linked to bovine serum albumine via the mixed anhydride method.

Both antisera were used in a micro-enzyme immunoassay on Terasaki plates and were found to react strongly with monensin-protein conjugates in an indirect test and to a lesser extent with free monensin in a competitive test. No cross-reactivity was detected against CTX but the successful development of a miniaturised immunoassay for monensin based on a monoclonal reagent provided the basis for the development of similar assays for polyether haptens.

A procedure requiring only 100 μg of hapten is under current investigation with the brevetoxin PbTx-3 and with CTX. The results of recent experiments are very promising and immunisation products are being screened.

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Structures and the origin of toxins involved in ciguartera

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Structural determination of the toxins involved in ciguatera has been a difficult but excitingly challenging target for natural product chemists. Obtaining a large amount of toxic fish for extraction was difficult. The extremely low concentration of the toxins in fish made the purification procedure laborious and tedious. Tons of fish yielded only a tiny amount of pure toxins. The complexity of the toxin molecules added to the difficulty.

Nevertheless, assisted by the rapid technological advances in spectroscopy and collaboration from many friends, we were able to determine the structures of several important toxins. We are quite convinced that the structure of most ciguatera toxins will be elucidated very soon. Then what will come after structures? Many difficult tasks still await chemists: developing analytical methods, preparing antigens, and toxin synthesis. But there are several other aspects which will be interesting to biologists.

We now know the structure of ciguatoxin (CTX) isolated from moray eels and that of CTX-4B isolated from *Gambierdiscus toxicus* growing in the wild. The resemblance in the skeletal structure of the two toxins indicates that CTX-4B is the precursor of CTX and that a series of oxidative modifications to the CTX-4B molecule takes place in the fish liver. The oxidative process reminds us of the role played by hemoprotein P450 which oxidises lipophilic toxins (e.g. afla-

toxin) so that the resulting hydrophilic metabolites can be eliminated into urine. The oxidation of CTX-4B to CTX could thus be regarded as a kind of detoxification process.

What actually happens to CTX-4B is the opposite of detoxification; the toxicity of the oxidised product (CTX) is in fact enhanced ninefold.

Therefore, we can say that moray eels are more toxic than parrotfish, both because the former are at a higher trophic level and because they accumulate toxins in the most toxic forms. If someone finds the enzymes which catalyse the oxidation, such enzymes will not only help us understand the metabolic fate of toxins, but will also help chemists run reactions which they cannot run with reagents.

Recently we have determined the structure of maitotoxin (MTX), the biggest natural product ever to be elucidated. MTX is nearly three times bigger than CTX, having a molecular formula $C_{164}H_{256}O_{68}S_2Na_2$ and a molecular weight of 3422 Da.

It consists of a C142 carbon chain, 32 ether rings, 28 hydroxyls, and 21 methyls. Analogous with CTX, most of the ether rings in MTX are fused in a ladder shape. Nevertheless, the two toxins are entirely different molecular entities. MTX does not contain CTX as a part of its structure.

Therefore, it is quite clear that there will be no possibility of converting MTX to CTX by feeding MTX to fish or bacteria, as speculated earlier by some people.

Also in our recent work, one *G. toxicus* strain was confirmed as producing CTX analogues in cultures. Structures of CTX-3C and CTX-4A in the cultures were unambiguously confirmed by spectroscopic measurements. This result puts to an end the long-running argument about whether *G. toxicus* is the true source of ciguatera toxins or not. The strain (RAI1 strain) was one of six tested.

If renewed effort were made to collect and screen more strains, there would be chances of finding other strains producing CTX analogues, hopefully in even higher yields than our RAI1 strain. As future progress on ciguatera studies depends on an adequate supply of toxins, and as the current supply from fish is very limited, the prospect of obtaining toxins by algal cultures is very encouraging.

We can even dream that some day we will be getting CTX by oxidising CTX precursors produced by the alga with liver enzymes. We would like to urge biologists to collect and test as many *G. toxicus* strains as possible for production of valuable toxins.

Evaluation of Hawaiian reef fishes with the solid-phase immunobead assay (SPIA)

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This study was published in the *Journal of Clinical Laboratory Analysis* in 1993. It presents data on the evaluation of a laboratory-made ciguatera testing system based on the solid-phase immunobead assay (SPIA) for the detection of ciguatoxin and related polyethers in Hawaiian reef fishes. The SPIA was performed on fish caught by volunteer fishermen throughout the State of Hawaii.

A total of 1,067 fish representing 61 different species was tested by the SPIA system, as reported in the *Journal of Clinical Laboratory Analysis* in 1990. Of the 1,067 fishes tested, 510 were from the island of Oahu, 402 from Hawaii (Big Island), and 75 from Maui. Other fish included 23 from Molokai, 20 from Kauai and 7 from Lanai. Twenty per cent of the total fish tested were positives, 41 per cent borderlines and 39 per cent negatives in the SPIA assay. The highest percentages of SPIA-positive fish were from the island of Hawaii (27%), followed by Oahu (19%) and Kauai (15%).

These results correlate with the incidents reported from the State Department of Health of actual ciguatera fish poisoning in the State of Hawaii.

Unfortunately fish in all categories were eaten, though warnings strongly emphasised that all borderline and positive SPIA-tested fish were *not* to be eaten. All 332 negative fish eaten (80% of 416 fish) caused no poisoning, therefore *no false negatives.*

However, of the 201 borderline SPIA value fish eaten (46% of 433 fish), 4 caused ciguatera