Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 218–224
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Conversion of major ginsenoside Rb1 to 20(*S*)-ginsenoside Rg3 by *Microbacterium* sp. GS514

Le-Qin Cheng [a,b], Ju Ryun Na [a], Myun Ho Bang [a], Myung Kyum Kim [a], Deok-Chun Yang [a,*]

[a] *Department of Oriental Medicinal Material and Processing, College of Life Science, Kyung Hee University, 1 Seocheon-dong, Kiheung-gu Yongin, Kyunggi-do 449-701, Republic of Korea*
[b] *Department of Pharmacy and Applied Chemistry, Jilin Institute of Chemical Technology, Jilin, PR China*

Received 1 February 2007; received in revised form 27 April 2007
Available online 30 August 2007

Abstract

Ginseng saponin, the most important secondary metabolite in ginseng, has various pharmacological activities. Many studies have been directed towards converting major ginsenosides to the more active minor ginsenoside, Rg3. Due to the difficulty in preparing ginsenoside Rg3 enzymatically, the compound has been mainly produced by either acid treatment or heating. A microbial strain GS514 was isolated from soil around ginseng roots in a field and used for enzymatic preparation of the ginsenoside Rg3. Blast results of the 16S rRNA gene sequence of the strain GS514 established that the strain GS514 belonged to the genus *Microbacterium*. Its 16S rRNA gene sequence showed 98.7%, 98.4% and 96.1% identity with those of *M. esteraromaticum*, *M. arabinogalactanolyticum* and *M. lacticum*. Strain GS514 showed a strong ability to convert ginsenoside Rb1 or Rd into Rg3. Enzymatic production of Rg3 occurred by consecutive hydrolyses of the terminal and inner glucopyranosyl moieties at the C-20 carbon of ginsenoside Rb1 showing the biotransformation pathway: Rb1 → Rd → Rg3.

Keywords: Conversion; 20(*S*)-ginsenoside Rg3; Ginsenoside Rb1; *Microbacterium* sp. GS514

1. Introduction

The ginseng saponin (ginsenoside) is one of the most important secondary metabolites in ginseng and has various pharmacological activities (Yokozawa et al., 1985; Kenarova et al., 1990; Kimura et al., 1988; Ota et al., 1991). To date, about 50 kinds of ginsenosides including malonyl Ra1/Ra2/Ra3/Rb1/Rb2/Rc/Rd, Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2 (R,S), Rg3 (R,S), Rg5, Rg6, Rh1 (R,S), Rh2 (R,S), Rh3, Rh4, Rk1, Rk2, Rk3, R1, R2, F2, Rs1, Rs2, Rs3 (R,S), Rs4, Rs5, Rs6, Rs7, Ro, F1, F2, F4, compound K/Y/O, notoginsenoside R1, and quinquenoside R1 have been isolated and identified from *Panax ginseng* C. A. Meyer. Among these ginsenosides, Rg3 (**1**) exerts many pharmacological activities such as tumor-suppressing (Shinkai et al., 1996), antimetastatic (Mami Mochizuki et al., 1995), anticarcinogenic (Li et al., 2005), hepatoprotective (Lee et al., 2005), neuroprotective (Tian et al., 2005), immune-stimulating (Wang and Meng, 1999) and vasodilating effects (Kim et al., 2003). In addition, Rg3 (**1**) is a precursor for ginsenoside Rh2, which also has a very strong antitumor effect. But the concentration of ginsenoside Rg3 (**1**) is extremely low in normal ginseng (Kitagawa et al., 1983). Thus, production of ginsenoside Rg3 (**1**) would be very important and many studies have been aimed at converting major ginsenosides to the more active minor ginsenoside Rg3 (**1**).

Ginsenoside Rg3 (**1**) exists as S (**1a**) and R (**1b**) optical isomers depending on the spatial arrangement of the hydroxyl group on the chiral carbon (C-20) of the aglycone (Fig. 1). The isomers exhibit different physical properties

* Corresponding author. Tel.: +82 31 201 2688; fax: +82 31 2022 687.
E-mail address: deokchunyang@yahoo.co.kr (D.-C. Yang).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.06.035

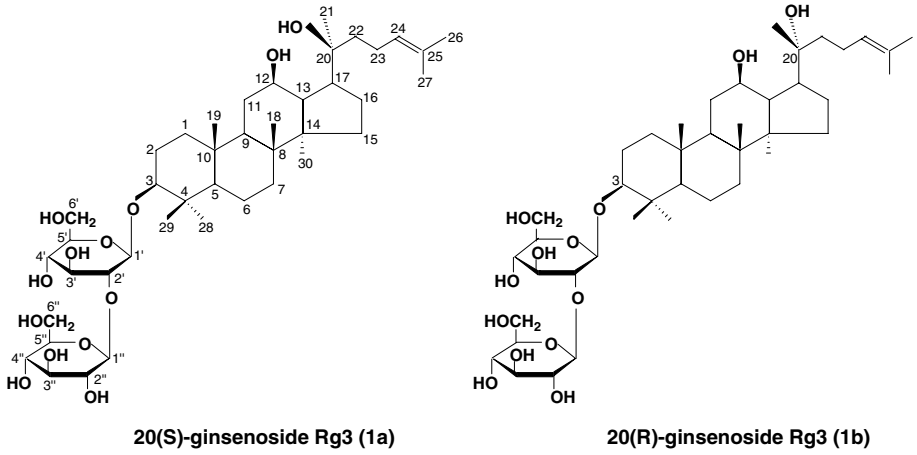

Fig. 1. Structures of 20(S)- and 20(R)-ginsenoside Rg3 (1a and 1b).

