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THE 1994 JAPAN-U.S. SEMINAR ON BIOORGANIC MARINE CHEMISTRY, MEETING REPORT

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ABSTRACT.—The third Japan-U.S. Seminar on Bioorganic Marine Chemistry was held 3–8 July, 1994, in Numazu, Japan, sponsored by the Japan Society for the Promotion of Sciences and the U.S. National Science Foundation. Japanese organizers were K. Tachibana and N. Fusetani (University of Tokyo); the U.S. organizer was K.L. Rinehart (University of Illinois, Urbana-Champaign). In attendance were twelve Japanese participants and fourteen Japanese observers, nine U.S. participants and one U.S. observer, and two Japanese student assistants.

The third Japan-U.S. Seminar on Bioorganic Marine Chemistry was held 3–8 July, 1994 in Numazu, Japan, sponsored by the Japan Society for the Promotion of Sciences and the U.S. National Science Foundation. Japanese organizers were K. Tachibana and N. Fusetani, University of Tokyo, and the U.S. organizer was K.L. Rinehart, University of Illinois, Urbana-Champaign. This seminar followed previous seminars on the same topic held in Okinawa in 1986 and Hawaii in 1990. Attending were twelve Japanese participants and fourteen Japanese observers, nine U.S. participants and one U.S. observer and two Japanese student assistants. The 27 presentations described a highly diverse range of topics, but a few major themes were apparent. Compared to the two earlier Japan-U.S. Seminars on the subject, relatively more attention was paid to the pharmacological activities (especially antitumor activity) and mode of action of compounds from a variety of marine sources. In this connection, emphasis was placed on the possibility that many of the marine compounds apparently derived from macroorganisms are in fact microbial products. A related topic dealt with species interactions with one another. Other topics covered, though in less detail, were biosynthesis and synthesis of marine natural products and marine-derived toxins.[1]

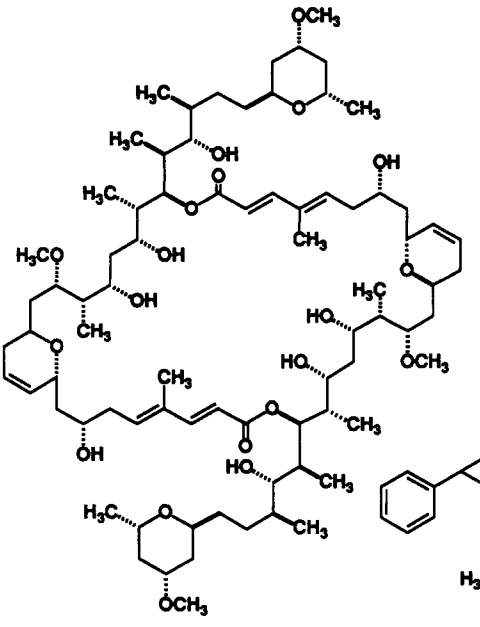
The next joint seminar is planned for Santa Cruz, California, in December 1997, or January 1998, with P. Crews the U.S. organizer and T. Kusumi the Japanese organizer.

MICROBIAL ORIGINS.—M. Kobayashi and I. Kitagawa (Osaka University) provided indirect evidence that many sponge natural products are actually microbial products. In particular, cyanobacteria grow commensally with the sponge *Theonella swinhoei*, which produces swinholides, dimeric macrolides; manzamine-type alkaloids have been isolated from both *Xestospongia* and *Haliclona* species growing in the same area; the potent cytotoxic depsipeptide arenastatin A, isolated from *Dysidea arenaria* is very closely related to cryptophycin B, from a *Nostoc* (cyanobacteria) species.

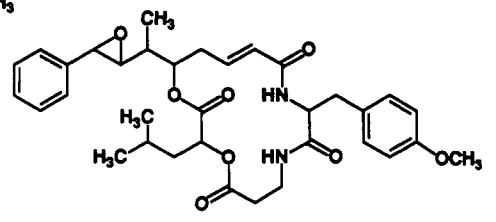
P. Crews (University of California, Santa Cruz) described a program designed to isolate compounds of interest from marine fungi growing on sponges. Thus far, an unidentified fungus from the sponge *Jaspis johnstoni* produced the new chloriolins A–C and the known coriolin B and dihydrocoriolin C, while another unidentified fungus, from a sponge of the genus *Pleiocheata* produced nectriapyrone B.

G. Carter (Lederle Laboratories, American Cyanamid Co.) described a program in

[1]Common names are used where they exist. The few unnamed compounds are numbered.



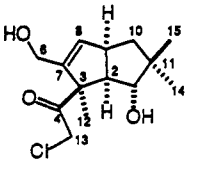
Swinholide A



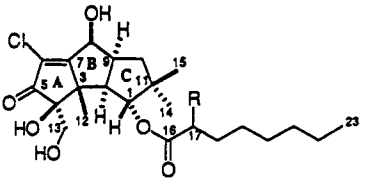
Arenastatin A

which marine microorganisms, mainly Gram-negative bacteria, were tested for a variety of activities. Actinomycetes proved to be the best source of antibacterial compounds, but fungi produced more compounds with cytotoxicity or cardiovascular activity. Examples of cytotoxic compounds isolated include bioxalomycin (naphthyridinomycin-like), also active in vivo, and compounds (BK223-A,B,C; NG-012) from the fungus *Hypoxylum oceanicum*.

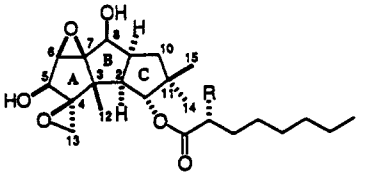
N. Imamura (Marine Biotechnology Institute, Shimizu) described their program for testing extracts of marine bacteria and fungi against marine microalgae. A halophilic bacterium, *Vibrio gazogenese* ATCC 29988, produced magnesidin A, active against the dianophyte *Prorocentrum micans*, while the marine fungus *Fusarium* sp. FE-71 produced halymecin A, which showed strong activity against the diatom *Skeletonema costatum*.



