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The Metabolites of a Marine Mollusk *Mytilus edulis* : Isolation of Taurine and Compositions of Free Fatty Acids and Free Amino acids

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Abstract—The metabolites of marine mollusk *Mytilus edulis* were isolated and characterized, revealing the presence of the rare free fatty acids and proteinogenic amino acids together with a non-proteinogenic free amino acid, taurine. The free fatty acids in this organism were particularly interesting for the presence of both unusual n-6 acid [20:4 (n-6)] and triple bond containing acid (2,5-octadecadiynoic acid). In addition to the proteinogenic amino acids commonly found in proteins, non-proteinogenic free amino acid taurine was isolated and the structure was determined by its physicochemical properties. Recently taurine has been given much interest in the molecular level because of diverse biological activities and the medicinal properties. Furthermore, the result of the analyses of free amino acids showed that glycine, glutamic acid, serine and alanine, which were considered to be related to the taste of this organism, are predominantly present.

Keywords—Marine mollusk · *Mytilus edulis* · free fatty acid · triple bond containing fatty acid · free amino acid · taurine

The marine mollusk *Mytilus edulis* (Phylum: Mollusca, Class: Pelecypoda, Order: Dysodonta, Family: Mytilidae) is one of the marine organisms that inhabits broadly in Korean coast, and it is also edible and greatly consumed in several countries. The tentative constituents of *M. edulis* were studied because they are necessary as reference materials in cultivation and processing industries in a view point of investigation of proteinous resources (Choi, 1970). In addition, isolation of marine toxins, such as domoic acid (Nijjar *et al.*, 1991), okadaic acid(Luckas and Meixner, 1988), and gonyautoxin (Chang *et al.*, 1987), from this organism were reported, and identification of these toxins is important for prevention of disasters by food poisoning as well as development of physiological and pharmacological agents. As a part of search for new biologically active substances toward an exploitation and development of medicinal resources from the marine organisms, we have examined the metabolites of the marine mollusk *M. edulis*. Here, we describe the isolation of non-proteinogenic free amino acid taurine together with both distribution and characterization of free fatty acids and free amino acids.

Experimental

Instrumentations and reagents - IR spectra were obtained using a Shimadzu IR-408 spectrometer. NMR spectra were measured with Bruker AM-300 (300 MHz) spectrometer with Me_4Si or TSP [3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, sodium salt] as the internal

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standards. GC charts were recorded on a Hitachi Gas Chromatograph model of G-3000 with FID (3% SE-30 on Chromosorb GAW DMCS, 80-100 mesh, 1 m x 3 mm , column temp. 230°C, carrier gas He at flow rate 30 ml/min) and GC-MS were taken on Hewlett Packard 5890 HP 5970 MSD spectrometer equipped with a 25 m x 0.2 mm Ultra-2 capillary column crosslinked with 5% diphenyl and 35% dimethylpolysiloxane. The temperature program was as follows: increased at 10°/min from 130° to 280° and then maintained for 20 min. The amino acid analyses were performed with a Hitachi Amino Acid Analyzer model 835 with ninhydrin method. Si gel 60 (Merck, 70-230 mesh), Dowex 50W x 8 (H^+) (Sigma, 50-100 mesh), Dowex 1 x 2-100 (Cl^-) (Sigma, 50-100 mesh), and alumina WB-5 (basic) (Sigma) were used on column chromatography and TLC was carried out on Merck Si gel $60F_{254}$ and the spots were checked with 1% $Ce(SO_4)_2$ in 10% H_2SO_4 or 5% ninhydrin in EtOH followed by heating.

Extraction - The marine mollusk *M. edulis* was purchased at Masan area (Jindong) and Jinhae area (Annkol) of Kyungnam Prefacture. The flesh (6.9 kg wet weight) separated from fresh purchased specimen was finely cutted, and extracted once with Me_2CO and twice with MeOH at r.t. The extracts were combined and concentrated under reduced pressure to obtain an aqueous suspension, which was extracted with EtOAc. The EtOAc phase was evaporated to dryness to give EtOAc extract (7.0 g). While, the H_2O phase was extracted with *n*-BuOH, and each solvents of *n*-BuOH and H_2O soluble portions were removed under vacuo to give *n*-BuOH extract (4.7 g) and H_2O extract (61.9 g).

Isolation of free fatty acid fractions, and GC and GC-MS analyses - The EtOAc extract (4 g) was chromatographed on Si gel column eluting with mixtures of *n*-hexane - EtOAc (15:1 → 5:1) in increasing polarity.

