# Investigation of the Model Enzyme Oxidation of Phenol by Peracetic Acid

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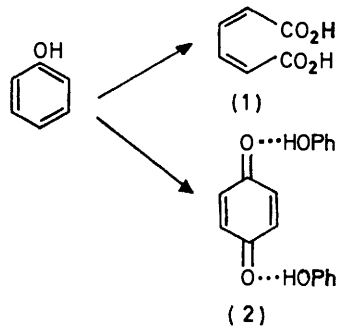
The pathway of the reaction of phenol with peracetic acid to yield hexa-2,4-dienedioic acid (1) and phenoquinone (*p*-benzoquinone–2 phenol) (2) has been investigated by chromatographic, radiochemical, spectroscopic, and kinetic techniques. These have shown that pyrocatechol and *o*-benzoquinone are discrete intermediates on the route to the dicarboxylic acid (1), and hydroquinone and *p*-benzoquinone on the route to phenoquinone. The rate of the side reaction of *o*-benzoquinone with water is slow compared with that of its oxidation by peracetic acid. However, its side reaction with pyrocatechol is more rapid, leading to a reduction in the yield of hexa-2,4-dienedioic acid. The oxidation of the dihydroxybenzenes to their quinones is catalysed by copper(II) ions. It has been concluded that peracetic acid is a poor model for hydroxylating and ring-cleaving enzymes, but may provide a useful parallel for the oxidation of pyrocatechol by *o*-diphenol–oxygen oxidoreductase.

PHENOL reacts with peracetic acid to give hexa-2,4-dienedioic acid (1) and phenoquinone (*p*-benzoquinone–2 phenol) (2), indicating that oxidation takes place in the *ortho*- and *para*-positions of the phenol molecule.[1] Comparable ring cleavage by peracetic acid also occurs with




SCHEME 1

pyrocatechol,[2] *o*-benzoquinone,[3] and coumaric acid [4] to give the same diacid (1). *o*- and *p*-Cresols,[5] protocatechuic acid,[6] 1- and 2-naphthols,[7] and 1,2-naphthoquinone [8] react in a similar manner with the splitting of an aromatic ring.

The oxidation of phenol to hexa-2,4-dienedioic acid is of interest because it is similar to various *in vivo* reactions. Thus *o*-diphenol oxidase [9] (1.10.3.1, *o*-diphenol–oxygen oxidoreductase; tyrosinase) catalyses the hydroxylation of phenol and substituted phenols to dihydroxybenzenes and their subsequent oxidation to *o*-quinones.[10] Also catechol 1,2-oxygenase [9] (1.13.1.1, catechol–oxygen 1,2-oxidoreductase; pyrocatechase) catalyses the aerobic oxidation of pyrocatechol, which is an important intermediate in aromatic degradation,[11] to the diacid (1).[12] Therefore, if the *in vitro* system proceeds from phenol *via* pyrocatechol to the diacid (1), we have a direct parallel to two well known enzymic reactions.

## RESULTS

*Identification of Intermediates.*—Some hours after the addition of phenol to 1·3M-peracetic acid the solution developed a golden brown colour and after several days a white solid precipitated. This was identified as hexa-2,4-dienedioic acid, which after three weeks at room temperature gave a yield of *ca.* 13%. Samples of the above solutions were removed and examined by t.l.c. using chloroform–methanol, which had previously been shown to give a good separation of the expected stable intermediates in the oxidation. Pyrocatechol, hydroquinone, and *p*-benzoquinone were identified by $R_F$ values and, following elution, by their u.v. spectra. In addition, analyses were carried out with $^{14}$C-labelled phenol in peracetic acid solution and the eluted sample from the chromatograph in each case diluted with inactive carrier. Recrystallised pyrocatechol, hydroquinone, and *p*-benzoquinone showed the predicted radioactivity, and autoradiography of the chromatogram confirmed the analysis.