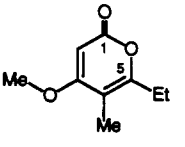
Chloriolin A



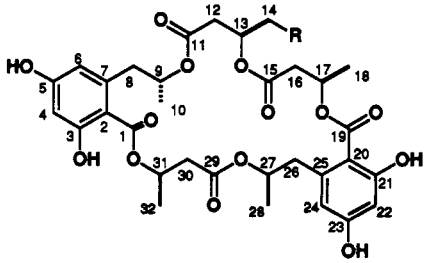
Chloriolin B R=OH
Chloriolin C R=H



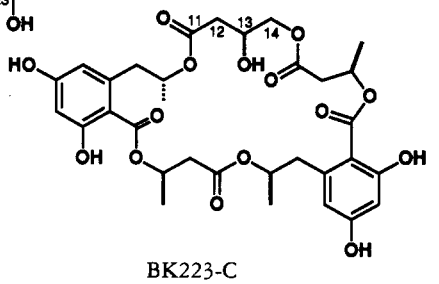
Coriolin B R=H
Dihydrocoriolin C R=OH



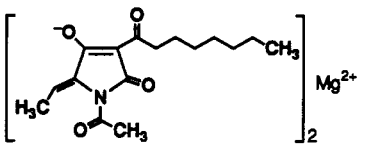
Nectriapyrone B



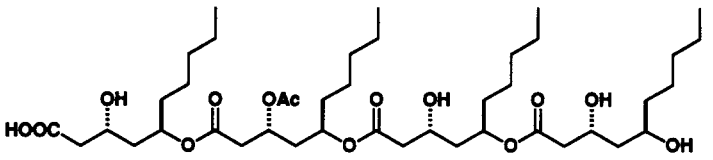
BK223-A R=OH
BK223-B R=H



BK223-C

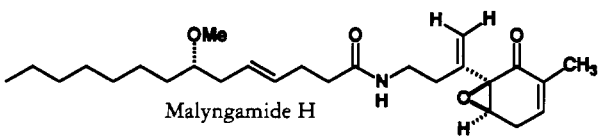


Magnesidin A

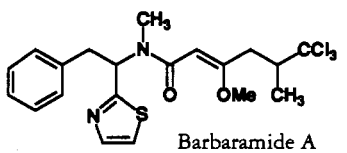


Halymecin A

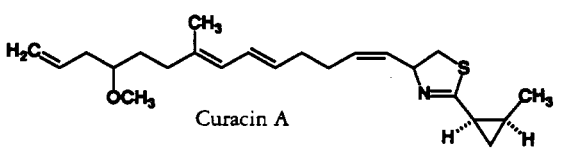
W.H. Gerwick (Oregon State University) noted that extracts of the marine cyanobacterium *Lyngbya majuscula* show three distinct bioactivities: ichthyotoxicity (LC_{50}=25–50 μg/ml vs. *Carassius auratus*) due to malyngamide H; molluscan (snail) toxicity (LD_{50}<100 μg/ml) due to barbaramide A; and potent cytotoxicity (ID_{50}=1–10 nM) due to curacin A, which inhibits tubulin polymerization.



Malyngamide H

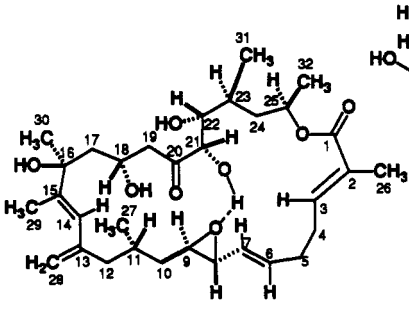


Barbaramide A

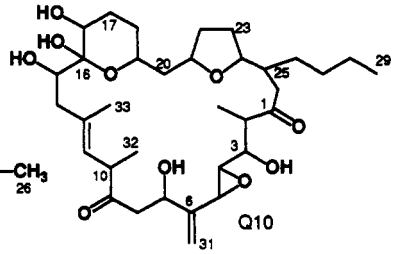


Curacin A

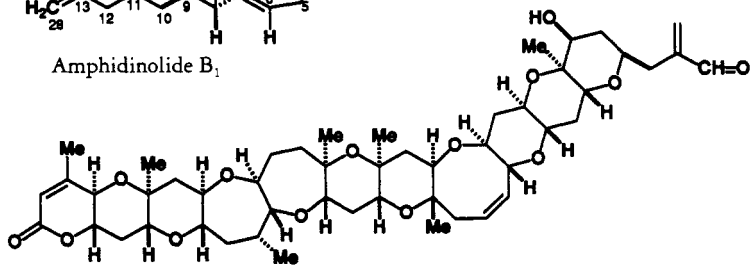
Y. Shimizu (University of Rhode Island) has directed his attention toward improving the yield of bioactive compounds from dinoflagellates, with the goal of making them useful sources of drugs. Potent cytotoxic compounds have recently been isolated from a dinoflagellate, an *Amphidinium* species. However, amphidinolide B and Q10 are produced in only minute amounts, requiring 5,000 liters of culture for preliminary in vivo tests. Brevetoxin (BTX-B) yields have been improved fourfold using semi-heterotrophic cultures and very large tanks are now employed.



Amphidinolide B_1



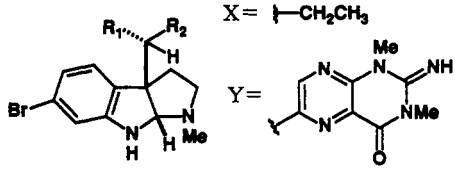
Q10



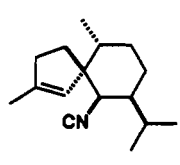
BTX-B

SPECIES INTERACTIONS.—A second major theme dealt with marine macroorganisms and compounds of importance to them, including those which allow species to communicate, either with themselves or with other organisms.

