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In vivo rat metabolism and pharmacokinetic studies of ginsenoside Rg_3

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Abstract

Metabolism of an anti-tumor active component of *Panax ginseng*, ginsenoside (20*R*)-Rg_3, was studied for better understanding its pharmacokinetics in rat. LC–MS was used to determine Rg_3 and its metabolites in rat plasma, urine and feces samples. An average half-life of 18.5 min was obtained after the ginsenoside was intravenously dosed at 5 mg/kg. However, Rg_3 was not detected in rat plasma collected after oral administration at 100 mg/kg. Only 0.97–1.15% Rg_3 of the dosed amount was determined in feces. Hydrolysis and oxygenated metabolites were detected and identified in feces collected after oral administration by using LC–MS and MS–MS.

Keywords: Ginsenoside Rg_3; Rat metabolism; Pharmacokinetics; Metabolite identifications; LC–MS.

1. Introduction

Panax ginseng has been frequently used in traditional Chinese medicine to treat many disorders, such as debility, ageing, stress, diabetes, insomnia and sexual inadequacy [1]. The major biological active components of ginseng are ginsenosides [2,3] that have shown various biological activities, including anti-inflammatory and anti-tumor effects [4–6]. It has been reported that biological activities are mainly generated from the biotransformation of ginsenosides by human intestinal bacteria [7–9]. Ginsenoside Rg_3 (3β, 12β, 20(*R*)-dihydroxydammar-24-ene 3-*O*-[β-D-glucopyranosyl (l → 2)-β-D-glucopyranoside]), a minor ginsenoside from the *Panax ginseng*, has been shown to inhibit tumor metastasis in mice as well as the invasion and metastasis of several tumors of rat and human in vitro [6,10]. Bae et al. [11] reported that the cytotoxicity of ginsenosides against tumor cell lines increased when Rg_3 was metabolized to Rh_2 (3β, 12β, 20(*R*)-dihydroxydammar-24-ene 3-*O*-β-D-glucopyranoside) or protopanaxadiol (3β, 12β, 20(*R*)-dihydroxydammar-24-ene). The metabolic pathways to protopanaxadiol also increased the anti-*Helicobacter pylori* activity. The results suggested that the natural ginsenoside may be the prodrug and the hydrolysis products, such as ginsenoside Rh_2 and protopanaxadiol could play an important role in the therapeutic activities. The metabolism study of ginsenosides is thus, of great interest for better understanding their pharmacology and pharmacokinetics.

(20*R*)-ginsenoside Rg_3 has been approved to be used as the major active component in a Category-I drug of anti-tumor and anti-cancer in China. Because the therapeutic effects of Chinese ginseng have been well recognized over thousands of years and its safety has been proved based on the historical experience, little pre-clinical and clinical investigation of ginsenoside Rg_3 was required. Wang et al. [12] recently reported some pharmacokinetic (PK) parameters of Rg_3 after oral administration of the ginsenoside in human at 3.2 mg/kg. The human plasma was prepared with solid-phase extraction and the extract was analyzed by using HPLC-UV for Rg_3 concentrations. Human PK parameters, including C_{max} of 15.67 ± 6.14 ng/ml and t_{max} of 0.66 ± 0.01 h were obtained from the oral experiments [12]. However, no data on intravenous dosing was reported.

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LC–MS has been proved to be a powerful and reliable analytical approach for supporting studies on active components in Chinese medicines [13–19]. Both positive and negative ESI-MS and MS–MS have been applied for analyzing various ginsenosides extracted from Oriental and North American ginsengs [20–23]. Recently, a sensitive and specific LC–MS method with quadrupole-time of flight (Q-TOF) detection was developed to support rat PK study of Rg_3 [13]. The preliminary results of the Rg_3 study on rat showed quite different PK profiles as those reported from the human study. The half-life of Rg_3 with intravenous administration on rat was only 14 min. While the in vitro metabolism results were reported on the ginsenoside, no detailed in vivo metabolism study was carried out [13]. To investigate the short half-life of Rg_3 obtained from the rat administrations, in vivo metabolism study involving the metabolic clearance of the ginsenoside is needed. Metabolism data may also be valuable for better understanding pharmacologic activities of the anti-tumor component, given that the ginsenoside has a very short half-life in vivo. However, little data of the in vivo metabolism of ginsenoside Rg_3 is seen from literature search. This study aims to study the in vivo metabolism of the ginsenoside Rg_3 with rat experiments. The identification of both deglucosylated and oxygenated metabolites of Rg_3 in biological samples by using LC–MS and MS–MS is described. To the best of our knowledge, the detection of oxygenated metabolite of Rg_3 in vivo was not yet reported.

2. Experimental

2.1. Chemicals

(20*R*)-ginsenoside Rg_3 (purity 99%) was provided by The Laboratory of Chinese Medicine, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Jilin, China. HPLC-grade methanol was purchased from Acros Organics (New Jersey, USA). The water used in the experiments was collected from a Mili-Q Ultra-pure water system (Millipore, Billerica, USA). Other chemicals (analytical grade) were purchased from Sigma (St. Louis, MO, USA).

2.2. Plasma calibration curve

External calibration method was used for the quantitative analysis. The calibration curve was obtained from the LC–MS analyses of calibration standards based on the method as described previously [13]. Briefly, Rg_3 stock solution was prepared in a solution of methanol and water (9:1) at concentration level of 4.65 μM and stored at −20 °C until use. Calibration standard solutions were prepared by adding 10, 20, 40, 60 and 80 μl of stock solution into 100 μl of blank rat plasma and well mixed. One mililiter of methanol was added into each standard solution and mixed well to precipitate the protein and to extract Rg_3. The sample was then centrifuged at 8200 × *g* for 20 min. The supernatant was transferred into another tube and dried under a stream of nitrogen. The residue was reconstituted with 100 μl of methanol and then centrifuged at 8200 × *g* for 20 min. Eight microliters of the supernatant was analyzed by LC–MS to obtain the calibration curve for the quantification of Rg_3 in plasma.