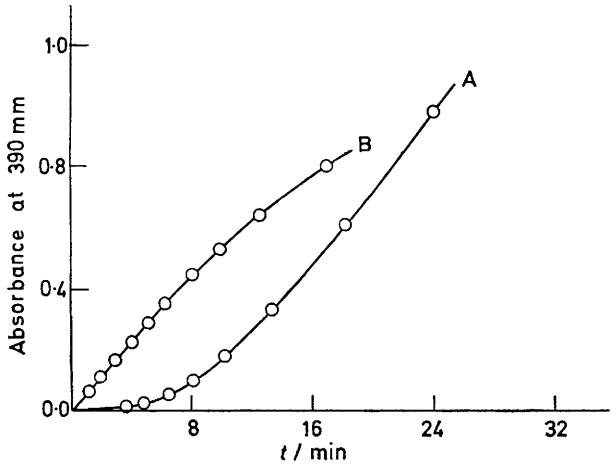
*o*-Benzoquinone decomposes in air at room temperature as well as in water,[13] so it is not expected to be isolable chromatographically. However, u.v. and visible spectra of phenol–peracetic acid solutions showed a peak at 390 nm corresponding to *o*-benzoquinone.[14] The rate at which the absorption increased was observed for different concentrations of phenol. The form of the plot of absorbance against time in each case indicated that an intermediate was formed between phenol and *o*-benzoquinone (curve A, Figure).[15] As pyrocatechol has already been shown to be an intermediate it is reasonable to assume that phenol is first oxidised to pyrocatechol, which is then oxidised to *o*-benzoquinone.

As confirmation of this hypothesis the oxidation of pyrocatechol by peracetic acid was examined by u.v. absorption spectroscopy. An absorption peak at 390 nm confirmed the formation of *o*-benzoquinone as an intermediate formed directly from pyrocatechol (curve B, Figure). The reaction was complete after *ca.* 18 h.

*Kinetics of the Reactions of Phenol, Pyrocatechol, and Hydroquinone.*—The progress of the oxidation of phenol by peracetic acid was observed by determining the peracid concentration by adding an excess of tin(II) chloride, followed by excess of iron(III) alum and titrating the resulting iron(II)

[1] J. Boeseken and R. Engelberts, *Proc. Acad. Sci. Amsterdam*, 1931, **34**, 1292.
[2] J. Boeseken, *Proc. Acad. Sci. Amsterdam*, 1932, **35**, 750.
[3] J. Boeseken and G. Sloof, *Proc. Acad. Sci. Amsterdam*, 1929, **32**, 1043.
[4] C. Grundman, *Ber.*, 1936, **69**(2), 1755.
[5] J. A. Elvidge, R. P. Linstead, and P. Sims, *J. Chem. Soc.*, 1951, 3386.
[6] L. R. Morgan, *J. Org. Chem.*, 1962, **27**, 1208.
[7] J. Boeseken and L. von Konigsfeldt, *Rec. Trav. chim.*, 1935, **54**, 313.
[8] J. Boeseken and G. Sloof, *Rec. Trav. chim.*, 1930, **49**, 91.
[9] ' Enzyme Nomenclature,' International Union of Biochemistry, Elsevier, London, 1965, p. 29.
[10] D. Kertesz and R. Zito in ' Oxygeneases,' ed. O. Hayaishi, Academic Press, New York, 1962, p. 307.
[11] D. T. Gibson, *Science*, 1968, **161**, 1093.
[12] O. Hayaishi in ' The Enzymes,' eds. P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press, New York, 1963, vol. 8, p. 353.
[13] ' Dictionary of Organic Compounds,' eds. I. Heilbron, A. H. Cook, H. M. Bunbury, and O. H. Hey, Eyre and Spottiswoode, London, 4th edn., 1965, p. 352.
[14] H. S. Mason, *J. Biol. Chem.*, 1949, **181**, 803.
[15] K. J. Laidler, ' Chemical Kinetics,' McGraw-Hill, New York, 1965, p. 324.

ion with potassium dichromate.[16] The decrease in peracid concentration was slow and a plot against time gave a straight line over 4 h; the second-order rate constant ($k_1$) for the reaction was calculated from the gradient as $4 \times 10^{-6}$ l mol$^{-1}$ s$^{-1}$.




