The 1990 United States--Japan Seminar on Bioorganic Marine Chemistry, Meeting Report

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THE 1990 UNITED STATES–JAPAN SEMINAR ON BIOORGANIC MARINE CHEMISTRY, MEETING REPORT

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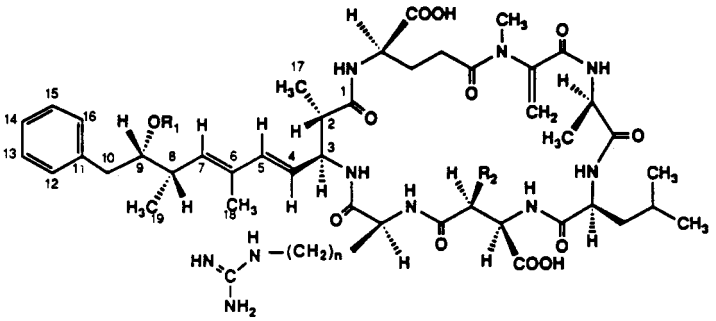
ABSTRACT.—The second U.S.–Japan Seminar on "Bioorganic Marine Chemistry" was held in Honolulu, Hawaii, 3–7 December, 1990. Twenty-one invited lecturers and fourteen observers from the two countries attended. Recent results in the areas of (a) new bioactive natural products, (b) biological and pharmacological activity of marine natural products, and (c) biosynthesis of marine natural products were presented and discussed. Summaries of the presentations and relevant chemical structures are presented in this report.

The first U.S.–Japan joint seminar on marine natural products was held in Okinawa, Japan, in 1986, as a means of exchanging ideas and information in a rapidly developing field and establishing contacts for cooperation and collaboration between scientists from the United States and Japan. In the interim, developments in the broad area of bioorganic marine chemistry have continued apace. Hence it was thought that a second U.S.–Japan joint seminar would be beneficial to exchange information, discuss current developments, and encourage contacts between scientists from these two countries, who are responsible for a majority of the papers published in this area. The second U.S.–Japan Seminar on Bioorganic Marine Chemistry was held at the East-West Center, University of Hawaii, Honolulu, Hawaii, 3–7 December 1990. The topics of discussion covered the broad areas of isolation and structure determination of bioactive compounds from marine organisms, biology and pharmacology of marine natural products, biosynthesis, and synthesis. There were eleven invited lecturers from the U.S. and ten from Japan plus fourteen observers, most of whom gave brief presentations. The following report is based on abstracts and short written papers submitted by the participants. Collaborators' names, when provided, are indicated in footnotes.

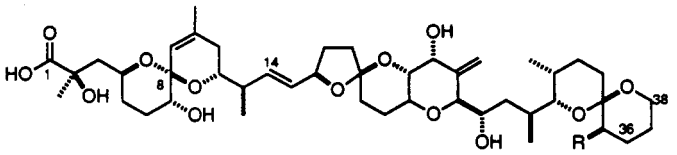
NEW BIOACTIVE MARINE NATURAL PRODUCTS

The early part of the seminar was devoted to newly discovered bioactive metabolites. Kenneth L. Rinehart,[1] University of Illinois, Urbana, Illinois, reviewed the pharmacological and pharmaceutical potential of selected marine natural products under investigation in his laboratories; he discussed in particular the use of fabms/ms in elucidating structures. Fabms/ms was utilized in assigning structures to three new cyclic heptapeptides **1–3** of the microcystin family from a *Nostoc* species of blue-green algae growing in Finnish lakes (1). Although these compounds may be too toxic for clinical use, nodularin and the microcystins are now recognized as potent inhibitors of protein phosphatases (2–4). Investigation of the cytotoxic and antiviral properties of a *Phakellia* sp. sponge from the Gulf of Maine (depth 30 m) led to the isolation of the known okadaic acid [**4**] (5), dinophysistoxin [**5**] (6), and the previously unknown 14,15-dihydrodinophysistoxin, all presumably produced by an as yet unidentified marine microorganism. The crambescidins **6–9** are antiviral (Herpes simplex, type 1) and cytotoxic (98% in-

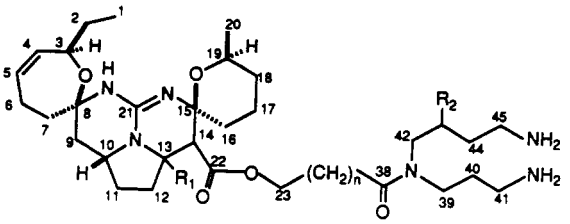
[1]With R. Sakai, M. Namikoshi, E.A. Jares-Erijman, and A. Lithgow-Bertelloni.



1 [ADAMAdda^5]microcystin-LR: $R_1 = \overset{20}{C}O\overset{21}{C}H_3$, R_2 = Me, n = 3
2 [ADMAdda^5]microcystin-LHar: R_1 = Ac, R_2 = Me, n = 4
3 [D-Asp^3,ADMAdda^5]microcystin-LR: R_1 = Ac, R_2 = H, n = 3

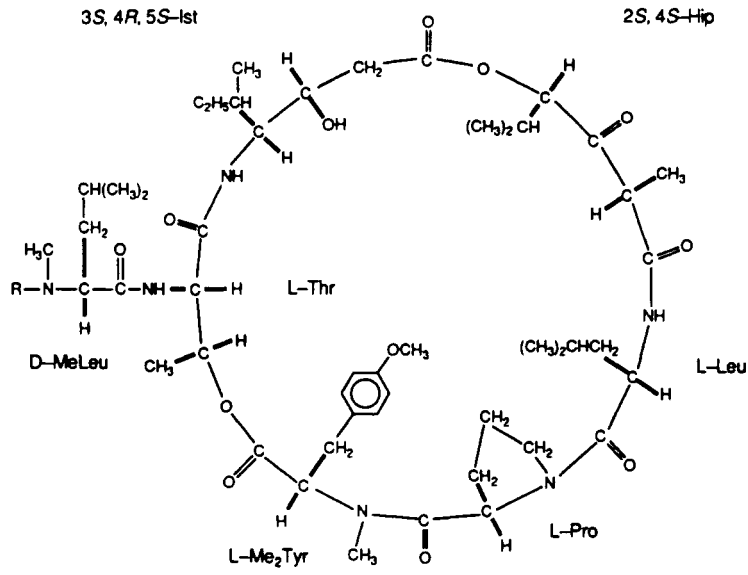


4 R = H
5 R = Me



6 Crambescidin 816: $R_1 = R_2$ = OH, n = 14
7 Crambescidin 800: R_1 = H, R_2 = OH, n = 14
8 Crambescidin 830: $R_1 = R_2$ = OH, n = 15
9 Crambescidin 844: $R_1 = R_2$ = OH, n = 16

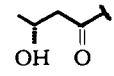
hibition of L1210 leukemia cells at 0.1 μg/ml) polycyclic guanidines isolated from the sponge *Crambe crambe* collected in the Mediterranean. The cyclic depsipeptide didemnin B [**10**] (7) isolated from the Caribbean tunicate *Tridemnum solidum* has survived nearly 3 years of Phase 2 clinical trials by the National Cancer Institute as an anticancer agent and so remains a leading pharmaceutical candidate among marine natural products. Three new didemnins, X [**11**], Y [**12**] (8), and dehydrodidemnin B [**13**] (9), were reported. Dehydrodidemnin B, isolated from the Mediterranean colonial tunicate *Aplidium albicans* (different taxonomic family from *Tridemnum solidum*), is three to five times as active in vivo as didemnin B and is effective in treating leukemia (T/C 210),



10 Didemnin B: R = CH_3CHOHC(L-Lac)—N(L-Pro)—C(=O)—

11 Didemnin X: R = Hydec—(L-Gln)$_3$—L-Lac—L-Pro—

12 Didemnin Y: R = Hydec—(L-Gln)$_4$—L-Lac—L-Pro—

Hydec = *n*-C_7H_{15} 

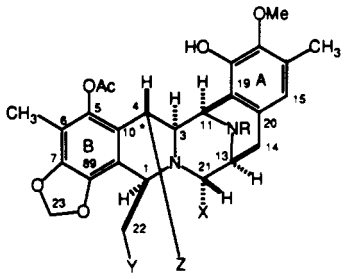
13 Dehydrodidemnin B: R = $CH_3C(=O)C(=O)$—(Pyruv)—N(L-Pro)—C(=O)—

melanoma (T/C 218), and lung tumors, all at 160 μg/kg/injection in mice. Two new members of the ecteinascidin family (10) were reported (11), namely, **14** and **15**; each contains a tetrahydro-β-carboline unit in place of one of the tetrahydroisoquinoline units present in the other ecteinascidins. These new ecteinascidins have in vivo antitumor activities comparable to those of the ecteinascidins reported earlier.

Isao Kitagawa,[2] Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan, discussed the details of the structures of swinholides B [**16**] and C [**17**] and isoswinholide A [**18**] (12), including the absolute stereochemistries. Isoswinholide A shows distinctly weaker cytotoxicity (IC_{50} 1.1 μg/ml) against the KB cell line than swinholides A, B, and C (IC_{50} 0.04–0.05 μg/ml).

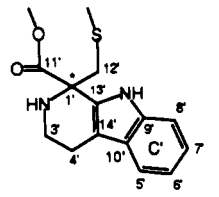
Frank E. Koehn, reporting for the Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Inc., Ft. Pierce, Florida, described a suite of polyoxygenated sterol disulfates, orthoesterol disulfates A [**19**], B [**20**], and C [**21**], isolated from the shallow water Caribbean sponge *Petrosia weinbergi*. These have a novel oxygenation pattern incorporated into an orthoester moiety. These disulfates exhibit in vitro antiviral activity (feline leukemia virus), but are devoid of antifungal, antitumor,

[2]With M. Kobayashi.

