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Inhibition of Mouse Ear Edema by Steroidal and Triterpenoid Saponins

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Certain steroids and triterpenoids isolated from diverse plant families were known to possess anti-inflammatory activity. In the course of finding new anti-inflammatory natural products, some steroidal and triterpenoid saponins were isolated and evaluated for their anti-inflammatory activity using *in vivo* mouse ear edema test. At the oral dose of 100 mg/kg, several steroidal saponins and triterpenoid saponins such as hederagenin glycosides showed significant inhibition of ear edema (20~37% inhibition), though less potent than indomethacin and hydrocortisone.

Key words : Steroidal saponin, Triterpenoid saponin, Hederagenin, Anti-inflammation, Ear edema, Medicinal plant

INTRODUCTION

Wide use of nonsteroidal anti-inflammatory drugs (NSAID) and steroidal anti-inflammatory drugs (SAID) has been limited by their frequent adverse side effects. Therefore, it may be fruitful to evaluate anti-inflammatory properties of natural products showing relatively low incidence of side effects. Among plant constituents, steroids and triterpenoids are most common and widely distributed in plant kingdom. Certain steroid and triterpenoid derivatives were known to possess anti-inflammatory activity *in vivo* (Lewis, 1989; Kang, 1996; Safayhi and Sailer, 1997). Some of them also showed immunoregulatory activity *in vitro* and *in vivo* suggesting their therapeutic potential for inflammation-related diseases. During our search for new anti-inflammatory principles from medicinal plants (Moon *et al.*, 1997; Lee *et al.*, 1998), we have isolated 7 steroidal saponins and 8 triterpenoid saponins from 6 different medicinal plants. In this investigation, these compounds were evaluated for anti-inflammatory activity *in vivo* using mice ear edema assay. And it was found that dioscin, gracillin, smilaxin A, smilaxin B and some of hederagenin glycosides showed significant anti-inflammatory activity *in vivo*.

MATERIALS AND METHODS

Dioscin, gracillin and methyl protogracillin were isolated from the rhizomes of *Smilax china* and identified according to the previously published method of Kim *et al.* (1989). Spicatoside A was obtained from tubers of *Liriope spicata* (Lee *et al.*, 1989). Smilaxin A, B, and C were purified from subterranean parts of *Smilax sieboldii* following the procedure of Woo *et al.* (1992). Suavissimoside R1 and coreanoside F1 were isolated from roots of *Rubus parvifolius* (Choi *et al.*, 1991; Ohtani *et al.*, 1990). Akebia saponin PA, HN-saponin F and akebia saponin D were isolated from *Dipsacus asper* and structurally identified according to the previous procedures of Higuchi and Kawasaki (1976), Kizu *et al.* (1985), and Higuchi and Kawasaki (1972), respectively. Loniceroside A was isolated from *Lonicera japonica* by the method of Son *et al.* (1994). Dipsacus saponin B and C were isolated and identified according to the procedure of Jung *et al.* (1993). The chemical structures of these derivatives were represented in Fig. 1. Male ICR mice were purchased from Korea Experiment Animal Co. (Seoul, Korea). The animals were maintained in specific pathogen free (SPF) animal facility (KNU) at least for 1 week, under conditions of 22±1°C, 55±5% relative humidity and 12 h/12 h (L/D) cycle. Mice were fed with mouse lab. chow (Purina Korea) and water *ad libitum*. For measuring inhibitory activity of ear edema, mice (18~22 g) were randomly grouped. Ear edema was provoked by topical application of 2% arachidonic acid (AA) or 2.5% croton oil and thickness of ears was measured with dial thickness gauge (Lux Scientific Instrument). The tested compounds were administered orally 1 h prior to topical application of each inflam-

