Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 946–957

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

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

Song-Lin Li[a], Shuk-Fan Lai[a], Jing-Zheng Song[a], Chun-Feng Qiao[a], Xin Liu[a], Yan Zhou[a], Hao Cai[b], Bao-Chang Cai[c], Hong-Xi Xu[a,*]

[a] Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Shatin, N.T., Hong Kong, PR China
[b] Institute of Chinese Medicines, Nanjing University of Chinese Medicine, Nanjing, PR China
[c] Haichang Chinese Medicine Co. Lit., Nanjing, PR China

ARTICLE INFO

Article history:
Received 18 May 2010
Received in revised form 28 June 2010
Accepted 1 July 2010
Available online 25 July 2010

Keywords:
UPLC–Q-TOF-MS/MS
Du–Shen–Tang
Decoction
Ginsenosides
Panax ginseng

ABSTRACT

An UPLC–Q-TOF-MS/MS based chemical profiling method was developed to evaluate decocting-induced chemical transformations in Du–Shen–Tang, the decoction of the root of *Panax ginseng*. Under the optimized UPLC and Q-TOF-MS/MS conditions, over 50 peaks were separated and detected in Du–Shen–Tang within 18 min. The components were identified by comparing the mass spectra and retention time with that of reference compounds, and/or tentatively assigned by elucidating low energy CID fragment ions as well as matching empirical molecular formula with that of the published known compounds. Totally 45 major ginsenosides were identified in Du–Shen–Tang, 21 of which were determined to be newly generated during the decoction of ginseng. The mechanisms involved were further deduced to be hydrolysis, dehydration, decarboxylation and addition reactions of the original ginsenosides in white ginseng through analyzing mimic decoctions of 13 pure reference ginsenosides. Significant difference in chemical profiles between decoctions of two batches of white ginseng suggested that storage duration or other factors significantly influenced the quality consistency of not only the crude drug but also the decoction (Du–Shen–Tang) of white ginseng.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Decoction, also called *Tang-Ji* in Chinese, is the traditional prescription of traditional Chinese medicines (TCMs). Decoctions are usually prepared by boiling with water one single herb or several kinds of herbs prescribed based on TCM theory. Although decoction is a common prescription type of TCM, studies on the holistic chemical profiles of decoctions were attempted only in recent years [1,2].

Du–Shen–Tang, the decoction of the root of single *Panax ginseng*, is a commonly used TCM prescription. The history of Du–Shen–Tang could be traced back to about 1600 years ago [3]. This traditional prescription has been documented in many books on traditional Chinese medicine, such as "*Shi Yao Quan Shu*" (Book on Ten Magic Herbs) and "*Fang Ji Xue*" (Formulas of Chinese Medicines). According to TCM theory, ginseng has actions of reinforcing *qi*, restoring normal pulse, benefiting the spleen and lungs, promoting the production of body fluids and anchoring the mind [4], and has been traditionally used as a tonic and a panacea that promotes longevity.

During the last several decades, great progress has been made on the research of the chemistry, bioactivity and clinical efficacy of ginseng. As summarized in many review articles [5–13], ginsenosides, which were generally classified into four groups, namely protopanaxadiol, protopanaxatriol, ocotillol and oleanolic acid type, were found to be the major components of ginseng with many bioactivities responsible for the panacea effects. The "adaptogenic" actions were recently clarified to be related to the compositional ratio between some individual ginsenosides with opposite activities [14]. Immune system modulation, anti-stress activities, anti-hyperglycemic activities, and cancer preventive effects are among the most notable features of ginseng in preclinical studies and in clinical trials. Nowadays, ginseng has been mainly used to increase resistance to physical, chemical, and biological stress and boost general vitality [10].

However, all pharmacological and clinical studies mentioned above were related to pure ginsenosides or extracts without clear indications of standardized decoctions. It should be accepted that the efficacy of decoctions are contributed by the holistic actions of multi-components in it. Any individual ginsenosides could not

* Corresponding author. Tel.: +852 3406 2873; fax: +852 3551 7333.
E-mail addresses: xuhongxi@hkjcicm.org, songlinli64@126.com (H.-X. Xu).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.07.001

contribute to the holistic efficacy of Du–Shen–Tang, and standardized decoctions can increase the consistency of quality, bioactivity and clinical efficacy of Du–Shen–Tang. To the best of our knowledge, no systematic studies on the chemistry, bioactivity, clinical efficacy and standardization of Du–Shen–Tang has been reported, although there has been some reports from TCM practitioners of the effects of Du–Shen–Tang in the treatment of cardiogenic shock [15], expansion of myocardium [16], and stimulation of parturition [17], etc. In order to systematically evaluate the bioactivity, safety and efficacy as well as standardization and quality control of Du–Shen–Tang, the chemical profiles of Du–Shen–Tang should be investigated first.

Many modern hyphenated techniques such as HPLC–UV (ELSD), GC–MS, LC–(Q/TQ)MS, LC–(IT)MS^n and UPLC–(Q)TOF-MS(/MS), etc. have been used for the qualitative or quantitative analysis of ginseng, as shown in some representative papers [18–23] and summarized in recently published review articles [10,24]. Among these techniques UPLC-Q-TOF-MS(/MS) is the state-of-the-art technique for rapid chemical profiling of medicinal herbs. The sub-2 μm particle short columns have enhanced retention time reproducibility, increased chromatographic resolution, improved sensitivity and increased operation speed of UPLC. Furthermore, the accurate mass values and low energy collision-induced dissociation (CID) of Q-TOF-MS have made UPLC–Q-TOF-MS(/MS) a powerful tool for finding characteristic markers to discriminate four medicinal *Panax* species (*P. ginseng*, *P. japonicus*, *P. quinqueflium* and *P. notoginseng*) [19,20], identification and quantification of ginsenosides in *P. notoginseng* [21], comparison of different parts of *P. notoginseng* [22], and determination of major ginsenosides in biological matrix [23].

The aim of the present study is to develop an UPLC–Q-TOF-MS/MS method for revealing the chemical profiles of Du–Shen–Tang. The chemical conversions and the possible mechanisms involved in the preparation of Du–Shen–Tang, and the holistic quality consistency of Du–Shen–Tang derived from different batches of white ginseng were also investigated and evaluated.

2. Experimental

2.1. Chemicals, standards and samples

HPLC–MS grade acetonitrile from TEDIA Company Inc. (Fairfield, USA), MS grade formic acid from Sigma–Aldrich (Steinheim, Germany) were purchased, other solvents and chemicals were of analytical grade. Purified water was prepared using Milli-Q SP Reagent water system (Millipore, Bedford, MA, USA).

The reference standards of ginsenoside Rg_1 (4), Re (5), Rf (9), 24(*S*)-pesudoginsenoside F_{11} (10), ginsenoside Rg_2 (12), Rb_1 (15), Ro (16), Rc (18), Rb_2 (22), Rb_3 (23), Rd (28), 20(*R*)-Rg_3 (37) and Rh_2 (47) were provided by Hong Kong Jockey Club Institute of Chinese Medicine (Hong Kong, China), their purities were determined to be higher than 95% by UPLC–MS analysis.

The commercial white ginseng samples of CMED-0087-16 (Batch No. 20031123) and CMED-0087-24 (Batch No. 20091229) were purchased from different herbal shops in China, and were authenticated according to the standard documented in Chinese Pharmacopoeia [3]. The voucher specimens were deposited in Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine.

2.2. Liquid chromatography

UPLC was performed with a Waters ACQUITY UPLC™ system (Waters Corp., MA, USA), equipped with a binary solvent delivery system, auto-sampler, and a PDA detector. The chromatography was performed on a Waters ACQUITY HSS T3 column (100 mm × 2.1 mm, 1.8 μm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) ACN containing 0.1% formic acid. The UPLC elution condition was optimized as follows: linear gradient from 10 to 32% B (0–10 min), 32 to 80% B (10–20 min) and isocratic at 80% B (20–21 min). The flow rate was at 0.5 ml/min. The column and auto-sampler were maintained at 35 and 10 °C respectively. Each wash cycle consisted of 200 μl of strong solvent (80% ACN) and 600 μl of weak solvent (30% ACN). The injection volume of standard and sample was 2 μl.

2.3. Mass spectrometry

Mass spectrometry was performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) equipped with electrospray ionization (ESI) source operating in negative mode. The desolvation gas flow rate was set to 700 l/h at temperature of 400 °C, the source temperature was 100 °C. The capillary and cone voltages were set at 3500 and 45 V, respectively. The Q-TOF acquisition rate was 0.2 s and the inter-scan delay was 0.02 s. Argon was employed as the collision gas at a pressure of 7.066×10^{-3} Pa.

The energies for collision-induced dissociation (CID) were set at 5 V for precursor ion and 45 V for product ion information, respectively.

2.4. Accurate mass measurement

All MS data were acquired using the LockSpray™ to ensure mass accuracy and reproducibility. The molecular masses of the precursor ion and of product ions were accurately determined with leucine-enkephalin (*m*/*z* 554.2615) in negative electrospray ionization mode at the concentration of 50 pg/μl and the infusion flow rate was 10 μl/min. Centroided data were acquired for each sample from 150 to 1400 Da and dynamic range enhancement (DRE™) was applied in the MS experiment to ensure accurate mass measurement over a wide dynamic range.