2.3. In vivo study with intravenous and oral administrations

Rg_3 pharmacokinetic study on rat was carried out by using the procedure as reported previously [13]. Rg_3 was dissolved in 370 μl of 5% β-cyclodextrin and sonicated for 1 h, then was diluted to 1 ml by adding 630 μl saline to the solution. Intravenous dose (5 mg/kg) with 1 ml Rg_3 dosing solution was given to each of three male Sprague–Dawley rats (body weight 200–220 g) within 1 min. Blood sample (0.2 ml) was withdrawn via the cannular at 0, 10, 20, 30, 40, 60, 90 min and 2, 3, 4, 6, 8, 10, 24 h after the intravenous administration. Oral administration of Rg_3 (100 mg/kg) was conducted on two rats. Blood samples were obtained and prepared for the LC–MS analysis by using the same procedure described above.

2.4. Feces sample preparation

Rat feces samples were collected from 0 to 24 h after the oral administration of Rg_3. The feces sample of each rat was suspended in water and then extracted with *n*-butanol [11]. The extract was dried and the residue was dissolved in 1 ml methanol. After centrifugation at 8200 × *g* for 20 min, 2 μl of the supernatant was analyzed by using LC–MS for both Rg_3 and its metabolites. The concentration of Rg_3 in rat feces sample was determined by comparing its peak area with that of a Rg_3 stock solution that had been prepared in a blank rat feces by using the same procedure. The obtained Rg_3 concentration in rat feces samples should be viewed as semi-quantitative because only one single point comparison was used for the determination.

2.5. Urine sample preparation

Rat urine samples were collected from 0 to 24 h after the intravenous and oral administrations of Rg_3. The urine sample was each extracted with *n*-butanol [11]. The extract was dried and the residue was dissolved in 1.0 ml methanol and then centrifuged at 8200 × *g* for 20 min. The supernatant (2 μl) was analyzed by using LC–MS.

2.6. LC–ESI-MS analysis

HPLC separation of the ginsenoside and its metabolites was carried out on a HP 1100 system (Agilent Technologies. Palo Alto, CA, USA) with conditions same as those reported previously [13]. A reversed-phase column (Waters. Xterra MS-C8. 2.1 mm × 100 mm, 3.5 μm) was used. The mobile phases consisted of water (A) and methanol (B) with a flow

rate of 100 μl/min. Initial mobile phase condition was 40% B and held for 4 min, then changed to 90% B within 1 min and hold for 30 min. The effluent from the LC column was diverted to waste for the first 12 min following the injection in order to avoid the non-volatile salts and interference background in the sample from contaminating the MS spectrometer.

Negative ESI ion mode was used to analyze Rg_3 and its metabolites in all rat plasma and feces samples unless noted specially. All mass spectrometric experiments were performed on a quadrupole-time of flight (Q-TOF) tandem mass spectrometer (API Q-STAR Pulsar *i*, Applied Biosystems, Foster City, USA). The following parameters of the turbo-ionspray were used: ionspray voltage, −4200 V; declustering potential 1 (DPI), −30 V; focusing potential (FP), −150 V and declustering potential 2 (DP2), −12 V The ion source gas 1 (GS1), gas 2 (GS2), curtain gas (CUR) and collision gas (CAD) were 20, 15, 25 and 3, respectively. The temperature of GS2 was set at 400 °C.

Full-scan mass spectra at a mass range of *m/z* 100–850 were acquired. Mass chromatogram for the $[M-H]^-$ ion of ginsenoside Rg_3 at the *m/z* 783 was integrated and the peak areas were used for quantitation. Molecular ion masses of potential metabolites were examined and the corresponding extracted mass chromatograms were recorded. Tandem mass spectrometric experiments were conducted on the ginsenoside Rg_3 and its metabolites. Parent ions of $[M-H]^-$ were selected for the MS–MS analyses of Rg_3 and the detected metabolites. The collision energy (CE) was set at −35 eV for the ESI-MS–MS analysis.

3. Results and discussion

3.1. LC–MS analysis of Rg_3 in rat biological samples

The in vivo plasma samples from both oral and intravenous administrations were analyzed by using the developed LC–MS method. The method validation of the quantitative assay was described previously [13]. Negative ESI was used in this study, although positive ion ESI also provided good sensitivity for the detection of ginsenosides [14–16,18–23]. It was found that the application of negative ion mode provided significant advantages for analyzing the ginsenoside in the biological samples, especially for the feces samples. Negative ion mode ESI produced much lower background and less interference for the detection, which ultimately provided lower detection limits. Ginsenoside Rg_3 was not detected in the plasma and urine samples collected from each of the two rats subjected to the oral administration. Although the relatively high dosing levels (100 mg/kg) were orally given to rats, the ginsenoside was not detected in the plasma samples with the detection limits of 0.03 μg/ml, indicating that Rg_3 might be metabolized quickly in gastrointestinal tract. Rg_3, however, was detected in the feces sample. The semi-quantitative analysis showed that the amount of Rg_3 detected in the feces was only 0.97–1.15% of the dosing amount, indicating that majority of dosed Rg_3 might have been metabolized (see Section 3.2 for metabolism study). Rg_3 was detected in the plasma samples collected within 0–1.5 h after the administration when the ginsenoside was dosed at 5 mg/kg intravenously. The levels of Rg_3 in the plasma samples were determined by using LC–MS. The results obtained from three rats were averaged and used for investigating the pharmacokinetics of Rg_3. Fig. 1 shows the plasma profiles of Rg_3 concentration versus sample collection time after the intravenous administration. PK parameters calculated from this curve included $t_{1/2}$ = 18.5 min, AUC = 32.15 μg/(ml min), MRT = 18.6 min and CL = 31.10 ml/min. No Rg_3 was detected in the plasma samples collected after 1.5 h. The ginsenoside was also not seen in the urine samples collected from 0 to 24 h after the intravenous dosing. The average half-life calculated from the PK curve was similar to that reported from previous study ($t_{1/2}$ = 14 min) [13]. Slightly longer half-life obtained for the present study may be due to the difference between the rat groups or Rg_3 solubility in dosing solutions. In previous study, Rg_3 was dissolved in 5% β-CD only by short vortex. In the present study, however, the ginsenoside was dissolved thoroughly by sonicating the dosing solution for 1 h. Nevertheless, both previous and present studies have demonstrated short half-life of Rg_3 after the intravenous administrations. The short half-life again indicated that ginsenoside Rg_3 was probably metabolized quickly in rat. The fast metabolic rate agrees with the results of metabolism studies reported in recent publications [11,13,24]. Rg_3 and other ginsenosides were reported to be metabolized intensively in human intestine and fecal microflora, with the products from the removal of glucose residues as the major metabolites [11]. For oral administration, ginsenosides may be easily metabolized under the acidic conditions by the stomach and intestinal flora [24].

