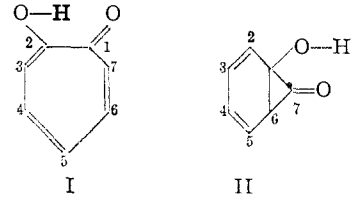
MOLECULAR STRUCTURE OF TROPOLONE

Sir:

Doering and Knox[1] have recently reported the synthesis of the interesting and important compound tropolone, I, which had been previously conceived by Dewar[2] as a structural unit having special resonance stability. The chemical and spectroscopic evidence obtained by Doering and Knox,



I II

they remark, is consistent with I but does not exclude the alternative structure II. We wish to report the preliminary results of an electron diffraction investigation of tropolone which (a) exclude II and (b) constitute direct evidence in support of I.

The sample, kindly supplied by Dr. Doering, was vaporized in a boiler and photographs were prepared in the usual way[3] with an electron wave length of about 0.06 Å. and a camera distance of about 11 cm. The radial distribution curve calculated[4] from our visual interpretation of the photographs is shown in Fig. 1. The following interpretations of the peaks

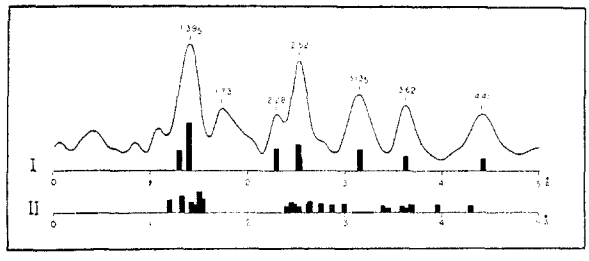


Fig. 1.—Radial distribution function for tropolone: vertical bars indicate distances calculated for models of structural types I and II; heights of bars are proportional to weights of distances.

of the radial distribution curve may be made in terms of I, if, just for purposes of orientation, the molecule is assumed to be approximately coplanar, and to have approximately all bonded C–C distances alike, bonded C–O distances alike, C–C–C angles alike, and corresponding O–C–C angles alike. The peak at 1.39(5) Å. is due to bonded C–C and bonded C–O interactions, which are too close together to be resolved. The peak at 2.28 Å. may be assigned to the distances of the type $O_1\cdots C_2$ and $O_1\cdots C_7$, that at 2.52 Å. to $C_1\cdots C_3$ and $O_1\cdots O_2$ (unresolved), and the remaining peaks at 3.13_5, 3.62, and 4.41 Å. to $C_1\cdots C_4$, $O_1\cdots C_3$ and $O_1\cdots C_6$, and $O_1\cdots C_4$ and $O_1\cdots C_5$, respectively. The peak at 1.73 Å. does not correspond to any possible distance in I or in any other reasonable model of tropolone, and must be considered as due to error in interpretation of the photographs. The interatomic distances for the completely symmetrical version of I defined in Table I correspond closely with the observed peak positions as may be seen in the figure. On the other hand, the distances for a reasonable model of II (C_1–C_2 = 1.54 Å., C_2–C_3 = 1.33 Å., C_3–C_4 = 1.46 Å., C_1–C_7 = C_1C_6 = 1.52 Å., C_1–O_1 = 1.43 Å., C=O = 1.21 Å., $\angle C_1C_3C_2$ = 125°, $\angle OC_1C_2$ = $\angle OC_1C_6$ = 109.5°, and plane of 3-membered ring bisecting $\angle OC_1C_2$) do not correspond at all well. Indeed, examination of the general situation indicates that it is extremely unlikely that a fit to the radial distribution curve can be obtained with models of II even on assumption of unreasonable values for the structural parameters.

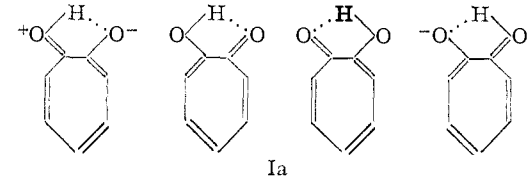
TABLE I

TROPOLONE. COMPARISON OF OBSERVED AND CALCULATED DISTANCES

The calculated distances are for a coplanar model of symmetry C_{2v}, with regular seven-membered ring, C–C = 1.40 Å., C–O = 1.30 Å., and $\angle C_1C_2O_2 = 115^3/_4°$.

Identification	Rel. weight	Distances Obsd.	Calcd.
C–C	100	1.39(5)	1.40 (assumed)
C–O	41		1.30 (assumed)
$O_1\cdots C_2$	23	2.28	2.28
$O_1\cdots C_7$	23		2.28
$C_1\cdots C_3$	56	2.52	2.52
$O_1\cdots O_2$	28		2.53
$C_1\cdots C_4$	44	3.13(5)	3.15
$O_1\cdots C_3$	15	3.62	3.62
$O_1\cdots C_6$	15		3.62
$O_1\cdots C_4$	12	4.41	4.42
$O_1\cdots C_5$	12		4.42

Our discussion of the data in terms of a symmetrical model for I might seem to imply that the tropolone molecule actually is symmetrical with the enolic hydrogen atom occupying a position midway between the two oxygen atoms as would correspond to symmetrical contributions from all the resonance structures including the typical ones shown in Ia. Presumably such a symmetrical



Ia

structure is attained by the somewhat analogous azulene molecule. It must be emphasized, however, that notwithstanding the excellent agreement shown in Table I, the radial distribution curve for tropolone does *not* exclude an unsymmetrical structure with unequal C–C bond distances and unequal C–O bond distances corresponding to unsymmetrical contributions of the resonance structures (Dewar[2] at first discussed only the symmetrical possibility but later favored an unsymmetrical structure) provided that in each group the distances do not differ by more than about 0.1 Å. Further, although the radial distribution curve definitely excludes large deviations of the heavy atom structure from coplanarity, small

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deviations are not ruled out. Such small deviations cannot be regarded as likely, however.

