The Chemical Nature of Scaritoxin

YONG-GOE JOH and PAUL J. SCHEUER

ABSTRACT—Two toxins were isolated from Scarus sordidus *(Family Scaridae) collected at Tarawa atoll, Republic of Kiribati. Both toxins were present in the flesh and viscera, but in different ratios. They correspond to the previously described scaritoxin and ciguatoxin. The toxins can be interconverted and evoke parallel symptoms in mice. Scaritoxin is probably identical with less polar ciguatoxin, but lack of material prevented unequivocal proof by spectral comparison.*

Introduction

One of the many questions associated with ciguatera research has been that of the multiple nature of the toxins that give rise to ciguatera symptoms in man. The lipophilic ciguatoxin (Scheuer et al., 1967) and the hydrophilic maitotoxin (Yasumoto et al., 1976) are distinct and reasonably well characterized molecules, though their specific contributions to the ciguatera syndrome have not been assessed.

Bagnis et al. (1974) conducted an epidemiological survey of ciguatera intoxication in the Gambier islands and observed that the most frequently implicated fish were parrotfish (Scaridae). Afflicted persons would, in addition to the conventional immediate ciguatera symptoms, suffer significantly from delayed (by 5-10 days) and prolonged (1 or more months) episodes of disturbed equilibrium, locomotor difficulties, and kinetic tremors.

An obvious explanation is the presence in parrotfish of a new toxin called scaritoxin, or of two distinct toxic entities (Chungue et al., 1977a, b). From the flesh of *Scarus gibbus*, Chungue et al. (1977a, b) successfully separated two toxins by chromatography on DEAE cellulose. A toxin eluted with chloroform and designated SG-1 evoked sluggishness and severe hind limb paralysis in mice, different from typical ciguatoxin symptoms. A second toxin eluted with chloroform-methanol (1:1) and designated SG-2 produced conventional ciguatoxin symptoms in mice, such as diarrhea, lachrymation, salivation, and respiratory difficulties.

Both toxin entities were further purified on Sephadex LH-20. SG-1 was a yellowish oil with an LD_{50} of 0.03 mg/kg. The polar toxin SG-2 was compared chromatographically with moray eel ciguatoxin and was found to be indistinguishable. Both toxins, SG-1 and SG-2, responded negatively to reaction with iodine and to Dragendorff and Jaffé reagents. A chromatographic estimate of the molecular weight of the new, less polar SG-1 toxin was approximately 800 daltons.

Chungue (1977) in a detailed investigation substantiated these results for six additional species of parrotfish. She concluded that scaritoxin is a ciguatera-causing toxin that is characteristic of the Scaridae.

In a more recent study, Nukina et al. (1984) showed that ciguatoxin isolated from moray eel, *Lycodontis javanicus* (≡ *Gymnothorax*), viscera can be separated on basic alumina into two entities, differing in polarity but indistinguishable in symptoms or in LD_{50} (i.p. mice). The two toxins show only minor differences in their high field ^1H NMF spectra. It was further shown that the two toxins are interconvertible. It occurred to us that these two chromatographic forms of ciguatoxin might be identical with the scaritoxin-ciguatoxin pair recognized in the flesh of parrotfish by Chungue (1977). We had an opportunity to collect parrotfish, *S. sordidus*, on Tarawa atoll, Republic of Kiribati, and thus subject this hypothesis to an experimental test.

The authors are with the Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, HI 96822. The permanent address of Yong-Goe Joh is: Department of Food Science and Nutrition, Dong-A University, Pusan, Korea.

Materials and Methods

Fish were collected 19-27 May 1983 at Taborio, Betio, Bikenibeu, and Teaoraereke (Fig. 1). Small-scale extraction and testing showed that only *S. sordidus* caught at Bikenibeu were toxic. Flesh (5.34 kg) and viscera (0.876 kg) were worked up separately.

Fish flesh was mixed with acetone and reduced to a brei in a 1-gallon Waring Blendor[1]. The mixture was transferred to a 4 liter Erlenmeyer flask and allowed to stand in excess acetone for 2 days, then with fresh acetone for another 4 days. The combined acetone extracts were concentrated to an aqueous suspension, which was treated twice with equal volumes of hexane. The hexane layer was back-washed with 3× 100 ml of methanol/water, 8:2. The washings were added to the aqueous suspension, and the combined aqueous material was extracted three times with equal volumes of ethyl acetate. Evaporation of ethyl acetate resulted in crude toxin.

