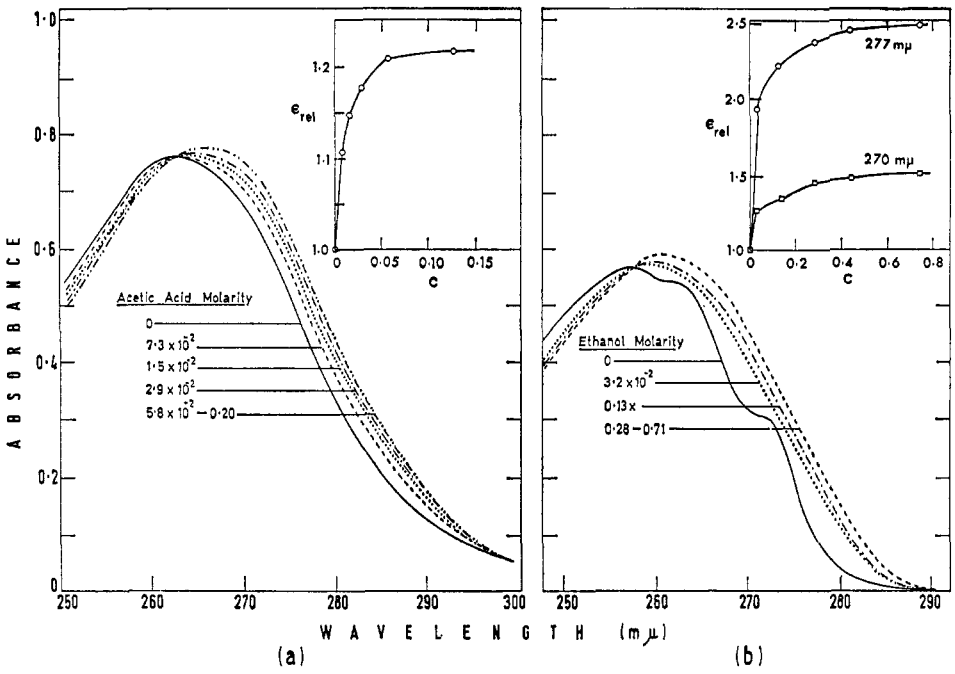

Figure 2. Effect of hydrogen bonding on the absorption spectrum of 9-ethyladenine. (a) Solvent, chloroform; perturbant, acetic acid; (b) solvent, isooctane; perturbant, ethanol. Insets show change of molar absorptivity, relative to the unperturbed value, with molar concentration of added hydrogen-bonding agent. The concentrations of adenine are approximately 8×10^{-5} and 6×10^{-5} *M*.

drogen-bonding agents. In A, however, there is a strong effect. Figure 2 shows the effect of hydrogen bonding to acetic acid in chloroform solution and to ethanol in isooctane. An absorbance increase, as well as a red shift, is noted. Calculations of association constants are precluded by the dimerization of the added hydrogen-bonding agent. However, the insets (Figure 2) show that this change is substantially complete at a perturbant concentration of *ca.* 3% and is not therefore a solvent effect. The hydrogen-bonding acceptor, dioxane, gives much lower association for similar concentrations. Absorbance increases on hydrogen bonding are common,[11,12] though the opposite effect is also known.[11] It may be noted that the two important transitions in the purine base absorption band, suggested[13] to be derived from the benzenoid $A_{1g} \rightarrow B_{2u}$ and $A_{1g} \rightarrow B_{1u}$ bands, undergo an approximately parallel change on addition of solvent (*cf.* ref 11). The possibility that the hypochromic effect is merely a hydrogen-bonding phenomenon, resulting from increased interaction of the π orbitals with nonbonding orbitals in the hydrogen-bonding substituent groups, appears to be excluded by the results of Figure 2. The examination of nuclear magnetic resonance spectra of similar systems[14] appears to exclude any question of base stacking in such solvents.

