

5th European Conference on Marine Natural Products

16-21 September 2007
ISCHIA, NAPLES, ITALY

*The Conference
is dedicated to the memory
of Prof. Pierre Potier
and Prof. Luigi Gomez-Paloma*

Welcome

On behalf of the local organizing committee and conference convenors, I warmly welcome fellow researchers from all over the world to Ischia for the 5th European Conference on Marine Natural Products. In the next five days, the meeting will host more than 150 presentations from almost 200 delegates from 33 countries. We are especially happy for the large participation of young researchers from many European countries.

Following the mainstream of the previous editions and acknowledging the major endorsement from PharmaMar S.A. and the European Network of Excellence on Marine Biodiversity and Functioning (MARBEF), the main aim of the 5th ECMNP is to report research on the basic and applied aspects of MNP studies, including the role in marine ecosystem functioning, the development of research tools devoted to defense and conservation of marine environments, and the application for pharmaceutical and cosmaceutical industry as well as marine aquaculture. The spaces of the Hotel Continental Terme will offer opportunities to share multidisciplinary knowledge among chemists, marine and molecular biologists, biochemists, ecologists, pharmacologists and those in related areas. I hope that the coming days may contribute to foster Marine Natural Products studies in Europe as much as the previous events did.

Naples is not new in hosting conferences on Marine Natural Products, having already organized two MANAPRO symposia held in Capri and Sorrento in 1992 and 2004, respectively. The organisation of this Conference started soon after the excellent EuroConference which was held in Paris in 2005. Having explored the availability of conference facilities, hotels, manpower, etc., we have decided to host the 5th EuroConference in Ischia, one of the most beautiful islands in the Gulf of Naples. I have received enormous support from many colleagues and friends during the preparation of this conference. Thanks to their efforts, we are very happy to be able to open the Conference activities on 16 Sept. I wish that both the conference and your stay in Ischia will be memorable for all participants.

Angelo Fontana

5th ECMNP Convenor and Chair of the organising committee

Background

Taking off from Athens in 1997, and continuing with Santiago de Compostela in 1999, Elmau in 2002, Paris in 2005, we are now arriving at the 5th Euroconference on Marine Natural Products taking place in Ischia. Almost 200 participants have set the basis for another successful meeting of this widely accepted series of European Conferences and the prospect of a 6th meeting in Portugal excites all of us.

The idea for the establishment of a European Series was born in 1997 after the realization that, mainly for financial reasons, not many European young scientists were able to participate in the major international conferences on Marine Natural Products. Europe used to be in the forefront of sciences and it seemed absolutely necessary to stimulate the interest of the young scientists in the fascinating areas of marine organisms' research and simultaneously promote interactions with colleagues around the world.

The 5th European Framework Program and the Training and Mobility of Researchers activity supported the organisation of the 1st event in Athens in November 2007 with over 50 fellowships for young scientists. This generous financial support continued till the 4th Conference that was self-financed but equally successful with the preceding events. In the Euroconferences, outstanding plenary lecturers are sharing the floor with young scientists presenting their work and getting the experience of the international scientific atmosphere. It is most encouraging to see that after 10 years and 4 events many of the young, in the initial events, scientists are now established and active cells in the European body.

Since the initial event it was decided that this series of Euroconferences should be organised in alternating years with the Gordon Conferences on Marine Natural Products. Of course, every three years the MaNaPro Symposia remain the most important appointment for all scientists interested in Marine Chemistry.

It is a great satisfaction to welcome in Ischia more than 100 participants that are below 35 years old, a fact that makes everybody confident that Europe will continue for a long time to furnish prestigious contributions in Marine Chemistry.

Guido Cimino and Vassilios Roussis

The organization and sponsors

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Financial support was also gratefully received from:



A special acknowledgement is due to

Ufficio Paesi Industrializzati Organismi Internazionali
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and Dipartimento di Progettazione Molecolare of Consiglio Nazionale delle Ricerche

Useful information

Registration Desk and Local Secretariat: The registration desk will be located in the Foyer of the Conference Centre. The desk will be opened from 3.00 p.m. to 6.30 p.m. on 16 September. The 5th ECMNP Local Secretariat will be based in the same place and will be opening every day from 8.30 a.m to 12.30 a.m and from 14.30 p.m to 17.30 p.m.

Oral Presentation: Invited lectures are allotted a total time of 30 min, including 5 min for questions. Other communications are allotted a total time of 20 min, including 5 min for questions. Speakers are invited to leave the electronic files at the Secretariat desk not later than the evening before the presentation. Operating systems available for oral presentations supports are Microsoft platforms (Windows[®] XP, 2000, NT[®], Me, 98, and 95) for PowerPoint[®] slides. Apple users are kindly asked to convert MacOS files into formats compatible with the above platforms or to contact the secretariat desk the day before the presentation.

Posters: Posters are divided in two groups scheduled from 5.30 p.m. to 7.30 p.m. on 17 Sept. (Green Session) and 18 Sept. (Blue Session) To promote discussion and dissemination, the authors are warmly encouraged to leave their contributions on the wall from Sunday 16th to Thursday 20th Sept. after the coffee break. Posters are to be fixed by push pins that are available at the Secretariat desk.

Poster parade. During the two poster sessions, a qualified committee will select 24 posters that will be promoted for oral presentations. These communications will be allotted a total time of 5 minutes for a 3-slide presentation. The selected authors are invited to leave the electronic files at the Secretariat desk not later than 12.30 a.m. of 19 Sept. Operating systems are all Microsoft platforms (Windows[®] XP, 2000, NT[®], Me, 98, and 95) supporting PowerPoint[®] slides.

Internet access: Free internet access via wireless LAN is available in the Conference Centre. Two computers are also accessible in the Conference Centre below the Foyer (near “Nitrodi” Rooms).

Social activities:

16 September: Welcome Cocktail (included)

17 September: Poster Pub Green

18 September: Poster Pub Blue

19 September: Poster Parade – Snack PUB (included)

20 September: Social Dinner (not included)

PROGRAMME AT A GLANCE

SEPT 16 TH	15.00-18.00	REGISTRATION
	18.00	OPENING CEREMONY
	18.30	OPENING LECTURE
SEPT 17 TH	09.00 – 13.10	ISOLATION & STRUCTURE ELUCIDATION <i>Chairmen: Marie-Lise Bourguet-Kondracki and Vassilios Roussis</i>
	15.00 – 17.20	ORGANIC SYNTHESIS <i>Chairman: Manuel Norte</i>
	17.30 – 19.00	POSTER SESSION GREEN
SEPT 18 TH	09.00 – 11.10	MOLECULAR SCIENCE & BIOSYNTHESIS <i>Chairman: Gabriele König</i>
	11.40 – 13.10	CHEMICAL ECOLOGY <i>Chairman: Cécile Debitus</i>
	15.00 – 16.50	CHEMICAL ECOLOGY <i>Chairman: Adrianna Ianora</i>
	17.30 – 19.00	POSTER SESSION BLUE
SEPT 19 TH	18.00	NPR AWARD LECTURE
	19.00 – 21.00	POSTER PARADE <i>Chairman: Joseph Pawlik</i>
SEPT 20 TH	9.00 – 13.00	BIOPROSPECTING <i>Chairmen: Rob Capon and Ted Molinski</i>
	15.00 – 17.20	MARINE TOXINS <i>Chairman: John Blunt</i>
SEPT 21 ST	9.00 – 10.20	NEW PERCEPTIONS <i>Chairman: Ernesto Fattorusso</i>
	10.20	CLOSING LECTURE
	11.00 – 12.00	CLOSING REMARKS

5th ECMNP SCIENTIFIC PROGRAMME

SEPTEMBER 16th

15.00-18.00	REGISTRATION
18.00	OPENING CEREMONY
18.30	BILL FENICAL OPENING LECTURE: NEW ANTITUMOR ANTIBIOTICS FROM DEEP OCEAN BACTERIA

SEPTEMBER 17th

MORNING SESSION: ISOLATION & STRUCTURE ELUCIDATION

CHAIRMAN: MARIE-LISE BOURGUET-KONDRACKI

09.00	GRAZIANO GUELLA	EXPERIMENTS AND THEORY IN THE STRUCTURAL DETERMINATION OF ARSENIC-CONTAINING NATURAL PRODUCTS
09.30	RÉMI LAVILLE	PACHYCHALINES A, B, C: ISOLATION OF A NEW FAMILY OF 3-ALKYLPYRIDINIUMS FROM THE CARIBBEAN MARINE SPONGE <i>PACHYCHALINA</i> SP
09.50	HARTMUT LAATSCH	NITRO COMPOUNDS FROM ARCTIC ICE BACTERIA AND OTHER SOURCES: METABOLIC VARIETY WITH UNUSUAL STRUCTURES
10.10	JÉRÔME APPENZELLER	NEW NONBROMINATED PYRROLE-2 AMINOIMIDAZOLES ALKALOIDS FROM <i>AGELAS</i> CF <i>MAURITIANA</i> PACIFIC SPONGE
10.30	MICHÈLE R. PRINSEP	RECENT STUDIES ON ALKALOIDS FROM NEW ZEALAND MARINE BRYOZOANS
10.50	GÉRALD CULIOLI	PHYTOCHEMICAL STUDIES OF BROWN ALGAE BELONGING TO THE CYTOSSEIRACEAE FAMILY
11.10		COFFEE BREAK

CHAIRMAN: VASSILIOS ROUSSIS

11.40	JUN'ICHI KOBAYASHI	CHEMICAL BIOLOGY OF OKINAWAN MARINE NATURAL PRODUCTS
12.10	ALFONSO MANGONI	SIMPLEXIDE REVISITED AND OTHER GLYCOLIPID STORIES
12.30	YUE-WEI GUO	BIOACTIVE NATURAL PRODUCTS FROM CHINESE TROPICAL MARINE INVERTEBRATES AND PLANTS
12.50	ALI MUHAMMAD SHAIQ	DITERPENOIDS FROM MARINE BROWN ALGA <i>DICTYOTA DICHOTOMA</i> COLLECTED FROM KARACHI COASTS OF ARABIAN SEA (PAKISTAN)
13.30		LUNCH

AFTERNOON SESSION: ORGANIC SYNTHESIS

CHAIRMAN: MANUEL NORTE

- 15.00 ALI AL-MOURABIT MARINE PYRROLE-2-AMINOIMIDAZOLE METABOLITES : FROM THE BIOMIMETIC SYNTHESIS TO THE IMPORTANT CHEMILUMINESCENT DIKETOPIPERAZINES
- 15.30 DIRK TRAUNER MAPPING THE CHEMISTRY OF HIGHLY UNSATURATED POLYKETIDES
- 16.00 CARLOS JIMÉNEZ CHEMICAL STUDIES OF VANCHROBACTIN, A SIDEROPHORE ISOLATED FROM THE BACTERIAL FISH PATHOGEN *VIBRIO ANGUILLARUM* SEROTYPE O2: CHARACTERIZATION OF ITS FERRIC/GALLIUM COMPLEXES AND SYNTHESIS OF SEVERAL ANALOGUES
- 16.20 THOMAS LINDEL BIOMIMETIC SYNTHESIS OF FLUSTRAMINE C: ORIGIN OF THE INVERSE 3-PRENYLATION
- 16.40 TED MOLINSKI SYNTHESIS-AIDED STRUCTURAL ASSIGNMENT OF NITROGENOUS MARINE NATURAL PRODUCTS
- 17.00 INES BRUNO TOWARDS THE DISCOVER OF A NEW CLASS OF SYNTHETIC PLA₂ INHIBITORS BASED ON γ -HYDROXYBUTENOLIDE SCAFFOLD
- 17.30 – 19.00 POSTER SESSION GREEN - POSTERS 1-50
PUB SNACK
-

SEPTEMBER 18TH

MORNING SESSION A: MOLECULAR SCIENCE & BIOSYNTHESIS

CHAIRMAN: GABRIELE KÖNIG

- 09.00 RUSSELL HILL BACTERIAL SYMBIONTS OF MARINE INVERTEBRATES ARE AN IMPORTANT RESOURCE FOR DRUG DISCOVERY
- 09.30 ARIEL KUSHMARO A GLIMPSE AT THE “UNCULTURED” MICROBIAL MAJORITY- FROM IDENTIFICATION TO DOMESTICATION
- 09.50 ADELE CUTIGNANO POLYKETIDE BIOSYNTHESIS IN MEDITERRANEAN OPISTHOBRANCHS: FURTHER STEPS TOWARDS THE CHARACTERIZATION OF PKS ASSEMBLY IN MARINE MOLLUSCS
- 10.10 KATJA MARIA FISCH SEARCH OF POLYKETIDE GENE CLUSTERS IN SPONGE METAGENOMES
- 10.30 PIERANGELO LUPORINI COLD-ADAPTED WATER-BORNE SIGNAL (PHEROMONE) PROTEINS FROM AN ANTARCTIC MICRO-EUKARYOTE, THE CILIATE *EUPLOTES NOBILII*
- 10.50 MATTHIAS KÖCK MASSADINE CHLORIDE: A BIOSYNTHETIC PRECURSOR OF MASSADINE AND STYLISSADINE
- 11.10 COFFEE BREAK

MORNING SESSION B: CHEMICAL ECOLOGY

CHAIRMAN: CÉCILE DEBITUS

- 11.40 **ALLAN CEMBELLA** CHEMICAL ECOLOGY OF DINOFLAGELLATE INTERACTIONS IN THE SEA: PHARMACOLOGY DOES NOT FOLLOW FUNCTION
- 12.10 **GEORG POHNERT** YOU ARE WHAT YOU EAT? FOOD QUALITY CONSEQUENCES OF RAPID LIPID METABOLISM OF DIATOMS
- 12.30 **GIOVANNA ROMANO** LOX-INDUCED LIPID PEROXIDATION AS MECHANISM RESPONSIBLE FOR THE DETRIMENTAL EFFECT OF MARINE DIATOMS ON ZOOPLANKTON GRAZERS
- 12.50 **JONATHAN SPENCER** STEREOCHEMICAL UNIFORMITY IN MARINE POLYETHER LADDERS – IMPLICATIONS FOR THE BIOSYNTHESIS AND STRUCTURE OF MAITOTOXIN
- 13.30 LUNCH

AFTERNOON SESSION: CHEMICAL ECOLOGY

CHAIRMAN: ADRIANNA IANORA

- 15.00 **JULIA KUBANEK** CHEMICALLY-MEDIATED INTERACTIONS AMONG MARINE COMPETITORS, HOSTS, AND PATHOGENS
- 15.30 **CONXITA AVILA** ANTARCTIC MARINE CHEMICAL ECOLOGY: WHAT HAS BEEN DONE AND WHAT'S NEXT?
- 15.50 **JOSEPH R. PAWLIK** TESTING AN HYPOTHESIS ON SPONGE CHEMICAL DEFENSES USING THE WRECK OF THE *USS SPEIGEL GROVE*
- 16.10 **ERNESTO MOLLO** THE CONTRIBUTION OF CHEMICAL ECOLOGY TO AN UNDERSTANDING OF FUNCTIONAL MORPHOLOGY AND EVOLUTION
- 16.30 **OLIVIER THOMAS** SPONGE CHEMICAL DEFENCES IN STRESS CONDITIONS: THE CASE STUDY OF THE LAST DISEASE OUTBREAK OBSERVED IN THE NW MEDITERRANEAN
- 17.30 – 19.00 POSTER SESSION BLUE - POSTERS 51-103
PUB SNACK

SEPTEMBER 19TH

- 18.00 **TAN REN XIANG** NPR 2007 AWARD LECTURE: METABOLITES OF MARINE AND TERRESTRIAL SYMBIONTS

POSTER PARADE SESSION

CHAIRMAN: JOSEPH PAWLIK

- 19.00-21.00 POSTER PARADE
PIZZA A VOLONTÀ
-

SEPTEMBER 20TH

MORNING SESSION: BIOPROSPECTING

CHAIRMAN: ROB CAPON

- 09.00 **ANA MARTINEZ** FROM THE SEA TO THE TREATMENT OF ALZHEIMER'S DISEASE
- 09.30 **GIUSEPPE BIFULCO** DESIGN, VIRTUAL SCREENING AND SYNTHESIS OF NEW HDAC INHIBITORS
A NATURAL PEPTIDE FROM THE MARINE SPONGE *GEODIA*
- 09.50 **MARISA RANGEL** *CORTICOSTYLIFERA* ACTS UPON MORPHOLOGY, MIGRATION AND INVASION OF BREAST CANCER
- 10.10 **MARIA CHIARA MONTI** MOLECULAR BASIS OF HUMAN GROUP IIA PHOSPHOLIPASE A₂ INHIBITION BY MARINE NATURAL PRODUCTS
- 10.30 **JOSE JIMENO** YONDELIS® (TRABECTEDIN): MAJOR CLINICAL IMPACT OF A MARINE ANTICANCER COMPOUND IN THE ERA OF TARGETED THERAPIES
- 11.10 COFFEE BREAK

CHAIRMAN: TED MOLINSKI

- 11.40 **HEONJOONG KANG** LIGANDS OF NUCLEAR RECEPTORS AS POTENTIAL DRUG LEADS
- 12.00 **JULIA JACOB** ANTIBIOTIC ALTERSOLANOL DERIVATIVES FROM A MANGROVE-DERIVED FUNGUS
- 12.20 **EBEL RAINER** NEW CYTOTOXIC A-PYRONE DERIVATIVES FROM THE SPONGE-DERIVED FUNGUS *PETRIELLA* SP.
- 12.40 **HENDRIK GREVE** NEW IANTHERANS FROM THE MARINE SPONGE *IANTHELLA QUADRANGULATA*: NOVEL AGONISTS OF THE P2Y₁₁ RECEPTOR
- 13.30 LUNCH

AFTERNOON SESSION: MARINE TOXINS

CHAIRMAN: JOHN BLUNT

- 15.00 **MICHIO MURATA** YESSOTOXIN AND SYNTHETIC POLYETHER MODELS – INTERACTION WITH MEMBRANE PEPTIDES
- 15.30 **PHILIPPE HESS** BIOACTIVE COMPOUNDS IN SHELLFISH - DRUG DISCOVERY OR FOOD SAFETY MANAGEMENT?
- 16.00 **ANTONIO DARANAS** NOVEL POLYETHER METABOLITES FROM DINOFLAGELLATES
- 16.20 **MARTINO FORINO** RECENT INSIGHTS INTO HARMFUL ALGAL BLOOMS ALONG THE ADRIATIC COASTLINE

16.40	CARMELA DELL' AVERSANO	THE GENOA 2005/2006 OUTBREAKS. DETERMINATION OF PUTATIVE PALYTOXIN AND ITS ANALOGUE IN <i>Ostreopsis ovata</i> BY A NEW LC-MS/MS METHOD
17.00	MANUEL LOLO	ADVANCED LC-MS/MS METHODS FOR THE ANALYSIS OF MARINE TOXINS
20.00		SOCIAL DINNER

SEPTEMBER 21ST

MORNING SESSION: NEW PERCEPTIONS

CHAIRMAN: ERNESTO FATTORUSSO

09.00	PETER KARUSO	REVERSE CHEMICAL PROTEOMICS WITH MARINE NATURAL PRODUCTS: A DIRECT LINK BETWEEN PHENOTYPE AND GENOTYPE VIA PHAGE DISPLAY
09.30	WERNER MULLER	NEW BIOMATERIALS: BIOFABRICATION OF BIOSILICA-GLASS BY SPONGES
10.00	SANDRA LOSS	SMALL VOLUME NMR FOR MARINE NATURAL PRODUCTS ANALYSIS
10.20	MURRAY MUNRO	CLOSING LECTURE: MOVING ON THE FUTURE SCALE AND SCOPE OF MARINE NATURAL PRODUCTS RESEARCH
11.00-12.00		CLOSING REMARKS

OPENING LECTURE

Sunday
16 September, 2007

NEW ANTITUMOR-ANTIBIOTICS FROM DEEP OCEAN BACTERIA

William Fenical^{1,2}, Paul R. Jensen¹ Victor Nizet² and Co-workers

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The rise in antibiotic resistance has created a medical emergency in the US and worldwide. While once simple bacterial infections were completely curable, these same pathogens have developed resistance to the most effective of our current antibiotic drugs. As a result, typical outpatient treatments for pneumonia, for example, often fail and require extended hospital treatment using intravenous drug therapies often with the last line of antibiotics such as vancomycin. The rise in drug resistance, while emerging in the 1950s, is at its highest level now, and it is further being exacerbated by the lack of new antibiotic drugs being discovered. In the mid 1990s, most of the pharmaceutical industry abandoned their previously productive microbe-based drug discovery programs in favor of combinatorial chemistry and other strategies to create chemical diversity. One ramification of this change was that the traditionally antibiotic producing microbial sources were no longer being explored. Because of this, and because the economics of antibiotic marketing did not meet the new demands of the pharmaceutical investor community, the majority of the pharmaceutical industry abandoned antibiotic discovery. In my research program, we have continued to develop marine microorganisms as a new source for drug discovery. While our first studies focused on drug discovery for cancer, we have come to realize that antibiotic drug discovery is an important area for development. Given the lack of interest by the pharmaceutical industry, we made a commitment to discover and develop new antibiotics at the UCSD campus through collaborations between scientists in UCSD's Skaggs School of Pharmacy and Pharmaceutical Sciences, as well as the UCSD School of Medicine. Reported in this presentation are the first of a set of new antibiotics discovered in that program.

ISOLATION & STRUCTURE ELUCIDATION

Monday
17th September, 2007

SESSION PROGRAMME

09.00	IL-1	GRAZIANO GUELLA	EXPERIMENTS AND THEORY IN THE STRUCTURAL DETERMINATION OF ARSENIC-CONTAINING NATURAL PRODUCTS
09.30	OR-1	RÉMI LAVILLE	PACHYCHALINES A, B, C: ISOLATION OF A NEW FAMILY OF 3-ALKYLPYRIDINIUMS FROM THE CARIBBEAN MARINE SPONGE <i>PACHYCHALINA</i> SP
09.50	OR-2	HARTMUT LAATSCH	NITRO COMPOUNDS FROM ARCTIC ICE BACTERIA AND OTHER SOURCES: METABOLIC VARIETY WITH UNUSUAL STRUCTURES
10.10	OR-3	JÉRÔME APPENZELLER	NEW NONBROMINATED PYRROLE-2 AMINOIMIDAZOLES ALKALOIDS FROM <i>AGELAS</i> CF <i>MAURITIANA</i> PACIFIC SPONGE
10.30	OR-4	MICHÈLE R. PRINSEP	RECENT STUDIES ON ALKALOIDS FROM NEW ZEALAND MARINE BRYOZOANS
10.50	OR-5	GÉRALD CULIOLI	PHYTOCHEMICAL STUDIES OF BROWN ALGAE BELONGING TO THE CYSTOSEIRACEAE FAMILY
11.10			COFFEE BREAK
11.40	IL-2	JUN'ICHI KOBAYASHI	CHEMICAL BIOLOGY OF OKINAWAN MARINE NATURAL PRODUCTS
12.10	OR-6	ALFONSO MANGONI	SIMPLEXIDE REVISITED AND OTHER GLYCOLIPID STORIES
12.30	OR-7	YUE-WEI GUO	BIOACTIVE NATURAL PRODUCTS FROM CHINESE TROPICAL MARINE INVERTEBRATES AND PLANTS
12.50	OR-8	ALI MUHAMMAD SHAIQ	DITERPENOIDS FROM MARINE BROWN ALGA <i>DICTYOTA DICHOTOMA</i> COLLECTED FROM KARACHI COASTS OF ARABIAN SEA (PAKISTAN)

EXPERIMENTS AND THEORY IN THE STRUCTURAL DETERMINATION OF ARSENIC-CONTAINING NATURAL PRODUCTS

Graziano Guella

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Simple alkylated derivatives of monoarsenic compounds have been rarely isolated from marine organisms such as brown algae, mollusks, arthropods and vertebrates¹ mainly in the form of volatile (like alkyl arsines) or non-volatile methyl arsine oxides (methylarsonic acid). Even seldom, arsenic has also been found in polar betaines, cholines, carbohydrate and lipid derivatives.² Despite its toxicity, While detoxification and energy generation are the primary processes, the discovery that organoarsenicals are involved in cellular functions suggests that Arsenic is readily used by a great diversity of organisms for cell growth and metabolism.

As a breakthrough in the chemistry of natural products containing arsenic, we have recently found that a poecilosclerid sponge from the north-eastern coast of New Caledonia, *Echinochalina bargibanti* (Hooper and Lévi, 1993) provides new polyarsenic compounds³.

The steps followed to arrive at the structural definition of arsenicin A (Figure) will be discussed in detail underlining the difficulties found with these compounds. In fact, the lack of spectroscopic data (NMR chemical shifts, IR frequencies and MS fragmentation routes) for polyarsenic containing compounds rules out the possibility to retrieve helpful reference data from literature. Thus, we were forced to deal with theoretical approaches able to provide, from “first principles”, calculated molecular properties for all the hypothetical structures initially guessed from experimental NMR, MS and IR data. In particular, *ab initio* calculations of the frequencies for its vibrational modes proved to be very helpful to establish the true connectivity and to discriminate among isomeric structures. A nice agreement between experimental (from FT-IR and Raman spectra) and theoretical infrared frequencies and intensities allowed us to rule out other spectrally compatible structures for arsenicin A. The structure was finally demonstrated by the synthesis of a model compound with known X-ray structure and a comparative *ab initio* simulation of its IR, Raman and NMR spectra.

Arsenicin A and other minor metabolites isolated from the same sponge wherein sulphur atoms are embedded in polyarsenicals are endowed of potent bactericidal and fungicidal activities on human pathogenic strains. All this may revive pharmacological interest in arsenic compounds while prompting to rethink about the arsenic cycle in nature.

1. R. Bentley, T. G. Chasteen, *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 250-271.
2. a) W. R. Cullen, K. J. Reimer, *Chem. Rev.* **1989**, *89*, 713-764; b) J. S. Edmonds, K. A. Francesconi, R. V. Stick, *Nat. Prod. Rep.* **1993**, *10*, 421-428.
3. I. Mancini, G. Guella, M. Frostin, E. Hnawia, D. Laurent, C. Debitus, F. Pietra *Chemistry Eur J.* **2006**, *12*, 8989-8994.

CHEMICAL BIOLOGY OF OKINAWAN MARINE NATURAL PRODUCTS

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Marine macro- and micro-organisms collected in Okinawa are good sources of compounds with intriguing structures and interesting biological activities.

Synthetic hybrid molecules of caffeine and eudistomin D from a tunicate *Eudistoma* sp. were found to show better potency as adenosine receptor ligands than caffeine. Potent cytotoxic polyene macrolides from a tunicate *Cystodytes* sp. were found to be potent osteoclast inhibitors and to inhibit V-ATPase *in vitro*. Pynadines and nakinadines are novel bis-pyridine alkaloids from sponges *Cribrochalina* sp. or *Amphimedon* sp., while agesamides and nagelamides are new bromopyrrole alkaloids from sponges *Agelas* sp. Theonezolid A, a long-chain polyketide from a sponge *Theonella* sp., induces a drastic shape change in platelets by reorganization of microtubules. The stereochemistry of many chiral centers in theonezolid A was elucidated by spectral data and chemical means. Amphidinolactones A and B are new macrolides from a dinoflagellate *Amphidinium* sp., and a potent cytotoxic macrolide from another strain was found to target actin cytoskeleton.

In this conference, the structures and bioactivities of these interesting marine natural products will be described.

1. Kobayashi, J. et al., *Bioorg. Med. Chem.* **2006**, *14*, 1063-1067.
2. Kobayashi, J. et al., *Tetrahedron Lett.* **2006**, *47*, 997-998.
3. Kobayashi, J. et al., *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1364-1370.
4. Kobayashi, J. et al., *Tetrahedron Lett.* **2006**, *47*, 4369-4371.
5. Kobayashi, J. et al., *Org. Lett.* **2006**, *8*, 4235-4238.
6. Kobayashi, J. et al., *Bioorg. Med. Chem.* **2006**, *14*, 8415-8419.
7. Kobayashi, J. et al., *J. Nat. Prod.* **2007**, *70*, 423-424.
8. Kobayashi, J. et al., *Bioorg. Med. Chem.* **2007**, *15*, 3235-3240.
9. Kobayashi, J. et al., *Org. Lett.* **2007**, *9*, 2369-2371
10. Kobayashi, J. et al., *Tetrahedron Lett.* **2007**, *48*, 4983-4986.

PACHYCHALINES A, B, C: ISOLATION OF A NEW FAMILY OF 3-ALKYLPYRIDINIUMS FROM THE CARIBBEAN MARINE SPONGE *PACHYCHALINA* SP

Rémi Laville,¹ Olivier P. Thomas,¹ Fabrice Berru ,¹ Rogelio Fernandez,² and Philippe Amade¹

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Marine sponges of the order Haplosclerida are well-known to produce a large variety of secondary metabolites derived from 3-alkylpyridines. Most of them, isolated from *Stelletta*, *Callyspongia*, *Niphates*, *Amphimedon*, *Reniera*, or *Haliclona* genus, are usually macrocyclic compounds which show cytotoxic and ichthyotoxic activities. Chemical studies on the genus *Pachychalina* are scarce in the literature. Recently, the group of Berlinck described the isolation of new alkaloids biogenetically derived from 3-alkylpyridiniums from the sponge *Pachychalina alcaloidifera*, collected off the coast of Brazil.¹

The chemical study of *Pachychalina* sp., collected off the Martinique Island coast in 2003, led us to isolate a novel acyclic family of 3-alkylpyridiniums, named pachychalines. Characteristic features of this new family include the terminal primary amine and/or norspermidine moieties. Their structures were confirmed by both exhaustive HRESIMS-MS studies and total synthesis.

The isolation of this new family of natural products raises the burning issue of 3-alkylpyridinium biosynthesis. The hypothesis is based on the oxidative cyclisation of the norspermidine into a 3-alkylpyridine or 3-alkylpyridinium.

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NITRO COMPOUNDS FROM ARCTIC ICE BACTERIA AND OTHER SOURCES: METABOLIC VARIETY WITH UNUSUAL STRUCTURES

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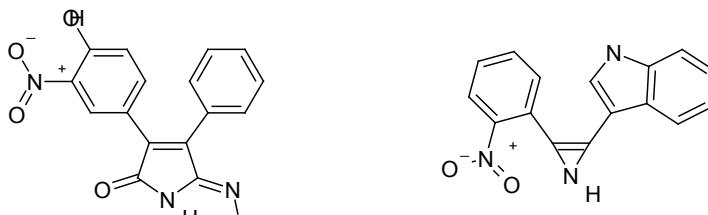
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In contrast to freshwater ice, seawater ice is not a dense and homogenous mass, but a sponge-like conglomerate of ice crystals surrounded by brine-filled channels open at the bottom and in contact and exchange with the seawater. Due to the translucent nature of the ice, algae can grow within these brine channels, and form a unique community together with bacteria and fungi. The population density may be extremely high and can even exceed those of algal blooms.

Genetic fingerprinting of the cultivable bacteria has revealed a large variety of new species and even new genera. In parallel, our chemical and biological screening indicated a broad range of cytotoxic, antimicrobial, antimalarial or phytotoxic activities. One drawback in natural products research with these cultures are their nutrient requirement and their low growth rates, resulting in low yields of secondary metabolites. Structure elucidation revealed chemically simple compounds derived from amino acid metabolism, but some possess unique features. A surprisingly high variety of aromatic nitro, dinitro and trinitro compounds was found in extracts from an arctic *Salegentibacter* strain. Their structural similarity with iron chelators on the basis of nitrosophenols may indicate a potential function in iron transport.



Nitro compounds were not only found in bacteria from cold habitats. A *Vibrio* species, isolated from the Red Sea soft coral *Simularia polydactyla*, showed even higher biosynthetic capabilities e.g. unusual nitrated azirines and maleinimides. Some of these show structural similarities with arcylarubins from myxomycetes and didemnimides from ascidians. Despite nitro compounds have not only been found in bacteria, but also in plants and fungi, such a structural variety of nitro derivatives, however, has never been isolated from a single organism before.

NEW NONBROMINATED PYRROLE-2-AMINOIMIDAZOLES ALKALOIDS FROM *AGELAS CF MAURITIANA* PACIFIC SPONGE

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In the frame of the CRISP research program, a sponge, identified as *agelas cf mauritiana*, was collected on Guadalcanal reefs. The chemical study of the methanolic extract has led to the identification of new pyrrole-2-aminoimidazole alkaloids related to the C₁₁N₅ family. These compounds exhibit a large molecular diversity with various substitutions, oxydation states and cyclisation modes. It is noteworthy than, in contrast with many members of the C₁₁N₅ family, none of the isolated compounds from this sponge bears bromine on the pyrrole carboxamide moiety.¹

Figure 1 shows two examples of this new nonbrominated alkaloids.

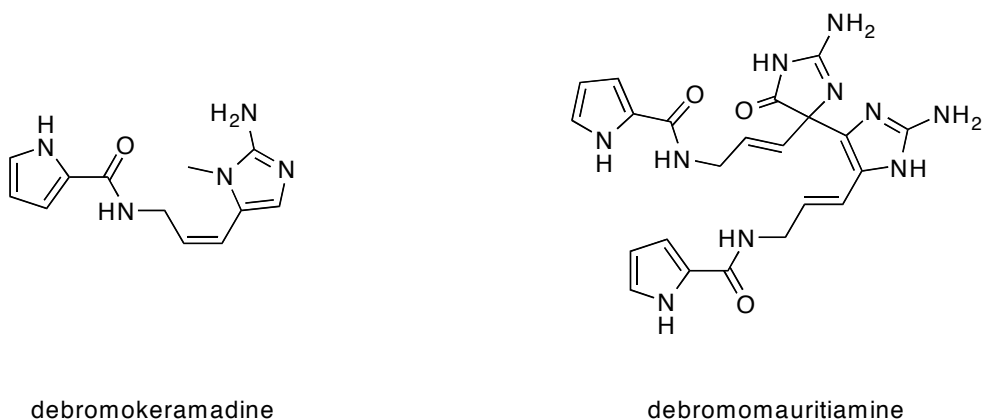


Figure 1

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RECENT STUDIES ON ALKALOIDS FROM NEW ZEALAND MARINE BRYOZOANS

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New Zealand marine bryozoans have yielded several families of alkaloids with novel structures and/or interesting bioactivity.¹ These alkaloid families include the β -carboline alkaloids,² the amathaspiramides,³ the euthyroideones⁴ and the pterocellins.⁵⁻⁷ This paper will present some of our recent results on the chemistry, bioactivity, distribution and detection of some of these alkaloids, including the structures of some novel compounds.

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PHYTOCHEMICAL STUDIES OF BROWN ALGAE BELONGING TO THE CYSTOSEIRACEAE FAMILY

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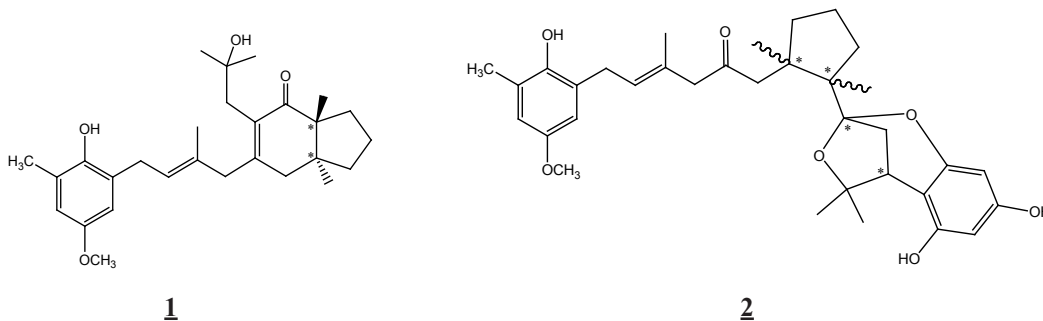
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Brown algae of the Cystoseiraceae family, more precisely those belonging to the *Cystoseira* genus, are known to contain a large number of structurally diverse terpenoids¹. A particular emphasis could be made for linear and cyclic meroditerpenoids isolated from such species, even if only few of them displayed significant biological activity, because they have proven to be very effective as chemotaxonomic tools^{2,3}. Furthermore, due to global change and intensification of human activities, a long-term decline of the populations of several *Cystoseira* species has been observed⁴ leading to an urgent need in the description of their chemodiversity.



1

2

In this work, we report on the isolation and structure determination of several new meroditerpenoids, some of them belonging to a new chemical family. From a Moroccan specimen of *Cystoseira baccata*, six new bicyclic prenyltoluquinols (e.g.: **1**) have been isolated, surprisingly for all these compounds, which possess a bicyclo[4.3.0]nonane ring system, a *trans* fusion of the bicyclic system was deduced by stereochemical studies (Noe, Mosher,...) even though such compounds isolated from *Cystoseira* species are known to have a typical *cis* orientation for the bridgehead methyls². Moreover, the phytochemical study of *Cystoseira tamariscifolia* harvested along the Algerian coasts allowed us to describe a series of monocyclic meroditerpenes together with a new family of natural compounds: these “phloromeroditerpenoids” are the result of the junction of a monocyclized meroditerpenic moiety with a phloroglucinol unit (e.g.: **2**).

1. Blunt J.W., Copp B.R., Hu W.-P., Munro M.H.G., Northcote P.T., Prinsep M.R. *Nat. Prod. Rep.* **2007**, *24*, 31-86 (and previous reports in this series).
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SIMPLEXIDE REVISITED, AND OTHER GLYCOLIPID STORIES

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In the last ten years, glycolipids for marine organisms (sponges in particular) have been shown to be an exceptionally rich source of chemical diversity. These compounds often show peculiar biological activities and, for example, agelasphin, an α -galactosylceramide first isolated from the sponge *Agelas mauritiana*, is the reference ligand for the CD1d protein, an antigen presenting molecule which binds and presents glycolipids to T lymphocytes. The most recent contributions in this field from our group will be presented.

Simplexide (**1**), glycoside of a very-long-chain secondary alcohol, has been isolated several years ago from the Caribbean sponges *Plakortis simplex*. We have now completed the total synthesis of simplexide, and shown that it is a potent stimulus for endogenous IL-10 production by Peripheral Blood Mononuclear Cells (PBMC), and more generally induces a unique profile of cytokine release. Vesparioside B (**2**) is a new glycosphingolipid isolated from the sponge *Sphēciospongia vesparia*, characterized by a complex hexasaccharide chain with three sugars in the furanose form. Its structure elucidation led to establish a new method to correlate the relative configuration of the ring carbons to vicinal proton-proton coupling constant values in the five-membered furanose sugar. The same method was then used for the structure elucidation of another complex glycosphingolipid from a *Terpios* sponge.

BIOACTIVE NATURAL PRODUCTS FROM CHINESE TROPICAL MARINE INVERTEBRATES AND PLANTS

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The oceans contain a vast biological diversity of species that have so far been utilized by mankind mainly as a source of protein. In the last few decades, however, natural products chemists have started to discover the wealth of bioactive secondary metabolites that are produced by marine invertebrates such as sponges, soft corals, molluscs and others.¹

Much of our research on marine natural products is centered in the tropics water (South China Sea) where marine diversity of invertebrates is abundant. Biotic stress factors due to predation (e.g. by fishes) are usually severe in the tropics which in turn has shaped the secondary metabolism of potential prey organisms such as sponges. This results in the accumulation of structurally diverse bioactive metabolites which offer interesting challenges to natural products chemists as well as to biologists and pharmacologists alike.