Crude toxin was dissolved in a minimum amount of chloroform and applied to the top of a column packed with Silicar (200-425 mesh, Mallinckrodt, 28 g/g sample). Successive elution with chloroform (10 ml/g silica), chloroform/

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

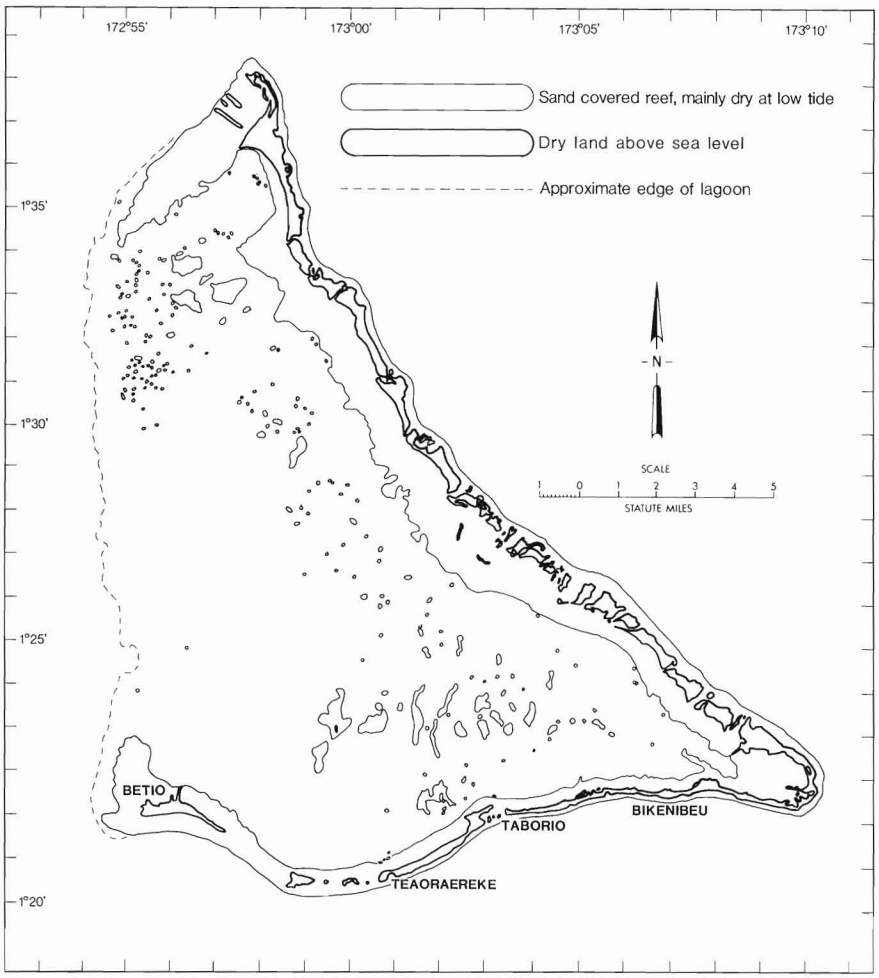

Figure 1.—Tarawa atoll, Republic of Kiribati.

methanol (9:1, 14 ml/g silica), chloroform/methanol (1:1, 10 ml/g silica), and methanol (10 ml/g silica) separated the toxin. All fractions were evaporated to dryness immediately after elution.

The chloroform/methanol fraction (9:1) was further purified on a DEAE celulose column (Cellex D, acetate form, BioRad Laboratories, 9.3 g/g sample), which was prepared as previously described (Rouser et al., 1963). Eluting solvents were chloroform (60 ml/g adsorbent), chloroform/methanol (1:1, 90 ml/g adsorbent), and methanol (60 ml/g adsorbent). Guaiazulene (a blue pigment, Aldrich Chemical Co.) was used to determine the bed volume and to detect imperfections in the packing.

An alumina (activity grade I, Woelm) column was prepared by suspending alumina (100 g/g sample) in chloroform. Toxic fractions were added as chloroform solutions and were eluted successively with chloroform/methanol (100:1, 9:1, 1:1), methanol, methanol/water (1:1), 375 ml/g alumina.

Alumina activity grade V was prepared according to the Brockmann scale. Grade I and V alumina columns (18.5 × 0.7 cm) were packed with 10 g alumina each and the toxins were eluted with chloroform (40 ml), chloroform/methanol (36 + 4 ml), chloroform/methanol (1:1), methanol (40 ml), and methanol/water (1:1). The eluates were concentrated and the residues were dissolved.

The toxic fractions (polar toxin, 450 ng; less polar toxin (810 ng) were further purified on a Sephadex LH 20 (Pharmacia) column (103 × 1.4 cm) by elution with chloroform/methanol (2:1). Fractions (4.9 ml) were collected every 10 minutes. Elution was monitored at 254 nm (ISCO Model UA-5).