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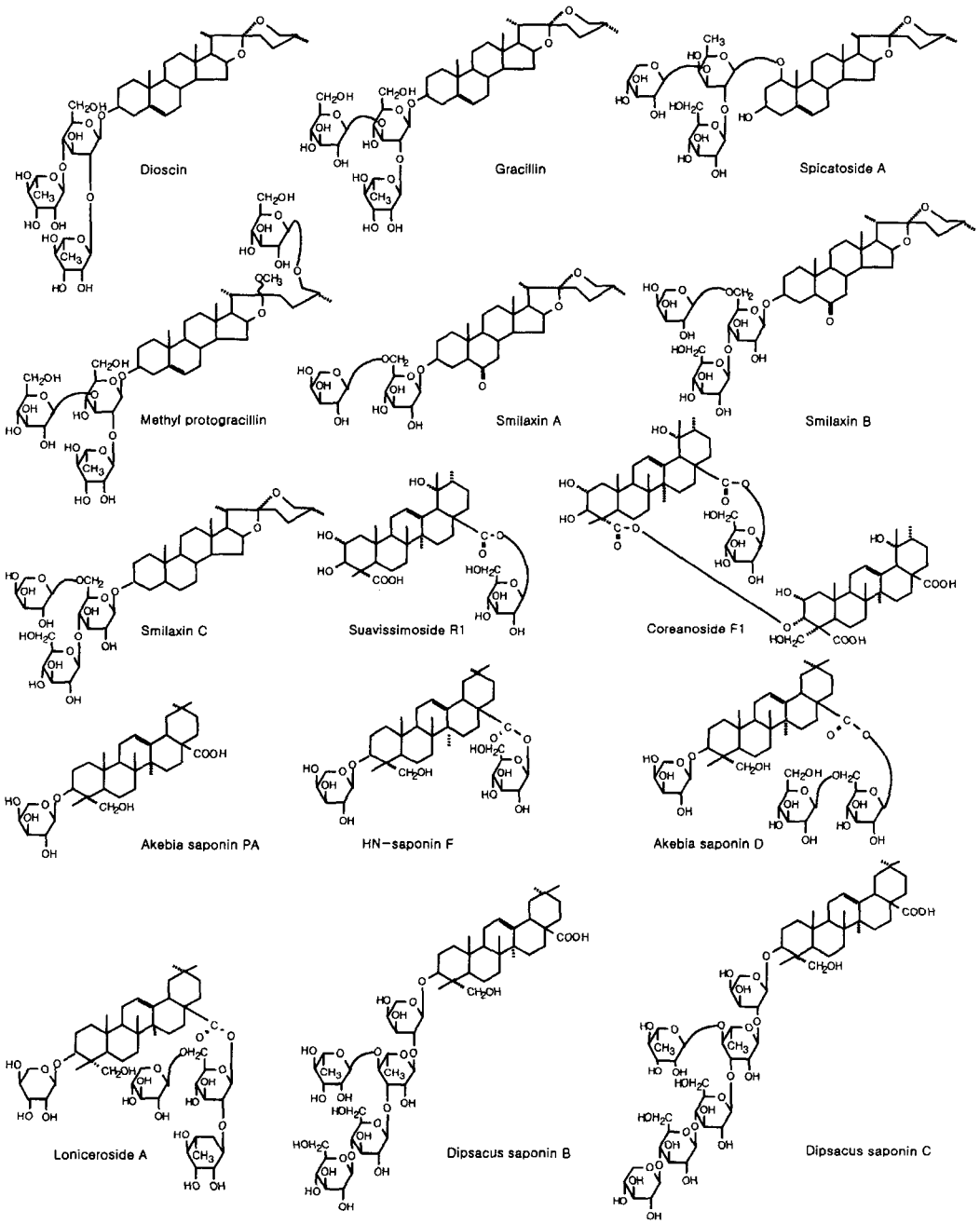

Fig. 1. Chemical structures of steroidal and triterpenoid saponins used in this study

magen. All procedures of mouse ear edema bioassay were carried out following the established method of Kim *et al.* (1993). The statistical analysis was performed by one-way ANOVA and P values less than 0.01 were considered as significantly different.