2.5. Sample preparation

2.5.1. Reference standards solutions

Store solutions: a certain amount of ginsenoside Re, Rg_1, Rf, Rg_2, Rb_1, Ro, Rc, Rb_2, Rb_3, Rd, 20(*R*)-Rg_3, Rh_2 and 24(*S*)-Pseudoginsenoside F_{11} were dissolved with methanol respectively to get thirteen reference standards store solutions (0.2–1.0 mg/ml), and were stored under 4 °C.

Reference standards mixture solution: a certain amount of above thirteen reference standards store solutions were mixed, and diluted with methanol to get reference standards mixture solution (about 125 ng/ml for each compounds), and the solution was filtered by a 0.2 μm PTFE syringe filter before UPLC–Q-TOF-MS/MS analysis.

Mimic decoctions of reference standards: 0.5 ml of each reference standard store solution was rotary evaporated to dryness, the residue was refluxed with 0.5 ml of water at 100 °C for 40 min. Then the water solution was rotary evaporated, the residues were dissolved with 1.0 ml of 70% methanol, the solution was filtered by a 0.2-μm PTFE syringe filter before UPLC–Q-TOF-MS/MS analysis.

2.5.2. Extracts of white ginseng

Methanol extracts: Each white ginseng sample was accurately weighed (approximately 0.2 g) and ultrasonic-extracted with 8.0 ml of methanol for 60 min at room temperature. The extract was then filtered by a 0.2 μm PTFE syringe filter before UPLC–Q-TOF-MS/MS analysis.

70% aqueous methanol extracts: Each white ginseng sample was accurately weighed (approximately 0.2 g) and ultrasonic-extracted

with 8.0 ml of 70% aqueous methanol for 60 min at room temperature. The extract was then filtered by a 0.2-μm PTFE syringe filter before UPLC–Q-TOF-MS/MS analysis.

Decoctions: Each white ginseng sample was accurately weighed (approximately 0.2 g) and refluxed with 8.0 ml of water at 100 °C for 40 min. The extract was rotary evaporated at 50 °C, and then ultrasonic-extracted with 8.0 ml of 70% aqueous methanol for 60 min at room temperature. The extract was then filtered by a 0.2-μm PTFE syringe filter before UPLC–Q-TOF-MS/MS analysis.

2.6. Establishment of in-house library and peak assignment

By searching from data bases, such as PubMed of the U.S. National Library Medicine and the National Institutes of Health, Scifinder Scholar of American Chemical Society, ScienceDirect of Elsevier and Chinese National Knowledge Infrastructure (CNKI) of Tsinghua University, all components reported in the literatures on *Panax* species were summarized in a Microsoft Office Excel table to establish a in-house library, which includes the name, molecular formula, molecular weigh, chemical structures and literatures of each published known compound. The "Find" function of Microsoft Office Excel was used to match the empirical molecular formula with that of published known compounds in the library. The empirical molecular formula was deduced from and short listed by comparing the accurately measured mass value to the theoretical exact mass value of putative deprotonated molecular ions $[M-H]^-$ and/or fragment ions at the mass accuracy of less than 5 ppm.

3. Results and discussion

3.1. Chromatographic conditions and Q-TOF-MS/MS method development

Peak capacity is very important for herb chemical profiling studies using the LC–MS approach. In previous studies on UPLC analysis of ginseng, ACQUITY BEH C_{18} column was used to separate ginsenosides [19–22]. It was reported that ACQUITY HSS T3 column was superior to ACQUITY BEH C_{18} column for hydrophilic compounds because of its higher peak capacity and stronger retention ability [25]. In preliminary studies, we compared the effectiveness of the two columns in the analysis of white ginseng decoctions and found that ACQUITY HSS T3 (100 mm × 2.1 mm, 1.8 μm) was more suitable for the analysis of white ginseng decoctions because more hydrophilic compounds were retained and separated, and under the optimal chromatographic condition, over 50 peaks were detected within 18 min (Fig. 2).

Both positive and negative ion modes were tested. It was found that compared to the positive ion mode, ginsenosides had not only higher sensitivity but also clearer mass spectra in the negative ion mode, which made it easier to detect ginsenosides of lower content in Du–Shen–Tang, and easier to confirm molecular ions or quasi-molecular ions in the identification of each peak. As such, data monitored in negative ion mode was used for the component detection and characterization.

3.2. Identity assignment and confirmation of the detected ginsenosides in Du–Shen–Tang

Under the present chromatographic and MS conditions, a total of 45 major ginsenosides were identified from the decoctions of white ginseng (Figs. 1 and 2), 11 of which (Re, Rg_1, Rf, Rg_2, Rb_1, Ro, Rc, Rb_2, Rb_3, Rd and 20 (*R*)-Rg_3) were confirmed by comparing the mass spectra and retention times with that of reference compounds, while the others were tentatively assigned by matching the empirical molecular formula with that of the published known ginsenosides, and/or further confirmed by elucidating the low energy CID fragment ions, in particular for those isomeric ginsenosides. In addition, the chromatographic behaviors of some ginsenosides in the literatures were considered as complementary data for the identity confirmation of isomers. The details of identified ginsenosides were summarized in Table 1 .

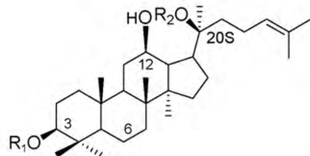
As shown in Table 1, the mass accuracy for all molecular ions, quasi-molecular ions and fragment ions were less than 5 ppm, indicating that the empirical molecular formula well match the pudative deprotonated ions, quasi-molecular ions and fragment ions. Formic acid was added to the mobile phase not only as a chromatographic modifier, but also to generate adduct ion $[M-H+HCOOH]^-$, which was helpful for the confirmation of deprotonated molecular ions $[M-H]^-$, and could also be used to distinguish the ginsenoside type of each peak. For example, malonyl-ginsenosides, acetyl-ginsenosides and oleanolic acid type ginsenosides could not generate adduct ions $[M-H+HCOOH]^-$, while other protopanaxadiol and protopanaxatriol-type ginsenosides did under the present conditions (Table 1).

Ginsenoside Re and Rg_1, two not easily separated ginsenosides in most of the previous studies [19,21,26–28], were also co-eluted under the present chromatographic conditions. Their deprotonated ions $[M-H]^-$, adduct ions $[M-H+HCOOH]^-$ and low energy CID fragment ions (such as ion *m/z* 783.4895 of Re) were used to confirm the existence of these two ginsenosides in the decoctions of white ginseng. Similarly, low energy CID fragment ions confirmed that Du–Shen–Tang contains ginsenoside Rf (fragment ions *m/z* 637.4301 and 475.3791), not 24(*S*)-Pseudoginsenoside F_{11} (fragment ions *m/z* 653.4269, 635.4140 and 491.3716). The retention sequence of isomeric ginsenosides Ma-Rc, Ma-Rb_2 and Ma-Rb_3 was deduced from the chromatographic features of their corresponding demalonylated ginsenosides Rc, Rb_2 and Rb_3, which have been confirmed with reference standards. Due to unavailable reference standards, the isomers Chikusetsusaponin IVa/Zingibroside R_1 [29], Rg_6/F_4/Rk_1/Rg_5, Rk_3/Rh_4, 20(*S*)-Rg_3/20(*R*)-Rg_3/F_2, 20(*S*)-Rs_3/20(*R*)-Rs_3 and Rs_5/Rs_4 [30] were analyzed based on their chromatographic features published in the literatures [29,30]. It should be noted that ginsenoside Rh_2, one of the artifact ginsenosides of red ginseng (steamed ginseng) [10], was not detected in the decoctions of these two white ginseng samples. It was astonishing to find that two major peaks (Peaks 1 and 2), which were assumed to be sulfur containing compounds through accurate molecular weight matching, were detected in the decoction and 70% aqueous methanol extract of the sample CMED-0083-24 (Fig. 2D, E and Table 1). The identities of these two compounds need further confirmation.

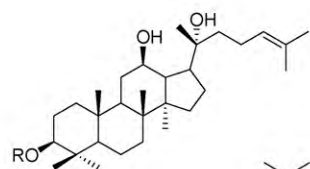
3.3. Decocting-induced chemical transformations in Du–Shen–Tang

Methanol extracts, 70% aqueous methanol extracts and decoctions of two batches of white ginseng were compared by the established UPLC–Q-TOF-MS/MS based chemical profiling method. It was found that the chemical profiles of methanol and 70% aqueous methanol extracts of the same batch of sample were similar to each other, thus 70% aqueous methanol was used as solvent to extract samples for further study. As demonstrated in Fig. 2B and D, there was significant difference between the chemical profiles of 70% aqueous methanol extracts from two batches of white ginseng. The peaks 6, 7, 8, 17, 21, 25, 27 were detected only in the sample (CMED-0087-24) produced in the year 2009 (Batch No. 20091229), and the peak areas of peaks 20, 24 and 29 were significantly higher than that in the sample (CMED-0087-16) produced in the year 2003 (Batch No. 20031123) (Fig. 2B and D). All these peaks were identified as malonyl-ginsenosides, as demonstrated in Table 1, supporting the previous findings that