and biological activities. The 20(*S*)-Rg3 (**1a**) isomer is more water-soluble and more bioavailable than the 20(*R*)-Rg3 isomer (**1b**) (Ni et al., 2005). 20(*S*)-Rg3 (**1a**) but not 20(*R*)-Rg3 (**1b**), can inhibit Ca^{2+}, K^{+} and Na^{+} channels and inhibit coronary artery contraction in endothelium-denuded (Jeong et al., 2004; Kim et al., 2006).

Major ginsenosides such as ginsenoside Rb1, Rb2, Rc and Rd can be readily converted into a mixture of 20(*R*)- and 20(*S*)-ginsenoside Rg3 (**1b**/**1a**) by either acid treatment or heating (Han et al., 1982; Park, 2004). But the isolation of each isomer from the racemic mixture is a time-consuming and complicated process (Bae et al., 2002). 20(*S*)-Rg3

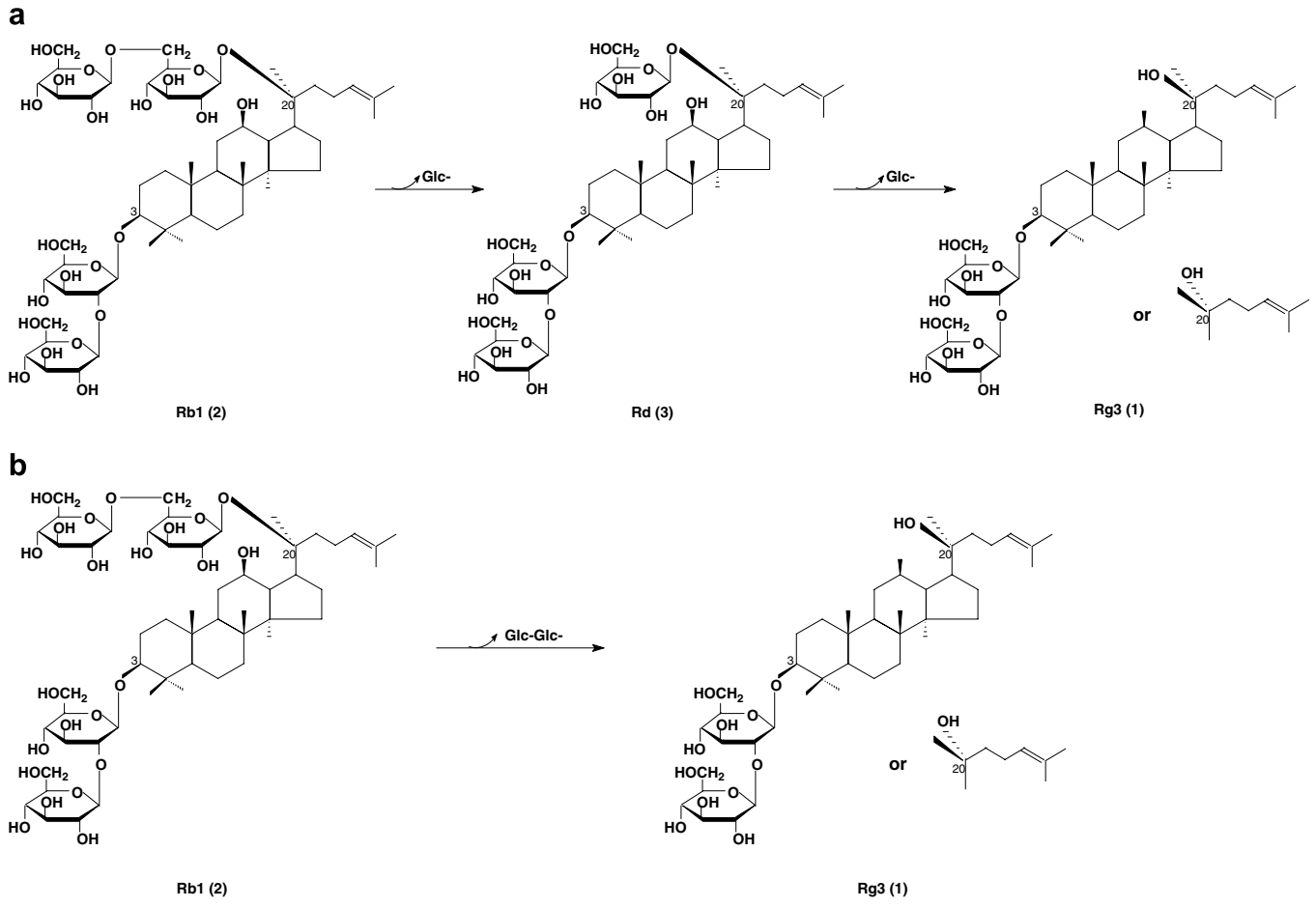

Fig. 2. Biotransformation pathway for production of ginsenoside Rg3 **(1)** from Rb1 **(2)**: (a), indirectly, in this case ginsenoside Rb1 (**2**) was converted to ginsenoside Rg3 (**1**) with an intermediate Rd (**3**); (b), directly, in this case ginsenoside Rb1 (**2**) was converted to ginsenoside Rg3 (**1**) without any intermediates.

