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BIOTRANSFORMATION OF GINSENOSIDE Rb_1 INTO F-2 AND COMPOUND K BY BACTERIUM *Sphingomonas* sp. BG 25

L. N. Ten, S. M. Chae, and S.-A. Yoo*

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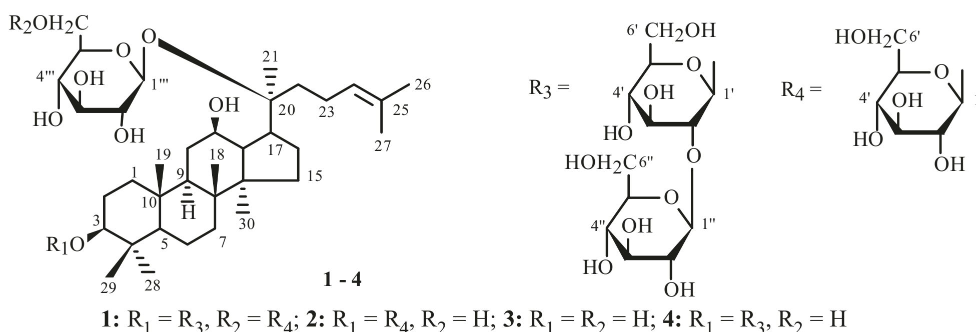
Triterpenoid saponins and ginsenosides, the majority of which contain protopanaxadiol or protopanaxatriol as the aglycon, are important biologically active compounds of *Panax ginseng* C. A. Meyer. One of the principal ginsenosides, Rb_1 (**1**), has β-sophorose and β-gentiobiose residues in the C-3 and C-20 positions, respectively, of 20(*S*)-protopanaxadiol. The minor ginsenoside F-2 (**2**) with glucose residues on C-3 and C-20 and compound K (**3**) with only one glucose on C-20 have structures close to that of Rb_1 and are practically absent in ginseng roots [1]. Saponins **2** and **3** have broader spectra of biological activity than Rb_1. For example, compound K inhibits growth of cultivated melanoma B16-B6, hepatocellular carcinoma Hep-G2, and lung carcinoma 95-D cells whereas F-2 induces apoptosis of breast cancer stem cells [1, 2].



1: $R_1 = R_3$, $R_2 = R_4$; **2:** $R_1 = R_4$, R_2 = H; **3:** $R_1 = R_2$ = H; **4:** $R_1 = R_3$, R_2 = H

Therefore, biotransformation of ginsenoside Rb_1 into F-2 and compound K, in particular using microorganisms, seemed of practical importance [3]. Microorganisms with amylase, xylanase, and cellulase activity were isolated earlier from soil samples taken from a ginseng field using specially synthesized substrates [4, 5]. Among these, strains showing high β-glucosidase activity in cultivation on agar media with added esculin were identified. Herein we communicate data on the transformation of ginsenoside Rb_1 into F-2 and compound K through the action of one of these strains, *Sphingomonas* sp. BG 25.

Standard ginsenosides Rb_1, Rd, F-2, and compound K were purchased from Faces Biochemical Co., Ltd. (Wuhan, PRC). The isolation of **1** from ginseng roots, analysis of biotransformation products, and their preparative isolation and identification were carried out as before [6] with the exception that PMR and ^{13}C NMR spectra were recorded on a Varian Unity Inova AS 400 spectrometer at operating frequencies of 400 and 100 MHz, respectively.