Variation of absorbance at 390 nm with time for: A, solution of 1·71M-phenol in 1·84M-peracetic acid; B, solution of 0·0182M-pyrocatechol in 1·07M-peracetic acid

The second-order rate constant for the oxidation of 0·15M-pyrocatechol by peracetic acid, $k_2$, was calculated from the slope of the plot of the increase of *o*-benzoquinone concentration with time (Figure) at zero time; its variation with peracetic acid concentration is given in Table 1.

TABLE 1

Variation of the second-order rate constant $k_2$ for the reaction of peracetic acid with pyrocatechol, with peracetic acid concentration

| [Peracetic acid]/M | $10^5k_2$/l mol$^{-1}$ s$^{-1}$ |
|---|---|
| 4·47 | 0·10 |
| 1·5 | 1·4 |
| 1·2 | 1·8 |
| 0·92 | 2·7 |
| 0·61 | 3·2 |
| 0·31 | 5·1 |
| 0·15 | 5·5 |

The oxidation of 0·18M-hydroquinone by 1·1M-peracetic acid was followed by measuring the increase in absorbance at 410 nm due to the formation of *p*-benzoquinone.[17] The resultant plot was of a form similar to curve B in the Figure indicating that there was no stable long-lived intermediate between hydroquinone and *p*-benzoquinone. The second order rate constant $k_3$ was calculated as $4 \times 10^{-4}$ l mol$^{-1}$ s$^{-1}$.

*Kinetics of the Reactions of* o-*Benzoquinone.*—Since *o*-benzoquinone, which has been shown to be an intermediate in the reaction, is known to react with water and with pyrocatechol,[18,19] the rates of these reactions were investigated so that their significance compared with the reaction with peracetic acid could be assessed. When small amounts (*ca.* 1 mg) of *o*-benzoquinone were added to $8·9 \times 10^{-2}$ and 0·19M-peracetic acid solutions, the absorption peak at 390 nm due to *o*-benzoquinone disappeared with the formation of a new peak at 260 nm attributed to hexa-2,4-dienedioic acid.[20] The rate of decrease in absorbance at 390 nm was then observed in peracetic acid solutions of different concentration. The reaction was shown to be approximately first order with respect to both *o*-benzoquinone and peracetic acid and the appropriate second-order rate constant $k_4$ was calculated. For the more dilute peracetic acid solutions the rate of the reaction of the *o*-benzoquinone with water became significant; a value for this, calculated at the appropriate pH (see Discussion section), was therefore subtracted in the estimation of the rate constant $k_4$ (Table 2).

TABLE 2

The second-order rate constant for the reaction of *o*-benzoquinone with peracetic acid

| [Peracetic acid]/M | $10^3k_4$/l mol$^{-1}$ s$^{-1}$ |
|---|---|
| 1·33 | 6·9 |
| 0·77 | 7·8 |
| 0·58 | 7·0 |
| 0·41 | 8·5 |
| 0·21 | 8·5 |
| 0·039 | 9·6 |

A similar technique was used to determine the rate of disappearance of *o*-benzoquinone in water at four pH values. The reaction was found to be first order with respect to *o*-benzoquinone and the pseudo-first-order rate constant $k'_5$ was calculated (Table 3).

The rate of reaction of pyrocatechol with *o*-benzoquinone was investigated in a similar manner at pH 0·5 and with a large excess of pyrocatechol. The second-order rate constant $k_6$ was found to vary with pyrocatechol concentration (Table 4).

*Effect of Copper*(II) *Ion.*—The addition of copper(II) acetate to phenol–peracetic acid solutions had no visible effect on the reaction rate. However, when a concentrated solution was added to pyrocatechol–peracetic acid mixtures, they effervesced, gave out heat, and produced a fine black suspension. A similar reaction was brought about by other copper(II) salts and also by some other metal ions [cobalt(II) and titanium(IV)].