GATES AND CRELLIN LABORATORIES
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA 4, CALIFORNIA EDGAR HEILBRONNER
CONTRIBUTION NO. 1477 KENNETH HEDBERG

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ISOLATION OF CRYSTALLINE PYROPHOSPHATASE FROM BAKER'S YEAST

Sir:

The presence in yeast of an enzyme capable of catalyzing the hydrolysis of inorganic pyrophosphate to orthophosphate has been established by Bauer[1] in 1936. The enzyme was named "pyrophosphatase." Several attempts have been made by various workers to purify the enzyme. The most notable advance in the purification of the enzyme was made in 1944 by Bailey and Webb.[2] They have been unsuccessful, however, in their attempt to obtain the enzyme in crystalline form. The enzyme has now been crystallized from Fleischmann's baker's yeast, in the form of fine needles and thin rectangular prisms.

The method of isolation consists essentially of the following steps: 1. Plasmolysis of compressed yeast with toluene at 38–40°, followed by extraction with water, at 5°. 2. Concentration and fractionation between 0.5 and 0.7 saturation of ammonium sulfate. 3. Removal of inert components by autolysis at 5°, accompanied by precipitation of the enzyme with ammonium sulfate. 4. Further removal of impurities by adsorption on $Ca_3(PO_4)_2$ gel, followed by precipitation of the enzyme with ammonium sulfate. 5. Removal of electrolytes by dialysis against distilled water at 5°. 6. Crystallization in dilute ethyl alcohol solution at −8°.

Crystalline pyrophosphatase is a soluble, colorless protein of the albumin type, free of phosphorus (C, 54.5; H, 7.4; N, 16.2; S, 0.14; ash, 0.36).

Details of the method of isolation, also a description of some of the physico-chemical and catalytic properties of the newly isolated crystalline enzyme, are to be submitted for publication in the *Journal of General Physiology.*

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ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
66TH STREET AND YORK AVENUE
NEW YORK 21, N. Y. M. KUNITZ

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PAPER CHROMATOGRAPHY OF HYDROXY AND KETOACIDS[1]

Sir:

Paper chromatography has been applied in the last few years to the detection of small amounts of various types of organic acids. Various procedures have been reported for the saturated aliphatic acids,[2–6] and Lugg and Overell[7] have developed an excellent method for the paper chromatography of polycarboxylic and other non-volatile acids. They employed butanol–water or other solvents, in combination with a volatile organic acid, such as formic or acetic, in order to decrease the ionization of the test acids and thus prevent streaking or "tailing."

In connection with an investigation of fatty acid metabolism in this laboratory, it was necessary to develop a technique for the separation and identification of small quantities of certain hydroxy and ketoacids of intermediate chain length. In attempting to apply a paper chromatographic method, it was found that the solvent system of Lugg and Overell was not suitable for most of these acids: they exhibited very high R_f values and poor resolution; furthermore, their moderate degree of volatility limited the length of time permissible to carry out the procedure.

It has been found, however, that a solvent system composed of toluene–acetic acid–water provides an excellent method for the analysis of many hydroxy and ketoacids. A mixture of 100 cc. of toluene and 5 cc. of acetic acid is equilibrated with 60 cc. of distilled water; after separation of the layers, an additional 4 cc. of acetic acid is added to the toluene layer. Whatman No. 1 filter paper is used without prior washing or other treatment. The papers are run in the descending manner for several hours, depending on the particular acids being chromatographed. After the removal of the paper from the chamber, it is dried several hours in a current of air. The test acids are located in a novel manner: the dried papers are exposed a few minutes to ammonia vapor in a closed chamber, the excess ammonia is removed by allowing the paper to stand 30 minutes, and the spots of ammonium salts are then located by dipping the paper in Nessler solution. Small, intensely orange spots against a light background result.

Since the mobile solvent is allowed to overrun the paper during the chromatographing, R_f values do not apply, but the distances the acids move from the starting point are equally characteristic. The excellent resolution obtained is indicated by the following data from a 6-hour chromatogram: α-hydroxyvaleric acid and α-hydroxycaproic acid move 5.3 cm. and 14.5 cm. from the starting point, respectively; β-hydroxycaproic acid moves 10.5 cm.; and α-ketovaleric acid moves 8.6 cm.

Preliminary experiments indicate the method of color development described above may be used for quantitative estimation of the test acids: After exposing to ammonia, the material can be eluted with water, Nesslerized, and the intensity of color determined in a photoelectric colorimeter or spectrophotometer.

(1) This work was supported in part by funds granted by the National Dairy Council on behalf of the American Dairy Association, and by the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

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