acter depends on the original disruptive method. This would mean that the 12 unit length deduced by Haworth[1] for the dextrin chain is an average value, and may vary with the disruptive method used.

Form factors less than unity are very seldom encountered and in the present work may be due to the uncertainties involved in the extrapolation of the diffusion constant to zero time. On the other hand, if further work should prove the extrapolation to be valid, a form factor less than unity may indicate a molecule with the shape of a prolate or oblate spheroid with rudders to stabilize an otherwise unstable orientation in the liquid.[9]

(9) C. W. Oseen, "Hydrodynamik," Akademische Verlagsgesellschaft m. b. H., Leipzig, Germany, 1927, p. 188.

Summary

1. The molecular weights of α-amylo dextrins prepared from substrates dispersed by three different methods from the same sample of potato starch varied from 8600 to 29,100.

2. The heterogeneity of the dextrins closely paralleled that of the amylose precursors.

3. In general they exhibited less departure from sphericity than the original amyloses. The evidence is interpreted to indicate the presence of an unattackable nucleus in the molecules of the amylose dispersion, the character of which varies widely depending upon the original methods utilized for disruption of the granule.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

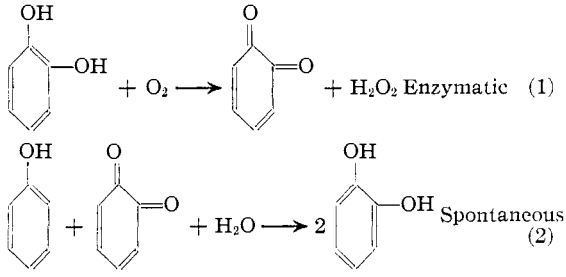
On the Oxidation of *p*-Cresol by Means of Tyrosinase

BY CHARLES A. BORDNER AND J. M. NELSON

The oxidase, tyrosinase, generally is regarded as capable of catalyzing two essentially different aerobic oxidations: first, the insertion of a hydroxyl group in a monohydric phenol, ortho to the one already present, and second, the oxidation of certain *o*-dihydric phenols to their corresponding *o*-quinones.

The rate of this enzymatic oxidation of an *o*-dihydric phenol, such as catechol, has a maximum value at the beginning of the reaction, while the initial rate in the case of a monohydric phenol is usually autocatalytic. This initial lag in the rate of oxygen uptake in the oxidation of monohydric phenols can be overcome by the addition of a trace of an *o*-dihydric phenol, such as catechol. This influence of catechol in accelerating the initial rate of oxidation of a monohydric phenol, such as *p*-cresol, has given rise to several theories concerning the mechanism of the reaction or reactions involved.

Among these theories that of Onslow and Robinson[1] seems to have been most widely accepted.[2] According to this view the monohydric phenol is oxidized spontaneously to the *o*-dihydric condition by *o*-quinone formed by the enzymatic oxidation of catechol.



Califano and Kertesz[3] even claim to have confirmed reaction (2) experimentally.

On the other hand, Pugh[4] claims that *o*-quinone, formed by oxidizing catechol in the presence of *p*-cresol by peroxidase and hydrogen peroxide, is unable to oxidize the cresol. Unfortunately her claim is weakened due to *o*-quinone and hydrogen peroxide being incompatible under these conditions as shown by Dawson and Ludwig.[5]

To overcome this objection, Miss Pugh's experiment has been repeated by oxidizing catechol to *o*-benzoquinone in the presence of phenol using an oxidase from sweet potatoes, *Ipomoea batatas*, instead of peroxidase and hydrogen peroxide. This oxidase has practically no action on phenol. By using the Warburg respirometer, it was found that the same amount of oxygen, 2.09 atoms per mole of catechol, was consumed when 1 mg. of

(1) M. W. Onslow and M. E. Robinson, *Biochem. J.*, **22**, 1327 (1928).

(2) (a) D. Keilin and T. Mann, *Proc. Roy. Soc.* (London), **125B**, 187 (1938); (b) F. Kubowitz, *Biochem. Z.*, **299**, 32 (1938).

(3) L. Califano and D. Kertesz, *Nature*, **142**, 1036 (1938).

(4) C. E. M. Pugh, *Biochem. J.*, **23**, 456 (1929).

(5) C. R. Dawson and B. Ludwig, THIS JOURNAL, **60**, 1617 (1938).