(**1a**) has also been prepared by chemical synthesis from 20(*S*)-dammer-24-en-3α, 12β, 20-triol (betulafolienetriol), but the synthetic steps were complicated and the overall yield was low (Anufriev et al., 1997).

The enzymatic conversion through sugar hydrolysis at a specific position of ginsenoside is desirable for the production of 20(*S*)-Rg3 (**1a**). Until now, no-one has reported production of ginsenoside Rg3 (**1**) by using microbial enzymes. In this study, we isolated a β-glucosidase-producing microorganism GS514 from soil around ginseng roots in a field using esculin-R2A agar, investigated the activity transforming ginsenoside Rb1 into Rg3, and identified its related metabolites.

2. Results and discussion

2.1. Screening and identification of microorganisms producing ginsenoside Rg3 (*1*)

A total of 200 isolates of β-glucosidase-producing microorganisms were isolated from soil around the ginseng roots in a field using esculin-R2A agar. Among these isolates, strains GS342, GS514 and GS3072 showed an ability to convert ginsenoside Rb1 (**2**) into Rg3 (**1**) in nutrient broth (Fig. 2). The strain GS514 showed the strongest activities to convert ginsenoside Rb1 (**2**) to ginsenoside Rg3 (**1**) and its rRNA gene sequence was blasted in the NCBI database. The strain GS514 belonged to the genus *Microbacterium* and its 16S rRNA sequences showed 98.7%, 98.4% and 96.1% similarities with those of *M. esteraromaticum*, *M. arabinogalactanolyticum* and *M. lacticum*.

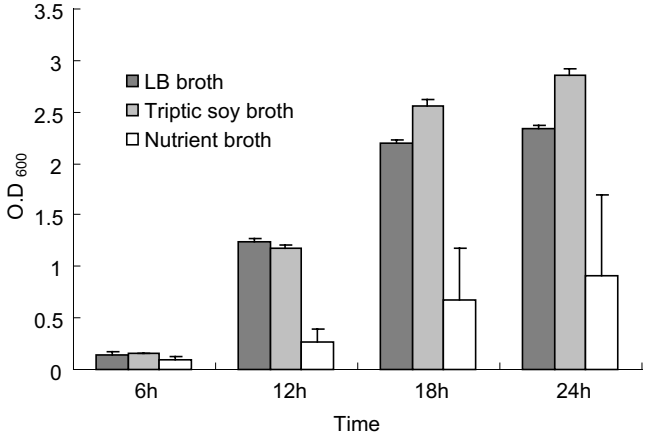

Fig. 3. Growth of the strain GS514 cultured in various media.

2.2. Selection of culture media

Growth of the strain GS514 was very slow in nutrient broth and the activity producing Rg3 (**1**) was very weak. In order to compare the effect of various media, strain GS514 was cultured in LB broth, tryptic soy broth and nutrient broth for 24 h. Samples were collected at 6, 12, 18 and 24 h, and O.D was measured at 600 nm. The O.D of LB broth, tryptic soy broth and nutrient broth samples at 24 h were 2.346, 2.849 and 0.910, respectively. The growth rates in LB broth and tryptic soy broth were significantly higher than in the nutrient broth (Fig. 3). Each suspension culture of the strain GS514 at 12 h was mixed with the same volume of ginsenoside Rb1 (**2**) and then incubated for 10 h in a shaking incubator. We observed that cultures in LB broth and tryptic soy broth converted ginsenoside Rb1 (**2**) into Rg3 (**1**), but the culture in nutrient broth only converted Rb1 (**2**) to Rd (**3**) (Figs. 2 and 4). This suggested that LB broth and tryptic soy broth were suitable for the growth of the strain GS514 and also for production of Rg3 (**1**). Therefore, LB broth, which is a costs lower price than other media, was chosen for further study.

2.3. Biotransformation pathway

Theoretically, there are two pathways for the bioconversion of Rb1 (**2**) to Rg3 (**1**) (Fig. 2). One is through the ginsenoside Rd (**3**) by sequential hydrolyses of two glucoses at C-20 of Rb1. The other is direct hydrolysis of the inner glucose at C-20 of Rb1 (**2**). In order to investigate the

