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Note

# *In Vitro* Anticomplementary Activity of Hederagenin Saponins Isolated from Roots of *Dipsacus asper*

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Anticomplementary activity of hederagenin and related saponins isolated from *Dipsacus asper* was investigated *in vitro*. HN saponin F (**3**) was most potent with $IC_{50}$ value of $3.7\times10^{-5}$ M followed by 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyrano side (**8**), 3-*O*-β-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**5**), dipsacus saponin A (**4**), and hederagenin (**1**) on the classical pathway (CP) of complement system, while the saponins **3-5** did not show the inhibition of hemolysis and rather increase the hemolysis on the alternative pathway (AP). However, all of C-3 monodesmosides [prosapogenin CP (**2**), dipsacus saponin B (**6**), and dipsacus saponin C (**7**)] evoked hemolysis directly on the erythrocytes.

**Key words :** Anticomplementary activity, Hederagenin, Hederagenin saponins, *Dipsacus asper*

## INTRODUCTION

The complement system is a major effector of the humoral immunity involved in the host defence. However, when the complement system is excessively activated, active peptides (anaphylatoxins, C3a, C4a, and C5a) of the components and protein complex (membrane attack complex, MAC) cause a variety of diseases and act fatally on organ transplantation (Kuby, 1994). Therefore, the modulation of complement activity can be useful in the therapy of inflammatory diseases.

In the investigations for anticomplementary compounds from medicinal plants, we have found that some triterpenoids such as citrostadienol (Lee *et al.*, 1997) from the fruits of *Shizandra chinensis* Baill (Shizandraceae) and ginseng saponins (Kim *et al.*, 1998) from Korean red ginseng showed a potent anticomplementary activity on complement system. Recently, several saponins were isolated from the roots of *Dipsacus asper* Wall (Dipsacaceae) (Jung *et al.*, 1993a, 1993b) and some of them were found to have an anti-inflammatory activity in mouse ear edema assay (Jung, 1994). *Dipsacus asper* is a perennial herb used in traditional Chinese medicine as an analgesic, anti-inflammatory agent and for enhancement of liver function. In this paper we report the *in vitro* anticomplementary activity of the triterpenoids isolated from *D. asper*.

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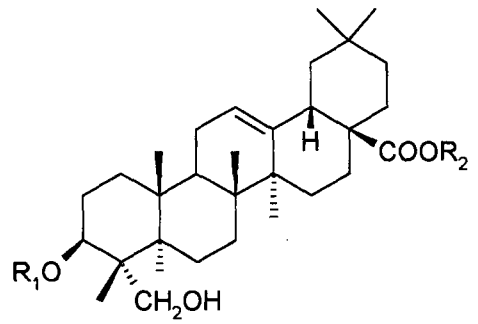
## MATERIALS AND METHODS

### Saponins

The dried roots of *D. asper* (5 kg) were refluxed with hot MeOH (3 times) and concentrated to give a residue (1.28 kg). which was suspended in $H_2O$ and extracted with $CHCl_3$, EtOAc and *n*-BuOH, successively. Compound **1**, compounds **2-4**, and compounds **5-8** were isolated from the $CHCl_3$ extracts, the EtOAc extracts, and the *n*-BuOH extracts, respectively. Detailed isolation procedures of them were described in the previous paper (Jung *et al.*, 1993a, 1993b).

### Complement titration

Complement activity was assessed by the modified method of Mayer as described previously (Oh *et al.*, 1998). Normal human serum (NHS) from a healthy volunteer was used as the complement source and the serum was diluted for the $CH_{50}$ (CP) and $APH_{50}$ (AP) just before sample treatment. Each compound was dissolved in DMSO and the solution was diluted to 2.5% in $GVB^{++}$ (CP) or GVB (AP). For the CP assay,




| | $R_1$ | $R_2$ |
|---|---|---|
| 1 | H | H |
| 2 | Ara | H |
| 3 | Ara | Glc |
| 4 | H | Glc(1→6)Glc |
| 5 | Ara | Glc(1→6)Glc |
| 6 | Glc(1→4)[Rha(1→6)]Glc(1→3)Rha(1→2)Ara | H |
| 7 | Xyl(1→4)Glc(1→4)Glc(1→3)[Rha(1→4)]Rha(1→2)Ara | H |
| 8 | Glc(1→3)Rha(1→2)Ara | Glc(1→6)Glc |