It is important to note that the A···U pairing need not be of Watson–Crick type, but may instead follow the Hoogsteen scheme;[15] indeed, in mixed A + T crystals this is the form which occurs.[15] From the two types of pairing, for reasonable transition moment directions,[2] it is qualitatively obvious that resonance interaction (corresponding transitions) should be greater in the Watson–Crick form. We are unable to comment categorically on the relevance of these observations to helical nucleic acids in aqueous solution, but a "horizontal" contribution to the hypochromic effect may be envisaged as a possibility under some circumstances.[16]

Acknowledgment. We thank Miss L. Lewis for help with the syntheses, and Professor Sir John Randall for facilities.

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Received July 25, 1966

The Formation of Polyenic Dialdehydes in the Photooxidation of Pure Liquid Benzene

Sir:

While the radiolysis and photolysis of aqueous benzene solutions in the presence and absence of oxygen have been studied in detail, particularly by Stein and Weiss[1a-f] and Dorfman, *et al.*,[1g] the photooxidation of dry liquid benzene has not been reported.[2] We have

(1) (a) G. Stein and J. Weiss, *J. Chem. Soc.*, 3245 (1949); (b) I. Loeff and G. Stein, *Nature.*, **184**, 901 (1959); (c) G. Stein and J. Weiss, *J. Chem. Soc.*, 3254 (1949); (d) G. Stein and J. Weiss, *ibid.*, 3265 (1951); (e) M. Daniels, G. Scholes, and J. Weiss, *ibid.*, 832 (1956); (f) I. Loeff and G. Stein, *ibid.*, 2623 (1963); (g) L. M. Dorfman, I. A. Taub, and R. E. Buhler, *J. Chem. Phys.*, **36**, 3051 (1962); L. M. Dorfman, I. A. Taub, and D. A. Harter, *ibid.*, **41**, 2954 (1966).
(2) For references for photooxidation mechanisms, see the papers of K. Gollnick and G. Schenck, and C. Foote summarized in "Photochemistry," J. G. Calvert and J. N. Pitts, Jr., Ed., John Wiley and Sons,

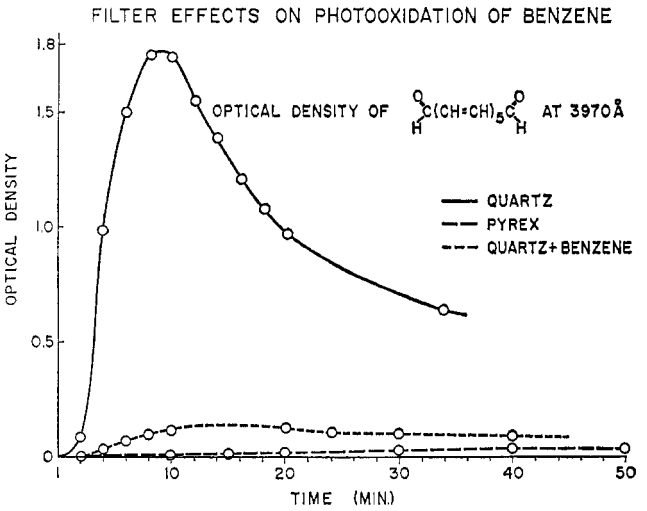

Figure 1.

investigated the latter system at room temperature and among the several products that are formed in low yields have isolated two polyenic dialdehydes, *trans*, *trans*-2,4-hexadienedial (mucondialdehyde, I) and 2,4,6,8,10-dodecapentaenedial ($OHC(CH{=}CH)_5CHO$, II).

