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Anti-elastase, anti-urease and antioxidant activities of (3–13)-monohydroxyeicosanoic acid isomers

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Abstract: A series of (3–13)-monohydroxyeicosanoic acid isomers were evaluated for their anti-elastase, anti-urease and antioxidant activities for the first time in this study. All the test compounds exhibited anti-elastase, anti-urease and antioxidant activities. According to the obtained results, the hydroxyeicosanoic acid isomers in which the hydroxyl group is located in the middle or close to the middle of the chain showed higher anti-elastase, anti-urease and antioxidant activities than that of the other isomers. Therefore, (3–13)-monohydroxyeicosanoic acid isomers can be used in agriculture, pharmacy and cosmetic industries due to their excellent anti-elastase, anti-urease and antioxidant activities.

Keywords: anti-elastase; anti-urease; antioxidant; hydroxyeicosanoic acid; enzyme; inhibition.

INTRODUCTION

Elastases are a group of serine proteases that possess the ability to cleave the important connective tissue protein elastin, which is widely distributed in vertebrate tissues, and is particularly abundant in the lung, arteries, skin, and ligaments. These proteases include the neutrophile elastase (NE), also known as leukocyte elastase, the pancreatic elastase (PE), the macrophage elastase (NMP-12) and the fibroblast elastase.[1–3] There has been increasing interest in elastases in recent years because of their possible involvement in diseases of the connective tissues.[4] Elastase activity increases significantly with age and results in reduced skin elastic properties, aging and sagging.[5] Inhibition of the elastase activity could be employed as a useful target to protect against skin aging.[6]

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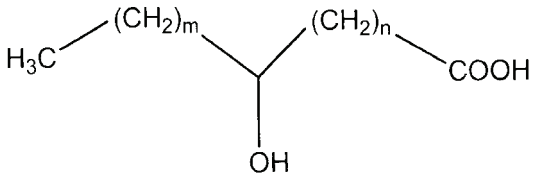
The metalloenzyme urease (urea amidohydrolase; EC 3.5.1.5) catalyzes the hydrolysis of urea into ammonia and carbon dioxide. It is present in a variety of plants, algae, fungi, bacteria and in soil enzymes.[7] Urease is involved in the pathogenesis of hepatic encephalopathy, hepatic coma urolithiasis, pyelonephritis, ammonia and urinary catheter encrustation.[8] It is also a major cause of pathologies induced by *Helicobacter pylori* (HP) as this allows bacteria to survive at the low pH of the stomach and hence plays an important role in producing peptic and gastric ulcers.[9] In agriculture, high urease activity releases abnormally large amounts of ammonia into the atmosphere after urea application and causes significant environmental problems and economical loss. The study of urease inhibition is of medical, agricultural and environmental significance. In the near past, a number of compounds have been proposed as urease inhibitors to reduce environmental problems and enhance the uptake of urea nitrogen by plants.[10,11]

Recently, interest in finding antioxidants for foods, cosmetics and medicines has increased considerably. Nowadays, antioxidants have become one of the major areas of scientific research. Antioxidants have been extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress. Scientists in many different disciplines have become more interested in new compounds, either synthesized or obtained from natural sources, that could provide active components to prevent or reduce the impact of oxidative stress on cells.[12] Chemical compounds and endogenous metabolic processes in the human body or in food systems might produce highly reactive free radicals, especially oxygen-derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Presently, synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary-butylhydroquinone (TBHQ) are the most commonly used antioxidants. However, their uses have been limited as they may be responsible for liver damage and carcinogenesis.[13,14] For these reasons, this problem has been overcome by new synthetic or natural compounds.

Hydroxy fatty acids are ubiquitous in nature and have been found as constituents of triacylglycerols, waxes, cerebrosides, and other lipids in plants, animals, insects and microorganisms.[15–18] The hydroxy fatty acids mentioned in the literature are valuable starting compounds used in the preparation of numerous textile auxiliaries, detergents, dispersion and emulsion reagents[19] and play an important role in cancer chemotherapy.[20] Hydroxy fatty acids exhibit an antitumor[21,22] effect at neutral and acidic pH values in human lung cancer (A 549) cells.[23] In the literature, there is no data on the anti-elastase, anti-urease and antioxidant activities of monohydroxyeicosanoic acid isomers. In this study, the anti-elastase, anti-urease and antioxidant activities of (3–13)-monohydroxyeicosanoic acid isomers were determined for the first time.

