Pure & Appl. Chem., Vol. 61, No. 3, pp. 293–302, 1989.
Printed in Great Britain.

Bioactive substances of marine animals: polyoxygenated substances

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Abstract - The symbiosis and food chain in the field of marine microorganisms and animals will be discussed. Many polyoxygenated bioactive substances, which show chemically and biologically interesting properties, have been isolated from marine animals. Recently, some symbiotic microorganisms associated with marine animals have been proved to produce some physiollogically active compounds included in the host animals. Especially marine sponges are regarded as rich sources for these compounds, which would be originated from dinoflagellates and blue green algae by symbiosis and food chain. Thus, the bioactive compounds of the sponges have been found in some kinds of nudibranches, the feeders of these animals.

INTRODUCTION

The chemical studies on the constituents of the terrestrial organisms, particularly on those of microorganisms and plants, have long been carried out: the development in this field has been remarkable owing to the progress of the chemical instrumentations after the World War II. Much work on the constituents of animals such as vitamins, hormones, and pheromones has also been reported.

In the early stage in the history of natural product chemistry in Japan, the scientific investigations on the constituents of marine organisms had been performed: studies on sodium glutamate (Ajinomoto) from a sea tangle by Ikeda and on tetrodotoxin from puffer fish by Tahara were published at the start of 20th century (ref. 1).

In this lecture the recent results on the studies of polyoxygenated bioactive substances from marine organisms, in particular marine animals, which have been actively done in Japan will be summarized.

CHARACTERISTIC FEATURES OF SUBSTANCES FROM MARINE ORGANISMS

The living environment of marine organisms differs much from that of the terrestrial organisms: the formers live in water and the latters live in the air. In the sea the variation of the environments such as the temperature occurs to a relatively small extent. Foods and nutritions are obtained from the surface of the body in some marine organisms, and are aquired by symbiosis in many other marine organisms. Owing to the differences in the living conditions between terrestrial and marine organisms described above as well as many other factors, the constituents in the marine organisms differ considerably from those of the terrestrial organisms.

(1) The marine organisms (among others algae) contain abundantly halogenated organic compounds, in particular brominated compounds (So far few fluoro compounds have been detected.). The guanidine compounds are abundant in marine organisms.
(2) Among the marine organisms there are many species of blue green algae and dinoflagellates. They produce structurally and biologically interesting compounds: some of them become symbiotics to other organisms such as sponges and produce metabolites, which are isolated as the bioactive constituents of the host organisms.
(3) As to the origin of the constituents of marine organisms, it must be considered that these constituents are produced by the symbiotics and are accumulated in the organisms by the food chain.
(4) Many marine organisms contain water-soluble constituents, particularly polyoxy or polyether substances. The characteristic features of these polyoxygenated compounds are that they show interesting chemical reactivities and bioactivities, in particular toxicity.

I will discuss polyoxygenated bioactive substances such as toxic substances, tetrodotoxins and palytoxins and potent antineoplastic substances, halichondrins and amphidinolides.

TETRODOTOXINS

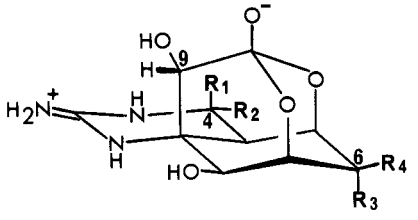
Puffer fish is known to be delicious and also to be strongly toxic. Scientific investigation on the toxic constituent of puffer fish had been carried out since the beginning of this century (ref. 1). Isolation of the toxin was difficult, because the toxin which has high oxygen content is insoluble in organic solvents except for water in the impure state, although its molecular weight is not large. In 1950, however, the crystalline toxin was isolated from the ovaries of Fugu rubripes rubripes by Yokoo (ref. 2). The structure determination of the toxin was also difficult because of the high oxygen content and the unprecedented unusual structure. Large scale isolation of the toxin could be realized by employing the ion exchange resin (Amberlite IRC 50). The availability of the NMR (60 MHz) spectroscopy as well as the X-ray diffraction method using computer led three research groups (Tsuda's, Woodward's and our groups) to the success in determining the structure of the toxin in 1962, the results of which were disclosed at the Third International Symposium on the Chemistry of Natural Products in Kyoto in 1964 (ref. 3, 4, 5). The synthesis of the toxin was achieved by Kishi and his coworkers in 1972 (ref. 6).