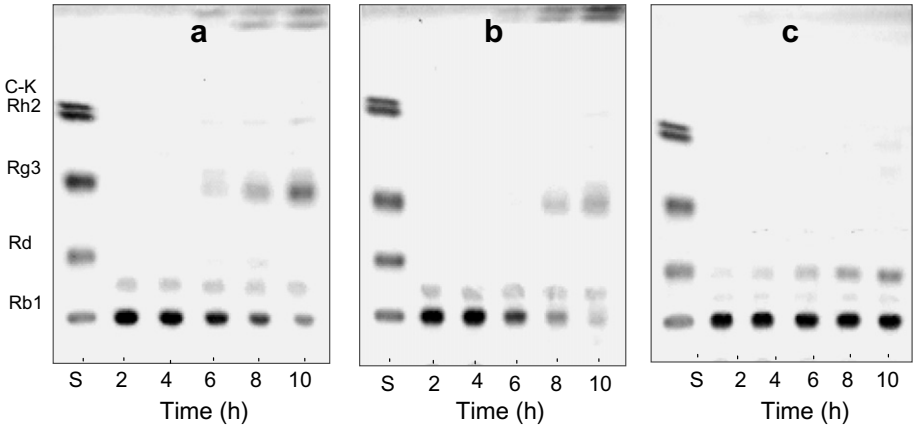

Fig. 4. TLC analysis of metabolites of ginsenoside Rb1 (**2**) converted by the strain GS514 cultured in different media, (a) LB broth, (b) tryptic soy broth and (c) nutrient broth. Developing solvent: $CHCl_3$/MeOH/H_2O (65:35:10, by vol., lower phase). S: saponin standards.

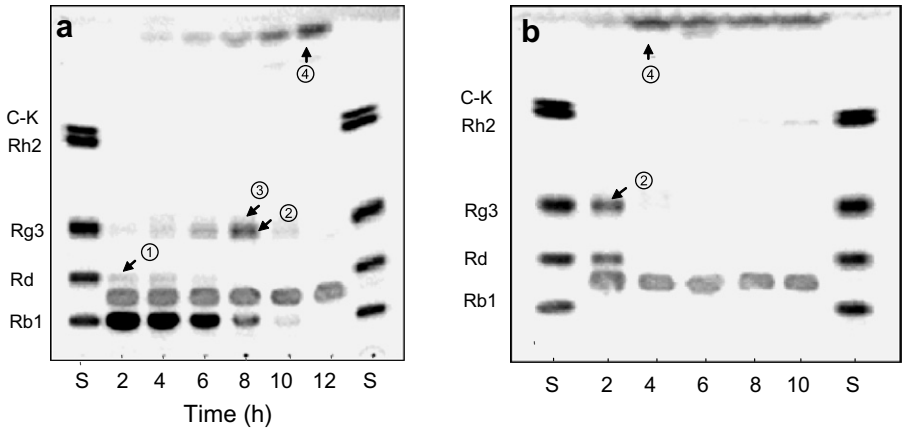

Fig. 5. Time-course TLC analysis of metabolites of ginsenoside Rb1 (**2**) and Rd (**3**) converted by the strain GS514. (a), ginsenoside Rb1 (**2**) was used as substrate; (b), ginsenoside Rd (**3**) was used as substrate. ①, ginsenoside Rd (**3**); ②, ginsenoside Rg3 (**1**); ③ ginsenoside F2; and ④ protopanaxadiol. Developing solvent: $CHCl_3$/MeOH/H_2O (65:35:10, by vol., lower phase). S: saponin standards.

biotransformation pathway of ginsenoside Rb1 (**2**) into Rg 3 (**1**), the suspension culture of strain GS514 was mixed with a ginsenoside Rb1 (**2**) and Rd (**3**) solution, respectively. As shown in Fig. 5, strain GS514 converted ginsenoside Rb1 (**2**) into metabolites Rd (**3**), Rg3 (**1**), F2 (Cheng et al., 2006) and presumably protopanaxadiol (aglycone) according to the high R_f value (Ma et al., 2001; Yu et al., 1999). When ginsenoside Rb1 (**2**) was added to the culture broth of the strain GS514, the content of ginsenoside Rb1 (**2**) and Rd (**3**) gradually decreased and that of Rg3 (**1**) gradually increased from 2 h to 8 h, (Fig. 5a). This proved that metabolite Rd (**3**) is a precursor of ginsenoside Rg3 (**1**). Similarly, when ginsenoside Rd (**3**) was added to the culture broth of the strain GS514, Rg3 (**1**) was also produced and the content of the Rg3 (**1**) remarkably decreased after 4 h (Fig. 5b). Conversion of Rd (**3**) into Rg3 (**1**) was faster than conversion of Rb1 (**2**) into Rg3 (**1**). This suggested that Rb1 (**2**) was converted by different enzymes secreted by the strain GS514 in the following sequence: Rb1 (**2**) → Rd (**3**) → Rg3 (**1**), as shown in the pathway (a) in Fig. 4. The enzymes hydrolyzed the terminal glucose and consecutively inner glucose at the C-20 position. Interestingly, after 10 h of the reaction (Fig. 5a), the content of the ginsenoside Rg3 (**1**) rapidly decreased. This may have occurred due to the precursors (Rb1 (**2**) and Rd (**3**)) being converted into ginsenoside Rg3 (**1**) so the rate of production of Rg3 (**1**) was slower than the rate of degradation of Rg3 (**1**).

The HPLC profile of the reaction mixture of ginsenoside Rb1 (**2**) and the strain GS514 after 8 h incubation is shown in Fig. 6. In the HPLC chromatogram, the peaks with retention time 40.33, 45.10, 53.17 and 57.12 min correspond to ginsenoside Rb1 (**2**), Rd (**3**), F2 and Rg3 (**1**). When 222 μg (0.2 μmol) of ginsenoside Rb1 (**2**) was reacted, 180 μg of Rb1 (**2**) was degraded (81.2% conversion) and 65 μg of Rg3 (**1**) was produced (yield 41.4%). The yield of enzymatic production of Rg3 (**1**) (41.4%) was higher than that of organic synthesis (12.8%) (Anufriev et al., 1997) and similar to that of acid treatment (42.4%) (Bae et al., 2002). In conclusion, a strong ability of the strain GS514 to produce ginsenoside Rg3 (**1**) in a short period of 8 h was observed.

