JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Studies on the Chemical Transformation of 20(*S*)-Protopanaxatriol (PPT)-Type Ginsenosides R_e, R_{g2}, and R_f Using Rapid Resolution Liquid Chromatography Coupled with Quadruple-Time-of-Flight Mass Spectrometry (RRLC-Q-TOF-MS)

Wei Wu,[†] Qiujie Qin,[†] Yingying Guo,[†] Jinghui Sun,[†,‡] and Shuying Liu*[,†,§]

[†]Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun 130117, People's Republic of China

[‡]Pharmaceutical College, Beihua University, Jilin 132013, People's Republic of China

[§]Changchun Center of Mass Spectrometry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China

ABSTRACT: A rapid resolution liquid chromatography coupled with quadruple-time-of-flight mass spectrometry (RRLC-Q-TOF-MS) method was developed for analysis of chemical transformation of 20(*S*)-protopanaxatriol (PPT)-type ginsenosides R_e, R_{g2}, and R_f in acidic conditions. The transformation products were identified by comparing the retention time of the standard compounds, the accurate mass measurement, and the fragment ions obtained from RRLC-Q-TOF-tandem mass spectrometry (MS/MS) analyses. The specific product ions of aglycone PPT (m/z 475), C-24- and C-25-hydrated PPT (m/z 493), and Δ20(21) or Δ20(22) dehydration PPT (m/z 457) by MS/MS were discussed for structural characterization. Experiments demonstrated that chemical transformation mechanisms of 20(*S*)-PPT-type ginsenosides in acidic conditions include hydrolysis of saccharide substitution, Δ20(21) or Δ20(22) dehydration, and hydration addition reactions at C-24 and C-25. The chemical transformation pathway for 20(*S*)-PPT-type ginsenosides was summarized. The developed RRLC-Q-TOF-MS method was also applied for comparative analysis of 20(*S*)-PPT ginsenoside and related chemical transformation products in ginseng products.

KEYWORDS: *RRLC-Q-TOF-MS, ginsenoside, chemical transformation, Panax ginseng*

INTRODUCTION

Ginseng, the root of *Panax ginseng* C. A. Meyer, has been well-known as an important medicinal resource in traditional Chinese medicine (TCM) for thousands of years. It is also used as an adaptogenic remedy or functional food available on the commercial market to help increase resistance to environmental stressors and reduce fatigue in health care.[1,2] Modern pharmacological studies and phytochemical analyses have revealed the wide range of therapeutic and pharmacological applications to improve the quality of life[3,4] and the chemical basis of ginseng.[5] Ginsenoside is considered as the major pharmacologically active ingredient of ginseng, which is responsible for most of the activities of ginseng, including antioxidant, anti-inflammatory, antiapoptotic, immunostimulant properties, etc.[6,7] Thus far, about 50 kinds of ginsenosides have been identified from the ginseng roots and related products, which are defined as types of protopanaxadiol (PPD), protopanaxatriol (PPT), and oleanolic acid according to aglycone skeleton.[5,8] With the increasing interests in ginseng for health care, the need to elucidate the chemical composition differences in ginseng cultivation, classification, and processing ways to ensure the quality, safety, and efficacy is necessary.

Recently, mass spectrometry (MS) has become a powerful tool in ginsenoside analysis because of its high sensitivity, rapid analysis, and low sample consumption.[9−11] High-performance liquid chromatography−mass spectrometry (HPLC−MS) provides effective analyses in a complex extract system, which can rapidly determine the compounds in mixtures by yielding information on their molecular weights and structures.[12−14] Especially, the rapid development of the ultraperformance liquid chromatography (UPLC) and rapid resolution liquid chromatography (RRLC) coupled with quadruple-time-of-flight mass spectrometry (Q-TOF-MS) allow for efficient separation, high resolution, sensitivity, and high-speed detection of ginseng.[15,16]

The ginseng products are often taken orally as functional food in thermally prepared forms, such as dried ginseng, red ginseng, and ginseng extracts. Because thermal processing (decoction and steaming) and biological metabolism of ginseng may lead to variety and content changes of ginsenosides, it is necessary to determine the changes for understanding the bioactive forms of the ginsenosides and their pharmacological mechanisms. The degradation products in gastrointestinal lumen, the absorption from the gastrointestinal tract, and the transformation in liver and intestine have been studied to explain the safety and pharmacological activity mechanisms.[17,18] Studies of pharmacokinetics and metabolism of ginsenosides demonstrated that some of the ginsenosides are transformed in the gastrointestinal tract by gastric acid or microflora.[19] In additional, processing procedures and decoction with different herbs can result in deviation in the amount of active ingredients

Received: June 24, 2012
Revised: September 19, 2012
Accepted: September 19, 2012
Published: September 19, 2012

dx.doi.org/10.1021/jf302638f | *J. Agric. Food Chem.* 2012, 60, 10007−10014

of ginseng and related pharmacological effects. Quantitative chemical analysis of some ginsenosides has been adopted for quality control.[20,21] The variety and content changes of ginsenosides have been studied when ginseng combined with different herbs, which may lead to great differences in clinical medication.[22−24] Analysis of type and content changes of ginsenoside in steam-processed ginseng roots has been undertaken.[25,26] Moreover, the studies of ginsenoside hydrolyzation mechanisms have been carried out,[27−30] which showed that large-molecular-weight ginsenosides, such as R_{b1}, R_{b2}, R_c, R_e, etc., can be hydrolyzed to smaller molecular-weight ginsenosides, such R_{g3}, R_{g2}, F_2. An UPLC-Q-TOF-tandem mass spectrometry (MS/MS)-based chemical profiling method was developed to evaluate decocting-induced chemical transformations in the decoction of the root of *P. ginseng*, and 21 kinds of ginsenosides were determined as newly generated ginsenosides during the decoction of ginseng.[31] Therefore, the research of ginsenoside transformation in ginseng processing is useful for revealing mechanisms of ginsenosides and is important in the quality and safety control of drugs and heath foods. Previous studies on ginsenoside transformation mainly focused on 20(*S*)-PPD-type ginsenosides. In this study, the chemical transformation of 20(*S*)-PPT-type ginsenosides R_e, R_{g2}, and R_f were investigated systematically using RRLC-Q-TOF-MS/MS. To speed up the transformation reaction and simulate the complex condition of ginseng decoction, the experiments were conducted in acidic conditions at pH 2.0. Furthermore, the developed method was applied for qualitative and quantitative analysis of 20(*S*)-PPT ginsenoside R_e, R_{g2}, and R_f in ginseng products.

MATERIALS AND METHODS

Chemicals and Materials. Authentic standards of ginsenosides 20(*S*)-R_e, 20(*S*)-R_{g2}, 20(*R*)-R_{g2}, 20(*S*)-R_f, 20(*S*)-R_{h1}, 20(*R*)-R_{h1}, and F_1 were obtained from Jilin University (Changchun, China). HPLC-grade acetonitrile was bought from Fisher (Waltham, MA). HPLC-grade formic acid (96%) was purchased from Tedia (Fairfield, OH). Ultrapure water (18 MΩ/cm) was prepared by a Milli-Q system (Millipore, Bedford, MA). Other reagents and chemicals were of analytical grade. *P. ginseng* (5 years old) was collected from Jilin Fusong. The specimen has been stored in the Jilin Ginseng Academy of the Changchun University of Chinese Medicine.