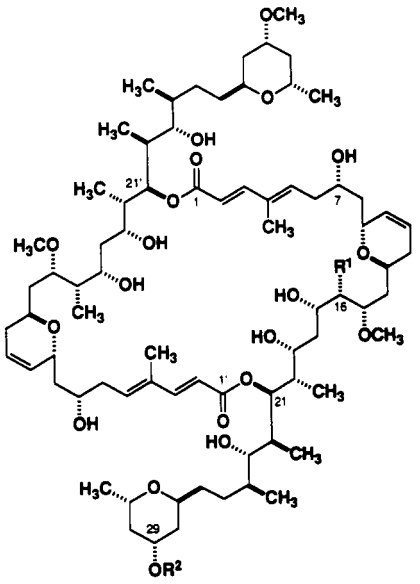


14 Et 736: X = OH, R = Me
15 Et 722: X = OH, R = H

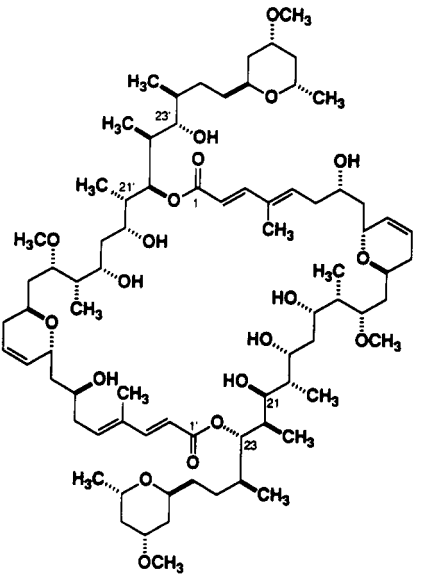
Y,Z =



*stereochemistry not determined



16 R^1 = H, R^2 = Me
17 R^1 = Me, R^2 = H



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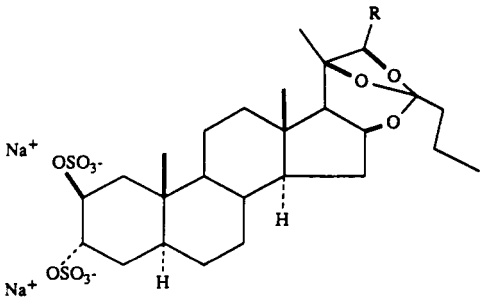
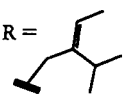
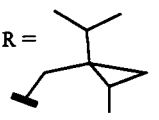


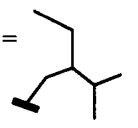
19 R =



20 R =

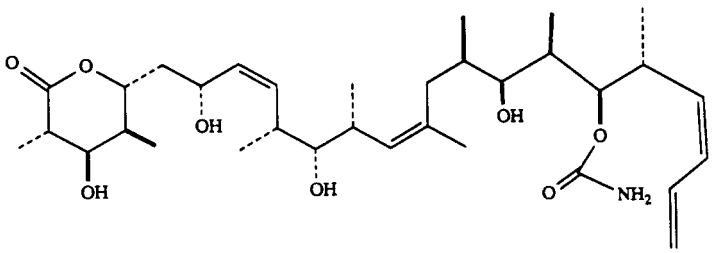


21 R =



and immunomodulatory activity. Biological activities of discodermolide [**22**] (13), a potent immunosuppressive agent, were discussed. It was noted that when activity is compared to cyclosporine in the murine in vivo graft vs. host reaction assay, discodermolide is active at a dose approximately one-tenth that of cyclosporine.

The Harbor Branch group has collected sponges in many different areas of the West Indies and over a wide range of depths. A comparison was made of the natural products chemistry of three categories of sponges: (a) truly deep water West Indian sponges; (b) sponges that were collected in deep water in the West Indies and have shallow water counterparts; and (c) shallow water sponges studied in other laboratories. The comparison revealed (I) unique chemistry in truly deep water sponges; (II) similar chemistry between taxonomically related but zoogeographically distinct deep and shallow water sponges; and (III) similar chemistry between taxonomically unrelated and zoogeographically distinct deep and shallow water sponges, and between deep water sponges and unrelated taxa.



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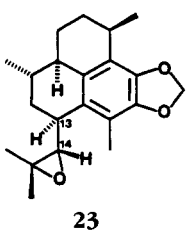
Tatsuo Higa,[3] Department of Marine Sciences, University of The Ryukyus, Okinawa, Japan, reported the isolation (from *Heliopora coerula*) of a group of compounds designated helioporins A [**23**], B [**24**], C [**25**], D [**26**] and E [**27**], which are related to the pseudopterosins. Other metabolites described were echinofuran [**28**] from the octocoral *Echinogorgia praelonga* and 8-isocyano-10-cycloamphilectene [**29**], compound **30**, and 4-thiocyanatoneopupukeanane [**31**], all from an unidentified sponge of the *Adociidae* family.

Jun'ichi Kobayashi, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan, reviewed recent results from his laboratory on bioactive metabolites from Okinawan marine organisms. These included three bromopyrrole alkaloids, ageliferin [**32**], bromoageliferin [**33**], and dibromoageliferin [**34**], all of which are potent actomyosin ATPase activators isolated from the Okinawan marine sponge *Agelas*



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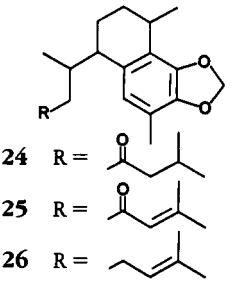

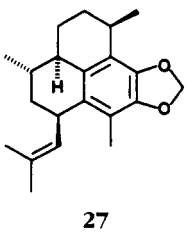

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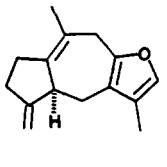
[3]With J. Tanaka and T. Uchida.



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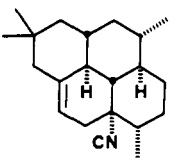

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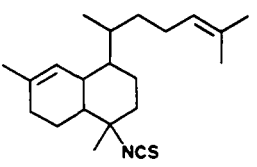

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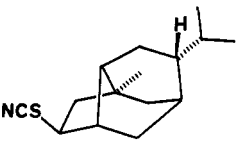

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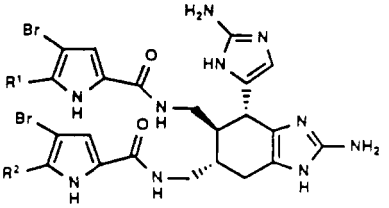
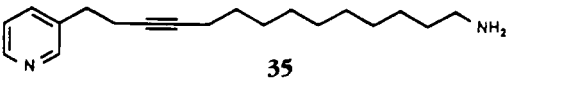
sp. (14). Niphatesines A [**35**], B [**36**], C [**37**], and D [**38**] are new antineoplastic pyridine alkaloids from the Okinawan marine sponge *Niphates* sp. (15). Purealin A [**39**], isolated from *Psammaplysilla purea*, is a new antileukemic bromotyrosine derivative (16) related to purealin, a known ATPase modulator. Rigidin [**40**], a novel pyrrolopyrimidine alkaloid with calmodulin antagonistic activity, was isolated from the Okinawan tunicate *Eudistoma* cf. *rigida* (17). Eudistomidins B [**41**], C [**42**], and D [**43**], novel β-carboline alkaloids, were obtained from the Okinawan tunicate *Eudistoma glaucus* (18). Eudistomidin B [**41**] inhibited Na^+,K^+-ATPase but activated actomyosin ATPase, while eudistomidin C [**42**] showed calmodulin-antagonistic activity. Eudistomidin D [**43**] induced Ca^{2+} release from sarcoplasmic reticulum. Amphidinolide E [**44**], a novel antileukemic 19-membered macrolide, has been isolated from the cultured symbiotic dinoflagellate *Amphidinium* sp. (19).



32 $R^1 = R^2 = H$
33 $R^1 = Br$, $R^2 = H$
34 $R^1 = R^2 = Br$



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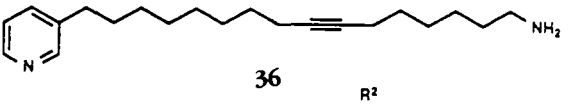

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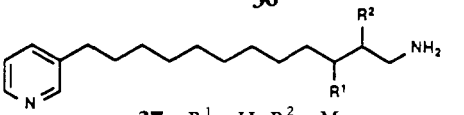
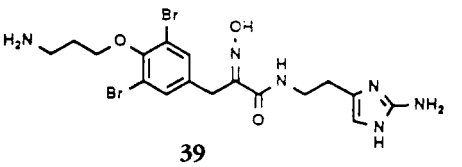


37 $R^1 = H$, $R^2 = Me$
38 $R^1 = Me$, $R^2 = H$



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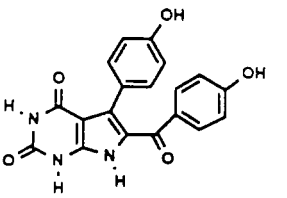

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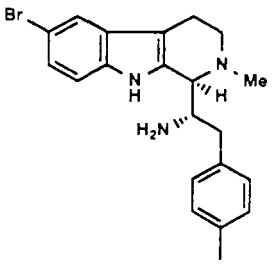

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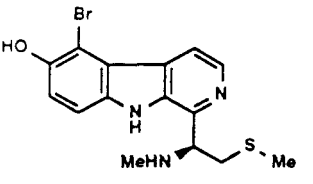

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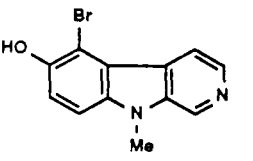

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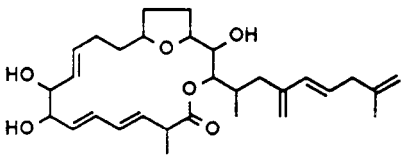

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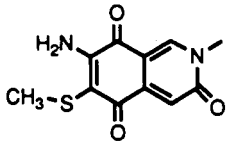
Francis J. Schmitz described the novel isoquinoline quinone **45** (20) obtained from a bryozoan, *Membranipora perfragilis*, from S. Australia. Several additional members **46–49** of the malabaricane triterpene family (21) isolated from a *Jaspis* sp. sponge, all of which show cytotoxicity, were discussed (22).



