FISHERIES RESEARCH BOARD OF CANADA
Translation Series No. 588

Extractive compounds of marine algae (Review)

by Kiichi Murata

Original title: Kaiso no ekisu seibun

From: Bulletin of the Japanese Society of Scientific Fisheries
29: 1962

Translated by Translation Bureau (YT)
Foreign Languages Division
Department of the Secretary of State of Canada

Fisheries Research Board of Canada

11 pages typescript

DEPARTMENT OF THE SECRETARY OF STATE
TRANSLATION BUREAU

MULTILINGUAL SERVICES DIVISION

CANADA

SECRÉTARIAT D'ÉTAT
BUREAU DES TRADUCTIONS

DIVISION DES SERVICES MULTILINGUES

TRANSLATED FROM – TRADUCTION DE	INTO – EN
Japanese	English

AUTHOR – AUTEUR

Kiichi Murata

TITLE IN ENGLISH – TITRE ANGLAIS

Extractive compounds of marine algae (Review)

TITLE IN FOREIGN LANGUAGE (TRANSLITERATE FOREIGN CHARACTERS)
TITRE EN LANGUE ÉTRANGÈRE (TRANSCRIRE EN CARACTÈRES ROMAINS)

Kaiso no ekisu seibun

REFERENCE IN FOREIGN LANGUAGE (NAME OF BOOK OR PUBLICATION) IN FULL. TRANSLITERATE FOREIGN CHARACTERS.
RÉFÉRENCE EN LANGUE ÉTRANGÈRE (NOM OU LIVRE OU PUBLICATION), AU COMPLET, TRANSCRIRE EN CARACTÈRES ROMAINS.

Bulletin of the Japanese Society of Scientific Fisheries

REFERENCE IN ENGLISH – RÉFÉRENCE EN ANGLAIS

PUBLISHER – ÉDITEUR / PLACE OF PUBLICATION – LIEU DE PUBLICATION	DATE OF PUBLICATION / DATE DE PUBLICATION: YEAR / ANNÉE	VOLUME	ISSUE NO. / NUMÉRO	PAGE NUMBERS IN ORIGINAL / NUMÉROS DES PAGES DANS L'ORIGINAL; NUMBER OF TYPED PAGES / NOMBRE DE PAGES DACTYLOGRAPHIÉES
	1962	29	2	

REQUESTING DEPARTMENT
MINISTÈRE-CLIENT

TRANSLATION BUREAU NO.
NOTRE DOSSIER Nº ?&&(6779

BRANCH OR DIVISION
DIRECTION OU DIVISION

TRANSLATOR (INITIALS)
TRADUCTEUR (INITIALES) YT

PERSON REQUESTING
DEMANDÉ PAR

YOUR NUMBER
VOTRE DOSSIER Nº 769-18-14

DATE OF REQUEST
DATE DE LA DEMANDE

Extractive compounds of marine algae (Review)

Kiichi Murata*

Marine algae are of a far lower order than land plants in the evolution system of plants. They have various colours such as green, yellow, brown, purple and red, which are influenced by enviroment. The mechanism of photosynthesis seems quite different from tha that of land plants. Marine algae sometimes absorb plenty of I, Mg, Si, Fe, Mn and S which are required for metabolism, as well as N, P and K from the environmental sea. The organic compounds produced in marine algae intermediatly or finally are quite specific. For example, alginic acid, laminarine and fucoizine produced by Laminaria japonica and Hibamata (Japanese), agar-agar of Japanese jelly plant and Ogonori (Japanese), phycoerythrin and phycocyan of Porphyra tenera are the compounds characteristic of marine algae. Extractive compounds of marine algae also have a specific which is not observed in land plants. These compounds are intermediate products of metabolism rather than final ones. To study these organic compounds is very interesting not only for comparative biochemistry but also to discover the secret of life. In Japan marine algae are widely used for food and luxury, ad she is surrounded by sea and is abundant in marine algae. This is quite unique in the world. Accordingly, investigation of extractive compounds is required for food chemistry, and to complete it is very important. Recently everyday eating of marine algae is recommended for the cure of high blood pressure.

