A BIOLOGICALLY ACTIVE ACID HYDROLYSIS PRODUCT OF SAXITOXIN

V.E. Ghazarossian, E.J. Schantz, H.K. Schnoes and F.M. Strong

Departments of Biochemistry and Food Microbiology and Toxicology
College of Agricultural and Life Sciences
University of Wisconsin-Madison
Madison, Wisconsin 53706

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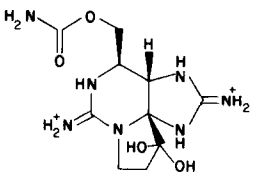
SUMMARY: The preparation, purification, and biological and chemical properties of a decarbamylated product of saxitoxin are described.

Saxitoxin (I), the structure of which was determined by x-ray crystallography in a cooperative study between our laboratory and laboratories at Iowa State University (1), originates in the dinoflagellate Gonyaulax catenella and is one of the most potent nonprotein neurotoxins known. Recently others have confirmed the structure I (2).

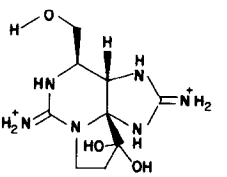
It has been established that the toxin inhibits the development of action potentials in the electrically excitable membranes of nerve and skeletal muscle by preventing inward passage of sodium ions through sodium channels (3,4). Because of this selective blocking property, I is being widely used as a tool in neurophysiological and pharmacological studies (5,6,7,8). These studies together with others on tetrodotoxin, a toxin with different structure but with similar biological properties, have resulted in an hypothesis concerning the structure and mechanism of action at the binding site (9).

Several derivatives of tetrodotoxin have been prepared and tested for biological activity (10). Apparently no systematic effort has been made to study the structure-activity relationships of I. We wish to report here the preparation, spectral properties, toxicity and probable structure of an acid hydrolysate of I. We propose II as the structure of this product, which we will call decarbamylsaxitoxin.

METHODS AND RESULTS: Decarbamylsaxitoxin was generated by heating purified I (11) in 7.5 N hydrochloric acid at 100° C. The toxicity of the reaction mixture was assayed with mice as previously described (12). After the third hour the



I



II

toxicity had dropped to about 60 ± 10% of that present originally and remained at that level up to seven and one-half hours of heating. The resulting brown solution was evaporated to dryness in a rotary evaporator, the dark brown to black residue taken up in 1-2 ml of absolute methanol, and chromatographed on a 2.3 x 130 cm Sephadex LH-20 (Pharmacia Fine Chemicals) column with absolute methanol as solvent. The toxic fractions were collected, concentrated, and rechromatographed several times in the same manner.

Typically 33 mg of purified II was obtained from 50 mg of I (75% yield). The product, a slightly yellow amorphous solid, showed $[\alpha]_D^{20}$ + 126.1° (methanol) and a specific toxicity of 3700 ± 400 MU/mg. The signs of death in mice were identical to those produced by I. I and II were chromatographically indistinguishable on silical gel G (EM Reagents) thin layer plates (0.25 mm) in six

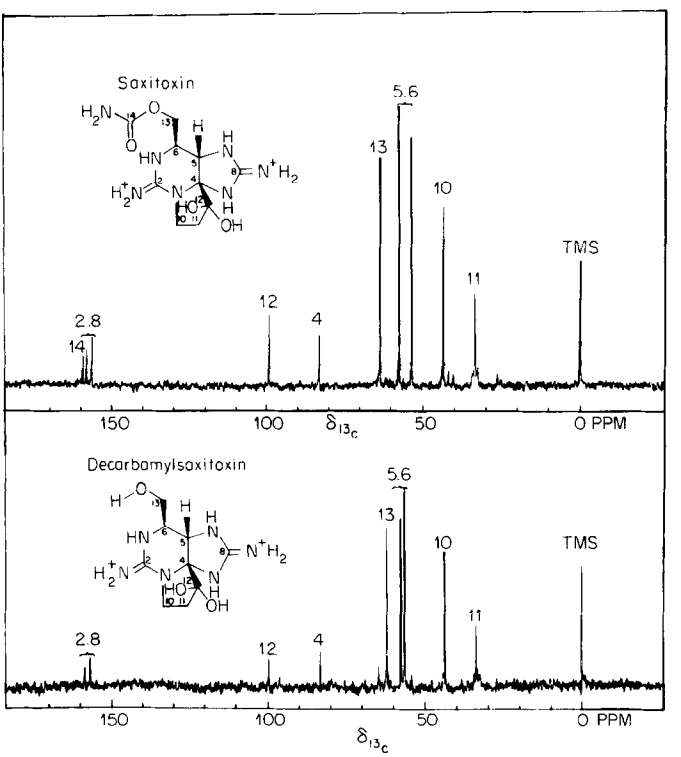


Fig. 1. ^{13}C NMR spectra of saxitoxin (55 mg) and decarbamylsaxitoxin (30 mg) at 90 MHz in 0.4 ± 0.1 ml 99.8% D_2O.