In more dilute solutions, when the copper(II) ion was present in merely catalytic quantities, the only permanent change in the u.v. and visible spectrum was that which could be attributed to the removal of pyrocatechol and the formation of hexa-2,4-dienedioic acid. The rate constant for the oxidation of pyrocatechol, $k_2$, which is that for the rate-determining step, was calculated and its increase shown to be approximately proportional to the copper(II) ion concentration (Table 5). The corresponding rate constant for the oxidation of *o*-benzoquinone was unaffected by the presence of this metal ion.

TABLE 3

Variation of the pseudo-first-order rate constant for the reaction of *o*-benzoquinone with water at different pH values

| pH | $10^3k_5'$/s$^{-1}$ |
|---|---|
| 7·0 | 0·78 |
| 4·0 | 0·56 |
| 2·2 | 0·14 |
| 1·7 | 0·13 |

Copper(II) ion also accelerated the oxidation of hydroquinone; with concentrated solutions greenish crystals,

[16] A. I. Vogel, 'A Textbook of Quantitative Inorganic Analysis,' Longmans, London, 3rd edn., 1962, p. 309.
[17] 'Organic Electronic Spectral Data,' eds. H. E. Ungnade and M. J. Kamlet, Interscience, New York, 1960.
[18] C. R. Dawson and J. M. Nelson, *J. Amer. Chem. Soc.*, 1938, **60**, 245.
[19] J. Doskocil, *Coll. Czech. Chem. Comm.*, 1950, **15**, 780.
[20] A. Sistrom and R. Y. Stanier, *J. Biol. Chem.*, 1954, **210**, 821.

possibly quinhydrone,[21] settled out within a few minutes. The second-order rate constant for the oxidation of hydroquinone by 1·1M-peracetic acid was calculated and the quantity of metal ion shown to have a marked effect (Table 5).

TABLE 4

The second-order rate constant for the reaction of *o*-benzoquinone with pyrocatechol

| [Pyrocatechol]/M | $k_6$/l mol$^{-1}$ s$^{-1}$ |
|---|---|
| 0·15 | 0·11 |
| 0·077 | 0·13 |
| 0·037 | 0·18 |
| 0·020 | 0·22 |

TABLE 5

The effect of copper(II) acetate upon the second-order rate constants for the oxidation of pyrocatechol and hydroquinone by peracetic acid

| [Copper(II)]/M | $k_2$ or $k_3$/l mol$^{-1}$ s$^{-1}$ |
|---|---|
| Pyrocatechol | |
| 0·00 | $4·2 \times 10^{-5}$ |
| 1·06 | $6·9 \times 10^{-5}$ |
| 4·22 | $14 \times 10^{-5}$ |
| 8·45 | $24 \times 10^{-5}$ |
| Hydroquinone | |
| 0·00 | $0·45 \times 10^{-3}$ |
| 10·0 | $3·0 \times 10^{-3}$ |
| 20·1 | $5·8 \times 10^{-3}$ |

## EXPERIMENTAL

*Identification of Products and Intermediates.*—Phenol (10 g, 1 equiv.) was dissolved in 1·42M-peracetic acid (225 ml, 3 equiv.) and after 3 weeks the white precipitate (2 g, 13%) was collected, washed, and dried. The fine white powder was insoluble in acid, but soluble in sodium hydrogen carbonate from which it liberated carbon dioxide. The m.p. of the unrecrystallised hexa-2,4-dienedioic acid was 194 °C (lit., 194 °C), but was lower on recrystallisation owing to part conversion into the *cis,trans*-isomer.[22] The u.v. absorption spectrum gave maxima at 260 and 227 nm in agreement with the literature.[20]

Of many solvent systems tried the most suitable for separating phenol and its oxidation products chromatographically was chloroform–methanol (24 : 1). The $R_F$ values for plated silica gel ($HF_{254}$) were hydroquinone (0·92), phenol (0·79), pyrocatechol (0·48), resorcinol (0·38), and *p*-benzoquinone (0·30). Portions (10 μl) were removed from mixtures of phenol (1 equiv.) and 1·3M-peracetic acid (3 equiv.) and chromatograms were developed with the solvent run in an ascending movement. Spots were identified by their u.v. fluorescence and eluted so that electronic spectra could be recorded: pyrocatechol,[23] $\lambda_{max.}$ 274 nm; hydroquinone,[17] $\lambda_{max.}$ 289 nm; and *p*-benzoquinone,[17] $\lambda_{max.}$ 244 nm. In addition to the above solvent system, a similar portion of the reaction mixture was developed in benzene–methanol–acetic acid (45 : 8 : 4) together with a pyrocatechol marker ($R_F$ 0·48). Again the eluted spot confirmed the presence of pyrocatechol.