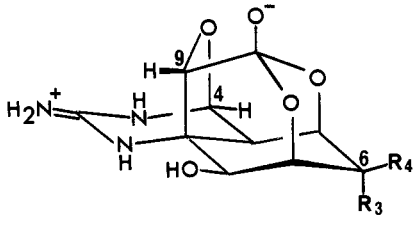
When the toxin once becomes crystalline after purification, unexpectedly it is insoluble in water except that it is soluble in acidic water, although it contains hydroxyl groups and a guanidine group.

Tetrodotoxin was found to inhibit specifically the sodium ion permeability of the membrane (ref. 7, 8). The main action of tetrodotoxin is paralysis of the peripheral nerves. It has been observed that animals possessing tetrodotoxin and puffer fish are highly resistant toward the toxin. These observations are presumably due to the fact that the puffer nerve is at least 1,000 times more resistant than the frog nerve. The taricha nerve is at least 30,000 times more resistant than the frog nerve (ref. 8).

Tetrodotoxin was also isolated from the California newt (ref. 10), the goby fish (ref. 11), the atelopia (ref. 12), and the blue ringed octopus (ref. 13). Recently tetrodotoxin and its congeners (tetrodotoxins) were isolated from various biota (ref. 14), from amphibians to bacteria about 43 species exclusive of puffer. As described above, many kinds of puffer also contain tetrodotoxin (ref. 15). Interestingly, however, a kind of originally toxic puffer becomes nontoxic when cultured in a laboratory for a long time. Now we believe that tetrodotoxin is produced not by puffers themselves, but by lower organisms.

From the Japanese newt Yasumoto has isolated several kinds of tetrodotoxins, namely tetrodotoxin (TTX) (**1**), 4-epiTTX (**2**), 4,9-anhydro-4-epiTTX (**3**), 6-epiTTX (**4**), 4,9-anhydro-4-epi-6-epiTTX (**5**), 11-deoxyTTX (**6**), 11-deoxy-4-epiTTX (**7**), and 4,9-anhydro-4-epi-11-deoxyTTX (**8**) (ref. 16). In the future, other kinds of tetrodotoxins will be found in nature. The biosynthesis of tetrodotoxin will be a problem of great interest.





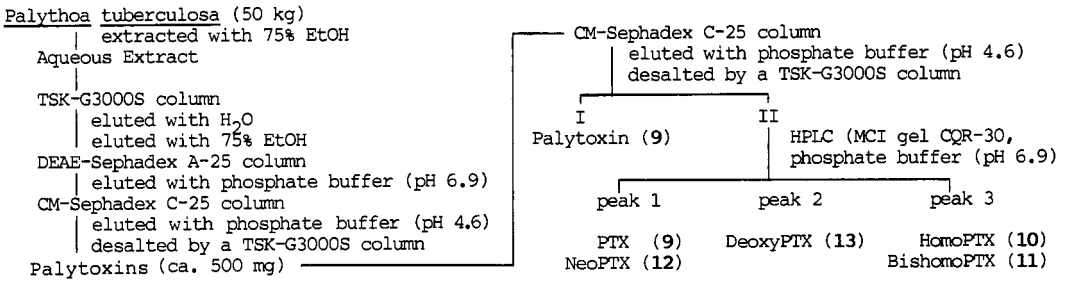
		R_1	R_2	R_3	R_4
1	TTX	H	OH	OH	CH_2OH
2	4-epiTTX	OH	H	OH	CH_2OH
4	6-epiTTX	H	OH	CH_2OH	OH
6	11-deoxyTTX	H	OH	OH	CH_3
7	11-deoxy-4-epiTTX	OH	H	OH	CH_3

		R_3	R_4
3	4,9-anhydro-4-epiTTX	OH	CH_2OH
5	4,9-anhydro-4-epi-6-epiTTX	CH_2OH	OH
8	4,9-anhydro-4-epi-11-deoxyTTX	OH	CH_3

Fig. 1. Tetrodotoxin analogues from the Japanese newt Cynops ensicauda (ref. 16)

PALYTOXINS

Palythoa tuberculosa (Coelenterata, Zoanthidae), a species of hexacorallia living on the coral reefs in tropical and subtropical regions, contains palytoxin (**9**) well-known as the most powerful toxin among those obtained from marine animal sources (LD_{50} in dog is 25ng/kg i.v.)(ref. 17). The toxin has been found to be extremely active against cardiovascular systems, particularly the coronary arteries (ref. 18). Furthermore, PTX induces profound vasoconstriction and increases in systematic blood pressure (ref. 19). This toxin is strongly positive in the irritant and tumor-promoter testings, but is negative in the test of inducing ODC (ornithine decarboxylase) activity (ref. 20).