In the course of our research activities²⁻⁹ on the marine invertebrates and plants (**mangroves, algae etc.**) from South China Sea, we discovered diverse classes of secondary metabolites showing significant biological activities including anti-inflammatory, anti-tumor, and antibacterial. In this communication the structures and activities of these new natural products, which might be useful as biological tools and biomedical agents, will be described.

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DITERPENOIDS FROM MARINE BROWN ALGA *DICTYOTA DICHOTOMA* COLLECTED FROM KARACHI COSTS OF ARABIAN SEA (PAKISTAN)

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During the search of new natural products, which may have some biological activity for the possible discovery of drugs, we have selected marine source. Among the marine sources, seaweeds are the rich source of secondary metabolites having novel carbon skeletal and sometimes with halogens. We have worked on various species of Red, Brown and Green algae collected from Karachi costs of Arabian Sea (Pakistan).

Presently, we are involved in the search of bioactive metabolites from brown algae. In the presentation, the isolation, characterization and some biological studies of 11 diterpenoidal constituents from brown alga *Dictyota dichotoma* would be described. They include:

- Three C-16 oxidized *seco*-dolastanes.
- Two enone containing moiety in dolastane-diterpenoids.
- Three ring-A hydroxylated dolastane-diterpenoids.
- Three *seco*-dolastanes.

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ORGANIC SYNTHESIS

Monday
17th September, 2007

SESSION PROGRAMME

15.00	IL-3	ALI AL-MOURABIT	MARINE PYRROLE-2-AMINOIMIDAZOLE METABOLITES: FROM THE BIOMIMETIC SYNTHESIS TO THE IMPORTANT CHEMILUMINESCENT DIKETOPIPERAZINES
15.30	IL-4	DIRK TRAUNER	MAPPING THE CHEMISTRY OF HIGHLY UNSATURATED POLYKETIDES
16.00	OR-1	CARLOS JIMÉNEZ	CHEMICAL STUDIES OF VANCHROBACTIN, A SIDEROPHORE ISOLATED FROM THE BACTERIAL FISH PATHOGEN <i>VIBRIO</i> <i>ANGUILLARUM</i> SEROTYPE O2: CHARACTERIZATION OF ITS FERRIC/ GALLIUM COMPLEXES AND SYNTHESIS OF SEVERAL ANALOGUES
16.20	OR-2	THOMAS LINDEL	BIOMIMETIC SYNTHESIS OF FLUSTRAMINE C: ORIGIN OF THE INVERSE 3-PRENYLATION
16.40	OR-3	TED MOLINSKI	SYNTHESIS-AIDED STRUCTURAL ASSIGNMENT OF NITROGENOUS MARINE NATURAL PRODUCTS
17.00	OR-4	INES BRUNO	TOWARDS THE DISCOVER OF A NEW CLASS OF SYNTHETIC PLA ₂ INHIBITORS BASED ON γ -HYDROXYBUTENOLIDE SCAFFOLD

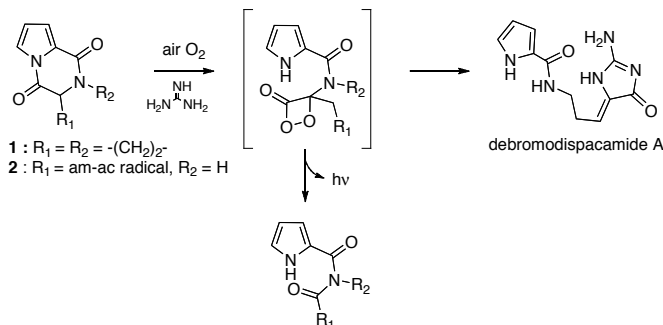
MARINE PYRROLE-2-AMINOIMIDAZOLE METABOLITES : FROM THE BIOMIMETIC SYNTHESIS TO THE IMPORTANT CHEMILUMINESCENT DIKETOPIPERAZINES

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Marine sponges belonging to Agelasidae and Axinellidae families are remarkable for their abilities to produce an interesting and increasingly large group of biologically active Pyrrole-2-AminoImidazole (P-2-AI) metabolites.¹ Their typical marine structural diversity is based on the extraordinarily reactive 2-aminoimidazole part. There is no direct biosynthetic evidence for the chemical pathways involved in the formation of this family of alkaloids from their early precursors.² Since our observation that the skeleton of the natural debromodispacamide A could be formed from the diketopiperazine (DKP) 1 and guanidine by spontaneous air oxidation,³ we presumed an important role of proline DKP as key precursors of motif in natural metabolites. The reaction seems to be general and concerns other DKP derived families of marine metabolites. Molecular oxygen reacts easily with the DKPs of type 2 to give various metabolites already isolated from sponges. The same reaction can lead to a chemiluminescent decarboxylation.



Considering that the natural aminoacides derived DKP could be the key structure involved in the biogenesis of a large number of marine metabolites, an extensive search of the expected derivatives combining structure guided metabolites isolation from phylogenetically related sponges and biomimetic synthesis was conducted. Beyond the discovery of new molecules and reactions, the biomimetic studies of the molecular reactivity can also suggest a great explanation of ecological purposes. Mechanistic studies of a spontaneous oxidation of selected DKPs by oxygen and their chemiluminescent properties will be discussed.

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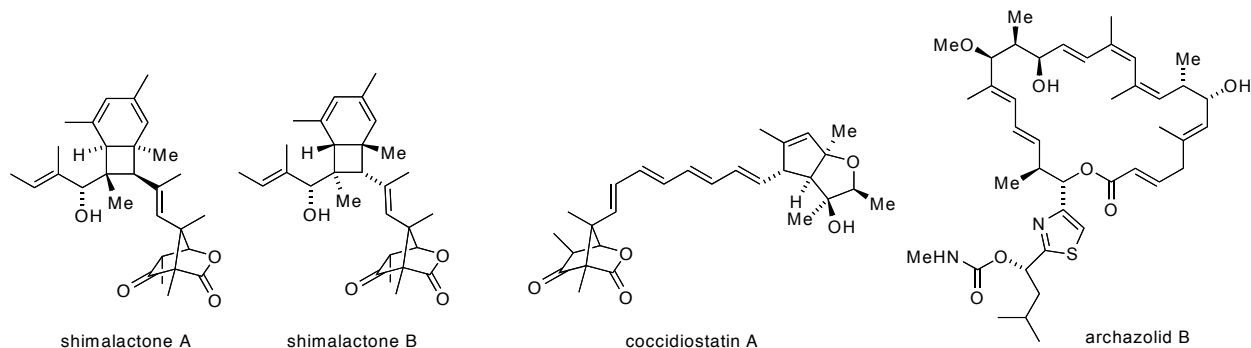
MAPPING THE CHEMISTRY OF HIGHLY UNSATURATED POLYKETIDES

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Highly unsaturated polyketides have a rich and rewarding chemistry that often includes fascinating reaction cascades.¹ I will present synthetic studies on complex natural products of this type, in particular the shimalactones, coccidiostatine A, and archazolide B. The shimalactones are neurotogenic natural products isolated from the marine fungus *Emericella varicolor*,² whereas the structurally related anticoccidial compound coccidiostatin A stems from *Penicillium rugulosum*.³ The archazolides, isolated from the myxobacterium *Archangium gephyra*, are highly potent and selective inhibitors of mammalian V-ATPases.⁴ Our highly convergent and stereoselective synthesis of archazolide B features a relay ring-closing metathesis and can be easily adapted for further biological exploration.⁵



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5. Roethle, P.A.; Chen, I.T.; Trauner, D. *J. Am. Chem. Soc.* **2007**, *129*, 8960-8961.

CHEMICAL STUDIES OF VANCHROBACTIN, A SIDEROPHORE ISOLATED FROM THE BACTERIAL FISH PATHOGEN *VIBRIO ANGUILLARUM* SEROTYPE O2: CHARACTERIZATION OF ITS FERRIC/GALLIUM COMPLEXES AND SYNTHESIS OF SEVERAL ANALOGUES

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Many marine bacteria use siderophores, which are low molecular weight (300 to 2000 Da) ferric specific ligands, to scavenge and transport Fe (III) into the cell. It is known that the ability to sequester iron is a key factor in the virulence of the fish pathogen bacteria [1].

In the course of our studies into siderophores from marine microorganisms, vanchrobactin (**1**) was isolated from the bacterial fish pathogen *V. anguillarum* serotype O2. This bacterium is the causative agent of vibriosis, an extremely fatal hemorrhagic septicaemia that results in considerable economic losses in aquaculture farming worldwide. The planar structure of the monocatecholate siderophore **1** was established by extensive NMR studies on the natural sample and the absolute configuration was unequivocally determined by chiral capillary electrophoresis comparison of the natural product with a synthetic sample which was prepared by a short and efficient method. [2].

In order to understand the mechanism of the iron uptake process by *V. anguillarum* and to know how the Fe (III) is bounded to the siderophore to acquire iron, we prepared its Ferric/Gallium complexes. Spectrophotometric and potentiometric measurements of the iron (III)-vanchrobactin complex, which allowed us to determine its stoichiometry and affinity constant, along with exhaustive NMR studies of the gallium (III)-vanchrobactin complexes, were very helpful to determine their structures and the iron chelation sites. On other hand, the synthesis of several isomers of **1** and the evaluation of the siderophore activity, allowed us to deduce some structure-activity relationships which are very useful to develop the Trojan Horse strategy in order to design antibacterial agents against vibriosis.

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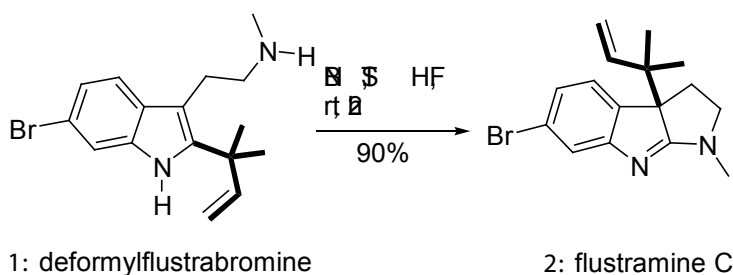
BIOMIMETIC SYNTHESIS OF FLUSTRAMINE C: ORIGIN OF THE INVERSE 3-PRENYLATION

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The bryozoan *Flustra foliacea* has been a rich source of brominated indole alkaloids bearing isoprenyl substituents at various positions. The discovery of deformylflustrabromine (**1**) as a major metabolite of *F. foliacea* collected near the North Sea island of Helgoland prompted us to investigate the chemical link between **1** and the pyrrolo[2,3-*b*]indole flustramine C (**2**).



Biological activity. Deformylflustrabromine (**1**) affects the nicotinic acetylcholine receptor (nAChR). Flustramine C (**2**) shows elevated concentrations in exposed parts of *F. foliacea*, it is secreted into the surrounding water and could play an important role in ecological interactions. It has also been suggested that the *Flustra* alkaloids are important for the bryozoan by controlling bacterial growth on its surface.

Biogenesis. Three major pathways may be discussed regarding the biogenesis of flustramine C. Aza-Claisen rearrangement of an indole-*N*-prenylated precursor could occur. Alternatively, direct introduction of an inverse prenyl group by S_N2' reaction at C3 may occur, without involvement of inverse prenylation at C2, as proposed by Harrison for the biosynthesis of roquefortine. Inverse prenyl groups at C3 may also arise via 1,2-shift from C2, as proposed by Barrow and by Gorst-Allman for the biosynthesis of roquefortine, and by Williams for paraherquamide A.

Result. Our investigation shows for the first time that, from a chemical perspective, the biogenesis of flustramine C (**2**) may indeed proceed via inverse prenylation of the indole 2-position in an oxidative process. The conversion of **1** (synthesized within 6 steps from tryptamine) to **2** on treatment with *t*BuOCl was monitored by ^1H , ^{15}N NMR spectroscopy shedding light on the mechanism. Best yields (90%) were obtained when *t*BuOCl was replaced by NBS.

SYNTHESIS-AIDED STRUCTURAL ASSIGNMENT OF NITROGENOUS MARINE NATURAL PRODUCTS

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Marine natural products with complex structures containing stereogenic centers provide challenges for structure elucidation. Molecules with acyclic segments and isolated stereocenters lacking functional groups are particularly difficult with respect to stereochemical analysis. Typically, their configurations are not amenable to general solutions and must be addressed on a case-by-case basis. In this talk I will present case studies of new natural products recently isolated in our laboratories and unique configurational analyses, based largely upon exploitation of weak CD effects and synthesis of suitable model compounds. The soft-tissue sponge *Phorbis amaranthus*, found in the tropical waters of Key Largo, Florida, shows high antifeedant properties against spongivorous fish such as *Thalassoma bifasciatum*.² Earlier, we reported ring-A contracted steroids as principal components of the lipophilic sponge extract obtained from fractionation of the whole extract.³ Extension of this a bio-assay guided isolation lead to an antifeedant fraction that was purified to provide a novel steroidal alkaloid, amaranzole A, along with congeners. Partial syntheses of amaranzole A side analogs allowed the use of CD for determination of the configuration of amaranzole A. Some observations will be presented of comparison of empirical interpretations of weak-CD effects compared to 'quantitative' DFT calculations.

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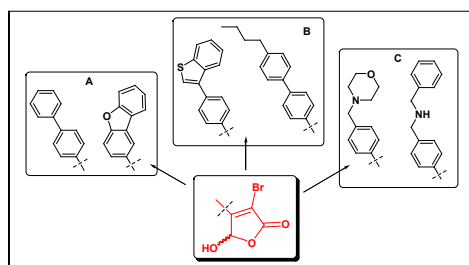
TOWARDS THE DISCOVER OF A NEW CLASS OF SYNTHETIC PLA₂ INHIBITORS BASED ON γ -HYDROXYBUTENOLIDE SCAFFOLD

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Marine sesterterpenes containing a γ -hydroxybutenolide ring are well known to specifically inhibit phospholipase A₂ (PLA₂) [1-5], a key enzyme involved in the inflammation pathway. Among this class of PLA₂ inhibitors, petrosaspongiolide M (PM, **1**), isolated in 1997 by our group from the marine sponge *Petrosaspongia nigra* [6], has been the subject of extensive investigation in our laboratory, allowing us to shed light on its mechanism of action at the molecular level [7]. Such relevant information drove us to generate a new class of γ -hydroxybutenolide-based synthetic products, taking advantage also of computational techniques of *de novo* rational design, starting from crystallographic data of substrate-PLA₂ complexes.

Thus a focused collection of simplified analogues of PM, belonging to four different structural groups were efficiently synthesized and finally subjected to pharmacological screening; this led us, quite unexpected, to the discovery of a promising inhibitor of prostanoid production acting by the selective modulation of microsomal prostaglandin E synthase-1 expression (mPGES-1), an attractive target deeply involved in the inflammatory process. Finally, we report a regioselective entry to 3-bromo and 4-bromo-5-hydroxy-5H-furan-2-ones by photooxidation of 3-bromofurane, a commercially available compound, with singlet oxygen in the presence of a suitable base. By this procedure a variety of 3- and 4-substituted γ -hydroxybutenolides have become for the first time easily accessible [8].



Petrosaspongiolide M (PM, **1**)

Fig. 1

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MOLECULAR SCIENCE & BIOSYNTHESIS

Tuesday
18th September, 2007

SESSION PROGRAMME

09.00	IL-5	RUSSELL HILL	BACTERIAL SYMBIONTS OF MARINE INVERTEBRATES ARE AN IMPORTANT RESOURCE FOR DRUG DISCOVERY
09.30	OR-1	ARIEL KUSHMARO	A GLIMPSE AT THE “UNCULTURED” MICROBIAL MAJORITY- FROM IDENTIFICATION TO DOMESTICATION
09.50	OR-2	ADELE CUTIGNANO	POLYKETIDE BIOSYNTHESIS IN MEDITERRANEAN OPISTHOBRANCHS: FURTHER STEPS TOWARDS THE CHARACTERIZATION OF PKS ASSEMBLY IN MARINE MOLLUSCS
10.10	OR-3	KATJA MARIA FISCH	SEARCH OF POLYKETIDE GENE CLUSTERS IN SPONGE METAGENOMES
10.30	OR-4	PIERANGELO LUPORINI	COLD-ADAPTED WATER-BORNE SIGNAL (PHEROMONE) PROTEINS FROM AN ANTARCTIC MICRO-EUKARYOTE, THE CILIATE <i>EUPLOTES NOBILII</i>
10.50	OR-5	MATTHIAS KÖCK	MASSADINE CHLORIDE: A BIOSYNTHETIC PRECURSOR OF MASSADINE AND STYLISSADINE

BACTERIAL SYMBIONTS OF MARINE INVERTEBRATES ARE AN IMPORTANT RESOURCE FOR DRUG DISCOVERY

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Marine sponges and corals harbor large assemblages of novel bacteria. We have done 16S rRNA gene-based bacterial community analysis on ten species of marine sponges from three different oceans and in all cases the sponges contained highly diverse bacterial assemblages that included many novel bacteria. *Actinobacteria* were well represented in the sponge-associated communities. In several sponges, over 25% of 16S rRNA gene clones were derived from *Actinobacteria* and the presence of actinobacterial cells in the sponge mesohyl has been confirmed by fluorescent in situ hybridization studies. Diverse bacterial assemblages, including *Actinobacteria*, have also been found associated with corals by molecular community analysis, in mucus from the solitary coral *Fungia scutaria* and the massive coral *Platygyra lamellina* from the Gulf of Eilat, northern Red Sea. A combination of molecular and conventional microbiological techniques is being used to culture many invertebrate-associated bacteria to make them accessible for drug screening programs. In cases where marine invertebrates are known to contain important bioactive compounds, we are exploring the possibility that these drug leads may be produced by symbiotic bacteria. In two case studies, we have isolated bacteria that produce manzamines and kahalalides. *Micromonospora* sp. strain M42 from the Indonesian sponge *Acanthostrongylophora* produces manzamine A, an antimalarial lead first found in sponges. A symbiotic *Vibrio* sp. that produces kahalalide F was isolated from the mollusk *Elysia rufescens*. The isolation of symbionts will facilitate economic production of these compounds. Molecular approaches are utilized to isolate the biosynthetic clusters encoding the natural products. The outcomes of our work are two-fold: 1. Isolation of novel bacteria, including *Actinobacteria*, to provide an important resource for drug screening programs and 2. An approach to solve the limited supply of marine natural products by developing methods that can consistently be applied to culturing symbionts that produce bioactive compounds of biomedical importance.

A GLIMPSE AT THE “UNCULTURED” MICROBIAL MAJORITY- FROM IDENTIFICATION TO DOMESTICATION

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The application of molecular, sequence-based methods has revealed vast majority of previously uncharacterized and uncultivated microorganisms. Despite this, the lack of cultured microorganisms represents a bottleneck in advancement in the field of microbiology. The development of novel culturing techniques therefore, is a crucial step in our understanding of microbial diversity in general, and in its role in the environment in particular. This study presents an innovative high-throughput method for cultivating previously uncultured microorganisms by encapsulating them in agar spheres that are then encased in a polysulfonic polymeric membrane and incubated in simulated or natural environment. This method stimulates growth of the entrapped microorganisms by allowing them access to essential nutrients and cues from the environment and allows the retrieval of microorganisms from dilutions that are ten to one hundred fold greater than using conventional plating techniques. Analysis of microorganisms grown in such spheres incubated in and on a number of substrates yielded numerous novel ribotypes. For example spheres incubated on the mucus surface of a Fungiid coral, yielded numerous ribotypes. Of these ribotypes 50% shared 85%, to 96% similarity to previously identified microorganisms suggesting that they represent novel species and genera. Repeated transfers through agar spheres, and subsequent plating resulted in “bacterial domestication”. This novel double encapsulation technique combined with high throughput screening methods may provide a new revolutionary tool providing us with access to previously unknown microbial diversity and access bioactive compounds from the “uncultured” majority of microbial life.

POLYKETIDE BIOSYNTHESIS IN MEDITERRANEAN OPISTHOBRANCHS: FURTHER STEPS TOWARDS THE CHARACTERIZATION OF PKS ASSEMBLY IN MARINE MOLLUSCS

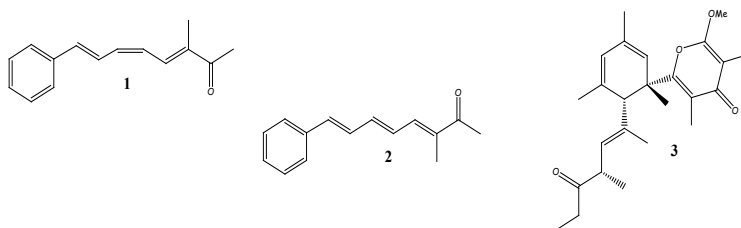
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During the past few years increasing interest has been paid to understand the origin of secondary metabolites in opisthobranch molluscs. These apparently harmless invertebrates are rarely prey of fish or other potential predators because of efficient strategies of defence that include the use of chemical weapons. Although often derived from diet, in few cases the defensive compounds are the end products of a synthetic ability acquired in the course of evolution. Polyketides are typical metabolites of species belonging to the order of Cephalaspidea and Sacoglossa, being usually recovered as regular or mixed acetate/propionate skeletons and occasionally featured by unusual starter unit. To date, the *de novo* origin of opisthobranch polyketides has been investigated only in few species, principally by using radiolabelled tracers. Recently we have discussed the involvement of PKS-like activities for alkylpyridine assembly in cephalaspidean species by feeding experiments with stable (²H, ¹³C) precursors.¹ In this communication we will present application of this technique for the elucidation of the biosynthesis of the polyketides from two Mediterranean molluscs, i.e. lignarenones (**1-2**) from the cephalaspidean *Scaphander lignarius*² and elysione (**3**) from the sacoglossan *Elysia viridis*.³ The results give account of biosynthetic pathways different from those described in fungi and most likely related to bacterial and animal PKS, providing further data on polyketide synthases in molluscs.



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SEARCH OF POLYKETIDE GENE CLUSTERS IN SPONGE METAGENOMES

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Many natural products from marine sponges have been suspected to be produced by symbiotic bacteria. Their cultivation usually fails, thus making their direct study and exploitation for sustainable drug production difficult. We follow a metagenomic approach to gain insights into the biosynthesis of polyketides and mixed polyketides/nonribosomal peptides. Total animal DNA containing all genomes is cloned to generate complex libraries, which are then PCR-screened for the gene clusters of interest. From *Theonella swinhoei*, a sponge harbouring enormous numbers of diverse bacteria, genes involved in the biosynthesis of the onnamides [1] were isolated from an 860,000 clone library [2]. Various versions of the onnamide system were found, which might explain why a single sponge contains numerous onnamide congeners. A bacterium of the phylum *Chloroflexi* has been identified as likely onnamide source. Large metagenomic libraries of the sponges *Cacospongia mycofijiensis*, *Suberites domuncula* and *Psammocinia* sp. have been constructed to study biosynthesis of further sponge polyketides. An unexpected finding was the isolation of a NRPS-PKS gene located on the genome of *S. domuncula*, suggesting that not all polyketides in sponges are made by symbionts. A discussion of ongoing work on the antitumor polyketide laulimalide and the antitumor mixed polyketide/nonribosomal peptide psymberin will highlight opportunities and technical challenges of current sponge metagenomics [3].

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**COLD-ADAPTED WATER-BORNE SIGNAL (PHEROMONE)
PROTEINS FROM AN ANTARCTIC MICRO-
EUKARYOTE, THE CILIATE *EUPLOTES NOBILII***

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The stably freezing (-1.8 °C) waters of the Antarctic coasts are inhabited by a huge variety of protozoan ciliates, among which *Euplotes* species dominate. Samples of these species can easily be collected and expanded into massive laboratory cultures, that feed on algae or bacteria in cold rooms where they reproduce true-to-type virtually indefinitely. They thus provide excellent experimental material to identify and characterize structurally and functionally cold-adapted molecules of eukaryotic origin. In this context, we have recently focused most interest on a family of small (from 52 to 63 residues), globular (4 intrachain disulfide bonds) signal proteins (designated as “pheromones” and having mitogenic and sexual activities), that strains of *E. nobilii* constitutively synthesize and secrete into the extracellular environment from which these molecules can rapidly be purified in good abundance. For three members (i. e., *En-1*, *En-2*, and *En-6*) of this protein family, we have determined the 3-D structures in solution by NMR spectroscopy. The comparison of these *En* structures with the NMR solution structures previously determined for a set of proteins representing the *Er* pheromone family (produced by *E. raikovi*, a species of temperate waters that phylogenetically is closely allied to *E. nobilii*) reveals how these protein families have strictly retained a common compact three-helix bundle core, on which the *En* molecules show to have adaptively imposed (to overcome the thermal constraints of the Antarctic waters) two unique, extended non-structured regions, one of which (spanning 10-12 residues) forms the molecule N-terminal extremity and the other (of 8-10 residues) connects the helices 1 and 2. By substantially improving, locally and globally, the flexibility of the molecular backbone, these two regions appear to greatly help the effective docking and binding of the *En* molecules to their target receptor proteins.

MASSADINE CHLORIDE: A BIOSYNTHETIC PRECURSOR OF MASSADINE AND STYLISSADINE

Achim Grube,¹ Stefan Immel,² Phil S. Baran,³ and Matthias Köck¹

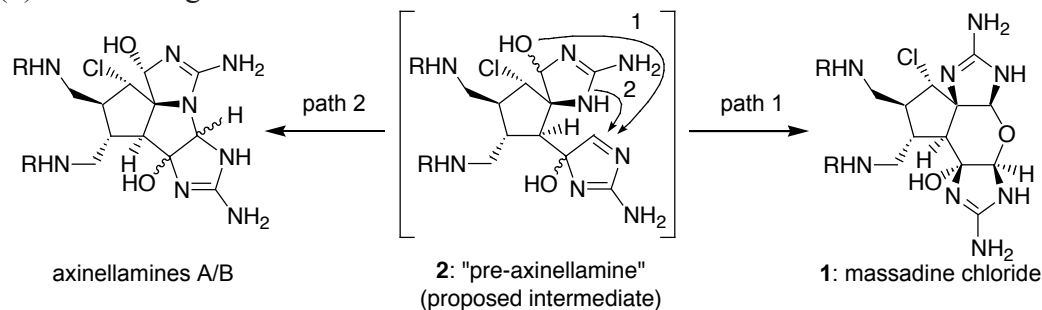
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To date about 30 dimeric pyrrole-imidazole alkaloids (PIA's) are known from the marine sponge families Agelasidae, Axinellidae, Dictyonellidae, and Hymeniacidonidae. The dimeric PIA massadine was isolated in 2003 from the marine sponge *Stylissa* aff. *massa*.^[a] Massadine is unique among the dimeric PIA's since a hydroxyl group resides in position 14 instead of a chlorine atom as in axinellamines^[b] (C-13) or palau'amines^[c]/styloguanidines^[d]/konbu'acidins^[e] (C-17). Therefore, the question arose if massadine is the natural product or the hydrolysis product of massadine chloride (**1**). The biosynthetic implications of isolating massadine chloride (**1**) would be two-fold. First, it would provide substantial evidence for a biosynthetic link to the axinellamines, palau'amines, and styloguanidines via "pre-axinellamine" **2**. "Pre-axinellamine" (**2**) is simply a ring-chain tautomer of massadine chloride (**1**) and axinellamine (see Scheme). Secondly, it would explain the existence of both the unusual hydroxyl group at C-14 of massadine and the stylissadines A and B^[f], which are the pinnacle of complexity within the PIA family. The chemical stability of the isolated massadine chloride (**1**) was investigated under different conditions.



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CHEMICAL ECOLOGY

Tuesday
18th September, 2007

SESSION PROGRAMME

11.40	IL-6	ALLAN CEMBELLA	CHEMICAL ECOLOGY OF DINOFLAGELLATE INTERACTIONS IN THE SEA: PHARMACOLOGY DOES NOT FOLLOW FUNCTION
12.10	OR-1	GEORG POHNERT	YOU ARE WHAT YOU EAT? FOOD QUALITY CONSEQUENCES OF RAPID LIPID METABOLISM OF DIATOMS
12.30	OR-2	GIOVANNA ROMANO	LOX-INDUCED LIPID PEROXIDATION AS MECHANISM RESPONSIBLE FOR THE DETRIMENTAL EFFECT OF MARINE DIATOMS ON ZOOPLANKTON GRAZERS
12.50	OR-3	JONATHAN SPENCER	STEREOCHEMICAL UNIFORMITY IN MARINE POLYETHER LADDERS – IMPLICATIONS FOR THE BIOSYNTHESIS AND STRUCTURE OF MAITOTOXIN
13.30			LUNCH
15.00	IL-7	JULIA KUBANEK	CHEMICALLY-MEDIATED INTERACTIONS AMONG MARINE COMPETITORS, HOSTS, AND PATHOGENS
15.30	OR-4	CONXITA AVILA	ANTARCTIC MARINE CHEMICAL ECOLOGY: WHAT HAS BEEN DONE AND WHAT'S NEXT?
15.50	OR-5	JOSEPH R. PAWLIK	TESTING AN HYPOTHESIS ON SPONGE CHEMICAL DEFENSES USING THE WRECK OF THE <i>USS SPEIGEL GROVE</i>
16.10	OR-6	ERNESTO MOLLO	THE CONTRIBUTION OF CHEMICAL ECOLOGY TO AN UNDERSTANDING OF FUNCTIONAL MORPHOLOGY AND EVOLUTION
16.30	OR-7	OLIVIER THOMAS	SPONGE CHEMICAL DEFENCES IN STRESS CONDITIONS: THE CASE STUDY OF THE LAST DISEASE OUTBREAK OBSERVED IN THE NW MEDITERRANEAN

CHEMICAL ECOLOGY OF DINOFLAGELLATE INTERACTIONS IN THE SEA: PHARMACOLOGY DOES NOT FOLLOW FUNCTION

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Dinoflagellates represent an ancient lineage of eukaryotic microorganisms (protists) with many unique features including multiple nutritional modes and the capacity to synthesize unusual natural products. Free-living marine dinoflagellates are a rich source of secondary metabolites – unusual sterols and a diversity of highly bioactive metabolites, such as the phycotoxins associated with harmful algal blooms (HABs). These phycotoxins comprise a variety of guanidine derivatives, secondary amino acids, cyclic imines and linear and macrocyclic polyether toxins. The pharmacological and toxicological properties of most phycotoxins are well described, as Na-channel blockers, Ca-channel activators, glutamate agonists, phosphatase inhibitors, etc. Nevertheless, despite recent advances in structural elucidation and the determination of biosynthetic pathways, relatively little is known about the structural/functional relationships of these secondary metabolites. Based upon their high specific potency, various hypotheses relating to their *in situ* role in chemical ecology have been advanced. These hypotheses include putative roles as allelopathic agents against competitors, as pheromones (sexual attractants), and as chemical defence mechanisms against predators. Comparison of toxin composition among dinoflagellate populations typically reveals a high degree of structural polymorphism and the expression of geographically distinct profiles. Yet current evidence suggests that allelochemical potency of dinoflagellates is not directly correlated with cell quota of phycotoxins.

Dinoflagellates pose severe challenges for whole genomic sequencing for toxicogenomic and gene expression studies because nuclear DNA complement can exceed 200 gbp. Therefore the limited genomic approach involving “expressed sequence tags” (ESTs) and microarrays have proven most promising for specific gene hunting and expression studies. Genetic analysis based on EST sequencing has revealed several putative genes for polyketide synthase (PKS), the key enzymes involved in the biosynthesis of polyether toxins in marine dinoflagellates. A definitive function for known phycotoxins has not yet been attributed, but hypotheses concerning evolutionary significance and chemical ecology are being addressed.

CHEMICALLY-MEDIATED INTERACTIONS AMONG MARINE COMPETITORS, HOSTS, AND PATHOGENS

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How individual species come to be dominant members of planktonic or benthic communities is not deeply understood; however, it is thought that chemistry plays a substantial role. For example, some red tide-forming dinoflagellates produce toxic secondary metabolites that are hypothesized to enhance dinoflagellate fitness by acting as grazer deterrents, allelopathic agents, or antimicrobial defenses. Similarly, benthic macroalgae produce a diversity of natural products that could serve as protection against pathogens or biofouling.

Marine pathogens frequently target benthic macroalgae in a species-specific manner, with some seaweeds appearing to escape disease altogether despite the prevalence of pathogenic microbes in the ocean. We have identified 20 novel bioactive natural products from collections of the Fijian red macroalga *Callophycus serratus*, all of which share a diterpene-shikimate biosynthetic origin. Analysis of multiple *C. serratus* populations revealed 2 distinct chemotypes, each consisting of 10 compounds. Compounds of both chemotypes provided protection against infection by a pathogenic marine fungus.

Recent field and lab experiments have shown that the red tide dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*) is allelopathic to several co-occurring phytoplankters, but that *K. brevis* natural products other than well-known brevetoxins are responsible for suppressing most of these species. Mechanisms of action of *K. brevis* allelopathy include increased cell membrane permeability and reduced photosynthetic output. At least one phytoplankton competitor, *Skeletonema costatum*, retaliates against *K. brevis*, reducing its allelopathic effects and degrading waterborne brevetoxins. Our results indicate that chemically-mediated interactions are reciprocal, and that ecosystem-level consequences of red tides (such as fish kills caused by waterborne toxins) may depend upon which non-blooming phytoplankton species are present.

YOU ARE WHAT YOU EAT? FOOD QUALITY CONSEQUENCES OF RAPID LIPID METABOLISM OF DIATOMS

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Lipid and fatty acid composition are considered key parameters determining nutritive quality of phytoplankton diets for zooplanktonic herbivores.¹ Fitness, reproduction and physiology of the grazers are influenced by these factors. The trophic transfer of lipids and fatty acids from algal cells has been typically studied using simple extraction and quantification approaches, which, as we argue here, do not reflect the actual situation in the plankton. We show that cell disruption as it occurs during grazing by a predator on diatoms can drastically change the lipid and fatty acid content of the food. In some algae, a rapid depletion of polyunsaturated fatty acids is observed within the first minutes after cell disruption. This fatty acid depletion is directly linked to the production of polyunsaturated aldehydes, molecules discussed as being involved in the chemical defence of the algae.² The biosynthesis of these aldehydes follows diverse pathways, which will be discussed here,^{3,4} involving fatty acids that are considered to be essential to the herbivores. Diatoms are even capable of transforming lipids from other sources if these are available in the vicinity of the wounded cells. Fluorescent staining reveals that the enzymes involved in lipid transformation are active in the foregut of copepods therefore linking the depletion processes directly to the food uptake. Incubation experiments with the calanoid copepod *Temora longicornis* showed that PUFA depletion in PUA-producing diatoms is correlated to reduced hatching success and can be compensated by externally added single fatty acids.

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LOX-INDUCED LIPID PEROXIDATION AS MECHANISM RESPONSIBLE FOR THE DETRIMENTAL EFFECT OF MARINE DIATOMS ON ZOOPLANKTON GRAZERS

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During last few years evidences has been reported showing that many marine diatoms species negatively impact the reproduction of their grazers such as copepods (1-4). This phenomenon has been explained in terms of chemical defence due to grazer-induced synthesis of polyunsaturated aldehydes (PUAs) that, unlike other deterrent metabolites, are of low acute toxicity to adult or mature predators but depress viability of gametes and offspring.

More recently this explanation turn out to be inadequate to describe the effect of diatom diet on copepod reproduction, mostly in those cases in which, despite the lack of PUAs, diatoms are able to induce egg hatching reduction and abnormalities in newborns (6, 7). We propose a new and more comprehensive explanation to this phenomenon, including a wide plethora of oxylipin products and hydroperoxide produced by lipoxygenase activities on C₁₆ and C₂₀ polyunsaturated fatty acids in three marine diatom species, namely *Chaetoceros affinis*, *Chaetoceros socialis* and the PUA producing diatom species *Skeletonema marinoi*. Synthesis of these molecules is species-specific even if the biochemical pathways of the three diatoms involve a parallel set of transformations that, after crushing of the cells, trigger an oxidative stress status characterized by massive lipid peroxidation and the presence of highly reactive oxygen species. Our study represents the first documentation of oxidative stress as the major metabolic mechanism for the reproductive dysfunction of grazing copepods.

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STEREOCHEMICAL UNIFORMITY IN MARINE POLYETHER LADDERS – IMPLICATIONS FOR THE BIOSYNTHESIS AND STRUCTURE OF MAITOTOXIN

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The largest known, non-polymeric natural product, maitotoxin, is a 3422 Da polyketide-derived polycyclic ether isolated from the marine dinoflagellate, *Gambierdiscus toxicus*. The structure of maitotoxin consists of four extended ring-fused systems termed polyether ladders. The family of marine polyether ladders can be grouped into 14 backbone structures and has gained much notoriety in being responsible for countless cases of human food poisoning and massive fish kills. Maitotoxin itself displays the highest toxicity of any non-proteinaceous natural product isolated thus far. Indeed, toxicity is characteristic of this family of natural products. Brevetoxin is the most studied of the ladder polyethers, and a hypothetical model for its biosynthesis was first proposed by Shimizu and Nakanishi. This was based upon the widely accepted polyepoxide cascade mechanism for monensin biosynthesis, a terrestrial polyether antibiotic, as put forward by Cane, Celmer and Westley. Examination of all known polyether ladders has revealed a uniform stereochemical feature that has allowed us to propose a simple model, using the polyepoxide premise, which can account for their biosynthesis. This model has also exposed a stereochemical inconsistency in one ladder section of maitotoxin, which we propose should prompt a re-examination of its assigned structure.

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ANTARCTIC MARINE CHEMICAL ECOLOGY: WHAT HAS BEEN DONE AND WHAT'S NEXT?

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Antarctic ecosystems are exposed to unique environmental characteristics resulting in communities being structured both by biotic interactions such as predation and competition, as well as abiotic factors such as seasonality and ice scouring. It is important to understand how ecological factors may trigger chemical mechanisms in marine Antarctic organisms as a response for survival. However, very little is known yet about the evolution of chemical compounds in Antarctic organisms. Chemical ecology investigations have demonstrated over the last years that defensive metabolites have evolved in numerous representative Antarctic species. This contradicts early theories concerning biogeographic variation in predation and chemical defenses. As reviewed here, a number of interesting natural products have been isolated from Antarctic organisms. However, we believe many more are still to be discovered. Currently, many groups such as microorganisms, planktonic organisms and deep fauna remain almost totally unknown regarding their natural products. Furthermore, for many described compounds, ecological roles have yet to be evaluated. In fact, much of the research carried out to date has been conducted in the laboratory, and only in a few cases in an ecologically relevant context. Therefore, there is a need to extend experiments to the field, as done in tropical and temperate marine ecosystems, or at least, to test the activity of the chemicals in natural conditions and ecologically meaningful interactions. Defense against predators is always one of the main topics when talking about natural products roles in species interactions, but many other interesting aspects, such as competition, chemoattraction, fouling avoidance and UV-protection, also deserve further attention. In our opinion, challenging future developments are to be expected for Antarctic marine chemical ecology in the years to come.