3.2. Metabolism study of ginsenoside Rg_3 in rat feces sample

Six possible metabolites were detected in rat feces samples collected from 0–24 h after oral administration of Rg_3. The detection of the metabolites were conducted from the LC–MS analyses and confirmed with the MS–MS experiments. Oxygenation and the removal of glucose residues were found to be the major metabolic pathways (Fig. 2).

Fig. 3 shows the extracted mass chromatograms of Rg_3 for the $[M-H]^-$ ion at *m/z* 783, its monooxygenated metabolite at *m/z* 799 (16 Da higher than that of Rg_3) and deoxygenated metabolite at *m/z* 815 (32 Da higher than that of Rg_3). Several oxygenated metabolites of Rg_3 were detected in the rat feces sample by using LC–MS in negative ESI mode. However, only the major monooxygenated product at the retention time of 17.32 min (Fig. 3B) and the dioxygenated metabolite at 16.90 min (Fig. 3C) were confirmed by the negative ion ESI-MS–MS analyses. Other minor peaks (Fig. 3B and C) were not confirmed because no good-quality MS–MS spectra

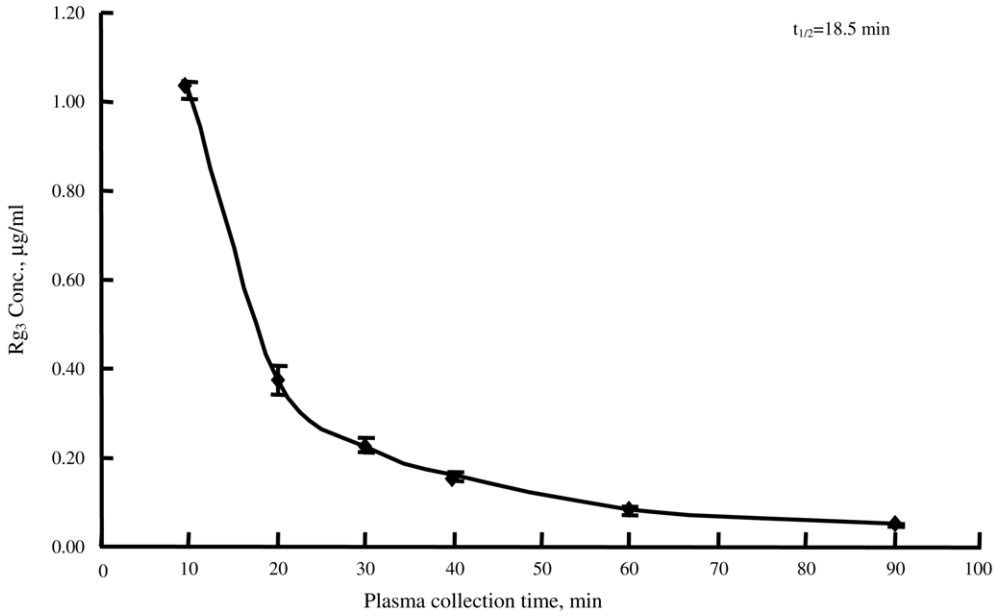

Fig. 1. Plasma concentration–time profiles of ginsenoside Rg_3 after intravenous administration of 5 mg/kg to SD rats ($n = 3$). The half-life calculated from the curve was 18.5 min. Rg_3 was not detected in plasma samples collected more than 1.5 h after the dosing.

could be obtained from the ion peaks that had very low intensity. The MS–MS spectra in Fig. 4 demonstrated the fragmentations of the parent Rg_3 (Fig. 4A), the major monooxygenated metabolite (m1, Fig. 4B) and dioxygenated metabolite of Rg_3 (m2, Fig. 4C). The MS–MS spectra of the metabolites were interpreted and compared with that of ginsenoside Rg_3. Interpretation of the Rg_3 MS–MS spectrum indicated that major fragment ions at *m/z* 621 and *m/z* 459 resulted from the loss of one and two sugars from the deprotonated molecular ion. The pattern of sugar loss was also observed for the metabolites m1 and m2. Fig. 4B showed the fragment ions from the loss of one and two sugars at *m/z* 637 and *m/z* 475, while the corresponding *m/z* 653 and *m/z* 491 were seen for m2 in Fig. 4C. In addition, the deprotonated ion peak of glucose moiety at *m/z* 161 was observed in the MS–MS spectra of parent ginsenoside Rg_3 and the two metabolites, providing further confirmation of the metabolites detected in the feces sample.