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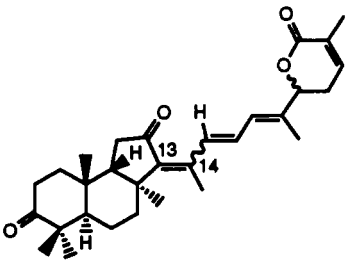
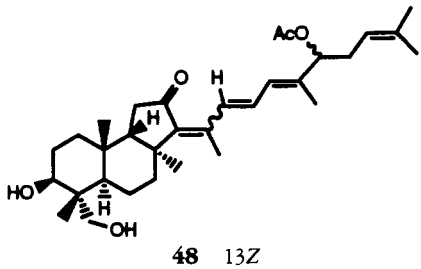


46 13*Z*
47 13*E*

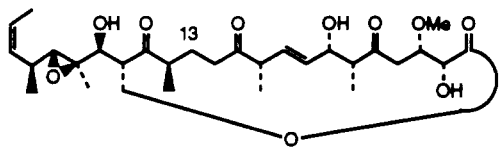


48 13*Z*
49 13*E*

Nobuhiro Fusetani, Laboratory of Marine Biochemistry, University of Tokyo, Tokyo, Japan, reviewed his group's work on cytotoxic compounds and enzyme inhibitors. Four potent cytotoxins were isolated from the sponge *Mycale adhaerens*. Two proved to be the known mycalolides A and B (23). The major compound was 13-deoxytedanolide [**50**], which showed good antitumor activity (T/C 190% at 0.125 mg/kg for P-388). The fourth cytotoxin isolated was the new bromoisocoumarin **51**. Since calyculin A [**52**] (24) has been found to be a potent protein phosphatase 2A inhibitor (25), large-scale re-isolation was undertaken; this resulted in the isolation of the new calyculins E and F (double bond isomers of calyculin A) and G and H (double bond isomers of calyculin C) (26). Calyculins A, B, E, and F are interconvertible in solution when exposed to light, while C, D, G, and H undergo similar interconversions. All these calyculins have similar inhibitory activity against protein phosphatase 2A (IC_{50} 0.9–6.0 nm). Results reported previously (27) on H^+,K^+-ATPase inhibitors were briefly reviewed. In the search for serine protease inhibitors, which are important in developing drugs for treating diseases related to aging, potent antithrombin cyclic peptides named cyclotheonamides A [**53**] and B [**54**] were isolated from a sponge, *Theonella* sp. (28). These peptides contain two new amino acids.

Jon Clardy,[4] Department of Chemistry, Cornell University, Ithaca, New York, re-

[4]With T.J. Stout and G.O. Van Duyne.



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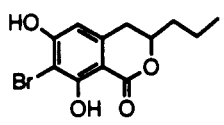

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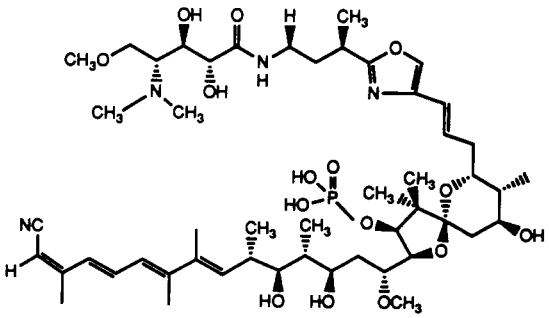

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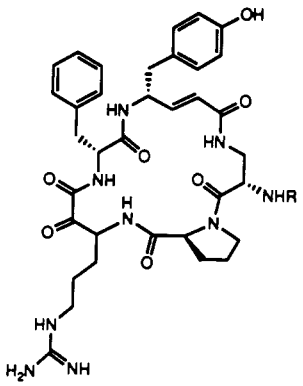


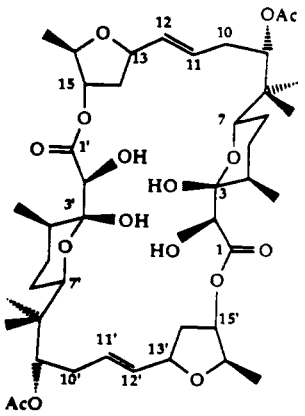
53 R = CHO
54 R = Ac

ported structural studies and molecular mechanics calculations on some marine natural products being investigated in a joint effort with William Fenical's group at Scripps Institution of Oceanography. From an actinomycete isolated from a sandy sediment sample, several closely related compounds were separated. One of these was deboroaplasmomycin C [**55**]. Two other very similar, but chromatographically separable, compounds proved to be conformational isomers of aplasmomycin C [**56**]. These were studied by single crystal X-ray diffraction and nmr techniques in order to understand their conformational differences (29). Also described were diazonamides A [**57**] and B [**58**], which were isolated from a marine ascidian from Siquijor Island, Philippines (30). The diazonamides represent an entirely new class of halogenated, highly unsaturated cyclic peptides. Diazonamide A has potent in vitro activity against HCT-116 human colon carcinoma and B-16 murine melanoma cancer cell lines with IC_{50} values of less than 15 ng/ml.

D. John Faulkner,[5] Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, reported work by his group and that from collaborative efforts with SmithKline Beecham Pharmaceuticals on the chemistry of some Pacific ascidians. Two potent HIV-1 protease inhibitors, didemnaketals A [**59**] and B [**60**], were isolated from a Palauan ascidian of the genus *Didemnum*.

In the course of identifying two aromatic alkaloids from an unidentified ascidian from Palau, this group confirmed that the previously reported ascidian metabolites kuanoniamines C [**61**] and D [**62**] (31) were identical to the sponge metabolites named dercitamides (32) and that structures in the dercitamide series must be revised to conform to the kuanoniamine skeleton. Polycyclic aromatic alkaloids that have the 1,10-

[5]With H. He, B.M.C. Potts, J.A. Chan, G.C. Simolike, P. Offen, M.E. Hemling, and T.A. Francis.



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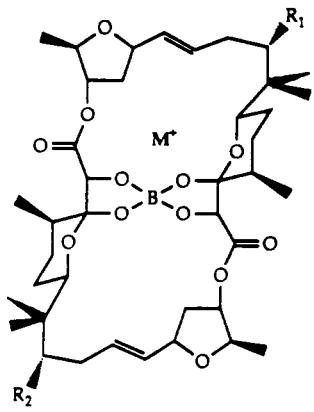
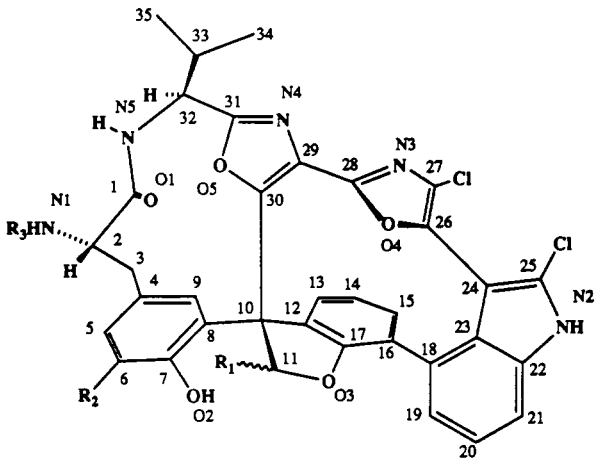
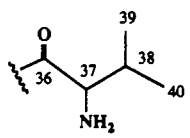
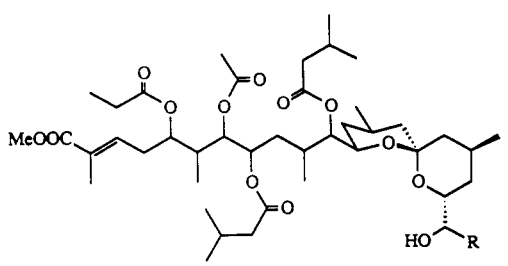


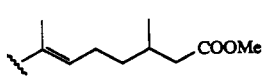
56 $R_1 = R_2 = OAc$



57 $R_1 = OH$, $R_2 = H$, $R_3 =$ 

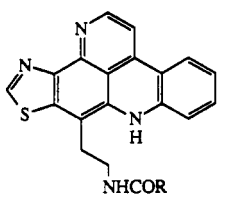
58 $R_1 = OH$, $R_2 = Br$, $R_3 = H$



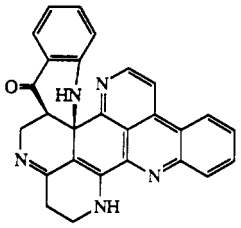
59 R = Ac **60** R = 

phenanthroline substructure as in **61** and **62** can be detected by their ability to form metal complexes. Nmr and fluorescence spectroscopy studies provided evidence for the formation of 2:1 complexes between these alkaloids and divalent metal ions such as Zn^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+}, and Hg^{2+}. The fluorescence spectrum of **62** underwent a significant change when calf thymus DNA was added. These data suggest that the alkaloids in this series may owe their cytotoxicity to a mechanism that involves an interaction with DNA.

