

CHEMICAL ECOLOGY OF SHALLOW WATER OCTOCORALS.  
OCCURRENCE OF CEMBRANOLIDES IN *PSEUDOPLEXAURA*  
AND *EUNICEA*.

by

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ABSTRACT

Gorgonians of the genus *Pseudoplexaura*, from a variety of geographical locations, yield crassin acetate as the major cembranolide. The cembranolides found in *Eunicea* collected at different locations vary. Eunicin is found in *Eunicea mammosa* from Bimini, and from a number of other locations. Its position isomer Jeunicin, has been found in the *Eunicea* from Jamaica. *Eunicea* from Curacao yields an epimer of eunicin, Cueunicin, while that from Puerto Rico yields Eupalmerin acetate, and Peunicin occurs in *Eunicea* collected off Panama. The cembranolides are toxic to various aquatic organisms. They inhibit ciliary action in the ciliate *Tetrahymena pyriformis*, and in the larvae of the nudibranch *Phestilla sibogae*.

Gorgonians or horny corals are coelenterates of the Class Anthozoa, Subclass Octocorallia, or Alcyonaria, Order Gorgonacea. They are prominent members of the sessile shallow water coral reef fauna in the Caribbean. They have few predators and hold their own in the competition for "space" on solid substrate in coral reef areas. I feel that the success of gorgonians against predation and against overgrowth by competing organisms is in part due to the secretion of defense substances (CIERESZKO 1960, 1973). These include diterpenoid compounds related to cembrene, a diterpene with a 14-membered ring.

The cembranolides are of ecological significance as they are toxic to a variety of organisms and inhibit ciliary motion (CIERESZKO 1962; PERKINS 1970, 1973, 1974). HADFIELD of Kewalo Marine Laboratory, University of Hawaii (personal communication), reports that in larvae of the nudibranch *Phestilla sibogae* velar cilia begin to disappear immediately when exposed to 10 ppm eunicin (II) in sea water; all larvae are dead in 24 hr. If exposed to this concentration for as brief a period as 15 minutes and then transferred back to clean sea water, most larvae still died within the following 48 hrs. When exposed to 5 ppm of peunicin (VI), larvae stop swimming within 10 minutes but continue to live for at least 16 hours; after 24 hours larvae are dead and disintegrating:

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However, disintegrating larvae are devoured by very active ciliate protozoa.

Some cembranolides have shown activity in the KB test, an *in vitro* assay using cultures of human carcinoma cells and in the PS test, an *in vivo* assay against P-388 lymphocytic leukemia (WEINHEIMER 1975).

I should like to comment on the occurrence and geographical distribution of the cembranolides in various species of *Pseudoplexaura* and *Eunicea*.

Crassin acetate I has been found to occur in all specimens of three species of *Pseudoplexaura*: *P. porosa*, *P. wagenari* and *P. flagellosa* collected at Belize, Bermuda, Bimini, Curacao, Florida, Grand Cayman, Jamaica, Puerto Rico, San Andres Isla (Colombia), St. Thomas and St. Croix (U. S. Virgin Islands), and St. Vincent.

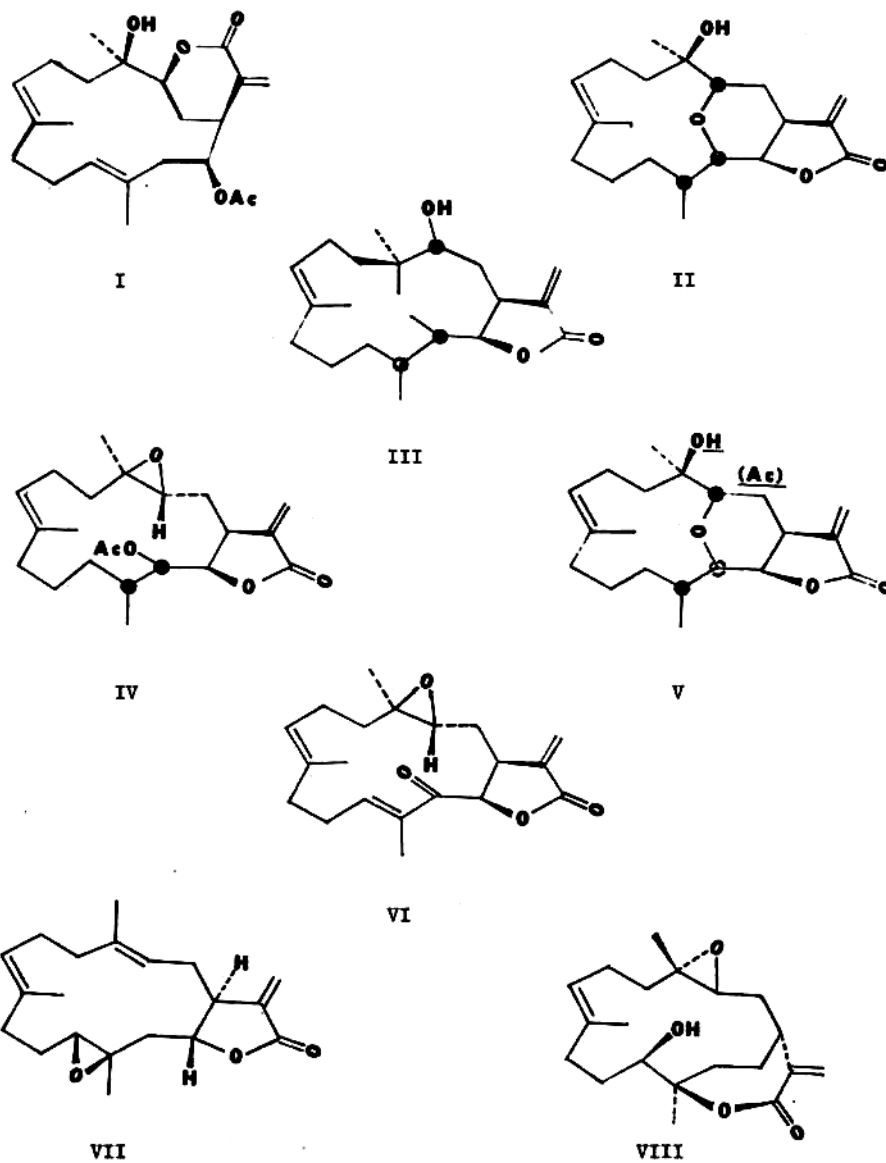
Specimens of *Eunicea mammosa* and *E. succinea* have yielded five other cembranolides depending upon their geographical origin. *Eunicea mammosa* from Bimini yielded eunicin II as did the same species collected at Ambergris Cay (Belize), at Isla Providencia (Colombia), St. Thomas, St. Vincent, and Grand Cayman. *Eunicea* from Jamaica, collected in 1960 yielded pure peunicin III, an isomer of eunicin. Later collections yielded both jeunicin and eunicin, with the eunicin more common in recent collections. *Eunicea mammosa* and *E. succinea* var. *plantaginea* collected in 1962 at Margot Fish Shoal near Miami, Florida, yielded still another lactone, later named eupalmerin acetate IV (REHM 1971, EALICK 1975). REHM (1971) found eupalmerin acetate in *Eunicea palmeri* BAYER collected near the Ragged Keys of Florida, but eunicin in the same species of *Eunicea* was collected further south, in the Florida Keys, near Molasses Key and Spanish Harbor. We have found the *Eunicea succinea* collected off La Parguera, Puerto Rico, to be the richest source of eupalmerin acetate.