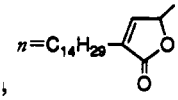
H. Hirota (Research Development Corporation of Japan, JRDC) described studies on fouling by marine organisms (barnacles, mussels, tunicates, hydroids, etc.). Chemical signals are important to the organisms both in promoting their larval settlement and inhibiting that of other organisms. For example, urochordamine A, isolated from the adult tunic of *Ciona savignyi* (tunicate), promoted not only larval settlement but also larval metamorphosis of *C. savignyi* at 1 ng/ml. On the other hand, axisonitrile-3 from the nudibranch *Phyllidia pustulosa* inhibited larval settlement of the barnacle *Balanus amphitrite*.



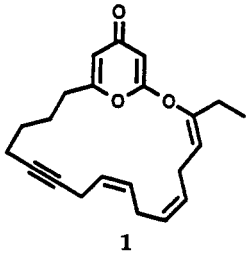
Urochordamine A R_1=X, R_2=Y



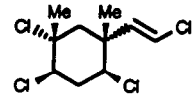
Axisonitrile-3

D. Uemura (Shizuoka University) argued that biofilm (slime) from Gram-negative bacteria promotes fouling of ship bottoms, harbor construction and fishnets by macroorganisms. An alkyl γ-lactone,  , promotes fouling, while certain diterpenes inhibit it.

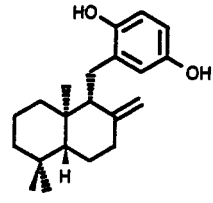
K. Sakata (Shizuoka University) described studies of the feeding preference of gastropods (*Haliotis discus*, abalone; *Turbo cornutus*, turban shell; *Omphalius pfeifferi*, topshell) on various algae. While algal glycerolipids like digalactosyldiacylglycerol stimulated feeding generally, feeding preference was determined by inhibiting secondary metabolites. Examples of inhibitors were the macrocyclic γ-pyrone **1** from *Phacelocarpus labillardieri* (red alga), aplysiaterpenoid A from *Plocamium leptophyllum* (red alga), zonarol from *Dictyopteris prolifera* (brown alga), and the diterpene **2** from *Turbinaria ornata* (brown alga).



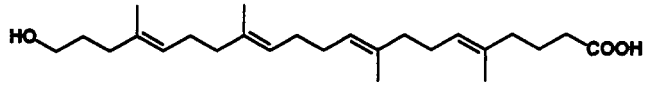
1



Aplysiaterpenoid A

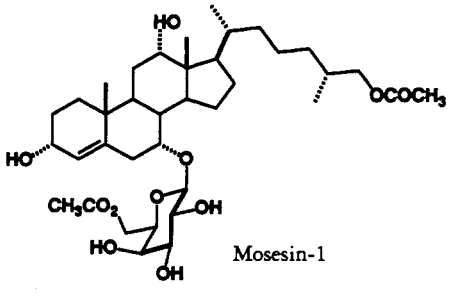


Zonarol

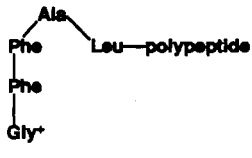


2

In a continuation of studies on the Moses sole (*Pardachirus marmoratus*) components as shark repellents through cell surface membrane attachment, K. Tachibana (University of Tokyo) has developed a liposome-based assay that releases a measurable solute (e.g., calcein tetrasodium). Compounds studied included pardaxin M-1 (30 times as active as its analogue lacking the four *N*-terminal amino acids). Mosesin-1 showed a synergistic effect with pardaxin M-1 in this assay but apparently did not bind to cholesterol, instead sensitizing the membrane to pardaxin.

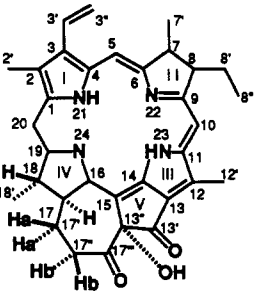


Mosesin-1

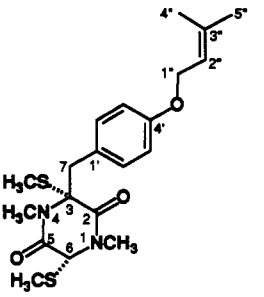


Pardaxin M-1

Antioxidants serve the function of protecting an organism against mutagens. A survey of antioxidants in marine bivalves by N. Watanabe and K. Sakata (Shizuoka University) showed that a number of molluscs (clams, scallops, oysters, and abalone) employ pyropheophorbide-related compounds like chlorophyllone a as antioxidants, perhaps derived from diatoms. Marine bacteria and fungi on the other hand employ a wider variety of compounds as antioxidants, e.g., indole, 3-hydroxyindolin-2-one, uric acid, and 3,4-dimethoxyphenol from bacteria and *cis*-bis(methylthio)silvatin and ergosterol from fungi.



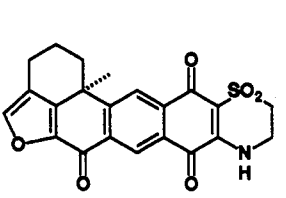
Chlorophyllone a



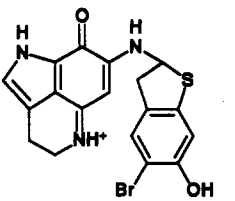
cis-bis(Methylthio)silvatin

S.A. Pomponi (Harbor Branch Oceanographic Institution, Fort Pierce, FL) described efforts to culture sponge species known to produce bioactive metabolites (e.g., *Hymeniacidon* and *Ptilocaulis* species). Important problems addressed included cryopreservation of cells, control of microbial contamination, development of a culture medium, and study of growth factors. Stimulation of cell doubling and metabolite production by phytohemagglutinin was observed. Currently the process has been scaled up to spinner flasks with cell doubling in suspension cultures.

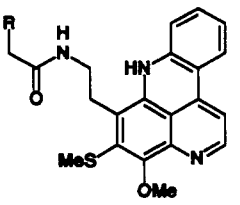
PHARMACOLOGICAL ACTIVITY.—Marine organisms are now recognized to be potentially rich sources of pharmacologically active compounds and considerable attention was directed toward such activity, especially potential anticancer activity, from in vitro bioassays to in vivo studies to clinical trials.



