did not react.

The reaction pathway for the conversion of ginsenoside Rb₁ into high bioactive ginsenoside Rg₃ and its derivatives by FeCl₃ should be that the only 20-O-sugar-moiety of ginsenoside Rb₁ was decomposed into the minor ginsenosides Rk₁ and Rg₅ with newly produced C-20 ethylene bands; but also hydrolyzed into 20(S)-Rg₃ and 20(R)-Rg₃; subsequently, the C-24(25) ethylene bands of 20(S)-Rg₃ and 20(R)-Rg₃ were hydrated to 20(S)-25-OH-Rg₃ and 20(R)-25-OH-Rg₃ as shown

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**Fig. 2. Three Bands of Products from Rb₁ Separated by Silica Gel Column Chromatography**

(A) The separated three products, samples I, II and III, in TLC. Rg₅, Rk₁, 20(S)-Rg₃, 20(R)-Rg₃ and 20(S)-25-OH-Rg₃ are standards. I. Separated product sample III. 2. Separated product sample II. 3. Separated product sample-I. (B) HPLC chromatograms of separated product samples I and II. (C) HPLC-ELSD chromatogram of separated product sample III.
in Fig. 3.

Conclusion
Six types of minor-ginsenosides Rk1, Rg5, 20(S)-Rg3, 20(R)-Rg3, 20(S)-25-OH-Rg3 and 20(R)-25-OH-Rg3 are successfully prepared from ginsenoside Rb1 by FeCl₃ catalysis using conditions similar to the enzymatic reaction conditions at a much lower cost than can be achieved with the enzymatic method.

FeCl₃ has low toxicity, and the reaction conditions are similar to enzymatic reaction conditions, and FeCl₃ is much less expensive than the enzyme, making this method suitable for the development of ginseng-based drugs.

The problems associated with the separation of 20(S)-25-OH-Rg3 and 20(R)-25-OH-Rg3 involving the lack of peaks observed with HPLC with a photodiode array detector, but the presence of peaks from the mixture of 20(S)-25-OH-Rg3 and 20(R)-25-OH-Rg3 with the Rk1, Rg5, 20(S)-Rg3, and 20(R)-Rg3 requires further study.

Experimental

Materials
The ginsenoside Rb1 and standard ginsenosides 20(S)-Rg3, 20(R)-Rg3, Rg5, Rk1 and 20(S)-25-OH-Rg3 were obtained from GreenBio Co., Ltd. (Dalian, P. R. China) and Tianle Co., Ltd. (Shenyang, P. R. China). The 60-F 254 silica gel plates were Merck (Darmstadt, Germany) to use for the TLC analysis. The AB-8 macroporous resin and D-280 anion exchange resin were obtained from Nankai University, Tianjin, P. R. China.

General Experimental Procedures
The ginsenoside Rb1 and standard ginsenosides 20(S)-Rg3, 20(R)-Rg3, Rg5, Rk1 and 20(S)-25-OH-Rg3 were obtained from GreenBio Co., Ltd. and Tianle Co., Ltd. The 60-F 254 silica gel plates were Merck (Darmstadt, Germany) to use for the TLC analysis; and the content ratio of ginsenosides in the silica gel plate was determined by scanning the TLC spots using a Shimadzu CS-930 TLC scanner (Shimadzu, Kyoto, Japan). The products 20(S)-Rg3, 20(R)-Rg3, Rk1 and Rg5 from ginsenoside Rb1 by FeCl₃ were examined by HPLC (Waters 2695 Separations Module with Waters 2996 Photodiode Array Detector, Waters Corp., Milford, U.S.A.) according to previously reported method. A Zhonghuida C-18 chromatographic column (5 μm, ϕ4.6 mm×250 mm, Zhonghuida Corp., Dalian, China) was used for separation using mobile phase A (acetonitrile) and B (water) as follows: 0–20 min, 20% A; 20–31 min, A from 20 to 32%; 31–40 min, A from 32 to 43%; and 40–70 min, A from 43 to 100%. The unknown product-I and -II from ginsenoside Rb1 conversion by FeCl₃ were examined by HPLC-ELSD (Waters 2695 Separations Module with Waters 2424 Evaporative Light Scattering Detector Waters Corp., Milford, U.S.A.). A Zhonghuida C-18 chromatographic column (5 μm, ϕ4.6 mm×250 mm, Zhonghuida Corp.). The mobile phase A