The exact oxygenation site could not be determined for the metabolites m1 and m2 from the results of the MS–MS experiments. The MS–MS data, however, provided evidence of possible oxygenation on the top-right aliphatic chain. The interpretation and comparison of the MS–MS spectra indicated that oxygenation might occur on the $-CH_2CH_2CH{=}C(CH_3)_2$ group as illustrated in Fig. 2. Fragment ions related to the loss of the $CH_2CH_2CH{=}C(CH_3)_2$ moiety (a mass of 84) were observed in the MS–MS spectrum of ginsenoside Rg_3 as shown in Fig. 4A. The loss of 84 Da was not produced directly from the $[M - H]^-$ molecular ion, which was probably due to the favorable cleavage of the glucose moiety (Fig. 4A). However, evidence of losing the $CH_2CH_2CH{=}C(CH_3)_2$ moiety was shown from the major fragment ions at *m/z* 621 and *m/z* 459. The fragment ions of $[M - glc\text{-}84\text{-}H]^-$ and $[M - glcglc\text{-}84\text{-}H]^-$ were detected at *m/z* 537 and *m/z* 375 in the spectrum, respectively. The same two *m/z* values were also seen in Fig. 4B for the monooxygenated metabolite. However, they must respectively correspond to the ions of $[M - glc\text{-}100\text{-}H]^-$ and $[M - glcglc\text{-}100\text{-}H]^-$ because the molecular mass of the monooxygenated metabolite is 16 Da higher than that of Rg_3. Furthermore, the $[M - 100\text{-}H]^-$ ion at *m/z* 699 resulted from the loss of both $CH_2CH2CH{=}C(CH_3)_2$ and an additional oxygen was detected in Fig. 4B for the metabolite, indicating that the oxygenation may occur on the aliphatic side chain. A positive ion ESI-MS–MS experiment on the $[M + Na]^+$ ion of the monooxygenated metabolite also produced the $[M - 100 + Na]^+$ (spectrum not shown), confirming the presence of the monooxygenated $CH_2CH_2CH{=}C(CH_3)_2$ group in the metabolite detected in the rat feces sample.

Detection of a major monooxygenated metabolite was previously reported from in vitro metabolism study of ginsenoside Rg_3 with rat liver S9 fraction [13]. A 24,25-epoxy compound was proposed as a major metabolite of ginseng sapogenins after the in vitro incubation with rat liver enzymes, such as microsomal cytochrome P450 [13,24] due to the detection and interpretation of the particular fragment ion of $[M - 58 + Na]^+$ (i.e., loss of 58 Da from the $[M + Na]^+$ ion) in the MS–MS spectrum of the in vitro monooxygenated metabolite. Although positive ion mode ESI-MS–MS analysis of $[M + Na]^+$ ion of the in vivo monooxygenated metabolite indicated that the site of the monooxygenation existed on the chain of $CH_2CH_2CH{=}C(CH_3)_2$, the exact structure of the in vivo metabolite detected in the rat feces may be different from the in vitro monooxygenated metabolite. The particular fragment ion peak corresponding the loss of 58 Da from the $[M + Na]^+$ ion was not detected in the MS–MS spectrum of the in vivo monooxygenated metabolite. Instead, the