Sample Preparation. Ginsenoside standards 20(*S*)-R_e, 20(*S*)-R_{g2}, and 20(*S*)-R_f were prepared in 50% (v/v) methanol/water. The solution pH was adjusted to 2.0 by the addition of formic acid. The standard solutions of ginsenosides were heated to reflux (60 °C) for 2 h. Red ginseng (5-year-old *P. ginseng* processed at 100 and 120 °C) was powered and immersed with 80% methanol to extract ginsenosides. Then, the extract solution was passed through a 0.45 μm filter before RRLC-Q-TOF-MS analysis.

Quantitation. The stock solution of a mixture of ginsenoside standards 20(*S*)-R_e (0.02 mg/mL), 20(*S*)-R_{g2} (0.01 mg/mL), and 20(*S*)-R_f (0.01 mg/mL) was prepared in 50% (v/v) methanol/water. The calibration curves were made for each compound drafted by plotting the peak area versus the compound concentration. The recovery was determined by standard additions to the plant powder, and then the powder was extracted as described in the Sample Preparation section.

Apparatus. The LC analysis was carried out using an Agilent 1200 RRLC system equipped with a binary pump, a microdegasser, an autoplate sampler, and a thermostatically controlled column apartment. The chromatography separation was performed on an Agilent SB-C18 column (3.0 × 100 mm, 1.8 μm, 600 bar) at a temperature of 35 °C. Formic acid (0.1%, v/v) and acetonitrile were used as the mobile phases A and B, respectively. The gradient elution was programmed as follows: 0−13 min (18−46% B), 13−20 min (46−50% B), 20−23 min (50−90% B), and 23−25 min (90% B). The flow rate was set at 0.3 mL/min. The injected sample volume was 5 μL. This RRLC system was connected to an Agilent 6520 Q-TOF mass spectrometer equipped with an electrospray interface. The scan range for MS acquisition was from *m/z* 100 to 2200 in both positive and negative modes. The conditions of the electrospray ionization (ESI) source were as follows: drying gas, N_2; flow rate, 10.0 L/min; drying gas temperature, 350 °C; nebulizer, 37 psi; capillary voltage, 3500 V; and fragmentor, 180 V. The data analysis was performed by Mass Hunter Qualitative (MHQ) software, version B.03.01 (Agilent Technologies, Santa Clara, CA).

RESULTS AND DISCUSSION

Analysis of 20(*S*)-R_e, 20(*S*)-R_{g2}, and 20(*S*)-R_f by RRLC-Q-TOF-MS. The nomenclature for fragmentation used in this paper is based on that described by Costello and co-workers and Liu et al.[10,32,33] Ions retaining the charge at the reducing terminus are termed Y and Z (glycoside cleavages) and X (cross-ring cleavages), whereas those ions retaining the charge at the non-reducing terminus are termed B and C (glycoside cleavages) and A (cross-ring cleavages). Cross-ring cleavage ions are designated by superscript numbers, indicating the two bonds cleaved. As an example, the fragmentation of 20(*S*)-R_e in the negative-ion mode by RRLC-Q-TOF-MS/MS was shown in Scheme 1. 20(*S*)-R_e, 20(*S*)-R_{g2}, and 20(*S*)-R_f were first analyzed by RRLC-Q-TOF-MS and MS/MS to obtain information about the retention time, accurate molecular mass, and fragmentation, which is helpful for elucidating structures. The mass accuracy for all molecular ions and fragment ions was less than 10 ppm. In the following discussion, the *m/z* value of the ion was expressed as a integer number, and the accurate mass measurement was listed in

Scheme 1

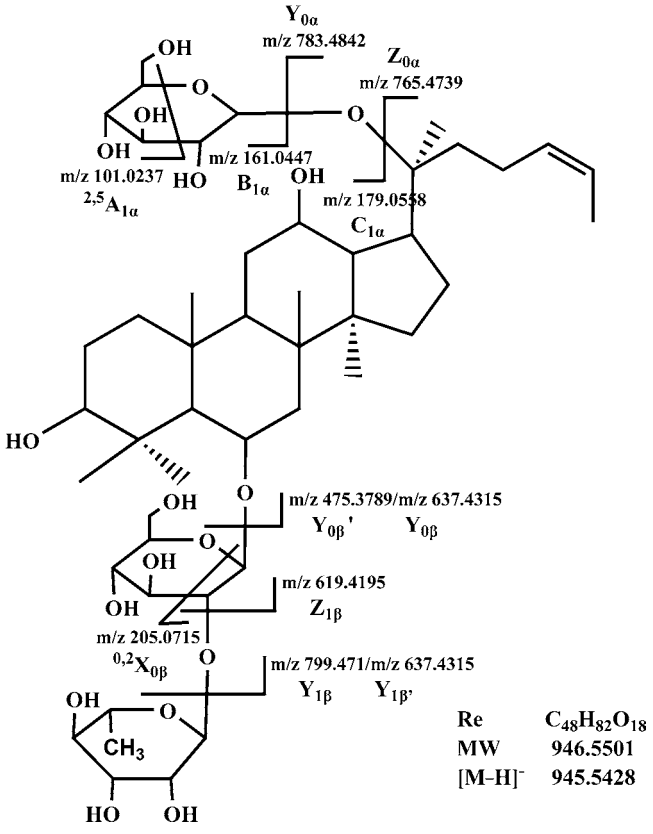

Table 1. Identified Chemical Transformation Products of 20(*S*)-PPT Ginsenosides R_e, R_{g2}, and R_f

