Chemistry of Natural Compounds, Vol. 50, No. 3, July, 2014 [Russian original No. 3, May–June, 2014]

TRANSFORMATION OF GINSENOSIDE Rc INTO (20*S*)-Rg_3 BY THE BACTERIUM *Leuconostoc* sp. BG78

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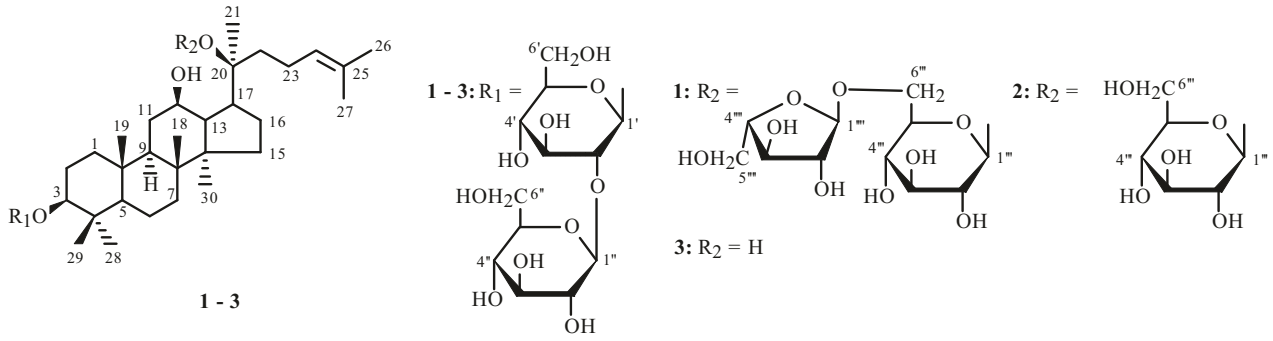
Ginsenoside (20*S*)-Rg_3 is one of the most active minor dammarane glycosides of *Panax ginseng* C. A. Meyer and exhibits a broad spectrum of biological activity including hepatoprotective, immunostimulating, neuroprotective, and antitumor. It inhibits cell growth of malignant A549 lung carcinoma, U937 lymphoma, LNCaP prostate carcinoma, and SK-HEP-1 hepatoma and is of interest in medicine as a potential antitumor agent [1]. One factor limiting the application of ginsenoside (20*S*)-Rg_3 is its low content in the total saponins of *Panax ginseng*. (20*S*)-Rg_3 differs from the principal dammarane glycosides Rb_1, Rb_2, Rc (**1**), and Rd (**2**) that have the aglycon protopanaxadiol only by the lack of a carbohydrate on C-20 [2]. This enables it to be produced by selective incomplete deglycosylation of these saponins, including by using various microorganisms [3, 4].



We reported earlier the use of several bacteria with β-glucosidase activity for transformation of ginsenoside Rb_1 into the minor glycoside F-2 and compound K [5] and Rd into the aforementioned ginsenoside (20*S*)-Rg_3 [6]. Herein we communicate data on the transformation of another principal glycoside, Rc (**1**) with a C-3 β-sophorose and a C-20 α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside, into (20*S*)-Rg_3 by the bacterium *Leuconostoc* sp. BG78.

Standard ginsenosides Rc, Rd, and (20*S*)-Rg_3 were purchased (Faces Biochemical Co., Wuhan, PRC). Glycoside **1** was isolated from six-year *P. ginseng* roots as described before [7] using in the final step preparative HPLC over an OptimaPak C18 column (250 × 10 mm, 10 μm). PMR, ^{13}C NMR, and mass spectra of isolated Rc agreed with those published for this compound [4, 7]. The Rc biotransformation products were analyzed using TLC and solvent system $CHCl_3$–MeOH–H_2O (65:35:10, lower phase) and HPLC over a Zorbax Eclipse XDB-C18 reversed-phase column. Mass spectra and NMR spectra (^1H and ^{13}C) were recorded as before [8].

Bacterium *Leuconostoc* sp. BG78 was cultivated in 200-mL Ehrlenmeyer flasks in MRS liquid growth medium for 24 h at 37°C with constant stirring. Then, cells were harvested by centrifugation (3000 g, 15 min, 4°C), rinsed twice with phosphate buffer (20 mM, pH 7.0), and suspended in the same buffer (20 mL). The resulting bacterium suspension (10 mL each) was placed into two sterile flasks and treated with freshly prepared MRS medium (40 mL) and an aqueous solution of ginsenoside Rc (50 mL, 1 mM). The first mixture was incubated for 48 h; the second, for 96 h at 37°C with constant stirring. Analytical samples were taken every 6 h. After the incubation was finished, each mixture was extracted twice with water-saturated BuOH (100 mL). The resulting extract was evaporated *in vacuo*. The residue was used to isolate the biotransformation products.

