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Inhibitory Effect of Hexane Fraction from *Myagropsis myagroides* on Pancreatic α-Amylase *In Vitro*

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A *Myagropsis myagroides* (Mm) methanol extract showed α-amylase inhibitory activity of 13% at a concentration of 5 mg/ml. Results showed that the hexane fraction from the Mm methanol extract exhibited α-amylase inhibitory activity with an IC_{50} value of 4.24 mg/ml. The hexane fraction was separated using silica-gel column chromatography, and six subfractions were obtained. The fraction eluted with $CHCl_3$:MeOH = 50:1 showed the highest α-amylase inhibitory activity with an IC_{50} value of 0.72 mg/ml. This fraction was purified using Sephadex LH-20 column chromatography and an octadecyl silica (ODS) Sepak cartridge, obtaining seven subfractions. Fraction (Fr.) 4 also showed a strong α-amylase inhibitory activity with an IC_{50} value of 0.75 mg/ml. Fr. 4 was purified by Sephadex LH-20 column chromatography and ODS Sepak cartridge, obtaining six subfractions. Fr. 4-2 was identified as sargachromanol I with an IC_{50} value of 0.40 mg/ml, and the inhibition pattern analyzed from Lineweaver-Burk plots revealed it to be an uncompetitive inhibitor. These results suggest that Mm has potential as a natural antidiabetes agent.

Keywords: α-Amylase, *Myagropsis myagroides*, sargachromanol I

Introduction

α-Amylases (α-1,4-glucan-4-glucanohydrolase, E.C. 3.2.1.1) catalyze the hydrolysis of α-D-(1,4) glycosidic linkages in starch and glycogen [7, 29]. α-Amylases (α-1,4-glucan-4-glucanohydrolase, E.C. 3.2.1.1) are widely distributed in plants, mammal tissues, and microorganisms. Starch is hydrolyzed into glucose by α-amylase in the intestine and maltase in the small intestine, which is more easily absorbed in the body as carbon and energy sources [7, 15]. However, elevated amylase may cause high blood sugar and insulin levels, leading to diabetes, obesity, and acidosis [23, 24]. Inhibitors of α-amylase affect the α-amylase kinetics of glucose absorption and blood glucose distribution by slowing the rate of starch digestion, resulting in an increase in the effect of dietary treatment of patients with diabetes mellitus or obesity due to a disorder of carbohydrate metabolism [21, 22].

Acarbose is a well-known α-amylase inhibitor with potent α-amylase inhibitory activity. However, acarbose has undesirable side effects, especially flatulence and diarrhea, and its safety has become questionable [22]. Therefore, researchers are seeking α-amylase inhibitors from natural products that have fewer side effects. An α-amylase inhibitor isolated from buckwheat was first reported by Chrzaszcz and Janicki [5]. Other researchers have also reported α-amylase inhibitors from terrestrial plants such as wheat [11], walnut [33], rye [8], and the bacterial strains *Streptomyces* sp. [10, 25–28], *Cladosporium* sp. [32], and *Actinoplanes* sp. [32]. However, there are few studies on α-amylase inhibitors isolated from seaweeds. Some α-amylase inhibitors isolated from *Ishige okamurae* [12], *Ecklonia cava* [20], and *Eisenia bicyclis* [4] have been reported.

The brown seaweed, *Myagropsis myagroides*, belongs to the family *Sargassaceae* in Phaeophyta and most commonly inhabits the southern Korea and Japan coastlines. Studies on *M. myagroides* have mostly been focused on its anti-inflammatory [18], antihypertensive [3], and anticoagulant

activities [2], and protective effect on liver damage [34]. Despite abundant investigation, to date, there is no research on the α-amylase inhibitory effect of *M. myagroides*. Therefore, the aim of this study was to investigate the α-amylase inhibitory activity of *M. myagroides* extracts and isolate the active compound responsible for this effect.