Phenol (50 μCi; specific activity 32 mCi mmol$^{-1}$; Radiochemical Centre, Amersham) was dissolved in water to give a bulk solution. 10 μCi Were then added to a phenol–peracetic acid solution and samples (10 μl) removed as above. The spots on the chromatograms corresponding to pyrocatechol and hydroquinone were eluted with water and inactive carrier (*ca.* 2 g) added. After recrystallisation from aqueous solution the radioactivity of each solid was measured with a plastic phosphor scintillation counter connected to an I.D.L. Scaler 1700 with EHT 1700 V and a discriminator bias of 40 V. *p*-Benzoquinone was identified similarly, after elution with ethanol. The autoradiographs were produced with Ilford Industrial G fast *X*-ray film and an exposure time of 3 weeks.

U.v. and visible spectra were recorded on a Unicam SP 800 spectrophotometer and absorbances at specific wavelengths on a Unicam SP 500 or an E.E.L. spectrophotometer. Temperature was maintained constant where necessary by an Adkin cell holder.

*Kinetics.*—Phenol (12 g, 1 equiv.) was dissolved in 1·42M-peracetic acid (100 ml, 1 equiv.) and portions (5 ml) were removed at intervals. These were made up to 50 ml with water and 5 ml was added to acidified tin(II) chloride (0·0595M) (20 ml). The excess of tin(II) ion was then estimated by addition of excess of iron(III) and titration with potassium dichromate (0·00816M) (diphenylaminesulphonic acid as indicator).[16] All kinetic experiments were carried out at 25 ∓ 0·5 °C.

*o*-Benzoquinone was prepared by oxidising an ethereal solution of pyrocatechol with dry silver oxide.[24] Yields of between 40 and 80% were obtained by cooling the ethereal solution to −70 °C, rather than evaporating the ether. The red crystals were recrystallised twice by dissolving them in ether at room temperature and cooling to −70 °C. The solid was stable at this temperature for several weeks.

*o*-Benzoquinone blackened rapidly when removed from solution at room temperature, so that crystals (*ca.* 1 mg) were removed from their cold ethereal solution and added directly to the reactant (25 ml) without weighing. The ether introduced into the solution was negligible. The concentration of the *o*-benzoquinone was always so low compared with that of the other reactants that pseudo-first-order kinetic equations were applicable. A logarithmic plot of absorbance at 390 nm against time gave a straight line and it was thus possible to calculate the pseudo-first-order rate constant without a knowledge of the exact initial *o*-benzoquinone concentration.

The pH of the aqueous solution was adjusted with a Cambridge buffer tablet or by the addition of hydrochloric acid and was checked with an E.I.L. direct reading pH meter (model 23A).

Peracetic acid (40% aqueous) was obtained from Laporte Limited and was stored in the dark at 0 °C. It was handled with great care because of its corrosive properties and explosive nature at high temperatures. It was analysed by addition of excess of sodium iodide and titration of the liberated iodine with sodium thiosulphate.[25] All other reagents were B.D.H. AnalaR except for pyrocatechol, hydroquinone, and copper(II) acetate, which were recrystallised before use.

## DISCUSSION

Pyrocatechol, hydroquinone, and *p*- and *o*-benzoquinone have all been identified in mixtures of phenol and

[21] 'Chemistry of Carbon Compounds,' ed. E. H. Rodd, Cleaver-Hume, London, 1956, vol. IIIB, p. 709.

[22] J. A. Elvridge, R. P. Linstead, P. Sims, and B. A. Orkin, *J. Chem. Soc.*, 1950, 2235.