peak	identity	molecula formula	MW	$[M - H]^-$ measured value (mass accuracy < 10 ppm)	MS/MS fragment ions of $[M - H]^-$ (mass accuracy < 10 ppm)
	R_e	$C_{48}H_{82}O_{18}$	946.5501	945.5421	799.471 ($Y_{1\beta}$), 783.4842 ($Y_{0\alpha}$), 765.4739 ($Z_{0\alpha}$), 637.4315 ($Y_{1\beta'}/Y_{0\beta}$), 619.4195 ($Z_{1\beta}$), 475.3789 ($Y_{0\beta'}$), 205.0715 ($^{0,2}X_{0\beta}$), 179.0558 ($C_{1\alpha}$), 161.0447 ($B_{1\alpha}$), 101.0237 ($^{2,5}A_{1\alpha}$)
	R_{g2}	$C_{42}H_{72}O_{13}$	784.4973	783.4852	637.4296 ($Y_{1\beta}$), 619.4164 ($Z_{1\beta}$), 475.3795 ($Y_{0\beta}$), 391.2843 ($Y_{0\beta}$-C_6H_{12}), 205.0709 ($^{0,2}X_{0\beta}$)
	R_f	$C_{42}H_{72}O_{14}$	800.4922	799.4799	637.4265 ($Y_{1\beta}$), 619.4254 ($Z_{1\beta}$), 475.3787 ($Y_{0\beta}$), 391.2866 ($Y_{0\beta}$-C_6H_{12}), 221.0654 ($^{0,2}X_{0\beta}$), 161.0442 ($B_{1\beta}$), 101.0237 ($^{2,5}A_{1\beta}$)
1	20(*S*)-R_{f2}	$C_{42}H_{74}O_{14}$	802.5079	801.4965	655.4418 ($Y_{1\beta}$), 637.4288 ($Z_{1\beta}$), 493.3865 ($Y_{0\beta}$), 205.0710 ($^{0,2}X_{0\beta}$)
2	20(*S*)-R_{g2}	$C_{42}H_{72}O_{13}$	784.4973	783.4852	637.4296 ($Y_{1\beta}$), 619.4164 ($Z_{1\beta}$), 475.3795 ($Y_{0\beta}$), 391.2843 ($Y_{0\beta}$-C_6H_{12}), 205.0709 ($^{0,2}X_{0\beta}$)
3	20(*R*)-R_{g2}	$C_{42}H_{72}O_{13}$	784.4973	783.4877	637.4301 ($Y_{1\beta}$), 619.4208 ($Z_{1\beta}$), 475.3769 ($Y_{0\beta}$), 391.2840 ($Y_{0\beta}$-C_6H_{12}), 205.0713 ($^{0,2}X_{0\beta}$)
4	20(*S*)-R_{h1}	$C_{36}H_{62}O_9$	638.4394	637.4258	475.3786 ($Y_{0\beta}$), 391.2845 ($Y_{0\beta}$-C_6H_{12}), 161.0441 ($B_{1\beta}$)
5	20(*R*)-R_{h1}	$C_{36}H_{62}O_9$	638.4394	637.4313	475.3790 ($Y_{0\beta}$), 391.2830 ($Y_{0\beta}$-C_6H_{12}), 161.0445 ($B_{1\beta}$)
6	F_1	$C_{36}H_{62}O_9$	638.4394	637.4310	475.3782 ($Y_{0\beta}$), 391.2862 ($Y_{0\beta}$-C_6H_{12}), 161.0451 ($B_{1\beta}$)
7	R_{g6}	$C_{42}H_{70}O_{12}$	766.4867	765.4723	619.4202 ($Y_{1\beta}$), 601.4108 ($Z_{1\beta}$), 457.3578 ($Y_{0\beta}$), 205.0702 ($^{0,2}X_{0\beta}$)
8	R_{g4}	$C_{42}H_{70}O_{12}$	766.4867	765.4750	619.4212 ($Y_{1\beta}$), 601.4102 ($Z_{1\beta}$), 457.3667 ($Y_{0\beta}$), 205.0712 ($^{0,2}X_{0\beta}$)
9	20(*S*)-R_{f3}	$C_{42}H_{74}O_{15}$	818.5028	817.4918	655.4382 ($Y_{1\beta}$), 637.4318 ($Z_{1\beta}$), 493.3887 ($Y_{0\beta}$), 221.0658 ($^{0,2}X_{0\beta}$), 179.0561 ($C_{1\beta}$), 161.0448 ($B_{1\beta}$)
10	R_{g8}	$C_{42}H_{70}O_{13}$	782.4816	781.4692	619.4209 ($Y_{1\beta}$), 601.4105 ($Z_{1\beta}$), 457.3684 ($Y_{0\beta}$), 221.0651 ($^{0,2}X_{0\beta}$), 161.0446 ($B_{1\beta}$), 101.0235 ($^{2,5}A_{1\beta}$)
11	R_{g9}	$C_{42}H_{70}O_{13}$	782.4816	781.4728	619.4163 ($Y_{1\beta}$), 601.4107 ($Z_{1\beta}$), 457.3671 ($Y_{0\beta}$), 221.0649 ($^{0,2}X_{0\beta}$), 161.0450 ($B_{1\beta}$)

Table 1. Figure 1 shows the MS/MS spectra of 20(*S*)-R_e, 20(*S*)-R_{g2}, and 20(*S*)-R_f in negative-ion mode. $Y_{0\beta'}$ (m/z 475) produced by the loss of α and β saccharide substitutions (R_e) or $Y_{0\beta}$ (m/z 475) produced by the loss of β saccharide substitutions (R_{g2} and R_f) from the intact molecular ions was the characteristic ion of PPT aglycone. In Figure 1a, ions at m/z 799 ($Y_{1\beta}$) and 783 ($Y_{0\alpha}$) are produced by losing a deoxyhexose (146 Da) and a hexose (162 Da), respectively, from the $[M - H]^-$ ion at m/z 945 of 20(*S*)-R_e, indicating two different terminal residues in the saccharide chain substitutions. For 20(*S*)-R_{g2} (m/z 783) and 20(*S*)-R_f (m/z 799) (panels b and c of Figure 1), the observation of the $Y_{1\beta}$ ion at m/z 637 provided clear evidence of the presence of terminal rhamnose (146 Da) and glucose (162 Da) residues, respectively. The neutral loss (308 and 324 Da) from the $[M - H]^-$ ion at m/z 783 and 799 to yield the aglycone ion at m/z 475 suggests that 20(*S*)-R_{g2} and 20(*S*)-R_f contained the glucose−rhamnose and glucose−glucose substitutions, respectively. Accurate mass measurement for molecular ions and fragment ions provides information of elementary composition, which is complementary for structural identification. The characteristic fragment ions were summarized in Table 1.

Characterization of Chemical Transformation Products by RRLC-Q-TOF-MS. According to ref 27, the chemical transformation experiment condition was selected in acidic conditions (pH 2.0). The transformation products were analyzed by RRLC-Q-TOF-MS to identify the components. Because 0.1% formic acid solution was employed as the mobile phase, each ginsenoside showed the deprotonated ion $[M - H]^-$ and adduct ion $[M + HCOO]^-$ in negative-ion mode, providing information about the molecular mass. Then, MS/MS was performed for determination of the aglycone type and sequences of sugar chains according to characteristic fragmentation and accurate mass measurement, which provide structural information for elucidation of analytes.

