Histamine-Release Effectors from *Angelica dahurica* var. *dahurica* Root

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Six compounds isolated from the EtOAc fraction of the dried roots of *Angelica dahurica* var. *dahurica* inhibited compound 48/80-induced histamine levels in mouse peritoneal cavity (in vivo). Bergapten, oxypeucedanin hydrate, and byakangelicin inhibited compound 48/80-induced histamine elevation at a dose of 25 mg/kg. Conversely, *sec*-*O*-acetylbyakangelicin [8-(2-acetoxy-3-hydroxy-3-methylbutoxy)-5-methoxypsoralen] enhanced compound 48/80-induced histamine elevation at a dose of 25 mg/kg, while phellopterin and oxypeucedanin had no effect.

The dried roots of *Angelica dahurica* Benth et Hook. var. *dahurica* Benth et Hook. (Umbelliferae) has been listed in a large number of Chinese and Japanese herbal prescriptions[1] and is claimed to be effective in the treatment of acne, eruption, and erythema. In addition, it is used as an aromatic sedative.

Because histamine is a chemical mediator that plays a central role in allergic reactions and consequently causes eruption and erythema in the skin,[2−4] a study was undertaken to isolate and characterize the effects of a series of coumarins from this crude drug on histamine release from mouse peritoneal cavity cells, in vivo.

Results and Discussion

It was found that histamine release in the peritoneal cavity fluids reached a maximum level 10 min after the administration of compound 48/80 (condensation products of *N*-methyl-*p*-methoxyphenylethylamine with formaldehyde) and declined to the basal level within 30 min (Figure 1). It has been reported that compound 48/80 causes histamine release from isolated mast cells by degranulation.[5] It is well known that histamine is released from the granules of mast cells[5] and basophils;[6] therefore, it is suggested that the histamine in the peritoneal cavity fluids is released from the mast cells. On the other hand, the histamine level in the mesentery was not changed by compound 48/80 (Figure 1). In the case of the mobility of the cells in the peritoneal cavity fluids after exposure to compound 48/80, the number of polymorphonuclear leukocytes and macrophages increased time dependently in the peritoneal cavity, while the number of mast cell was not affected (Figure 2). The effect of isolated coumarins on the histamine level was measured 10 min after an intraperitoneal administration of the inducer.

As shown in Table 1, the Et_2O and EtOAc extracts (each 100 mg/kg, ip) of the roots inhibited the induced elevation of histamine level in the peritoneal cavity fluids, while the MeOH extract (100 mg/kg, ip) showed no effect.

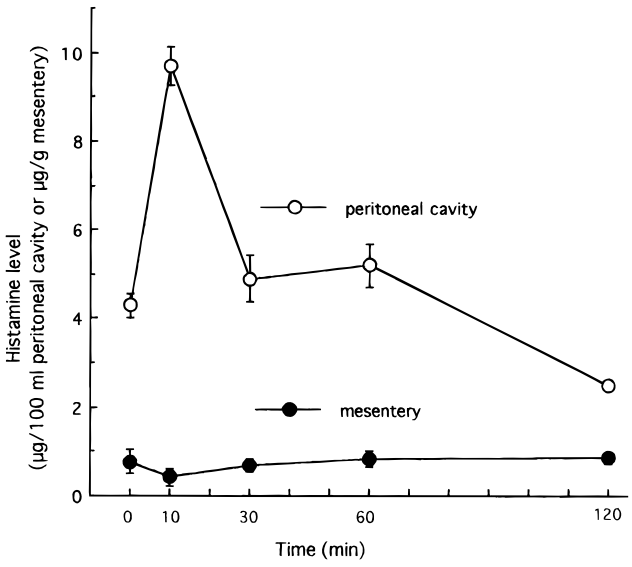

Figure 1. Time-change of histamine level induced by compound 48/80 in the mouse peritoneal cavity. Values are expressed as the mean ± S. E. of six mice.

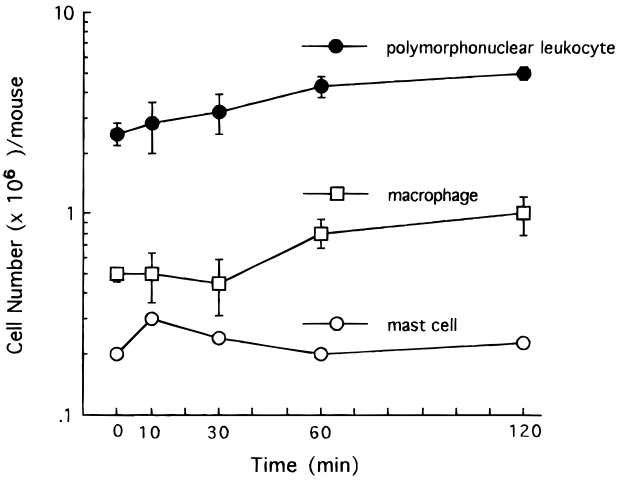

Figure 2. Time-change of cell classification and number induced by compound 48/80 in the mouse peritoneal cavity. Values are expressed as the mean ± S. E. of six mice.