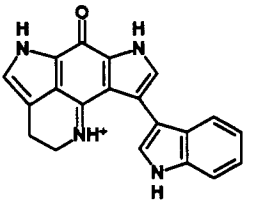
Adociaquinone B



Makaluvamine F



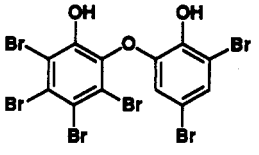
Varamine A R=CH_3
Varamine B R=H



Wakayin

C.M. Ireland (University of Utah) described a mechanism-based screening program designed to discover antitumor agents which inhibit topoisomerase I or II. These mechanism-based assays have identified the compounds shown as topoisomerase inhibitors from sponges—a *Xestospongia* sp. (e.g., adociaquinone B) and *Zyzzya marsailis* (e.g., makaluvamine F), and several ascidians—*Lissoclinum, Diplosoma, Cystodytes* (e.g., varamines A and B), and *Clavelina* (e.g., wakayin) species.

F.J. Schmitz (University of Oklahoma) described early results from a screen employing inhibition of the enzyme inosine monophosphate dehydrogenase as a predictor of anticancer activity. Examples of modestly active inhibitors (IC_{50}=ca. 1–20 mM) identified from sponges are brominated phenoxyphenols (e.g., **3** from a *Dysidea* sp.), unsaturated fatty acids (e.g., **4** from *Pellina triangulata*), tetronic acids (e.g., fasciculatin), and bastadins (e.g., bastadin 8, from *Ianthella basta*).



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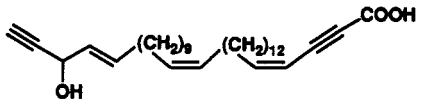

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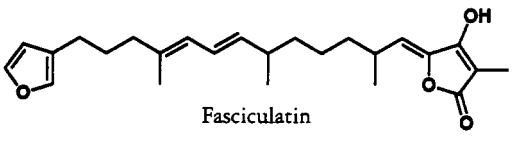
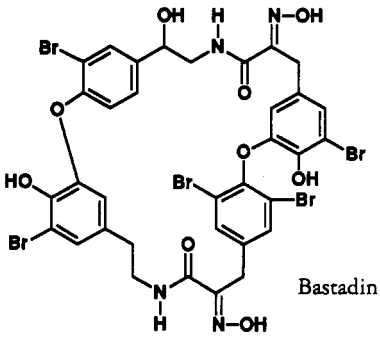
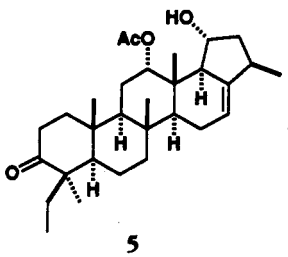


Fasciculatin



Bastadin 8

T. Higa (University of the Ryukyus, Okinawa) described a variety of cytotoxic compounds, including a new bishomosesterterpene [**5**] and the known geranylgeranylhydroquinone (IC_{50} 0.3 mg/ml vs. P-388) from a *Dysidea* sp. (sponge), as well as new sulfated sesterterpene hydroquinones (e.g., **6**) from a *Hippospongia* sp.



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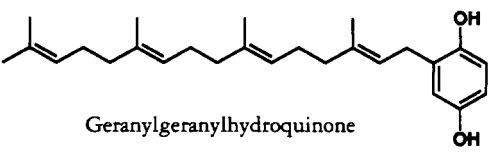
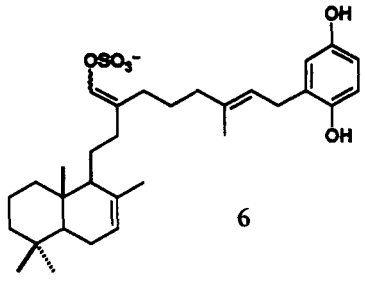


Geranylgeranylhydroquinone



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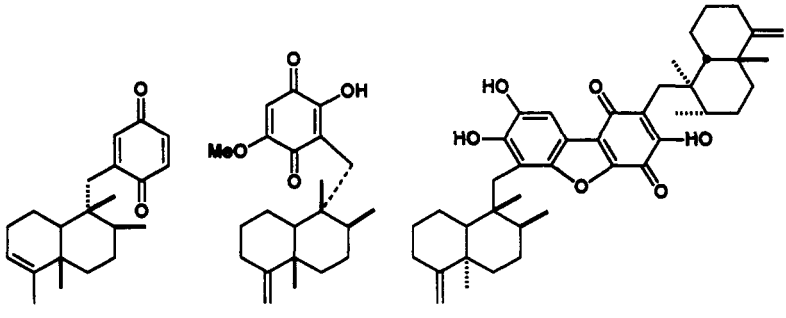
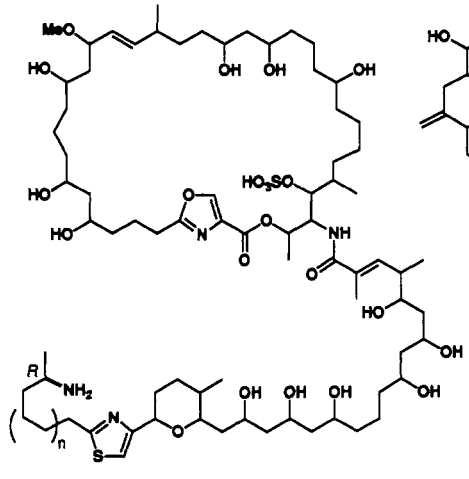
P. Scheuer (University of Hawaii) reviewed the structures and bioactivities of compounds related to avarone, which include ilimaquinone (anti-HIV activity) and popolohuanone E (antitopoisomerase-II activity).

