support (A. E.), and Mr. B. O'Leary for technical assistance with the irradiation.

DEPARTMENT OF PHYSICAL CHEMISTRY A. EKSTROM
THE UNIVERSITY OF NEW SOUTH WALES J. L. GARNETT
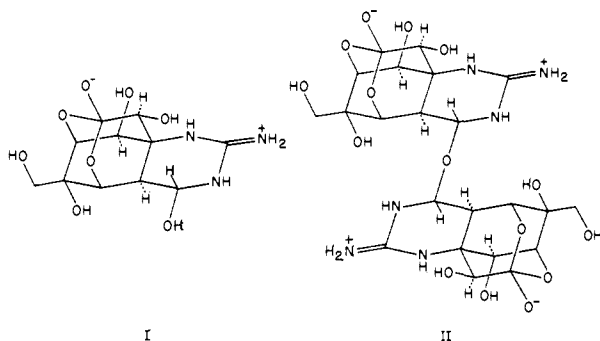KENSINGTON, N.S.W., AUSTRALIA

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 suggestion[3] that the toxin possesses the structure II, in which two units of the structure I are joined together by an

I II

ethereal 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 C_{11} structure I rather than a C_{22} 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
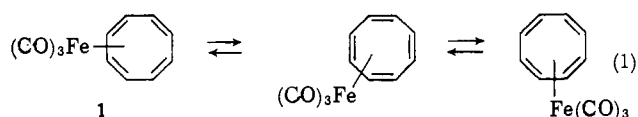
(1) Lecture presented on April 14, 1964, in Kyoto at the 3rd International Symposium on the Chemistry of Natural Products; R. B. Woodward, *Pure Appl. Chem.*, **9**, 49 (1964). The structure there presented is actually the mirror image of I. Professor Nitta and his colleagues reported at the same symposium 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*, **72**, 771 (1952); *cf.* also H. Kakisawa, Y. Okumura, and Y. Hirata, *J. Chem. Soc. Japan*, **80**, 1483 (1959).

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 parallelopipeds 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 $\leq$ 0.06 mm.), it was possible to mount several of them for single crystal X-ray diffraction studies, using Cu Kα 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 C_2^2-P2$_1$, since tetrodotoxin is optically active, and systematic extinctions were found only for $0k0$, k odd ($k \leq 13$), in Weissenberg photographs. Precession photographs taken of the $h0l$ and $h1l$ reciprocal lattice planes established the axial lengths a = 6.72 ± 0.04 Å., c = 6.49 ± 0.02 Å., and the interaxial angle β = 113°56' ± 22'. The length of the unique axis, b = 14.66 ± 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 ± 0.006 g./cm.^{3} by the flotation method; this is almost certainly a minimum value, since observations were made on aggregates of the parallelopipeds, 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 ± 4 Å.^{3}, and the weight is 632 ± 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 × 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.

CHEMICAL LABORATORIES R. B. WOODWARD
HARVARD UNIVERSITY J. ZANOS GOUGOUTAS
CAMBRIDGE, MASSACHUSETTS 02138

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

diffraction, by the proposal that in solution the iron tricarbonyl moiety is rapidly shifting around the ring[1] (eq. 1). This concept has since been used to explain

spectra of complexes 2,[2] 3,[3] and, e.g., 4[4] in terms of similar nonfixation of the metal to the large ring.
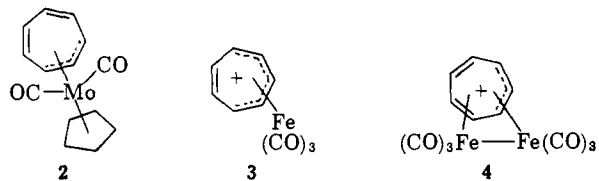

This type of mobility could have interesting consequences in the chemistry of linear and cross-conjugated polyene–iron tricarbonyl complexes. We have accordingly investigated this possibility in a linear triene–iron tricarbonyl complex and are prepared to report the following.

(a) The two isomeric olefin–iron tricarbonyl complexes 7 and 8 which differ only in the position of the iron tricarbonyl residue have been prepared.

(b) They are chemically distinct species, having different melting points, a depressed mixture melting point, and similar but nonidentical infrared spectra.

(c) They retain their identity indefinitely at room temperature.