The EtOAc extract (25 g) was purified by chromatography on a column of Si gel (400 g), using *n*-hexane−EtOAc (3:2) as the eluent. The eluate was divided into

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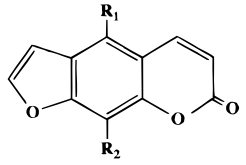
Table 1. Effects of *Angelica dahurica* var. *dahurica* Root Extracts on Compound 48/80-Induced Histamine Level in Mouse Peritoneal Cavity[a]

groups	no. of animals	hitamine contents (μg/100 mL cavity fluids) mean ± S. E.	Δ histamine release[b] (mg/100 mL cavity fluids)	% of control
normal	6	3.80 ± 0.21[c]	0	
compound 48/80 (2.5 mg/kg, ip)	6	12.5 ± 0.98	8.70	100
compd 48/80 + Et_2O extract (100 mg/kg, ip)	6	5.80 ± 0.32[c]	2.00	22.3
compd 48/80 + EtOAc extract (100 mg/kg, ip)	6	4.90 ± 0.87[c]	1.10	12.6
compd 48/80 + MeOH extract (100 mg/kg, ip)	6	15.3 ± 2.49	11.5	132.2

[a] Values are expressed as the mean ± standard error (S. E.) of six mice. [b] ΔHistamine release is expressed as the means of the histamine contents of compound 48/80-treated groups minus the spontaneous histamine contents of normal groups. [c] Significantly different from compound 48/80 -treated groups: $p < 0.01$.



Bergapten (**1**): R_1= OCH_3, R_2= H

Phellopterin (**2**): R_1= OCH_3, R_2= OCH_2-CH=C(CH_3)$_2$

Oxypeucedanin (**3**): R_1= OCH_2-CH(O)-C(CH_3)$_2$, R_2=H

Oxypeucedanin hydrate (**4**): R_1= OCH_2-CH(OH)-C(OH)(CH_3)$_2$, R_2=H

Byakangelicin (**5**): R_1= OCH_3, R_2= OCH_2-CH(OH)-C(OH)(CH_3)$_2$

sec-O-Acetylbyakangelicin (**6**): R_1= OCH_3, R_2= OCH_2-CH($OCOCH_3$)-C(OH)(CH_3)$_2$

Figure 3. Structures of 6 coumarins isolated from the roots of *Angelica dahurica* var. *dahurica*.

fractions 1 (0.6 g), 2 (2.1 g), 3 (4.3 g), 4 (5.4 g), 5 (3.3 g), 6 (2.8 g), and 7 (2.0 g). Fractions 3 (4 g) and 4 (4 g) inhibited compound 48/80-induced histamine elevation in mice (data not shown) and were chromatographed once again on a column of Si gel (100 g) with *n*-hexane−EtOAc (3:2). Bergapten (**1**), phellopterin (**2**), oxypeucedanin (**3**), oxypeucedanin hydrate (**4**), byakangelicin (**5**), and *sec-O*-acetylbyakangelicin (**6**) were obtained from the active fractions and identified by comparison with authentic samples[7,8] (Figure 3). As shown in Tables 2 and 3, bergapten (**1**), oxypeucedanin hydrate (**4**), and byakangelicin (**5**) significantly inhibited compound 48/80-induced histamine elevation in the peritoneal cavity at a dose of 25 mg/kg (ip), but was inactive at a dose of 10 mg/kg. On the other hand, *sec-O*-acetylbyakangelicin (**6**) significantly enhanced compound 48/80-induced histamine elevation at a dose of 25 mg/kg, as compared with the control. Whether *sec-O*-acetylbyakangelicin contributes to the biological effects of the roots of *A. dahurica* var. *dahurica* is in question, since Kozawa *et al.*[7] have reported that *sec-O*-acetylbyakangelicin might be an artifact formed from byakangelicol during extraction with hot EtOAc. This finding suggested that *sec-O*-acetylbyakangelicin does not contribute to the inhibitory effects of the above crude drug on compound 48/80-induced histamine release in mice. Because we could not obtain byakangelicol from this crude drug in this study, the biological effects of byakangelicol on histamine release are unknown. Additionally, further work is needed to clarify the actions of byakangelicol and the mechanisms for action of various coumarins. The number of polymorphonuclear leukocytes, macrophages, and mast cells was not changed by the administration of these coumarins in the peritoneal cavity (data not shown).

