

# Recent Studies on the Chemistry of Ginseng Saponins

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The isolation and the structure-determination of physiologically active dammarane-type saponins of ginseng roots have been established; ginsenosides-Rb<sub>1</sub>, -Rb<sub>2</sub>, -Rc, Rd [common sapogenin: 20(S)-protopanaxadiol(1)] and -Re, -Rf, -Rg<sub>1</sub> [common sapogenin: 20(S)-protopanaxatriol(2)]<sup>1,2)</sup>. In the search for cheaper materials for production of these saponins, we have investigated chemical constituents of aerial parts of ginseng and its related *Panax* spp.<sup>3,4,5)</sup>

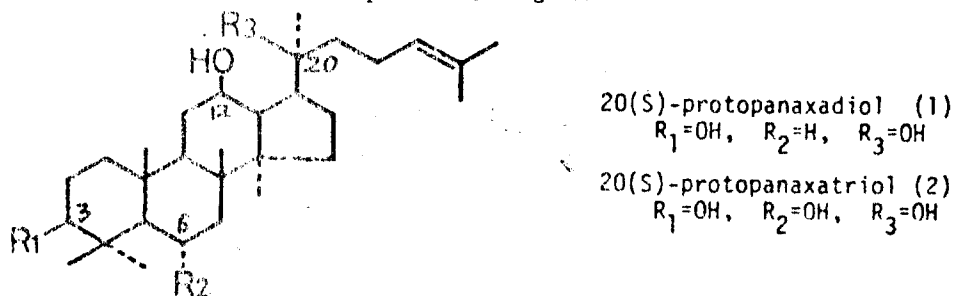
In connection with this study, several modern procedures for structure-elucidation and identification of saponins of this type have been developed in our laboratory.

## 1. Enzymatic Hydrolysis

It has been reported by our research group that the dammarane-type aglycones of ginseng saponins are very unstable to acid treatment, resulting in epimerization of C-20-OH, ring closure of the side chain(formation of tetrahydropyran ring), and dehydration etc.<sup>6)</sup> Accordingly, in order to obtain the genuine aglycones, mild hydrolysis such as Smith's degradation, deMayo's degradation<sup>1,2)</sup> and hydrolysis by soil bacteria were used formerly. Since most of these procedures are troublesome, low-yield, and not always effective, we have searched for more efficient method for hydrolysis of the saponins. Kohda found that crude preparation of hesperidinase hydrolyzed these

Chart 1.

Saponins of Ginseng Roots

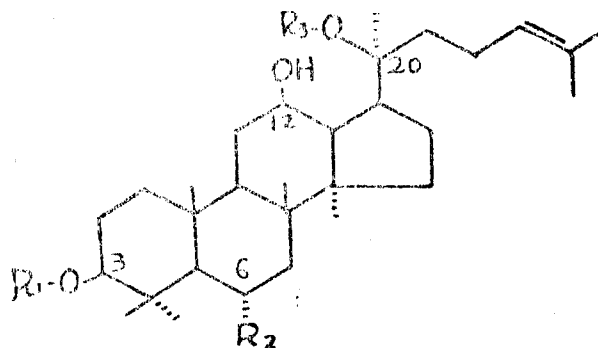


	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
ginsenoside Rd	-0-glc <sup>2</sup> -glc	-H	-0-glc
" Rb <sub>1</sub>	-0-glc <sup>2</sup> -glc	-H	-0-glc <sup>6</sup> -glc
" Rb <sub>2</sub>	-0-glc <sup>2</sup> -glc	-H	-0-glc <sup>6</sup> -ara(pyr.)
" Rc	-0-glc <sup>2</sup> -glc	-H	-0-glc <sup>6</sup> -ara(fur.)
" Re	-OH	-0-glc <sup>2</sup> -rha	-0-glc
" Rf	-OH	-0-glc <sup>2</sup> -glc	-OH
" Rg <sub>1</sub>	-OH	-0-glc	-O-glc
" Rg <sub>2</sub>	-OH	-0-glc <sup>2</sup> -rha	-OH

glc: β-O-glucopyranose rha: α-L-rhamnopyranose ara: α-L-arabinose

### Saponins of Ginseng Leaves

(ginsenosides-F<sub>1</sub>, -F<sub>2</sub>, -F<sub>3</sub>)



