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Isolation of Angiotensin Converting Enzyme Inhibitors from Compositae Plants

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Abstract

Thirty plants of the Compositae family were screened for inhibitory activity of angiotensin converting enzyme (ACE). Among them, *Chrysanthemum boreale* Makino and *Ixeris dentate* Nakai were selected for further investigation since they had the highest inhibitory activity. Crude water extracts of the flowers of *Chrysanthemum boreale* Makino and the roots of *Ixeris dentate* Nakai were prepared by heating at 95°C and 60°C for 2 hr, respectively, followed by centrifugation at 8000×g for 30 min. Crude extracts were then filtered using YM-3 and YM-1 membranes. The ACE inhibitors were isolated using consecutive chromatographic methods including: Sephadex G-15, ion exchange, FPLC, and reverse phase HPLC. The inhibitors were identified to have molecular masses of 204 and 283 daltons, respectively, by mass spectrometry. These results demonstrate that crude extracts of Compositae plants may be useful as functional food ingredients with anti-hypertensive properties.

Key words: ACE inhibitors, chromatography, Compositae plants, mass spectrometry

INTRODUCTION

Angiotensin converting enzyme (ACE, peptidyldipeptide hydrolase, EC3.4.15.1) converts angiotensin I into angiotensin II by cleaving the C-terminal dipeptide (His-Leu) of angiotensin I. ACE also inactivates bradykinin, a potent vasodilative peptide with hypotensive effects. The first ACE inhibitor was reported as a natural peptide isolated from a Brazilian snake's venom (1,2). Using this peptide as a model system, specific ACE inhibitors such as captopril and enalapril have been synthesized and commercially used in the treatment of hypertension and prevention of chronic heart failure. However, these synthetic drugs have side effects such as cough, taste disturbances, and skin rash. Therefore, various food sources (3-12), which were generally recognized as safe, were screened for ACE inhibitory activity. Compositae plants have been used in oriental medicine in Korea. To assess the efficacy of Compositae plants for hypertension, extracts of Compositae plants were prepared and their ACE inhibitory activities were measured and screened. We report here the isolation of ACE inhibitors from *Chrysanthemum boreale* Makino and *Ixeris dentate* Nakai.

MATERIALS AND METHODS

Materials

Freshly harvested *Chrysanthemum boreale* Makino and *Ixeris dentata* Nakai were purchased in Gongju and Yangpyung, Korea, respectively.

Preparation of crude extracts

Crude water extracts of the flowers of *Chrysanthemum boreale* Makino and the roots of *Ixeris dentate* Nakai were obtained by steeping in water at 95°C and 60°C for 2 hr, respectively, followed by centrifugation at 8,000 ×g for 30 min.

Isolation of an ACE inhibitor

Crude extracts were filtered using a series of YM-3 (Mw 3000 cut-off) and YM-1 (Mw 1000 cut-off) membranes. The membrane-filtered solutions were subjected to consecutive chromatographic separations using, Sephadex G-15, ion exchange, FPLC, and normal and reverse phase HPLC. For *Chrysanthemum boreale* Makino, the membrane-filtered solution was loaded on Sepahdex G-15 (1.5 ×100 cm) pre-equilibrated with phosphate buffer (10 mM, pH 7.0). The eluate was monitored by the absorbance at 254 nm. The highest inhibitory fraction was further purified using a normal phase HPLC amino column (Shiseido capcell pak, 4.6×250 mm). The elution was performed on the condition of solvent A (97% acetonitrile containing 0.1% trifluroacetic acid (TFA)) and solvent B (30% acetonitrile containing 0.1% TFA), having a gradient of 0% to 30% at 0.5 mL/min. The highest inhibitory fraction was further purified using FPLC with an anion exchange column (Resource® RPC, Amersham Pharmacia Co.). The column was equilibrated with a 20 mM Tris-HCl

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buffer (pH 8.0) and eluted with linear gradient of NaCl in the buffer from 0 to 1 M at a flow rate of 0.5 mL/min. Finally, to further purify an ACE inhibitor, the highest inhibitory fraction from the ion exchange chromatography was loaded onto the reverse phase HPLC with a C_{18} column (μBondapak C_{18}, 3.9 mm×300 mm, Waters Co.). The elution was performed on the condition of solvent A (0.1% trifluroacetic acid, TFA) and solvent B (acetonitrile containing 0.1% TFA), having a gradient of 0% to 20% at 0.3 mL/min.