Experimental Section

Materials. Compound 48/80 (condensation products of *N*-methyl-*p*-methoxyphenylethylamine with formaldehyde, lot no. C2313) was purchased from Sigma Co. Other chemical reagents were of the highest grade available. Test compounds were suspended in 0.9% NaCl containing 2% EtOH.

Plant Materials. The crude drug "Byaku-shi" (Japanese name) or "Pai-chi" (Chinese name), which is the dried root of *A. dahurica* Benth et Hook. var. *dahurica* Benth et Hook. (Umbelliferae), was purchased from Mikuni & Co., Ltd. (Osaka, Japan), and voucher samples are stored at the 2nd Department of Pharmacognosy, Osaka University, Pharmaceutical Sciences.

Chemical Analysis. IR and ORD spectra were measured on a Shimadzu IR-400 spectrometer and JASCO ORD/UV-5 spectrometer. ^{1}H-NMR (200 MHz) and ^{1}H-NMR (300 MHz) spectra were recorded in $CDCl_3$ and $CDCl_3$ + D_2O on a JEOL-FX and Varian XL-300 spectrometer. Tetramethylsilane was used as internal standard, and chemical shifts are reported on the δ scale (ppm and Hz). Column chromatography was performed using Si gel 60 (70−230 mesh, ASTM, Merck Co.) as the adsorbent. Melting points, determined on a Yamato MP-21 capillary apparatus, were uncorrected.

Animals. Male ddY mice (7 weeks old) weighing 30−35 g, were housed at 25 ± 1°C with 60% relative humidity and given free access to food and water. The room was illuminated for 12 h each day starting at 7:00 am, and the animals were allowed to adapt for 7 days before the experiments were performed.

Measurement of Histamine Content Induced by Compound 48/80 in the Peritoneal Cavity Fluid and Mesentery of Mice. To evaluate the activities of the extracts and compounds isolated from the roots of *A. dahurica* var. *dahurica*, the test materials were administered intraperitoneally to mice (six animals in each groups) 15 min prior to the administration of compound 48/80 (2.5 mg/kg, ip). The mice were killed by decapitation 10 min after the administration of compound 48/80, and the calcium-free Tyrode's solution (pH 7.4, 5 mL/mouse) containing heparin (1 U/mL) was injected into the peritoneal cavity, the abdomen was massaged for 10 s, then the peritoneal cavity fluid was collected from the peritoneal cavity, and mesentery was quickly removed from the small intestine. The cell classification and number were determined by Giemsa staining and light microscopy. Polymorphonuclear leukocytes, macrophages, and mast cells were more than 80% viable as judged by trypan blue exclusion. After the peritoneal cavity fluids were centrifuged at 1630 × *g* for 10 min at 4 °C, histamine in the supernatant fluid was assayed by the method of Shore *et al.*[9] using histamine HCl as a standard.

Table 2. Effects of Bergapten, Phellopterin, and Oxypeucedanin on Compound 48/80-Induced Histamine Level in Mouse Peritoneal Cavity[a]

groups	no. of animals	hitamine contents (μg/100 mL cavity fluids) Mean ± S. E.	Δhistamine release[b] (μg/100 mL cavity fluids)	% of control
normal	6	3.90 ± 0.31[c]	0	
compound 48/80 (2.5 mg/kg, ip)	6	10.0 ± 0.79	6.10	100
compd 48/80 + Bergapten (10 mg/kg, ip)	6	10.2 ± 0.62	6.30	108.2
compd 48/80 + Bergapten (25 mg/kg, ip)	6	7.28 ± 0.87[d]	3.38	55.4
compd 48/80 + Phellopterin (10 mg/kg, ip)	6	10.6 ± 1.13	6.70	109.8
compd 48/80 + Phellopterin (25 mg/kg, ip)	6	12.3 ± 1.03	8.40	137.7
compd 48/80 + Oxypeucedanin (10 mg/kg, ip)	6	12.6 ± 1.75	8.70	142.6
compd 48/80 + Oxypeucedanin (25 mg/kg, ip)	6	12.2 ± 1.00	8.30	136.1

[a] Values are expressed as the mean ± standard error (S. E.) of six mice. [b] ΔHistamine release is expressed as the means of the histamine contents of compound 48/80-treated groups minus the spontaneous histamine contents of normal groups. [c] Significantly different from compound 48/80 -treated groups: $p < 0.01$. [d] Significantly different from compound 48/80-treated groups: $p < 0.05$.

