

Hemolytic and Its Protective Activity of Ginseng Saponins

Ginseng is one of the most important Chinese drugs which has widely been used from the Han Dynasty in a traditional Chinese medicine. Main effective component of ginseng has recently been considered to be saponins according to the chemical studies by Shibata's group¹⁾ and the pharmacological studies by Takagi, *et al.*,²⁾ and also through the studies of Elyakov, *et al.*,³⁾ Brekhman, *et al.*⁴⁾ and Petkov.⁵⁾

It has been reported that ginseng saponins showed no hemolytic activity by Yonekawa,⁶⁾ using crude saponin preparation and by Kotake,⁷⁾ using partially purified saponins. In the present study, we discovered that some fractions of ginseng saponins had potent hemolytic activity and the others had protective activity against the hemolysis by the former fractions and other hemolytic reagents.

Total saponin was prepared from lateral root of ginseng cultivated in Korea. The saponin was subjected to the silica gel column and fractionated with the solvent system, ethyl acetate saturated with water-methanol. As is shown in Fig. 1, the fractions I—IX were analysed by thin-layer chromatography (TLC) and each spot of these chromatograms was identified by the analyses of products of acid hydrolysis and by the comparison of *R_f* values with those of the authentic samples which are kindly provided by Shibata and Shoji.

Hemolytic activity was measured according to the method of Roth, *et al.*⁸⁾ using human red cells in place of sheep ones. Protective activity of saponin was measured against hemolysis by the reagents such as saponaria saponin (Merck), deoxycholate, lecithin and lysolecithin which was prepared from yolk lecithin by hydrolyzing it with phospholipase A of snake venom according to the method of Wakui, *et al.*⁹⁾

Hemolytic activity and protective activity against saponaria saponin- and lecithin-hemolysis of ginseng saponin fractions (Fr. I—IX) were measured, as is shown in Table I. Total saponin had no hemolytic activity. However, Fr. I (Rh and Rg) had almost the same potent hemolytic activity (25 µg/ml) as saponaria saponin which is well known as one of the

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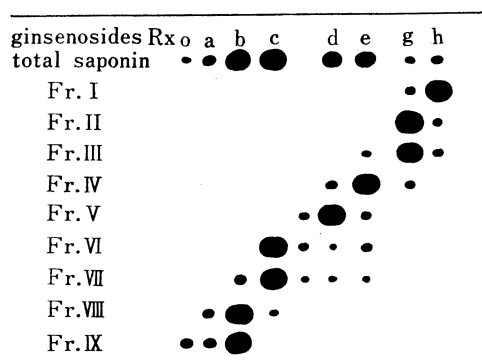


Fig. 1. Thin-layer Chromatogram of Ginseng Saponin

Shibata *et al.*¹⁰ designated the ginseng saponin as ginsenosides Rx ($x=0, a, b, \dots, h$) according to the sequence of *Rf* values from lower to higher on TLC.

TLC was carried out on silica gel with *n*-butanol-acetic acid-water (4:1:5, upper layer) as solvent. Another solvent system, chloroform-methanol-water (65:35:10, lower layer), was used for distinguishing ginsenoside Rb₁ from Rb₂.

strongest hemolytic reagent. Hemolytic activities of Fr. II, III, IV, and V were observed in descending order and no activities of Fr. VI, VII, and VIII were found. Fr. IX was slightly hemolytic, which is probably due to the hemolytic activity of the contaminated ginsenoside Ro¹⁰ (sapogenin: oleanolic acid).

With regard to the protective activity of ginseng saponin against saponaria saponin and lecithin-hemolysis, total saponin showed a weak activity, but Fr. VI, VII, and VIII showed marked protective activity. No protective activity was observed in the hemolytic fractions, Fr. I—V. Hemolysis by lysolecithin (19 $\mu\text{g/ml}$) was protected by Fr. VII (main component: Rc and Rb₂), one of the protective fractions, in a final concentration of 180 $\mu\text{g/ml}$ (P_{50}), but hemolysis by deoxycholate (570 $\mu\text{g/ml}$) was not affected by this fraction. Hemolysis by Fr. I (25

$\mu\text{g/ml}$) was protected by Fr. VII in a final concentration of 156 $\mu\text{g/ml}$ (P_{50}).