Bacterium *Sphingomonas* sp. BG 25 was cultivated in Ehrlenmeyer flasks in Luria–Bertani liquid medium (500 mL) for 48 h at 30°C with constant stirring. Then, bacterial suspension (125 mL) of concentration $8 \cdot 10^6$ CFU/mL (colony-forming units/mL) was placed into three sterile flasks and treated with an aqueous solution (125 mL, 1 mM) of Rb_1 ginsenoside. The first mixture was incubated for 24 h; the second, 72 h; the third, 96 h at 30°C with constant stirring. Analytical samples were taken every 6 h. After incubation, each mixture was extracted with water-saturated BuOH (250 mL). The resulting extract was evaporated *in vacuo*. The residue was analyzed. TLC and HPLC showed the presence in the first mixture of starting Rb_1 and its transformation product **4**, the spectral characteristics of which agreed fully with those of ginsenoside Rd that were published by us [6]. The main constituents in the second and third mixtures were two other Rb_1 biotransformation products, **2** and **3**, which had the same R_f values on TLC and HPLC retention times as standard ginsenoside F-2 and compound K. Both compounds were isolated from the obtained extracts by preparative HPLC over an OptimaPak C18 column (250 × 10 mm, 10 μm) and identified by spectral methods in order to confirm this.

Department of Biology and Medicinal Science, Pai Chai University, 155-40 Baijae-ro, Seo-Gu, Daejeon, 302-735, Republic of Korea, fax: (8242) 520 53 80, e-mail: lten@pcu.ac.kr, *say1000@pcu.ac.kr. Translated from *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2013, pp. 1002–1003. Original article submitted July 29, 2013.

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TABLE 1. ^{13}C NMR Spectral Data for Rd (**4**), F-2 (**2**), and Compound K (**3**) (δ, ppm)

C atom	Rd	F-2	K	C atom	Rd	F-2	K	C atom	Rd	F-2	K
1	39.6	39.0	39.4	17	52.0	51.5	51.6	3′	78.5	78.8	
2	26.9	26.5	28.2	18	16.4	16.1	16.3	4′	72.0	71.8	
3	89.3	88.7	78.2	19	16.7	15.8	16.0	5′	78.4	78.3	
4	40.0	39.5	39.5	20	83.6	83.2	83.2	6′	63.2	63.1	
5	56.7	56.1	56.3	21	22.7	22.3	22.3	1″	106.3		
6	18.8	18.2	18.7	22	36.5	35.8	36.1	2″	77.3		
7	35.5	34.9	35.1	23	23.6	23.1	23.2	3″	79.5		
8	40.4	39.8	40.0	24	126.2	125.7	125.9	4″	72.0		
9	50.6	49.9	50.2	25	131.1	130.8	130.8	5″	78.2		
10	37.3	36.7	37.3	26	26.1	25.6	25.7	6″	63.2		
11	31.3	30.6	30.7	27	18.2	17.6	17.6	1‴	98.5	98.1	98.2
12	70.5	70.1	70.1	28	28.5	27.9	28.6	2‴	75.4	75.0	75.0
13	49.6	49.5	49.4	29	16.9	16.6	16.4	3‴	78.6	79.0	79.3
14	51.8	51.2	51.4	30	17.8	17.1	17.3	4‴	72.0	71.7	71.6
15	31.2	30.4	30.9	1′	105.3	106.7		5‴	78.5	78.5	78.0
16	27.0	26.4	26.6	2′	83.7	75.6		6‴	63.0	62.7	62.8

A comparison of the $[M + Na]^+$ ions in mass spectra of Rd (**4**) [6] and **2** showed that one glucose was cleaved from **4**. Comparison of the PMR and ^{13}C NMR spectra of these compounds indicated that this cleavage occurred from the *β*-sophorose residue in the C-3 position of Rd. In fact, the ^{13}C NMR spectrum of **2** contained resonances for only two anomeric C atoms (106.7 and 98.1 ppm). The C-2′ resonance in the carbohydrate residue on C-3 (75.6 ppm) underwent a strong-field shift compared with that in the *β*-sophorose residue of **4** (83.7 ppm) (Table 1). Resonances of anomeric C atoms (106.7 and 98.1 ppm) in **2** were analogous to those of C-1′ (105.3 ppm) and C-1‴ (98.5 ppm) of **4**, which indicated the presence in **2** of glucose residues in the C-3 and C-20 positions and; correspondingly, that it was identical to the known ginsenoside F-2 [1, 2]. A comparison of the same spectral data of F-2 and **3** indicated that the latter contained only one glucose in the C-20 position because a strong-field shift of C-3 (78.2 ppm) in **3** was observed compared with that in **2** (88.7 ppm). Hence, **3** was the known compound K [2]. This was also confirmed by the fact that PMR and ^{13}C NMR spectra of **2** and **3** were identical to those of F-2 and compound K, respectively, that were published before [7].