For *Ixeris dentate* Nakai, the membrane-filtered solution was loaded onto FPLC with a Resource® RPC column (6.5 mm×100 mm, Amersham Pharmacia Co.). FPLC was performed on the condition of solvent A (0.1% trifluroacetic acid, TFA) and solvent B (acetonitrile containing 0.1% TFA), having gradient of B from 0% to 50%. The eluate was monitored by the absorbance at 214 nm. The highest inhibitory fraction from FPLC was further purified using anion exchange chromatography with a Resource® Q column (6.4 mm×30 mm, Amersham Pharmacia Co.). The column was equilibrated with 20 mM Tris-HCl buffer (pH 8.0) and eluted with a linear gradient of NaCl in the buffer from 0 to 0.2 M at a flow rate of 0.5 mL/min. Finally, to further purify an ACE inhibitor, the highest inhibitory fraction from anion exchange chromatography was loaded onto the reverse phase HPLC with a C_{18} column (μBondapak C_{18}, 4.6 mm×250 mm, Waters Co.). The elution was performed on the condition of solvent A (0.1% trifluroacetic acid, TFA) and solvent B (ethanol containing 0.1% TFA), having a gradient of 30% to 32% at 0.2 mL/min.

ACE assay

ACE inhibitory activity was measured by the method of Cushman and Cheung (13) with modifications established in our laboratory (4-8). The reaction mixture contained 150 μL of 5 mM Hip-His-Leu as a substrate, 50 μL of rabbit lung ACE powder (5 munit) in a 50 mM sodium borate buffer (pH 8.3), and 50 μL of the sample solution. The reaction was carried out at 37°C for 30 min, and terminated by adding 250 μL of 1 N HCl, and 1 mL of ethylacetate. After centrifugation, the absorbances of the supernatants were measured at 228 nm.

Mass spectrometry

The molecular mass of the purified ACE inhibitor was determined using an ESI tandem mass spectrometer (JEOL, JMS HX-110A, Japan).

RESULTS AND DISCUSSION

Preparation of crude extracts

Thirty Compositae plants were screened for ACE inhibitory activity. Among them, *Chrysanthemum boreale* Makino and *Ixeris dentate* Nakai had the highest inhibitory activity. Each part of the plants was examined for ACE inhibitory activity. The flowers of *Chrysanthemum boreale* Makino and the roots of *Ixeris dentate* Nakai had the highest inhibitory activity (data not shown). Crude extracts were serially membrane-filtered using YM-3 and YM-1 membranes. Noh and Song (8) previously reported that membrane-filtration with a 1000 dalton MW cut-off could be a useful processing method for the purification of ACE inhibitors, since most potent ACE inhibitory peptides have molecular weights below or around 1000 daltons (3).

Isolation of an ACE inhibitor from *Chrysanthemum boreale* Makino

YM-1 membrane-filtered solution of crude extracts of *Chrysanthemum boreale* Makino was concentrated and loaded onto Sephadex G-15 column, and resolved into five fractions (Fig. 1). Among them, the F1 fraction exerted the highest inhibitory activity of 45.2% (Table 1). The F1 fraction was then pooled and loaded onto normal phase HPLC with amino column for further purification. There were five major peaks eluted from the column (Fig. 2). The F13 fraction, having the highest inhibitory activity (52.12%), was pooled and subjected to ion exchange chromatography with Resource® RPC column. F133 fraction, having the highest inhibitory activity (56.45%), from the column was then pooled (Fig. 3) and further purified.

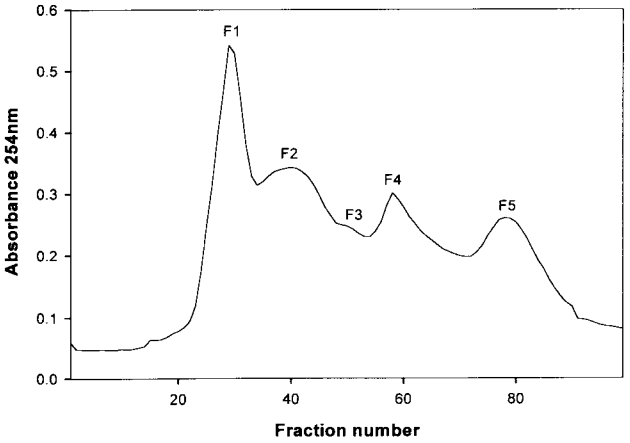

Fig. 1. Elution profile of GPC using crude extracts of *Chrysanthemum boreale* Makino.

