ΒΙΟΔΡΑΣΤΙΚΑ ΠΡΟΪΟΝΤΑ ΘΑΛΑΣΣΙΑΣ ΠΡΟΕΛΕΥΣΗΣ

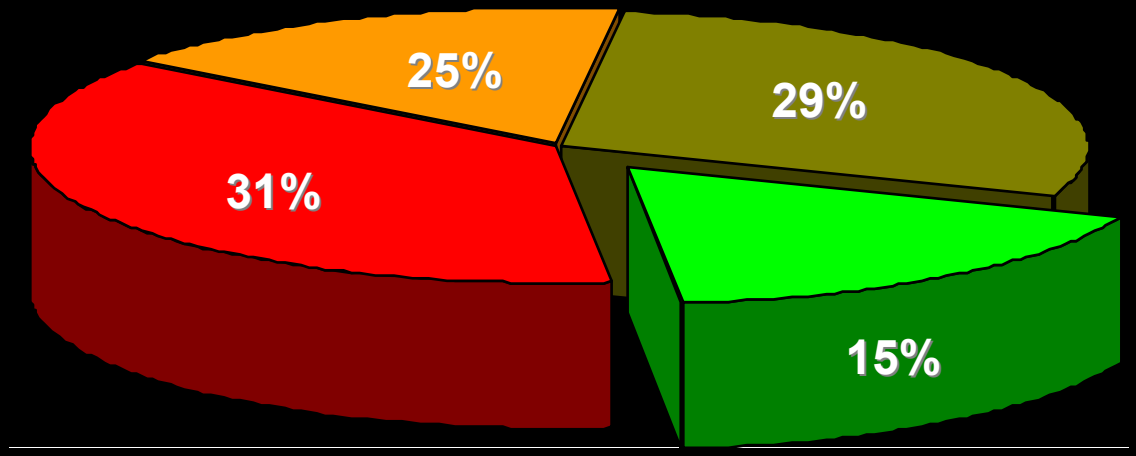
Βασίλης Ρούσσης

ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

Τμήμα Φαρμακευτικής

Τομέας Φαρμακογνωσίας & Χημείας Φυσικών Προϊόντων

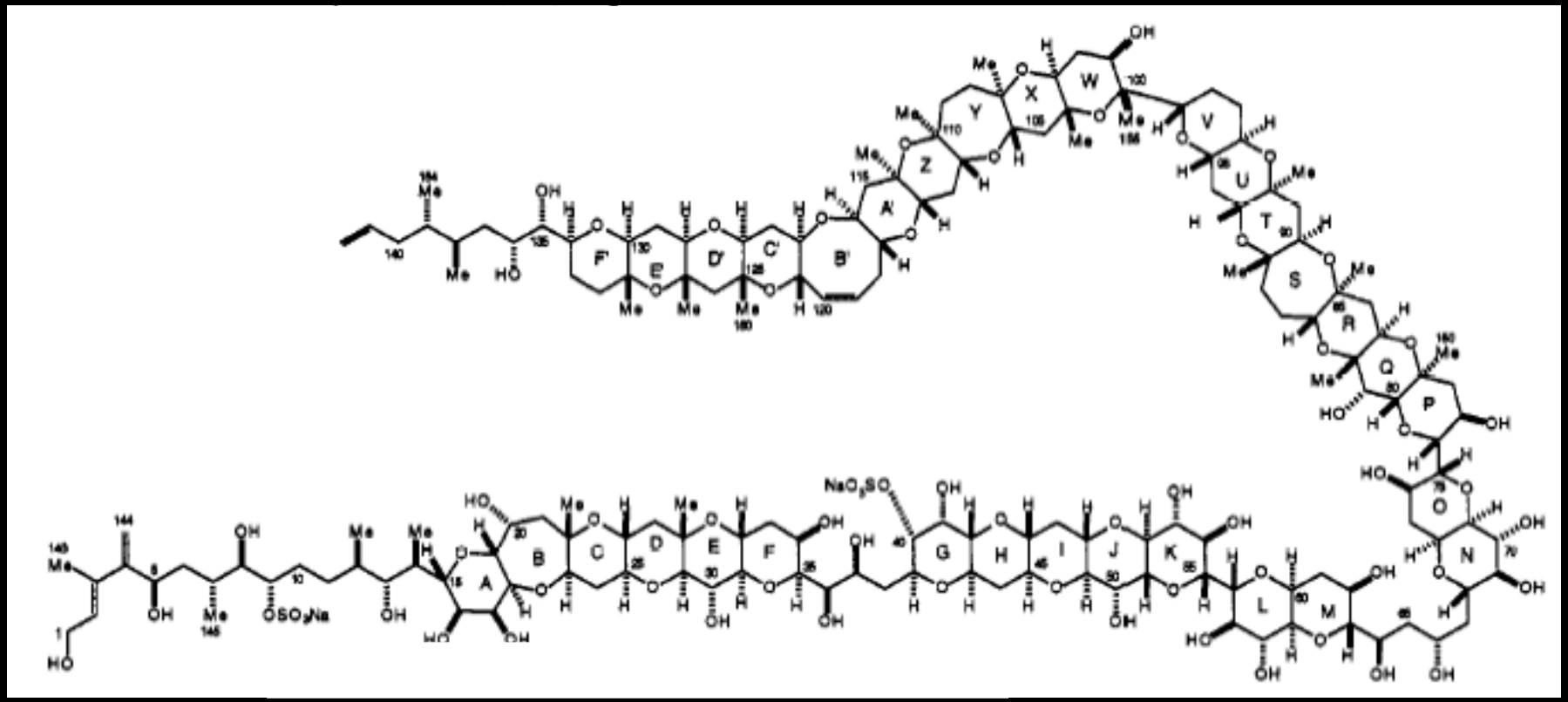
Αντικαρκινικά Φάρμακα (NCI)

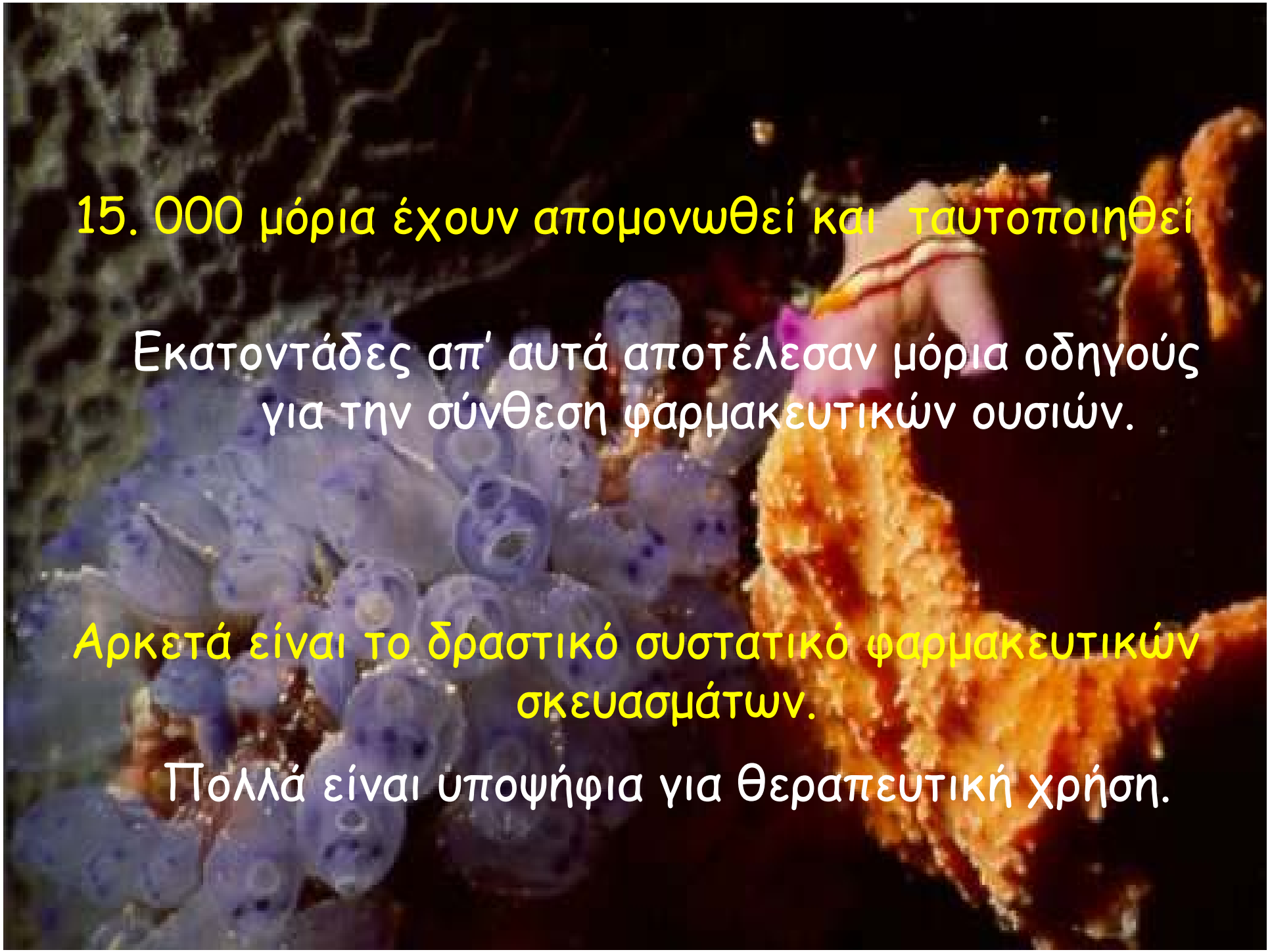


- Φυσικά Προϊόντα
- Ημισυνθετικά
- Συνθετικά Ανάλογα
- Συνθετικά

Πλεονεκτήματα

Μειονεκτήματα





15. 000 μόρια έχουν απομονωθεί και ταυτοποιηθεί

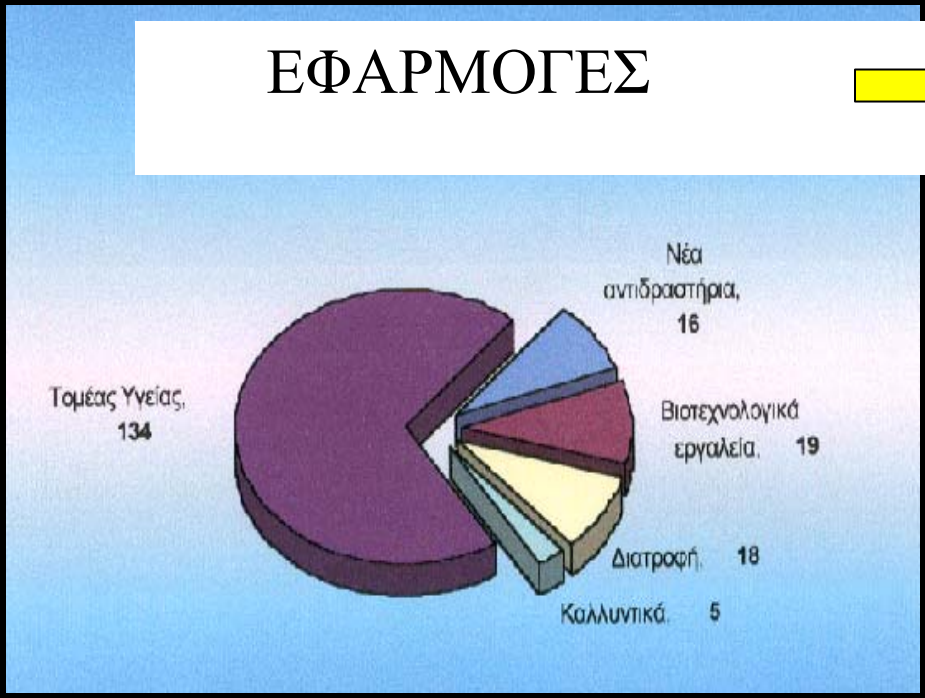
Εκατοντάδες απ' αυτά αποτέλεσαν μόρια οδηγούς για την σύνθεση φαρμακευτικών ουσιών.

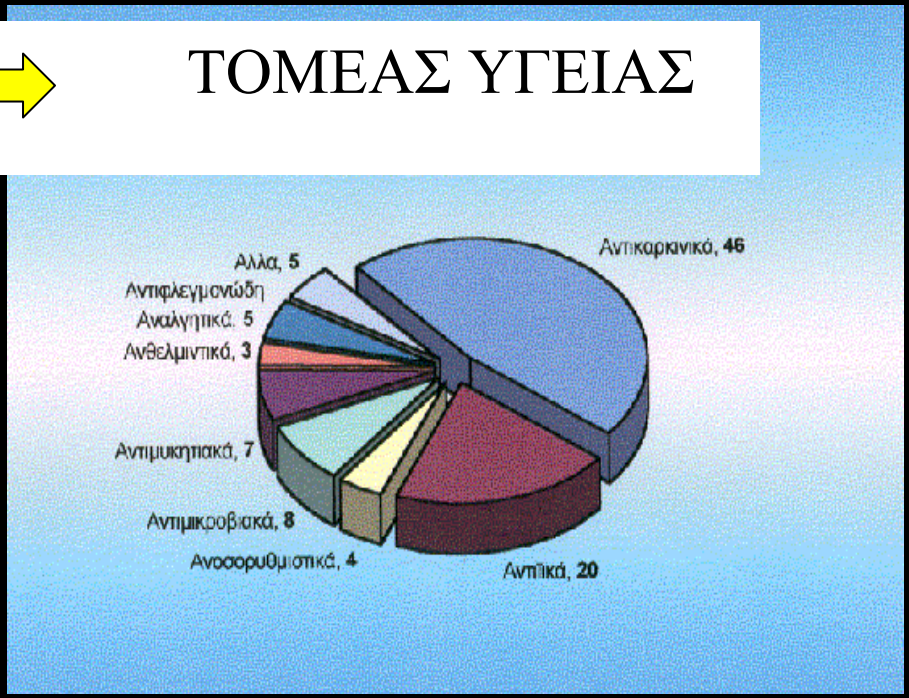
Αρκετά είναι το δραστικό συστατικό φαρμακευτικών σκευασμάτων.

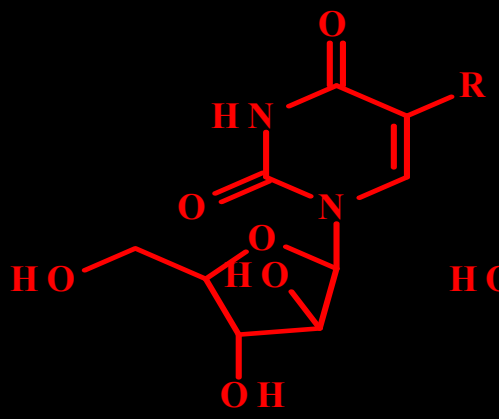
Πολλά είναι υποψήφια για θεραπευτική χρήση.

Φυσικά προϊόντα από θαλάσσιους οργανισμούς

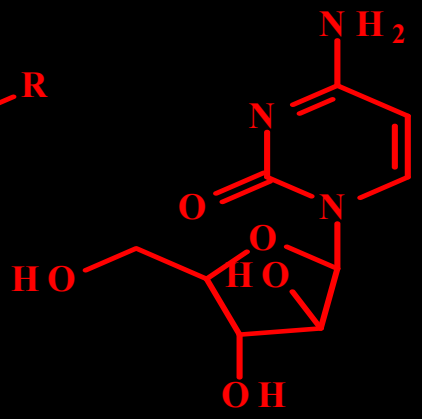
ΕΦΑΡΜΟΓΕΣ → ΤΟΜΕΑΣ ΥΓΕΙΑΣ



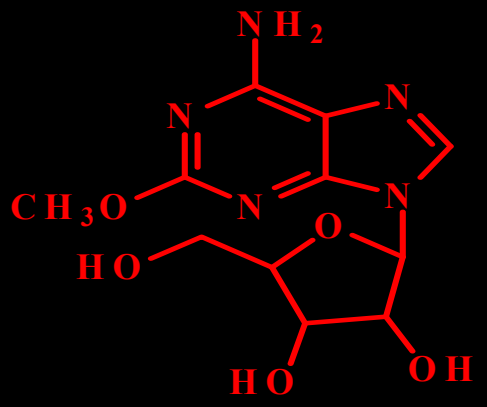




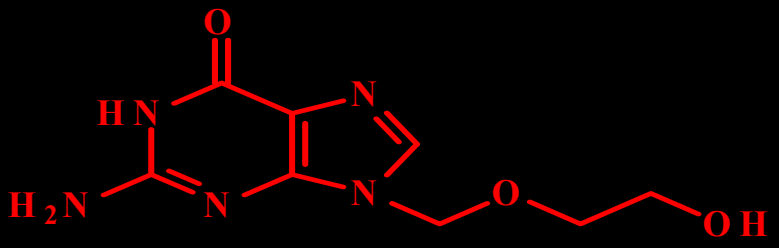
R : A) H, B) CH_3



ARA C

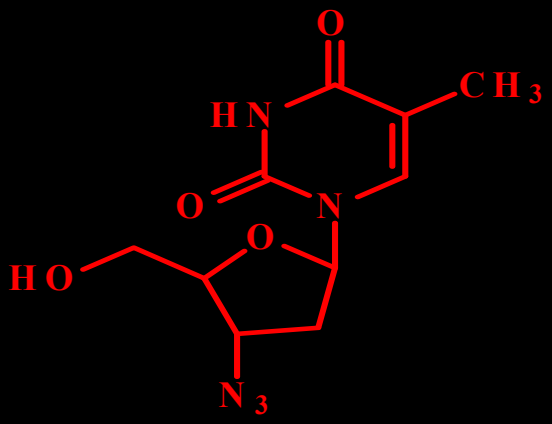


Spongosine



9-(2-Hydroxyethoxymethyl)guanine

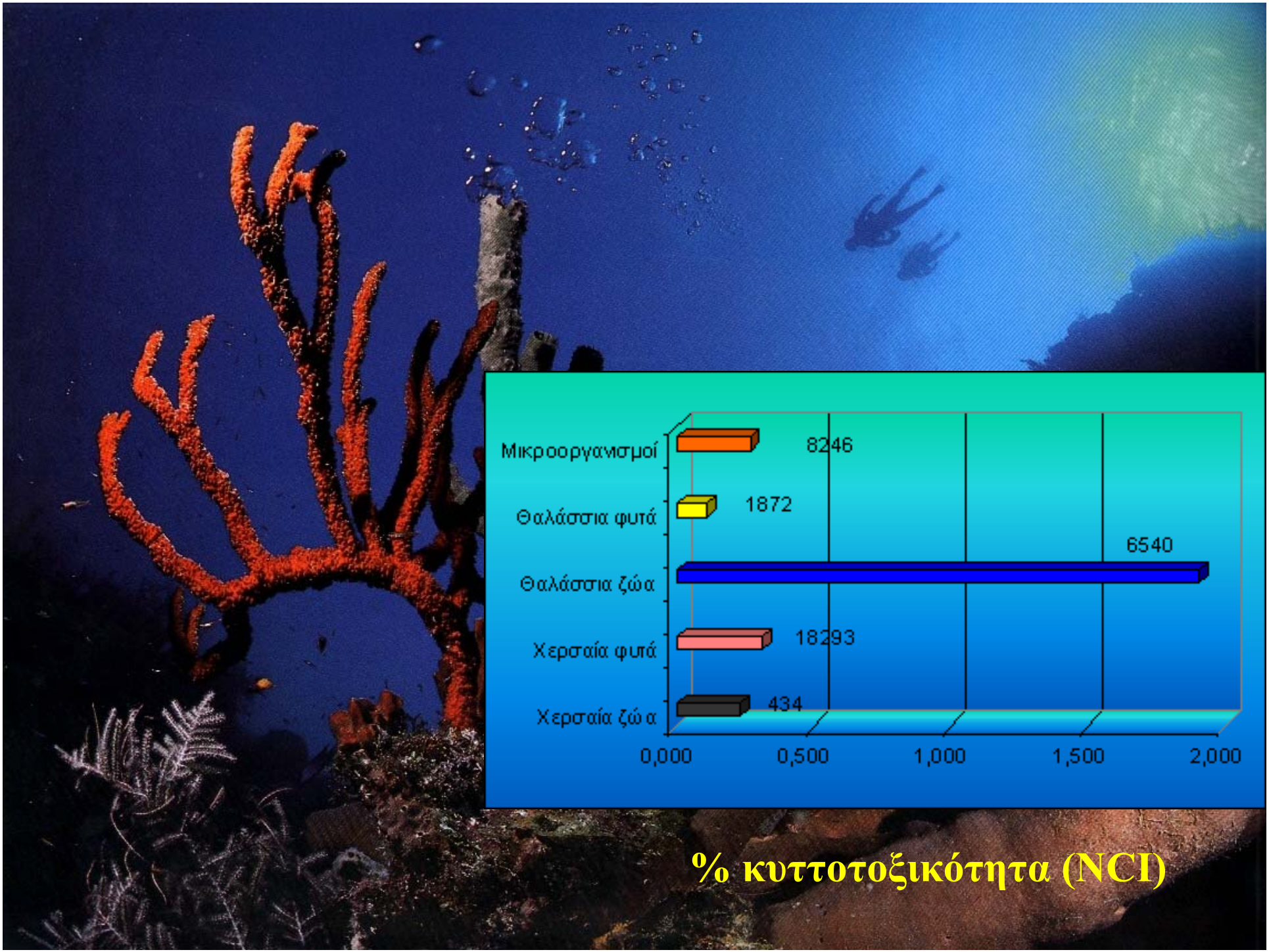
Zovirax

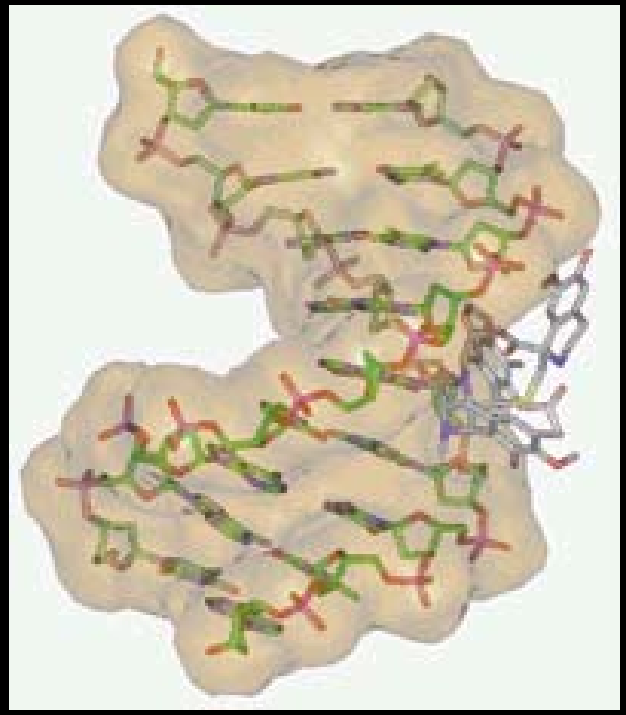


3'-Azidothymidine

Retrovir

Compound Name	Source	Chemical Class	Company	Disease Area	Status
Compounds targeting ion channels					
Ziconotide	Cone snail	Peptide	Neurex	Chronic pain	Phase III
AM-336	Cone snail	Peptide	AMRAD	Chronic pain	Phase I/II
GTS-21	Nemertine worm	Anabaseine-derivative	Taiho	Alzheimer/Schizophrenia	Phase I/II
Compounds targeting enzymes					
(a) Protein kinase inhibitors					
Bryostatin-1	Bryozoan	Polyketide	GPC Biotech	Cancer	Phase II
(b) PLA_2 inhibitors					
OAS-1000	Soft Coral	Diterpene-pentoseglycoside	OsteoArthritis Sciences	Wound Healing/Inflammation	Phase I/II
(c) Methionine aminopeptidase inhibitors					
LAF-389	Sponge	Amino acid derivative	Novartis	Cancer	Phase I
Microtubule-interfering agents					
Dolastatin-10	Sea slug	Peptide	NCI/Knoll	Cancer	Phase II
ILX-651	Sea slug	Peptide	Ilex Oncology	Cancer	Phase I
Cemadotin	Sea slug	Peptide	Knoll	Cancer	Phase II
Discodermolide	Sponge	Polyketide	Novartis	Cancer	Phase I
HTI286	Sponge	Tripeptide	Wyeth	Cancer	Phase I
DNA-interactive agents					
Yondelis™	Sea squirt	Isoquinolone	PharmaMar/Johnson&Johnson	Cancer	Phase II/III
Oxidative stress inducers					
Aplidin™	Sea squirt	Cyclic depsipeptide	PharmaMar	Cancer	Phase II
Lysosomotropic compounds					
Kahalalide F	Sea slug/Alga	Cyclic depsipepetide	PharmaMar	Cancer	Phase II
Small G Protein antagonists					
ES-285	Clam	Anti-β-amino alcohol	PharmaMar	Cancer	Phase I
Immunostimulatory agents					
KRN-7000	Cone snail	α-Galactosylceramide	Kirin	Cancer	Phase I
Calcium-binding protein antagonists					
Squalamine lactate	Shark	Aminosteroid	Genaera	Cancer	Phase II
Compounds with unknown mechanism of action					
IPL-512602	Sponge	Steroid	Inflazyme/Aventis	Inflammation/Asthma	Phase II


Μικροοργανισμοί
8246
Θαλάσσια φυτά
1872
Θαλάσσια ζώα
6540
Χερσαία φυτά
18293
Χερσαία ζώα
434
0,000
0,500
1,000
1,500
2,000
% κυττοτοξικότητα (NCI)

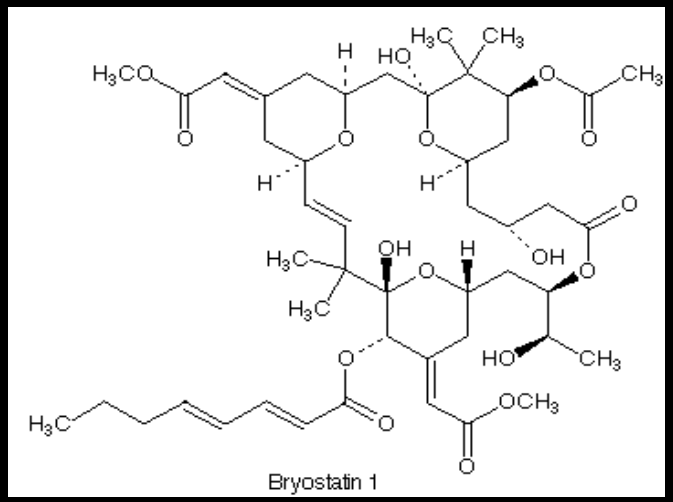


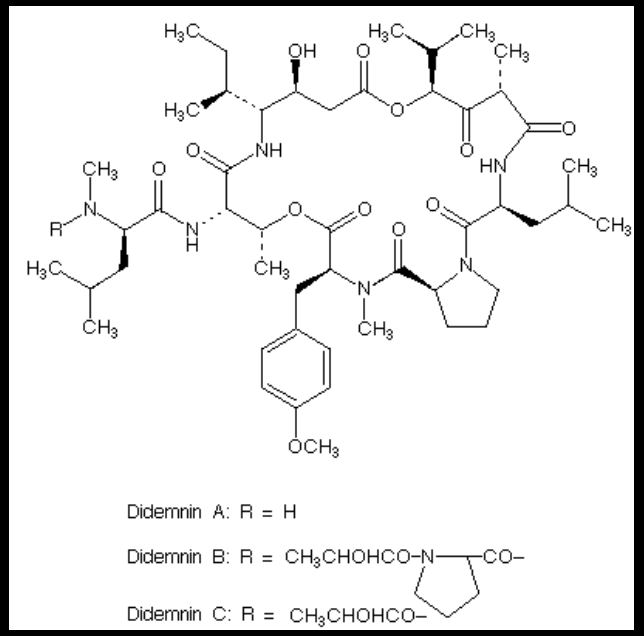
ET-743 (Ecteinascidin-743)

PharmaMar

Yondelis®

Διαθεσιμότητα





Limulus polyphemus

L A L TEST

- **Calcitonin** (Ανθρώπινη)
  MB: 3417 $C_{151}H_{226}N_{40}O_{45}S_3$

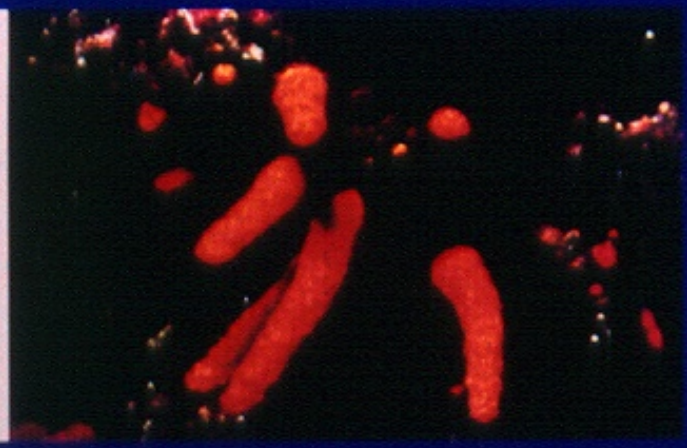
- **Calcitonin** (Σολωμός)
  MB: 3431 $C_{145}H_{240}N_{44}O_{48}S_2$

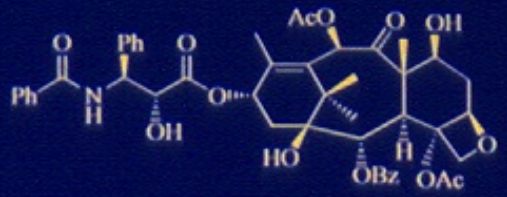
25 φορές ισχυρότερη της ανθρώπινης με διάρκεια δράσης 5 φορές μεγαλύτερη.
-- Πιθανότητα αλλεργιών.

ΚΟΡΑΛΙΑ

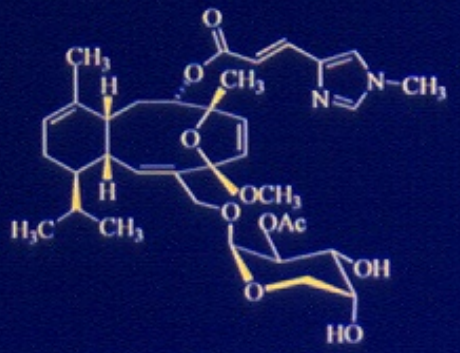
Σχηματισμοί Υδροξυαπατίτη δομής παρόμοιας με των οστών.

- Παρασκευάσματα για μεταμόσχευση οστών ή υποκατάσταση μοσχευμάτων. (Pro Osteon Implant 500).
- Παρασκευάσματα στην οδοντιατρική.





Taxol



Eleutherobin

Drugs from the Sea

Studying chemical defense systems of ocean life may prove lifesaving to man

In the lukewarm waters of coral reefs, where the abundance and variety of life create a competition for food and space that is keener perhaps than that found in any other ecosystem in the ocean, it takes something special to stay alive.

It's an environment where predators lurk at every level of the food chain and, in order to survive, many species of plants and animals have developed chemical defense systems.

A group of scientists in a chemical ecology program at Scripps Institution of Oceanography (SIO) are studying these chemicals in an effort to understand how the various species adapt and react to each other in their overcrowded environment.

The work the scientists do is a classic example of basic research in marine chemistry, but their discoveries are making important contributions to the art and science of medicine.

Two SIO professors, D. John Faulkner, a specialist in marine natural products chemistry at the Ocean Research Division, and William Fenical, a chemist in the Institute of Marine Resources, head the research. They are backed by a core research group of about 20 graduate students, postdoctoral researchers, technicians and visiting scholars. The group collaborates with pharmacologists at UC Santa Barbara, and the program is funded primarily by the Department of Commerce's Sea Grant College Program, with related support provided by the National Science Foundation and the National Institutes of Health.

Student investigators study organisms in the ocean.

Students who enroll in the marine chemistry group commit themselves to a five-year, broadly-based doctoral program that applies techniques from sophisticated x-ray crystallography and nuclear magnetic resonance to classical degradation chemistry.