---

* Lab. of Sea Food Chem., Fac. of Fish., Hokkaido Univ., Hakodate, Japan.

Consequently marine algae are considered as becoming more important as a food. From these standpoints let us consider the extractive compounds of marine algae.

A. Free Amino Acids

In the past 10 years many new amino acids were discovered in various plants by Fowden[1, 6] Gmelin[7, 11], Morris[12, 16], Thompson[12, 16], Virtanen[17, 20], and others, using ionexchange chromatography, method of Partridge. From marine algae too, some amino acids have been isolated. That is, Murakami, Takemoto and others (1953)[21] isolated L-α-kainic acid, m.p. $251^{\circ}C$, and its stereoisomer, L-α-allokainic acid, m.p. $231^{\circ}C$ from the Digenea simplex. The structure of these is as follows: 3-carboxymethyl-4-isopropenylproline. These substances were synthesized by Ueno, Tanaka and others[22]. Daigo[23] isolated 3-carboxy-methyl-4-(2-carboxy-1-methylhexa-1,3-dienyl) proline, m.p. $217^{\circ}C$ from Chondria armata (1959). This was named domoic acid. These amino acids showed a yellow colour with ninhydrin and had anthelmintic effects.

In addition, the author and others[24] isolated Chondrine, a new amono acid, having a thiazane radical, L-1, 4-thiazane, 3-carbox- /190
ylic acid S-oxide, from Chondria crassicaulis. This amino acid showed at first cobalt colour, later deep blue and then brown with ninhydrin. The melting point of it was $255 - 257^{\circ}C$ in a sealed tube. Optical rotation was considerably larger,

(c=2, 6N HCl). Desulfuric reduction of Chondrine with raney nickel produced N-ethylalanine and a little alanine, and oxidation with hydrogen peroxide produced a bit of taurine. The amino acid was usually not contained in marine algae and was found only in Desmarestia ligulata[25] and Chondrus ocellatus[26], besides Chondria crassicaulis. For this reason neither the synthetic process of the amino acid in a living body nor its role in metabolism were known.

Moreover, rhodoic acid, N-1-carboxy-ethyl-taurine, m.p. $258^{\circ}C$, $[\alpha]_D^{13} = -1.15$ (c=5, N NaOH) was isolated by Kuriyama[27] from red algae such as Chondrus ocellatus, Neodilsea yendoana and Iridaea cornucopiae. Though this acid was not sensitive ot ninhydrin, it showed a pale purple colour. This acid was synthesized from taurine and α-brompropionic acid. A conversion from rhodoic acid to chondrine seems possible, but coexistence of the two substances has not been found, except in Chondrus ocellatus. No enzyme catalyzing the conversion is known.

Table 1. Forms of nitrogen in various algae.

Forms of nitrogen in various algae are shown in Table 1[28]. It can be seen that 10 to 33% of the total nitrogen is extractable by ethanol. In regard to free amino acids in ethanolic extract, Ogino[29] discovered that glutamic acid, aspartic acid, alanine, cystine and arginine were most commonly found in marine algae. Coulson[30] concluded, after inspecting 9 brown and red algae, that glutamic acid, aspartic acid, glycine, alanine, valine, leucine and proline were quite commonly found, cystine, serine, threonine, phenylalanine, arginine and lysine were missing in some kinds of algae, and tyrosine and histidine did not exist in the form of free acid. Tsuchiya and Suzuki[31] proved by inspecting free amino acids in Porphyra tenera that the main origin of savor was free glutamic acid and alanine. (Table 2).

Table 2. Free amino acids in Prophyra tenera.

