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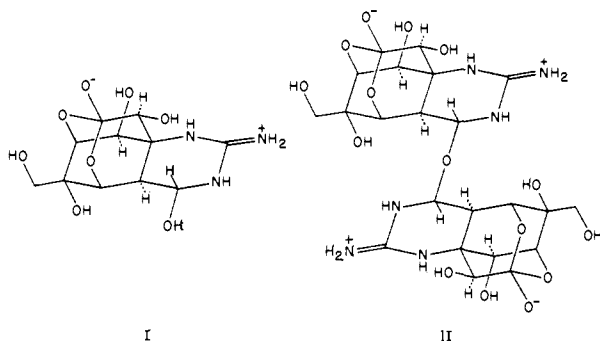
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J. L. GARNETT

RECEIVED JULY 13, 1964

The Structure of Tetrodotoxin

Sir:

Recently we presented the evidence which led us to deduce the structure I for the *Spheroides* poison tetrodotoxin.^{1,2} The very interesting alternative suggestion³ that the toxin possesses the structure II, in which two units of the structure I are joined together by an



etheral oxygen atom, deserves serious consideration. The problem of differentiating between the two structures is not accessible to study by the usual methods of solution chemistry, since tetrodotoxin is all but completely insoluble in all solvents except acids, and the latter might well effect cleavage of the ether link in II, whose lability would have to be accepted in view of the fact that all transformation products of established constitution are derived from the C₁₁ structure I rather than a C₂₂ molecule. We now wish to present evidence which permits an unequivocal decision in favor of the structure I for tetrodotoxin.

Tetrodotoxin is ordinarily crystallized by addition of ether and ethanol or methanol to solutions of the toxin in dilute aqueous acetic acid.⁴ The aggregates of very small crystals prepared in this way are not suitable for fundamental crystallographic studies. However, when material thrice recrystallized by the

standard procedure was dissolved in dilute aqueous acetic acid, the solvent removed *in vacuo*, the residue taken up in a minimum quantity of pure water, and the solution allowed to stand for three weeks, completely transparent, beautifully formed, individual parallelepipeds separated. This material was shown to be tetrodotoxin through the identity of its infrared spectrum (KBr) and Debye-Scherrer diagram with those of an authentic sample. Although the crystals were still very small (maximum extension ≤ 0.06 mm.), it was possible to mount several of them for single crystal X-ray diffraction studies, using Cu K α radiation.

The crystals were found to be monoclinic through observation of C_{2h} diffraction symmetry in oscillation and precession photographs, and to belong to space group C₂²-P2₁, since tetrodotoxin is optically active, and systematic extinctions were found only for 0k0, k odd (k ≤ 13), in Weissenberg photographs. Precession photographs taken of the h0l and h1l reciprocal lattice planes established the axial lengths a = 6.72 \pm 0.04 Å., c = 6.49 \pm 0.02 Å., and the interaxial angle β = 113°56' \pm 22'. The length of the unique axis, b = 14.66 \pm 0.02 Å., was determined through observation of the 0k0 reciprocal lattice line, after suitable reorientation of the same crystal which provided the above information. The standard deviations were ascertained through superimposing lead nitrate powder diagrams upon the precession photographs. Satisfactory confirmatory values were obtained for all three axial lengths from oscillation photographs taken on different crystals mounted along each of the crystallographic axes. The density of the crystals was found to be 1.792 \pm 0.006 g./cm.³ by the flotation method; this is almost certainly a minimum value, since observations were made on aggregates of the parallelepipeds, rather than on the exceedingly small and very difficultly observable single crystals.

The volume of the unit cell of the tetrodotoxin crystal is therefore 585 \pm 4 Å.³, and the weight is 632 \pm 6 on the atomic scale. Further, for a nonpolymeric substance, the crystal symmetry requires the presence within the unit cell of an even number of complete, discrete, unconnected molecules. These conditions are fully met by a cell containing two molecules of the structure I (2 \times mol. wt. = 638), and cannot in any way be reconciled with the alternative ether structure II, which would require a minimum cell weight of 1240.

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R. B. WOODWARD
J. ZANOS GOUGOUTAS

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Concerning "Bond-Fixation" in Olefin-Iron Tricarbonyl Complexes

Sir:

The unusual nuclear magnetic resonance spectrum of cyclooctatetraene-iron tricarbonyl (1) has been reconciled with its structure, as determined by X-ray

(1) Lecture presented on April 14, 1964, in Kyoto at the 3rd International Symposium on the Chemistry of Natural Products; R. B. Woodward, *Pure Appl. Chem.*, **9**, 49 (1964). The structure there presented is actually the mirror image of I. Professor Nitta and his colleagues reported at the same symposium that extension of their X-ray crystallographic studies on bromoanhydrotetrodoic lactone hydrobromide [Y. Tomiie, A. Furusaki, K. Kasami, N. Yasuoka, K. Miyaki, M. Haisa, and I. Nitta, *Tetrahedron Letters*, **30**, 2101 (1963)] had enabled them to deduce the absolute configuration of that derivative, in the sense corresponding to I for tetrodotoxin itself.

(2) For independent studies leading to consideration of the same structure, cf. T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata, *ibid.*, 779, 1831 (1964), and K. Tsuda, C. Tamura, R. Tachikawa, K. Sakai, O. Amakasu, M. Kawamura, and S. Ikuma, *Chem. Pharm. Bull. (Tokyo)*, **12**, 634 (1964). It is also of much interest that tarichatoxin, from the California salamander *Taricha torosa*, has recently been shown to be identical with tetrodotoxin; H. S. Mosher, F. A. Fuhrman, H. D. Buchwald and H. G. Fischer, *Science*, **144**, 1100 (1964).

(3) K. Tsuda, R. Tachikawa, K. Sakai, C. Tamura, O. Amakasu, M. Kawamura, and S. Ikuma, *Chem. Pharm. Bull. (Tokyo)*, **12**, 642 (1964).

(4) K. Tsuda and M. Kawamura, *J. Pharm. Soc. Japan*, **73**, 771 (1952); cf. also H. Kakisawa, Y. Okumura, and Y. Hirata, *J. Chem. Soc. Japan*, **80**, 1483 (1959).