Biologically Active Principles of Crude Drugs. Anti-allergic Principles in “Cnidii monnieri”

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The anti-allergic effects of “Cnidii monnieri” and its constituents were examined. Imperatorine, a coumarin derivative in “Cnidii monnieri,” showed anti-allergic activity.

Keywords—anti-allergic effect; picryl chloride; passive cutaneous anaphylaxis; Cnidii monnieri Fructus; coumarin derivative

Cnidii monnieri Fructus (Japanese name, “Ja-sho-shi”), the fruit of Cnidii monnieri CUSSON (Umbelliferae) is a natural medicine which has been used as a topical agent for such skin conditions as eczema and pruritus. Since a possible anti-allergic effect of Cnidii monnieri may exist, based on its traditional usage, it is of interest to examine the anti-allergic effects of Cnidii monnieri and its constituents. They were tested for effect on contact dermatitis induced by picryl chloride (PC) in mice, used as an experimental model for a delayed allergic reaction, and on the passive cutaneous anaphylaxis (PCA) reaction in guinea pigs, as an experimental model for an acute allergic reaction. The results are described below.

Materials and Methods

Cnidii monnieri, of Chinese origin, was purchased from a local market in Osaka. It was macerated in about 5 volumes of methanol, and kept for 3 d at room temperature. After filtration, a dried extract was obtained by evaporation under reduced pressure at below 50 °C. The extract was then fractionated into 4 fractions by silica gel column chromatography (eluent, benzene : acetone = 50:1→5:1). Fraction 2 (25.98 g), which was found to be effective in the screening procedure of contact dermatitis induced by PC, was further fractionated by column chromatography (eluent, n-hexane : ethyl acetate = 4:1→3:1→2:1) into 5 fractions as follows; CM-a (2.97 g), CM-b (1.00 g), CM-b’ (50 mg), CM-c (476 mg) and CM-c’ (86 mg). They were characterized by measurements of mp and proton nuclear magnetic resonance (1H-NMR) and carbon-13 nuclear magnetic resonance (13C-NMR) spectra.

Effect on Contact Dermatitis—The method used was the same as that reported by Imazumi et al.31 Male BDF1 mice weighing about 20 g were divided into groups of 10. Each animal was shaved in the abdominal area, and 24 h later an antigen solution (about 0.2 ml) of 7% PC (Wako Junyaku, extra pure grade)-ethanol (stored in molecular sieves, Nakarai Chemical Co.) was applied twice to the shaved area using a felt-covered roller. The 7% PC-ethanol solution was freshly prepared at the time of application. The second application was done 5 d after the first application, using the same procedure.

Each test drug was suspended in 5% acacia and given orally twice a day (about 10 a.m. and 5 p.m.) starting immediately after the first application of the antigen solution until 24 h prior to the skin reaction test (a total of 20 times). The control group that received an application of the antigen solution and the untreated group that was only shaved without any other treatment were administered water. The other groups also received one oral administration of test drug 6 h after the skin reaction test.

The skin reaction (contact dermatitis) was induced by pinching each ear once with a surgical clamp, which had tied to its tips a piece of felt (6 mm x 12 mm), soaked with 1% PC-olive oil (Nakarai Chemical Co.). This was done
24 h after administration of each of the test drugs. The control group and the untreated group went through the same procedure. Measurement of the thickness of the ear was done just prior to the induction of reaction and 12, 18, 24 and 36 h later. The differences between the values after the reaction and the values prior to the reaction were calculated, and the effect of each test drug was expressed as percentage inhibition, compared to that of the control. The thickness of the ear was measured with a dial thickness gauge (PEACOC-G, smallest division of 0.01 mm, Ozaki Seisakusho) to the level of 0.01 mm, and the increase in thickness was expressed in the unit of $10^{-3}$ cm. The value given at each time is the average of 20 measurements.

**Effect on PCA Reaction**—Male Hartley guinea pigs weighing about 300 g were divided into groups of 5. They were administered orally a test drug suspended in 5%, acacia. Antibody (antiserum) was then injected intradermally (physiological saline for the control group) at three sites on the back of the animals (0.1 ml/site). After 3 h, antigen (2 mg of bovine serum albumin (BSA) plus 5 mg of Evans blue in 1 ml of physiological saline) was injected intravenously into a foreleg. Each animal was sacrificed by means of a blow on the head 30 min later, and the skin on the back was removed. Each area affected by PCA reaction was traced on a sheet of tracing paper and the area was measured with a planimeter. The change in the area was expressed as a percentage compared to the control.

**Results**

**Identification and Characterization of Constituents**

CM-a, CM-b and CM-b' were identified as osthol, imperatorine and isopimpinelline, respectively, on the basis of mp and $^1$H-NMR and $^{13}$C-NMR spectra.