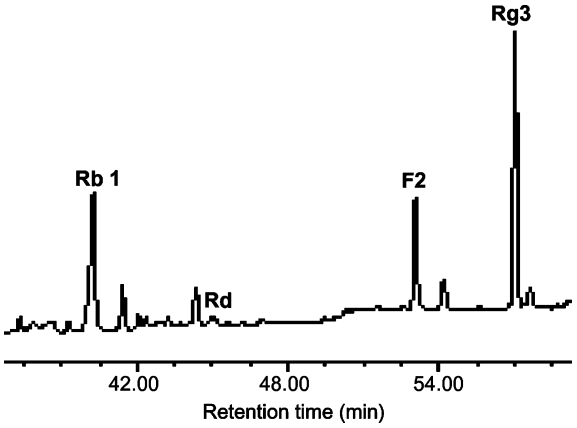

Fig. 6. HPLC profile of the metabolites of ginsenoside Rb1 (**2**) converted by strain GS514. Suspension culture of the strain GS514 in LB broth was mixed with 1 mM ginsenoside Rb1 (**2**) and then incubated for 8 h, extracted by *n*-BuOH, evaporated vacuo and analyzed by HPLC after dissolved in MeOH.

2.4. Structural identification

Among the metabolites of ginsenoside Rb1 (**2**), compounds ① and ③ (Fig. 5) were previously identified by ^{1}H NMR and ^{13}C NMR spectroscopy as Rd and F2, respectively (Cheng et al., 2006). Because the R_f value and the retention time of metabolite ② were the same as those of standard Rg3 (**1**) in TLC and HPLC analysis, compound ② was assumed as ginsenoside Rg3 (**1**), but we could not confirm whether the Rg3 (**1**) was in the *R*-form or *S*-form. To confirm the spatial configuration of ginsenoside Rg3 (**1**) by NMR, more than 100 fractions were obtained using silica gel column chromatography. The 51st to the 94th fractions, which contained only one component of the same R_f values as the standard Rg3 (**1**), were collected and evaporated *in vacuo* to obtain 22.59 g of metabolite ②. This

compound was dissolved in pyridine-d_5 and analyzed by ^{1}H NMR and ^{13}C NMR spectroscopy.

In the ^{1}H NMR spectrum of metabolite ②, (Fig. 5) the signals for the anomeric proton appeared at δ 4.91 ppm (1H, *d*, $J = 7.2$ Hz) and δ 5.36 ppm (1H, *d*, $J = 7.2$ Hz), which is indicative that the metabolite ② harbored two β-D-glucose moiety; the anomeric proton signals were similar to the anomeric proton signals of the 3-*O*-inner-glucopyranosyl and 3-*O*-outer-glucopyranosyl moieties of ginsenoside Rb1 (**2**), which appeared at δ 4.88 ppm (1H, *d*, $J = 7.6$ Hz) and δ 5.34 ppm (1H, *d*, $J = 7.6$ Hz) (Dong et al., 2003).

The ^{13}C NMR spectra of the two isomers of the ginsenoside Rg3 (**1**) are very similar, but there are obvious differences. For example, the signals for C-17 and C-21 of 20(*S*)-Rg3 (**1a**) appeared downfield than those for 20(*R*)-Rg3 (**1b**), and the signal for C-22 of 20(*S*)-Rg3 (**1a**) appeared upfield than that for 20(*R*)-Rg3 (**1b**) in the ^{13}C NMR spectrum (Teng et al., 2000). The ^{13}C NMR analysis result of metabolite ② is shown in Table 1. A comparison of the ^{13}C NMR spectrum of metabolite ② with that of ginsenoside Rb1 (**2**) showed that the signal for the C-20 was remarkably shifted upfield from δ 83.5 ppm to δ 73.0 ppm. This may mean that glucose moiety at C-20 position was removed. Meanwhile, the signal for the C-3 appeared at δ 88.9 ppm, which is similar to δ 89.0 ppm of Rb1 (**2**), which means that there is no change of glucose moiety at the C-3 position (Dong et al., 2003). The signals for C-17, C-21 and C-22 of the metabolite ② appeared at δ 54.9 ppm, δ 28.2 ppm and δ 36.0 ppm, respectively, which are similar to those for the same carbons in 20(*S*)-Rg3 (**1a**) (see Table 1). Based on the observations, we confirmed that metabolite ② is 3-*O*-[β-D-glucopyranosyl-(1,2)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol, which is identical to ginsenoside 20(*S*)-Rg3 (**1a**).