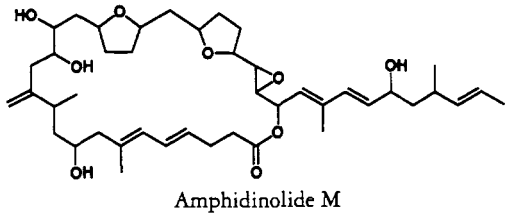


Avarone Ilimaquinone Popolohuanone E

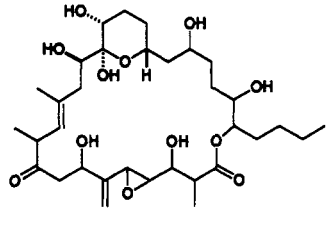
M. Ishibashi and J. Kobayashi (Hokkaido University, Sapporo) reported new compounds from a variety of sources—theonezolides A–C from a *Theonella* sp. (sponge),



Theonezolides A (n=3), B (n=2), C (n=4)



Amphidinolide M



Amphidinolide N

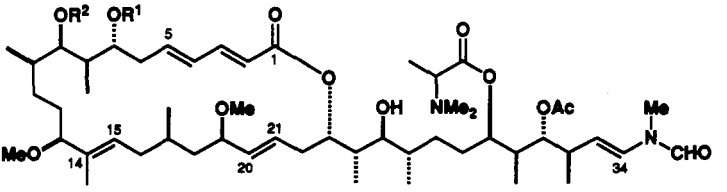
Shimofuridin A

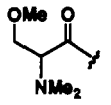
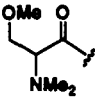
shimofuridins A–G from *Aplidium multiplicatum* (compound tunicate), amphidinolides M and N (new) from an *Amphidinium* sp. (dinoflagellate), together with the known amphidinolide B. Amphidinolide N was especially cytotoxic (IC_{50}=0.05 ng/ml vs. L-1210 cells).

N. Fusetani (University of Tokyo) discussed the status of his group's continuing work on the polytheonamides, polypeptides of high cytotoxicity (IC_{50} ca. 0.08 ng/ml) from the sponge *Theonella swinhoei*. The polytheonamides are believed to form β-turns and to be pore-formers. Amino acids were determined by nmr and sequences by nmr and chemical degradation. The stereochemistry of the amino acids includes many D-amino acids, which usually alternate with L-amino acids.

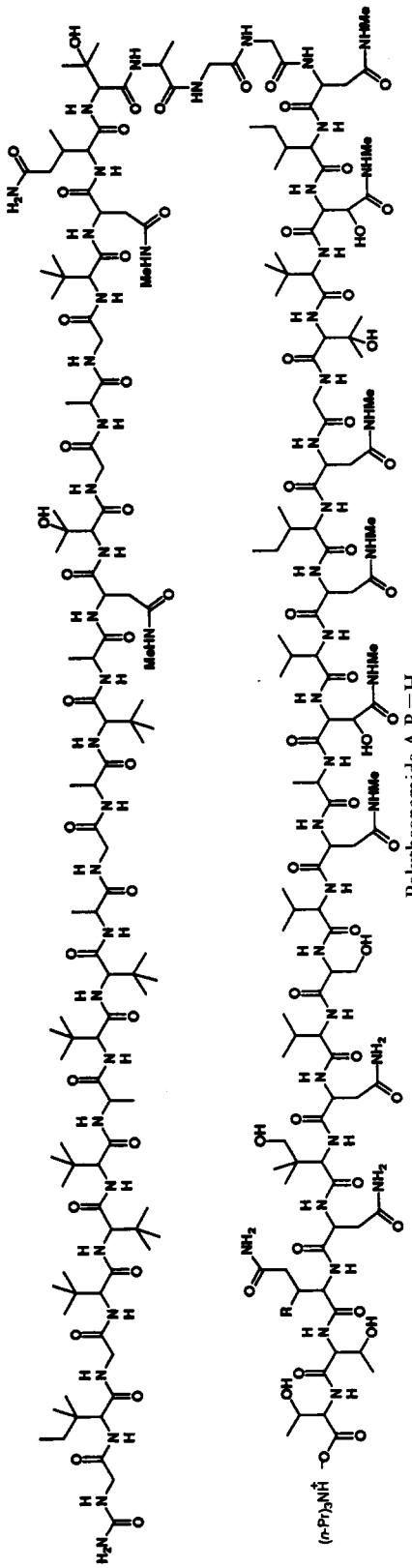
New members of the cephalostatin class of antitumor compounds, the ritterazines, from the tunicate *Ritterella tokioka*, were also described, with IC_{50} values of 0.018–9.6 ng/ml vs. P-388 cells.

Especially promising new antitumor macrolides, the aplyronines, from the sea hare *Aplysia kurodai*, were described by K. Yamada (Nagoya University). Aplyronine A is remarkably active in vivo, with T/C 545% vs. P-388 murine leukemia and 255% vs. colon 26 carcinoma at 80 μg/kg, as well as T/C 556% vs. Lewis lung carcinoma, 398% vs. Ehrlich carcinoma and 201% vs. B16 melanoma at 40 μg/kg. The activity is highly dependent on the presence and location of the trimethylserine substituent. The structures were assigned by nmr studies and stereoselective syntheses of oxidative fragments. Aplyronine itself has now been synthesized as well, in 75 steps.

