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Anti-Allergic and Anti-Asthmatic Activity of Helioscopinin-A, a Polyphenol Compound, Isolated from *Euphorbia helioscopia*

PARK, KWAN HA[2], DONGSOO KOH[3], SEUNGHO LEE[4], ILLMIN JUNG[5], KYUNG HYUN KIM[6], CHUL-HOON LEE[7], KYE-HOON KIM[8], AND YOONGHO LIM[1]*

[1]*Department of Applied Biology and Chemistry, Konkuk University, Seoul 143-701, Korea*
[2]*Department of Marine Biomedical Sciences, Kunsan National University, Chonbuk 573-702, Korea*
[3]*Department of Applied Chemistry, Dongduk Women's University, Seoul 136-716, Korea*
[4]*College of Pharmacy, Yeungnam University, Kyongpook 712-749, Korea*
[5]*Department of Crop Science, Konkuk University, Seoul 143-701, Korea*
[6]*Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea*
[7]*Department of Medical Genetics and Institute of Biomedical Science, College of Medicine, Hanyang University, Seoul 133-791, Korea*
[8]*Department of Environmental Horticulture, The University of Seoul, Seoul 130-743, Korea*

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Abstract During the course of searching for anti-allergic substances from unexplored plant sources, an inhibitor of leukotriene D_4-induced tracheal contraction was isolated from *Euphorbia helioscopia*. This isolated polyphenol compound, known as helioscopinin-A, showed a certain inhibitory activity on capillary permeability in passive cutaneous anaphylaxis responses of rats and also on antigen-induced bronchial constriction in an experimental asthma model of guinea pigs. The compound at a high concentration weakly inhibited histamine release from isolated mast cells of rats. It is suggested that this compound is an anti-allergic and anti-asthmatic which exerts its activity through antagonism on leukotriene D_4-induced responses. A partial inhibition of allergic mediator release may also be involved.

Key words: *Euphorbia helioscopia*, helioscopinin-A, anti-asthma, anti-allergy

One-hundred-fifty aqueous, ethyl acetate, and methylene chloride extracts derived from 50 different plant species were selected on the basis of Korean traditional medicinal use as anti-allergic or anti-inflammatory agents. Of these, the ethyl acetate fraction of *Euphorbia helioscopia* was found to be active in inhibiting the leukotriene D_4 (LTD_4)-induced contraction of isolated guinea pig trachea in the primary screening.

E. helioscopia, widely distributed in Cheju Island of Korea as well as in Europe, is identified as a biannual herbaceous plant. There are only a few reports on the plant pharmacology of *E. helioscopia*: molluscidal activity [2]; skin irritating activity [24]; and efficacy for chronic bronchitis [11].

To identify the chemical nature of the active principle in *E. helioscopia*, an activity-guided fractionation of crude extract and its partitioned fractions was employed. Pharmacological characterization on the final constituent, helioscopinin-A [17], was performed, and it was found that the compound has dual inhibitory activities on the leukotriene D_4-induced response and on allergic mediator release from mast cells as well.

Materials and Methods

General Analytical Equipments

Mass and NMR spectra were measured with the VG Micromass Autospec and Bruker ARX400 NMR spectrometer, respectively [16, 18, 21, 23].

Plant Material

The whole plants of *E. helioscopia* were collected in 1997 from the farmhouse in the College of Pharmacy, Yeungnam University, Kyongpook, Korea. The botanical identification was carried out by Seungho Lee and the voucher specimen was deposited in College of Pharmacy, Yeungnam University, Kyongpook, Korea.