An unidentified ascidian from the Seychelles yielded two novel octacyclic alkaloids **63** and **64**.



61 R = Et
62 R = Me



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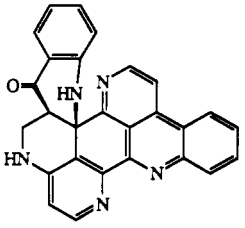
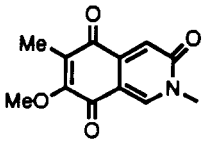


64

Paul J. Scheuer, Department of Chemistry, University of Hawaii, Honolulu, Hawaii, described the chemistry of mollusks and their diet. Mimosamycin [**65**], first isolated in 1977 from a *Streptomyces lavendulae* strain of terrestrial origin, was isolated from the nudibranch *Jorunna funebris* and its sponge host, a *Xestospongia* sp. (33). Mimosamycin has also been isolated from a *Reniera* sp. of sponge. A series of 3-alkylpyridines, the most abundant of which is ikimine A [**66**], were isolated from an unidentified Micronesian sponge (34). Ikimine A is cytotoxic to KB cells (IC_{50} 5 μg/ml). A new cyclic depsipeptide, $C_{43}H_{63}N_5O_9$, was isolated from the nocturnal mollusk, *Philinopsis speciosa*. The ultimate source of the depsipeptide is not known, since *P. speciosa* is reported to feed on another mollusk, *Bulla vernicosa*, which in turn is said to graze on green algae, *Enteromorpha* sp.

Takenori Kusumi,[6] Department of Chemistry, University of Tsukuba, Tsukuba, Japan, reported on three different ongoing projects. The first concerned the chemical properties of ptilomycalin A [**67**], a cytotoxic, antiviral, and antifungal marine alkaloid obtained from a Caribbean sponge *Ptilocaulis spiculifer* and the Red Sea sponge *Hemimycale* sp. (35). It was determined that a derivative of this alkaloid, di(trifluoroacetyl)ptilomycalin A trifluoroacetate, possesses remarkable affinity for fatty acids; e.g., the affinity of ptilomycalin A for isobutyric acid was much larger than that of quinine. The affinity for carboxylic acids was studied by shaking a $CDCl_3$ solution of di-TFA-Ptilo A with an aqueous saturated solution of a salt of a carboxylic acid for a few minutes and then analyzing the $CDCl_3$ layer by nmr (500 MHz) spectroscopy.



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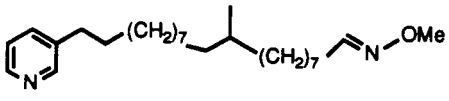


66

[6]With I. Ohtani and H. Kakisawa.

In other work, two novel tetraterpenes, methyl sarcophytoate [**68**] and a hydrochlorinated analogue were isolated from an Okinawan soft coral, *Sarcophytum glaucum* (36). The carbon framework is the same as that of methyl sartortuate (37), and it is speculated that these compounds are formed by Diels-Alder reactions of still unknown cembranoids.

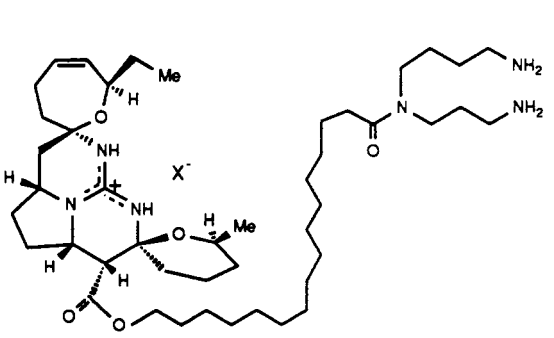
The third research effort outlined by this group concerned results from the application of Mosher's method using high field nmr spectroscopy for the determination of the absolute configuration of marine terpenoids (38–41).

Brief reports were given by a number of observers. Daisuke Uemura,[7] Chemical Research Laboratory, Faculty of Liberal Arts, Shizuoka University, Shizuoka, Japan, reported isolation of the cytotoxic alkaloids halichonines A [**69**], B [**70**], C [**71**], and D [**72**] from the sponge *Halichondria okadai*, the same sponge from which okadaic acid and the potent antitumor polyether macrolides, the halichondrins (42), have been isolated.

Masahiro Murakami,[8] Laboratory of Marine Biochemistry, The University of Tokyo, Tokyo, Japan, described a novel macrolide **73** obtained from a cultured *Oscillatoria* sp. and goniodomins B–D, minor metabolites isolated along with goniodomin A [**74**] (43) from the dinoflagellate *Alexandrium hiranoi*, which was collected in a tide pool.

Kazuo Iguchi, Tokyo College of Pharmacy, Tokyo, Japan, reported a new ichthyotoxic sterol, xestosterol A [**75**], from a sponge, *Xestospongia* sp.

Yasukatsu Oshima, Department of Food Chemistry, Tohoku University, Sendai,



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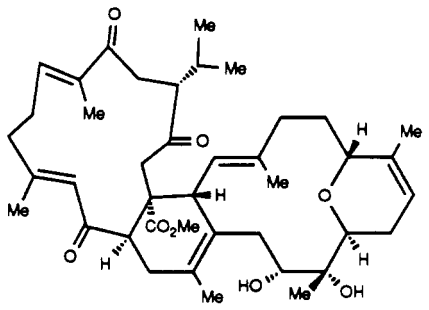

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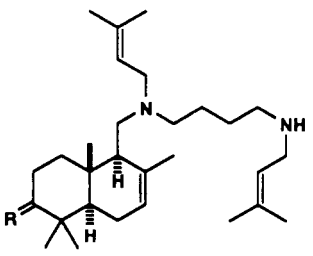
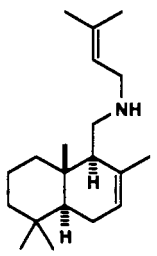


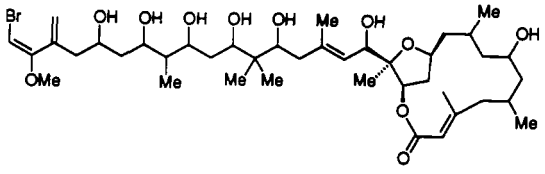
69 R = O
70 R = H_2
71 R = H, HO



72

[7]With T. Chiba, Y. Hayashi, and J. Aiso.

[8]With H. Matsuda and K. Yamaguchi.



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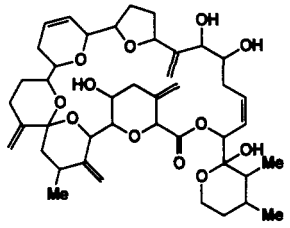

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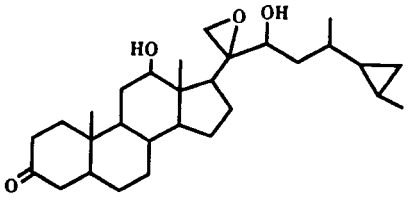

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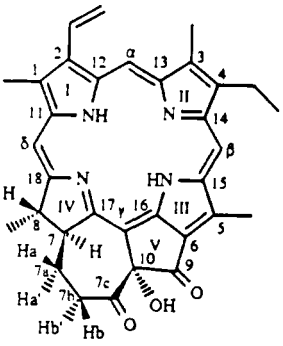
Japan, reported on the mixtures of saxitoxin derivatives detected in strains of *Gymnodinium catenatum* from Spain, Australia and Japan. K. Sakata,[9] Research Laboratory of Marine Biological Science, Mochimune, Shizuoka, Japan, described several chlorophyll-A-related compounds, i.e., chlorophyllone a [**76**] (44), chlorophyllone-lactone a [**77**] and a related α-diketone analogue **78** (45), isolated as antioxidants from marine bivalves. There is evidence to suggest that these compounds are produced by microalgae.



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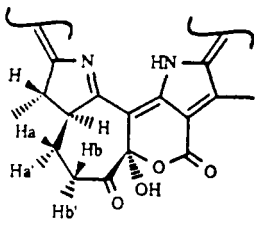

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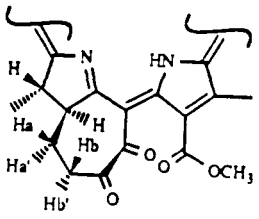
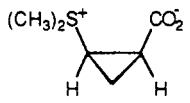


78

Hideshi Nakamura, Department of Chemistry, Hokkaido University, Sapporo, Japan, reported on biologically active substances related to the circadian rhythm of the dinoflagellate *Gonyaulax polyedra*. The bioluminescent system is controlled by a circadian clock consisting of a luciferin, luciferase, and luciferin-binding protein. The structure of the luciferin was determined to be a tetrapyrrole (46). A period-shortening substance, gonyauline [**79**], was isolated from *G. polyedra* (47), and its structure has been confirmed by synthesis (48). Creatine isolated from bovine muscle also causes period shortening.

