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MARINE BIOTOXINS: LABORATORY CULTURE AND MOLECULAR STRUCTURE

Annual Report

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Toxic cultures of Gambierdiscus toxicus have been established. Bioassays (mouse lethality) and pharmacological profiles indicate that the toxins are maitotoxin and a toxin which has the pharmacological profile of ciguatoxin but has reduced lethality. These results must be confirmed by replication. Small amounts of ciguatoxin are being purified from mooray eel viscera procured on Tarawa atoll, Kiribati, and from locally caught yellow amberjack, Seriola dumerilii.

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FOREWORD

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____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Paul Reese Oct 18, 1989

PI - Signature DATE

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I. INTRODUCTION

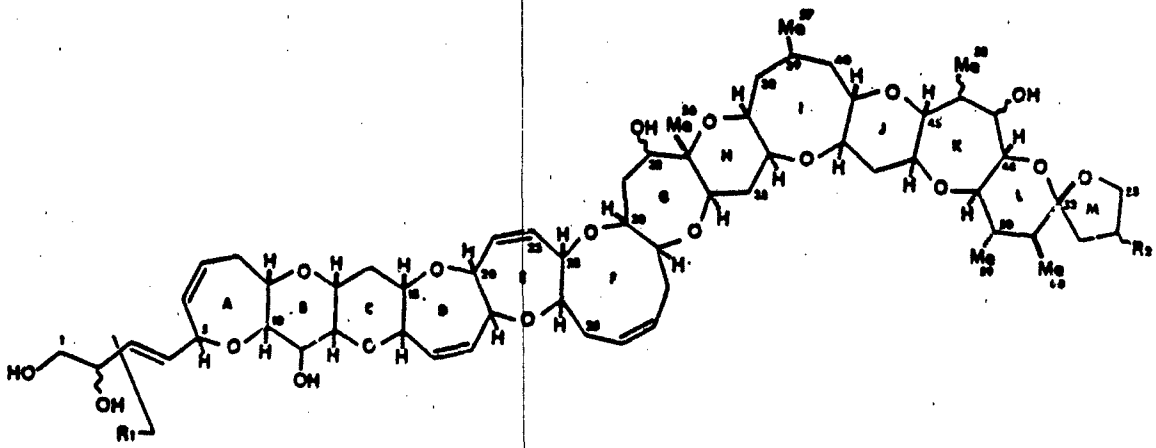
A. The Problem

Marine biotoxins are among the most potent naturally occurring toxins known. Their physiological actions are diverse, as are their molecular structures, not all of which have been fully determined. Some of these toxins constitute a hazard to human health and safety. It is therefore imperative to elucidate their structures and to make pure toxins available for the study of pharmacological properties and mechanisms of action.

In this study we are principally concerned with two toxins that are associated with the human fish intoxication known as ciguatera, ciguatoxin and maitotoxin, and with palytoxin. Each of the three toxins presents a different set of problems.

1. Ciguatoxin (CTX)

Its molecular structure of ciguatoxin (1) has recently been determined by Yasumoto and coworkers (Personal Communication). The compound is a 55-carbon chain fashioned into 13 contiguous ether rings, reminiscent of okadaic acid and the brevetoxins.



1 R_2 = OH (Moorea eel)

2 R_1 = CH_3=CH- (*G. toxicus*)
R_2 = H

1 2

Laboratory culture and continued extraction of eel viscera are necessary in order to

a. study the poorly known pharmacology and chemistry of the toxin
b. devise a monoclonal antibody-based diagnostic test to detect ciguatoxic fish
c. determine the absolute stereochemistry of the compound
d. initiate molecular modelling studies directed toward drug development and therapy.

2. Maitotoxin

In contrast to ciguatoxin, which is soluble in organic solvents, maitotoxin is water-soluble. It was first described from the gut of herbivorous toxic fishes, where it occurs in low concentration. It has been produced in a number of laboratories from cultured G. toxicus. Its molecular structure is partially known.

3. Palytoxin

This toxin, whose structure has been known for several years and which has recently been synthesized[1], has so far been isolated from toxic zoanthid corals, Palythoa sp. Preliminary evidence suggests that the producer of this toxin is not the coral, but an epiphyte, possibly a bacterium.

B. Current Status

1. Ciguatoxin

The PI reviewed the status of ciguatera research at the Mycotoxins and Phycotoxins Symposium in August, 1988 in Tokyo, Japan. A copy of the paper is attached as Appendix II. The principal development since then is the

structural elucidation of ciguatoxin by Yasumoto and coworkers (Personal Communication). As a result, ciguatoxin production can now be devoted to the tasks outlined under Section A.1 above. For work in Hawaii the best toxin source remains eel viscera from Tarawa atoll, Republic of Kiribati, despite formidable communication and transportation problems.

There are preliminary indications (see Section II a, Table I and Figure 2) that our laboratory culture of G. toxicus produces a toxin that has a pharmacological profile of ciguatoxin but is less toxic than CTX. If these experiments can be replicated, production of this toxin will be an important goal.

2. Maitotoxin

Replication of experiments to evaluate our G. toxicus cultures (see Section IIA), followed by quantification and upscaling will allow us to begin maitotoxin production in the near future.

3. Palytoxin

We have not yet initiated research on this phase of the contract. We will recruit appropriate personnel within the next few months.

C. References

1. Kishi, Y. et al., J. Am. Chem. Soc. 1989, 111, 7525, 7530.

II. Results During Year II

A. DINOFLAGELLATE CULTURE

1. INTRODUCTION

Previous reports have detailed the attempts to establish cultures of the benthic dinoflagellate Gambierdiscus toxicus and associated dinoflagellates under laboratory conditions. The results of these attempts are now presented, with emphasis on the progress achieved since the last Quarterly Report was submitted.

2. MATERIALS AND METHODS

2.1 Population densities of benthic dinoflagellates

The methodology used to determine the population densities of dinoflagellates growing attached to macroalgae is as described previously. Briefly, species of macrolgae were harvested in approximately 50g quantities from Kahala Beach, Oahu. The algal samples were shaken in seawater and the biodetrital material so obtained was filtered successively through nylon filters of mesh size 250, 100 and 37 μm. The number of cells of Gambierdiscus toxicus and other dinoflagellates in 1 mL aliquots of the biodetritus fraction (100 - 37 μm) was determined using a calibrated water counting chamber.

2.2 Culture of dinoflagellates

Batch and clonal cultures of Gambierdiscus toxicus and associated dinoflagellates have been initiated as follows. Cells of dinoflagellate

species are removed from the algal biodetritus with sterile micropipettes and are washed in sterile seawater. For clonal cultures, single cells are transferred to sterile test tubes containing 5 mL of ES medium (Provasoli, 1968). For batch cultures 20 cells are placed in 10 mL of ES medium. The cultures are maintained at 27°C on a 14:10 light:dark cycle at 1.0×10^{16} quanta cm^{-2} s^{-1} provided by "Cool White" fluorescent tubes (General Electric). At three weeks, the cells are transferred to 125 mL flasks containing 50 mL of ES medium. Cultures so established are maintained as stock cultures in ES medium and subcultured every 3 - 4 weeks.

Axenic cultures of each clonal and batch stock culture are obtained by the following method. Samples of each stock culture (100 - 200 cells) are washed in sterile medium using glass spotting dishes and transferred to ES medium supplemented with antibiotics (Penicillin:Streptomycin; 2:1). After 48 h, the cells are placed in a 50 mL flask containing 20 mL of normal ES medium. This process is being repeated at 3 - 4 week intervals.

2.3 Harvesting and extraction of dinoflagellates

For toxicity studies, batch and clonal cultures of Gambierdiscus toxicus were prepared as follows. Fernbach flasks each containing 1.5 L of ES medium were inoculated with seed cultures of the dinoflagellate (approx. 200 mL; 200 cells/mL). The cells were maintained for 3 - 4 weeks as decribed previously, at which time the cell density had reached $1 - 1.5 \times 10^6$ cells/mL.

The cells were harvested by filtration onto glass fibre filters (Whatman GF/A), washed with deionized distilled water to remove salts and lyophilized.

