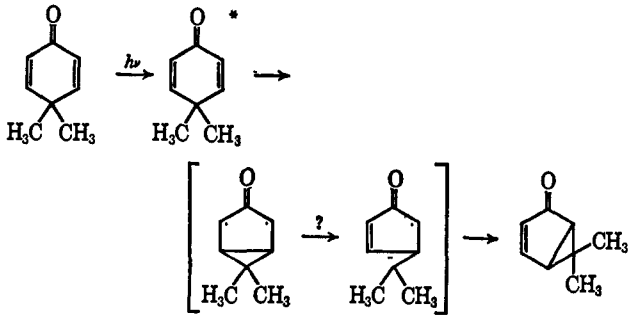
cess.[7] Irradiation of IIa in cyclohexane solution gave IIIa in 70% yield. These products are, without exception, those that could have been predicted from the well-documented studies of Zimmerman and his coworkers on Ib.[4,8]

Irradiation of Ia in the gas phase was carried out at 3660 Å at a temperature of 85° and a pressure of 6 torr.[9,10] *Exactly as with Ib, the initial product of the photoisomerization of Ia was IIa only.* As soon as about 6% of IIa had built up in the system, IIIa began to appear. The mass balance was excellent up to about 15% conversion of Ia. There is no doubt that Ia leads to IIa which in turn gives IIIa. After prolonged irradiation there were small amounts of other carbonyl compounds besides IIa and IIIa, but there was no detectable amount of the phenolic products IVa or Va. In relation to the yields of IVa and Va in aqueous dioxane, their yields in the gas-phase reaction can be estimated to be less than 0.01. Addition of methanol vapor was seen to decrease the yield of IIIa but not of IIa.

The quantum yield for the formation of IIa from Ia in the vapor phase was 0.40 ± 0.03 at 3660 Å. This may be compared to the quantum yield for the formation of IIb from Ib of 0.85 in this wavelength region.[11]

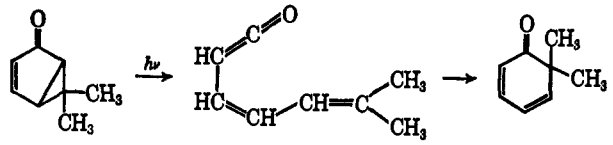
The scheme in terms of which the photoisomerization of Ib to IIb has been explained[11] involves a mesoionic intermediate[12] which is not likely to be involved in the gas-phase reaction. It follows that Ia can be transformed to IIa *via* nonionic intermediates in at least this instance.

The conversion of Ia to IIa is formally a specific example of the divinylmethane to vinylcyclopropane rearrangement which is known to be a general photochemical process.[13] A mechanism based on this idea would be as follows.



The transformation of IIa to IIIa should also involve a nonionic pathway at least when it takes place in the vapor phase, whereas the formation of the phenols IVa and Va from IIa probably involves zwitterionic intermediates since it is observed only in a polar medium. The latter route is likely to be the same as the one that has been proposed for the conversion of IIb to IVb and Vb in a polar solvent.[14]

The formation of IIIa is reduced by the presence of methanol which supports the intermediacy of a ketene in this reaction. This ketene, in turn, can cyclize to give IIIa.[15]



It is hardly surprising that in the vapor phase the formation of the phenols IVa and Va from IIa is not to be observed since these transformations require the intramolecular migration of a methyl group by a nonfree-radical process. In fact, there appears to be no well-documented instance of any gas-phase interaction in which a methyl group undergoes such a shift. Among the reactions of 2,5-cyclohexadienones that have been discussed here, this seems to be the only process that *demands* the intermediacy of polar intermediates.

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(7) In contrast, neither phenolic product was detectable in the photolysate in cyclohexane solution.

(8) H. E. Zimmerman, R. Keese, J. Nasielski, and J. S. Swenton, *J. Am. Chem. Soc.*, 88, 4895 (1966).

(9) Qualitatively, the same results were obtained when the irradiation was carried out at 25° at ~0.5 torr. Under both conditions the vapor pressure of ketone Ia was well below its saturation vapor pressure so that any possibility that the material may have been irradiated as a liquid film can be discounted.