[9]With K. Yamamoto, H. Ishikawa, N. Watanabe, H. Itoh, A. Yagi, and K. Ina.



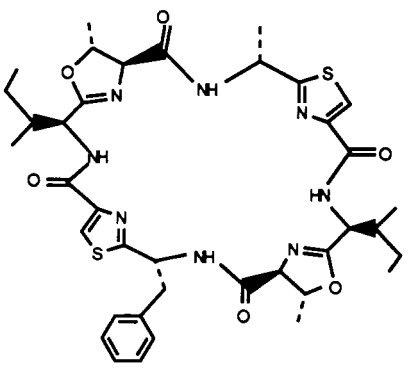
79

BIOLOGICAL AND PHARMACOLOGICAL ACTIVITY OF MARINE NATURAL PRODUCTS

Robert S. Jacobs,[10] Department of Biological Sciences, University of California–Santa Barbara, Santa Barbara, California, described results of studies on the effect of marine natural products on the growth of multidrug-resistant CH^R-C5 cells. In chemotherapy, one major obstacle to successful treatment is the inherent ability of cancer cells to evade or overcome the cytotoxic nature of anticancer drugs (49–51). It has recently been suggested that drug resistance may be mediated, at least in part, by a phenomenon known as multiple-drug resistance or MDR. This is a process whereby cytotoxins are actively extruded from the interior of resistant cells. A membrane-bound glycoprotein (*p*-glycoprotein or *p*-170) acts to pump a wide variety of structurally and functionally unrelated cytotoxins from the cell's interior (49), e.g., *Vinca* alkaloids, colchicine, anthracyclines. Of the dozen marine natural products, which varied widely in structural type, that were tested in this screen, only one, patellamide D [**80**] (52), showed activity equivalent to verapamil, the standard drug used in the assay for MDR. One other similar cyclic peptide and two other unrelated aromatic alkaloids were partially active.

Yasushi Ohizumi, Faculty of Pharmacy, Tohoku University, Sendai, Japan, described results of the effect of maitotoxin (MTX) on calcium channels. Maitotoxin, a principal toxin of ciguatera seafood poisoning, caused Ca^{2+}-dependent excitatory effects on excitatory membranes such as skeletal, smooth, or cardiac muscle. These effects were markedly suppressed by verapamil or divalent cations but were slightly affected by tetrodotoxin, various receptor blockers, or reserpine. The electrophysiological experiments performed by the whole-cell patch-clamp method showed that MTX did not increase the usual Ca^{2+} channel current of a guinea pig cardiac myocyte. Instead, MTX produced a steadily flowing current, which was stopped by Ca^{2+}.

MTX also exhibited excitatory effects on nonexcitable cell membranes such as platelets. MTX (3–10 ng/ml) induced concentration-dependent aggregation of

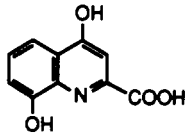


80

[10]With A.B. Williams.

platelets from rabbits and humans. This effect was absent in a Ca^{2+}-free medium and was increased linearly with Ca^{2+} concentrations between 0.1 and 3 mM. These and other results suggest that MTX-induced platelet aggregation is caused by direct action on the cell membrane due to an increase in Ca^{2+} influx which occurred through Ca^{2+} channels. A possible explanation of these observations is that MTX may activate a new class of voltage-insensitive Ca^{2+} channels, not only in the excitable cell membrane but also in the non-excitable membrane.

Yoko Naya,[11] Suntory Institute for Bioorganic Research, Shimamoto-cho, Mishima-gun, Osaka, Japan, reviewed progress in the study of the regulation of ecdysteroidogenesis in crustaceans. Earlier work (53,54) had established that xanthurenic acid [**81**] was a regulator of ecdysteroidogenesis in crustacean molting glands (Y-organ) and that 3-hydroxy-L-kynurenine (3-OHK) [**82**], an endogenous precursor of **81**, exhibited molt-inhibitory effects in vivo upon injection into the eyestalks of ablated crayfish, *Procambarus clarkii*. In recent work the presence of the receptor system for 3-OHK in the Y-organ has been confirmed by specific incorporation of [^{3}H]-3-OHK. It has also been demonstrated that the excised Y-organs from *P. clarkii* synthesized 3-dehydroecdysone as a major product.



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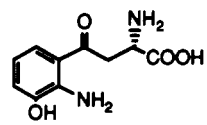


82

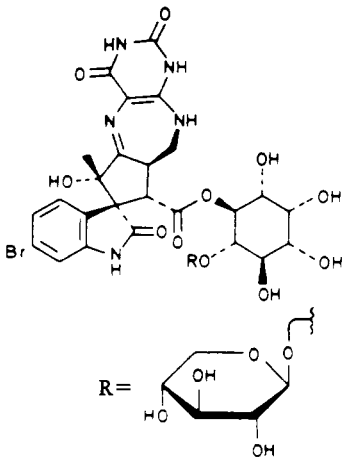
In order to understand molt inhibition, the effects of **81** and **82** on the excised prothoracic glands of silkworms, *Bombyx mori*, were studied. The experiments showed that the production of ecdysone in the prothoracic glands of the silkworm was inhibited at high concentrations of **81**. The overall conclusions at this point are that **81** functions as an inhibiting agent on ecdysteroidogenesis in both excised molting glands from the silkworm (10^{-2} M) and the crayfish. The combined evidence suggests the same molecular mechanism for the inhibition but different modes of transportation in the two organisms. The results indicate that **81** has no biological function in the insect.

Kazuo Tachibana,[12] Department of Chemistry, Faculty of Science, The University of Tokyo, Tokyo, Japan, discussed results of studies on the mode of action of pardaxins, a congeneric series of surfactant peptides responsible in large part for shark repellent and other bioactive properties shown by the defense secretion of soles, *Pardachirus* sp., such as the Moses sole in the Red Sea. The phospholipid bilayer structure of protoplasmic membrane is regarded as the primary target of the pardaxins. The present conclusions, deduced from conformational and structure-activity studies, are that the hydrophobic amino terminus assists by binding the molecule to the membrane, while the middle helical region is essential for the permeabilizing action.

Kuniro Tsuji, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan, reported that neosurugatoxin [**83**] (55) is a useful tool for neuronal studies. It suppressed nicotinic receptors at parasympathetic ganglia without affecting those in skeletal muscle. The toxin also reduced the dimethylphenylpiperazinium-induced release of [^{3}H]dopamine from striatal synaptosomes and the binding of

[11]With M. Ohnishi, M. Ikeda, and K. Nakanishi.

[12]With C.J. Barrow and M.G. Zagorski.

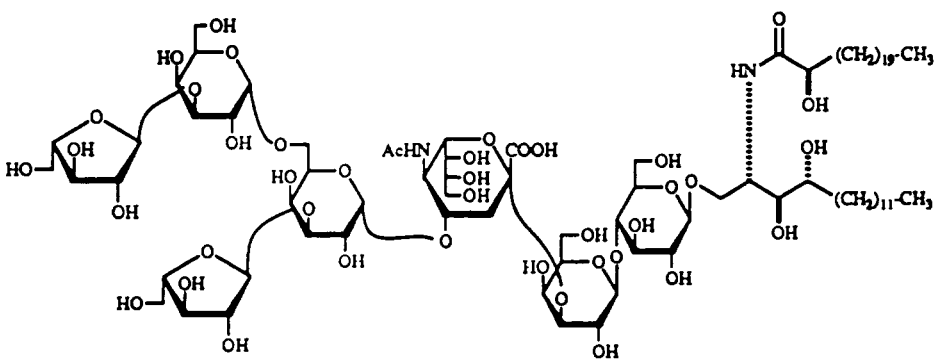


83

[^{3}H]nicotine to brain. The antinociceptive response to nicotine was attenuated by subcutaneous injection of neosurugatoxin. Injection of neosurugatoxin into the ventricles also decreased the secretion of luteinizing hormone mediated by hypothalamic nicotinic receptors. It also inhibits the secretion of catecholamines from cultured bovine adrenal medullary cells in response to nicotine and carbachol. The high affinity and selectivity of neosurugatoxin make the toxin a useful probe for studying nicotine receptors in nerve tissues.

R. Higuchi,[13] Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan, described the structure and biological activity of starfish *Asterina pectinifera* gangliosides, one of which was obtained in pure form, asterinaganglioside A [**84**]. One of the ganglioside fractions was found to support the survival of cultured cerebral cortex cells, most of which were neuronal cells of rat fetuses.

Tadeusz F. Molinski, Department of Chemistry, University of California at Davis, Davis, California, described an assay that selects for ergosterol-dependent antifungal activity against *Candida albicans*. Amphotericin B [**85**], one of the most widely used antifungals for therapy against disseminated *C. albicans*, disrupts lipid structure in fun-



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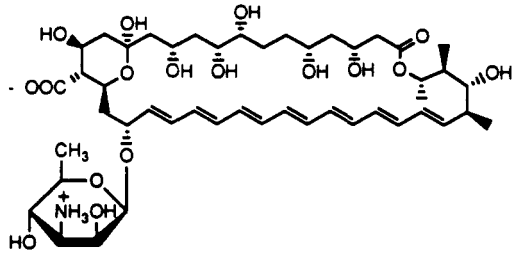
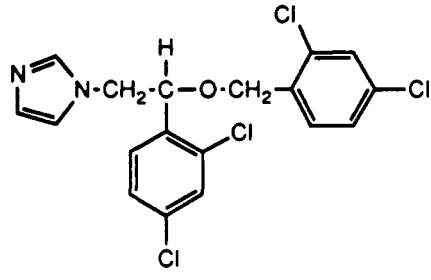
[13]With K. Inagaki, T. Natori, T. Komori, and S. Kawajiri.