The washed, dried cells were sonicated in acetone and extracted in acetone over 48 h. The extract suspension was filtered and the acetone-insoluble material extracted in methanol over 48 h. Solvent was removed from both the acetone and methanol extracts under vacuum (Buchi, Rotavapor) and the residues taken to dryness under a stream of nitrogen. The crude extracts are being stored at -20°C.

2.4 Mouse biossay

Male and female mice (Swiss white; 18-23g) were injected i.p. with doses of the dinoflagellate extracts prepared as fine suspensions in 1% Tween 80 in physiological saline. Signs expressed in the mice following administration of the extracts were identified according to the criteria detailed in Hoffman et al. (1983) and recorded at 1 h and then at intervals up to 48 h. The mice were used and sacrificed in accordance with ethical standards.

2.5 Cardiotoxic effects of extracts of Gambierdiscus toxicus

The cardiotoxic effects of selected extracts prepared from cultured G. toxicus have been evaluated using the following method. Male guinea-pigs (300-500g) were sacrificed, the hearts excised, and placed in oxygenated Krebs-bicarbonate solution. The left and right atria were dissected free and used separately to study the effects of the G. toxicus extracts upon the electrically-stimulated and spontaneously-elicited contraction, respectively. The left atrium was stimulated by rectangular pulses (1.5 threshold voltage, 4 msec duration and at 1.5 Hz) delivered through a Grass SD9 stimulator. The isometric responses of both the left and right atria were measured using force transducers and recorded on a Grass polygraph. The crude extracts were

dissolved in 10% methanol and 100 µL aliquots of the solutions added to the organ bath to give a final concentration of 10 µg/mL.

3. RESULTS

3.1 Population densities of benthic dinoflagellates

The population density of Gambierdiscus toxicus and Ostreopsis lenticularis growing attached to macroalgae collected at Kahala Beach, Oahu, has been monitored from September, 1988 to August, 1989. The results of this survey are presented in Fig. 1. There has been a gradual increase in the number of both G. toxicus and O. lenticularis growing attached to the red alga Spyridia filamentosa since March, 1989, with the maximum number of cells of each species of dinoflagellate being recorded in August, 1989. Analysis of biodetrital fractions obtained in June and August, 1989 revealed the presence of both G. toxicus and O. lenticularis growing attached to Acanthophora spicifera and Dictyota sp. On one previous occasion, O. lenticularis had been found in the biodetritus obtained from A. spicifera, whereas G. toxicus had been found growing only on S. filamentosa. Other workers (Bomber et al., 1989); Gillespie et al., 1985) have reported that G. toxicus displays an apparent preference for certain species of macroalgae, particularly those that possess a filamentous and/or branched morphology and therefore a large surface area. However, it has also been demonstrated that G. toxicus will utilize a wide variety of algal substrates, including species from different divisions (Bomber et al., loc. cit., Gillespie et al., loc. cit.).

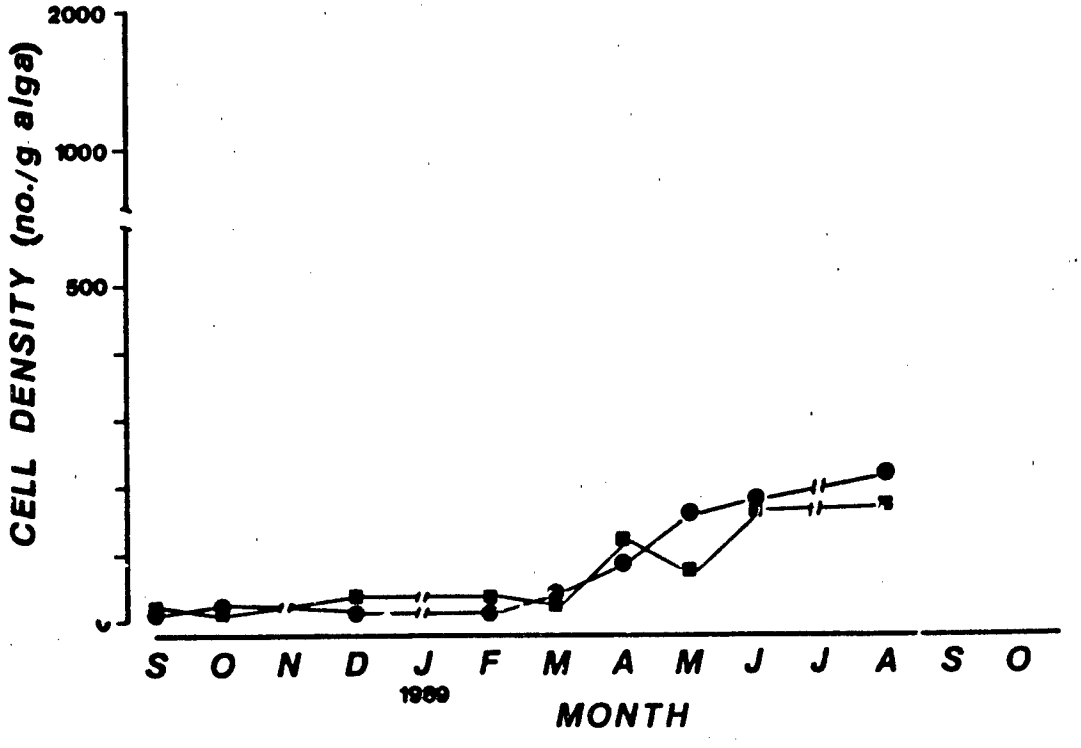


Fig. 1 The mean density of Gambierdiscus toxicus [•] and Ostreopsis lenticularis [▪] growing attached to the alga Spyridia filamentosa. Counts were made of the dinoflagellates present in biodetrital material (37 - 100 μm fraction) obtained from plants to S. filamentosa collected from Kahala Beach, Oahu. The mean densities were calculated from cell counts obtained from five plants of S. filamentosa. Standard error bars are not shown.

3.2 Dinoflagellate culture

An inventory of the dinoflagellate cultures currently being maintained in the Chemistry Department is presented in Appendix I. Six species of benthic dinoflagellate are being cultured, with the emphasis on the culture of both batch and clonal cultures of Gambierdiscus toxicus and Ostreopsis lenticularis. Cultures of G. toxicus have been initiated from biodetrital samples collected from Hawaiian and Tahitian waters. A total of eight batch (non-clonal) and 34 clonal cultures of G. toxicus and one batch and 28 clonal cultures of O. lenticularis are currently being maintained in ES medium. Certain cultures have been found to acclimate better to laboratory conditions than others. Cultures of G. toxicus that are growing well under the conditions described in the Materials and Methods section include clonal cultures GT 41, 156, 174, 177, 181, 186 and 188 and TGT Stn4/2. Division rates of 0.42 divisions/day have been recorded for clone GT 188, while for the other cultures the division rates range from 0.23 divisions/day to 0.38 divisions/day. Frequent subculture of all cultures is undertaken so as to achieve stable growth under laboratory conditions. The other four species of benthic dinoflagellate being grown are Prorocentrum lima, P. concavum, Amphidinium sp. and Coolia monotis. Partially axenized cultures of all batch and clonal cultures have been obtained. Whilst there is no evidence of large-scale injurious bacterial contamination of the stock cultures, efforts will continue to obtain totally axenic cultures.

3.3 Toxicity of extracts of cultured dinoflagellates

Extracts obtained from both batch and clonal cultures of Gambierdiscus toxicus and cultures of Ostreopsis lenticularis and Prorocentrum lima, have

been evaluated for toxicity using the mouse bioassay. Data pertaining to the cell and crude extract yields for one liter cultures of these dinoflagellates are detailed in Table I. The weight of lyophilized cells obtained has been found to vary both for liter cultures derived from the same stock culture and for those grown from different stock cultures. These discrepancies may reflect the degree of bacterial contamination associated with individual stock cultures. For example, cultures that are only partially axenized tend to produce large amounts of mucous and the weight of cells obtained is considerably higher than that recorded for cultures such as GT 41 and G. tox., KT 18/1 that are testing bacteria-free. Both the methanol and acetone extracts of cultured G. toxicus have proved to be toxic when tested in the mouse bioassay. In the case of the acetone extracts, the only sign of toxicity evident in the mice have been the onset of severe diarrhea, with all of the mice surviving the 48 h test period. The methanol extracts have proved to be far more toxic, inducing a range of toxic signs in the test animals including ataxia, wobbling gait, reduced forelimb grip, loss of body tone with associated hypothermia, cyanosis and breathing difficulties. Death accompanied by terminal convulsions has been recorded at 4 h post-administration of the extracts, with the primary cause of death being respiratory paralysis. Methanol extracts prepared from cultures of Prorocentrum lima have also proved to be lethal to mice at a dose (i.p.) of 10 mg/kg. Extracts of one culture of Ostreopsis lenticularis were not toxic to mice.

