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ANTICOMPLEMENTARY ACTIVITY OF GINSENG SAPONINS AND THEIR DEGRADATION PRODUCTS

DONG SEON KIM, SEI RYANG OH, IM SEON LEE, KEUN YOUNG JUNG, JONG DAE PARK,* SHIN IL KIM* and HYEONG-KYU LEE†

Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yusong, Taejon 305-600 Korea; *Korea Ginseng and Tobacco Research Institute, Taejon 305-345, Korea

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Key Word Index—*Panax ginseng*; *Araliaceae*, saponin; ginsenoside; anticomplementary; dammarane; oleanene.

Abstract—The anticomplementary activity of ginseng saponins and their degradation products obtained by chemical treatment of Korean red ginseng saponins was investigated. The total saponin and its major components showed strong anticomplementary activity and their structure–activity relationship was evaluated. © Elsevier Science Ltd. All rights reserved

INTRODUCTION

Saponins isolated from about 50 plants showed anti-inflammatory activity in several experimental models of inflammation in mice and rats [1]. The mechanisms considered included indirect (many saikosaponins) and direct (saikosaponin d and ginsenosides) corticomimetic activity and inhibition of enzymatic formation and release of inflammation mediators (ginsenoside Rb_2, Rc and Re) [2]. In a previous study [3], we prepared several ginseng saponins and sapogenins derivatives by chemical treatment and evaluated the cytotoxic activities of these compounds. During investigations of the biological activities of ginseng total saponin, we found that it exhibited a strong anticomplementary effect. Therefore anticomplementary activity of these substances was investigated.

The complement system consists of over 20 serum proteins which are activated by a cascade mechanism of the classical (CP) or alternative pathway (AP). Activation of the system may contribute to or evoke pathological reactions in a variety of inflammatory and degenerative diseases, for example, asthma, allergy, atopy's dermatitis [4–7], arthritis [8, 9] systemic lupus erythematosus [10] and adult respiratory distress syndrome [11]. Moreover, its effects are normally beneficial for the host, but they can also cause adverse effects depending on the site, extent and duration of complement activation. Therefore, the modulation of complement activity can be important to treatment of inflammations.

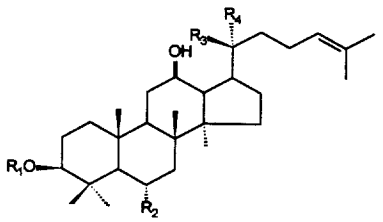
† Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Total saponin fraction of red ginseng showed strong anticomplementary activity on the CP (IC_{50}, 154 $\mu g\ ml^{-1}$), but scarcely at all on AP at the various concentrations between 50 $\mu g\ ml^{-1}$ and 500 $\mu g\ ml^{-1}$ (data not shown). Similarly, most of major ginsenosides (Ro, Rb_1, Rc, Rd, Re, Rf and Rg_1) except ginsenoside Rb_2, isolated from the total saponins, also showed strong anticomplementary activity (Table 1). Unfortunately, in the case of dammarane saponins, it was difficult to evaluate the structure–activity relationship in detail because some of them caused hemolysis. However, it was interesting that the activity was dependent on sugar components at two positions of their aglycone. That is, the dammarane type saponins and sapogenins showed anticomplementary activity with the following order: according to sugar moiety at C-6, glucosyl ≅ glucosyl-glucosyl > glucosyl-rhamnosyl, and at C-20, hydroxyl > glucosyl ≅ glucosyl-arabinosyl > glucosyl-glucosyl.

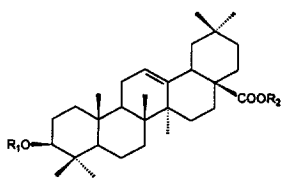
The anticomplementary activities of oleanene type saponins were stronger than that of the dammarane type saponins. Ginsenoside Ro (IC_{50} = 58 μM) and oleanolic acid (IC_{50} = 69 μM) showed the strongest anticomplementary activity among the tested ginseng saponins. These compounds have been reported to have a strong antiinflammatory activity [12, 13]. Therefore, we suggest that their antiinflammatory activity is related to, at least partly, anticomplementary action through the classical pathway.