85

gal membranes by binding to ergosterol. The assay described involves first assaying for *C. albicans* activity using a conventional agar disk diffusion assay. Compounds or extracts exhibiting activity against *C. albicans* but not *Escherichia coli* were tested further on agar-tryptic broth plates to which varying amounts (0–100 ppm) of ergosterol had been added. At 50 ppm of ergosterol, amphotericin B activity was reduced to 50%. Miconazole [**86**] activity was undiminished in the presence of ergosterol, which is consistent with the different mechanism of action it follows. Assay results indicated that the mechanism of action of the sponge metabolite jaspamide (=jasplakinolide) [**87**] (56,57) depends on its ability to bind to ergosterol, while kabiramide C [**88**] (58) is unaffected by ergosterol.



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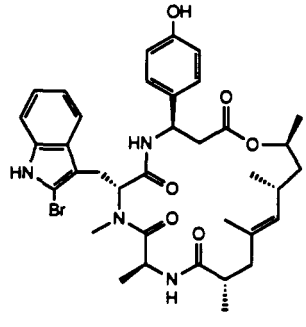

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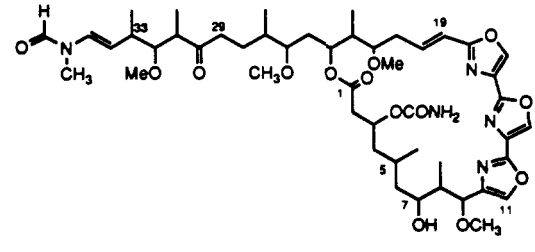

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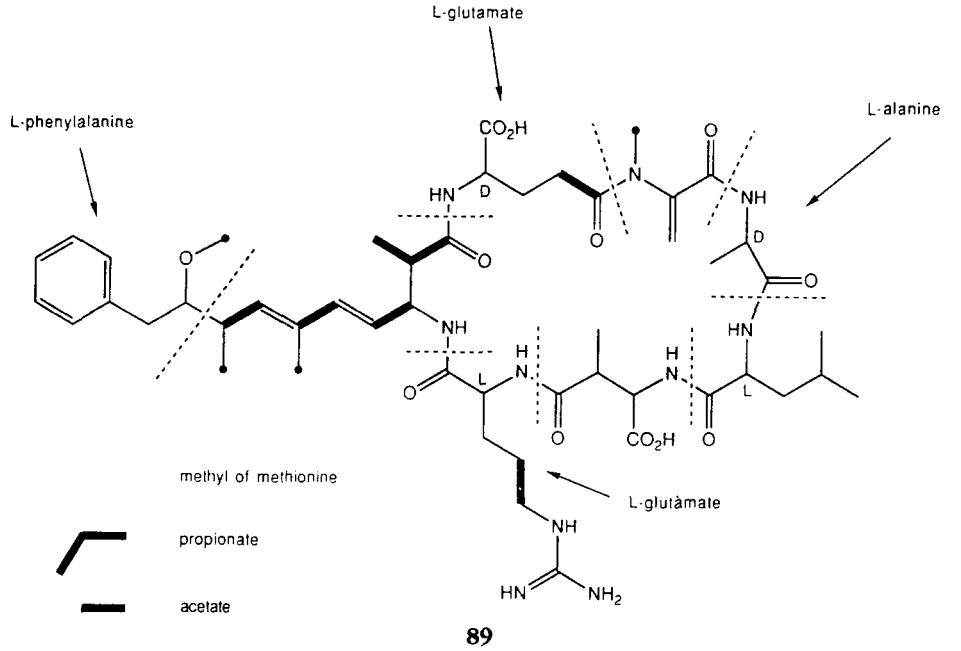
BIOSYNTHESIS

Richard E. Moore, Department of Chemistry, University of Hawaii, Honolulu, Hawaii, discussed progress in the study of the biosynthesis of the blue-green algal metabolites microcystin LR [**89**] (59) and tolytoxin [**90**] (60). Microcystin LR is one

member of a group of cyclic heptapeptides which are responsible for the potent hepatotoxicity of certain blue-green algae (cyanobacteria). Microcystin LR has recently been found to be a highly effective inhibitor of protein phosphatases 1 and 2A (61,62), and this activity is believed to be closely associated with its hepatotoxicity. Preliminary results on the biosynthesis of microcystin LR using *Microcystis aeruginosa* are summarized in Scheme 1. Efforts were focused on the origin of the carbons in the unusual β-amino acid Adda (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid and D-β-Masp (*erythro*-3-methyl-Asp) units. Feeding experiments using [1,2-^{13}C] acetate confirmed intact incorporation of acetate units in Adda and unexpectedly showed that acetate as well as propionate was serving as precursor of C-1 and C-2 of Adda. The origin of the carbons in the Masp unit is presently unknown. Results of a feeding experiment with [1,2-^{13}C]glycine (intact incorporation of C-1 and C-2 into the Mdha unit and comparable incorporation of C-2 of glycine into C-3 of Mdha) suggest that Mdha results from loss of H_2O from a serine unit.



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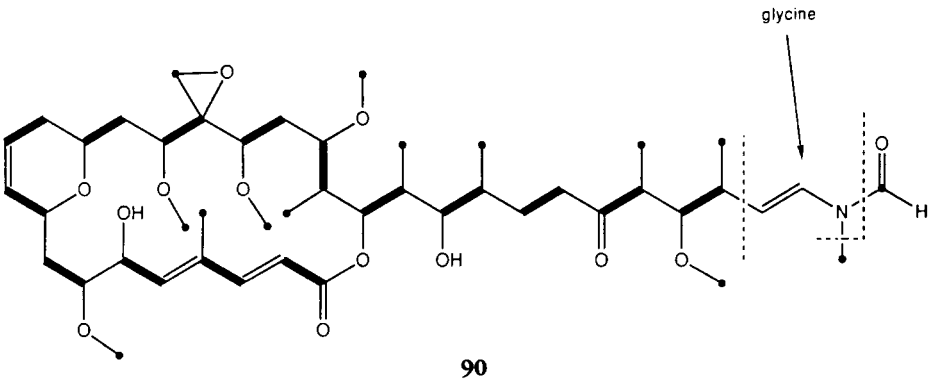
SCHEME 1. Biogenesis of microcystin LR [**89**].

Preliminary results on the biosynthesis of tolytoxin [**90**] (Scheme 2) show that (1) the polyketide chain is formed from glycine as the starter unit (C-31, C-32, and N-32) and acetate for the remaining 15 units of the chain, and that (2) all of the C_1 branches on this polyketide chain, as well as the OMe and NMe carbons, arise from the methyl carbon of methionine.

Yuzuru Shimizu, Department of Pharmacognosy and Environmental Health Sciences, The University of Rhode Island, Kingston, Rhode Island, discussed the problems of culturing microalgae, especially dinoflagellates, which are source organisms for many important secondary metabolites found in the marine environment. Field studies are being conducted to determine environmental conditions which affect the growth of these organisms. Blooms of *Dinophysis* spp. coincide with a high concentration of organic nitrogen. Amino acids such as aspartate and leucine can tremendously enhance the growth of *Gymnodinium breve* (source of brevetoxins and hemibrevetoxins), and a change in toxin profile was also observed.



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▬ [^{13}C-1,2]acetate

[^{13}C-methyl]methionine

SCHEME 2. Biogenesis of tolytoxin [**90**].

Based on the results of a variety of labeled precursor feeding experiments with *G. breve,* Shimizu and coworkers propose that these microalgae are engaged in heterotrophy, but that they first metabolize the compounds to basic fragments such as ammonia or acetate outside the cells and then utilize these basic units. It is not clear whether these metabolic conversions are made by enzymes bound to the outside of the organisms or by the bacteria associated with the organisms, although a similar amino acid conversion has been recently reported for a chrysophyte by Palenic and Morel (63).

Takeshi Yasumoto,[14] Faculty of Agriculture, Tohoku University, Sendai, Japan, discussed structures and biosynthesis of bioactive products of the dinoflagellate *Prorocentrum lima.* Metabolites isolated earlier from this alga were okadaic acid (64), dinophysistoxin-1,7-deoxyokadaic acid (65), and prorocentrolide (66). A search for minor components resulted in the isolation of 2-deoxyokadaic acid and two prorocentrolide analogues. The biosynthesis of okadaic acid and prorocentrolide was studied by culturing *P. lima* in media containing [1-^{13}C], [2-^{13}C], and [1,2-^{13}C]acetic acid. ^{13}C-nmr analyses of isolated okadaic acid revealed that of 44 carbons, 39 were labeled. Only 16 carbons were accounted for by the polyketide pathways. Eight acetic acid molecules were found to be incorporated into okadaic acid via a hydroxymethyl glutarate derivative (Figure 1). Incorporation of ^{13}C was even lower in prorocentrolide. Only 44 of 56 carbons were labeled. Unlike the case of okadaic acid, two carbons (C-43, C-50) could be labeled by either $^{13}CH_3COOH$ or $CH_3{}^{13}COOH$. Biosynthesis of prorocentrolide seems to involve polyketides, succinic acid, and hydroxymethyl glutaric acid (Figure 2), but many parts remain to be studied further.