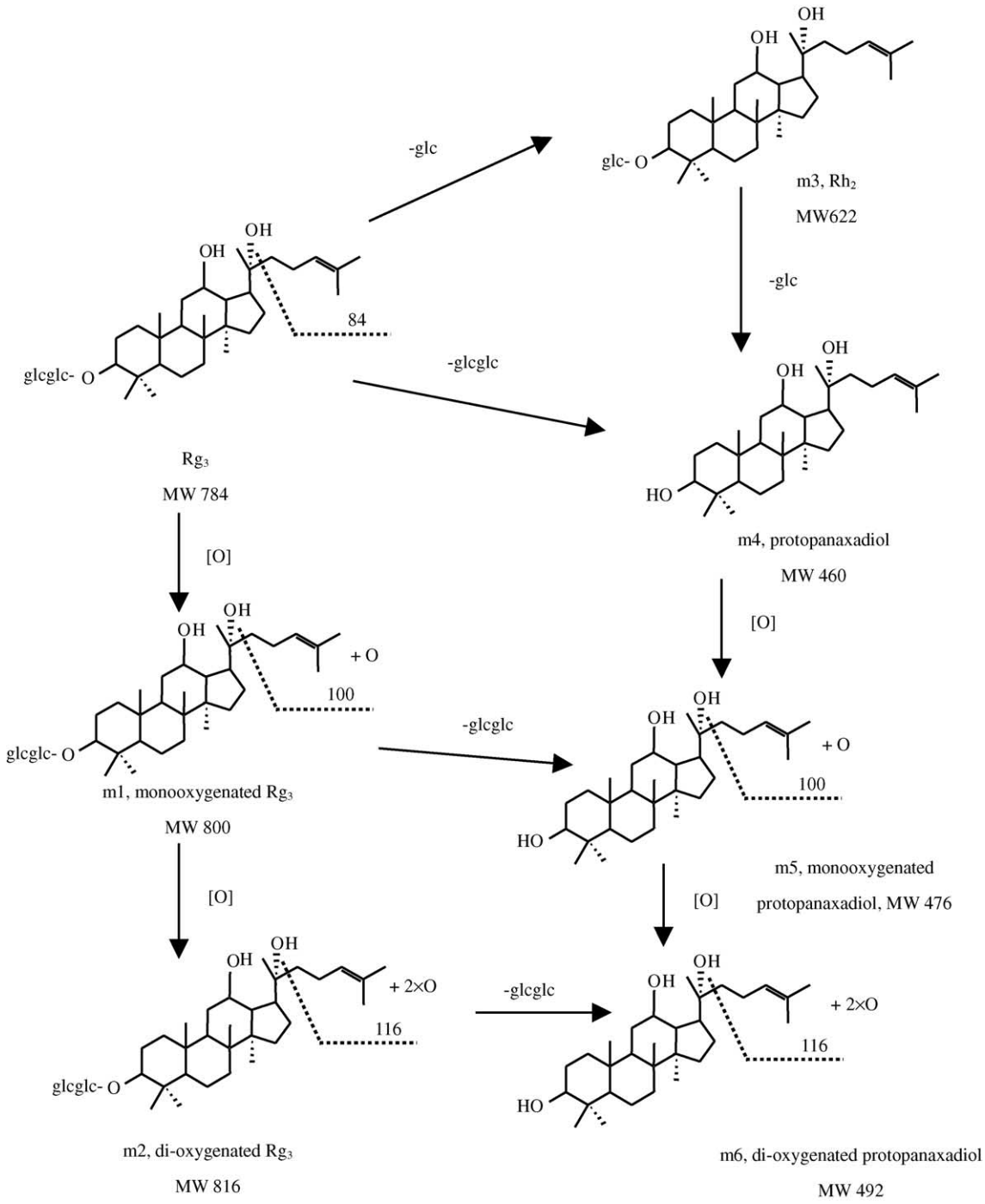

Fig. 2. Proposed metabolic pathway of Rg_3 in rat gastrointestinal tract.

loss of 100 Da was seen for the in vivo metabolite (Fig. 4B). which only indicated the monooxygenation on the chain of $CH_2CH_2CH{=}C(CH_3)_2$ not the particular 24,25-epoxy structure. Karikura et al. [25] reported the detection of hydroperoxide and hydroxide products of ginsenoside Rb_2 in rat stomach and cecum after oral administration. Rb_2 hydroperoxides were initially formed but decomposed gradually into the corresponding hydroxides. Another ginsenoside Rb_1 was found to metabolize to a monooxygenated product in rat stomach with the oxygenation occurred on the aliphatic side chain [26]. These results supported our proposed oxygenation pathway of Rg_3 in rat feces after oral administration.

The MS–MS spectrum of the major dioxygenated metabolite as shown in Fig. 4C was interpreted in a similar way. In addition to the presence of $[M - glc\text{-}116\text{-}H]^-$ and $[M - glcglc\text{-}116\text{-}H]^-$ ions as detected at *m/z* 537 and *m/z* 375, the base peak of $[M - 116\text{-}H]^-$ ion was observed, indicating that oxygenations might occur on the $CH_2CH_2CH{=}C(CH_3)_2$ group.

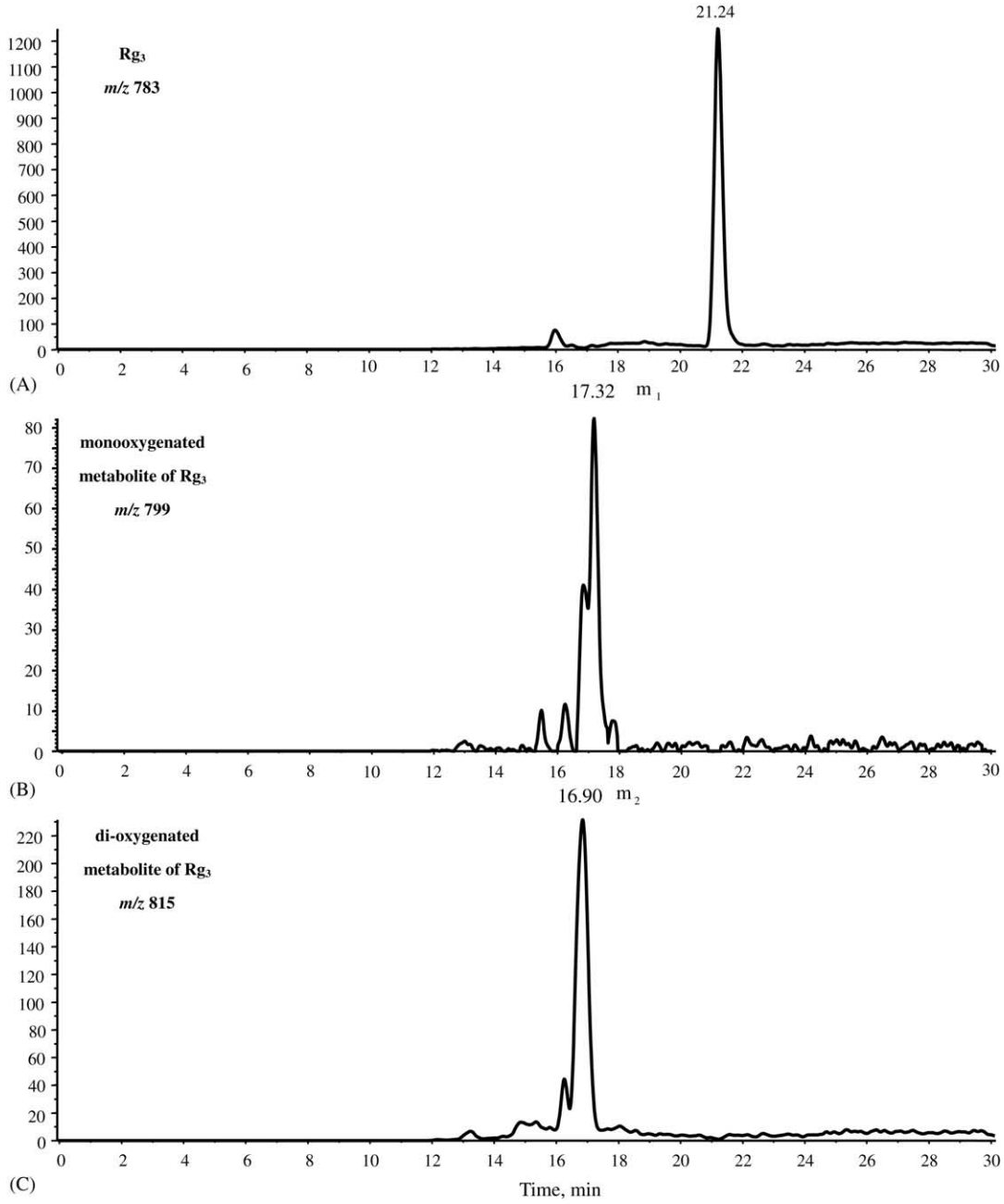

Fig. 3. LC–MS chromatograms obtained from the analysis of the rat feces sample after oral administration: ginsenoside Rg_3 at *m*/*z* 783 (A), monooxygenated metabolite of Rg_3 at *m*/*z* 799 (B), and dioxygenated metabolite of Rg_3 at *m*/*z* 815 (C).