Table I. Cell and Extract Yields, Toxicity to Mice of Crude Extracts from Cultural Dinoflagellates*

CULTURE	YIELD OF CELLS (mg)	YIELD OF EXTRACT (mg) A	YIELD OF EXTRACT (mg) M	TOXICITY TO MICE A	TOXICITY TO MICE M
BATCH CULTURE					
G. tox.:KB 1/18					
(A)	23.4	13.0	24.0	*	*(L)
(B)	35.6	1.6	5.0	*	*(L)
(C)	72.8				
G. tox.:KB 2/28	110.2				
G. tox.:KB 6/7 Acanthophora	75.5				
TG. tox.; Stn 4	22.2	13.0	13.4	*	*(L)
Q. lent.; KB 1/18	67.5	11.4	21.8	-	-
CLONAL CULTURES					
TGT Stn4/2	232.1	17.0	14.2		
GT 41	72.0				
GT 174	242.2	22.0	14.0		
GT 177	269.9	12.0	16.0	*	*(L)
PL 4 (A)	90.3	41.3	7.9	-	*(L)
PL 4 (B)	108.3	12.1	6.4	-	*(L)

+ The yield of cells denotes the weight (mg) of washed lyophilized cells obtained per liter of dinoflagellate culture. The symbols A, B and C refer to separate liter cultures of the same stock culture of a particular dinoflagellate species. The coding system used for the dinoflagellates is as listed in the inventory of dinoflagellate cultures (Appendix 1). The yield of both the acetone (A) and methanol (M) extracts is expressed in mg. The toxicity of each extract was evaluated using the mouse bioassay. The dose of the crude extract administered i.p. to the mice was 10 mg/kg. The symbol * indicates that the extract induced toxic signs in the test animal, while the symbol L indicates that the extract proved to be lethal.

3.4 Cardiotoxic properties of G. toxicus extracts

Both the acetone and methanol extracts of Hawaiian Gambierdiscus toxicus (Batch G. tox., KB 18/1) have been found to be cardiotoxic when tested upon the isolated guinea-pig atrium. Representative traces obtained in the course of these experiments are presented in Fig. 2. The acetone extract (A, 10 µg/mL) of G. toxicus exerted a positive inotropic effect upon both the electrically-stimulated (Fig. 2; panel 1) and spontaneously-beating atria (Fig. 2, panel 3). This extract also elicited a positive chronotropic effect upon the spontaneously beating atrium (Fig. 2; panel 3). The positive inotropic effects of the acetone extracts of G. toxicus were blocked in the presence of the adrenergic blockers propanalol and phentolamine and the sodium ion channel blocking agent tetrodotoxin. The effects of the G. toxicus acetone extract upon the isolated guinea-pig atria closely resemble those described for ciguatoxin and for extracts of certain species of toxic fish (Miyahara et al., 1989). The effects of the methanol extracts (M; 10 µg/mL) of G. toxicus upon the electrically-stimulated guinea-pig atrium are shown in Fig. 2, panel 2. The extract induces a positive inotropic response similar to that recorded previously for maitotoxin (Prof. Y. Hokama, pers. comm.).

4. CONCLUSIONS AND ONGOING STUDIES

In previous reports, it was noted that problems encountered in providing a suitable environment for the culture of benthic dinoflagellates, was hampering attempts to establish cultures of these microalgae in the laboratory. These problems have now been largely overcome and cultures of G. toxicus and associated dinoflagellates are being successfully maintained in

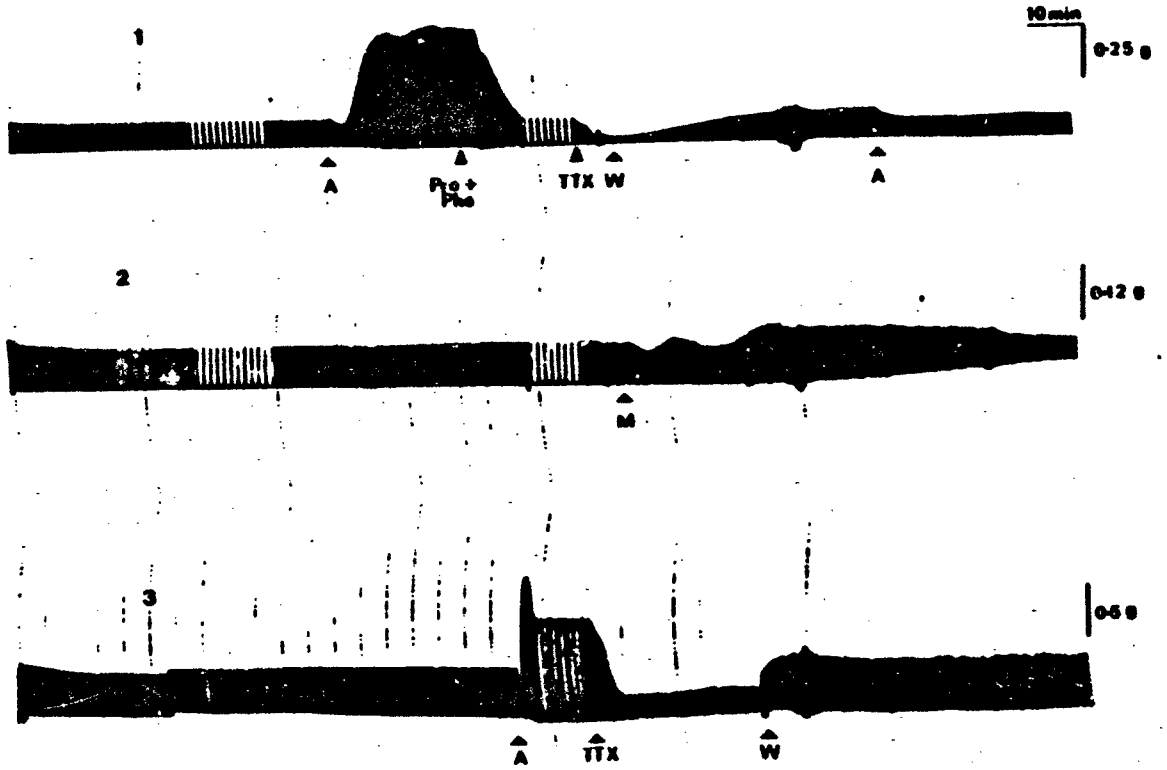


Fig. 2. The inotropic and chronotropic effects of the acetone and methanol extracts of Gambierdiscus toxicus. Panels 1 and 3 show the effects of the acetone (A, 10 μg/mL) extract on the electrically-stimulated atrium (panel 1) and spontaneously-beating atrium (panel 3) of the guinea-pig. The effects of the addition of tetrodotoxin (TTX, 5×10^{-7} M) and propranolol and phentolamine (Pro, 1×10^{-6} M; and Phe, 5×10^{-7} M) on the extract-induced responses of the atria are shown. Panel 2 shows the effects of the methanol extract of G. toxicus (M, 10 μg/mL) on the electrically-stimulated guinea-pig atrium.

the laboratory. The primary objective of the ongoing work is to optimize the growth of these cultures and to screen as many as possible for the presence of toxic substances. Those cultures that have already proved to be toxic in the mouse bioassay are being cultured in larger volumes so as to provide sufficient quantities of the crude extract for the purification of the toxic components to be attempted. Results obtained using the isolated guinea-pig atrium suggest that a ciguatoxin-like compound(s) may be present in extracts of Hawaiian G. toxicus. It is also likely that cultures of G. toxicus are producing maitotoxin as has been reported for cultures of G. toxicus grown elsewhere. Further experimentation is needed to ensure that this is indeed the case. It is also planned to continue to study the optimization of the growth of cultures that prove to be toxic in the bioassays. Experiments dealing with modifications to the culture media supporting the growth of the dinoflagellates have been initiated. Another series of experiments designed to evaluate the use of different substrates to facilitate the growth of G. toxicus are planned. Of particular interest here will be the use of microcarriers; these are small beads composed of a variety of materials including glass and plastic, that have been used in cell culture to facilitate the growth of anchorage-dependent cell lines. If these microcarriers can be used in a similar fashion for the growth of G. toxicus, this would greatly improve cell yields and thereby increase the supply of extract required for the purification of the toxic components.