Scheme 1. Isolation of palytoxins

Isolation and structural determination of PTX were also reported by Scheuer and Moore (ref. 17, 21), whose results are not described here owing to the limitation of the space provided. We have isolated PTX as the main toxic constituent of the Okinawan Palythoa tuberculosa possessing eggs and have determined its structure (ref. 22). Further, we have isolated six congeners of PTX (**10**, **11**, **12**, **13**, and **14**), which were difficult to separate owing to their chromatographically similar properties and their structures were elucidated in 1981 (ref. 23).

(1) Purification of PTXs was extremely difficult, because they are unstable and soluble only in water, and have high molecular weights, and in addition they are a mixture of congeners. Fortunately, the porous polymer resin became available, which can be used for efficient separation of polar compounds in aqueous solutions. The use of the porous polymer resin made possible the separation of PTXs as well as the workup of the periodate oxidation of PTX.

(2) The structural elucidation of PTX could not be achieved solely by utilizing the NMR and mass spectroscopy because of the structural complexity and the high molecular weight. PTX was subjected to ozonolysis or to periodate oxidation on the porous polymer resin, and those oxidation products were separated and their structures were elucidated by the NMR and mass spectral means as well as by the X-ray diffraction method. On the basis of the information obtained from the partial structures of those oxidation products described above, the whole structure of PTX could finally be determined.

(3) The molecular weight of PTX was determined by the plasma desorption mass spectroscopy (PDMS) in collaboration with Macfarlane in 1980 (ref. 24).

(4) Availability of high-field NMR (600, 400, and 270 MHz) made possible the structural determination of PTX.

(5) The absolute configurations of each oxidation product mentioned above (cf. item (2)) could be determined by comparison of the oxidation product with the compound having the known absolute configurations, the latter being synthesized stereospecifically from the material with the established absolute configurations (collaboration with Kishi, 1982)(ref. 25).

