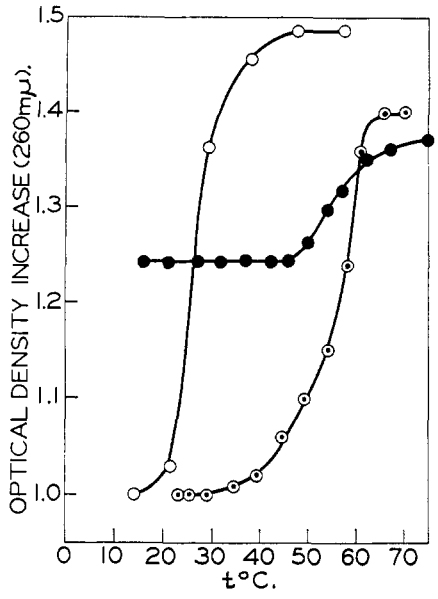


Fig. 1.—Optical density (260 mμ) increases as a function of temperature with *d*-pGpGpG: Curve 1 (-⊙-⊙-), 0.25 *M* phosphate buffer, pH 6.8; Curve 2 (-●-●-) recycling of the solution from Curve 1 after two weeks at room temperature; Curve 3 (-O-O-) melting of a sample (0.01 ml.) of the stock solution of the trinucleotide in 0.25 *M* phosphate buffer (100 optical density units/ml. at 260 mμ) after dilution to 2 ml. in 0.25 *M* succinate–hydrochloride buffer (*p*H 6.55). All readings were taken in cells with 1-cm. light path.

58°. The melting apparently corresponds to reversion to the "monomeric" trinucleotide form. Behavior in the ultracentrifuge (Spinco Model E, ultraviolet optics) further supports the above conclusions. The sample of trinucleotide with T_m of 58° has in the same buffer S_{20} 10–12 whereas after denaturation with alkali and dissolution in the same buffer, its behavior is as expected for the "monomeric" trinucleotide (S_{20} <0.5). The denatured sample on storage at room temperature yields again a high molecular weight aggregate (S_{20} 12–16).

The optical density changes occurring on heating the tetranucleotide (*d*-pGpGpGpG) aggregate are shown in Fig. 2. In 0.25 *M* phosphate buffer complete denaturation was not realized up to 95° (Curve 1). In the 0.25 *M* succinate buffer (Curve 2) and in sodium chloride (0.2 *M*)–phosphate buffer (0.0013 *M*) (Curves 3 and 4) the T_m were much lower. Renaturation to an ordered structure was much faster in the case of the tetranucleotide than with the trinucleotide. Curve 4 shows the recycling of the solution of Curve 3 after keeping at 4° for about two days.

Although detailed studies of other homologs of the above compounds remain to be done, evidence for aggregate formation in the pentanucleotide (*d*-pGpGpGpGpG)[3] was obtained by sedimentation studies (S_{20} 8–10).

(3) The same sample when centrifuged in cesium sulfate equilibrium gradient bands at the buoyant density similar to that observed for ribosomal RNA under the same conditions. We are grateful to Dr. W. Szybalsky for this experiment.

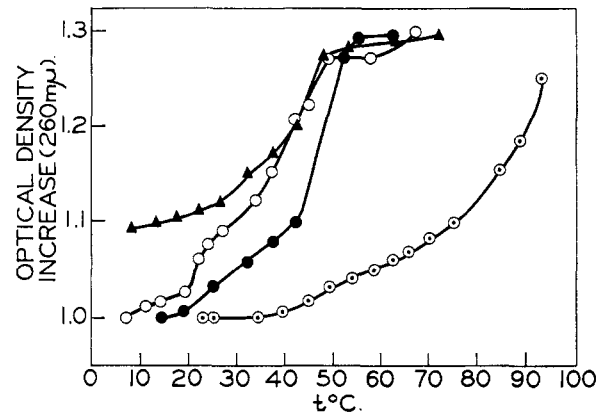


Fig. 2.—Optical density (260 mμ) increases as a function of temperature with the tetranucleotide, *d*-pGpGpGpG: Curve 1 (-⊙-⊙-) in 0.25 *M* phosphate buffer, pH 6.8; Curve 2 (-●-●-) in 0.25 *M* succinate buffer, pH 6.55; Curve 3 (-O-O-) in 0.0013 *M* phosphate + 0.2 *M* sodium chloride; Curve 4 (-▲-▲-) recycling of the solution of Curve 3 after two days at 4°. All readings were taken in cells with 1-cm. light path.

We are grateful to Dr. R. M. Bock and Mr. Wendell Stanley, Jr., for the sedimentation data. This work has been supported by grants from the National Cancer Institute of the National Institutes of Health and the National Science Foundation.

