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Research Article

Methanol-involved heterogeneous transformation of ginsenoside Rb1 to rare ginsenosides using heteropolyacids embedded in mesoporous silica with HPLC-MS investigation

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ABSTRACT

Background: The biological activity and pharmacological effects of rare ginsenosides have been proven to be superior to those of the major ginsenosides, but they are rarely found in ginseng.
Methods: Ginsenoside Rb1 was chemically transformed with the involvement of methanol molecules by a synthesized heterogeneous catalyst 12-HPW@MeSi, which was obtained by the immobilization of 12-phosphotungstic acid on a mesoporous silica framework. High-performance liquid chromatography coupled with mass spectrometry was used to identify the transformation products.
Results: A total of 18 transformation products were obtained and identified. Methanol was found to be involved in the formation of 8 products formed by the addition of methanol molecules to the C-24 (25), C-20 (21) or C-20 (22) double bonds of the aglycone. The transformation pathways of ginsenoside Rb1 involved deglycosylation, addition, elimination, cycloaddition, and epimerization reactions. These pathways could be elucidated in terms of the stability of the generated carbenium ion. In addition, 12-HPW@MeSi was able to maintain a 60.5% conversion rate of Rb1 after 5 cycles.
Conclusion: Tandem and high-resolution mass spectrometry analysis allowed rapid and accurate identification of the transformation products through the characteristic fragment ions and neutral loss. Rare ginsenosides with methoxyl groups grafted at the C-25 and C-20 positions were obtained for the first time by chemical transformation using the composite catalyst 12-HPW@MeSi, which also enabled cyclic heterogeneous transformation and facile centrifugal separation of ginsenosides. This work provides an efficient and recyclable strategy for the preparation of rare ginsenosides with the involvement of organic molecules.

1. Introduction

Ginseng, the root of *Panax ginseng* Mayer, has been widely used as a functional food and health-enhancing supplement for thousands of years. It has been shown to have an extensive range of pharmacological properties, such as antistress, antioxidative, and antifatigue activities as well as cancer-preventive effects [1–3]. Ginsenosides are considered to be the main components of ginseng with biological activity and pharmacological effects [4–6]. Major ginsenosides refer to the most abundant ones in ginseng, including ginsenoside Rb1, Rb2, Rc, Rd, Re, and Rg1, which were determined to constitute more than 80% of the total ginsenosides. In particular, the protopanaxadiol (PPD)-type ginsenoside Rb1 accounts for 23.8% of the total ginsenoside [7]. And the rest ginsenosides, which are the minority or absent in ginseng, are called rare ginsenosides, such as ginsenoside Rg3, C–K, and Rh2 [8,9].

There has been considerable interest in rare ginsenosides, as their bioactivity and clinical efficacy are proven to be superior to the major ginsenosides [10]. In particular, dehydrated ginsenosides have been proven to have various biological activities and pharmacological effects [11–13]. For example, ginsenosides Rk1 and Rg5 can exert sedative and hypnotic effects and ameliorate memory impairment, and have been implicated in the regulation of anti-inflammatory, anti-tumor activities and anti-adipogenic activities [14–16]. However, many rare ginsenosides are barely present or even absent in natural ginseng. They can only be prepared from the major ginsenosides by the transformation process [17,18]. The transformation methods of the rare ginsenosides, such as

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heating, acid or alkali treatment, and enzymatic conversion, have been extensively studied [19–22]. In particular, heteropolyacids (HPAs) are considered good candidates for the chemical transformation of ginsenosides due to their strong acidity and tunable structures [23–25].

Nevertheless, the main disadvantages of HPAs for catalytic applications are their low specific surface area and high solubility in polar solvents, which limit their catalytic activity and separation from the reaction system [26]. Many studies focused on dispersing them on suitable host materials to form insoluble host-guest complexes, which would increase the surface area and reduce the solubility [27–30]. The silica-based host materials have attracted attention for their large specific surface area, high thermal stability, and moderate acidity and alkalinity. The HPA-SiO_2 composite catalysts typically could exist in the solution phase in a non-homogeneous form, which makes them easy to recover and reuse. For example, the $TiW_{11}Ti/SiO_2$ composite synthesized by Ai et al. [27] exhibits good catalytic activity and recyclability for the degradation of dye X–3B. However, there are few reports on the application of heterogeneous HPA catalysts for the preparation of rare ginsenosides.

High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) has been employed as a powerful tool for the separation and identification of ginsenosides. It provides abundant structure information and enables rapid qualitative and quantitative analysis of ginsenosides with high sensitivity and selectivity [31–34]. In our previous study, multistage tandem MS and high-resolution MS were used to identify the product structure of homogeneous transformation of ginsenoside Re by raw 12-phosphotungstic acid [35]. At present, much attention has been paid to the study of the bioactivity of ginsenoside transformation products. The effect of the reaction solvent on the transformation of ginsenosides and the acquisition of novel rare ginsenosides has rarely been reported.