"In the traditional products chemistry program, researchers would just receive a bag of leaves from a tropical plant, never having seen it alive, and work on that to isolate and analyze its chemical components and then determine any biomedical properties," Faulkner said in a recent interview. "Our students actually go out into the field and base their collections on what they observe.

"Specifically, they look for organisms without a visible means of defense that might be chemically protected, collect them and bring them back for analysis. Thus, the student investigator knows the interactions of the organism in the ocean and can relate its chemistry to what was seen in the field."

Coral reefs make ideal collecting spots. Coral is composed of myriads of skeletons of tiny marine organisms. In most cases, its sharp and brittle texture of calcium carbonate protects it from predators. However, the abundance of certain soft corals, such as sea fans, sea whips and sea pens, which possess no external hard structure, shows just how successful chemical defenses can be.

To avoid being eaten, these spongy and seemingly easy prey employ a system of defense based on what the scientists describe as chemical messenger compounds.

Chemical messenger compounds are used by marine life to transmit information to other individuals of their own species, to ward off potential predators, and to attract prey. They affect both the behavior of the individual animals and the structure of marine communities.

"A characteristic of chemical messenger compounds is that they are effective at low concentrations, thus possessing a very pronounced effect on the sensory apparatus of the target organisms," Faulkner said. "It's this rather strong effect that clues us that the compound may be worth screening for pharmaceutical properties."

What the scientists have discovered is that, genetically, most marine organisms are totally distinct from terrestrial plants and animals. Their biosynthetic processes are distinct as well, partly because the chemistry of their environment - seawater - is different.

An example, Fenical said, are the terpenes, the agents that produce the distinctive odor of pine trees and that are used commercially to produce turpentine. In the oceans, terpenes are produced that contain bromine and chlorine because of the presence of these substances in seawater.

Indo-Pacific soft corals contain chemicals that defend against predators.

Working on the prolific red seaweeds of the genus Laurencia, the scientists have isolated more than 400 terpenoids representing 26 structure classes, 16 of which are novel structure groups found only in the marine environment.

Among the other discoveries made by the scientists is that a surprisingly large number of marine products are cytotoxins—chemicals that inhibit cell development and that might eventually be used in cancer chemotherapy.

Some sponges and soft corals have been found to possess anti-inflammatory agents that may be effective in treating arthritis; and some sea whips contain lophotoxin, a neurotoxin that inhibits muscular activity and that is under investigation at more than a dozen medical school laboratories as a molecular probe to study neuromuscular disease.

Another discovery, monoalide, found in tropical sponges, was isolated at SIO and characterized pharmacologically at UC Santa Barbara. It has potent anti-inflammatory qualities. It has been tested in animals and Allergan Inc. has received approval from the FDA to test its effectiveness in treating psoriasis in humans.

Other discoveries may have agricultural possibilities. Naturally occurring halogenated organic chemicals have been found that appear to ward off or kill other marine organisms, similar to the synthetic varieties that are used in herbicides and insecticides.

"Terrestrial natural products rarely contain halogens, whereas more than 600 halogenated products are fabricated by marine bacteria, red algae, some coelenterates, and sponges," Fenical said.

The University of California owns all the patents on the discoveries made and it will collect the profits, if any. Although the discovery of new drugs is a biomedical success story in itself, the researchers continually insist on the basic research aspect of the work that they do. Some of the discoveries that the group have made are "incredibly useful in research," Fenical said, by providing " a new mechanism to study biochemical processes that no one understood before." An example of that, he said, is tetrodotoxin, the puffer fish toxin that is far too toxic to use as a pharmaceutical agent, but it has been used successfully for understanding how nerve transmission takes place.

But the important thing, as far as Fenical is concerned, is the basic research. "We want to know how marine organisms adapt and why they survive."

Therefore, the scientists in the group distance themselves from the research leading to the biomedical applications of the discoveries they make. "Our role is to provide sufficient screening on a pure marine product to allow the pharmaceutical company to decide whether that compound should be studied by them," Faulkner said. "The company can then embark on the long and expensive process of transforming a physiologically active compound into a useful pharmaceutical agent."

UCSD

By Chuck Colgan, a senior public information representative at UCSD's Scripps Institution of Oceanography.

ANTI-CANCER DRUG DEVELOPMENT: THE PROCESS

1 ON-SITE SPECIMEN COLLECTION *(1–2 years)*

SPECIMENS REDUCED TO SERIES OF CHEMICAL EXTRACTS *(1–2 years)* 2

3 INITIAL SCREENING OF EXTRACTS vs CANCER CELL LINES *(1–2 years)*

CHEMICAL ISOLATION OF ACTIVE COMPOUND *(2–3 years)* 4

5 PRE-CLINICAL ANALYSIS – STRUCTURAL AND CHEMICAL ANALYSIS OF COMPOUND *(2–4 years)*

FORMULATION – DOSAGES AND DELIVERY SYSTEMS DEVISED *(1 year)* 6

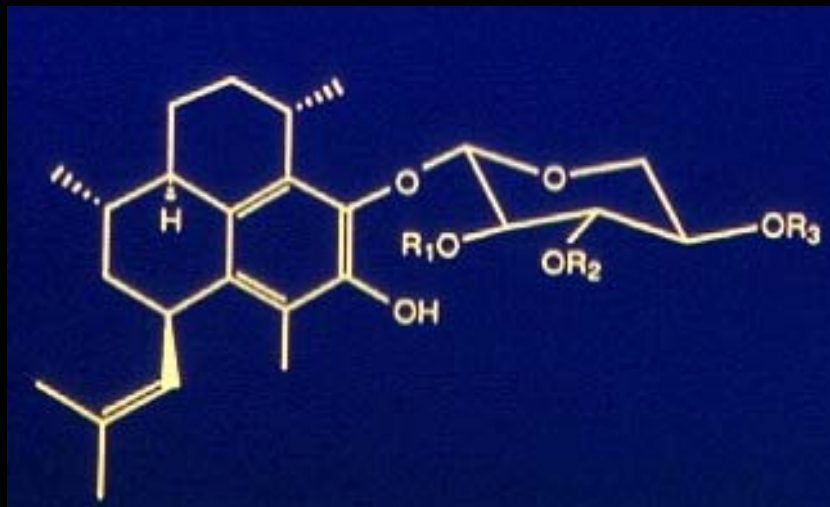
7 ANIMAL SAFETY STUDIES *(1 year)*

PHASE 1 CLINICAL TRIALS *(1–2 years)* 8

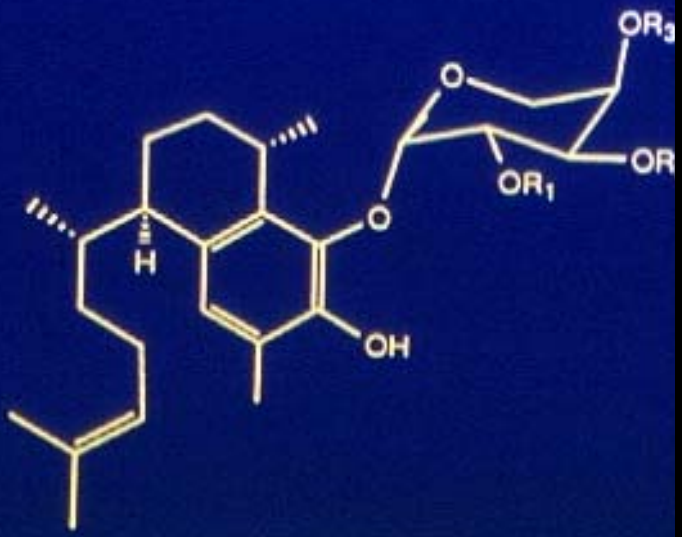
9 PHASE 2 CLINICAL TRIALS *(1–2 years)*

PHASE 3 CLINICAL TRIALS *(2–3 years)* 10

11 INTRODUCTION TO STANDARD TREATMENT REGIMENS FOR LOCAL PATIENT POPULATIONS.



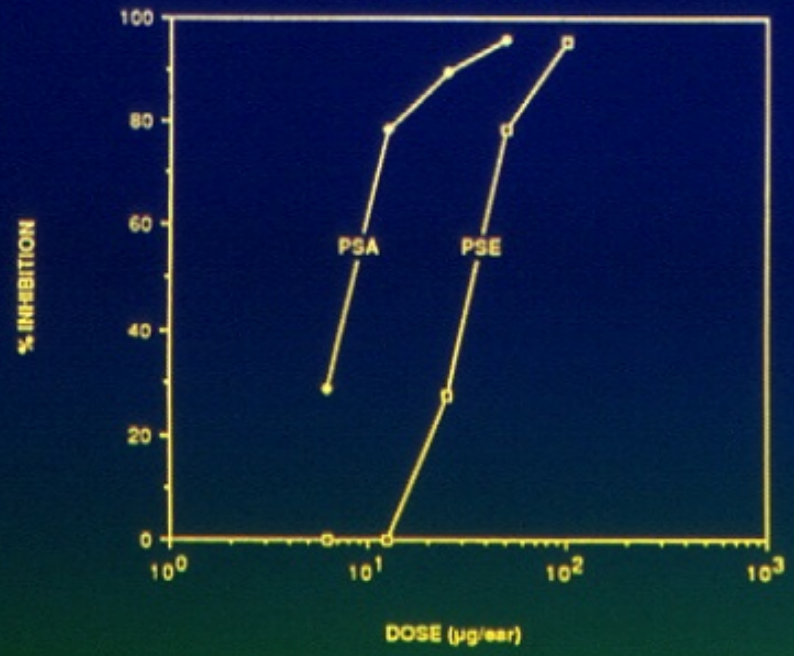
Pseudopterosin-A : R_1=R_3=H, R_2=Ac
Pseudopterosin-B : R_1=R_2=H, R_3=Ac
Pseudopterosin-C : R_1=R_2=R_3=H
Pseudopterosin-D : R_2=R_3=H, R_1=Ac



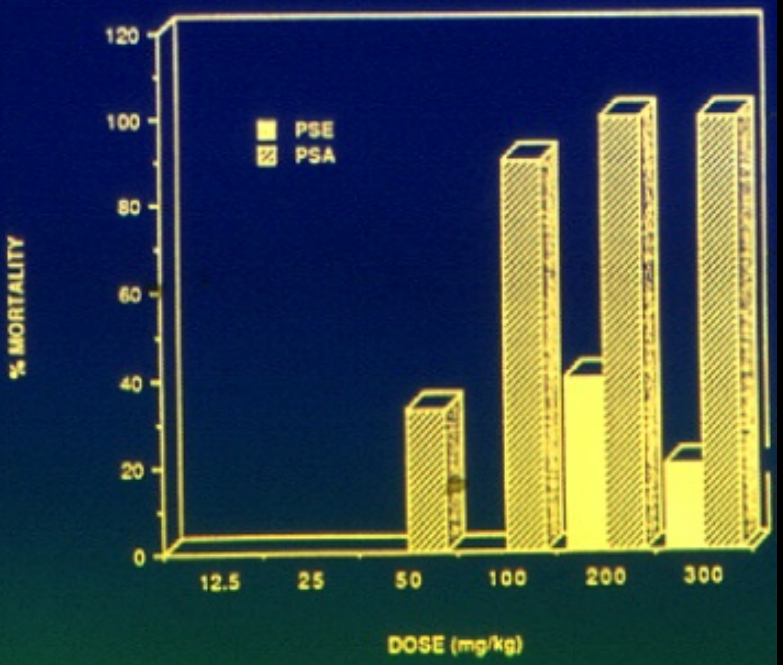
Seco-A : R_1=R_2=R_3=H
Seco-B : R_2=R_3=H, R_1=Ac
Seco-C : R_1=R_3=H, R_2=Ac
Seco-D : R_1=R_2=H, R_3=Ac

INHIBITION OF PMA INDUCED INFLAMMATION

(Topical Application)

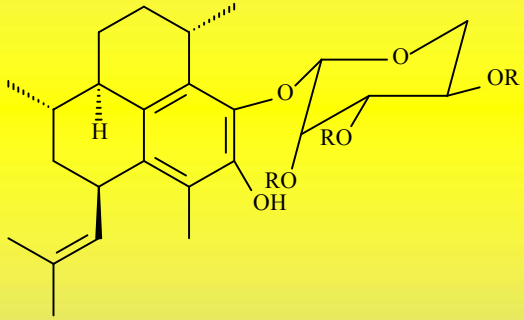


TOXICITY OF PSA AND PSE



LD50 PSE > 300 mg/kg

LD50 PSA - 57 mg/kg



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Elastin Refirming Cremme

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Conus geographus

ω-Conotoxins

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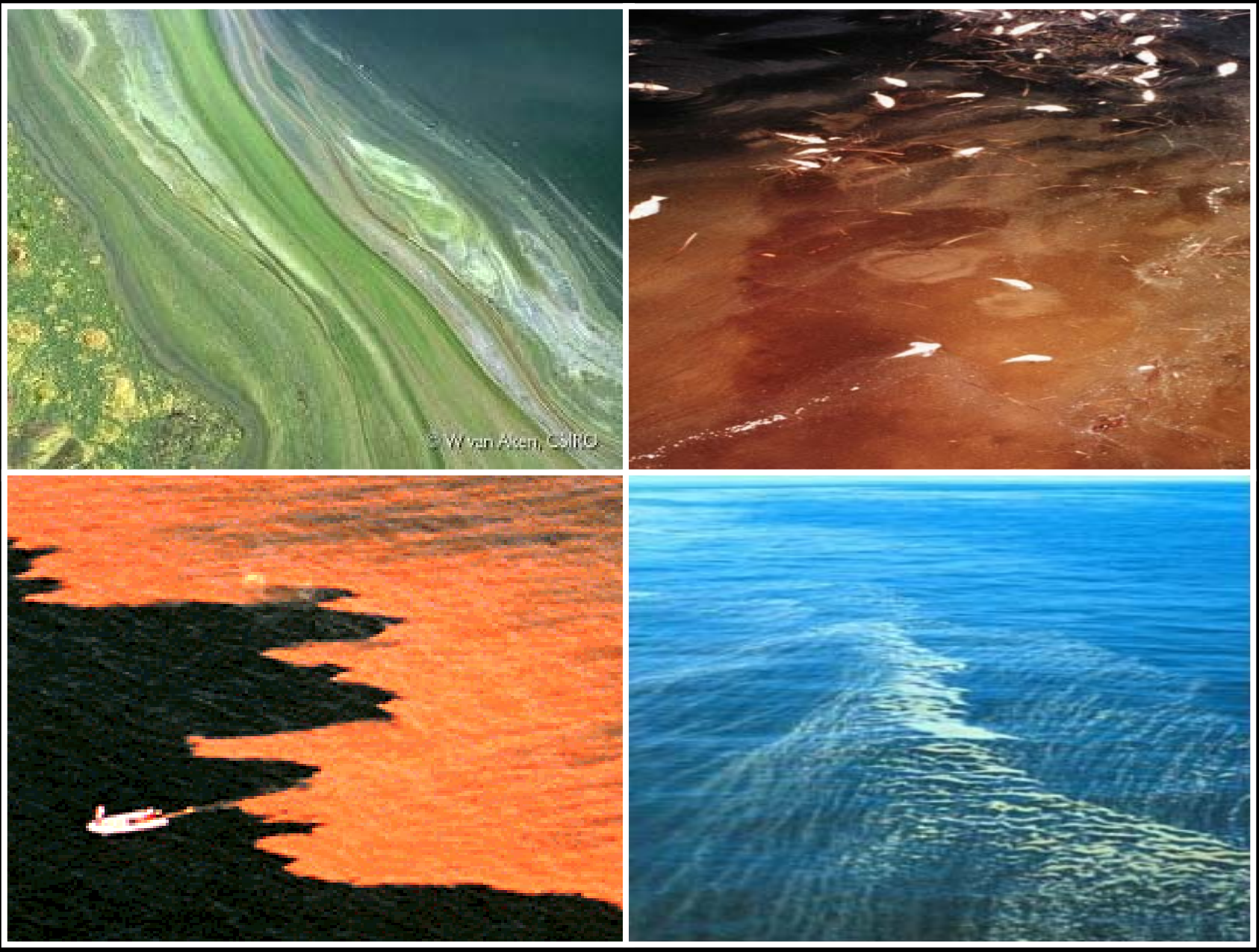
ΚΩΝΟΤΟΞΙΝΕΣ

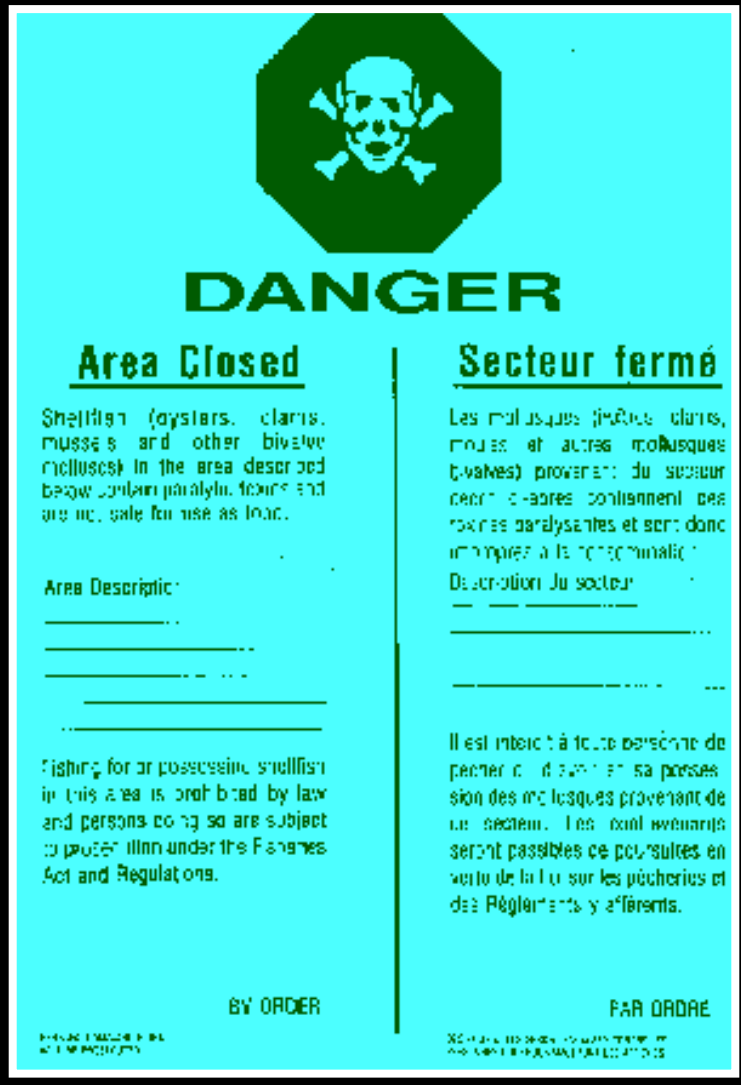
Πεπτίδια 12-30 αμινοξέων με αρκετούς δισουλφιδικούς δεσμούς.

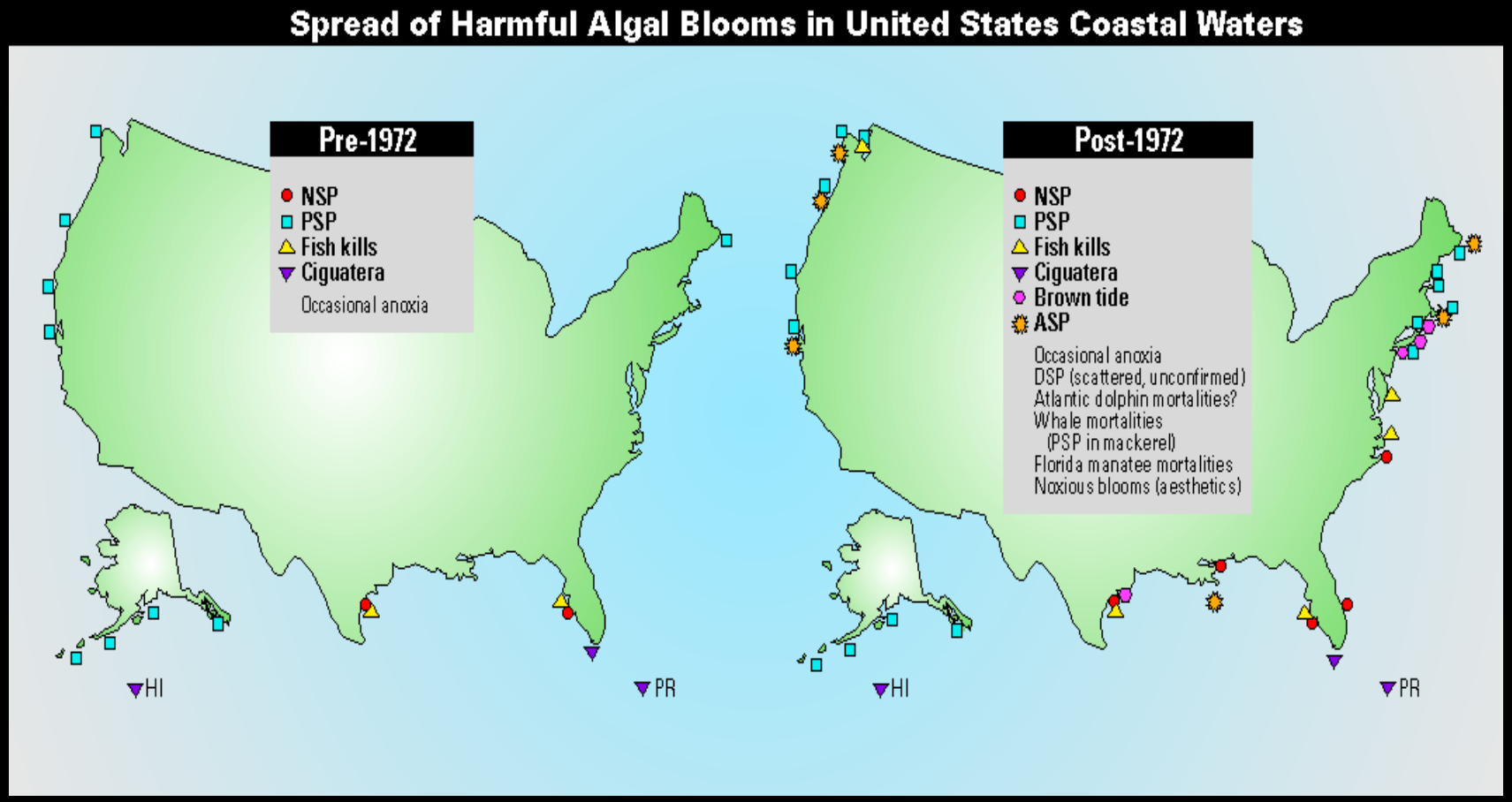
Δρουν αποκλείοντας τις νευρικές συνάψεις.

- *α*-κωνοτοξίνες (νικοτινικοί υποδοχείς).
- *δ*-κωνοτοξίνες (δίαυλοι νατρίου).
- *κ*-κωνοτοξίνες (δίαυλοι καλίου).
- *ω*-κωνοτοξίνες (δίαυλοι ασβεστίου).


© W van Aken, CSIRO


WARNING . . .
TOXIC SHELLFISH
SHELLFISH FROM THIS AREA ARE UNSAFE TO EAT DUE TO PARALYTIC SHELLFISH TOXIN.
DO NOT EAT CLAMS, OYSTERS, MUSSELS OR SCALLOPS.
Red Tide Hotline
1-800-562-5632
Health


DANGER
Area Closed
Shellfish (oysters, clams, mussels and other bivalve molluscs) in the area described below contain paralytic toxins and are not safe for use as food.
Area Description:
Fishing for or possession of shellfish in this area is prohibited by law and persons doing so are subject to prosecution under the Fisheries Act and Regulations.
BY ORDER
Secteur fermé
Les mollusques (huîtres, clams, moules et autres mollusques bivalves) provenant du secteur décrit ci-après contiennent des toxines paralysantes et sont donc impropres à la consommation.
Description du secteur
Il est interdit à toute personne de pêcher ou d'avoir en sa possession des mollusques provenant de ce secteur. Les contrevenants seront passibles de poursuites en vertu de la Loi sur les pêcheries et des Règlements y afférents.
PAR ORDRE



Algal blooms have increased significantly in recent years in U.S. coastal waters, causing massive fish kills and leading scientists to ponder the cause.

ΚΑΤΑΤΑΞΗ – ΔΙΑΚΡΙΣΗ .

1: P.S.P. Paralytic Shellfish Poisoning

2: D.S.P. Diarrhetic Shellfish Poisoning

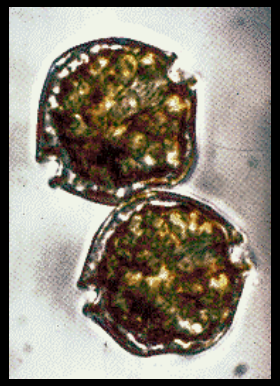
3: N.S.P. Neurotoxic Shellfish Poisoning

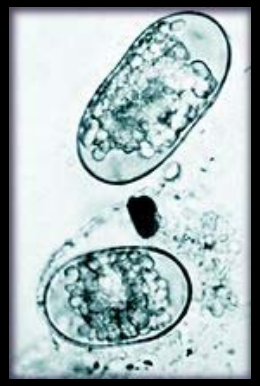
4: A.S.P. Amnesic Shellfish Poisoning

5: Ciguatera Fish Poisoning

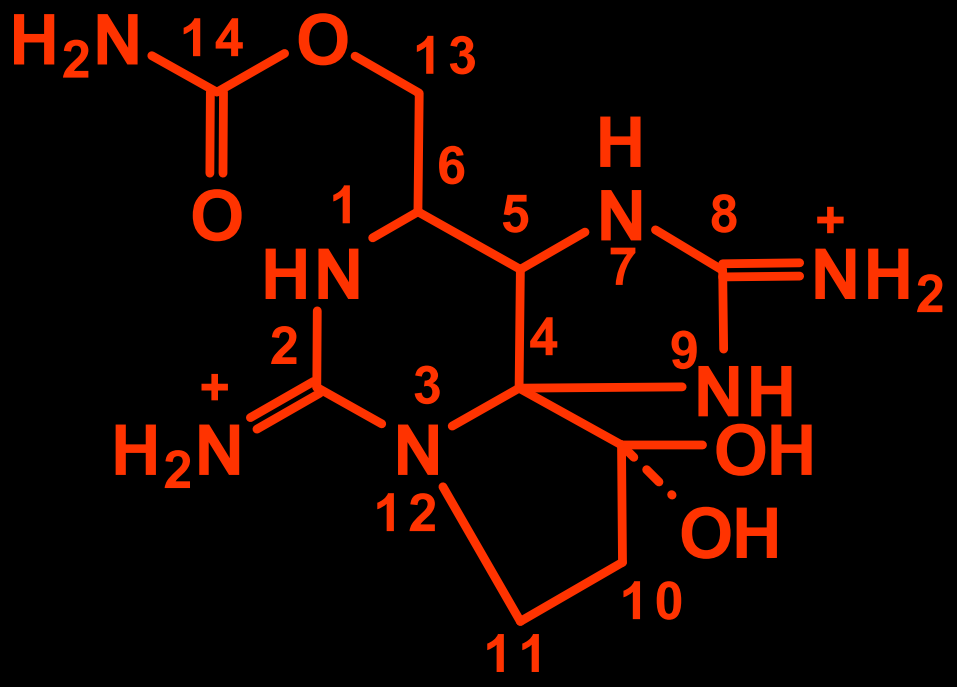
6: Pfiesteria Toxins

P.S.P.

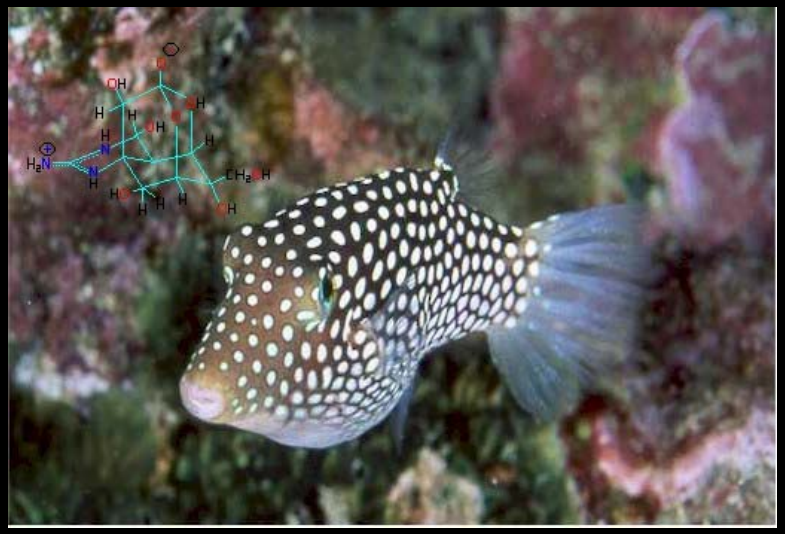


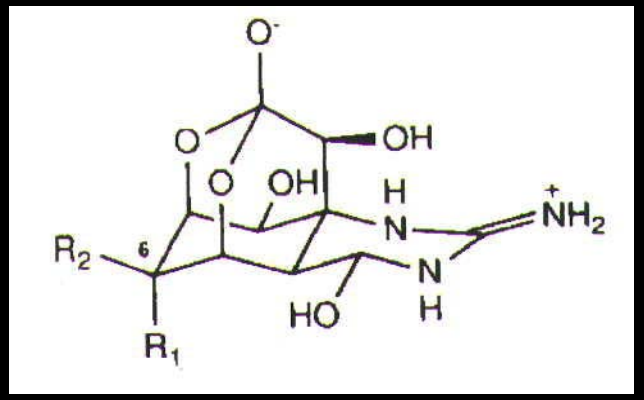


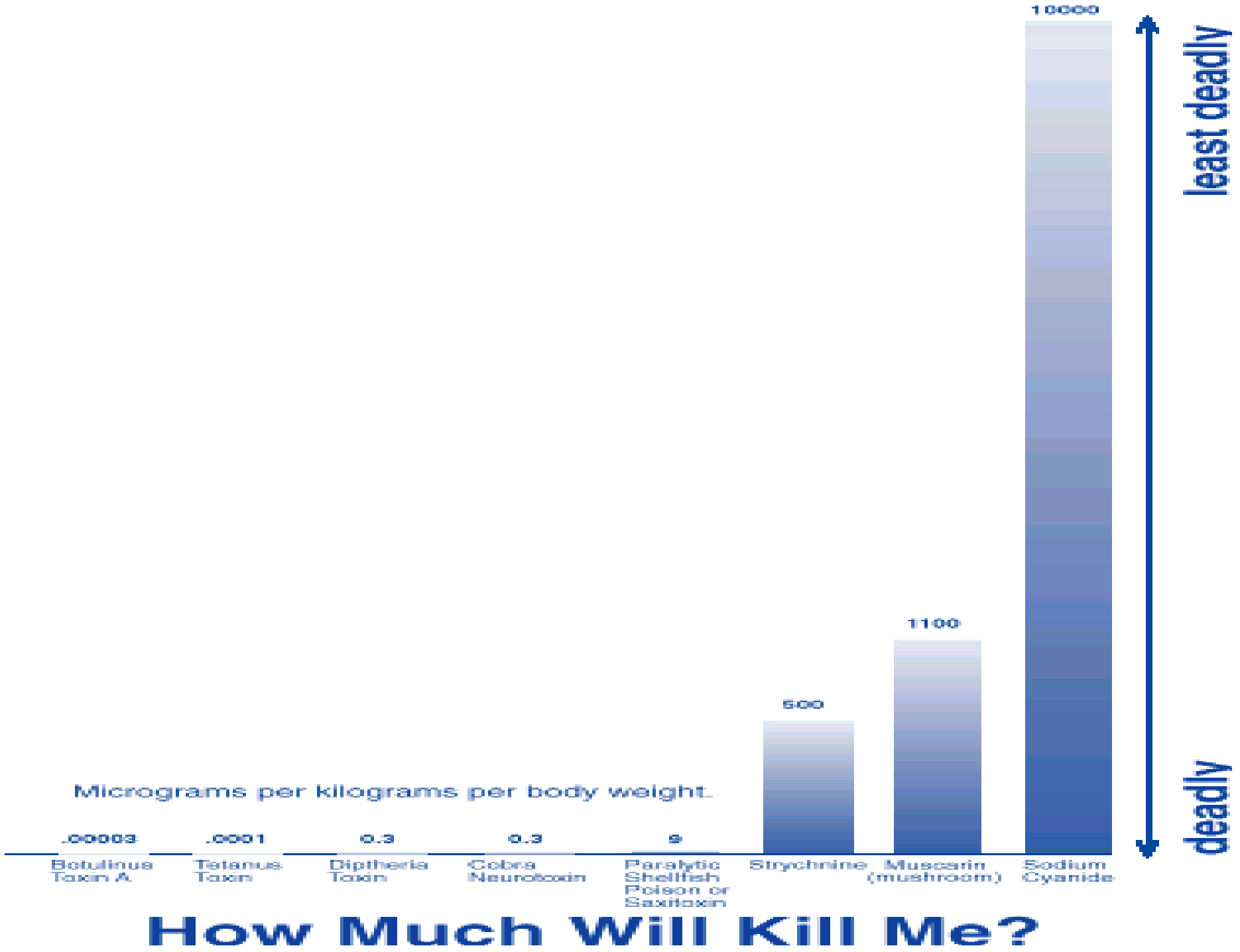
***Moraxella* sp (*Alexandrium*)**



Saxitoxin



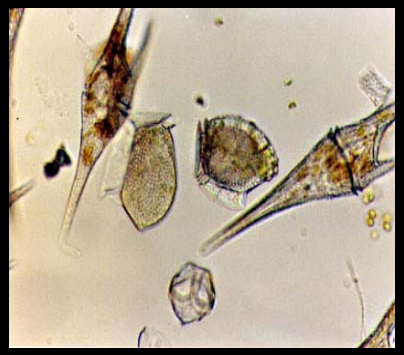

O⁻
O
OH
O
OH
H
N
NH₂
R₂
6
N
H
HO
R₁



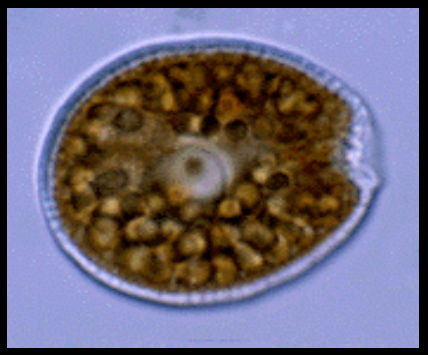
How Much Will Kill Me?