F<sub>1</sub>: R<sub>1</sub>=H, R<sub>2</sub>=OH, R<sub>3</sub>=β-glc

F<sub>2</sub>: R<sub>1</sub>=β-glc, R<sub>2</sub>=H, R<sub>3</sub>=β-glc

F<sub>3</sub>: R<sub>1</sub>=H, R<sub>2</sub>=OH, R<sub>3</sub>=β-glc-<sup>6</sup>-<sup>1</sup>B-arabinopyranose

saponins effectively: ginsenosides-Rb<sub>1</sub>, -Rb<sub>2</sub>, -Rc, and -Rd → 20-0-β-glucosyl-20(S)-protopanaxadiol(compound k) and -Re, -Rf, -Rg<sub>1</sub>, -Rg<sub>2</sub> → 20(S)-propanaxatriol(2)<sup>7)</sup>. This crude enzyme is now used extensively for micro-identification and structure determination of saponins.

## 2. Mass Spectrometry

Komori(Kyushu University) in co-operation with our research group reported the mass spectrometry of some of the acetylated ginseng saponins<sup>8)</sup>. Recently, Kasai *et al.* of our group investigated the mass spectrometry of pertrimethylsilylated ginseng sapogenins and saponins. Complete trimethylsilylation(even sterically hindered 20-OH) was achieved by the action of trimethylsilylimidazole. The mass spectra of trimethylsilylated 1 and 2 exhibited characteristic fragmentations around the C-20 as shown in Fig. 1, which are diagnostic for structure determination of the side chain.

In references to the mass spectrometry of trimethylsilylated oligosaccharides, the mass spectra of trimethylsilylated ginseng saponins were also analyzed.<sup>9)</sup> (see Fig. 2). Recently, gas chromatographic and mass spectrometric analysis of trimethylsilylated ginseng saponins was reported also by Bombardelli *et al.*<sup>10)</sup>

### 3. <sup>13</sup>C Nuclear Magnetic Resonance Spectroscopy

<sup>13</sup>C NMR spectroscopy has been expected to offer excellent advantage over all other spectroscopic and chemical procedures for structure elucidation and identification of ginseng saponins. In our laboratory, Asakawa, Kasai and Yamasaki have explored the assignments of carbon signals of 1,2, and their related dammarane-type triterpenes as shown in Table I.<sup>11)</sup> Further, Kasai and Yamasaki *et al.* have investigated <sup>13</sup>C NMR of numerous type aliphatic glycosides, establishing the regularities of chemical shift displacements of both sugar and aglycone moieties on glycosylation<sup>12)</sup>. It should be noted that by <sup>13</sup>C NMR, structure of genuine aglycone including stereochemistry and the location of glycoside-linkage to the aglycone can readily be determined without any loss of the sample. Furthermore, the structure of sugar moiety including the configuration of the anomeric centre can be elucidated by <sup>13</sup>C NMR with the aid of the enzymatic hydrolysis, mass spectrometry, and <sup>1</sup>H NMR.

### 4. Isolation and Structure-Determination of Saponins of Aerial Parts of *Panax* spp.

Takagi *et al.* reported the physiological activities of the glycoside fraction of the leaves of *Panax ginseng*.<sup>13)</sup> Recently, Yahara *et al.*<sup>3)</sup> isolated the known saponins, ginsenosides-Rb<sub>1</sub>(0.4%), -Rc(0.2%), -Rd(1.2%), -Re(1.2%), and -Rg<sub>1</sub>(1.2%) from the leaves of this plant. Besides these root-saponins, there were isolated new saponins named ginsenosides-F<sub>1</sub>(0.4%), -F<sub>2</sub>(0.2%), and -F<sub>3</sub>(0.2%), the structures of which were established by the modern procedures mentioned above. From the flower-buds of *Panax ginseng*, Matsuura and Yahara isolated the known saponins, ginsenosides-Rb<sub>1</sub>(0.2%), -Rb<sub>2</sub>(0.2%), -Rc(0.2), -Rd(0.5%), -Re(2.8%), and -Rg<sub>1</sub>(0.3%).<sup>4)</sup> The new saponins of the flower-buds were also isolated and the structure elucidation are under progress. The high contents of the saponins, especially ginsenosides-Rd, -Re, and -Rg<sub>1</sub> in the leaves and the flower-buds indicate the significance of the aerial parts as important medicinal sources.

