Anti-allergic Activities of Anthricin and Its Structure Elucidation

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The present research aimed at obtaining anti-allergic agents from farmhouse-cultivatable wild herbs and farm crops. In clinically applied chemicals, safety concern is far more important than their efficacy. It is therefore a reasonable approach to screen out active principles from natural products and use them as a basis for new drug development. Substances derived through this method will probably have different chemical structures from those developed by foreign companies and thus will be free from patent problems. Cultivation of pharmaceutically useful plants along with the traditional agriculture in farm-houses will clearly provide expectation of prosperous rural economy. A total of 300 samples, extracted with methylene chloride, ethyl acetate, and water from 100 plants, were screened for anti-allergic activity using the rat passive cutaneous anaphylaxis test. When effective extracts were found, compounds were isolated by separation and purification processes, and their structures were determined. From the above screening, CH_2Cl_2 extracts of *Anthriscus sylvestris* showed potent anti-allergic activities. Active single compound was isolated and its structure was determined as follows.

Isolation of anthricin and its biological activity. *Anthriscus sylvestris* was dissolved in 1:1 solution of CH_2Cl_2 and MeOH. After filtration and evaporation, the residue was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was evaporated, and the residue was purified with silica gel column (acetone/n-hexane, 15-20%) to give five fractions which were used for biological tests. The second fraction turned out to be active.

Biologically active single molecule (**AS2-3**) was isolated from the second fraction by successive Sephadex LH20 CC (80% MeOH), reverse phase C18 CC (65% MeOH), MCI gel CHP 20p (90% MeOH), and silica gel column (0.5% MeOH/MC). Pharmacological activities of **AS2-3** as an anti-allergic were examined using *in vivo* models of experimental animals. *In vivo* anti-allergic activity was assessed with passive cutaneous anaphylaxis (PCA) in rats. Male Wistar rats (about 250 g) were immunized i.m. with 1 mg of recrystallized ovalbumin (Sigma, Grayson, Georgia, USA) along with 0.8 ml of pertussis antigen i.p. (Difco, St. Louis, Missouri, USA) as an adjutant. Rat serum containing anti-ovalbumin IgE was taken on day 12 and diluted to a concentration that produces cutaneous Evans blue diffusion of 10-20 mm in diameter by rat PCA test. For PCA test, naive Wistar rats were passively sensitized by intradermal

Table 1. Anti-allergic activities of *Anthriscus sylvestris extracts* by PCA method.

fraction		dose	number of test	results*
CH_2Cl_2 fraction		50 mg/kg, ip	5	55.2 ± 13.9
CH_2Cl_2	fr. 1	15 mg/kg, iv	5	103.4 ± 21.3
	fr. 2	20 mg/kg, iv	4	46.0 ± 109
	fr. 3	30 mg/kg, iv	4	80.6 ± 10.9
	fr. 4	45 mg/kg, iv	5	110.2 ± 11.5
	fr. 5	40 mg/kg, iv	5	112.7 ± 17.5
Fr. 2	AS2-1	15 mg/kg, iv	4	70.8 ± 8.9
	AS2-2	15 mg/kg, iv	5	74.2 ± 8.7
	AS2-3	5 mg/kg, iv	5	59.8 ± 9.5
		15 mg/kg, iv	5	38.8 ± 7.1
	AS2-4	10 mg/kg, iv	4	110.2 ± 25.1

*percent effectiveness (percent reduction in Evans blue diameter) compared to control.

Table 2. ^{1}H-NMR and ^{13}C-NMR data of AS2-3 (anthricin) and their assignments.

no.	δc	Multiplicity (DEPT)	δ_H of directly attached protons (HMQC)	^{13}C assignments
1	174.5	s		13
2	153.8	s		16/18
3	147.6	s		2
4	147.3	s		3
5	139.1	s		17
6	136.9	s		14
7	131.5	s		10
8	129.1	s		9
9	111.0	d	6.59 (s)	4
10	109.8	d	6.7 (s)	15/19
11	108.7	d	6.54 (s)	1
12	101.3	t	5.43 (d, J=11.4Hz)	11
13	71.5	t	3.75 (dd, J=14.6, 8.3Hz)/ 3.15 (dd, J=9.3, 8.3Hz)	12
14	60.5	q	3.89 (s)	21
15	56.3	q	3.61 (s)	20/22
16	47.4	d	2.08 (dd, J=3.9, 4.9Hz)	6
17	44.3	d	2.48 (m)	5
18	32.9	t	2.31 (dd, J=14.6, 5.4Hz)	8
19	32.7	d	2.00 (dd, J=5.4, 11.0Hz)	7

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Abbreviations: DEPT, distortionless enhancement of polarization transfer; PCA, passive cutaneous anaphylaxis.

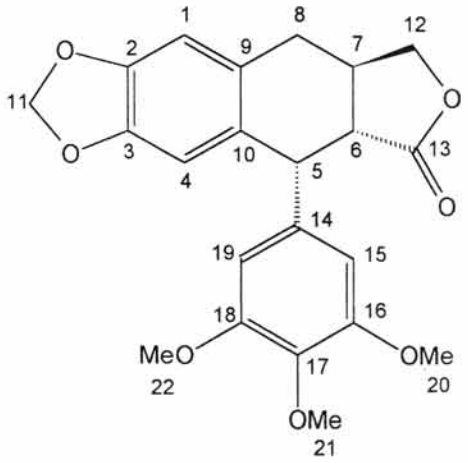

Fig. 1. The Structure of AS 2-3 (Anthricin)

injection (0.1 ml/site, 2 sites) of IgE-containing rat serum. After 48 hr animals were challenged with ovalbumin (5 mg/rat) dissolved in 0.25% Evans blue solution (0.5 ml). Animals were sacrificed 30 min after the ovalbumin challenge, and the degree of dye diffusion was measured. Evans blue in the back skin tissue was also quantified spectrophotometrically at 620 nm according to Katayama *et al.*[1]

Identification of anthricin. Several NMR spectra such as ^{1}H NMR, ^{13}C NMR, Distortionless Enhancement of Polarization Transfer (DEPT),[2] Correlated Spectroscopy (COSY),[3] Homonuclear Hartmann Hahn Spectroscopy (HOHAHA),[4] Heteronuclear Multiple Quantum Coherence (HMQC), and Heteronuclear Multiple Bond Connectivity (HMBC)[5] were collected on Bruker Avance 400 (9.4 T). The existence of aromatic ring was identified based on ^{1}H-NMR and ^{13}C-NMR. Even though the ^{13}C-NMR spectrum showed nineteen peaks, three peaks with double intensity were observed whereby the number of carbons were determined to be 22. The signals shown in the ^{13}C-NMR spectrum are listed in Table 2.

The DEPT experiments revealed the compound included two methyl, three methylene, six methine, and eight quaternary carbons. The attached protons determined by HMQC are listed in Table 2. Using the database supplied by Chapman & Hall,[6] **AS2-3** was identified as anthricin.[7,8] Its structure is shown in Fig. 1.

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