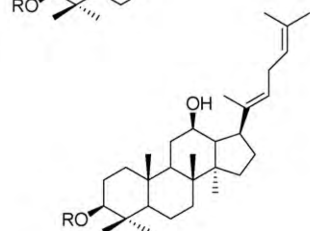
Protopanaxadiol-type ginsenosides



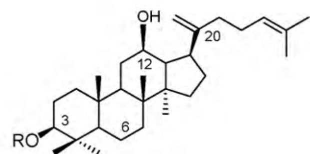
Ginsenoside	R_1	R_2
Rb_1	-Glc²-¹Glc	-Glc⁶-¹Glc
Ma-Rb1	-Glc²-¹Glc⁶-Malonyl	-Glc⁶-¹Glc
Rb_2	-Glc²-¹Glc	-Glc⁶-¹Ara(p)
Ma-Rb_2	-Glc²-¹Glc-Malonyl	-Glc⁶-¹Ara(p)
Rb_3	-Glc²-¹Glc	-Glc⁶-¹Xyl
Ma-Rb_3	-Glc²-¹Glc⁶-Malonyl	-Glc⁶-¹Xyl
Rc	-Glc²-¹Glc	-Glc⁶-¹Ara(f)
Ma-Rc	-Glc²-¹Glc⁶-Malonyl	-Glc⁶-¹Ara(f)
Rd	-Glc²-¹Glc	-Glc
Ma-Rd	-Glc²-¹Glc⁶-Malonyl	-Glc
F_2	-Glc	-Glc
Ra_1	-Glc²-¹Glc	-Glc⁶-¹Ara(p) ⁴-¹Xyl
Ra_2	-Glc²-¹Glc	-Glc⁶-¹Ara(f) ²-¹Xyl
20(*S*)-Rg_3	-Glc²-¹Glc	-H
20(*S*)- Rs_3	-Glc²-¹Glc⁶-Ac	-H
Rh_2	-Glc	-H



Ginsenoside	R
20(*R*)-Rg_3	-Glc²-¹Glc
20(*R*)-Rs_3	-Glc²-¹Glc⁶-Ac

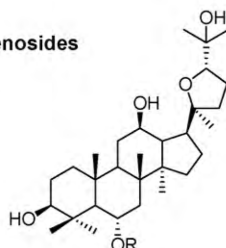


Ginsenoside	R
Rg_5	-Glc²-¹Glc
Rs_4	-Glc²-¹Glc⁶-Ac



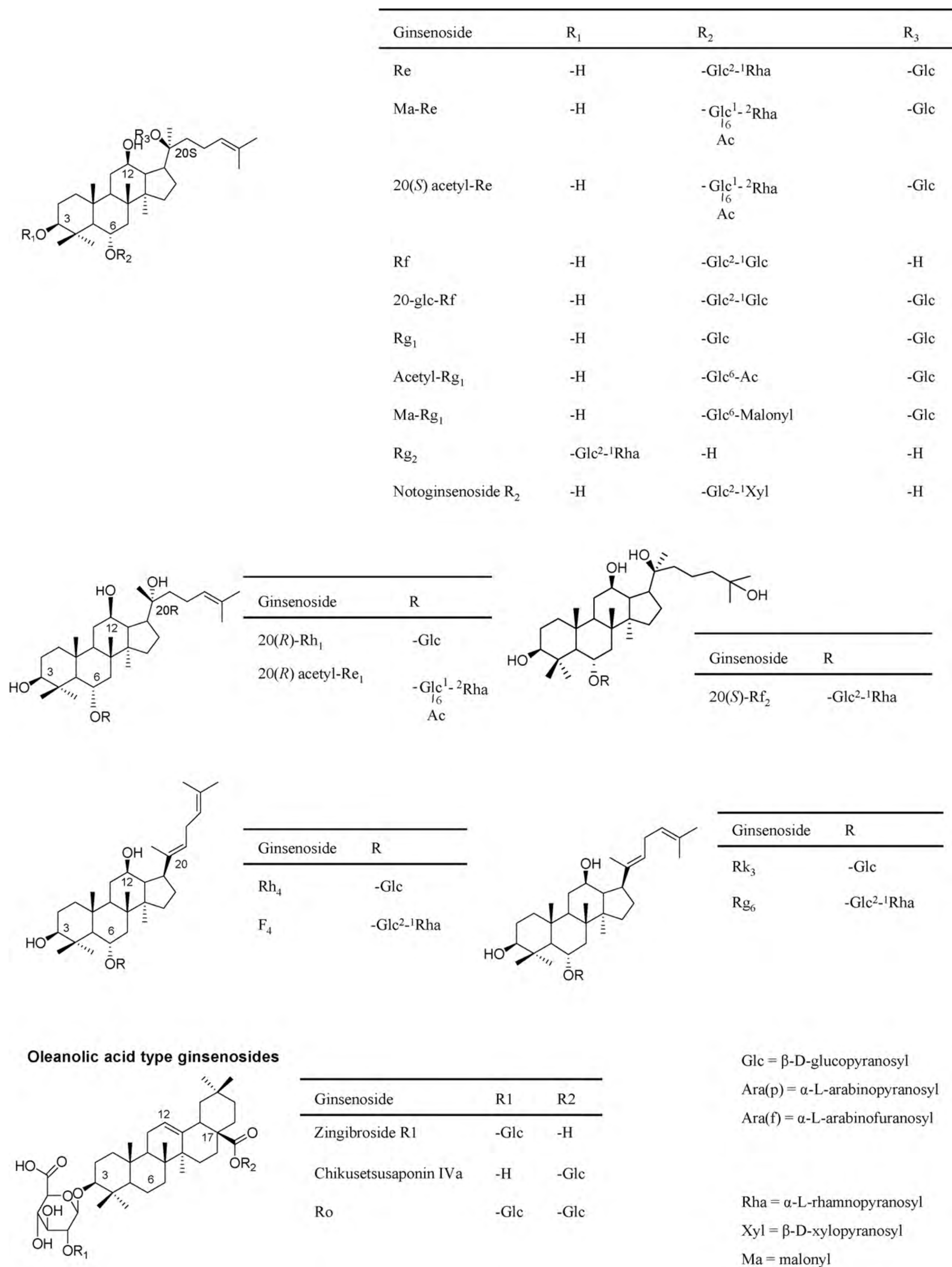
Ginsenoside	R
Rk_1	-Glc²-¹Glc
Rs_5	-Glc²-¹Glc⁶-Ac

Ocotilloll-type ginsenosides



Ginsenoside	R
24(*S*)- Pseudo F_{11}	-Glc²-¹Rha

Fig. 1. Major components identified from Du–Shen–Tang of white ginseng.



Ginsenoside	R_1	R_2	R_3
Re	-H	-Glc^{2}-^{1}Rha	-Glc
Ma-Re	-H	-Glc^{1}-^{2}Rha (6: Ac)	-Glc
20(*S*) acetyl-Re	-H	-Glc^{1}-^{2}Rha (6: Ac)	-Glc
Rf	-H	-Glc^{2}-^{1}Glc	-H
20-glc-Rf	-H	-Glc^{2}-^{1}Glc	-Glc
Rg_1	-H	-Glc	-Glc
Acetyl-Rg_1	-H	-Glc^{6}-Ac	-Glc
Ma-Rg_1	-H	-Glc^{6}-Malonyl	-Glc
Rg_2	-Glc^{2}-^{1}Rha	-H	-H
Notoginsenoside R_2	-H	-Glc^{2}-^{1}Xyl	-H

Ginsenoside	R
20(*R*)-Rh_1	-Glc
20(*R*) acetyl-Re_1	-Glc^{1}-^{2}Rha (6: Ac)

Ginsenoside	R
20(*S*)-Rf_2	-Glc^{2}-^{1}Rha

Ginsenoside	R
Rh_4	-Glc
F_4	-Glc^{2}-^{1}Rha

Ginsenoside	R
Rk_3	-Glc
Rg_6	-Glc^{2}-^{1}Rha

Ginsenoside	R1	R2
Zingibroside R1	-Glc	-H
Chikusetsusaponin IVa	-H	-Glc
Ro	-Glc	-Glc

Glc = β-D-glucopyranosyl
Ara(p) = α-L-arabinopyranosyl
Ara(f) = α-L-arabinofuranosyl
Rha = α-L-rhamnopyranosyl
Xyl = β-D-xylopyranosyl
Ma = malonyl

Fig. 1. (*Continued*).

Table 1
Components identified from Du–Shen–Tang of white ginseng.