Aluminum plates coated with silica gel 60F-254 (0.2 mm) and glass plates spread with silica gel H were used for thin-layer chromatography (TLC). Plates were developed with chloroform/methanol/water/acetic acid (90:9.5:0.3:0.2) and visualized with iodine vapor. Spots or bands were scraped off and extracted with chloroform/methanol, 4:1 for the less polar and 1:1 for the polar toxin. For bioassay the recovered TLC fractions were homogenized in 1 percent Tween 80.

For bioassay, each sample was dissolved in a known volume of methanol. Aliquots were removed from this stock solution and solvent was removed by a stream of nitrogen. Tween 80 (0.5 ml of 1 percent or 0.1 ml of 5 percent) was added and homogenized in a Vortex mixer. Swiss Webster mice (male or female, 16-22 g) were injected intraperitoneally. Death times of less than 3 hours were used to estimate toxicity as described by Tachibana (1980).

Results

Small-scale extractions and bioassay showed that *S. sordidus* was the only toxic species; *S. frenatus*, *S. scaber*, and *S. pectoralis* were nontoxic.

Yields and lethalities of extracts and chromatographic fractions are shown in Tables 1 (flesh) and 2 (viscera). The viscera proved to have a higher concentration of toxin, a phenomenon that had previously been observed in the moray eel (Yasumoto and Scheuer, 1969). Toxin could be eluted from silicic acid cleanly in chloroform/methanol (9:1). The toxin was separable into polar and less polar entities on DEAE cellulose. In *S. sordidus* flesh the polar toxin predominated, while in the viscera the reverse was so. When the chloroform/methanol (1:1) fraction from the DEAE cellulose column was rechromatographed on alumina activity grade I and

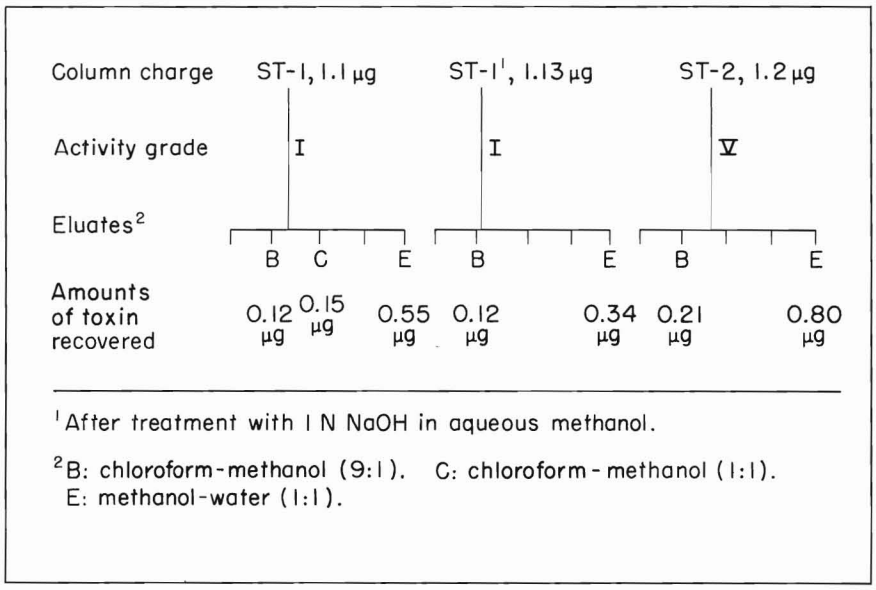

Figure 2.—Interconversion of *Scarus sordidus* flesh toxins on alumina.

Table 1.—Yields and toxicity of extracts and of chromatographic fractions of *S. sordidus* flesh (5.34 kg).

Purification state	Yield (g)	LD_{50} (mg/kg)	Total toxicity (M.U.)
Ethyl acetate	8.99		
Silicic acid			
Chloroform	3.52	Nontoxic	
Chloroform/methanol (9:1)	1.53	23.0	3,319
(1:1) (9:1)	0.63	12.5	2,520
(1:1)	1.13	Nontoxic	
Methanol	2.20	Nontoxic	
DEAE-cellulose			
Chloroform	2.74	29.0	556
Chloroform/methanol (1:1)	0.52	22.5	1,160
Methanol	0.01	Nontoxic	

Table 2.—Yields of extracts and of chromatographic fractions of *S. sordidus* viscera (0.876 kg).

