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Note

Antioxidative and Antihyaluronidase Activities of Some Constituents from Foeniculi Fructus (Fruit of *Foeniculum vulgare* MILLER)

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The inhibitory effects of some constituents which were previously isolated from the MeOH extract of Foeniculi Fructus (Fruit of *Foeniculum vulgare* MILLER) were investigated on the oxidation of linoleic acid and on the activation of inactive hyaluronidase induced by compound 48/80. Among the test compounds, six stilbene trimers, miyabenol C, *cis*-miyabenol C, foeniculoside I, foeniculoside II, foeniculoside III and foeniculoside IV, exhibited greater antioxidative activities than BHA. Furthermore, miyabenol C and *cis*-miyabenol C showed strong hyaluronidase inhibitory effects.

Keywords: antioxidative activity, antihyaluronidase activity, Foeniculi Fructus, *Foeniculum vulgare*, miyabenol C, foeniculoside

Recently, it is becoming recognized that active oxygen species and free radicals cause various human diseases such as cancer (Yagi, 1987; Yoshikawa *et al.*, 1994). It was also reported that some antiallergic drugs showed antioxidative activities due to scavenging of superoxide radicals (Yoshikawa *et al.*, 1989). Because hyaluronidase is related to histamine release from mast cells, the inhibitory effect of this enzyme is one of the indexes of the anti type I allergy (Sakamoto *et al.*, 1980; Kakegawa *et al.*, 1985).

Foeniculum vulgare MILLER (Umbelliferae) is widely cultivated around the world, and its fruit (Foeniculi Fructus) is used as flavoring, spice and in folk medicine (Mabey *et al.*, 1990).

During the course of screening for the antioxidative compounds from various crude drugs and edible plants (Ono *et al.*, 1995a), the MeOH extract of Foeniculi Fructus was found to show strong activity.

In the preceding paper, we reported the isolation and structure elucidation of eighteen compounds, miyabenol C (**1**), *cis*-miyabenol C (**2**), foeniculoside I (**3**), foeniculoside II (**4**), foeniculoside III (**5**), foeniculoside IV (**6**), icariside F_2 (**7**), syringin (**8**), sinapyl alcohol 1,3′-di-*O*-*β*-D-glucopyranoside (**9**), *threo*-anethole glycol (**10**) and *erythro*-anethole glycol (**11**), zizibeoside I, adenosine, and foeniculosides V-IX from the MeOH extract of Foeniculi Fructus (Ono *et al.*, 1995b, 1996) (Fig. 1). This paper deals with the antioxidative activities of **1**-**11** and the inhibitory effects of **1**-**6** on the activation of inactive hyaluronidase induced by compound 48/80 (Kakegawa *et al.*, 1985).

Materials and Methods

Hyaluronidase (from bovine testis, Type IV-S, 760 units/mg, Lot 100H8270) and compound 48/80 were purchased from Sigma Chemical Co., St. Louis, MO, USA and other reagents were purchased from Nacalai Tesque, Inc., Kyoto.

Isolation and structure elucidation of test samples The details of the isolation and structure elucidation of the test samples were reported in the preceding papers (Ono *et al.*, 1995b, 1996).

Powdered fruit was extracted with MeOH under reflux. The MeOH extract was defatted with *n*-hexane to afford an *n*-hexane soluble fraction (fr. 1) and a residue (fr. 2). Fraction 2 was subjected successively to Diaion HP 20, silica gel, Sephadex LH 20 column chromatographies and high performance liquid chromatography to give **1**-**11**.

Assay of antioxidative activity The antioxidative activity of the test samples was evaluated based on the ferric thiocyanate method as described in the previous paper (Ono *et al.*, 1995a).

Assay of antihyaluronidase activity Test samples were dissolved in dimethylsulfoxide, and each solution was diluted with 0.1 M acetate buffer (pH 4.0) to ten volumes. Hyaluronidase, hyaluronic acid potassium salt and compound 48/80 were dissolved with the same buffer. Hyaluronidase activity was determined by the method of Kakegawa *et al.* (1985).