RESULTS AND DISCUSSION

As represented in Table 1, dioscin and gracillin among steroidal saponins showed significant inhibitory activity against AA-induced ear edema (25 and 20% inhibition, respectively), but not against croton oil-induced ear edema. In contrast, smilaxin A and B showed inhibition against ear edema induced by croton oil (25 and 20% inhibition, respectively), but not by AA. At the dose of 100 mg/kg, other steroidal saponins were not significantly active against ear edema induced by either AA or croton oil. Tigogenin, a sapogenin of smilaxin C, was previously reported to show potent inhibition of rat paw edema induced by λ-carrageenan by intraperitoneal injection (Peana *et al.*, 1997). However, in our study, smilaxin C did not show the significant inhibition

Table I. Inhibition of mouse ear edema by steroids and triperpenoids from plants[a]

Compounds[b]	AA-induced edema[c] % Inhibition	Croton oil-induced edema[c] % Inhibition
Steroidal saponins		
Dioscin	25±4*	<15
Gracillin	20±4*	<15
Spicatoside A	<15	<15
Methyl protogracillin (Furostanol)	<15	<15
Smilaxin A	<15	25±6*
Smilaxin B	<15	20±4*
Smilaxin C	<15	NT
Triterpenoid saponins		
Suavissimoside R1	<15	<15
Coreanoside F1	19±8	<15
Akebia saponin PA	<15	<15
HN-saponin F	16±6	<15
Akebia saponin D	<15	<15
Loniceroside A	34±6*	31±4*
Dipsacus saponin B	20±1*	20±2*
Dipsacus saponin C	37±6*	36±6*
Reference compounds		
Control[d]	0±8	0±5
Indomethacin	62±8*	31±8*
Hydrocortisone	25±8*	77±10*
Hederagenin	23±4*	20±2*

[a]Results of one of three separate sets of experiments were represented here.
[b]All compounds were dissolved in 0.5% carboxymethyl cellulose (CMC) and administered orally at the same dose of 100 mg/kg, except control group receiving only vehicle (CMC).
[c]Five animals were used in each group (n=5) and data were represented as arithmetic mean±S.E.
[d]Ear thickness increased in control group with inflammagen were 2.1±0.2 mm and 2.5±0.1 mm for AA-induced and croton oil-induced ear edema, respectively., NT: Not tested., *: $P<0.01$, significantly different from the control group.

against AA-induced ear edema, which may reflect its steroidal action since SAIDs usually do not possess potent inhibitory activity on this animal model. In a group of triterpenoid saponins, suavissimoside R1 and coreanoside F1 were not significantly active against ear edema induced by either AA or croton oil. Among glycosides of hederagenin, loniceroside A, dipsacus saponin B and C having more than four sugar residues showed significant anti-inflammatory activity against both ear edema induced by AA or croton oil (20~37% inhibition). However, mono (akebia saponin PA), di (HN-saponin F) and triglycoside derivative (akebia saponin D) were not significantly active. Hederagenin, a sapogenin, was found to possess anti-inflammatory activity (Bhargava *et al.*, 1970), and hederagenin tetraglycoside (loniceroside A) was previously reported to possess anti-inflammatory activity against acute as well as chronic inflammation (Lee *et al.*, 1995). We do not know why glycosylated hederagenins having one to three sugar units did not show the considerable anti-inflammatory activity by oral administration. No structural activity relationship could be found. It is speculated that the anti-inflammatory activities of hederagenin glycosides may be mainly depending on their bioavailability and/or metabolism *in vivo*, not on their intrinsic activity. And it is noteworthy that hederagenin glycosides possess wide range of anti-inflammatory activity in both AA-induced and croton oil-induced ear edema. In conclusion, some steroidal and triterpenoid saponins such as diosgenin glycosides, laxogenin glycosides and hederagenin glycosides showed significant anti-inflammatory activity *in vivo*. However, they were less active than currently used anti-inflammatory drugs.

