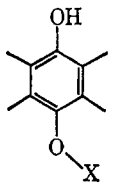

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oxidation proceed with breaking of the phosphorus–oxygen bond (rather than the carbon–oxygen bond), so that the phosphate moiety is eliminated as metaphosphate or its equivalent and can be used to phosphorylate an acceptor. However, studies on the 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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(5) W. Dürckheimer and L. A. Cohen, *Biochemistry*, **3**, 1948 (1964).
(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 $C_{10}H_{14}O_2$ (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. Oney and M. Caplow, *ibid.*, **89**, 6972 (1967).

John Wodak
Department of Biochemistry, University of Alberta
Edmonton, Alberta, Canada
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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-

ing which there is considerable disagreement. The expected errors in measurements of dipole moments of 1,4-cyclohexadiene and its 1,4-dichloro derivative preclude conformation assignments,[1,2] and the vibrational or the pure rotational Raman spectra of 1,4-cyclohexadiene do not distinguish a planar or near-planar conformation from a rapidly interconverting boat conformation.[3,4] A boat conformation of 1,4-cyclohexadiene has been calculated to be more stable by 1.5 kcal/mol on the basis solely of angle strain and interactions between nonbonded hydrogen atoms.[5]

In the present investigation, the planar conformation of 1,4-cyclohexadiene indicated by the biological activity and nuclear magnetic resonance studies of certain 1,4-cyclohexadiene derivatives has been established conclusively in the structure determination of 1,4-cyclohexadiene-1-glycine by X-ray diffraction.

Because of the possibility that the ring of 1,4-cyclohexadiene-1-alanine could exist in a planar conformation, which would greatly enhance its potential for activity as a phenylalanine antagonist, the analog was prepared[6] and found to be one of the most effective of the many known phenylalanine antagonists.[7] The exceptionally effective competitive antagonism of phenylalanine strongly suggests that the 1,4-cyclohexadienyl ring is essentially planar.

In nmr studies of 1,4-cyclohexadiene, methylene protons are found to be equivalent even at −150°, which is consistent only with a planar conformation or rapidly inverting boat conformation with a low energy barrier. The inability of a 3-phenyl substituent to alter the chemical shifts of protons in the *cis* or *trans* position on C-6 of 1,4-cyclohexadiene even when steric considerations would be expected to stabilize one form of an inverting boat conformation suggests a planar or very near planar conformation.[8]

A modified[9] equation[10] for vicinal coupling *vs.* dihedral angle predicts that the coupling constant between an olefinic proton and adjacent methylene protons in ring systems becomes smaller as the ring becomes more planar. Calculations indicate that a rapidly inverting boat with conformations such that the dihedral angle ranges from 95 to 25° would have a coupling constant approximately 70% greater than the planar conformation. Such coupling constants are 2.1 and 1.2 for the nonplanar cyclopentene and the presumed planar cyclopentadiene, respectively, and 3.1 and 1.5 for cyclohexene and 1,4-cyclohexadiene, respectively. These results strongly suggest a planar or very near planar conformation of 1,4-cyclohexadiene in solution.

In order to provide more conclusive evidence for the conformation of the ring, DL-1,4-cyclohexadiene-1-glycine[11] was selected for X-ray diffraction studies. The space group was determined to be $P2_1/c$ with four molecules per unit cell, and the unit cell dimensions are: $a = 15.864$, $b = 4.810$, $c = 10.022$ Å; $\beta = 98.03°$. The structure was solved by the Karle symbolic addition procedure,[12] and the agreement index, R, is 9.3% for 625 independent reflections. All of the hydrogen atoms were found in a difference density map, and the molecule was found to exist as a zwitterion with extensive intermolecular hydrogen bonding. The carbon–carbon double bonds in the ring average 1.347 Å, and the carbon–carbon single bonds average 1.485 Å; the estimated standard deviations in the bond lengths are no greater than 0.019 Å. Since the average distance of an atom in the ring to the best fit plane of the ring is 0.007 Å, the 1,4-cyclohexadiene ring is planar within experimental error.