TESTING AN HYPOTHESIS ON SPONGE CHEMICAL DEFENSES USING THE WRECK OF THE *USS SPIEGEL GROVE*

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On 11 June 2002, the 155 m long, decommissioned U.S. Navy Landing Ship Dock *Spiegel Grove* was intentionally sunk as an artificial reef in 40 m of water off Key Largo, Florida, USA. After discovering that sponges had recruited to the wreck, we surveyed the three decks of the vessel in November 2006. On average, sponges covered 5.7, 12.0 and 6.8% of the fore, mid, and aft decks, respectively. The most abundant sponges were *Iotrochota birotulata* and *Holopsamma helwigii* (2.3 and 2.1%), followed by *Niphates erecta* and *Callyspongia* spp. (0.3 and 0.2%), with other species occurring <0.05%. Five of the largest sponges of each of the 8 most abundant species were collected and their volumes determined. Individuals of *Callyspongia* spp. were largest, with a mean of 1900 ml, followed by *Holopsamma helwigi* (587 ml), *Mycale laxissima* (572 ml), and *Iotrochota birotulata* (347 ml). The most abundant and fastest growing sponges on the wreck were those that are not chemically defended from fish predators¹, yet the adjacent reefs have a mixed population of chemically defended and undefended sponges. In addition to providing important data on sponge growth rates, these results corroborate the resource allocation trade-off hypothesis that two classes of sponges occur on Caribbean reefs: chemically undefended species that grow rapidly and reproduce prolifically, and chemically defended species that grow more slowly and reproduce less frequently^{2,3}.

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THE CONTRIBUTION OF CHEMICAL ECOLOGY TO AN UNDERSTANDING OF FUNCTIONAL MORPHOLOGY AND EVOLUTION

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Chemical ecology has greatly contributed to the understanding of evolutionary patterns and processes amongst the so-called “sea slugs” (Mollusca: Gastropoda: Opisthobranchia). Chemical defense has played a primary role in the evolution of the group, and preceded the regression of the external shell and its function in mechanical defense.¹⁻⁴ With the complete loss of the shell in nudibranchs, the primitive function of the mantle of producing the shell has been lost. In some groups this has led to great elaboration of the mantle with the development of papillae, cerata and tubercles, while in others there has been a tendency towards a reduction in its size and importance. Within the genera of the family Chromodorididae, a gradual mantle skirt reduction in relative size can be observed in a series from *Glossodoris* and *Chromodoris*, through *Hypselodoris* and *Risbecia*, to *Ceratosoma* where the mantle skirt is almost completely absent. Chromodorid mantle glands, for which a defensive role has been suggested, are also arranged in a characteristic way and in distinct areas of the mantle in each genus.⁵⁻¹¹ In this report we describe recent findings on chromodorids collected along the Chinese coast. A combination of chemical analysis on different mantle sections and feeding deterrence assays allowed us to clarify the functional significance of the diversified mantle morphologies within the Chromodorididae.

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SPONGE CHEMICAL DEFENCES IN STRESS CONDITIONS: THE CASE STUDY OF THE LAST DISEASE OUTBREAK OBSERVED IN THE NW MEDITERRANEAN

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The increase in frequency of epizootic events and mass mortalities has recently been connected with the warming of the NW Mediterranean. This study focuses on *Spongia officinalis* particularly threatened by the climatic warming. The general goal was to acquire fundamental data about the biology of this sponge and on the damage mechanism to its health. We studied the expression of indicators of the resistance capability such as heat shock proteins (HSP), a chaperon protein essential in maintaining homeostasis. We also studied the production of secondary metabolites implied in chemical defences. We looked for these early markers in samples taken during the diseases of summer 2003.

The analysis of the two markers showed opposite trends. The HSP70 expression increases 2.5 times in the disease context while the natural toxicity of secondary metabolites decreases 1.8 times. To go further into the comprehension of the fluctuation of the natural toxicity, chemical studies were undertaken. Two known furanoterpenes were isolated as the major chemical constituents of the sponge. The fluctuation of their production was followed by quantitative HPLC and the data obtained showed that the expression of these secondary metabolites was indeed lowered during the disease context. We concluded that *S. officinalis* allocates more energy in maintaining homeostasis than in producing toxic compounds. A reduction of the defence capability of the sponge would then favour the virulence of pathogens.

The disease considered in this study occurred during a positive thermal anomaly in late summer 2003. The observed effects on HSP 70 expression, on natural toxicity, and on the production of chemical defences could therefore be connected to the increase of temperature.

**NPR 2007
AWARD**

Wednesday
19th September, 2007

METABOLITES OF MARINE AND TERRESTRIAL SYMBIONTS

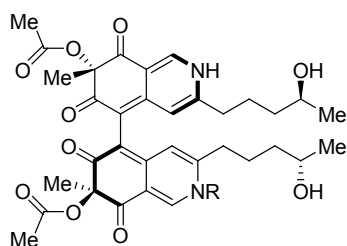
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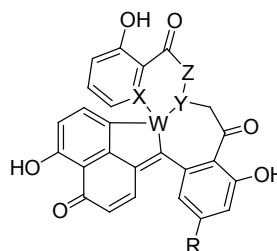
Growing evidences indicate that the symbiont seems to be widespread in a plant or animal species. Biologically, a symbiont is either bacterium (including representatives in actinomycetes) or fungus spending the whole or part(s) of their life span by colonizing inter- and/or intra-cellularly inside normal host tissues. It has been well documented that the symbiont plays a multiple physiological and ecological roles in the process of microbe-host and symbiont-host-environment interactions. The colony of some symbionts has been disclosed to be able to improve or initiate the host growth through improving the tolerance of the host to environmental stresses such as drought, salinity, heavy metals as well as attacks of or consumptions by microbial pathogens, nematodes, insects and mammal herbivores. Biochemically, an expanding pile of data has demonstrated that the ‘host-helping’ effects of symbionts are ascribable to the production of bioactive compounds. In the viewpoint of bio-resource, symbionts could be accepted as a slightly opened reservoir of ‘special microorganisms’ that must be a rich source of new bioactive natural products.

This presentation will mainly deliver the new findings about the topic as exemplified by the given novel bioactive compounds, and try to highlight the trend and significance of the field.



chaetomiumin A R = H

chaetomiumin B R = -CH₂CH₂OH



daldine A, W-X=—, Y-Z=—, R=H

daldine B, W-X=—, Y-Z=—, R=OH

daldine C, W-X=·····, Y-Z=·····, R=H

daldine D, W-X=·····, Y-Z=·····, R=OH



Ren Xiang Tan, born in 1960 in Jiangsu, China. China Pharmaceutical University (B 1983; M 1986), Lanzhou University (Ph D 1990, Prof. Z. J. Jia), Technical University of Berlin (Visiting Ph D candidate, 1989-1990, Prof. F. Bohlmann), University of Lausanne (Visiting scholar, 1995 and 1997, Prof. K. Hostettmann), University of California, San Diego (Visiting scholar, 2001 and 2003, Prof. W. Fenical), Nanjing University (Associate Professor, 1992; Professor, 1994-present), International Society for the Development of Natural Products (President, 2005-2007). Research filed: natural product chemistry & microbe-host chemical communication/interaction.

BIOPROSPECTING

Thursday
20th September, 2007

SESSION PROGRAMME

09.00	IL-8	ANA MARTINEZ	FROM THE SEA TO THE TREATMENT OF ALZHEIMER'S DISEASE
09.30	OR-1	GIUSEPPE BIFULCO	DESIGN, VIRTUAL SCREENING AND SYNTHESIS OF NEW HDAC INHIBITORS
09.50	OR-2	MARISA RANGEL	A NATURAL PEPTIDE FROM THE MARINE SPONGE <i>GEODIA CORTICOSTYLIFERA</i> ACTS UPON MORPHOLOGY, MIGRATION AND INVASION OF BREAST CANCER
10.10	OR-3	MARIA CHIARA MONTI	MOLECULAR BASIS OF HUMAN GROUP IIA PHOSPHOLIPASE A ₂ INHIBITION BY MARINE NATURAL PRODUCTS
10.30	IL-9	JOSE JIMENO	YONDELIS® (TRABECTEDIN): MAJOR CLINICAL IMPACT OF A MARINE ANTICANCER COMPOUND IN THE ERA OF TARGETED THERAPIES
11.10			COFFEE BREAK
11.40	OR-4	HEONJOONG KANG	LIGANDS OF NUCLEAR RECEPTORS AS POTENTIAL DRUG LEADS
12.00	OR-5	JULIA JACOB	ANTIBIOTIC ALTERSOLANOL DERIVATIVES FROM A MANGROVE-DERIVED FUNGUS
12.20	OR-6	EBEL RAINER	NEW CYTOTOXIC α -PYRONE DERIVATIVES FROM THE SPONGE-DERIVED FUNGUS <i>PETRIELLA</i> SP.
12.40	OR-7	HENDRIK GREVE	NEW IANTHERANS FROM THE MARINE SPONGE <i>IANTHELLA QUADRANGULATA</i> : NOVEL AGONISTS OF THE P2Y ₁₁ RECEPTOR

FROM THE SEA TO THE TREATMENT OF ALZHEIMER'S DISEASE

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During the last twenty years, approximately the half of the drugs introduced in the market derived directly or indirectly from small molecules of natural origin.¹ Most of these drugs were isolated from terrestrial sources but in the recent years the marine environment has been shown to provide a very rich source of chemical structures with promising biological activities in antitumour, anti-inflammatory, analgesia, immunomodulation, allergy and antiviral assays. A growing number of these compounds are entering clinical trials and thus, the impact of this field on the biomedical industry is increasing.² Moreover, new therapeutic fields, such neurology, are finding new promising drugs from the sea.^{3 4}

Alzheimer's disease (AD), the most common cause of dementia among the elderly, is a progressive, degenerative disorder of the brain characterised by the loss of memory, and intellectual ability, accompanied by behavioural disturbances and decreasing ability to perform basic activities of daily living. Worldwide, the disease is estimated to affect more than 15 million individuals, being alarming the social and economic consequences of the disease due to the increase in life expectancy. Although the key neuropathological features of the disease are well known, that are extracellular β -amyloid (A β) containing plaques, intracellular neurofibrillary tangles composed of abnormally hyperphosphorylated tau, and degeneration of cholinergic neurons of the basal forebrain, no efficient therapeutic treatment exists in the market up to now. However, great efforts are performing in the search for effective therapeutic treatments that interfere with the neurodegenerative process.

Marine or marine-derived compounds have recently started pharmaceutical development programs in this field like GSK-3 inhibitors,⁵ BACE inhibitors, neuroprotectants or nicotinic modulators offering a great promise for the future treatment of Alzheimer's disease.

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YONDELIS® (TRABECTEDIN): MAJOR CLINICAL IMPACT OF A MARINE ANTICANCER COMPOUND IN THE ERA OF TARGETED THERAPIES

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Yondelis has obtained a positive opinion from the EMEA as therapy for patients with advanced pre-treated sarcoma (STS). This is the first regulatory approval in this setting in the past 25 years: A comparative study in advanced pre-treated STS has confirmed a major impact of the proposed dose (1.5 mg/m²) and schedule (24 hours iv infusion) thus validating the previous phase II results. The median survival, 13.8 months, attained with the infusional regimen, is better than the one achievable with first line therapy; the progression free survival at 6 months, 37%, is superior to the 14% proposed as cut-off for active agents; 50% of the cases treated with the infusional schedule have shown its tumour shrinkage. The safety profile continues to sustain it's feasible for chronic administration with a lack of hair loss, mucositis, diarrhea and complicated neutropenia. There is now major interest to dissect it's potential in specific sarcoma sub-types. New data demonstrates a dramatic impact in patients with advanced pre-treated mixoid liposarcoma. A 90% rate of long lasting, median progression free survival of 14 months, objective remissions and tumour control has been recently reported. Mixoid liposarcoma relates to a reciprocal 12:16 translocation that leads to a chimeric, FUS-CHOP, DNA binding protein that act as a transcription factor. Early data indicates the capability of Yondelis to down- regulate the activity of genes that are FUS dependent. A new project is seeking to characterize the clinical potential of Yondelis in other translocation related sarcomas. Additionally phase II results demonstrate a 70% rate of long lasting tumour control in advanced relapsed ovarian ca and a phase III registration study comparing liposomal doxorubicin+Yondelis vs Yondelis has been completed. Moreover the pharmacogenomic program has confirmed a molecular signature, based on a non functional homologous recombinant DNA repair and an effective nucleotide excision repair that clusters both sensitivity and resistance to Yondelis. The transtumoral impact of this specific DNA repair profiling is being evaluated, in prostate and in breast cancer, including a patient's enrichment clinical study focusing on triple negative and BRCA mutant patient. In conclusion Yondelis is the standard of care in pre-treated sarcoma represents a major step ahead in contemporary anticancer intervention.

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DESIGN, VIRTUAL SCREENING AND SYNTHESIS OF NEW HDAC INHIBITORS

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Raffaele Riccio and Giuseppe Bifulco

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In the last years the epigenetic therapy has played a key role in carcinogenesis and tumour progression, and one of most important enzyme involved in these processes, the histone deacetylase (HDAC) ¹ has recently been the target of several research groups. Our study has been focused on the virtual screening of synthetic analogous of azumamide E, ² a new marine cyclotetrapeptidic inhibitor of HDAC, with the aim of designing high affinity derivatives. The structural design has been based on SAR considerations derived from the three dimensional model of azumamide E (1) bound to the histone deacetylase like-protein (HDLP).³ While the macrocyclic structure and the side chain has been left unaltered, position 12 (D-Phe) and 24 (D-Val) have been modified, and nine different skeletons have been designed in order to have a faster synthetic protocol. The affinity constants of this library have been calculated using Autodock 3.0.5⁴ software, and have been compared to the K_i of the natural compound (1) calculated at the same level. Such virtual screening has suggested the synthesis of derivatives **2** and **3**, which have been shown to be the most promising for their predicted activity.

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A NATURAL PEPTIDE FROM THE MARINE SPONGE *GEODIA CORTICOSTYLIFERA* ACTS UPON MORPHOLOGY, MIGRATION AND INVASION OF BREAST CANCER CELLS

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Cell lines grown in three-dimensional (3D) culture conditions recapitulate essential features of the tissue *in vivo*. Tissue manipulated in 3D conditions is considered tractable cell-based models that allow investigations of possible new drugs and therapies. We are currently investigating effects of the depsipeptide geodiamolide H, isolated from the Brazilian sponge *Geodia corticostylifera*, on cancer cell lines growth in a 3D environment. As shown previously the geodiamolide H disrupts actin cytoskeleton in both sea urchin eggs and breast cancer cell monolayers ¹. We used a normal mammary epithelial cell line MCF10A that in 3D assay results formation of polarized acini-like spheroids. We also used cell lines derived from breast tumors with different degrees of differentiation and malignancy: MCF-7 positive for estrogen receptor and the pleomorphic Hs578T, negative for hormone receptors. Cells were grown embedded into Matrigel. Spheroids obtained from these cultures were treated at the 10th day with the geodiamolide H (20, 120 and 360 nM) during 48h. Control and treated samples were analyzed by light microscopy (H&E), and by laser scanning confocal microscopy of whole-mount preparations stained with rodamine-phalloidin. The geodiamolide H affected only the poorly differentiated and aggressive Hs578T cells grown in Matrigel. The peptide induced phenotypic modifications in this cell line, mostly characterized by disruption of the actin cytoskeleton. This result has prompted us to investigate the effect of the geodiamolide H on the migration and invasion of MCF10A and Hs578T cells through time-lapse video microscopy and invasion assays. Time-lapse microscopy showed that the peptide inhibited the migration of Hs578T in a dose-dependent manner, and invasion assays using modified Boyden chambers revealed that the geodiamolide H induced a 30% decrease on the invasive behavior of Hs578T cells. MCF10A migration and invasion patterns were not altered by treatment. Our results indicate that the geodiamolide H preferentially affected a more aggressive breast cancer cell lineage in 3D conditions, which resemble *in vivo* tissue. Furthermore, the peptide reduced Hs578t cells migration and invasion features, characteristics related to metastasis processes *in vivo*. The fact that normal cell lines were not significantly influenced by the treatment with the geodiamolide H stimulates new studies towards medicinal use for this peptide.

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MOLECULAR BASIS OF HUMAN GROUP IIA PHOSPHOLIPASE A₂ INHIBITION BY MARINE NATURAL PRODUCTS

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and Raffaele Riccio

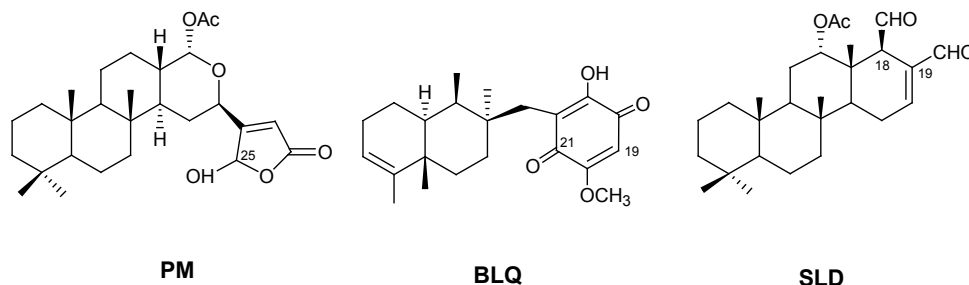
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Marine terpenoids containing γ -hydroxybutenolide, hydroxyquinone or functional aldehydes moieties are well known to specifically inhibit PLA₂ enzymes^[1]. Among them, Petrosaspongiolides M-R (PM-PR), Bolinaquinone (BLQ) and Scalaradial (SLD) has been investigated in our laboratory to clarify their mechanism of bee venom PLA₂ (*bv*PLA₂) and human group IIA PLA₂ (sPLA₂-IIA) inhibition. The ultimate goal of our study is a deep comprehension of the action of these agents for a rational design of simplified analogs as selective PLA₂ inhibitors. All these bioactive metabolites were pharmacologically screened: PM showed an IC₅₀ of 1.6 μ M toward sPLA₂-IIA and it was able to reduce PGE₂, TNF α , and LTB₄ levels and to modulate inducible COX-2 and iNOS expression^[2].

Firstly, we studied the *in vitro* inactivation of *bv*PLA₂, revealing the key step of the inhibition as the non-covalent hydrophobic anchoring into the enzyme active site, guided by the catalytic calcium ion chelation. Besides, a minor role was depicted for the covalent reaction due to the binding of a *bv*PLA₂ amino group with the electrophilic moieties of our molecules.

More recently, the inhibition mechanism of sPLA₂-IIA by PM has been investigated by circular dichroism, fluorescence, surface plasmon resonance, mass spectrometry and *in silico* docking calculation. From the gathered data we postulate an unusual mode of PM action, through a dual inhibition. Indeed, we propose that the non-covalent recognition process, based on VDW/electrostatic contacts, involves the active site of sPLA₂-IIA, whereas the covalent modification of PM occurs at the enzyme-membrane interfacial binding surface.



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LIGANDS OF NUCLEAR RECEPTORS AS POTENTIAL DRUG LEADS

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The farnesoid X-activated receptor (FXR) and the liver X receptors (LXRs), and the peroxisome proliferator-activated receptors (PPARs), nuclear receptors, regulate glucose and lipid metabolism in human.¹ Absorbed and accumulated dietary cholesterol is metabolized to bile acids in liver, and they are excreted into the intestine and removed through fecal elimination. In this metabolism, CYP7A1 gene, the rate limiting enzyme in bile acid biosynthesis, is stimulated by activated LXRs, while bile acids-activated FXR represses the expression of CYP7A1 and increases re-absorption of bile acids from the intestine to the liver. LXR agonists and FXR antagonists thus can be drug leads for treatment of dyslipidemia. Among the three PPAR subtypes (α , γ , and δ), targeted activation of PPAR δ in adipocytes and skeletal muscle showed that PPAR δ serves as a widespread regulator of fat burning and a key transcriptional factor regulating muscle fiber plasticity.² It is therefore a novel drug target to treat metabolic syndrome through increasing oxidative capacity in skeletal muscle.

We have discovered fifteen FXR antagonists and one LXR agonist from marine organisms, some of which are more potent than well-known FXR antagonists, guggulsterones, in clinical use in India. A combination of natural product isolation, chemical modification and automated bioassay also led to identification of several compounds as agonists with nano-molar affinities for the nuclear receptor PPAR δ . The ligands have over 100,000-fold selectivity toward PPAR δ over the other subtypes, PPAR α and PPAR γ . Pharmacological treatment of mice with newly developed PPAR δ -selective agonists mimicked physical exercise for the first time through altering muscle fiber type, thus increasing physical endurance. The treated mice increased running time and distance 2.9- and 3.0-fold, respectively, compared to control mice.

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ANTIBIOTIC ALTERSOLANOL DERIVATIVES FROM A MANGROVE-DERIVED FUNGUS

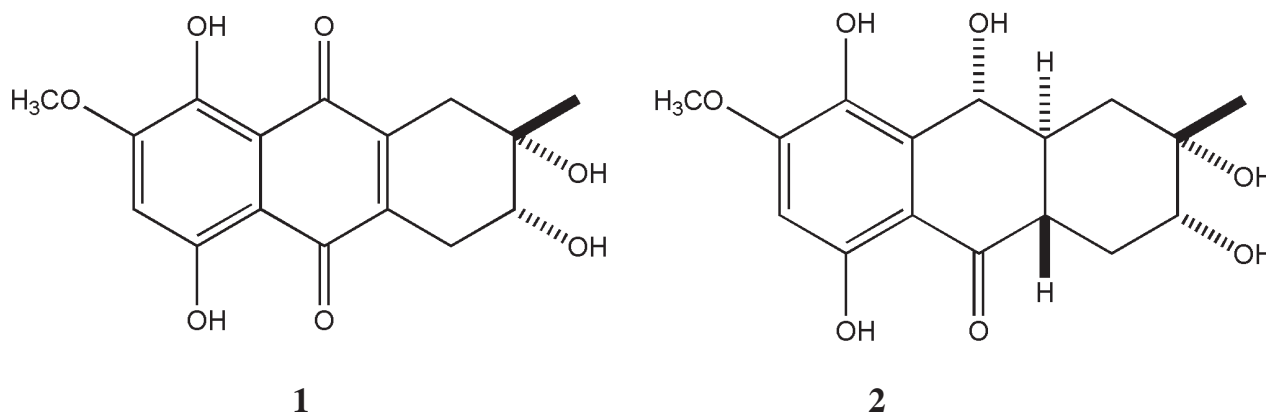
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Screening for antibiotic and antifungal activities of crude extracts of fungal strains obtained from the mangrove plant *Bruguiera sexangula* collected in Dongzhai Mangrove Garden, Hainan, China, revealed one extract from an as yet unidentified endophytic fungus, that exhibited good antibiotic activity against *Bacillus subtilis*.

Chemical analysis showed the presence of anthracinone derivatives, the known deoxybostrycin **1** [1] and the hitherto unreported semichinone **2**. The structure and relative configuration of **2** was assigned by analysis of MS, 1D and 2D NMR data, which showed close structural similarity to the previously described tetrahydroaltersolanol B [2, 3]. Further biological tests revealed that the activity observed for the crude extract was due to the presence of both compounds **1** and **2**.



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NEW CYTOTOXIC α -PYRONE DERIVATIVES FROM THE SPONGE-DERIVED FUNGUS *PETRIELLA* SP.

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During a systematic evaluation of fungal strains obtained from the Mediterranean sponge *Suberites domuncula* collected at different locations in the Adriatic Sea near Rovinj, Croatia, an isolate of a previously undescribed *Petriella* sp. caught our attention due to the striking cytotoxic activity of its crude extract. Chemical analysis revealed the presence of the known cyclic tetrapeptide WF-3161 [1] (**1**) and three new α -pyrone derivatives (**2**, **3** and **4**) related to infectopyrone which had previously been described from *Alternaria infectoria* [2]. When tested for their cytotoxic properties, compound **2** exhibited pronounced activity against the L5178Y cell line, while congeners **3** and **4** only showed moderate activity. WF-3161 (**1**) displayed exceptionally strong activity with an ED₅₀ value < 0.1 ug/ml.

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NEW IANTHERANS FROM THE MARINE SPONGE *IANTHELLA QUADRANGULATA*: NOVEL AGONISTS OF THE P2Y₁₁ RECEPTOR

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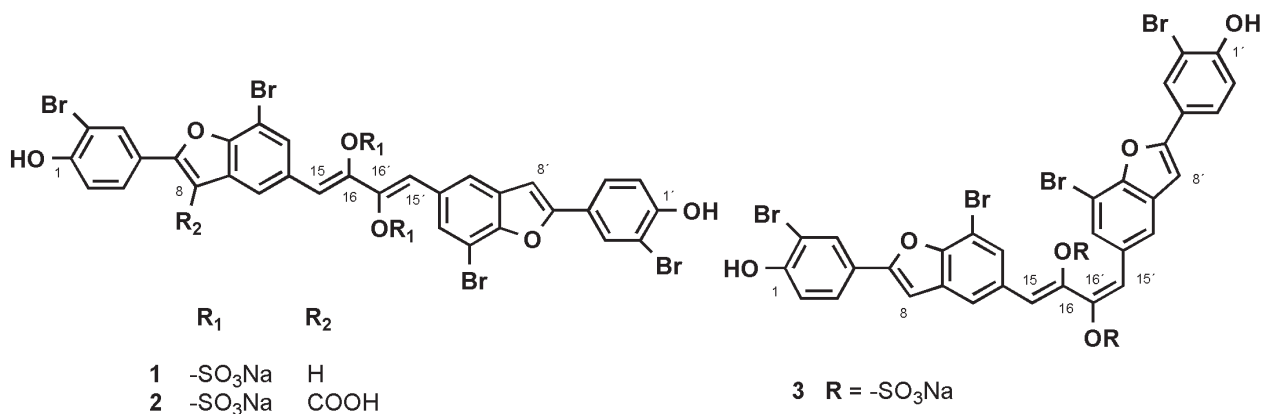
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Human P2Y₁₁ receptors belong to the G-protein coupled purine receptors.¹ P2Y₁₁ receptors are naturally activated by ATP and seem to play a role in the human immune system.^{2,3} To date hardly any selective ligands for these receptors are known.

The newly identified iso-iantheran A (**1**) and 8-carboxy-iso-iantheran A (**2**), isolated from the polar extract of the marine sponge *Ianthella quadrangulata*, exhibited agonist activity at P2Y₁₁ receptors with EC₅₀ values of 1.29 μM and 0.48 μM, respectively. Compound **2** showed selectivity for P2Y₁₁ over P2Y₁ and P2Y₂ receptors. **1** and **2** are the first secondary metabolites acting as agonists at P2Y₁₁ receptors and provide a new structural skeleton for further ligand development.

The new iantherans, iso-iantheran A (**1**), 8-carboxy-iso-iantheran A (**2**) and iso-iantheran B (**3**) possess a dimeric benzofuran skeleton including a 2,3-dihydroxy-1,3-butadiene disulfate moiety which is a unique feature of iantherans.⁴



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MARINE TOXINS

Thursday
20th September, 2007

SESSION PROGRAMME

15.00	IL-10	MICHIO MURATA	YESSOTOXIN AND SYNTHETIC POLYETHER MODELS – INTERACTION WITH MEMBRANE PEPTIDES
15.30	IL-11	PHILIPPE HESS	BIOACTIVE COMPOUNDS IN SHELLFISH - DRUG DISCOVERY OR FOOD SAFETY MANAGEMENT?
16.00	OR-1	ANTONIO DARANAS	NOVEL POLYETHER METABOLITES FROM DINOFLAGELLATES
16.20	OR-2	MARTINO FORINO	RECENT INSIGHTS INTO HARMFUL ALGAL BLOOMS ALONG THE ADRIATIC COASTLINE
16.40	OR-3	CARMELA DELL'AVERSANO	THE GENOA 2005/2006 OUTBREAKS. DETERMINATION OF PUTATIVE Palytoxin AND ITS ANALOGUE IN <i>Ostreopsis ovata</i> BY A NEW LC-MS/MS METHOD
17.00	OR-4	MANUEL LOLO	ADVANCED LC-MS/MS METHODS FOR THE ANALYSIS OF MARINE TOXINS

YESSOTOXIN AND SYNTHETIC POLYETHER MODELS

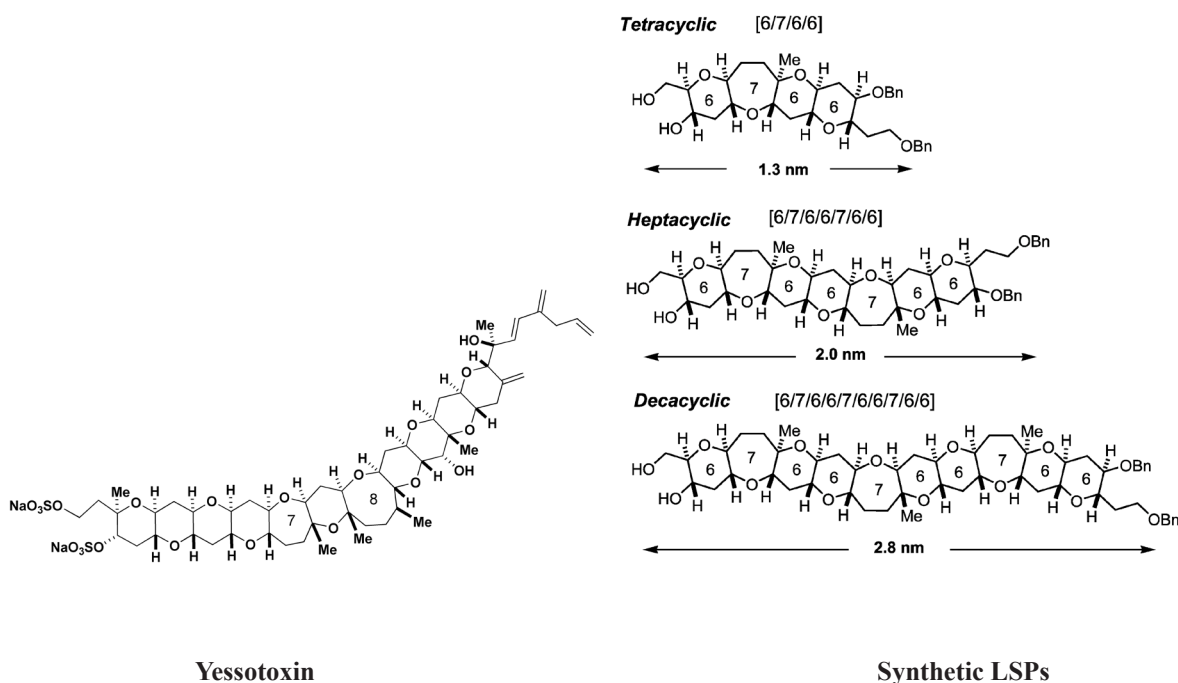
– INTERACTION WITH MEMBRANE PEPTIDES –

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Ladder-shaped polyether (LSP) compounds, unique products of Dinophyceae and Haptophyceae, comprise continuous trans-fused cyclic ethers, and all of those known so far possess potent toxicity. Among LSPs, yessotoxin was first discovered in association with toxic shellfish poisoning¹ and later found to be a metabolite of *Protoceratium* species dinoflagellate.² As a model compound of LSP, we adopted desulfated yessotoxin and examined its interaction with membrane-integral proteins.³ Besides natural products, synthetic LSPs were prepared by a newly developed method⁴ and their interactions with membrane proteins were examined by using surface plasmon resonance and saturation-transfer difference NMR.



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BIOACTIVE COMPOUNDS IN SHELLFISH - DRUG DISCOVERY OR FOOD SAFETY MANAGEMENT?

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Shellfish accumulate a variety of bioactive marine natural products (MNPs) as well as contaminants of anthropogenic nature. Mechanisms of uptake of bioactives in shellfish may be active (i.e. filter-feeding) or passive (through partitioning), and can therefore be related or unrelated to the octanol/water-partition coefficient of the compound. Thanks to this wide array of uptake mechanisms, shellfish can be used as biomarkers of pollution and of MNPs in the marine environment.

Some MNPs, such as unsaturated fatty acids from marine algae, have a positive nutritional value for human consumption of shellfish. However, many MNPs were discovered in shellfish through their adverse effects in humans following consumption of shellfish, hence, *in-vivo* assays have extensively been used in food safety programmes¹. This paper reviews recent studies on the bioactivity of phycotoxins, including azaspiracids and dinophysistoxins^{2,3,4}.

Shellfish have also been used for long-term monitoring of anthropogenic persistent organic pollutants (POPs). Many POPs have been shown to be toxic to humans or the ecosystem, however, concentrations are typically low, and *in-vivo* assays are not suitable for the surveillance of these compounds. Further anthropogenic contaminants include pesticides, insecticides and veterinary drug residues. The potential presence of these known compounds must be taken into account to reduce the rate of false positive hits in biodiscovery screening using *in-vitro* assays. Analytical techniques are discussed for screening of large numbers of knowns in shellfish. The paper summarises typical MNPs and POPs monitored and their levels of bioactivity. The comparison of *in-vivo* and *in-vitro* methods leads to the conclusion that *in-vivo* assays may be more useful for biodiscovery than for the protection of public health. The uptake of MNPs in shellfish depends heavily on seasonal occurrence of phytoplankton, and metabolic conversion of the originally produced MNP may occur in the shellfish. Therefore, the use of passive samplers is also described as an effective tool for biodiscovery and the study of distribution pathways of bioactives in the marine food web.

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NOVEL POLYETHER METABOLITES FROM DINOFLAGELLATES

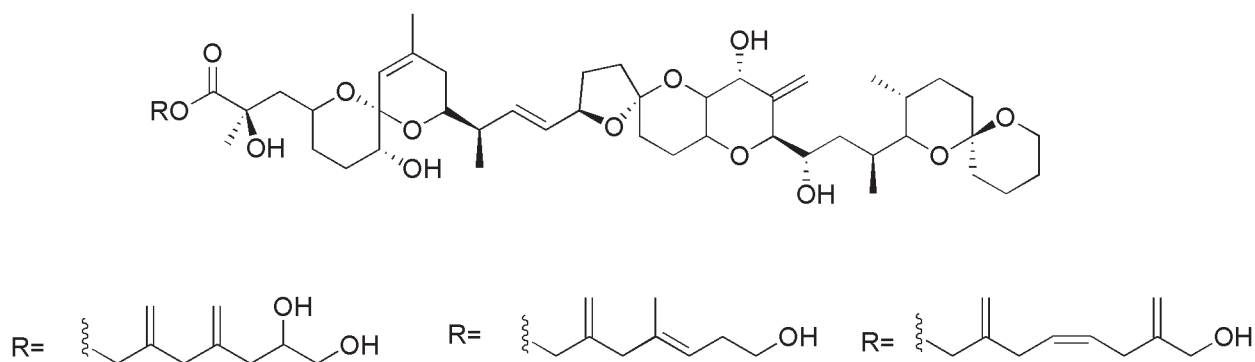
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Over the last years our research group has been focused on the study of microalgal metabolites, especially those related with red tide phenomena, as a source of possible bioactive compounds. Among them, the most commonly found toxins in the Spanish shores belong to the DSP (Diarrhetic Shellfish Poisoning) and the PSP (Paralytic Shellfish Poisoning) types. DSP toxins have unique chemical features and after their isolation focused natural product chemist attention, not only because of their public health repercussion and economical impact on the shellfish industry, but also to establish the real origin of the poisoning. Moreover the isolation and the structure elucidation of minor new toxins is imperative for designing proper countermeasures such as their detection in contaminated samples and for the determination of their biosynthetic pathway. In addition, marine toxins are more than just tools of biological chemistry; they are also powerful molecular probes that shed light on the molecular details of important cellular events. In this way, for example, the remarkable selectivity of okadaic acid to inhibit some serine-threonine protein phosphatases lead directly to the discovery and characterization of some members of that family of soluble proteins.

The DSP phenomena are due to the proliferation of marine dinoflagellates belonging to genera *Prorocentrum* and *Dinophysis*, real producers of this type of toxins. Artificial cultures of dinoflagellates has been undertaken in our laboratory by inoculating 80 L tanks of Guillard K medium with 5 L of *P. lima* (PL2V strain) or *P. belizeanum* cultures. Cell extracts were afterwards successively chromatographed yielding a series of new derivatives of okadaic acid. Their structures will be discussed on the basis of their spectroscopical data, essentially obtained by 2D NMR techniques, in combination with molecular modelling studies. In addition the utility of these new compounds to understand the structural requirements necessary to inhibit protein phosphatases will be commented.



RECENT INSIGHTS INTO HARMFUL ALGAL BLOOMS ALONG THE ADRIATIC COASTLINE

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In the late '80s for the first time we afforded unambiguous evidence over the occurrence of okadaic acid (OA) in the Adriatic Sea¹. Since then, our study on marine biotoxins has been proven a cornerstone in analyzing outbreaks of algal blooms along the Italian coastline. Our research on Italian marine toxic events has so far depicted an ever changing toxin profile so complex that is hardly matched across the world². In particular, over the last decade, yessotoxins (YTXs) have been the most occurring Adriatic toxins, while OA predominant in the early '90s has slowly subsided as of 1995 until disappearing around the turn of the new millennium². OA is a potent tumor promoter³, while YTX is of significantly lower oral toxicity⁴; that's why the EU has recently set up a new protocol capable of separating YTXs and DSP-toxins in distinct layers⁵. However, in samples of toxic Adriatic mussels we have recently individuated two new desulfocarboxyhomoyessotoxins that get co-extracted in the same layer as okadaic acid; thus a revision of the official protocol would be now in order⁶. Very recently, a massive occurrence of *Alexandrium ostenfeldii* in the Adriatic plankton was detected, with an ensuing high concentration of spirolides that are now the most abundant toxins⁷. Our studies led to characterize a number of major spirolides, some of them never reported before⁸.

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THE GENOA 2005/2006 OUTBREAKS. DETERMINATION OF PUTATIVE PALYTOXIN AND ITS ANALOGUE IN *OSTREOPSIS OVATA* BY A NEW LC-MS/MS METHOD

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Over the last few years a new treat due to harmful algal blooms has been impending in the Mediterranean Sea: microalgae belonging to *Ostreopsis* genus have spread along the Italian coasts, mostly in the Tyrrhenian Sea, causing serious concerns to both environment and public health. The phenomenon was recorded for the first time during summer 1998 along the north-western Tuscany coasts,¹ but reached alarming proportions in late July 2005, when about 200 people exposed to marine aerosols on the beach and promenade of Genoa required extended hospitalization. Respiratory distress observed in humans was concurrent with a massive bloom of *Ostreopsis ovata* along Genoa coasts and disappeared when the *O. ovata* population decreased. Human illness were recorded also in summer 2006, when *O. ovata* populations spread along the whole Tirrenian coasts from Liguria to Sicily and in the Southern Adriatic Sea. In the present work, we report on the investigation of plankton samples collected along Liguria coasts during the 2005/2006 outbreaks. The application of LC-MS/MS methods we have developed so far for the analyses of a number of liposoluble and hydrosoluble phycotoxins²⁻⁵ prompted us to exclude the presence of such toxins in the samples. Then, since some *Ostreopsis* strains are regarded as the producing organisms of palytoxin,⁶ one of the most potent marine toxin so far known, a new LC-MS/MS method for detection of palytoxin was set up.⁷ It allows the rapid and specific determination of palytoxin in selected ion monitoring (SIM) and multiple reaction monitoring (MRM) modes. The minimum detection levels for matrix-free toxin on-column were estimated to be 125 pg and 200 pg in MRM and SIM mode, respectively. Spiking experiments before and after extraction allowed to assess limits of detection and quantitation for palytoxin in matrix, accuracy, intra-day and inter-day reproducibility. Relying on the newly developed method, we were able to detect the presence of putative palytoxin and its analogue in the plankton samples and to infer them as the likely cause of the Genoa 2005/2006 outbreaks.