Examination of *Eunicea mammosa* collected in Curacao led to the discovery of a second isomer of eunicin, cueunicin V, which occurs largely as the acetate (GROSS 1974). Collection of specimens of *E. succinea* var. *plantaginea* off Isla Buenavista, near Portobelo, Panama, led to the discovery of still another cembranolide, "peunicin" IV (CHANG 1977).

Cembranolides have been sought for and found in Indo-Pacific octocorals. TURSCH (1974) found lobophytolide VII in the soft coral *Lobophytum cristagallio* and sinulariolide VIII in the soft coral *Sinularia flexibilis*, both collected in Indonesia off Sewaru (Leti Island). Other cembrane derivatives have been found in the Pacific soft corals, *Litophyton viridia* (TURSCH 1975) and *Nephthea* sp. (SCHMITZ 1974).

The concentration of cembranolide in the octocorals is high. *Pseudoplexaura* usually contains at least 2% of its dry weight of crassin

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acetate. *Eunicea* yield 0.5-2% of their dry weight of cembranolide. We have observed crystalline crassin acetate and eunicin in the brownish fluid expressed from *Pseudoplexaura porosa* and *Eunicea mammosa* by squeezing the live gorgonian. Crystalline crassin acetate has also been observed in the tissues of *P. porosa* by RICE (1970). I have prepared fairly pure crassin acetate by partially breaking up pieces of cortex from live *P. porosa* (Bermuda) in a Waring Blendor, straining off the coarse animal debris on a plastic kitchen sieve, allowing the denser calcareous spicules to settle and collecting the crystals from the suspension of finer materials on a nylon paint sieve which passes zooxanthellae.

I believe that the outer tissue fluids of the gorgonians are supersaturated with respect to the cembranolides and that these diterpenoids are being continuously secreted. The cembranolides are odorless, but one can smell other terpenes peculiar to the gorgonians as soon as the animals are taken from the sea.

I should point out that PERKINS (1973) has produced *Tetrahymena pyriformis* resistant to crassin acetate, but this resistant strain is killed by eunicin or jeunicin.

The production of the variety of cembranolides in *Eunicea* from different locations may point to differences in the existing symbiotic algae (zooxanthellae). The sesquiterpene hydrocarbons found in *Eunicea mammosa* by YOUNGBLOOD (1969) vary with geographical origin. This is not the case with *Pseudoplexaura prosa*.

PROCEDURE FOR PREPARATION OF CEMBRANOLIDES

Gorgonians were collected by hand and drip-dried on a line during the sunny part of the day. Drying was completed in a stream of warm air from a blower. The dried ground gorgonian cortex was extracted in a continuous percolator-extractor (CIERESZKO 1966) with commercial n-hexane, changing the hexane in the boiling flask at intervals. On standing, partially concentrated extracts deposited insoluble material, often in crystalline form. The precipitates were washed with fresh hexane by decantation to remove some pigment and oil, and were then taken up in benzene. The benzene solution was poured into a column of dry Florisil (magnesium silicate adsorbent, Floridin Co.). Elution was carried out with benzene. Concentration of the benzene solutions from the column yielded crystalline solid which was recrystallized from benzene-hexane. The time of elution varied with the different cembranolides. The hydroxylated cembranolides eunicin, jeunicin, crassin acetate and cueinicin took considerably longer time than those lacking free hydroxyl groups, such as cueunicin acetate and eupalmerin acetate.

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