Table 1. ACE inhibitory activity of fractions isolated from crude extracts of *Chrysanthemum boreale* Makino

Purification step	ACE inhibition (%)
Membrane filtration	41.12
GPC (F1)	45.20
Normal phase HPLC (F13)	52.12
Anion exchange (F133)	56.45
Reverse phase HPLC	63.23

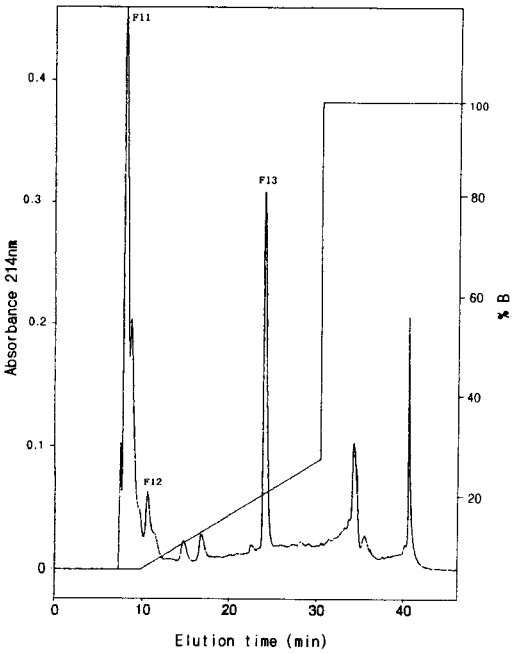

Fig. 2. Elution profile of normal phase HPLC using F1 in Fig. 1.

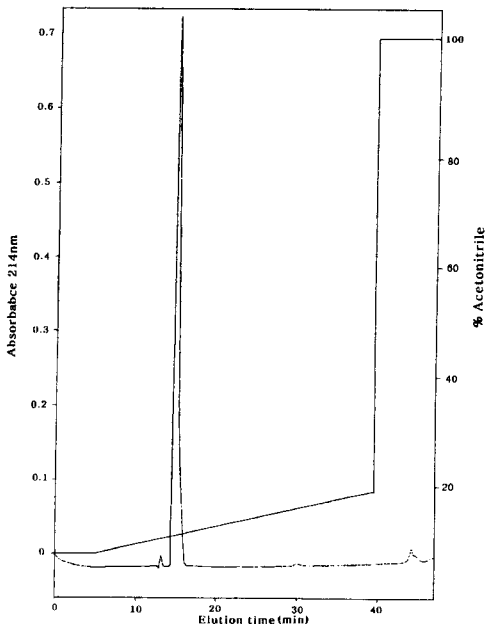

Fig. 4. Elution profile of reverse phase HPLC using F133 in Fig. 3.

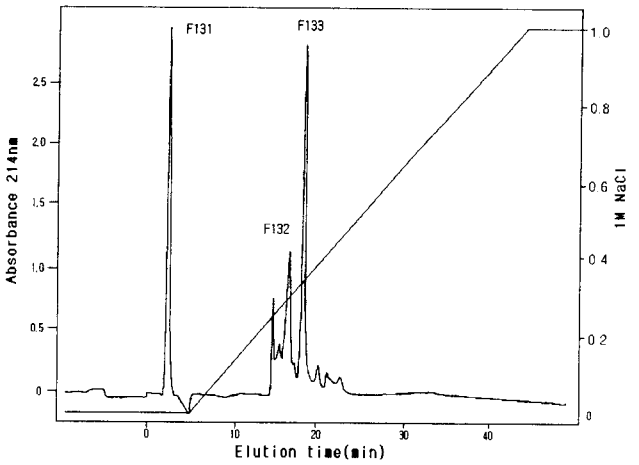

Fig. 3. Elution profile of anion exchange chromatography using F13 in Fig. 2.