Extraction and Isolation of the Active Compound

The compound showing LTD_4 antagonism was extracted, separated, and purified from the whole plants of *E. helioscopia*

*Corresponding author
Phone: 82-2-450-3760; Fax: 82-2-453-3761;
E-mail: yoongho@konkuk.ac.kr

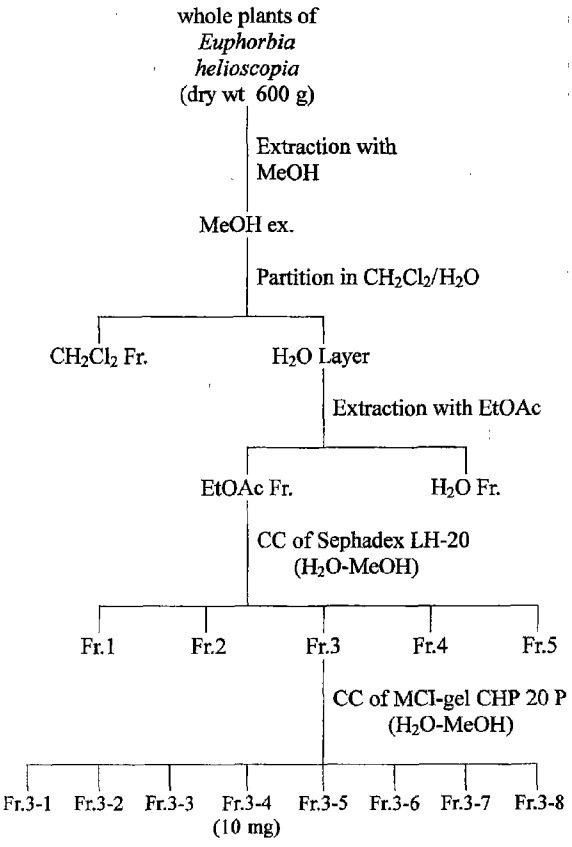

Fig. 1. The procedure for the extraction, separation, and isolation of the active compound.

(dry wt 600 g), as shown in Fig. 1. The final active compound (about 10 mg) was obtained as a yellow crystal.

Identification of the Active Compound

The melting point ranged between 245°C and 247°C. The compound was freely soluble in acetone. Its molecular ion by FAB/MS was identified at *m/z* 953.7 (MH^+). NMR spectra such as ^{1}H-NMR, ^{13}C-NMR, Distortionless Enhancement of Polarization Transfer (DEPT) [8], Correlated Spectroscopy (COSY) [4], Homonuclear Hartmann Hahn Spectroscopy (HOHAHA) [3], Heteronuclear Multiple Quantum Coherence (HMQC) [5], and Heteronuclear Multiple Bond Correlation (HMBC) [6] were collected. Since 72 peaks were observed in the ^{13}C-NMR spectrum, this observation did not agree with the result of MS. However, the ^{1}H-NMR spectrum, indicated that this compound is a mixture of 1:0.8. Therefore, the number of carbons in the compound could not be 72. Only half of these numbers could belong to one isomer. All peaks, except two peaks, were observed between 116.3 and 170.2 ppm and they were singlet peaks based on DEPT, where the compound could be assumed as being one of the polyphenols. This assumption was clarified by the ^{1}H-NMR spectrum which did not show a great number of peaks. Several compounds such as 12-deoxyphorbol-13-phenylacetate-20-acetate [24], hyperoside [1], *m*-hydroxyphenylglycine [19], 3,5-dihydroxyphenylglycine [19], helioscopinin-A [17], and helioscopinin-B [17] have been found in *E. helioscopia*. Among those, NMR and MS data of helioscopinin-A matched with those of our compound. Its structure is shown in Fig. 2.