Herein, we describe the chemical transformation of ginsenoside Rb1 with the involvement of methanol molecules using a host-guest catalyst system with 12-phosphotungstic acid immobilized on a mesoporous silica solid (12-HPW@MeSi). The mesoporous silica host offered a favorable environment for the stabilization and insolubility of the loaded 12-phosphotungstic acid in polar solvents, which can be readily separated from the catalytic system and recycled. Ginsenoside Rb1 underwent deglycosylation, epimerization, elimination, and addition reactions to produce 18 rare ginsenosides in the resulting heterogeneous environment. All the products were rapidly differentiated and identified by HPLC coupled with multistage tandem and high-resolution MS. Notably, for the first time, rare ginsenosides grafted with organic solvent molecules at the C-25 and C-20 positions of the aglycone are generated via chemical transformation.

2. Experimental

2.1. Chemicals

Tetraethyl orthosilicate (TEOS), hexadecyl trimethyl ammonium bromide (CTAB) and ammonium hydroxide ($NH_3{\cdot}H_2O$) were purchased from Shanghai Macklin Biochemical Co., Ltd. TEOS and CTAB were of analytical grade and $NH_3{\cdot}H_2O$ was of electronic grade. $H_3PW_{12}O_{40}{\cdot}6H_2O$ (12-HPW) of analytical grade and ginsenoside authentic standards of Rb1, 20(S)-Rg3, 20(R)-Rg3, Rg5 and Rk1 with over 98% purity were bought from Shanghai Yuanye Biological Technology Co., Ltd (Shanghai, China). HPLC-grade acetonitrile and methanol were acquired from Tedia (Fairfield, USA). HPLC-grade formic acid was acquired from Thermo Fisher (Waltham, USA). Distilled water was prepared by a Milli-Q system (Millipore, Bedford, MA).

2.2. Instruments

The powder X-ray diffraction (XRD) patterns were recorded on a TDM-10 X-ray diffractometer (Dandong Tongda Technology Co., Ltd., Liaoning, China) using Cu Kα radiation (λ = 1.5418 Å) over the 2θ range of 0.6–40°. Transmission electron microscopic (TEM) images were generated with a JEOL JEM-2100f instrument at an accelerating voltage of 200 kV. HPLC analysis was carried out using an Ultimate 3000 system (Thermo Scientific, San Jose, CA, USA). The chromatographic separation was performed on a Thermo Syncronis C18 column (100 mm × 2.1 mm, 1.7 μm) at a temperature of 35 °C. 0.1% formic acid in water (v/v) and acetonitrile were used as the mobile phases A and B, respectively, at a flow rate of 0.2 mL min^{-1}. The gradient elution was programmed as follows: 0–5 min (30% B); 5–8 min (30–36% B); 8–15 min (36–48% B); 15–20 min (48–70% B); 20–25 min (70–90% B) and 28–34 min (25% B). The injected sample volume was 2 μL. The mass spectra were recorded on Q Exactive hybrid quadrupole-orbitrap mass spectrometer and LTQ XL mass spectrometer (Thermo Scientific, San Jose, CA, USA), both equipped with an electrospray ionization (ESI) source. The conditions of the ESI source were as follows: sheath gas, 35 arb units; auxiliary gas, 10 arb units; capillary voltage, −3200 V.

2.3. Sample preparation

2.3.1. Preparation of MeSi

1.76 g of CTAB and 3.20 mL of $NH_3{\bullet}H_2O$ were dispersed in 500 mL of distilled water and stirred for 30 min. Under vigorous stirring, 9.33 mL of TEOS was slowly added to the above solution followed by a further 2 h of stirring. The gel mixture was allowed to age for 20 h at room temperature. The resulting solid product was recovered by filtration, washed thoroughly with 50% (v/v) ethanol aqueous solution to remove the residual reagents and dried at 80 °C overnight. The sample obtained was then calcined in air at 550 °C for 4 h prior to use.

2.3.2. Preparation of 12-HPW@MeSi

0.7 g of 12-HPW was dissolved in 60 mL of distilled water followed by the addition of 2 g of the synthesized MeSi. The resulting mixture was stirred vigorously for 22 h at room temperature. The reaction system was then heated at 50 °C to slowly evaporate the solvent. Afterwards, it was dried at 100 °C overnight and calcined in a muffle furnace at 300 °C for 2 h.

2.4. Transformation of ginsenoside Rb1

2.0 mg of ginsenoside Rb1 authentic standard and 44.0 mg of synthesized 12-HPW@MeSi were accurately weighed and dissolved in 2 mL of 70% methanol aqueous solution. The solution was heated in a shaking bath at 80 °C for 4 h, and then cooled to room temperature. After centrifugation at 3000 rpm for 2 min, 200 μL of the clear reaction solution was successively aliquoted, diluted to 1 mL, filtered through a 0.22 μm membrane and subjected to HPLC-MS analysis. The precipitate was collected and washed with 50% (v/v) methanol aqueous solution to remove the residual ginsenoside. The resulting solid was dried under vacuum at 50 °C and then reused for the transformation of Rb1.