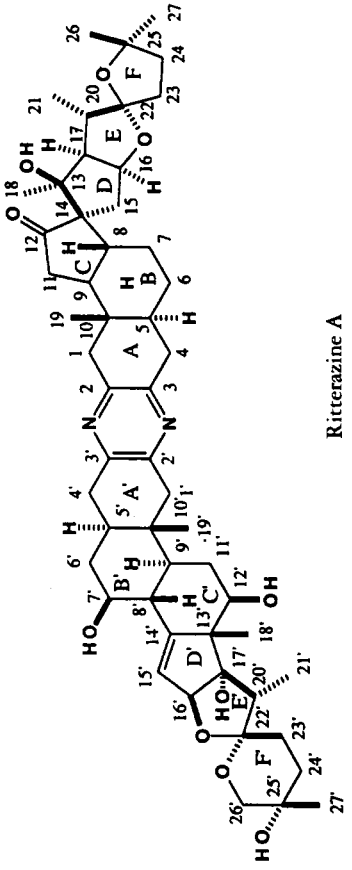


	R^1	R^2
Aplyronine A		H
Aplyronine B	H	
Aplyronine C	H	H

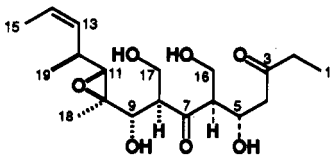
K. Rinehart (University of Illinois, Urbana-Champaign) discussed cytotoxic compounds in varying stages of promise as antitumor agents. The newly discovered myriaporones (IC_{50}=100 ng/ml vs. L-1210 cells) are of structural interest due to their close relation to tedanolide. Ecteinascidins (T/C 213% vs. P-388 leukemia; 241% vs. B16 melanoma; 210% vs. M5076 ovarian sarcoma; 0.0% vs. Lewis lung cancer, and showing some cures against advanced stage MX-1 mammary tumor xenografts), are currently in preclinical development. Didemnin B, in Phase II clinical trials, is showing some activity against non-Hodgkin's lymphoma and a CNS tumor (astrocytoma/glioblastoma), and its analogue, dehydrodidemnin B, is approximately six times as potent. Recently identified apparent biosynthetic precursors to ecteinascidins were also described (e.g., Et's 596, 597).



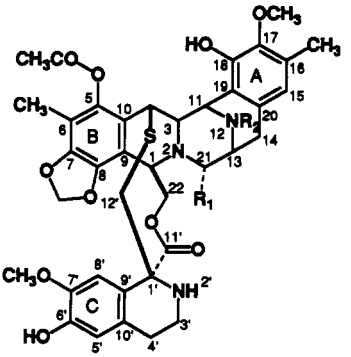
Polytheonamide A R=H
Polytheonamide B R=H
Polytheonamide C R=Me



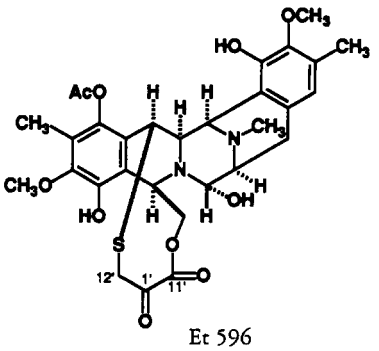
Ritterazine A



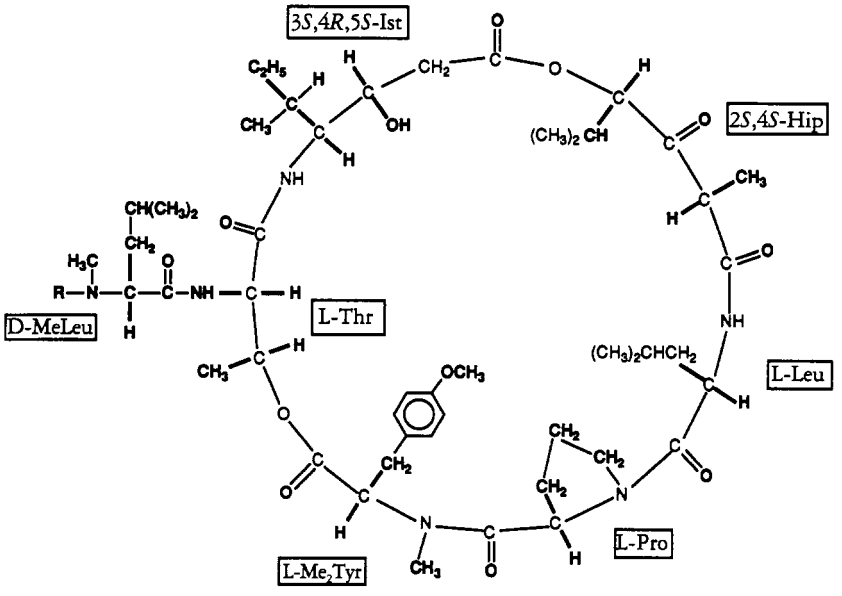
Myriaporone 4

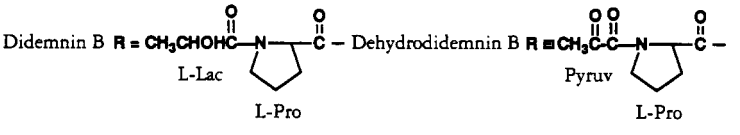


Ecteinascidin 743 R_1=OH, R_2=CH_3



Et 596
Et 597 1′-amino

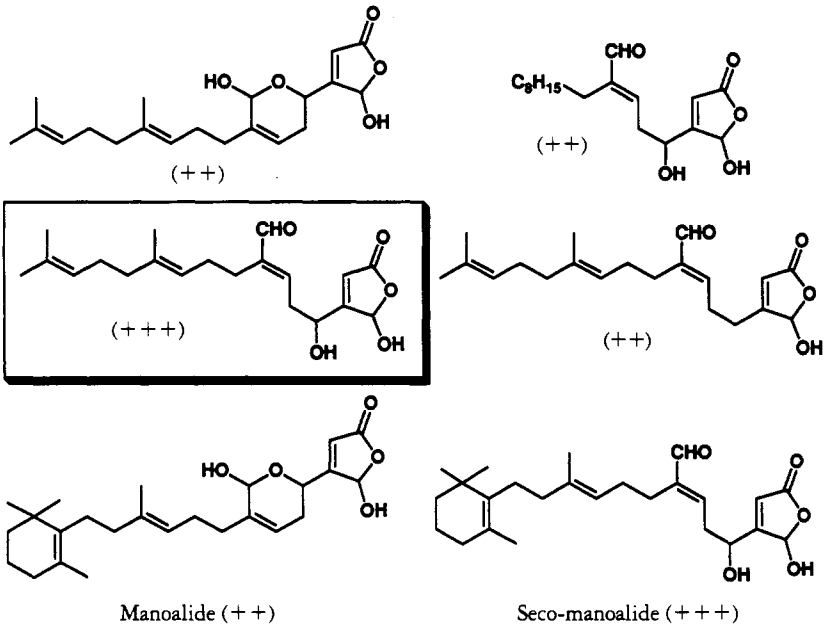




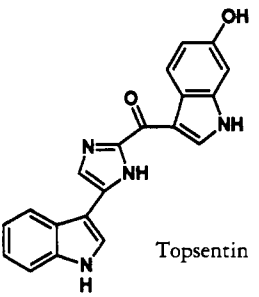
OTHER MODE OF ACTION STUDIES.—In addition to antitumor activity, marine-derived compounds often have other pharmacological activities and the mechanisms of some—anti-inflammatory activity and neurotoxicity—were described.