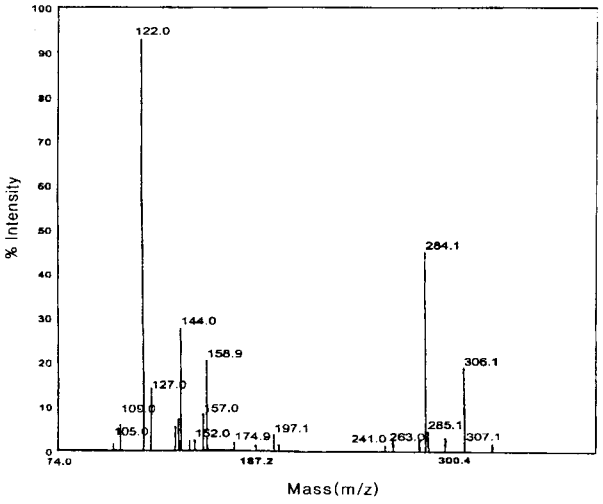

Fig. 5. Mass spectrum of the purified ACE inhibitor from *Chrysanthemum boreale* Makino.

Final purification of the ACE inhibitor utilized reverse phase HPLC with a μBondapak C_{18} column, from which a single peak was obtained (Fig. 4). The ACE inhibitor was identified using an ESI tandem mass spectrometer. The ACE inhibitor was determined to have a molecular mass of 283 daltons (Fig. 5). This low molecular weight molecule has not been previously reported, although there have been studies on ACE inhibitors isolated from plant sources (3,8). Also, this is the first report of an ACE inhibitor isolated from *Chrysanthemum boreale* Makino. ACE inhibitory activity was predictable in crude extracts of *Chrysanthemum boreale* Makino, since the flowers have been traditionally used as an oriental medicine treatment for patients having hypertension in Korea.

Isolation of an ACE inhibitor from *Ixeris dentate* Nakai

YM-1 membrane-filtered solution of *Ixeris dentate* Nakai extract was concentrated and loaded onto FPLC with a Resource® RPC column and resolved into five major fractions (Fig. 6). Among them, the F4 fraction exerted the highest inhibitory activity of 66%, while the F2 fraction had an inhibition of 45% (Table 2). F4 fractions were then pooled and separated by anion exchange column chromatography for further purification. There were three major peaks eluted from the column (Fig. 7). The F42 fraction had the highest inhibitory activity and was eluted at a concentration of 0.1 M NaCl. Finally, to purify the ACE in-

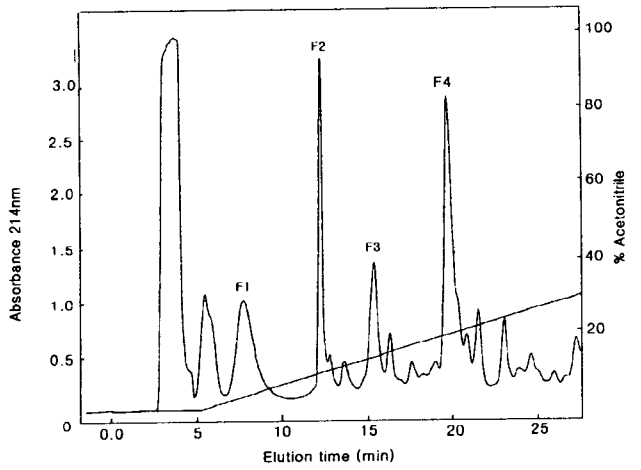

Fig. 6. Elution profile of FPLC using crude extracts of *Ixeris dentate* Nakai.

Table 2. ACE inhibitory activity of fractions isolated from crude extracts of *Ixeris dentate* Nakai

Purification step	ACE inhibition (%)
Membrane filtration	49.35
FPLC (F4)	65.82
Anion exchange (F42)	71.20
Reverse phase HPLC	84.16

hibitor, reverse phase HPLC with a μBondapak C_{18} column was used and a single peak was detected at the 31% ethanol gradient (Fig. 8). Purification was achieved using an ethanol gradient instead of acetonitrile, indicating that the change in solvent polarity could improve the purification of the inhibitor. The ACE inhibitor was identified using an ESI Tandem mass spectrometer. The ACE inhibitor was determined to have a molecular mass of 204 daltons (Fig. 9).

