Title
The Biomedical Potential of California Marine Organisms

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The Biomedical Potential of California Marine Organisms (R/MP-87)
Final Narrative

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Introduction

The search for new pharmaceuticals continues to demand our greatest efforts. Despite tremendous advances in medical science, there are no comprehensive cures for HIV or AIDS, certain forms of cancer, arthritis and other inflammatory conditions, and a large assortment of viral and fungal diseases. In fact, the incidence of some previously curable illnesses, such as tuberculosis, is on the rise due to the spread of drug-resistant infections. Currently, about half of the medicines prescribed are derived from natural sources such as terrestrial plants and micro-organisms. In addition, many drugs that are now produced by total synthesis were originally derived by modification of natural products. Although few marine natural products are currently used medicinally or are in clinical trials, marine organisms comprise the greatest unexploited source of potential pharmaceuticals. Because of the unusual diversity of chemical structures isolated from marine organisms, there is great interest in screening marine natural products in new mechanism-based bioassays.

An examination of the marine natural products literature reveals that most studies of metabolites from California marine organisms were performed during the 1970s, when very few compounds were systematically evaluated for their biological or pharmacological activity. Looking back on that era, it is apparent that chemists could easily publish their results on the basis of the novelty of the structures rather than the bioactivity of the compounds. Yet it was those same novel structures that were used to justify programs to discover "Drugs from the Sea." However, during the time of these early programs that preceded the start of Sea Grant funded collaborative program in 1979, marine organism collections had begun to focus more on tropical environments with greater biodiversity and less on California. Was this a mistake? From the viewpoint of biodiversity of sessile invertebrates, concentration on tropical Indo-Pacific locations appears to have been well justified, but those who have studied marine organisms from temperate waters have recently reported a number of bioactive compounds.

The goal of this project was to revisit California waters and to screen extracts of California marine organisms in mechanism-based bioassays that are far superior to those used in the earlier investigations. In addition to the goal of discovering new pharmaceutical agents, we also proposed to provide data that would help others to assess the value of California's marine biodiversity. Just as the commercial value of seafood can be predicted and used to justify conservation, biomedical screening will provide some idea of the potential value of California's marine invertebrates and algae. Our efforts can be summarized as follows:

[†] Deceased

2001–2002

Just over a year into the project, the withdrawal of the trainee (Michael McCoy) from the graduate program had an adverse effect on our progress. Collecting marine organisms from the California coast is a seasonal operation but we had several projects under way, some of which look quite promising. For "conservation" reasons we examined the feasibility of collecting small samples of each specimen for screening, followed by re-collection of active samples to complete the chemical studies. This strategy proved to be less effective than making the normal sized collections.

At this point, we had collected and screened 114 specimens, mainly from the Channel Islands. Each specimen was partitioned into organic and aqueous fractions and rescreened. Initial screening in the cytotoxicity assay showed that we have three "priority 1" (potent and selective) and sixl "priority 2" (potent but less selective) extracts. Three extracts showed activity in the HIV-1 integrase assay. Three extracts showed antifungal activity. Bioassay-guided fractionation had begun on the cytotoxic "priority 1" extracts.

2002–2003

45 collections were made (43 sponges, 1 ascidian, 1 hydrozoan). Collections were made by SCUBA in the subtidal zone or by hand in the intertidal zone. Specimens were collected mostly from Point Loma, Boomer's Beach, and Casa Cove tide pools in San Diego. We have activity profiles (antimicrobial, antifungal, HCT-116, and DNA-binding) for each crude methanol extract. Voucher samples from each collection were crudely fractionated on reverse phase to give five fractions of decreasing polarity. We have ^{1}H-NMR spectra and activity profiles for each fraction.

There were several Haplosclerid sponges that have similar activity profiles (antimicrobial, HCT-116, and PQ-37) and crude 1H-NMR spectra with one another and with a sponge that was ranked priority 1 by the NCI in 1999-2000. These compounds are extremely active on the HCT-116 assay. 01-231 is a full collection (620 g wet wt). A liquid/liquid partition (ethyl acetate and water) used to separate the polar and nonpolar compounds, followed by reverse-phase medium and high pressure liquid chromotrography resulted in the isolation of the known diterpenes Macfarlandins A and B.