| C-No. | Product-I | Product-II | C-No. | Product-I | Product-II |
|-------|-----------|------------|-------|-----------|------------|
| C-1   | 39.34     | 39.27      | C-23  | 19.33     | 18.86      |
| C-2   | 26.91     | 26.80      | C-24  | 45.89     | 45.74      |
| C-3   | 89.12     | 89.03      | C-25  | 69.92     | 69.83      |
| C-4   | 39.88     | 39.84      | C-26  | 30.07     | 30.06      |
| C-5   | 56.58     | 56.50      | C-27  | 30.37     | 30.35      |
| C-6   | 18.64     | 18.58      | C-28  | 28.30     | 28.25      |
| C-7   | 35.38     | 35.32      | C-29  | 16.79     | 16.73      |
| C-8   | 40.21     | 40.15      | C-30  | 17.23     | 17.46      |
| C-9   | 50.59     | 50.51      | 3-O-Glc- |
| C-10  | 37.12     | 37.05      | 1"    | 105.26    | 105.27     |
| C-11  | 32.24     | 32.30      | 2"    | 83.54     | 83.66      |
| C-12  | 71.20     | 70.98      | 3"    | 78.40     | 78.38      |
| C-13  | 48.77     | 49.42      | 4"    | 71.79     | 71.79      |
| C-14  | 51.92     | 51.89      | 5"    | 78.08     | 78.10      |
| C-15  | 31.61     | 31.54      | 6"    | 63.00     | 62.99      |
| C-16  | 27.07     | 26.88      | 3-O-Glc-(1→2)-Glc |
| C-17  | 54.88     | 50.87      | 1"    | 106.15    | 106.24     |
| C-18  | 16.03     | 15.97      | 2"    | 77.26     | 77.31      |
| C-19  | 16.57     | 16.52      | 3"    | 78.46     | 78.47      |
| C-20  | 73.55     | 73.43      | 4"    | 71.83     | 71.79      |
| C-21  | 27.44     | 23.01      | 5"    | 78.23     | 78.25      |
| C-22  | 36.73     | 44.21      | 6"    | 62.86     | 62.84      |
(acetonitrile) and B (water) was as follows: 0–20 min, 20% A; 20–31 min, A from 20 to 32%; 31–40 min, A from 32 to 43%; and 40–70 min, A from 43 to 100%. The injection volume was 20 \( \mu \)L, the flow rate was 3.2 mL/min, the column temperature was 35°C, the carrier gas was nitrogen under 0.1 MPa pressure, the ELSD drift tube temperature was 110°C, and the Impact device was Off. All reagents were chromatographic grade and were obtained from Merck, U.S.A.

The product mixture from ginsenoside Rb1 conversion by FeCl3 was separated using AB-8 macroporous resin and D-280 anion exchange resin (Nankai University, Tianjin, P. R. China) column. The single band in TLC from product mixture from ginsenoside Rb1 conversion by FeCl3 were separated using a silica gel (Qingdao Haiyang Chemical Co., Ltd., P. R. China) column. The structures of the unknown product-I and II were analyzed using NMR. Each of these was dissolved in pyridine-d5 and the NMR (Nuclear Magnetic Resonance) spectra were recorded using the BrukeAVANCE 600 (\( ^1 \)H: 600 MHz; \( ^13 \)C: 150 MHz) NMR spectrometer (Switzerland).

**Conversion Condition of Ginsenoside Rb1 by FeCl3**

A 2% Rb1 was dissolved in 35–40% ethanol–water, and mixed with the same volume of 1.3 mol FeCl3 solution in 35–40% ethanol–water, and reacted at 40°C for different time. The reaction mixture was diluted with 3 times the volume of water and extracted by water-saturated n-butanol; the water-saturated n-butanol layer was washed with water (3 times) and dried by vacuum distillation to obtain the reacted ginsenosides.