DSP

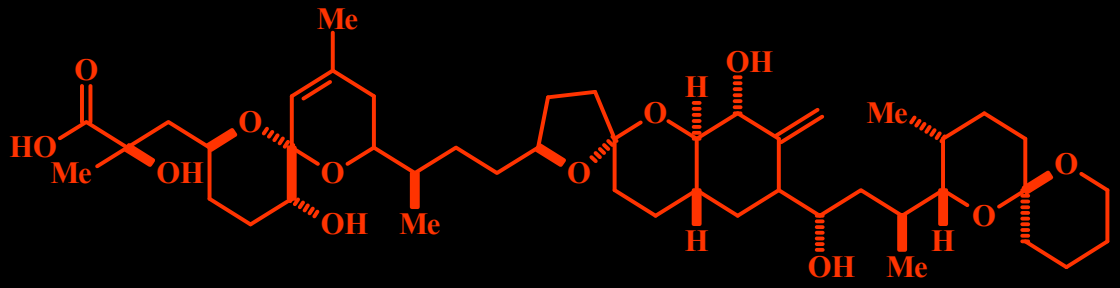
Halichondria okadai



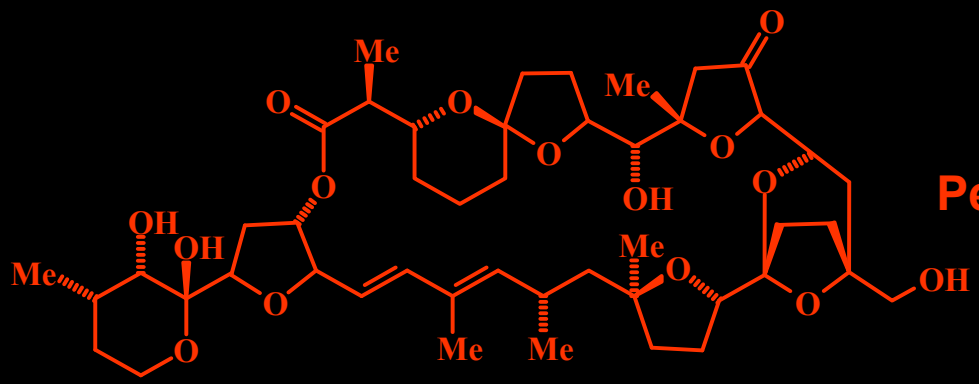
Dinophysis sp



Prorocentrum sp



Okadaic Acid



Pectenotoxin

DSP (*D*iarrhetic *S*hellfish *P*oisoning)

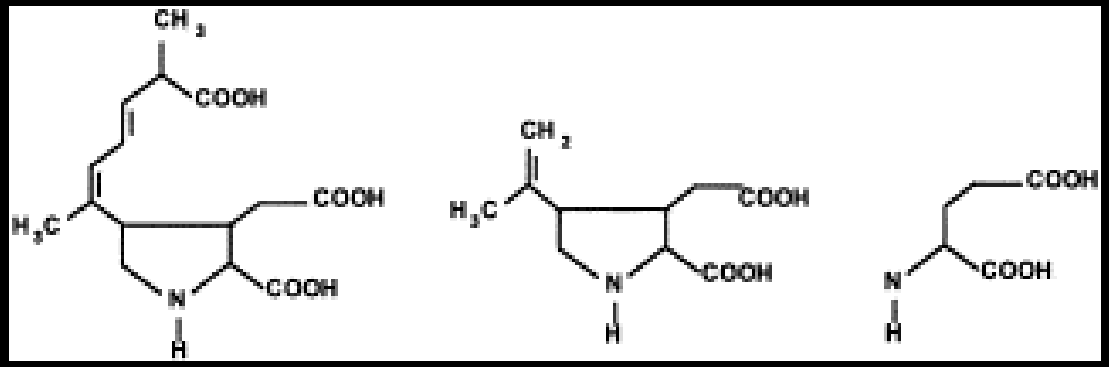
ΤΟΞΙΝΕΣ ΜΕ ΣΥΜΠΤΩΜΑΤΑ ΑΠΟ ΤΟ ΓΑΣΤΡΕΝΤΕΡΙΚΟ ΣΥΣΤΗΜΑ

Παραγωγοί οργανισμοί: Δινομαστιγωτά

Συμπτώματα: διάρροια,ναυτία, έμετοι και πόνοι στην κοιλιακή χώρα. (Κατανάλωση ακόμη και 5 μολυσμένων οστράκων είναι αρκετή για να εμφανισθεί το σύνδρομο αυτό).

Σοβαρές κοινωνικοοικονομικές επιπτώσεις σε χώρες όπως η Ιαπωνία, οι ΗΠΑ, η Ισπανία (αναφέρονται 5.000 περιστατικών μόνον κατά το 1981), η Ολανδία και οι χώρες της Βορειοδυτικής Ευρώπης.

A.S.P. (Amnesic Shellfish Poisoning)



Δομοϊκό οξύ

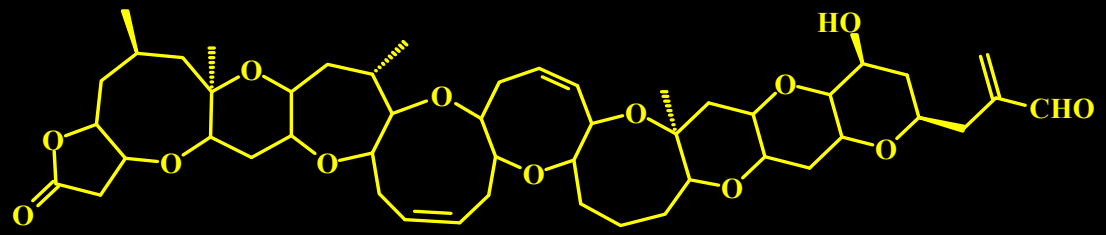
Απομόνωση: Από ροδοφύκη της οικ: rodomelaceae, μαζί με το Καϊνικό οξύ.(1958)

Προέλευση: *Pseudonitzschia* sp (Bacillariophyceae)

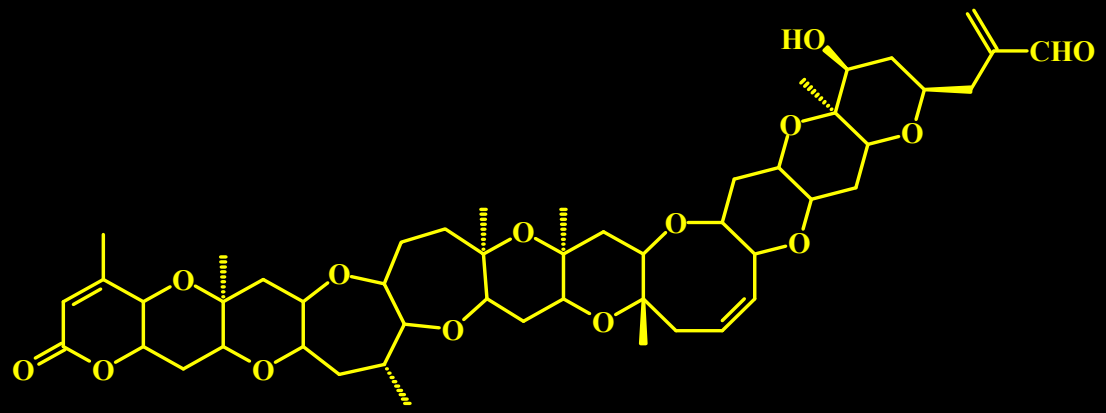
Συμπτώματα: εκτός άλλων και Αμνησία.

Συνδέεται με γλουταμικούς και N-μεθυλ-D-ασπαρτικούς υποδοχείς των νευρώνων του εγκεφάλου με τελικό αποτέλεσμα τον θάνατο των νευρικών κυττάρων.

Neurotoxic Shellfish Poisoning (NSP)



Μπρεβετοξίνη A



Μπρεβετοξίνη B

«ΣΙΓΚΟΥΑΤΕΡΑ»

Σύνδρομο τροφικής δηλητηρίασης από κατανάλωση τροφών Θαλάσσιας προέλευσης.

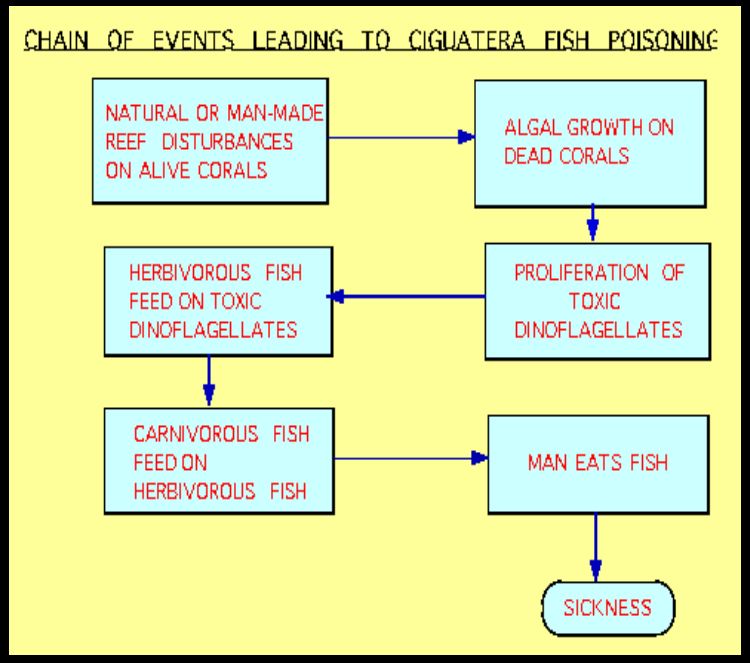
Παρουσιάζει ποικίλα και πολλές φορές διαφοροποιημένα συμπτώματα από το γαστρεντερικό αλλά και το κυκλοφοριακό και νευρικό σύστημα.

Πρωταρχική πηγή παραγωγής το επιφυτικό μαστιγοφόρο *Gambierdiscus toxicus,* και άλλοι παρόμοιοι οργανισμοί.

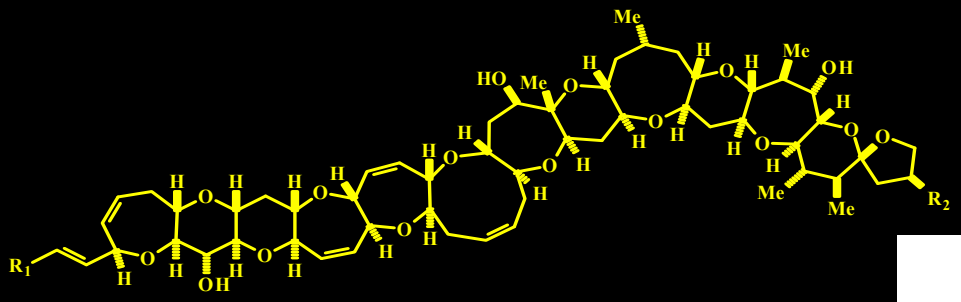
Δια μέσου της τροφικής αλυσίδας τα τοξικά συστατικά μεταφέρονται μέχρι και τον άνθρωπο.

Κύριοι εκπρόσωποι είναι η **Σιγκουατοξίνη** και η **Μαϊτοτοξίνη**.

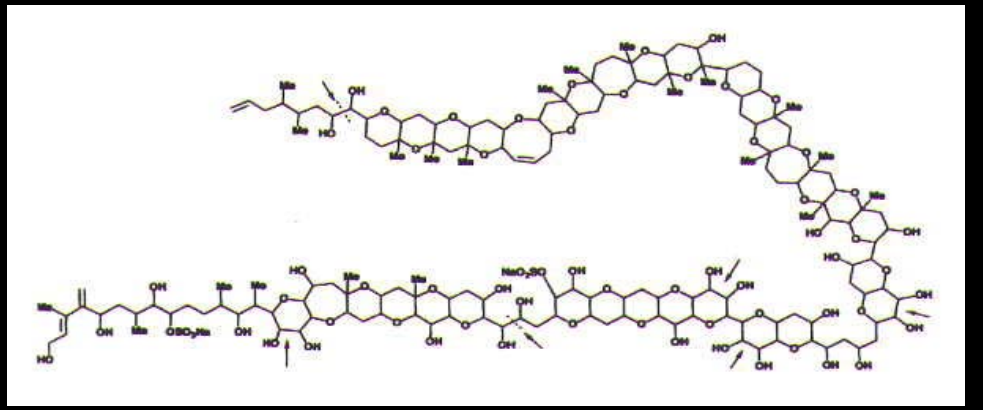
CIGUATERA FISH POISONING



Gambierdiscus toxicus



Ciguatoxin: $R_1 = -CH(HO)-CH_2OH$, $R_2 = OH$

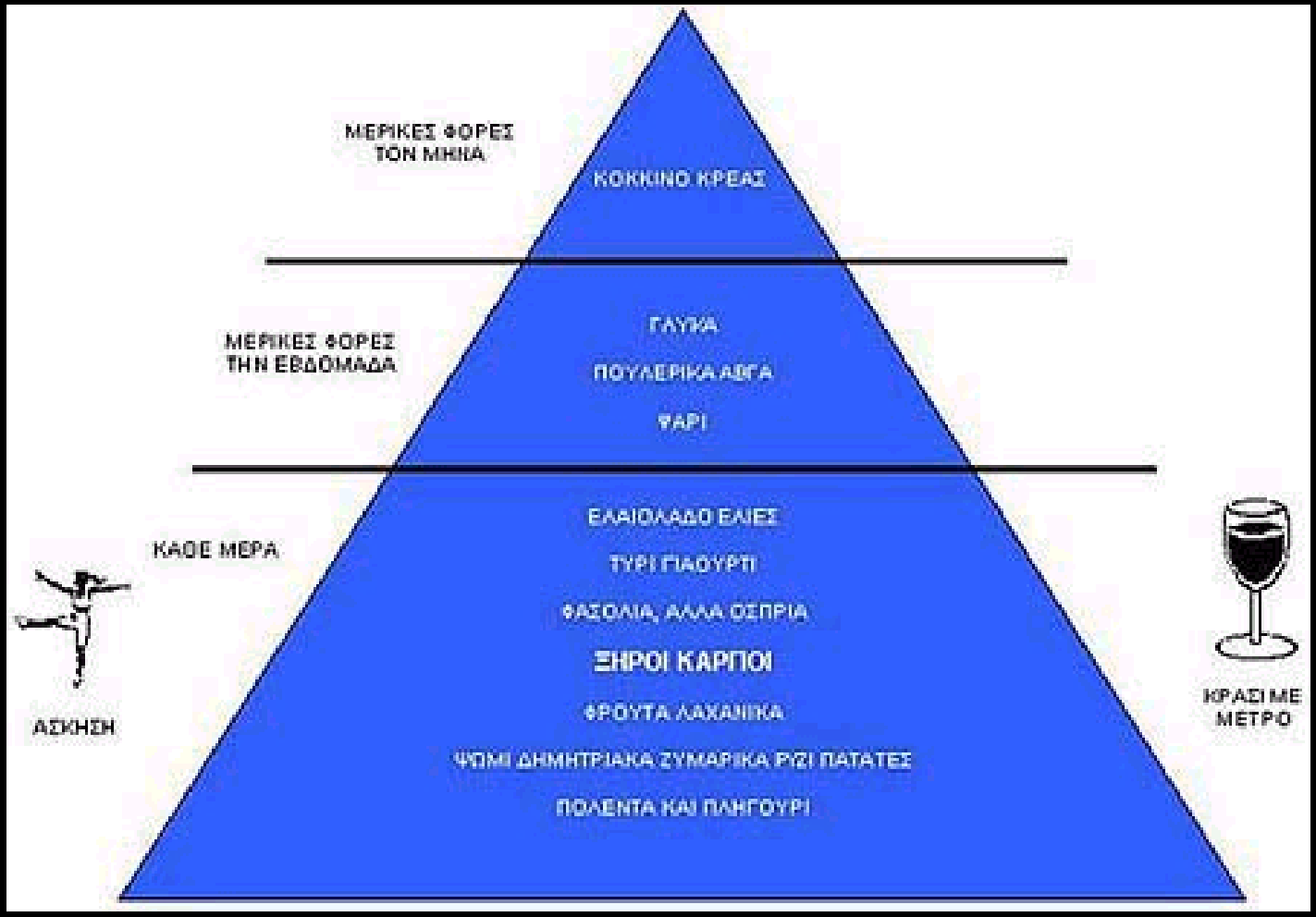


Maitotoxin

ΣΥΜΠΕΡΑΣΜΑΤΑ-ΑΝΑΓΚΑΙΟΤΗΤΕΣ

- Προσδιορισμός των φυσικών, χημικών και βιολογικών παραγόντων που προκαλούν την εμφάνιση τέτοιων φαινομένων.
- Βελτίωση των μεθόδων άμεσης αναγνώρισης των υπεύθυνων οργανισμών
- Καταγραφή φαινομένων HABs και συσχετισμός με την εμφάνιση συμπτωμάτων.
- Ανάπτυξη μεθόδων ανίχνευσης στις τροφές .
- Διερεύνηση του μηχανισμού δράσης των τοξινών – Ανάπτυξη αντιδότων – Θεραπεία.
- Ειδική εκπαίδευση των αρμοδίων φορέων υγείας.

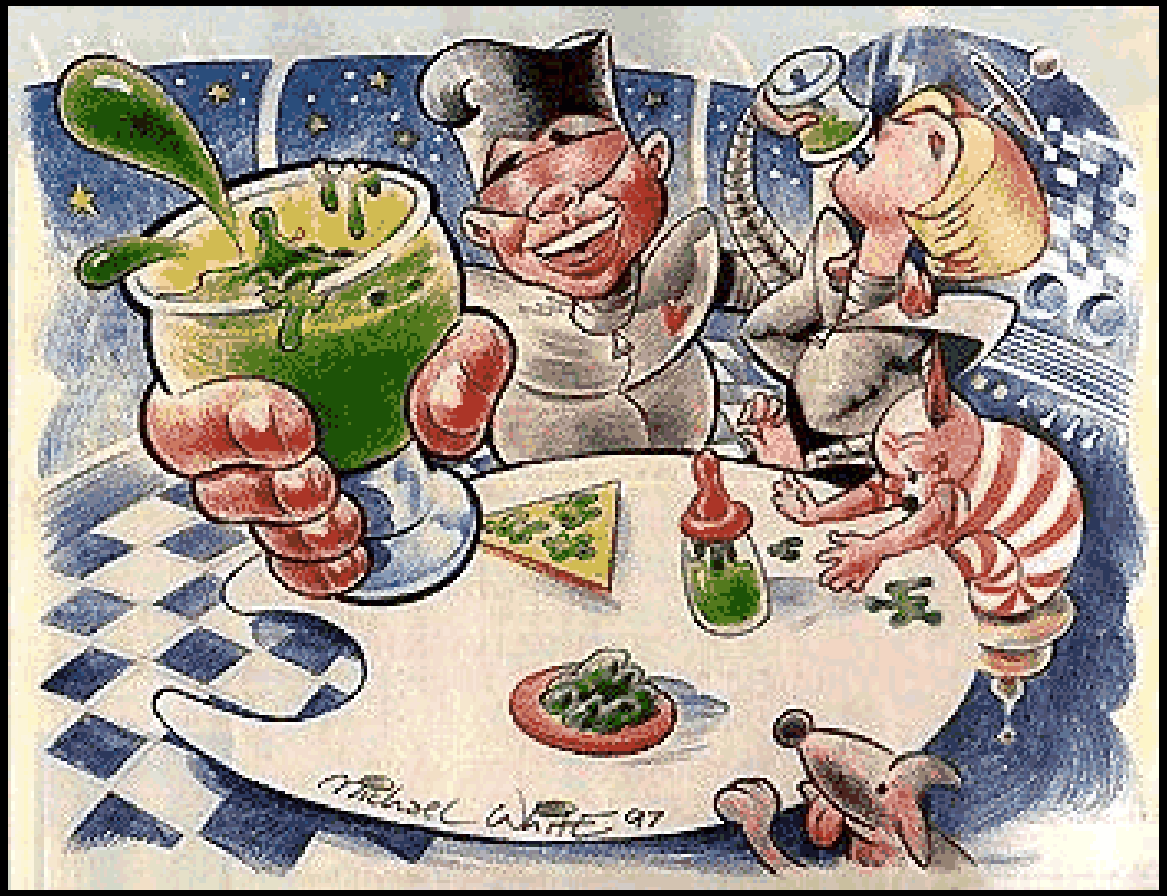
ΜΕΣΟΓΕΙΑΚΗ ΔΙΑΙΤΑ



Φαρμακευτικές ιδιότητες:

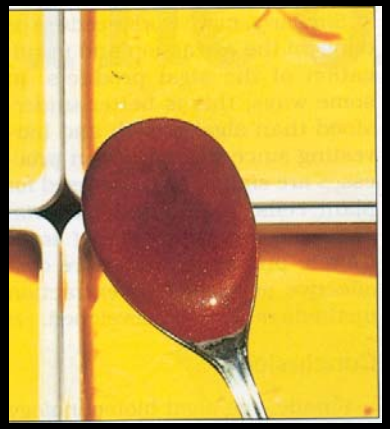
- Ενεργούν προφυλακτικά στα **καρδιοαγγειακά νοσήματα**, ρίχνοντας την χοληστερόλη και παράλληλα ανεβάζοντας την HDL, βοηθώντας έτσι με ισορροπημένο τρόπο ένα επιθυμητό προφίλ λιπιδίων του αίματος.
- Συμβάλλουν στην αποφυγή της ανάπτυξης αρκετών μορφών **καρκίνου**.
- Μειώνουν τις πιθανότητες πρόκλησης **ρευματοειδούς αρθρίτιδας**.
- Υποβοηθούν την οστική μεταλλική πυκνότητα και την προάσπιση από την **οστεοπόρωσης**
- Βοηθούν στη **μείωση της συγκέντρωσης γαστρικών υγρών**.
- Μειώνουν την **δυσπεψία**
- Εχουν θεραπευτική δράση στο **έλκος του δωδεκαδακτύλου**.
- Βελτιώνουν την **κινητικότητα του παχέος εντέρου**.
- Προλαμβάνει **ασθένειες του ήπατος**.
- Παρουσιάζουν αξιόλογη ευεργετική δράση στην **θεραπεία του διαβήτη**.
- Προστατεύουν τον οργανισμό από τη **δημιουργία θρομβώσεων**.

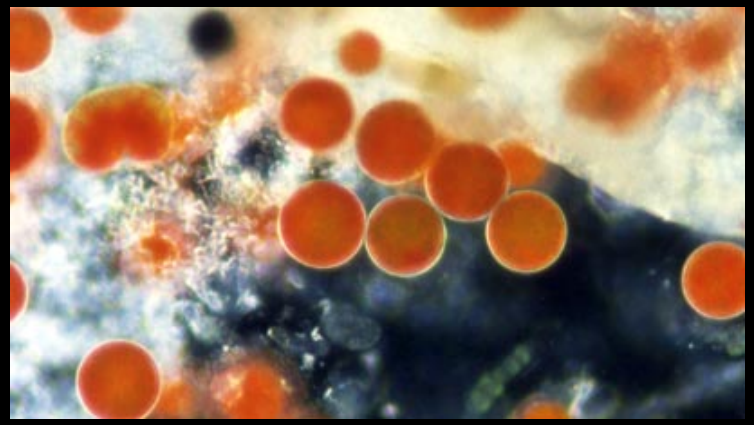
Επιδρούν ευνοϊκά στην **ανάπτυξη του κεντρικού νευρικού συστήματος, στην δομή των ιστών του εγκεφάλου και του αγγειακού συστήματος**.



Summary of Alga tablet

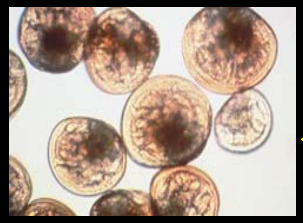
- Χρωστικές
- Λιπίδια
- Βιοδραστικοί Μεταβολίτες





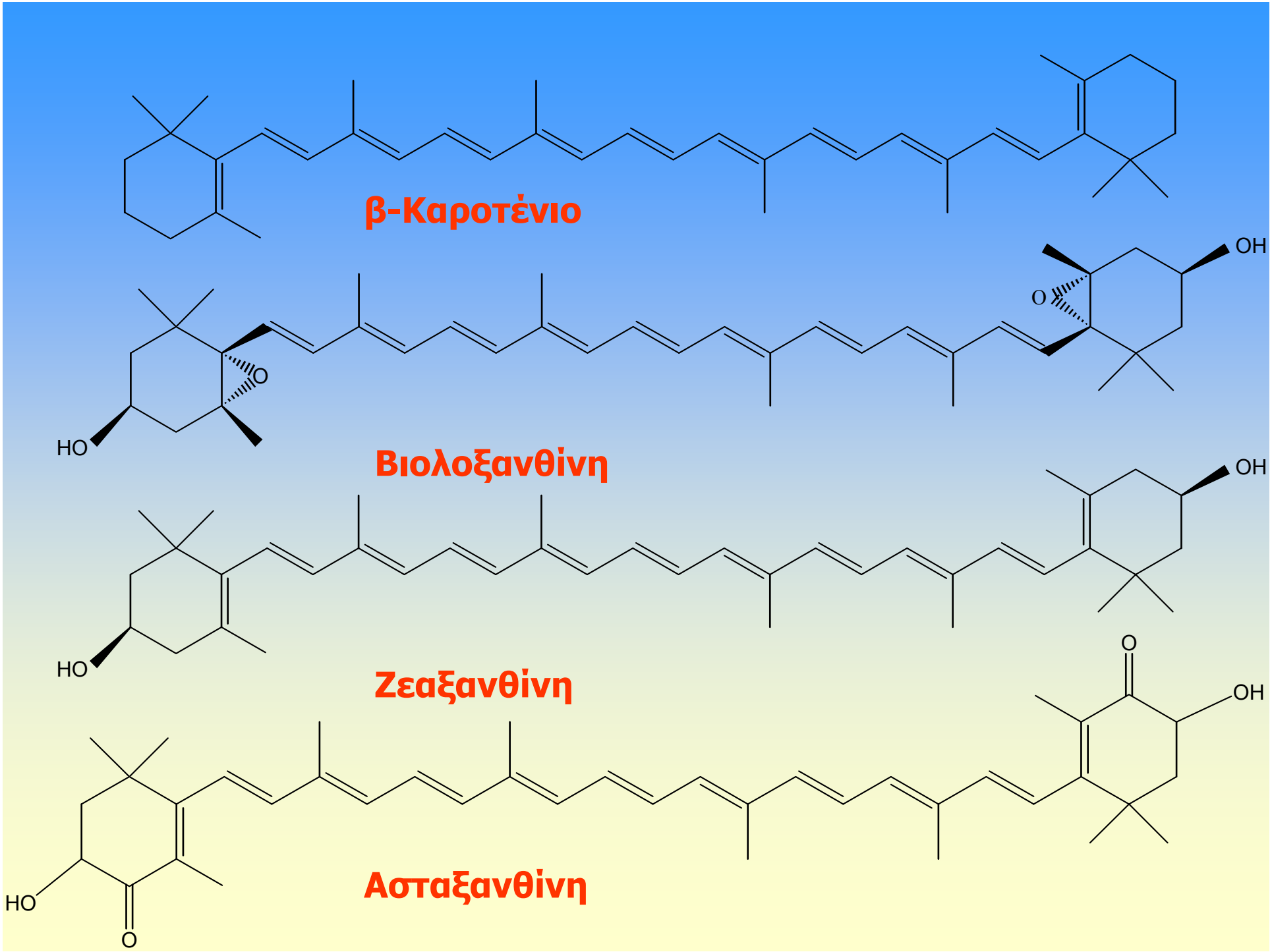
ΔΙΑΤΡΟΦΗ & ΒΙΟΜΑΖΑ

- Υδατοκαλλιέργιες
- Συμπληρώματα Διατροφής



Τα μικροφύκη ως πηγές ειδικών ουσιών

ΠΡΟΪΟΝ	ΑΞΙΑ US$/Kg	ΑΓΟΡΑ (in US$ Millions)	ΠΕΡΙΕΚΤΙΚΟΤΗΤΑ (% D.W.)
ΒΙΟΜΑΖΑ			
Health Food	20-30	10-100	100
Feed additive	30-130	100-1.000	100
ΑΝΤΙΟΞΕΙΔΩΤΙΚΑ			
Tocopherol	25	60	>2
S.O.D	>1000		>1
(ΠΟΛΥΑΚΟΡΕΣΤΑ ΛΙΠΑΡΑ)			
Arachidonic acid	400-10.000	>1.000	1-2
Eicosapentaenoic acid	160.000	>1.000	1-2
gamma-linolenic acid	400-10.000	>1.000	1-2
ΕΚΧΥΛΙΣΜΑ			
High pressure extract	2.000-3.000	1-2	1-2
Ethanolic extract	300-500	3-4	7-8
Aquatic extract	40	3-4	55
ΧΡΩΣΤΙΚΕΣ			
Beta-carotin	>1.000	10-100	10
Xanthophylls	200-500	10-100	1
Astaxanthin	>1.500	>50	1-6
Canthaxanthin	1.000		>1
Phycocyanin	>100	>10	1-5
Phycoerhythrin	>10.000	2	1-5


β-Καροτένιο
OH
O
HO
O
Βιολοξανθίνη
OH
HO
Ζεαξανθίνη
O
OH
HO
O
Ασταξανθίνη

PHAEODACTYLUM TRICORNUTUM

ΔΙΠΛΑΣΙΑΣΜΟΣ της περιεκτικότητας σε DHA και ARA

NANNOCHLOROPSIS OCULATA

Αύξηση του EPA από 33.7% σε 41.5%.