The transformed products were tentatively identified by comparison to the retention time (t_R) of the authentic standards, the accurate mass, the isotopic ratio pattern, and the characteristic fragment ions obtained from RRLC-Q-TOF-MS/MS analyses. The identified transformation compounds were summarized in Table 1. The mass accuracies for all molecular ions and fragment ions were less than 10 ppm. The compound peaks produced by chemical transformation were well-separated using RRLC-Q-TOF-MS, and the extracted ion chromatogram (EIC) was applied for peak identification. Figure 2 shows the typical RRLC-Q-TOF-MS EICs of the chemical transformation products of ginsenoside 20(*S*)-R_e, 20(*S*)-R_{g2}, and 20(*S*)-R_f in the negative-ion mode. Eight product compounds (peaks 1−8) for 20(*S*)-R_e, five product compounds (peaks 1, 4, 5, 7, and 8) for 20(*S*)-R_{g2}, and five product compounds (peaks 4, 5, 9, 10, and 11) for 20(*S*)-R_f were identified (Table 1). The peaks 4 and 5 were produced in reaction solution of all of the three ginsenosides 20(*S*)-R_e, 20(*S*)-R_{g2}, and 20(*S*)-R_f, exhibiting ion $[M + HCOO]^-$ at m/z 683. From analyses of the MS/MS spectra, the characteristic ion at m/z 475 of PPT-type ginsenoside was observed by the loss of the hexose residue (162 Da). When the retention time was compared to that of the standard compound and ref 34, which reported that the 20(*S*) epimer eluted earlier than its relevant 20(*R*) epimer, peaks 4 and 5 are identified as 20(*S*)-R_{h1} and 20(*R*)-R_{h1}, respectively. It was shown that 20(*S*)-R_{h1} and 20(*R*)-R_{h1} were produced by the hydrolysis of the terminus glycosylation moiety at C-6 for 20(*S*)-R_{g2} and 20(*S*)-R_f and the terminus glycosylation moiety at C-6 and C-20 for 20(*S*)-R_e. In Figure 2a, it was found that 20(*S*)-R_e could be hydrolyzed to 20(*S*)-R_{g2} (peak 2) and its epimer 20(*R*)-R_{g2} (peak 3) by the loss of the oligosaccharide chain at C-20 of the aglycone, which coincided with the previously reported result.[27] Peak 6 corresponding to the $[M - H]^-$ ion at m/z 637 was identified as F_1 by comparison to the reference standard. Peaks 1, 7, and 8 were found in transformation products of both 20(*S*)-R_e and 20(*S*)-R_{g2} (panels a and b of Figure 2) corresponding to $[M - H]^-$ ions at m/z 801, 765, and 765. The three compounds were tentatively assigned as C-24- and C-25-hydrated PPT ginseno-

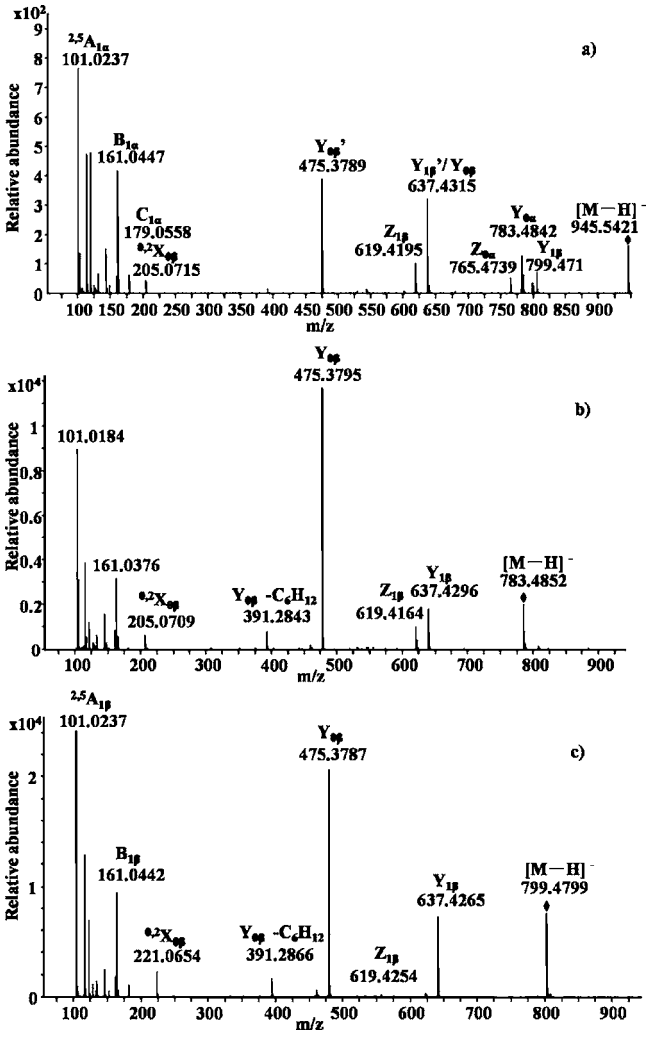

Figure 1. ESI-Q-TOF-MS/MS spectra of ginsenosides in negative-ion mode: (a) 20(*S*)-R_e, (b) 20 (*S*)-R_{g2}, and (c) 20(*S*)-R_f.

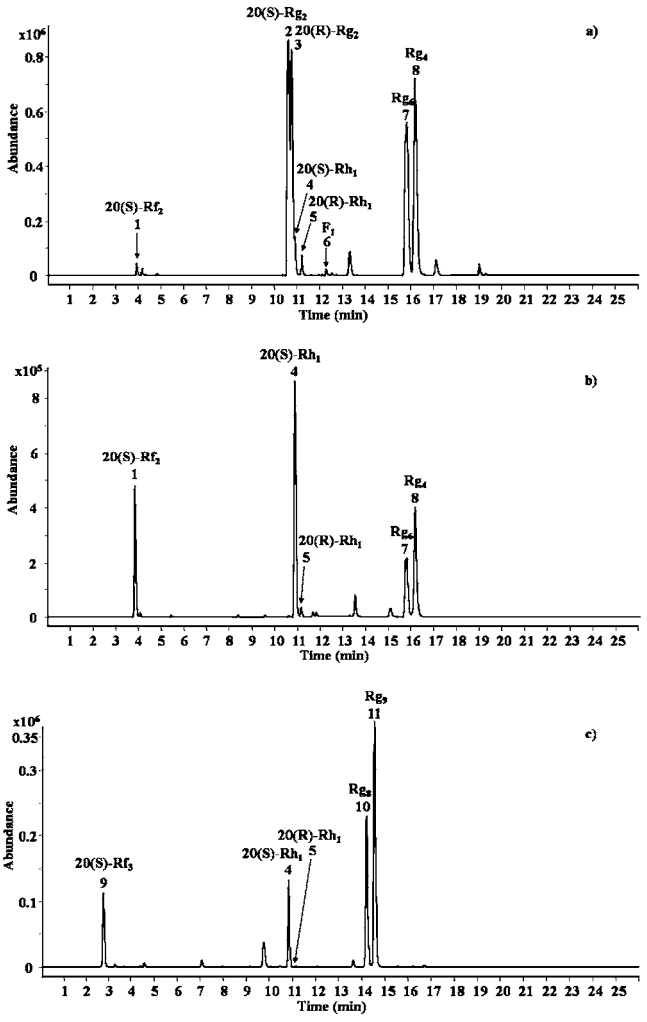

Figure 2. RRLC-Q-TOF-MS extracted ion chromatograms of the ginsenoside chemical transformation products in negative-ion mode: (a) 20(*S*)-R_e, (b) 20(*S*)-R_{g2}, and (c) 20(*S*)-R_f.