5. LITERATURE CITED

BOMBER, J.W., RUBIO, M.G. and NORRIS, D.R. (1989) Epiphytism of dinoflagellates associated with the disease ciguatera: substrate specificity and nutrition. Phycologia, (in press).

GILLESPIE, N.C., HOLMES, M.J., BURKE, J.B. and DOLEY, J. (1985) Distribution and periodicity of Gambierdiscus toxicus in Queensland, Australia. In: Toxic Dinoflagellates (Ed by D.M. Anderson, A.W. White and D.G. Baden) pp 183 - 188. Elsevier Science Publishing Co., Amsterdam.

HOFFMAN, P.A., GRANADE, H.R., and McMILLAN, J.P. (1983) The mouse ciguatoxin bioassay: A dose - response curve and symptomatology analysis. Toxicon 21, 363 - 369.

MIYAHARA, J.T., KAMBAYASHI, C.K. and HOKAMA, Y. (1989) Pharmacological characterization of the toxins in ciguateric fishes. In: Mycotoxins and Phycotoxins 88 (Ed by S. Natori, K. Hashimoto and Y. Ueno) pp 399-406. Elsevier Science Publishing Co., Amsterdam.

PROVASOLI, L. (1968) Media and prospects for the cultivation of marine algae. In: Cultures and Collections of algae. Jpn. Soc. Pl. Physiol. pp. 63 - 75.

B. Ciguatoxin Isolation

Approximately 500 kg of Yellowtail Amberjack, Seriola dumerilii, was extracted whole to shorten the extraction process and maximize total ciguatoxin yield. A total of 70 μg of crude ciguatoxin was obtained as determined by toxic reactions in mice. However, further purification and testing indicated that substances in the crude isolate other than ciguatoxin had contributed to the toxicity. Thus whole fish extraction yielded roughly the same net amount of ciguatoxin as extraction of the fish viscera only. Since the purification process had to be lengthened, whole fish extraction became more time-consuming than visceral extraction. It was then decided that subsequent extractions would be performed on the viscera only.

Subsequent visceral extractions of S. dumerilii also yielded small amounts of toxin and required many purification steps. Thus in June, 1989, it was concluded that only small quantities of toxin could be isolated from S. dumerilii, and only at great effort.

In June, 1989, we resumed procurement of eel viscera from Tarawa despite continuing communication and transportation problems. A shipment arriving on July 5, 1989, contained 1.65 kg of moray eel viscera, from which 8.5 μg of crude ciguatoxin was isolated. The concentration of ciguatoxin isolated per kilogram is roughly equivalent to that derived several years ago. Thus ciguatoxin levels in eels from Tarawa has remained constant, and Tarawa remains an excellent source of ciguatoxin-containing eels.

Locally, recent ciguatera outbreaks had been reported, which prompted a collection of moray eels from the Hyatt Regency Hotel beach in Kona and Anae Hoomalu Bay, Hawaii. These eels were received via Dr. Hokama's laboratory and

were extracted for ciguatoxin. The mouse assay tests of the crude extracts resulted in ciguatera-like symptoms in the mice, indicating the presence of the ciguatoxin, but in small concentrations. These findings confirm a possible ciguatera outbreak on the Big Island of Hawaii and also indicate further exploration a source of ciguatoxin.

Recently the question as to the stability of the ciguatoxin over time was addressed. In September, 1989, extraction was made of eels from Tarawa that had remained unrefrigerated in transit for more than one week. The toxicity of this extract proved to be the same as the toxicity of extracts from freshly obtained eels. This demonstrated that ciguatoxin remains stable in unrefrigerated eels for at least one week.

Table II. Ciguatoxin Isolated from Seriola dumerilii and Moray eels from the Period October, 1988, to September, 1989.

Extract	Date	wt.(kg)	toxin(μg)
fish flesh and viscera	10/88 to 9/89	170	~12
eel viscera (Tarawa)	7/11/89	1.65	8.5
eel viscera (Big Island)	8/30/89	0.96	~1.5
eel viscera (Tarawa)	10/1/89	5.10	54

A total of approximately 160 μg of toxin previously and currently accumulated has been prepared for HPLC purification.

III. Conclusions

Gambierdiscus toxicus culture is now well established. Replication of recent toxicity assays and scaling up of toxin production are our immediate tasks.

Accumulation of ciguatoxin from mooray eels will continue to provide material for the determination of the stereochemistry of the molecule; for monoclonal antibody production; and for pharmacology, including drug design.

Palytoxin research will be initiated.

APPENDIX 1

DINOFLAGELLATE CULTURES, CHEMISTRY DEPARTMENT, UNIVERSITY OF HAWAII

SEPTEMBER, 1989

CULTURE	SOURCE	ALGAL SUBSTRATE	DATE	COLLECTOR
BATCH CULTURES				
HAWAII				
G. toxicus	Lahala B.	Spyridia filamentosa	1/18/89	E. McCaffrey
G. toxicus	Lahala B.	Spyridia filamentosa	3/31/89	E. McCaffrey
G. toxicus	Lahala B.	Spyridia filamentosa	4/27/89	E. McCaffrey
G. toxicus	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
G. toxicus	Lahala B.	Dictyota sp.	6/07/89	D. Corgiat
G. toxicus	Lahala B.	Acanthophora spicifera	6/07/89	D. Corgiat
TAHITI				
G. toxicus	Hitiaa, sta	Jania sp.	5/30/89	E. McCaffrey
G. toxicus	Hitiaa, sta.4	Jania sp.	5/30/89	E. McCaffrey
CLONAL CULTURES				
Gambierdiscus toxicus				
HAWAII				
GT 36	Lahala B.	Spyridia filamentosa	1/18/89	E. McCaffrey
GT 41	Lahala B.	Spyridia filamentosa	1/18/89	E. McCaffrey
GT 155	Lahala B.	Spyridia filamentosa	4/27/89	E. McCaffrey
GT 156	Lahala B.	Spyridia filamentosa	4/27/89	E. McCaffrey
GT 157	Lahala B.	Spyridia filamentosa	4/27/89	E. McCaffrey
GT 158	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 164	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 170	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 174	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 177	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 178	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 181	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 186	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 187	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 188	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 189	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 193	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 201	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 206	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 207	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 209	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 215	Lahala B.	Spyridia filamentosa	6/07/89	E. McCaffrey
GT 232	Lahala B.	Spyridia filamentosa	6/07/89	E. McCaffrey
GT 228	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 234	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 239	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
GT 244	Lahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
TAHITI				
TGT STN 4/2	Hitiaa, STN 4	Jania sp.	5/30/89	E. McCaffrey

TGT 11	Hitiaa, motu	Jania sp.	5/30/89	R. McCaffrey
TGT 15	Hitiaa, motu	Jania sp.	5/30/89	R. McCaffrey
TGT 70	Hitiaa, motu	Jania sp.	5/30/89	R. McCaffrey
TGT 79	Hitiaa, motu	Jania sp.	5/30/89	R. McCaffrey
TGT 80	Hitiaa, motu	Jania sp.	5/30/89	R. McCaffrey
TGT 86	Hitiaa, motu	Jania sp.	5/30/89	R. McCaffrey