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 0009-3130/14/5003-0562

TABLE 1. ^{13}C NMR Spectra of Ginsenosides **1**, **2**, and **3** (125 MHz, δ, ppm)

C atom	**1**	**2**	**3**	C atom	**1**	**2**	**3**	C atom	**1**	**2**	**3**
1	39.2	39.6	39.1	19	16.1	16.7	16.5	1″	106.0	106.3	106.1
2	26.7	26.9	26.8	20	83.4	83.6	73.0	2″	77.1	77.3	77.2
3	89.0	89.3	89.0	21	22.4	22.7	28.0	3″	79.3	79.5	78.4
4	39.7	40.0	39.8	22	36.2	36.5	36.1	4″	71.7	72.0	71.7
5	56.4	56.7	56.4	23	23.3	23.6	23.1	5″	78.0	78.2	78.2
6	18.4	18.8	18.5	24	125.9	126.2	126.3	6″	62.7	63.2	62.7
7	35.2	35.5	35.3	25	130.9	131.1	130.8	1‴	98.0	98.5	
8	40.0	40.4	40.1	26	25.9	26.1	25.9	2‴	75.0	75.4	
9	50.2	50.6	50.4	27	18.0	18.2	17.8	3‴	78.0	78.6	
10	37.0	37.3	37.0	28	28.2	28.5	28.2	4‴	71.7	72.0	
11	30.8	31.3	32.1	29	16.8	16.9	16.7	5‴	76.5	78.5	
12	70.3	70.5	71.0	30	17.5	17.8	17.5	6‴	68.5	63.0	
13	49.5	49.6	48.7	1′	105.1	105.3	105.1	1⁗	110.0		
14	51.4	51.8	51.8	2′	83.3	83.7	83.4	2⁗	83.4		
15	30.9	31.2	31.5	3′	77.9	78.5	78.0	3⁗	78.9		
16	26.7	27.0	27.0	4′	72.1	72.0	71.8	4⁗	86.0		
17	51.6	52.0	54.9	5′	78.3	78.4	78.3	5⁗	62.7		
18	16.4	16.4	15.9	6′	62.9	63.2	62.8				

TLC and HPLC showed that the samples of the first mixture contained starting Rc (R_f 0.27) and two transformation products, **2** (R_f 0.38) and **3** (R_f 0.57). The Rc concentration decreased in proportion to the incubation time. The concentration of **2** increased, reaching a maximum after 36 h, and then decreased gradually. This was accompanied by a proportional increase in the concentration of **3**. This was clearly visible in the analyses of samples taken from the second mixture after incubation for 36 h. The R_f values on TLC and retention times in HPLC of compounds **2** and **3** corresponded to those of standard ginsenosides Rd and (20*S*)-Rg_3. Compounds **2** and **3** were isolated from the extracts obtained from the first and second mixtures, respectively, using preparative HPLC over an OptimaPak C18 column. They were identified by mass and NMR spectroscopy. The spectral properties of **2** agreed fully with those of ginsenoside Rd reported by us [8]; of **3**, with those of ginsenoside (20*S*)-Rg_3 described by us [6].

The difference in the *m/z* values for the $[M + Na]^+$ molecular ions of starting ginsenoside Rc (1101) and its transformation product **2** (*m/z* 969) [8] corresponded to an α-L-arabinofuranose residue. This indicated that it was cleaved from Rc during the biotransformation. A comparison of the ^{13}C NMR spectra of these compounds confirmed this conclusion. In particular, the ^{13}C NMR spectrum of **2** (Table 1) contained resonances for only three anomeric C atoms (98.5, 105.3, and 106.3 ppm). The resonance of C-6‴ in the C-20 carbohydrate of **2**-R_2 (63.0 ppm) underwent a strong-field shift relative to that in the **1**-R_2 residue of **1** (68.5 ppm). An analogous comparison of the spectral characteristics of **2** and **3** [6] indicated that (20*S*)-Rg_3 formed as a result of the cleavage from Rd of the C-20 glucose.

Thus, incomplete deglycosylation of **1** by *Leuconostoc* sp. BG78 involved formation of Rd and its subsequent transformation into (20*S*)-Rg_3 (70–75% yield). Rd was formed more rapidly from Rc than its transformation into (20*S*)-Rg_3. This was probably related to the more active α-L-arabinofuranosidase, which catalyzes the first step, than β-glucosidase, which is responsible for the biotransformation of Rd into (20*S*)-Rg_3.

The results indicated that *Leuconostoc* sp. BG78 could be used to transform principal glycoside Rc into the biologically more active minor ginsenoside (20*S*)-Rg_3. This distinguished it from other *Leuconostoc* bacterial strains that transform Rc into other minor dammarane glycosides [3].

3-*O*-[β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]-3β,12β,20β-trihydroxydammar-24-ene (1). $C_{53}H_{90}O_{22}$. White powder, mp 198–201°C. ^1H NMR spectrum (500 MHz, Py-d_5, δ, ppm, J/Hz): 0.79 (3H, s, CH_3-19), 0.93 (6H, s, CH_3-18, 30), 1.07 (3H, s, CH_3-29), 1.25 (3H, s, CH_3-28), 1.59 (3H, s, CH_3-26), 1.61 (3H, s, CH_3-21), 1.64 (3H, s, CH_3-27), 4.90 (1H, d, J = 7.5, H-1′), 5.11 (1H, d, J = 7.5, H-1‴), 5.35 (1H, d, J = 7.5, H-1″), 5.62 (1H, d, J = 7.2, H-1⁗) (only characteristic proton resonances are given). FAB mass spectrum: *m/z* 1101 $[M + Na]^+$.

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