CHAETOCHEROS GRACILIS

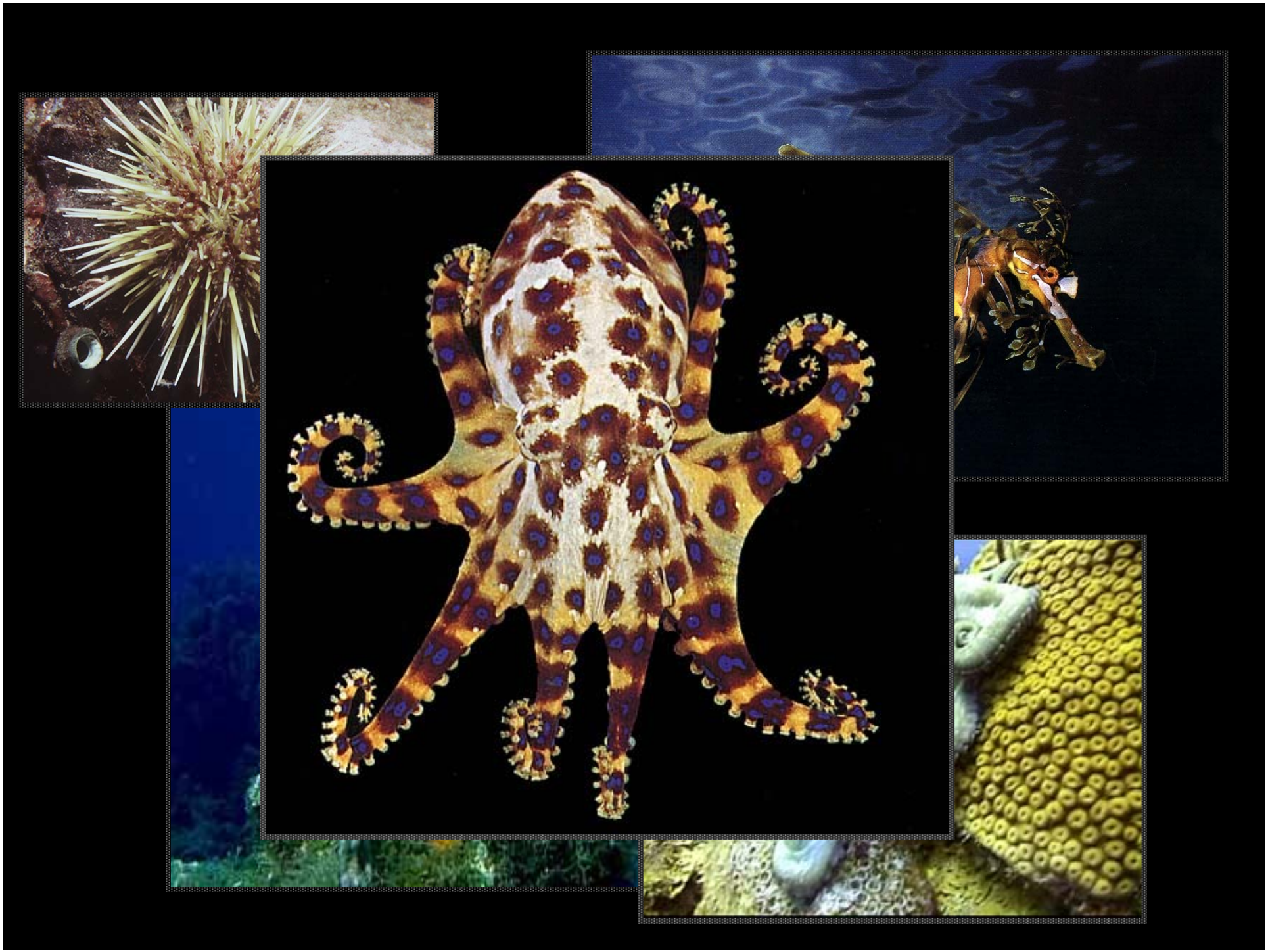
Αύξηση σε σημαντικά επίπεδα όλων των επιθυμητών συστατικών

CHLORELLA MINUTISSIMA

ΤΡΙΠΛΑΣΙΑΣΜΟΣ της περιεκτικότητας σε EPA.

ISOCHRYSIS aff. GALBANA

ΤΡΙΠΛΑΣΙΑΣΜΟΣ της περιεκτικότητας σε EPA και ο **ΔΙΠΛΑΣΙΑΣΜΟΣ** της περιεκτικότητας σε DHA.

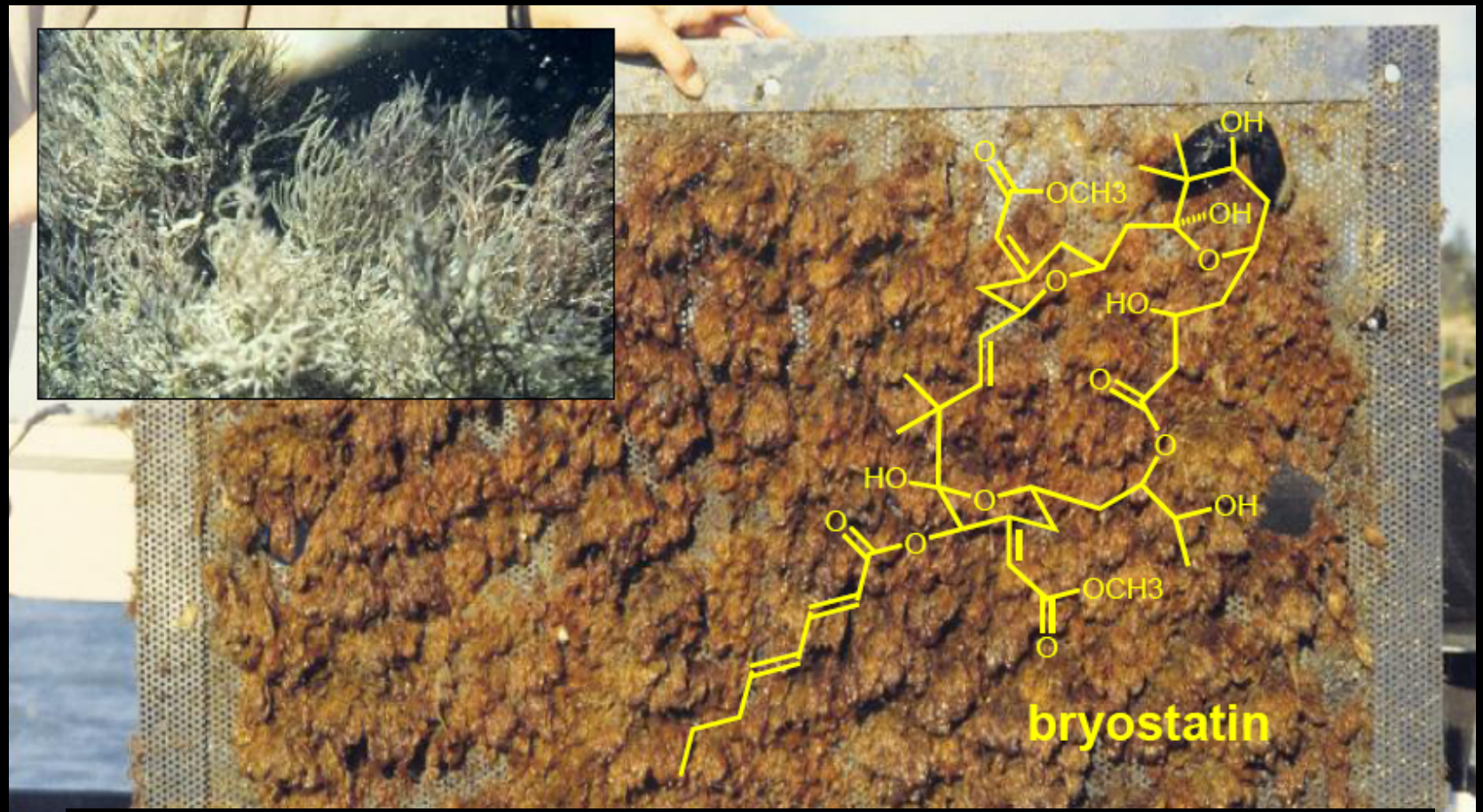


ANTI-PREDATORY CHEMICAL DEFENSES

Pre-1980s: Assessed by correlation or toxicity studies

Subsequent: Development of more ecologically relevant bioassays

- -Feeding assays
- -Realistic consumers
- -Natural concentrations of metabolites



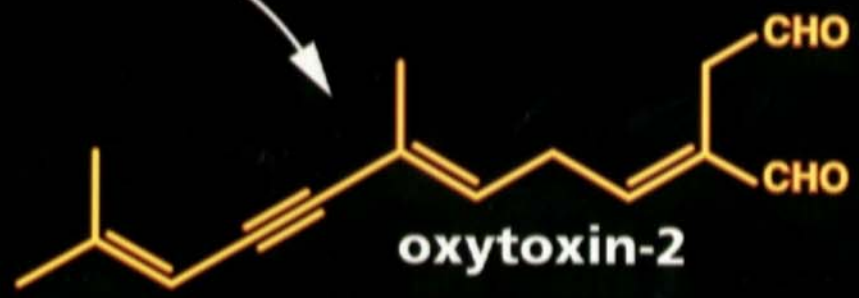
Bryostatins are isolated from the bryozoan, *Bugula neritina*, but are produced by a symbiotic bacterium. The bryostatins are concentrated in the bryozoan's larvae and protect the larvae from predation by fishes. Bryozoans are filter feeding invertebrates.

Soft fleshy seaweeds found where herbivorous fishes and invertebrates are abundant typically deter herbivory through the production of distasteful secondary metabolites.

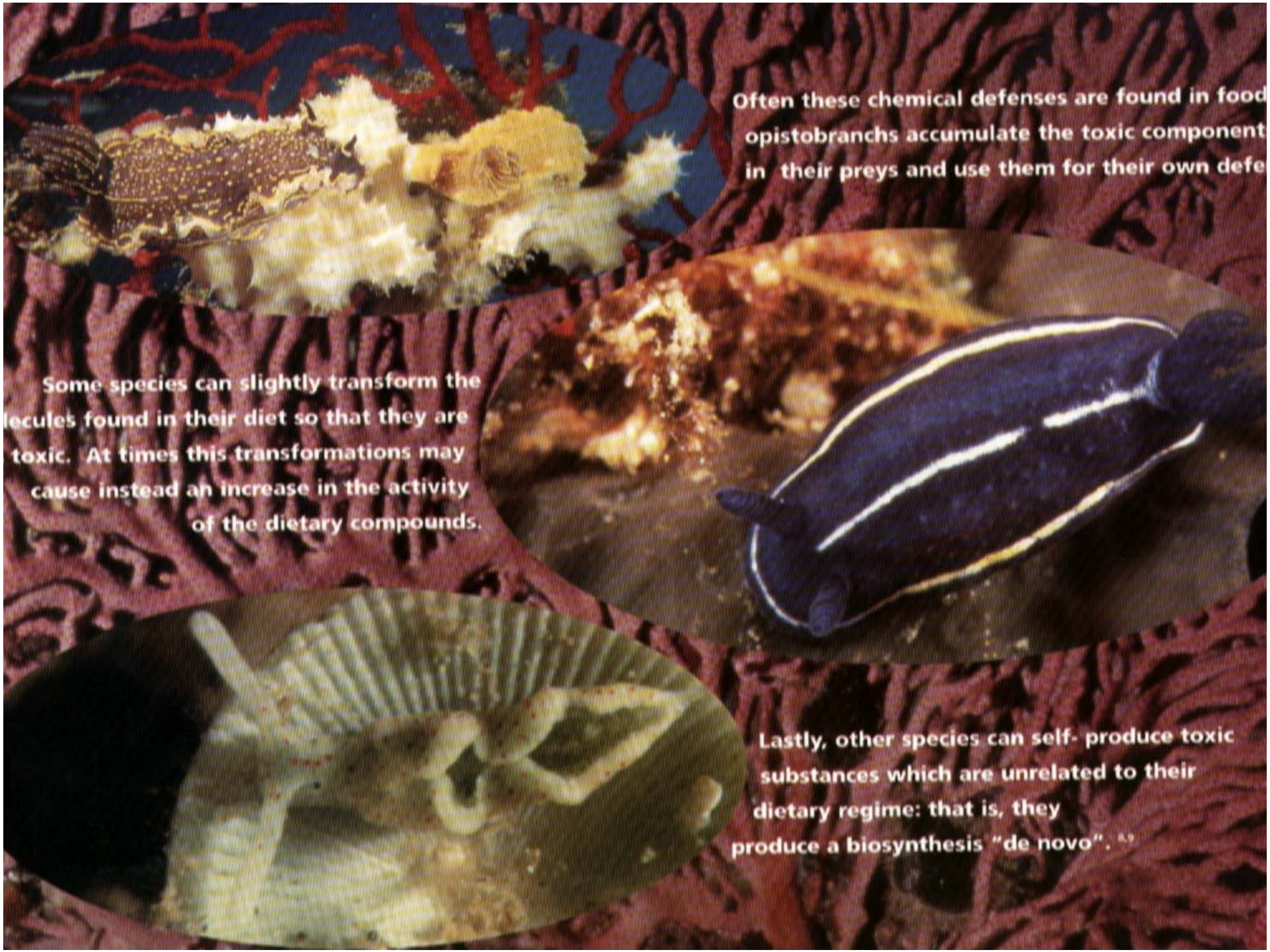
Nudibranchs, also called sea slugs, are a commonly cited example of how organisms with potent chemical defenses have little need for a physical defense, such as a hard shell, to protect against potential predators.
Nudibranchs typically get their defensive chemistry from the sponges, bryozoans, and sea squirts that they eat.

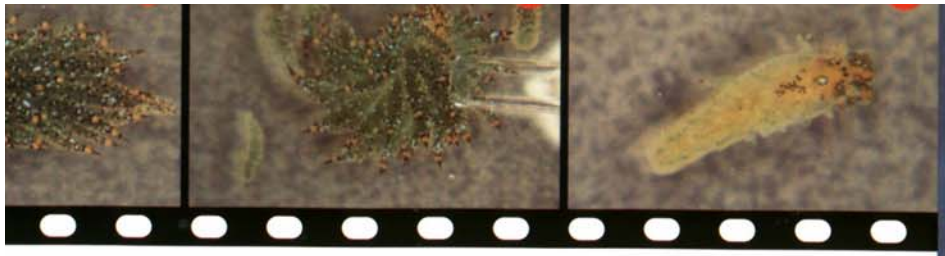
Nudibranches also put defensive compounds in their soft egg ribbons.

Even if Opistobranchs do not have the mechanical protection that a shell would provided, they are not, however, exposed to attacks by predators. They defend themselves by releasing a mucous secretion containing small organic molecules which are both toxic and repellent. [3,4,5]



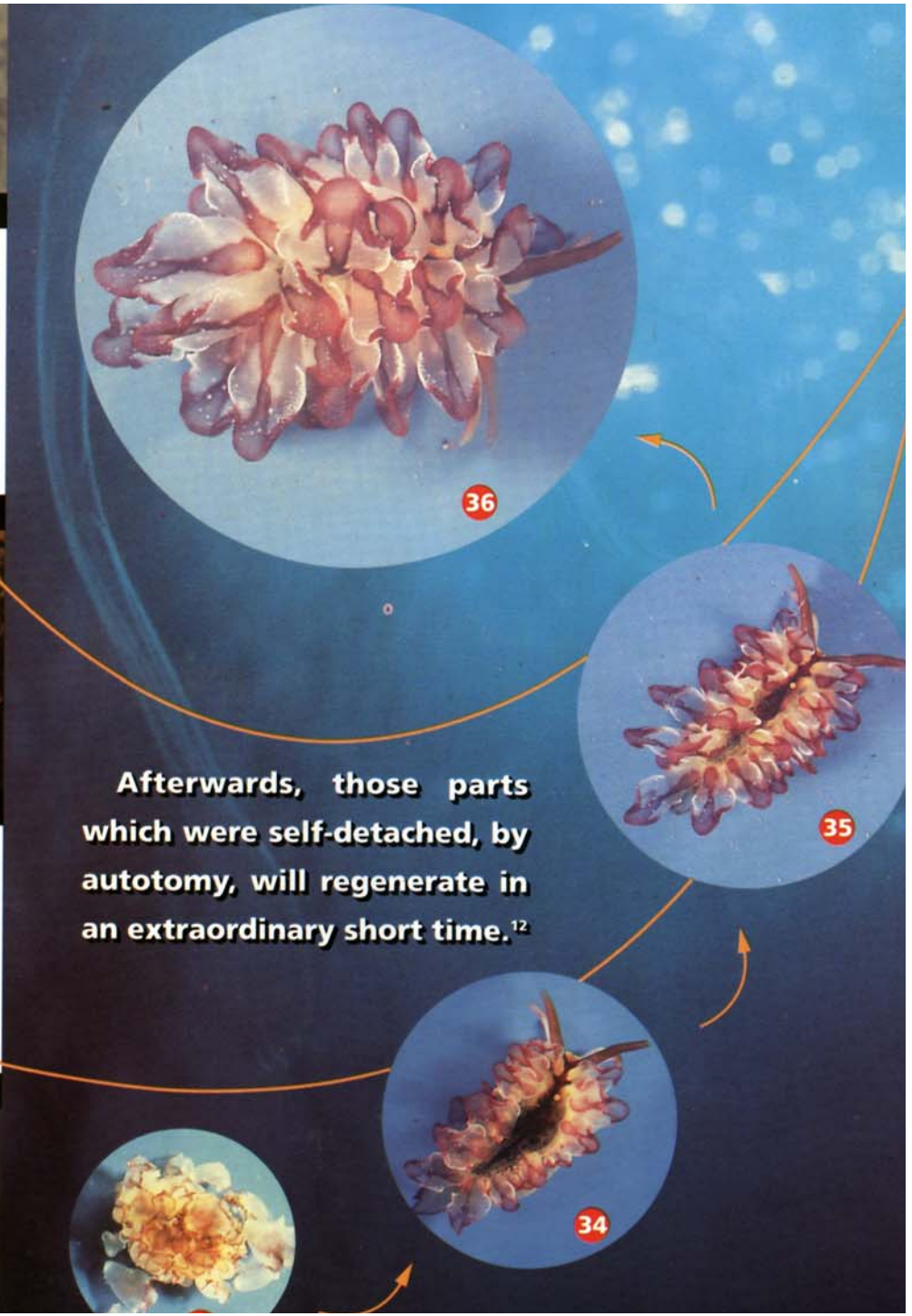
oxytoxin-2

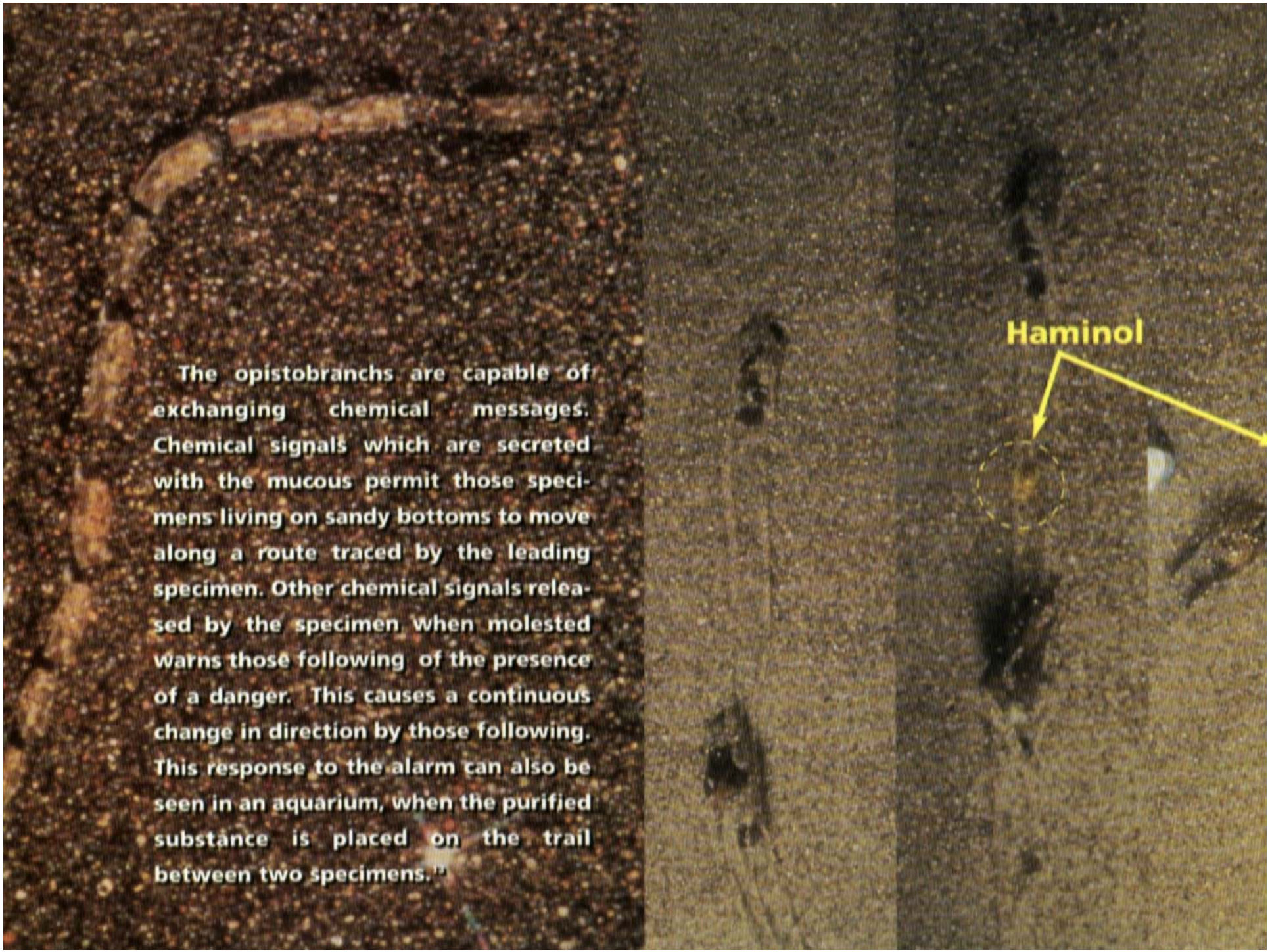

Often these chemical defenses are found in food
opistobranchs accumulate the toxic component
in their preys and use them for their own defe
Some species can slightly transform the
lecules found in their diet so that they are
toxic. At times this transformations may
cause instead an increase in the activity
of the dietary compounds.
Lastly, other species can self- produce toxic
substances which are unrelated to their
dietary regime: that is, they
produce a biosynthesis "de novo". 8,9



t from secreting toxic substances when molested, cked some opistobranchs are capable of self- ng, by autotomy, those parts of their body which ticularly exposed.

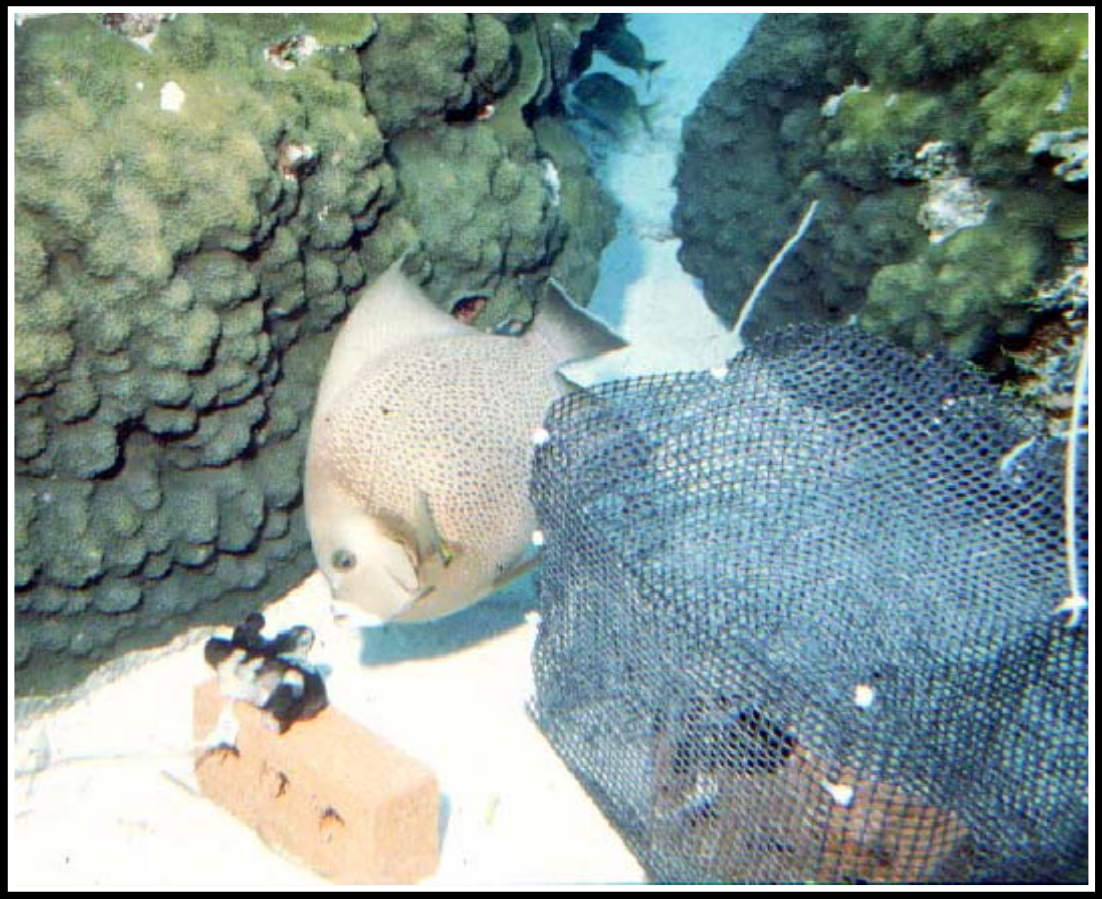
autotomy, associated with the secretion of toxic , form another valid instrument of defense. In some the detached appendages will continue to contract e time thereby distracting the aggressor.[11]





The opistobranchs are capable of exchanging chemical messages. Chemical signals which are secreted with the mucous permit those specimens living on sandy bottoms to move along a route traced by the leading specimen. Other chemical signals released by the specimen when molested warns those following of the presence of a danger. This causes a continuous change in direction by those following. This response to the alarm can also be seen in an aquarium, when the purified substance is placed on the trail between two specimens."

Caging experiments help to further illustrate the role of predators in eliminating poorly defended prey from habitats, like coral reefs, where predators are abundant.



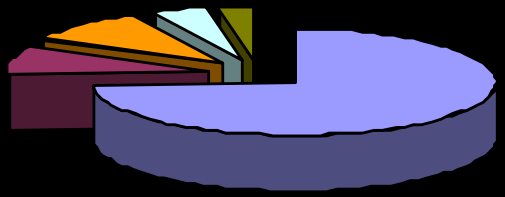
Research on Marine Natural Products

ADVANTAGE

Remarkable variety of secondary metabolites

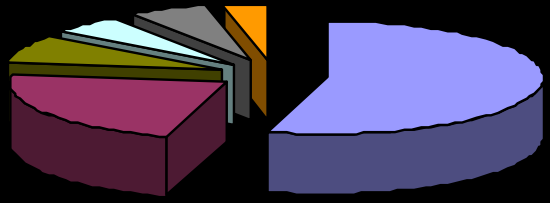
Applications of Marine Natural Products

Applications

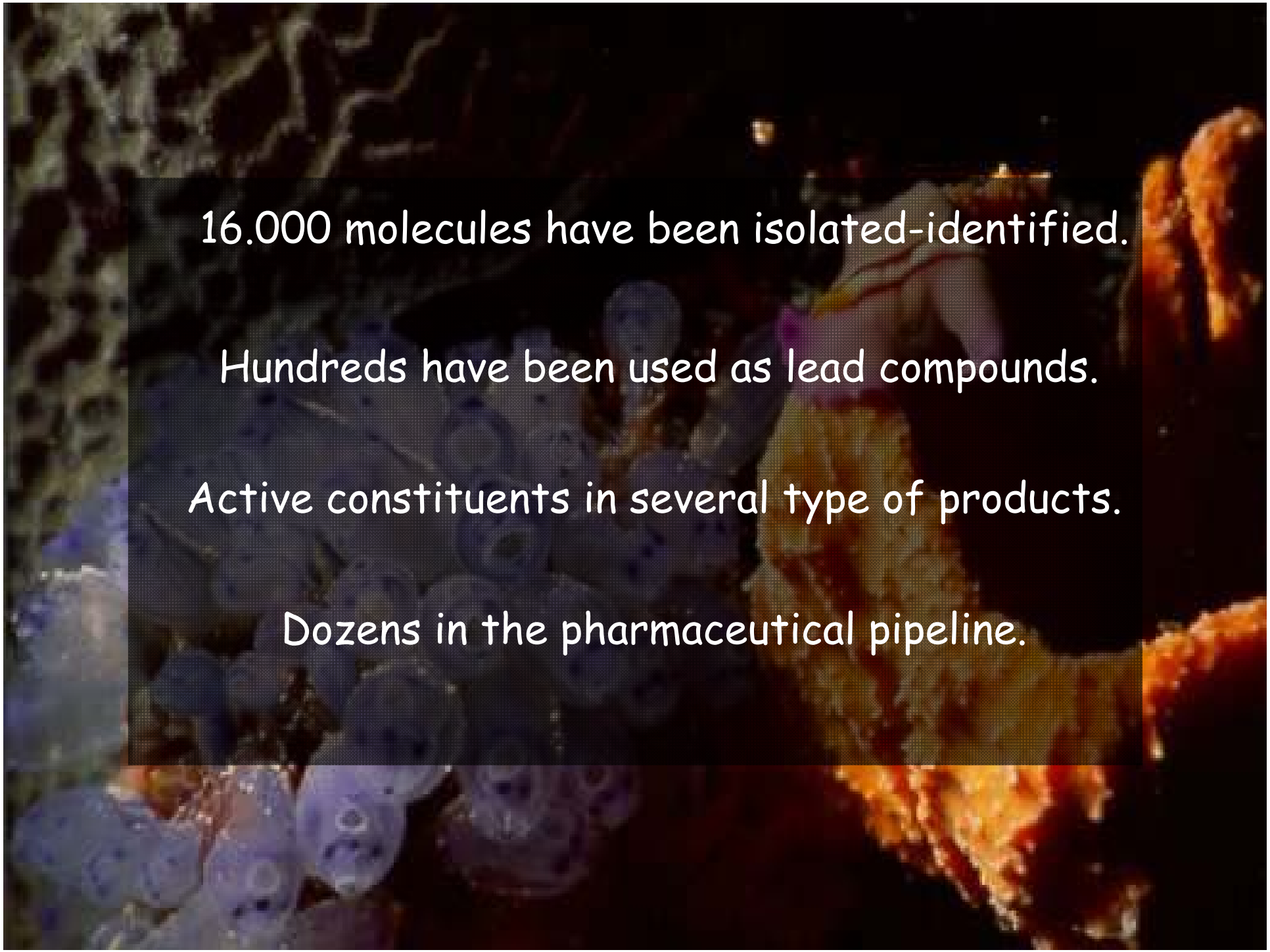


- Health
- New material
- Biotechnological tools
- Nutrition
- Cosmetics

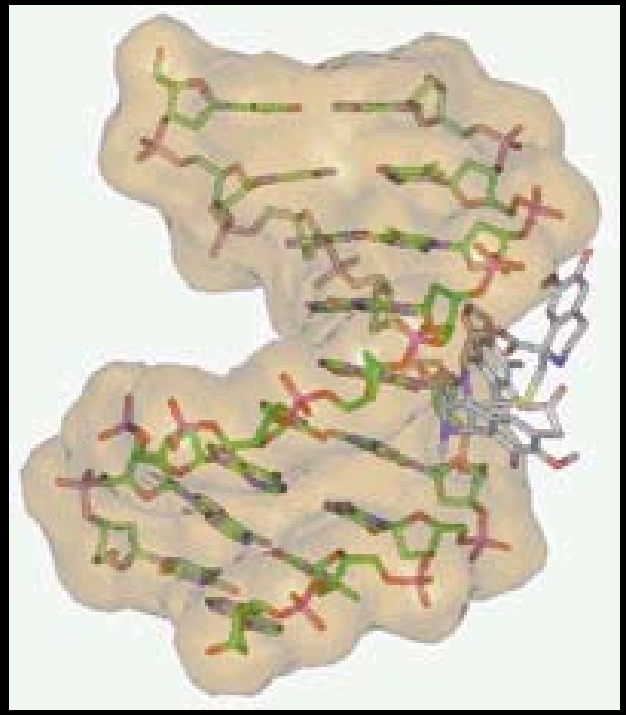
Health Sector



- Anticancer
- Antiviral
- Antifungal
- Analgesic
- Antiinfalatory
- Anthelminthic


16.000 molecules have been isolated-identified.
Hundreds have been used as lead compounds.
Active constituents in several type of products.
Dozens in the pharmaceutical pipeline.