Ostreopsis lenticularis

HAWAII

BATCH CULTURES

O. lenticularis	Kahala B.	Spyridia filamentosa	1/18/89	R. McCaffrey

CLONAL CULTURES

OL 51	Hawaii Kai	Spyridia filamentosa	12/07/89	R. McCaffrey
OL 120	Kahala B.	Acanthophora spicifera	6/07/89	D. Corgiat
OL 121	Kahala B.	Acanthophora spicifera	6/07/89	D. Corgiat
OL 123	Kahala B.	Acanthophora spicifera	6/07/89	D. Corgiat
OL 127	Kahala B.	Acanthophora spicifera	6/07/89	D. Corgiat
OL 130	Kahala B.	Dictyota sp.	6/07/89	D. Corgiat
OL 131	Kahala B.	Dictyota sp.	6/07/89	D. Corgiat
OL 132	Kahala B.	Dictyota sp.	6/07/89	D. Corgiat
OL 134	Kahala B.	Dictyota sp.	6/07/89	D. Corgiat
OL 135	Kahala B.	Dictyota sp.	6/07/89	D. Corgiat
OL 138	Kahala B.	Dictyota sp.	6/07/89	D. Corgiat
OL 140	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 142	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 144	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 146	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 147	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 149	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 151	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 152	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 153	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 155	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 156	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 158	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 159	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 160	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 162	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 165	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat
OL 178	Kahala B.	Spyridia filamentosa	6/07/89	D. Corgiat

Coolia monotis

CM 1	Kahala B.	Spyridia filamentosa	4/27/89	R. McCaffrey
CM 2	Kahala B.	Spyridia filamentosa	4/27/89	R. McCaffrey

Prorocentrum lima

PL 1	Hitiaa, motu	*Jania* sp.	5/30/89	E. McCaffrey
PL 2	Hitiaa, motu	*Jania* sp.	5/30/89	E. McCaffrey
PL 3	Hitiaa, motu	*Jania* sp.	5/30/89	E. McCaffrey
PL 4	Hitiaa, motu	*Jania* sp.	5/30/89	E. McCaffrey

Prorocentrum concavum

P. concavum (Batch)	Lahala B.	*Acanthophora spicifera*	3/31/89	E. McCaffrey

Amphidinium sp.

AMP. 1	Hitiaa, motu	*Jania* sp.	5/30/89	E. McCaffrey
AMP. 2	Hitiaa, motu	*Jania* sp.	5/30/89	E. McCaffrey

APPENDIX 2

S. Natori, K. Hashimoto and Y. Ueno (Eds), *Mycotoxins and Phycotoxins '88* 265
A Collection of Invited Papers Presented at the Seventh International IUPAC Symposium on Mycotoxins and Phycotoxins, Tokyo, Japan, 16-19 August 1988.

CIGUATERA - WHAT WE KNOW AND WHAT WE WOULD LIKE TO KNOW

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SUMMARY

The discovery of Gambierdiscus toxicus in 1977 was the turning point in ciguatera research. During the ensuing decade great strides have been made in our knowledge and understanding of ciguatera. Major advances and remaining unsolved problems are the highlights of the following discussion.

INTRODUCTION

A recent lead article in The Medical Journal of Australia (ref. 1) is entitled, "What on earth is ciguatera? " The author answers the question by stating, "Ciguatera is an impressive type of fish poisoning which, once experienced, is never forgotten..." In the same journal issue Gillespie and coworkers (ref. 2) render a concise account of our current knowledge and understanding of ciguatera with emphasis on the situation in Australia. In other parts of the world, recent symposia and conferences have generated an abundant ciguatera literature (refs. 3-7), which at times has seemed to outpace new findings and insights. This surge of interest may not necessarily mean that ciguatera has become more prevalent. It may merely be a reflection on a tendency of scientists to be attracted to puzzling problems. In this paper I will highlight in roughly chronological order the major advances and the principal unsolved riddles, rather than attempt to present yet another "comprehensive" review of ciguatera.

HISTORICAL NOTES

Modern scientific inquiry originated in Japan prior to World War II with Hiyama's (ref. 8) detailed report on poisonous fishes in the South Pacific. After the war, following a few early fragmentary studies (e.g. ref. 9) by the late Professor Hashimoto of Tokyo University ciguatera research in Japan vigorously resumed in the mid-1960's, an effort that Hashimoto's student, Professor Yasumoto of Tohoku University, has continued with great distinction.

World War II, specifically United States involvement in the Pacific, sparked Bruce Halstead's interest in ciguatera and led to his landmark three volume treatise published between 1965 and 1970 (ref. 10). It remains the definitive source of the history of ciguatera. The large list of potentially

ciguateric fishes (more than 400), frequently quoted, perhaps reflects the global distribution of ciguatera, but many of the cited reports have not been rigorously confirmed by modern techniques. Hiyama's estimates (ref. 8) of fewer than a hundred poisonous species is probably much closer to the mark. Similarly, the late Professor Banner's military service in the Pacific also roused his interest in ciguatera. He initiated a research program at the University of Hawaii in the mid-1960's. Although trained in descriptive marine biology Professor Banner recognized the multifaceted nature of ciguatera and recruited a staff that included ecologists, pharmacologists, and chemists. I became one of his early recruits.

DISCOVERY OF GAMBIERDISCUS TOXICUS

Intuitive guesses and empirically supported hypotheses of a diet-derived toxin were based on observations of the sporadic occurrence of intoxications and of the anatomy and feeding habits of reef fishes that had been proven toxic by incontrovertible evidence, viz. human consumption. Randall's hypothesis (Ref. 11) was the first comprehensive and well articulated theory of the origin of ciguatera. It was the result of extensive field studies, both ecological and ichthyological, in the Society Islands and in the Caribbean. From his observations, from interviews with local people, and from literature research, Randall concluded that three types of fish tend to become ciguateric - herbivorous reef fish, detritus-feeding reef fish, and large carnivores that feed on these two types of reef fish. Hence he postulated that the toxic organism would be benthic and "would most likely be an alga, a fungus, a protozoan, or a bacterium." Furthermore, an alga would have to be fine, perhaps a blue-green (cyanophyte). In order to account for the seemingly random outbreaks of ciguatera in previously nontoxic areas Randall also suggested that the appearance and growth of the toxic organism was the consequence of new or denuded surfaces produced by natural or man-made events. However, subsequent field studies designed to obtain experimental proof for the new surface theory during the 1960's at a ciguatoxic reef in Tahiti failed to provide evidence. No additional field or laboratory studies have been put forth to bolster or refute the hypothesis. Yet circumstantial evidence in support of such a new surface theory continues to come to light.

Discovery of the predicted toxic precursor proved to be elusive for nearly twenty years. Despite many diligent efforts, which included examination of stomach contents of numerous toxic fishes (ref. 12) and careful ecological, ichtyological, and biochemical scrutiny of herbivorous surgeon fishes (ref. 13) which are often the cause of human intoxication, no toxic algae, fungi, or bacteria were found. The decisive breakthrough by Yasumoto and coworkers (ref. 14) initiated a new phase in ciguatera research

Microscopic examination of a sample of coral detritus from a toxic area in the Gambier Islands, French Polynesia, revealed that toxicity of the sample was proportional to the number of cells of a new dinoflagellate, Diplopsalis sp., subsequently named Gambierdiscus toxicus (ref. 15). Fractionation of the detritus and purification of the dinoflagellate toxin to an LD of 56 μg/kg showed that two toxins could be separated, and that they exhibited chromatographic behavior that paralleled those of ciguatoxin and maitotoxin. In terms of mouse units, maitotoxin predominated by a ratio of 3.8:1. Since maitotoxin is approximately three times more toxic than ciguatoxin (ref. 16), the two toxins contributed to toxicity on a nearly equal weight basis. At the time, both toxins were but incompletely characterized. By far the major effort had been directed toward structural elucidation of ciguatoxin, the toxin that accumulates in large toxic carnivorous fish. Maitotoxin first described from the stomach contents of a Tahitian surgeonfish (maito) (ref. 17) was known and recognized by its water solubility, but because of the small size of the surgeonfish was even more difficult to procure than ciguatoxin.

Not all wild populations of G. toxicus elaborate ciguatoxin. In Australia, Gillespie et al. (ref. 18) found that G. toxicus from Flinders Reef (27° latitude) in Southern Queensland contained high concentrations of water-soluble but no lipid-soluble toxin. In a 19-month study they also demonstrated that G. toxicus populations peaked in September, coinciding with low water temperatures.

