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NOTE

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Isolation and identification of histamine-release inhibitors from *Pistacia weinmannifolia* J. Pisson ex. Franch

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Abstract Seven histamine-release inhibitors were isolated from *Pistacia weinmannifolia* J. Pisson ex. Franch. They were identified as gallic acid, 3-*O*-galloylquinic acid, methyl gallate, ethyl gallate, penta-*O*-galloyl-*β*-D-glucopyranoside, myricetin 3-*O*-*α*-L-rhamnopyranoside, and myricetin- 3-*O*-(3″-*O*-galloyl)-*α*-L-rhamnopyranoside. These compounds suppressed the compound 48/80-induced histamine release from rat peritoneal mast cells.

Key words Allergy · Histamine-release inhibition · Peritoneal mast cell · *Pistacia weinmannifolia* · Tannins

Introduction

The number of people who suffer from allergic diseases, such as atopic dermatitis and hay fever, is increasing rapidly. Allergies are categorized into four types. Both atopic dematitis and hay fever belong to the Type 1 allergy [1], which can be linked to the amount of histamine release from mast cells.

In a preliminary study, we found that extracts of *Pistacia weinmannifolia*, which are used as a folk medicine in China, have a potential histamine-release inhibitory activity. The leaves of *P. weinmannifolia* are used for treating dysentery, enteritis, influenza, traumatic bleeding, headache, and lung cancer [2, 3]. To date, however, there has not been reported that *P. weinmannifolia* extracts have show potent histamine-release inhibitory effects. Furthermore, active compounds in *P. weinmannifolia* has not been identified. In this paper, we demonstrate the presence of histamine-release inhibitors in *P. weinmannifolia*.

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Materials and methods

Materials

All chemicals used in this study were purchased from Wako Pure Chemical (Osaka, Japan), and they were used without further purification. Leaves of *P. weinmannifolia* (6 kg) were collected in the Shirin region in the Yunnan province of China.

Analytical methods

^{1}H and ^{13}C NMR were measured using JMR EX270 (JEOL, 270 MHz), MS by GCMS 9100mk (Shimadzu), IR spectra by FTIR8200 (Shimadzu) and UV spectra by a DU640 Spectrophotometer (Beckman-Coulter).

Isolation of active compounds

The leaves of *P. weinmannifolia* were dried, ground up and extracted with 60% aqueous ethanol at room temperature for 12 h. Following filtration to remove debris, the solvent was evaporated and the resulting concentrate was spray-dried at 146–154°C to obtain a powdered material. The powder was then extracted with methanol (MeOH) for 1 day, and the concentrated MeOH extract was subsequently chromatographed over Diaion HP-20 (Mitsubishi Chemical, Japan) using water and MeOH as eluents to obtain five fractions: aqueous fraction, 25%

MeOH fraction, 50% MeOH fraction, 75% MeOH fraction, and MeOH fraction. The 25% MeOH fraction and the 50% MeOH fraction showed higher histamine-release inhibitory effects. Using a preparative HPLC, we isolated compounds **1** (6.0 mg) and **2** (132.4 mg) from the 25% MeOH fraction (0.4 g), and compounds **3** (24.7 mg), **4** (235.8 mg), **5** (91.3 mg), **6** (74.9 mg), and **7** (48.9 mg) from the 50% MeOH fraction (1.8 g). The structures of the isolated compounds with histamine-release inhibitory effect are shown in Fig. 1.

Evaluation of the histamine-release inhibitory effect

The histamine-release inhibitory effects of the isolated compounds were evaluated as described elsewhere [4] with minor modifications. Briefly, rat peritoneal mast cells were collected, and the number of mast cells was adjusted to 6×10^6 cells ml^{-1}. Each test compound (5 μM) was pre-incubated with mast cells for 10 min in a 2 ml test tube, and then compounds 48/80 (20 μg/ml) was added to the test tube, which was then incubated for 10 min at 37°C. After the incubation, the mixture was put in an ice-acetone bath to quench the reaction, and then centrifuged at 2000 *g* to obtain a supernatant. The concentration of histamine in the supernatant was measured by HPLC after a diazo-derivatization. The diazo-derivative of histamine was detected as follows;Cosmosil $5C_{18}$ AR-II ODS column 4.6x150 mm; oven temperature, 40°C; detector, 460 nm; gradient mode [eluent A, 5% aqueous acetonitrile containing 0.1% trifluoroacetic acid; eluent B, acetonitrile (20–38% linear gradient), 1.0 ml min^{-1}]. Under these conditions, the detected peak area and histamine content was well correlated from 0.05 μg ml^{-1} to 5.0 μg ml^{-1} (data not shown).

The assay was repeated at least three times using mast cells from one animal and, in order to confirm the assay data in general, we used three different animals. Histamine release inhibitory effects was calculated with following equation:

$$A = ((C - S) - (T - S))/(C - S) \times 100 \quad (1)$$

where, "A" is histamine-release inhibitory activity, "T" is histamine release from sample-treated mast cells, "S" is spontaneous histamine release from mast cells, and "C" is histamine release from compound 48/80-treated cells.