3. Results and discussion

3.1. Structural characterization of ginsenoside Rb1 transformation products by HPLC-MS

The XRD and TEM characterization of 12-HPW@MeSi (Figs. S1 and S2) is shown in the supporting information. It has been demonstrated that 12-HPW is successfully immobilized in the mesoporous pores of the MeSi framework and retains its Keggin-type structure, resulting in the heterogeneous catalyst 12-HPW@MeSi. Ginsenoside Rb1 was then chemically transformed in 70% methanol aqueous solution using the synthesized 12-HPW@MeSi at 80 °C for 4 h. The resulting products were detected by an established HPLC-MS method. As shown in Fig. 1A of the total ion chromatogram (TIC), there are 18 newly generated peaks with distinct retention time, which are designated as compound 1 to 18. A

comprehensive analysis on the structures of the transformation products was further conducted using the multistage tandem and high-resolution MS.

The molecular mass of compounds 3, 4, 5, and 7 are all calculated to be 816.5, corresponding to the molecular formula of $C_{43}H_{76}O_{14}$, indicating the presence of a quadruple isomer in the transformation products. Among them, the fragment ions in the tandem MS spectra of compounds 3 and 4, 5 and 7 are the same, respectively, suggesting that they are two pairs of epimers resulting from the chiral carbon at the C-20 position (Fig. 1B).

Taking compound 3 as an example, the MS^2 spectrum on the $[M-H]^-$ ion at *m/z* 815.5 is shown in Fig. 2. The identical neutral loss of 162.0 Da between the three adjacent ions at *m/z* 815.5, *m/z* 653.5, and *m/z* 491.4 suggests that compound 3 is composed of two glucose substituents and one aglycone ion at *m/z* 491.4. The ions at *m/z* 621.4 and *m/z* 459.4 have a neutral loss of 32.0 Da with the ions at *m/z* 653.5 and *m/z* 491.4, respectively. 32.0 Da corresponds to the molecular formula of CH_3OH, from which it can be inferred that the structure of compound 3 contains at least one methoxyl group. The neutral loss of 58.1 Da between the ions at *m/z* 401.3 and *m/z* 459.4 corresponds to the molecular formula of C_3H_8O, suggesting that an isopropanol group is formed at the C-25 position through the hydration reaction of the double bond at the C-24(25) position. This indicates that compound 3 is the deglycosylation and hydration product of Rb1 and contains a hydroxyl group at the C-25 position.

The aglycone ion of compound 3 at *m/z* 491.4 differs from that of PPD at *m/z* 459.4 by +32.0 Da. Taking into account the +18.0 Da produced by the hydration of a water molecule, there is still a mass difference of 14.0 Da between their aglycones. This suggests that one of the hydroxyl groups on the PPD aglycone is substituted by the involved methoxyl group. Since the substituent at the C-20 position is more reactive than those at the C-3 and C-12 positions and is easier to dissociate, the substitution of the hydroxyl group occurred at the C-20 position, which also rationalizes the intense peaks of the methoxyl-removal ions at *m/z* 621.4 and *m/z* 459.4. It can be concluded that compound 3 consists of a methoxyl group at the C-20 position, a hydroxyl group at the C-25 position, a single bond between C-24 and C-25, and two glucose substituents at the C-3 position. Since the ginsenoside 20(*S*)-epimer elutes from the C18 column earlier than its 20(*R*) counterpart [35,36], compounds 3 and 4 are identified as 20(*S*)–OCH_3-25-OH-Rg3 and 20(*R*)–OCH_3–25-OH-Rg3, respectively, the structures of which are shown in Fig. 2, S3.

Similarly, the structures of compounds 5 and 7 are identified using compound 5 as an example. As shown in Fig. 3A, the only two ions at *m/z* 653.5 and *m/z* 491.4 in the MS^2 spectrum indicate that compound 5 has the same glycosyl substituents and aglycone ion as compound 3. The MS^3 analysis is performed on the aglycone ion at *m/z* 491.4, and the result is shown in Fig. 3B. The neutral loss of 32.0 Da between the product ion at *m/z* 459.4 and the aglycone ion suggests that compound 5

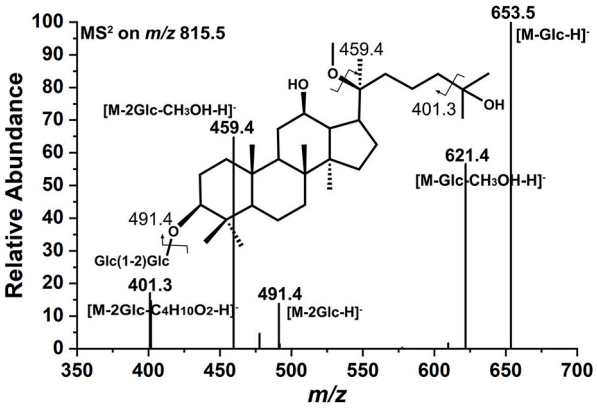


Fig. 2. MS^2 spectrum of the $[M-H]^-$ ion at *m/z* 815.5 from the ginsenoside 20(*S*)–OCH_3-25-OH-Rg3.