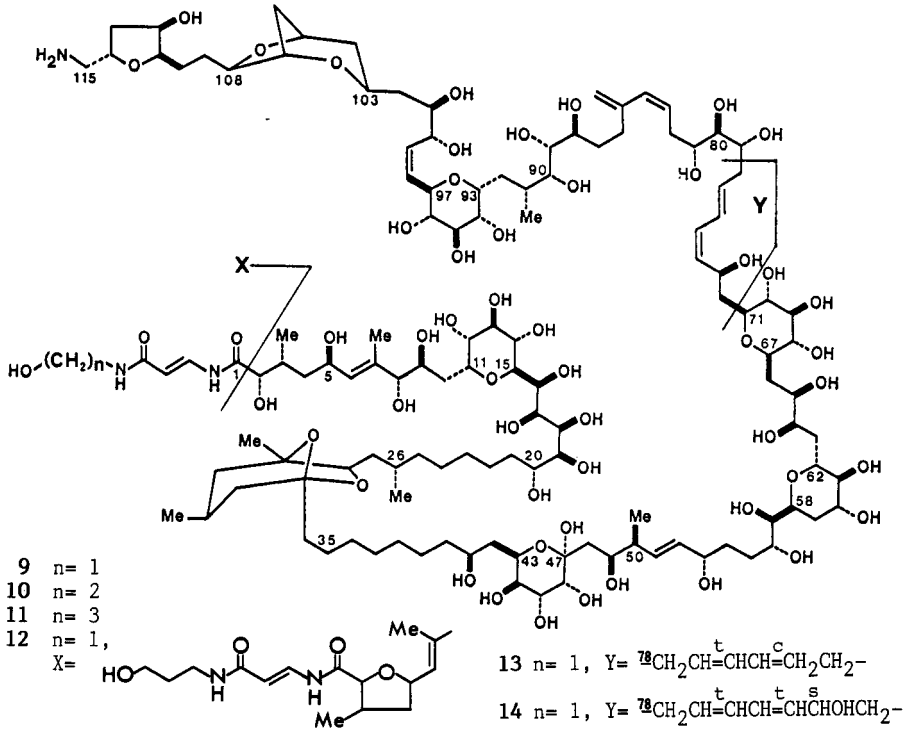


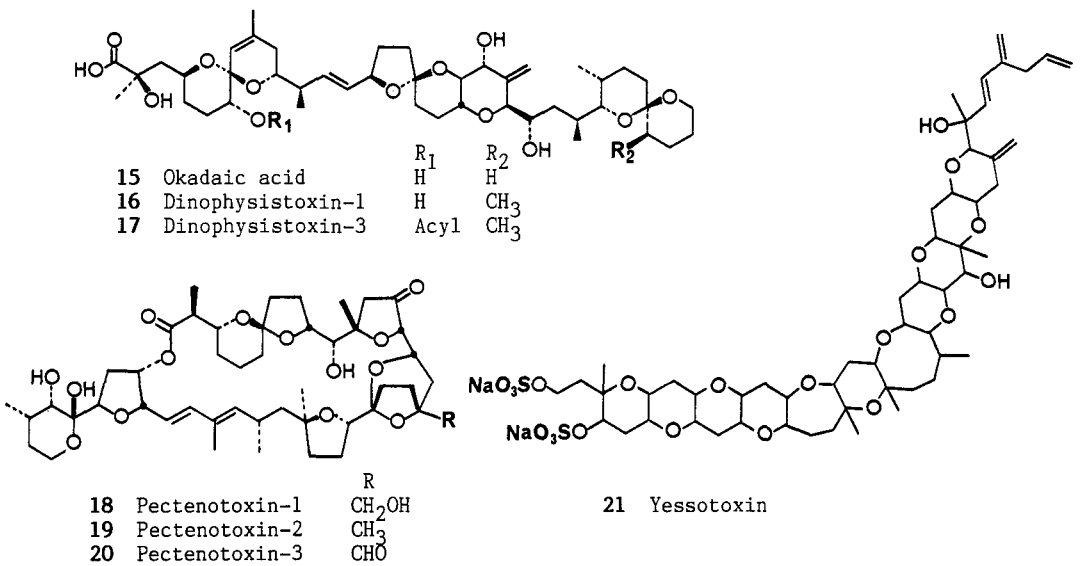
Fig. 2. Structures of palytoxins

It is uncertain whether PTXs are produced by the Palythoa sp. or not. There are a number of Palythoa sp.: The Okinawan Palythoa tuberculosa shows strong toxicity only when it possesses eggs; the Hawaiian Palythoa toxica is toxic throughout the year and its toxicity is much stronger than that of the Okinawan Palythoa tuberculosa. These findings are presumably due to the difference in the contents of PTXs and in the variety of PTXs. It is interesting to note that a kind of PTXs was found in the alga Chondria armata Kuzing Okamurai collected at the Yakushima island of Japan (ref. 26): this finding is interesting in connection with the origin of PTXs. Recently, causative food poisoning occurred by parrot fishes in Aichi prefecture in Japan (ref. 27), by trigger fishes in Micronesia (ref. 28), and by crabs in Philippines (ref. 29), and all the cases were found to be caused by PTXs. The causative poisoning described above is considered to be due to PTXs contained in the Palythoa sp., which the fishes and the crabs took as foods (food-chain).

POLYETHERS FROM MICROORGANISMS AND SPONGES

Dinoflagellates and blue green algae have been well known to produce biologically and chemically interesting metabolites and much attention has been paid to these microorganisms (ref. 30).

Tachibana, Scheuer, Tsukitani, and Schmitz and the co-workers isolated okadaic acid (**15**) from Halichondria okadai Kadota and its structure was elucidated by X-ray crystallographic analysis (ref. 31). From a dinoflagellate, at the same time, Schmitz obtained acanthiofolicin which is regarded as the 9,10-epi-sulfide of okadaic acid (ref. 32). Diarrhetic Shellfish Poisoning (DSP), which is caused by ingestion of bivalves feeding on toxic dinoflagellates, has been one of the most widely occurred natural food poisonings from marine source. Recently, Yasumoto and the coworkers isolated seven compounds classified into three groups from the dinoflagellate which causes diarrhetic shellfish poisoning and determined their structures. One of the three groups includes okadaic acid (ref. 33), and its congeners (**16** and **17**)(ref. 34, 35). Other two groups include pectenotoxin-1 (**18**), -2 (**19**), and -3 (**20**)(ref. 36), and yessotoxin (**21**)(ref. 37), respectively. Although okadaic acid was originally isolated as an antitumor substance from Halichondria okadai, the practical utility of this substance can not be expected owing to its toxicity. Some other biological activities such as tumor promoting activity have been found for okadaic acid. Synthesis of okadaic acid has been made by Isobe and the coworkers (ref. 38).