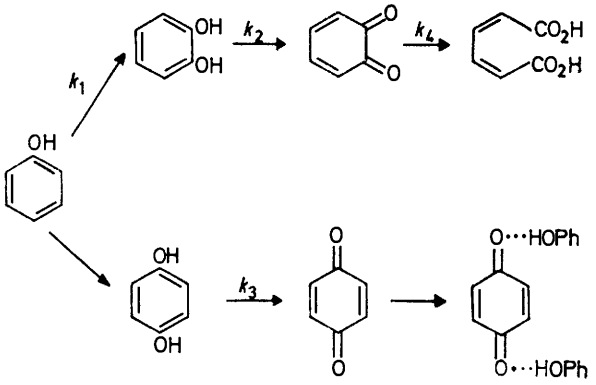
[23] L. Doub and J. M. Vandenbelt, *J. Amer. Chem. Soc.*, 1949, **71**, 2414.

[24] R. Willstätter and A. Pfannenstiel, *Ber.*, 1904, **37**, 4744; R. Willstätter and E. Muller, *ibid.*, 1908, **41**, 2581; E. Dyer and O. Baudisch, *J. Biol. Chem.*, 1932, **95**, 483.

[25] Ref. 16, p. 363.

peracetic acid and the existing evidence points to these compounds being intermediates in the phenol oxidation. Radioactive labelling of phenol confirmed that pyrocatechol, hydroquinone, and *p*-benzoquinone were produced from phenol and were not impurities or oxidation products of impurities. The increase of the rate of formation of *o*-benzoquinone with time (Figure) is compatible with the existence of an intermediate between the *o*-quinone and phenol, namely pyrocatechol. In addition, each of the identified intermediates has been shown to react with peracetic acid to give the next product in the scheme. It is thus reasonable to propose the reaction steps shown in Scheme 2.

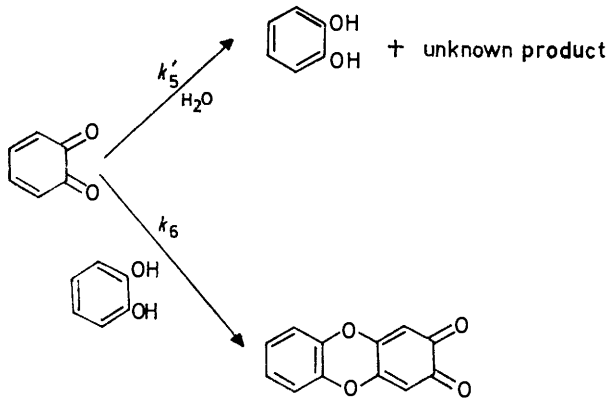
The second order rate constants for the reactions with *ca.* 1M-peracetic acid are: $k_1 = 4 \times 10^{-6}$, $k_2 = 2 \times 10^{-5}$, $k_3 = 4 \times 10^{-4}$, and $k_4 = 7 \times 10^{-3}$ 1 mol$^{-1}$ s$^{-1}$. The determination of the first of these is possibly affected by interference from products, but it provides an estimate




SCHEME 2

for the rate of hexa-2,4-dienedioic acid formation. The other reactions may be affected by pH or solvent effect on changing the peracetic acid concentration or could be of a more complex order. However, the numerical differences are sufficiently large for it to be stated that the first step (phenol to pyrocatechol) is the rate-determining step in the oxidation of phenol to the dicarboxylic acid (1). Similarly, in the reaction of pyrocatechol the oxidation of this reactant to the *o*-quinone is the rate-determining step.