Peak No.	Identity	t_R (min)	Molecular formula	$[M-H]^-$			$[M-H+HCOOH]^-$ (mass accuracy, ppm)	Fragment ions of $[M-H]^-$ at low energy (45 eV) CID (mass accuracy, ppm)	References
				Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)			
1	Unknown	1.82	$C_{42}H_{71}O_{17}S$	879.4415	879.4412	0.3	–	717.3884 $[M-H-(Glc-H_2O)]^-$ (0) 699.3777 $[M-H-Glc]^-$ (−0.1)	
2	Unknown	1.93	$C_{42}H_{73}O_{17}S$	881.4600	881.4568	3.6	–	719.4016 $[M-H-(Glc-H_2O)]^-$ (−2.7) 701.3922 $[M-H-Glc]^-$ (−1.9)	
3	20-glc-Rf	2.31	$C_{48}H_{82}O_{19}$	961.5402	961.5372	3.1	1007.5565 (3.4)	799.4808 $[M-H-(Glc-H_2O)]^-$ (−4.5) 637.4357 $[M-H-2(Glc-H_2O)]^-$ (4.9)	[33]
4	Rg_1[a]	2.52	$C_{42}H_{72}O_{14}$	799.4833	799.4844	−1.4	845.4913 (1.7)	637.4304 $[M-H-(Glc-H_2O)]^-$ (−4.0) 475.3797 $[M-H-2(Glc-H_2O)]^-$ (1.2)	[33]
5	Re[a]	2.47	$C_{48}H_{82}O_{18}$	945.5423	945.5391	−3.2	991.5515 (3.7)	799.4844 $[M-H-(Rha-H_2O)]^-$ (0.0) 783.4895 $[M-H-(Glc-H_2O)]^-$ (0.1) 637.4316 $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$ (−4.4) 475.3792 $[M-H-2(Glc-H_2O)-(Rha-H_2O)]^-$ (1.1)	[10]
6	Ma-Rg_1/isomer	2.62	$C_{45}H_{74}O_{17}$	885.4868	885.4848	2.3	–	841.4921 $[M-H-CO_2]^-$ (−3.3)	[33]
7	Ma-Re	2.78	$C_{51}H_{84}O_{21}$	1031.5427	1031.5427	0	–	987.5551 $[M-H-CO_2]^-$ (2.2)	[10]
8	Ma-Rg_1/isomer	2.84	$C_{45}H_{74}O_{17}$	885.4855	885.4848	0.8	–	841.4945 $[M-H-CO_2]^-$ (−0.5)	[33]
9	Rf[a]	4.85	$C_{42}H_{72}O_{14}$	799.4838	799.4844	−0.8	845.4898 (−0.1)	637.4301 $[M-H-(Glc-H_2O)]^-$ (−2.4) 475.3791 $[M-H-2(Glc-H_2O)]^-$ (0.8)	[33]
10	24(*S*)-Pseudoginsenoside F_{11}[a]	4.92	$C_{42}H_{72}O_{14}$	799.4853	799.4844	1.1	845.4897 (−0.2)	653.4269 $[M-H-(Rha-H_2O)]^-$ (0.6) 635.4140 $[M-H-(Rha-H_2O)-H_2O]^-$ (0.5) 491.3716 $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$ (−4.3)	[10]
11	Notoginsenoside R_2	5.51	$C_{41}H_{70}O_{13}$	769.4774	769.4738	4.7	815.4818 (3.1)	637.4328 $[M-H-(Xyl-H_2O)]^-$ (1.9) 475.3782 $[M-H-(Xyl-H_2O)-(Glc-H_2O)]^-$ (−1.1)	[27]
12	Rg_2[a]	6.40	$C_{42}H_{72}O_{13}$	783.4872	783.4895	−2.9	829.4954 (0.6)	637.4301 $[M-H-(Glc-H_2O)]^-$ (−1.9) 475.3815 $[M-H-2(Glc-H_2O)]^-$ (3.9)	[29]
13	20 (*R*)-Rh_1/F_1	6.62	$C_{36}H_{62}O_9$	637.4343	637.4316	2.7	683.4355 (−2.2)	475.3796 $[M-H-(Glc-H_2O)]^-$ (2.6)	[33]
14	Ra_1/Ra_2	6.90	$C_{58}H_{98}O_{26}$	1209.6307	1209.6268	3.2	1255.6179 (−3.5)	1077.5891 $[M-H-(Xyl-H_2O)]$ (2.8)	[34]
15	Rb_1[a]	7.32	$C_{54}H_{92}O_{23}$	1107.5948	1107.5951	−0.3	1153.5947 (4.1)	945.5423 $[M-H-(Glc-H_2O)]^-$ (−1.4) 783.4895 $[M-H-2(Glc-H_2O)]^-$ (−0.1) 621.4326 $[M-H-3(Glc-H_2O)]^-$ (−3.1)	[33]
16	Ro[a]	7.95	$C_{48}H_{76}O_{19}$	955.4909	955.4903	0.6	–	793.4373 $[M-H-(Glc-H_2O)]^-$ (0.1)	[10]
17	Ma-Rb_1	8.02	$C_{57}H_{94}O_{26}$	1193.5976	1193.5955	0.8	–	1149.6057 $[M-H-CO_2]^-$ (−5.0)	[28]
18	Rc[a]	8.63	$C_{53}H_{90}O_{22}$	1077.5862	1077.5846	1.5	1123.5868 (3.7)	945.5422 $[M-H-(Ara(f)-H_2O)]^-$ (−0.1) 915.5361 $[M-H-(Glc-H_2O)]^-$ (3.0) 783.4803 $[M-H-(Ara(f)-H_2O-(Glc-H_2O)]^-$ (−4.2) 621.4360 $[M-H-(Ara(f)-H_2O-2(Glc-H_2O)]^-$ (2.3)	[10]
19	Ra_1/Ra_2	8.82	$C_{58}H_{98}O_{26}$	1209.6218	1209.6268	−4.1	1255.6256 (1.5)	1077.5853 $[M-H-(Xyl-H_2O)]$ (1.7)	[34]
20	Ma-Rc	9.52	$C_{56}H_{92}O_{25}$	1163.6218	1163.6268	−4.1	–	1119.5961 $[M-H-CO_2]^-$ (4.6)	[28]
21	Ma-Rb_1/isomer	9.92	$C_{57}H_{94}O_{26}$	1193.5917	1193.5955	−3.2	–	1149.6057 $[M-H-CO_2]^-$ (−2.4)	[28]
22	Rb_2[a]	10.20	$C_{53}H_{90}O_{22}$	1077.5891	1077.5846	4.2	1123.5890 (4.2)	945.5454 $[M-H-(Ara(p)-H_2O)]^-$ (3.1) 915.5359 $[M-H-(Glc-H_2O)]^-$ (3.7) 783.4914 $[M-H-(Ara(p)-H_2O)-(Glc-H_2O)]^-$ (4.9)	[30]
23	Rb_3[a]	10.80	$C_{53}H_{90}O_{22}$	1077.5842	1077.5838	−0.4	1123.5831 (−0.4)	945.5463 $[M-H-(Xyl-H_2O)]^-$ (4.2) 915.5368 $[M-H-(Glc-H_2O)]^-$ (4.1) 783.4912 $[M-H-(Xyl-H_2O)-(Glc-H_2O)]^-$ (4.9)	[30]
24	Ma-Rb_2	11.00	$C_{56}H_{92}O_{25}$	1163.5809	1163.5849	−3.4	–	1119.5951 $[M-H-CO_2]^-$ (3.6)	[28]
25	Ma-Rc/Rb_2/Rb_3/isomer	11.31	$C_{56}H_{92}O_{25}$	1163.5779	1163.5849	−6.0	–	1119.5946 $[M-H-CO_2]^-$ (3.5)	[28]
26	Chikusetsusaponin IVa	11.60	$C_{42}H_{66}O_{14}$	793.4373	793.4374	0.1	–	631.3728 $[M-H-(Glc-H_2O)]^-$ (−2.9)	[10]
27	Ma-Rb_3	11.88	$C_{56}H_{92}O_{25}$	1163.5859	1163.5849	0.9	–	1119.5951 $[M-H-CO_2]^-$ (3.6)	[28]
28	Rd[a]	12.02	$C_{48}H_{82}O_{18}$	945.5467	945.5423	4.7	991.5554 (4.3)	783.4895 $[M-H-(Glc-H_2O)]^-$ (4.0) 621.4314 $[M-H-2(Glc-H_2O]^-$ (−4.6)	[28]
29	Ma-Rd	12.21	$C_{51}H_{84}O_{21}$	1031.5391	1031.5427	−4.9	–	987.5558 $[M-H-CO_2]^-$ (4.9)	[28]
30	Rg_6	13.46	$C_{42}H_{70}O_{12}$	765.4805	765.4789	2.1	811.4857 (1.6)	619.4372 $[M-H-(Rha-H_2O)]^-$ (1.5)	[30]
31	F_4	13.64	$C_{42}H_{70}O_{12}$	765.4780	765.4789	−1.2	811.4895 (4.3)	619.4395 $[M-H-(Rha-H_2O)]^-$ (2.7) 457.3419 $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$ (3.6)	[30]
32	Rk_3	13.80	$C_{36}H_{60}O_8$	619.4215	619.4210	0.3	665.4243 (−3.3)	–	[30]
33	Rh_4	14.10	$C_{36}H_{60}O_8$	619.4196	619.4210	−2.3	665.4272 (1.1)	–	[30]

Table 1 (*Continued*)