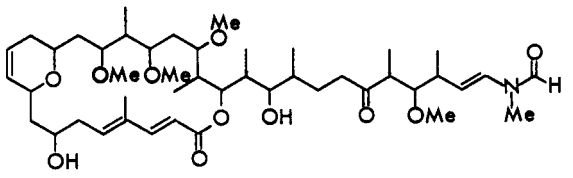
		R_1	R_2
15	Okadaic acid	H	H
16	Dinophysistoxin-1	H	CH_3
17	Dinophysistoxin-3	Acyl	CH_3

		R
18	Pectenotoxin-1	CH_2OH
19	Pectenotoxin-2	CH_3
20	Pectenotoxin-3	CHO

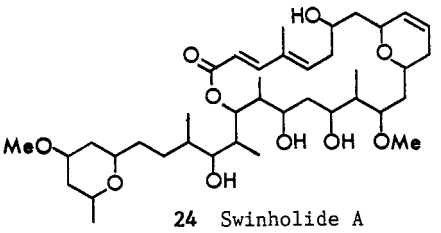
21 Yessotoxin

Fig. 3. DSP substances originated from dinoflagellates

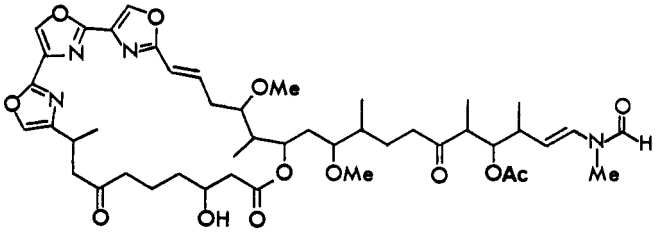
Konosu isolated a polyether macrolide, goniodomin A (**22**) as an antifungal component from a different kind of dinoflagellate (ref. 39). Moore and his coworkers isolated five antitumor compounds, scytophycin A, B, C (**23**), D and E from the cultured terrestrial blue green algae (ref. 40). They are structurally and biogenetically related to the marine natural products, swinholide A (**24**) from the sponge (ref. 41) and ulapualide A (**25**)(ref. 42)(similar to kabiramide C (ref. 43)) from eggmasses of nudibranch.



23 Scyptophycin C



24 Swinholide A

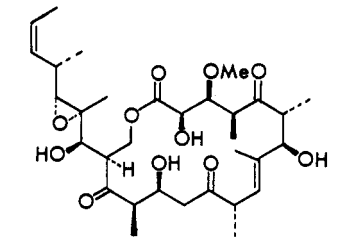


25 Ulapualide A (similar to Kabiramide A)

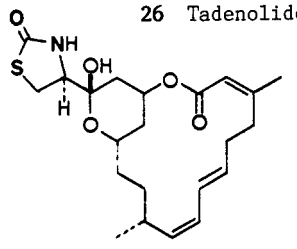
Fig. 4. Polyether macrolides from a microorganism, a sponge and a nudibranch

Sponges together with coelenterates are major organisms in the coral reefs and have a large number of variety. Sponges are interesting organisms in view of the fact that they keep many symbionts (microorganisms occupy the 40% volume of the host sponges in some cases).
Chemical studies on sponges have been made extensively, because various kinds of sponges are found and novel compounds have been isolated. Structurally and biologically interesting constituents of sponges have been isolated, and, in particular, there have been obtained many bioactive polyethers and polyether macrolides such as okadaic acid. Some of them are considered to be originated from symbiosis or food chain.

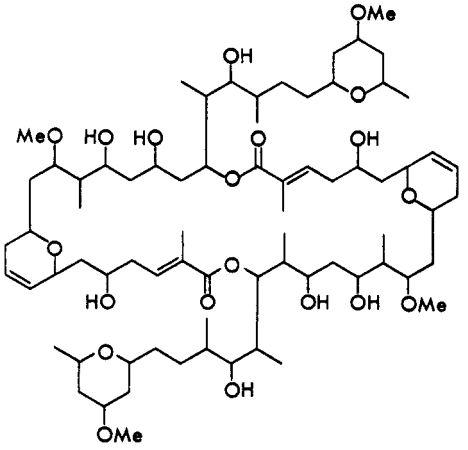
For example, potent antineoplastic substances have been isolated and their structures have been determined: tadenolide (**26**) by Schmitz (ref. 44), latrunculin A (**27**)(ref. 45), and swinholide A (**24**)(ref. 41) by Kashman. Fusetani, Higa and Kashman and their coworkers reported isolation of bistheonilide A (**28**) and B from a sponge Theonella sp. as the egg development inhibitor of starfish or sea urchin (The structure of bistheonilide A was revised from the previously proposed monomeric macrolide structure (misakinolide A) to its dimeric macrolide structure (ref. 46)). This assay system is very useful in search for bioactive, especially antitumor substances.