3. Conclusions

Previously, preparation of pharmacologically active minor ginsenosides from major ginsenosides has been done by chemical or physical methods such as acid treatment and heating. The enzymatic conversion process may occur in milder reaction conditions than chemical or physical conversion and selective enzymatic conversion to *S* isomer is possible. But until now there is no study on the enzymatic production of Rg3 (**1**). The enzymatic conversion of ginsenoside Rb1 (**2**) into Rg3 (**1**) is not easy because enzymatic hydrolysis of glycosidic bond at C-3 is easier than at C-20. C-20 of ginsenoside Rb1 (**2**) is a tertiary carbon with larger steric crowding, which inhibits approaches of microbial enzymes, whereas C-3 is a secondary carbon with more free space. If the glycosidic bond at C-3 is hydrolyzed, ginsenoside Rg3 (**1**) cannot be produced (Kim et al., 1998).

In this study, we isolated more than 200 β-glucosidase-producing microorganisms from soil of a ginseng field and identified one strain, *Microbacterium* sp. GS514 showing a strong ability to convert the ginsenoside Rb1 (**2**) into 20(*S*)-Rg3 (**1a**). The bioconversion pathway to produce Rg3 (**1**) followed the sequence: Rb1 (**2**) → Rd (**3**) → Rg3 (**1**). The suspension culture of the strain GS514 used in this study contained also the enzymes to degrade Rg3 (**1**) to other metabolites and so the production yield was

Table 1
^{13}C NMR comparison of metabolite ② produced by the strain GS514 with 20(*R*)- and 20(*S*)-Rg3 (**1b** and **1a**) (100 MHz, solvent: pyridine-d_5)

Carbon site	20(*R*)-Rg3[a] (ppm)	20(*S*)-Rg3[a] (ppm)	Metabolite ② (ppm)	Carbon site	20(*R*)-Rg3[a] (ppm)	20(*S*)-Rg3[a] (ppm)	Metabolite ② (ppm)
C-1	39.2	39.2	39.1	C-22	43.3	36.0	36.0
C-2	26.7	26.8	26.9	C-23	22.7	23.1	23.1
C-3	89.0	89.0	88.9	C-24	126.1	126.4	126.3
C-4	39.8	39.8	39.8	C-25	130.8	130.8	130.8
C-5	56.4	56.5	56.4	C-26	25.9	25.9	25.9
C-6	18.5	18.5	18.5	C-27	17.7	17.1	17.8
C-7	35.2	35.3	35.2	C-28	28.2	28.2	28.2
C-8	40.0	40.1	40.1	C-29	16.6	16.7	16.7
C-9	50.4	50.5	50.4	C-30	17.4	17.8	17.1
C-10	37.0	37.0	37.0	1′	105.2	105.2	105.1
C-11	32.2	32.1	32.2	2′	83.5	83.5	83.4
C-12	70.9	71.0	71.0	3′	78.0	78.0	78.0
C-13	49.3	48.7	48.7	4′	71.8	71.8	71.6[b]
C-14	51.9	51.8	51.8	5′	78.1	78.3	78.3[b]
C-15	31.5	31.4	31.5	6′	62.9	62.9	62.8
C-16	26.8	26.9	27.2	1″	106.1	106.1	106.1
C-17	50.7	54.9	54.9	2″	77.2	77.2	77.2
C-18	15.9	15.9	15.9	3″	78.4	78.4	78.3[b]
C-19	16.4	16.4	16.5	4″	71.7	71.7	71.6[b]
C-20	73.0	73.0	73.0	5″	78.3	78.2	78.2
C-21	22.8	28.2	27.2	6″	62.8	62.8	62.7

[a] Ref. Teng et al., (2000).
[b] Overlapped with other signals.

relatively low. So, the isolation of the enzymes responsible for the production of ginsenoside Rg3 (**1**) is needed to increase the yield.

4. Experimental

4.1. Materials

Ginsenoside Rb1 (**2**) was isolated from *Panax quinquefolius*. Standard ginsenosides including 20(*S*)-Rb1, 20(*S*)-Rd, 20(*S*)-Rg3, 20(*S*)-Rh2 and compound-K were obtained from KT&G, in Daejeon, Korea. R2A agar, LB broth, tryptic soy broth and nutrient broth were purchased from Difco. The 60 F-254 Silica gel plate (Merck) was used for thin-layer chromatography (TLC) and silica gel 60 (70–230 mesh, Merck) was used for column chromatography.

4.2. Screening and identification of microorganisms producing ginsenoside Rg3

The isolation of β-glucosidase-producing microorganisms from soil around ginseng roots in a field was performed according to a previously published method (Cheng et al., 2006; Kim et al., 2005). Each microbial suspension cultured in nutrient broth was added to the same volume of aqueous 1 mM ginsenoside Rb1 (**2**) solution and then incubated on a rotary shaker (200 rpm) at 30 °C for 48 h. The reaction mixture was extracted with butanol saturated with H_2O and then analyzed by thin layer chromatography (TLC). Eight microlitres of the ginseng extract solution was spotted on a TLC plate and developed to 5.5 cm distance in a chamber with $CHCl_3$/MeOH/H_2O (65:35:10, v/v/v, lower phase) as the mobile phase. Bands on the TLC plates were detected by spraying 10% H_2SO_4, followed by heating. The 16S rRNA gene sequences of the microorganisms producing ginsenoside Rg3 was sequenced by Genotec (Daejeon, Korea). The 16S rRNA gene sequences were blasted in the NCBI database (Accession number is EU036992).