TABLE I

COMPARISON OF ^{13}C NMR DATA FOR SAXITOXIN (I) AND DECARBAMYLSAXITOXIN (II)[a]

C atom	I[b]	II
C-14	159.7	----
C-2[c]	158.5	158.5
C-8[c]	156.8	156.7
C-12	99.4	99.4
C-4	83.2	83.1
C-13	64.0	62.0
C-5[d]	57.8	57.3
C-6[d]	53.8	56.3
C-10	43.8	43.6
C-11	33.8	33.6

[a]Ppm relative to Me_4Si (TMS) as standard contained in a 1 mm i.d. melting point capillary centered in the NMR tube.
[b]Assignments based on predicted chemical shifts and comparison of derivatives (14).
[c]These two assignments may be interchanged.
[d]These two assignments may be interchanged.

different solvent systems (proportions, v/v): 1. n-butanol:water:formic acid (60:35:15); 2. ethanol:pyridine:water:acetic acid (60:40:30:10); 3. ethanol: formic acid (60:50); 4. ethanol:water:acetic acid (100:40:25); 5. ethanol: isopropyl alcohol:formic acid:water (50:40:30:40); 6. methanol:12.4 N HCl (60:40). In these systems, I and II had identical Rf values of 0.23, 0.68, 0.61, 0.56, 0.66, and 0.75, respectively, and a mixture of the two appeared as a single spot. Chromatography on precoated carboxymethyl cellulose plates (Brinkmann Instruments, Inc., type MN 300 CM, 0.1 mm) using pH 4 sodium acetate buffer as solvent also did not resolve I from II. Both compounds gave orange spots of Rf 0.58 with the Jaffe reagent (13).

In Fig. 1 and Table I is presented the proton noise decoupled natural abundance ^{13}C NMR spectrum (Bruker Spectrospin, Model HX90E) of II as compared to that of I. It shows that C-14, which has been assigned to the carbamyl carbon of I at 159.7 ppm (Table I), is absent in II. The peaks assigned to C-13, C-5 and C-6 have shifted in the spectrum of II (see Table I for comparison). A comparison of the IR spectra of I and II obtained in 99.8% D_2O in CaF_2 cells is shown in Fig. 2 and clearly shows that the carbonyl absorption at 1705 cm^{-1} of the carbamate moiety in I is absent in II. On the basis of these data we propose II as the structure for decarbamylsaxitoxin.

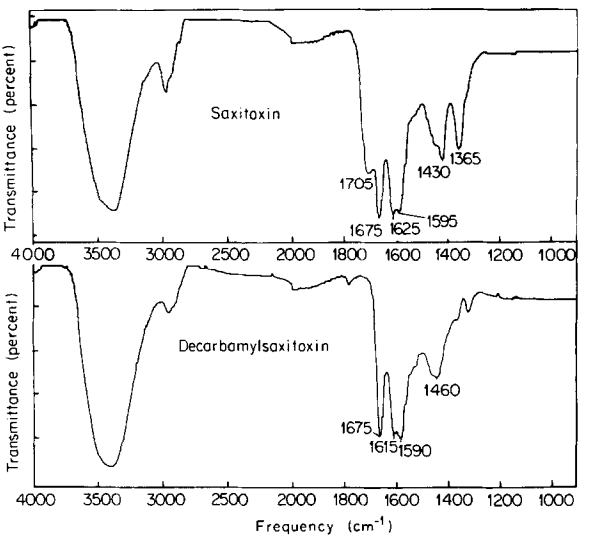


Fig. 2. IR spectra of saxitoxin and decarbamylsaxitoxin in 99.8% D_2O in 0.1 mm CaF_2 cells.

Also, II reacts with alkaline picrate (Jaffe reagent) under the conditions established for I (15) to form a product with the same orange-red color, maximum absorption at 480 nm, and a similar extinction coefficient. The colored products obtained from these reactions are not toxic to mice. Treatment of II with sodium borohydride in absolute ethanol reduced its toxicity to less than 1% of the original level. This derivative presumably is reduced II. Sodium borohydride treatment of I in ethanol (14) as well as catalytic reduction (16) yields nontoxic products.

DISCUSSION: It is significant that II still possesses biological activity in mice similar to that of I. This is in accord with Hille's hypothesis for the mode of binding of I to the sodium channel (9). The guanidinium moiety required for insertion into the postulated selectivity filter of the sodium channel is still intact in II. The C-12 carbon and its surrounding environment are also not altered, and are available for the proposed nucleophilic attack by the receptor (9). The loss of the carbamyl group of I may be offset by formation of a strong hydrogen bonding hydroxyl function in II.

The biological activity of II suggests that this product may be a suitable derivative of saxitoxin for the development of a radioimmunoassay for saxitoxin and affinity chromatography and affinity labeling of the toxin receptor.

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