Peak No.	Identity	t_R (min)	Molecular formula	$[M-H]^-$			$[M-H+HCOOH]^-$ (mass accuracy, ppm)	Fragment ions of $[M-H]^-$ at low energy (45 eV) CID (mass accuracy, ppm)	References
				Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)			
34	Zingibroside R_1	14.18	$C_{42}H_{66}O_{14}$	793.4398	793.4374	3.0	–	631.3714 $[M-H-(Glc-H_2O)]^-$ (−4.2)	[10]
35	20(*S*)-Rg_3	14.72	$C_{42}H_{72}O_{13}$	783.4866	783.4895	−3.7	829.4960 (0.5)	621.4366 $[M-H-(Glc-H_2O)]^-$ (−2.4) 459.3865 $[M-H-2(Glc-H_2O)]^-$ (1.7)	[30]
36	Acetyl-Rg_1/isomer	14.92	$C_{44}H_{74}O_{14}$	825.5007	825.5000	0.8	–	783.4924 $[M-H-C_2H_2O]^-$ (3.7) 621.4377 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (1.8) 459.3834 $[M-H-C_2H_2O-2(Glc-H_2O)]^-$ (−0.9)	[30]
37	20(*R*)-Rg_3	14.98	$C_{42}H_{72}O_{13}$	783.4921	783.4895	3.3	829.4963 (0.5)	621.4365 $[M-H-(Glc-H_2O)]^-$ (−2.4) 459.3838 $[M-H-2(Glc-H_2O)]^-$ (−1.0)	[30]
38	Acetyl-Rg_1/isomer	15.02	$C_{44}H_{74}O_{14}$	825.5027	825.5000	3.3	–	783.4912 $[M-H-C_2H_2O]^-$ (2.2) 621.4341 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (−4.0) 459.3833 $[M-H-C_2H_2O-2(Glc-H_2O)]^-$ (−1.1)	[30]
39	20(*S*) acetyl-Re_1	15.21	$C_{44}H_{74}O_{14}$	825.5039	825.5000	4.7	–	783.4911 $[M-H-C_2H_2O]^-$ (2.0) 621.4388 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (3.5) 459.3835 $[M-H-C_2H_2O-2(Glc-H_2O)]^-$ (−0.7)	[35]
40	20(*R*) acetyl-Re_1	15.38	$C_{44}H_{74}O_{14}$	825.5044	825.5000	4.4	–	783.4897 $[M-H-C_2H_2O]^-$ (0.3) 621.4397 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (5.0) 459.3855 $[M-H-C_2H_2O-2(Glc-H_2O)]^-$ (4.4)	[35]
41	20(*S*)-Rs_3	15.88	$C_{44}H_{74}O_{14}$	825.5029	825.5000	3.5	871.5055 (0.0)	783.4882 $[M-H-C_2H_2O]^-$ (−1.7) 621.4337 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (−4.7) 459.3835 $[M-H-C_2H_2O-2(Glc-H_2O)]^-$ (−0.7)	[30]
42	20(*R*)-Rs_3	16.04	$C_{44}H_{74}O_{14}$	825.4998	825.5000	−0.2	871.5059 (0.5)	783.4901 $[M-H-C_2H_2O]^-$ (0.8) 621.4389 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (4.7) 459.3835 $[M-H-C_2H_2O-2(Glc-H_2O)]^-$ (−0.7)	[30]
43	Rk_1	16.45	$C_{42}H_{70}O_{12}$	765.4788	765.4789	−0.1	811.4865 (2.6)	603.4261 $[M-H-(Glc-H_2O)]^-$ (−0.2)	[30,33]
44	Rs_5	16.62	$C_{44}H_{72}O_{13}$	807.4933	807.4895	4.7	–	765.4817 $[M-H-C_2H_2O]^-$ (3.7) 603.4241 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (−3.3)	[30]
45	Rg_5	16.78	$C_{42}H_{70}O_{12}$	765.4803	765.4789	1.8	811.4841(−0.4)	603.4265 $[M-H-(Glc-H_2O)]^-$ (0.7)	[30,33]
46	Rs_4	16.80	$C_{44}H_{72}O_{13}$	807.4902	807.4895	0.9	–	765.4811 $[M-H-C_2H_2O]^-$ (2.8) 603.4258 $[M-H-C_2H_2O-(Glc-H_2O)]^-$ (−0.5)	[30]
47	Rh_2[a]	17.28	$C_{36}H_{62}O_8$	621.4371	621.4366	0.8	667.4429 (1.2)	621.4367 $[M-H]^-$ (−2.4) 459.3851 $[M-H-(Glc-H_2O)]^-$ (0.6)	[30]
48	F_2	13.41	$C_{42}H_{72}O_{13}$	783.4867	783.4895	−3.6	829.4952 (0.4)	621.4378 $[M-H-(Glc-H_2O)]^-$ (2.0)	[10]
49	20(*S*)-Rf_2	2.36	$C_{42}H_{74}O_{14}$	801.5032	801.5000	4.0	847.5062 (0.8)	655.4424 $[M-H-(Rha-H_2O)]^-$ (0.5) 637.4337 $[M-H-(Rha-H_2O)-H_2O]^-$ (3.3) 493.3911 $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$ (3.6)	[32]

[a] Identified with reference standard.

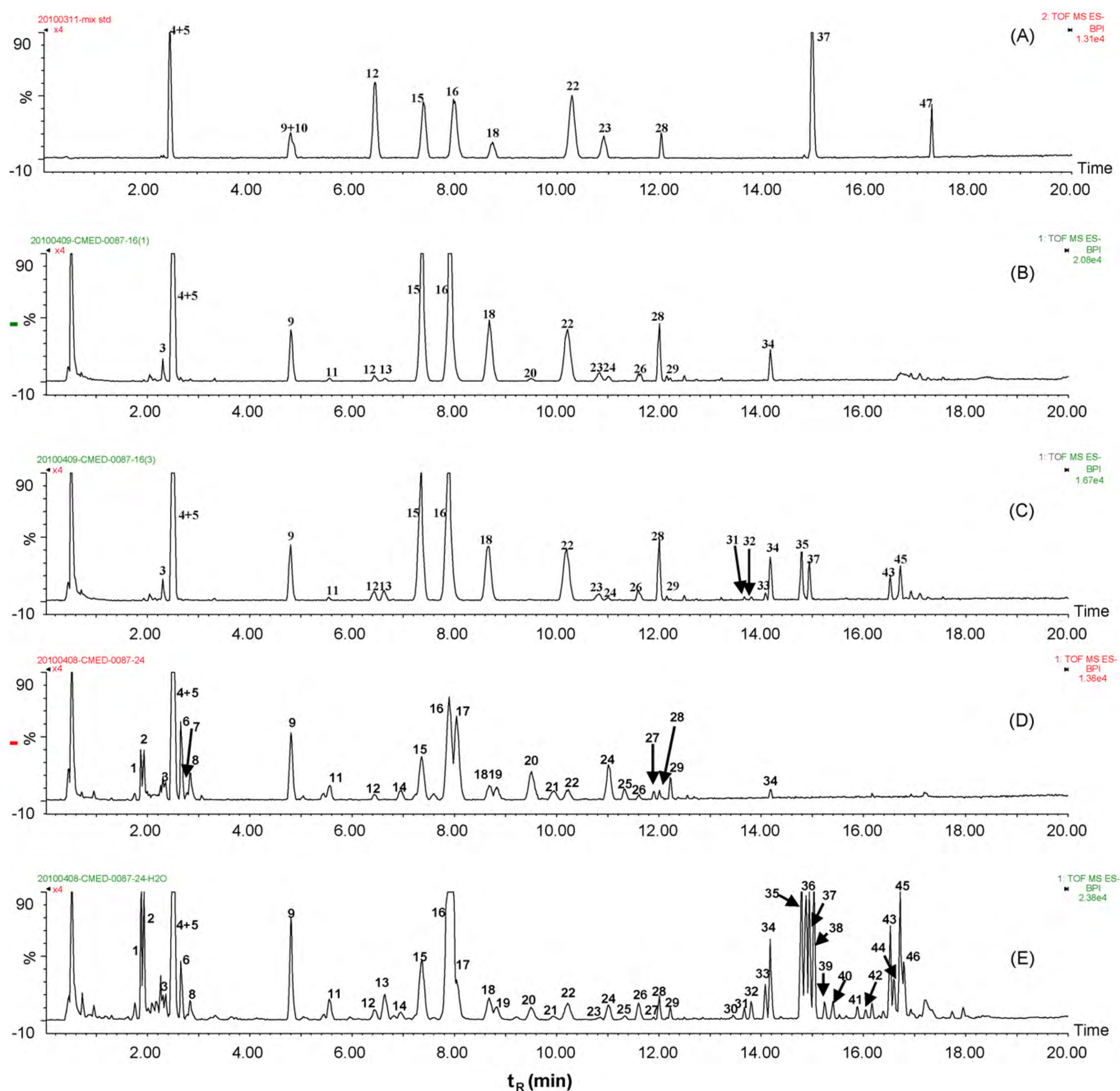


Fig. 2. Representative chromatograms of white ginseng: (A) reference compounds; (B, C) white ginseng CMED-0087-16; (D, E) white ginseng CMED-0087-24; and (B, D) 70% aqueous methanol extract; (C, E) decoctions.

the content of malonyl-ginsenosides decreased during storage of white ginseng [31], the mechanisms of which were deduced to be enzyme-involved hydrolysis of malonyl-ginsenosides into their corresponding neutral ginsenosides [10]. Compared to the 70% aqueous methanol extracts (Fig. 2B and D) of two batches of white ginseng, more peaks (peaks 30–33, 35–46) with t_R>13 min were detected in the decoctions (Fig. 2C and E), indicating that chemical conversions were induced during the decoction process of Du–Shen–Tang. Furthermore, it was interesting to note that there was also significant difference between the chemical profiles of decoctions derived from these two batches of white ginseng (Fig. 2C and E). Peaks 36, 38, 39, 40, 41, 42, 44 and 46 were only detected in the decoction derived from the sample CMED-0087-24 and were identified as acetyl-ginsenosides (Table 1). All aforementioned results suggested that storage duration may be one of the factors that can significantly influence the quality of not only the crude drug but also decoctions derived from white ginseng, although many other factors such as collection place, harvest time, post-harvest handling, etc. may also affect the chemical profiles of ginseng. Whether or not these changed chemical profiles can significantly influence the bioactivity of Du–Shen–Tang need further investigation.