Chemical investigation of the deep-water sponge *Plakortis nigra* resulted in the isolation of eight compounds with novel structures: four brominated tryptophan-derivatives with a β-carboline backbone (Plakortamine A-D), two cyclic peroxide acids (Epiplakinic acid G-H), and two γ-lactones. MALDI-FTMS was used to determine the exact mass for each compound. Structures and relative stereochemistry were determined by NMR and ESI-MS.[1]

The sponge *Haliclona lunisimilis* from Point Loma, California, contained six known chlorinated acetylenes, previously isolated from the dorid nudibranch *Diaulula sandiegensis*, and three new metabolites, (1*Z*,3*Z*)-1-chlorohexadeca-1,3-diene-5,7-diyne-14-ol, (1*Z*,3*E*,9*Z*)-15-acetoxy-1-chlorohexadeca-5,7-diyne-1,3,9-triene, and (1*Z*,3*E*)-14-acetoxy-1-chlorohexadeca-

[1] *J. Nat. Prod.* **65**, 1258 (2002)

1,3-diene-5,7-diyne. The structures of the new compounds were elucidated by interpretation of spectroscopic data.[2]

We have screened our library of marine invertebrate extracts to identify natural products that are selectively toxic to budding yeast cell lines (*Saccharomyces cerevisiae*) with defined alterations in cell-cycle checkpoint and DNA-damage repair genes. Budding yeast are a good model system due to the high degree of conservation of DNA repair pathways and the relative ease of handling (for more information on this, please refer to *Cancer Research*, 2000, 60, 328–333).

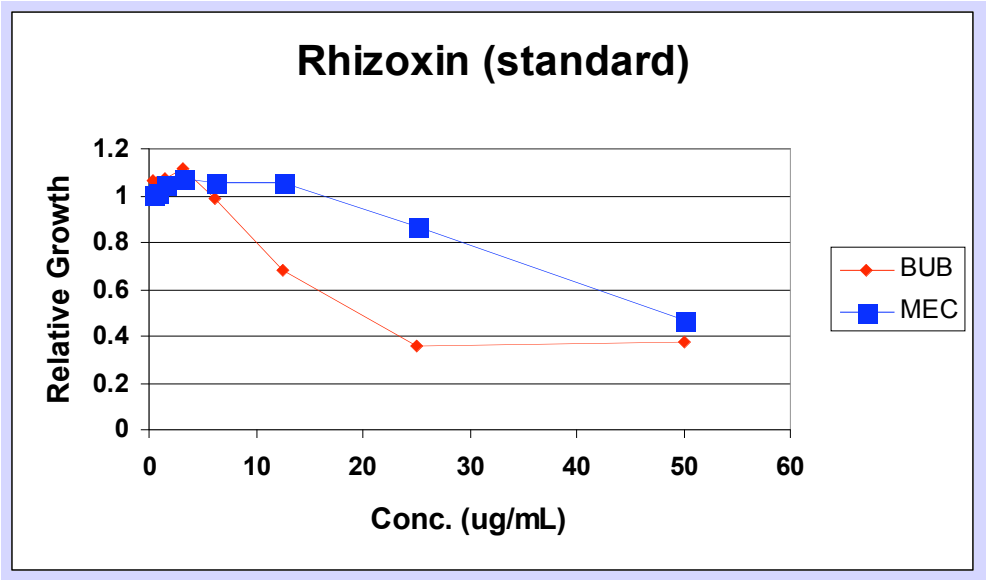
This assay was successfully used to identify novel compounds from the organic extract of a marine sponge. The marine sponge *Erylus lendenfeldi* was collected in February 2000 in the Red Sea just north of Hurghada. From this sponge we have isolated three steroidal glycosides, erylosides A, K, and L, which exhibited selective activity against a *Δrad50* yeast strain. The methanol extract of the frozen sponge was subjected to HP-20 flash chromatography with aqueous acetone. The 50% acetone fraction was purified by reversed-phase HPLC with 75% methanol to yield erylosides A, K, and L.[3]