is also the methanol adducted product. There are only two possible pathways for the addition of methanol molecules to aglycones. One is the addition with the C-24(25) double bond and the other is the substitution of the hydroxyl group at the C-20 position as in compound 3. The ion at *m/z* 375.3 is the characteristic fragment ion of PPD-type ginsenosides, formed by the cleavage of the C-20(22) single bond to dissociate the olefin chain, leaving the residual charge on the aglycone. Its presence indicates that the hydroxyl group at the C-20 position has not been substituted by methoxyl. Moreover, the neutral loss of 116.1 Da between the ion at *m/z* 375.3 and the aglycone ion corresponds to the molecular formula of $C_7H_{16}O$, which refers to the alkene chain of PPD-type ginsenosides added by one methanol molecule. Thus, the methanol molecules are supposed to be added with the C-24(25) double bond. The electrophilic addition of alkene in an acidic environment follows Markovnikov's rule, so that the methoxyl is apparently attached at the C-25 position, resulting in the 2-methoxypropyl group. It can be seen that compounds 5 and 7 are generated from Rb1 by hydrolyzing the disaccharide substituent at the C-20 position and adding one methanol molecule at the C-24(25) double bond. They are therefore identified as 20(*S*)-25-OCH_3-Rg3 and 20(*R*)-25-OCH_3-Rg3, respectively, and their structures are shown in Fig. 3, S4.

Compounds 9 and 10 are isomers with the same molecular mass of 830.5. As shown in the MS^2 spectrum (Fig. 4A), only one product ion at *m/z* 667.5 is obtained by removing one glucose substituent from the parent ion at *m/z* 829.5. MS^3 on the ion at *m/z* 667.5 is further carried out and the result is shown in Fig. 4B. The ion at *m/z* 505.4 is the aglycone ion generated by the removal of one more glucose substituent from the precursor ion.

The neutral loss between the precursor ion at *m/z* 667.5 and the product ions at *m/z* 635.5 and *m/z* 603.4 are 32.0 Da and 64.1 Da, respectively, corresponding to one and two methanol molecules. This

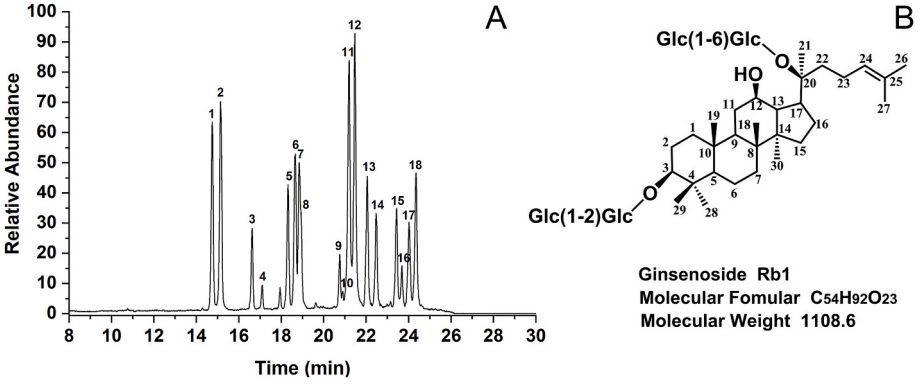


Fig. 1. TIC of the transformation products (A) and the chemical structure (B) of ginsenoside Rb1.

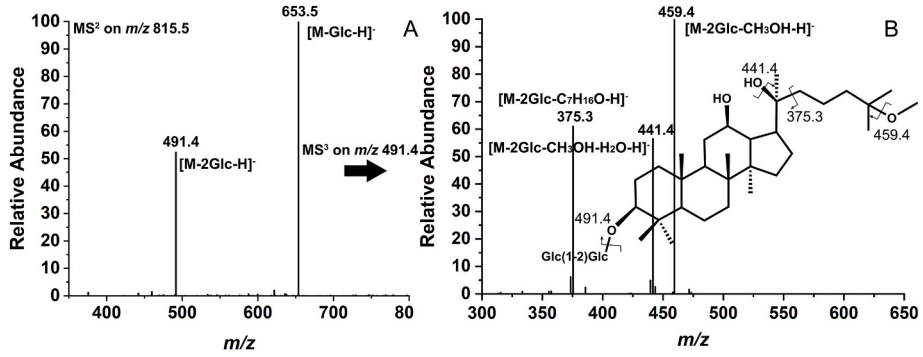


Fig. 3. MS^2 spectra of the ions at *m/z* 815.5 from the $[M - H]^-$ ion of ginsenoside 20(*S*)-25-OCH_3-Rg3 (A). Fragmentation pathways and MS^3 spectra of the ions at *m/z* 491.4 from the $[M - H]^-$ ion of ginsenoside 20(*S*)-25-OCH_3-Rg3 (B).