This is the first report of the isolation of ACE inhibitors from Compositae plants, although there are several other

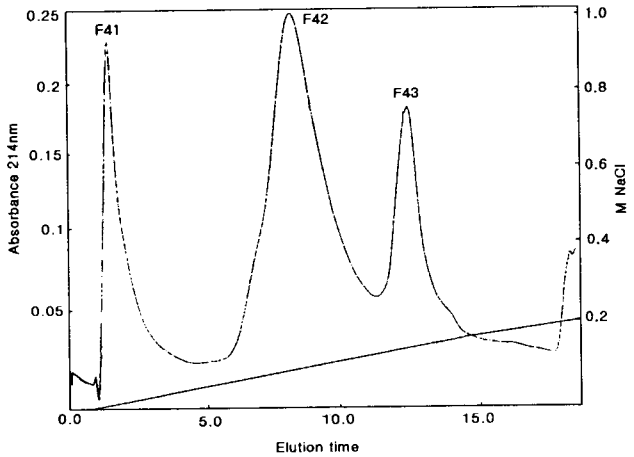

Fig. 7. Elution profile of anion exchange chromatography using F4 in Fig. 6.

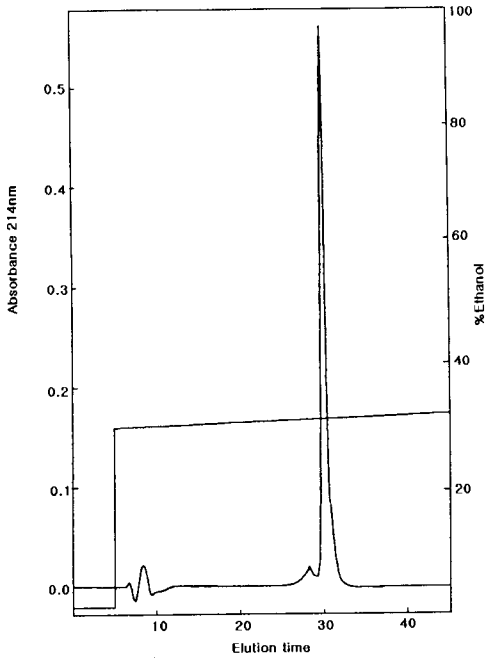

Fig. 8. Elution profile of reverse phase HPLC using F42 in Fig. 7.

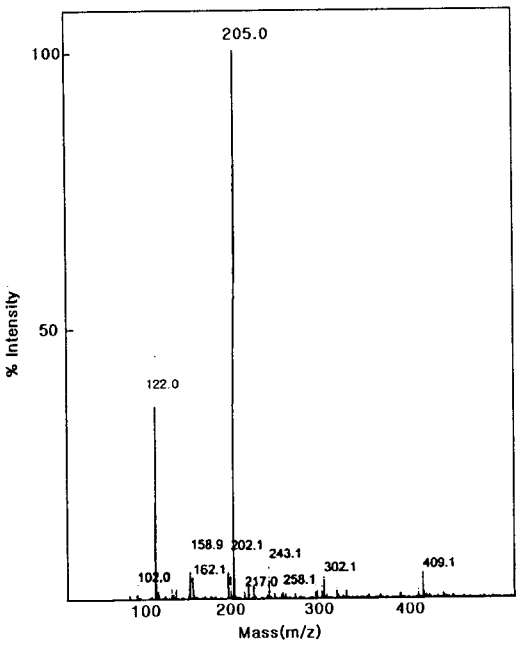

Fig. 9. Mass spectrum of the purified ACE inhibitor from *Ixeris dentate* Nakai.

reports of ACE inhibitors isolated from different herbal products (3,8). Although *in vivo* experiments using spontaneously hypertensive rats (SHR) are needed; the purified inhibitors of small molecular weight inhibitors from Compositae plants are quite promising as active ingredients in the manufacture of functional food beverages for the treatment of hypertension. The simplicity of processing is especially attractive since membrane filtration with a 1000 dalton mo-

lecular weight cut-off can produce highly purified and concentrated ACE inhibitor. Further characterization of the inhibitor and the development of manufacturing processes, that preserve activity and produce efficacious extracts, are subjects of ongoing investigation.

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