**Fig. 1.** Structures of the saponins from *Dipsacus asper*

**Table I.** *In vitro* anticomplementary activity of hederagenin and its saponins isolated from *Dipsacus asper* on the complement system

| Compound | Classical pathway $IC_{50}$ | Alternative pathway % inhibition[a] |
|---|---|---|
| 1 | $1.6\times10^{-4}$ M | 18.0 |
| 2 | –[b] | –[b] |
| 3 | $3.7\times10^{-5}$ M | -44.3±3.0c |
| 4 | $1.1\times10^{-4}$ M | -32.3±2.8c |
| 5 | $9.9\times10^{-5}$ M | -41.4±5.3c |
| 6 | –[b] | –[b] |
| 7 | –[b] | –[b] |
| 8 | $7.9\times10^{-5}$ M | -42.9±3.0c |
| Ginsenoside Ro[d] | $5.8\times10^{-5}$ M | 28.0±1.0 |

[a]Measured at the concentration of 125 /ml.
[b]Hemolysis occurred over 90% at the concentration of 30 μg/ml.
[c]Negative values denote enhancement of hemolysis from standard condition.
[d]Used as a positive control (Kim *et al*, 1998).

sensitized erythrocytes (EA) were prepared by incubation of sheep erythrocyte suspension ($4.0\times10^8$ cells/ml) with an equal volume of optimally diluted hemolysin (S-1389, Sigma Co.) at 37 for 30 min and restored to formal concentration prior to use. The 80 μl of optimally diluted NHS was pre-incubated with 80 μl of sample solution at 37 for 30 min, and then 40 μl of EA were added to them. After incubation under the same conditions, the mixture was centrifuged and the optical density of the supernatant (100 μl) was measured at 405 nm. For the AP assay, rabbit erythrocyte suspension ($3.0\times10^8$ cells/ml) in Mg-EGTA-GVB (8 mM of $MgCl_2$ and 4 mM of EGTA in GVB) was used and the overall procedure was the same with that of CP assay.

### Anticomplementary activity

The % hemolysis was calculated by ($A_i/A_{max}\times100\%$), where $A_i$ and $A_{max}$ indicated OD 405 nm values of a given titration and maximum lysis by $H_2O$ from the corresponding background. The anticomplementary activity was determined as a mean of triplicate tests per concentration and expressed as a percent inhibition from complement-dependent hemolysis of the control.

## RESULTS AND DISCUSSION

Table I shows the activity of the isolated compounds from *D. asper* on the immunohemolysis. Hederagenin (**1**), the genin of the isolated saponins, exhibited an anticomplementary activity and this result was in good agreement with those of β-boswellic acid (Knaus *et al.*, 1996) and oleanolic acid (Kim *et al.*, 1998). These triterpenoic acids commonly showed a potent anticomplementary activity on the CP of complement system and the carboxylic group of them was known to be an essential feature for the activity. However, all of the C-3 monodesmosides (**2**, **6**, and **7**) of **1** evoked over 90% hemolysis at 30 μg/ml (data not shown). In the 12-oleanene saponins, C-3 monodesmosides with a carboxylic acid in the genin were characteristically found to have a strong hemolytic activity regardless of the number of sugars (Kang, 1997). Contrary to the results of C-3 monodesmosides, C-28 monodesmoside (dipsacus saponin A, **4**) did not exhibit hemolytic activity but showed a significant anticomplementary activity on the CP of complement system. Moreover, C-3,28 bisdesmosides of **1** (**3**, **5**, and **8**) did not evoke hemolysis any more, and rather showed a strong anticomplementary activity on the CP. Compound **3** showed the most potent anticomplementary activity and followed by compounds **8**, **5**, and **4**. These results indicate that glycosylation on the carboxylic acid of the genin is essential for the anticomplementary activity and addition of sugars on **3** seems to reduce the potency. In view of hemolytic activity, the results also suggest that the presence of a free carboxylic acid as well as attachment of sugar(s) at C-3 of the genin are responsible for the hemolytic activity.