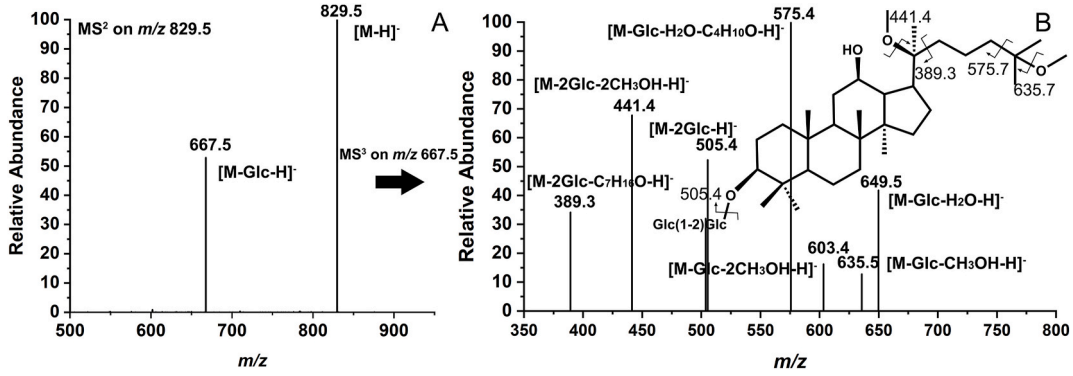


Fig. 4. MS^2 spectra of the ions at *m/z* 829.5 from the $[M - H]^-$ ion of ginsenoside 20(*S*)–OCH_3-25-OCH_3-Rg3 (A). Fragmentation pathways and MS^3 spectra of the ions at *m/z* 667.5 from the $[M - H]^-$ ion of ginsenoside 20(*S*)–OCH_3-25-OCH_3-Rg3 (B).

indicates the presence of two methoxyl groups in the structure of compound 9. The neutral loss of 74.1 Da between the product ions at *m/z* 649.5 and *m/z* 575.4 corresponds to the $C_4H_{10}O$ molecule, which indicates the characteristic 2-methoxypropane group at the C-25 position as in compound 5. It suggests the addition of one methanol molecule with the C-24(25) double bond and is responsible for one of the methoxyl groups. The other methoxyl group is considered to be at the C-20 position. This is because, after the addition of one methanol molecule (+32.0 Da), there is still a mass difference of 14.0 Da between the aglycone ions of compound 9 and PPD, indicative of substitution of the hydroxyl group at the C-20 position by the methoxyl group, as in compound 3. Moreover, the ion at *m/z* 389.3 is generated by the cleavage of the alkene chain at the C-20 position, dissociation of the $C_7H_{16}O$ molecule from the aglycone ion at *m/z* 505.4, and retention of the residual charge on the aglycone. It is 14.0 Da higher than the corresponding ion at *m/z* 375.3 of PPD. This is further evidence that the other methoxyl group is located on the aglycone end but not on the alkene chain. Compound 9 is therefore identified as 20(*S*)–OCH_3-25-OCH_3-Rg3. Compound 10 is the epimer of compound 9 as their MS spectra are identical and therefore assigned to 20(*R*)–OCH_3–25-OCH_3-Rg3, the structure of which is shown in Fig. S5.

Compounds 15 and 16 are isomers, having the same molecular mass of 798.5 and MS spectra. Fig. S6 demonstrates the tandem MS spectra of compound 15. The product ions at *m/z* 635.5 and *m/z* 473.4 are formed by removing two glucose substituents sequentially from the $[M - H]^-$ ion. The characteristic neutral loss of 74.1 Da ($C_4H_{10}O$ molecule) between the product ions at *m/z* 617.4 and *m/z* 543.3 (Fig. S6A) indicates the 2-methoxypropane group at the C-25 position as in compounds 5 and 9. The ion at *m/z* 441.4 is 32.0 Da lower than the aglycone ion at *m/z* 473.4 (Fig. S6B), which further confirms the presence of one methoxyl group. In addition, the aglycone ion at *m/z* 473.4 has a +14.0 Da mass difference from that of PPD, indicating a dehydration reaction of the hydroxyl group at the C-20 position. Therefore, a new double bond is expected to be located at the C-20(21) or C-20(22) positions, resulting in the Δ20(21) and Δ20(22) isomers. Based on the relative retention time of these position isomers, compounds 15 and 16 are consequently assigned as 25-OCH_3-Rk1 and 25-OCH_3-Rg5 with structures shown in Figs. S6 and S7, respectively.

Compounds 13 and 14 are isomers with a molecular mass of 784.5. The MS^2 spectrum of compound 13 shows two product ions at *m/z* 401.3 and *m/z* 375.3 (Fig. S8). The neutral loss between them and the aglycone ion at *m/z* 459.4 is 58.1 Da and 84.1 Da, respectively. As discussed above, the former neutral loss corresponds to the C_3H_6O molecule, implying the isopropanol group at the C-25 position, and the latter corresponds to the C_6H_{12} molecule, implying the olefin chain at the C-20 position. The coexistence of the two characteristic neutral loss suggests that the products are the cycloaddition products of the hydroxyl group at the C-20 position and the double bond at the C-24(25) position. Therefore, compounds 13 and 14 are identified as (20*S*, 25)-epoxy-Rg3 and (20*R*, 25)-epoxy-Rg3, the structures of which are shown in Figs. S8 and S9, respectively.

The tandem MS spectra of compounds 1, 2, 6, and 8 are shown in Figs. S10–S13, respectively. Based on the presence of the characteristic neutral loss of 58.1 Da and the mass difference with PPD in the aglycone ion, compounds 1, 2, 6, and 8 are identified as 20(*S*)-25-OH-Rg3, 20(*R*)-25-OH-Rg3, 25-OH-Rk1, and 25-OH-Rg5, respectively. Moreover, compounds 11, 12, 17, and 18 are identified as 20(*S*)-Rg3, 20(*R*)-Rg3, Rk1, and Rg5 by comparison of their retention time and MS spectra with those of authentic standards (Figs. S14–S17). The characteristic fragment ions of all the transformation products are summarized in Table S1.