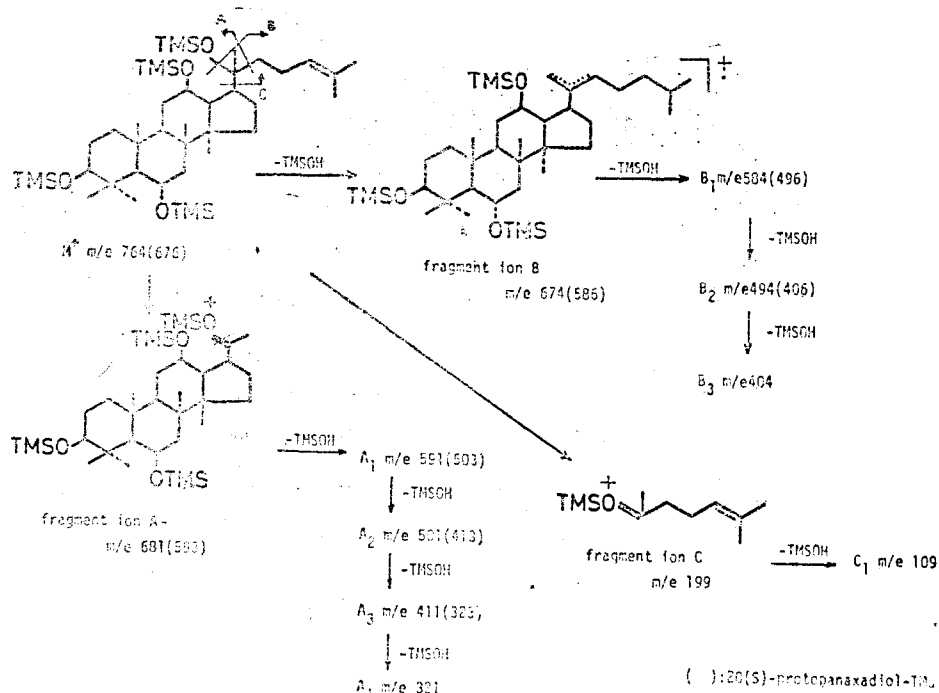


Fig. 1. Fragmentation pathway of 20(S)-potopanaxadiol-TMS and 20(S)-protopanaxatriol-TMS