Results and Discussion

Antioxidative activities of MeOH extract, fr. 1 and fr. 2 The MeOH extract of Foeniculi Fructus showed a stronger antioxidative activity than *t*-butylhydroxyanisole (BHA), which is a synthetic antioxidant, using linoleic acid as the substrate by the ferric thiocyanate method (Fig. 2). Furthermore, fr. 2 indicated a stronger antioxidative activity than fr. 1 (Fig. 3).

Antioxidative activities of ***1***-***11*** The antioxidative

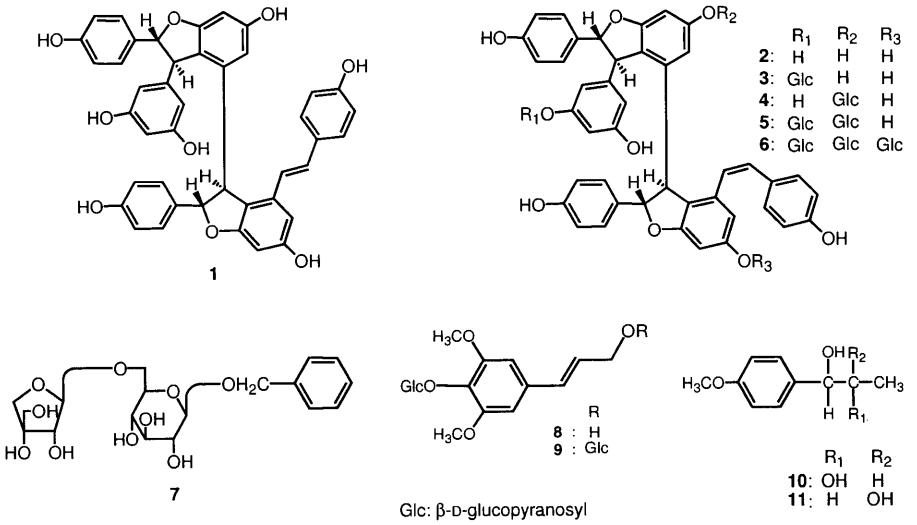

Fig. 1. Structures of **1**–**11**.

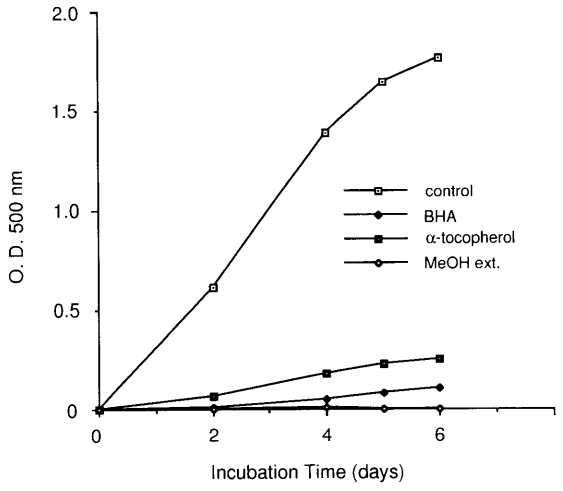

Fig. 2. Antioxidative activity of MeOH ext.

activities of **1**–**11** from fr. 2 were investigated as the same manner as that for the MeOH extract. As a result, **1**–**6** showed stronger antioxidative activities than BHA (Fig. 3), and these activities were presumed to come from phenolic hydroxyl groups in **1**–**6** (Kikuzaki & Nakatani, 1993). However, the antioxidative activity of fr. 2 was stronger than those of **1**–**6**. This might be due to a synergistic effect.

Antihyaluronidase activities of 1–6 Compounds **1**–**6** were assayed for their inhibitory effects on the activation of inactive hyaluronidase induced by compound 48/80 (Kakegawa *et al.*, 1985). The inhibition ratios of **1**–**6** were 100, 98, 41, 37, 8 and 3%, respectively, at a final concentration of 0.4 mM. These inhibitory effects appeared to decrease with the number of glucose groups attached to the phenolic hydroxyl groups in **2**. Therefore, it was presumed that the inhibitory effects on hyaluronidase of **1**–**6** were related to the number of phenolic hydroxyl groups. Especially, the IC_{50} values of **1** and **2** were estimated to be 135 μM (92 μg/ml) and 60 μM (41 μg/ml), respectively (Fig. 4). Maeda *et al.* (1991) reported that the

