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BIOTRANSFORMATION OF GINSENOSIDE Rd INTO 20(*S*)-Rg_3 BY BACTERIUM *Flavobacterium* sp. BGS36

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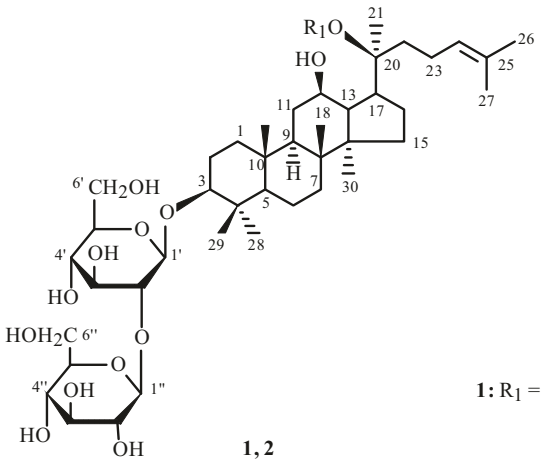
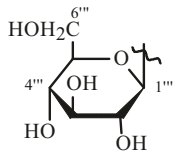
Triterpenoid glycosides, i.e., ginsenosides, are the main active principles of *Panax ginseng* C. A. Meyer and are responsible to the various pharmacological properties of this unique plant [1]. One of the most active minor constituents of *P. ginseng* is ginsenoside 20(*S*)-Rg_3, which possesses a broad spectrum of biological activity, in particular, growth inhibition of malignant A549 lung cancer cells, U937 lymphoma, LNCaP prostate carcinoma, and SK-HEP-1 hepatoma. It is viewed as a potential anticancer drug among *P. ginseng* saponins [2]. 20(*S*)-Rg_3 differs from the principal ginseng glycosides Rb_1, Rb_2, Rc, and Rd, which have protopanaxadiol as the aglycon, only by the lack of a carbohydrate group on C-20 [1]. This enables it to be prepared by selective incomplete deglycosylation of these saponins, including the use of various microorganisms [3].

We communicated earlier the isolation from soil samples taken from a ginseng field of several bacteria with β-glucosidase activity [4] and the use of several of them to convert ginsenosides Rb_1 into Rd [5] and Rd into the minor glycoside F-2 and compound K [6]. In continuation of these studies, we present data on the biotransformation of Rd (**1**), which has β-sophorose and β-D-glucose residues in the C-3 and C-20 positions, respectively, of 20(*S*)-protopanaxadiol, into ginsenoside 20(*S*)-Rg_3 (**2**) using the bacterium *Flavobacterium* sp. BGS36.



1, 2

1: R_1 = 

2: R_1 = H

Standard ginsenosides Rd (**1**) and 20(*S*)-Rg_3 were purchased (Faces Biochemical Co., Wuhan, PRC). The preparation of Rd for deglycosylation experiments; the analysis of its biotransformation products using TLC and $CHCl_3$:MeOH:H_2O (65:35:10, lower phase) and HPLC on a Zorbax Eclipse XDB-C18 reversed-phase column, and the recording of mass spectra and PMR and ^{13}C NMR spectra were carried out as previously described [5].

The bacterium *Flavobacterium* sp. BGS36 was cultivated in Ehrlenmeyer flasks in R2A liquid medium (200 mL) for 24 h at 30°C with constant stirring. Then, bacterial suspension (100 mL) with cell concentration 6×10^6 CFU/mL (colony-forming units/mL) was mixed with an aqueous solution of Rd (100 mL, 1 mM). The mixture was incubated for 24 h at 30°C with constant stirring. Analytical samples were taken every 3 h. When the incubation was finished, the mixture was extracted with water-saturated BuOH (2×200 mL). The resulting extract was evaporated in *vacuo*. The residue was used to isolate the biotransformation product. TLC and HPLC showed the presence in the collected samples of starting Rd (R_f 0.38) and its transformation product **2** (R_f 0.57). Thus, the concentration of the first glycoside decreased whereas that of the second increased in proportion to the incubation time.

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TABLE 1. ^{13}C NMR Spectra of Ginsenosides Rd (**1**) [5], **2**, and 20(*S*)-Rg_3 [7], δ, ppm*