Phillip Crews, Department of Chemistry and Institute of Marine Sciences, University of California–Santa Cruz, Santa Cruz, California, reviewed aspects of the chemotaxonomy of sponges. He highlighted situations where the distribution of secondary metabolites appears to be inconsistent with reported systematics. An explanation may be that the systematics in those cases are incorrect. One cited case was the reported occurrence of isonitrile sesquiterpenes from taxa within different orders of sponges. This apparent anomaly disappeared after a very recent phylogenetic reorganization proposed by Van Soest *et al.* (67); according to the new classificatiion scheme all isonitrile

[14]With K. Torigoe.

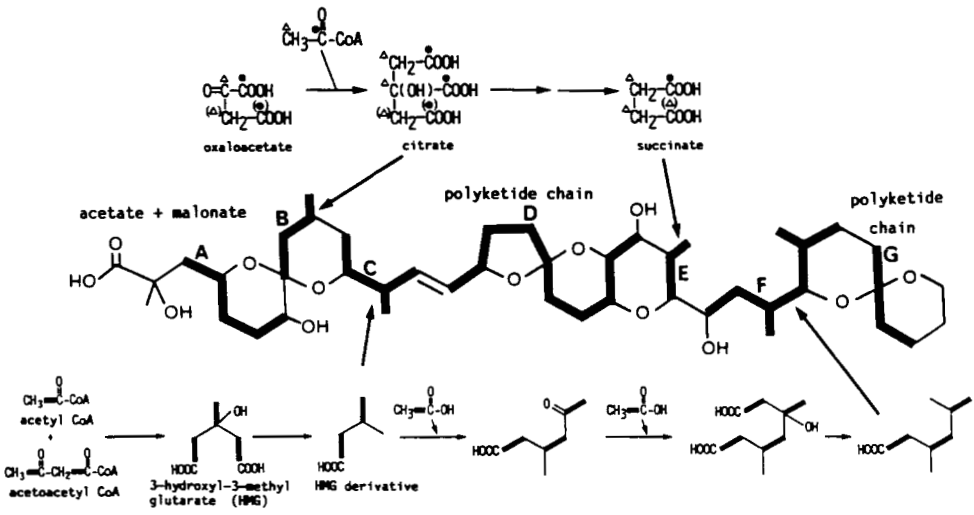


FIGURE 1. Probable intermediates of okadaic acid.

sesquiterpenes are in families belonging to the same order. On the other hand, the occurrence of unexpected metabolites in sponges may be attributed to microbial symbionts, such as algae or bacteria. In analyzing the metabolites isolated from a particular sponge belonging to the family Spongidae, Crews's group has noted substantial variation in the types of secondary metabolites with changing geographic locale of specimen collection. Chlorophyll analysis in the sponge specimens has been negative; hence algae symbionts would not appear to be the cause of metabolite variation. Bacteria from this sponge are now being cultured.

SYNTHESIS OF MARINE NATURAL PRODUCTS

Minoru Isobe, Laboratory of Organic Chemistry, School of Agriculture, Nagoya University, Nagoya, Japan, discussed synthesis of the east sphere of okadaic acid and progress towards a synthesis of optically active tetrodotoxin. One of the intermediates from Isobe's earlier stereocontrolled total synthesis (68) of okadaic acid was readily converted to **91**, referred to as the east sphere of okadaic acid. This compound was five

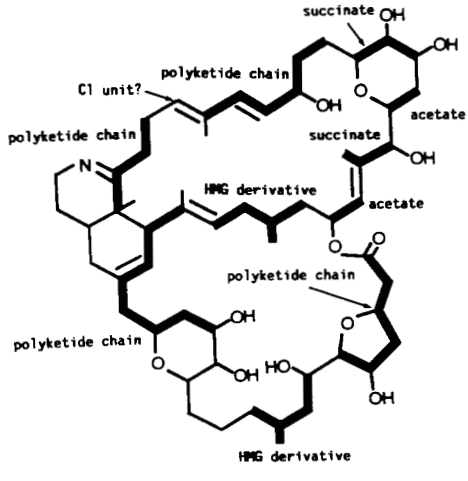
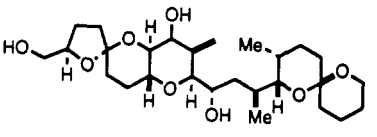


FIGURE 2. Probable intermediates of prorocentrolide.

times as reactive as okadaic acid in the immunoassay developed for okadaic acid; on the other hand, it did not inhibit protein phosphatase Type IIa, which okadaic acid does at very low concentrations. Regarding progress toward synthesis of optically active tetrodotoxin, Isobe reviewed a recently published synthesis (69) of the guanidine-containing moiety **92** and described steps leading to the highly functionalized intermediate **93** using the same Diels-Alder strategy that was used for synthesizing **92**.

Yasuji Yamada, Tokyo College of Pharmacy, Tokyo, Japan, described the synthesis of marine cyclopentanoids starting with chiral 4-hydroxy-2-cyclopenten-1-one [**94**], which can be obtained in both the *R* and *S* form from tartrate. This approach has been used for synthesis of marine prostanoids such as the clavulones (70), chloroclavulones (71), and punaglandins (72). Synthesis (73) of halimedatrial [**95**], a biologically active diterpenoid isolated from a calcareous alga, starting with (*S*)-4-hydroxy-2-cyclopenten-1-one yielded (+)-halimedatrial, the enantiomer of the natural product.



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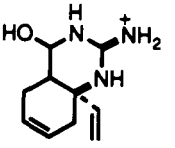

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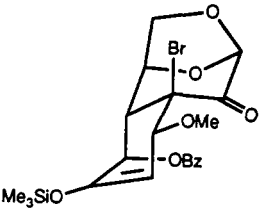

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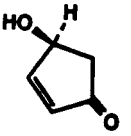

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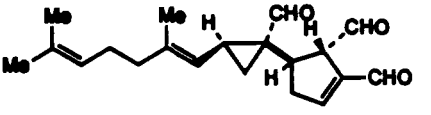

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LITERATURE CITED

1. M. Namikoshi, K.L. Rinehart, R. Sakai, K. Sivonen, and W.W. Carmichael, *J. Org. Chem.*, **55**, 6135 (1990).
2. C. MacKintosh, K.A. Beattie, S. Klumpp, P. Cohen, and G.A. Codd, *FEBS Lett.*, **264**, 187 (1990).
3. S. Yoshizawa, R. Matsushima, M.F. Watanabe, K.-I. Harada, A. Ichihara, W.W. Carmichael, and H. Fujiki, *J. Cancer Res. Clin. Oncol.*, **116**, 609 (1990).
4. R.E. Honkanen, J. Zwiller, R.E. Moore, S.L. Daily, B.S. Khatra, M. Dukelow, and A.L. Boynton, *J. Biol. Chem.*, **265**, 19401 (1990).
5. K. Tachibana, P.J. Scheuer, Y. Tsukitani, H. Kikuchi, D. Van Engen, J. Clardy, Y. Gopichand, and F.J. Schmitz, *J. Am. Chem. Soc.*, **103**, 2469 (1981).
6. J. Hescheler, G. Mieskes, J.C. Rüegg, A. Takai, and W. Trautwein, *Pflügers Arch.*, **412**, 248 (1988).
7. K.L. Rinehart Jr., J.C. Cook Jr., R.C. Pandey, L.A. Gaudioso, H. Meng, M.L. Moore, J.B. Gloer, G.R. Wilson, R.E. Gutowsky, P.D. Zierath, L.S. Shield, L.H. Li, H.E. Renis, J.P. McGovern, and P.G. Canonico, *Pure Appl. Chem.*, **54**, 2409 (1982).
8. K.L. Rinehart Jr., R. Sakai, and J.G. Stroh, U.S. Patent 4,948,791, Aug. 14, 1990; *Chem. Abstr.*, **114**, 214413h (1991).
9. K.L. Rinehart, British Patent Application #8922026.3, filed Sept. 29, 1989.
10. K.L. Rinehart, T.G. Holt, N.L. Fregeau, J.G. Stroh, P.A. Keifer, F. Sun, L.H. Li, ad D.G. Martin, *J. Org. Chem.*, **55**, 4512 (1990).
11. R. Sakai, K.L. Rinehart, and F. Sun, *J. Am. Chem. Soc.*, (submitted).