Compound Name	Source	Chemical Class	Company	Disease Area	Status
Compounds targeting ion channels					
Ziconotide	Cone snail	Peptide	Neurex	Chronic pain	Phase III
AM-336	Cone snail	Peptide	AMRAD	Chronic pain	Phase I/II
GTS-21	Nemertine worm	Anabaseine-derivative	Taiho	Alzheimer/Schizophrenia	Phase I/II
Compounds targeting enzymes					
(a) Protein kinase inhibitors					
Bryostatin-1	Bryozoan	Polyketide	GPC Biotech	Cancer	Phase II
(b) PLA_2 inhibitors					
OAS-1000	Soft Coral	Diterpene-pentoseglycoside	OsteoArthritis Sciences	Wound Healing/Inflammation	Phase I/II
(c) Methionine aminopeptidase inhibitors					
LAF-389	Sponge	Amino acid derivative	Novartis	Cancer	Phase I
Microtubule-interfering agents					
Dolastatin-10	Sea slug	Peptide	NCI/Knoll	Cancer	Phase II
ILX-651	Sea slug	Peptide	Ilex Oncology	Cancer	Phase I
Cemadotin	Sea slug	Peptide	Knoll	Cancer	Phase II
Discodermolide	Sponge	Polyketide	Novartis	Cancer	Phase I
HTI286	Sponge	Tripeptide	Wyeth	Cancer	Phase I
DNA-interactive agents					
Yondelis™	Sea squirt	Isoquinolone	PharmaMar/Johnson&Johnson	Cancer	Phase II/III
Oxidative stress inducers					
Aplidin™	Sea squirt	Cyclic depsipeptide	PharmaMar	Cancer	Phase II
Lysosomotropic compounds					
Kahalalide F	Sea slug/Alga	Cyclic depsipepetide	PharmaMar	Cancer	Phase II
Small G Protein antagonists					
ES-285	Clam	Anti-β-amino alcohol	PharmaMar	Cancer	Phase I
Immunostimulatory agents					
KRN-7000	Cone snail	α-Galactosylceramide	Kirin	Cancer	Phase I
Calcium-binding protein antagonists					
Squalamine lactate	Shark	Aminosteroid	Genaera	Cancer	Phase II
Compounds with unknown mechanism of action					
IPL-512602	Sponge	Steroid	Inflazyme/Aventis	Inflammation/Asthma	Phase II



ET-743 (Ecteinascidin-743)

PharmaMar

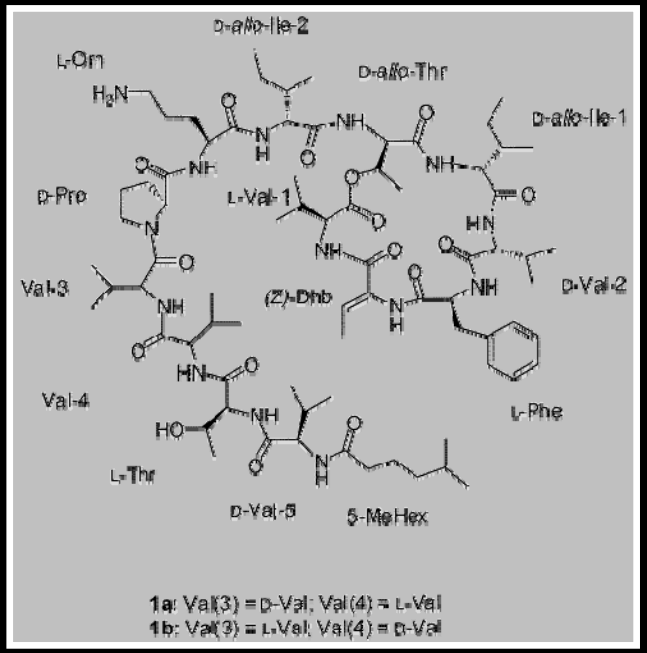
Yondelis®

Conus geographus

ω-Conotoxins

COGNETIX®

Kahalalide F

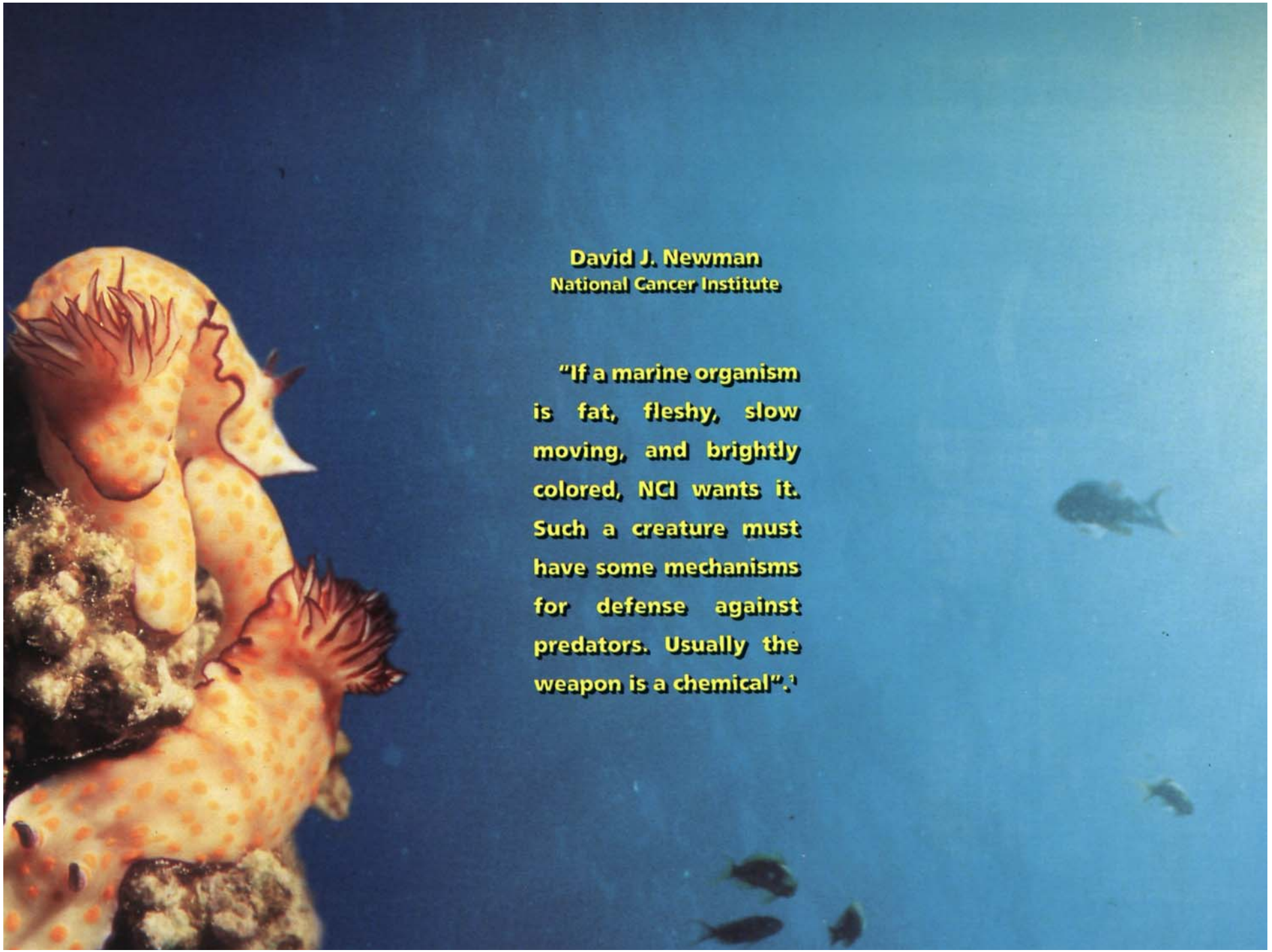


Elysia rufescens

Bryopsis **sp.**

- Kahalalide F is currently undergoing Phase II clinical trials in various solid tumours: melanoma, non-small cell lung cancer and hepatocellular carcinoma. A Phase II trial for the treatment of patients with severe psoriasis is also ongoing.

- Kahalalide F alters the function of the lysosomal membrane, a mechanism that distinguishes it from all other known anti-tumour agents. Studies demonstrate that Kahalalide F induces cell necrosis *in vivo* (oncosis) and shows selectivity for tumor compared with normal cells *in vitro*. Its activity is independent of multidrug resistance (MDR) expression.



David J. Newman
National Cancer Institute

"If a marine organism is fat, fleshy, slow moving, and brightly colored, NCI wants it. Such a creature must have some mechanisms for defense against predators. Usually the weapon is a chemical".[1]

Alien sea slugs in the Mediterranean

In Porto Germeno, Greece specimens of Lessepsian opisthobranch gastropods were collected feeding on their new dietary sources.

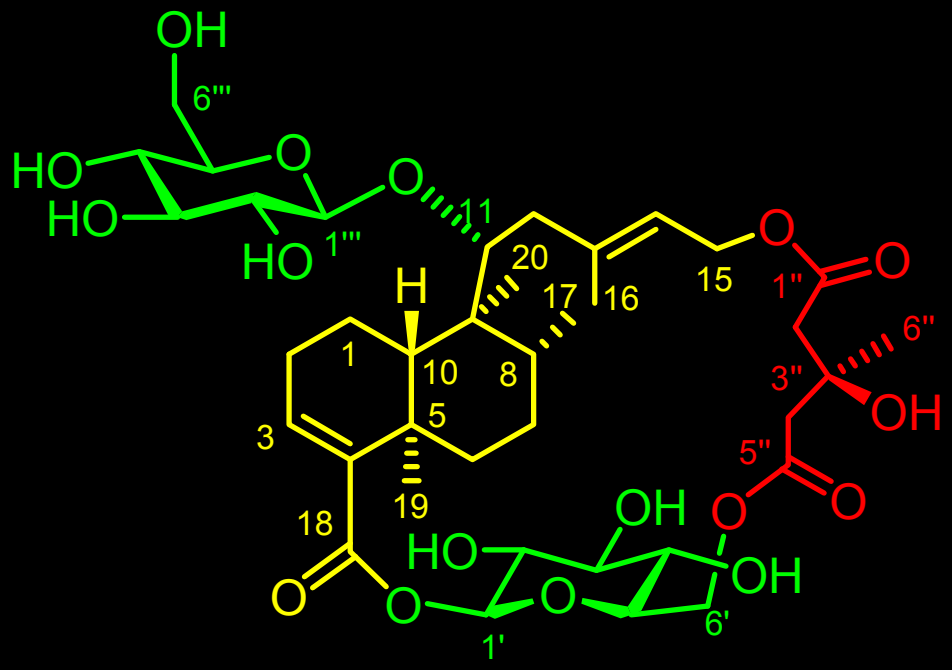
Haminoea cyanomarginata

Melibe viridis

Syphonota geographica

Syphonoside displays an unprecedented macrocyclic skeleton with an original conformation.

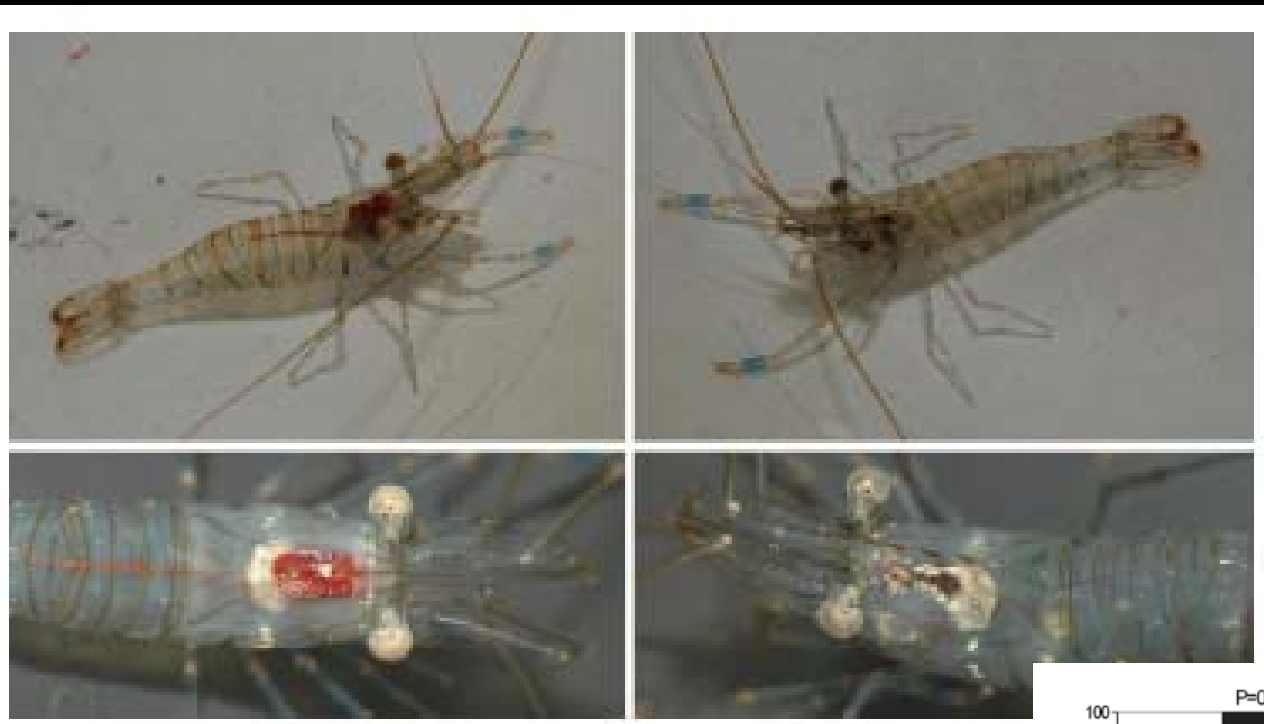
The resulting internal cavity could host hydrophobic molecule, stabilized though hydrogen bond interactions, indicating potential technological applications.



Syphonoside

Gavagnin M., Carbone M., Amodeo P., Mollo E., Vitale R. M., Roussis V., Cimino G.

J. Org. Chem. **2007**, *72*, 5625-5630



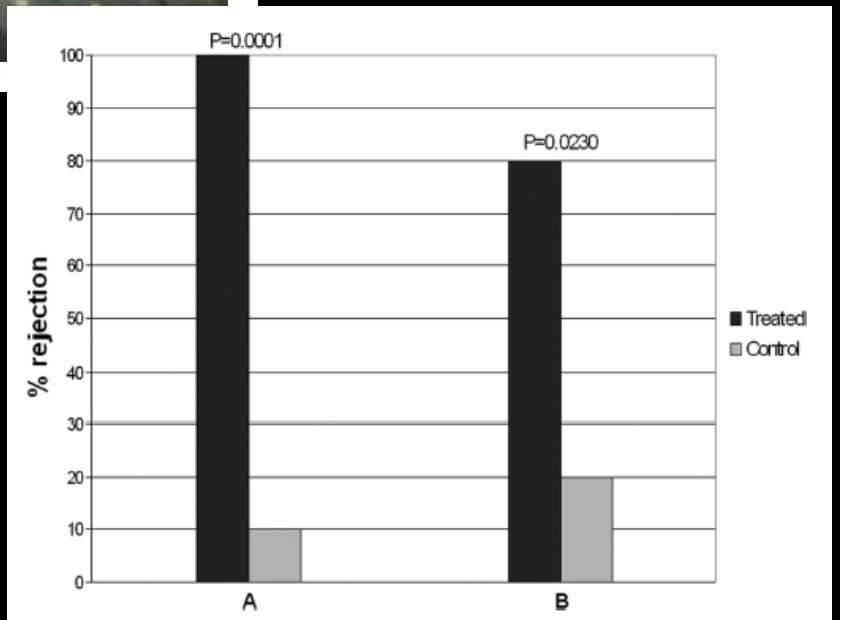


Fig. 3. *P. elegans* alimentary response to food pellets treated with compound **2** (A) and **4** (B) at the volumetric concentration of 2.3 mg/ml and 1.0 mg/ml, respectively. Two-tailed Fisher's exact test: $P < 0.05$ vs. control; $n = 10$ for each bar.

Proc. Nat. Acad. Sci., **2008**, 105, 4582-4586.

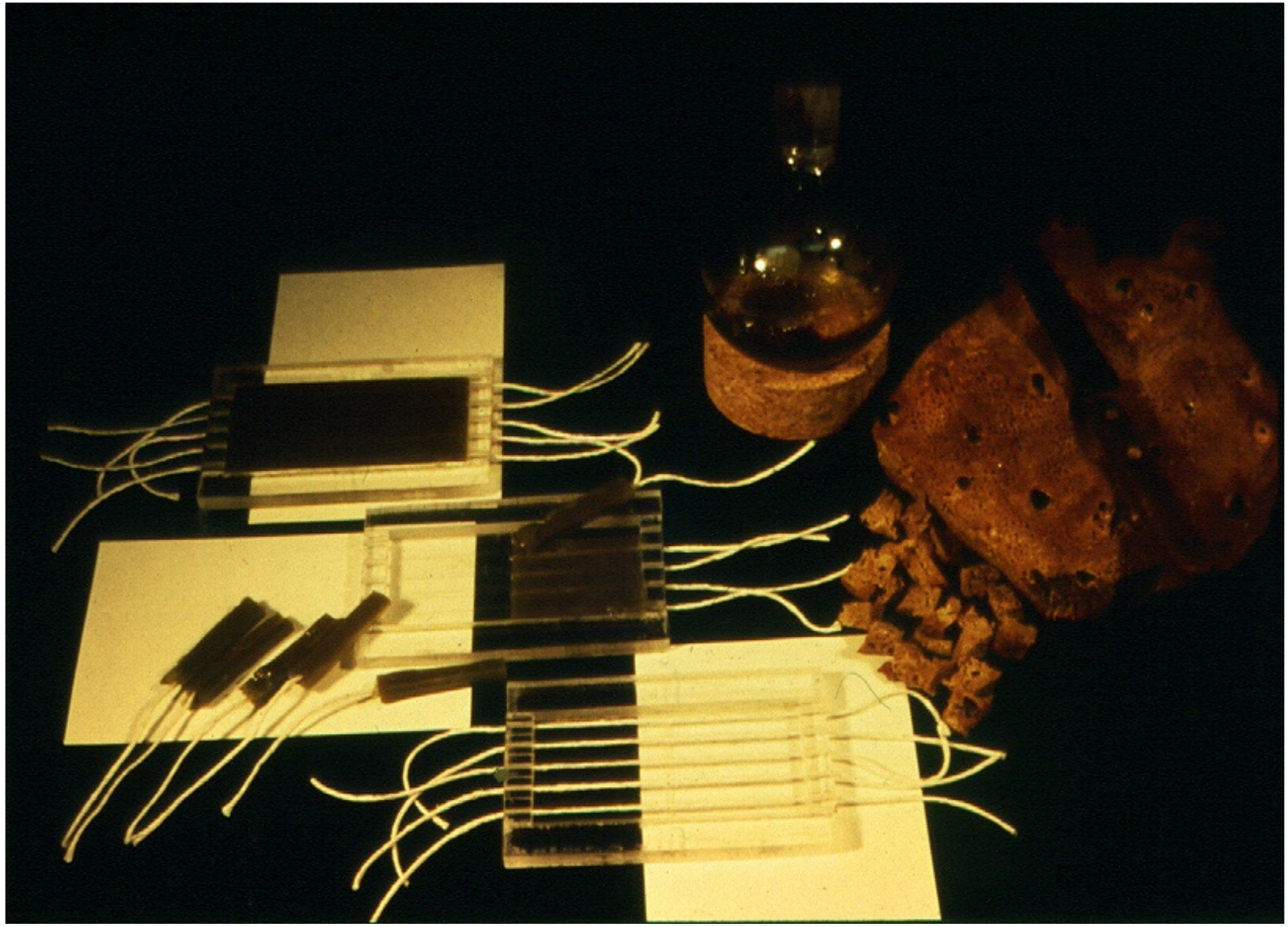
BIOFOULING

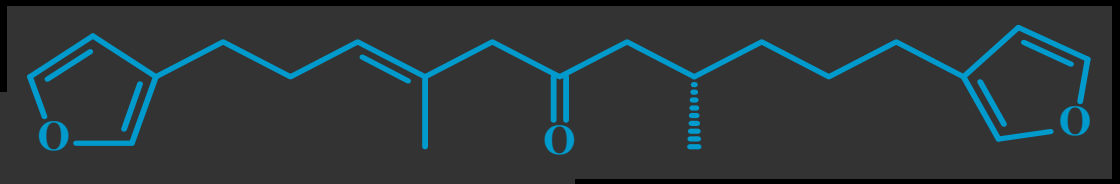

WALLENIUS WILHELMSEN

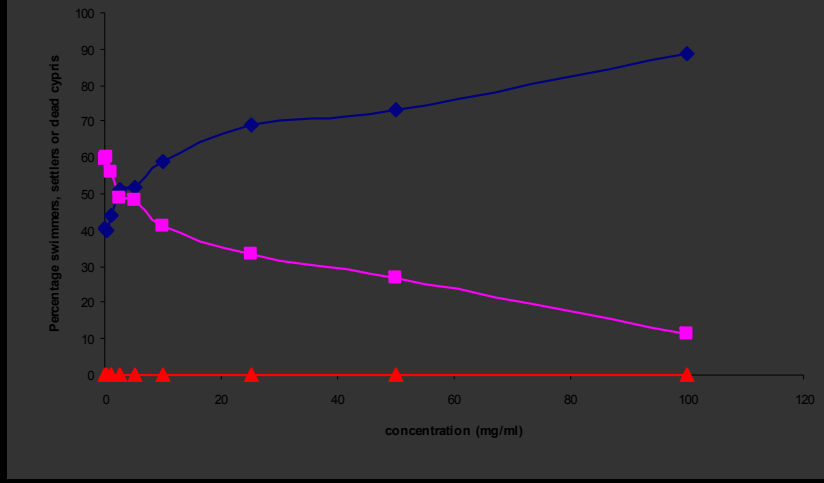

ΕΠΟΙΚΙΣΜΟΣ ΜΑΚΡΟΟΡΓΑΝΙΣΜΩΝ
ΕΠΟΙΚΙΣΜΟΣ ΜΙΚΡΟΟΡΓΑΝΙΣΜΩΝ
Τριτοταγείς επoικιστές
Δευτεροταγείς επoικιστές
Πρωτοταγείς επoικιστές
Οργανική μεμβράνη
Υπόστρωμα
1 Λεπτό
1-24 Ώρες
1
2-3 Εβδομάδες
Προσκόλληση οργανικών σωματιδίων (γλυκοπρωτεϊνών)
Βακτήρια
(e.g. Pseudomonas putrefaciens, Vibrio alginolyticus)
Διάτομα
(e.g. Achnantes brevipes, Amphiprora paludosa, Amphora coffeaeformis, Licmophora abbreviata, Nitzschia pusilla)
Σπόρια μακροφυκών
(e.g. Enteromorpha intestinalis, Ulothrix zonata, -Chlorophyta-)
Πρωτόζωα
(e.g. Vaginicola sp., Vorticella sp., Zoothamnium sp., -Ciliata-)
Προνύμφες ασπόνδυλων
(e.g. Balanus amphitrite -Crustacea- Electra crustulenta -Bryozoa- Laomedia flexuosa -Coelenterata- Mytilus edulis -Mollusca- Spirorbis borealis -Polychaeta- Styela coriacea -Tunicata-)

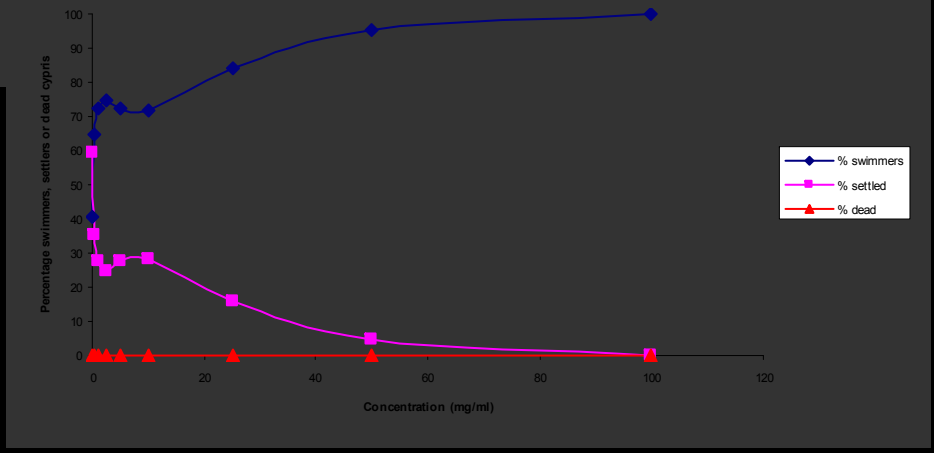
Determination of antifouling activity

- Inhibition of attachement and germination of spores and zygotes of macroalgae
- Inhibition of the growth of phytoplancton
- Inhibition of the growth of marine fungi
- Inhibition of the growth of bacteria
- Inhibition of phenoloxidase activity
- Inhibition of barnacles larval settlement

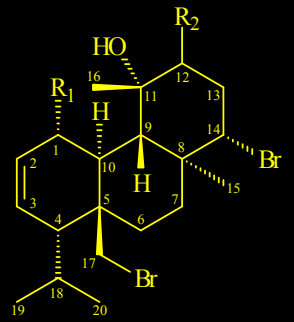


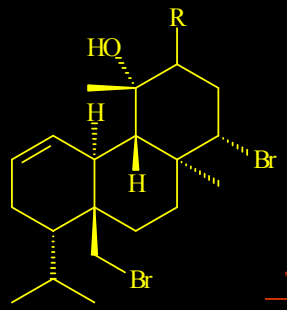



Percentage swimmers, settlers or dead cypris
concentration (mg/ml)
% swimmers
%settled
%dead


Percentage swimmers, settlers or dead cypris
Concentration (mg/ml)
% swimmers
% settled
% dead

Sphaerococcus coronopifolius





1: R_1= H R_2= H

2: R_1= OOH R_2=⋯OH

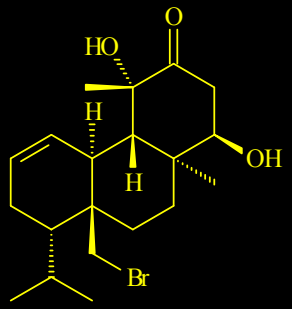
3: R_1= OOH R_2=▬OH

4: R_1= OH R_2= H

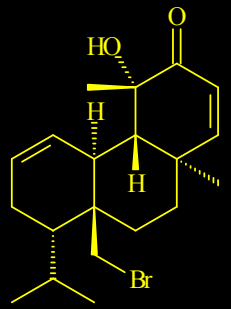
5: R=⋯OH

6: R=▬OH

7: R= H



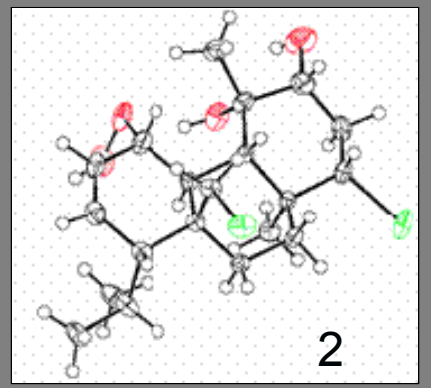
8

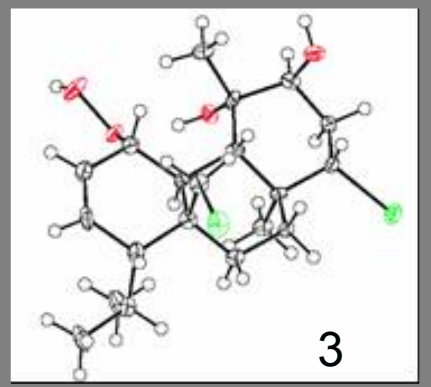


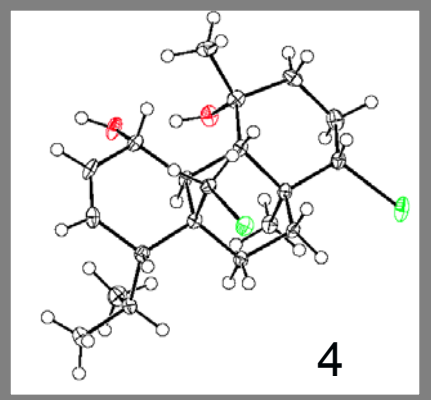
9

Table 3. *In vitro* cytotoxicity of metabolites **1 - 9**.