3.2. Pathways and mechanisms of the chemical transformation of Rb1 in methanol solution

In summary, a total of 18 ginsenosides are obtained from the chemical transformation of Rb1 in a methanol aqueous solution catalyzed by 12-HPW@MeSi. The observed transformation pathways of Rb1 are shown in Scheme 1. It is described based on the structures of the products and mainly involves deglycosylation, epimerization, addition, elimination, and cycloaddition reactions.

There are two disaccharide substituents attached to the C-3 and C-20 positions of the reactant Rb1, respectively. The C-20 is a quaternary carbon, which is more reactive than the tertiary carbon at C-3. In other words, the tertiary carbenium ion generated by the cleavage of glycosidic bond at C-20 is more stable than the secondary carbenium ion at C-3. In the acid environment generated by 12-HPW@MeSi, the disaccharide substituent at the C-20 position is therefore more susceptible by hydrolysis than its C-3 counterpart, producing the main products of 20(*S*)-Rg3 and 20(*R*)-Rg3 via deglycosylation. The disaccharide substituent at the C-3 position is quite stable even after 10 h of chemical transformation. It is noteworthy that the peak areas of 20(*S*)-Rg3 and 20(*R*)-Rg3 are almost identical, as seen in the TIC (Fig. 1). This is due to the non-selective attack of the hydroxyl group on the sp^2 hybridized carbenium ion intermediate with trigonal planar geometry, leading to equal amounts of epimers with tertiary hydroxyl groups of different configurations [37]. The generated Rg3 epimers are used as intermediate products for further reactions.

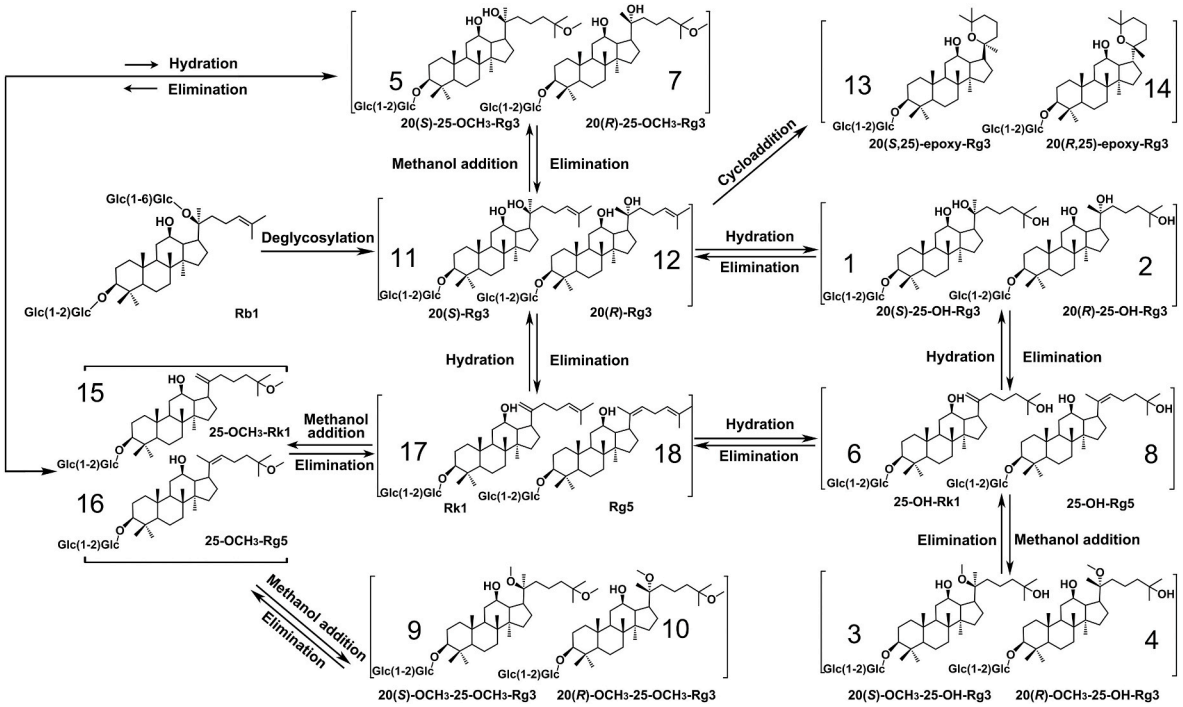
The double bond at the C-24(25) position is susceptible to addition reactions with electrophilic reagents in an acid environment, yielding products with an electrophilic group added at the C-25 position. This process follows the Markovnikov's rule for electrophilic addition to alkenes, which can be rationalized theoretically in terms of the stability of the carbenium ion intermediate [38]. In the methanol-involved chemical transformation of Rb1, both water and methanol molecules can serve as electrophilic reagents. They react with the C-24(25) double bond to form the isopropanol and 2-methoxypropane groups, respectively, which demonstrate neutral losses of 58.1 Da and 74.1 Da in the tandem MS analysis. In addition, the newly formed double bonds in Rk1 and Rg5 at the C-20(21) and C-20(22) positions are also capable of addition reaction with water and methanol molecules. In this case, the hydroxyl and methoxyl groups are attached at the C-20 position to form products such as 20(*S/R*)–OCH_3–25-OH-Rg3 and 20(*S/R*)–OCH_3–25-OCH_3-Rg3. The addition reactions of water and methanol molecules with the double bond are carried out in competition. The outcome of the competition depends mainly on their acidity. Uniquely, the hydroxyl group at the C-20 position can also interact as an electrophilic group with the double bond at the C-24(25) position. This pathway leads to the cycloaddition products 20(*S/R*, 25)-epoxy-Rg3 with oxygenated six-membered ring.