Interestingly, methyl esterification of ginsenoside Ro increased remarkably anticomplementary activity (IC_{50} = 10 μM), whereas methyl esterification of ole-



	R_1	R_2	R_3	R_4
(20R)-protopanaxadiol	H	H	CH_3	OH
(20S)-protopanaxadiol	H	H	OH	CH_3
ginsenoside Rb_1	-Glc^2-Glc	H	-Glc^6-Glc	H
ginsenoside Rb_2	-Glc^2-Glc	H	-Glc^6-Ara	H
ginsenoside Rc	-Glc^2-Glc	H	-Glc^6-Ara†	H
ginsenoside Rd	-Glc^2-Glc	H	Glc	H
(20S)-ginsenoside Rg_3	-Glc^2-Glc	H	OH	CH_3
(20S)-ginsenoside Rh_2	-Glc	H	OH	CH_3
(20R)-protopanaxatriol	H	OH	CH_3	OH
(20S)-protopanaxatriol	H	OH	OH	CH_3
ginsenoside Re	H	-O-Glc^2-Rha	-Glc	CH_3
ginsenoside Rf	H	-O-Glc^2-Glc	OH	CH_3
ginsenoside Rg_1	H	-O-Glc	-Glc	CH_3
(20S)-ginsenoside Rh_1	H	-O-Glc	OH	CH_3

†Furanose configuration; all other suger rings have pyranose configuration.



oleanolic acid R_1=H R_2=H
ginsenoside Ro R_1=-Glucuronic acid^2-Glc
R_2=-Glc

Fig. 1. The structures of ginsenosides.

anolic acid caused haemolysis. Ginsenoside Ro methyl ester is expected to have potent antiinflammatory action, and methyl esterification of further oleanolic saponins, which have been used as a therapeutic agent, also expected to give compounds that show more potent activity.

EXPERIMENTAL

Preparation of total saponin fraction. The dried red ginseng (1 kg) which was prepd by steaming from fresh six-year-old ginseng provided by Korea Ginseng and Tobacco Corporation was cut into pieces and extracted $\times 3$ with MeOH (3 l.) at room temp. for 3 days. A suspension of the MeOH extract (185 g) in water (2 l.) was washed $\times 3$ with petrol (1 l.) and extracted $\times 2$ with *n*-BuOH (1 l.). The BuOH layer was concd to dryness to give the total saponin (105 g).

Separation and preparation of saponins, prosapogenins, and sapogenins. Isolation and prepn of saponins, prosapogenins, and sapogenins from the above total saponin fr. followed the methods given in a previous study [3].

Determination of anticomplementary activity through the classical pathway. Anticomplementary activity was determined by the modified method of Mayer [14] as described previously [15]. A diluted soln of normal human serum (80 μl) was mixed with gelatin veronal buffer (80 μl) with or without sample. The mixt. was preincubated at 37° for 30 min and sensitized sheep red blood cells (40 μl) were added. After incubation under the same conditions, the mixt. was centrifuged (800 *g*, 4°, 4 min) and the optical density of the supernatant (100 μl) was measured at 405 nm. Anticomplementary activity was determined as a mean of triplicates.

Determination of anticomplementary activity through the alternative pathway. Rabbit red blood cells and normal human serum were diluted with gelatin veronal buffer which was prepd by EGTA and Mg^{2+}. The rest of the process was the same as the assay for anticomplementary activity through the classical pathway.

Table 1. Anticomplementary activity of ginseng saponins and sapogenins on classical pathway

Ginseng saponin and sapogenin	IC_{50} (μM)
Dammaranes	
Ginsenoside Rb_1	289
Ginsenoside Rb_2	113
Ginsenoside Rc	115
Ginsenoside Rd	109
Ginsenoside Re	580
Ginsenoside Rf	97
Ginsenoside Rg_1	180
(20*R*)-Ginsenoside Rg_2	109
(20*S*)-Ginsenoside Rg_2	230
(20*R*)-Ginsenoside Rg_3	—*
(20*S*)-Ginsenoside Rg_3	—
Δ^{20}-Ginsenoside Rg_3	—
(20*S*)-Ginsenoside Rh_1	105
(20*R*)-Ginsenoside Rh_2	230
(20*S*)-Ginsenoside Rh_2	—
Ginsenoside Rh_4	112
(20*R*)-Protopanaxadiol	—
(20*S*)-Protopanaxadiol	—
(20*S*)-Protopanaxatriol	—
(20*R*)-Protopanaxatriol	—
(20*R*)-Panaxatriol	800
(20*R*)-Panaxadiol	—
Oleanenes	
Ginsenoside Ro	58
6′-*O*-Methyl-ginsenoside Ro	10
Oleanolic acid	69
28-*O*-Methyl oleanolic acid	—
Total saponin fraction	154 (μg ml^{-1})
† Rosmarinic acid	180

* Haemolysis occurred on intact sheep red blood cells.
† This compound was tested as a positive control.

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