By the treatment of acid hydrolysis, hemolytic fractions (Fr. I—IV) gave panaxatriol and protective fractions (Fr. VI—VIII) gave panaxadiol. In so far as ginseng saponin is concerned, the saponins, whose genuine sapogenins are 20-S-protopanaxatriol, were found to

TABLE I. Hemolytic and Protective Activity of Fractions

Fractions	Hemolytic dose (HD_{50} : $\mu\text{g/ml}$)	Protective dose (P_{50}) against ($\mu\text{g/ml}$)	
		Lecithin ^{a)}	Saponaria saponin ^{b)}
Saponaria saponin	10	no activity	—
Total saponin	no activity ^{c)}	290	2600
Fr. I	25	no activity	no activity
Fr. II	63	no activity	no activity
Fr. III	118	no activity	no activity
Fr. IV	215	—	—
Fr. V	800	no activity	no activity
Fr. VI	no activity ^{c)}	58	545
Fr. VII	no activity ^{c)}	42	440
Fr. VIII	no activity ^{c)}	62	305
Fr. IX	2500	79	1600

Hemolytic dose (HD_{50}): The final concentration of the sample which gives 50% hemolysis, based on the absorbance at 550 $m\mu$ when complete hemolysis was caused by the excess addition of saponaria saponin (Merck, 170 $\mu\text{g/ml}$).

Protective dose (P_{50}): To the sample solution of various concentrations was added the solution of such a hemolytic reagent as lecithin or saponaria saponin whose amount was HD_{50} in a final concentration and its hemolytic activity was measured under the same condition as that of HD_{50} measurement, except that the time of incubation was 80 min in the case of lecithin-hemolysis. Based on the absorbance at 550 $m\mu$ when the ginseng saponins were omitted as a control, the protective activities of the samples were estimated by measuring the final concentration (P_{50}) which gives 50% protection of the control hemolysis.

Concentration of a) lecithin and b) saponaria saponin was $3.3 \times 10^{-4}M$ and 10 $\mu\text{g/ml}$, respectively. c) Activity was not detected with saturated solution of the sample.

be hemolytic and the saponins, whose genuine saponins are 20-S-protopanaxadiol, were protective from the hemolysis by the former saponins and by other hemolytic reagents. Panaxadiol and panaxatriol, which are the artifacts derived from the genuine saponins during the acid hydrolysis of saponines, did not show any activity of hemolysis and protection either. Studies on the correlation between hemolytic and protective activities and chemical structure of saponin in general are now in progress.

Takagi, *et al.*²⁾ reported that Rg group stimulated the central nervous system and Rb group showed sedative effect. In our present study, we found that some fractions of ginseng saponins were hemolytic and the others were contrarily anti-hemolytic. Both components were present in one Chinese crude drug and, with deep interest, both activities are apparently masked in a crude preparation owing to their ballanced counter-activity. The facts may explain the principle of the double-faced effects in the traditional Chinese medicine where the same drugs were properly applied, dependent upon the constitution and condition of patients of different diseases.

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A New Synthetic Pathway to $\Delta^{\alpha,\beta}$ -Butenolide from γ -Butyrolactone¹⁾

Various substances containing saturated and unsaturated γ -butyrolactone moiety in each molecule have been well found to occur widely in the plant kingdom. In the course of our studies on chemistry of physiologically active sesquiterpenolides, we have recently devised an interesting method being useful for transformation of α , β -unsaturated γ -butyrolactone from the corresponding saturated homologue.

The present communication describes a preliminary account for the synthetic pathway to $\Delta^{\alpha,\beta}$ -butenolide from α -methyl- γ -butyrolactone involving a new sequence of carboxylation-bromination-dehydrobromination reactions. The application of the ordinary synthetic operation for bromination of the ester or lactone grouping brings about little promise, even in the case of apparent simple lactones. So the possible introduction of the carboxyl group at α -position of the lactonic carbonyl would be first envisaged for an attempted modification. As far as we are aware in the references, there are no precedences for carboxylation of the lactones. Now it is of special interest to note that alkoxy-carboxylation of the simplest γ -

1) Presented to the 92nd annual meeting of the Pharmaceutical Society of Japan, Osaka, April 1972; paper abstract II, p. 191.