The author and others[32] determined the composition of free amino acids in Porphyra tenera by microbiological determination, and found that glustamic acid, aspartic acid, proline and alanine were much contained, and glutamic acid content varied according to kind, part, place and qual- /191
ity. Takagi and Kuriyama[33] determined free amino acids in ethanolic extracts of several marine algae by using Dowex 50 and ionexchange chromatography, method of Stein and Moore. The results are shown in Table 3.

Table 3. Free amino acids in various algae.

The findings show nitrogen of free amino acids as percentages of total amino N.

It is obvious that the ratio of the amino acids in the 4 extracts are quite different. That is, for Laminaria japonica and Iridaea cornucopiae, glutamic acid and alanine are remarkably high, 41-44% of total amino N. This tendency is also found in Porphyra tenera, but the ratio of glutamic acid and alanine are reversed. In Porphyra tenera and Ulva pertusa aspartic acid exceeds glutamic acid. This is in contrast with Laminaria Japonica and Iridaea cornucopiae. In Ulva pertusa arginine content is far higher than others. This fact might be connected with the physiological function of the alga which lacks asparagine. Prophyra ten- /192 era peculiarly containes citrulline and a little tryptophan. Green and brown algae such as Ulva pertusa and Laminaria japonica contain much proline. On the other hand red algae contain none as little of it.

The above-mentioned facts are not more than examples, i.e. the free amino acid content of algae largely depends on the picking season. Tsuchiya and Suzuki[31] studied the seasonal variation of alanine and glycine in Porphyra tenera. It is shown in Table 4 that alanine and glycine are maximum in January.

Table 4. Seasonal variation of alanine and glycine in Porphyra tenera.

Ogino[34] studied the seasonal variation of free arginine in Prasiola japonica and found that it became maximum in December, then rapidly decreased. (Figure 1).

Figure 1. Seasonal variation of free arginine N. of Prasiola japonica.

He explained that the rapid increase of arginine was not only because it was needed for spore formation but also because it has the same role as asparagine and glutamic acid in higher plants. Kuriyama[26] compared free amino acids in Chondrus ocellatus picked in January and April, and reported that citrulline and asparagine were contained in large amounts in January, proline in April, and chondrine appeared for the first time in April. Takagi and others[35] confirmed the existence of chondrine in Chondrus ocellatus in May. Appearance of chondrine in Chondrus ocellatus is not fully understood, (like that of) 1-aminocyclopropanecarboxylic acid in a pear 3 to 4 weeks before its maturity, and it is not known whether it has an important role in metabolism or any special reason of existence.

In connection with sulfur containing free amino acids in algae, Ogino[29] and Takagi[37] reported the existence of cystine in certain marine algae. Coulson[30] reported cysteic acid in Rhodymenia palmata, Gigartina stellata, Laminaria cloustoni. Takagi and Kuriyama[32] determined the quantity of cysteic acid in Ulva pertusa, Laminaria japonica, Iridaea cornucopiae and Porphyra tenera. The author and others isolated, as mentioned above, chondrine, a new sulfur containing amino acid, from Chondria crassicaulis. The acid was later found in Desmarestia ligulata and Chondrus ocellatus. Although found to be present in small quantity, it was not found generally. Kuriyama[27] isolated taurine, rhodoic acid and unknown B from Chondrus ocellatus, Iridaea cornucopiae and Neodilsea yendoana, using Amberlite IR-45 and Dowex 2. Distribution of these amino acids is shown in Table 5.

Table 5. The distribution of taurine and newly isolated amino acids in various algae.