Results of a study in Hawaii (ref 19) paralleled those from French Polynesia (ref. 20). The water-soluble predominated over the ether-soluble toxic fraction in a ratio of 26:1, but the overall level of toxicity was distinctly lower. It is not known whether this is an environmental or genetic phenomenon. Another interesting difference came to light in the Hawaii investigation. In a survey in French Polynesia, Yasumoto and coworkers (ref. 21) observed preferential settling of G. toxicus on a red alga, Jania sp., while the Hawaii workers documented preferential settling on the red alga Spyridia filamentosa.

Wild populations of G. toxicus from the Caribbean (ref. 22) also furnished lipid-soluble and water-soluble toxic fractions, but the lipid-soluble material did not elicit symptoms in mice which are normally associated with those produced by fish-extracted ciguatoxin.

G. TOXICUS CULTURE

Throughout the twenty year span between the Randall hypothesis (ref 11) and the discovery of G. toxicus, the bane of ciguatera research was the lack of toxin for research in Chemistry and Pharmacology. It was well recognized that the liver and viscera of the large carnivores, as e.g. moray eels, proved

to be the richest raw material for the extraction of ciguatoxin (ref. 23), but even these sources contained toxin averaging no more than 1×10^{-6}%. When one adds to this the formidable factors of logistics and finances, spearing or trapping the eels and transporting them to a well-equipped laboratory, the obstacles are indeed daunting. Suddenly, these problems would be a thing of the past. The discovery of the toxic dinoflagellate G. toxicus, the demonstration that it elaborates the ether-soluble ciguatoxin and the water-soluble maitotoxin (ref. 24), heralded a new era in ciguatera research - ample toxin would be available from dinoflagellate culture. There was sound precedent for this optimistic prognosis: other toxic marine dinoflagellates, notably Gonyaulax spp. and Gymnodinium sp., had been successfully cultured for many years.

The state of euphoria was short-lived. First, the experience gained from the culture of free-swimming dinoflagellates did not readily extrapolate to the benthic epiphytic G. toxicus, which could not be grown in large tanks nor could its growth be enhanced by aeration or agitation. Secondly, and more seriously, early cultures produced maitotoxin but only minimal traces of ciguatoxin (ref. 20). Despite culturing efforts in different laboratories over the past ten years this disappointing situation has remained essentially unchanged. Among recent experiences are those of Durant-Clement (ref. 25), who after considerable experimentation established cultures of G. toxicus that were consistently toxic and produced water-soluble, maitotoxin-like, and lipid-soluble, ciguatoxin-like, fractions in ratios that varied from 9:1 to 7.5:2.5.

Miller and coworkers (ref. 26) succeeded in growing G. toxicus from a Caribbean collection on a moderately large scale (20 liter carboys). An assessment of the nature of the toxins derived from this culture is difficult since the LD_{50} of the lipid extract after four chromatographic purification steps was 4.95 mg/kg, or only 0.01% of the lethality of pure ciguatoxin. Their water-soluble toxin was purified to an LD_{50} of 1.1 mg/kg.

Ciguatoxin production from cultured G. toxicus remains to be achieved. There is sufficient circumstantial evidence that genetic factors - existence of nontoxic strains of G. toxicus (ref. 18) - and environmental parameters - preferential settling on macroalgae - play a role.

MOLECULAR STRUCTURE

Production of maitotoxin from G. toxicus cultures has been successful. Good progress has been made toward structural elucidation of this toxin, which is approximately three times as large as ciguatoxin. Most if not all of this has been achieved in Professor Yasumoto's laboratory. I will restrict this

account to the ciguatoxin structure, which has been our concern for some time.

While the total structure is still unknown, Tachibana (ref. 27) isolated chromatographically pure toxin with an LD_{50} of 0.45 μg/kg. This toxin subsequently and serendipitously crystallized in an NMR tube enroute from New Jersey to Hawaii. While attempts to determine a ^{13}C NMR spectrum had failed, the crystallinity of the toxin (Fig. 1) constituted additional and welcome proof of its homogeneity. Extensive ^{1}H NMR spectral analyses of ciguatoxin at 600 MHz (ref. 28) resulted in establishment of the first unambiguous standard of comparison for all workers in the field.

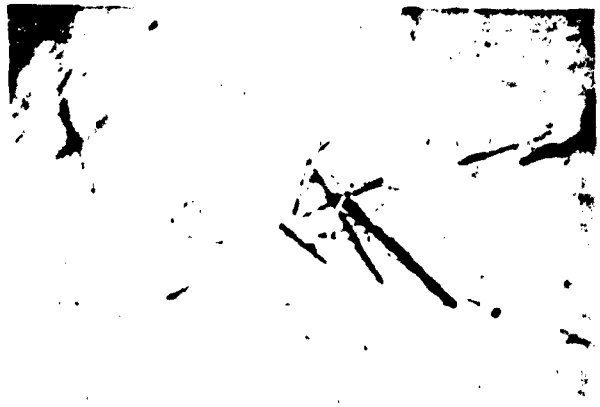
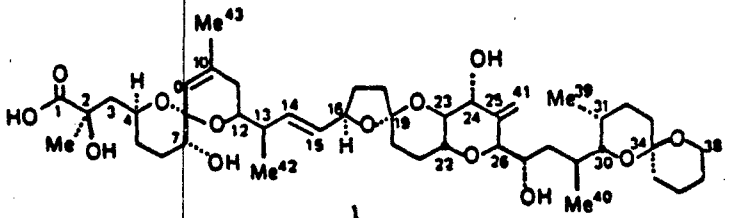


Figure 1: Crystals of ciguatoxin from methanol. Approximate length, 1 mm.

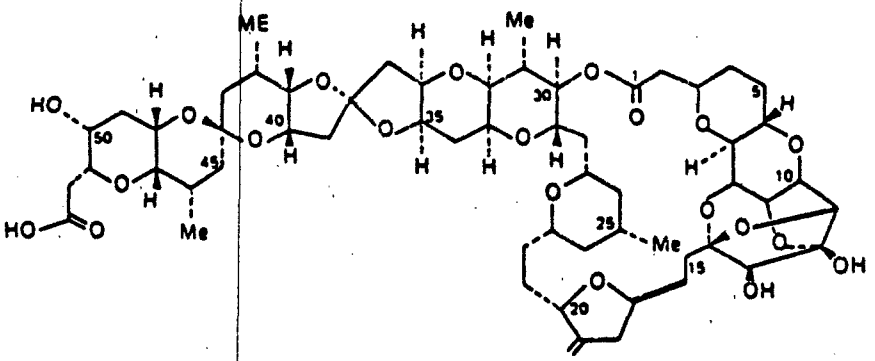
While the mass spectral technology of the time did not allow high resolution mass measurements, a reliable molecular ion at m/z 1,111.7 ± 0.3 dalton was compatible with ^{1}H NMR data. This number, combined with ^{1}H NMR spectral interpretation, suggests $C_{59}H_{85}NO_{19}$ as the most plausible molecular formula of ciguatoxin.

Yasumoto's discovery that okadaic acid, which he had isolated from the marine dinoflagellate, Prorocentrum lima (ref. 29), had the same chromatographic mobility as ciguatoxin, placed the compound in the class of polyethers. Since ciguatoxin possesses only five hydroxyl groups, the bulk of its oxygen atoms must be part of ether or ketal linkages. A comparison of the known structural parameters of ciguatoxin with those of okadaic acid (1), first isolated from the sponge Halichondria okadai (ref. 30),

norhalichondrin-A (2) from the same sponge (ref. 31), and prorocentrolide (3) from P. lima (ref. 32) is presented in Table 1. It is worth noting that ciguatoxin is by far the most potent toxin among these four compounds and the as yet unknown molecular architecture must account for this.