The hydroxyl group at the C-20 position in Rg3 readily forms a protonated alcohol when catalyzed by acid. This weakens the C–O bond and promotes monomolecular elimination reactions to form alkenes. The carbenium ion intermediate is also formed during the reaction, following the E1 mechanism, where the reaction rate is only dependent on the reactant concentration [39]. Therefore, at the beginning of the Rb1 transformation, the higher concentration of Rg3 favors the preferential formation of the products Rk1 and Rg5, which formed double bonds at the C-20(21) and C-20(22) positions via elimination reactions of the hydroxyl group at the C-20 position. Furthermore, hydration of the double bond and dehydration of the hydroxyl group are reversible reactions under acid conditions. This indicates a dynamic chemical equilibrium between the addition products of the water and methanol molecules and the olefin.

3.3. Effects of reaction conditions on the chemical transformation of ginsenoside Rb1

To investigate the effect of thermal hydrolysis on the transformation, ginsenoside Rb1 was transformed in 70% methanol solution at 80 °C without the participation of 12-HPW@MeSi. The content of ginsenoside Rb1 at different reaction time are shown in Fig. S18. After 10 h of reaction, the content of ginsenoside Rb1 remained and no transformation product was observed. This indicates that ginsenoside Rb1 was free from thermal hydrolysis at 80 °C and that the 18 transformed products were obtained due to the participation of 12-HPW@MeSi.

As shown in Fig. 5A, the unreacted Rb1 and the main products 20(*S/*



Scheme 1. Chemical transformation pathway of ginsenoside Rb1 catalyzed by 12-HPW@MeSi.

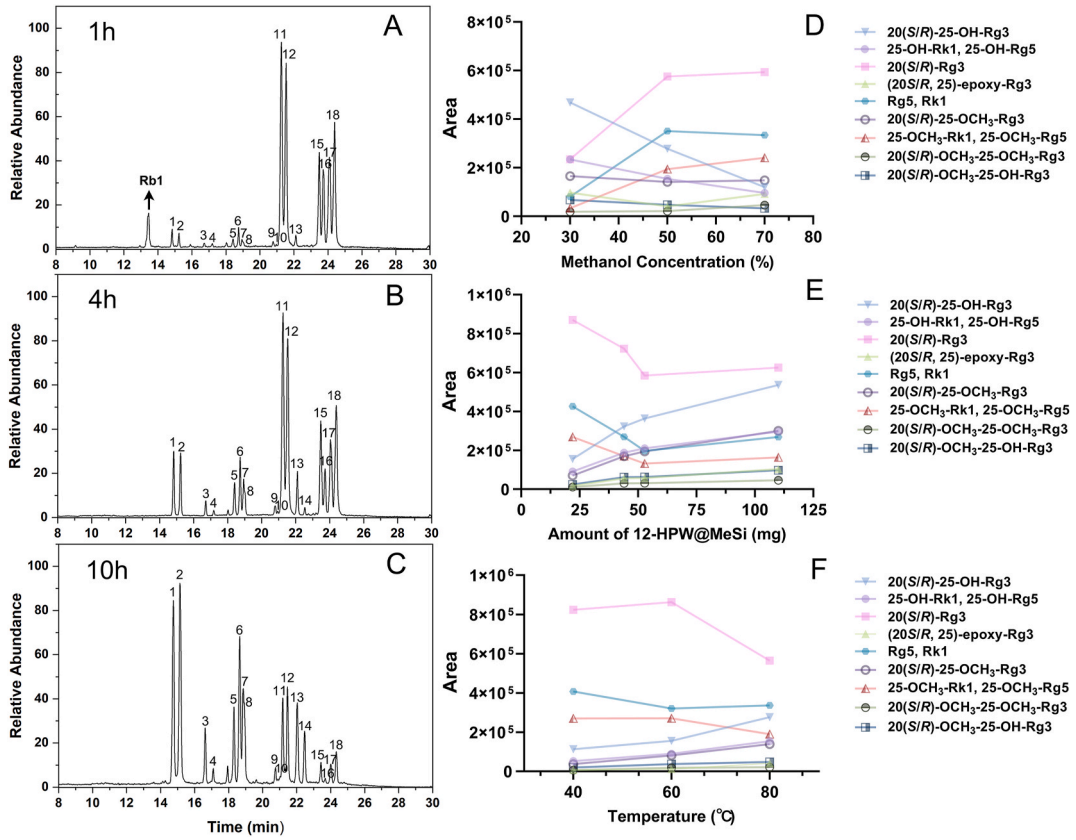


Fig. 5. TIC of the transformation products of ginsenoside Rb1 catalyzed by 12-HPW@MeSi for 1 h (A), 4 h (B), and 10 h (C). Peak areas of the transformation products catalyzed by 12-HPW@MeSi at varied methanol concentration (D), catalyst amount (E), and temperature (F).