On the basis of the ultraviolet spectra of the di-*p*-nitrophenylhydrazone derivative, Loeff and Stein have reported that mucondialdehyde is a primary product when air-saturated solutions of benzene *in water* are irradiated with a 2534-A Hg resonance lamp.[1f] However, they did not isolate or further characterize the dialdehyde. The twelve-carbon compound, II, has not been previously reported as a photooxidation product; in fact, only one reference to its synthesis was found.[3] This involved a complex, multistep process and only an elemental analysis and the melting point of II, 173–174°, were given. No other data were cited for this spectroscopically interesting, strongly absorbing, yellow compound. However, it was noted that derivatives of II and its analogs possessed antiviral properties.[2]

In our experiments, we utilized unfiltered radiation from a medium-pressure mercury lamp (Hanovia 673 A36-550W) to irradiate at room temperature pure, dry, liquid benzene through which oxygen was continuously bubbled. The irradiated benzene was chromatographed twice on silica gel, giving three fractions. The most polar fraction, a complex mixture of phenol and other products (possibly including pyrocatechol[3,1f]), was not investigated further. The first fraction, eluted with an 80:20 pentane–ether solution, contained mucondialdehyde (I). The second fraction, eluted with a 70:30 pentane–ether solution, contained dialdehyde II.

Dissolved oxygen is known to extend substantially the absorption of benzene toward longer wavelengths,[5] and we also observed a shift of the onset of absorption from 2900 A in 10 cm of degassed benzene to 3500 A in 10 cm of benzene saturated with O_2 at 1 atm. We found, however, that only radiation shorter than about 2700 A was effective in this photooxidation (see Figure 1). With unfiltered radiation, the reaction is fast and the maximum concentration of II, as determined by its absorbance at 3970 A, occurs at about 8 min under our experimental conditions (Figure 1). The observed optical density at this time corresponds to a concentration of about 3.5 mg/l. of compound II and a 0.001% conversion.

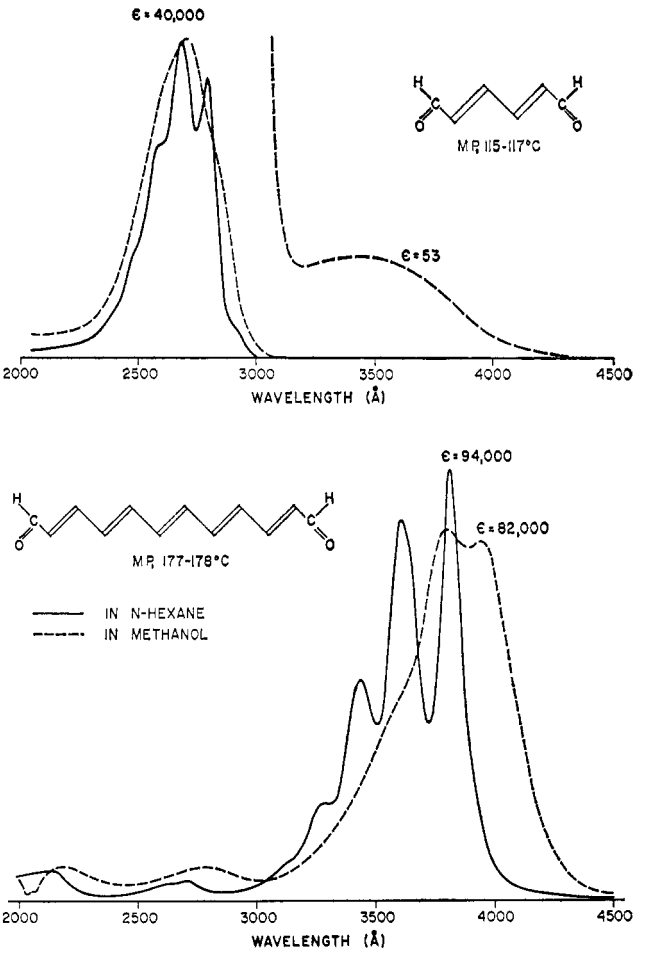

Figure 2. Absorption spectra of compounds I and II.