1



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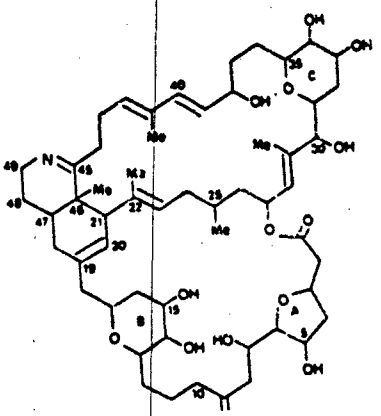

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TABLE 1

Comparison of structural features and lethality of several dinoflagellate toxins

	Okadaic Acid (1)	Norhalichiondrin-A (2)	Prorocentrolide (3)	Ciguatoxin
Molecular Formula	$C_{44}H_{68}O_{13}$	$C_{59}H_{82}C_{21}$	$C_{56}H_{85}NO_{13}$	$C_{59}H_{85}NO_{19}$
Molecular Wt.	804	1,126	981	1,111
Unsaturation	11	19	15	18
Chain Length	38	53	49	?
No. of Rings	7	15	7	?
Methyls	5	4	5	5
Exomethylenes	1	1	1	1
Hydroxyls	4	3	8	5
Ethers	1	7	3	?
Acetal/Ketal	3	4	-	?
Carboxyl, Ester	1	2	1	?
Other Functions	2 C = C	-	5 C = C, 1 C = N	3 C = C
LD_{50} (mice)	0.19 mg/kg	?	0.4 mg/kg	0.45 µg/kg

DETECTION

The topics in the foregoing discussion - origin of the toxin, dinoflagellate culture, molecular structure - are of fundamental scientific importance. In fact, ciguatera is an extraordinary ecological phenomenon that spans trophic levels from unicellular algae to man. But if one were to poll a cross section of people who are familiar with ciguatera - fish eaters, fishmongers, fishermen, scientists - in an attempt to pinpoint that aspect of ciguatera that is of greatest concern, no doubt the leading answer would be, detection of the toxin. A random event that occurs without a tell-tale red tide, that infects a broad spectrum of food fishes, that produces toxins which are tasteless, odorless, and survive cooking, is, as was quoted in the INTRODUCTION, an impressive type of fish poisoning. Hence development of a rapid, simple, and inexpensive test that sounds the alarm for the first person who handles the fish after it is caught and tells that person whether the fish is safe to eat, would help the consumer who needs uncontaminated fish as well as the scientist who needs the toxic fish for research. While the day of a

test that is comparable to the color change of litmus paper has not yet arrived, great strides have been made toward this long sought-after goal.

Hokama and coworkers (ref. 33) first described a radioimmunoassay (RIA) for the detection of ciguatoxin in 1977. In their assay ciguatoxin was conjugated to human serum albumin and the resulting conjugate was injected into sheep. Ciguatoxin antibody produced by the sheep was purified and coupled to radio-iodine (^{125}I). The resulting radio-labelled antibody was used successfully to screen more than 5,000 samples of ciguatera-prone amberjack (Seriola dumerilii, kahala) prior to commercial sales in Hawaii between 1979 and 1981 (ref. 34).

This was a major breakthrough since previous bioassays either depended on feeding a portion of a suspect fish to a susceptible animal (cat, mongoose) or on lengthy extraction and partial purification of fish tissue, followed by injection into a mouse. The RIA used small amounts of fish (10-20 µg) and no longer depended on assay animals, but it still required a sophisticated laboratory and a relatively long (4 hours) experimental protocol. It was clearly not an invention that was readily adaptable for use on a fishing boat or in a fish market.

Hokama and coworkers (ref. 35) next turned to the development of an enzyme immunoassay (EIA). They coupled their sheep anti-ciguatoxin to horseradish peroxidase. This conjugate plus a suitable marker could be evaluated by measuring absorbance at 405 nm in a spectrophotomer. The new test proved to be simpler and faster, but still depended on instrumentation. It also appeared that compounds that are structurally related to ciguatoxin, as e.g. okadaic acid, would also bind to the sheep anti-ciguatoxin. This is not necessarily a drawback, since okadaic acid, though less toxic, may well be present in fish and contribute to the toxic syndrome.

The third stage of the quest for a ciguatera detection test led Hokama (ref. 36) to his socalled stick test, in which a coated bamboo skewer is inserted into the fish. The stick is then rapidly prepared for insertion into the test (i.e. sheep anti-ciguatoxin horseradish peroxidase) solution and visually evaluated. While this test is still not wholly specific and the visual evaluation of a color involves subjective factors, it is rapid, inexpensive, and can be used in the field.

THERAPY

The success achieved in the long quest for a rapid, simple, inexpensive test for ciguatoxic fish, described in the previous section, was the result of a deliberate research program based on immunological techniques and spanning more than a decade. Another unsolved ciguatera problem, what to do once

intoxication has occurred, has apparently also found a solution. In contrast to the carefully charted and executed program toward development of the stick test, the newly suggested ciguatera treatment (ref. 37) was an empirical discovery. Its importance to the people who live in endemic ciguatera regions is underlined by the fact that the description of the therapy (ref. 37) was preceded by a notice in the lay press (ref. 38) and was followed by an editorial in a Honolulu newspaper (Fig. 2). The highly effective use of

Miracle drug

ADD mannitol to the list of miracle drugs along with aspirin and penicillin. Mannitol, commonly used to reduce brain swelling, is an artificially produced sugar compound that has been found to banish permanently all effects of the fish poisoning ciguatera.

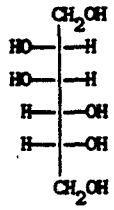
The antidote, discovered and tested by University of Hawaii physicians, has been used on 40 seafood eaters who were seriously ill from the poisoning. The affliction is common throughout the Pacific, in Florida and the Caribbean. Ciguatera poisoning is rarely fatal, but its symptoms — numbness, vomiting and diarrhea — make victims miserable and can cause unconsciousness.

Dr. Neal Palafox, a graduate of the UH John A. Burns School of Medicine practicing in the Marshall Islands, is credited with discovering mannitol's effect on ciguatera patients.

Although the drug hasn't been used in Hawaii, there is no reason to doubt it wouldn't perform just as well here. Seafood lovers can eat a little easier now

Fig. 2 Honolulu Star-Bulletin, June 6, 1988

intravenous infusion of D-mannitol (4) with dramatic results was foreshadowed by similar, though ineffective, calcium gluconate therapy in Fiji (ref. 39). The very much lower dose of calcium gluconate infusion may well account for its failure at the time. Both gluconic acid (5) and mannitol are simple hexose derivatives which differ in only two of the six carbons. This coincidence may prove to be of theoretical interest once the structures of ciguatoxin and maitotoxin are known. Computer modelling studies may then shed light on the mechanism by which intoxication takes place.



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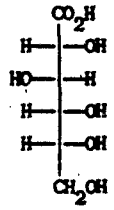

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CONCLUSION

The decade since the discovery of Gambierdiscus toxicus has seen impressive progress in ciguatera research: G. toxicus is being cultured and the cultures are producing maitotoxin; ciguatoxin has been spectrally characterized and has been obtained as a crystalline compound; a simple, rapid, inexpensive test is at hand; and an empirical therapy appears to be effective. Still poorly understood are the ecology of G. toxicus; the factors - genetic and/or environmental - that govern ciguatoxin biosynthesis in wild and in cultured populations; and the interrelationship of ciguatoxin and maitotoxin at all trophic levels.

REFERENCES

1 S.K. Sutherland, What on earth is ciguatera, Med. J. Australia, 145(11/12) (1986) 558-559.

2 N.C. Gillespie, R.J. Lewis, J.H. Pearn, A.T.C. Bourke, M.J. Holmes, J.B. Bourke and W.J. Shields, Ciguatera in Australia, Med. J. Australia 145(11/12)(1986) 584-590.

3 E.P. Ragelis (Ed.), Seafood toxins, ACS Symposium Series 262, American Chemical Society, Washington, DC, 1984, pp. 217-329.

4 T. Higerd, D. Ballantine, D. Jollow and P. Scheuer (Eds), The second international conference on ciguatera, Marine Fish. Rev. 48(4) (1986) 1-59.

5 R. Bagnis and P.J. Scheuer (Eds.) Ciguatera and other reef seafood poisoning, in: Proceedings of the Fifth International Coral Reef Congress, Tahiti, 1985, Vol. 4, Antenne Museum-Ephe, Moorea, French Polynesia, 1985, pp. 401-502.