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REFERENCES CITED

Bhargava, K. P., Gupta, M. B., Gupta, G. P. and Mitra, C. R., Anti-inflammatory activity of saponins and other natural products. *Indian J. Med. Res.*, 58, 724-730 (1970).

Choi, Y. S., Son, K. H. and Do, J. C., Triterpenoids from the roots of *Rubus parvifolius*. *Arch. Pharm. Res.*, 14, 225-230 (1991).

Higuchi, R. and Kawasaki, T., Seed saponins of *Akebia quinata* Decne. II. Hederagenin 3,28-O-bisglycosides. *Chem Pharm. Bull.*, 20, 2143-2149 (1972).

Higuchi, R. and Kawasaki, T., Pericarp saponins of *Akebia quinata* Decne. I. Glycosides of hederagenin and oleanolic acid. *Chem. Pharm. Bull.*, 24, 1021-1032 (1976).

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

Kang, S. S., Triterpenoid saponin. Kang, S. S. (ed.), 1996, Seoul National University Press, Seoul, pp67-140.

Kim, H. K., Namgoong, S. Y. and Kim, H. P., Anti-inflammatory activity of flavonoids: Mice ear edema inhibition. *Arch. Pharm. Res.*, 16, 18-24 (1993).

Kim, S. W., Chung, K. C., Son, K. H. and Kang, S. S., Steroidal saponins from the rhizomes of *Smilax china*. *Kor. J. Pharmacogn.*, 20, 76-82 (1989).

Kizu, H., Kitayama, S., Nakatani, F., Tomimori, T. and Namba, T., Studies on Napalese crude drugs. III. On

the saponins of *Hedera napalensis* K. Koch. *Chem. Pharm. Bull.,* 33, 3324-3329 (1985).

Lee, D. Y., Son, K. H., Do, J. C. and Kang, S. S., Two new steroidal saponins from the tubers of *Liriope spicata*. *Arch. Pharm. Res.,* 12, 295-299 (1989).

Lee, S. J., Shin, E. J., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P., Anti-inflammatory activity of the major constituents of *Lonicera japonica*. *Arch. Pharm. Res.,* 18, 133-135 (1995).

Lee, S. J., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P., Anti-inflammatory activity of *Lonicera japonica*. *Phytotherapy Res.,* 12, 445-447 (1998).

Lewis, D. A., Anti-inflammatory drugs from plants and marine sources. Agents and Actions, Suppl. 27, Lewis, D. A. (ed.), 1989, Berkhauser Verlag, Basel, pp167-286.

Moon, T. C., Chung, K. W., Chung, K. C., Son, K. H., Kim, H. P., Kang, S. S. and Chang, H. W., Screening of inflammatory phospholipase A_2 inhibitors from medicinal products. *Yakhak Hoeji,* 41, 565-570 (1997).

Ohtani, K., Miyajima, T., Takahasi, T., Kasai, R., Tanaka, O., Hahn, D. R. and Naruhashi, N., A dimeric triterpene-glycoside from *Rubus coreanus*. *Phytochemistry,* 29, 3275-3280 (1990).

Peana, A. T., Moretti, M. D. L., Manconi, V., Desole, G. and Pippia, P., Anti-inflammatory activity of aqueous extracts and steroidal sapogenins of *Agave americana*. *Planta Med.,* 63, 199-202 (1997).

Safayhi, H. and Sailer, E.-R., Anti-inflammatory actions of pentacyclic triterpenes. *Planta Med.,* 63, 487-493 (1997).

Son, K. H., Jung, K. Y., Chang, H. W., Kim, H. P. and Kang, S. S., Triterpenoid saponins from the aerial parts of *Lonicera japonica*. *Phytochemistry,* 35, 1005-1008 (1994).

Woo, M. H., Do, J. C. and Son, K. H., Five new spirostanol glycosides from the subterranean parts of *Smilax sieboldii*. *J. Nat. Prod.,* 55, 1129-1135 (1992).