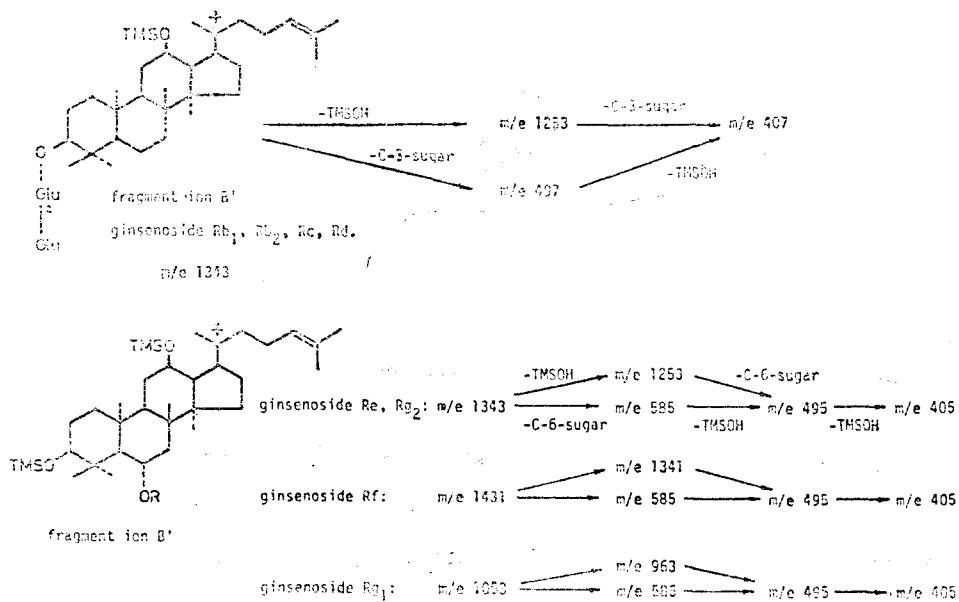


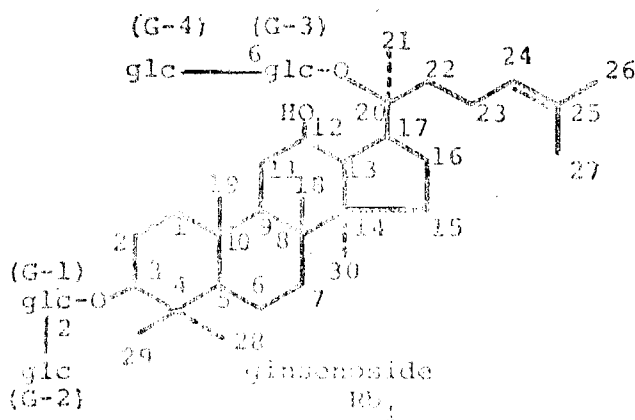
Fig. 2. Fragmentation pathway of per-O-trimethylsilylated ginsenosides (B series)

**Table 1.** Chemical shift and assignment for CMR spectra of ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re and Rg<sub>1</sub> in C<sub>5</sub> D<sub>5</sub> N

Compound	ppd	Rb <sub>1</sub>	Rb <sub>2</sub>	Rc	Rd	ppt	Re	Rg <sub>1</sub>
Carbon								
1	39.5	39.1 (-0.4)	39.6 (+0.1)	39.0 (-0.5)	39.1 (-0.4)	39.3	39.4 (+0.1)	39.5 (+0.2)
2	28.2	26.6 (-1.6)	26.6 (-1.6)	26.6 (-1.6)	26.7 (-1.5)	28.0	27.4 (-0.6)	27.6 (-0.4)
3	77.9	89.2 (+11.3)	89.1 (+11.2)	89.0 (+11.1)	88.9 (11.0)	78.3	78.7 (+0.4)	78.6 (+0.3)
4	39.5	39.6 (+0.1)	39.6 (+0.1)	39.6 (+0.1)	39.6 (+0.1)	40.2	39.8 (-0.4)	40.1 (-0.1)
5	56.3	56.3 ( 0 )	56.4 (+0.1)	56.3 ( 0 )	56.4 (+0.1)	61.7	60.7 (-1.0)	61.3 (-0.4)
6	18.7	18.6 (-0.1)	18.3 (-0.4)	18.3 (-0.4)	18.5 (-0.2)	67.6	74.6 (+7.0)	77.8 (+10.2)
7	35.2	35.1 (-0.1)	35.1 (-0.1)	35.1 (-0.1)	35.2 ( 0 )	47.4	45.7 (-1.7)	44.9 (-2.5)
8	40.0	39.9 (-0.1)	39.9 (-0.1)	39.9 (-0.1)	40.0 ( 0 )	41.1	41.0 (-0.1)	41.0 (-0.1)
9	50.4	50.1 (-0.3)	50.1 (-0.3)	50.1 (-0.3)	50.2 (-0.2)	50.1	49.4 (-0.7)	49.9 (-0.2)
10	37.3	36.8 (-0.5)	36.8 (-0.5)	36.8 (-0.5)	36.9 (-0.4)	39.3	39.4 (+0.1)	39.5 (+0.2)
11	32.0	30.8 (-1.2)	30.7 (-1.3)	30.7 (-1.3)	30.8 (-1.2)	31.9	30.6 (-1.3)	30.8 (-1.1)
12	70.9	70.1 (-0.8)	70.1 (-0.8)	70.2 (-0.7)	70.2 (-0.7)	70.9	70.4 (-0.5)	70.3 (-0.4)
13	48.5	49.2 (+0.8)	49.4 (+0.9)	49.2 (+0.7)	49.4 (+1.1)	48.1	48.8 (+0.7)	48.9 (+0.8)
14	51.6	51.3 (-0.3)	51.3 (-0.3)	51.3 (-0.3)	51.4 (-0.2)	51.6	51.3 (-0.3)	51.3 (-0.3)
15	31.3	30.8 (-0.5)	30.8 (-0.6)	30.7 (-0.6)	30.8 (-0.5)	31.3	30.6 (-0.7)	30.6 (-0.7)
16	26.8	26.6 (-0.2)	26.4 (-0.4)	26.6 (-0.2)	26.7 (-0.1)	26.8	26.5 (-0.3)	26.4 (-0.4)
17	54.7	51.6 (-3.1)	51.6 (-3.1)	51.6 (-3.1)	51.7 (-3.0)	54.6	51.8 (-2.8)	51.6 (-3.0)
18	16.2 <sup>a</sup>	16.2 <sup>a</sup> ( 0 )	16.2 <sup>a</sup> ( 0 )	16.2 <sup>a</sup> ( 0 )	16.2 (-0.1)	17.5 <sup>a</sup>	17.4 <sup>a</sup> (-0.1)	17.4 <sup>a</sup> (-0.1)
19	15.8 <sup>a</sup>	15.9 (+0.1)	15.9 (+0.1)	15.9 (+0.1)	15.8 <sup>a</sup> ( 0 )	17.4 <sup>a</sup>	17.4 <sup>a</sup> ( 0 )	17.4 ( 0 )
20	72.0	83.5 (+10.6)	83.5 (+10.6)	83.1 (+10.2)	83.4 (+10.5)	72.9	83.2 (+10.3)	83.3 (+10.4)
21	26.9	22.6 (-4.3)	22.2 (-4.7)	22.2 (-4.7)	22.4 (-4.5)	26.9	22.4 (-4.5)	22.3 (-4.6)
22	35.8	36.1 (+0.3)	36.3 (+0.5)	36.0 (+0.2)	36.0 (+0.2)	35.7	35.8 (+0.1)	35.9 (+0.2)
23	22.9	23.1 (+0.2)	23.1 (+0.2)	23.1 (+0.2)	23.2 (+0.3)	22.9	23.3 (+0.4)	23.2 (+0.3)
24	126.2	125.8 (-0.4)	125.8 (-0.4)	125.9 (-0.3)	125.9 (-0.3)	126.2	125.7 (-0.5)	125.8 (0.4)
25	130.6	131.0 (+0.4)	131.0 (+0.4)	130.9 (+0.3)	130.9 (+0.3)	130.6	130.9 (+0.3)	130.9 (+0.3)
26	25.8	25.8 ( 0 )	25.8 ( 0 )	25.7 (-0.1)	25.8 ( 0 )	25.8	25.7 (-0.1)	25.7 (-0.1)
27	17.6	17.9 (+0.3)	17.9 (+0.3)	17.8 (+0.2)	17.2 (+0.2)	17.7	17.9 (+0.2)	17.9 (+0.2)
28	28.6	28.0 (-0.6)	28.0 (-0.6)	28.0 (-0.6)	28.9 (-0.6)	31.9	32.0 (+0.1)	31.6 (-0.3)

