Chinese Chemical Letters Vol. 16, No. 3, pp 359-361, 2005
http://www.imm.ac.cn/journal/ccl.html

A New Saponin Transformed from Ginsenoside Rh_1 by *Bacillus subtilis*

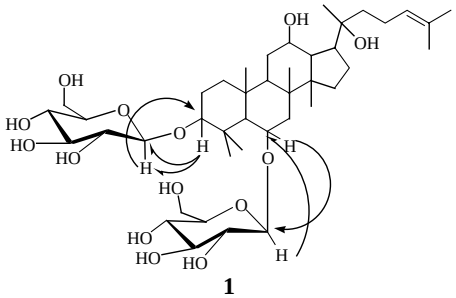
Guo Hong LI[1], Yue Mao SHEN[2], Ke Qin ZHANG[1]*

[1] Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming 650091
[2]The State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204

Abstract: A novel saponin was isolated from the transformed products of ginsenoside Rh_1 by *Bacillus subtilis*. It's structure was determined to be 3-*O*-β-D-glucopyranosyl-6-*O*-β-D-glucopyranosyl-20 (*S*)-protopanaxatriol on the basis of the spectral data.

Keywords: Ginsenoside Rh_1, novel saponin, biotransformation.

Biotransformation is an efficient way to produce new structural products. In our experiments, ginsenoside Rh_1 was biotransformed by *Bacillus subtilis* and a new triterpene saponin, 3-*O*-β-D-glucopyranosyl-6-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxatriol (**1**) was isolated from the transformed products. The saponin was linked with two β-D-glucopyranosyls at C-3 and C-6.



1

Experimental

Bacillus subtilis was inoculated in 250 mL flask contained 100 mL wort medium. 120 mg ginsenoside Rh_1 was dissolved in ethanol and equally accede to three flasks when the strain was inoculated in, then the disposal was cultured at 30 °C for 96 hours. The

* E-mail: Kqzhang1@yahoo.com.cn

Table 1 The NMR data of compound **1** in C_5D_5N (δ ppm)

Position	δ_C	δ_H	HMBC	^1H-^1H COSY
1	39.4	0.99, s	C-9, C-5, C-4	H-1, H-2
		1.65, s	C-19, C-2	H-1, H-2
2	27.9	1.90, d, 10.8	C-4	H-1, H-2
		1.85, d, 9.4		H-1, H-2
3	89.2	4.23, dd, 9.0, 15.2	C-5, C-1′ (w)	/
4	40.3	/	/	/
5	61.5	1.40, m	C-19, C-3	H-6
6	80.3	4.36, m	C-1″	H-5, H-7
7	45.3	2.46, d, 12.4	C-18, C-9, C-8, C-5	H-7,
		1.90, s	C-18, C-9(w), C-8, C-5	H-5, H-7
8	41.2	/		/
9	50.2	1.59, s	C-11, C-8	H-11
10	39.7	/	/	/
11	32.1	2.29, m	C-13(w), C-12	H-9, H-12
12	71.1	3.91, m		H-13
13	48.3	2.02, s	C-30, C-16(w) ,C-14,C-12	H-12
14	51.7	/	/	/
15	31.3	1.63, s	C-30, C-16	H-16
		1.02, s	C-30, C-14	
16	26.9	1.35, s	C-20, C-17, C-14	H-17, H-15
		1.77, s		H-15, H-17
17	54.8	2.28, m	C-15, C-13	H-16, H-15
18	17.4	1.16, s	C-30, C-9, C-8, C-5(w)	/
19	17.7	1.05, s	C-9, C-5, C-1	/
20	73.0	/	/	/
21	27.1	1.39, s	C-22, C-20, C-17	/
22	35.9	2.02, m	C-21, C-20, C-17	/
		1.68, m	C-21	
23	23.0	/	/	
24	126.4	5.32, m	C-27, C-26, C-23	/
25	130.8	/	/	/
26	25.8	1.56, s	C-27, C-25, C-24	/
27	17.6	1.50, s	C-26 (w)	/
28	31.7	2.02, s	C-19, C-5, C-4	/
29	16.3	1.52, s	C-28, C-5, C-4, C-2	/
30	16.8	0.78, s	C-15, C-14, C-13, C-8	
	3-glc			
1′	105.8	4.94, d, 7.7	C-3, C-3′, C-4′, C-5′	H-2′
2′	75.6	4.13, m	C-1′, C-3	H-3′, H-1′
3′	80.3	4.36, m	C-1′, C-6′	H-2′, H-4′
4′	72.0	3.91, m	C-1′	H-3′
5′	78.6	3.49	C-3′	
6′	62.5	4.47, m	/	H-6′
		4.32, m		H-6′
	6-glc			
1″	103.1	5.91, d, 8.0	C-3″	H-2″
2″	74.8	4.18, m	C-1″, C-3″	H-1″
3″	77.6	3.87, m	/	/
4″	71.0	4.21	C-1″, C-2″, C-6″	/
5″	77.6	3.87 m	/	
6″	62.6	4.47, m	/	H-6″
		4.32, m		H-6″

