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Leukotriene D_4 Antagonistic Activity of a Stilbene Derivative Isolated from the Bark of *Pinus Koraiensis*

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A phenolic substance was isolated from the bark of *Pinus koraiensis*. It was identified to be pinostilbenoside. Its anti-LTD_4 activity was evaluated using isolated guinea pig trachea, and its half maximal bronchodilating activity (EC_{50}) was 593 ± 56 μg · ml^{-1}. Because it is not known that stilbene derivatives possess anti-asthmatic activity through LTD_4 antagonism, here we report the result.

Key words: Pinus koraiensis, *stilbene derivative, anti-asthmatic, Leukotriene D_4.*

Pinus koraiensis is an evergreen tree and widely distributed throughout the Korean districts. Its seeds are edible, and its flowers, leaves and resin have been used in oriental medicine for *diabetes mellitus*, tuberculosis, and asthma therapy.

LTD_4 is a metabolite derived from the 5-lipoxygenase pathway and accounts for the key physiological response ascribed to slow-reacting substance of anaphylaxis.[1)] LTD_4 is a potent bronchoconstrictor causing the major symptom in allergic asthma and thus that aLTD_4 antagonist would be a novel therapeutic agent for the disease.

Recently, developments for drugs of new type using LTD_4 antagonism are being tried by several pharmaceutical companies. We examined whether *Pinus koraiensis* shows LTD_4 antagonism and it was proved that a compound isolated from the bark of *Pinus koraiensis* has such activity. The compound was identified to be pinostilbenoside, **1**.

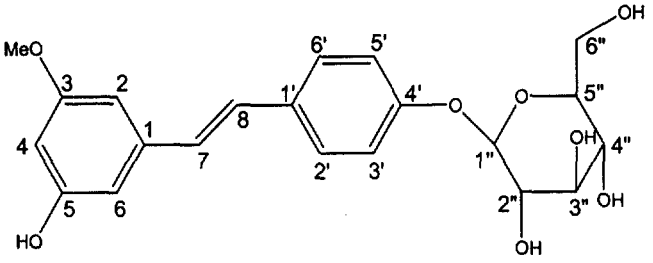

Fig. 1. The structure of the compound.

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Abbreviations: LTD, leukotriene D; DEPT, distortionless enhancement of polarization transfer; COSY, correlated spectroscopy; HOHAHA, homonuclear Hartmann Hahn spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond correlation; FAB/MS, fast atom bombardment mass spectrometry.

Materials and Methods

General analytical equipments. Mass spectrum, and NMR spectra were measured with VG Micromass Autospec and Bruker Avance 400 NMR spectrometer, respectively.

Plant material. A 25 years old *Pinus koraiensis* in Moogap Mt., Kyunggi-Do, Korea was cut in April 1997. After timber cutting its bark was peeled off and dried under the shade. The botanical identification was carried out by Hong Keun Song (one of authors) and the voucher specimen was deposited in the Dept. of Forest and Resources, Konkuk University, Seoul, Korea.

Extraction and isolation of the compound. The bark was ground by Wiley mill. The powdered white pink bark was extracted with acetone : water (7 : 3 v/v). The extract was freeze-dried and dissolved in minimum amount of water. The resulting aqueous extract was extracted with $CHCl_3$ (3300 m*l*) and EtOAc (3300 m*l*), successively the EtOAc extract (1 g) was dissolved in EtOH : H_2O (9 : 1 v/v, 1 m*l*) and chromatographed on a sephadex LH-20 column (2.045 cm) eluted with 99.8% EtOH (150 + 500 m*l*). After eluting 150 m*l* of EtOH, each 100 m*l* of EtOH was collected by gravity (fraction I, II, III, IV, V). The fraction III was purified on a Toyo-pearl TSK gel-40F column (2.67 cm) and eluted with ethanol : H_2O (9 : 1 v/v), at a flow rate of 1.5 m*l*/min. The eluents were collected for every minute and detected by 280 nm of UV. The fraction between 33 and 35 min, was developed in EtOAc : $CHCl_3$: 88% formic acid (6 : 3 : 1 v/v) for silica gel TLC and developed in two dimensional TLC in *tert*-buthanol : acetic acid : water (3 : 1 : 1 v/v) and 6% acetic acid for cellulose TLC. It gave a blue fluorescent spot under UV light (366 nm). The value of R_f was 0.28 on silica gel, TBA 0.64, 6% AcOH and 0.21 on cellulose.

