Effects of Phyllodulcin, Hydrangenol, and Their 8-O-Glucosides, and Thunberginols A and F from *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO on Passive Cutaneous Anaphylaxis Reaction in Rats

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We examined the antiallergic effects of phyllodulcin, hydrangenol, and their 8-O-glucosides, and thunberginols A and F isolated from the processed leaves (*Hydrangea Dulcis* Folium) and dried leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO using the passive cutaneous anaphylaxis (PCA) reaction. With the exception of phyllodulcin, these constituents were found to significantly inhibit the PCA reaction. Although thunberginol A showed the most potent inhibitory effect, hydrangenol was considered to be the principal antiallergic component in the processed leaves, after taking into account their contents.

Key words Hydrangea Dulcis Folium; *Hydrangea macrophylla* var. *thunbergii*; hydrangenol; passive cutaneous anaphylaxis reaction; thunberginol A

Hydrangea Dulcis Folium (H. D. F.), a rare natural medicine indigenous to Japan, is prepared from the leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (H. M. T., Saxifragaceae) via several processing procedures. This natural medicine is listed in the Japanese Pharmacopoeia XIII and is extensively used in confectionery, drinks, and foods as an oral refrigerant and as a sweetener. With regards to the chemical constituents, two dihydroisocoumarins, phyllodulcin (1) and hydrangenol (3), have been isolated from this natural medicine (the processed leaves) as the principal components (both amount to ca. 2% yield of the natural medicine), while their 8-O-glucosides (2, 4) were isolated from the dried leaves in ca. 2% and 3% yields, respectively.

As part of our studies on bioactive constituents of medicinal foodstuffs, the methanolic extract of H. D. F. was found to show potent antiallergic, antibacterial, antioxidantive, antinflammatory, and choleagocic activities. Recently, we characterized the active constituents such as thunberginols A (5, 0.009%) and F (6, 0.003%) by monitoring the inhibitory effects on *in vitro* Schütz-Dale (SD) reaction, and histamine release from rat mast cells. The principal constituents 1 and 3 in H. D. F. had less activity than 5 and 6 in the *in vitro* SD reaction and mast cell degranulation tests. Moreover, their glucosides 2 and 4 showed no activity in these tests. However, the *in vivo* anti-type 1 allergic activity of these compounds was left uncharacterized. In this study, we compared the *in vivo* inhibitory effects of the important constituents (1–6) isolated from H. D. F. and H. M. T. using PCA reaction. Moreover, we describe the principal constituents that contribute to the anti-type 1 allergic activity of H. D. F. and H. M. T.

MATERIALS AND METHODS

**Animals** Rats were purchased from Kiwa Laboratory Animals Co., Ltd. (Wakayama, Japan) and housed in an air-conditioned room at 23±2°C for more than 3 d. Standard laboratory chow (MF, Oriental Yeast Co., Ltd.) and tap water were given freely.

**Materials** Anti-dinitrophenyl (Anti-DNP) IgE was purchased from Seikagaku Co., Ltd., and divided into small volumes and stocked at −20°C. Dinitrophenylated bovine serum albumin (DNP-BSA) was prepared from bovine serum albumin fraction V (Sigma) and sodium dinitrobenzene sulfonate (Tokyo Kasei Organic Chemicals Co., Ltd.) according to the method of Tada *et al.* Tranilast was obtained from Kissei Pharmaceutical Industries Co. The methanol extracts of H. D. F. (yield: 20.8%) and H. M. T. (yield: 28.2%) were obtained at reflux and room temperature, respectively. Compounds 1, 3, 5, and 6 were isolated from the ethyl acetate soluble portion of the H. D. F. extract. Phyllodulcin 8-O-glucoside (2) and hydrangenol 8-O-glucoside (4) were isolated from the butanol soluble portion of the H. M. T. extract.