EXPERIMENTAL

(3–13)-Hydroxyeicosanoic acids (Scheme 1) were synthesized with high purity by Celik *et al.*[24,25]. The elastase activity was examined using *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (STANA) as a substrate and by the measuring the release of *p*-nitroaniline at 410 nm.[26] Urease inhibitory activity was determined according to van Slyke and Archilbald.[27] The cupric reducing antioxidant capacity of the monohydroxyeicosanoic acids was determined according to the method described by Apak *et al.*[28]



Scheme 1. Structure of the studied monohydroxyeicosanoic acid isomers; *n*: 1, 2, 4, 5, 6, 7, 8, 9, 10, 11; $m = 17 - n$.

RESULTS AND DISCUSSION

Fatty acids with one hydroxyl group, called α-hydroxy and β-hydroxy fatty acids, are healing agents in cosmetic and clinical use against skin diseases.[29] Hydroxy acids, mainly α-hydroxy acids and polyhydroxy acids, remain timeless in their ability to modulate skin structure and performance, providing both clinical and cosmetic benefits to skin. α-Hydroxy acids (AHAs) are a class of compounds derived from food sources that have become increasingly popular as skin rejuvenating agents. At higher concentrations (50–70 %), AHAs are used for superficial skin peeling, while at low concentrations (8–30 %), they have been reported to act as moisturizing agents and can cause a decrease in corneocytic attachment.[30,31] β-Hydroxy fatty acids are formed during mitochondrial β-oxidation of fatty acids in mammalian tissues. Increased concentrations of free 3-OH fatty acids in body fluids are indicative of disorders in fatty acid oxidation, which is important for tissues with a high-energy demand.[32]

According to a literature survey, no positional fatty acid isomers have been examined to date for their elastase inhibition. This work demonstrates the importance of the positional effect on elastase inhibition. The elastase inhibition activities of the monohydroxy C_{20} acids are given in Table I. The elastase inhibitory activities of monohydroxy C_{20} acids were found to increase in a dose dependent manner, the results are expressed as half maximal inhibitory concentrations (IC_{50}) values, calculated from the regression equations prepared from the concentrations of samples. Amongst them, the best inhibition was found for the 10-hydroxyeicosanoic acid, followed by the 11- and 9-hydroxyeicosanoic acid isomers as seen in Table I. A higher elastase inhibitor activity is associated with a lower IC_{50} value. A high elastase inhibition (55.08±0.95 %) was seen at 1 μg mL^{-1} for the 10-hydroxy C_{20} acid and the IC_{50} value was 0.59±0.004 μg mL^{-1}. A low elastase inhibition (24.28±0.63 %) was seen at 1 μg mL^{-1} for the 12-hydroxy C_{20} acid and IC_{50} value of the 12-hydroxy C_{20} acid was 7.69±1.77 μg mL^{-1}.

The (3–13)-hydroxyeicosanoic acid isomers showed good elastase inhibition. The 3-, 4-, 6-, 7-, 8-, 9-, 11- and 13-monohydroxy C_{20} acids showed nearly the same inhibition (Table I).

TABLE I. The inhibition of elastase activity (mean±*SD*) by the monohydroxyeicosanoic acid isomers at a concentration of 1 μg mL^{-1}

Hydroxyl position	Inhibition, %	IC_{50} / μg mL^{-1}
3	48.94±1.80	1.24±0.42
4	48.47±0.73	1.43±0.33
6	48.72±0.51	1.19±0.10
7	44.84±0.39	2.10±0.04
8	45.35±1.87	1.42±0.29
9	49.41±1.00	1.08±0.18
10	55.08±0.95	0.59±0.004
11	51.04±0.37	0.88±0.02
12	24.28±0.63	7.69±1.77
13	26.84±2.79	4.9±2.19