side 20(*S*)-R_{f2}, Δ20(21) and Δ20(22) geometric ginsenoside isomers R_{g6} and R_{g4}, respectively, by accurate MS/MS information and comparison to refs 27, 31, and 34, which reported that Δ20(21) geometric isomers were eluted earlier than the relevant Δ20(22) isomers. Panels a and b of Figure 3 show the MS/MS spectra of 20(*S*)-R_{f2} and R_{g6} for structural elucidation. The specific $Y_{0\beta}$ ions at *m*/*z* 493 and 457 give information of aglycone with +18 and −18 Da mass differences when compared to the PPT aglycone ion at *m*/*z* 475, which indicated the addition and dehydration reaction occurring in PPT aglycone. The neutral loss of 146 Da from $[M - H]^-$ ions producing $Y_{1\beta}$ ions (*m*/*z* 655 and 619) suggests the deoxyhexose (rhamnose) terminus substitution, and the subsequent loss of 162 Da producing $Y_{0\beta}$ ions (*m*/*z* 493 and 457) showed that the saccharide substitution chain was glucose−rhamnose. R_{g4} has similar MS/MS fragmentations to R_{g6}, and the MS/MS spectrum is not shown. Thus, it could be deduced that these three compounds were generated through the addition and dehydration reaction of 20(*S*)-R_{g2}, as mentioned in refs 27, 31, and 34. With regard to 20(*S*)-R_f, the chemical transformation has not been systematically studied to our knowledge. Peaks 9, 10, and 11 were specific chemical transformation products of 20(*S*)-R_f and first detected (Figure 2c). From MS analysis, $[M - H]^-$ ions at *m*/*z* 817, 781, and 781 for peaks 9, 10, and 11 were produced in negative-ion mode. Furthermore, MS/MS was carried out to investigate the structures. The MS/MS spectrum of peak 9 is shown in Figure 3c. Product ions at *m*/*z* 655 and 493 were observed, which are produced by the loss of 162 and (162 + 162) Da from the ion at *m*/*z* 817. The results indicated that the terminus substitution is glucose and the compound consists of a disaccharide (glucose−glucose), which are consistent with the structure of 20(*S*)-R_f. Accurate mass measurement of the product ion at *m*/*z* 493 matched with the C-24- and C-25-hydrated PPT type of aglycone. On the basis of our results and the reports in refs 27 and 31, peak 9 could be deduced to be generated through the addition reaction of 20(*S*)-R_f. Thus, it could be tentatively identified as C-24- and C-25-hydrated 20(*S*)-R_f, named as 20(*S*)-R_{f3}. Peaks 10 and 11 are isomers providing similar fragmentations. As an example, Figure 3d shows the MS/MS of peak 10. The product ion at *m*/*z* 619 was produced by the loss of the glucose residue (162 Da) from the ion at *m*/*z* 781, and the characteristic ion at *m*/*z* 457 indicated the aglycone type as Δ20(21)- or Δ20(22)-PPT, which was produced by the loss of the glucose−glucose residue (162 + 162 Da) from the precursor ion. According to MS/MS fragmentation information, accurate mass measurement, and refs 31 and 34, it could be deduced that peaks 10 and 11 were produced by

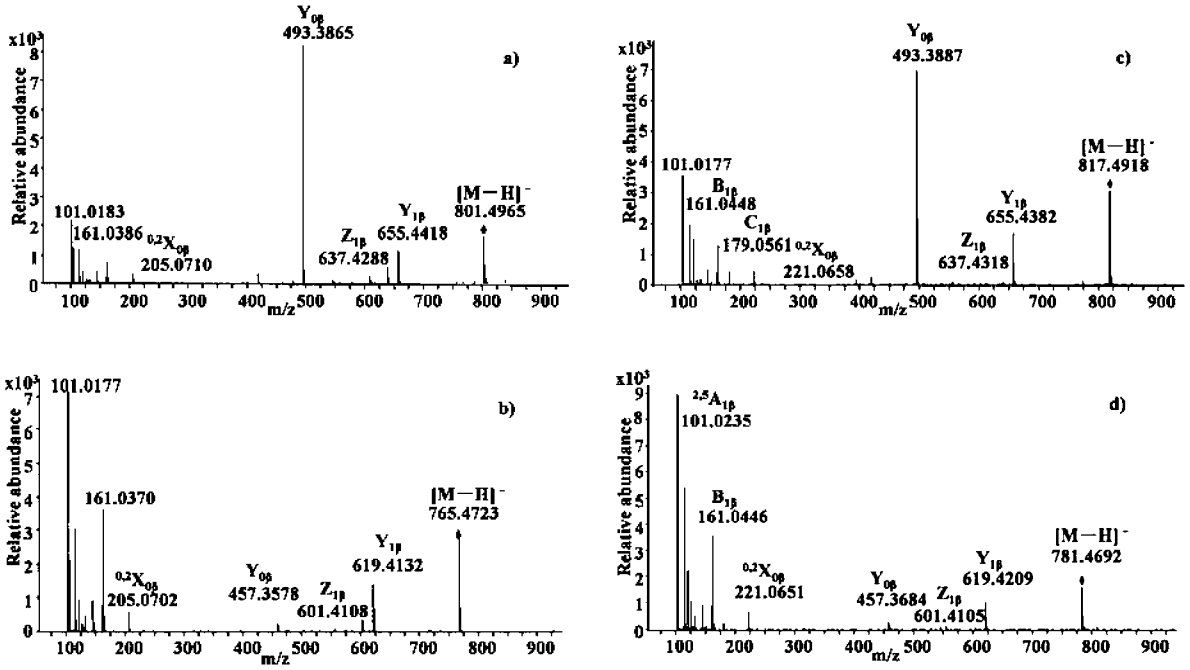

Figure 3. ESI-Q-TOF-MS/MS spectra of ginsenosides in negative-ion mode: (a) 20(*S*)-R_{f2}, (b) R_{g6}, (c) 20(*S*)-R_{f3}, and (d) R_{g8}.