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THE ANALYSIS OF MARINE TOXINS USING A HYBRID TRIPLE QUADRUPOLE LINEAR ION TRAP LC/MS/MS SYSTEM

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Marine toxins originate from algal blooms and are found in shell fish. They can be separated into several classes; these include marine toxin which induce Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poison (DSP such as Okadaic Acid, Dinophysistoxins, Pectenotoxins Yessotoxins, and Azaspiroacids) and the Amnesic Shellfish Poison (ASP, Domoic Acid). The toxicity of the toxin and its biological effect vary with class and identity

Traditional techniques such as Liquid Chromatography with Ultra-Violet or Fluorescence detection (LC/UV or LC/FL) and Gas Chromatography Mass Spectrometry (GCMS) have been shown to be insensitive, non-specific and they often require rigorous sample prep with derivitisation often needed. Other techniques such as the Mouse Bioassay test are not specific and it has issues with cross contamination often producing false positive results. This traditional test is also expensive and has some ethical problems.

Over recent years Liquid Chromatography Mass Spectrometry (LC/MS) has started to be used for these analyses. It has several benefits over the more traditional techniques in that it is highly selective, sensitive and accurate and helps eliminates false positives. It can provide both qualitative and quantitative analysis in a single run which meets all requirements for modern residue analysis but has the added advantage of requiring less sample preparation.

This presentation describes the use of a hybrid triple quadrupole linear ion trap LC/MS/MS system as a screening tool for the detection and confirmation of trace levels of marine toxins. The combination of a standard triple quadrupole multiple reaction monitoring (MRM) scan used as a trigger to generate linear ion trap Enhanced Product Ion (EPI) spectra of specific marine toxins provides both MRM quantitation and library searchable spectra for confirmation and identification of toxins at trace levels. The analysis of several marine toxins classes is discussed.

NEW PERCEPTIONS

Friday
21th September, 2007

SESSION PROGRAMME

09.00	IL-12	PETER KARUSO	REVERSE CHEMICAL PROTEOMICS WITH MARINE NATURAL PRODUCTS: A DIRECT LINK BETWEEN PHENOTYPE AND GENOTYPE VIA PHAGE DISPLAY
09.30	IL-13	WERNER MULLER	NEW BIOMATERIALS: BIOFABRICATION OF BIOSILICA-GLASS BY SPONGES
10.00	OR-1	SANDRA LOSS	SMALL VOLUME NMR FOR MARINE NATURAL PRODUCTS ANALYSIS

REVERSE CHEMICAL PROTEOMICS AND BIOMIMETIC SYNTHESIS IN THE DISCOVERY OF BIOACTIVE MARINE NATURAL PRODUCTS

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Marine natural products provide a unique source of new leads in drug discovery due to their novel structures and biological activity. A technique that could simultaneously and quickly identify potential protein binding partner of a natural product would validate marine natural products as a source for drug leads and facilitate drug development. Similarly, the total synthesis of natural products is a noble pursuit but all too often the routes chosen are based on highlighting a particular reaction rather than devising the most efficient route. This lecture will outline a platform technology we call “reverse chemical proteomics” that can be used by chemists to link small molecules with their protein receptors and the genes for those proteins. This technique has the potential to unlock the secrets behind the activities of many marine natural products and expand the reach of natural products chemistry in defining gene, cell and organism functions in health and disease. A second aspect of this presentation is on the biomimetic synthesis of natural products to access small focused libraries of compounds for biological screening. Here we are interested in the oroidin group of alkaloids and the myriad of biological activities these alkaloids have been reported to possess. As reactions in Nature are biased towards function, it follows that every natural product should have a biological receptor; only if one accepts this premise can the enormous biochemical expense of producing natural products be rationalised. Even though natural products may not have co-evolved with human proteins, they have emerged in nature to interact with biomolecules from a range of other species. As Jerrold Meinwald succinctly put it, “Natural products have evolved to interact with something, and that something may not be so different from human proteins”^[1]. This assertion is supported by a recent survey^[2], which found that 61% of the 877 new small molecule chemical entities introduced as drugs worldwide during 1981-2002 were either natural products, natural product derivatives or natural product mimics. The percentages were even higher when considering only the antibacterial (79%) and anticancer (74%) compounds. Though few marine natural products have made it to market, there are many that show huge potential, particularly in cancer and infectious diseases^[3]. Clearly, marine natural products are a rich source of drugs and drug leads. However, the cellular target and mode of action of these compounds are rarely identified. This is also true of many natural products that are currently in clinical trials or have already been approved as pharmaceuticals. Because the lack of definitive protein targets for marine natural products hinders the validation of the marine environment as a source of new drugs, there is a great need for new techniques to facilitate the rapid identification of cellular targets. By combining approaches from chemistry and biology we have been able to isolate drug targets from large libraries and thus expand the reach of marine natural products in defining gene, cell, and organism function in health and disease. Chemical proteomics (Fig. 1A)^[4] is a powerful tool for isolating and identifying cellular receptors for biologically active natural products, thereby facilitating subsequent rational drug design, and often providing valuable information regarding underlying biochemical and cellular processes. The key to chemical proteomics is the construction of an affinity probe. In reverse chemical proteomics (Fig. 1B)^[5], the starting point is a transcriptome of a phenotype of interest. A cDNA library is cloned into an expression system and the resulting tagged proteome is screened against a small molecule. To date, only phage display^[6] and ribosomal display^[7] has been used in a reverse chemical proteomics context, although other methods, such as RNA-display, bacterial and yeast cell-surface display, are also possible, but need to be developed further. In phage display, the proteome is expressed, one protein at a time on the surface of the phage particle and individual members can be isolated based on their affinity to some probe, such as an immobilised marine natural product. However, the real power of phage display comes from the fact that isolated sub-libraries can be amplified by transfection into *E. coli* and then subjected to further rounds of affinity selection. In this lecture, we will report on our work to improve the method^[8] and in the first isolation of a human receptor for a marine natural product and the application of biomimetic organic synthesis to easily access analogues of bioactive marine natural products. Our first target is the anticancer marine natural product ageladine A^[9], which has prompted us to look at the synthesis of small libraries based on this unique structure.

[1] A. M. Rouhi, *Chemical & Engineering News* **2003**, *81*, 77; [2] D. J. Newman, G. M. Cragg, K. M. Snader, *Journal of Natural Products* **2003**, *66*, 1022; [3] D. J. Newman, G. M. Cragg, *Chimica Oggi* **2006**, *24*, 42; [4] D. A. Jeffery, M. Bogyo, *Current Opinion in Biotechnology* **2003**, *14*, 87; [5] A. M. Piggott, P. Karuso, *Combi. Chemistry & High Throughput Screening* **2004**, *7*, 607; [6] P. P. Sche, K. M. McKenzie, J. D. White, D. J. Austin, *Chemistry and Biology* **1999**, *6*, 707; [7] A. Rothe, R. J. Hosse, B. E. Power, *Expert Opinion on Biological Therapy* **2006**, *6*, 177; [8] A. M. Piggott, P. Karuso, *Marine Drugs* **2005**, *3*, 36; [9] S. R. Shengule, P. Karuso, *Organic Letters* **2006**, *8*, 4083.

NEW BIOMATERIALS: BIOFABRICATION OF BIOSILICA-GLASS BY SPONGES

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Biosilicification is an evolutionary old and widespread type of biomineralization both in unicellular and multicellular organisms including sponges, diatoms, radiolaria, choanoflagellates, and higher plants. In the last few years combined efforts in molecular biology, cell biology, and inorganic and analytical chemistry allowed first insights in the molecular mechanisms by which these organisms form an astonishing structural variety of siliceous structures not reached by chemical methods. The skeletal elements of two classes of sponges, Demospongiae and Hexactinellida, the siliceous spicules, consist of glassy amorphous silica (biosilica). The demosponges exhibit the unique ability to synthesize biosilica using a novel group of enzymes which have been termed silicateins. Silicateins have been isolated, cloned and sequenced both from marine sponges and freshwater sponges. Sponges also possess a silicase, which mediates the dissolution of amorphous silica. Diatoms, which belong to the Protista, have used an independent strategy to utilize silica for the construction of their skeletal elements. The recombinant silica enzymes (protected by patents worldwide [W.E.G. Müller and H.C. Schröder]) are of high interest and potential importance for a variety of medical and technical applications, e.g. surface modification of glasses and other materials including biomaterials, the preparation of resins, insulators, mesoporous molecular sieves and catalysts. First, strategies have been designed towards the application of these enzymes for surface modification (coating) of biomaterials. The advantage of these bio-based approaches compared to conventional technical procedures is the fact that the enzymatic silicatein reaction occurs under mild physiological conditions, whereas physical-chemical methods require high temperatures or pressures, and the use of caustic chemicals which possibly damage the organic (bio)materials used. Such biomaterials may include collagen used in tissue engineering and in bone replacement materials. The use of biocatalytically (silicatein) formed silica may also be a suitable approach for coating metal implants to increase biocompatibility or to attach bioactive substances to the relatively inert metal surface. The same strategy could be employed for the encapsulation of drugs, hormones and other bioactive molecules and the controlled release of these compounds. In addition, the recombinant silica enzymes may also be applied for the synthesis of the nanostructures of amorphous silica. In industry, micro- and mesoporous silicas are used as reinforcing fillers in plastics, paints, sealants and rubber materials, as adsorbents and catalyst supports, as desiccant agent and as filters in separation technologies. Such applications require silicas with specific mechanical strength, pore volume, surface area and pore-size distribution. Finally, the application of techniques in lithography based on enzyme (silicatein)-mediated biosilicification may represent an innovative approach in the field of fabrication of microelectronics. Besides the use of the recombinant enzymes, biomimetic approaches have been undertaken to exploit the biosynthetic potential of the natural biosilicification mechanisms, using either naturally occurring polyamines and their analogues (spermine, spermidine and putrescine homologues, mimicking the biomineralization process in diatoms) or bifunctional molecules acting as catalysts for silica formation at neutral pH. In addition, block copolypeptides have been used to mimic catalytic activity of silicatein.

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SMALL VOLUME NMR FOR MARINE NATURAL PRODUCTS ANALYSIS

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Structural elucidation of natural products is a research field of growing importance but due to the very limited sample amounts also poses a problem to the analyst. NMR is a very powerful tool in this field but lacked the possibility to investigate these small sample amounts in a reasonable timescale due to a comparably poor sensitivity. This problem was partially solved some years ago, when the 1mm MicroProbe was developed that is capable of running all standard experiments with outstanding sensitivity on only 5ul solution. However, due to the low intrinsic sensitivity and natural abundance of C¹³ even this Probe did not allow the measurement of carbon spectra – an experiment that may be sometimes needed, especially if many quaternary carbon atoms are present in the structure, which makes the observation using inverse methods like the HSQC or HMBC difficult or even impossible. This is a very typical problem of marine natural products, which often contend bromine as substituent.

To overcome this problem, a new MicroProbe was developed that has a slightly bigger sample tube diameter of 1.7 mm, resulting in a total volume of 30 ul needed for the analysis. This Probe combines the highest mass sensitivity on the carbon and proton channel, making the direct observation of carbon spectra feasible. To get even higher sensitivity it is also available as CryoProbe.

On the examples of marine natural products the characteristics of these Probes as well as some useful NMR experiments for the structural elucidation will be presented.

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CLOSING LECTURE

Friday
21th September, 2007

MOVING ON: THE FUTURE SCALE AND SCOPE OF MARINE NATURAL PRODUCTS RESEARCH

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Move back 30 to 40 years and imagine two young chemists on a windswept beach sorting through the washed up red alga. Many kilograms of each alga were collected, sun-dried, ground and extracted with dichloromethane. From column chromatography on silica of the multi-grams of extract the young chemists found that each species yielded many new and interesting halogenated terpenoids.¹ This is of course a true story: the collectors were Blunt and Munro and the beach was at Kakanui on the East Coast of the South Island of New Zealand. Those young men are no longer callow youth and are now semi-retired professors whose careers have spanned those decades from then until now. That was the scale and the style of marine natural products in the 1970s. As we move towards the second decade of the 21st Century the scale of research operations has changed markedly, as has the equipment, and also the scope of what is now described as marine natural products.

For the space of this talk we shall describe some aspects of marine natural products as studies in this field move forward. We will focus especially on the scale aspects as this is where we have made some contributions, also on the role of databases and conclude by trying to pick what might be likely research topics for future graduate students.

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POSTER SESSION GREEN COMMUNICATIONS 1-50

PRELIMINARY RESULTS ON SECONDARY METABOLITES IN *PHYLLODESMIUM* SPP. (AEOLIDOIDEA, OPISTHOBRANCHIA, GASTROPODA, MOLLUSCA)

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The aeolid genus *Phyllodesmium* comprises about 25 species which feed on soft corals. Unlike other members of the Aeolidioidea, which mainly feed on hydrozoans, *Phyllodesmium* species do not take up cnidocysts from their prey, though a functional cnidosac is present. Incorporation of cnidocysts in these sacs and their expelling is the usual defensive strategy of aeolids against predators. However, *Phyllodesmium* species take up zooxanthellae from the corals, which they store in the cells of the highly branched digestive gland. With these incorporated zooxanthellae the slugs are able to survive for a certain time without feeding, living exclusively on the photosynthetic products of the algae. Moreover, it has been shown for three species, that they take up terpenes from their host corals, which they store in the cerata. One of these terpenes, i.e. 11- β -acetoxypukalide, has also been positively tested for feeding deterrent activity¹.

To further our understanding of the evolutionary scenario concerning the host switch from Hydrozoa to Octocorallia, the symbiotic relationship of slug and zooxanthellae, as well as the sequestration of defensive chemical compounds from the prey, we examine natural compounds in *Phyllodesmium* species and identify the localisation, as well as the source of secondary metabolites. Here we present preliminary results of this study.

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CALCIUM CHANNELS BLOCKERS FROM MARINE SPONGES

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Voltage-dependent calcium channels (VDCC) blockers have traditionally been used for treatment of cardiovascular diseases. However, a body of evidence has recently been accumulated showing their ability to modify neurotransmission and/or calcium neurotoxicity in several neurological disorders (Parkinson's, Alzheimer's diseases) opening the possibility for therapeutic use of these drugs in other neurological pathologies, such as neuropathic pain, or some neurodegenerative disorders. Within this framework, there is still a need for new compounds able to modulate VDCC which could be useful in the treatment of cognitive or neurodegenerative diseases¹.

The oceans are the source of a large group of structurally unique natural products that are mainly present in invertebrates. Several of these compounds show pronounced pharmacological activities and are interesting candidates for new drugs in several therapeutic areas². Thus, our purpose is to find marine compounds able to modulate VDCC as potential therapeutic agents for treating nervous system diseases.

In an attempt to identify new VDCC blockers from marine organisms we have found that the isopropanolic extract from different species of sponges from the *Verongia* genus, shows VDCC blocker activity in cell-based assays using human SH-SY5Y neuroblastoma cells. The initial cell-based screening assay is performed in two steps: first, intracellular Ca⁺⁺ overload is induced by KCl treatment and the different marine extracts are tested for its ability to rescue from this KCl-induced neuronal cell death; second, those positive extracts are then directly tested for their ability to specifically block VDCC by measuring its effect on the Fluo-4 intracellular fluorescence. Fractionation and purification of active components from these extracts, guided by biological activity assays, resulted in the isolation and identification of a huge variety of natural bromotyrosine-compounds, with various potencies as VDCC blockers. Interestingly, the compound with the most potent activity has not yet been described previously.

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**DEFENSIVE COMPOUNDS OF THE OPISTHOBRANCH MOLLUSC
AUSTRODORIS KERGUELENENSIS: THE FIRST EXAMPLE OF
DE NOVO BIOSYNTHESIS IN AN ANTARCTIC ORGANISM**

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The common Antarctic nudibranch *Austrodoris kerguelensis* (Bergh) contains diterpene diacylglycerides only present in its external body parts. These compounds provide chemical defense against sympatric predators, such as the seastar *Odontaster validus* Koehler. Bioassays conducted with *O. validus* revealed that live nudibranchs, mantle tissue, and EtO₂ extract of the *A. kerguelensis* mantle deterred feeding by the seastar. Further bioassays testing organic fractions of the EtO₂ mantle extract showed that the diterpene diacylglycerides, as well as the corresponding monoacylglycerides and monoacylglycerides of regular fatty acids, were responsible for the feeding deterrence in *O. validus*. We suggested that *A. kerguelensis* derives the bioactive diacylglycerides by *de novo* biosynthesis rather than by sequestration from its sponge diet, since the mollusc does not contain active metabolites in the viscera, and neither the active compounds nor precursors were detected in the sponge diet (Iken et al., 2002). *A. kerguelensis* was also previously suggested to *de novo* biosynthesize its defensive compounds, but no experimental evidence existed so far to support this hypothesis. We will report here for the first time direct evidence of this biosynthesis occurring in *A. kerguelensis*, by the injection of labeled precursors into live slug specimens in Antarctica. Two types of precursors: 1-¹³C-glucose and 1-¹³C-acetate, were injected into the animals. After several days the slugs had produced a variety of terpenoid glycerid compounds, which were identified by NMR, HPLC, and LC-MS. The incorporation of the labeled compounds is very low but supports both the *de novo* biosynthesis of the diacylglycerides, as well as the mevalonic origin of the terpenoidic part of the molecules. It is noteworthy however that our data indicate that there could be different biochemical transformations in different animals, even from the same populations, although the variety of compounds found in the different specimens suggests a theoretical biosynthetic pathway. To our knowledge, this is the first testimony of *de novo* biosynthesis of natural products in an Antarctic organism.

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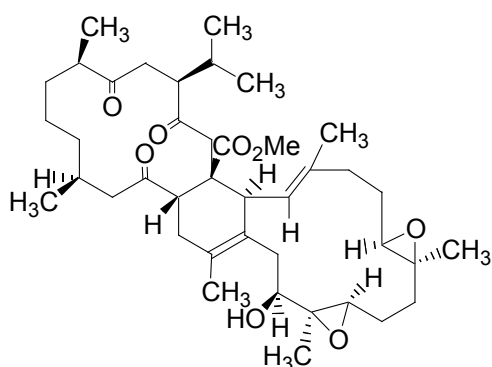
THREE NEW BISCEMBRANOIDS, AND THEIR MONOMERIC COUNTERPART CEMBRANOID, A BIOGENIC DIELS-ALDER PRECURSOR, FROM THE SOFT CORAL *SARCOPHYTON* SP

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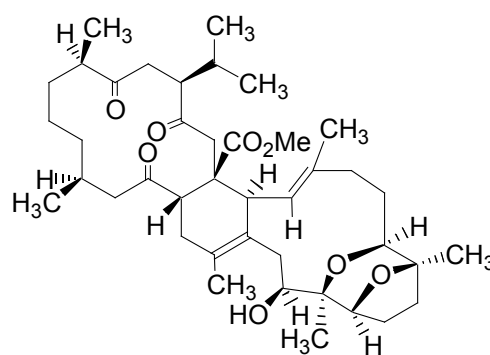
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Two new cembranoids, methyl tetrahydrosarcoate (**1**) and methyl tetrahydroisosarcoate (**2**) were isolated from the soft coral *Sarcophyton* sp., collected at Kitangambwe, Kenya, together with four biscembranoids, the known nyalolide (**3**) and the unprecedented, desacetylnyalolide (**4**), diepoxynyalolide (**5**) and dioxanyalolide (**6**). The structures and relative stereochemistry of the compounds were elucidated by interpretation of MS, 1D NMR and COSY, HSQC, HMBC and NOESY experiments. Compound **1** is most likely the dienophile affording by a Diels-Alder reaction the four biscembranoids. Dioxanyalolide (**6**) possesses anti-bacterial activity against *Escherichia coli* at a concentration of 1.25 µg/ml. Methyl tetrahydrosarcoate (**1**) and diepoxynyalolide (**5**) exhibited LC₅₀ values of 1.5 µM in brine shrimp bioassay while desacetylnyalolide (**4**) was only mildly active.



Diepoxynyalolide



Dioxanyalolide

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FUSARIPYRONES, NOVEL POLYPROPIONATES FROM THE MEDITERRANEAN MOLLUSC *HAMINOEA FUSARI*

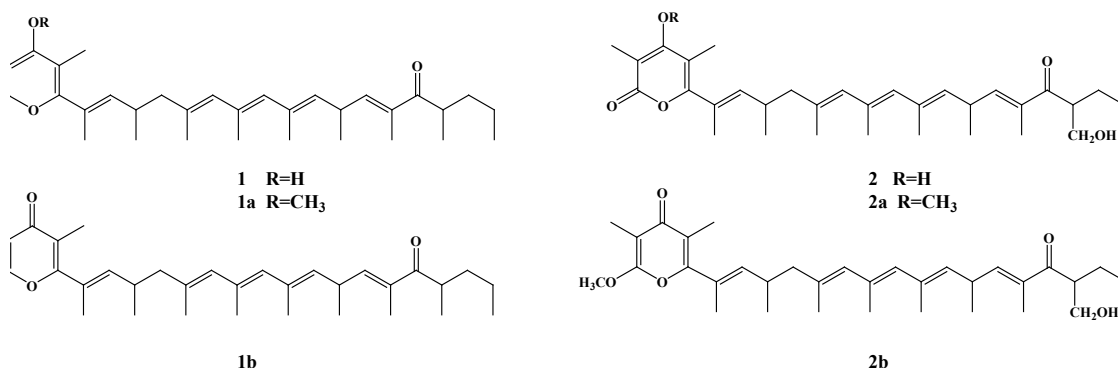
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Polypropionates are secondary metabolites rather common in a few groups of marine molluscs of the subclasses Pulmonata and Opisthobranchia.^{1,2} Within this latter group of invertebrates, acyclic and pyrone-based polypropionate skeletons have been mainly reported from sacoglossans, where they may exert a photoprotective role,³ and from cephalaspideans, in which a defensive function has been invoked.⁴ In the course of our continuing studies on secondary metabolites from Mediterranean molluscs, we have reinvestigated the organic extracts of *Haminoea fusari* (Cephalaspidea, Gastropoda, Opisthobranchia). Along with known alkyl-pyridine alkaloids, namely haminols 1-6, previously reported from the mantle of the mollusc,⁵ we have detected two new minor pyrone-containing polypropionates, which we named fusaripyrones A and B (**1** and **2**), isolated as natural forms and methyl derivatives (**1a/1b** and **2a/2b**). Here we present the structural elucidation of the compounds by chemical and advanced spectroscopical methods. Furthermore, biogenetic relationships with other polypropionates from related cephalaspideans will be discussed. The two metabolites show an interesting antifouling activity in settlement inhibition assay with laboratory reared *Balanus amphitrite* larvae ($EC_{50} = 9.5\text{ppm}$).



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COMPARISON OF THE BIOLOGICAL EFFECTS OF POLYETHER COMPOUNDS ISOLATED FROM THE MARINE DINOFLAGELLATE *KARENIA BREVIS*

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Karenia brevis is marine dinoflagellate most associated with red-tides in Gulf of Mexico. The compounds responsible for the toxic effects of *K. brevis* blooms are the brevetoxins. The brevetoxins are polyether ladder toxins that bind to site 5 of the voltage sensitive sodium channels (VSSC) in mammalian nervous tissue ($K_i=4.3$ nM for PbTx-3) (1). Brevetoxins kill fish at nM (1,3) concentrations in water and cause mammalian respiratory effects at pM concentrations (2).

K. brevis is cultured in large quantities and regularly harvested each week to produce a continuous supply of brevetoxins for research purposes. Along with the brevetoxins other polyethers such as brevenals (3) and brevisin (4) have been isolated and identified from our cultures. The brevenals bind ($K_i=400$ nM) to the same binding site as the brevetoxins and act as functional antagonist to brevetoxins in all assays tested including; fish bioassay (1,3), sheep bronchoconstriction assay (2), and comet assay (5). Brevisin binds to the same site on the VSSC as brevetoxins but with less affinity; no other biological effects are known. Currently, enough brevenal and brevisin are produced to make simple derivatives of (*e.g.* reduction and oxidation products) and to run a series of bioassays.

The bioassays used in these experiments are: [³H]-PbTx-3 synaptosome binding assays, bronchoconstriction in sheep, fish bioassay, neuroblastoma cytotoxicity assay, calcium flux assay, and electrophysiology patch clamp experiments. The results of the bioassays for the brevenals and brevisins will be compared to similar derivatives of brevetoxin. Supported by the State of North Carolina and NIEHS grant PO1 ES010594.

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UNUSUAL STEROID GLYCOSIDES FROM THE CARIBBEAN MARINE SPONGE *PANDAROS* SP.

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During the last thirty years, marine organisms have appeared as potential sources of bioactive substances and have been able to provide novel lead compounds for the pharmaceutical industry. As part of the French West Indies in the Caribbean, with both influences of the Caribbean Sea and the Atlantic Ocean, the marine biodiversity of the Martinique Island is known to be extremely rich. To evaluate the bio- and chemiodiversity of the marine invertebrates of this area, samplings were organized during an expedition in 2003.

We present our results concerning a very little studied sponge *Pandaros* sp. (Poecilosclerida: Microcionidae). Only one polyether carboxylic acid has been reported in a species of *Pandaros*. in 1981. Nevertheless, the chemical profile of the organic extracts of our specimen was very promising and we decided to undertake the full characterization of its chemical constituents.¹ After extraction and a first fractionation by C18 flash chromatography, two fractions showed very interesting and complex HPLC profiles. The reversed-phase HPLC purification of these fractions allowed us to isolate ten compounds having the same UV pattern. The NMR study of the major compound led us to postulate a sugar moiety linked to a steroid part. Extensive 2D NMR and HRESIMS studies showed that all compounds shared the same aglycone moiety. Differences appeared in the number and the structures of the glycosides, but also in the terminal part of the aglycone moiety. The originality of this new family of compounds arises from the cyclopentenone ring of the steroid part. Such an original pattern has only been reported in a plant belonging to the family Asclepiadaceae: *Solenostemma argel*.²

This new family of marine steroid glycosides isolated from *Pandaros* sp. showed moderate antitumoral activities.

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***IN-VITRO* CYTOTOXICITY FROM VENEZUELAN MARINE SPECIES**

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The search for new compounds with potential biological activity remains the main objective of natural products development. New exciting substances are regularly found and the introduction of novel advanced techniques speed up the possibilities of good achievements in short term¹⁻³. In recent years, an increasing interest has developed in works concerning untouched geographical areas⁴. In this context, Venezuela's rich biodiversity offers a huge potential for this purpose. A variety of extracts from marine organisms were analysed for cytotoxic activity using human cancer cell lines as model of cytotoxicity. A total of 26 crude extracts were obtained from a variety of marine organisms (Sea stars, sea urchins, holothurian, and clams) collected in Playa Tocuchare, Estado Sucre, Venezuela. Each specimen was divided into two equivalent portions, A and B, and immediately fixed after collection; those in portion A were preserved in 100% methanol [MeOH], while groups in portion B were added to dichloromethane [DCM]. Marine organisms with calcium carbonate skeletons were despoiled, tissues sliced, and extracted further. All crude extracts were assessed for cytotoxicity. The in-vitro cytotoxic activity was analysed through the protein-staining sulphorhodamine B (SRB) assay⁵. Data was registered, normalised and analysed through linear regression. All the extracts analysed display their highest activity at the maximum exposure time of 72h tested. The marine extracts tested exhibited variable degrees of activity against the two cell lines analysed. DCM extracts displayed higher activity than MeOH extracts ($P \geq 0.01\%$), suggesting that the active compounds are mainly of low polarity. The cell line MCF7 showed more susceptibility to the action of the extracts than CORL23 ($P = 0.05$). The species **Anadara notabilis**, **Acanthaster plancii**, **Echinometra lucunter** and **Lythechinus variegatus** displayed the highest cytotoxic activity, with a range of IC_{50} values between 25.37 and 73.66 $\mu\text{g/ml}$. The recovery test showed that the cells lost the ability to recover after treatment with the DCM extracts at concentrations higher than 30 $\mu\text{g/ml}$ for the 66.7% of the species analysed. On the contrary, the methanolic extracts obtained from the same species displayed very low cytotoxic activity in the two models of cytotoxicity utilised, indicating a direct relationship between the cytotoxicity and the polarity of the compounds present in the extracts.

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NEW RESORCYLIC MACROLIDES ISOLATED FROM THE MARINE FUNGUS *THIELAVIA SP.*

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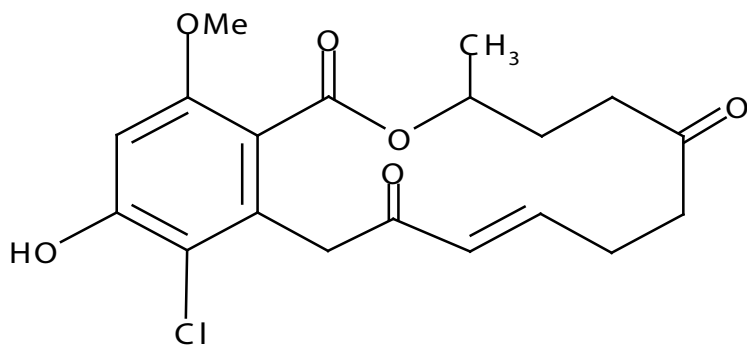
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Historically, terrestrial fungi have been a rich source of pharmaceutically important compounds [1]. More recently, marine fungal species have been exploited as a source of novel bioactive metabolites [2,3].

In the course of our search for novel bioactive metabolites from marine sources, we have isolated a new cytotoxic resorcylic macrolide CH0046 (1) from a marine fungus *Thielavia* sp. Other related compounds such as radicicol, LL-Z-1640s, monocillines, nordinone and aigialomycins have previously been isolated from different species of fungi and a broad spectrum of activities have been described for them [4].



(1)

Details of the producer microorganism, isolation and spectroscopic data leading to the structure determination of the CH0046 and the minor compounds CH0051 and CH0052, its *in vitro* biological activities will be presented.

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DIATOM-DERIVED POLYUNSATURATED ALDEHYDES HAVE A DIFFERENTIAL EFFECT ON DIFFERENT MARINE BACTERIA

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Diatoms produce polyunsaturated aldehydes (PUAs) which are teratogenous for the copepods feeding on them. These secondary metabolites are also able to reduce other phytoplankton growth by inhibiting cell division and inducing death by apoptosis. We have tested three of these aldehydes (*2E,4E-decadienal*, *2E,4E-octadienal* and *2E,4E-heptadienal*) on 32 cultured strains of marine bacteria. Three kind of responses were observed: a) no effect even with very high PUA concentrations, b) reduction in growth rate upon PUA exposure, c) increase of growth with respect to the control (with no PUA added). The three responses were found within each taxonomical group, and no recognizable trend could explain these differences. However, representatives of the Flavobacteria and gamma-Proteobacteria showed a remarkable resistance to PUAs, suggesting an adaptation probably related to frequent exposure. We also tested the effect of these PUAs on a natural bacterial community from a coastal area of the Mediterranean Sea during incubation experiments. Cell concentrations, respiration and total production were reduced in samples inoculated with the three aldehydes or with a mixture of them. This confirms previous results on isolated marine strains in culture. However, it is possible that only part of the community was responsible for the drop in average values. To test this hypothesis, microautoradiography has been used coupled with FISH using five bacterial group-specific probes to investigate the occurrence of differential toxicity of these aldehydes on different bacterial groups. The preliminary results show that gamma-Proteobacteria are able to grow faster following an initial toxic effect. Further analyses are in due course to verify if other groups show a similar response upon aldehyde exposure. These results are very relevant to natural conditions during blooms of aldehyde-releasing diatoms, as some bacterial group may profit of the toxic effect on growth to outgrow competitors for the same resources and dominate at times. Some groups of bacteria may also possess resistance or detoxification mechanisms against these compounds, probably maintained by their close association with diatoms.

NEW HEXAHYDROBENZOPYRAN DERIVATIVES ISOLATED FROM THE MARINE-DERIVED FUNGUS *EUTYPELLA SCOPARIA* OBX

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The marine environment represents the source of an impressive variety of biologically active chemical compounds isolated from all forms of life.¹ Marine microorganisms such as bacteria and fungi inhabit every environment of the sea, and are rich sources of chemically and biologically diverse compounds.² The interest towards marine microorganisms has grown due to the possibility of their fermentation to provide large amounts of secondary metabolites for further studies especially in the case of those microbial products, pharmacologically active, biogenetically related to metabolites isolated from difficult-growth marine invertebrates.³

In this communication we describe the chemical investigation of the secondary metabolite pattern of the culture broth of the fungus *Eutypella scoparia*. This strain was isolated from the external part of the Chinese marine pulmonate *Onchidium* sp, collected in the upper part of the intertidal zone of Hainan coasts (China). The ethyl acetate extract of the culture filtrate was characterized by the presence of two new hexahydrobenzopyran derivatives and by the already reported cytochalasin B, cyclo-(D)-Pro-(D)Leu and cyclo-(D)-Pro-(D)-Phe. The new compounds possess a carbon framework characterized by a polyketide portion linked to a terpene unit, and a rare cyclic carbonate moiety. The structure elucidation was carried out by means of NMR and mass techniques.

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HOMOPHYMINES, A NEW FAMILY OF CYCLODEPSIPEPTIDES WITH ANTI-HIV ACTIVITY FROM A NEW CALEDONIAN LITHISTIDE SPONGE

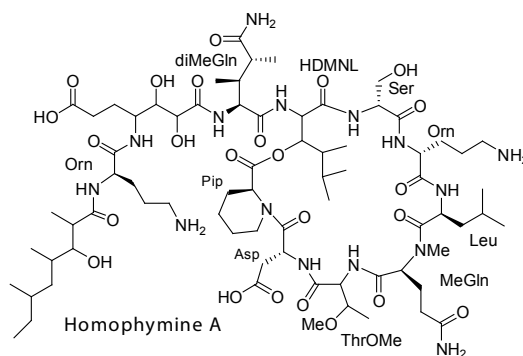
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Sponges belonging to Lithistid order are recognized as a rich source of compounds with unique structures and intriguing biological activities. Within this group of marine metabolites is a family of cyclic peptides with potent anti HIV-properties which comprises callipeltins,^a papuamides A-C,^b microspinamide^c and neamphamide,^d all sharing a more or less degree of structural homology suggesting a common pharmacophore. More recently we had the opportunity to study the polar extracts of the sponge *Homophymia* sp., a new genus belonging to order Lithistida, that showed cytotoxic and anti-HIV activity. Chromatographic procedures allowed us the isolation of three new compounds named homophymine A-C. Homophymines are cyclodepsipeptides and possess the C-terminus lactonized with a 3-hydroxy-4,5-dimethylnorleucine (HDMNL) residue whereas the N-terminus is involved in an amidic linkage with an unusual β -hydroxyacid. The most intriguing structural feature of homophymines is the presence of several non-ribosomal aminoacid units, two of them not previously isolated from natural sources. Structural characterization was performed through extensive application of NMR techniques and MS/MS experiments whereas stereochemical determination was based on an integrated approach which combined NMR methods and LC/MS analysis.



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THE POTENTIAL OF METABOLOMIC FINGERPRINTING USING ¹H NMR SPECTROSCOPY AND HPLC/ELSD IN COMBINATION WITH PCA

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The main focus of our research is the isolation and identification of unknown marine natural products with biological activity. Marine microorganisms such as fungi and myxobacteria are cultured under different conditions (e.g. different media, diverse temperatures, pH-values), according to the OSMAC (One Strain – Many Compounds) concept [1], leading to altered patterns in metabolite production. Metabolomic fingerprinting has the potential to be used for the comparison of diverse extracts and the detection of unknown natural products [2]. In this context we focus on two methods namely ¹H NMR spectroscopy and HPLC/ELSD. The evaporative light scattering detection (ELSD) is universally applicable after chromatographic separation as any compound of sufficient concentration is detected. Statistic analysis of ¹H NMR spectra and chromatograms with principal component analysis (PCA) clearly shows any difference between samples.

To establish this methodology in our laboratory we started working with plant-derived extracts. In order to obtain consistent data the experimental procedure was standardised after a series of preliminary experiments. The dried and ground biological material was extracted first with dichloromethane and subsequently with methanol. The ¹H NMR spectra and the HPLC/ELSD chromatograms of the extracts were recorded and statistically analysed by PCA.

The chemical diversity of the metabolome offers major opportunities for the discovery of novel drugs and bioactive molecules.

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NEW INSIGHTS FOR HYDROGEN PRODUCTION FROM THE MARINE THERMOPHILIC *THERMOTOGA NEAPOLITANA*

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Lately, there's a considerable tendency in the world to increase the use of renewable sources of energy to limit the dependence of the conventional fossil sources and, in the same time, to face the environmental problems due to their use. In the last years, attention has been increasingly paid to study the hydrogen production by microorganisms, both prokaryotes and eukaryotes, which are able to use many metabolic processes, as the dark fermentation, the light fermentation and the photosynthesis.¹ In this context, one of the best hydrogen-producer microorganism is *Thermotoga neapolitana*, initially isolated from hot spring in the Gulf of Naples. It is a thermophilic microaerophilic bacterium, able to produce hydrogen throughout dark fermentation in anaerobic conditions.^{2,3} Here we summarize our results on commercial *Thermotoga neapolitana* (DSMZ 4359), regarding the improvement of culture condition and the use of complex feedstocks to increase hydrogen production. Many parameters such as hydrogen production, pH, growth conditions, consume of glucose and levels of organic acids, were monitored. The data are used to build a model to study metabolic pathways connected with hydrogen production.

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STUDY OF POLYETHER TOXINS FROM DINOFLAGELLATE CULTURES

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Some species of dinoflagellates have interest because they are producers of potent biotoxins, responsible for the “Harmful Algal Blooms” (HABs) also known as red tides. In recent years an increase in number of events has been reported. This in turn has a great impact on the environment as well as on the public health, either by consumption of contaminated shellfish, alternatively by inhalation of sea water aerosols or by direct contact with the toxin. In the coastal waters of Europe, the toxins most frequently found are liposoluble polyethers with high toxicity.

However, the study of these toxins does not deal solely with the repercussions on public health. Another important issue is that these substances have diverse and complex structures and activities, making them valuable tools for studying cellular processes. In fact, has been widely used in pharmacological studies.

For all those reasons, it is desirable to develop cultures of dinoflagellates. Therefore, we have initiated the production of large scale artificial cultures in order to optimize the isolation and detection of such toxins.