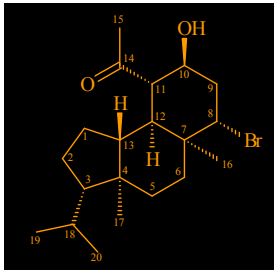
Compound	IC_{50} (μg/mL) Cell line	
	NSCLC-N6-L16	A549
1	inactive	inactive
2	9.5	12
3	6	5
4	inactive	inactive
5	>30	>30
6	inactive	>30
7	inactive	inactive
8	5	4
9	inactive	inactive







Smyrniotopoulos, Quesada, Vagias, Moreau, Roussakis, Roussis. *Tetrahedron*, ***2008***, *64, 5184-5190.*



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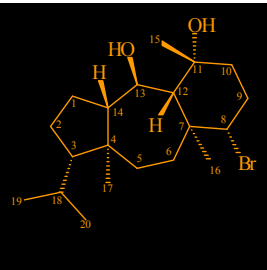

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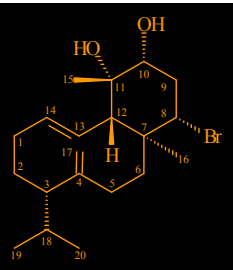

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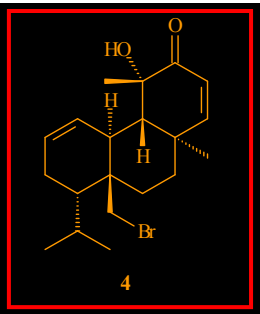

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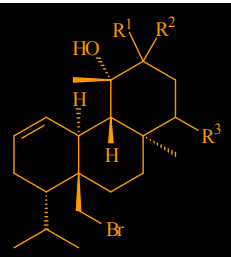
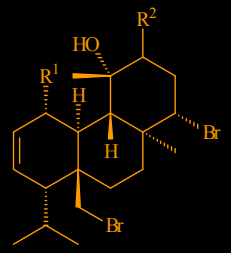


5 $R^1=R^2=H$, $R^3=\alpha$-Br

6 $R^1=\beta$-OH, $R^2=H$, $R^3=\alpha$-Br

7 $R^1=\alpha$-OH, $R^2=H$, $R^3=\alpha$-Br

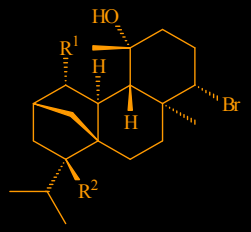
8 $R^1,R^2=O$, $R^3=\beta$-OH



9 $R^1=\alpha$-OH, $R^2=H$

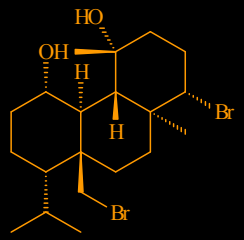
10 $R^1=\alpha$-OOH, $R^2=\beta$-OH

11 $R^1=\alpha$-OOH, $R^2=\alpha$-OH



12 $R^1=H$, $R^2=OH$

14 $R^1=OH$, $R^2=H$



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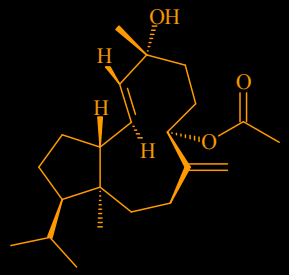

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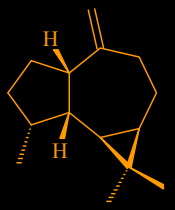


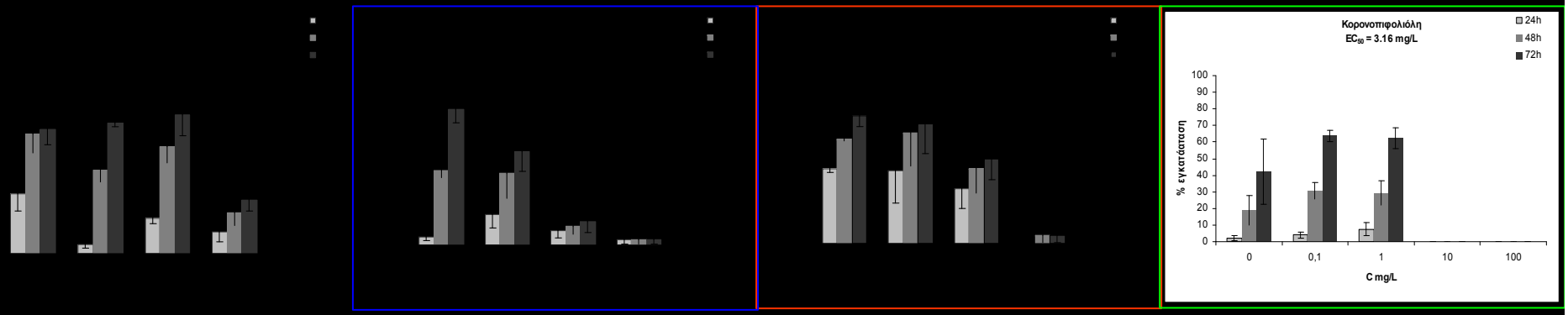
16

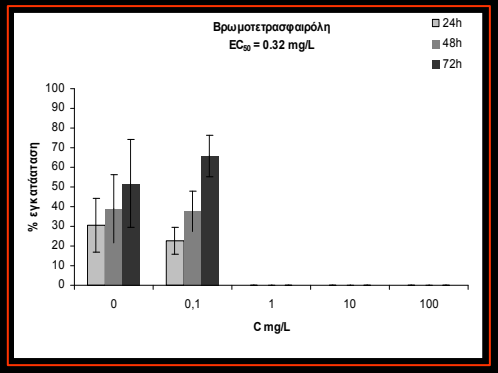
Structure and in vitro antitumor activity evaluation of brominated diterpenes from the red alga *Sphaerococcus coronopifolius*

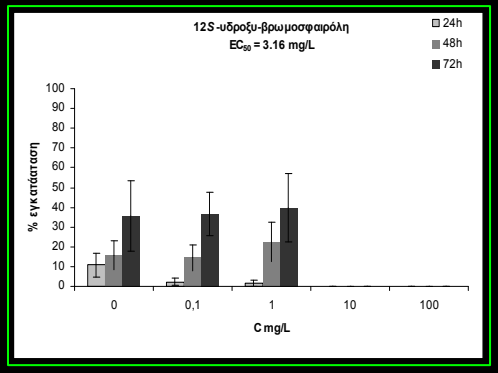
Smyrniotopoulos V., Vagias C., Bruyère C., Lamoral-Theys D., Kiss R., Roussis V. Under Review

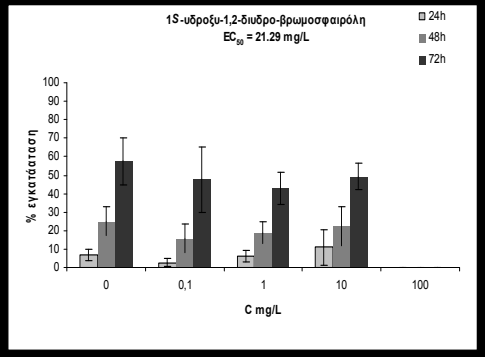
Compounds	IC_{50} *in vitro* growth inhibitory concentrations (μM)					
	U373	A549	OE21	SKMEL-28	PC-3	LoVo
1	32	42	30	38	31	28
2	83	99	58	94	73	65
3	60	64	33	63	48	25
4	3	4	3	5	4	3
5	30	35	28	34	31	24
6	16	20	19	22	12	9
7	25	28	25	29	26	27
8	7	19	8	22	8	5
9	21	24	15	31	28	20
10	22	28	27	28	28	28
11	32	40	25	32	30	21
12	75	63	64	100	43	56
13	25	27	21	27	26	23
14	34	38	33	43	31	27
15	19	43	34	58	34	23
16	72	79	84	100	35	63

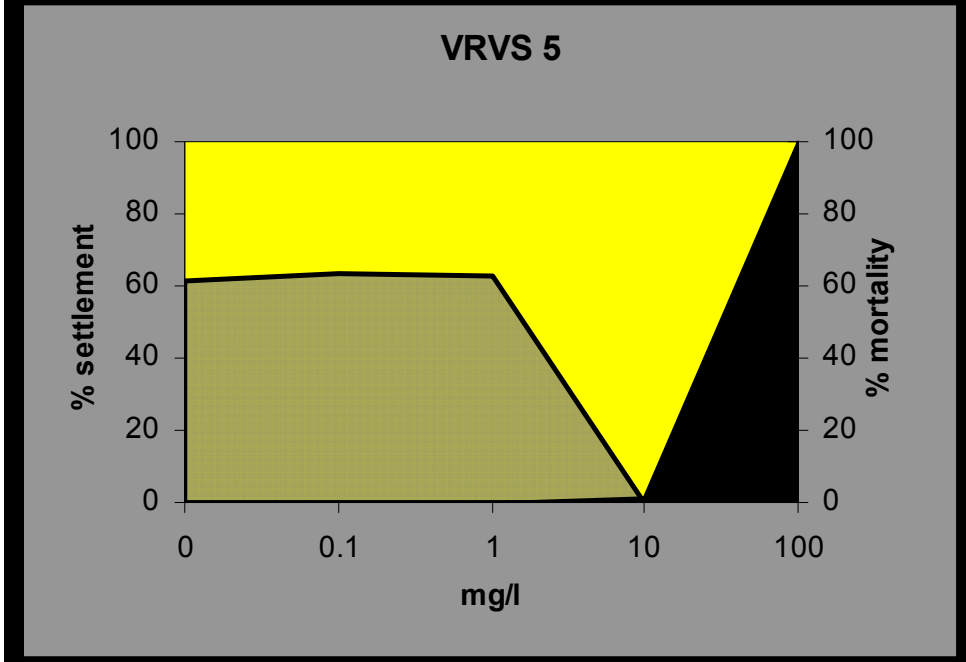
Settlement Inhibition of *Balanus amphitrite*

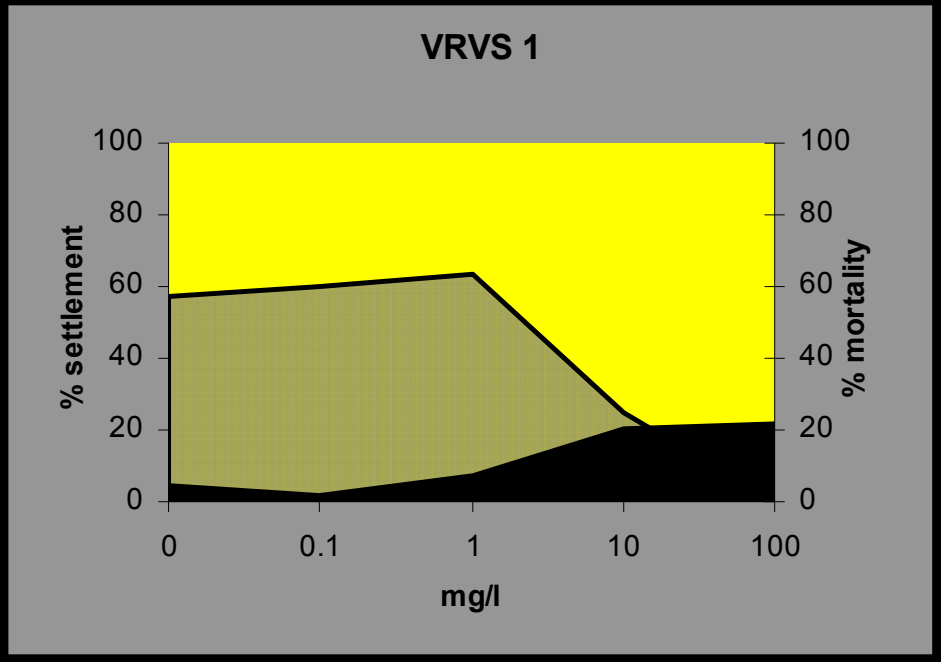


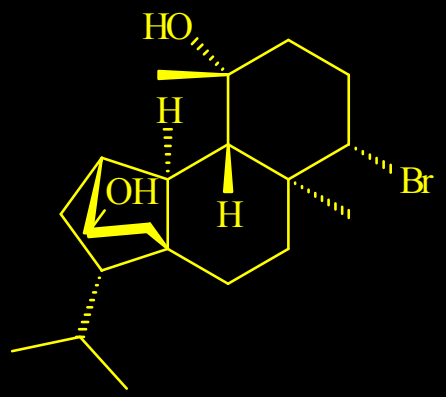


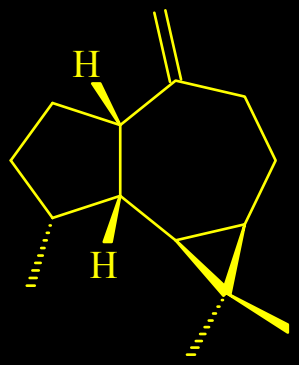










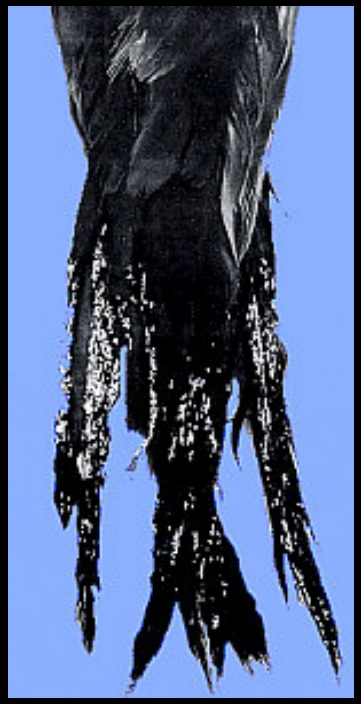


Coronopifoliol

(-)*allo*-aromadendrene


Bulgaria
FYROM
Albania
Turkey
Black Sea
Sea of Marmara
Aegean Sea
Ionian Sea
Sea of Crete
Mediterranean Sea
Orestiás
Sérrai
Drámа
Xánthi
Komotiní
Kilkís
Édhessa
Flórina
Kastoría
Kavála
Alexandroúpolis
Thessaloníki
Políyiros
Thásos
Samothráki
Mt. Athos
Véroia
Kozáni
Grevená
Kalabáka
Ioánnina
Kérkira
Igoumenítsa
Tríkala
Lárisa
Kardhítsa
Vólos
Northern Sporades
Límnos
Lésvos
Mitilíni
Árta
Levkás
Karpenísion
Lamía
Skíros
Évvoia
Khalkís
Agrínion
Mesolóngion
Ámfissa
Levádhia
Khíos
Kefallinía
Argostólion
Pátrai
Mégara
Athens
Zákinthos
Kórinthos
Pírgos
Ándros
Tínos
Sámos
Vathí
Ikaría
Dodecanese
Cyclades
Trípolis
Návplion
Náxos
Kos
Kalámai
Spárti
Yíthion
Thíra
Kíthira
Ródhos
Rhodes
Kárpathos
Khaniá
Kastéllion
Iráklion
Réthimnon
Áyios Nikólaos
Sitía
Crete

Chemical communication of marine insects. The case of *Halobates*



Investigated species

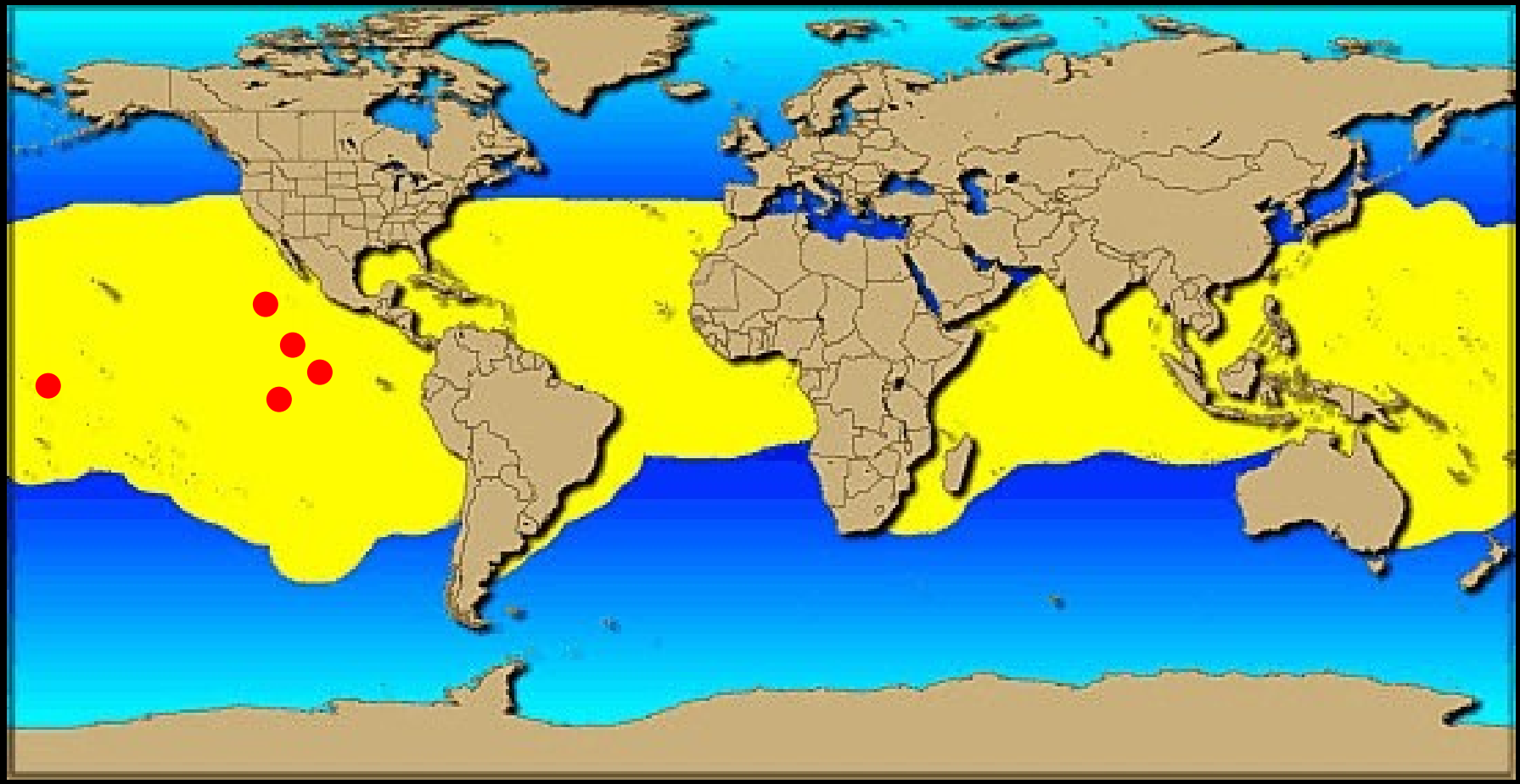
Halobates hawaiiensis
Halobates micans
Halobates sobrinus

Adults (Males & Females) & Nymphs

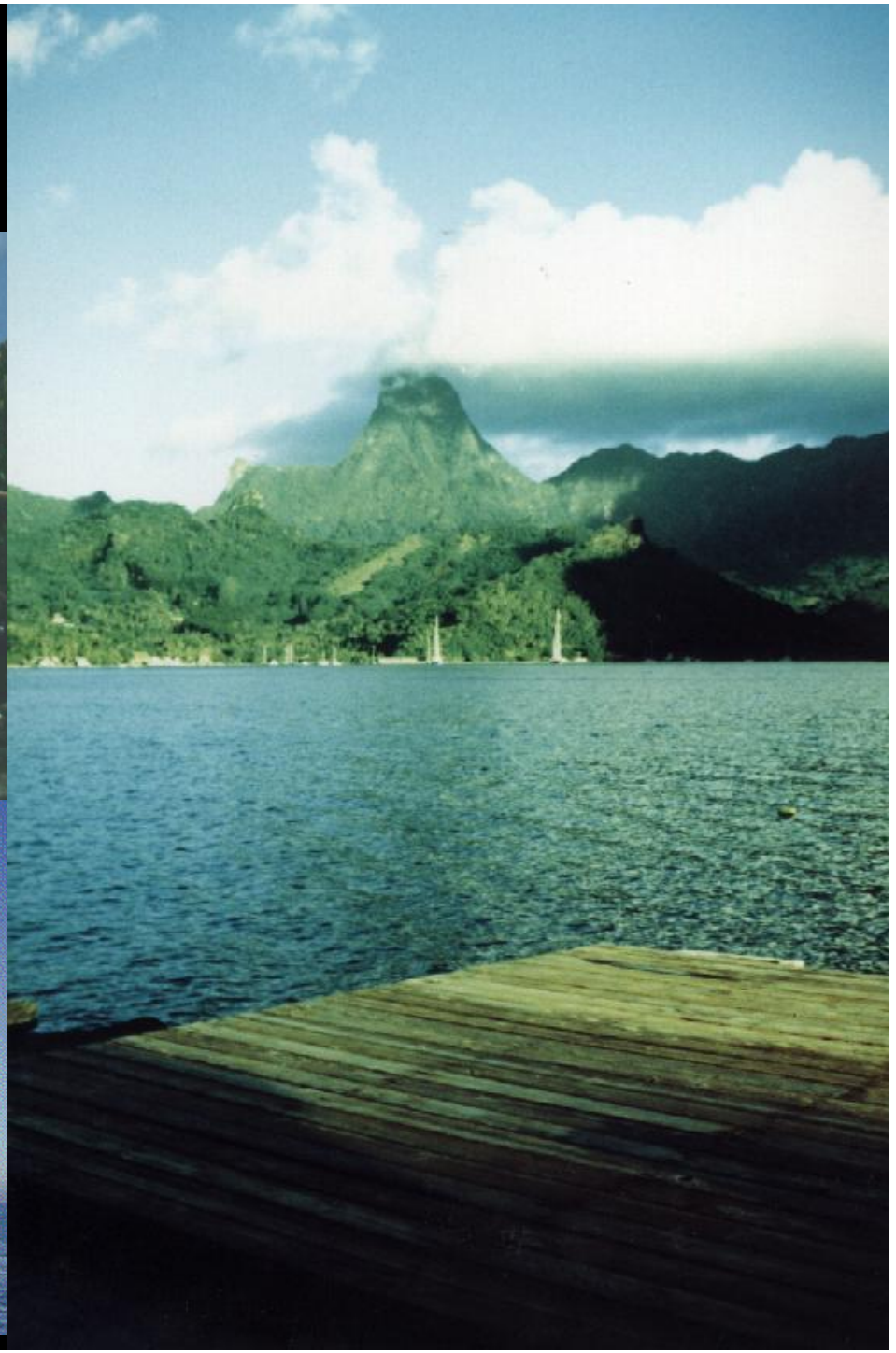
5 Geographic areas

Total extract, Cuticle lipids

Distribution of the genus *Halobates*



Insect collection



Chemotropism assays

Behavioural responses

Sex	Extract	Reaction	Concentration
Female	Male	Attraction	≥ 0.2
Female	Female	Repellence	0.2
Male	Male	Repellence	≥ 2.0
Male	Female	Attraction	≥ 2.0

M. Tsoukatou, L. Cheng, C. Vagias and V. Roussis. Chemical composition and behavioural responses of the marine insect *Halobates hawaiiensis.Z. Naturforsch.* 56c, 597, 2001

Metabolites candidates for pheromonic activity

Pentadecanoic acid methyl ester (*H. sobrinus*)

Octadecenoic acid tetradecyl ester (*H. micans*)

9-Octadecanamide (*H. micans*)

Hexadecanol (*H. hawaiiensis*)

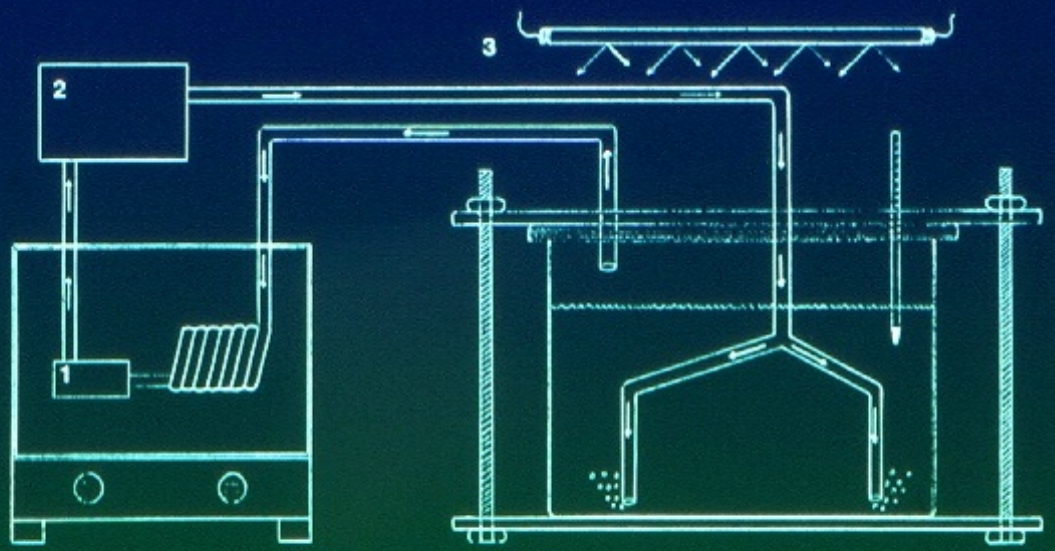
2-Cycloheptenone (*H. hawaiiensis*)

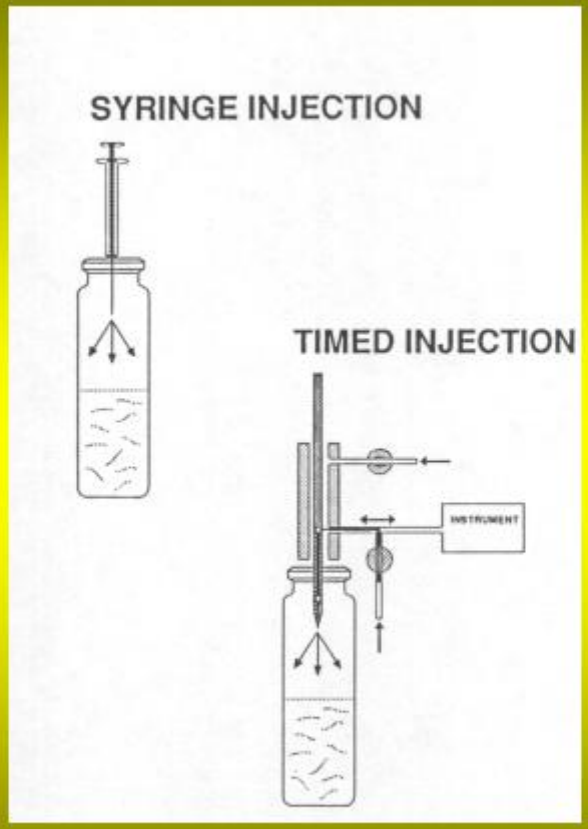
ΠΤΗΤΙΚΟΙ ΜΕΤΑΒΟΛΙΤΕΣ

Ulva rigida

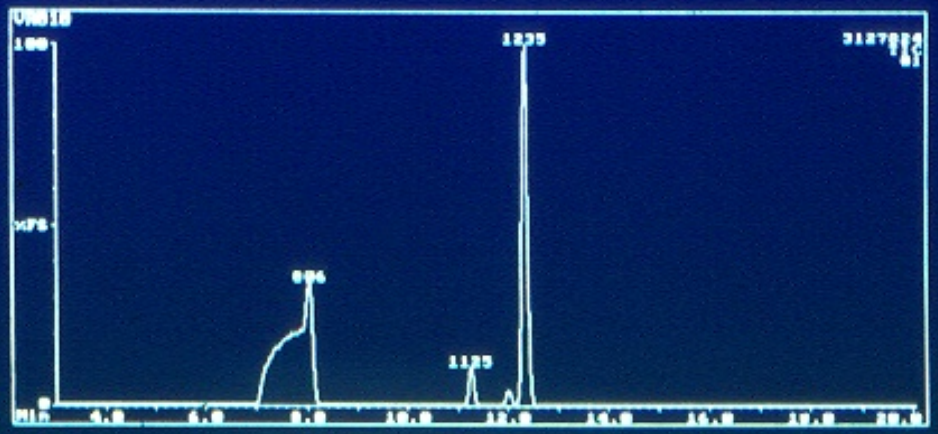
CLOSED LOOP SETUP FOR THE COLLECTION OF THE VOLATILE METABOLITES

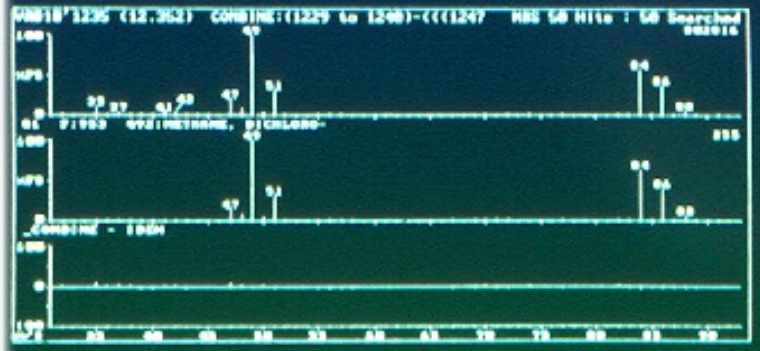
1. TRAP
2. AIR PUMP
3. LIGHT SOURCE

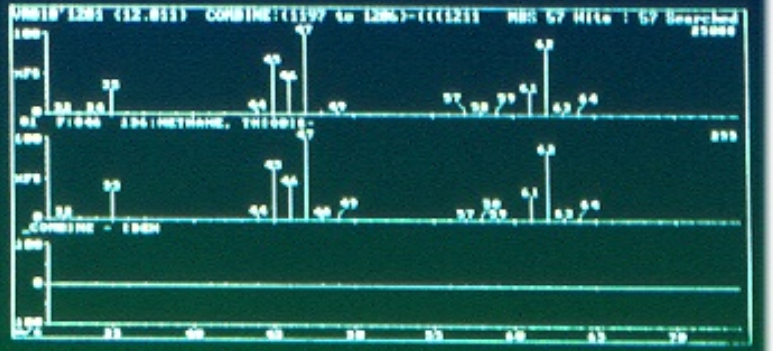



SYRINGE INJECTION
TIMED INJECTION
INSTRUMENT

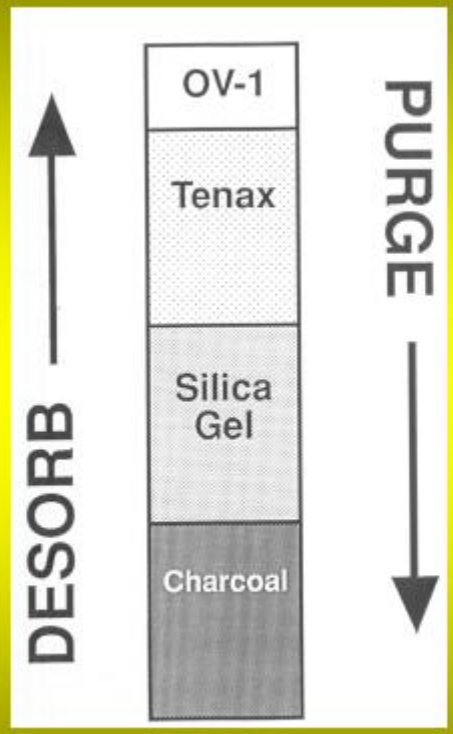
HEAD SPACE METABOLITES OF THE ALGAL ESSENTIAL OIL

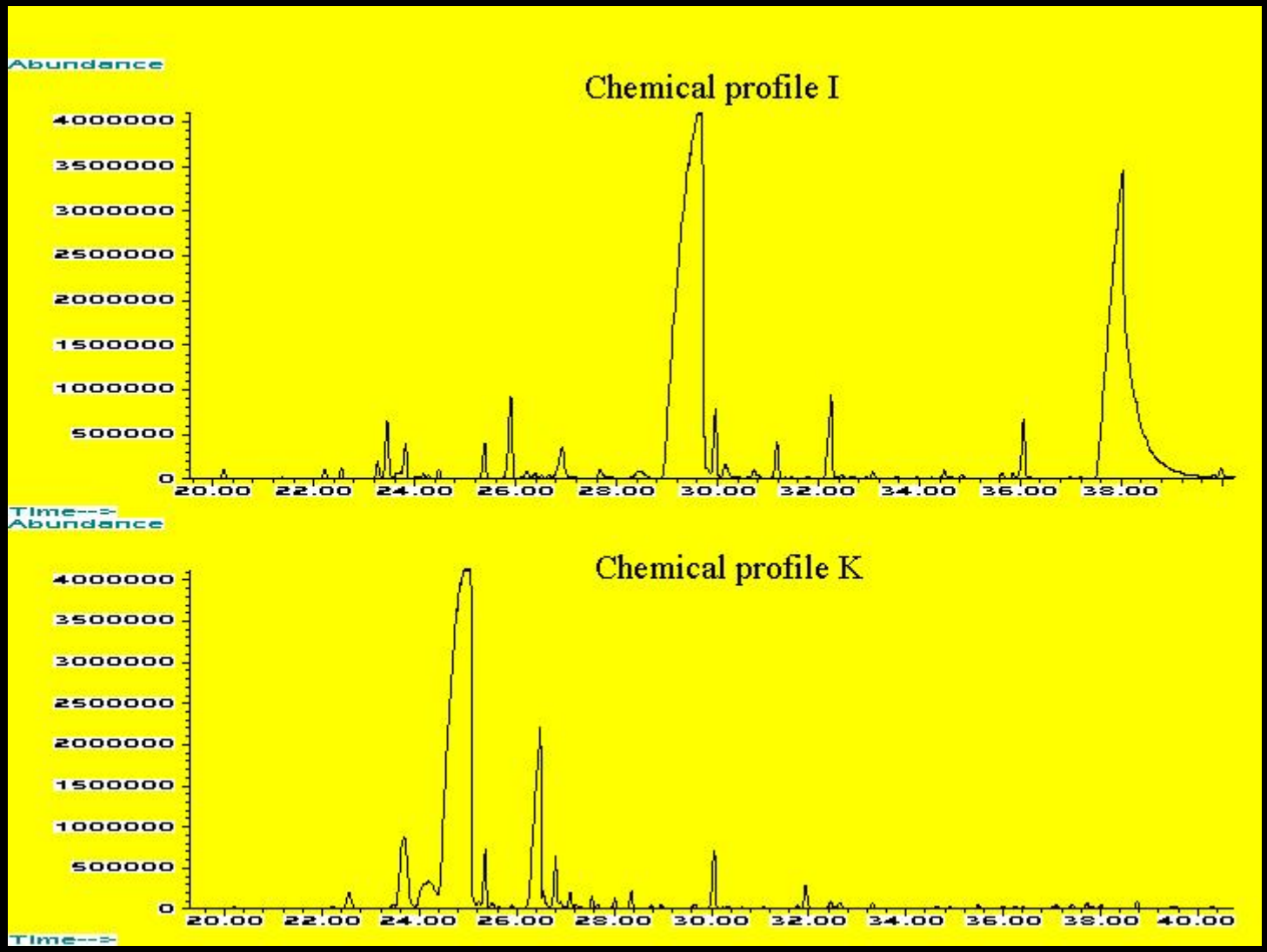






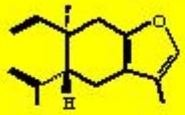
ΧΗΜΕΙΟΤΑΞΙΝΟΜΙΚΕΣ ΜΕΛΕΤΕΣ


OV-1
Tenax
Silica Gel
Charcoal
DESORB
PURGE

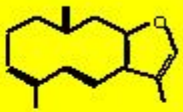

Abundance
Chemical profile I
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00
Time-->
Abundance
Chemical profile K
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00
Time-->

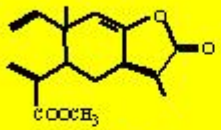
Characteristic components of the recognized chemical profiles assigned to the genus *Gorgonia*.