3.4. Possible mechanisms involved in the decocting-induced chemical changes in Du–Shen–Tang

In order to identify the mechanisms underlying the decocting-induced chemical changes in Du–Shen–Tang, 13 available pure ginsenosides i.e., Re, Rg_1, Rf, Rg_2, Rb_1, Ro, Rc, Rb_2, Rb_3, Rd, 20(*R*)-Rg_3, Rh_2 and 24(*S*)-Pseudoginsenoside F_{11} underwent decocting respectively in the same way as in the preparation of Du–Shen–Tang. The mimic decoctions of pure ginsenosides were subjected to UPLC–Q-

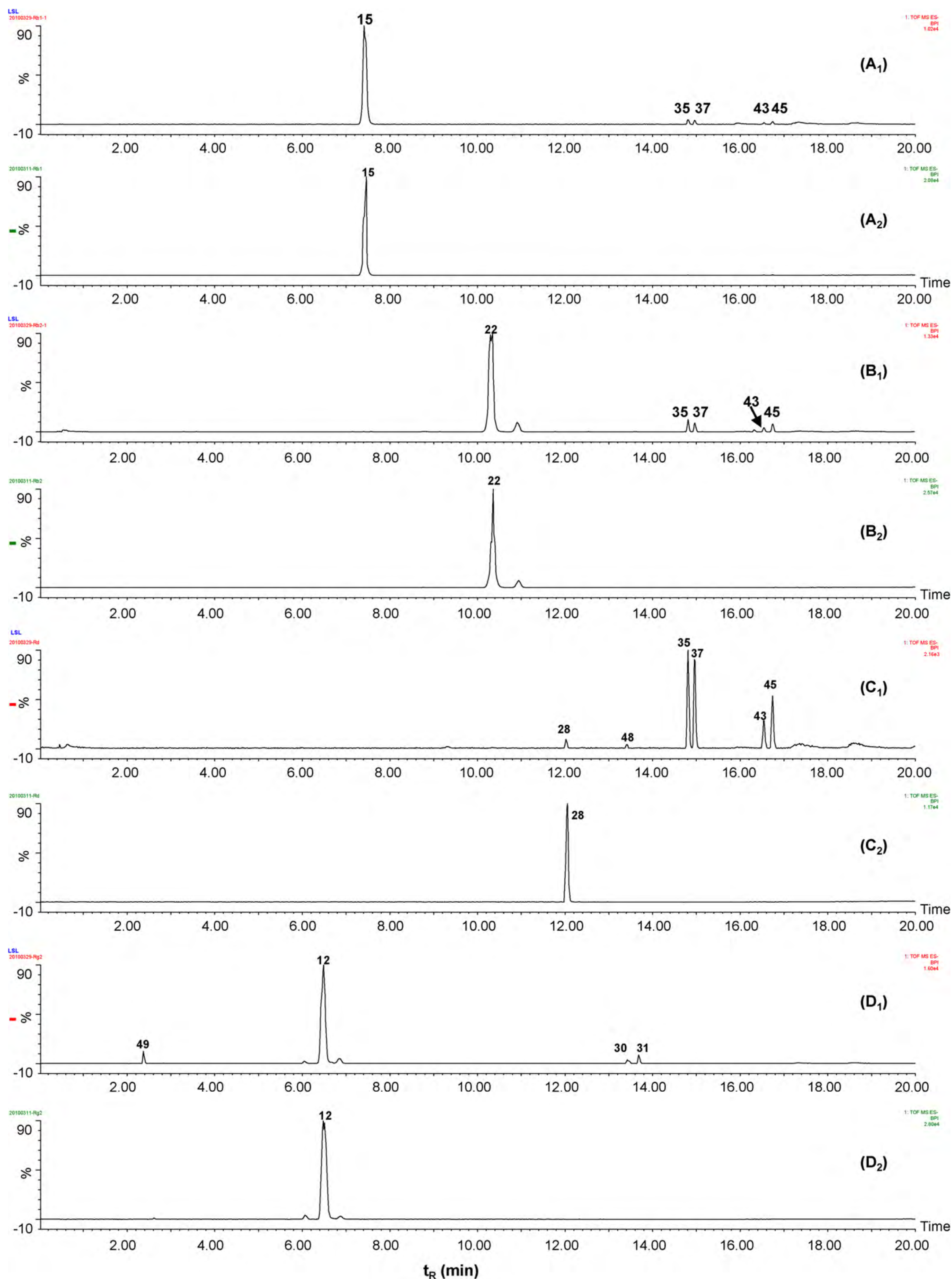


Fig. 3. Representative chromatograms of ginsenosides (A1, B1, C1, D1) 70% aqueous methanol solutions of four reference compounds; (A2, B2, C2, D2) mimic decoctions of four reference standards; (A) ginsenoside Rb_1; (B) ginsenoside Rb_2; (C) ginsenoside Rd; and (D) ginsenoside Rg_2.

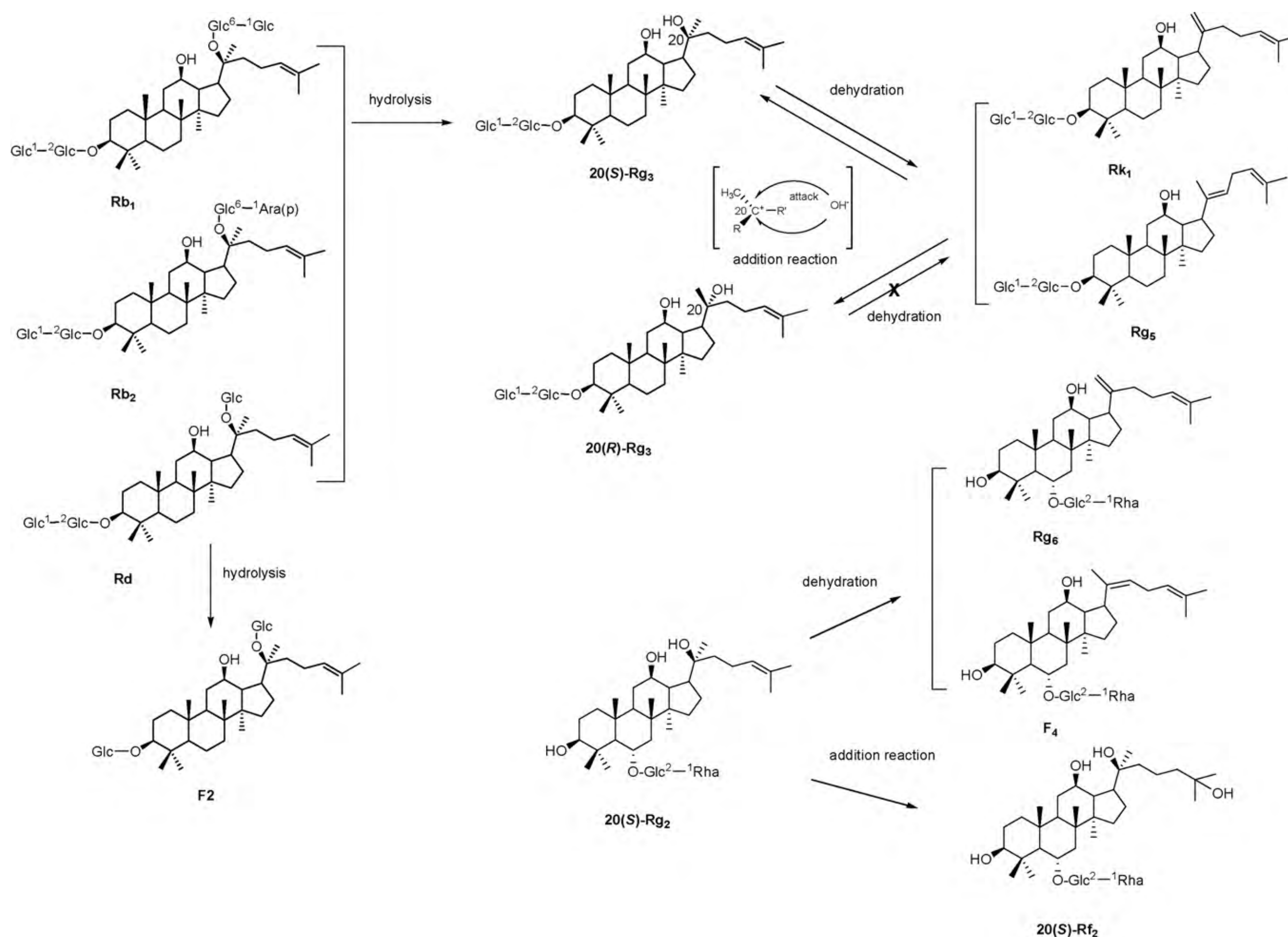


Fig. 4. Possible mechanisms involved in decocting-induced chemical conversions of ginsenosides Rb_1, Rb_2, Rd and Rg_2.

TOF-MS/MS analysis and the results were shown in Fig. 3. It was found that no significant chemical changes happened for most of ginsenosides tested (data not shown), except for ginsenosides Rb_1, Rb_2, Rd and Rg_2. Extra compounds were detected in the mimic decoctions of ginsenosides Rb_1, Rb_2, Rd and Rg_2, among which, Rd seemed much more easily changed during decocting (Fig. 3).