In an effort to isolate novel cell-cycle inhibitors, our library of microbial extracts was screened for compounds that are selectively cytotoxic to yeast strains that are deficient in a DNA-damage checkpoint gene (*MEC2*/*RAD53*) or a mitotic spindle checkpoint gene (*BUB1*) as compared to parent strains. Thus far, ~3,000 extracts have been screened, and 25 confirmed hits against the *Δbub1* strain have been identified. Only one confirmed hit against the *mec2-1* strain has been identified, suggesting that this may not be a drugable target. Producing strains of my confirmed hits are now being regrown, but the active components of two extracts have been identified as staurosporine derivatives (**see next page**). This validates the assay and suggests that it is capable of distinguishing selectively bioactive compounds from nonspecific cytotoxins (staurosporine is a known protein kinase inhibitor).

Conclusion

The goals of this project were to screen extracts of California marine organisms for pharmaceutical activity and to provide data that would help others to assess the value of California's marine biodiversity. We have successfully surveyed the invertebrate population of Southern California. Several of our samples have yielded novel and exciting compounds that have been reported in peer-reviewed technical articles. Southern California is a wellspring for bioactive marine invertebrates that has been long overlooked. The rocky intertidal zones and kelp forests unique to California serve as a haven for sponges, ascidians, and opisthobranch molluscs. We are thankful for the opportunity to have explored this unique realm.

[2] *J. Nat. Prod.,* **66**, 671 (2003)
[3] *Tetrahedron* **61**, 1199 (2005)


Rhizoxin (standard)
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Conc. (ug/mL)
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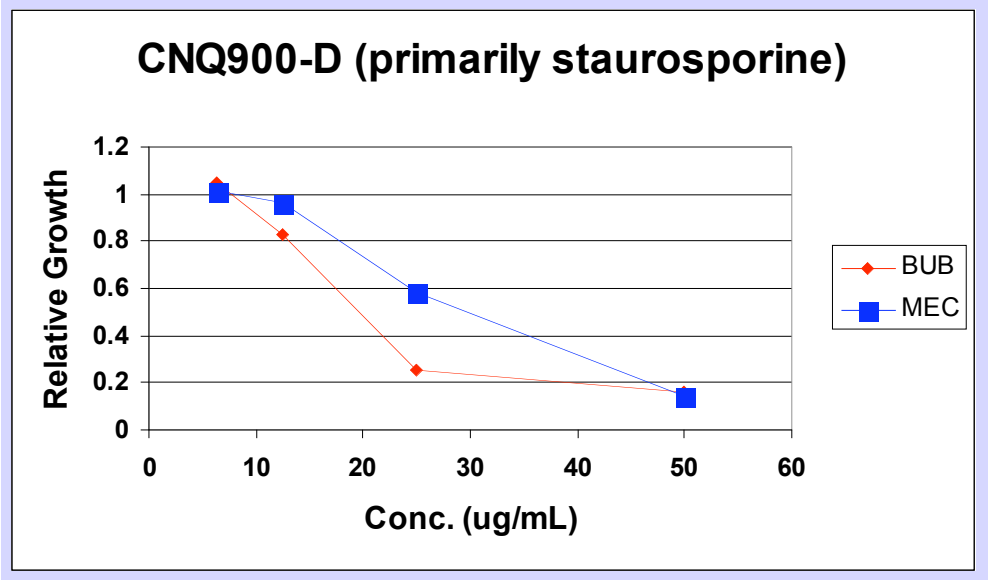
CNQ900-D (primarily staurosporine)
Relative Growth
1.2
1
0.8
0.6
0.4
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Conc. (ug/mL)
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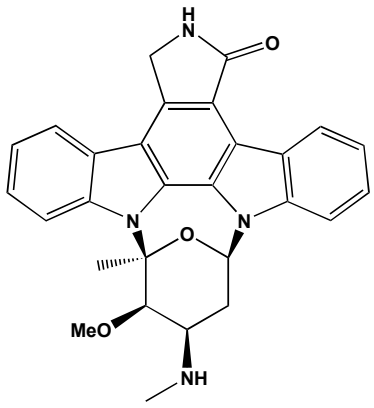
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