C atom	**1**	**2**	20(*S*)-Rg_3	C atom	**1**	**2**	20(*S*)-Rg_3
1	39.6	39.1	39.2	25	131.1	130.8	130.8
2	26.9	26.8	26.8	26	26.1	25.9	25.9
3	89.3	89.0	89.0	27	18.2	17.8	17.1
4	40.0	39.8	39.8	28	28.5	28.2	28.2
5	56.7	56.4	56.5	29	16.9	16.7	16.7
6	18.8	18.5	18.5	30	17.8	17.5	17.8
7	35.5	35.3	35.3	1′	105.3	105.1	105.2
8	40.4	40.1	40.1	2′	83.7	83.4	83.5
9	50.6	50.4	50.5	3′	78.5	78.0	78.0
10	37.3	37.0	37.0	4′	72.0	71.8	71.8
11	31.3	32.1	32.1	5′	78.4	78.3	78.3
12	70.5	71.0	71.0	6′	63.2	62.8	62.9
13	49.6	48.7	48.7	1″	106.3	106.1	106.1
14	51.8	51.8	51.8	2″	77.3	77.2	77.2
15	31.2	31.5	31.4	3″	79.5	78.4	78.4
16	27.0	27.0	26.9	4″	72.0	71.7	71.7
17	52.0	54.9	54.9	5″	78.2	78.2	78.2
18	16.4	15.9	15.9	6″	63.2	62.7	62.8
19	16.7	16.5	16.4	1‴	98.5		
20	83.6	73.0	73.0	2‴	75.4		
21	22.7	28.0	28.2	3‴	78.6		
22	36.5	36.1	36.0	4‴	72.0		
23	23.6	23.1	23.1	5‴	78.5		
24	126.2	126.3	126.4	6‴	63.0		

*20(*R*)-Rg_3 [7]: δ_C 50.7 (C-17), 22.8 (C-21), and 43.3 (C-22).

Compound **2** corresponded to standard ginsenoside 20(*S*)-Rg_3 according to TLC R_f value and HPLC retention time. Compound **2** was isolated from the obtained extract by preparative HPLC over an OptimaPak C18 column (250 × 10 mm, particle size 10 μm) and identified using mass and NMR spectroscopy in order to confirm this.

The difference in the *m*/*z* values of the $[M + Na]^+$ molecular ions of starting Rd (*m*/*z* 969) [5] and its transformation product **2** (*m*/*z* 807) corresponded to a glucose residue. This indicated that it was cleaved from Rd during the biotransformation. A comparison of the PMR and ^{13}C NMR spectra of these compounds provided evidence that this cleavage occurred at the C-20 glycoside of Rd. In fact, the ^{13}C NMR spectrum of **2** contained resonances for only two anomeric C atoms (105.1 and 106.1 ppm), the chemical shifts of which were similar to those for the C-3 β-sophorose of Rd (Table 1). Also, C-20 underwent a strong-field shift in **2** (73.0 ppm) compared with that in Rd (83.6 ppm), indicating that C-20 in **2** lacked a carbohydrate component. The presence in the PMR spectrum of **2** of resonances for only two anomeric protons at 4.90 and 5.35 ppm and their agreement with those of the β-sophorose residue in Rd [5] also confirmed the aforementioned conclusion. Therefore, the Rd biotransformation product was the known triterpenoid glycoside Rg_3, which had a β-sophorose residue in the C-3 position, like Rd, but differed from it by the lack of a glucose on C-20 [1]. This ginsenoside is known to be capable of existing as two different stereoisomers, 20(*S*)-Rg_3 and 20(*R*)-Rg_3, the ^{13}C NMR spectra of which, despite their similarity, have noticeable differences in the chemical shifts of the C-17, C-21, and C-22 resonances [7, 8].

Table 1 shows that these C atoms in the ^{13}C NMR spectrum of **2** had chemical shifts of 54.9, 28.0, and 36.1 ppm, respectively, which indicated unambiguously that it was identical to 20(*S*)-Rg_3. Obviously, incomplete deglycosylation of **1** by bacterium *Flavobacterium* sp. BGS36 occurred stereospecifically to cleave the β-D-glucose on C-20, leading to the formation of only one stereoisomer, 20(*S*)-Rg_3. An analogous phenomenon was observed during enzymatic transformation of ginsenosides Rb_1 and Rd into 20(*S*)-Rg_3 through the action of recombinant β-glucosidase from *Microbacterium esteraromaticum* [3].

The results indicated that *Flavobacterium* sp. BGS36 can be used for biotransformation of one of the principal ginseng glycosides Rd into the biologically more active minor ginsenoside 20(*S*)-Rg_3.

3-*O*-[β-D-Glucopyranosyl(1→2)-β-D-glucopyranosyl]-3β,12β,20β-trihydroxydammar-24-ene (2). $C_{42}H_{72}O_{13}$. Yield 84%, white powder, mp 292–294°C. ^{1}H NMR (500 MHz, Py-d_5, δ, ppm, J/Hz): 0.79 (3H, s, CH_3-19), 0.94 (3H, s,

CH_3-30), 0.95 (3H, s, CH_3-18), 1.08 (3H, s, CH_3-29), 1.27 (3H, s, CH_3-28), 1.42 (3H, s, CH_3-21), 1.61 (3H, s, CH_3-27), 1.64 (3H, s, CH_3-26), 4.90 (1H, d, J = 7.0, H-1′), 5.35 (1H, d, J = 7.4, H-1″) (only characteristic proton resonances are given). Mass spectrum: FAB, *m*/*z* 807 $[M + Na]^+$.

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