Table 3. Effects of Oxypeucedanin Hydrate, Byakangelicin, and *sec-O*-Acetylbyakangelicin on Compound 48/80-Induced Histamine Level in Mouse Peritoneal Cavity[a]

groups	no. of animals	hitamine contents (μg/100 mL cavity fluids) Mean ± S. E.	Δhistamine release[b] (mg/100 mL cavity fluids)	% of control
normal	6	3.95 ± 0.29[c]	0	
compound 48/80 (2.5 mg/kg, ip)	6	11.4 ± 0.38	7.45	100
compd 48/80 + oxypeucedanin hydrate (10 mg/kg, ip)	6	12.5 ± 1.57	8.55	114.8
compd 48/80 + oxypeucedanin hydrate (25 mg/kg, ip)	6	9.10 ± 0.66[d]	5.15	69.1
compd 48/80 + byakangelicin (10 mg/kg, ip)	6	11.8 ± 0.58	7.85	105.4
compd 48/80 + byakangelicin (25 mg/kg, ip)	6	8.20 ± 0.45[d]	4.25	57.0
compd 48/80 + *sec-O*-acetylbyakangelicin (10 mg/kg, ip)	6	10.9 ± 0.47	6.95	93.2
compd 48/80 + *sec-O*-acetylbyakangelicin (25 mg/kg, ip)	6	15.2 ± 0.52[d]	11.25	151.0

[a] Values are expressed as the mean ± standard error (S. E.) of six mice. [b] ΔHistamine release is expressed as the means of the histamine contents of compound 48/80-treated groups minus the spontaneous histamine contents of normal groups. [c] Significantly different from compound 48/80 -treated groups, $p < 0.01$. [d] Significantly different from compound 48/80-treated groups, $p < 0.05$.

Histamine levels in the mesentery of mice were similarly measured as described above. Briefly, the mesentery was homogenized with calcium-free Tyrode's solution (pH 7.4) at 4 °C, and histamine was measured.

Data and Statistical Analysis. Values are expressed as mean ± standard errors of the mean. Statistical analysis was performed with Student's *t*-test.

Bergapten (Compound 1). Colorless needles were obtained from a mixture of *n*-hexane and EtOAc: mp 192−193 °C (yield, 0.002%). The melting point showed no depression upon admixture with an authentic sample of bergapten. The IR and ^{1}H-NMR spectral data were identical with those reported for bergapten.[7,8]

Phellopterin (Compound 2). Colorless needles were obtained from a mixture of *n*-hexane and EtOAc: mp 102−103 °C (yield, 0.002%). The melting point showed no depression upon admixture with an authentic sample of phellopterin. The IR and ^{1}H-NMR spectral data were identical with those reported for phellopterin.[7,8]

Oxypeucedanin (Compound 3). Colorless needles were obtained from a mixture of *n*-hexane and EtOAc: mp 141−142 °C (yield, 0.005%). The melting point showed no depression upon admixture with an authentic sample of oxypeucedanin. The IR and ^{1}H-NMR spectra were identical with those reported for oxypeucedanin.[7,8]

Oxypeucedanin Hydrate (Compound 4). Colorless needles were obtained from a mixture of *n*-hexane and EtOAc: mp 136−137 °C (yield, 0.01%). The melting point showed no depression upon admixture with an authentic sample of oxypeucedanin hydrate. The IR and ^{1}H-NMR spectra were identical with those reported for oxypeucedanin hydrate.[7,8]

Byakangelicin (Compound 5). Pale yellow needles were obtained from a mixture of *n*-hexane and EtOAc: mp 123−124 °C (yield, 0.25%). The melting point showed no depression upon admixture with an authentic sample of byakangelicin. The IR and ^{1}H-NMR spectra were identical with those reported for byakangelicin.[7,8]

***sec-O*-Acetylbyakangelicin (Compound 6).** Pale yellow needles were from a mixture of *n*-hexane and EtOAc: mp 129−131 °C (yield, 0.025%); ORD(*c* 1.08, EtOH)[α]^{16}(nm) +11.11° (589), +13.90° (550), +27.80° (500), +41.67° (450), +61.12° (410). The IR and ^{1}H-NMR spectra were identical with those reported for *sec-O*-acetylbyakangelicin.[7, 8]

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