Identification of the compound. The compound is freely soluble in MeOH. Its molecular ion by FAB/MS was identi-

Table 1. The NMR data and assignments of the compound.

$\delta^{13}C$	CHn[a]	$\delta^{1}H(J_{Hz})$[b]	HMBC	COSY	Assignments
55.84	q	3.75(s)	-		$-OCH_3$
61.57	t	3.49, 3.71	-	H6"a/H6"b, H5" H6"b/H6"a, H5"	C6"
70.59	d	3.19(m)	-	H4"/H3", H5"	C4"
74.11	d	3.26(m)	C2"/H3"	H2"/H1", H3"	C2"
77.48	d	3.28(m)	C3"/H4", H2"	H3"/H2", H4"	C3"
77.93	d	3.34(m)	-	H5"/H6"a, H6"b, H4"	C5"
101.15	d	4.89(d 7.4)	C1"/ H2"	H1"/H2"	C1"
101.52	d	6.25(t 2.2)	C4/H6(H2)	H4/H2(H6), H6(H2)	C4
103.57	d	6.60(t 1.33, 2.04)	C2(C6)/H7	H2(H6)/H4	C2(C6)
106.89	d	6.56(t 1.64 1.55)	C6(C2)/ H7	H6(H2)/H4	C6(C2)
117.29	d	7.03(d 8.8)	-	H3', H5'/H2', H6'	C3', C5'
127.78	d	6.98(d 16.4)	C7/H2(H6)	H7/H8	C7
128.50	d	7.52(d 8.8)	C2', C6'/H8	H2', H6'/H3', H5'	C2', C6'
128.80	d	7.1(d 16.4)	C8/H2', H6'	H8/H7	C8
131.62	s	-	C1'/H7', H3', H5'	-	C1'
140.05	s	-	C1/H8	-	C1
157.93	s	-	C4'/H2', H6'	-	C4'
159.46	s	-	-	-	C5
161.50	s	-	C3/$-OCH_3$	-	C3

[a]data determined by DEPT.
[b]data determined by HMQC.

fied at m/z 405.5 (MH^+). NMR spectra such as ^1H NMR, ^{13}C NMR, DEPT,[2] COSY,[3] HOHAHA,[4] HMQC,[5] and HMBC[6] were collected in DMSO-d_6. The data and their assignments are listed in Table 1.[7]

Pharmacology 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 m*l*-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 bubbling 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 tracheal contraction with 5 nM LTD_4 was attained. Activity was expressed as the concentration to induce 50% relaxation (ED_{50}).

Results and Discussion

It has been observed that a series of natural or synthesized stilbene derivatives were active in isolated trachea, receptor binding and experimental asthma models as well. Since a partial structure of the compound, stilbene, is common in the above-mentioned related compounds which possess LTD_4 receptor antagonistic activity, we examined its activity against LTD_4-induced contraction of isolated guinea pig trachea. It was observed that compound is a weak, but unique inhibitor against the contraction induced by LTD_4 in trachea.

Table 2. The inhibitory activity of the compound in isolated guinea pig trachea.

Test substance	n	anti-LTD_4 activity[a]
The compound	5	539 ± 56 μM
Resveratrol	5	52 ± 7 μM

[a]EC_{50}
n: number of test set.

The inhibitory activity of the compound in isolated guinea pig trachea was weak as shown in Table 2. The reference compound, another known stilbene derivative being found from various plants, resveratrol, was at least 10 times more potent than the current compound in antagonizing the contraction evoked by LTD_4.

It is currently accepted that LTD_4 plays an important role in the pathological process of allergic asthma and thus that a LTD_4 antagonist can be a novel candidate for asthma therapy. Some stilbene compounds including resveratrol are feasible anti-allergic agents applicable to asthma therapy. Although we did not test in the present study, it is very likely that the present compound would possess anti-asthmatic activity.

From this and previous experiments, it is postulated that the intrinsic LTD_4 antagonistic activity of stilbene nucleus is markedly diminished by the glycosidic substitution at the 4-position. From earlier days some parts of *Pinus koraiensis*

have been utilized as an anti-asthmatic folkloric medicine. The weakly active pinostilbenoside might be one of the active principles in the plant which exert anti-asthmatic action.

In summary, this paper describes a unique stilbene LTD_4 antagonist isolated from an evergreen plant, *Pinus koraiensis*. Although its activity is very weak, it is significant in that we identified an LTD_4 antagonist composed of a chemical structure unrelated to any reported LTD_4 antagonists. The observed anti-LTD_4 activity may explain why this plant has been efficaciously used for asthma patients in oriental countries.

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