**Passive Cutaneous Anaphylaxis (PCA) Reaction** The PCA reaction was performed according to the method reported previously. Namely, male Wistar rats weighing about 200 g had their back hair shaved and 100 μl of anti-DNP IgE diluted (×6250) in phosphate buffered saline (PBS, pH 7.4) was injected intracutaneously into their skin. After 46 h, test samples which were suspended with 5% acacia (Nacalai Tesque Co., Ltd.) in water were administered orally. After 2 h, 0.5 ml of PBS which contained 0.75 mg of DNP-BSA and 1% Evans blue (Tokyo Kasei Organic Chemicals Co., Ltd.) was injected into the vain. Thirty min thereafter, rats were sacrificed and the back skin peeled. The blue spot area was measured with a digital planimeter (Uchida Yoko Co., Ltd.).

**Chart 1.** Chemical Constituents in H. D. F. and Dried Leaves of H. M. T.

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RESULTS

Inhibitory Effects of Methanolic Extracts from H. D. F. and H. M. T. Figure 1 shows a comparison of the anti-type I allergic effects of H. D. F. and H. M. T. The extract of H. D. F. suppressed PCA reaction dose-dependently from 1000 to 2000 mg/kg, its effect was significant at 2000 mg/kg. On the other hand, H. M. T. slightly suppressed PCA reaction, but not significantly.

Inhibitory Effects of the Constituents Isolated from H. D. F. and H. M. T. Figure 2 shows the effects of 1—6 on rat PCA reaction. Compound 1 slightly inhibited the PCA reaction at 300 and 500 mg/kg by 2 h pretreatment, but not significantly. Compound 3 dose-dependently inhibited PCA reaction at doses of 300 and 500 mg/kg. On the other hand, 2 and 4 also significantly suppressed PCA reaction at 300 and 500 mg/kg, but tended to show less activity than 3. Compound 5 strongly inhibited PCA reaction at doses of 300 and 500 mg/kg, and tended to show more potent activity than tranilast. Compound 6 also significantly inhibited PCA reaction at a dose of 500 mg/kg, but showed less activity than tranilast.

DISCUSSION

We previously reported that the methanolic extract of H. D. F. suppressed PCA reaction at 2000 mg/kg and active constituents seemed to be contained in the less polar fraction. Moreover, principal constituents in this fraction such as 1 and 3 showed no significant effect by oral administration at less than 250 mg/kg. As a result of this examination, although 1 lacked significant activity, 3 dose-dependently suppressed PCA reaction at the high doses of 300 to 500 mg/kg. We previously reported that 3 was present in the methanolic extract of H. D. F. at more than 10% concentration. Thus, 3 seems to significantly contribute to the anti-allergic activity of the methanolic extract from H. D. F. On the other hand, 1, which is structurally similar to 3, lacked activity at 500 mg/kg. As a result of this experiment, the lack of an OH group at the 3'-position or the presence of a methoxyl group at the 4'-position seemed to reduce the anti-allergic activity. The glucoside 4 significantly inhibited PCA reaction, but tended to show less activity than 3. We had already reported that these glucosides (2 and 4) showed no inhibitory activity on mast cell degranulation induced by histamine secretagogues. Since 3, the aglycon of 4, suppressed PCA reaction, 4 was suspected to show the activity following hydrolysis after digestion. On the other hand, 2 showed suppressive effects, but its aglycon 1 lacked a significant effect. This result indicated that the glucoside 2 showed antiinflammatory effects or was metabolized to unknown active compounds. The potent histamine release inhibitors 5 and 6 suppressed PCA reactions at doses of 300 and 500 mg/kg, respectively. The activity of 5 was stronger than 2, 3, and 4. Compound 6, which showed potent inhibitory activity in the mast cell degranulation test, displayed lower activity than 2, 3, 4, and 5 in this in vivo examination.

In conclusion, 3 appears to be the principal anti-type I allergic constituent in H. D. F. Compound 5, a potent histamine release inhibitor from H. D. F., also showed a strong effect in this examination, but 6 showed less activity. Since the extract of H. M. T. and the principal constituent 4 tended to show less activity than the extract of H. D. F. or 3, the processing of H. M. T. to H. D. F. must be important for expression of
anti-type I allergic activity.

REFERENCES