Meanwhile, the anticomplementary saponins on the CP did not show inhibition of hemolysis but showed enhancement of hemolysis on the AP of complement system. Because they did not exhibit an intact hemolysis up to 250 μg/ml, the enhancement of hemolysis seemed to be caused by the positive effect of them on the complement activation. The opposite effect on the immunohemolysis between the CP and the AP of complement system has been known as a complement modulating property and found in some flavonoid glycosides (Cimanga *et al.*, 1995, 1997) and triterpenoic acid methyl-

esters (Lee *et al.*, 1997), although the mechanism has not been clearly understood.

This is the first report that the C-28 monodesmoside (dipsacus saponin A) and C-3,28 bisdesmosides of hederagenin have an anticomplementary activity and the present results demonstrate that the anticomplementary activity of hederagenin and the related saponins could be one of the causative constituents for anti-inflammatory effect of *D. asper*.

## REFERENCES CITED

Cimanga, K., de Bruyne, T., Lasure, A., Poel, B. V., Pieters, L., Berghe D. V. and Vlietinck, A., *In vitro* anticomplementary activity of constituents from *Morinda morindoides*. *J. Nat. Prod.*, 58, 372-378 (1995).

Cimanga, K., de Bruyne, T., Poel, B. V., Ma, Y., Claeys, M., Pieters, L., Kambu, K., Tona. L., Bakana, P., Berghe, D. V. and Vlietinck, A. J., Complement-modulating properties of a kaempferol 7-*O*-rhamnosylsophroside from the leaves of *Morinda morindoides*. *Planta Medica*, 63, 220-223 (1997).

Jung, K. Y., Do, J. C. and Son, K. H., Triterpene glycosides from the roots of *Dipsacus asper*. *J. Nat. Prod.*, 56, 1912-1916 (1993a).

Jung, K. Y., Son, K. H. and Do, J. C., Triterpenoids from the roots of *Dipsacus asper*. *Arch. Pharm. Res.*, 16, 32-35 (1993b).

Jung, K. Y., Studies on the terpenoid constituents from the roots of *Dipsacus asper* Wall. Ph. D. Thesis, Yeungnam University (1995).

Kang, S. S., *Triterpenoid saponins*. Seoul National Univ. Press, Seoul, pp 72-76 (1997).

Kim, D. S., Oh, S. R., Lee, I. S., Jung, K. Y., Park, J. D., Kim, S. I. and Lee, H.-K. Anticomplementary activity of ginseng saponins and their degradation products. *Phytochem.*, 47, 397-399 (1998).

Knaus, U. and Wagner, H., Effects of boswellic acid of *Boswellia serrata* and other triterpenic acids on the complement system, *Phytomed.* 3, 77-81 (1996).

Kuby, J., *Complement* in 'Immunology' 2nd ed., W. H. Freeman Company, New York, pp. 336-355, 1994.

Lee, H. -K., Oh, S. R., Kim. D. S., Lee, I. S., Jung, K. Y., Park, S. H., Lee, J. J. and Kim, J. H., *Abstracts, International Symposium on Natural Medicines*, Kyoto, Japan, October, 3P-030, p 272, 1997.

Lee, I. S., Oh, S. R., Jung, K. Y., Kim, D. S., Kim J. H. and Lee, H.-K., Anticomplementary activity and complete $^{13}C$ NMR asignment of citrostadienol from *Schizandra chinensis*. *Int. J. Pharmacogn.*, 35, 358-363 (1997).

Oh, S. R., Kim, D. S., Lee, I. S., Jung, K. Y., Lee, J. J. and Lee, H.-K., Anticomplementary activity of constituents from the heartwood of *Caesalpinia sappan*. *Planta Medica*, 64, 456-458 (1998).