The exact structure of the metabolite could not be determined from the MS–MS analysis. NMR analysis should be conducted if adequate amount of metabolite could be obtained.

The detection of major deglucosylated metabolites of Rg_3 is illustrated in Fig. 5. LC–MS and MS–MS analyses as well as their comparisons with the authentic standards confirmed the deglucosylated products as ginsenoside Rh_2 and protopanaxadiol. In addition, major oxygenated products of protopanaxadiol were also detected. Fig. 5 showed that the extracted mass chromatograms of the deglucosylated metabolites Rh_2 at *m*/*z* 621 (Fig. 5A), protopanaxadiol at *m*/*z* 459 (Fig. 5B), as well as the monooxygenated product of protopanaxadiol at *m*/*z* 475 (Fig. 5C) and the dioxygenated product of protopanaxadiol at *m*/*z* 491 (Fig. 5D).

The detection of protopanaxadiol as one of the major metabolites of Rg_3 was confirmed by MS–MS analysis. The obtained MS–MS spectrum from the rat feces sample (Fig. 6A) was found to match well with that of the protopanaxadiol standard. Similar to the MS–MS confirmation of the two major oxygenated metabolites of Rg_3, the detection of the major monooxygenated and dioxygenated products of protopanaxadiol were confirmed by MS–MS analyses of their $[M - H]^-$ ions at *m*/*z* 475 and *m*/*z* 491. Fig. 6B presented the MS–MS spectrum of the major monooxygenated

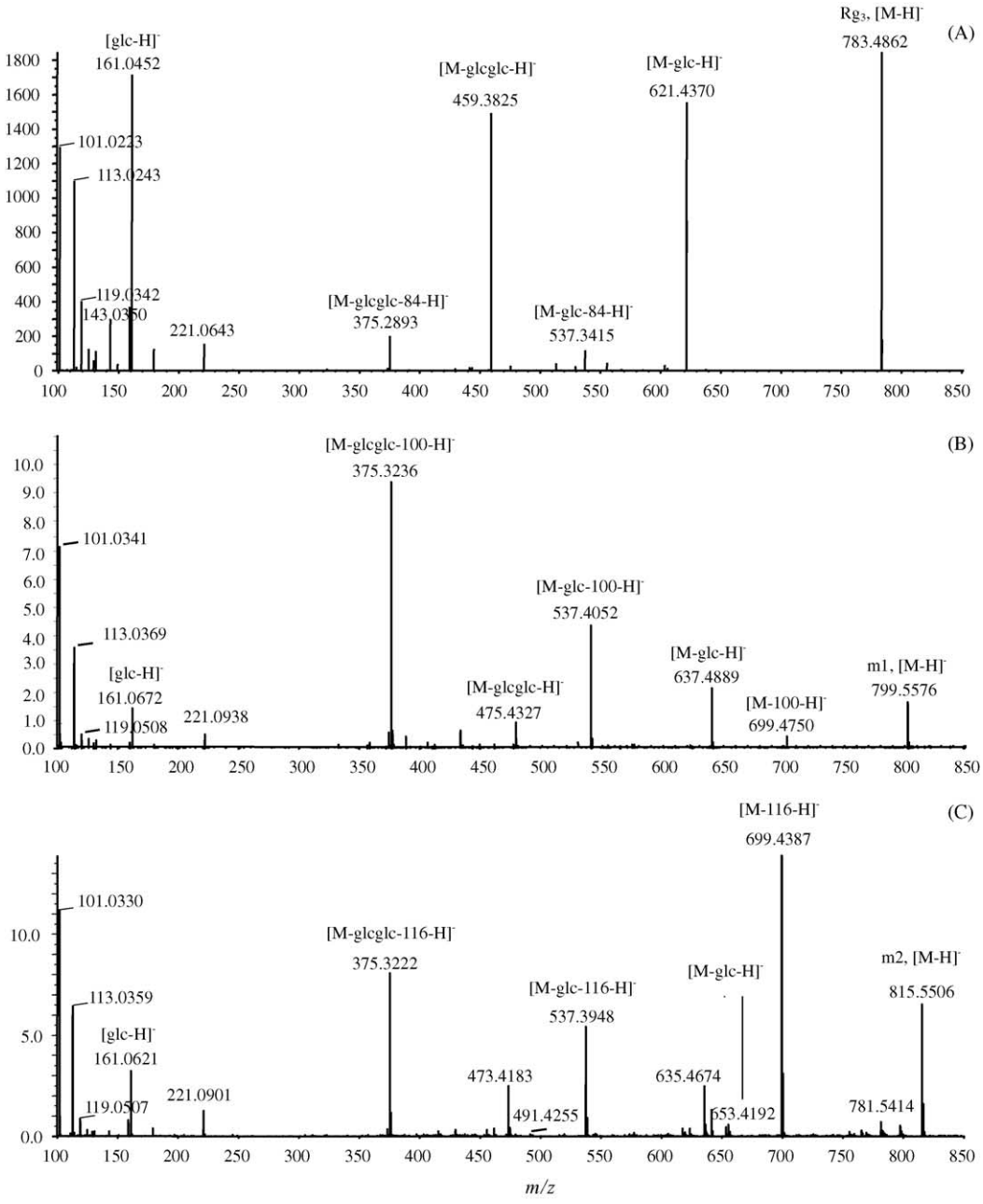

Fig. 4. Negative ion ESI-MS–MS spectra of ginsenoside Rg_3 (A), the monooxygenated metabolite of Rg_3 (B), and dioxygenated metabolite of Rg_3 (C).