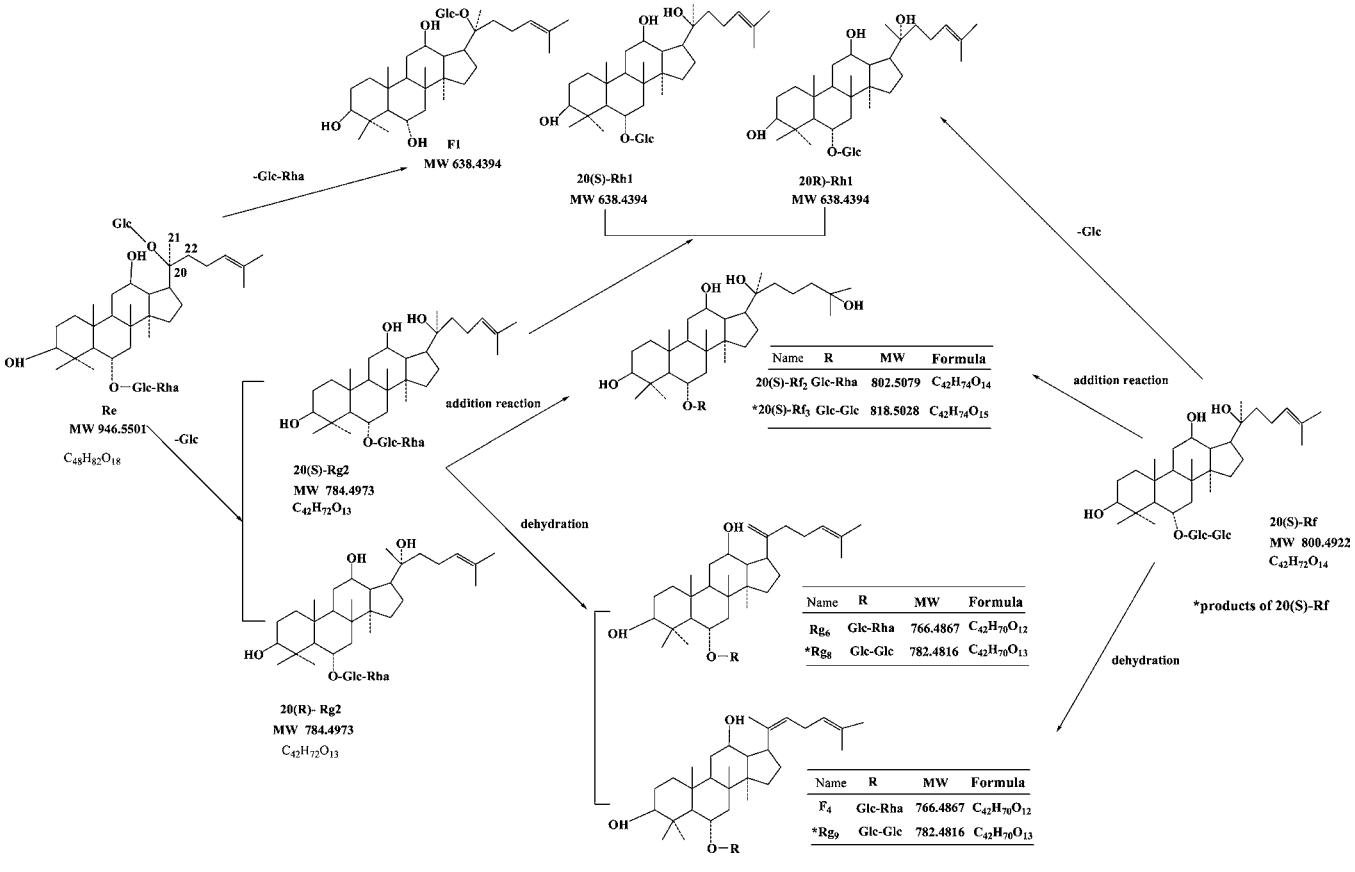

Figure 4. Chemical transformation pathways of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f.

dehydration of 20(*S*)-R_f to produce Δ20(21) or Δ20(22) dehydration products, here named as R_{g8} and R_{g9}. The experiment demonstrated that the chemical transformation of 20(*S*)-R_f in acidic solution was similar to that of 20(*S*)-R_{g2}, involving hydrolysis to form 20(*S*)-R_{h1} and 20(*R*)-R_{h1}, the hydration addition reaction at C-24 and C-25 to form 20(*S*)-R_{f3}, and Δ20(21) or Δ20(22) dehydration to produce R_{g8} and R_{g9}. The chemical transformation pathways of 20(*S*)-PPT ginsenoside R_e, R_{g2}, and R_f are summarized in Figure 4.

Comparative Analysis of 20(*S*)-PPT Ginsenosides and Related Chemical Transformation Products in Ginseng Products by RRLC-Q-TOF-MS. Ginseng has been used as a

dx.doi.org/10.1021/jf302638f | *J. Agric. Food Chem.* 2012, 60, 10007−10014

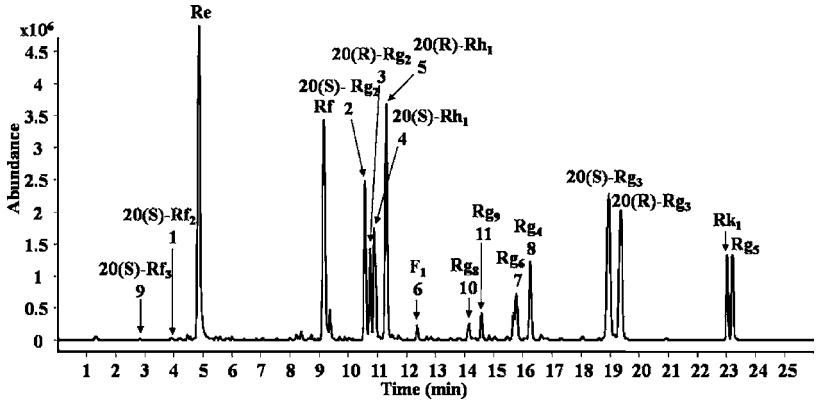

Figure 5. RRLC-Q-TOF-MS extracted ion chromatogram of the identified 20(*S*)-PPT ginsenoside products by chemical transformation in red ginseng (100 °C steamed) in negative-ion mode.

tonic herb to boost energy and reduce stress for centuries, and it is usually used in TCM formulations or as nutritional supplements to adjust the balance of the human body. The chemical complexity and a wide range of pharmacological activities call for good-quality control and bioactive component definition. One of processed ginsengs used popularly in China and South Korea is red ginseng, which is steamed and then dried after harvest. Refs 34 and 35 have reported the chemical changes of 20(*S*)-PPD ginsenosides after steaming (thermal processing), such as the increased content of 20(*S*)-R_{g3} and 20(*R*)-R_{g3} and the generation of R_{k1} and R_{g5}, as shown in Figure 5. In this study, the processing-related 20(*S*)-PPT ginsenoside changes in red ginseng (100 and 120 °C steamed) were discussed. The chemical transformation products of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f as discussed above were all detected, assigned as peaks 1−11 (summarized in Table 1) in both processed ginseng products. As an example, the extracted ion chromatogram of chemical transformation products in red ginseng (100 °C steamed) extract is shown in Figure 5. The constituents were well-separated and identified using their retention time, accurate mass measurement, and MS/MS characteristic ions. In Figure 5, 20(*S*)-R_{g3}, 20(*R*)-R_{g3}, R_{k1}, and R_{g5} were also observed, which were reported as the transformation products of 20(*S*)-PPD ginsenosides.[34,35] The experiment demonstrated that the chemical transformation of 20(*S*)-PPD and 20(*S*)-PPT ginsenosides occurred during the processing procedure of ginseng, including hydrolysis of saccharide substitution [20(*S*)-R_{g3}, 20(*R*)-R_{g3}, 20(*S*)-R_{h1}, 20(*R*)-R_{h1}, 20(*S*)-R_{g2}, and 20(*R*)-R_{g2}], Δ20(21) or Δ20(22) dehydration (R_{k1}, R_{g5}, R_{g6}, Rg_4, R_{g8}, and R_{g9}), and hydration addition at C-24 and C-25 [20(*S*)-R_{f2} and 20(*S*)-R_{f3}]. These compounds generated during the processing procedure of ginseng may be helpful to explain the variation of pharmacological effects. Further studies should be made to attempt to systematically study the structure−activity relationship of these ginsenosides.