Establishment of the conformation of the cyclohexadiene ring is of importance to theoretical studies of structure[5] and other chemical aspects such as assignments of configurations to isomers,[13] and in addition it can be anticipated that the isosteric relationship of the 1,4-cyclohexadienyl and phenyl group will be general for biologically active compounds. Preliminary studies, particularly with plant hormones, have demonstrated that high activity can be anticipated in analogs of biologically active compounds with this isosteric alteration of structure.[14]

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(1) W. D. Kumler, R. Boikess, P. Bruck, and S. Winstein, *J. Am. Chem. Soc.*, **86**, 3126 (1964).

(2) I. Miyagawa, Y. Morino, and R. Riemschneider, *Bull. Chem. Soc. Japan*, **27**, 177 (1954).

(3) B. J. Monostori and A. Weber, *J. Mol. Spectry.*, **12**, 129 (1964).

(4) H. D. Stidham, *Spectrochem. Acta*, **21**, 23 (1965).

(5) F. H. Herbstein, *J. Chem. Soc.*, 2292 (1959).

(6) DL-1,4-Cyclohexadiene-1-alanine was prepared by reducing DL-phenylalanine (5 g) with lithium (2 g) in liquid ammonia. The residue remaining after evaporation of the ammonia was repeatedly recrystallized from water to obtain 3.2 g of product, mp 234–236° dec. *Anal.* Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.47; H, 7.86; N, 8.36. The nmr spectrum shows no aromatic protons, three olefinic protons (τ 4.20, two protons; τ 4.43, one proton), and four doubly allylic protons (τ 7.35).

(7) In growth studies, the ratio of concentrations of antagonist to phenylalanine necessary for inhibition of visible growth for 18 hr ranged from 1 to 10 for *Escherichia coli* 10876, 9723, and W grown in inorganic salts–glucose media and for *Lactobacillus plantarum* 8014 and *Leuconostoc dextranicum* 8086 grown in a previously described synthetic media (J. M. Ravel, L. Woods, B. Felsing, and W. Shive, *J. Biol. Chem.*, **206**, 391 (1954); J. Edelson, P. R. Pal, C. G. Skinner, and W. Shive, *J. Am. Chem. Soc.*, **79**, 5209 (1957)). The analog is 50 to 100 times as active as 1-cyclohexene-1-alanine with *L. dextranicum*.

(8) Reported spectra show the equivalence of methylene protons in 3-phenyl-1,4-cyclohexadiene and the same chemical shift, τ 5.20, for the 6 proton in both *cis*- and *trans*-3-phenyl-6-trityl-1,4-cyclohexadienes (L. J. Durham, J. Studebaker, and H. S. Perkins, *Chem. Commun.*, 456 (1965).

(9) K. L. Williamson and W. S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961).

(10) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(11) Synthesized by the same procedure as the alanine derivative from phenylglycine, mp 280–282 dec. *Anal.* Calcd for $C_8H_{11}NO_2$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.78; H, 7.25; N, 9.24.

(12) J. Karle and I. Karle, *Acta Cryst.*, **21**, 849 (1966).

(13) D. H. Hey, M. J. Perkins, and G. H. Williams, *J. Chem. Soc.*, 110 (1965).

(14) Since this manuscript was submitted, a report has appeared on the structure of 1,4-cyclohexadiene by electron diffraction (G. Dallinga and L. H. Toneman, *J. Mol. Structure*, **1**, 117 (1967–1968)) with the conclusion that the planar conformation is the most probable, but the possibility that other nearly planar conformations are present cannot be precluded with certainty.

(15) Rosalie B. Hite Predoctoral Fellow, 1962–1965.

(16) NDEA Fellow, 1965–1967; NIH Trainee, 1964–1965.

B. A. Shoulders, Robert M. Gipson[15]
Ronald J. Jandacek,[16] S. H. Simonsen, William Shive
Clayton Foundation Biochemical Institute and the Department of Chemistry
The University of Texas, Austin, Texas 78712

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Dicyclopenta[*ef*,*kl*]heptalene (Azupyrene)[1,2]

Sir:

Extensions of the Hückel rule, while remarkably satisfactory for catacondensed polycyclic systems, have not

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