Materials and Methods

Materials

M. myagroides was collected from the subtidal zone at Song-Jung, Busan, Korea. The samples were washed with tap water, lyophilized, and pulverized. The powder was stored at −20°C. Porcine pancreatic amylase (E.C. 3.2.1.1, type VI) and potato starch (type IV) were purchased from Sigma Co. (St. Louis, MO, USA).

Extraction

The dried *M. myagroides* (1.5 kg) was extracted three times with methanol for 24 h at room temperature. The extracts were filtered, centrifuged, and concentrated using a rotary evaporator (RE 200; Yamato Co., Tokyo, Japan) at 37°C. The dried methanol extract was stored at −20°C until use.

Isolation of Sargachromanol I

The methanol extract was suspended in distilled water and partitioned successively with hexane, chloroform, ethyl acetate, and butanol. The hexane fraction (13 g) was separated by flash silica gel column chromatography (70–230 mesh; Merck Art, Darmstadt, Germany; 5 × 10 cm column, $CHCl_3$:MeOH, 100:1−1:1 (v/v)). The fraction eluted with $CHCl_3$:MeOH (50:1) was subjected to Sephadex LH-20 column chromatography (Amersham Pharmacia Biotech AB, Uppsala, Sweden; 2.5 × 90 cm column, $CHCl_3$:MeOH, 1:1) and two fractions were separated on silica gel thin-layer chromatography (TLC; No. 5744, Merck; Darmstadt, Germany) plates with hexane-ethylacetate (2:1 (v/v)). Fr. 2 was subjected to octadecyl silica (ODS) Sepak cartridge (SPE C18 10 g; Grace, IL, USA; 60−90% methanol) and five fractions (Fr. 3 to Fr. 7) were isolated. Fr. 4 was successively subjected to Sephadex LH-20 column chromatography (methanol) and an ODS Sepak cartridge (50−60% methanol). The six fractions (Fr. 4-1−Fr. 4-6) were separated using ODS high-performance liquid chromatography (HPLC; ODS column i.d. 4.6 × 150 mm, 50−100% aqueous methanol gradient, 1 ml/min) and the fraction 4-2 was isolated using preparative HPLC (Cosmosil 5C18-MS II, 10 × 150 mm column; Nacalai Tesque, Kyoto, Japan; 70% aqueous methanol, 9 ml/min) and found to be a compound, sargachromanol I.

Sargachromanol I: ^{1}H NMR (600 MHz, $CDCl_3$) 6.37 (^{1}H, d, 1.4 Hz), 5.27 (1H, s), 6.47 (^{1}H, d, 2.8 Hz), 5.11 (^{1}H, br t, 6.5 Hz), 4.87 (^{1}H, dd, 9.7, 3.4 Hz), 3.91 (^{1}H, d, 3.4 Hz), 4.97 (^{1}H, br d, 9.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) 75, 31.3, 22.4, 121.1, 112.6, 147.9, 115.6, 127.1, 39.5, 22.1, 124.8, 134.3, 39.4, 25.2, 33.4, 41.2, 214.6, 74.3, 120.9, 140, 25.9, 18.6, 16, 15.6, 24, 16.

α-Amylase Inhibitory Activity

The α-amylase inhibitory activity was measured following the method of Ali *et al*. [1] with a slight modification. Diluted samples (40 μl) and porcine pancreatic amylase (200 μl, 16 unit/ml) were added into test tubes and incubated at 25°C for 5 min. A volume of 400 μl of 0.5% potato starch was added to the mixture for 3 min. Subsequently, 200 μl of this reactant and 100 μl of 96 mM DNS (potassium sodium tartrate in 2 M NaOH) were mixed for 15 min at 85°C. The reaction was stopped by adding ultra pure water (900 μl) and the absorbance was measured at 540 nm using a UV/visible spectrophotometer (GENESYS 10 UV; Rochester, NY, USA). Acarbose (Sigma Co.) was used as a positive control and the inhibitory activity was calculated according to the formula given below, and the IC_{50} value (the concentration of the extract that results in 50% inhibition of maximal activity) was determined.