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INVESTIGATIONS ON THE BIOSYNTHETIC GENE CLUSTER OF SIPHONAZOLE

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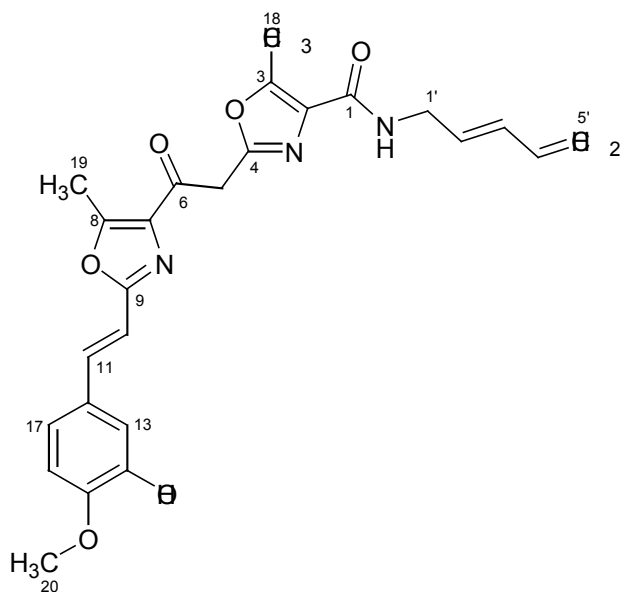
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Herpetosiphon species are gram-negative filamentous heterotrophs with the ability to glide on solid surfaces. *Herpetosiphon* organisms have been found in soil, freshwater and sewage treatment plants. In contrast to other bacteria, very little is known about the secondary metabolite production in these organisms.

In a screening approach for new compounds from *Herpetosiphon* strains, siphonazole (**1**) was isolated [1]. The structure of this secondary metabolite represents a polyketide with incorporated amino acids (threonine, glycine) and an unusual diene containing side chain.

This suggests the involvement of nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKS). Both types of enzymes are large, multifunctional protein complexes, which are organized in modules [2].



For the characterisation of the siphonazol biosynthetic genes, a genomic library was generated using the cosmid vector SuperCosI. The cosmid gene library of the *Herpetosiphon* sp. was screened with *pks*- and *nrps*- primers for the corresponding gene cluster.

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A COCKTAIL OF *SCYLLO*-INOSITOL GLYCOLIPIDS IN THE SPONGE *DRAGMACIDON DURISSIMA*

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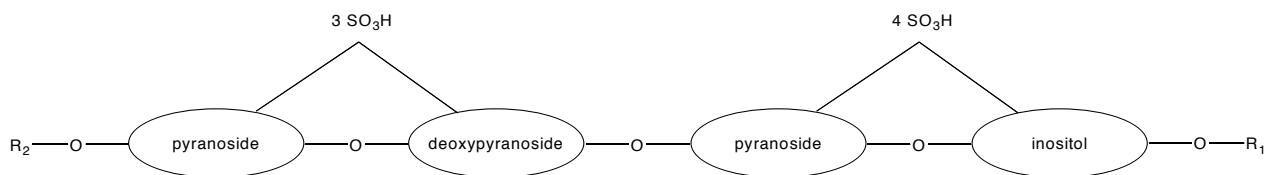
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In the course of our continued program on the isolation of new bioactive metabolites from marine organisms, the methanolic extract of the marine sponge *Dragmacidon durissima* from Mauritius Island, afforded a mixture of 32 glycolipids, which we have named dragmacinosides. Nine of those compounds were isolated using the XBridge column with triethylammonium acetate buffer at pH 7, without chemical modifications.

The structure elucidation of the 4 major compounds, which differ only in the branching and length of acyl chains, was carried out on the basis of 1D and 2D NMR experiments, and of MS data in MALDI and ESI. Dragmacinosides consist of four sugars, e.g., a *scyllo*-inositol, a fucose and 2 galactoses. Each derivative bears 7 sulfates groups and two (*Z*)- α - β unsaturated fatty acids (Figure 1). The lengths of the fatty acids chains vary from C₁₈ to C₂₂ for R₁ and from C₁₀ to C₁₄ for R₂. Due to LC/MS and LC/MS/MS data, partial chemical structures of the 23 minor compounds could be obtained.



Scheme 1 global composition of dragmacinosides

One example of similar *scyllo*-inositol has been isolated from the sponge *Axinella infundibula*.¹ The structure determination of these derivatives will be discussed.

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LIPOXYGENASE ACTIVITY IN DIATOMS

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Contrary to the traditional belief that diatoms provide a rich food base for their main grazers, calanoid copepods, these unicellular algae can in fact negatively impact copepod reproduction.¹ The enzyme lipoxygenase plays a major role in the formation of the metabolites deemed responsible for the reduction in reproductive success. It converts free fatty acids into lipid hydroperoxides², which are then transformed into a complex mixture of secondary metabolites further downstream. Therefore studying the activity of this enzyme contributes to a better understanding of the effect diatoms have on copepods and can be a useful tool in field studies to model the influence the phytoplankton population has on copepod reproduction.

In this methodological study lipoxygenase activity in three different diatom species were analysed by two different methods. The colorimetric assay³ is based on the detection of product formation, whereas the polarographic method⁴ measures reactant consumption. Changes in activity between the species as well as differences between the methods are discussed. Furthermore comparisons are made between frozen and fresh samples because in future these assays will be applied to the study of lipoxygenase activity in frozen field samples.

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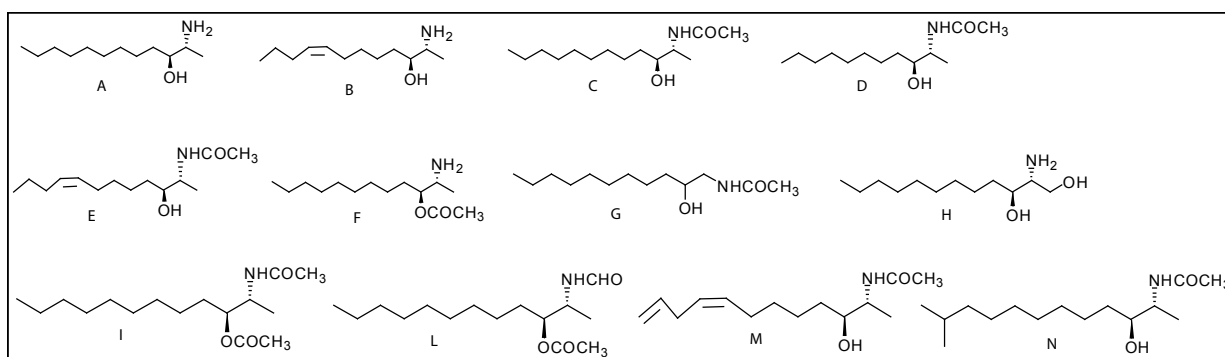
CLAVAMINOLS A-N, NOVEL CYTOTOXIC AMINO ALCOHOLS FROM THE TUNICATE *CLAVELINA PHLEGRAEA*: STRUCTURES, ABSOLUTE CONFIGURATION AND PHARMACOLOGICAL ACTIVITY

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2-Amino-alkanols are commonly encountered in tunicates and some sponges. The structures of these molecules are generally related to the widely distributed amphiphilic sphingosine, the central structural element of sphingolipids; their carbon chain lengths vary from C₁₂ to C₃₀ and polyunsaturated variants have been also reported. Ascidians from *Pseudodistoma* and *Clavelina* genera have been prolific in the production of 2-amino-3-hydroxyhydrocarbons¹⁻⁷. During our search for new biologically active and significant metabolites from Mediterranean marine ascidians, we have examined specimens of the tunicate *Clavelina phlegraea* Salfi, 1929 collected in the Bay of Naples. This study led to the isolation of twelve new cytotoxic compounds, clavaminols A – N, whose stereostructures were established by analysis of spectroscopic data and chemical conversion. Most of new compounds have been tested for their cytotoxic and pro-apoptotic properties and clavaminol A was shown to be the more potent cytotoxic compound of this series inducing cell death through activation of the apoptotic machinery.



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NATURAL PRODUCTS FROM *LATRUNCULIA* SPECIES SPONGES: GEOGRAPHIC AND BIOSYNTHETIC VARIATION

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The discorhabdins are a class of cytotoxic alkaloids found in the marine sponges of the families Latrunculiidae and Acarnidae. As part of a structure-activity relationship investigation of discorhabdin B, several known discorhabdins and five new compounds were isolated from New Zealand and Antarctic specimens of *Latrunculia* species. Naturally occurring (-) and (+) enantiomers of discorhabdins B, G*, L and W are presented along with the observed spatial variation in compound production. A revised discorhabdin biosynthetic tree is proposed, with makaluvamine F and discorhabdin B as precursor compounds to analogues with the common discorhabdin B, D, U and W skeleton. Biological activities of all new natural products and enantiomeric pairs is presented.

ANTIFOULING CHEMICAL DEFENSE MECHANISM USING SECONDARY METABOLITES IN THE ASCIDIAN *PHALLUSIA NIGRA*.

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The solitary ascidian *Phallusia nigra* is common in both the Mediterranean and the Red Sea. It grows to an average height of 10 cm. and its black surface is free of fouling all year long¹. This is in contrast to other solitary ascidian species such as *Herdmania momus*, a co-occurring solitary ascidian that is naturally fouled. Secondary metabolites extracted from *P. nigra* were field-tested against a wide range of epibionts in their natural environment using methods adapted to local conditions.

The antifouling activity of the extracts was tested by settlement assays at a number of locations in the Mediterranean Sea. Multi-well tissue culture plates containing gel surfaces as settlement surfaces were deployed for 4 weeks at a time. The wells contained either control gel surfaces or gels with *P. nigra* extract^{2,3}.

P. nigra extract was found to reduce barnacle abundance in settlement experiments (Replicated goodness of fit test, one tailed, $p < 0.001$, $df = 20$). Experiments also showed that polychaete settlement was significantly lower on surfaces containing *P. nigra* extracts (Replicated goodness of fit test, one tailed, $p < 0.005$, $df = 19$). Algae settlement was found to be significantly lower on surfaces containing extracts than on control surfaces (Replicated goodness of fit test, one tailed, $p < 0.005$, $df = 8$).

These results concur with the results of *P. nigra* extract tested in the lab on brine-shrimp in a 24 hour assay. *P. nigra* extract was found to be active against brine-shrimp resulting with 82% mortality of brine shrimp at a concentration of 1.667 mg/ml. The same concentration of *H. momus* extract resulted in only 7% mortality which is similar to the controls that were tested (DMSO & water).

This research shows the necessity of field experiments conducted in the organism's natural environment to test the activity of extracts. The results of this research clearly indicate that *P. nigra* extracts have antifouling activities. More work has to be done to isolate and identify the active compounds.

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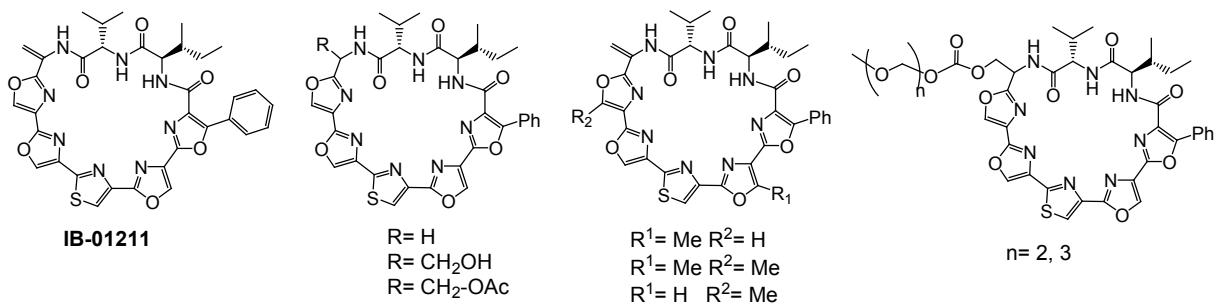
SYNTHESIS OF IB-01211 DERIVATIVES BIOLOGICAL ACTIVITIES

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The metabolic and physiological capabilities of marine organisms that allow them to survive on their unique habitats also provide a great potential for production of metabolites. Marine invertebrates produce important antibiotic, anti-inflammatory, antitumor, antiviral, insecticidal, and herbicidal effects. In the last two decades, unprecedented natural products containing directly linked 2,4-azoles, have been isolated from natural sources.¹⁻³ Recently, a new cyclic peptide, IB-01211 was isolated from the marine microorganism strain ES7-008, which is phylogenetically close to *Thermoactinomyces* genus. This peptide is strongly cytotoxic to several tumor cell lines. IB-01211, contains four oxazoles, one thiazole, and a tri-peptide that includes a didehydroamino acid residue.⁴ An efficient and versatile synthesis of IB-01211 based on a combination of peptide (dehydration of serine and phenylserine containing peptides) and heterocyclic chemistry (Hantzsch synthesis) has been described.⁵ This synthesis allows access to novel structural analogues for further biological evaluation. The preparation of several analogues of IB-01211 and its activities will be described.



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NEW DOLASTANES FROM THE BROWN ALGA *DILOPHUS SPIRALIS*

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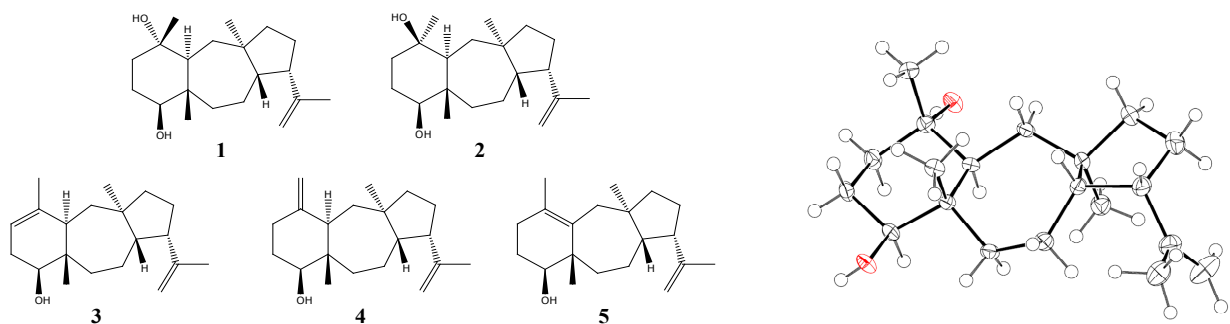
Brown algae belonging to the family Dictyotaceae are known to produce a wide range of diterpenes of different carbon frameworks, e.g. dolabellanes, dolastanes, xenicanes or sesquiterpenoid skeletons with an additional prenyl unit on the side chain. Many of these metabolites have proved to possess antibacterial, algicidal, cytotoxic, ichthyotoxic and antifeedant activities.

In continuation of our studies on bioactive natural products from marine organisms of the Greek Seas, we investigated the chemical composition of *Dilophus spiralis* collected from Elafonisos island, south of Peloponnese.

Extraction of the freeze-dried alga with dichloromethane, followed by a series of chromatographic separations led to the isolation of a number of known metabolites, along with five new dolastane derivatives (**1-5**).

The structural elucidation of the isolated compounds and the assignment of their relative stereochemistry was based on analyses of their spectroscopic data, mainly information obtained through 1D and 2D NMR experiments and HR-MS. The proposed structure of metabolite **1** was confirmed by single crystal X-ray diffraction analysis. The absolute configuration of the new natural compounds is currently under investigation using the modified Mosher's method.

Pharmacological evaluation of metabolites **1-5** proved them to possess moderate *in vitro* cytotoxic activity against a panel of cancer cell lines.



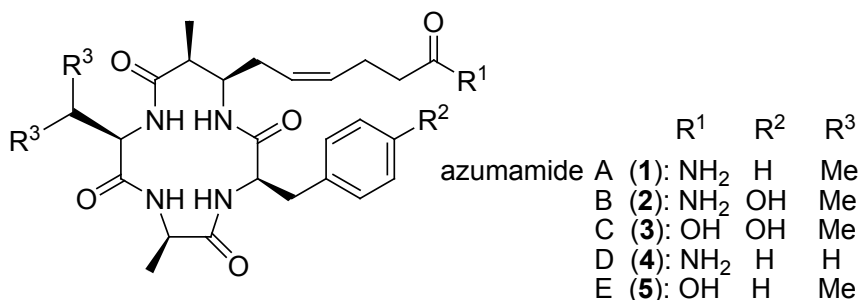
SYNTHESIS OF A CYCLODEPSIPEPTIDE ANALOG OF AZUMAMIDE E

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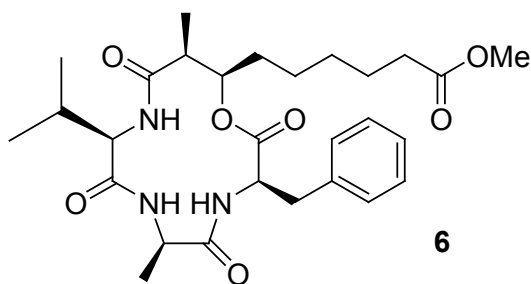
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The azumamides (1-5) are five cyclopeptides recently isolated by Fusetani and coworkers from the marine sponge *Mycale izuensis*.¹ These compounds show interesting HDAC inhibitory activity which makes the azumamide A and E, the most active members, promising antitumor agents.



Azumamide A and E have been recently synthesized.² Our studies on interaction of azumamides with the receptor binding pocket³ prompted us to start the design and the virtual screening of azumamides analogs in order to find more potent and synthetically accessible compounds. In this communication we wish to report the synthesis of a cyclodepsipeptide simplified analog (6) of azumamide E.



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ANTIFUNGAL ACTIVITY EVALUATION OF THE CONSTITUENTS OF *HALICLONA BAERI* AND *HALICLONA CYMAEFORMIS*, COLLECTED FROM THE GULF OF THAILAND

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A new compound maleimide-5-oxime was isolated, together with 3,4-dihydroxybenzoic acid, tetillapyrone from the ethyl acetate extract of the marine sponge *Haliclona baeri* while tetillapyrone, nortetillapyrone, *p*-hydroxybenzaldehyde and phenylacetic acid were isolated from the ethyl acetate extract of *Haliclona cymaeformis*, collected from the Gulf of Thailand. The structures of tetillapyrone and nortetillapyrone, previously isolated from the marine sponge *Tetilla japonica*¹ have been re-examined. Maleimide-5-oxime, tetillapyrone and nortetillapyrone were found to be inactive against three human tumor cell lines (the estrogen-dependent ER(+) MCF-7, the estrogen-independent ER(-) MDA-MB-231 and NCI-H460. Maleimide-5-oxime, *p*-hydroxybenzaldehyde, phenylacetic acid, tetillapyrone and nortetillapyrone were evaluated for their growth inhibitory effect against seven yeasts and eight filamentous fungi. Only nortetillapyrone exhibited antifungal activity, with a preponderance on the dermatophytic filamentous fungi.

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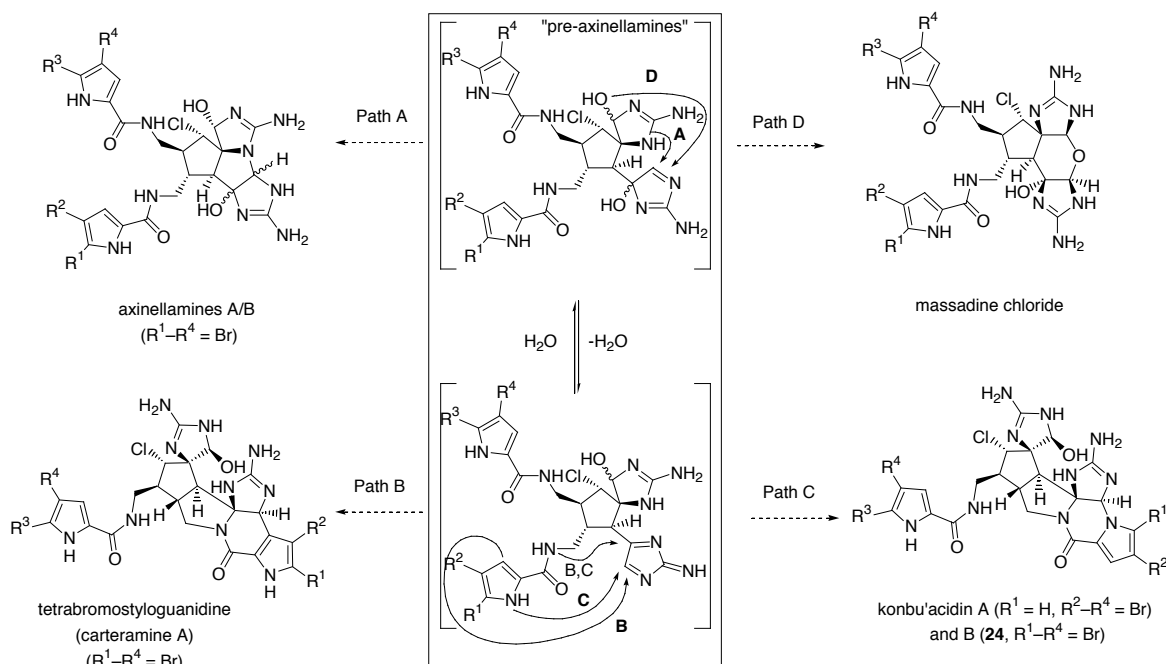
COMMON BIOSYNTHETIC ORIGIN OF ALL DIMERIC PYRROLE-IMIDAZOLE ALKALOIDS

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Very recently the relative configuration of palau'amine was revised.^[a] This has important implications for the presumed biogenesis of cyclized dimeric pyrrole-imidazole alkaloids.^[b] As illustrated in the scheme, the proposition can be made that all of the members of the family owe their existence to a single precursor. This is a significant departure from the previously proposed origins of these alkaloids, although they share some common features. The hypothetical central intermediate "pre-axinellamine" might follow one of four reaction pathways (A – D, see scheme). Pathways A and D lead to molecules with a tetracyclic core (axinellamines and massadines), B and C to molecules with a hexacyclic core (palau'amines, styloguanidines, and konbu'acidins). The ramifications of these hypotheses will be discussed.



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CORRELATION BETWEEN THE LIPOXYGENASE ACTIVITY OF DIATOMS AND THE REPRODUCTIVE FAILURE OF THEIR GRAZERS

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In the last years, the beneficial role of diatoms in the marine food web has been questioned on the basis of laboratory and field studies showing the antiproliferative effect of these microalgae on the reproduction of their grazers, the copepods. This effect was initially correlated to polyunsaturated aldehydes isolated from diatoms,^{1,2} but recently we report that other diatom-derived fatty acid derivatives, generically named phycoxylinins, produced by lipoxygenases with different regio- and stereo-specificity have a similar or stronger effect on copepod reproduction.^{3,4} Here we show data on lipoxygenase activity and levels of phycoxylinin and aldehydes in four different diatom species (*Skeletonema marinoi*, *Skeletonema pseudocostatum*, and two strains of *Thalassiosira rotula*).⁵ These parameters were compared to the effect on the reproduction (egg production rates and hatching viability) of copepods reared on the microalga. Analysis of the data suggests the deleterious effect of diatoms on copepod reproduction is not necessarily dependent on the presence of volatile aldehydes, whereas the diatom-induced effects on copepods can be due to a complex mixture of components and massively influenced by the generation of transient species, such as fatty acid hydroperoxides (FAHs) and reactive oxygen species (ROS), capable to trigger an highly oxidative status. This process might be responsible to DNA damage that well explains the parental effects observed in nauplii generated by copepod fed on marine diatoms.^{5,6}

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**INFLUENCE OF CULTURE CONDITIONS ON THE
PRODUCTION OF KNOWN AND NEW BACILLAENES BY
A MARINE SPONGE-DERIVED *BACILLUS SUBTILIS***

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The extract of a *Bacillus subtilis* isolated from the marine sponge *Oscarella lobularis* was found to exhibit pronounced antimicrobial activity against a selection of phytopathogenic bacteria. Chemical investigation using HPLC-UV and -MS revealed the presence of polyene antibiotics belonging to the bacillaene class of compounds.

The present study shows the influence of varying culture conditions on diversity and concentration-time profile of bacillaenes; using optimized conditions an 100fold increase in bacillaene production was achieved.

Besides the known metabolites bacillaene and dihydrobacillaene also new members of this intriguing class of polyketide natural products were found, purified, and structurally elucidated.

POTENT ANTI-INFLAMMATORY ACTIVE COMPOUNDS FROM MARINE BROWN ALGAE (SARGASSACEAE, FUCALES) FROM THE SOLOMON ISLANDS, SOUTH PACIFIC

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This project is part of the Coral Reef Initiative in the South Pacific (CRISP), and involves research into active compounds isolated from marine organisms.

With the objective of making valuable use of the high biomass of Sargassaceae species found in tropical areas [1, 2], 4 species of *Sargassum* and 3 species of *Turbinaria* were collected in the Solomon Islands. In this study, potent anti-inflammatory activity of the polar and non-polar extracts was screened by evaluating their anti-phospholipase A₂ activity [3]. Only dichloromethane-methanol crude extracts from 6 species showed a significant activity (> 80% of inhibition). Among these 6 species, we selected extracts from *Turbinaria conoides* and *Sargassum crassifolium* for further active compounds isolation.

For this purpose, silica-gel gravity chromatography [4] was performed, and each fraction obtained was tested for its anti-phospholipase A₂ activity. The most active fractions were the MeOH ones, which 3 fatty acids commonly present in it both species. Isolation and identification of the active compounds will be presented and discussed.

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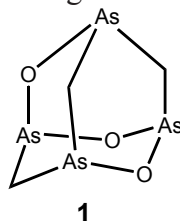
THE FIRST SULFURATED POLYARSENIC COMPOUNDS DISCOVERED IN NATURE: ARSENICIN B AND -C FROM THE NEW CALEDONIAN SPONGE *ECHINOCHALINA BARGIBANTI*

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We recently described arsenicin A as the first polyarsenic compound ever found in nature. It was isolated from the sponge *Echinochalina bargibanti* from the northeastern coast of New Caledonia. Backbreaking experimentation, culminated in the synthesis of an analogue, allowed us to attribute the adamantane-type structure **1** to this compound.¹ Less abundant sulfurated polyarsenic compounds were also isolated from the same bioassay-guided fractionation of the ethanol extract of this sponge. The compositions C₃H₆As₄OS for arsenicin B and C₃H₆As₄S₂ for arsenicin C were deduced from HR-EI-MS experimentation. The structural assignment is supported by i) a pattern of tandem MS fragmentation similar to that observed for arsenicin A in soft-ionization atmospheric pressure chemical ionization (APCI) experiments, and ii) matching of NMR spectral data with those obtained from calculations. Although the literature on natural sulfurated arsenicals is scanty,² the structures of arsenicin B and C are in line with the high affinity of arsenic for sulfur. These metabolites join arsenicin A to constitute an unprecedented new family of compounds that pose anew the challenge of the cycle of arsenic in nature. On the other hand, their bactericidal activity on human pathogenic strains surpasses that by arsenicin A, which already defeated the clinical antibiotic gentamycin to this respect. Coupled to the availability of synthetic analogues, these compounds furnish models for a new class of antibacterial agents.



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NEW CYCLIC PEPTIDES FROM THE CYANOBACTERIUM *NOSTOC INSULARE*

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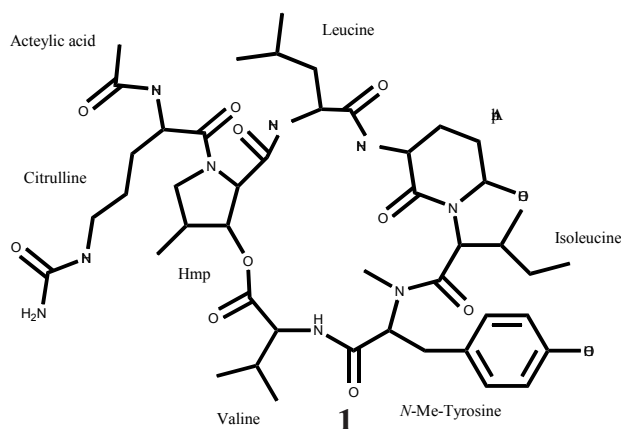
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Cyanobacteria belong to the oldest forms of life on earth and populate nearly every habitat of our planet. Because of the wide spectrum of secondary metabolites and their outstanding bioactivities, cyanobacteria are of enormous pharmaceutical interest.

Cyanopeptolines, a large group of cyanobacterial peptides, are 19-membered cyclic depsipeptides cyclized by an ester-linkage of the threonine hydroxyl group with the carboxy terminus of the C-terminal amino acid precursor. All contain the unusual amino acid Ahp (3-amino-6-hydroxy-2-piperidone) [1]. Cyanopeptolines are inhibitors of serine-proteases and were part of cocrystallizing studies with trypsin [2] and elastase [3].

The current project focuses on the chemistry and bioactivity of the extract of the cyanobacterium *Nostoc insulare*. In a screening of cyanobacterial extracts against human leucocyte elastase this extract showed a prominent activity with an IC₅₀ value of 9.03±0.62 µg/ml. To date we could isolate five new cyanopeptolines, e.g. Noi-H-1-4 (**1**) from this extract. Especially noticeable is the occurrence of citrulline in some of these peptides, which is extremely rare in cyanobacterial cyclic peptides. Apart from Ahp some of the isolated compounds contain the unusual amino acid Hmp (3-hydroxy-4-methyl-proline). The elucidation of stereochemistry and the bioactivity of individual peptides is in progress.



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SUBINHIBITORY CONCENTRATIONS OF ANTIBIOTICS INDUCE PHENAZINE PRODUCTION IN A *STREPTOMYCES* SP.

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Recent studies showed that antibiotics, regardless of their mode of action, cause at subinhibitory concentrations considerable transcription activation in various bacteria¹. In a study aiming to reveal, if also the secondary metabolite production of bacteria is affected by low antibiotic concentrations, we treated sixteen actinomycetes with several antibiotics. The most rewarding results were obtained for a marine *Streptomyces* sp. isolate. Under conditions of treatment with the protein synthesis inhibitor tetracycline, three new phenazines were induced. When using subinhibitory concentrations of the cell wall inhibitor bacitracin another new phenazine was produced. The careful analysis disclosed that these new phenazines were biosynthesized in traces also in the untreated culture. The new phenazines were produced earlier and in much higher concentrations in the “antibiotic-stimulated” cultures than in the untreated fermentations.

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MARINE NATURAL PRODUCTS OF SEA CUCUMBERS AND SEA STARS IN ARGENTINA: BIOACTIVITY AND CHEMICAL ECOLOGY

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The search for natural marine products in South America is at this moment at an important developing stage and is arousing great interest. The first stage –isolation and structural elucidation of new molecules– has been enriched with the possibility of generating important biotechnological and pharmacological applications as well as with the recent development of a marine chemical ecology research line that intends to understand the role that secondary metabolites play in the organism, in close relationship of prey-predator.

This work presents an updated checklist of the results obtained from interdisciplinary research lines in Argentina on the isolation, evaluation of biological activities and ecological role of secondary metabolites from the sea stars *Allostichaster inaequalis*, *Anasterias minuta*, *Cosmasterias lurida*, *Heliaster helianthus*, *Labidiaster annulatus* and *Luidia ludwigi*, the ophiuroids *Astrotoma agassizii*, *Ophiactis asperula*, *Ophiacantha vivipara*, *Ophiocoma echinata*, *Ophioplocus januari*, *Ophionotus victoriae* and *Gorgonocephalus chilensis*, and the sea cucumbers *Hemiodema spectabilis*, *Psolus patagonicus*, *Staurocucumis liouvillei* and *Athyonidium chilensis*.

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CHEMICAL ECOLOGY RELATIONS BETWEEN DIATOMS AND CRUSTACEANS: THE LIPIDIC PROFILE OF THE BENTHIC DIATOM *COCCONEIS SCUTELLUM*

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Diatoms of the genus *Cocconeis* play a particular ecological role in the marine benthos: they influence the sex reversal of the decapod *Hippolyte inermis*¹. This shrimp is a protandric crustacean (*i.e.* individuals experience a male stage before switching to female, about a year after hatching²). In nature this is a frequent phenomenon happening in autumn, in the absence of diatoms, by apoptosis in the androgenic gland of the male shrimps. Contrastingly, in spring (the period of maximum diatom development in their habitat) the shrimp populations are represented by both males and females. These last females do not go through the usual previous male phase, but they directly derive from differentiation of the postlarvae³. The direct differentiation into females is produced by the diatoms they feed upon, which do contain a factor(s) triggering the early programmed cell death of the androgenic gland in the postlarvae. Thus, *Cocconeis* diatoms speed up the apoptosis mechanisms that the shrimps would undergo one year later⁴. *Cocconeis scutellum* is one of the most active diatom foods for *H. inermis*, hence these algae have been studied from the chemical point of view. *In vivo* experiments have demonstrated that the ethereal extract of *C. scutellum* is the fraction with the main apoptotic activity. Current bioactivity experiments are being carried out in order to identify the active compound(s) within this extract. The diatom lipidic profile has been determined by GC/MS analysis and it consists of sterols, fatty acids and fatty alcohols. Interestingly, we have not detected the occurrence of any of the aldehydes previously reported to be responsible for teratogenic effects of planktonic diatoms on copepod crustaceans^{5,6}. Separation of the components of the ethereal fraction is in progress.

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NEW METABOLITES FROM MARINE ORGANISMS OF THE CANARY ISLANDS

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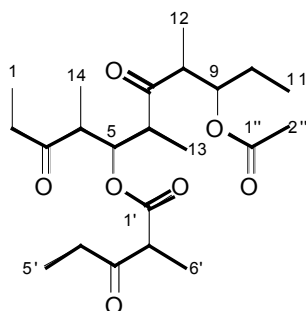
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During the past few years our investigation has focused in the study of new substances from macro and marine microorganisms. Although our interest has been focused specially in polyether toxins related to the red tides, another polyethers group has called our attention powerfully. In effect, the red seaweed of the *Laurencia* genus is a prolific source of polyethers squalene derivatives, which are characterized for a strong antitumor activity, as well as, for being specific protein-phosphatases enzymes inhibitors. The lead compounds into this group of metabolites are thyrseferol and venustatriol. A large number of congeners have been identified by our group from *Laurencia viridis* collected in the south of the Island of Tenerife.^{1,2}

On the other hand, gastropod molluscs have been a prolific source of structurally-novel natural products for over forty years.²⁻⁴ A wide range of secondary metabolites derived from the iterative condensation of methylmalonyl-CoA units have been isolated from opisthobranch and pulmonate molluscs. Most of them possesses a contiguous carbon skeleton as would be expected from regular polyketide biosynthesis and only a few examples have been isolated with a non-contiguous polypropionate chains. Selected examples of these metabolites are baconipyrones isolated from the pulmonate *Siphonaria baconi* and siserrone A from *S. serrata*^{3,4}.

The results obtained in the isolation of new polyethers from a collection of *L. viridis* as well as new examples of non-contiguous polypropionate derivatives from the marine gastropod *Micromelo undata* will be discussed.



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ISOLATION OF NEW SECONDARY METABOLITES FROM MYXOBACTERIA

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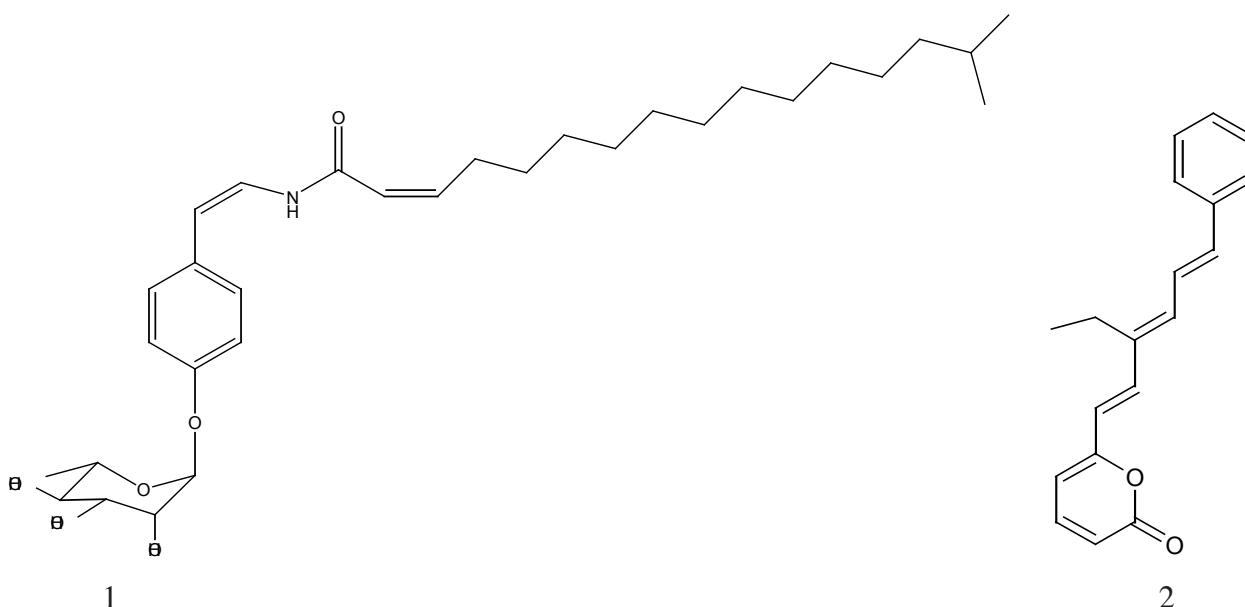
Myxobacteria are gram-negative rod shaped bacteria which form swarm-like colonies and spread over surfaces. They feed on other microorganisms or organic macromolecules, e. g. cellulose. The most outstanding feature of myxobacteria is their ability to form fruiting bodies by which the different genera can be distinguished.

In the last years myxobacteria have been in the focus of natural products research and have become known as potent producers of secondary metabolites. The best-known myxobacterial compounds are the epothilones which are in clinical trial as anticancer agents [1].

Searching for new natural products, we isolated and screened myxobacterial strains. Two of them, strain 150 and strain 131, were singled out due to the results of NMR and LC-MS analyses. The cultivation of each of the two strains led to the isolation of new compounds.

The structures were elucidated by applying different one and two dimensional NMR-techniques as well as mass spectrometry and UV- and IR- spectroscopy.

The extract of strain 150 (*Nannocystis* spec.) contains an unusual polyene (**1**) and two derivatives thereof. The other strain, presumably a *Myxococcus* spec., was found to produce two rhamnosides (e. g. **2**) which differ only in the length of the attached fatty acid. Both structural types are unprecedented.