12. M. Kobayashi, J. Tanaka, T. Katori, and I. Kitagawa, *Chem. Pharm. Bull.*, **38**, 2960 (1990).
13. S.P. Gunasekera, M. Gunasekera, and R.E. Longley, *J. Org. Chem.*, **55**, 4912 (1990).
14. J. Kobayashi, M. Tsuda, T. Murayama, H. Nakamura, Y. Ohizumi, M. Ishibashi, M. Iwamura, T. Ohta, and S. Nozoe, *Tetrahedron*, **46**, 5579 (1990).
15. J. Kobayashi, T. Murayama, S. Kosuge, F. Kanda, M. Ishibashi, H. Kobayashi, Y. Ohizumi, T. Ohta, S. Nozoe, and T. Sasaki, *J. Chem. Soc., Perkin Trans. 1*, 3301 (1991).
16. M. Ishibashi, M. Tsuda, Y. Ohizumi, T. Sasaki, and J. Kobayashi, *Experientia*, **47**, 299 (1991).
17. J. Kobayashi, J.-F. Cheng, Y. Kikuchi, M. Ishibashi, S. Yamamura, Y. Ohizumi, T. Ohta, and S. Nozoe, *Tetrahedron Lett.*, **31**, 4617 (1990).
18. J. Kobayashi, J.-F. Cheng, T. Ohta, S. Nozoe, Y. Ohizumi, and T. Sasaki, *J. Org. Chem.*, **55**, 3666 (1990).
19. J. Kobayashi, M. Ishibashi, T. Murayama, M. Takamatsu, M. Iwamura, Y. Ohizumi, and T. Sasaki, *J. Org. Chem.*, **55**, 3421 (1990).
20. F.J. Schmitz, F.S. DeGuzman, Y.-H. Choi, M.B. Hossain, S.K. Rizvi, and D. v.d. Helm, *Pure Appl. Chem.*, **62**, 1393 (1990).
21. B.N. Ravi and R.J. Wells, *Aust. J. Chem.*, **35**, 39 (1982), and references cited therein.
22. E.O. Pordesimo, "I. An Investigation of the Diterpenoid Chemistry of the Caribbean Gorgonians *Erythropodium caribaeorum* and *Briareum* sp. II. Studies on the Chemistry of the Sponges *Jaspis stellifera* from Guam and *Jaspis* sp. from New Guinea. III. New Bastadin-type Metabolites from the Sponge *Ianthella basta* from Guam." Ph.D. Dissertation, University of Oklahoma, Norman, 1989.
23. N. Fusetani, K. Yasumuro, S. Matsunaga, and K. Hashimoto, *Tetrahedron Lett.*, **30**, 2309 (1989).
24. Y. Kato, N. Fusetani, S. Matsunaga, K. Hashimoto, S. Fujita, and T. Furuya, *J. Am. Chem. Soc.*, **108**, 2780 (1986).
25. M. Suganuma, H. Fujiki, H. Suguri, S. Yoshizawa, S. Yasumoto, Y. Kato, N. Fusetani, and T. Sugimura, *Cancer Res.*, **50**, 3521 (1990).
26. Y. Kato, N. Fusetani, S. Matsunaga, K. Hashimoto, and K. Koseki, *J. Org. Chem.*, **53**, 3930 (1988).
27. N. Fusetani, *New J. Chem.*, **14**, 721 (1990).
28. N. Fusetani, S. Matsunaga, H. Matsumoto, and Y. Takebayashi, *J. Am. Chem. Soc.*, **112**, 7053 (1990).
29. T.J. Stout, J. Clardy, I.C. Pathirana, and W. Fenical, *Tetrahedron*, **47**, 3511 (1991).
30. N. Lindquist, W. Fenical, G.D. Van Duyne, and J. Clardy, *J. Am. Chem. Soc.*, **113**, 2303 (1991).
31. G.P. Gunawardana, S. Kohmoto, and N.S. Burres, *Tetrahedron Lett.*, **30**, 4359 (1989).
32. A.R. Carroll and P.J. Scheuer, *J. Org. Chem.*, **55**, 4426 (1990).
33. N.K. Gulavita, P.J. Scheuer, and E.D. Silva, in: "International Conference on Bioactive Compounds from Marine Organisms." Ed. by M.-F. Thompson, Oxford IBH Publishing Private Ltd., (in press).
34. A.R. Carroll and P.J. Scheuer, *Tetrahedron*, **46**, 6637 (1990).
35. Y. Kashman, S. Hirsh, O.J. McConnell, I. Ohtani, T. Kusumi, and H. Kakisawa, *J. Am. Chem. Soc.*, **111**, 8923 (1989).
36. T. Kusumi, M. Igari, M.O. Ishitsuka, A. Ichikawa, Y. Itezono, N. Nakayama, and H. Kakisawa, *J. Org. Chem.*, **55**, 6286 (1991).
37. S. Jingyu, L. Kanghou, P. Tangsheng, H. Cun-heng, and J. Clardy, *J. Am. Chem. Soc.*, **108**, 177 (1986).
38. T. Kusumi, I. Ohtani, Y. Inouye, and H. Kakisawa, *Tetrahedron Lett.*, **29**, 4731 (1988).
39. I. Ohtani, T. Kusumi, O.M. Ishitsuka, and H. Kakisawa, *Tetrahedron Lett.*, **29**, 4731 (1988).
40. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Org. Chem.*, **56**, 1296 (1991).
41. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1991).
42. D. Uemura, K. Takahashi, T. Yamamoto, C. Katayama, J. Tanaka, Y. Okumura, and Y. Hirata, *J. Am. Chem. Soc.*, **107**, 4796 (1985).
43. M. Murakami, K. Makabe, K. Yamaguchi, S. Konosu, and M.R. Wälchli, *Tetrahedron Lett.*, **29**, 1149 (1988).
44. K. Sakata, K. Yamamoto, H. Ishikawa, A. Yagi, H. Etoh, and K. Ina, *Tetrahedron Lett.*, **31**, 1165 (1990).
45. K. Sakata, K. Yamamoto, H. Ishikawa, N. Watanabe, H. Etoh, A. Yagi, and K. Ina, *Tennen Yuki Kagoubutsu Toronkai Koen Yoshishu*, **32**, 57 (1990).
46. H. Nakamura, Y. Kishi, O. Shimomura, D. Morse, and J.W. Hastings, *J. Am. Chem. Soc.*, **111**, 7607 (1989).
47. T. Roennenberg, H. Nakamura, L.D. Cramer III, K. Ryan, Y. Kishi, and J.W. Hastings, *Experientia*, **47**, 103 (1991).
48. H. Nakamura, *J. Chem. Soc., Perkin Trans. 1*, 3219 (1990).

49. W.T. Beck, *Biochem. Pharmacol.*, **36**, 2879 (1987).
50. W.T. Bellamy, W.S. Dalton, J.M. Kailey, M.C. Gleason, and T.M. McCloskey, *Cancer Res.*, **48**, 6303 (1988).
51. N.A. Bruno, L.A. Carver, and D.L. Slate, *Cancer Commun.*, **2**, 151 (1990).
52. F.J. Schmitz, M.B. Ksebati, J.S. Chang, J.L. Wang, M.B. Hossain, D. van der Helm, M.H. Engel, A. Serban, and J.A. Silfer, *J. Org. Chem.*, **54**, 3463 (1989).
53. Y. Naya, K. Kishida, M. Sugiyama, M. Murata, W. Miki, M. Ohnishi, and K. Nakanishi, *Experientia*, **44**, 50 (1988).
54. Y. Naya, M. Ohnishi, M. Ikeda, W. Miki, and K. Nakanishi, *Proc. Natl. Acad. Sci. USA*, **86**, 6826 (1989).
55. T. Kosuge, K. Tsuji, K. Yamaguchi, T. Okamoto, and Y. Iitaka, *Tetrahedron Lett.*, **22**, 3417 (1981).
56. T.M. Zabriskie, J.A. Klocke, C.M. Ireland, A.H. Marcus, T.F. Molinski, D.J. Faulkner, C. Xu, and J. Clardy, *J. Am. Chem. Soc.*, **108**, 3123 (1986).
57. P. Crews, L.V. Manes, and M. Boehler, *Tetrahedron Lett.*, **27**, 2797 (1986).
58. S. Matsunaga, N. Fusetani, K. Hashimoto, K. Koseki, and M. Noma, *J. Am. Chem. Soc.*, **108**, 847 (1986).
59. K.L. Rinehart, K. Harada, M. Namikoshi, C. Chen, C.A. Harvis, M.H.G. Munro, J.W. Blunt, P.E. Mulligan, V.R. Besley, A.M. Dahlem, and W.W. Carmichael, *J. Am. Chem. Soc.*, **110**, 8557 (1988).
60. S. Carmeli, R.E. Moore, and G.M.L. Patterson, *J. Nat. Prod.*, **53**, 1533 (1990).
61. C. MacKintosh, K.A. Beattie, S. Klumpp, P. Cohen, and G.A. Codd, *FEBS Lett.*, **264**, 187 (1990).
62. R.E. Honkanen, J. Zwiller, R.E. Moore, S.L. Daily, B.S. Khatra, M. Dukelow, and A.L. Boynton, *J. Biol. Chem.*, **266**, 6614 (1991).
63. B.P. Palenik and F.M.M. Morel, *Limnol. Oceanogr.*, **35**, 260 (1990).
64. Y. Murakami, Y. Oshima, and T. Yasumoto, *Bull. Jpn. Soc. Sci. Fish.*, **48**, 69 (1982).
65. T. Yasumoto, N. Seino, Y. Murakami, and M. Murata, *Biol. Bull.*, **172**, 128 (1987).
66. K. Torigoe, M. Murata, and T. Yasumoto, *J. Am. Chem. Soc.*, **110**, 7876 (1988).
67. R.W.M. Van Soest, M.C. Diaz, and S.A. Pomponi, *Beaufortia*, **40**, 15 (1990).
68. M. Isobe, Y. Ichikawa, D. Bai, H. Masaki, and T. Goto, *Tetrahedron*, **43**, 4767 (1987).
69. M. Isobe, Y. Fukuda, T. Nishikawa, P. Chabert, T. Kawai, and T. Goto, *Tetrahedron Lett.*, **31**, 3327 (1990).
70. H. Kikuchi, Y. Tsukitani, K. Iguchi, and Y. Yamada, *Tetrahedron Lett.*, **23**, 5171 (1984).
71. K. Iguchi, S. Kaneta, K. Mori, Y. Yamada, A. Honda, and Y. Mori, *Tetrahedron Lett.*, **26**, 5787 (1985).
72. B.J. Baker, R.K. Okuda, P.T.K. Yu, and P.J. Scheuer, *J. Am. Chem. Soc.*, **107**, 2976 (1985).
73. H. Nagaoka, H. Miyaoka, and Y. Yamada, *Tetrahedron Lett.*, **31**, 1573 (1990).

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