Table 1. The free fatty acids from *Mytilus edulis*

Fatty Acid	Abundance (%)
Tetradecanoic acid (14:0)	3.0
Pentadecanoic acid (15:0)	0.1
Hexadecanoic acid (16:0)	15.7
11*z*-Hexadecenoic acid (16:1)	5.5
8*E*-Octadecenoic acid (18:1)	12.5
11*z*-Eicosenoic acid (20:1)	13.1
5*z*, 8*z*, 11*z*, 14*z*-Eicosatetraenoic acid (20:4)	23.9
2,5-Octadecadiynoic acid (18:2)	20.5

The resulting fractions were combined according to their NMR spectra, some of which showed the presence of complex fatty acid mixtures (250 mg). The mixture of fatty acids was methylated with CH_2N_2 followed by Lobar column (Si 60) purification eluting with *n*-hexane to give a mixture of fatty acid methyl esters, which was analyzed by GC and GC-MS, and its ratio was obtained by comparison of peak area for each of the esters (Table I).

Tetradecanoic acid methyl ester : t_R (min.) = 1.65, GC-MS, m/z (rel. int.) 242[M^+](25), 211(18), 199(30), 143(33), 87(70), 74(100).

Pentadecanoic acid methyl ester : t_R (min.) = 2.60, GC-MS, m/z (rel. int.) 256[M^+](4), 255(19), 213(17), 143(23), 87(70), 74(100).

Hexadecanoic acid methyl ester : t_R (min.) = 2.81, GC-MS, m/z (rel. int.) 270[M^+], 239, 227, 143, 87, 74(100).

11z-Hexadecenoic acid methyl ester : t_R (min.) = 4.53, GC-MS, m/z (rel. int.) 268[M^+](8), 236(34), 194(24), 152(23), 123(27), 110(32), 97(57), 83(57), 74(70), 69(81), 55(100).

8E-Octadecenoic acid methyl ester : t_R (min.) = 4.82, GC-MS, m/z (rel. int.) 296[M^+](6), 295(11), 263(59), 223(13), 180(18), 124(23), 97(64), 83(54), 69(75), 55(100).

11z-Eicosenoic acid methyl ester : t_R (min.) = 7.55, GC-MS, m/z (rel. int.) 324[M^+](9), 323(14), 292(39), 249(28), 208(29), 125(27), 111(42), 97(65), 83(74), 69(93), 55(100).

5Z,8Z,11Z,14Z -Eicosateiraenoic acid methyl ester : t_R (min.) = 7.88, GC-MS, m/z (rel. int.) 318[M^+](16), 247(7), 220(15), 203(26),

Table 2. The proteinogenic free amino acids from *Mytilus edulis*

Composition	%[a]	Composition	%[a]
Glycine	19.74	Phenyl alanine	4.04
Glutamic acid	12.46	Isoleucine	3.56
Serine	10.46	Tyrosine	3.32
Alanine	10.42	Cysteine	3.05
Aspartic acd	8.13	Arginine	2.89
Threonine	6.35	Leucine	2.39
Lysine	5.99	Methionine	1.03
Valine	5.39	Histidine	0.79

[a]denote relative content (%).

180(25), 150(54), 133(32), 119(50), 106(45), 91(81), 79(100).

2,5-Octadecadiynoic acid methyl ester : t_R (min.) = 13.01, GC-MS, m/z (rel. int.) 201(7), 133(31), 119(56), 105(51), 91(79), 79(100), 67(52), 55(22), 41(35).

Isolation of Taurine and Analysis of Free Amino Acids - The H_2O extract (10 g) of *M. edulis* was subjected to a positive ion exchange resin column (Dowex 50W x 8, H^+) eluting with H_2O and then alkali solution (2M NH_4OH) to afford H_2O elute and alkali elute (4.9 g). The H_2O elute was loaded on a negative ion exchange resin column (Dowex 1 x 2, Cl^-) with elution of H_2O and then acid (0.3M AcOH). The fraction (1.1 g) eluted with acid (0.3M AcOH) was further purified by a alumina (basic form) cc (20% MeOH), followed by recrystallization with aq. EtOH to give compound-1 (66 mg) (0.7% from the H_2O extract).

Compound-1 (taurine): Colorless needles, IR, ν_{max}^{KBr} 3400 (br)(-NH_2) and 1175, 1034 (-SO_3H) cm^{-1} ; ^{1}H-NMR, (300 MHz, D_2O) δ 3.44, 3.26 (each 2H, t, J = 6.5 Hz); ^{13}C-NMR, (75 MHz, D_2O) δ_c: 50.2 (t), 38.3 (t).

Alkali elute (free amino acid fraction) was analyzed quantitatively in an automatic amino acid analyzer using 0.02N HCl (Table II).