4.3. Selection of culture media

The strain GS514 was cultured in LB broth on a rotary shaker (160 rpm) at 27 °C for 24 h then inoculated in nutrient broth, LB broth or tryptic soy broth at 1% (v/v) and shaken for 36 h under the same conditions. The $O.D_{600}$ of suspension cultures was periodically measured.

4.4. Biotransformation pathway

The strain GS514 was cultured in LB broth on a rotary shaker (160 rpm) at 27 °C for 24 h and then was added to the same volume of 1 mM ginsenoside Rb1 (**2**) aqueous solution and then incubated on a rotary shaker (200 rpm) at 30 °C for 48 h. The Reaction mixtures were extracted with *n*-BuOH saturated with H_2O every 2 h and analyzed by TLC and HPLC.

4.5. HPLC analysis

HPLC was performed using a NS 3000 system (FUTECS Co., Ltd.), equipped with a UV/Vis detector and gradient pump. The detection wavelength was 203 nm. The column used was a C18 (250 × 4.6 mm, ID 5 μm), a 20-ml sample volume was injected, and the mobile phase utilized gradient conditions with CH_3CN (solvent A) and distilled H_2O (solvent B). The solvent A/solvent B ratios were as follows: 15:85, 21:79, 58:42, 90:10, 90:10, 15:85 and 15:85, with run times of 0–5, 5–25, 25–70, 70–72, 72–82, 82–84 and 84–100 min, respectively, at a 1.6 ml/min flow rate.

4.6. Structural identification

An amount of 72 ml of 1 mM (0.072 mmol) ginsenoside Rb1 (**2**) was treated with the suspension culture of microorganism producing Rg3. The reaction mixture was extracted twice with *n*-BuOH saturated with H_2O and evaporated *in vacuo*. The residue was dissolved in MeOH and then evaporated *in vacuo* to dryness after adding a little silica gel. The silica gel, which adsorbed an equal amount of sample, was used in silica gel cc (φ 4.5 × 25 cm) to isolate ginsenoside Rg3 (**1**). The eluent was $CHCl_3$/MeOH/H_2O (9:3:1, by vol., lower phase) and the collection volume was 20 ml per fraction. From the fractions, the same metabolite was collected *via* TLC analysis and evaporation *in vacuo*. The residue was dissolved in pyridine-d_5, and the structure was analyzed *via* ^{1}H NMR and ^{13}C NMR, using an FT-NMR spectrometer (Varion Inova AS 400, Varion USA. 400 MHz).

4.6.1. Metabolite ② (ginsenoside Rg3 (**1a**))

^{1}H NMR (pyridine-d_5, 400 MHz): m.p: 248–250 °C (dec.), δ 0.77 ppm (3H, *s*, H-19), δ 0.93 ppm (6H, *s*, H-18, H-30), δ 1.08 ppm (3H, *s*, H-29), δ 1.26 ppm (3H, *s*, H-28), δ 1.44 ppm (3H, *s*, H-21), δ 1.59 ppm (3H, *s*, H-27), δ 1.62 ppm (3H, *s*, H-26), δ 4.91 ppm [1H, *d*, $j = 6.8$ Hz, H-3-glc (inner)-1H′], δ 5.36 ppm [1H, *d*, $j = 7.2$ Hz, H-3-glc (outer)-1H″], for ^{13}C NMR (pyridine-d_5, 100 MHz) data see Table 1.

Acknowledgements

This study was supported by the Technology Development Program for Agriculture and Forestry (TDPAF), Ministry of Agriculture and Forestry, Republic of Korea.

References

Anufriev, V.P., Malinovskaya, G.V., Denisenko, V.A., Uvarova, N.I., Elyakov, G.B., Kim, S.I., Baek, N.I., 1997. Synthesis of ginsenoside Rg3, a minor constituent of Ginseng radix. Carbohydr. Res. 304, 179–182.

Bae, E.A., Han, M.J., Choo, M.K., Park, S.Y., Kim, D.H., 2002. Metabolism of 20(*S*)- and 20(*R*)-ginsenoside Rg3 by human intestinal bacteria and its relation to in vitro biological activities. Biol. Pharm. Bull. 25, 58–63.

Cheng, L.Q., Kim, M.K., Lee, J.W., Lee, Y.J., Yang, D.C., 2006. Conversion of major ginsenoside Rb(1) to ginsenoside F(2) by *Caulobacter leidyia*. Biotechnol. Lett. 28, 1121–1127.

Dong, A.L., Ye, M., Guo, H.Z., Zheng, J.H., Guo, D., 2003. Microbial transformation of ginsenoside Rb_1 by *Rhizopus stolonifer* and *Curvularia lunata*. Biotechnol. Lett. 25, 339–344.

Han, B.H., Park, M.H., Han, Y.N., Woo, W.S., Sankawa, U., Yahara, S., Tanaka, O., 1982. Degradation of ginseng under mild acidic condition. Plant Med. 44, 146–149.

Jeong, S.M., Lee, J.H., Kim, J.H., Lee, B.H., Yoon, I.S., Lee, J.H., Kim, D.H., Rhim, H., Kim, Y., Nah, S.Y., 2004. Stereospecificity of ginsenoside Rg3 action on ion channels. Mol. Cells 18, 383–389.