CHEMICAL PROFILE A


curzerene


furanotriene


isofuranotriene



elemanolide


furanodiene

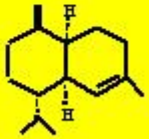
CHEMICAL PROFILE B

Curzerene
Elemanolide
Furanotriene
Isofuranotriene
Furanodiene
Ledol

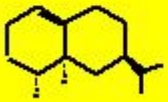
ledol

CHEMICAL PROFILE C

γ-Muurolene
Valencene
Furanotriene
Isofuranotriene
Furanodiene
Elemanolide
Curzerene

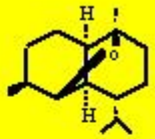


γ-muurolene


valencene


Bicyclogermacrene

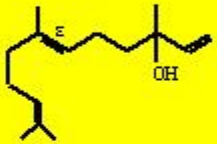
CHEMICAL PROFILE D



5,10-epoxymuurolene

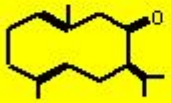
CHEMICAL PROFILE E

Furanotriene
Isofuranotriene
E-Nerolidol



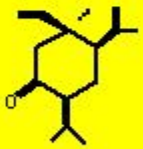
E-nerolidol

CHEMICAL PROFILE F

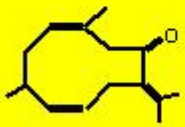


germacrone

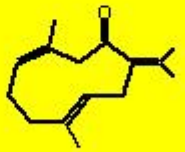
trans-β-elemenone



cis-β-elemenone



cis, cis-germacrone



cis-C3-C4 *trans*-C7-C8-germacrone

CHEMICAL PROFILE G

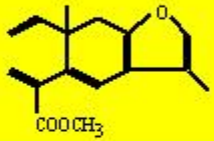
α-santalene

12,13-epoxy-α-santalene

Dihydro-α-santalen-12-one

CHEMICAL PROFILE H

Isosericenine



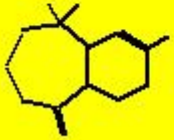
CHEMICAL PROFILE I

5,10-Epoxymuurolane
Isosericenine
Elemanolide

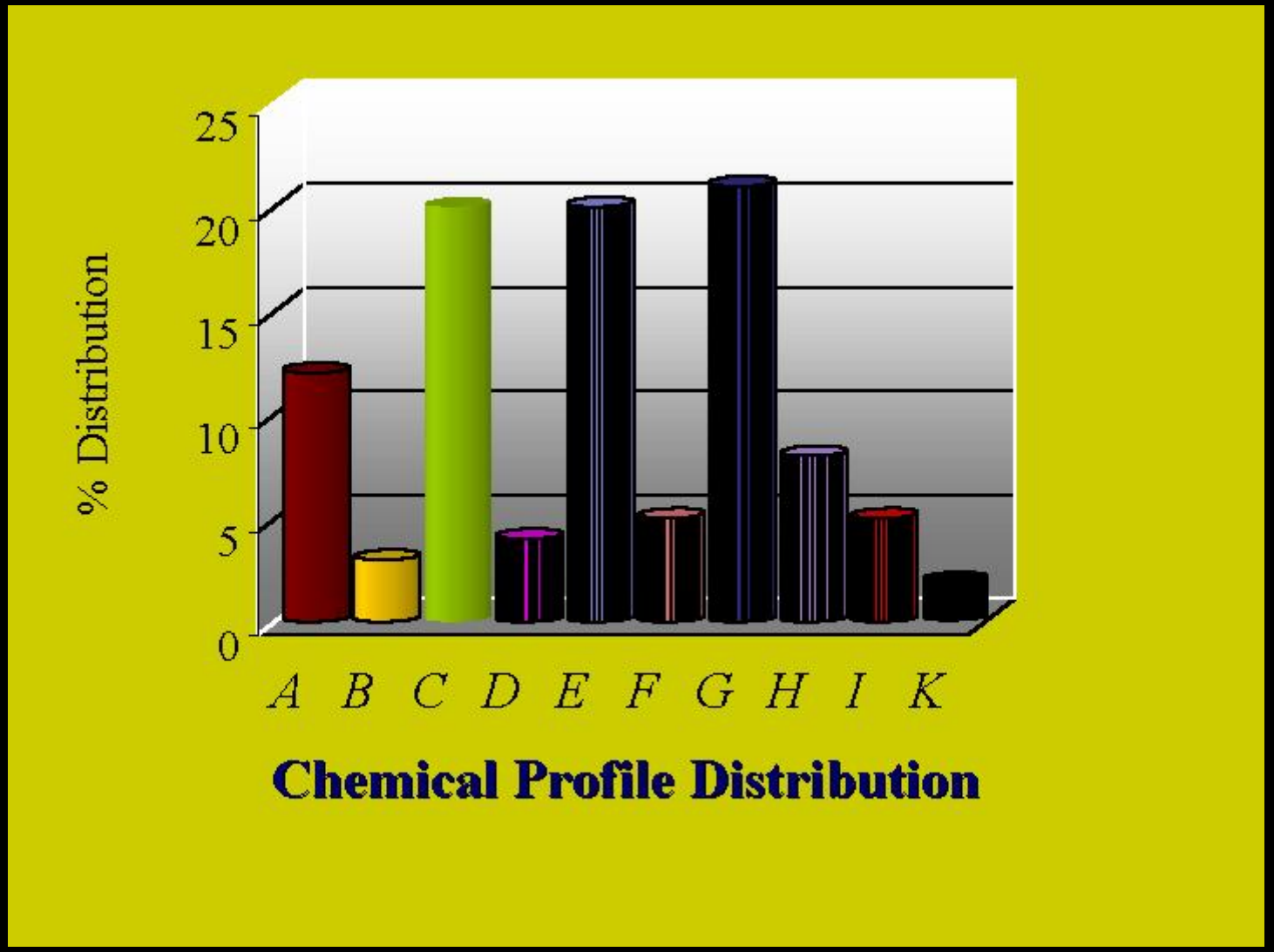
CHEMICAL PROFILE K

α-Gurjunene
γ-Muurolene
α-Himachalene

α-gurjunene



α-himachalene


% Distribution
25
20
15
10
5
0
A B C D E F G H I K
Chemical Profile Distribution

BIOACTIVE METABOLITES WITH INDUSTRIAL INTEREST FROM MARINE BACTERIA

Environmental Remediation

Oil Spills

Sewage treatment

Toxic metals

Polychlorinated Phenols

Petroleum - emulifying agent from marine bacteria

ALASKA 1989

Exon Valdez ran aground off the coast of Alaska, spilling more than 11 million gallons of oil into Prince William Sound.

Marine Bacteria to Combat Metal Pollution

- Spores obviate the need for viable bacteria to be introduced into the environment;
- Spores are naturally resistant to harsh environmental conditions;
- The spores studied bind, accumulate, or precipitate a variety of metals;
- Metal precipitation occurs over a wide range of environmentally relevant conditions (pH, temperature, metal concentration, and in both fresh and saline waters);
- Spores have a high capacity for binding ions at their surface, as well as high metal affinity and specificity;
- Spores can be re-used after their metal coats have been stripped.

Chitosan

a product extracted from shellfish waste, is being used to treat wheat seeds to reduce fungal infestation, with resultant increases in germination and ultimate yield (up to 10%).

•chitosan can trigger genes in plants that fight disease

• has a history of about three decades of use in detoxifying water.

•spread over the surface of water, it absorbs greases, oils, heavy metals and other potentially toxic substances.

•SELECTIVELY ABSORB FATS EVEN IN A WATER MEDIUM.

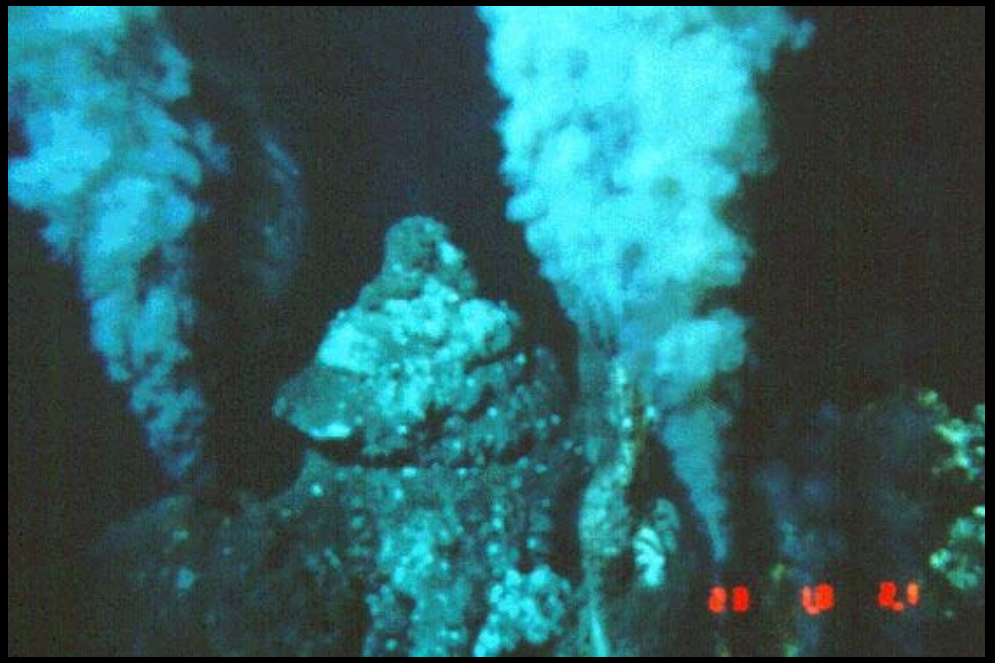
LSU Ref.: 9625 [Dr. Vadake Srinivasan]
Status: US Patent 5,739,015

Description	Applications	Advantages
• Bacteria transforms chitin to the more soluble, useful product chitosan	• Biomedical: wound healing, burn treatment • Agricultural • Pharmaceutical • Cosmetics	• Economical and environmentally-friendly process: does not require strong alkali, nor high temperatures; does not generate a pollutant stream of alkaline liquor • Bacteria grow faster than fungi, and do not need to be purified, as do enzymes

Cyanobacteria produce cellulose

HYDROTHERMOPHILIC MICRO-ORGANISMS

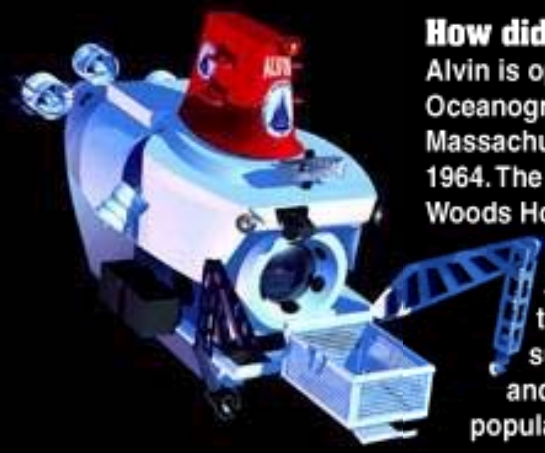
- Xylanase-type enzymes operate at harsh conditions for the transformation of pulp for paper.
- Better digestion of animal feeds



About Hydrothermal Vent Research

Along mid-ocean ridges, sea water can percolate through cracks into the sea floor rock. As it reaches progressively deeper depths, the fluid is heated from contact with hot (but solidified) rocks. At the water flows deeper and the temperature increases the fluids begin to react with the rocks resulting in a fluid chemistry much different than the starting sea water. Eventually, the fluids become so hot, their density forces them to rise up through the oceanic crust, where they are released at the sea floor as deep sea hot springs or **"black smokers."** These black smokers can reach temperatures **>400 degrees** Celsius. Fantastic animal communities including giant tube worms and mussels have been found living near these black smokers. These animals do not live in the ~350 degree water, but live in very nearby cooler areas where the hydrothermal waters have mixed with sea water to form cooler ~5-60C "diffuse flow fluids." Chemicals in these "diffuse flow fluids" provide the energy that the animals need to live on the bottom of the ocean.

The goal of a cruise, for the water chemists, is to collect black smoker and "diffuse flow fluids" in order to analyze their chemistries. Then try to understand the chemical relationship between the high temperature vents and the diffuse flow. Is diffuse flow simply a mix of black smoker fluids with sea water? Or are there other chemical reactions occurring that result in the chemistry of "diffuse flow?" Understanding the of hydrothermal fluids at mid-ocean ridges is important to understanding how animals can live in these regions, completely isolated from the light of the sun.



How did *Alvin* get its name?

Alvin is operated by the Woods Hole Oceanographic Institution in Woods Hole, Massachusetts. The sub was christened in 1964. The Deep Submergence Group at Woods Hole named *Alvin*. It was both a contraction of their colleague Allyn Vine's name (he worked tirelessly to make the submersible a reality) and a reference to the popular cartoon chipmunk.

How big is *Alvin*?

The sub (its official title is "deep submergence vehicle") is about 23 feet long and 12 feet high. The 6-foot-diameter sphere in which the scientists work is made of titanium.

What equipment does *Alvin* have?

Its tools include video cameras, an underwater telephone, a computer/data display/recording system, sonar, sediment corers, temperature probes, a magnetometer, and more. *Alvin* has long, clawed arms called manipulators that are used to collect scientific specimens and place them in the sub's plexiglass collecting basket. The manipulators can lift up to 250 pounds and reach up to 75 inches.

Alvin *Statistics*

Length: 23 feet, 4 inches
Height: 11 feet, 10 inches
Draft: 7 feet, 6 inches
Gross Weight: 37,400 pounds
Operating Depth: 13,124 feet
Payload: 1,800 pounds
Dive Duration: 6 – 10 hours
Life Support: 216 man-hours
Complement: 3 people
Propulsion: Five hydraulic thrusters

Tubeworm

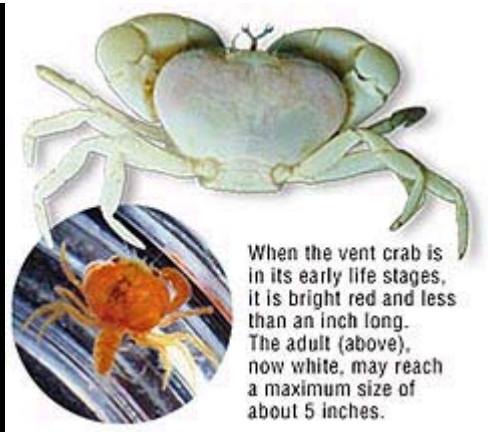
Resembling giant lipsticks, tubeworms *(Riftia pachyptila)* live over a mile deep on the Pacific Ocean floor near hydrothermal vents. They may grow to about 3 meters (8 ft) long. The worms' white tube home is made of a tough, natural material called *chitin* (pronounced "kite-in").

Tubeworms have no mouth, eyes, or stomach ("gut"). Their survival depends on a symbiotic relationship with the billions of bacteria that live inside of them. These bacteria convert the chemicals that shoot out of the hydrothermal vents into food for the worm. This chemical- based food-making process is referred to as *chemosynthesis.*

Since a tubeworm has no mouth, how do bacteria enter the worm? Scientists have found that, during its earliest stages, the tubeworm does have a mouth and gut for bacteria to enter. But as the worm grows, these features disappear!

While the tubeworm depends on the bacteria that live in its body for energy and food, sometimes tubeworms provide food for other deep-sea dwellers. Fish and crabs may nibble off the tubeworm's red plume.

Vent Crab



When the vent crab is in its early life stages, it is bright red and less than an inch long. The adult (above), now white, may reach a maximum size of about 5 inches.

The hydrothermal vent crab, *Bythograea thermydron,* is a top predator at vent sites in the Pacific Ocean.

This crab is present in such high densities that scientists actually use it as an indicator that they are approaching an active vent field.

The vent crab is typically found among dense clusters of tubeworms at an average depth of 1.7 miles and can tolerate a temperature gradient that ranges from 77°F in the tubeworm clumps, to 36°F, which is the temperature of the water surrounding the vent sites.

Because vent fields may be separated by hundreds of miles, scientists have many questions about how they are colonized by the crabs. At the University of Delaware College of Marine Studies, scientists are examining the crab's life stages and reproductive biology to look for clues.

Going Crabbing in the Deep Sea!

To collect a small number of adult crabs for laboratory study, scientists deploy modified minnow traps on the seafloor with the help of the deep-sea sub *Alvin.* Younger crabs are captured indirectly by collecting clumps of tubeworms at the vent site.

How Do You Keep a Vent Crab Happy in Delaware?

Because the vent crab is found at an average depth of about 2.7 kilometers (1.7 mi), it experiences about 250 times the atmospheric pressure that we do. While scientists have found that young vent crabs can live for months in the lab at atmospheric pressure, the adult crabs must be kept under high-pressure conditions similar to those back home or they will die.

The pressure chambers shown above were designed by University of Delaware scientists to keep the vent crabs alive. The aquaria house different life stages of the crabs. A hydrostatic pump keeps the system at a pressure of 1,500 pounds per square inch, or about 150 atmospheres — not quite as great as what the crabs experience at the vents, but close enough to keep them happy.

The crabs are fed weekly when the tanks are cleaned. Their diet is mussels, which are collected at the vents and frozen for later use.

Pompeii Worm

Deep-Sea Vents Harbor
Earth's Hottest Animal

University of Delaware marine scientist Craig Cary recently discovered that an inhabitant of the deep sea is the most heat-tolerant animal on Earth. The Pompeii worm *(Alvinella pompejana)* can survive an environment as hot as 80° C (176° F) — nearly hot enough to boil water. How the worm survives this heat remains a mystery.

Formerly, the Sahara desert ant was believed to be the most heat-hardy creature, foraging briefly in the desert sun at temperatures up to 55° C (131° F).

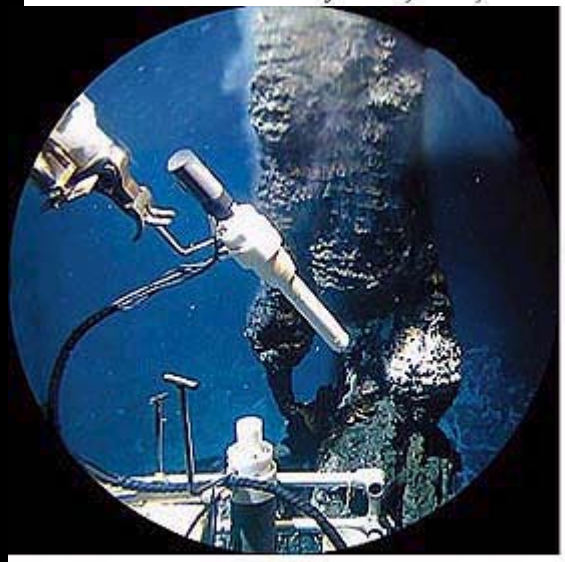
Cary's research was conducted onboard the deep-sea submersible *Alvin* at hydrothermal vent sites in the Pacific Ocean west of Costa Rica. Using a long temperature probe called "the Mosquito," he found that the worm's rear end sits in water as hot as 80° C (176° F), while its head, which sticks out of the worm's tube home, rests in water that is much cooler, about 22° C (72° F).

Covering the Pompeii worm's back is a fleece of bacteria that can also "take the heat." **These bacteria are of particular interest to industry because they may harbor enzymes that are useful in such high-temperature applications as processing food and drugs, making paper, and dislodging oil inside wells.** By learning more about the unique biology of the Pompeii worm and other "extremophiles" — organisms that thrive in extreme temperature and pressure conditions — scientists may open the door to beneficial new products and processes.

At the lower right-hand quadrant of this photo, you can see a Pompeii worm extending its dark-red feathery head and paler body from its tube home. The worm is about 13 centimeters (5 in) long.

Ancient Bacteria

Magnified view of microbes on the surface of pyrite. The bacteria are about 4 microns long. Photo by Kirk Czymmek.



This wand extended from the deep-sea sub *Alvin* houses a thermometer, electrodes for taking chemical measurements, and an apparatus called the Sipper for collecting small water samples at hydrothermal vents.

Recent evidence suggests that life originated in extreme environments, for example, at high temperatures. The National Science Foundation (NSF) has initiated a program called Life in the Extreme Environment (LExEn) that is dedicated to finding new and exciting organisms that live in harsh environments.

The Extreme 2000 research expedition, at hydrothermal vent sites in the Sea of Cortés, is led by marine scientists from the University of Delaware and Portland State University and their chief objective is to make real-time chemical measurements at the vents using microsensors, which will guide the microbiologists and molecular biologists in finding organisms that are descendants of early life forms.

Chemical Detective Work at the Bottom of the Sea

Are hydrothermal vents home to the closest relatives of the oldest life on Earth? Using special tools housed in a wand on the sub *Alvin,* researchers will be testing the chemistry of vent water in search of microscopic organisms. The wand houses a thermometer, an apparatus called "the Sipper" to collect small water samples, and a super-sensitive chemical analyzer.

The analyzer is like a sophisticated underwater "snooper." It can be used near the vents and, from its chemical readings, tell scientists what kind of microbes might live there. While our food chain is based on energy from the sun, the sun's rays never reach the deep sea. There, organisms must rely on a different energy source: the chemicals that rocket out of the vents.

During a previous expedition, the Extreme 2000 scientific team found that the presence of two compounds — hydrogen sulfide (H_2S) and iron monosulfide (FeS) — may be an important indicator of the oldest microscopic vent life. These compounds react to form the mineral pyrite ("fool's gold") and hydrogen gas.

The hydrogen provides the energy that these microbes need to grow.

With the analyzer's help, marine scientists may be able to track down the nearest descendants of the first life on Earth, and perhaps on other planets.

Europa, one of the moons of Jupiter, is covered in ice. However, recent findings suggest that portions of the ice move, which is strong evidence that liquid water lies beneath the ice. The water may be maintained in its liquid state by hydrothermal vents. If hydrothermal vents exist on Europa, there's a possibility that ancient microbes could live there, too.

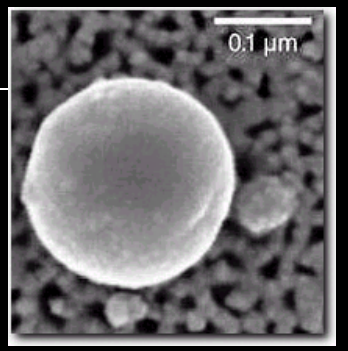
Thermophilic Bacteria

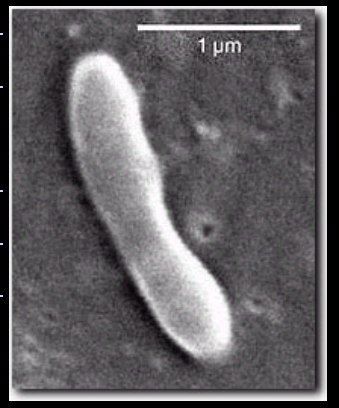
- *Thermus thermophilus*
- From hydrothermal vents 2 Km deep in the Pacific Ocean

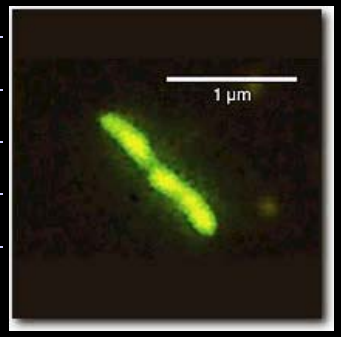
New Cosmetic Products
from
Sederma

New Antarctic taxa

Taxa	Isolated from
Antarctobacter heliothermus	Hypersaline lake
Flavobacterium hibernum	Freshwater lake
Glaciecola punicea	Sea ice
Glaciecola pallidula	Sea ice
Colwellia demingiae	Sea ice
Colwellia rossensis	Sea ice
Colwellia hornerae	Sea ice
Colwellia psychrotropica	Marine salinity lake
Octadecobacter antarcticus	Sea ice, seawater
Gelidibacter algens	Sea ice
Psychroserpens burtonensis	Marine salinity lake
Psychrobacter glacincola	Sea ice, seawater,
Methylosphaera hansonii	Marine salinity lake
Pseudoalteromonas antarctica	Seawater
Psychroflexus torquis	Sea ice
Hymenobacter roseosalivarius	Sandstone liths, soil
Polaribacter franzmanii	Sea ice, seawater
Polaribacter irgensii	Sea ice, seawater
Poleribacter glomeratus	Marine salinity lake
Polaribacter filamentus	Sea ice, seawater
Planococcus mcmeekinii	Sea ice
Pseudoalteromonas prydzensis	Sea ice







All photos are courtesy of David M. Karl, et al

Psychrophilic enzymes

typically have maximal catalytic activity at temperatures below 40° C

Applications

- in cleaning agents,
- leather processing,
- degradation of xenobiotic compounds in cold climes,
- food processing (fermentation, etc)
- molecular biology (heterologous gene expression).

Structural characteristics that confer increased flexibility and cold-adaptation in proteins.

Amino acid substitutions in critical areas near the active site

Increased incidence of glycine residues near functional domains

Fewer salt bridges

Less packed hydrophobic cores

Reduction of proline residues in loop structure

Less interaction between aromatic sidechains

Less hydrogen bonding

Lower levels of arginine

Less charge–dipole interactions in helices

Higher surface hydrophilicity (from additional charged sidechains)

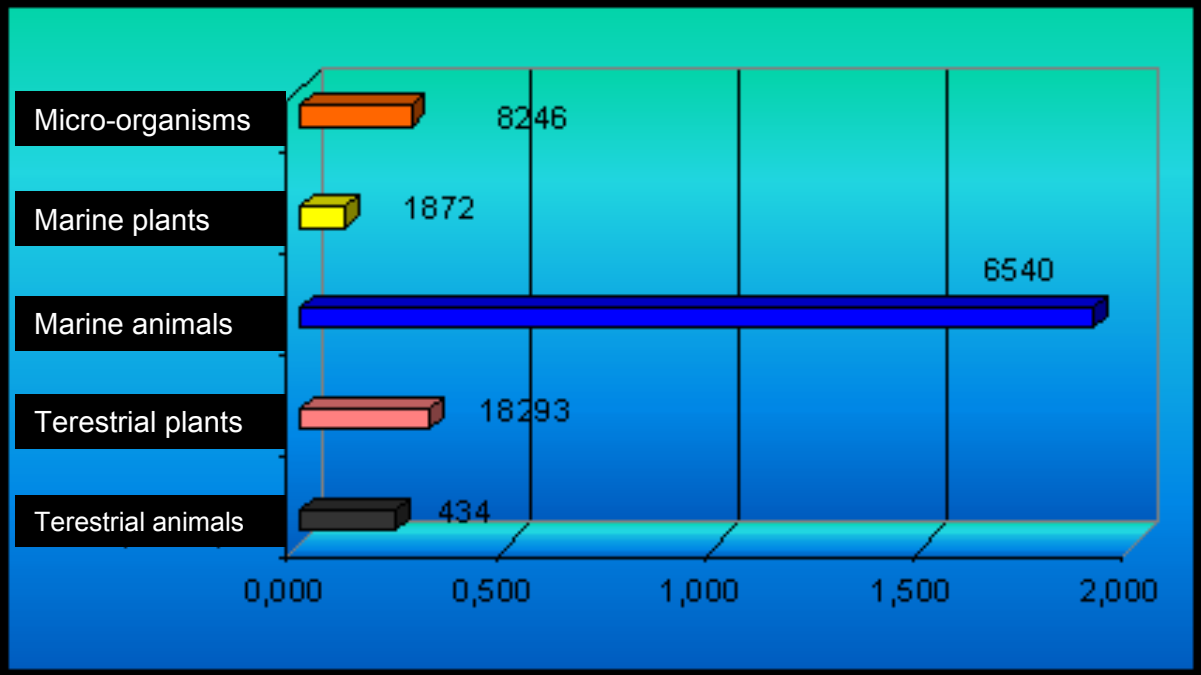
Looser coordination of Ca^{2+} ions

Protein folding

Optimal temperature and relative activity of psychrophilic enzymes from a variety of Antarctic sea-ice bacteria.