Infrared, ultraviolet, and nmr techniques were used to determine the physical and spectroscopic properties of I and II. Confirmatory evidence was obtained by mass spectrometry and by microhydrogenation which, in the case of II, yielded a product identical with an authentic sample of 1,12-dodecanediol. The physical and spectroscopic properties of I, including comparison of its infrared spectrum with that of the three possible isomers,[6] confirm that I has the all-*trans* configuration. The close similarity of the carotenoidlike ultraviolet spectrum of II with that of all-*trans* 4,9-dimethyl-2,4,6,8,10-dodecapentaenedial,[7] together with its nmr spectrum which indicates a symmetrical dialdehyde with an α-*trans* double bond, and other data, strongly suggest the all-*trans* structure for II. Ultraviolet spectra for I and II in cyclohexane and methanol are given in Figure 2.

Inc., New York, N. Y., 1966, p 548; see also the review by K. Gollnick and G. Schenck in "1,4-Cycloaddition Reactions," J. Hamer, Ed., Academic Press Inc., New York, N. Y., 1967, p 255.

(3) S. M. Makin, G. A. Lapitskii, and R. V. Strel'tsov, *J. Gen. Chem. USSR*, 64 (1964).

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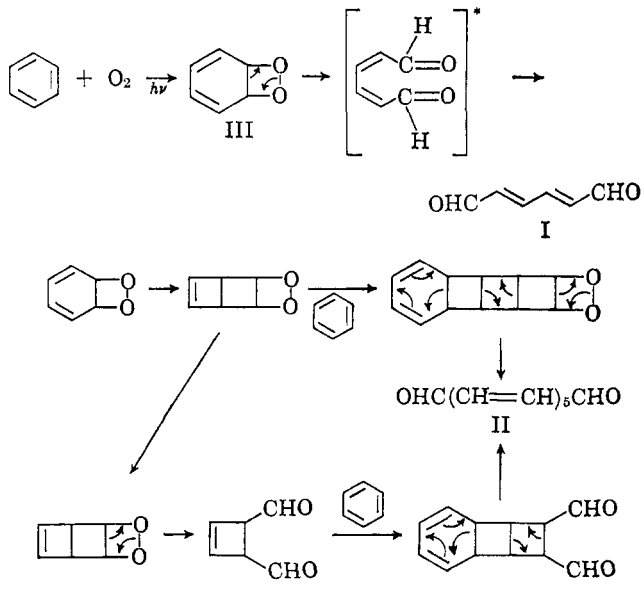
It is premature to speculate on the detailed mechanism of the reaction or the excited states of the species involved (including possibly a benzene isomer or excimer[8] and/or singlet oxygen). However, the reaction sequences given in Chart I[9] have an analogy[10] in known benzene photochemistry.[11] This photoinitiated

Chart I



attack of oxygen on benzene leading to ring opening and the formation of strongly absorbing, long-chain, conjugated dialdehydes has interesting implications in molecular biology. Thus, for example, enzymatic ring opening of pyrocatechol by oxygen gives *cis,cis*-muconic acid[12] while in another enzymatic oxygen fixation process with tryptophan, the oxygen opens the pyrrole ring to form N-formylkynurenine.[13] It is interesting to note that such attacks by "enzymatically activated oxygen"[13a] lead to ring opening and products similar to those we have observed in a photooxidation process.

Finally, the "yellowing" of polystyrene has long been a subject of investigation and speculation.[14] Mechanisms proposed to date concern reactions only of the skeletal chain. Our results with liquid benzene photooxidation suggest that ring opening by oxygen also may be involved.

Acknowledgments. We are grateful to Professor Robert Livingston and Dr. Kurt Schaffner for helpful discussions and to Grant AP 00109 of the National Center for Air Pollution Control, U. S. Public Health Service, for support of this research. J.-C. M. acknowledges a NATO Fellowship and a Fullbright Grant.