26 Tadenolide



27 Latrunculine A



28 Bistheonilide A (Misakinolide A)

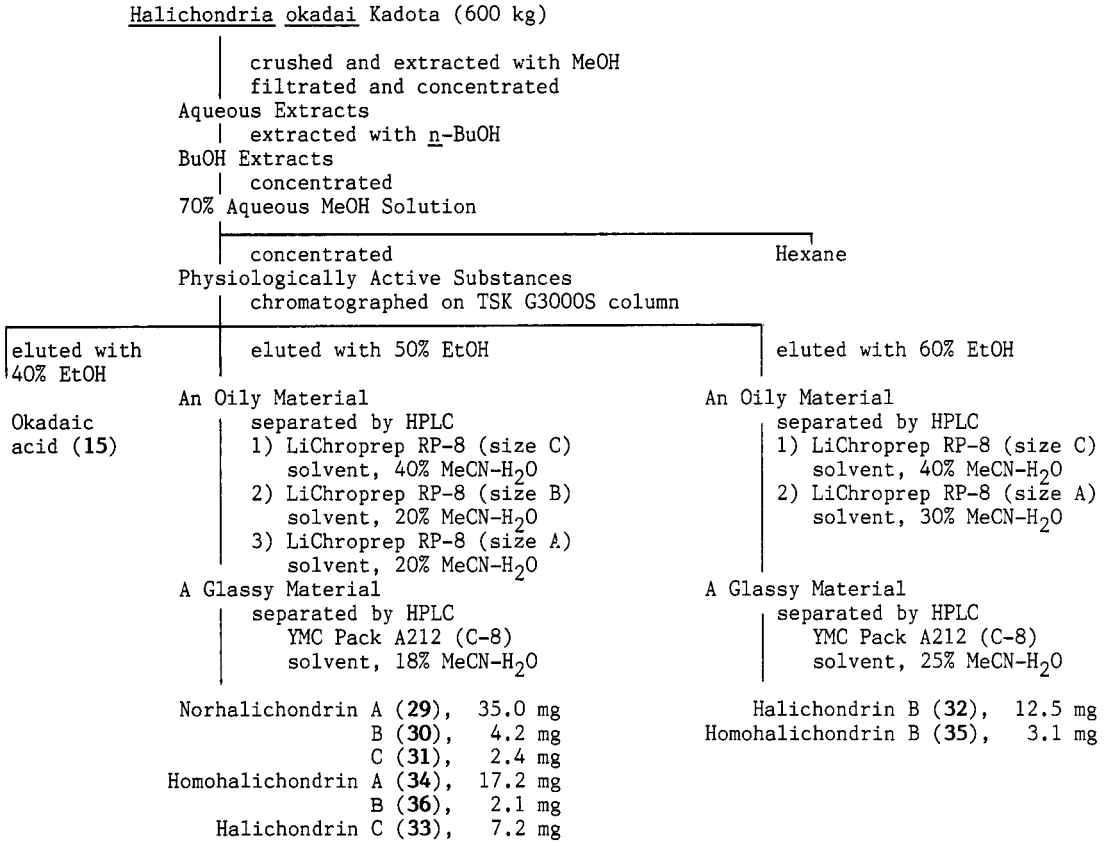
Fig. 5. Antineoplastic substances from sponges

HALICHONDRINS

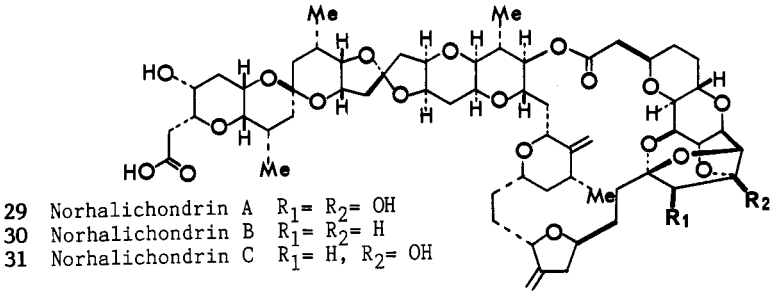
A black sponge Halichondria okadai Kadota commonly found along the coast of Japan is well-known for containing okadaic acid. We have isolated eight new compounds, halichondrins (**29** - **36**) together with known okadaic acid from this sponge by using the porous polymer resin. We have also isolated glycookadaic acid, which consists of okadaic acid and glycine, and okadaic acid congeners from the sponge.