Inhibitory activity (%) = 100 − [(enzyme activity of test/enzyme activity of control)]

Kinetics of Inhibition

The inhibition was measured using increasing concentrations of starch as a substrate (5, 8, and 10 mM) in the presence of different concentrations (0, 0.8, and 1 mM) of sargachromanol I. The type of inhibition was determined using Lineweaver−Burk plot analysis of the data that were calculated from the results according to Michaelis−Menten kinetics.

Results and Discussion

α-Amylase Inhibitory Activity of *M. myagroides* Extracts

As shown in Table 1, the methanolic extract had a lower inhibitory activity (13%) at 5 mg/ml than acarbose. The methanolic extract was fractionated with *n*-hexane, chloroform, ethyl acetate, butanol, and water. The inhibitory effects of the fractions were higher than those of the methanolic extract in the order of chloroform > hexane > ethyl acetate, with IC_{50} values of 2.79, 4.24, and 4.28 mg/ml, respectively. However, all fractions were weaker than the positive

Table 1. α-Amylase inhibitory activity of solvent extraction fractions from *Myagropsis myagroide*s methanol extract.

	Inhibitory activity (%)			IC_{50} (mg/ml)
	5 mg/ml	2.5 mg/ml	1 mg/ml	
Hexane	59.01 ± 0.75	25.03 ± 0.79	19.17 ± 0.84	4.24 ± 0.05
Chloroform	67.39 ± 0.52	45.05 ± 1.57	22.68 ± 0.93	2.79 ± 0.12
Ethyl acetate	58.43 ± 0.67	38.82 ± 0.79	19.29 ± 1.18	4.28 ± 0.05
Butanol	-	=	=	=
Water	-	=	=	=
Methanol	13.24 ± 1.10	=	=	=

-: less than 5%.
=: Not done.

Table 2. α-Amylase inhibitory activity of subfractions from the hexane fraction of *Myagropsis myagroide*s obtained by silica gel column chromatography.

	Inhibitory activity (%)			IC_{50} (mg/ml)
	2.5 mg/ml	1 mg/ml	0.5 mg/ml	
$CHCl_3$	22.96 ± 1.67	21.39 ± 0.79	=	=
CM100:1	84.62 ± 0.83	70.84 ± 0.98	32.40 ± 0.95	0.74 ± 0.04
CM50:1	72.35 ± 0.79	57.06 ± 1.59	43.39 ± 0.18	0.72 ± 0.22
CM20:1	42.43 ± 0.16	8.41 ± 1.11	=	2.77 ± 0.24
CM5:1	-	=	=	=
CM1:1	11.96 ± 1.25	=	=	=
Hexane	25.03 ± 0.79	19.17 ± 0.84	=	4.24 ± 0.05

-: less than 5%.
=: Not done.

Table 3. α-Amylase inhibitory activity of subfractions (CM = 50:1) from the hexane fraction of *Myagropsis myagroide*s obtained by sephadex LH-20 column chromatography and ODS Sepak cartridge.

	Inhibitory activity (%)		IC_{50} (mg/ml)
	1 mg/ml	0.5 mg/ml	
1	33.56 ± 0.81	-	=
2	59.39 ± 0.57	12.03 ± 0.83	0.84 ± 0.01
3	29.84 ± 0.40	-	=
4	66.37 ± 0.47	17.98 ± 0.58	0.75 ± 0.01
5	30.51 ± 0.82	-	=
6	45.03 ± 0.47	14.15 ± 0.83	1.11 ± 0.01
7	9.05 ± 0.16	-	=
CM50:1	57.06 ± 1.59	43.39 ± 0.18	0.72 ± 0.22

-: less than 5%.
=: Not done.

control acarbose. The chloroform fraction showed the highest inhibition of α-amylase among the fractions, and this fraction was subjected to successive silica gel column chromatography. However, the most active subfraction obtained was not further analyzed owing to a low yield. Thus, the hexane fraction was selected for use in further experiments.