6 First International Workshop in Ciguatera, Beverly, MA, 1985; Biol. Bull. 172(1) (1987) pp. 89-153.

7 M. Nukina, K. Tachibana and P.J. Scheuer, Recent developments in ciguatera research, in: J.B Harris (Ed.), Natural toxins, Clarendon Press, Oxford, UK, 1986, pp. 46-55.

8 Y. Hiyama, Report of an investigation on poisonous fishes in the south seas (in Japanese), Nissan Fisheries Institute, Odawara, 1943. [English translation: W.G. van Campen, Special Scientific Report - Fisheries, No. 25, U.S. Fish and Wildlife Service, 1950, pp. 1-221.]

9 Y. Hashimoto, A note on the poison of a barracuda, Sphyraena picuda Bloch & Schneider, Bull. Jap. Soc. Sci. Fish. 21(11) (1956) 1153-1157.

10 B.W. Halstead, Poisonous and venomous marine animals of the world, U.S. Government Printing Office, Washington, DC, 1965, 1967, 1970.

11 J.E. Randall, A review of ciguatera, tropical fish poisoning, with a tentative explanation of its cause, Bull. Marine Sci. Gulf Carib. 8(3) (1958), 236-267.

12 P. Helfrich, T. Piyakarnchana and P.S. Miles, Ciguatera fish poisoning I. The ecology of ciguateric reef fish in the Line Islands, Occasional Papers of Bernice P. Bishop Museum, Honolulu, HI, 23(14) (1968), pp. 305-382.

13 T. Yasumoto, Y. Hashimoto, R. Bagnis, J.E. Randall and A.H. Banner, Toxicity of surgeonfishes, Bull. Jap. Soc. Sci. Fish 37(8) (1971) 724-734.

14 T. Yasumoto, I. Nakajima, R. Bagnis and R. Adachi, Finding of a dinoflagellate as a likely culprit of ciguatera, Bull. Jap. Soc. Sci. Fish. 43(8) (1977), 1021-1026.

15 R. Adachi and Y. Fukuyo, The thecal structure of a marine toxic dinoflagellate Gambierdiscus toxicus gen. et sp. nov. collected in a ciguatera-endemic area, Bull. Jap. Soc. Sci. Fish. 45(1) (1979), 67-71.

16 T. Yasumoto, Recent progress in the chemistry of dinoflagellate toxins, in: D.M. Anderson, A.W. White and D.G. Baden (Eds.) Toxic dinoflagellates, Elsevier, New York, 1985, pp. 259-270.

17 T. Yasumoto, R. Bagnis and J.P. Vernoux, Toxicity of the surgeonfishes 2. Properties of the principal water-soluble toxin, Bull. Jap. Soc. Sci. Fish. 42(3) (1976), 359-365.

18 N. Gillespie, R. Lewis, J. Burke and M. Holmes, The significance of the absence of ciguatoxin in a wild population of G. toxicus, in: Proceedings of the Fifth International Coral Reef Congress, Tahiti, 1985, Vol. 4, Antenne Museum-Ephe, Moorea, French Polynesia, 1985, pp. 437-441.

19 Y. Shimizu, H. Shimizu, P.J. Scheuer, Y. Hokama, M. Oyama and J.T. Miyahara, Gambierdiscus toxicus, a ciguatera-causing dinoflagellate from Hawaii, Bull. Jap. Soc. Sci. Fish. 48(6) (1982), 811-813.

20 T. Yasumoto, K. Fujimoto, Y. Oshima, A. Inoue, T. Ochi, R. Adachi and Y. Fukuyo, Ecological and distributional studies on a toxic dinoflagellate responsible for ciguatera, Report to the Ministry of Education, Tokyo, Japan, 1984, 50 pp.

21 T. Yasumoto, A. Inoue, R. Bagnis and M. Garcon, Ecological survey on a dinoflagellate possibly responsible for the induction of ciguatera, Bull. Jap. Soc. Sci. Fish. 45 (3) (1979), 395-399.

22 J.P. McMillan, P.A. Hoffman and J.R. Granade, Gambierdiscus toxicus from the Caribbean: A source of toxins involved in ciguatera, Marine Fish. Rev. 48(4) (1986), 48-52

23 T. Yasumoto and P.J. Scheuer, Marine toxins of the Pacific - 7-Ciguatoxin from moray eel livers, Toxicon 7(4) (1969), 273-276.

24 R. Bagnis, S. Chanteau, E. Chungue, J.M. Hurtel, T. Yasumoto and A. Inoue, Origins of ciguatera fish poisoning: a new dinoflagellate, Gambierdiscus toxicus Adachi and Fukuyo, definitely involved as a causal agent, Toxicon 18(2) (1980), 199-208.

25 M. Durant-Clement, Study of production and toxicity of cultured Gambierdiscus toxicus, Bio.. Bull. 172(1), (1987), 108-121.

26 D.M. Miller, R.W. Dickey and D.R. Tindall, Lipid-extracted toxins from a dinoflagellate, Gambierdiscus toxicus, in: E.P. Ragelis (Ed.), Seafood Toxins, ACS Symposium Series No. 262, American Chemical Society, Washington, D.C. 1984, pp. 241-255.

27 K. Tachibana, Structural studies on marine toxins, Ph.D. Dissertation, University of Hawaii, Honolulu, HI, 1980.
28 M. Nukina, L.M. Koyanagi and P.J. Scheuer, Two interchangeable forms of ciguatoxin, Toxicon 22(2) (1984), 169-176.
29 Y. Murakami, Y. Oshima and T. Yasumoto, Identification of okadaic acid as a toxic component of a marine dinoflagellate, Prorocentrum lima, Bull. Jap. Soc. Sci. Fish. 48(1) (1982), 69-72.
30 K. Tachibana, P.J. Scheuer, Y. Tsukitani, H. Kikuchi, D. Van Engen, J. Clardy, Y. Gopichand and F.J. Schmitz, Okadaic acid, a cytotoxic polyether from two marine sponges of the genus Halichondria, J. Am. Chem. Soc. 103 (9) (1981), 2469-2471.
31 D. Uemura, T. Takahashi, T. Yamamoto, C. Katayama, J. Tanaka, Y. Okamura and Y. Hirata, Norhalichondrin A: an antitumor polyether macrolide from a marine sponge, J. Am. Chem. Soc. 107(16) (1985), 4796-4798.
32 K. Torigoe, M. Murata and T. Yasumoto, Prorocentrolide, a novel polyether amine from the marine dinoflagellate, Prorocentrum lima, Abstracts, 16th International symposium on the Chemistry of Natural Products (IUPAC), May 29 - June 3, 1988, Kyoto, Japan, Pa 20.
33 Y. Hokama, A.H. Banner and D.B. Boylan, A radioimmunoassay for the detection of ciguatoxin, Toxicon, 15(4) (1977), 317-325.
34 L.H. Kimura, M.A. Abad and Y. Hokama, Evaluation of the radioimmunoassay (RIA) for detection of ciguatoxin (CTX) in fish tissues, J. Fish Biol. 21(6) (1982), 671-680.
35 Y. Hokama, M.A. Abad and L.H. Kimura, A rapid enzyme immunoassay (EIA) for the detection of ciguatoxin in contaminated fish tissues, Toxicon 21(6) (1983), 817-824.
36 Y. Hokama, A rapid, simplified enzyme immunoassay stick test for the detection of ciguatoxin and related polyethers from fish tissues, Toxicon 23(6) (1985) 939-946.
37 M.A. Palafox, L.G. Jain, A.Z. Pinano, T.M. Gulick, R.K. Williams and I.J. Schatz, Successful treatment of ciguatera fish poisoning with intravenous mannitol, J. Am. Med. Assoc. 259(18) (1988), 2740-2742.
38 G. Johnson, Marshalls' M.D. curing fish poison, Pacific Magazine 13(2) (1988), 51.
39 M. Sorokin, Ciguatera poisoning in north-west Viti Levu, Fiji Islands, Haw. Med. J. 34(6) (1975), 207-210.