As discussed above, the processing-related 20(*S*)-PPT-type ginsenoside changes occurred during the processing procedure of ginseng. It is important to quantitatively analyze the main 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f in ginseng-related products for quality control and pharmacological effect studies. The developed RRLC-Q-TOF-MS method for quantitative analysis of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f in white ginseng and red ginseng was validated with respect to linearity, intra- and interday precision, repeatability, stability, and recovery.

The linearity of the calibration curves was verified by the correlation coefficients, and the results are given in Table 2.

Table 2. Calibration Curve and Linear Range of Four 20(*S*)-PPT Ginsenosides

ginsenosides	calibration curve[a]	r^2	linear range (μg)
R_e	$y = 7.546x - 3.943$	0.996	0.02−0.20
R_f	$y = 2.865x - 2.084$	0.994	0.01−0.10
R_{g2}	$y = 3.153x - 2.325$	0.995	0.01−0.10

[a] y and x are the peak area and amount of the analyte, respectively.

Good linearities (regression coefficient between 0.994 and 0.996) were obtained. The limits of detection and quantitation (LOD and LOQ) were measured at a signal-to-noise ratio of 3 and 10 as criteria, and they were less than 0.8 and 1.1 ng mL^{-1} for the three ginsenosides, respectively. The injection precision was obtained by analyzing the peak area variations of six injections of a mixture of three standard ginsenosides. The intra- and interday (6 days) precisions are 0.6−2.04% (n = 6) and 1.1−2.68% (n = 6), respectively. The overall stability and repeatability variations were less than 2.12 and 2.10%, respectively. The recoveries of the ginsenosides were determined with spiked samples. The recoveries of all three ginsenosides were in the range of 90.46−101.60%, with relative standard deviations (RSDs) below 5.46% (n = 3). This validated method was used for comparative quantitation of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f in white ginseng and red ginseng (100 and 120 °C steamed). The contents of the three ginsenosides in ginseng samples are shown in Table 3. It was shown that the temperature affected the content of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f. The contents of 20(*S*)-R_e and 20(*S*)-R_f decreased largely in red ginseng (120 °C steamed), and the content of 20(*S*)-R_{g2} increased slightly in red ginseng (100 and 120 °C steamed). The results are consistent with the

Table 3. Content of 20(*S*)-PPT Ginsenosides in Different Ginseng Products

	content of ginsenosides (mg/g)[a]		
ginseng products	R_e	R_f	R_{g2}
white ginseng	10.99 ± 0.29	6.02 ± 0.10	2.71 ± 0.06
red ginseng (100 °C)	9.91 ± 0.21	5.02 ± 0.09	4.32 ± 0.06
red ginseng (120 °C)	1.27 ± 0.02	3.34 ± 0.13	3.98 ± 0.17

[a] Mean value ± standard deviation (n = 3).

above conclusion summarized in Figure 4. Ginsenosides 20(*S*)-R_e and 20(*S*)-R_f as original 20(*S*)-PPT ginsenosides existing in ginseng could be chemically transformed to derivatives, leading to the decrease of the contents, while 20(*S*)-R_{g2} as original and intermediate 20(*S*)-PPT ginsenosides could be chemically transformed to derivatives and produced from 20(*S*)-R_e and 20(*S*)-R_f, leading to the increase of the content slightly. The above experiments indicate that quantitative evaluation of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f should be strictly carried out to ensure the consistent quality and the therapeutic effect of ginseng and related products.

In this research, the chemical transformation products of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f were investigated systematically using RRLC-Q-TOF-MS and MS/MS. Ginsenosides with different aglycones can be determined rapidly. The chemical transformation mechanisms of 20(*S*)-PPT ginsenosides were discussed, involving hydrolysis, dehydration, and addition reactions. The experiment demonstrated that chemical transformation products existed in the crude extract of processed ginseng (red ginseng), providing new insights into monitoring and assessing bioactive compounds. The developed accurate and sensitive RRLC-Q-TOF-MS method was applied for qualitative and quantitative analysis of 20(*S*)-PPT ginsenosides R_e, R_{g2}, and R_f in ginseng products. The research result is valuable for quality control of ginseng and related processed products.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-431-86045155. Fax: +86-431-86045258. E-mail: syliu19@yahoo.com.cn or syliu@ciac.jl.cn.

Funding

We thank the Science and Technology Development Plan Project of Jilin Province (20096043) and the National Development and Reform Commission.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Xiang, Y.; Shang, H.; Gao, X.; Zhang, B. A comparison of the ancient use of ginseng in traditional Chinese medicine with modern pharmacological experiments and clinical trials. *Phytother. Res.* **2008**, *22*, 851−858.

(2) Shi, Y.; Sun, C.; Zheng, B.; Li, Y.; Wang, Y. Simultaneous determination of nine ginsenosides in functional foods by high performance liquid chromatography with diode array detector detection. *Food Chem.* **2010**, *123*, 1322−1327.

(3) Coleman, C. I.; Hebert, J. H.; Reddy, P. The effects of *Panax ginseng* on quality of life. *J. Clin. Pharm. Ther.* **2003**, *28*, 5−15.

(4) Liu, C. X.; Xiao, P. G. Recent advances on ginseng research in China. *J. Ethnopharmacol.* **1992**, *36*, 27−38.

(5) Angelova, N.; Kong, H.; Heijden, R. V. D.; Yang, S.; Choi, Y. H.; Kim, H. K.; Wang, M.; Hankemeier, T.; Greef, J. V. D.; Xu, G.; Verpoorte, R. Recent methodology in the phytochemical analysis of ginseng. *Phytochem. Anal.* **2008**, *19*, 2−16.

(6) Lü, J.; Yao, Q.; Chen, C. Ginseng compounds: An update on their molecular mechanisms and medical applications. *Curr. Vasc. Pharmacol.* **2009**, *7*, 293−302.

(7) Jia1, L.; Zhao, Y.; Liang, X. Current evaluation of the millennium phytomedicine—Ginseng (II): Collected chemical entities, modern pharmacology, and clinical applications emanated from traditional Chinese medicine. *Curr. Med. Chem.* **2009**, *16*, 2924−2942.

(8) Zhu, G.; Li, Y.; Hau, D. K.; Jiang, Z.; Yu, Z.; Fong, W. Protopanaxatriol-type ginsenosides from the root of *Panax ginseng*. *J. Agric. Food Chem.* **2011**, *59*, 200−205.

(9) Miao, X.; Metcalfe, C. D.; Hao, C.; March, R. E. Electrospray ionization mass spectrometry of ginsenosides. *J. Mass Spectrom.* **2002**, *37*, 495−506.

(10) Liu, S.; Cui, M.; Liu, Z.; Song, F. Structural analysis of saponins from medicinal herbs using electrospray ionization tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 133−141.

(11) Song, F.; Liu, Z.; Liu, S.; Cai, Z. Differentiation and identification of ginsenoside isomers by electrospray ionization tandem mass spectrometry. *Anal. Chim. Acta* **2005**, *531*, 69−77.