From 600kg of the sponge the largest amount of a halichondrin (norhalichondrin A (**29**)) was 35 mg, and halichondrin B (**32**) was isolated in the crystalline state (12 mg), which shows the most potent antitumor activity and the lowest toxicity. Although the most abundant norhalichondrin A is not crystalline, its crystalline p-bromobenzyl ester was subjected to the X-ray crystallographic analysis, which disclosed the structure: the absolute configuration of norhalichondrin A was determined by the CD study on the di-p-bromobenzoated derivative (1985 - 1986)(ref. 47).

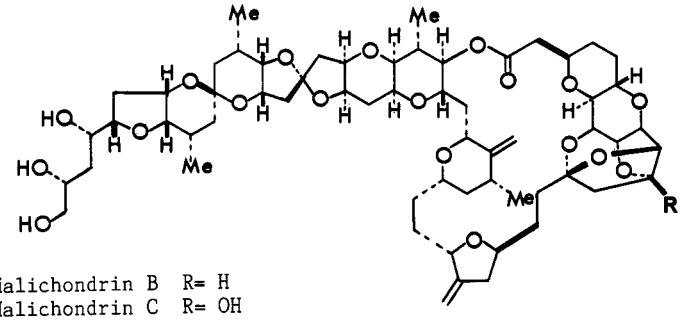
From the results of the in vivo animal testing halichondrin B was proved to have potent antitumor activity and low toxicity. Since the content of halichondrin B in the sponge is low and its synthesis would be difficult owing to its complex structure, the clinical use of halichondrin B can not be expected at present. Because halichondrins are considered to be produced by the symbionts of the sponge, we are trying to search for the symbionts, to separate them, and to cultivate them.



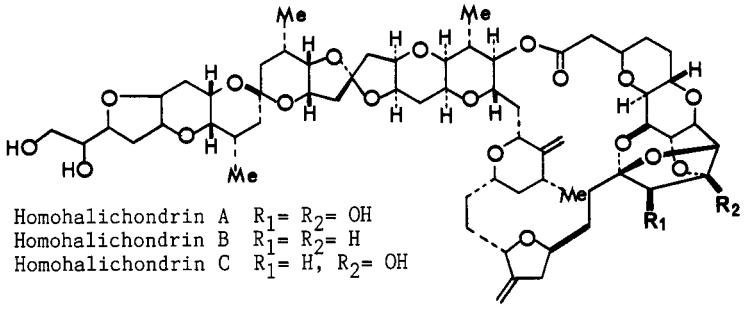
Scheme 2. Isolation and purification of halichondrins



29 Norhalichondrin A R_1= R_2= OH
30 Norhalichondrin B R_1= R_2= H
31 Norhalichondrin C R_1= H, R_2= OH



32 Halichondrin B R= H
33 Halichondrin C R= OH



34 Homohalichondrin A R_1= R_2= OH
35 Homohalichondrin B R_1= R_2= H
36 Homohalichondrin C R_1= H, R_2= OH

Fig. 6. Structures of halichondrins

CIGUATOXIN

Historically, mankind has suffered from poisoning caused by ciguatoxin. Symptoms of ciguatera poisoning are: nausea, diarrhea, headache, temperature reversal and so on. Chemical studies on ciguatoxin have been done by the Scheuer's group (ref. 48). They have isolated pure ciguatoxin and determined the molecular weight, molecular formula, and the partial structures.

Three difficulties of ciguatoxin studies can be pointed out.