(10) The identities of the products from the vapor-phase reaction were established from both their infrared spectra and their retention times.

(11) H. E. Zimmerman and J. S. Swenton, *J. Am. Chem. Soc.*, **89**, 906 (1967).

(12) Since irradiation of Ib gives IIb but scarcely any of its photoproducts, it has been argued that the excited state of the product IIb is not involved in this pathway. The same reasoning can be applied to Ia as well since the initial product in this case is only IIa.

(13) H. E. Zimmerman, R. W. Binkley, R. S. Givens, and M. A. Sherwin, *J. Am. Chem. Soc.*, **89**, 3932 (1967).

(14) This would be in line with the results obtained by H. E. Zimmerman and J. O. Grunewald [*ibid.*, **89**, 3354 (1967)] on the migratory aptitudes of substituted phenyl groups in IIb.

(15) For analogs of this process see ref 4 and 8 and also B. Miller and H. Margulies, *Chem. Commun.*, 314 (1965).

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The Enzymatic Oxidation of a Quinol Phosphate. Position of Bond Cleavage

Sir:

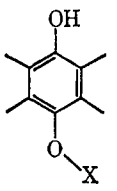
Their possible involvement in biological energy-transfer reactions has stimulated interest in the oxidative cleavage of monoesters of quinols (I, X = CH_3CO,[1] HSO_3,[2] or H_2PO_3[3]). The most important of these are the phosphate esters, partly because of the central role of phosphates in biological processes and partly because of evidence directly implicating quinones in oxidative phosphorylation.[4] Involvement of quinol phosphates in the latter process requires that their

(1) (a) J. W. Thanassi and L. A. Cohen, *J. Am. Chem. Soc.*, **89**, 5733 (1967); (b) C. A. Bunton and J. Hellyer, *ibid.*, **89**, 6252 (1967).

(2) S. W. Weidman, D. F. Mayers, O. R. Zaborsky, and E. T. Kaiser, *ibid.*, **89**, 4555 (1967).

(3) V. M. Clark, D. W. Hutchinson, G. W. Kirby, and A. R. Todd, *J. Chem. Soc.*, 715 (1961).

(4) R. A. Morton, Ed., "Biochemistry of Quinones," Academic Press Inc., New York, N. Y., 1965.



I

oxidation proceed with breaking of the phosphorus–oxygen bond (rather than the carbon–oxygen bond), so that the phosphate moiety is eliminated as metaphosphate or its equivalent and can be used to phosphorylate an acceptor. However, studies on the oxidation of these compounds using a variety of oxidizing agents and conditions have produced evidence that the major pathway for the reaction may involve C–O bond fission, with no more than 10–35% of the products coming from the "useful" P–O bond-fission pathway.[5-7]

Quinol phosphates, as substituted phenols, have the structural features required of hydrogen donors for peroxidase, and a study of the oxidation of duroquinol (2,3,5,6-tetramethylbenzoquinol) phosphate by horseradish peroxidase (HRP) and hydrogen peroxide has been made. This system has additional interest in that HRP is a hemoprotein related to several enzymes in the electron-transport chain, and its use as a mediator in this reaction might therefore be a satisfactory model for *in vivo* oxidation of quinol phosphates.

Duroquinol phosphate[8] is oxidized by HRP and hydrogen peroxide in aqueous solution (pH 4–6.5) at 25°; the sole detectable products are duroquinone (identified by its ir and mass spectra and assayed by its uv absorption) and inorganic phosphate (identified and assayed by the method of Fiske and SubbaRow[9]) in essentially quantitative yield. Reaction is very slow or negligible if either peroxide or enzyme is omitted from the reaction mixture, and 1.0–1.1 moles of peroxide is consumed for each mole of duroquinol phosphate oxidized. Although the over-all kinetics are complex, the initial velocity of the reaction, measured by following the absorbance increase in the uv, is directly proportional to the enzyme concentration.

