NOTES

Tetrodotoxin. III.[1] *A Revised Molecular Formula for Tetrodotoxin*

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The molecular formula of tetrodotoxin, a strongly toxic compound obtained from ovaries of swellfish (*Spheroides rubripes*), was said by Yokoo[2] in 1952 to be $C_{12}H_{17}O_{10}N_3$ on the basis of elementary analyses and the molecular weight determination by means of freezing point depression and the titration of its basic group. In 1953, Tsuda and Kawamura[3] reported on the analytical values of the toxin, but they did not give any definite molecular formula, since the values fluctuated considerably. Several years later, both Kakisawa et al.[4] and Tsuda et al.[5] gave a molecular formula, $C_{12}H_{19}O_9N_3$, based on elementary analyses and a titration equivalents.

Though it has previously been considered very difficult to purify tetrodotoxin (I), we have found that it can be purified through its nicely crystalline picrate II, which can be recrystallized from hot water. Acetylation of the toxin I with acetic anhydride and *p*-toluenesulfonic acid afforded, in a good yield, amorphous tetraacetyltetrodotoxin *p*-toluenesulfonate (III), which was further acetylated with acetic anhydride in the presence of pyridine to pentaacetylanhydrotetrodotoxin *p*-toluenesulfonate (IV).

The equivalent values of the purified toxin I determined by several runs of titration were between 311 and 324. The molecular weight of the crystalline pentaacetate IV was found to be 673±10 by means of the osmometer method[6] in a chloroform solution. These results, coupled with the analytical values of tetrodotoxin (I), the picrate II, and the acetates, III and IV, led to the molecular formula $C_{11}H_{17}O_8N_3$ for tetrodotoxin (I).

Experimental

Tetrodotoxin (I).—This toxin was purified by precipitating it with alcohol from its aqueous acetic acid solution according to the method of Tsuda and Kawamura[3] (samples 1 and 2), or through its crystalline picrate II, which had been treated with aqueous ammonia to precipitate the toxin (sample 3). The toxin I was obtained as microscopic crystals and darkened over 200°C without melting. Analytical samples were dried at 80~100°C for ca. 20 hr. in vacuo.

Found: (Sample 1) C, 40.21, 40.40; H, 5.62, 5.72; N, 12.52, 12.70;[7a] O, 41.43, 41.11, 41.00.[7b] (Sample 2) C, 40.62, 40.61; H, 5.75, 5.66; O, 41.73;[7c] titration equivalent (5 runs) 311 to 324. (Sample 3) C, 40.58, 40.87; H, 5.63, 5.94; N, 12.61, 12.60.[7d] Calcd. for $C_{11}H_{17}O_8N_3 \cdot \frac{1}{2}H_2O$: C, 40.24; H, 5.53; N, 12.80; O, 41.43%; mol. wt., 328.28.

Tetrodotoxin Picrate (II).—After this had been prepared from the toxin I and picric acid in water, it was recrystallized from water to give yellow needles which darkened over 200°C. An analytical sample was dried at 80~100°C for 20 hr. in vacuo.

Found: C, 36.25, 36.52; H, 3.95, 4.03; N, 14.24, 14.40.[7d] Calcd. for $C_{11}H_{17}O_8N_3 \cdot C_6H_3O_7N_3 \cdot H_2O$: C, 36.05; H, 3.92; N, 14.84%.

Tetraacetyltetrodotoxin *p*-Toluenesulfonate (III).—Tetrodotoxin (100 mg.) was suspended on a solution of *p*-toluenesulfonic acid hydrate (100 mg.) in acetic acid (2 ml.); the mixture was then allowed to stand overnight at room temperature. The resultant clear solution was evaporated to dryness under reduced pressure at 55°C (water-bath temp.), and the residue was treated with ice water containing sodium acetate (200 mg.) and then extracted

1) Part II: T. Goto, K. Kishi and Y. Hirata, This Bulletin, 35, 1244 (1962).
2) A. Yokoo, *Proc. Japan Acad.*, 28, 200 (1952).
3) K. Tsuda and M. Kawamura, *Pharm. Bull. (Japan)*, 1, 112 (1953).
4) H. Kakisawa, Y. Okumura and Y. Hirata, *J. Chem. Soc. Japan, Pure Chem. Sec. (Nippon Kagaku Zasshi)*, 80, 1483 (1959).
5) K. Tsuda, M. Kawamura and R. Hayatsu, *Chem. Pharm. Bull. (Japan)*, 8, 257 (1960).
6) A Mechrolab. Inc. osmometer, Model 301A, was used. We wish to thank Takeda Chemical Industries, Ltd., for this measurement.
7) The authors are indebted to the following laboratories and companies for the microanalyses: a) Scandinavian Microanalytical Lab. b) Takeda Chemical Ind., Ltd. c) Elementary Analysis Center, Kyoto University. d) Fujisawa Pharmaceutical Co., Ltd. e) Elementary Analysis Lab., Osaka City University.

with chloroform (three times). The combined extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in chloroform and precipitated by the addition of petroleum ether. The precipitate (110 mg.) was further purified by dissolving it in benzene and precipitating it with ether. The analytical sample was a white amorphous powder; m. p. 162~164°C.

Found: C, 46.97; H, 5.27; N, 6.44.[7e] Calcd. for $C_{11}H_{13}O_8N_3(CH_3CO)_4 \cdot C_7H_8O_3S$: C, 47.34; H, 5.04; N, 6.37%.

Pentaacetylanhydrotetrodotoxin *p*-Toluenesulfonate (IV). — The tetraacetate III (500 mg.) was dissolved in a mixture of pyridine (2 ml.) and acetic anhydride (2 ml.), and the mixture was allowed to stand overnight at room temperature. The reaction mixture was evaporated at 55°C (bath temp.) under reduced pressure, and the residue was dissolved in chloroform. Ether was added to the solution to precipitate the product, which was then recrystallized from ethanol to give 200 mg. of white needles; m. p. 208~210°C.

Found: C, 48.83; H, 5.20; N, 5.81;[7e] C, 48.53, 48.80, 48.77; H, 4.77, 5.03, 5.40; N, 6.32;[7d] N, 6.13;[7a] O, 35.42;[7c] mol. wt., 673±10 (0.6418% solution in chloroform). Calcd. for $C_{11}H_{10}O_7N_3 \cdot (CH_3CO)_5 \cdot C_7H_8O_3S$: C, 49.19; H, 4.87; N, 6.15; O, 35.11%; mol. wt., 683.62.

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