**Preparation and Separation of Products from Rb1 by FeCl3**

Using 35–40% ethanol as the solvent system, 1.3–1.4 M FeCl3 solution was mixed with the same volume of 2–2.2% ginsenoside Rb1 solution, and the mixture was reacted at 40°C for 12h. The reaction mixture was diluted with 3–6 times the volume of water, isolated using the previously reported methods of the AB-8 macroporous resin column and the anion exchange resin D280 column. The reaction mixture was eluted on the AB-8 macroporous resin column (the column volume was 20 times of dried ginsenoside weight).
to adsorb ginsenoside; then the FeCl₃ and sugar and other impurities were rinsed with water (about 5–8 times of the column volume) elution; then the column was eluted by 80–84% alcohol (about 4–5 times of the column volume) to wash the ginsenosides; the alcohol eluant was eluted on the anion-exchange resin D280 (volume was same with AB-8 column) to decolorize; then the decolorized solution was concentrated and dried by vacuum distillation to obtain the product mixture (with three bands in TLC) containing six types of minor ginsenosides Rg3 and derivatives.

The reaction product mixture from Rb₁ (with three bands in TLC) was separated using silica gel column. The mixture products were dissolved in methanol and chloroform, mixed with 80–100 mesh silica gels (2.3 times of the sample weight), constantly stirred, and dried to form powder; then the mixture powder was put in a column (diameter–height = 1:15 to 20) containing 300–400 mesh samplesilica-gel (20 times of sample weight); the top of column was put with 2 cm cotton. The column was first dried by 100% chloroform, then eluted with a solvent consisting of chloroform and methanol (8:8.1.2 (v/v)), the fractions were about 250 mL. In the elution, the mixture of Rg5 and Rk1 (top band of reaction mixture in TLC) was firstly eluted; second, the isomer mixture of 20(S)-Rg3 and 20(R)-Rg3 (middle band of reaction mixture in TLC); the last, the mixture of unknown product-I and -II (bottom band of reaction mixture in TLC) was eluted. After completely eluted the product-I and -II, the fractions with the same component by TLC were then collected and dried by vacuum distillation to obtain sample I containing Rg5 and Rk1 (top band in TLC), sample II containing 20(S)-Rg3 and 20(R)-Rg3 (middle band in TLC), and sample III containing unknown product-I and -II (bottom band in TLC).

The sample I of Rg5 and Rk1, and sample II of 20(S)-Rg3 and 20(R)-Rg3 were identified using HPLC by standards Rk₁, Rg₅, 20(S)-Rg₃ and 20(R)-Rg₃, and the monomers were purified by the preparative chromatography, respectively.

**Purification and Identification of Sample III Containing Unknown Product-I and -II**

The separated sample III with unknown product-I and II from silica gel column was purified by the crystallization method, and identified by the methods of TLC, HPLC-ELSD and NMR. The sample III containing product-I and -II was dissolved in 20 times volume for weight of 60% methanol, then stored at room temperature for about 2–3 days (with shaking 3–6 times of day to slowly evaporate small amount of solvent) to get white precipitate. Subsequently, the precipitate was collected by filtering method, washed with small amount of cold 50% methanol, dried to get purified product-II; the precipitation is repeated several times. After product-II precipitation, the remained solution was dried by vacuum distillation, dissolved in hot methanol, cooled in the ice pattern to precipitate, the experiment repeated several times to get product-I. The product-I was recognized by the HPLC-ELSD according to standard 20(S)-25-OH-Rg₃. The structures of purified product-I and -II were determined by the methods of HPLC-ELSD and NMR.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**References**