Table 5 shows that taurine and rhodoic acid are distributed extensivley in red algae. Distribution of unknown B is restricted. Chondria crassicaulis contains chondrine, but no taurine, rhodoic acid and unknown B. Brown algae such as Laminaria japonica, Alaria praelonga, Undaria pinnatifida and Pelvetia wrightii, and green algae such as Ulva pertusa and Chaetomorpha moniligera do not contain any traurine, rhodoic

acid and unknown B. Unknown B was isolated from Chondrus ocellatus and Iridaea cornucopiae by Kuriyama. The amino acid was prism srystal, m.p. $161^{\circ}C$ and showed red purple with ninhydrin, though it was not sensitive to it.(?) Rf value was 0.17 in butanol:acetic acid: water=4:2:1, 0.15 in 4:1:5, 0.60 in phenol saturated with water and 0.62 in collidine:lutidine=1:1. The structure of this amino acid is still unknown. Takagi and /193
others[34] confirmed the existence of unknown C, a sulfur-containing amino aicd, in Chondrus ocellatus, as a portion was adsorbed by Dowex 2. This acid showed at first brown, later red purple iwth ninhydrin. Rf value was 0.18 in phenol saturated with water, almost the same as for taurine, whereas in butanol:acetic acid:water=4:2:1, it was 0.04 and was clearly different from taurine. An attempt to isolate the amino acid from taurine was not su-cessful. Hence the structure is unknown. Lindberg[38] isolated taurine, N-methyl taurine and N-dimethyl taurine from Ptilota pectinata, Porphyra umbilicalis and Gelidium cartilagineum; on the other hand, taurine is marine algae.

Asparagine is generally contained in various brown and red algae such as Laminaria japonica, Desmarestia ligulata, Iridaea cornucopiae, Porphyra tenera, Neodilsea yendoana, Chondria crassicaulis, Chondruns ocellatus and Fujimatsumo (Japanese), but glutamic acid is not. In this respect marine algae are quite different from fresh-water algae such as Chlorella pyrenoidosa, C. vulgaris and Anaboena cylindrica. Citrulline and ornithine are distributed in red algae such as Porphyra tenera, Neodilsea yendoana, Iridaea cornucopiae, Chondria crassicaulis and Chondrus ocellatus. -Alanine certainly exists in Chlorella vulgaris[39] and

Scenedesmus sp.[40] but in marine algae its existence is quite doubtful. Takagi and aothers[35] isolated unknown D from Chondrus occellatus, which was considered to be a new amino acid. It is a hexagonal tablet crystal, m.p. 238-239°C in a sealed tube, and shows red purple with ninhydrin. Rf values were 0.77 in phenol saturated with water and 0.15 in butanol:acetic acid:water=4:2:1. The structure of the substance is unknown. Recently, new amino acids were discovered one after another in the vegetable kingdom, hence the existence of undiscovered amino acids in marine algae is quite possible. As peculiar amino acids are found in seeds of plants, it is quite reasonable to assume that in a spore of alga there might be some special amino acids. /194

B Peptides

Extracts from algae are treated with basic lead acetate for elimination of albumin. Then lead is precipitated with sulfuric acid. Barium hydroxide is added for elimination of excess sulfuric acid. After thickening in a vacuum, mercuric acetate is added, resulting in the preceipitation of peptide as a mercuric salt. The salt is reduced by hyrogen sulfide. After filtration of mercuric sulfide, the peptide is dried in a vacuum. Peptides isolated in this manner are shown chronologically in Table 6.

Table 6. Several peptides isolated from marine algae.

Haas and others [41, 42, 45] found peptides in Fucus vesiculosus, F. serratus and Ascophyllum nodosum, besides Pelvetia, and determined composing amino acids in the following peptides. (Table 7).

Table 7. Qualitative composition of peptides of marine algae.

Channing and Young[46] succeeded in separating peptides from Pelvetia canaliculata as a N-dinitrophyenyl derivative.

Haas[45] studied the seasonal variation of a peptide in Griffithsia flosculosa, of the Kazashigusa (Japanese) genus, and concluded that the deficiency of light was one of the main causes of formation of peptide in a living body, because in the summer buiret reaction was little or not observed. Again he assumed that, in Corallina officinalis and C. squamata, of the Sanagomo (Japanese) family, whish were covered with a calcareous sheath preventing permeation of light, peptides were produced as a result of photsynthesis of abnormal metabolism. /195

Figure 2 is a diagram illustrating zonation of marine algae[47] in which the apparent effect of sunshine is shown; that is, its close connection with the formation of peptides.