Elastase has a serine residue with a free hydroxyl group in its active center. This hydroxyl group may form an ester bond with the carboxyl group of the hydroxyeicosanoic acid isomers. In a previous study, lactones and their derivatives were used as elastase inhibitors.[33,34] This inhibition was explained by a new transesterification between the hydroxyl group of the serine and carboxyl group of the lactone ring. In the present study, some hydroxyeicosanoic acid isomers were examined for their elastase inhibition activity (Table I). The hydroxyl and carboxyl groups of these isomers may be effective in the inhibition of elastase. The carboxyl group may be more active than the hydroxyl group according to the literature data on the elastase inhibition activity of some saturated and unsaturated fatty acids.[35,36] The inhibition effect of the hydroxyeicosanoic acid isomers may be explained by the formation of an ester bond between the carboxyl group of the hydroxy acid isomers with the hydroxyl group of serine located in the active center. However, the experimental results obtained in this study showed that the position of the hydroxyl group of the hydroxy acid isomers plays a role on the inhibition effect indicating to a second effect resulting from an interaction between the hydroxyl group of the hydroxy acid isomers and the hydroxyl group of the serine. The best elastase inhibition was found for the 10-hydroxy isomer. This isomer has a symmetrical structure and is like an arrow. The hydroxyl group of the 10-hydroxy isomer is not sterically hindered and is free. The other hydroxy acid isomers showed very similar inhibition, but their degree of inhibition was lower than that of the 10-hydroxy acid isomer. These isomers are less free and more sterically hindered than the 10-hydroxy acid isomer. The 12- and 13-monohydroxy hydroxyeicosanoic acid isomers having the hydroxyl group closer to the end exerted the lowest inhibition. The molecule structure at

these positions may sterically prevent the formation of the hydrogen bridge between the hydroxyl groups of the serine and hydroxy acid isomers, affected probably by van der Waals forces. The hydroxyl group of the 12- and 13-hydroxy isomers is located very far from the carboxyl group. The hydroxyl group of the 3–8 isomers is very close to the carboxyl group. Another effect depending on the position of the hydroxyl may occur between the hydroxyl and carboxyl group. This position effect may be also active on elastase inhibition effect of the hydroxyeicosanoic acid isomers (Table I).

Certain synthetic compounds have shown potential urease inhibition, such as hydroxyurea, flurofamide and hydroxyamic acid. However, the *in vivo* use of some of these is prohibited because of their toxicity or instability, for example, acetohydroxyamic acid was demonstrated to be teratogenic in rats.[37] The discovery of potent and safe urease inhibitors is a very important area of pharmaceutical research because of the involvement of urease in different pathological conditions.

The urease inhibition activity of the monohydroxy C_{20} acids is given in Table II. The urease activity was detected at a lower concentration of the hydroxy acid isomers than the elastase activity. Many synthetic and natural apple polyphenols have shown inhibitory activity against urease activity.[38,39] Xiao *et al*. explained the urease inhibition activity of polyphenols by the ability of the hydroxyl group to form a complex with the nickel metal located at the active center of urease.[38] In this study, the urease inhibition activity of the monohydroxy C_{20} acid isomers was found to increase dose dependently. The results are expressed as IC_{50} values calculated from the regression equations prepared from the inhibition and the concentrations of the samples. A high urease inhibition (70.22±1.36 %) was seen at 0.1 μg mL^{-1} for the 8-hydroxy C_{20} acid. The IC_{50} value was 0.000012± ±0.0000092 μg mL^{-1}. A low urease inhibition (52.89±2.41 %) was seen at 0.1 μg mL^{-1} for the 6-hydroxy C_{20} acid. The IC_{50} value of the 6-hydroxy C_{20} acid was 0.0766±0.01 μg mL^{-1}. A number of novel synthetic and natural inhibitors of urease were investigated.[40,41] Lodhi *et al*. studied triacontanyl palmitate as an effective inhibitor of urease.[42] The 8-, 10- and 7-hydroxy C_{20} acid isomers, with the hydroxyl group located in the middle and close to the middle of the chain showed the best inhibition activities. These middle positions make these isomers successful in chelating and, as a result, in the urease inhibition. Moving away from these positions, the urease inhibitory activity decreased due to the molecule symmetry and steric hindrance of the alkyl groups. The activity against urease is the complex building ability of the hydroxyl group with nickel metal.[37] The urease inhibition activity of the hydroxy acid isomers in this study can also be attributed to the complex building ability of the hydroxy acid isomers with nickel active center of the urease. The presence of –OH and –COOH group of mono-

hydroxyeicosanoic acid isomers in this study may play together a great role in the inhibition of urease activity.