Bacterial strains	Enzyme	T_{OPT} k_{cat}(° C)	% Activity	
			10° C	40° C
Colwellia demingae ACAM 607	Protease (azocasein)	28	75	25
	Protease (azoalbumin)	30	39	30
	Trypsin	14	90	29
	Phosphatase	23	90	85
Colwellia-like strain IC169	Trypsin	12	100	53
	Phosphatase	17	85	85
	-galactosidase	26	75	70
Cytophaga-like strain IC166	Protease (azocasein)	20	68	65
	Protease (azoalbumin)	27	70	55
	Trypsin	30	72	60
	-galactosidase	15	100	46
	-amylase	25	65	60

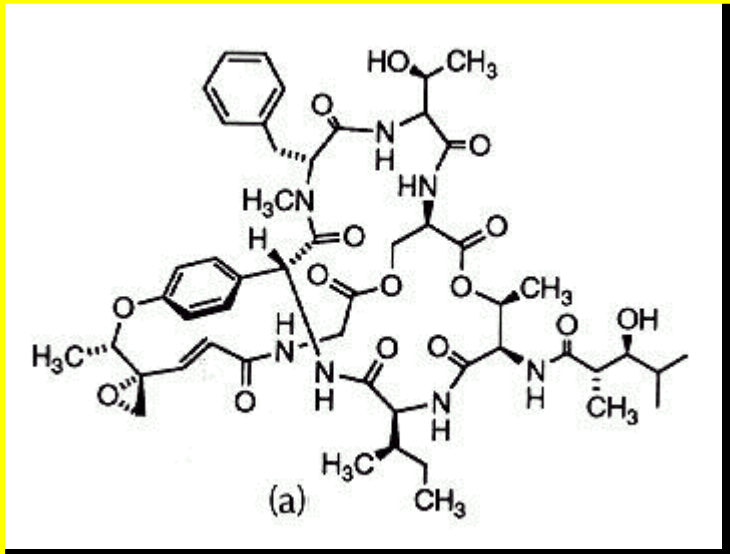
New pharmaceuticals from Marine Bacteria

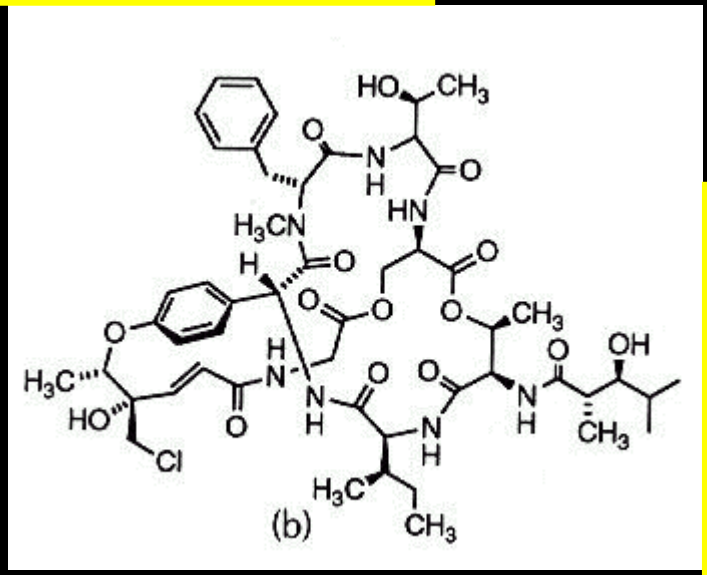


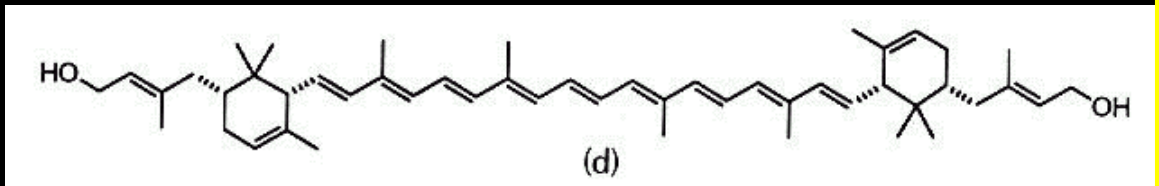
% cytotoxicity (NCI)

SPECIES	SOURCE	COMPOUND	ACTIVITY
Cephalosporium sp	Sea water near sewage outlet	Cephalosporins	Antibiotic
Aspergillus sp.	Italy floor mud	Gliotoxin	Antibiotic
Halocyphina villosa		Siccayne	Antibiotic
Corollospora pulchella	Sand grain	Melinacidins III and IV, Gancidin W	Antibiotic
Leptosphaeria oraemaris	Submerged wood	Culmorin	Atifungal
Leptosphaeria oraemaris		Leptospaerin	
Zopfiella marina	Ocean foor mud	Zophinol	
Asteromyces cruciatus	Decaying wood / algae	Gliovictin	
Leptosphaeria obiones	Salt marsh grass	Obionin A	Dopamine receptor antagonist
Dendryphiella salina		Dendryphiellins A-G	
Helicascus kanaloanus	Mangrove	Helicascolides A & B and Ochracin	
Preussia aurantiaca	Mangrove	Auranticins A & B	Antibacterial
Leptospaeria oraemaris	Plant	Leptospaerolide	
Stachybotrys sp	Brakish water	Stachybotrins A & B	Antimicrobial
Leptospaeria sp	Algae (Sargassum tortile)	Leptosins A -G	Cytotoxic / antitumour
Penicillium sp.	Algae (Enteromorpha intestinalis)	Communensins A & B	Cytotoxic
Phoma sp.	Shell or crab (Chionoecetes opilio)	Phomactins A, B, B1, B2, C& D	PAF antagonist
Aspergillus fumigatus	Gastrointestinal tract of fish (Pseudolabrus japonicus)	Fumiguanazolines A -C	Cytotoxic
Penicillium fellutanum	Gastrointestinal tract of fish (Apogon endekataenia)	Fellutamides A & B	Cytotoxic
Unidentified Leptospaeria sp.	Sponge	Nectriapyrones A & B	
Unidentified Leptospaeria sp.	Sponge (Jaspis sp.)	Chloriolins A-C	
Trichoderma harzianum	Sponge (Micale cecilia)	Trichoharzin	
Kallichrorma tethys		Culmorin type sesquiterpenes	Cytotoxic
Penicillium sp.		Epolactaene	Neurotrophic

Bacterial metabolites produced by isolates obtained from nutrient-rich invertebrates and vertebrates



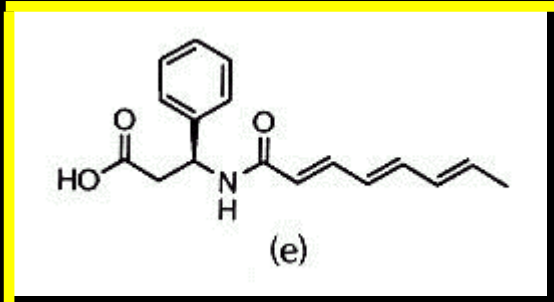




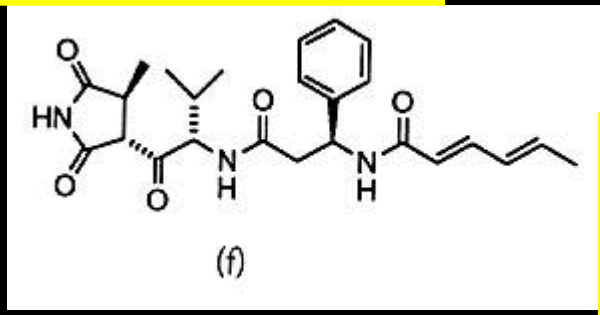
(a) Salinamide A and (b) salinamide B are bicyclic depsipeptides mechanism of action related to vancomycin.

(c) Vibrindole A, is a *bis*-indole derivative that exhibits antimicrobial activity.

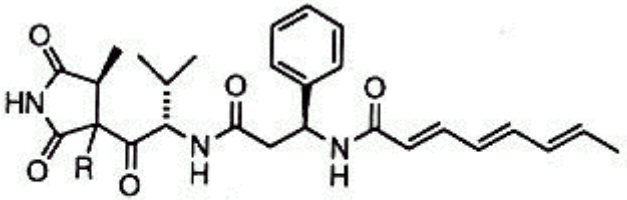
(d) Okadaxanthin is a new C_{50} carotenoid containing the structural unit of -carotene that is an excellent singlet oxygen quencher.



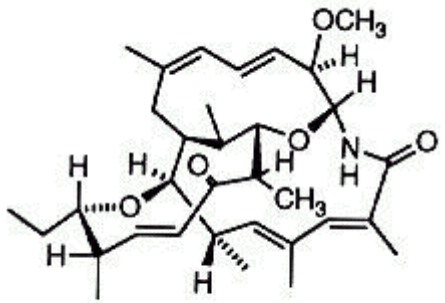
(e)



(f)



(g) R = α-OH
(h) R = β-H



(i)

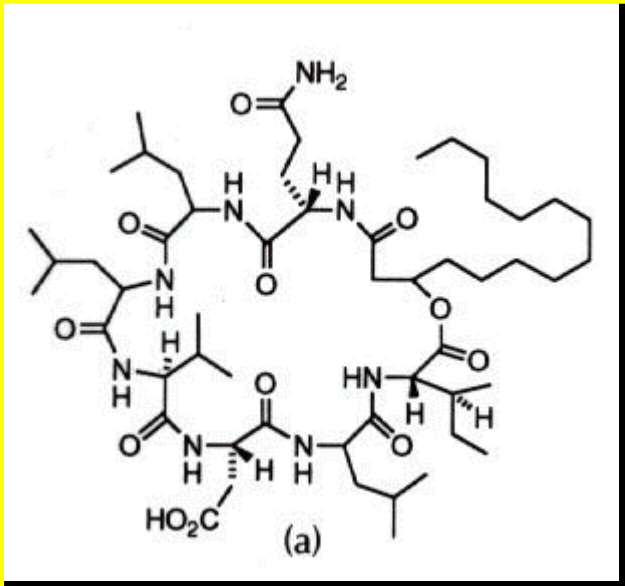
e) Moiramide A, (f)moiramide B, (g) moiramide C and (h) andramid
Isolated from a bacterium obtained from the surface of a marine tunicate.

Moiramide B exhibits an antimicrobial activity.

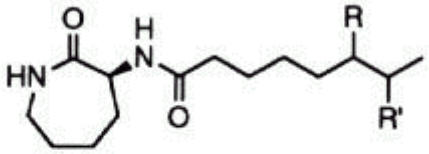
i) Halichomycin is a cytotoxic tricyclic macrolide

Isolated from a bacterium cultured from the intestinal tract of a marine fish.

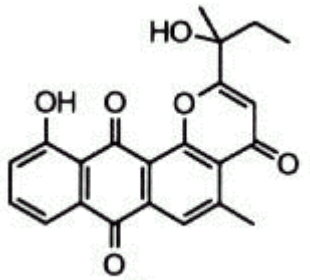
Compounds produced by bacterial isolates obtained from marine sediments and nutrient-poor seawater.



(a)



(b) R = H, R' = CH_3
(c) R = CH_3, R' = H



(d)

(a) Halobacillin is a cytotoxic acylpeptide related to surfactin, one of the most efficient surfactants known.

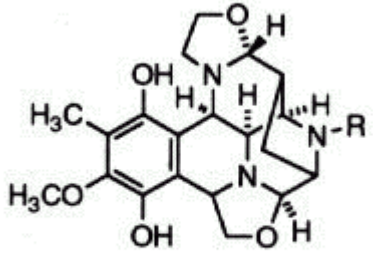
lacks the antimicrobial activity exhibited by surfactin.

(b) Caprolactin and (c) caprolactin B exhibit mild cytotoxicity and antiviral activity.

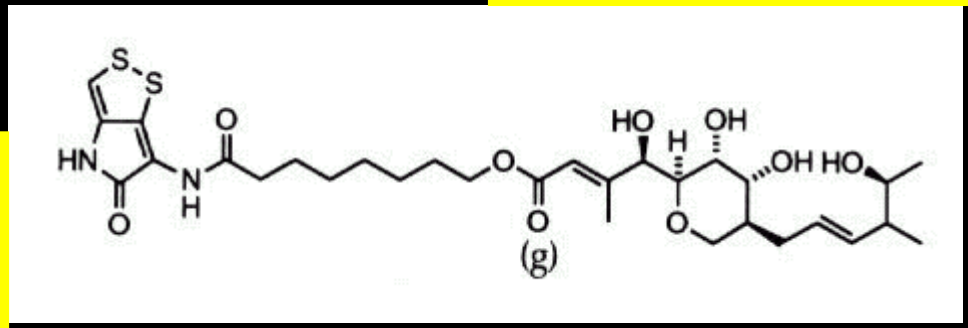
(d) -indomycinone is cytotoxic as aresult of DNA adduct formation.

(e) Bioxalomycin 1 and

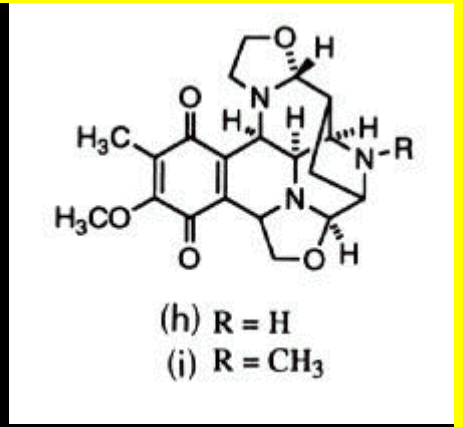
(f) bioxalomycin 2



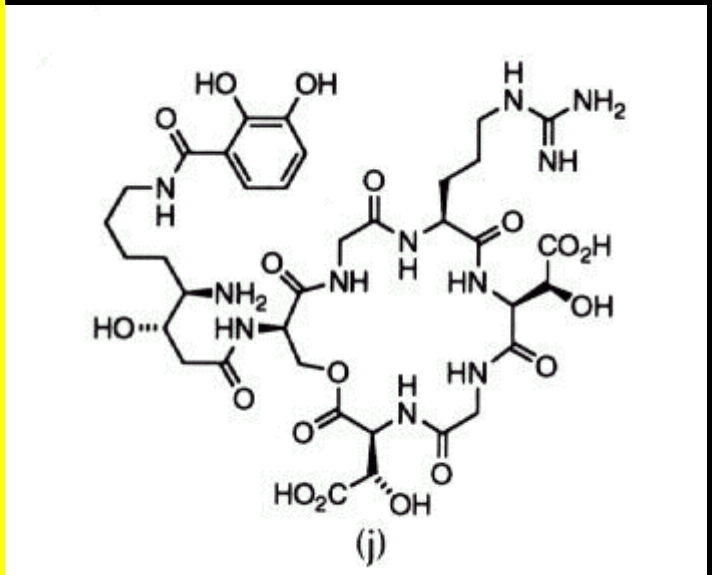
(e) R = H
(f) R = CH_3



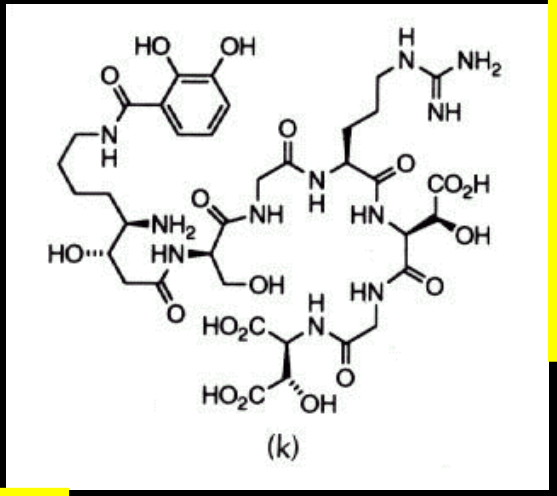
(g)



(h) R = H
(i) R = CH_3



(j)



(k)

(g) Thiomarinol exibits antibiotic activity.

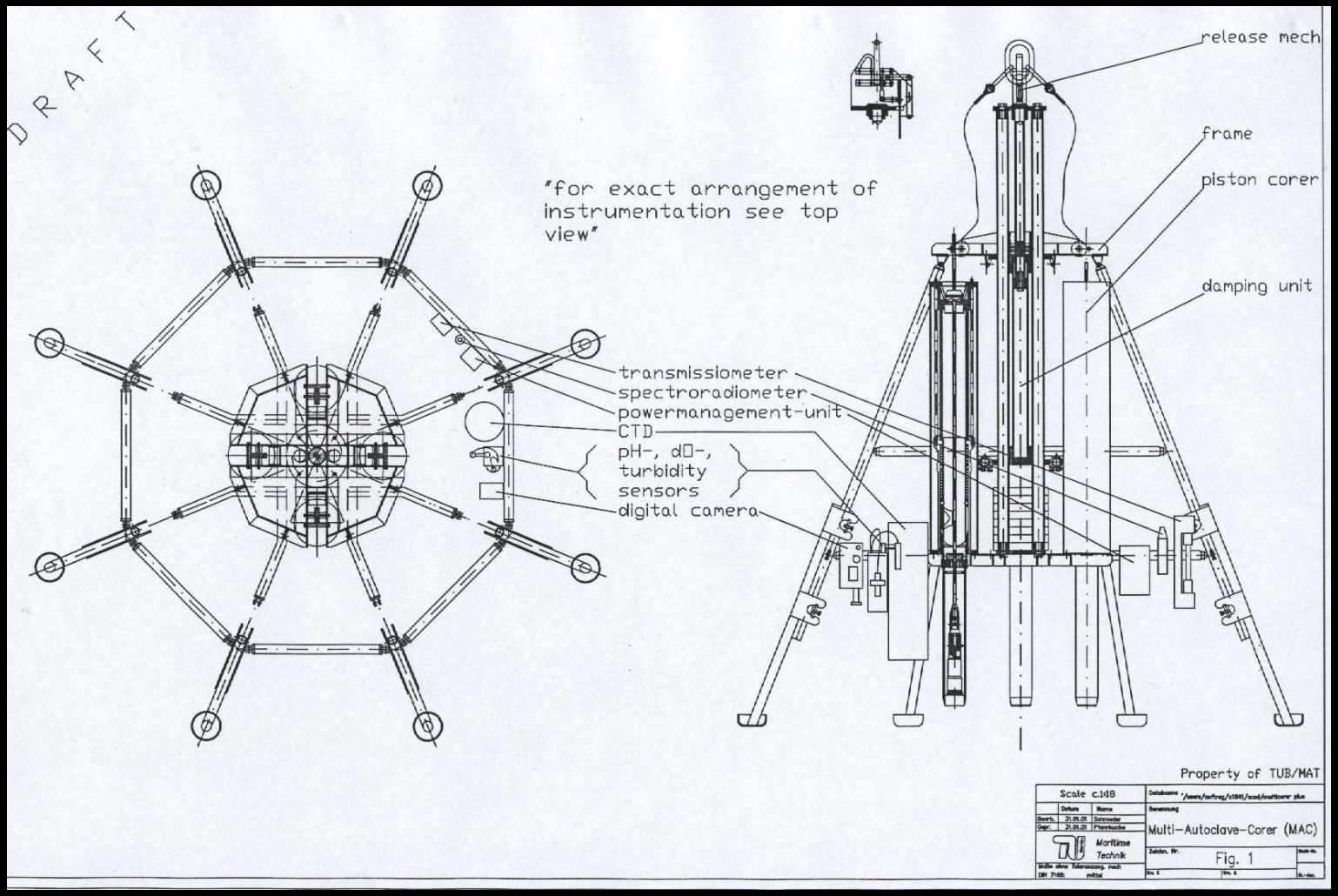
(h) Bioxalomycin 1 and (i) bioxalomycin 2, exhibit excellent *in vitro* and *in vivo* antimicrobial activity against Gram-positive bacteria.

(j)Alterobactin A (depsipeptide) (k) alterobactin B. Alterobactins bind with Fe^{3+}.

- ISOLATION OF STRAINS
- HIGH TROUGH PUT SCREENING
- BIOLOGICAL IDENTIFICATION
- CHEMICAL IDENTIFICATION
- ISOLATION OF METABOLITES

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

AUTOCLAVABLE SEDIMENT CORER



MAIN CHARACTERISTICS

Ships name: AEGAEO

Year of construction: 1985

Ship building company: Chalkis ship yard

Port of Registry: Chalkis

Class: + H 1000 A1

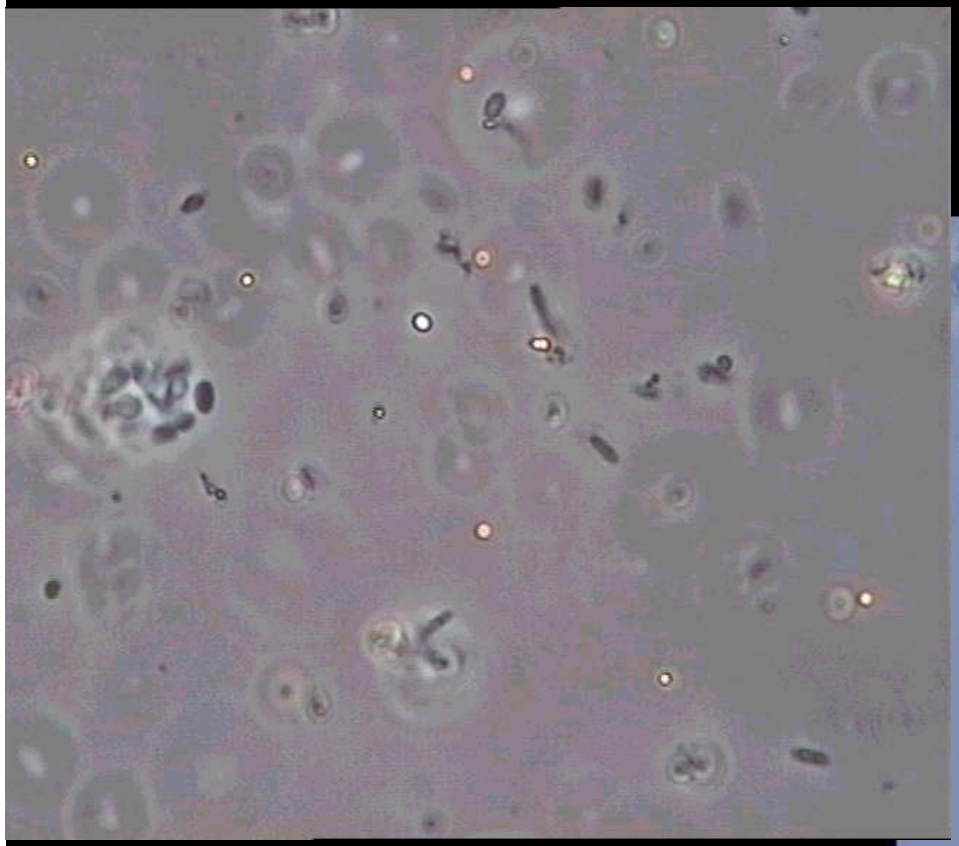
Overall Length: 61.51m

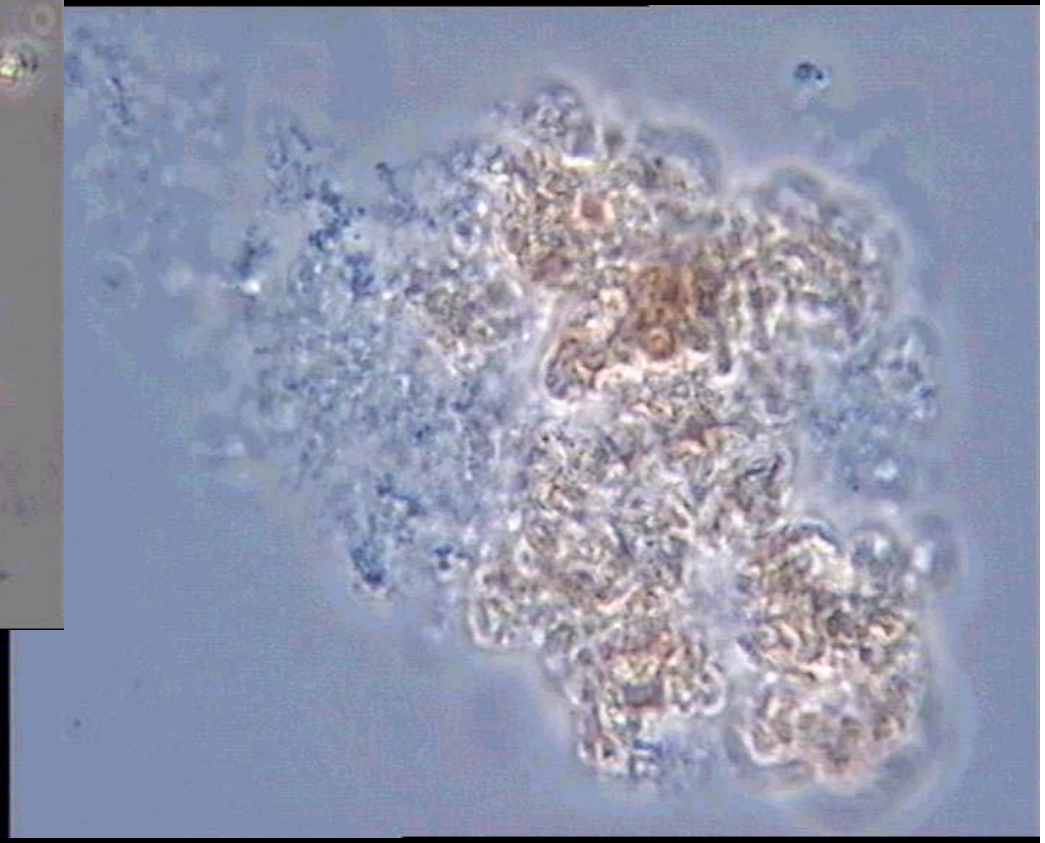
LBP: 57.2m

Max. Breadth: 9.60m

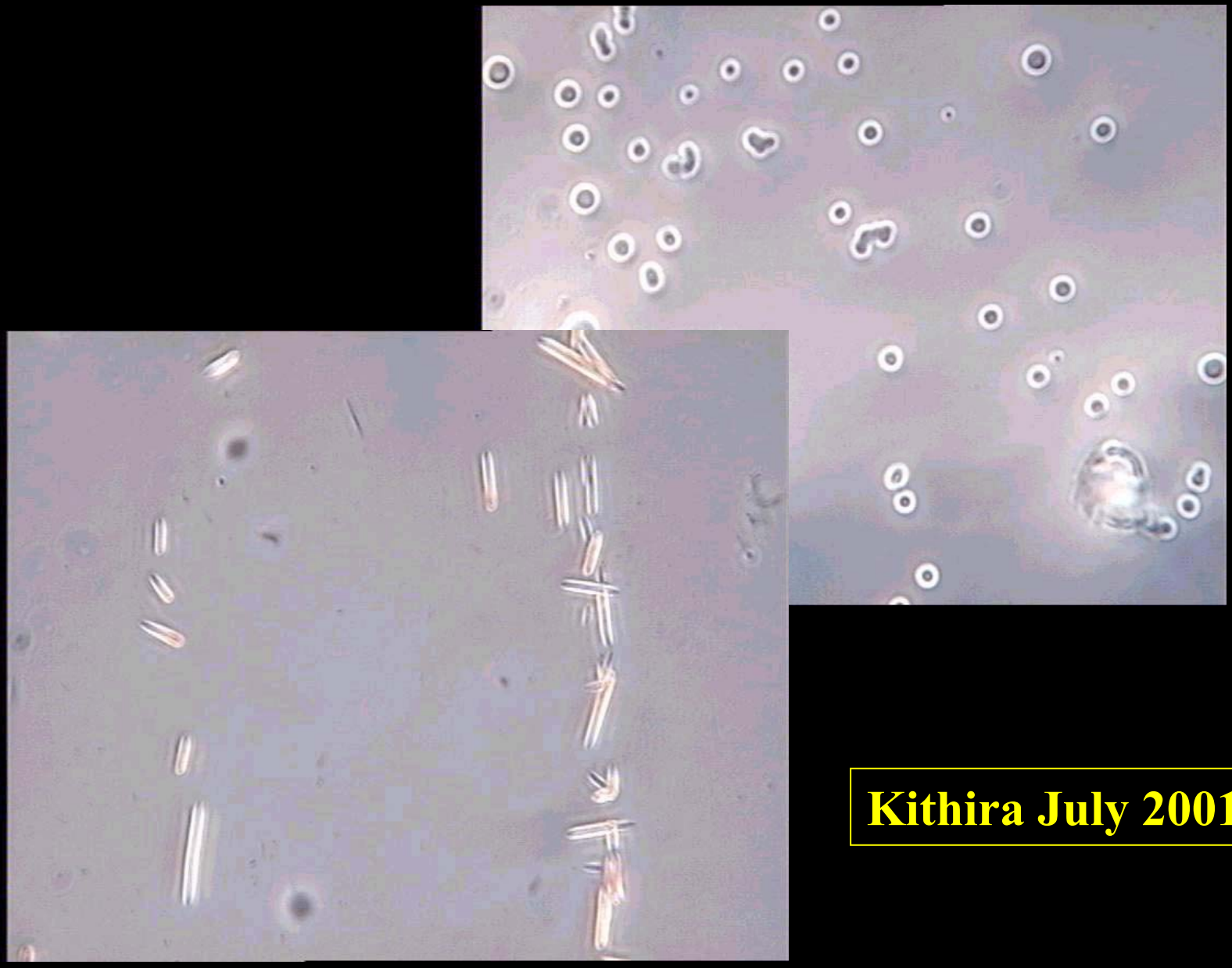
Summer Draught: 2.9m

Gross tonnage: REG. TON 778





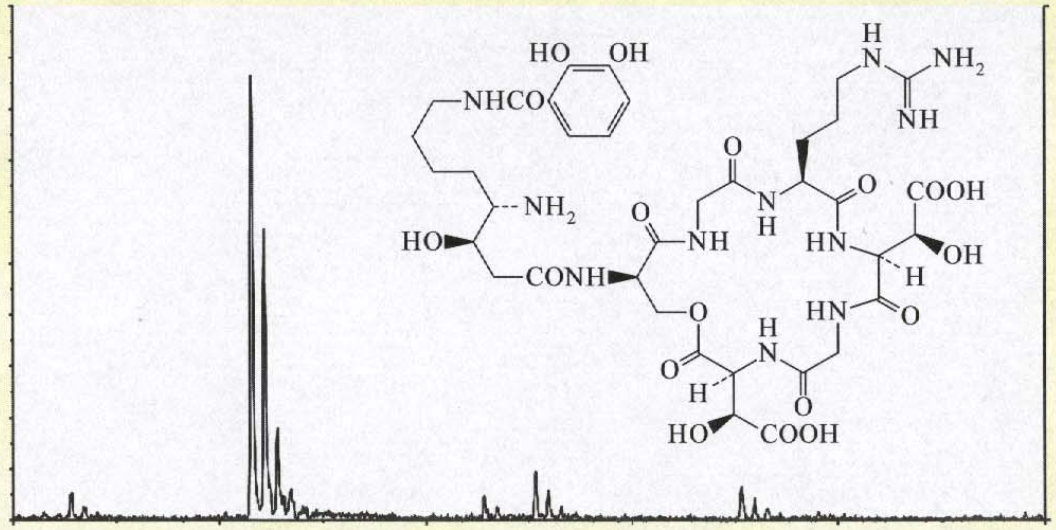
Corfu June 2001



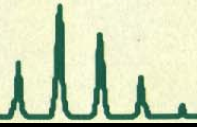
Kithira July 2001

Attachment 10:

MALDI TOF MS analysis of natural products



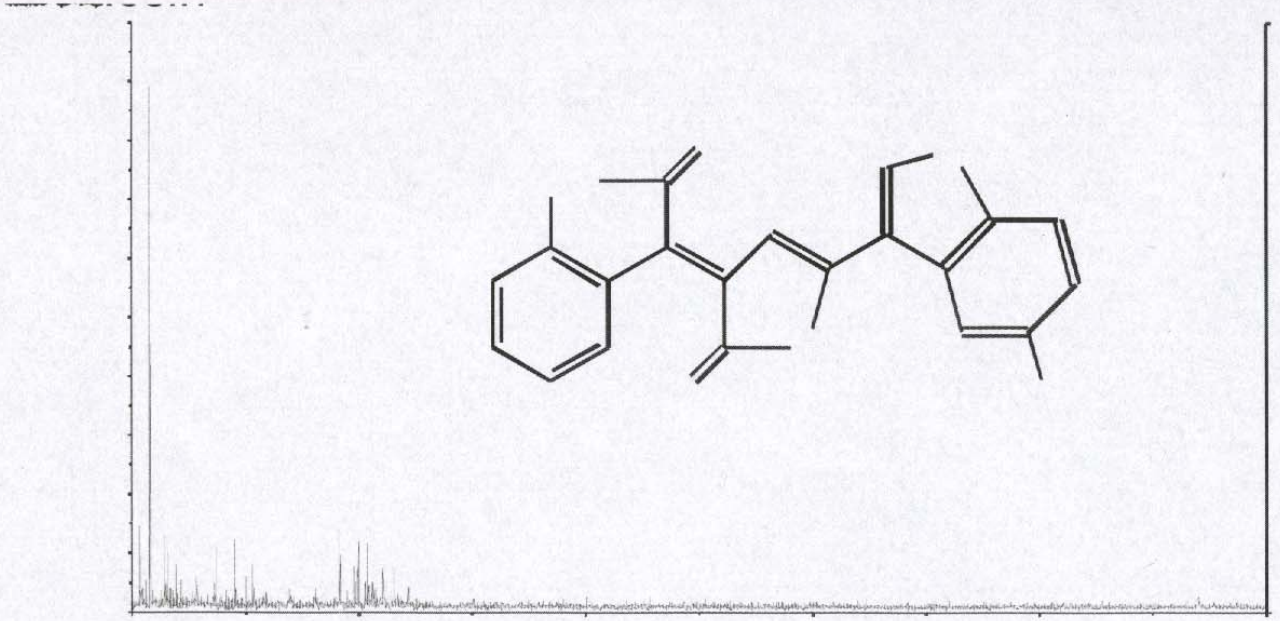
Detection of Alterobactins A and B (ring-opened form) as iron complexes (Isolate MM67).



Anagnostec

Anagnostec

MALDI TOF mass spectrometry of natural products



Violet pigment Violacein produced by isolate MM64.