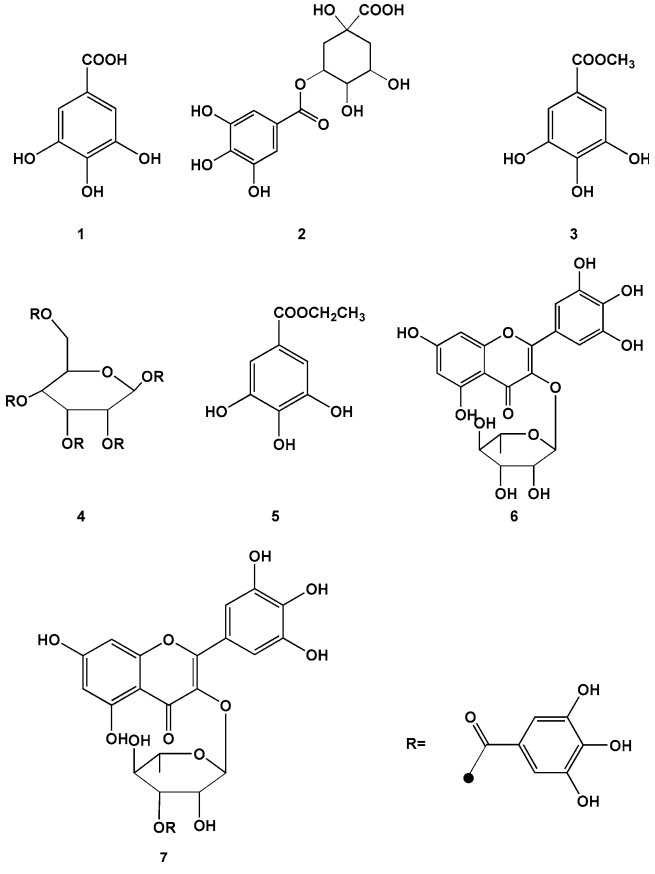

Fig. 1 Histamine-release inhibitors isolated from *Pistacia weinmannifolia*

Results and discussion

Based on the histamine-release inhibitory effects, we have fractionated the ethanol extract of *P. weinmannifolia* using a series of column chromatography, and seven compounds were isolated and identified (Fig. 1). Spectral data for 3-*O*-galloylquinic acid (**2**) [5], penta-*O*-galloyl-*β*-D-glucopyranoside (**4**) [6], myricetin 3-*O*-α-L-rhamnopyranoside (**6**) [7] and myricetin 3-*O*-(3''-*O*-galloyl)-α-L-rhamnopyranoside (**7**) [7] were confirmed by comparing their values to those found in literatures. The spectral data for the other compounds were confirmed by comparing them to authentic compounds purchased from Wako Pure Chemicals (Fig. 1).

Two flavonoids and five gallic acid derivatives were isolated and identified from the extracts of *P. weinmannifolia*. The inhibitory activities of these derivatives on histamine-release from rat peritoneal mast cells were then determined. When these derivatives were tested at a concentration of 5 μM, the histamine-release inhibitory effects of myricetin 3-*O*-α-L-rhamnopyranoside (**6**) (51.9%) was higher than that of myricetin 3-*O*-(3''-*O*-galloyl) -α-L-rhamnopyranoside (**7**) (32.8%). Gallic acid (**1**) showed a relatively low effect (12.7%) in inhibiting the release of histamines, however its derivatives showed higher effects [27.5% for methyl gallate (**3**), 36.7% for ethyl gallate (**5**), 30.9% for 3-*O*-galloylquinic acid (**2**), and 54.7% for penta-*O*-galloyl-*β*-D-glucopyranoside (**4**); Table 1].

In this study, we have demonstrated that *P. weinmannifolia* contains a number of compounds showing a potent histamine-release inhibitory effect. The compounds that we isolated show potent histamine-release inhibitory effect, and it is also likely that they improve allergic symp-

Table 1 Histamine-release inhibitory effects of the compounds isolated from *Pistacia weinmannifolia* (treatment: 5 μM)

[a]Yield (in parenthesis) is expressed as a percentage of the ethanol extract[b]Data are given as means ± standard errors of the means ($n = 9$)

Compounds (yield in %)[a]	Inhibition (%)[b]
(**1**) Gallic acid (1.0)	12.7 ± 0.52
(**2**) 3-*O*-galloylquinic acid (2.8)	30.9 ± 0.81
(**3**) Methyl gallate (0.4)	27.5 ± 1.00
(**4**) Penta-*O*-galloyl-*β*-D-glucopyranoside (3.9)	54.7 ± 2.56
(**5**) Ethyl gallate (1.5)	36.7 ± 1.33
(**6**) Myricetin 3-*O*-*α*-L-rhamnopyranoside (1.2)	51.9 ± 0.96
(**7**) Myricetin 3-*O*-(3''-*O*-galloyl)-*α*-L-rhamnopyranoside (0.8)	32.8 ± 1.35

toms [8–10]. Using HPLC, we determined that these histamine-release inhibitor are relatively major components of the ethanol extract of *P. weinmannifolia* (Table 1). We therefore suggest that these compounds are responsible for the anti-allergic effects of this plant. All of the active compounds were found to be phenolic compounds, and they may act on rat peritoneal mast cells with basically the same mode of action to reduce histamine release. However, it was beyond the scope of the present investigation to determine just how those compounds act as potent histamine-release inhibitors. Further study is necessary to elucidate possible mechanisms in order to understand and clarify the anti-allergic effect of other crude folk medicines like *P. weinmannifolia*.

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