compound	ppd	Rb <sub>1</sub>	Rb <sub>2</sub>	Rc	Rd	ppt	Re	Rg <sub>1</sub>
29	16.4 <sup>a</sup>	16.5 <sup>a</sup> (+0.1)	16.5 <sup>a</sup> (+0.1)	16.5 <sup>a</sup> (+0.1)	16.5 <sup>a</sup> (+0.1)	16.4 <sup>a</sup>	16.5 <sup>a</sup> (+0.1)	16.5 <sup>a</sup> (+0.1)
30	17.0	17.3 (+0.3)	17.3 (+0.3)	17.3 (+0.3)	17.2 (+0.2)	17.0	17.4 (+0.4)	17.4 (+0.4)
anomeric C								
G-1		105.6 (+0.1)	105.7 (+0.2)	105.6 (+0.1)	105.5 ( 0 )			
G-2		105.0 (-0.5)	105.0 (-0.5)	104.9 (-0.6)	104.9 (-0.6)			
G-3		97.9 (-7.6)	97.9 (-7.6)	97.9 (-7.6)	98.1 (-7.4)		98.1 (-7.4)	98.1 (-7.4)
G-4		105.0 (-0.5)						
G-5							101.6 (-3.9)	105.7 (+0.2)
A-1(pyr.)			104.5					
A-2(fur.)				109.9				
R-1							101.6 (-0.8)	

ppd: 20(S)-protopanaxadiol. ppt: 20(S)-protopanaxatriol, a: These signals may be reversed on vertical column.



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#### Other Works on Ginseng Saponins

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