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(8) Suggested by A. Chandross, Bell Telephone Laboratories, Inc.
(9) The valence isomerization of III should provide ample energy to lead to the all-*trans* form I.
(10) We are grateful to referees I and II for this approach.
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(13) (a) O. Hayaishi, "Oxygen in the Animal Organism," The Macmillan Co., New York, N. Y., 1964, p 151; (b) P. Feigelson, Y. Ishimura, and O. Hayaishi, *Biochem. Biophys. Acta*, **96**, 283 (1965); (c) B. Pullman and A. Pullman, "Quantum Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1963, p 327.
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Enzymatic Stereospecificity in the Hydration of Epoxy Fatty Acids. Stereospecific Incorporation of the Oxygen of Water[1]

Sir:

We have found that a soluble (100,000*g*) extract, prepared from a pseudomonad[2] (NRRL-2944), catalyzes the stereospecific hydration of the Δ^9-olefinic bond of oleic acid, yielding 10-D-hydroxystearic acid (or 10-*R*).[3-6] We now wish to report that the same enzyme preparation catalyzes the hydration of *cis*- and *trans*-9,10-epoxystearic acids, yielding *threo*- and *erythro*-9,10-dihydroxystearic acids,[7] respectively. These reactions are characterized by both substrate and product stereospecificity. The enzymatic hydration of the racemic epoxides proceeds only to the extent of ~50% utilization of the added substrates, yielding optically active dihydroxystearic acids.[8] In the case of the *trans*-epoxystearate the recovered, unreactive substrate after prolonged and repeated incubation with the enzyme preparation was also optically active.[9] This finding constitutes a clear example of the use of an enzyme to effect the resolution of a racemic epoxide, a result which would be difficult, if not impossible, to obtain by other methods.

These observations, indicative of notable stereospecificity with respect to substrate and product, raise an additional question: is the oxygen of water incorporated specifically at one carbon atom during the course of the enzymatic hydration of the epoxide? Accordingly, we have incubated the sodium salts of *cis*- and *trans*-9,10-epoxystearic acids with the enzyme preparation in water enriched with respect to ^{18}O. Mass spectrometry provides a powerful tool for the quantitation and localization of the isotope in the product. Methyl 9,10-dihydroxystearate (I), upon electron impact, does not give a significant molecular ion suitable for determination of the isotopic composition but it does give a significant peak at *m/e* 187 (III) corresponding to the fragment containing carbon atoms

(1) Supported by a grant (HE 09501) from the National Heart Institute, U. S. Public Health Service.
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(3) G. J. Schroepfer, Jr., and K. Bloch, *J. Biol. Chem.*, **240**, 54 (1965).
(4) G. J. Schroepfer, Jr., *J. Am. Chem. Soc.*, **87**, 1411 (1965).
(5) W. G. Niehaus, Jr., and G. J. Schroepfer, Jr., *Biochem. Biophys. Res. Commun.*, **21**, 271 (1965).
(6) G. J. Schroepfer, Jr., *J. Biol. Chem.*, **241**, 5441 (1966).
(7) Characterized as the methyl esters by mass spectrometric, infrared, and gas–liquid partition chromatographic (3.8% SE-30 on Diatoport S) analyses. The *threo* and *erythro* configurations of the respective diols were established by thin layer chromatographic analyses on silica gel G plates impregnated with boric acid (5%) using as solvent pentane–ether–acetic acid, 50:50:1. This system, a minor modification of that described by L. J. Morris (*Chem. Ind.* (London), 1238 (1962)), allows complete separation of the *threo* and *erythro* isomers.
(8) Methyl *threo*-9,10-dihydroxyoctadecanoate: $[\alpha]_{5890}$ + 27.0 ± 3.8° (*c* 0.74, methanol); methyl *erythro*-9,10-dihydroxyoctadecanoate: $[\alpha]_{2500}$ +1.38 ± 0.11° (*c* 1.71, methanol)
(9) *trans*-9,10-Epoxyoctadecanoic acid: $[\alpha]_{5890}$ −14.6 ± 0.4° (*c* 1.57, methanol).