Kenarova, B., Neychev, H., Hadjiivanova, C., Petkov, V.D., 1990. Immunomodulating activity of ginsenoside Rg1 from *Panax ginseng*. Jpn. J. Pharmacol. 54, 447–454.

Kim, C.S., Choi, K.J., Ko, S.Y., Sung, H.S., Lee, Y.G., Kim, S.C., 1998. Controls of the hydrolysis of ginseng saponins by neutralization of organic acids in red ginseng extract preparations. J. Ginseng Res. 22, 205–210.

Kim, J.H., Lee, J.H., Jeong, S.M., Lee, B.H., Yoon, I.S., Lee, J.H., Choi, S.H., Kim, D.H., Park, T.K., Kim, B.K., Nah, S.Y., 2006. Stereospecific effects of ginsenoside Rg3 epimers on swine coronary artery contractions. Biol. Pharm. Bull. 29, 365–370.

Kim, M.K., Lee, J.W., Lee, K.Y., Yang, D.C., 2005. Microbial conversion of major ginsenoside rb(1) to pharmaceutically active minor ginsenoside Rd. J. Microbiol. 43, 456–462.

Kim, N.D., Kim, E.M., Kang, K.W., Cho, M.K., Choi, S.Y., Kim, S.G., 2003. Ginsenoside Rg3 inhibits phenylephrine-induced vascular contration through induction of nitric oxide synthase. Brit. J. Pharmacol. 140, 661–670.

Kimura, Y., Okuda, H., Arichi, S., 1988. Effects of various ginseng saponins on 5-hydroxytryptamine release and aggregation in human platelets. J. Pharm. Pharmacol. 40, 838–843.

Kitagawa, I., Yoshikawa, M., Yoshihara, M., Hayashi, T., Taniyama, T., 1983. Chemical studies of crude drugs(1). Constituents of *Ginseng radix rubra*. Yakugaku Zasshi 103, 612–622.

Lee, H.U., Bae, E.A., Han, M.J., Kim, D.H., 2005. Hepatoprotective effect of 20(*S*)-ginsenosides Rg3 and its metabolite 20(S)-ginsenoside Rh2 on *tert*-butyl hydroperoxide-induced liver injury. Biol. Pharm. Bull. 28, 1992–1994.

Li, X., Guan, Y.S., Zhou, X.P., Sun, L., Liu, Y., He, Q., Fu, L., Mao, Y.Q., 2005. Anticarcinogenic effect of 20(*R*)-ginsenoside Rg3 on induced hepatocellular carcinoma in rats. Sichuan Da Xue Xue Bao Yi Xue Ban 36, 217–220.

Ma, J.S., Zhou, Q.L., Fei, X.F., Sun, Y., Wang, B.X., 2001. Metabolism of ginsenoside Rb1 and panaxadiol saponins by fungi. Yao Xue Xue Bao 36, 603–605.

Mochizuki, M., Yoo, Y.C., Matsuzawa, K., 1995. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside Rb2, 20(*R*)- and 20(*S*)-ginsenoside Rg3, of red ginseng. Biol. Pharm. Bull. 18, 1197–1202.

Ni, J.S., Xin, Y., Wang, X.R., Shi, B., Chen, D., Tian, K., Wu, J.X., 2005. Effect of 20(*S*)-ginsenoside Rg3 combined with cytotoxic agents on sarcoma 180 of mice. Ji Lin Da Xue Xue Bao (Yi Xue Ban) 31, 705–708.

Ota, T., Maeda, M., Odashima, S., 1991. Mechanism of action of ginsenoside Rh2: uptake and metabolism of ginsenoside Rh2 by cultured B16 melanoma cells. J. Pharm. Sci. 80, 1141–1146.

Park, J.H., 2004. Sun ginseng – A new processed ginseng with fortified activity. Food Industry Nutrition 9, 23–27.

Shinkai, K., Akedo, H., Mukai, M., Imamura, F., Isoai, A., Kobayashi, M., Kitagawa, I., 1996. Inhibition of in vitro tumor cell invasion by ginsenoside Rg3. Jpn. J. Cancer. Res. 87, 357–362.

Teng, R.W., Li, H.Z., Wang, D.Z., He, Y.N., Yang, C.R., 2000. NMR Complete assignments of three protopanaxadiol monodesmosides. Bo Pu Xue Za Zhi 17, 461–468.

Tian, J.W., Fu, F.H., Geng, M.Y., Jiang, Y.T., Yang, J.X., Jiang, W.L., Wang, C.Y., Liu, K., 2005. Neuroprotective effect of 20(S)-ginsenoside Rg3 on cerebral ischemia in rats. Neurosci. Lett. 374, 92–97.

Wang, T.F., Meng, M.Z., 1999. Experiment for immunity effects of ginsenoside Rg 3. Zhong Guo Yao Ke Da Xue Xue Bao 2, 55–57.

Yokozawa, T., Kobayashi, T., Oura, H., Kawashima, Y., 1985. Studies on the mechanism of the hypoglycemic activity of ginsenoside-Rb2 in streptozotocin-diabetic rats. Chem. Pharm. Bull. (Tokyo) 33, 869–872.

Yu, H.S., Ma, X.Q., Guo, Y., Jin, F.X., 1999. Purification and characterization of ginsenoside-β-glucosidase. J. Ginseng Res. 23, 50–54.