1) Choi H. K., Wen J. A., *Plant Syst. Evol.*, 224, 109–120 (2000).
2) “Biotransformation of Natural Products,” ed. by Jin F. X., Chapter 5, Chemical Industry Press, Beijing, 2009, pp. 74–113.
3) “Ginseng is the King of Herbs—Ginseng chemistry, Physiology activity and Pharmacokinetics,” ed. by Zhang J. T. Chemical Industry Press, Beijing, 2008, pp. 1–16, 34–44.
4) Christensen L. P., *Adv. Food Nutr. Res.*, 55, 1–99 (2009).
5) Liu C. Y., Song J. G., Li P. F., Yu H. S., Jin F. X., *J. Dalton Polytechnic Univ.*, 30, 79–82 (2011).
6) Hasegawa H., *J. Pharmacol. Sci.*, 95, 153–157 (2004).
7) Park C. S., Yoo M. H., Noh K. H., Oh D. K., *Appl. Microbiol. Biotechnol.*, 87, 9–19 (2010).
8) Jang H. A., Cho S., Kang S. G., Ko Y. H., Kang S. H., Bae J. H., Cheon J., Kim J. J., Lee J. G., *Urol. Int.*, 88, 463–469 (2012).
9) Kobashi K., *J. Trad. Med.*, 15, 1–13 (1999).
10) Hasegawa H., Lee K. S., Nagakura T., Tezuka Y., Uchiyama M., Katoda S., Saiki I., *Biol. Pharm. Bull.*, 23, 298–304 (2000).
11) Hasegawa H., Suzuki R., Nagakura T., Tezuka Y., Katoda S., Saiki I., *Biol. Pharm. Bull.*, 25, 861–866 (2002).
12) Tawab M. A., Bahr U., Karas M., Wurglins M., Schubert-Zsilavecz M., *Drug Metab. Dispos.*, 31, 1065–1071 (2003).
13) Lu P., Su W., Miao Z., Niu H., Liu J., Hua Q., *Chin. J. Integr. Med.*, 14, 33–36 (2008).
14) Kim M., Ahn B. Y., Lee J. S., Chung S. S., Lim S., Park S. G., Jung H. S., Lee H. K., Park K. S., *Biochem. Biophys. Res. Commun.*, 389, 70–73 (2009).
15) Kim S. N., Lee J. H., Shin H., Son S. H., Kim Y. S., *Planta Med.*, 75, 596–601 (2009).
16) Yang L. L., Hao J. R., Zhang J., Xia W. J., Dong X. F., Hu X. Y., Kong F., Cui X., *J. Pharm. Pharmacol.*, 61, 375–380 (2009).
17) Sunwoo H. H., Gujral N., Huebl A. C., Kim C. T., *Food Bioprocess Tech.*, 7, 1246–1254 (2014).
18) Yu H. S., Liu Q. M., Zhang C. Z., Lu M. C., Fu Y. Y., Im W. T., Lee S. Y., Jin F. X., *Process Biochem.*, 44, 772–775 (2009).
19) Chen G. I., Yang M., Song Y., Lu Z. Q., Zhang J. Q., Huang H. L., Wu L. J., Guo D. A., *Appl. Microbiol. Biotechnol.*, 77, 1345–1350 (2008).
20) Dong A. L., Ye M., Guo H. Z., Zheng J. H., Guo D., *Biotechnol. Lett.*, 25, 339–344 (2003).
21) Liu C. Y., Zhou R. X., Sun C. K., Jin Y. H., Yu H. S., Zhang T. Y., Xu L. Q., Jin F. X., *Ginseng Research*, 39, 221–229 (2015).
22) Liu C. Y., Jin Y. H., Yu H. S., Sun C. K., Guo P., Xiao Y. K., Zhang T. Y., Xu L. Q., Jin F. X., *Process Biochem.*, 49, 813–820 (2014).
23) Zhao H., Holladay J. E., Brown H., Zhang Z. C., *Science*, 316, 1597–1600 (2007).
24) Datraku S. L., Al-Afyouni M. H., Snyder B. E. R., Kneebone J. L., Neidig M. L., *J. Am. Chem. Soc.*, 136, 9132–9143 (2014).
25) Tanaka I., Kasai R., “Progress in Chemistry of Organic Natural Products, Vol. 46, Saponins of ginseng and related plants,” Springer, Berlin, 1984, pp. 1–76.