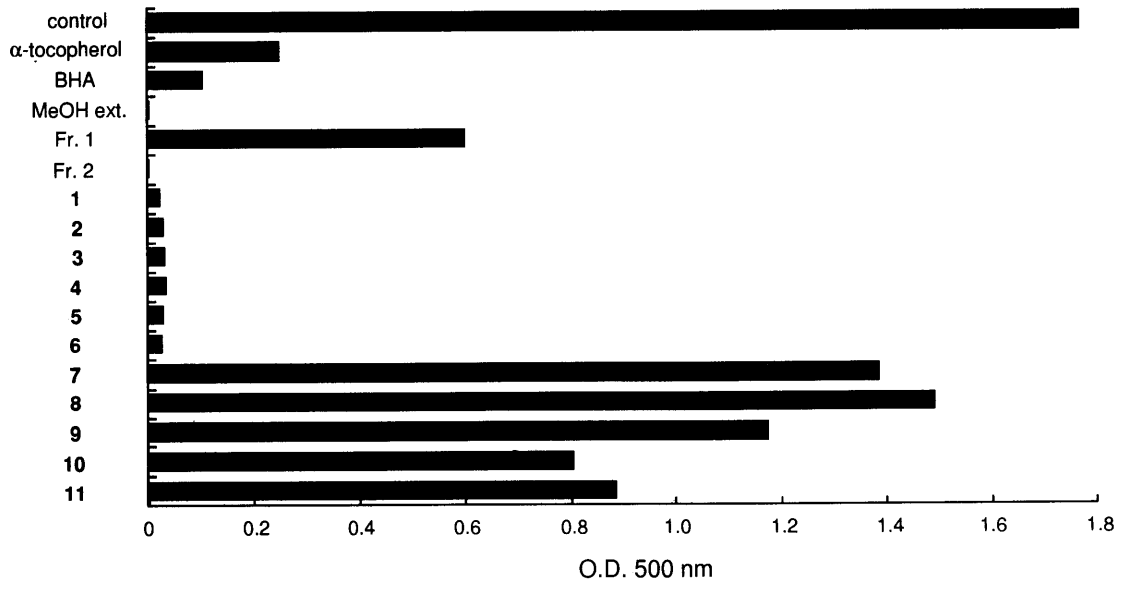

Fig. 3. Antioxidative activities on the 6th day of the lipid peroxidation.

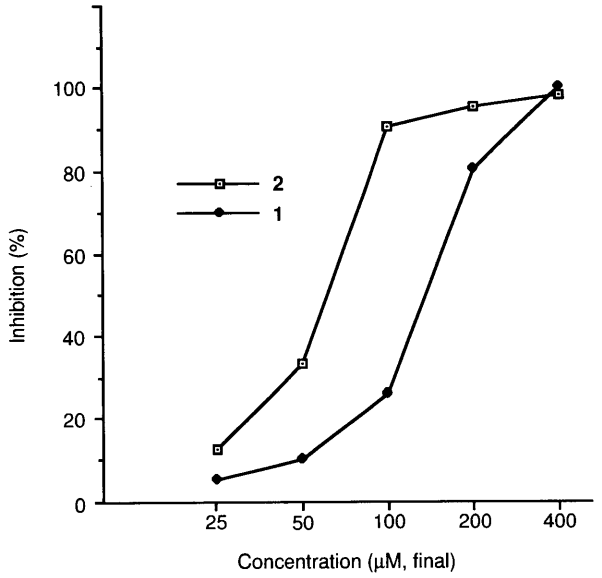

Fig. 4. Inhibitory effects of **1** and **2** on the activation of hyaluronidase.

IC_{50} value of the hyaluronidase inhibitory effect of an antiallergic agent, disodium chromoglycate (DSCG), was 110 μg/ml in the same manner as in this study. Therefore, **1** and **2** seemed to have almost the same hyaluronidase inhibitory activities as DSCG.

Although Kurihara *et al.* (1991) reported that **1** showed antibacterial activity against *Staphylococcus aureus* by the paper disk method, this is the first report on the antioxidative and antihyaluronidase effects of **1**–**6**.

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