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BIOACTIVE METABOLITES OF DINOFLAGELLATES FROM OKINAWAN CORAL REEFS

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Benthic dinoflagellates inhabiting coral reefs are well-known sources of various bioactive substances such as the okadaic acids from *Prorocentrum lima*, the ciguatoxins and maitotoxin from *Gambierdiscus toxicus*, the ostreocins from *Ostreopsis siamensis*, and amphidinols from *Amphidinium klebsii*¹⁻³. In view of the marked interspecies variation of bioproducts and the demands for these bioactives as biochemical probes as well as reference materials in analysis, we renewed our effort to collect benthic species from different sites of Okinawa, culture them in a large scale, and explore the possibility of new structures. Because of the heavy demands for the okadaic acids, we set our primary target on *P. lima*. In addition to the known okadaic acids, occurrence of new analogs was detected by LC-MS. Much significantly, we found three novel polyketides and determined the planar structures of two macrolides. The macrolides, named okilimalide-1 and -2 respectively, were cytotoxic to tumor cell lines. Okilimalide-1 was deduced to have a molecular formula C₄₃H₆₈O₉ and an 18-membered lactone ring in the molecule. Okilimalide-2 was an isomer having a molecular formula C₄₃H₆₈O₉ and a 17-membered lactone ring. The third compound was a large molecule having an [M+Na]⁺ ion at *m/z* 1566. The structural elucidation is under way. We also collected 29 clones of *G. toxicus* in Okinawa and explored the possible production of ciguatoxins by LC-MS and Na⁺-channel specific cytotoxicity assays. Ciguatoxins known from the Tahitian clone of *G. toxicus* were not detected but occurrence of new polyketides was suggested.

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AGELASINES J AND K FROM THE SOLOMON ISLANDS MARINE SPONGE *AGELAS* SP.

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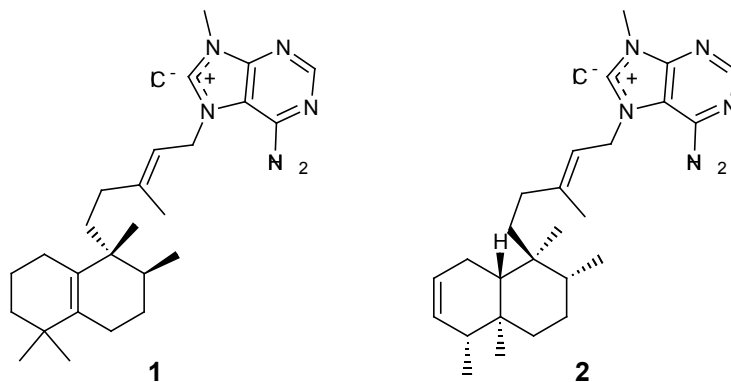
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Agelasidae sponges have been widely investigated, but still represent a good source of new marine metabolites.¹ In the course of our survey of bioactive substances from marine sources, we investigated different extracts of *Agelas* cf. *mauritiana* that have been collected in Solomon Islands. The dichloromethane extract displayed antimalarial activity and was found to be rich in purine diterpenes possessing 9-N-methyladeninium named agelasines.² These purine diterpenes mixture was shown to be responsible of this yet unreported property for those compounds. Nevertheless, some guanine base analogs have been described recently as lead compounds for antimalarial chemotherapy.³ The present paper deals with the isolation of two new Agelasines J (**1**) and K (**2**).



Agelasine J and K displayed mild activity on *P. falciparum* (FcB1-Columbia strain) with an IC₅₀ = 6.6 μM and 8.3 μM and a low cytotoxicity on human tumor cells MCF7 cells with IC₅₀ = 33 μM and 30 μM respectively.

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KINASES INHIBITION AND CYTOTOXICITY OF NOVEL INDIGOID ANALOGS OF MARINE INDIRUBINS

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Bis-indole indirubin, isolated from the marine gastropod mollusc *Hexaplex trunculus*, has been described more than 30 years ago as clinically active in the treatment of human chronic myelocytic leukaemia. Analysis of the molecular mechanisms underlying this pharmacological activity demonstrated that indirubin and its analogues are potent and selective inhibitors of cyclin-dependent kinases (CDKs) [1]. However, the natural product 6-bromoindirubin and its synthetic cell permeable derivative 6-bromoindirubin-3'-oxime, display increased selectivity for inhibition of glycogen synthase kinase 3 (GSK-3) versus CDKs.

In an effort to identify new pharmacological inhibitors of disease-relevant protein kinases with increased potency and selectivity, we synthesised and evaluated various new nitro / amino or-halogeno-indirubin derivatives [2]. The antiproliferative activity of these new substituted polyheterocyclic compounds was determined on various human carcinoma cell lines [3].

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NEW ISONITRILE-CONTAINING INDOLE ALKALOIDS FROM A SOIL SAMPLE OF THE CYANOBACTERIUM *FISCHERELLA* SP.

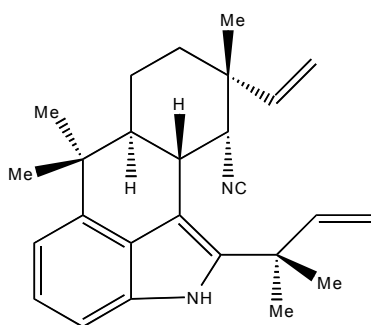
Raveh Avi and Carmeli Samuel

School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Ramat Aviv Tel-Aviv 69978, ISRAEL.

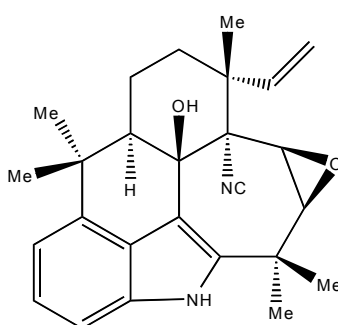
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Isonitrile-containing indole alkaloids are often presented in branched, filamentous cyanobacteria (blue-green algae) such as *Hapalosiphon* spp., *Fischerella* spp., *Westiellopsis* spp., *Calothrix* sp. and *Westiella* spp. These novel isonitrile-containing indole alkaloids are responsible for the antibacterial, antifungal and antimycotic activity associated with these cyanobacteria species. A cyanobacterium strain (IL-199-3-1) was isolated from a soil sample collected at the "Hakaktus" nursery in Herzliya, Israel, in August 1996. The isolate was identified as *Fischerella* sp. and was mass cultured in the laboratory in a modified BG-11 medium. The freeze-dried bacterium mass was first extracted with 7:3 MeOH/H₂O and then with the less polar 1:1 MeOH/CHCl₃ solvent mixtures. The polar crude extract showed antifungal activity, and was subjected to a reversed-phase column chromatography. Fraction 10 (9:1 MeOH/H₂O), which exhibited most of the antifungal activity (and mass), was subjected to a gel filtration on a sephadex LH-20 column. Three fractions from this column showed antifungal activity and were further separated on a C₁₈ HPLC column. Nine alkaloids were isolated from these fractions and their structure was elucidated using 1D and 2D-NMR, and MS techniques. Three of the alkaloids are new natural products namely, ambiguline H isonitrile, ambiguline I isonitrile and ambiguline J isonitrile. The other six are the following known compounds: ambiguline A isonitrile, ambiguline B isonitrile, ambiguline D, ambiguline E isonitrile, hapalindole H and 12-epi-hapalindole H.

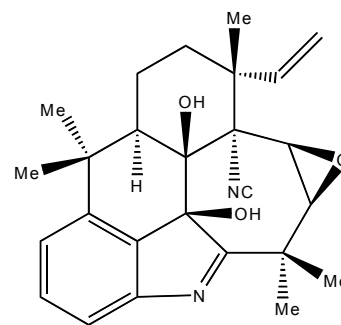
Ambiguline H isonitrile



Ambiguline I isonitrile



Ambiguline J isonitrile



NEW CYTOTOXIC STAUROSPORINE DERIVATIVES FROM THE TUNICATE *CYSTODYTES* SP.

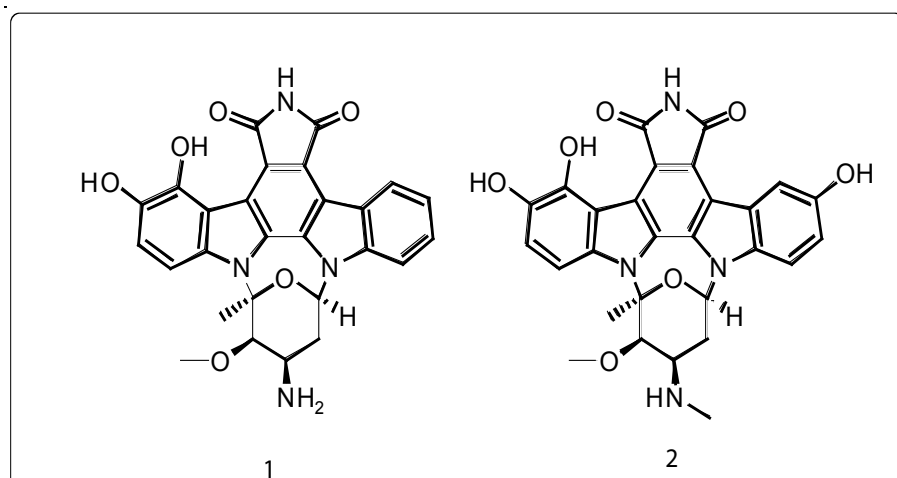
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The staurosporines are a group of complex structurally related indolo[2,3-*a*]carbazole alkaloids obtained from culture broths of actinomycetes¹ and extracts of the marine tunicate *Eudistoma* sp.,^{2,3} the prosobranch mollusc *Coriocella nigra*,⁴ the colonial tunicate *Eudistoma toalensis* and its predatory flatworm *Pseudoceros* sp.^{5,6} Cytotoxic and antitumour properties, as well as strong inhibition of protein kinases, are among the most relevant biological properties exhibited by compounds of this structural class.¹

Herein we report the isolation, structural characterization and cytotoxic activity of two new members of this family (**1** and **2**), obtained by bioassay-guided fractionation of extracts of the tunicate *Cystodytes* sp. The structures of both compounds were elucidated by analysis of their 1D and 2D-NMR spectra and comparison with data reported for other staurosporine derivatives.



Both compounds exhibited strong cytotoxicity against a panel of three human tumour cell lines, including lung (A549), colon (HT29), and breast (MDA-MB-231).

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CHEMICAL CONSTITUENTS OF THE MARINE ANGIOSPERM *CYMODOCEA NODOSA*

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The marine angiosperm *Cymodocea nodosa*, significantly influences the local ecosystem by amplifying the primary substrate and by providing a spatially diverse nursery habitat structure and resources for algal and animal communities¹.

The organic extract of *C. nodosa* was fractionated with a combination of chromatographic techniques to allow the isolation of 4 new and 15 known compounds. Two new compounds are diarylheptanoids (**1-2**), a chemical category known in ground plants and only once isolated from a marine organism². One meroterpenoid (**3**) and a brominated diterpene (**4**), possessing a ring system found only in the soft coral *Briareum sp.*³, complete the number of new secondary metabolites isolated from *C. nodosa*. Furthermore, six known steroids, pacifenol, 10-*epi*-2,3-dihydroaromaticin, 12-acetoxy farnesolacetate, copalic acid, (9*S*,10*E*,12*Z*,15*Z*)-hydroxyoctadeca-10,12,15-trienoic acid, phytol and three known carotenoids were isolated. The chemical structures were assigned on the basis of their NMR and MS spectroscopic data.

A number of the above metabolites were submitted for evaluation of their antibacterial activity, including multidrug resistant and methicillin-resistant strains of *Staphylococcus aureus*, as well as *Mycobacterium phlei*, *M. smegmatis* and *M. fortuitum*.

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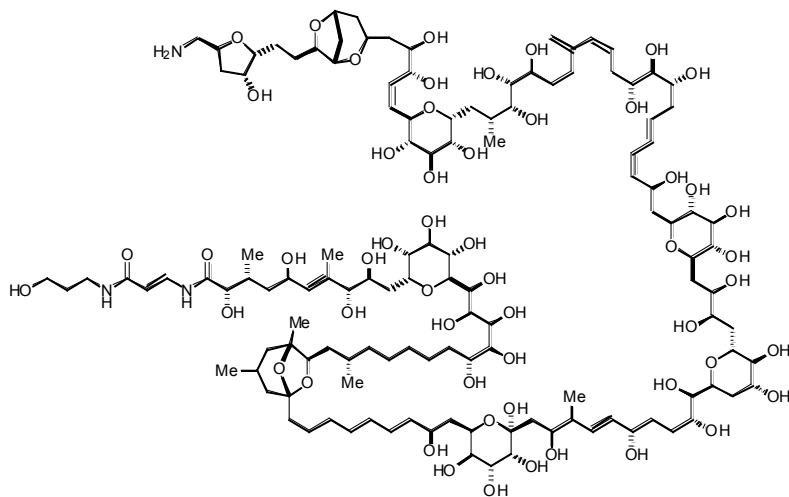
C-GLYCOSIDES: INTERESTING PALYTOXIN SUBUNITS WITH CYTOTOXIC PROPERTIES

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C-glycosides are glycosidic derivatives where the interglycosidic union between the saccharide and aglyconic units is through a C-C bond. These compounds can be found in the structure of several marine natural products. A good example is the marine toxin *Palytoxin*¹ that includes five C-glycosidic subunits in its structure and had been a source of inspiration in the work of many synthetic and carbohydrate chemists.² C-glycosides show high stability toward chemical and enzymatic hydrolysis due to the structural nature of the C-C bond. Moreover, their conformational properties show some similarity to their *O*-glycosidic analogues.³ All these properties make them good candidates as glycomimetics and many authors have taken advantage of these compounds to potentiate the bioactivity of some *O*-glycosidic substrates.⁴ In this communication we describe the synthesis of a large number of C-glycosides and their promising antitumoral and apoptotic properties.⁵ Some structure-activity relationships will be described.



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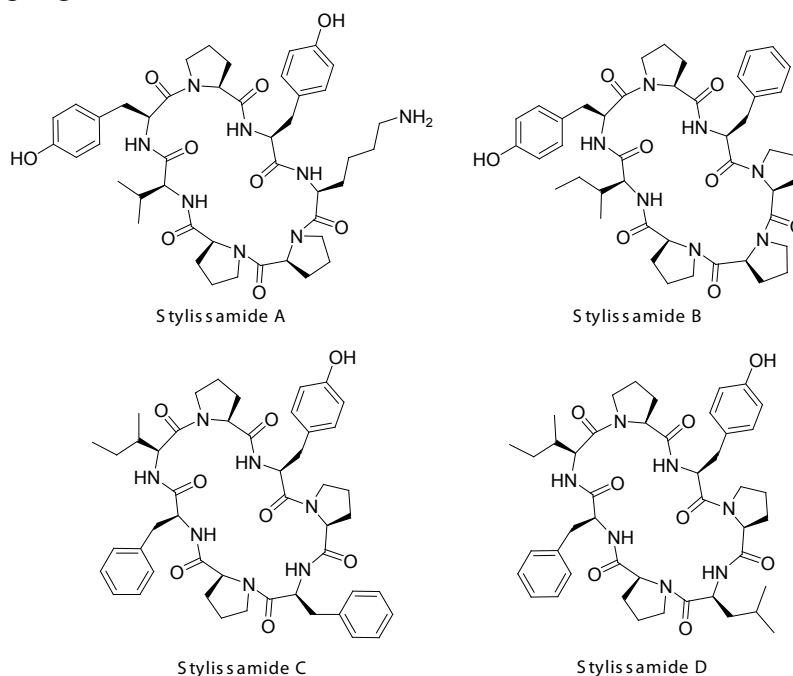
CONFORMATIONAL STUDIES ON STYLISSAMIDES, CYCLIC HEPTAPEPTIDES FROM *STYLISSA CARIBICA*

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The marine sponge *Stylissa caribica* investigated by a MS-guided fractionation revealed several new secondary metabolites. In addition to the six new pyrrole-imidazole alkaloids^[a] (including the first tetrameric derivatives stylissadines A and B)^[b] four cyclic heptapeptides stylissamide A to D were isolated from this sponge.^[c]



The structures of the stylissamides were elucidated using HMBC and NOESY correlations as well as MSⁿ results. The stylissamides A to D are related to the phakellistatins, the hymenamides, and the stylisins.^[d] NOESY experiments with mixing times of 100, 150, 200 ms served as basis for the conformational studies on the stylissamides. The NOE-derived interproton distances were used as distance restraints in the computational analysis using distance geometry and simulated annealing.

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NITROGENOUS COMPOUNDS FROM MARINE INVERTEBRATES

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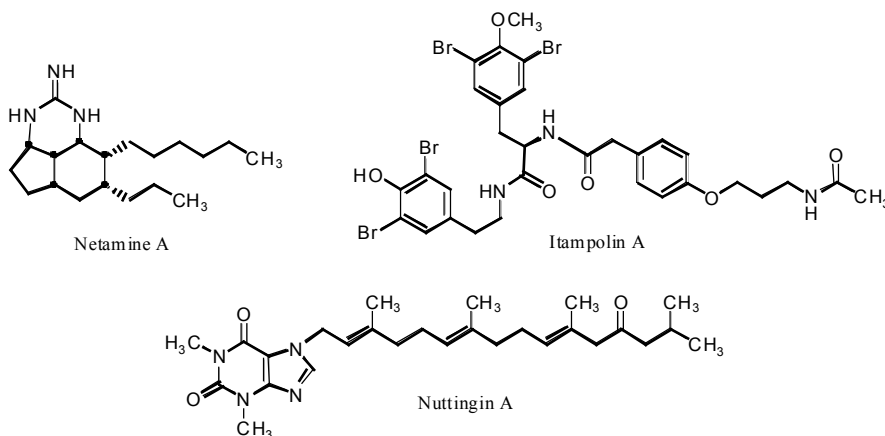
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In search for new compounds from marine invertebrates with bioactive potency, we have isolated the following nitrogenous compounds:

1. Seven new guanidine alkaloids, designated netamines A, B and E-G, from the Madagascar sponge, *Biemna laboutei*. Netamines A and B possess anti bacterial activity (*E. coli* *S. albus* and *B. subtilis*) in concentration of 1.25µg/ml, and netamines E and F induce apoptosis in transformed mammalian cells in concentration of 1.25µg/ml.¹
2. Two novel compounds, designated itampolin A and B, from the sponge *Iotrochota purpurea* collected at Itampolo, southwest of Tuléar, Madagascar.²
3. Six tetraprenylated purine alkaloids, Nuttingins A-F, from the gorgonian *Euplexaura nuttingi* collected in Tanzania. Nuttingins A-E displayed inhibitory activity of both K562 and UT7 tumor cell lines.³

The structures of all the compounds were elucidated on basis of spectroscopic data including ¹⁵N chemical shifts obtained from ¹H-¹⁵N HMBC and ¹H-¹⁵N HSQC-TOCSY spectra.



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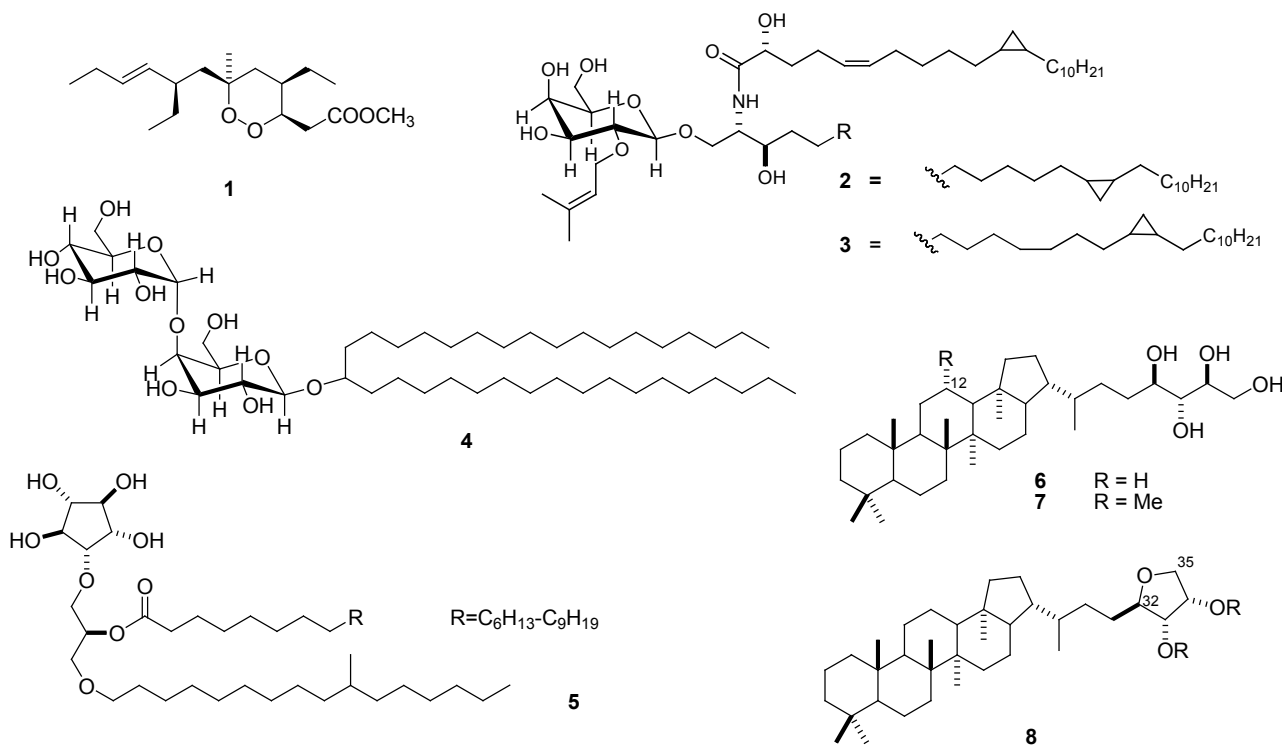
STORIES FROM THE CARIBBEAN SPONGE *PLAKORTIS SIMPLEX*: FROM STRUCTURE ELUCIDATION TO STUDIES OF BIOSYNTHETIC PATHWAYS

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Chemical analyses of *Plakortis simplex* sponge extract provided a number of structurally unique secondary metabolites, including the antimalarial polyketide plakortin,¹ the IL-10 inducing simplexide² and other unusual glycolipids, and hopanoids³ (**1-8**).



The hypothesis that they could be products of symbionts was explored by a multi-step strategy:⁴ i) cell separation of the fresh sponge tissue in combination with chromatographic and spectroscopic analysis; ii) PCR experiments to prove the presence of key enzymes of the biosynthesis of glycolipids and polyketides; iii) cultivation and screening of GSL-producing bacteria; iv) screening of metagenomic DNA of *P. simplex* in search of a PKS compatible with the structure of plakortin.

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UTILIZATION OF HYDROGEN-PRODUCING MARINE BIOMASSES AS SOURCE OF RAW CATALYTIC ACTIVITIES IN BIOCATALYSIS

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Marine sources are represented by marine microorganisms comprising bacterial extremophiles, and plants or animals. This ecosystem is considered a quite unexplored source of biological material and can be also a surprising font of enzymes endowed with new and interesting catalytic activities for applications in biocatalysis [1].

Thermotoga neapolitana (DSM 4359) is an extremophilic marine bacterium belonging to *Thermotogales* order for which the ability to produce hydrogen has been demonstrated. The use of this species has been recently described in a patent for the bioproduction of hydrogen, specifically in an environmental-friendly process using complex carbohydrates as feedstock [2]. Members of this order possess an array of important hyperthermophilic glycosyl hydrolases; [3] therefore, in the conditions adopted for hydrogen production, these enzymes can be well expressed.

Within our involvement in a research project (FIRS-MIUR 2005) based on biohydrogen production, we focused our attention on the catalytic activities for the synthesis of glycosidic linkage from *Thermotoga neapolitana*. Despite of the huge amount of literature that can be found on glycosyl hydrolases from *Thermotogales*, there are few articles dealing with synthetic characteristics of such interesting enzymes. The present communication is aimed to describe a series of enzymatic transglycosylation reactions performed using the crude homogenate easily produced from *Thermotoga neapolitana* biomass.

Xylosidase/xylanase activity seems to be the most abundant leading us to a convenient synthesis of interesting series of pure (β -1,4)-xylooligosaccharides of different aglycones such as 1-hexanol (producing promising candidates for new surfactants), 9-fluorene methanol (obtaining anti-HSV agents), 1,4-butanediol (for the synthesis of new glycolipids), and geraniol (producing aroma compounds). Furthermore it has been also investigated the regioselectivity during galactose, fucose, glucose and mannose enzymatic transfers. The knowledge of synthetic characteristic of all these enzymes will be useful in the feasibility evaluation of large scale processes for the biohydrogen production.

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NEW BROMINATED DITERPENES FROM THE RED ALGA *SPHAEROCOCCUS CORONOPIFOLIUS*

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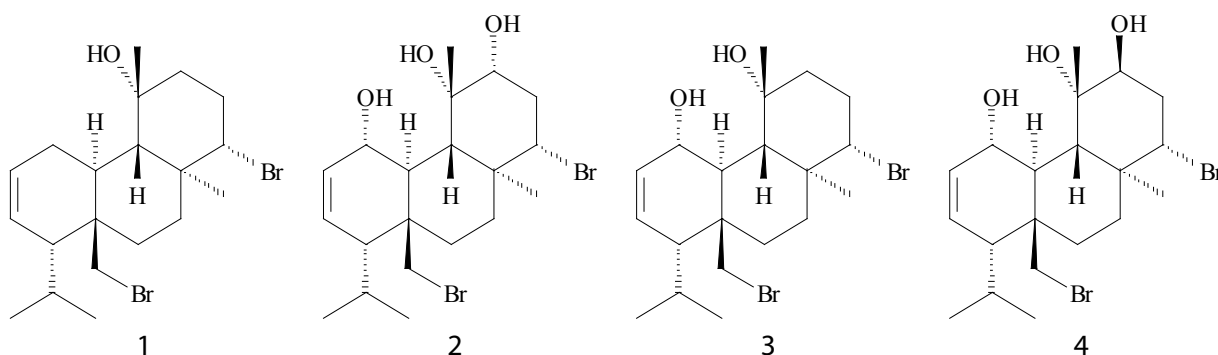
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There are only a few reports on the presence of brominated diterpenes in marine algae and most of them come from red seaweeds (Rhodophyta) of the genus *Laurencia* (order Ceramiales) and *Sphaerococcus coronopifolius* (order Gigartinales).

In the course of our investigations for the isolation of biologically active compounds from marine organisms of the Greek Seas, we recently studied the chemical composition of the red alga *S. coronopifolius*, collected from the north coasts of Corfu island.

Chromatographic separations of the organic extract of *S. coronopifolius* led to the isolation of the Δ^2 isomer of bromosphaerol (**1**), 12*R*-hydroxy-bromosphaerodiol (**2**), bromosphaerodiol (**3**)¹ and 12*S*-hydroxy-bromosphaerodiol (**4**)². The structures of the isolated metabolites, as well as their relative stereochemistry, were established by means of spectral data analysis, including 1D and 2D NMR experiments and HR-MS spectroscopy.

To the best of our knowledge, this is the first report of metabolites **1** and **2**. Additionally, the chemical shifts of the known compounds **3** and **4** were fully assigned.



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ANTIPLASMODIAL AND CYTOTOXIC ACTIVITIES OF HALOGENATED MONOTERPENES FROM *PLOCAMIUM CORNUTUM*

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Malaria is a disease that kills millions of people, especially children, every year.¹ Although drugs such as chloroquine, mefloquine, quinine, pyrimethamine and sulfadoxine are available for prophylaxis and treatment of malaria, their efficacy has greatly diminished in the recent years due to the development of resistance by the parasitic protozoan. In order to combat malaria there is a need for development of new and improved antimalarial compounds.² In the recent years the marine environment has been a source of biologically active compounds and in our search for antiplasmodial compounds from the South African marine coast several algae have been screened against chloroquine sensitive *Plasmodium falciparum* D10 strain.

Crude extracts of *Plocamium cornutum* collected from Kalk bay on the west coast of South Africa were screened against the chloroquine sensitive *P. falciparum* D10 strain. The hexane and DCM fractions showed good activity with $IC_{50} < 10 \mu\text{g/ml}$. This presentation reports the isolation and structure elucidation of two known and three new halogenated monoterpenes from the alga. Each of the pure compounds was tested against the D10 strain of chloroquine sensitive *P. falciparum* and was found to be active with IC_{50} values ranging from 4.6 to 64.0 $\mu\text{g/ml}$.

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POLYUNSATURATED FATTY ACIDS (PUFA) FROM CORAL ASSOCIATED PROTISTS

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The dynamic of microbial biota living in the coral mucus, tissues surface or tissue layers of many coral species may play important roles in their physiology. Coral associated protists may contribute to the coral's nutritional balance. In this study we succeeded in isolating various protists from the mucus of the hermatypic corals *Fungia granulosa* and *Favia sp.* from the Gulf of Eilat. These protists were identified morphologically and molecularly as belonging to the genera *Thraustochytrium* and *Aplanochytrium* (family *Thraustochytriidae*, order *Labyrinthulida*, phylum *Stramenopiles*). *Stramenopiles* are widely distributed marine heterotrophs and are known to be producers of polyunsaturated fatty acids (PUFA); including omega-3 (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)) that is considered essential for normal growth and development of higher organisms. The lipid profile of protists isolated from corals was characterized by gas chromatography coupled with mass spectrometer (GC-MS), demonstrated a diverse spectrum of long chain fatty acids that also include omega-3. Further research assessing their role in coral holobiont physiology is needed. Moreover, these coral associated *stramenopiles* may have potential value for various biotechnological applications.

**POSTER SESSION BLUE
COMMUNICATIONS 51-103**

NEW THERMOPHILIC HYDROGEN PRODUCING MARINE BACTERIA

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Over 80% of the energy consumed today in the world is derived from fossil fuels, which will eventually become depleted in the not too distant future. In addition, burning of fossil fuel contributes severely to the climate change, environmental deterioration and threatening of public health. For this reason there's a considerable tendency to increase the use of renewable sources of energy.¹ Hydrogen represents a potentially ideal fuel source. Thermophilic microorganisms, both *Bacteria* and *Archaea* have been recently selected for hydrogen production.^{2,3} In this contribution, we study hydrogen production from three natural samples isolated from hot marine springs, named "Safen" (collected at Ischia), "2" e "3" (collected at Lucrino). The isolates were cultivated at high temperature and controlled parameters. Anaerobic growing conditions were pointed out to obtain high levels of hydrogen. Lipid analysis of these strains could suggest a similarity of the isolates with members of *Thermotogales* order, even if a complete microbial characterization is still in progress. The identification of new hydrogen-producing microorganisms will be used for developing a reactor able to generate large quantities of hydrogen relatively easily and inexpensively.

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NATURAL PRODUCTS LIBRARY GENERATION – ASSIMILATING INTO THE PHARMACEUTICAL DRUG DISCOVERY PARADIGM

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High-throughput screening (HTS) drives drug discovery in today's pharmaceutical industry. Many of the platforms used in HTS are tailored to synthetically-prepared small molecule libraries. Furthermore, the discovery process is characterized by ever shortening timelines with rapid hit-to-lead development. Traditionally natural products programs have complemented synthetic drug discovery by providing biologically-active, structurally diverse compounds covering a unique chemical space. However, to be competitive, natural products programs must be able to assimilate into the current discovery paradigm beginning with HTS. Therefore, the design of a natural products screening library is of significant importance. Select tools and methodologies used in the generation and characterization of a high quality, multifaceted natural products library at Wyeth will be reviewed. Using case studies, both the benefits and the drawbacks of various sample types will be demonstrated.

CYTOTOXIC AND PRO-APOPTOTIC ACTIVITIES FROM THE MARINE RED ALGAE *SOLIERIA CHORDALIS* (C. AGARDH) J. AGARDH

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Marine algae represent an exceptionally rich source of bioactive compounds with various biological activities. Halogenated terpenoids from red algae represent the more promising macroalgae secondary metabolites in anticancer research. Dehydrothysiferol and “halomon”, extracted from *Laurencia viridis* sp. nov. [1] and *Portieria hornemanii* [2] respectively, have been developed to preclinical phase. Our study has focused on the abundant *Solieria chordalis* (Gigartinales) from Brittany coast, well investigated relating its primary metabolism [3,4] but little explored for its secondary metabolism and related bioactivity. The cytotoxic and pro-apoptotic effects of crude extracts and their fractions from *S. chordalis* have been examined on several human cell lines: Jurkat (leukaemia T cell), Daudi (Burkitt lymphoma, B cell) and A549 (non small cell lung cancer). Two crude extracts (CH₂CL₂ and CHCL₃) have demonstrated a rapid cell death in all these cell lines, revealed by Annexine V+/IP+ staining in flow cytometry. Apoptosis induced by 100 µg of these extracts was accompanied by exposure of membrane phosphatidylserines and caspases activation in all cells lines. Moreover, intracellular reactive oxygen species (ROS) have increased about up 20 to 60% in Jurkat and Daudi cells, and the mitochondrial potential has decreased 24 to 48h after treatment. The mitochondrial membrane protein Apo2.7 was overexpressed from 15 to 50% in Jurkat and Daudi cells. Preliminary analyses have suggested that is probably terpenes compounds from *S. chordalis* extracts that induced cytotoxic effects and apoptose in human cancer cell lines.

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PYRIDOACRIDINE ALKALOIDS WITHIN PURPLE MORPHS OF *CYSTODYTES* SPP. (ASCIDIACEA: POLYCITORIDAE)

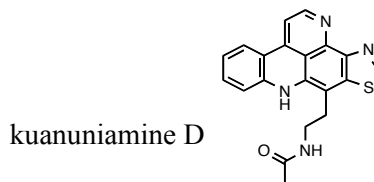
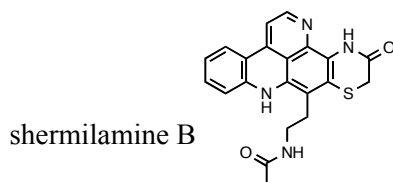
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Cystodytes (Della Valle, 1877) is a colonial ascidian genus widely distributed in both tropical and temperate waters. In the western Mediterranean, colonies of the genus *Cystodytes* have usually been assigned to the circumtropical species *C. dellechiajei* in spite of a noticeable variability in terms of colony color (e.g. green, blue, purple, white, brown), secondary metabolites, and spicule composition¹. In a previous study we described two chemotypes² which, on the basis of genetic and reproductive studies, were found to correspond to sibling species³. The chemotype described for the purple morph, contained the sulphur-containing pyridoacridines, shermilamine B and kuanuniamine D and their deacetylated forms.



In this work, we investigated 3 different purple species of *Cystodytes* collected in the Mediterranean, the Indic and the Pacific. Our results indicate that the purple color is due to the presence of shermilamine or kuanuniamine analogs. The extraction and purification of pyridoacridine alkaloids from *Cystodytes violatinctus* (Solomon Islands) and *Cystodytes aucklandicus* (Glorieuses Islands) led to the characterization of 8 pyridoacridines, 5 of which were new natural products. Structure elucidation was carried out by spectroscopic techniques (1D- and 2D-NMR, MS) and by comparison with existing data. The biological activities of the isolated alkaloids will also be discussed.

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HRMAS PROTON TOTAL CORRELATION APPLIED TO CULTURED MCF7 BREAST CARCINOMA CELL TREATED WITH ASCIDIDEMIN DEMONSTRATE EARLY AND PROFOUND ALTERATIONS OF CELL METABOLISM WHICH SUGGEST A SHUNT TOWARDS AN UNUSUAL METABOLIC PATHWAY

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The identification of drug response pathways and response biomarkers is critical during the development of new drugs. With the aim to determine the value of HRMAS-¹H-MRS in this application, HRMAS Proton Total Correlation was used for metabolomic analysis of tumor cell response to treatment by different drugs. In this study, we tested the changes in cell metabolism induced by the marine intercalating agent ascididemin (Asc). **Methods** Human MCF7 breast carcinoma cells were treated by Asc 5 μ M or the solvent (DMSO 0.5%) for 4, 6, 8, 24, 48 or 72 hrs and tested for apoptotic response (FACS) and metabolomic alterations by HRMAS-¹H-MRS. Spectra were acquired with a Bruker DX500 MHz equipped with a HRMAS probe. Sequences were used after water signal suppression. 1D spectra were used for assessment of metabolite profiles and preliminary identification of metabolic alterations. 2D TOCSY spectra were used for complete identification and quantification of metabolites. **Results** 32 metabolites were quantified at 6 and 24 hrs. Strong alterations of phospholipid and changes in mitochondrial metabolism were observed as early as 6hrs after cell-treatment. Consistent with FACS data, strong apoptotic response was observed after 48 hrs. At early steps, Asc promotes PUFA accumulation, profound alterations of phospholipids metabolism and, changes in the synthesis of metabolites involved in maintenance of cell volume homeostasis and in protection against oxidative stress. At 24 hrs, the biosynthesis of citrate, amino-acids, lactate was also profoundly altered evidencing important alterations of mitochondrial metabolism and, krebs-cycle blockade. Two response biomarkers have been identified: citrate and gluconate. This is the first identification of gluconate as a response biomarker. **Conclusion** Altogether our data, demonstrate profound alterations of cell metabolism which suggest that, MCF7-response to Asc involves oxidative stress, induction of apoptosis and, a shunt towards a very unusual metabolic pathway. This is the first time that this latter effect is reported. Tests in animal bearing tumor are planned to determine how the metabolic changes observed *in vitro* are conserved *in vivo*.

CALANOID COPEPOD *LIMNOCALANUS MACRURUS* FEEDING ON PLANKTON ALGAE AT THE GULF OF RIGA (BALTIC SEA)

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The calanoid copepod *Limnocalanus macrurus* is known as herbivore at smaller developmental stages and omnivore at latest stages. Its predaceous feeding habits began in the fourth copepodite stage. As the species feeding ecology are researched very scarce, this investigation is devoted to ascertain *Limnocalanus macrurus* feeding on phytoplankton at natural water environment in wintertime. Sampling was performed in January and February 2007 in the Gulf of Riga and samples collected at 0-10m, 10-20m, 20-30m, 30-40m and 40-50m water layers. With a view to study *Limnocalanus macrurus* diet at different depths algae species composition in the guts were identified. Dominated plankton algae in the central Gulf of Riga were diatoms, but phytoplankton species composition changes vertically. At the top layer main phytoplankton biomass made *Melosira arctica* and *Actinocyclus octonarius*, at 30m and 40m layers *Actinocyclus octonarius*, but at deepest 50m dominated *Actinocyclus octonarius* and *Thalassiosira baltica*. These algae species would be main components make *Limnocalanus macrurus* diet composition. The research will give view on vertical feeding patterns of the copepod species.

ILIMAQUINONE, A SESQUITERPENE QUINONE FROM THE MARINE SPONGE *SMENOSPONGIA* SP. INDUCES APOPTOSIS IN HUMAN METASTATIC MALIGNANT MELANOMA M4BEU CELLS BY TRIGGERING CERAMIDE SIGNALLING.