Four extra peaks (peaks 35, 37, 43 and 45) were detected in the mimic decoctions of ginsenoside Rb_1, Rb_2 and Rd (Fig. 3, A1, B1, C1), and were identified as 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1 and Rg_5, respectively [26]. The mechanisms involved were deduced to be the loss of glycosyl moiety at C-20-OH of Rb_1, Rb_2 and Rd through hydrolysis to generate 20(*S*)-Rg_3, then 20(*S*)-Rg_3 further undergo dehydration to generate ginsenosides Rk_1 and Rg_5. It should be noted that 20(*R*)-Rg_3 was detected in Du–Shen–Tang and mimic decoctions of ginsenoside Rb_1, Rb_2 and Rd, and its peak area always seemed similar to that of 20(*S*)-Rg_3 (Figs. 2C, E and 3, A1–C1), but no naturally occurring 20(*R*)-ginsenosides were reported so far, therefore 20(*R*)-Rg_3 was assumed to be generated from Rk_1 or Rg_5 via addition reaction. There might be a chemical equilibrium between 20(*S*)-Rg_3, 20(*R*)-Rg_3 and Rk_1 or Rg_5 (Fig. 4). Because Rk_1 and Rg_5 were not detected in the mimic decoction of 20(*R*)-Rg_3 (data not shown), the Rk_1 and Rg_5 found in Du–Shen–Tang were thought to be from the dehydration of 20(*S*)-Rg_3. In addition to these four artifacts, another compound (peak 48) was also detected in the mimic decoction of ginsenoside Rd (Fig. 3, C1). Since only one product ion *m*/*z* 621.4378 $[M-H-(Glc-H_2O)]^-$ was found in its low energy CID mass spectrum (Table 1), indicating that only one glycosyl moiety was attached at C-20, thus it was assigned to be ginsenoside F_2 [10]. It was assumed to be generated from Rd through hydrolysis of a glycosyl moiety at C–3–OH. The putative chemical conversion schemes were demonstrated in Fig. 4. Similarly, three extra peaks (peaks 31, 32 and 49) were detected in the mimic decoction of ginsenoside Rg_2 (Fig. 3, D1) and were assigned as ginsenoside Rg_6, F_4 [30] and 20(*S*)-Rf_2 [32], respectively, and were deduced to be generated through dehydration and addition reaction of Rg_2 (Fig. 4).

From the analysis of pure ginsenosides mimic decoctions described above, it could be concluded that those compounds newly generated in Du–Shen–Tang, such as 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1 and Rg_5 were mainly derived from Rb_1, Rb_2 and Rd, while Rg_6 and F_4 were from Rg_2 via hydrolysis, dehydration and addition reactions during the decoction of Du–Shen–Tang.

As for those acetyl-ginsenosides in Du–Shen–Tang, such as acetyl-Rg_1 [10], 20(*S*)-Rs_3, 20(*R*)-Rs_3, Rs_4, Rs_5 [30], 20(*S*)-acetyl Re or 20(*R*)-acetyl Re [33], etc., because they could only be detected in the decoction of white ginseng sample CMED-0087-24 which concurrently contained many kinds of malonyl-ginsenosides, but not in the decoction of white ginseng sample CMED-0087-16 which concurrently contained few malonyl-ginsenosides with very low content, these acetyl-ginsenosides were assumed to be generated from the malonyl-ginsenosides through decarboxylation, hydrolysis, dehydration and addition reactions (Fig. 5).

20(*S*)-Rs_3 was deduced to be generated from the hydrolysis of glycosyl moiety at C-20-OH and decarboxylation of malonyl moiety attached to glycosyl linkage at C-3-OH of malonyl-Rb_1, malonyl-Rb_2, malonyl-Rb_3, malonyl-Rc and malonyl-Rd. 20(*S*)-Rs_3 further underwent dehydration to generate Rs_4 and Rs_5. Like the conversions among 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1 and Rg_5, Rs_4 and Rs_5 may undergo addition reaction to generate 20(*S*)-Rs_3 and 20(*R*)-Rs_3,

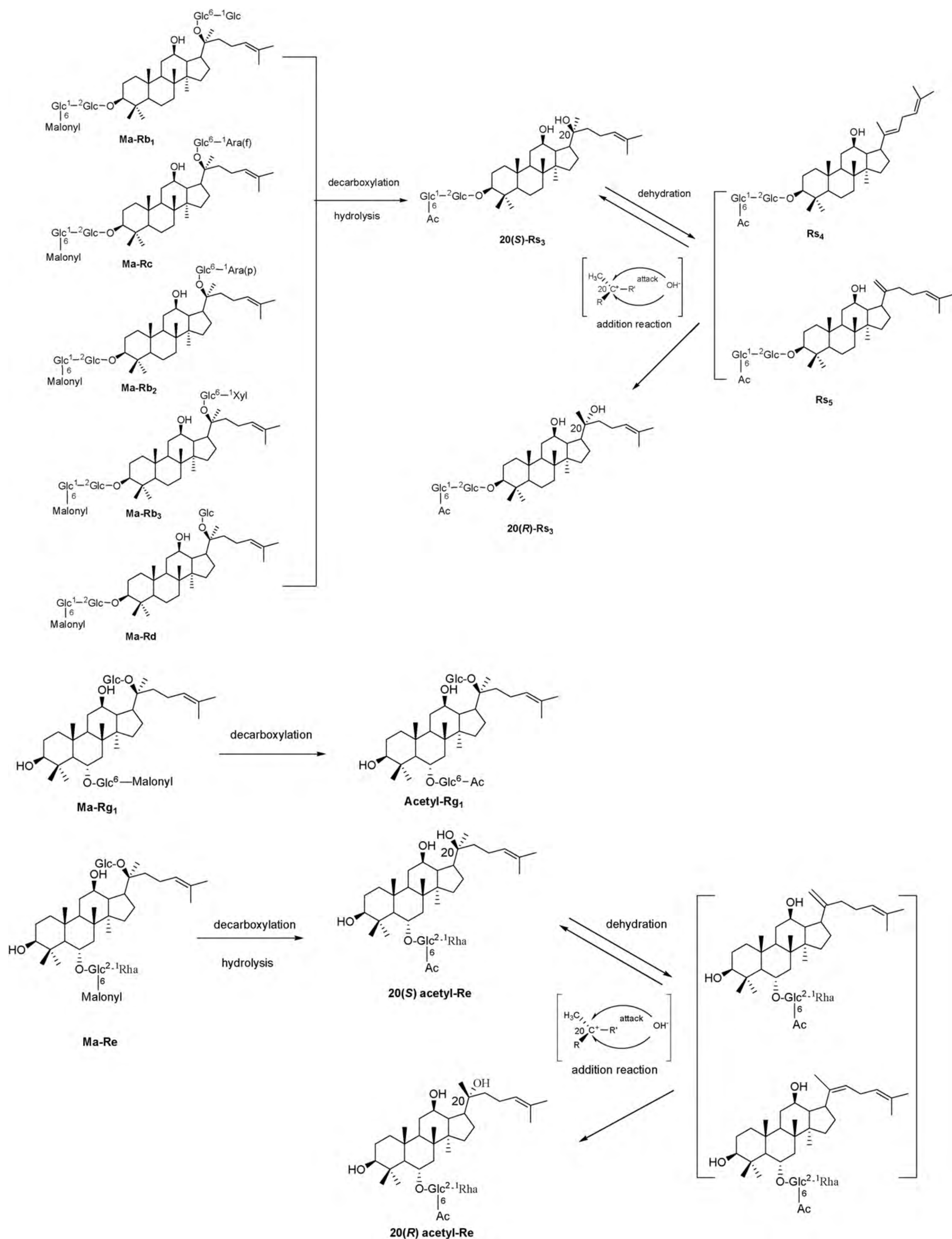


Fig. 5. Possible mechanisms involved in decocting-induced generation of acetyl ginsenosides in Du–Shen–Tang.

and chemical equilibrium may also occur among these four components. Similarly, acetyl Rg_1 was deduced to be produced from malonyl-Rg_1, and 20(*S*)-acetyl Re or 20(*R*)-acetyl Re from malonyl-Re, respectively (Fig. 5).

4. Conclusion

For the first time, the chemical profiles of Du–Shen–Tang derived from white ginseng were investigated by a newly established UPLC–Q-TOF-MS/MS based chemical profiling method. A total of 45 major ginsenosides were unambiguously identified and/or tentatively assigned in Du–Shen–Tang, among them 21 ginsenosides were detected to be newly generated during the decoction of white ginseng. The mechanisms involved in the chemical changes were assumed to be hydrolysis, dehydration, decarboxylation and addition reactions of the many original ginsenosides in white ginseng. Significant difference in chemical profiles was found between the decoctions of two batches of white ginseng, suggesting that storage duration or other factors significantly influenced the quality consistency of not only white ginseng but also its decoction Du–Shen–Tang. It can be concluded that UPLC–Q-TOF-MS/MS based chemical profiling is a rapid and powerful approach for holistic quality evaluation of Du–Shen–Tang, and should also be useful for the global quality investigation of decoctions derived from other herbal medicines.

Acknowledgement

This research was financially supported by the Hong Kong Jockey Club Charities Trust, and in part by the National Natural Science Foundation of China (No. 30940093) and the Natural Science Foundation of Jiangsu Province, China (No. BK2009495).