At first sight it is surprising to find that the reaction proceeds *via* *o*-benzoquinone, which is known to undergo two other reactions in this system (Scheme 3). However, the rate of decomposition of *o*-benzoquinone by water decreases with decreasing pH. At the pH of 1M-peracetic acid the pseudo-first-order rate constant for the reaction is *ca.* $1{\cdot}4 \times 10^{-4}$ s$^{-1}$, whereas the equivalent rate constant for the reaction with 1M-peracetic acid is $7 \times 10^{-3}$ s$^{-1}$. Thus little *o*-benzoquinone will be diverted from its path to the diacid (1) by reaction with water at low pH. Dawson and Nelson also noted a change in reaction rate of *o*-benzoquinone with water over the pH range 4·0—5·5.[18] Their data combined with those obtained here fit approximately the relationship $k_5' = 0{\cdot}011\ [OH^-]^{0{\cdot}16}$ over the pH range 1·7—7·0.

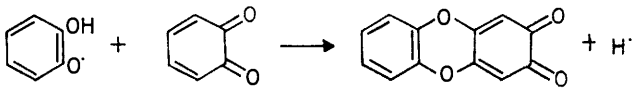



(3)

SCHEME 3

One of the products of the reaction of *o*-benzoquinone with water is pyrocatechol,[26] one molecule being produced for every two of the quinone which decompose.[19] The other product is not hydroxybenzoquinone,[19] which would have been expected if the reaction had followed a path comparable to the decomposition of 1,2-naphthoquinone.[27] However, at the concentrations of *o*-benzoquinone employed here ($\leqslant 10^{-4}$M) the secondary reaction between pyrocatechol and *o*-benzoquinone is not fast enough to affect the first-order integrated rate equation plot over the time periods studied.

A possible product of the reaction of pyrocatechol and *o*-benzoquinone is dibenzo[*b*,*e*]dioxin-2,3-quinone (3), the reaction proceeding possibly *via* the semiquinone radical (4).[28] E.s.r. spectra have shown the existence of the semiquinone radical and its mono- and di-protonated forms and their dependence upon pH.[29]



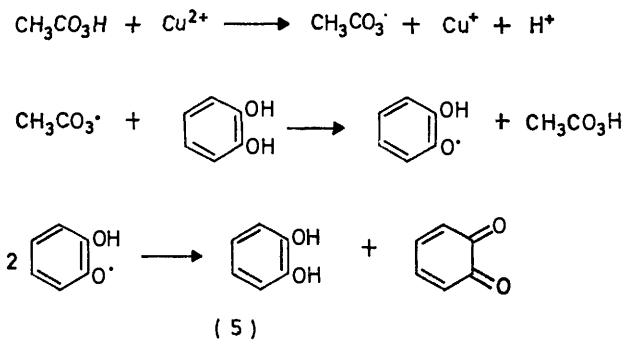

(4)

SCHEME 4

The catalytic effect of the copper(II) ion upon the reaction of peracetic acid and pyrocatechol can also be explained using the same semiquinone radical intermediate [30] (4), as shown in Scheme 5. The alternative mechanism involving a dihydroxybenzene–copper–peracid complex would usefully explain the pyrocatechol oxidation, but would not be applicable to that of hydroquinone.[31]

[26] O. Hayaishi, A. A. Patchett, and B. Witkop, *Annalen*, 1957, **608**, 158.
[27] L. F. Fieser and M. A. Peters, *J. Amer. Chem. Soc.*, 1931, **53**, 803; H. Wagreich and J. M. Nelson, *ibid.*, 1938, **60**, 1545.
[28] A. I. Scott, *Quart. Rev.*, 1965, **19**, 9; F. R. Hewgill, T. J. Stone, and W. A. Waters, *J. Chem. Soc.*, 1964, 408.
[29] I. C. P. Smith and A. Carrington, *Mol. Phys.*, 1967, **12–5**, 439.
[30] A. G. Davies, 'Organic Peroxides,' Butterworths, London, 1961, p. 177; A. R. Forrester, J. M. Hay, and R. H. Thomson, 'Organic Chemistry of Stable Free Radicals,' Academic Press, New York, 1968, p. 334.
[31] W. Brackman and E. Havinga, *Rec. Trav. chim.*, 1955, **74**, 937; 1021, 1070, 1100, 1107; E. Ochiai, *Tetrahedron*, 1964, **20**, 1831.