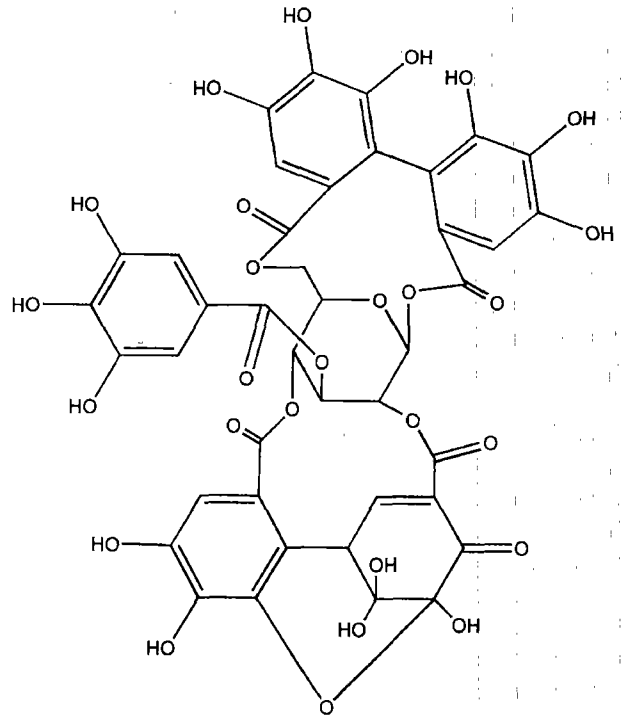

Fig. 2. Chemical structure of the isolated compound, helioscopinin-A.

Primary Screening of LTD_4 Antagonism

Male Hartley guinea pigs weighing 400–500 g were sacrificed by a sharp blow to the head and the trachea was removed. The trachea was opened by cutting along the ventral side, and two strips containing three cartilages each were sutured in parallel. The preparation was bathed in a jacketed 13 ml-organ bath filled with Krebs-Henseleit buffer (in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.6; $NaHCO_3$, 24.9; KH_2PO_4, 1.2; glucose, 11.0; pH 7.4 at 37°C). Contractile change was monitored by connecting the preparation to an isometric transducer and recorded on a chart-strip recorder. The bath was saturated by a continuous supply with 95% O_2 and 5% CO_2. Inhibitory effect of the test compounds on LTD_4 was examined by adding test compounds to the bath after the tracheal contraction with 5 nM LTD_4. Activity was expressed as the relaxant action induced by a test substance at fixed concentrations.

Pharmacology of Helioscopinin-A

Male Wistar rats (250–300 g) were treated with ovalbumin (1 mg, im) and 0.8 ml of pertussis vaccine (Difco, ip), and

IgE-containing serum was obtained 12 days later. Passive cutaneous anaphylaxis (PCA) test was performed with this serum as described by Katayama *et al.* [13]. For mast cell isolation, Wistar rats (male, about 250 g) were intraperitoneously injected with 20 ml of Tyrode solution and abdominal fluid was collected. Mast cells were purified by the Percoll density gradient method [9] and the effect of helioscopinin-A on histamine release from these cells was performed as described by Gomes *et al.* [12]. Inhibitory effect of helioscopinin-A on antigen-induced bronchial constriction was estimated in a passively sensitized asthma model of guinea pigs. Guinea pig anti-serum was prepared by injecting 1 mg of ovalbumin, emulsified in a 0.5 ml Freunds complete adjuvant, once a week for 4 weeks and taking serum 12 days later. Anti-ovalbumin serum (0.5 ml) was intravenously injected to fresh guinea pigs *via* the hind leg vein for production of asthma models [20]. Inhibitory effect of test substance on LTD_4-induced ileal contraction was measured, similarly to the tracheal preparations described above, except for a longitudinal ileal segment (approx. 1.5 cm, 2 g resting tension) instead of sutured tracheal sections.

RESULTS AND DISCUSSION

This study indicates that the polyphenol compound helioscopinin-A isolated from *E. helioscopia* possesses an anti-allergic and anti-asthmatic activity. This activity is explained by both the blockade of LTD_4-induced pharmacological action and a moderate inhibition of allergic mediator release.

Employing our standard assay system with isolated guinea pig trachea to discover inhibitors on LTD_4-induced functional responses, it was possible to isolate helioscopinin-A in a pure form after activity-guided fractionation. The inhibition rate in the overall steps was 43–65% at concentrations of 500–1,000 μg/ml in the fractions which possessed the best possible activity (Table 1). A weak inhibition was also observed in some additional fractions such as Fr-4, Fr-3-5, Fr-3-6, and Fr-3-7. Since we did not pursue any further on these moderately active additional fractions, it was not possible to judge whether this extra activity was caused by unidentified principle(s) or to

Table 1. Primary screening with the extracts of *E. helioscopia* on relaxant effects of LTD_4-induced contraction in isolated guinea pig trachea.