Purification state	Yield (g)	LD_{50} (mg/kg)	Total toxicity (M.U.)
Ethyl acetate	13.81		
Silicic acid			
$CHCl_3$	4.46	Nontoxic	
$CHCl_3$-CH_3OH (9:1)	5.05	29	8,763
$CHCl_3$-CH_3OH (1:1)	1.48	Nontoxic	
CH_3OH	1.58	Nontoxic	
DEAE-cellulose			
$CHCl_3$	2.94	29.5	5,034
$CHCl_3$-CH_3OH	1.00	27.8	1,799
CH_3OH	0.08	Nontoxic	

Table 3.—Comparison of R_f-values of *S. sordidus* flesh toxins with PCTX (Tachibana, 1980) and scaritoxin (Chungue, 1977). Aluminum plates were coated with silica gel 60F-254 (0.2 mm); solvent system was chloroform/methanol/water/acetic acid (90:95:0.2:0.3).

ST-1	ST-2	PCTX	Scaritoxin
0.60 –	0.30 –	0.28 –	0.78 –
0.75	0.54	0.54	0.92

eluted with a gradient beginning with chloroform/MeOH (100:1) and ending with methanol/water (1:1), 95.5 percent toxicity was eluted with methanol/water (1:1). The two toxins gave rise to parallel symptoms in mice.

In analogy with the recent demonstration (Nukina et al., 1984) that ciguatoxin from moray eel viscera can be separated into two distict entities of different polarity by alimina chromatography and that the two toxins are interconvertible, we were able to show that the two *S. sordidus* toxins obtained by DEAE cellulose chromotography can also be partially interconverted. Figure 2 shows that ST-1 is partially converted to ST-2 when passed through a column of highly active (Grade I) alumina. ST-2, on the other hand, can be partially converted to ST-1 by chromatography over deactivated (Grade V, 15 percent water) alumina. When ST-1 is first treated with aqueous methanolic sodium hydroxide, then chromatographed on alumina of activity I, partial conversion to ST-2 also takes place, but the loss of material is necessarily severe. Paucity of available toxin prevented us from carrying out a full-scale experiment. Results are shown in Figure 2. The less polar toxin is designated ST-1, the polar toxin, ST-2.

On subsequent chromatography on Sephadex LH 20, ST-1 and ST-2 are eluted in parallel fractions (88-110 ml for ST-1 and 86-108 ml for ST-2), thus demonstrating the identical size of the two toxins.

To show the relationship of the two *S. sordidus* toxins to ciguatoxin (Nukina et al., 1984) and to scaritoxin (Chungue, 1977), we carried out thin layer chromatography under Chungue's conditions. Within the normal variability of TLC with time and place, and with a four-solvent developer, ST-1 and scaritoxin are very likely to be identical, as are ST-2 and ciguatoxin (Table 3). The TLC spots are egg-shaped rather than circular. Significantly, though, the R_f-values do not overlap. Amounts of toxin sufficiently large for ^1H NMF comparison are needed for unequivocal proof.

Discussion

The demonstration of two chromatographically distinct toxins in a ciguatoxic fish, while interesting by itself, raised another question concerning the precursor(s) of the toxins. Parrotfish, *S. gibbus*, feed primarily on coral. To shed light on the origin of the toxin, Yasumoto in collaboration with workers in Tahiti (Yasumoto et al., 1977) examined the gut contents and liver of *S. gibbus*. The gut, surprisingly, contained no scaritoxin, but contained ciguatoxin and maitotoxin in addition to a fast-acting acetone-soluble paralysis-causing toxin. Only ciguatoxin was isolated from the liver. Since both ciguatoxin and scaritoxin are routinely isolated from flesh, these results suggest that the parrotfish may have the ability to transform ciguatoxin into scaritoxin, yet the absence of scaritoxin in the liver was puzzling.

Our findings that toxic parrotfish from Tarawa atoll come from a narrow geographic area (Fig. 1) is consistent with the traditional ciguatera phenomenon (e.g., Withers, 1982). We were both surprised, though, that of four species examined, only *S. sordidus* proved toxic. We were able to isolate two toxins, parallel with Chungue's (1977) work on *S. gibbus*. In contrast to Yasumoto's (Yasumoto et al., 1977) results, we were able to show that both toxins are present in the flesh and in the viscera, albeit in different proportions. We observed no evidence of a third acetone-soluble and fact-acting toxin (Yasumoto et al., 1977). This is not a critical point, as coral-feeding parrotfish have many opportunities to ingest other toxins. Bagnis' (1974) original observation of the different symptomology in man following intoxication by parrotfish remains unexplained.

Acknowledgments

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