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The median survival of advanced malignant melanoma is 6 to 7 months with a 5-year survival of c.a. 6%. Because of its rising incidence in Caucasian population, this malignancy has become an important health issue. Its low prognosis is largely due to rapid development of liver, brain and lung metastases. In this study, the rezasurin reduction test (a cytotoxicity assay) and FACS analysis were used to assess the antiproliferative effect of ilimaquinone, a sesquiterpene quinone previously isolated from the marine sponge *Smenospongia* sp. collected in the Red Sea,^{1,2} on human metastatic malignant melanoma M4Beu cells, human A549 lung carcinoma cells and primary human fibroblasts. Ilimaquinone exhibited preferential antiproliferative effects on M4Beu cells and, more pronounced antiproliferative effects on M4Beu and A549 cells than on primary fibroblasts suggesting a therapeutic margin. Inhibition of M4Beu proliferation was related to cell-cycle arrest in G1 and, at higher concentration induction of apoptotic cell death. Additional investigations correlated apoptosis with ceramide release and cleavage of poly (ADP-ribose)-polymerase. Ceramide release was reduced by co-treatment with myriocin (an inhibitor of serine-palmitoyl transferase) or with N acetyl cysteine (Nac) a precursor of glutathione (an inhibitor of neutral sphingomyelinase). Apoptosis was strongly impaired by Nac pre-treatment while pre-treatment by myriocin had little inhibitory effect. Altogether, these data indicate that ilimaquinone triggered ceramide signalling in human M4Beu cells and that the pool of ceramide induced by ilimaquinone treatment was due in part to “*de novo*” ceramide synthesis and, in part to sphingomyelin hydrolysis by neutral sphingomyelinase. Our data also showed that neutral sphingomyelinase activity was required for induction of apoptosis. Finally, the positive cytotoxicity index on M4Beu and A549 cells suggests a possible value of the sesquiterpene quinone derivatives from *Smenospongia* sp for treatment of metastatic melanoma and, justify further investigations to test this hypothesis.

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MARINE NATURAL PRODUCTS AT NATURAL HISTORY MUSEUMS IN THE NETHERLANDS

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Text of abstract – The Dutch National Museum of Natural History Naturalis and the Zoological Museum of Amsterdam have been active in the field of marine natural products and chemical ecology for almost two decades. Initially their role took the form of storage for voucher specimens of species that had been studied by natural product chemists and had been identified by the museum curators. The interest in secondary metabolites subsequently developed into research projects related to chemical ecology and chemo-taxonomy (e.g. Symbiosponge) with participation of MSc and PhD students. The curators at the two museums have collaborated with scientists from more than 20 institutes worldwide, resulting in co-authorship of over 50 peer-reviewed papers.

At present the two museums are in the process of fusing together with other institutes in the Netherlands into the National Centre for Biodiversity (NCB). In a poster we shall discuss the future aims the NCB and prospected research in marine natural products.

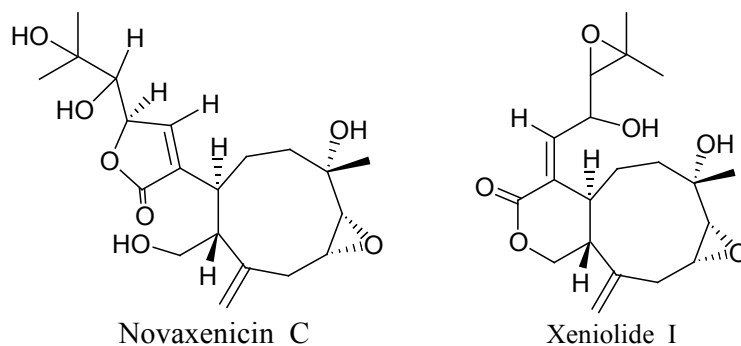
NOVAXENICINS A-D AND XENIOLIDES I-K, SEVEN NEW DITERPENES FROM THE SOFT CORAL *XENIA NOVAEBRITANIAE*

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Soft corals are a rich source of diterpenes, among which are the xenia metabolites. The latter compounds have originally been divided by us into three groups, the xenicins, xeniaphyllanes and xeniolides; other groups followed.¹ From the Kenya soft coral *Xenia novaebritaniae* we have been isolated seven new compounds, novaxenicins A-D (**1-4**) and xeniolides I-K (**5-7**).² The structures and relative stereochemistry of **1-7** have been established by detailed spectroscopic analysis including 2D NMR (COSY, HSQC, HMBC and NOESY experiments). The structure of novaxenicin A (**1**) was secured by an X-ray diffraction analysis. Compound **5** possesses anti bacterial activity in concentration of 1.25 $\mu\text{g/ml}$ (*E. coli* and *B. subtilis*) and compound **2** induces apoptosis in transformed mammalian cells in concentration of 1.25 $\mu\text{g/ml}$.



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ANTISETTLEMENT ACTIVITY OF CHEMICAL EXTRACTS OF 10 IRCINID SPONGES AGAINST *BALANUS AMPHITRITE* LARVAE

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Biofouling is the growth of sessile aquatic organisms on substrates, causing important economic costs on man-made submerged structures. The search for environmental friendly antifouling compounds has become a necessity, especially due to the total ban of tributyltin (TBT) established by International Marine Organization starting from 2008. Benthic sessile filter feeding organisms are under great selection pressure to avoid clotting of their openings by fouling organisms. Sponges, belonging to this diverse group, produce a variety of bioactive secondary metabolites, including antifouling activity^{1,2}. In the present study the chemical extracts of 10 Ircinid sponges (collected from the Mediterranean Sea at Tel Aviv, Israel and Naples, Italy) were evaluated in larval antisetlement bioassays. The settlement inhibition mechanism (toxic versus nontoxic) was analyzed. The sponges were extracted with methanol, and tested for both antifouling activity (settlement inhibition) and toxicity against laboratory reared *Balanus amphitrite* (ciprits and nauplii) larvae. According to the activities, the sponges were classified into 3 main groups. Extracts of six sponges exhibited antifouling activity; effective concentration that inhibited 50% larval settlement (EC_{50}) ranged from 0.77 to 3.40 $\mu\text{g/ml}$. Four of them were not toxic ($LC_{50} > 100 \mu\text{g/ml}$) while two were toxic ($LC_{50} = 1.74 \mu\text{g/ml}$). The other four sponges showed a low antifouling activity ($EC_{50} = 18.88-45.27 \mu\text{g/ml}$). The ecological relevance of the assays is discussed. The results encourage further work with the nontoxic antifouling sponges with the aim of isolation of the active compounds, and the potential use in antifouling paints.

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COMPARISON OF BACTERIAL DIVERSITY ASSOCIATED WITH AZOOXANTHELLATE HEXACORALS AND OCTOCORALS

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This study examined symbiotic microbiota of the azooxanthellate hexacoral *Cirrhopathes lutkeni* and the octocorals *Leptogorgia minimata*, *Swiftia exertia*, and *Iciligorgia schrammi* using traditional plate culture and fluorescence in situ hybridization (FISH). Absence of zooxanthellae in these corals was confirmed using PCR based techniques with dinoflagellate-specific primers and additional measurements of dinoflagellate specific chlorophyll absorbance patterns. FISH counts for *C. lutkeni* showed a major presence of γ -Proteobacteria (22%) and Actinobacteria (19%), followed by α -Proteobacteria (14%), Firmicutes (9%), Cytophaga-Flavobacterium (7%), β -Proteobacteria (6%), and Chloroflexi (2%). FISH counts for the three octocorals also showed a predominance of γ -Proteobacteria (up to 33% of the total population). The remaining bacterial components as probed with FISH, Actinobacteria, Cytophaga, Chloroflexi and Firmicutes were only minor elements, comprising only 2-4% of the overall microbial diversity. Cultures were found to be highly selective for γ -Proteobacteria, α -Proteobacteria, and Firmicutes for all coral specimens. The predominance of γ -Proteobacteria may be region- and not species-specific since Pacific coral species and Scleractinians show a different distribution pattern. Some γ -Proteobacteria may be symbiotic in nature and may thus be important for coral health as an imbalance in the bacterial population may lead to white band disease, yellow blotch disease, and coral bleaching amongst others. This is the first study comparing the microbiota of tropical hexa- and octocoral species, which grow at moderate depth (40-100m) in the absence of direct sunlight. The study provides further insight into coral microbial ecology and may enhance the search for novel marine bioactives from these organisms in the near future.

ANTICANCER ACTIVITY EVALUATION OF KUANONIAMINES A AND C ISOLATED FROM THE MARINE SPONGE *OCEANAPIA SAGITTARIA*; COLLECTED FROM THE GULF OF THAILAND

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In the course of our investigation on bioactive compounds from the marine sponges from the Gulf of Thailand, we have isolated 24 α -methylcholestanol, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic, phenylacetic acid, 3-formylindole, kuanoniamine A and kuanoniamine C from the ethyl acetate extract of the marine sponge *Oceanapia sagittaria* (Sollas), collected near Koh Samed island in the Gulf of Thailand. Kuanoniamine A and kuanoniamine C have been evaluated for their cytotoxic effect against five human tumor cell lines: MCF-7 (breast carcinoma, estrogen-dependent ER+), MDA-MB-231 (breast carcinoma, estrogen-independent ER-), SF-268 (glioma), NCI-H460 (non small cell lung cancer), UACC-62 (melanoma), and a non-tumour human cell line MRC-5 (diploid embryonic lung fibroblast), by the SRB method. Kuanoniamine A has shown to be a potent growth inhibitor of all the human tumor cell lines as well as the non-tumour cell line. Though kuanoniamine C was found to be much less potent than kuanoniamine A, it was found to possess a high selectivity toward the estrogen dependent (ER+) breast cancer cell line. Using the TUNEL assay, it was found that both kuanoniamines A and C have caused an increased of the apoptotic cells, indicating that their antiproliferative effects on the MCF-7 cancer cell lines were in part associated with the phenomenon of apoptosis.

NOVEL NATURAL PRODUCTS FROM THE NUDIBRANCHS *ACTINOCYCLUS PAPILLATUS* AND *CHROMODORIS SINENSIS*

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A wide range of secondary metabolites with peculiar structural characteristics and activities has been discovered in opisthobranch molluscs (Mollusca: Gastropoda: Opisthobranchia).^{1,2} The taxonomic value of these metabolites has been often underlined as a promising way to elucidate affinities within the different families and discriminate closely related taxa. However, many lacunas persist in the chemotaxonomical scenario of opisthobranchs. This is mainly due to the absence of chemical information about many species and some entire families. In addition, the chemicals that opisthobranchs often obtain from dietary sources could not be good taxonomic *markers*, because of geographic variations in the secondary metabolites of any species.^{1,3,4}

Moving in this frame of studies, in this report we describe the chemical profile of two never studied opisthobranch species collected along Chinese coasts, *Actinocyclus papillatus* and *Chromodoris sinensis*. The first is a dorid nudibranch that belongs to the family Actinocyclusidae for which no chemical data are available in the literature while the second is a member of an extensively chemically investigated genus belonging to the family Chromodorididae.

This study led to the characterization of new metabolites that, in the former case, represented the first chemical information on Actinocyclusidae, while in the latter indicated the presence of a typical diterpenoidic *Chromodoris* pattern, but with a novel variation on a known structural theme.

Chemical comparison between the extracts, obtained by different anatomical sections, allowed in both cases intriguing speculations regarding the origin and the ecological role of the isolated metabolites.

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AGE AND NUTRIENT LIMITATION ENHANCE POLYUNSATURATED ALDEHYDE PRODUCTION IN MARINE DIATOMS

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Recent evidence indicates that certain diatom species induce a drastic reduction in the reproductive response of copepods due to the production of bioactive polyunsaturated aldehydes (PUA). However, nothing is known about the regulation of the production of these potentially defensive compounds. Here, we investigate the production of 2,4-heptadienal, 2,4-octadienal, and 2,4,7-octatrienal in the diatom *Skeletonema marinoi* in culture under different growth conditions. In nutrient-replete cultures, PUA production increases from the exponential to the stationary phase of growth from 1.2 fmol cell⁻¹ (\pm 0.4 fmol cell⁻¹ SD) to 4.2 fmol cell⁻¹ (\pm 1.0 fmol cell⁻¹ SD), with 2,4-heptadienal as the dominant aldehyde. The plasticity of PUA production with age of the culture supports the hypothesis of a direct link between toxin production and cell physiological state. N- and P-limited cells in stationary phase produced 1.4 and 1.8 fold higher amounts of PUA than control cultures and 10.7 and 4.6 times higher PUAs when compared to their own exponential growth phase, respectively. The increase in PUA production in the nutrient-limited cultures was not paralleled by an increase in the total amount of precursor fatty acids indicating that physiological stress might trigger an enhanced expression or activity of the enzymes responsible for PUA production, i. e. chemical defense increase in aged and nutrient-stressed diatoms. If this holds true during blooms, grazers feeding at the end of a bloom would be more affected than early-bloom grazers.

NOVEL APPROACH TO THE SYNTHESIS OF THE POLYKETIDE ANTIBIOTIC BASILISKAMIDE A

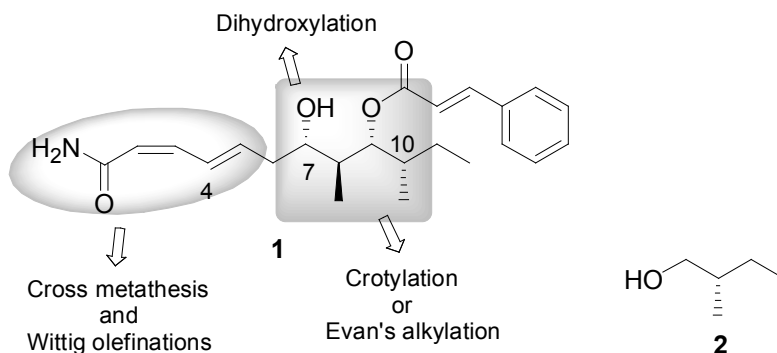
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The search for new antifungal agents represents a major challenge researchers concerned with combating mycotic diseases. Basiliskamide A **1**¹ was isolated in 2002 from marine bacterium PNG-276 off the coast of Papua New Guinea. Polyketide **1** showed activity against *Candida albicans* and *Aspergillus fumigatus* with MIC values of 1.0 and 2.5 µg/mL respectively. Although Basiliskamide A **1** had efficacy comparable to that Anphotericin B against *C. albicans*, it is less cytotoxic. The first total synthesis² was made in 2004 by Panek and co-worker.

Our synthetic strategy adopted stereoselective reactions as outlined in the Figure: crotylation³ or aldolic condensation,⁴ and subsequent asymmetric dihydroxylation⁵ would introduce stereochemical tetrad on C7-C10 starting from chiral commercially available alcohol **2**. Finally cross metathesis⁶ and Wittig olefinations⁷ would afforded (2*Z*,4*E*)-dienic system.



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This research was supported by Miur and by USB.

**PHYTOCHEMICAL ANALYSIS AND POTENT
ANTIOXYDANT, CYTOTOXIC AND PRO-APOPTOTIC
ACTIVITIES FROM THE TROPICAL MARINE BROWN ALGA
CHNOOSPORA MINIMA (HERING) PAPENFUSS FROM THE
FRENCH POLYNESIA ISLANDS, SOUTH PACIFIC**

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Organic compounds from marine organisms have extensively studied in the treatment of many diseases and serve as compounds of interest both in their natural form and as templates for synthetic modification. Over 15,000 novel compounds have been chemically determined since 1975. Focusing on bioproducts, recent trends in drug research from natural sources suggest that algae are a promising group to furnish novel biochemically active substances [1]. Halogenated compounds are naturally produced by brown algae and are dispersed in several different classes of primary and secondary metabolites including carbohydrates, terpenes and polyphenolic compounds as predominant metabolite classes. New promising phloroglucinol derivatives [2] and bisnorditerpene [3] are extracted from the scytosiphonaceae macroalgae secondary metabolites with potent activity in antioxidant and anticancer research. Our study has focused on the abundant brown alga [4] *Chnoospora minima* (Scytosiphonales) collected in French Polynesia Island. Radical scavenging/antioxidant activities of different extracts with a mixture of methanol/demineralized water (50/50, V/V) were assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical method. The antioxidant activity seems to depend on the degree of depolymerisation of phloroglucinol, small sulfated phlorotannins being more active generally than highly polymerised compounds. The cytotoxic and pro-apoptotic effects of crude extracts and their fractions from *C. minima* have been examined on several human cell lines: Jurkat (leukaemia T cell), Daudi (Burkitt lymphoma, B cell). Two crude extracts (CH_2Cl_2 and CHCl_3) have demonstrated a rapid cell death in all these cell lines, revealed by Annexin V+/IP+ staining in flow cytometry. Apoptosis induced by 100 μg of these extracts was accompanied by exposure of membrane phosphatidylserines and caspases activation in all cell lines. Preliminary analyses have suggested that it is probably the terpenes compounds from *C. minima* extracts that induced cytotoxic effects and apoptosis in human cancer cell lines.

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THE OXIDATION PROFILE AT C-18 OF FURANOCEMBRANOLIDES PROVIDES A TAXONOMICAL MARKER FOR SEVERAL GENERA OF OCTOCORALS

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Octocorals of the genera *Pseudopterogorgia*, *Alcyonium*, *Gersemia*, *Lophogorgia*, *Leptogorgia*, and *Sinularia* have the ability to biosynthesize furanocembranolides,¹ highly oxygenated diterpenoids based on the 14-membered carbocyclic cembrane skeleton into which a substituted furane ring and a γ -lactone subunit have become embedded. Studies to date indicate that certain members of the family exhibit potent neurotoxicity,² in addition to antiinflammatory,³ antifeedant activity⁴ and other biological properties.⁵ Furanocembranolides have also been popular targets for total synthesis.⁶ Herein we report on the isolation of three new furanocembranolides **1-3**. Compounds **1-3** were isolated from the eastern Pacific octocoral *Leptogorgia* spp and their structures determined by spectroscopic evidences. A NMR-based method using Pirkle's reagent at low temperature allowed us to determine the absolute configuration at C-10 of a γ -butenolide unit embedded in a flexible furanocembranolide network. The C-18 of furanocembranolides undergoes an oxidation cascade leading from a methyl group to a carboxylic acid/ester that appears to be genus-specific. We introduce the concept *genus-specific oxidation*, a feature that provides a chemotaxonomical marker for several genera of octocorals. This concept also allowed us to propose a biogenetic pathway for these compounds.

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ISOLATION OF ELATOL AS LEISHMANICIDAL COMPOUND FROM *LAURENCIA MICROCLADIA*

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Red algae of the genus *Laurencia* are some of the most prolific producers of secondary metabolites in the marine environment^{1,2}. From Mediterranean *Laurencia microcladia* predominantly sesquiterpenes (halogenated or not), have been isolated as secondary metabolites^{3,4,5,6}. The ethanolic extract of *Laurencia microcladia* from Southern Brazil displayed significant activity against *Leishmania* promastigotes⁷. The algae (1,5 kg) were collected in Bombinhas (SC, Brazil), affording 6 g of crude extract. Part of this (3,0 g) was submitted to chromatographic separation on silica gel with a gradient with hexane and ethyl acetate. A further separation using CHCl₃: ethyl acetate (9:1) afforded a colorless oil as pure compound. This was also tested for leishmanicidal activity and identified as the active compound of the extract. NMR spectra were acquired in CDCl₃, in a Bruker AM500. The total ¹³C-NMR spectrum (125 MHz) and DEPT indicated the presence of 15 carbon atoms: three methyl groups, five methylene groups, two methin groups and five quaternary carbon atoms. MS data show peak at m/z = 332, which suggested the molecular formula C₁₅H₂₂OBrCl. The HMQC and HMBC data, which allowed the proposal of partial structures who were combined to constitute the structure of the halogenated chamigrene known as elatol. This compound have been isolated from from other *Laurencia* spp^{8,9,10,11} but this is the first report for *L. microcladia*.

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SEVEN NEW TRYPSIN AND CHYMOTRYPSIN INHIBITORS FROM THE CYANOBACTERIA *MICROCYSTIS* SP.

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Microcystis sp. has been shown to be a rich source of unique and bioactive peptides such as micropeptins, microginins, aeruginosins, anabaenopeptins and microviridins. As part of our ongoing research, a bloom of *Microcystis* sp. (IL-342) was collected from a water reservoir adjacent to Kibutz Hulda, in the Autumn of 2004. Here we report the isolation and structure elucidation of nine compounds, seven of which are new natural products. The sample of the cyanobacterium was freeze-dried and extracted with 70% MeOH in H₂O. The extract was found to inhibit trypsin and chymotrypsin. Flash-chromatography on an ODS column yielded eight fractions that were eluted from the column, with 10-80% MeOH in H₂O. These latter fractions exhibited protease inhibitory activity and were further separated on a Sephadex LH-20 column. Finally, the compounds were purified on a reversed-phase HPLC column. The compounds that were isolated are: micropeptin HU895, micropeptin HU909, micropeptin HU975, micropeptin HU989, micropeptin HU1021, micropeptin HU1041 and micropeptin HU1069 (**1-7**). These compounds compose a new subgroup of micropeptins which contain the unnatural amino acid m-chloro-*N*-methyl-*O*-methyl tyrosine and its derivatives. These natural products were isolated along with the known compounds micropeptin 478-A (**8**) and micropeptin 478-B (**9**). The structure of the compounds was determined by homonuclear and inverse-heteronuclear 2D-NMR techniques and confirmed by mass spectra. The absolute configuration of the asymmetric centers were studied using Marfey's method for HPLC. These new peptides inhibit the serine-proteases trypsin and chymotrypsin.

NEW PREGNANES FROM THE SOFT CORAL *EUNICELLA CAVOLINI*

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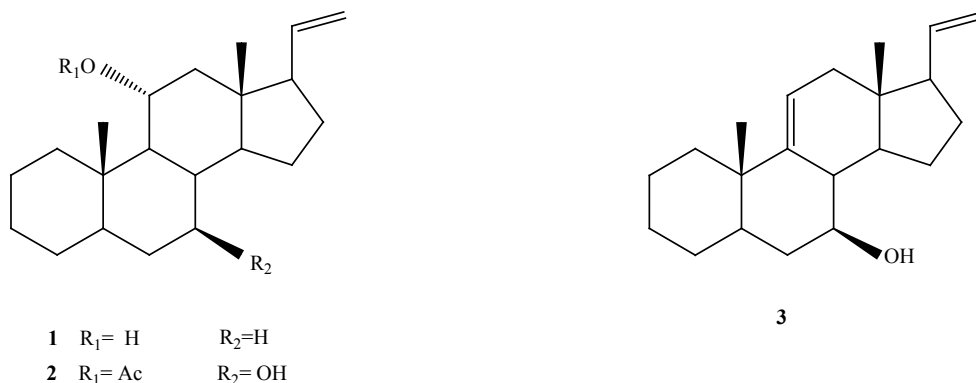
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The genus *Eunicella* has been proven a rich source of the structurally and pharmacologically interesting secondary metabolites. Previously isolated natural products from this genus include diterpenes¹⁻², pregnanes³⁻⁴ and tryptamine derivatives⁵.

In the present work, specimens of the soft coral *Eunicella cavolini* were collected by SCUBA diving from Maliakos gulf, Greece, and subsequently extracted with CH₂Cl₂/MeOH. The residue was subjected to a series of chromatographic separations to yield from the medium polarity fractions three new pregnanes (**1-3**), along with a number of known peroxy derivatives of ergosterol.

The structures of the new natural products, as well as their relative stereochemistry, were established by means of spectral data analyses, including 1D and 2D NMR experiments and HR-MS.

The cytotoxic activity of the new compounds is currently in progress.



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SECONDARY METABOLISM OF THE SPONGE GROUP HOMOSCLEROMORPHA : DIVERSITY AND VARIATION OF ITS EXPRESSION IN RELATION WITH BIOTIC AND ABIOTIC FACTORS

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Homoscleromorpha sponges are of particular interest due to their phylogenetic position.¹ Despite their “primitive” organisation plan, the representatives of the Homoscleromorpha group possess some cytological, biochemical and embryological characters, specific to the Eumetazoa and absent in the other sponge clades.^{2,3} In addition, they contain the characteristic cells that could be responsible for the production and/or storage of secondary metabolites. The systematics of this group is complex and often requires the study of cytological characters for the determination at species level (especially in the genus *Oscarella* which is devoid of spicules).⁴ A method based on chemical fingerprints, developed and applied to 8 mediterranean species of Homoscleromorpha, has proved its efficiency for the study of phylogenetic relationships within the sponges. Potential chemotaxonomic markers at the Homoscleromorpha group level and at species level are proposed. This method also allowed the evaluation of secondary metabolites diversity as well as its potential for valorisation in environmental and biomedical fields.

Chemical analysis of *O. tuberculata* has led to the identification of one major substance, the (5Z)-tetracos-5,x-dienoic acid (C24:2), an unsaturated fatty acid never described in previous studies of this species.^{5,6} The crude extract of *O. tuberculata* has displayed bioactivity on a microtox assay, and the fatty acid C24:2 along with other metabolites present may show antifouling properties. *Oscarella tuberculata* has also been chosen for the study of the natural fluctuations of the secondary metabolites production. The production of the secondary metabolites in *O. tuberculata* seems to be influenced by a complex set of biotic and abiotic factors. It is not only conditioned by the season or temperature. The increasing trend in the production was observed during the period of gametogenesis and embryogenesis, followed by a strong decline at the moment of the larvae expulsion.

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CORAL MUCUS AS A SOURCE OF BACTERIA WITH ANTIMICROBIAL ACTIVITY

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In the oligotrophic marine environment there are ecological niches rich in nutrients and diverse in bacterial populations. One such niche is the coral surface mucus layer. Interactions amongst microorganisms found in coral mucus may be symbiotic or competitive; competing over space and food. It has been hypothesized that the microbial communities found on the coral surface may play a role in the coral defense, possibly through the production of antimicrobial substances. To find potentially active compounds produced by coral-mucus bacteria, selected microorganisms isolated from mucus layer of a number of scleractinian coral species were grown using agar plating technique. Screening for antimicrobial substances was performed using overlay and drop techniques, and antibacterial activity was tested against indicator microorganisms. Results indicated that approximately 25% of the mucus-associated bacteria demonstrated bioactivity. Isolates related to the genus *Vibrio* and *Pseudoalteromonas* demonstrated high activity against both gram positive and gram negative bacteria. Isolates related to the genus *Shewanella* demonstrated activity against gram positive bacteria. Gram positive bacteria (*Bacillus*, *Planomicrobium*) demonstrated lower activity, primarily against gram positive bacteria. These results demonstrate the existence of microorganisms with antimicrobial activity on the coral surface, indicating that they may play a role in protecting the coral host against pathogens. Further isolation and characterization of these active substances may lead to novel substances for use in medical and biotechnological applications.

EPIPHYTIC BACTERIA AS PRODUCERS OF ANTIMICROBIAL SUBSTANCES

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Bacterial isolates were obtained from the marine brown alga *Laminaria saccharina* collected from the Baltic Sea. Strains, which inhibited the growth of at least one test strain were selected for further investigations, e.g. identification by 16S rDNA sequencing, determination of the activity against Gram-positive and Gram-negative bacterial strains as well as yeasts and chemical analysis of organic extracts.

About 100 bacterial isolates were identified. They affiliated with various different species including members of the *Alpha-*, *Beta-* and *Gammaproteobacteria*, the *Bacteroidetes*, *Actinobacteria* and *Firmicutes*. The comparison of the 16S rDNA sequences of the alga derived isolates with next related type strain sequences available in databases revealed, that at least 5 isolates might represent novel species and in some cases, even genera. The strains studied displayed 16 different antimicrobial activity patterns indicating different modes of microbial interactions. In some cases, the substances responsible for the antimicrobial effect were identified.

In conclusion, *L. saccharina* provides a rich source for the detection of various bacterial strains with antibiotic activity. Assumed ecological functions are stabilising the microbial community, habitat for fish pathogens, decomposing algal compounds and/or defending the algae against pathogens of predators.

STRUCTURE VALIDATION OF APLIDINONE A, A CYTOTOXIC TUNICATE METABOLITE, AND SYNTHESIS OF ITS STRUCTURAL DERIVATIVES FOR SAR STUDIES

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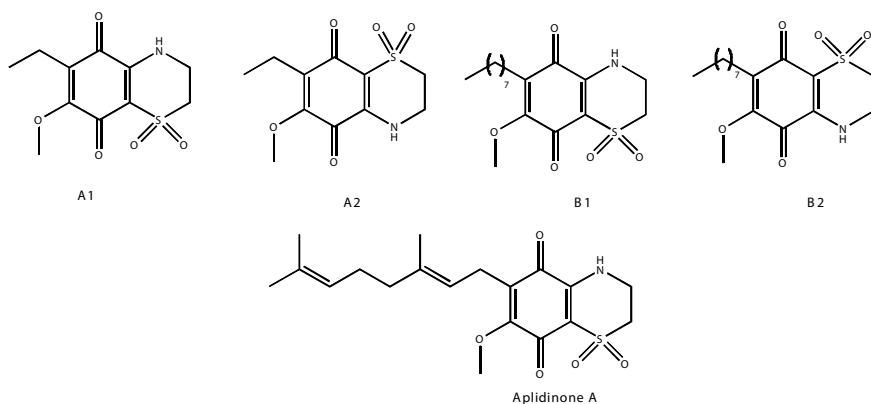
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Aplidinone A¹ is a natural product isolated from the Mediterranean ascidian *Aplidium conicum* with interesting pro-apoptotic properties; it is an example of terpene quinones containing the unusual 1,1-dioxo-1,4-thiazine ring. The structure of this compound had been determined by spectroscopic analysis while the regiochemistry of the thiazine ring was defined by a comparison of the experimental ¹³C chemical shifts with those predicted by GIAO² shielding calculations for the model compounds A-1 and A-2. Successively, the synthesis of compounds A1 and A2 allowed us to confirm the proposed regiochemistry and provided useful quantities of these compounds to initiate SAR studies. The designed synthetic procedure was also adopted to prepare other quinones analogues, compounds B1 and B2, which revealed a very interesting pharmacological activity. Specifically we found that B2 is endowed with potent pro-apoptotic activity in Jurkat (leukaemia) and AGS (gastric cancer cell) cell lines and also inhibits TNF α -induced NF- κ B activation.



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BIOACTIVE NORDITERPENE METABOLITES FROM THE INDIAN MARINE GORGONIAN *ACANTHOGORGIA TURGIDA*

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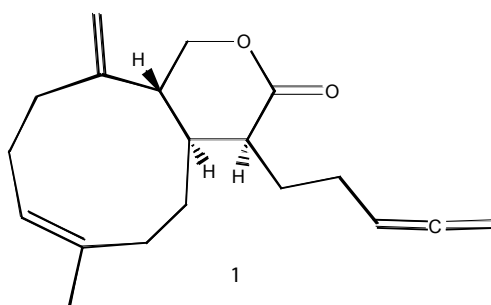
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Soft corals and gorgonians belonging to genera *Xenia*, *Capnella* and *Acalycigorgia* are characterized by the presence of xenicane diterpenoids (1-5). These compounds possess different chemical structures, showing in particular five framework types: xenicin, xeniolide, xeniaphyllane, azamilide and nine-membered monocarbocyclic skeleton. Many xenicane diterpenoids show very interesting cytotoxic activities against human tumoral cell lines (6).

We report here the results of the chemical investigation on a population of the gorgonian *Acanthogorgia turgida*, from Grandi Island (India), conducted in the frame of a scientific bilateral cooperation program between CNR (Italy) and CSIR (India).

The apolar extract of *A. turgida* has been found to contain a series of xeniolide norditerpenes (i.e. compound **1**). Two novel compounds have been also isolated and characterized by spectral methods (mono- and bi-dimensional NMR spectroscopy, mass spectrometry and IR).



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DIAZONAMIDES C AND D, NEW CYTOTOXIC METABOLITES FROM THE TUNICATE *DIAZONA* SP.

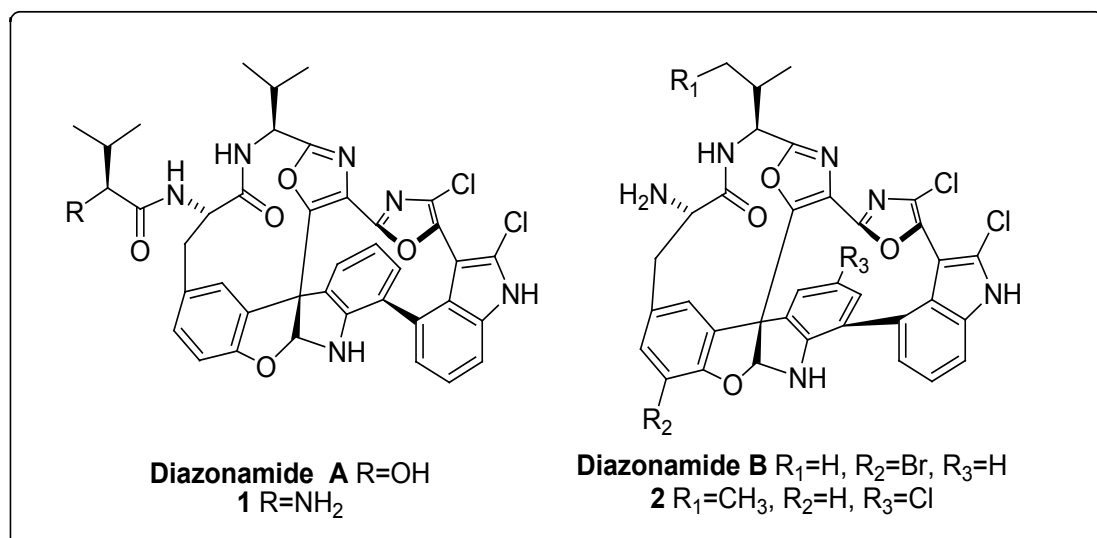
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The diazonamides are a family of novel macrocyclic peptides with potent cytotoxic activity isolated from the ascidian *Diazona angulata* (originally misidentified as *Diazona chinensis*) by Fenical and co-workers.¹ The first structural proposal by the Fenical's group was later revised and the final structures of the diazonamides A and B were established after reinterpretation of the X-ray data and total synthesis of diazonamide A.²⁻⁴

In the course of our continuing search for new antitumour drugs from marine organisms, diazonamide B and two new structurally related compounds, designated as diazonamides C (**1**) and D (**2**), were isolated from samples of a tunicate of the genus *Diazona* collected in Indonesia. Details on the isolation, structural characterization and cytotoxic properties of the new metabolites will be presented.



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ANTIOXIDANT ACTIVITY SCREENING OF EXTRACTS FROM SELECTED SEAWEEDS OF THE AZORES

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Free radical production occurs continuously in all cells as part of normal cellular function. However, oxidative stress may play a role in the pathophysiology of common diseases including atherosclerosis, chronic renal failure and diabetes mellitus (Young and Woodside, 2001). Therefore, prophylaxis and therapeutics could be supported by searching new natural sources of antioxidants, widely distributed in plant or seaweeds. The Azores archipelago is a rich environment concerning the ecology of algal communities. The aim of the present study was to evaluate the potential antioxidant activity of 15 extracts from 5 representative seaweed species from S. Miguel (*Ulva compressa*, *Gelidium microdon*, *Osmundea pinnatifida*, *Fucus spiralis* and *Cystoseira abies-marina*) using the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. Methanolic (ME), dichloromethane (DE) and hexane (HE) extract fractions were prepared for these five algae species. Commercial standards like Trolox and BHT were used as reference. Overall, the best results were obtained for the ME extracts (except for *G. microdon*) followed by the HE and DE extracts. Values obtained by the EC₅₀ parameter (substrate concentration needed to produce 50% reduction of DPPH) reveal maximum antioxidant activity on *F. spiralis* ME and HE fractions, showing the lowest EC₅₀ values (0.62 µg/mL and 2.0 µg/mL, respectively), even better than those obtained for Trolox or BHT standards (6.0 µg/mL and 31.0 µg/mL, respectively). Previous studies (Cérantola et al., 2006) also reported antioxidant activity by phlorotannins on methanolic extracts from *F. spiralis*. Further research will provide new data about chemical composition of *F. spiralis* n-hexane extract.

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CHEMICAL ECOLOGY AND BIOACTIVITY IN THE SPECIES *TRITONIA ODHNERI* (OPHISTOBRANCHIA, DENDRONOTACEA) AND *RENILLA* SP. (OCTOCORALLIA, PENNATULACEA) FROM PATAGONIA, ARGENTINA

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Recent interdisciplinary studies have been conducted on the nudibranch *Tritonia odhneri* (Marcus, 1959) and the sea pen *Renilla* sp. for the first time. These species coinhabit from 13 m up to 108 m in depth in San Jorge Gulf (45°25'57''S, 67° 05' 77''W, Chubut Province), being abundant as accompanying fauna of large fisheries¹. SEM analysis of stomach content demonstrated the predator-prey interaction, suggesting a single-species diet in San Jorge Gulf and a multi-species diet in soft-corals in the rest of the Magellanic Region^{2,3}. Chemical screening did not show this dietary relationship; the compounds mono-glyceryl ether, including 1-O-hexadecylglycerol (chimilic alcohol) and N-methylpicolinic acid (homarine), with defensive function, were found in *Tritonia*, whereas in *Renilla* sp. a group of new fatty acids with important antifungal activity was detected.

A checklist of dietary interactions, isolated chemical compounds, ecological role, and bioactivity known up to the present in dendronotacean species of the genera *Arminia*, *Mariona*, *Tritonia*, and *Tritoniella*, as well as in sea pens of the genus *Renilla* is presented^{4,5}.

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BIOASSAY-GUIDED SCREENING AND CHARACTERIZATION OF THE SECONDARY METABOLITE SPECTRUM OF THE MARINE FUNGUS *EMERICELLOPSIS* SP.

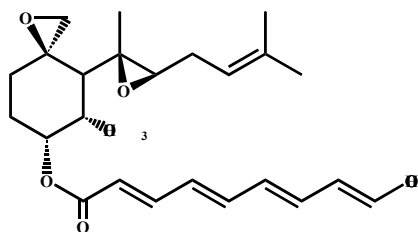
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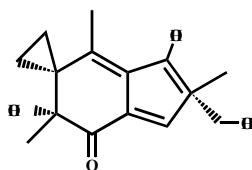
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Cytotoxic fungal metabolites and derivatives thereof are well known and some of them have reached clinical trials as promising candidates for anti-cancer drugs, e.g. illudin S and fumagillin.^{1,2}

Bioassay-guided screening has proven to be a logical and successful approach to search for new and biologically active natural products from microorganisms. In order to evaluate marine fungi as a source of such secondary metabolites, we isolated and cultivated different types of fungi from sponges and algae. The crude extracts were tested in a monolayer assay for cytotoxic activity. Promising samples were then further investigated and characterized. That way the focus was drawn to an *Emericellopsis* species, whose crude extract from a solid phase cultivation on a yeast medium showed strong cytotoxic activity in a six-cell line panel. Vacuum liquid chromatography of the crude extract led to 20 fractions, one of which showing strong cytotoxicity and selectivity against only two cancer cell lines. Deduction of the active principle is in the focus of the current investigation.