S. Katsumura (Kwansei Gakuin University) described results on inhibition of bovine pancreatic phospholipase A_2 by the terpene manoalide (from a sponge) and its analogues. The two aldehyde groups and a long side-chain are required for activity and

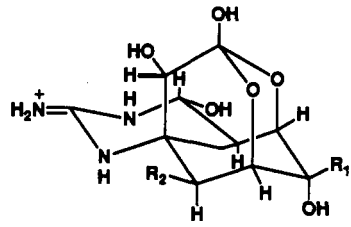
the 4-hydroxyl is important. The two aldehyde groups apparently react covalently with two lysine residues of the enzyme. Manoalide and some of the more active analogues are shown. The highlighted compound was used to elucidate the inactivation mechanism of bovine pancreatic PLA_2.



Topsentin, a bisindolylimidazole was reported by R. Jacobs (University of California, Santa Barbara) to be an even more potent inhibitor of phospholipase A_2 than manoalide in the in vivo mouse ear assay but was less active in inhibiting hydrolysis of phosphatidylcholine by bee venom PLA_2.



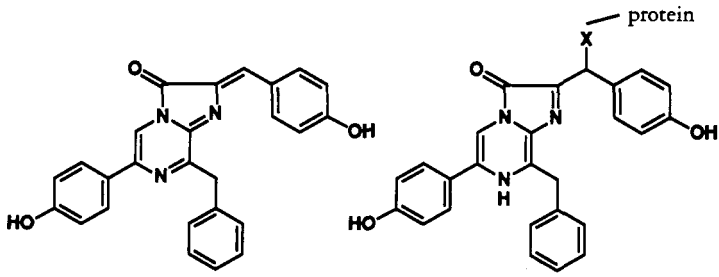
A structure-activity study by T. Yasumoto *et al.* (Tohoku University, Sendai) of tetrodotoxin (TTX) analogues in competition to binding by saxitoxin (STX) indicated that binding to the Na channel was favored by hydrophilic substituents, was influenced by stereochemistry, and was most strongly hindered by carboxylic substituents, which probably repel the Asp-177 carboxyl group. The dissociation constant of STX bound to pufferfish channels was much higher than for STX bound to bonito or rat channels, thus explaining the resistance of pufferfish to STX (TTX). Highly active analogues (equivalent to TTX) are shown below.

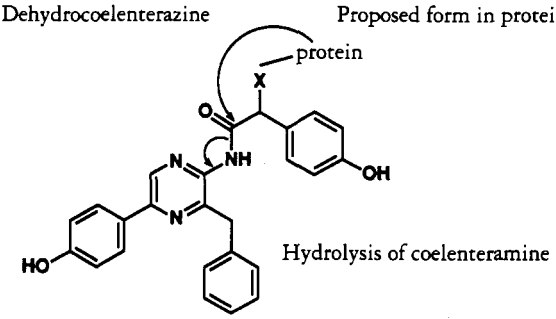


	R_1	R_2
TTX	CH_2OH	OH
Chiriquitoxin	*	OH
11-oxoTTX	CHO	OH
11-norTTX-6,6-diol	OH	OH

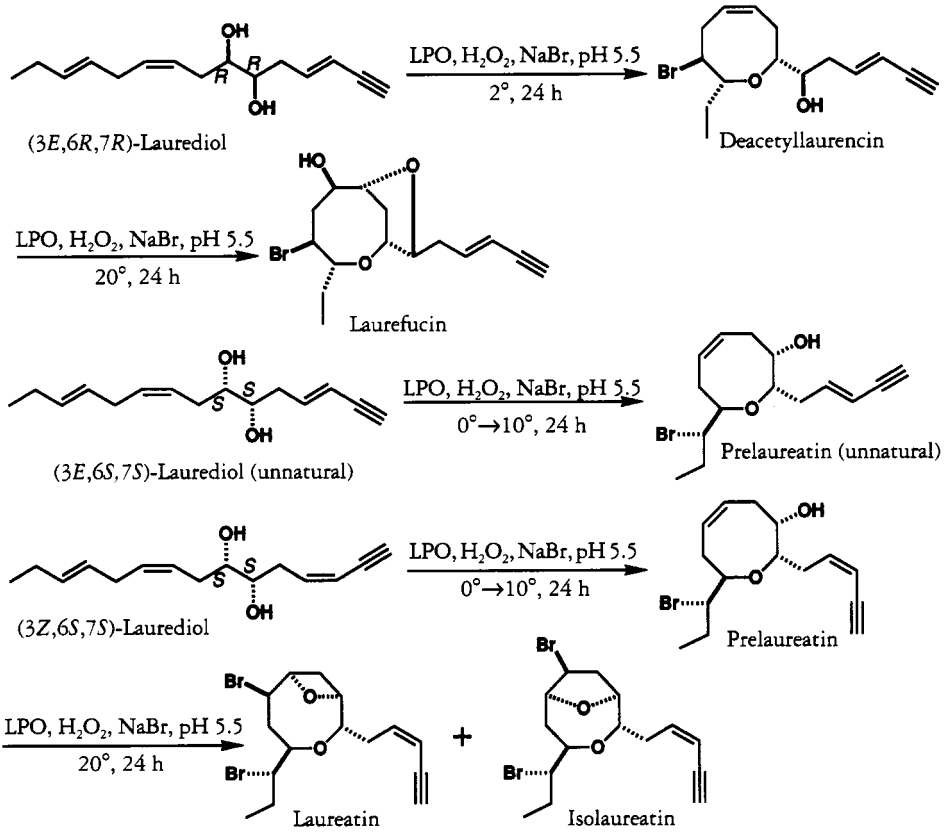
*$CH(OH)CH(NH_2)COOH$

M. Isobe (Nagoya University) proposed a mechanism for the bioluminescence of *Symplectoteuthis oualaniensis,* a luminous squid, involving dehydrocoelenterazine, a photoprotein, and coelenteramine.