Table 4. α-Amylase inhibitory activity of fraction 4 from hexane fraction of *Myagropsis myagroide*s by sephadex LH-20 column chromatography and ODS Sepak cartridge.

	Inhibitory activity (%)		IC_{50} (mg/ml)
	1 mg/ml	0.5 mg/ml	
4-1	42.39 ± 0.73	-	1.18 ± 0.02
4-2	27.01 ± 0.65	-	=
4-3	62.24 ± 0.29	9.39 ± 0.34	0.80 ± 0.00
4-4	65.61 ± 0.77	25.36 ± 0.32	0.76 ± 0.01
4-5	61.32 ± 0.38	30.08 ± 0.16	0.82 ± 0.01
4-6	70.02 ± 0.85	36.70 ± 0.32	0.68 ± 0.01
4	66.37 ± 0.47	17.98 ± 0.58	0.75 ± 0.01

-: less than 5%.
=: Not done.

α-Amylase Inhibitory Activity of Sargachromanol I

The hexane fraction was eluted with a chloroform/methanol mixture (100:1, 50:1, 20:1, 5:1, 1:1) using silica gel column chromatography. The fraction eluted from the chloroform/methanol mixture (50:1) exhibited the highest α-amylase inhibition, with an IC_{50} value of 0.72 mg/ml. The α-amylase inhibitory activity was higher than that of the positive control acarbose. The fraction eluted with the 50:1 chloroform/methanol mixture was submitted to a Sephadex LH-20 column with elution of a chloroform/methanol mixture, and to silica gel TLC with elution of a hexane/ethyl acetate mixture (2:1). Two fractions (Fr. 1 and Fr. 2) were obtained and Fr. 2 was subjected to an ODS Sepak cartridge with elution of 60–90% methanol. Five fractions (Fr. 3–Fr. 7) were obtained and Fr. 4 showed the highest α-amylase inhibitory activity, with an IC_{50} value of 0.75 mg/ml. Fr. 4 was purified using HPLC and six subfractions were obtained. Although Fr. 4-4 to Fr. 4-6 appeared to have potent α-amylase inhibitory activity, structural identification of these fractions was not possible owing to insufficient purity and amounts. The structures of Fr. 4-1 and Fr. 4-3 were also identified and their inhibitory activities will be presented in other papers. The sargachromanol I obtained from the Fr. 4-2 subfraction by preparative HPLC had a molecular mass of 428 Da. Sargachromanol I, with an IC_{50}

Table 5. α-Amylase inhibitory activity of the subfraction from the hexane fraction of *Myagropsis myagroides* methanol extract by HPLC.

	Inhibitory activity (%)			IC_{50}, mg/ml (mM)
	1 mg/ml	0.5 mg/ml	0.1 mg/ml	
Sargachromanol I	85.80 ± 1.10	62.10 ± 0.36	22.34 ± 0.68	0.40 ± 0.00 (0.93)
Acarbose	57.07 ± 3.70	38.48 ± 0.36	12.85 ± 1.23	0.88 ± 0.06 (1.36)

value of 0.93 mM, showed a more potent inhibitory activity against α-amylase than that of the acarbose.

Meroterpenoids of the chromene class, consisting of a polyprenyl chain attached to a hydroquinone or similar aromatic rings, are widely distributed among marine organisms. Among the meroterpenoids, sargachromanols have been reported to possess several biological activities, including anticancer [13], antioxidant [17], and anti-inflammatory [35] effects. Sargachromanol I was isolated from brown seaweed *Sargassum siliquastrum* [14] and its structure was similar to that of sargachromanol G [9]. To date, the biological activities of sargachromanol I on Na^+/K^+ ATPase inhibitory activity [6] and antioxidant activity [16] have been reported. Thus, to the best of our knowledge, this is the first report on its α-amylase inhibitory activity and isolation from *M. myagroides*. Sargachromanol I showed stronger inhibition against α-amylase than acarbose and may be an important contributor to the treatment of diabetes with α-amylase inhibitors.