1. Extremely low and variable concentration of the toxin in fish.
2. Variation in toxicity in seasons and places.
3. Structural complexity of the toxin

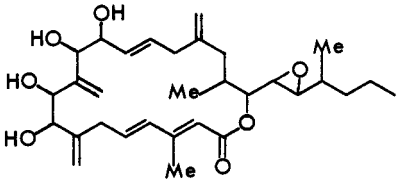
Molecular Weight 1111.7
Molecular Formula $C_{53}H_{77}NO_{24}$ or $C_{54}H_{78}O_{24}$

A red snapper, Lutjanus bohar, is a very common ciguateric fish in Okinawa. Poisoning caused by this species has been frequently observed. Viscera of a red snapper are suitable as sources of siguatoxin. The content of the toxin is very low, but it is possible to obtain the toxin constantly. A spectroscopic comparison of the Okinawan toxin with the ciguatoxin investigated by Scheuer (less polar ciguatoxin) was successfully performed. Structural studies of ciguatoxin are currently in progress.

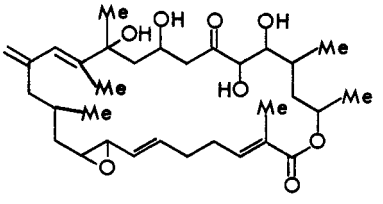
SYMBIOSIS AND FOOD CHAIN

As described previously, further research on the metabolites of the marine animals must be carried out considering both symbiosis and food chain. In fact, some marine microorganisms have been proved to produce some natural products previously obtained from hosts or higher animals. For example, Fusetani's group isolated kabiramide A - E (**25**)(ref. 43), which has the skeleton similar to that of swinholides (**24**) but possesses the novel tris-oxazole moiety, as antifungal macrolides from the egg masses of nudibranch. Later, these compounds were found to have egg development inhibitory activity. At the same time, the Scheuer's group isolated two new compounds ulapualides (**25**)(ref. 42) structurally similar to kabiramides from the egg of nudibranch. Recently, the Faulkner's group also obtained a compound, halichondramide similar to kabiramide, from a sponge of the South Pacific Ocean (ref. 49). It is reasonable to consider that the occurrence of compounds of this type from nudibranch and a sponge is due to food chain, because nudibranch eats sponges. Scytophycin A - E, the skeleton of which is similar to that of the above mentioned compounds, were isolated from a terrestrial blue green alga by Moore and his coworkers (ref. 40).

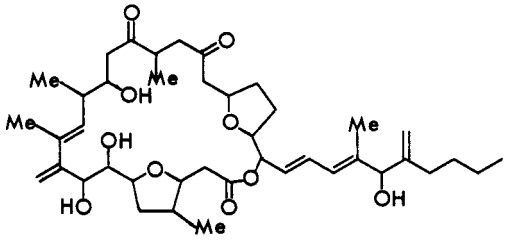
We have cultivated Amphidinium sp., a kind of dinoflagellate separated from a flat worm Amphiscopolopus sp., and have isolated amphidinolide A (**37**), B (**38**), C (**39**), and D (**38**) possessing potent antineoplastic activity. The structural determination of these compounds has been carried out using spectroscopic methods (IR, FABMS, EIMS, 2D-NMR) and chemical means, and the structures disclosed are quite novel. Amphidinolide A, B, C, and D possess 20-membered, 26-membered, 25-membered, and 26-membered lactone, respectively. The 25-membered lactone of amphidinolide C is the first example as a natural product. It is interesting to note that the potent antineoplastic compounds with structural diversity as to the size of the lactone ring and the substitution patterns were obtained from one dinoflagellate (ref. 50). So far, marine animals are known to keep various symbiotic organisms and symbiosis has an important role for both symbiotic organisms and host animals. Although symbiosis among marine organisms is indispensable to the development of coral reefs, little is known about the symbiotic organisms because of the difficulty in their separation and cultivation. When the separation and cultivation of the symbiotic organisms can be carried out rather easily, the studies on the constituents of the symbiotic organisms will be rapidly developed. In the case that a useful constituent such as halichondrin B is isolated in a minute amount from marine animals such as sponges, it is desirable that the symbiotic organisms are searched and their separation and cultivation are attempted and their chemical constituents are examined.



37 Amphidinolide A



38 Amphidinolide B or D (stereoisomer)



39 Amphidinolide C

Fig. 7. Structures of amphidinolides

As in the case of the relationship between nudibranch and a sponge described above, one must consider the food chain in conjunction with symbiosis, when the relationship and the origin of the constituents obtained from blue green algae, dinoflagelltes, sponges, nudibranches, and flat worms are discussed.

What is the origin of tetrodotoxin (TTX)? Symbiosis or food chain? Are palytoxins produced by Palythoa species? Does Halichondria okadai produce okadaic acid and halichondrins? These interesting problems will be solved in the future.

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