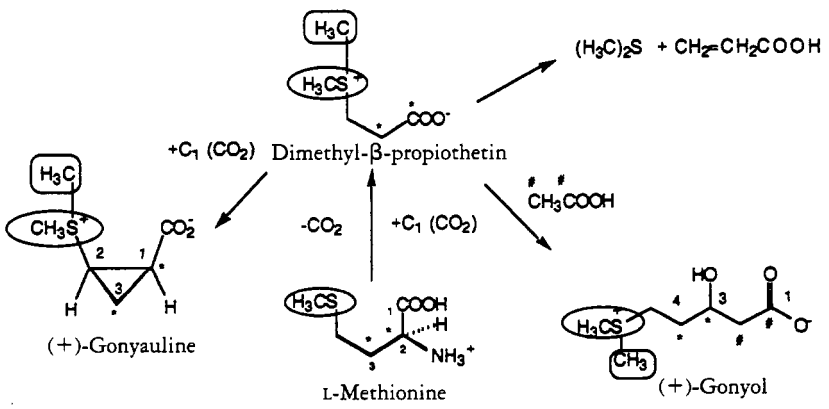




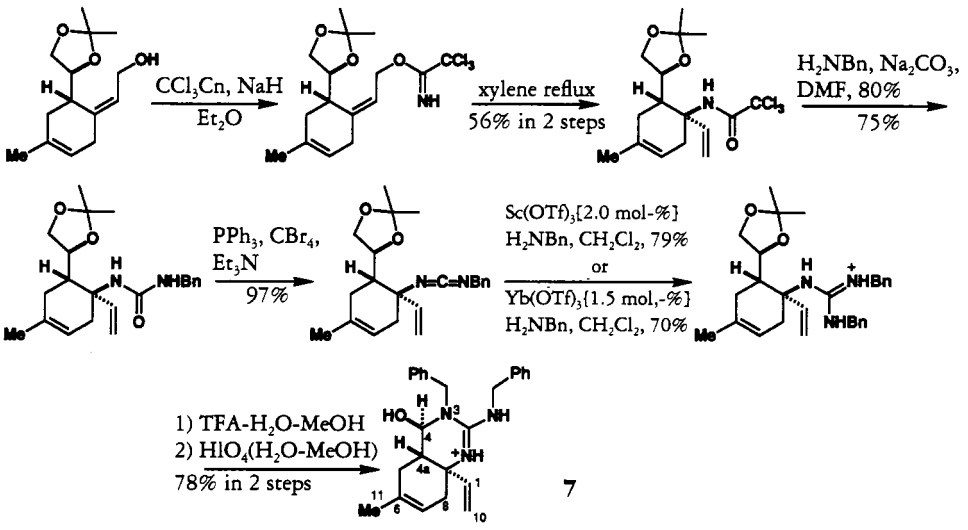
OTHER TOPICS: BIOSYNTHESIS, SYNTHESIS, STRUCTURAL TOOLS, TOXINS.—A. Murai (Hokkaido University, Sapporo) reported on biosynthetic studies aimed at brominated cyclic ethers found in *Laurencia nipponica* (red alga). The experiments employed lactoperoxidase (LPO) as a surrogate for the bromoperoxidase (BPO) in *L. nipponica.* Different results were obtained with different stereoisomers of laurediol. The (3*E*,6*R*,7*R*)-isomer gave the natural laurefucin via the intermediate deacetyllaurencin, while the mirror image gave the unnatural isomer of prelaureatin. The (3*Z*,6*S*,7*S*)-isomer gave the natural prelaureatin (an intermediate), laureatin, and isolaureatin.



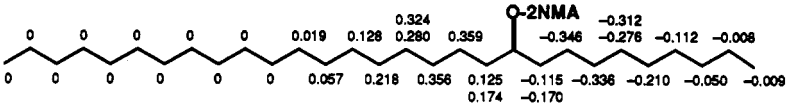
H. Nakamura (Hokkaido University, Sapporo) reported results on the biosynthesis of gonyauline, a cyclopropane derivative involved in the bioluminescence of *Gonyaulax polyedra's* luciferin. As shown, the compound is derived from L-methionine and a C_1 unit.



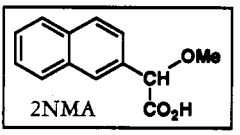
In synthetic studies, M. Isobe (Nagoya University) has developed from simple starting materials a useful synthesis of a guanidine [7] containing the partial structure of (−)-tetrodotoxin.



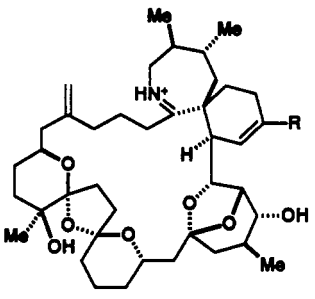
T. Kusumi (Tokushima University) reported on results employing modifications of Mosher's reagent to determine chirality. Using the best of the new compounds, 2NMA, it was possible to assign absolute stereochemistry to ginnol, whose chirality depends only on the length of two long alkyl groups. The figures represent $\Delta\delta$ values between the two diastereomeric ginnol 2NMA esters.



Ginnol *R*-2NMA ester

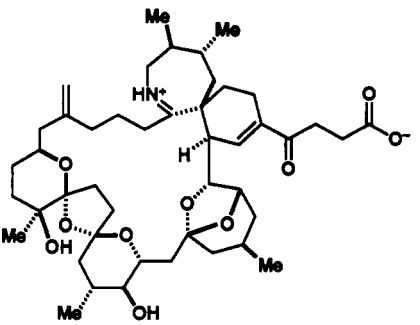


D. Uemura (Shizuoka University) described the toxicity, the isolation from *Pinna attenuata* and *P. atropurpurea*, and structures of the pinnatoxins, polycyclic ethers present in minute amounts (1–4 mg/kg) in the pen shells.



Pinnatoxin A R=-COO$^-$

Pinnatoxins B, C R=-$\overset{*}{C}$HCOO$^-$
OH



Pinnatoxin D

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