R)-Rg3, Rk1, Rg5, 25-OCH_3-Rk1, and 25-OCH_3-Rg5 could be observed, when it was transformed in 50% methanol aqueous solution for 1 h. Since there is only one deglycosylation step transformed from Rb1 to 20(*S*/*R*)-Rg3, 20(*S*/*R*)-Rg3 is predominated in the products. As the reaction time progresses, they are transformed to the other 12 products, whose peak areas gradually increase with the reaction time, as shown in Fig. 5B and C. The time course results suggest that the transformation of Rb1 begins with the deglycosylation, followed by the elimination of the C-20 hydroxyl group and the addition of the C-24(25) double bond.

The water molecule adducts 25-OH-Rk1, 25-OH-Rg5, and 20(*S*/*R*)-25-OH-Rg3 are clearly observed at the methanol concentration of 30%, as shown in Fig. 5D. When the concentration is increased to 50%, methanol appears to be more reactive than water with the C-24(25) double bond at the beginning of the transformation, leading to abundant methanol molecule adducts. By further increasing the concentration to 70%, the addition reaction of water molecules is further inhibited and fewer water molecule adducts are obtained. This implies that the addition products are dependent on the methanol concentration and that the addition of water and methanol molecules at the C-24(25) double bond is competitive.

As shown in Fig. 5E, as the amount of 12-HPW@MeSi is increased from 22.0 mg to 52.8 mg, the peak areas of the 6 main products show a clear decrease while those of the other 12 products increase significantly. This is an indication that the increase in the amount of 12-HPW@MeSi improves the reaction rate and allows for a more rapid transformation of the main products. However, when the catalyst amount is further increased to 110.0 mg, there is no significant change in the peak areas of all the products, suggesting that the catalyst has provided an excessive concentration of H^+ for the transformation.

The effect of temperature on the transformation is similar to that of the catalyst amount. As shown in Fig. 5F, after 4 h of transformation at 40 °C, the 6 main products such as 20(*S*/*R*)-Rg3 are absolutely dominant. It further confirms that the deglycosylation at the C-20 position, elimination of the C-20 hydroxyl group and addition of the C-24 (25) double bond proceed more readily. As the temperature is raised, the other 12 products gradually appear and the peak areas of the 6 main products are significantly reduced. This suggests that the transformation of Rb1 is thermodynamically favored and that the higher temperature is effective in accelerating the transformation process.

3.4. *Reusability of 12-HPW@MeSi in the transformation of Rb1*

The synthesized 12-HPW@MeSi is insoluble in water and capable of forming a heterogeneous catalytic environment. In the chemical transformation of Rb1, 12-HPW@MeSi can be rapidly separated from the solution by centrifugation and reused in the transformation after being dried. The reusability of 12-HPW@MeSi is evaluated by the conversion rate of Rb1 in 50% methanol aqueous solution at 80 °C for 4 h. As shown in Fig. S19. The newly synthesized 12-HPW@MeSi provides a 100% conversion rate of Rb1. As the recycle times increase, the conversion rate gradually decreases. This may be attributed to the release of the immobilized 12-HPW or the decomposition of the host MeSi framework. After 5 cycles, 12-HPW@MeSi is still able to transform 60.5% of Rb1 within 4 h, implying its moderate reusability, which renders it promising potential for practical applications.

4. Conclusion

The heterogeneous catalyst 12-HPW@MeSi is synthesized, characterized, and applied to the chemical transformation of ginsenoside Rb1 with the involvement of methanol molecules. A total of 18 transformation products are obtained and detected using HPLC coupled with tandem MS and high-resolution MS methods. Their structures are identified on the basis of accurate molecular mass, tandem MS patterns,

and characteristic neutral loss. In particular, methanol is involved in the generation of 8 transformation products, which are formed by the addition of methanol molecules at the C-24(25), C-20(21) or C-20(22) double bonds, giving rise to methoxyl groups at the C-25 and C-20 positions. For the first time, rare ginsenosides modified with methanol molecules, such as 20(*S/R*)–OCH_3–25-OH-Rg3, are obtained by chemical transformation. The transformation pathways involved deglycosylation, addition, elimination, cycloaddition, and epimerization reactions. Carbenium ion theory applies to each pathway, and the stability of the carbenium ion determines the direction of the transformation reaction. In addition, the synthesized 12-HPW@MeSi is conveniently recovered from the reaction solution and demonstrated moderate recyclability. Combining the heterogeneous catalytic system with a wide variety of organic molecules will provide an efficient and recyclable strategy for the preparation of more rare ginsenosides. Although the biological activities of the obtained methoxylated ginsenosides are unknown, we believe that their bioactivities are highly exploratory based on previous studies on rare ginsenosides. And the transformation method described in this study will lay the foundation not only for expanding the diversity of rare ginsenosides, but also for further study of their preparation and activities.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2024.01.007.

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