Illudin S



Fumagillin

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FEEDING REPELLENCE OF ORGANIC EXTRACTS FROM ANTARCTIC BENTHIC INVERTEBRATES AGAINST AN OMNIVOROUS SEA STAR

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The marine benthos of Antarctica has been the subject of many taxonomical, zoogeographical and ecological studies^{1,2,3,4}. However, little is known about the chemical ecology of the natural products from Antarctic marine invertebrates⁵. We are currently working in the chemical analysis of the natural products from selected species, their origin, location in the organisms, ecological role and pharmacological activity. Samples were collected in the Weddell Sea during the Antarctic cruise ANT XXI/2, (Nov. 2003-Jan. 2004) on board of R/V Polarstern (of the “Alfred-Wegener Institut for Polar and Marine Research”, Bremerhaven, Germany). We will show here the results of the tests developed to evaluate the ecological role of the organic extracts. These tests consisted in repellency experiments against predation by the sympatric omnivorous Antarctic sea star *Odontaster validus*. In the Antarctic marine environment, sea stars have replaced fish as the dominant predators of sessile and slow-moving invertebrates^{1,6}. In order to evaluate the chemical defenses against predation by *O. validus*, the extracts of 62 species of Antarctic benthic invertebrates were tested. **We carried out more than one hundred tests** in January 2006 using sea stars captured in the South Shetland Islands from the Spanish R/V BIO Hespérides. The experiments were done in the Spanish Antarctic base B.A.E. “Gabriel de Castilla” in Deception Island. The results provide information on which species, in the different taxonomical groups analyzed, do possess chemical defenses to avoid predation. Furthermore, they allow us to locate the defensive compounds in the organisms (by testing, *e.g.* external *vs.* internal parts, or basal *vs.* apical zones). Our data revealed that a high percentage of samples showed feeding repellence against *O. validus*, thus indicating the presence of chemical defenses in different taxonomical groups. These results support the assumption that chemical defenses are widespread in Antarctic benthic invertebrates. Further chemical analyses are in progress in order to identify the natural compounds responsible for these activities.

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PRELIMINARY RESULTS ON THE DIATOM SECONDARY METABOLITES IN THE SE BALTIC SEA

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Diatoms are the most important group of eukaryotic phytoplankton living in the marine environments and are responsible for near the half of primary production in the seas. For many years it was known that diatoms are good diet for the copepods. However, the copepods on the unialgal diet of diatoms could increase generation time, lower egg productivity and hatching success or increase mortality rates, etc. Only recently it was discovered that secondary metabolites – derivatives of fatty acids – are responsible for such phenomena.

In the south-east coast of the Baltic Sea the diatoms dominate mostly over the year. The seasonality and abundance of diatoms, potential producers of secondary metabolites harmful to copepods, are discussed in this study along with pilot results on the second metabolites, analyzed from the Baltic Sea (SE) phytoplankton community collected in early June 2007.

MOLECULAR DIALOGUE WITHIN THE MICROBIAL COMPARTMENT OF THE TEMPERATE CALCAREOUS SPONGE *LEUCONIA NIVEA*

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In a program devoted to study the role of sponge-associated bacteria, the cultivable heterotrophic bacterial flora was isolated from the calcareous sponge *Leuconia nivea* (class Calcarea, order Calcaronea, family Baeriidae) collected off Concarneau (Northeast Atlantic, France) and characterized. The antimicrobial activity of heterotrophic bacteria isolated from this sponge has been examined and their impact on the sponge chemical ecology has been studied.

The sponge *L. nivea* was selected after antimicrobial screening that revealed a permanent activity against Gram positive and Gram negative bacteria (biomedical and marine environmental strains) in the CH₂Cl₂ crude extract at all seasons from June 2005 through February 2007.

This activity was localized in the bacterial fraction of the sponge separated by differential sedimentation of the dissociated sponge cell suspension. Out of 150 bacterial strains, 16 revealed an activity against *Staphylococcus aureus* and/or *Vibrio splendidus*. The bioactive cultivable heterotrophic flora was abundant and displayed a high phylogenetic diversity, with 3 phyla represented throughout the year, suggesting the existence of mechanisms of regulation of each population: gamma-Proteobacteria, alpha-Proteobacteria and Firmicutes.

One of the most active strains was phylogenetically affiliated with the genus *Microbulbifer* (sub-class gamma-Proteobacteria, family Alteromonadaceae) with 99.8% sequence homology with the 16S RNA of *Microbulbifer arenaceous*.¹ Bacterial metabolites from this strain were purified, and structurally elucidated. The major natural antimicrobial substance produced *in vitro* was active against a large spectra of native strains, co-isolated from the sponge. Moreover this compound was also detected *in situ* by LC-MS in the host sponge extract at every season.

Results indicate that bioactive molecules from cultivable heterotrophic bacteria contribute to their sponge host antimicrobial activity and that they may be involved in the regulation of its bacterial compartment.

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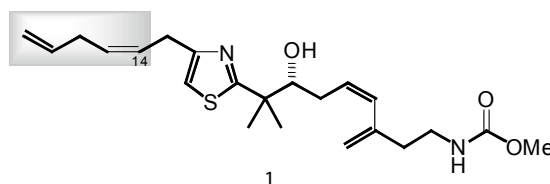
MYCOTHIAZOLE: APPROACH TO THE TOTAL SYNTHESIS OF THE CORRECTED STRUCTURE

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Mycothiazole (**1**) is a marine secondary metabolite isolated from *Spongia mycofijiensis*^{1a} and *Dactylospongia*^{1b} having an important activity throughout the 60 NCI human tumor cell line panel.² Very recently Crews et al. have revised the firstly proposed *E* configuration to *Z* of the Δ^{14} double bond, based on NOE measurements.³ Curiously, this structural feature was not found by the two reported total syntheses of **1**.⁴



During our attempt directed towards the total synthesis of **1** and analogs,⁵ we have synthesized two (*Z*)- Δ^{14} -configured intermediates which have provided additional corroboration of the new proposed structure of naturally derived mycothiazole.⁶ Our synthetic approach to the total synthesis of **1** is based in the preparation of the thiazolic core and the addition of the acyclic dienic side chains through an indium cross-couplings reaction.⁷

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1-METHYLADENINE - THE MAJOR ADENYLATE DERIVED SECONDARY METABOLITE OF THE MARINE SPONGE *GEODIA CYDONIUM*

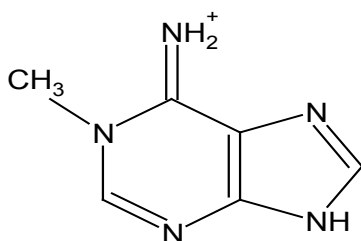
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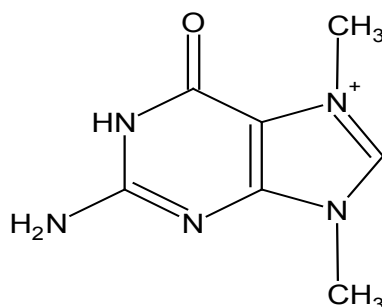
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A series of methylated purine derivatives have been isolated from marine organisms. For some of these derivatives pharmacological activity has been shown, but the role of most of these compounds for the particular host organism is unknown. However, the hormonal function of 1-methyladenine in marine invertebrates, Echinodermata, is well established. Analysis of the acid extract of the marine sponge *Geodia cydonium* revealed that high amounts of 1-methyladenine **1** were present among the extracted nucleotides. In case of the other species of the same genus, *Geodia barretti*, 7, 9-dimethylguanine **2** was the major constituent of the acid extract. In both cases the amount of the purine derivative exceeded that of the total adenylate nucleotides by an order of magnitude.



1



2

The putative precursors of **1** and **2**, 1-methyladenosine and 7-methylguanosine, are minor components of tRNA. Our experiments showed that **1** may also participate in the metabolism of deoxyribonucleic acids. The nucleosidase and deoxyribosyltransferase activities were found in crude extract of *Geodia cydonium*, using synthetic 1-methyladenine containing nucleosides as substrates. The reactions proceeded 6.6 times slower with 1-methyladenosine than with 1-deoxymethyladenosine.

ANTIPROLIFERATIVE CEMBRANE DITERPENES FROM THE SOFT CORAL *UMBELLULIFERA* SP.

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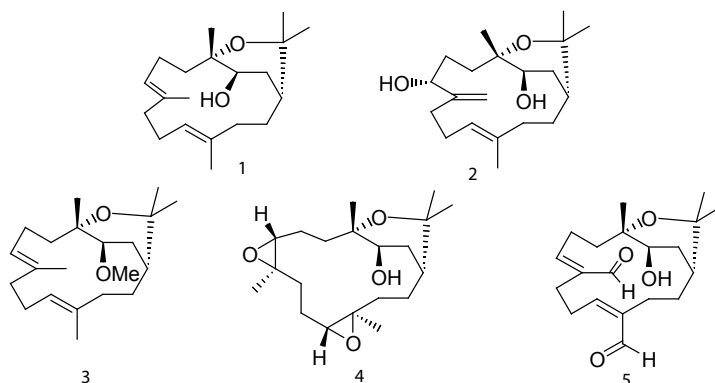
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Soft corals are known sources of terpenes, mainly diterpenes of the cembrane type, containing a 14-membered macrocycle ring. Cembranoids have been shown to exert a chemical defence role against predation from other reef organisms, while in biological systems several reports about cytotoxic, antiinflammatory, Ca-antagonistic and antimicrobial activities are present in the literature.

A specimen of *Umbellulifera* sp. (Alcyonacea, Nephtheidae) has been collected off the costs of Manado (North Sulawesi, Indonesia) and analyzed for chemical composition. The main constituent of the organic extract is decaryiol¹(**1**), previously isolated from *Sarcophyton decaryi*, accompanied by a series of new analogues, e.g. decaryiol B (**2**).

Decaryiol showed remarkable concentration-dependent inhibition of cell growth ($GI_{50} = 0.15 \mu\text{g/mL}$ against MCF7, breast adenocarcinoma) and it seems to act as cell cycle specific (G2/M) inhibitor of cell growth.² Stimulated by this results we have prepared a series of semi-synthetic derivatives of decaryiol (e.g. the methylated **3**, the diepoxide **4**, the dialdehyde **5**). Both natural and semi-synthetic analogues of decaryiol have been tested as growth-inhibition agents to gain information about the structure-activity relationships.



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NEW METABOLITES FROM THE RED ALGA *LAURENCIA GLANDULIFERA*

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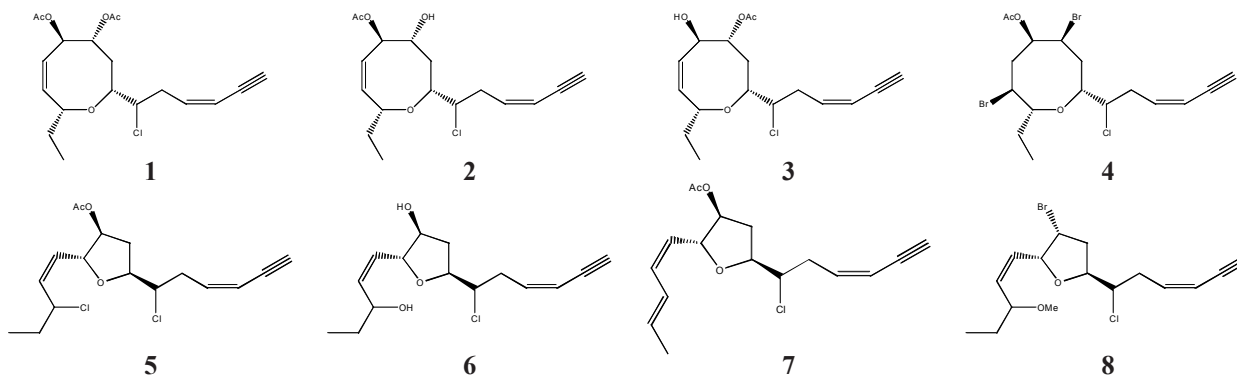
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Species of algae belonging to the genus *Laurencia* are found throughout the world and have been the subject of intensive research¹. The vast majority of *Laurencia* metabolites include sesquiterpenes, diterpenes, triterpenes and C₁₅ acetogenins^{2,3}. Many of these compounds exhibit biological properties and have been valuable in chemosystematic studies of the genus⁴.

In the course of our continuing investigations in marine natural products chemistry⁵, we studied the secondary metabolites of the red alga *L. glandulifera*, collected from the island of Crete. Fresh alga tissue was exhaustively extracted with CH₂Cl₂/MeOH (3/1). Following a combination of chromatographic techniques, compounds **1-8** were isolated in pure form. The structures of the new natural products, as well as their relative stereochemistry, were established by means of spectral data analysis.

The new natural products are C₁₅ acetogenin eight membered cyclic ethers (**metabolites 1-4**) and C₁₅ acetogenin tetrahydrofuran ethers (**metabolites 5-8**), with a characteristic terminal *cis* ene-yne group. **Metabolites 1-4** were evaluated for their antibacterial activity, showing minimum inhibitory concentration (MIC) values ranging from 8 to 256 µg/ml.



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EVALUATION OF ANTIFOULING ACTIVITY OF ALGAL METABOLITES

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Fouling is one of the most serious problems the maritime domain currently faces. It has been estimated that the growth of marine fouling organisms costs the shipping and other marine industries over \$6.5 billion per year.

Biofouling is considered to have distinct stages, the first one starting from the moment a man-made object is immersed in water. The surfaces of these objects quickly accumulate dissolved organic matter and gradually bacteria and single-cell diatoms settle and form a microbial film, to be colonized soon by algae, marine fungi and ciliate protozoa and in the final stage by other marine organisms, such as barnacles, tunicates, mussels, bryozoans, polychaetes and tubeworms.

Efficient antifouling paints are so far based on copper compounds and booster biocides that when submerged, release toxic compounds causing adverse environmental effects. In view of the prohibition of TBT containing paints in 2008, there is urgent need for the development of new non-toxic or environmentally benign antifouling alternatives that would be efficient against the most severe fouling organisms.

In the present study, the antifouling activity of six *Taonia atomaria*, four *Dilophus spiralis*, five *Laurencia microcladia* and two *L. glandulifera* metabolites was tested against the marine bacteria *Pseudoalteromonas elyakovii*, *Shewanella putrefaciens*, *Polaribacter irgensii*, *Vibrio aestuarianus* and *Cobetia marina*, the marine microalgae *Navicula jeffreyi*, *Cylindrotheca closterium*, *Chlorarachnion globosum*, *Pleurochrysis roscoffensis* and *Exanthemachrysis gayraliae* and the freshwater microalgae *Scenedesmus armatus*, *Cosmarium* sp. and *Fragilaria crotonensis*.

Noteworthy activity against the marine bacterial and microalgal strains was exhibited by two meroterpenoids from *T. atomaria*, a perydroazulene diterpene from *D. spiralis* and three cuparene sesquiterpenes from *L. microcladia*. Nevertheless, none of these compounds were active towards the freshwater microalgae.

C. VENTRICOSUS VENOM PEPTIDES PROFILING: INSIGHT INTO INTRASPECIFIC VARIATION

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Cone snails are marine mollusks equipped with a venom apparatus that produces a highly peptide-rich mixture (conopeptides) used to immobilize predators or competitors^{1,2}. Their low mobility and the extreme variety of prey have led to an evolutionary selection process resulting into hypervariable and highly pharmacologically active peptides (e.g., conotoxins), potent inhibitors of many physiological processes. As each cone snail species can potentially express approximately one hundred different peptides, distinct from that produced by any other species, it has been estimated that approx. 50000 structurally and pharmacologically different peptides can be expressed in the venoms of the living cone snails. The variety and pharmacological potency of *Conus* venom components makes them excellent starting points for drug development³⁻⁵. Until today, advanced genome and transcriptome techniques have been largely used in providing information on the potential conotoxin repertoire of single *Conus* species. On the basis of these data, the diversity of conopeptides has been attributed to different mechanisms, some of these considered remarkable, mechanistically unexplained and specific of the *Conus* peptide diversification⁶.

In order to characterize the actual expression profile of conopeptide diversity in the context of geographical habitat, sex and age, we recently started a project to analyze and compare at a high throughput level the peptide complement of the venom from single specimens of *Conus ventricosus* Gmelin, collected in four different Mediterranean areas. As a result, two novel conotoxins were discovered and variation in the venom composition of the biogeographical subspecies highlighted.

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“DETECTION OF TETRODOTOXIN AND ITS ANALOGUES IN DIFERENT FAMILIES OF MARINE FISH BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS) AND LC-MS/MS”

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Tetrodotoxin¹ (TTX, **1**) is one of the most potent neurotoxins ever known. This toxin is a powerful voltage-gated sodium channel blocker² which usually exists as a mixture of analogues. It is well known that TTX is distributed both in terrestrial and marine animals. It is important to identify the species of fishes which contain these dangerous substances and to determine the TTX composition in fishes located at different places.

In the last decade, liquid chromatography coupled with mass spectrometry (LC-MS) has proven to be the most valuable methodology for direct determination of toxins. In fact, these techniques have replaced the liquid chromatography-fluorescence detection (LC-FLD) as a method for detecting of TTX and its analogues. More specifically, by application of Hydrophilic Liquid Interaction Chromatography (HILIC)⁴ has enabled us to separate, detect and quantify TTX and a large number of analogues in several species of different families such as Tetraodontidae and Diodontidae collected in different countries around the World. It is worth to indicate that some of them are documented on human consumption and consequent poisoning.⁵

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IDENTIFICATION OF A GALACTOGLYCEROLIPID WITH ANTIFOULING ACTIVITY IN THE BROWN ALGA *SARGASSUM MUTICUM* (YENDO) FENSHOLT FROM BRITTANY (FRANCE)

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Marine algae produce a wide variety of chemically active metabolites, potentially as an aid to protect themselves against other settling organisms. Such metabolites have been stated as antibacterial, antifungal, antialgal and anti-macrofouling agents, effective in the prevention of biofouling [1, 2]. In our study we focused on the introduced brown macroalga *Sargassum muticum* (Yendo) Fensholt (Sargassaceae, Fucales) which is currently observed along the coasts of Brittany (France), where it represents high biomass [3]. This species was then collected in order to investigate potential antifouling activities with the goal of making valuable use of its biomass. Several polar and apolar extracts were submitted to bacterial, fungal and microalgal strains implicated in microfouling in marine environments [4]. Among active crude extracts, the most antibacterial CHCl₃ extract was studied for further fractioning. In parallel of all chemical steps, fractions were tested for their antibacterial activity. Extracts were purified using chromatographic methods, i.e. SiO₂ column chromatography and preparative high performance liquid chromatography. Thin-layer chromatography was also used to control the purity of resulting fractions. In the aim to characterize isolated compounds, pure fractions were analysed using ¹H and ¹³C NMR, GC/MS and DEP/MS. These investigations permitted us the isolation of a new galactoglycerolipid in *Sargassum muticum*. The characteristic fragment ions at m/z 169 and 211 allowed confirming the predominance of galactose moiety. GC/MS allowed us to identify linolenic acid as the main fatty acid. The structural elucidation of the new structure together with its biological activity and potential anti-microfouling industrial applications will be discussed.

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JASPAMIDE: RATIONAL DESIGN AND SYNTHESIS OF NEW POTENTIAL ACTIN-TARGETED INHIBITORS

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A large and ever-expanding number of cytotoxic marine natural products have been found to target actin filaments, disrupting its dynamic organization. Since actin filaments are involved in many vital cell functions, the identification of new agents affecting its cellular activities may be extremely useful in cancer chemotherapy.¹ One of the most studied actin-binding natural compound is jaspamide, a cyclodepsipeptide isolated exclusively from marine sponges.² There has been much interest in the biological properties of the natural compound since it showed a potent antitumor activity by inducing actin polymerization and/or inhibiting the depolymerization of actin filaments.³ Moreover, it has been found that jaspamide induces apoptosis in various transformed cell lines by a caspase-3-like-protease-dependent pathway.⁴ These results indicate that jaspamide represents an attractive target for the development of a novel class of anticancer agents. Recently, in order to identify the key structural elements that contribute to the biological activity of the natural molecule, we described the synthesis, the conformational and the biological properties of a collection of simplified jaspamide analogues.⁵ Subsequently, with the aim of discovering new more effective actin-targeted inhibitors, we have designed and synthesized by a combination of solution and solid phase techniques new structurally derivatives of the natural lead compound. The biological effects of this new collection of jaspamide analogues on actin microfilaments are currently under investigation.

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ENZYMATIC GLUCOSYLATION OF NARINGIN AND GRAPEFRUIT JUICE BY α -D-GLUCOSIDASE FROM THE MARINE MOLLUSC *APLYSIA FASCIATA*

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Marine organisms are a rich and almost unexplored source of enzymes with different specificities for formation and hydrolysis of glycosidic bonds [1]. Glycosyl hydrolases usually hydrolyze glycosidic bonds but, often, they are also able to catalyse the stereospecific formation of such linkages [2], finding application in the enzymatic synthesis of oligosaccharides and glycoconjugates. Recently, we focused our attention on sea hare *Aplysia fasciata* (Poiret 1789), a large mollusc belonging to order *Anaspidea* [3], very common in Mediterranean habitats. Many different glycosyl hydrolases activities were identified both in the hepatopancreas and in the visceral mass homogenates. An α -glucosydase activity was the most abundant found in the crude extract from the visceral mass, in which β -fucosidase, β -glucosidase, β -mannosidase activities are also present [4]. The characterization and the purification to homogeneity of α -glucosidase (EC 3.2.1.20) from *Aplysia fasciata* was previously attained. This enzyme acted on maltose, and relatively short maltooligosaccharides forming transglycosylation end-products containing α -1,6 linkage. Furthermore, good transglycosylation activity was observed when maltose was used as donor and cellobiose sucrose or pyridoxine as acceptors[5].

Here we report the enzymatic α -glucosylation of naringin, a flavonone glycoside responsible for bitter taste in grapefruit and possessing important pharmacological properties. Some reactions were performed using α -glucosidase from *Aplysia fasciata* and maltose at different concentrations. The regioselective formation of the β -gluco C6 α -monoglucoside derivative and of the corresponding isomaltosyl diglucoside was obtained with high yield and efficiency also at maltose low concentrations. The feasibility of a highly efficient glucosylation of naringin straight in grapefruit juice is also demonstrated at enzyme optimal pH. Mutation at enzyme active site could be useful to optimise the application of this food-compatible methodology at natural acidic juice pH in order to suggest this metod as an easy and biotechnologically interesting debittering grapefruit juice process.

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NEW SESQUITERPENES FROM THE SOFT CORAL *PSEUDOPTEROGORGIA RIGIDA*

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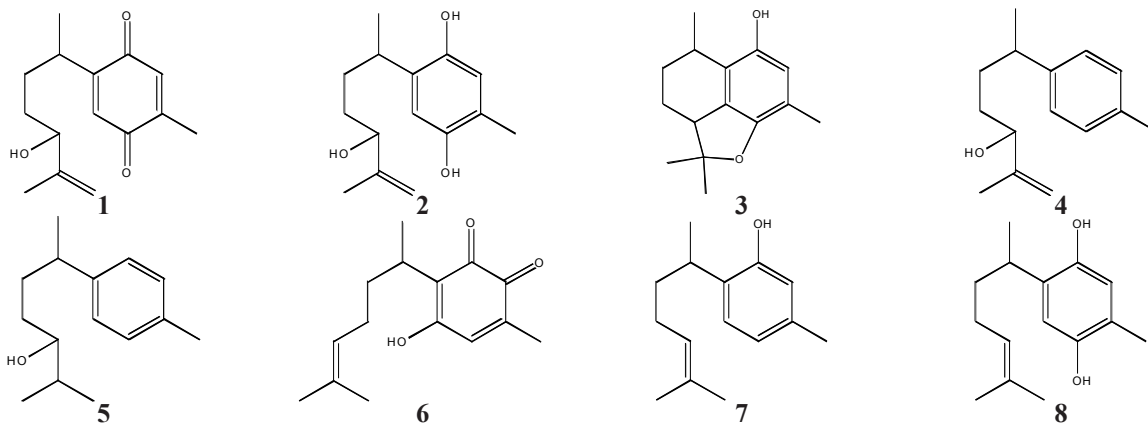
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A chemical reinvestigation of the colonial polypods of the tropical soft coral *Pseudopterogorgia rigida*, collected from the Caribbean Sea, afforded eight sesquiterpenoid metabolites.

The tissues of the gorgonian *P. rigida* were extracted with CH₂Cl₂/MeOH (3:1, v/v) to give an oily residue that was submitted to a number of chromatographic separations, including SiO₂ flash column chromatography (cHex / EtOAc) and HPLC (normal phase with EtOAc / cHex mixtures and reversed phase C₁₈ with MeOH / H₂O mixtures), to yield pure compounds **1-8**.

The structure determination was carried out by the combination of one- and two-dimensional NMR spectroscopy and high resolution mass spectrometry. To the best of our knowledge, this is the first report of metabolites **1-3**, whereas **4**¹ and **5**² are reported for the first time as natural products.

Additionally, the antifungal activity of the known sesquiterpenes rigidone (**6**)³, curcuphenol (**7**)⁴ and curcuhydroquinone (**8**)⁵ that were also isolated, was evaluated against *Candida albicans* MLR-62. Metabolites **6** and **8** exhibited significant activity, whereas the evaluation of the new compounds **1-5** is in progress.



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ANTIFOULING POLYMERIC 3-ALKYLPYRIDINIUM SALT FROM AN ANTARCTIC SPONGE *HALICLONA* SP.

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One of the most promising alternative technologies to antifouling paints based on toxic heavy metals biocides is the development of coatings whose active ingredients are natural eco-friendly compounds. In this study the potential antifouling efficacy of a poly-alkylpyridinium salt (poly-APS) from an Antarctic sponge *Haliclona* sp. was evaluated by laboratory-reared *Balanus amphitrite* larvae. The pure compound was obtained by ultrafiltration (3,000 Da MWCO) of the methanol-soluble part of the butanolic extract. The chemical characterisation was performed by NMR spectroscopy and mass spectrometry (ESI-TOF and MALDI-TOF). The monomeric unit was found to be a charged 3-octylpyridine, exactly the same as that of the Mediterranean *Reniera sarai* poly-APS¹. However, the MALDI-TOF mass spectra of the polymer displayed one main cluster centred at 3,675 Da (α -cyano matrix) and at 3,674 Da (DHB matrix), whereas those from *R. sarai* were larger, showing two main clusters centred at 5,520 and 18,900 Da. The cyprids settlement inhibition efficacy concentration (EC_{50}) of the *Haliclona* poly-APS was 0.06 $\mu\text{g/ml}$. The toxicity tests on *B. amphitrite* nauplius stage II showed a low toxicity level ($LC_{50} > 100 \mu\text{g/ml}$). The ratio between $EC_{50}:LC_{50}$ indicates a non-toxic settlement inhibition mechanism. The settlement inhibition is reversible: cyprids maintained for 3 days at a concentration of 100 $\mu\text{g/ml}$ of *Haliclona* poly-APS were able to settle again (45 %) when transferred to natural filtered sea water. Efforts have been made to chemically synthesise this class of compounds, but difficulties were encountered on arriving on a larger polymerisation numbers². The results show that the *Haliclona* poly-APS do have similar non toxic antifouling activities than the larger ones from *R. sarai*³. This encourages further studies for potential use of poly-alkylpyridinium salts as non-toxic antifoulants.

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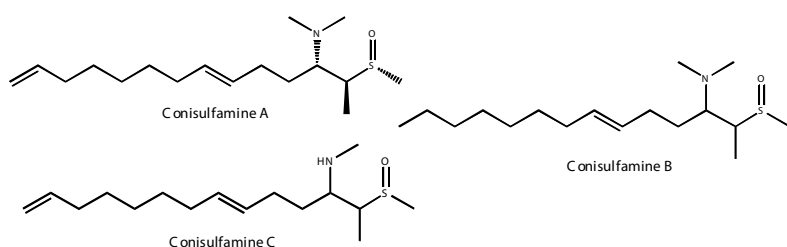
CONISULFAMINES A-C, NOVEL UNIQUE ALKYL SULFOXIDES FROM THE MEDITERRANEAN ASCIDIAN *APLIDIUM CONICUM*

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Ascidians belonging to the genus *Aplidium* of the Polyclinidae family are renowned for the variability of their metabolic content. A great chemiodiversity is devised from non-nitrogenous metabolites, mainly prenyl quinones or hydroquinones, to a vast array of nitrogenous metabolites.¹ Within a research project directed to the study of the chemistry of marine ascidians from the Mediterranean sea, our previous investigation of the ascidian *Aplidium conicum* Olivi collected along Sardinian coasts (Italy) resulted in the isolation of a number of novel bioactive compounds, comprising a number of antitumoral prenyl quinones derivatives,² cytotoxic secosterols,³ and an indole alkaloid with antihistaminic properties.⁴ In addition, a previous paper, a sample of *A. conicum* collected off Tariza Island (Spain) had been reported also to contain meroterpenoids but their structures were very different from those recovered in the sardinian sample. Recently, we analyzed a further sample of *A. conicum* collected in the Bay of Naples, which revealed a very different methabolic content. Surprisingly, this *A. conicum* sample is completely lacking in meroterpenoids, whilst the major component of the methanolic extract was showed to be a unique class of new compounds, the sulfoxide derivatives **1-3**, named conisulfamines A–C.



The structures of the novel metabolites have been elucidated through extensive spectroscopic analysis and the stereochemical analysis of conisulfamine A has been carried out in a three stages-approach, relying on the combination of NMR spectroscopy and computational methods.

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FOUR NOVEL TRYPSIN AND CHEMOTRYPSIN INHIBITORS FROM A WATER BLOOM OF THE CYANOBACTERIUM *MICROCYSTIS* SP.

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The cyanobacterium *Microcystis* sp. (IL-361) was collected from a fishpond in Kibbutz Maayan Zvi, Israel on July 2006. The cyanobacterial cells were freeze-dried and extracted with 7:3 MeOH/H₂O solvent mixture. The crude extract was separated using various chromatographic methods: flash-chromatography on an ODS column, gel-filtration on Sephadex LH-20 column and reversed-phase HPLC, in order to isolate the natural products. Four novel protease inhibitors: cyanopeptolin S', micropeptin MZ845, micropeptin MZ859 and micropeptin MZ939 were isolated from the crude extract of the cyanobacterium. Cyanopeptolin S' was previously detected by mass spectrometry in cell extracts of *microcystis* sp. bloom from Germany.¹ The structure of the pure natural products was elucidated using spectroscopic methods; MS, UV and one- and two-dimensional NMR. The absolute stereochemistry of the amino acids was established by Marfey's method for HPLC. The inhibitory activity of the compounds was determined against two serine proteases – trypsin and chemotrypsin.

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NEW JASPAMIDE DERIVATIVES WITH ANTIMICROFILAMENT ACTIVITY FROM THE SPONGE *JASPIS SPLENDANS*

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Jaspamide, a cyclodepsipeptide isolated from marine sponges of the *Jaspis* genus,^a is one of the most studied marine natural product. Jaspamide has many interesting biological properties including antifungal, anthelmintic, insecticidal and ichthyotoxic activities.¹ It has been shown to be active against 36 human solid tumour cell cultures.^b It is known that jaspamide binds to F-actin competitively with phalloidin,^c a well-known fungal toxin affecting actin dynamics, promotes actin polymerization under non polymerizing conditions and lowers the critical concentrations of actin assembly *in vitro*. In order to re-isolate further amounts of jaspamide for additional pharmacological studies, we examined the extracts of the sponge *Jaspis splendans*, still available in our laboratories. After careful sequential reversed-phase HPLC separation we obtained several very minor jaspamide derivatives modified in the polypropionate fragment or alternatively in the aminoacidic composition of the tripeptide unit. We report here details on isolation procedures, structural determination and biological activity of the new jaspamide derivatives. Furthermore, the pharmacological data on jaspamides allowed us to propose a structure-activity relationship.

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SCREENING OF SOUTH AFRICAN MARINE ALGAE FOR ANTIPLASMODIAL ACTIVITY

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Tuberculosis, human immunodeficiency virus (HIV) and malaria are the three major causes of death in Africa. Malaria causes fatalities in 3-5 million people and morbidity in 300-500 million people a year. The majority of the populations affected are children in the underdeveloped or developing countries.¹ Malaria is caused by the apicomplexan parasites of the *Plasmodium* genera of which the most harmful species is *Plasmodium falciparum*. Medicines that are currently available for the prevention and treatment of the disease such as chloroquine, mefloquine, pyrimethamine, sulfadoxine derivatives are becoming increasingly less effective as a result of resistance.² No resistance has been reported against artemisinin and its derivatives thus far, however they are expensive and thus are unaffordable in the countries where they are mostly needed, thus necessitating the development of new and improved antimalarial agents.

Over the last decade the search for biologically active compounds from the marine environment has grown. An investigation of the South African marine algae resulted in the collection and extraction of 20 algae. The extraction produced 82 crude extracts and these were screened for antiplasmodial activity against the chloroquine sensitive *P. falciparum* D10 strain. Good antiplasmodial activity was exhibited by 18% of the extracts with $IC_{50} < 10 \mu\text{g/ml}$. Furthermore, three extracts showed a selectivity index (SI) > 10 ($SI = IC_{50} \text{ antiplasmodial} / IC_{50} \text{ CHO cytotoxicity}$) indicating their selectivity for this strain of *Plasmodium*.

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INFLUENCE OF CERULENIN AND AMINO ACIDS IN *PROROCENTRUM LIMA* CULTURES

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Okadaic Acid and Dinophysitoxins are the main responsables of the red tide phenomenon known as Diarrhetic Shellfish Poisoning, produced by dinoflagellates belonging to the genera *Dinophysis* and *Prorocentrum*.

Dose toxins, are of great interest not only because of their toxic effects, but also because they are highly selective inhibitors of protein phosphatases tipe 1 (PP1) and 2A (PP2A). As well as to being potent tumor promoters. For these reasons, Okadaic Acid is an extremely useful tool to study cellular processes and an important standard for shellfish control.

In order to set deeper knowledge of the biosynthetic origin of Okadaic Acid, a series of experiments, feeding a where a selective inhibitor of polyketide sintases was added to batch cultures of *Prorocentrum lima*, were undertaken.

In addition, a number of selected amino acid were used, this study analyzes the effects of the inhibidor on the promotion of growth, biomass and toxin production in cultures of *Prorocentrum lima* of the PL2V strain in batch cultures.

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OCCURRENCE OF OKADAIC ACID IN THE FEEDING GROUNDS OF DUGONGS (*DUGONG DUGON*) AND GREEN TURTLES (*CHELONIA MYDAS*) IN MORETON BAY, AUSTRALIA

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Okadaic acid (OA) is a diarrhetic shellfish poison (DSP) produced by a number of marine organisms including benthic dinoflagellates *Prorocentrum lima*, which are often found on seagrass. As seagrass forms the basis of the diet of both dugong (*Dugong dugon*) and green turtle (*Chelonia mydas*), these herbivores may potentially be exposed to OA through ingestion of *P. lima* found on the seagrass. In this study, the abundance of epiphytic dinoflagellate, *P. lima*, on seagrass, and the concentration of OA produced by these epiphytes were measured from Moreton Bay, Queensland, Australia. *P. lima* were found on all four species of seagrass collected. OA was detected, with a maximum of 459.6 ng OA kg⁻¹ *Halophila spinulosa*_{-(wet weight)}. From this information, the estimated maximum daily intake of OA by an adult dugongs consuming 40 kg seagrass day⁻¹ was 18,383 ng OA day⁻¹, and an adult turtles consuming 0.2 kg seagrass day⁻¹ 92 ng OA day⁻¹ were estimated. HPLC/MS/MS analysis of dugong and turtle collection from 54 and 19 stranded animals respectively, did not yield OA above the detection limit of 10,000ng kg⁻¹.

ANTICANCER BIODISCOVERY FROM AUSTRALIAN MARINE BIODIVERSITY

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During a collaborative research project with PharmaMar (Madrid, Spain) (2004-2007) we screened the organic and aqueous extracts from a library of 2642 Australian marine organisms (sponges 88%, algae 7%, tunicates 4%, misc 1%), assessing their ability to inhibit the growth of a selection of human cancer cell lines. Of the 110 extracts that displayed promising *in vitro* potency and selectivity, the activity was localized in either the n-BuOH (73%) or the H₂O partition (21%), or was distributed between both the n-BuOH and H₂O partitions (6%). During the course of this project we examined the chemistry of 70 extracts, isolating both known and new marine natural products. To date we have discovered at least two novel families of marine natural product with nM *in vitro* potency and promising selectivity against important cancer cell lines over mammalian control cell lines. This presentation will describe some of the finding from this study.

MOLECULAR BIODISCOVERY AT THE UNIVERSITY OF QUEENSLAND

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In the past 17 months, 18 bioassays were identified, regrouped around six indications: Cancer, antibacterial, antiviral, diabetes/obesity, chronic-acute inflammatory conditions and CNS-related indications. Most assays are *in vitro* cell-based functional assays, with validated molecular targets of very high interest. The IMB also has carefully archived and annotated chemical libraries containing tens of thousands of yet undescribed chemical structures, available for screening. These include natural products (marine sponges-, cephalopods- and bacteria-extracts from Rob Capon research group), venom extracts and combichem libraries. Two examples of cell-based assays and one example of purified target-based assay, successfully used against IMB's extracts' libraries are presented: i/ glycine-gated chloride channel inhibition assay¹ (CNS-related indication), ii/ TNF α trafficking/delivery assay² (anti-inflammation indication) and iii/ pneumococcal surface antigen A (PsaA) competitive zinc binding assay³ (antibacterial). The first two assays are high-content-screening assays in 384 and 96-well plate format, respectively. A high frequency of active extracts was identified (0.5 to 0.8%), with promising chemistry. The last assay in 384 well plate format uses purified recombinant PsaA, a fundamental determinant of *Streptococcus pneumoniae* virulence. Active extracts, potentially containing new metalloprotein inhibitors were also identified.

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MARINE UNCULTURED BACTERIA AS POTENTIAL SOURCE FOR BIOACTIVE COMPOUNDS

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The emergence of pathogens resistant to currently available antibiotics, and the increasing threat of bio-terrorism urge the search for new efficient and safe antibiotics. One common approach to antimicrobial compound discovery is to screen natural products, for molecules that inhibit bacterial cell growth. These methods require high concentrations of test compounds and long incubation times. A recent study demonstrated that certain antibiotics trigger the SOS gene expression in bacteria, resulting in shutdown of DNA replication and transient dormancy and increasing the potential for survival of these antibiotic sensitive bacteria. It appears that many antibiotics, when used at sub-inhibitory concentrations, have the ability to activate or repress gene transcription, which is distinct from their inhibitory effects. We used this gene induction coupled with the use of reporter gene (e.g.. bioluminescent bioreporter) as a strategy for detection of sub-inhibitory concentrations of antimicrobial compounds. In our research, the detection of such concentrations of known as well as unknown antimicrobial agents produced by uncultivable microorganisms cultured by our novel culturing technology, e.g. the encapsulation of bacteria in agar spheres coated by polymeric membrane and the subsequent incubation of the spheres with the coral host, were studied using luminescent modified indicator bacteria. The emitted luminescence was measured repeatedly with a luminometer, and showed differences in the activity between different antibiotics. In addition using this methodology allowed us to detect sub-inhibitory concentrations in different dilutions of the agar from spheres containing microbiota associated with mucus from the corals *Fungia granulose*, *Favia* sp. and *Rehytisma fulvum fulvum*. Many of the microorganisms were identified as novel species that demonstrated bioactivity and can be useful resources for bioactive natural products.

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Edizioni Ziino - Italy
www.edizioniziino.com

Finito di stampare nel mese di settembre 2007