Note: The 1D-NMR data for compound **1** was recorded on Bruker AM-400(400 MHz), and 2D-NMR data for compound **1** was recorded on Bruker DRX-500(500 MHz), respectively.

cultures of *Bacillus subtilis* containing saponin were combined (300 mL) and filtered. The filtrate was exhaustively extracted four times with *n*-butanol and 4.5 g transformational residue of ginsenoside Rh_1 (TRh) was obtained from the filtrate. Fraction TRh was chromatographied on a silica gel (12 g) column and eluted with chloroform and methanol (6:1, v/v) and further subjected on Sephadex LH-20 eluting with methanol to obtain compound **1** (5 mg).

Compound **1**, the negative HRFABMS determined the molecular formula to be $C_{42}H_{72}0_{14}$ (*m/z* 799.4846 $[M - H]^-$, calcd. 799.4844), which was 162 amu greater than its substrate ginsenoside Rh_1. The data of ^1H- and ^{13}C-NMR showed compound **1** had two proton signals at δ 4.94 (d, J = 7.7 Hz), δ 5.91 (d, J = 8.0 Hz) and two methine carbon signals at δ 105.8、δ 103.1, and two terminal carbons at δ 62.5 and 62.6, hence one could speculate that compound **1** contained two β-glucopyranosyls. Compared with the spectral data[1] of the ginsenoside Rh_1, the C-3 chemical shift of compound **1** was obviously shifted to low field. Therefore the additional β-glucopyranosyl might be linked at C-3. The HMBC experiments showed the ^1H-^{13}C long-range correlations between the methine protons at δ 4.23 (H-3) and δ 105.8 (C-3-glc-1″), δ 4.63 (H-6) and δ 103.1 (C-6-glc-1′), δ 5.91 (H-6-glc-1′) and δ 80.3 (C-6), and between the methine protons at δ 4.94 (H-3-glc-1″) and δ 89.2 (C-3). The ROESY experiments showed ^1H-^1H correlation between the protons at δ 4.23 (H-3) and δ 4.94 (H-3-glc-1″). In comparison with the substrate ginsenoside Rh_1, compound **1** had additional β-glucopyranosyl linked with C-3, so its structure was determined to be 3-*O*-β-D-glucopyranosyl-6-*O*-β-D-glucopyranosyl-20 (*S*)-protopanaxatriol.

Compound **1**: white power, $[\alpha]_D^{22}$ +180 (*c* 0.1, CH_3OH), FAB-MS^- *m/z*: 799 ($[M-H]^-$, 100), 637 ($[M – H - 162]^-$, 6), 475($[M – H - 2×162]^-$, 14); IR (KBr) ν 3414, 2958, 1633, 1455, 1146, 1077, 1031 cm^{-1}. The ^1H- and ^{13}C-NMR spectral data see **Table 1.**

Acknowledgments

The authors gratefully acknowledge support for this work of the National Natural Science Foundation (No. 20362010) and are grateful to Mr. Y. N. He and Ms. H. L. Liang in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring NMR and MS data, respectively.

Reference

1. J. Zhou, M. Z. WU, S. Taniyasu, H. Besso *et al*., *Chem*. *Pharm*. *Bull*., **1981,** *29* (10), 2844.

Received 18 February, 2004