INSTITUTE FOR ENZYME RESEARCH
THE UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN
R. K. RALPH
W. J. CONNORS
H. G. KHORANA

RECEIVED APRIL 23, 1962

SAXITOXIN, THE PARALYTIC SHELLFISH POISON. DEGRADATION TO A PYRROLOPYRIMIDINE

Sir:

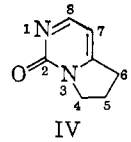
Saxitoxin, the paralytic poison isolated from toxic Alaska butter clams (*Saxidomus giganteus*), toxic mussels (*Mytilus californianus*), and the plankton *Gonyaulaux catenella*, is among the most toxic known substances. Its pharmacology[1] and biochemistry[2] have been summarized recently. Also, its isolation and purification[3,4] and some of its chemical and physical properties[5] have been described. The molecular formula $C_{10}H_{17}N_7O_4 \cdot 2HCl$ was clearly established[3,4] for saxitoxin, and its most significant degradation product was guanidinopropionic acid, obtained[5] by drastic oxidation with periodic acid or potassium permanganate.

We now wish to report[6] the degradation of saxitoxin to a pyrrolopyrimidine containing eight of the

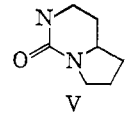
(1) E. F. Murtha, *Ann. N. Y. Acad. Sci.*, **90**, 820 (1960).
(2) E. J. Schantz, *ibid.*, **90**, 843 (1960).
(3) E. J. Schantz, J. D. Mold, D. W. Stanger, J. Shavel, F. J. Riel, J. P. Bowden, J. M. Lynch, R. S. Wyler, B. Riegel and H. Somner, *J. Am. Chem. Soc.*, **79**, 5230 (1957).
(4) J. D. Mold, J. P. Bowden, D. W. Stanger, J. E. Maurer, J. M. Lynch, R. S. Wyler, E. J. Schantz, and B. Riegel, *ibid.*, **79**, 5235 (1957).
(5) E. J. Schantz, J. D. Mold, W. L. Howard, J. P. Bowden, D. W. Stanger, J. M. Lynch, O. P. Wintersteiner, J. D. Dutcher, D. R. Walters, and B. Riegel, *Can. J. Chem.*, **39**, 2117 (1961).
(6) Our efforts were generously supported by the U. S. Army Chemical Corps.

ten carbon atoms initially present in the molecule. Saxitoxin,[7] when heated with phosphorus and hydriodic acid in acetic acid, gave a 57% yield of the weakly basic compound I, $C_8H_{10}N_2O$ (m.p. 100–102°. *Anal.* Found: C, 63.6; H, 6.8; N, 18.7) which contained one C–CH_3 group but no O–CH_3 or N–CH_3 groups. On oxidation with potassium permanganate, urea and guanidinoacetic acid[8] were obtained. Hydrogenation of I in the presence of platinum oxide (200 mole % hydrogen absorption) gave II, $C_8H_{14}N_2O$ (m.p. 129–131°. *Anal.* Found: C, 62.8; H, 9.4; N, 17.9) which also contained one C–CH_3 group. Strong acid hydrolysis of II led to the strongly basic, extremely hygroscopic oily diamine III, $C_7H_{16}N_2$, and III on heating with palladium-on-carbon formed a substance which readily gave a positive Ehrlich test for pyrroles.

On the basis of these data, we assumed that III was a pyrrolidine and that II was a saturated cyclic urea, consistent also with its lack of ultraviolet absorption and its strong infrared absorption at 3410 and 1635 cm.$^{-1}$ in chloroform. That the cyclic urea was part of a six-membered ring was clear from the ultraviolet absorption of I with $\lambda_{max}^{CH_3OH}$ 298 mμ (ϵ 6200), shifted to 305 mμ (ϵ 8900) on addition of acid. This is the same behavior observed for 1-methyl-2-pyrimidone [$\lambda_{max}^{C_2H_5OH}$ 302 mμ (ϵ 5400), shifted in acid to 313 mμ (ϵ 7100)].[9] Therefore, I was considered to be a fused pyrrolidine-pyrimidone with one nitrogen common to both rings, and IV and V, containing the new pyrrolo-[1,2-c]pyrimidine ring system, were adopted as working expressions for des-methyl I and des-methyl II, respectively.