Figure 2. Diagram illustrating typical zonation of a European rocky shore.

C Organic Bases

Existence of volatile organic bases such as trimethylamine was shown by Klebahn[48] Haas and Hill[49] Kapeller-Adler and Casto[50] and Shirahama[51]. Likewise, existence of stachydrine, an unvolatile base, in Porphyra umbilicalis was shown by Toda[52] and choline in 10 algae by Zeller[53]. Ogino[54] found stachydrine, choline and adenine, and presumed guanine and hypoxanthine in Laminaria sp. He also found[55] histidine and choline in Sargassum ringgoldianum, and isolated crystals considered to be trigonelline and lysine. Takagi[56] found adenine in Porphyra pseudolinearis and Undaria pinnatifida.

D Organic Acids and Monosaccharides

Existence of organic acids in algae was reported by Kylin[57] i.e. malic acid, citric acid and tartaric acid in the vacude of Desmarestia viridis, resulting in low PH in the cacude, though this is still in question. Creach[58] confirmed the existence of citric acid in 4 green algae, 15 browns and 13 reds especially in Himanthaliaceae, of the Hibamata (Japanese) family, and Cystoseira fibrosa, of the Yabane (Japanese) order, Hondawara (Japanese) family, (in higher content?). The content differed by parts, that is, in the tang family the leaf contained more than the stem did. The content also differed by stages of growth. In Gelidium attenuatum, one of the Japanese jelly plants, the young body contained only half as much as the matured body which has tetraspores. Hence citric acid in algae is considered to be connected not only with metabolism but also with spore formation. Bean and Hassid[59] inquired into the course of a carbon atom in the photosynthesis of marine algae by fixing $C^{14}O_2$ in Iridophycus flaccidum, and found glyceric acid, glycolic acid, malic acid and phosphate of monosaccharides such as fructose diphosphate, glucose phosphate, uridine diphosphate and uridne diphosphate galactose.

In this review stress was laid on free amino acids in marine algae. The author expresses his appreciation to Dr. Mituzo Takagi, associate professor of Hakkaido University, for his cooperation.

References

21 S. Murakami, T. Takemoto, N. Shimizu; Yakugaku Zasshi (Japanese), Journal of Phormacology, 73 1026 (1953)

23 K. Daigo; Yakugaku Zasshi (Japanese), Journal of Pharmacology, 79 353, (1959).

25 M. Takagi, D. Nakamura; M. Kuriyama; unpublished

26 M. Takagi; this journal, 27 689 (1961)

27 M. Takagi; this journal, 27 694, 699 (1961)

31 Y. Tsuchiya, Y. Suzuki; this journal, 20 1092 (1955)

32 K. Oishi, Y. Tamura, E. Kanai, A. Oyamatsu, S. Okumura, K. Murata; this journal, 27 601 (1961)

35 M. Takagi, S. Ishihara, H. Nishiide, Y. Yamada, H. Murayama, Y. Noto; on the meeting of the Japanese Society of Scientific Fisheries at Nagasaki (1961)

40 M. Takagi, S. Ishihara, M. Oashi; unpublished

43 T. Ohira; Noka (Japanese), Journal of Agricultural Chemistry, 15 370 (1939); 16 1, 293 (1940); 18 915 (1942)

51 K. Shirahama; Sapporo Norin Gakukaiho (Japanese), Journal of Sapporo Agricultural and Forestry Society, 29 234 (1937)

54 C. Ogino; this journal, 10 156 (1941)

55 C. Ogino; this journal, 12 48 (1943)

56 M. Takagi; Hokudai Suisan Iho (Japanese), Bulletin of the Faculty of Fishery, University of Hokkaido, 2 297 (1952)