product of protopanaxadiol at retention time at 21.77 min detected at *m/z* 475 in Fig. 5C. The MS–MS spectrum of the peak at 22.12 min from the same $[M-H]^-$ ion at *m/z* 475 was very similar to that of 21.77 min. Other minor peaks from Fig. 5C were not confirmed because their signal intensities were too low for MS–MS analyses. Similarly, MS–MS spectrum (Fig. 6C) was obtained only for the major dioxygenated product of protopanaxadiol at the retention time of 21.57 min as shown in Fig. 5D. Although only a few fragment ions were observed in the MS–MS spectra of the major oxygenated products due to the low quantity of the metabolites, the produced base fragment ion peaks at *m/z* 375 confirmed the metabolites detection. The fragment ions of $[M-100\text{-}H]^-$ and $[M-116\text{-}H]^-$ resulted from oxygenated $CH_2CH_2CH{=}C(CH_3)_2$ group for the mono- and dioxygenated metabolites, respectively. Again, the interpreted fragmentation pathways presented in Fig. 2 indicated that oxygenation might occur on the aliphatic side chain. The exact oxygenation site could not be determined because the generated amount of the metabolites were not enough for NMR and other instrumental analyses.

Fig. 2 summarizes the major metabolites of Rg_3 as detected in rat feces samples and its metabolic pathway in rat gastrointestinal tract. After oral administration, the ginsenoside was found to be biologically transferred to deglucosylated and oxygenated products. The immediate oxygenated

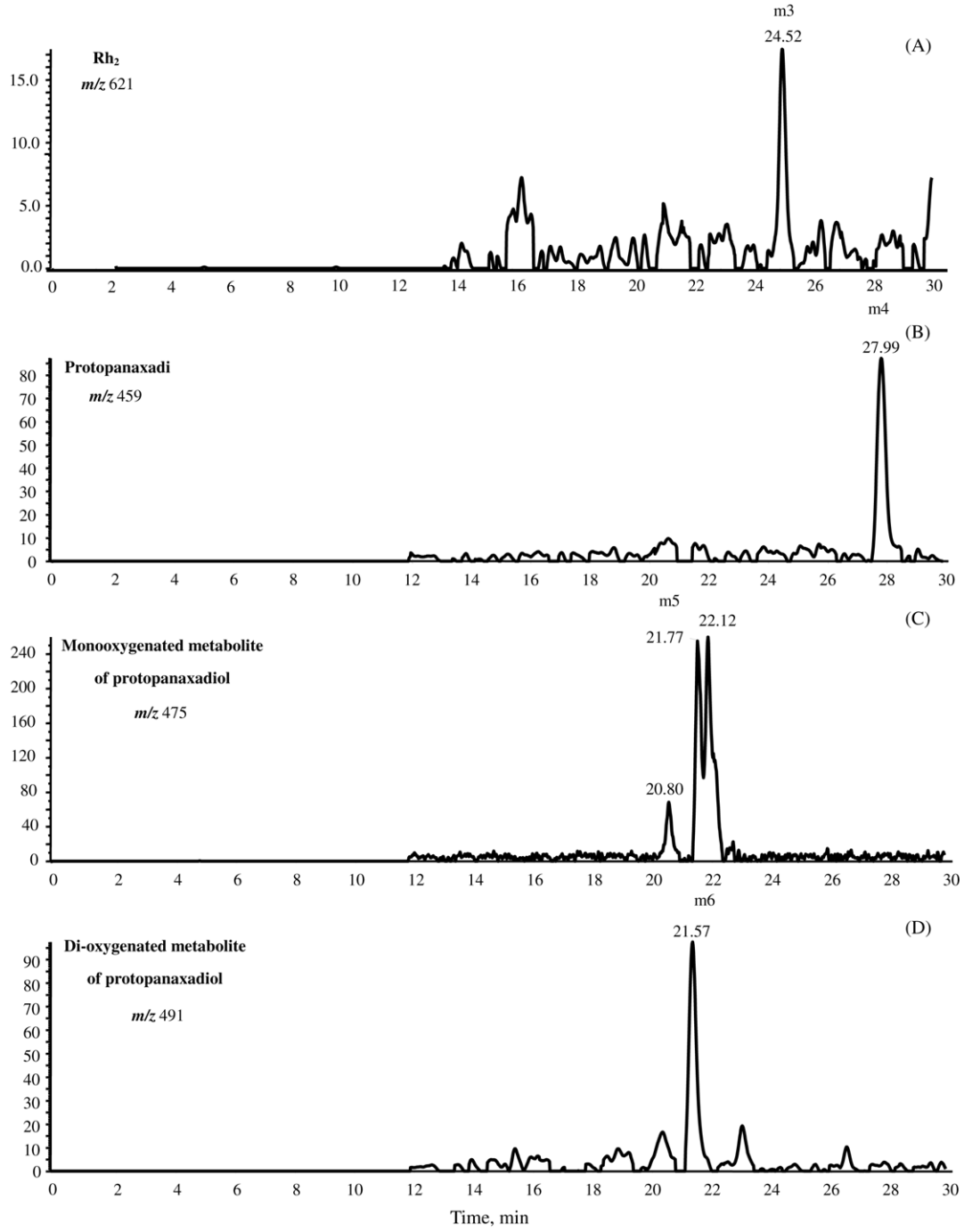

Fig. 5. LC–MS chromatograms of the metabolites of ginsenoside Rg_3 that were detected in the rat feces sample after oral administration: Rh_2 at *m*/*z* 621 (A), protopanaxadiol at *m*/*z* 459 (B), monooxygenated metabolite of protopanaxadiol at *m*/*z* 475 (C), and dioxygenated metabolite of protopanaxadiol at *m*/*z* 491 (D).

metabolites of Rg_3 (m1 and m2) and the deglucosylated products (m3 and m4) might be formed from the parent ginsenoside. The formation pathway of the oxygenated products of protopanaxadiol (m5 and m6), however, was unclear. The metabolites m5 and m6 might be produced either from protopanaxadiol via further oxygenation metabolism, or from the further deglucosylation of the Rg_3 oxygenated metabolite (Fig. 2).