IV



V

2 - Oxo - 2,4,5,6 - tetrahydropyrrolo[1,2-c]pyrimidine (IV) was synthesized from 2-(2-aminoethyl)-pyrrole[10] by reduction to 2-(2-aminoethyl)-pyrrolidine (b.p. 39–41° (0.7 mm.)) *Anal.* Found: C, 63.2; H, 12.3; N, 24.7), and this was cyclized by heating with diethyl carbonate to V (m.p. 127–128°. *Anal.* Found: C, 60.0; H, 8.5; N, 19.8). Oxidation with potassium permanganate at room temperature gave IV (m.p. 126–127°. *Anal.* Found: C, 61.2; H, 5.9; N, 21.4). The ultraviolet absorption of IV [$\lambda_{max}^{CH_3OH}$ 302 mμ (ϵ 6100), shifted in acid to 307 mμ (ϵ 7200)], was very similar to that of I, and in the infrared each compound had a broad band at 1660–1610 cm.$^{-1}$ which was split into two sharp peaks (1700, 1610 cm.$^{-1}$) in the hydrochloride. The nuclear magnetic resonance spectra[11] were practically identical except that IV had two aromatic proton peaks at τ 1.57 and 3.60 while I had only one at τ 3.73, indicating that position 8 was substituted by methyl which appeared as a singlet at τ 7.64.

To synthesize I, 2-aldehydopyrrole was condensed with nitroethane and the resulting 2-(2-methyl-2-nitrovinyl)-pyrrole (m.p. 82–83°. *Anal.* Found: C, 55.5; H, 5.4; N, 18.1) was reduced with lithium aluminum hydride to 2-(2-amino-1-propyl)-pyrrole (b.p. 68° (0.5 mm.), m.p. 30°. *Anal.* Found: C, 67.5; H, 10.0; N, 22.1). Catalytic hydrogenation gave 2-(2-amino-1-propyl)-pyrrolidine which, by heating with diethyl carbonate, was cyclized to 8-methyl-2-oxo-1,2,4,5,6,6a,7,8-octahydropyrrolo[1,2-c]pyrimidine (II), identical in infrared absorption with II derived from saxitoxin. Oxidation of synthetic II with permanganate at room temperature gave 8-methyl-2-oxo-2,4,5,6-tetrahydropyrrolo[1,2-c]pyrimidine (I), identical with I derived from saxitoxin in all respects (m.p., mixed m.p., ultraviolet, infrared, and nuclear magnetic resonance spectra; and R_f on paper).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA
WOLFGANG SCHUETT
HENRY RAPOPORT

RECEIVED APRIL 2, 1962

(7) Isolated from the siphons of toxic Alaska butter clams and supplied by Dr. T. C. Simmons of the Army Chemical Corps.

(8) C. J. West, *J. Biol. Chem.*, **34**, 187 (1918).

(9) D. J. Brown, E. Hoerger and S. F. Mason, *J. Chem. Soc.*, 211 (1955).

(10) W. Herz, *J. Am. Chem. Soc.*, **75**, 483 (1953).

(11) Taken in deuteriochloroform with tetramethylsilane as internal standard. We are grateful to Richard Moore for his assistance.

ADSORPTION ON INORGANIC MATERIALS. IV. CATION EXCHANGE PROPERTIES OF ZIRCONIUM ANTIMONATE[1,2]

Sir:

As first shown in previous papers[2,3] and since confirmed at a number of laboratories, dried (but hydrous) precipitates of Zr(IV) with polyvalent inorganic anions, such as phosphates, tungstates, molybdates, and arsenates have useful cation exchange properties including high uptakes of certain ions, reasonable exchange rates, and interesting selectivities. In a search for other inorganic materials which might exhibit unique cation exchange properties, the solid resulting from co-precipitation of Zr(IV) and Sb(V) was found particularly attractive. The material was obtained by mixing zirconium oxychloride solutions with excess of HCl solutions of antimony pentachloride, adding ammonia to decrease acidity and cause precipitation, filtering, washing, and drying at 25°. The material then was ground and screened for column use. Distribution coefficients, D (amount per kg. solid/amount per l. solution), were determined for a number of ions by batch equilibration experiments.

The material used in the experiments to be described had an Sb(V)/Zr(IV) ratio somewhat larger than 2 to 1, as established by radiometric analysis, and a water content of *ca.* 27%, as established by determination of weight loss on firing to 900°.

(1) This document is based on work performed for the U. S. Atomic Energy Commission at the Oak Ridge National Laboratory, operated by the Union Carbide Corporation, Oak Ridge, Tennessee.

(2) Previous paper: K. A. Kraus, H. O. Phillips, T. A. Carlson and J. S. Johnson, "Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy," United Nations, Geneva, 1958, Volume 28, p. 3.

(3) K. A. Kraus and H. O. Phillips, *J. Am. Chem. Soc.*, **78**, 694 (1956).