TABLE II. The inhibition of urease activity (mean±*SD*) by the monohydroxyeicosanoic acid isomers at a concentration of 1 μg mL^{-1}

Hydroxyl position	Inhibition, %	IC_{50} / μg mL^{-1}
3	59.18±0.64	0.0509±0.007
4	66.32±0.50	0.0174±0.0002
6	52.89±2.41	0.0766±0.01
7	67.41±2.00	0.0065±0.0038
8	70.22±1.36	0.000012±0.0000092
9	64.85±1.27	0.0297±0.00028
10	62.92±0.28	0.0049±0.0007
11	67.87±2.62	0.0234±0.0043
12	67.20±1.36	0.0301±0.000212
13	60.21±1.46	0.0297±0.0031

Antioxidants can be reductants, and deactiviation of oxidants by reductants can be described as redox reactions in which one reactions species is reduced at the expense of the oxidation of the other. As is known, transition metal ions, such as ferrous and cupric ions, accelerate lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals *via* the Fenton reaction.[43] Therefore, chelating agents, known as secondary antioxidants, are important to retard radical degradation. There are several methods for the determination of antioxidant activities. In this study, the cupric reduction antioxidant capacity test (CUPRAC method) was used.[28] The CUPRAC method is also used to determine the reducing power of antioxidant compounds.[28] This method is based on the reduction of Cu^{2+} to Cu^{+} by antioxidants in the presence of neocuprein.[44] In this method, a higher absorbance indicates a higher cupric ion reduction ability. The CUPRAC method is simultaneously cost effective, rapid, stable, selective and suitable for a variety of antioxidants regardless of the chemical type or hydrophilicity.[45] The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[44]

The cupric ion (Cu^{2+}) reducing abilities of the monohydroxyeicosanoic acid isomers are given in Table III. The compounds 12-, 9-, 6- and 13-monohydroxyeicosanoic acid showed the lowest cupric ions (Cu^{2+}) reducing capability (Table III). Compounds, 3-, 4-, 7-, 10- and 11-monohydroxyeicosanoic acids exhibited a moderate reducing power. The highest reducing capacity was found for 8-monohydroxyeicosanoic acid. All the monohydroxyeicosanoic acid isomers are less effective on cupric ions (Cu^{2+}) reducing ability than BHT. This method is based on the chelating ability of the hydroxyl group of some 3–13-monohydroxyeicosanoic acid isomers with Cu^{2+}. The position of the hydroxyl group is important for the chelating activity. The best reducing activity was found for the 8-hydroxy

isomer, in which the hydroxyl is located close to the middle of the chain. This position has no steric hindrance; therefore, the hydroxyl group is free and suitable for chelating with Cu^{2+}. This reducing effect decreased with 3–7- and 9–13-monohydroxyeicosanoic acid isomers because of the moleculear symmetry and steric hindrance of the alkyl groups.

TABLE III. The cupric ions reducing antioxidant capacity of the monohydroxyeicosanoic acid isomers (mean±*SD*) at a concentration of 100 μg mL^{-1}

Hydroxyl position	CUPRAC reducing power (absorbance)
3	0.35±0.008
4	0.36±0.004
6	0.23±0.004
7	0.37±0.004
8	0.57±0.006
9	0.27±0.001
10	0.37±0.002
11	0.44±0.004
12	0.32±0.002
13	0.24±0.003
BHT	1.65±0.045

CONCLUSIONS

A series of (3–13)-monohydroxyeicosanoic acid isomers were synthesized and their anti-elastase, anti-urease and antioxidant activities evaluated. The results showed that all the monohydroxyeicosanoic acid isomers exhibited anti-elastase, anti-urease and antioxidant activities. According to the obtained results, the monohydroxyeicosanoic acid isomers with the hydroxyl group located in the middle or close to the middle of the chain showed higher antioxidant, anti-urease and anti-elastase activities than the other isomers. These monohydroxyeicosanoic acid isomers could be used in the agriculture, pharmacy and cosmetic industries due to their excellent anti-elastase, anti-urease and antioxidant activities.

ИЗВОД

АНТИЕЛАСТАЗНА, АНТИУРЕАЗНА И АНТИОКСИДАТИВНА АКТИВНОСТ НЕКИХ (3–13)-МОНОХИДРОКСИЛНИХ ИЗОМЕРА ЕИКОЗАНСКЕ КИСЕЛИНЕ

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Испитана је антиеластазна, антиуреазна и антиоксидативна активност групе (3–13)-монохидроксилних изомера еикозанске киселине. Сва анализирана једињења су испољила све ове активности. Резултати су показали да највећу активност имају изомери код којих је хидроксилна група у средини или близу средине ланца. Према томе, (3–13)-

-монохидроксилни изомери еикозанске киселине, због својих својстава, могу наћи примену у пољопривреди, фармацији и козметичкој индустрији.

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