Results and Discussion

Free fatty acid composition - The principal fatty acids in the marine mollusk which have been reported are the saturated acids hexadecanoic (16:0) and octadecanoic (18:0), the monoene oleate (18:1, Δ^9), and polyunsaturated fatty acids belonging principally to the n-6 and n-3 series, such as 20:4 (n-6), and 22:5 (n-3) (Carballeira *et al.*, 1992). Furthermore, very characteristic of mollusk is also the presence of two C_{20} non-methylene interrupted dienes (NMID), namely 20:2, $\Delta^{5,11}$ and 20:2, $\Delta^{5,13}$ (Carballeira *et al.*, 1992). The NMR spectra of fatty acid fractions showed typical fatty acid spectra, i.e., δ 0.88 and 0.93 (each 3H, deformed -t) and δ_c 14.0 and 14.2 (each q) due to terminal methyls, intense peak at δ 1.26 and δ_c 20.5-34.1 (a mass of signals, each t) revealing methylenes in the fatty acid chain, double bond absorption observed at δ5.26-5.37 and δ_c127.0-132.0 (a mass of signals, each d). For further characterization, the total mixture was esterified with CH_2N_2 and the corresponding fatty acid methyl esters were analyzed by GC and GC-MS (Table I). The principal fatty acids in the mixture were hexadecanoic acid (16:0), 8*E*-octadecenoic acid (18:1), 11*Z*-eicosenoic acid (20:1), the polyunsaturated fatty acid eicosatetraenoic acid (20:4) of the n-6 family, and the triple bond containing fatty acid 2,5-octadecadiynoic acid (18:2). However, C_{20} non-methylene interrupted dienes (NMID), namely 20:2, $\Delta^{5,11}$ and 20:2, $\Delta^{5,13}$, which have been reported to be characteristic in the mollusk (Carballeira *et al.*, 1992), were not detect-

ed. The triple bond containing fatty acid is not common in the marine invertebrates. This is the first report of its presence in the marine mollusk *M. edulis*. Accordingly, its biological activities and roles are of interest.

Compound-1 (Taurine) - Among the constituents of the marine organisms, the systematic study for free amino acids was not active because of their polarity and water-solubility. However, in accordance with recent rapid progress and excellent results for the field of marine natural product chemistry, unique and bioactive non-proteinogenic amino acids, which have not yet been reported in terrestrial organisms, were isolated from the marine organisms. Examples were as follows: an anthelmintic α-kainic acid and domoic acid, a hypotensive (+)-2-hydroxy-3-aminopropanesulfonic acid, hormonal chondrine, and toxic N-acyl-2-methylene-β-alanine methyl ester (Shiba, 1979). Recently non-proteinogenic amino acids have been much interest in the various fields because of their importance in biosynthesis (Herbert, 1981), their use as enzyme inhibitors (Farrington *et al.*, 1987), their application to the investigation of enzyme mechanisms (Baldwin, 1985), and their medicinal properties (Csol, 1980).

The H_2O extract of *M. edulis* was column chromatographed on Dowex (50W x 8, H^+) followed by Dowex (1 x 2, Cl^-) and then alumina (basic form) to give compound-1 as colorless needles, which showed positive coloring test for ninhydrin. IR spectrum of compound-1 showed absorption bands due to amino group (3400 cm^{-1}) and sulfonate group (1175, 1034 cm^{-1}), whereas ^1H- and ^{13}C-NMR spectra showed signals ascribable to the two methylenes [δ3.44, 3.26 (each 2H, t, J = 6.5 Hz); δ_c38.3 (t), 50.2 (t)]. Compound-1 was also compared with an authentic taurine by TLC (isopropanol : H_2O = 10 : 1, R_f = 0.3). Based on the above evidences, compound-1 was determined as taurine. Taurine is a free amino acid which exists broadly in the tissues of a mammal, such as skeletal muscle, heart, and neuron (Garvin, 1960; Jacobsen and Smith, 1968). But, the importance of taurine in field of physiology and biochemistry was not fully investigated yet in spite of its broad distribution as well as high concentration in these tissues. Recently bioactivities of taurine on a central nervous system, heart, and muscle were reported, i.e., a possible inhibitory transmitter in the cerebellum (McBride and Frederickson, 1980) and antispasmodic activity in epilepsy (Goodman *et al.*, 1980), functions on both congestive heart failure (Huxtable and Bressler, 1974) and irregularities of the heart (Read and Welty, 1962), and both stabilization of membrane function and increase of calcium transfort in the muscle intracellular membrane (Huxtable and Bressler, 1973).

Hence, the significance of this organism is great because of high concentration of taurine and an important food item in our country as well. In addition, taurine will be utilized as a reference constituent in both development of functional food and quality control for this organism.

Free amino acid composition - Ion exchange chromatographic separation of the H_2O extract provided a free amino acid mixture which showed positive colors on TLC examination with ninhydrin. The composition of proteinogenic amino acids was analyzed and the result was shown in Table II. As the result, glycine (19.74%), glutamic acid (12.46%), serine (10.46%), and alanine (10.42%) were predominantly present. As glycine, serine and alanine are representive of delicious and sweet taste, these amino acids are attributed to the taste for this organism.

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