References

[1] Y. Wang, L. Yang, Y.Q. He, C.H. Wang, E.W. Welbeck, S.W.A. Bligh, Z.T. Wang, Characterization of fifty-one flavonoids in a Chinese herbal prescription Longdan Xiegan decoction by high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and photodiode array detection, Rapid Commun. Mass Spectrom. 22 (2008) 1767–1778.
[2] S.L. Li, J.Z. Song, C.F. Qiao, Y. Zhou, H.X. Xu, UPLC–PDA-TOFMS based chemical profiling approach to rapidly evaluate chemical consistency between traditional and dispensing granule decoctions of traditional medicine combinatorial formulae, J. Pharm. Biomed. Anal. 52 (2010) 468–478.
[3] C.J. Song, Du–Shen–Tang and “*Shi Yao Quan Shu*” (Book on Ten Magic Herbs), Ginseng Res. 17 (2005) 2–3.
[4] Chinese Pharmacopeia Commission, Pharmacopeia of the People’s Republic of China, vol. 1, 2010 edition, China Medical Science Publisher, Beijing, 2010, p. 8.
[5] S.F. Chu, J.T. Zhang, New achievements in ginseng research and its future prospects, Chin. J. Integr. Med. 15 (2009) 403–408.
[6] L. Jia, Y. Zhao, Current evaluation of the millennium phytomedicine–ginseng (I): etymology, pharmacognosy, phytochemistry, market and regulations, Curr. Med. Chem. 16 (2009) 2475–2484.
[7] L. Jia, Y. Zhao, X.J. Liang, Current evaluation of the millennium phytomedicine-ginseng (II): collected chemical entities, modern pharmacology, and clinical applications emanated from traditional Chinese medicine, Curr. Med. Chem. 16 (2009) 2924–2942.
[8] J.M. Lü, Q. Yao, C. Chen, Ginseng compounds: an update on their molecular mechanisms and medical applications, Curr. Vasc. Pharmacol. 7 (2009) 293–302.
[9] J.Z. Luo, L. Luo, Ginseng on hyperglycemia: effects and mechanisms, Evid. Based Complement. Alternat. Med. 6 (2009) 423–427.
[10] L.P. Christensen, Ginsenosides chemistry, biosynthesis, analysis, and potential health effects, Adv. Food Nutr. Res. 55 (2009) 1–99.
[11] D.J. Jang, M.S. Lee, B.C. Shin, Y.C. Lee, E. Ernst, Red ginseng for treating erectile dysfunction: a systematic review, Br. J. Clin. Pharmacol. 66 (2008) 444–450.
[12] J.K. Seida, T. Durec, S. Kuhle, North American (*Panax quinquefolius*) and Asian ginseng (*Panax ginseng*) preparations for prevention of the common cold in healthy adults: a systematic review, Evid. Based Complement. Alternat. Med. 6 (2009), 10.1093/ecam/nep068.
[13] M.S. Lee, E.J. Yang, J.I. Kim, E. Ernst, Ginseng for cognitive function in Alzheimer’s disease: a systematic review, J. Alzheimers Dis. 18 (2009) 339–344.
[14] P. Ying, K. Yue, N.K. Mak, Y.K. Cheng, K.W. Leung, T.B. Ng, D.T.P. Fan, H.W. Yeung, R.N.S. Wong, Pharmacogenomics and the Yin/Yang actions of ginseng: anti-tumor, angiomodulating and steroid-like activities of ginsenosides, Chin. Med. 2 (2007) 6.
[15] B.C. Liu, Two cases treatment of cardiogenic shock by Du–Shen–Tang, Pract. Clin. Comb. West. Trad. Chin. Med. 5 (2005) 57.
[16] F.H. Zhen, Twenty cases treatment of expanding myocardium by Du–Shen–Tang, Chin. J. Clin. Pharmacol. Ther. 2 (1997) 12.
[17] Y.X. Zhen, B.Y. Yang, Application of Du–Shen–Tang in obsterics, Chendu Med. 28 (2002) 215–216.
[18] T.W. Chan, P.P. But, S.W. Cheng, I.M. Kwok, F.W. Lau, H.X. Xu, Differentiation and authentication of *Panax ginseng*, *Panax quinquefolius*, and ginseng products by using HPLC/MS, Anal. Chem. 72 (2000) 1281–1287.
[19] G.X. Xie, Y. Ni, M.M. Su, Y.Y. Zhang, A.H. Zhao, X.F. Gao, Z. Liu, P.G. Xiao, W. Jia, Application of ultra-performance LC–TOFMS metabolite profiling techniques to the analysis of medicinal *Panax* herbs, Metabolomics 4 (2008) 248–260.
[20] G.X. Xie, R. Plumb, M.M. Su, Z.H. Xu, A.H. Zhao, M.F. Qiu, X.B. Long, Z. Liu, W. Jia, Ultra-performance LC/TOFMS analysis of medicinal *Panax* herbs for metabolomic research, J. Sep. Sci. 31 (2008) 1015–1026.
[21] M. Dan, G.X. Xie, X.F. Gao, X.B. long, M.M. Su, A.H. Zhao, T. Zhao, M.M. Zhou, Y.P. Qiu, W. Jia, A rapid ultra-performance liquid chromatography–electrospray ionization mass spectrometric method for the analysis of saponins in the adventitious roots of *Panax notoginseng*, Phytochem. Anal. 20 (2009) 68–76.
[22] M. Dan, M.M. Su, X.F. Gao, T. Zhao, A.H. Zhao, G.X. Xie, Y.P. Qiu, M.M. Zhou, Z. Liu, W. Jia, Metabolite profiling of *Panax notoginseng* using UPLC–ESI-MS, Phytochemistry 69 (2008) 2237–3344.
[23] X.Y. Wang, T. Zhao, X.F. Gao, M. Dan, M.M. Zhou, W. Jia, Simultaneous determination of 17 ginsenosides in rat urine by ultraperformance liquid chromatography-mass spectrometry with solid-phase extraction, Anal. Chim. Acta 594 (2007) 265–273.
[24] N. Angelova, H.W. Kong, R.V.D. Heijden, S.Y. Yang, Y.H. Choi, H.K. Kim, M. Wang, T. Hankemeier, J.V.D. Greef, G.W. Xu, R. Verpoorte, Recent methodology in the phytochemical analysis of ginseng, Phytochem. Anal. 19 (2008) 2–16.
[25] S.L. Li, J.Z. Song, F.F.K. Choi, C.F. Qiao, Y. Zhou, Q.B. Han, H.X. Xu, Chemical profiling of Radix Paeoniae evaluated by ultra-performance liquid chromatography/photo-diode-array/quatrupole time-of-flight mass spectrometry, J. Pharm. Biomed. Anal. 49 (2009) 253–266.
[26] X.Q. Ma, H.B. Xiao, X.M. Liang, Identification of ginsenosides in *Panax quinquefolium* by LC–MS, Chromatographia 64 (2006) 31–36.
[27] J.H. Liu, X. Wang, S.Q. Cai, K. Komatsu, T. Namba, Analysis of the constituents in the Chinese drug Notoginseng by liquid chromatography–electrospray mass spectrometry, J. Chin. Pharm. Sci. 13 (2004) 225–237.
[28] N. Fuzzati, B. Gabetta, K. Jayakar, R. Pace, F. Peterlongo, Liquid chromatography–electrospray mass spectrometric identification of ginsenosides in *Panax ginseng* roots, J. Chromatogr. A 845 (1999) 69–79.
[29] J. Li, H. Qi, L.W. Qi, L. Yi, P. Li, Simultaneous determination of main phytoecdysones and triterpenoids in Radix Achyranthis Bidentatae by high-performance liquid chromatography with diode array-evaporative light scattering detectors and mass spectrometry, Anal. Chim. Acta 596 (2007) 264–272.
[30] B.S. Sun, L.J. Cu, Z.M. Fang, C.Y. Wang, Z. wang, M.R. Lee, Z. Li, J.J. Li, C.K. Sung, Simultaneous quantification of 19 ginsenosides in black ginseng developed from *Panax ginseng* by HPLC–ELSD, J. Pharm. Biomed. Anal. 50 (2009) 15–22.
[31] X.W. Du, R.B.H. Will, D.L. Stuart, Changes in neutral and malonyl ginsenosdies in American ginseng (*Panax quinquefolium*) during drying, storage and ethanolic extraction, Food Chem. 86 (2004) 155–159.
[32] J.D Park, Y.H. Lee, S.I. Kim, Ginsenoside Rf_2, a new dammarane glycoside from Korean red ginseng (*Panax ginseng*), Arch. Pharm. Res. 21 (1998) 615–617.
[33] X.Q. Ma, X.M. Liang, Q. Xu, X.Z. Zhang, H.B. Xiao, Identification of ginsenosides in roots of *Panax ginseng* by HPLC–APCI/MS, Phytochem. Anal. 16 (2005) 181–187.
[34] P. Jiang, S.S. Dou, L. Liu, W.D. Zhang, Z.L. Chen, R.L. Xu, J.M. Ding, R.H. Liu, Identification of multiple constituents in the TCM-formula Shexiang Baoxin Pill by LC coupled with DAD–ESI-MS–MS, Chromtographia 70 (2009) 133–142.
[35] J.M. Jia, Z.Q. Wang, L.J. Wu, Two new acetylated ginsenosides from the roots of *Panax quinquefolium*, Chin. Chem. Lett. 19 (2008) 1099–1102.