Although no metabolites of Rg_3 were detected in the plasma and urine samples, the results of the metabolism study on rat feces provided explanation for the short half-life of Rg_3 obtained from the oral administrations, i.e., the high rate of metabolic clearance might be the reason. Furthermore, our metabolism data supported the assumption that the natural glycosidic ginsenosides, such as Rg_3 might be the prodrugs that can be easily transformed to active components by in-

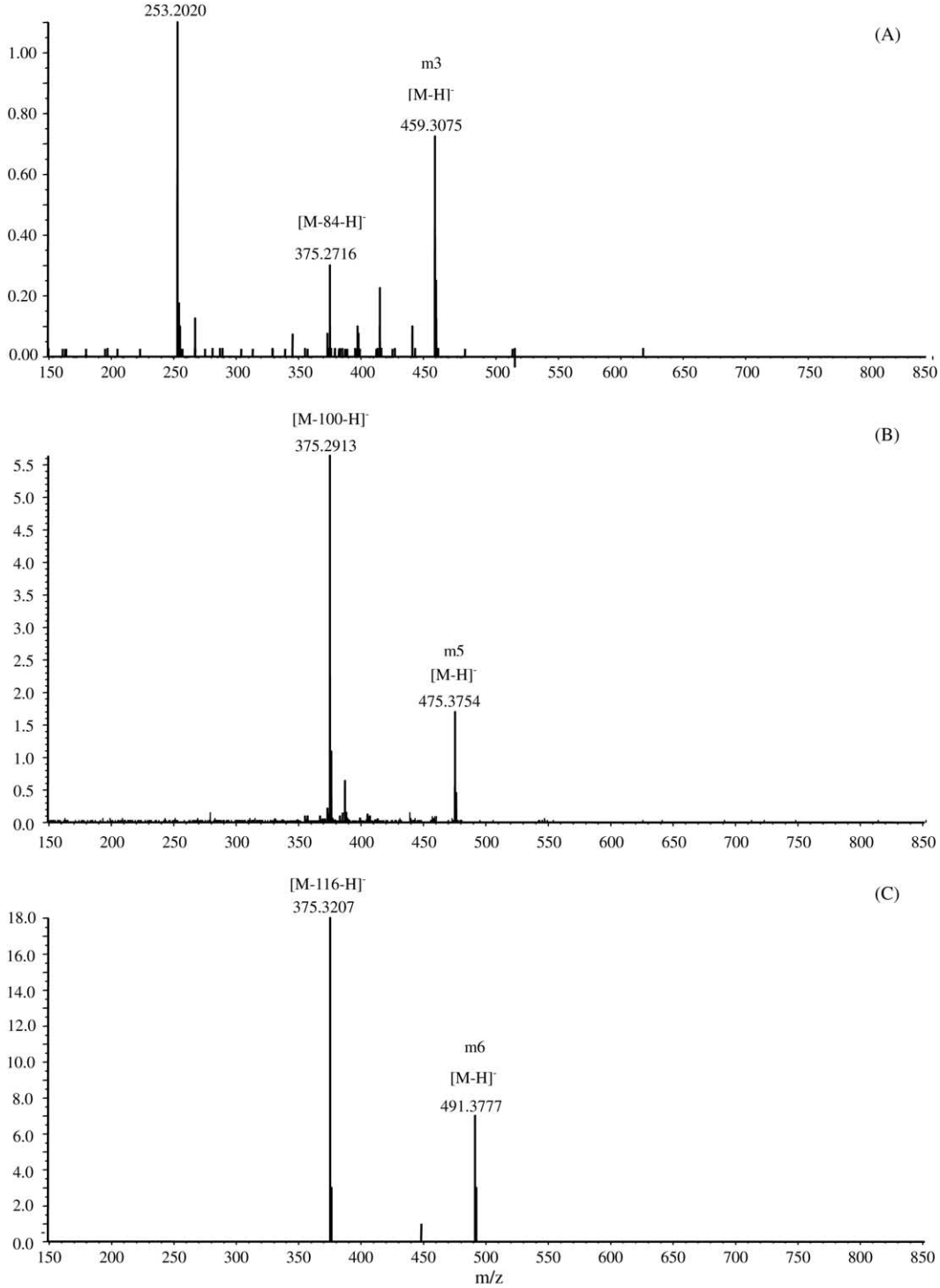

Fig. 6. Negative ion ESI-MS–MS spectra of the metabolites of ginsenoside Rg_3 detected in the rat feces sample: protopanaxadiol (A), monooxygenated metabolite of protopanaxadiol (B), and dioxygenated metabolite of protopanaxadiol (C).

testinal flora [11,20]. Rh_2 and protopanaxadiol were detected as intestinal metabolites of Rg_3 in our study. These two components have been shown to possess higher anti-tumor and other activities than Rg_3 [11,27–29], indicating that the two metabolites produced from Rg_3 might be the active form of the ginsenoside.

4. Conclusion

The short half-life of Rg_3 from the rat PK study indicated that the ginsenoside Rg_3 may be metabolized quickly after oral and intravenous administrations. Six metabolites of Rg_3 were detected from feces collected between 0 and 24 h after oral administration. The metabolites detection by LC–MS was confirmed by MS–MS analyses. Oxygenation and deglucosylation were found to be the major metabolic pathway of Rg_3 in rat gastrointestinal tract. The metabolite identification and suggested metabolic pathways may provide important information to the bioactive form of this ginsenoside.

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