It has been argued by Boeseken [2] that pyrocatechol is not an intermediate in the oxidation of phenol because



(5)

SCHEME 5

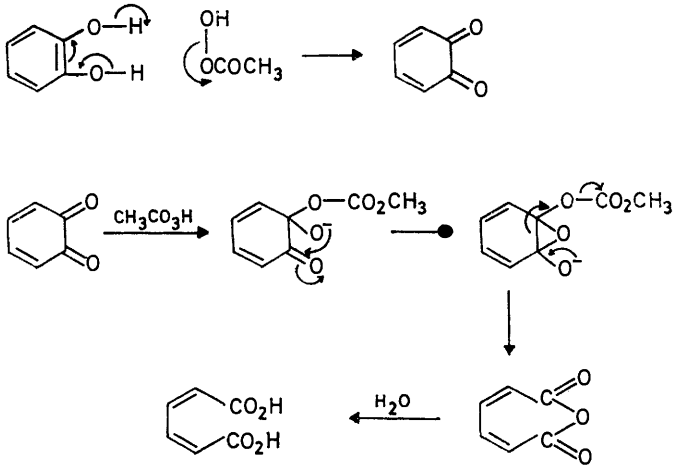
the yield of hexa-2,4-dienedioic acid is less from pyrocatechol than from phenol. However, a reduced yield would be expected from pyrocatechol because this would react more effectively with the intermediate *o*-benzoquinone. Under typical reaction conditions for the oxidation of phenol (0·5M-phenol; 1·3M-peracetic acid) the initial transient equilibrium concentration of pyrocatechol can be calculated to be *ca.* 0·1M. The rate of oxidation of *o*-benzoquinone by peracetic acid is therefore approximately comparable with that of the side reaction of *o*-benzoquinone with pyrocatechol. However, when the pyrocatechol concentration is as high as 1M as in the experiments of Boeseken [2] >90% of the *o*-benzoquinone would initially be diverted from its path to the diacid (1).

It should be noted that the yield of hexa-2,4-dienedioic acid will depend, amongst other factors, upon the phenol or pyrocatechol concentration, the peracetic acid concentration, the pH, and possibly the purity of the reactants with respect to metal ions.

The oxidation of phenols by peracetic acid can be compared superficially to two enzyme systems: first, to the copper-containing *o*-diphenol oxidase which aerobically converts phenol into *o*-benzoquinone *via* pyrocatechol; [10] secondly, to catechol 1,2-oxygenase which catalyses the absorption of one molecule of oxygen by pyrocatechol to give the diacid (1).[12] It would be particularly interesting to provide an analogy for this latter ring fission, because only a few methods of chemically splitting the benzene ring are known.[32] It is therefore disappointing to conclude that the first stages at least of the reactions are different. Thus we have established that *o*-benzoquinone is a discrete intermediate in the peracid oxidation of pyrocatechol whilst all attempts to detect this intermediate in the *in vivo* system have failed.[26,33] A mechanism put forward for this reaction involves a complex of iron, oxygen, and pyrocatechol on the enzyme surface with a peroxide intermediate.[11,34]

A likely mechanism for the *in vitro* oxidation of pyrocatechol is shown in Scheme 6, where the second step is similar to the path already established for the cleavage of benzils.[35]

Several model systems for aromatic hydroxylation have been carefully studied: namely Fenton's reagent [iron(II) ion and hydrogen peroxide],[36] Hamilton's system [hydrogen peroxide in acid solution with catalytic quantities of iron(III) or copper(II) ions and pyrocatechol],[37] Brackman's system [copper(II) ion, oxygen, and morpholine],[31] and Udenfriend's system [iron(II) ion, EDTA, ascorbic acid, and oxygen].[38] It has been pointed out that the most useful model would be one that starts with molecular oxygen as occurs with the



SCHEME 6

*in vivo* hydroxylation.[39] Peracetic acid is thus potentially a much less satisfactory model than that, for instance, of Udenfriend. However, the oxidation by peracetic acid of pyrocatechol to the *o*-quinone, particularly as it is accelerated by copper(II) ion, may be worth further investigation as a model for the action of *o*-diphenol oxidase.

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