An analysis of the concentration change dynamics of these compounds in the incubation medium showed that Rb_1 was biotransformed by *Sphingomonas* sp. BG 25 as follows: $Rb_1 \rightarrow Rd \rightarrow F\text{-}2 \rightarrow$ compound K. Limiting the incubation time to 72–78 h enabled **2** and **3** to be obtained simultaneously. The main product was **3** if it was increased. The results indicated that *Sphingomonas* sp. BG 25 could be used in addition to other microorganisms [3, 7] for biotransformation of Rb_1 into biologically more active ginsenoside F-2 and compound K.

3-*O*-(*β*-D-Glucopyranosyl)-20-*O*-(*β*-D-glucopyranosyl)-3*β*,12*β*,20*β*-trihydroxydammar-24-ene (2), white powder, mp 183–185°C. ^1H NMR spectrum (400 MHz, Py-d_5, δ, ppm, J/Hz): 0.81 (3H, s, CH_3-19), 0.95 (3H, s, CH_3-18), 0.96 (3H, s, CH_3-30), 1.00 (3H, s, CH_3-29), 1.30 (3H, s, CH_3-28), 1.59 (6H, s, CH_3-26, 27), 1.62 (3H, s, CH_3-21), 4.93 (1H, d, J = 7.6, H-1′), 5.18 (1H, d, J = 7.6, H-1‴) (only characteristic proton resonances are given). Mass spectrum: FAB, *m/z* 807 $[M + Na]^+$. $C_{42}H_{72}O_{13}$.

20-*O*-*β*-D-Glucopyranosyl-3*β*,12*β*,20*β*-trihydroxydammar-24-ene (3), white powder, mp 176–178°C. ^1H NMR spectrum (400 MHz, Py-d_5, δ, ppm, J/Hz): 0.85 (3H, s, CH_3-19), 0.92 (3H, s, CH_3-18), 0.96 (3H, s, CH_3-30), 1.01 (3H, s, CH_3-29), 1.21 (3H, s, CH_3-28), 1.58 (6H, s, CH_3-26, 27), 1.60 (3H, s, CH_3-21), 5.17 (1H, d, J = 7.6, H-1‴). Mass spectrum: FAB, *m/z* 645 $[M + Na]^+$. $C_{36}H_{62}O_8$.

REFERENCES

1. L. P. Christensen, *Adv. Food Nutr. Res.*, **55**, 1 (2009).
2. D. G. Popovich, C.-R. Yeo, and W. Zhang, *Int. J. Biomed. Pharm. Sci.*, **6**, 56 (2012).
3. C. S. Park, M. H. Yoo, K. H. Noh, and D. K. Oh, *Appl. Microbiol. Biotechnol.*, **87**, 9 (2010).
4. L. N. Ten, W.-T. Im, M.-K. Kim, M. S. Kang, and S.-T. Lee, *J. Microbiol. Methods*, **56**, 375 (2004).
5. L. N. Ten, W.-T. Im, M.-K. Kim, and S.-T. Lee, *Lett. Appl. Microbiol.*, **40**, 92 (2005).
6. L. N. Ten, S. M. Chae, and S.-A. Yoo, *Chem. Nat. Compd.*, **49**, 773 (2013).
7. L.-H. Quan, L.-Q. Cheng, H.-B. Kim, J.-H. Kim, N.-R. Son, S.-Y. Kim, H.-O. Jin, and D.-C. Yang, *J. Ginseng Res.*, **34**, 288 (2010).