Separation steps (Refer to Fig. 1)	Extract code	Concentration (μg/ml)	Relaxant activity (%)
Positive reference	FPL-55712$^+$	100	57.7±8.1
I	EH-water	1,000	48.8±7.8(*)
	EH-CH_2Cl_2		11.2±6.3
II	EH-EtOAc	1,000	52.3±9.4(*)
	EH-H_2O		10.3±6.2
III	Fr-1	500	0.0±0.6
	Fr-2		0.8±2.4
	Fr-3		43.3±8.1(*)
	Fr-4		15.5±4.5
	Fr-5		4.3±2.6
IV	Fr-3-1	500	0.3±2.2
	Fr-3-2		0.0±0.0
	Fr-3-3		2.6±2.4
	Fr-3-4		61.2±4.8
	Fr-3-5		25.0±6.7
	Fr-3-6		13.0±3.6
	Fr-3-7		10.2±0.4
	Fr-3-8		4.8±0.2

Data as mean±SEM of 3-5 determinations. (*) Fractions used for further separation steps. Fr-3-4: helioscopinin-A. + is known to be the first leukotriene antagonist, which is effective in blocking LTD_4-induced guinea pig tracheal smooth muscle contraction [14].

Table 2. Effects of helioscopinin-A on allergic responses and LTD_4-induced functions.

Tests	Parameters	Helioscopinin-A	n	Effect (% inhibition)
Rat PCA response	Dye diameter	45 mg/kg (i.v.)	5	79.3±7.3
	Dye content			67.5±7.1
Rat mast cell HA release	Compound 48/80-induced	1 mg/ml	7	13.4±2.2
	A 23187-induced			21.3±5.0
Asthmatic bronchial constriction	Maximal inflation pressure increase	30 mg/kg (i.v.)	5	38.7±2.4
LTD_4-induced tracheal contraction		0.1 mg/ml		12.5±0.9
		0.5 mg/ml		54.3±7.4
		1 mg/ml		68.8±5.9
LTD_4-induced ileal contraction		0.1 mg/ml		20.4±7.5
		1 mg/ml		32.2±6.6

Data as mean±SEM of n determinations. PCA: passive cutaneous anaphylaxis; HA: histamine.

helioscopinin-A being incompletely resolved during the separation procedures.

It has been proposed that LTD_4 is one of the most important mediators in allergic asthma [25]. Thus, it may be reasonable to suggest that an agent which possesses LTD_4 antagonism can be a candidate for an asthma therapeutant. However, we cannot presently distinguish whether helioscopinin-A inhibits at the receptor level or at steps ensuing LTD_4-LTD_4 receptor interaction, e.g. the contractile process of the smooth muscle. The test compound of helioscopinin-A also inhibited PCA responses in both dye exudation and tissue dye contents (Table 2). As PCA responses in experimental animals are mediated by several mediators such as histamine, leukotrienes, prostaglandins, serotonin (5-HT), or platelet-activating factor [15], the observed inhibitory effects of helioscopinin-A seem to be contributed both by direct LTD_4 antagonism and release suppression of these mediators.

Most anti-asthmatic agents have limitations in their efficacy, probably due to multiple pathophysiological mechanisms involved in its etiology. As promising anti-asthmatic agents, compounds which have dual action mechanisms may be desirable, since they might be more effective than those with a simple pharmacological mechanism.

Helioscopinin-A exhibited a relatively weaker inhibitory action on the LTD_4 response in the guinea pig ileum than in the trachea. There is a slight difference in LTD_4 receptors between those present in the bronchial smooth muscle and the ileum of guinea pigs [10]. Even though not strictly selective, a compound with organ-specific pharmacologic action is desirable.

Overall, our results suggest that helioscopinin-A may be useful in the therapy of allergic diseases, especially allergic asthma. Since its activity is not potent enough for clinical use, further chemical modification may be necessary.

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