![Chart 1. Structures of CM-a, CM-b and CM-b']

CM-c$_1$, colorless needles, mp 119—122 °C, was assigned the molecular formula, C$_{15}$H$_{16}$O$_4$, on the basis of the mass spectrum (MS) ($m/z$ 259, M$^+$ - H) and elementary analysis. Its $^{13}$C-NMR spectrum (in CDCl$_3$) signals due to $=\text{CH}-\text{OH}$ at $\delta$ 87.4 (d) and $=\text{CH}_2$ at $\delta$ 113.3 (t) instead of those due to $=\text{C}H=$ at $\delta$ 121.3 (d) and $=\text{C}-\text{CH}_3$ at $\delta$ 25.8 (q) observed in the spectrum of osthol (CM-a). Therefore, CM-c$_1$ was supposed to be substituted by $-\text{CH}_2-\text{CH}-(\text{CH})-(\text{CH}_3)=\text{CH}_2$ at C-4 on a coumarin framework. The $^1$H-NMR spectrum (in CDCl$_3$) exhibited signals due to 1'-H$_2$ (1H, dd, $J$ = 7, 14 Hz at $\delta$ 3.08 and 1H, dd, $J$ = 8, 14 Hz at $\delta$ 3.24), 2'-H (t, $J$ = 7 Hz, at $\delta$ 4.56), 3'-CH$_3$ (s, at $\delta$ 1.88), 3',CH$_2$ (d-like, $J$ = 7 Hz, at $\delta$ 4.84), 3-H (d, $J$ = 9 Hz, at $\delta$ 6.20), 4-H (d, $J$ = 9 Hz, at $\delta$ 7.62), 5-H (d, $J$ = 9 Hz, at $\delta$ 6.82) and 6-H (d, $J$ = 9 Hz, at $\delta$ 7.32), supporting the structure shown in Chart 2.

CM-c$_2$, colorless needles, mp 138—141 °C, showed a molecular ion (C$_{15}$H$_{16}$O$_4$) at $m/z$ 260 in the MS. Its $^1$H-NMR spectrum (in CDCl$_3$) showed signals ascribable to 3-, 4-, 5- and 6-H on coumarin at $\delta$ 6.42 (1H, d, $J$ = 10 Hz), 7.60 (1H, d, $J$ = 10 Hz), 6.84 (1H, d, $J$ = 8 Hz) and 7.30 (1H, d, $J$ = 8 Hz) respectively. Moreover, based upon the presence of the signal due to two methyl groups attached to the carbon bearing the hydroxyl groups at $\delta$ 1.48 and two vinyl
protons at δ 6.88, the structure for CM-c₂ was concluded to be as shown in Chart 3. The ¹³C-NMR spectrum (in CDCl₃) also supported the indicated structure: δ 24.7 (2 × q, 3ʹ-(CH₃)₂), 82.9 (s, C-3'), 117.4, 141.4 (each d, H -C¹ = C²⁻H), 162.5 (s, C-2), 112.4 (d, C-3), 145.2 (d, C-4), 127.8 (d, C-5), 152.5 (s, C-10), 56.4 (q, -OMe).

Effect on Allergic Reaction

Effect on Contact Dermatitis — The methanol extract (500 mg/kg, p.o.) of *Cnidii monnieri* significantly inhibited swelling caused by PC reaction as compared to the control (Fig. 1). Figure 2 shows that, among the 4 fractions of the methanol extract examined, fraction II (250 mg/kg, p.o.) significantly inhibited the swelling throughout the observation period as compared to the control. Fraction II was further fractionated into CM-a, CM-b, CM-b', CM-c₁, and CM-c₂. As shown in Fig. 3, CM-a (osthol) and CM-b (imperatorine) were examined, and imperatorine significantly inhibited the swelling, while osthol had no significant effect. CM-b', CM-c₁ and CM-c₂ were obtained in amounts too small for assay of

*Fig. 1. Effect of Cnidii Fructus on PC Reaction*

- ○, control; - △-, untreated control; - △-, Prednisolone, 2.5 mg/kg. - △-, Cnidii fructus MeOH ext., 500 mg/kg.

Each value is the mean with standard error obtained from 10 mice. Significantly different from the control at a) p < 0.01.

*Fig. 2. Effect of Cnidii Fructus Fraction on the PC Reaction*

- ○, control; - △-, untreated control; - △-, Cnidii fructus fr. I, 100 mg/kg. - △-, Cnidii fructus fr. II, 250 mg/kg. - △-, Cnidii fructus fr. III, 100 mg/kg. - △-, Cnidii fructus fr. IV, 250 mg/kg.

Each value is the mean with standard error obtained from 10 mice. Significantly different from the control at a) p < 0.05, b) p < 0.01.
the pharmacological effect.

**Effect on PCA Reaction**—As shown in Table I, imperatorine had a significant inhibitory effect on the PCA reaction. The effect of imperatorine, however, was weak as compared to that of a reference drug, prednisolone.

**Discussion**

There have been reports on coumarins present in *Cnidii monnieri*, but little is known of the pharmacological effects of coumarins except for reports of insecticidal and antibacterial effects. *Cnidii monnieri* has been used as an ingredient of various tonics, and there are several reports pertaining to this. In the present experiment, a possible anti-allergic effect of *Cnidii monnieri* and its constituents was examined. The results showed that imperatorine, a coumarin derivative in *Cnidii monnieri*, shows significant anti-allergic activity, whereas osthol, a main coumarin derivative, had no significant effect. This provides a pharmacological basis for one of the traditional medicinal effects of *Cnidii monnieri*. Anti-allergic and calcium-antagonistic effects of various coumarin derivatives including the two new coumarins isolated from *Cnidii monnieri* in the present experiment will be examined in the future.

**References**