(12) Fuzzati, N.; Gabetta, B.; Jayakar, K.; Pace, R.; Peterlongo, F. Liquid chromatography−electrospray mass spectrometric identification of ginsenosides in *Panax ginseng* roots. *J. Chromatogr., A* **1999**, *854*, 69−79.

(13) Wang, X.; Sakuma, T.; Asafu-Adjaye, E.; Shiu, G. K. Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS. *Anal. Chem.* **1999**, *71*, 1579−1584.

(14) Cui, M.; Song, F.; Zhou, Y.; Liu, Z.; Liu, S. Rapid identification of saponins in plant extracts by electrospray ionization multi-stage tandem mass spectrometry and liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1280−1286.

(15) Chan, E. C. Y.; Yap, S.; Lau, A.; Leow, P.; Toh, D.; Koh, H. Ultra-performance liquid chromatography/time-of-flight mass spectrometry based metabolomics of raw and steamed *Panax notoginseng*. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 519−528.

(16) Xie, G. X.; Ni, Y.; Su, M. M.; Zhang, Y. Y.; Zhao, A. H.; Gao, X. F.; Liu, Z.; Xiao, P. G.; Jia, W. Application of ultra-performance LC−TOFMS metabolite profiling techniques to the analysis of medicinal *Panax* herbs. *Metabolomics* **2008**, *4*, 248−260.

(17) Karikura, M.; Miyase, T.; Tanizawa, H.; Taniyama, T.; Takino, Y. Studies on absorption, distribution, excretion and metabolism of ginseng saponins. VI. The decomposition products of ginsenoside R_{b2} in the stomach of rats. *Chem. Pharm. Bull.* **1991**, *39*, 400−404.

(18) Karikura, M.; Miyase, T.; Tanizawa, H.; Taniyama, T.; Takino, Y. Studies on absorption, distribution, excretion and metabolism of ginseng saponins. VII. Comparison of the decomposition modes of ginsenoside-R_{b1} and -R_{b2} in the digestive tract of rats. *Chem. Pharm. Bull.* **1991**, *39*, 2357−2361.

(19) Akao, T.; Kanaoka, M.; Kobashi, K. Appearance of compound K, a major metabolite of ginsenoside R_{b1} by intestinal bacteria, in rat plasma after oral administration—Measurement of compound K by enzyme immunoassay. *Biol. Pharm. Bull.* **1998**, *21*, 245−249.

(20) Wang, Y.; Pan, J.; Xiao, X.; Lin, R.; Cheng, Y. Simultaneous determination of ginsenosides in *Panax ginseng* with different growth ages using high-performance liquid chromatography−mass spectrometry. *Phytochem. Anal.* **2006**, *17*, 424−430.

(21) Kim, N.; Kim, K.; Choi, B. Y.; Lee, D.; Shin, Y.; Bang, K.; Cha, S.; Lee, J.; Choi, H.; Jang, D. S.; Lee, D. Metabolojmic approach for age discrimination of *Panax ginseng* using UPLC-Q-TOF MS. *J. Agric. Food Chem.* **2011**, *59*, 10435−10441.

(22) Zhang, X.; Wang, L. N.; Song, F. R.; Liu, Z. Q.; Liu, S. Y. Study on the variation of chemical constituents during combination of ginseng with trogopteroum feces and semen raphani by high performance liquid chromatography mass spectrometry. *Chin. J. Anal. Chem.* **2007**, *35*, 559−563.

(23) Zhang, X.; Song, F.; Wang, L.; Liu, Z.; Liu, S. Studies on the content variation of chemical constituents during the combination of ginseng with *Veratrum nigrum* by ESI−MS and HPLC−ESI−MS. *Acta Chim. Sin.* **2007**, *65*, 829−833.

(24) Zhang, X.; Zhang, X.; Chen, G.; Liu, Z.; Liu, S. Analysis of compatibility mechanism of Radix Ginseng Rubra with Fructus Schisandrae Chinensis by HPLC−ESI−MS. *Chinese Traditional and Herbal Drugs* **2007**, *38*, 41−43.

(25) Lau, A.; Woo, S.; Koh, H. Analysis of saponins in raw and steamed *Panax notoginseng* using high-performance liquid chromatography with diode array detection. *J. Chromatogr., A* **2003**, *1011*, 77−87.

(26) Lau, A.; Seo, B.; Woo, S.; Koh, H. High-performance liquid chromatographic method with quantitative comparisons of whole

chromatograms of raw and steamed *Panax notoginseng*. *J. Chromatogr., A* **2004**, *1057*, 141−149.
(27) Zhang, X.; Song, F.; Cui, M.; Liu, Z.; Liu, S. Investigation of the hydrolysis of ginsenosides by high performance liquid chromatography−electrospray ionization mass spectrometry. *Planta Med.* **2007**, *73*, 1225−1229.
(28) Pietta, P.; Mauri, P.; Rava, A. Hydrolysis of ginsenosides in artificial gastric fluid monitored by high-performance of liquid chromatography. *J. Chromatogr.* **1986**, *352*, 291−297.
(29) Miyamoto, E.; Odashima, S.; Kitagawa, I. Stability kinetics of ginsenosides in aqueous solution. *J. Pharm. Sci.* **1984**, *73*, 409−410.
(30) Han, B. H.; Park, M. H.; Han, Y. N.; Woo, L. K. Degradation of ginseng saponins under mild acidic conditions. *Planta Med.* **1982**, *44*, 146−149.
(31) Li, S.; Lai, S.; Song, J.; Qiao, C.; Liu, X.; Zhou, Y.; Cai, H.; Cai, B.; Xu, H. Decocting-induced chemical transformations and global quality of Du−Shen−Tang, the decoction of ginseng evaluated by UPLC−Q-TOF-MS/MS based chemical profiling approach. *J. Pharm. Biomed. Anal.* **2010**, *53*, 946−957.
(32) Domon, B.; Costello, C. E. A systematic nomenclature for carbohydrate fragmentations in FAB−MS/MS spectra of glycoconjugates. *Glycoconjugate J.* **1988**, *5*, 397−409.
(33) Perreault, H.; Costello, C. E. Liquid secondary ionization tandem and matrix-assisted laser desorption/ionization time-of-flight mass spectrometric characterization of glycosphingolipid derivatives. *Org. Mass Spectrom.* **1994**, *29*, 720−735.
(34) Kwon, S. W.; Han, S. B.; Park, I. H.; Kim, J. M.; Park, M. K.; Park, J. H. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *J. Chromatogr., A* **2001**, *921*, 335−339.
(35) Ha, Y. W.; Lim, S. S.; Ha, I. J.; Na, Y.; Seo, J.; Shin, H.; Son, S. H.; Kim, Y. S. Preparative isolation of four ginsenosides from Korean red ginseng (steam-treated *Panax ginseng* C. A. Meyer), by high-speed counter-current chromatography coupled with evaporative light scattering detection. *J. Chromatogr., A* **2007**, *1151*, 37−44.