(d) They are smoothly interconverted on heating to 120°.
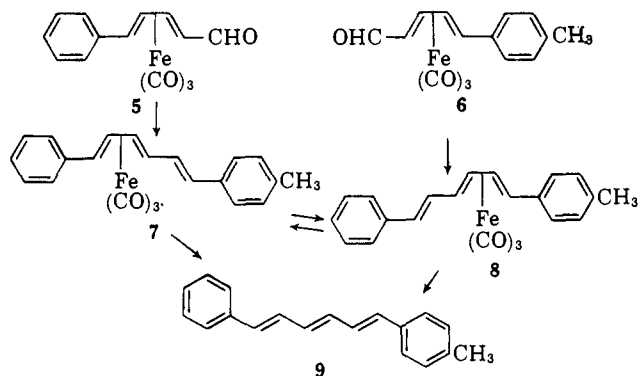
Reaction of 5-phenyl-2,4-pentadienal with iron pentacarbonyl in refluxing heptane for 3 days gave the iron tricarbonyl complex 5, m.p. 163–164°.[5] Similarly, 5-p-tolyl-2,4-pentadienal gave complex 6, m.p. 138–139°. Reaction of 5 with p-methylbenzylidene triphenylphosphorane in tetrahydrofuran at 0° for 1 hr. gave, in high yield, triene–iron tricarbonyl complex 7 as sheaves of orange plates, m.p. 125–126°, or as clumps of red rods, m.p. 129–131°. Complex 7 exhibits a band

(1) B. Dickens and W. N. Lipscomb, J. Chem. Phys., **37**, 2084 (1962).

(2) R. B. King, Tetrahedron Letters, 1137 (1963).

(3) J. E. Mahler, D. A. K. Jones, and R. Pettit, J. Am. Chem. Soc., **86**, 3589 (1964).

(4) G. F. Emerson, J. E. Mahler, R. Pettit, and R. Collins, ibid., **86**, 3590 (1964).

(5) All new compounds were characterized spectrally and by elemental analysis.

at 965 cm.[-1] in its infrared spectrum which is attributable to the uncomplexed trans double bond. Reaction of 7 with ferric chloride in acetonitrile at 25° gave trans,trans,trans-1-phenyl-6-p-tolyl-1,3,5-hexatriene[6] (9).

Reaction of 6 with benzylidene triphenylphosphorane gave the isomeric complex 8 as golden orange rods, m.p. 144–145°, which also exhibited a band at 965 cm.[-1] in its infrared spectrum and which was degraded to 9 with ferric chloride in acetonitrile. The infrared spectra of 7 and 8 are distinguishable chiefly by virtue of small shifts in the positions of the phenyl and p-tolyl out-of-plane CH deformation bands. A mixture of 7 and 8 had m.p. 114–138°. As an example of the isomerization of 7 and 8, 200 mg. of 7 was heated neat (molten) at 120–123° for 17 hr. Chromatography of the resulting viscous tar on silica gel gave 183 mg. of a mixture of 7 and 8. Repeated chromatography of this mixture and fractional recrystallization of the less polar fractions ultimately afforded 1.53 mg. of pure 8, m.p. 144–145°, shown to be identical with 8 as prepared above by mixture melting point and infrared. As expected for the proposed equilibrium, longer heating led to more isomerization, equilibrium could be approached from complex 8, and the isomerization could be carried out in dilute solution in n-decane.
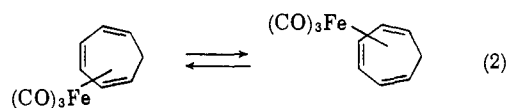
Although only qualitative evidence is as yet available as to the unimolecularity of the reaction 7 ⇌ 8, we suggest that the isomerization proceeds through an intermediate or transition state which may be represented as 10.[7] Production of this species is consistent with the sluggishness with which iron pentacarbonyl undergoes exchange of its carbonyl groups.[9]
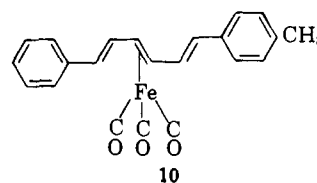
(6) B. M. Mikhailov, L. S. Povarov, and G. S. Ter-Sarkisyan, Stsintillyatory i Stsintillyats Materialy, Vses. Nauchn.-Issled. Inst. Khim. Reaktivov, Materialy 2-go Vtorogo Koordinats. Soveshch, 155, 1957; Chem. Abstr., **58**, 4443f (1963).

(7) It is interesting in this respect that the n.m.r. of cycloheptatriene-iron tricarbonyl[8] is consistent with its undergoing a slow or nonexistent shifting of the iron tricarbonyl residue. This may be a consequence of a "double-jump" mechanism being necessary in this case (eq. 2) whereas a "single-jump" mechanism is possible for complexes 1–4 (eq. 1).

(8) R. Burton, L. Pratt, and G. Wilkinson, J. Chem. Soc., 594 (1961).

(9) D. F. Keely and R. E. Johnson, J. Inorg. Nucl. Chem., **11**, 33 (1959).

DEPARTMENT OF CHEMISTRY   H. W. WHITLOCK, JR.
UNIVERSITY OF WISCONSIN   Y. N. CHUAH
MADISON, WISCONSIN 53706