Mode of α-Amylase Inhibition Type

The mode of inhibition of sargachromanol I on α-amylase was determined using the Lineweaver–Burk plot. Sargachromanol I displayed uncompetitive inhibition of the enzyme's activity (Fig. 3). In this case, the inhibitor only binds to the enzyme-substrate complex but not the free

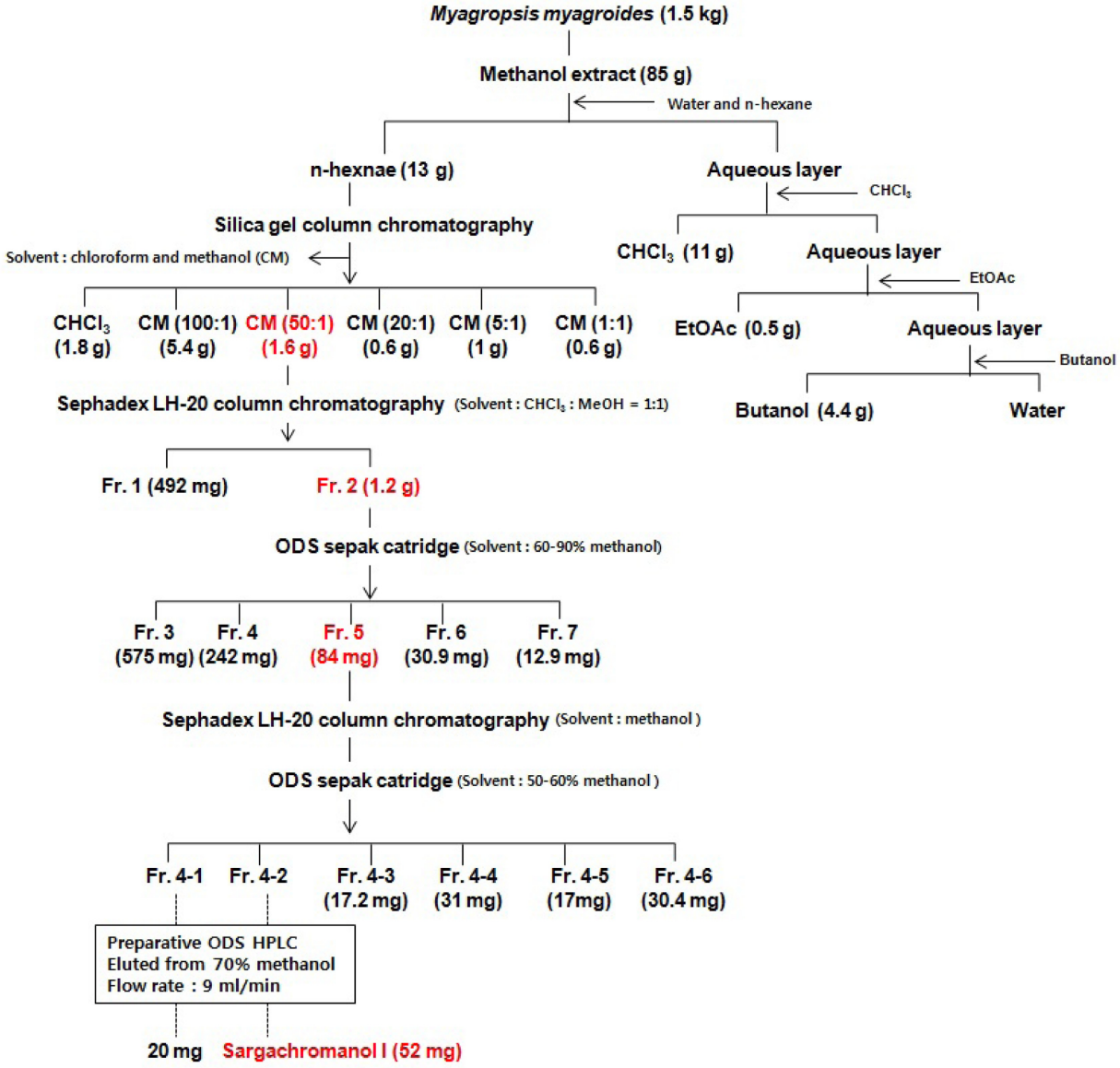

Fig. 1. Solvent fractionation of the *Myagropsis myagroides* extract.

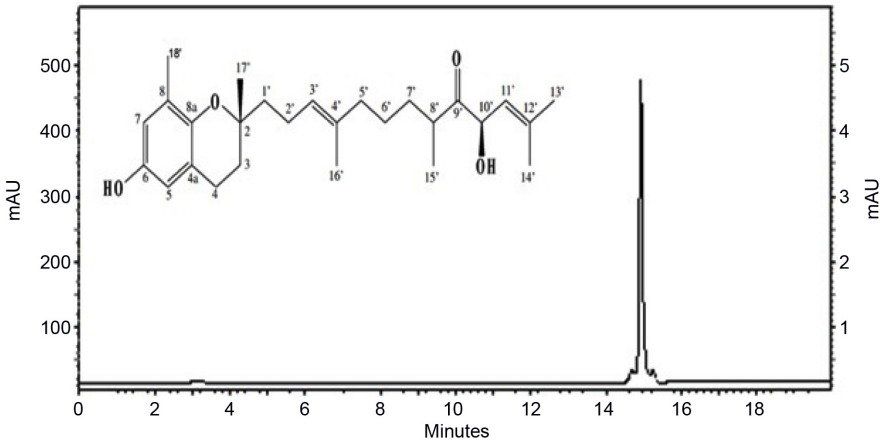

Fig. 2. HPLC profile of sargachromanol I isolated from *Myagropsis myagroides* methanol extract.

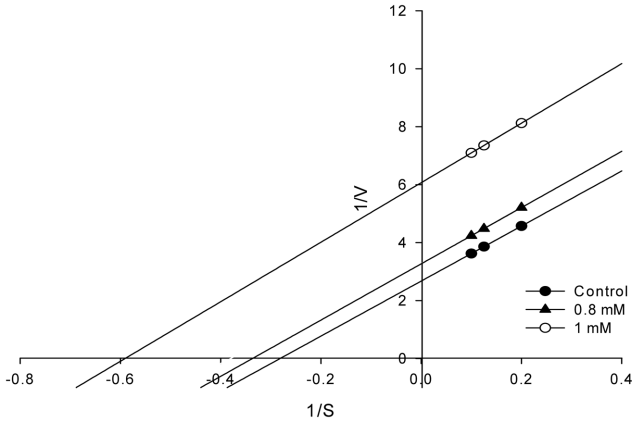

Fig. 3. Lineweaver-Burk plot for the inhibition of α-amylase by sargachromanol I.

Variable dextrin from starch concentrations (5, 8, 10 mM) at fixed concentrations of sargachromanol I [0 mM (●), 0.8 mM (▲) and 1 mM (○)].

enzyme. This plot suggests that sargachromanol I does not compete with the substrate for binding to the active site of the enzyme. Ponnusamy *et al*. [30] reported that bisdemethoxycurcumin isolated from the *Curcuma longa* rhizome showed uncompetitive inhibition against human pancreatic amylase. Lee and Lee [19] also reported that Chinese quince extract exhibited an uncompetitive inhibition mode.

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