Oxidations were carried out in water enriched with ^{18}O (4.5 atom % excess), and both products were isolated and purified for mass spectrometric analysis. The duroquinone was found to contain only the natural abundance of the heavy isotope;[10] the phosphate, after conversion to carbon dioxide by the method of Boyer, *et al.*,[11] was found to contain 1.22 ± 0.10 atom % ^{18}O, which corresponds to the incorporation of 0.91 ± 0.09 atom of oxygen per phosphate. Under these conditions, therefore, only an insignificant portion of the product could be formed *via* C–O bond fission.

The oxidation was also carried out in the presence of phosphate acceptors other than water. The choice of compounds for this function was limited by the requirement that the trapping agent be compatible with the HRP–H_2O_2 system, but it was found that the reaction would proceed in methanolic solution (up to 10 *M* methanol) and in up to 6.5 *M* ethylene glycol without affecting the yield of quinone. In the presence of methanol the yield of inorganic phosphate was reduced (Table I); treatment of the product with alkaline phosphatase (without removing the methanol) increased the concentration of inorganic phosphate; this is consistent with its being trapped as methyl phosphate. The presence of ethylene glycol did not affect the yield of inorganic phosphate. These results imply that the reaction proceeds *via* nucleophilic attack on the phosphorus before the P–O bond is broken rather than indiscriminate phosphorylation by metaphosphate, but the evidence may not be conclusive.[12]

Table I. Oxidation of Duroquinol Phosphate in Aqueous Methanol[a]

Methanol, *M*	Yield of inorganic phosphate,[b] %	Mole % trapped phosphate/mole % methanol[c]
0	100	
2.5	93.1	1.8
5.0	89.6	1.2
7.5	80.5	1.4
10.0	73.5	1.4

[a] HRP, 1 μM; duroquinol phosphate (initial), 73 μM; 0.01 *M* acetate buffer, pH (in the absence of methanol) 4.50; room temperature (23°). [b] Phosphate assays carried out in duplicate, agreeing to within 3%; yield expressed as percentage of maximum. [c] Mole % methanol calculated from data in J. Timmermans, "The Physico-Chemical Constants of Binary Systems," Vol. 4, Interscience Publishers, Inc., New York, N. Y., 1960, p 152.

The oxidation of quinol phosphates under physiological conditions can therefore be an efficient step in the transfer of high-energy phosphates. It is interesting that the HRP–hydrogen peroxide oxidation results exclusively in P–O bond cleavage whereas C–O bond fission appears to be the principal pathway in chemical oxidations. However, the complexity of the kinetics precludes direct comparison of these reactions, and a complete mechanism for the over-all process cannot be formulated without additional evidence.

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(6) A. Lapidot and D. Samuel, *Biochim. Biophys. Acta*, 65, 164 (1962).
(7) A. Lapidot and D. Samuel, *J. Am. Chem. Soc.*, 86, 1886 (1964).
(8) K. J. M. Andrews, *J. Chem. Soc.*, 1808 (1961).
(9) L. F. Leloir and C. E. Cardini, *Methods Enzymol.*, 3, 843 (1957).
(10) The M + 2 peak at *m/e* 166 could be resolved into two components under high resolution, one being the true isotopic M + 2 peak and the other being due to the species $C_{10}H_{11}O_2$ (see also S. Ukai, K. Hirose, A. Tatematsu, and T. Goto, *Tetrahedron Letters*, 4999 (1967)). The true M + 2 peak height was estimated after complete resolution of the doublet using a Du Pont 310 curve resolver.
(11) P. D. Boyer, D. J. Graves, C. H. Suelter, and M. E. Dempsey, *Anal. Chem.*, 33, 1906 (1961).
(12) (a) A. J. Kirby and A. G. Varvoglis, *J. Am. Chem. Soc.*, 89, 415 (1967); (b) I. Öney and M. Caplow, *ibid.*, 89, 6972 (1967).

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Conformation and Biological Activity of 1,4-Cyclohexadiene Derivatives

Sir:

Various studies of the 1,4-cyclohexadiene ring have not conclusively established its conformation concern-