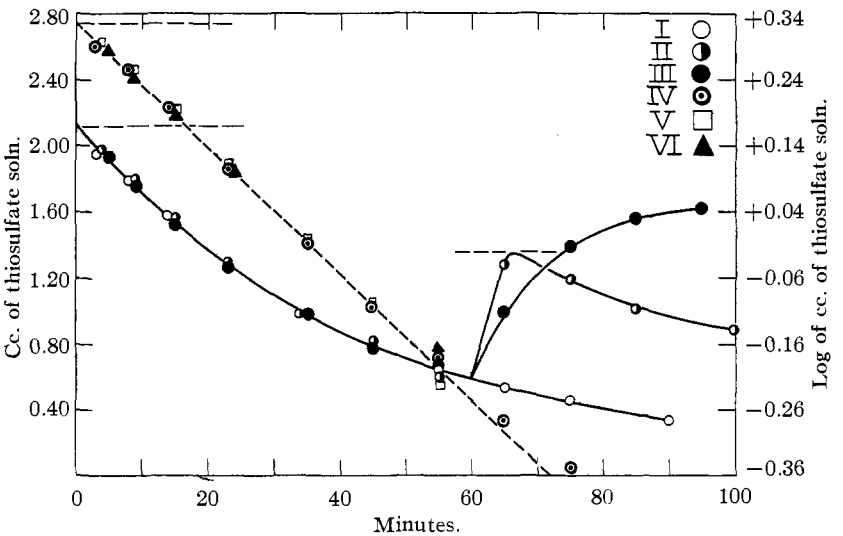


Fig. 1.—Showing that the rate of disappearance of homoquinone from an aqueous solution is not influenced by the presence of tyrosinase and *p*-cresol: temp., 25°; acetate buffer, *p*H 4.7. Curve I (ordinates to the left) represents the rate of disappearance of homoquinone in the absence of tyrosinase and *p*-cresol. Curve II shows the rate of disappearance of the homoquinone when tyrosinase was present. Curve III shows the rate of disappearance of the quinone in the presence of both tyrosinase and a mole equivalent of *p*-cresol.

The apparatus consisted of a reaction vessel (immersed in the thermostat) provided with three side-arms and a pipetting arrangement whereby samples could be withdrawn and introduced, under the surface of 25 cc. of sulfuric acid, contained in an Erlenmeyer flask, without coming into contact with air. A stream of oxygen-free nitrogen was used to stir the solution and in pipetting the samples. A stream of oxygen, instead of nitrogen, was passed through the reaction mixture at the end of sixty minutes. All the reaction mixtures contained, besides modifications indicated below, 10 cc. of freshly prepared homocatechol (17.22 mg.) solution, 0.1 *M* acetate buffer, and sufficient freshly boiled water to make the final reaction volume 300 cc. and *p*H 4.7. At zero time 6.15 cc. of 0.04512 *M* ceric sulfate (theoretical amount necessary to oxidize 17.22 mg. of homocatechol to homoquinone) in 0.18 *M* sulfuric acid and 8.85 cc. of water, previously mixed, were added from the side-arm. The system was freed of oxygen by evacuating five times to 25 mm. and refilling with nitrogen. At regular intervals 21.44-cc. samples were withdrawn and the homoquinone determined iodometrically according to the method of Dawson and Nelson (ref. 7). The theoretical amount of homoquinone (before any had disappeared) contained in the 21.44-cc. sample required 2.11 cc. of 0.00938 *N* sodium thiosulfate. The nitrogen used was freed from oxygen by first washing with 40% potassium hydroxide, followed by passing through concd. sulfuric acid and finally over heated copper.

Modifications of the reaction mixture described above. For curve I, no modifications. The admission of oxygen, at the end of sixty minutes, produces no change in the shape of the curve. For curve II, 5 cc. of tyrosinase preparation (70 cresolase units) in place of 5 cc. of water. The enzyme was added thirty seconds after the addition of the ceric sulfate. When oxygen was admitted at the end of sixty minutes, the amount of quinone increased to approximately one-half of the amount which had disappeared, indicating that homoquinone undergoes the same reaction in water as that observed by Wagreich and Nelson[12] in the case of *o*-benzoquinone. For curve III same as for curve II, except 15 cc. of water was replaced by 15 cc. of *p*-cresol (15 mg.) solution. The *p*-cresol was added from a side-arm at the same time as the enzyme. The admission of oxygen at the end of sixty minutes showed that the enzyme was still active.

Curves IV, V and VI were obtained by plotting the log of the number of cc. of thiosulfate solution used against time. The straight line obtained indicates that the disappearance of the homoquinone is a reaction of the first order.

The *p*-cresol was purified according to the method of Fox and Barker (*J. Soc. Chem. Ind.*, **37**, 268T (1918)). The purified product had b. p. 202° at 760 mm. The method of Hartmann and Gattermann (*Ber.*, **25**, 3531 (1892)) was used in the preparation of the homocatechol. The product was crystallized from a mixture of benzene and Skelly *D* gasoline, m. p. 62–63°.

The ceric sulfate had the composition $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$ and was obtained from the G. Frederick Smith Chemical Company. It was standardized against potassium dichromate, according to the method of R. P. Chapman (Dissertation, Columbia University, 1932, p. 23).

Tyrosinase preparations used throughout this study were prepared from the common mushroom, *Psalliota campestris*, by grinding the frozen mushroom, soaking in an equal volume of water, pressing out the water extract, precipitating with cold acetone, redissolving in water and precipitating with ammonium sulfate, redissolving in water and subjecting to fractional adsorption to alumina and finally dialyzing the elutate. The water solutions were practically colorless. Since tyrosinase acts both on monohydric phenols (*p*-cresol) and on *o*-dihydric phenols (catechol), the activity was determined with respect to both substrates. The preparations contained from 50–100 cresolase units and 200–500 catecholase units (Adams and Nelson units (ref. 14)) per mg. dry weight.

catechol was oxidized in the presence of 1 mg. of phenol and in the absence of phenol in the reaction mixture. When phenol alone was used practically no oxygen uptake occurred. Similar results were obtained when *p*-cresol was used in the place of the phenol. Thus the results obtained confirm the claim made by Miss Pugh that *o*-benzoquinone does not oxidize monohydric phenols and that reaction (2) above cannot be regarded as spontaneous.

Richter[6] has suggested that reaction (2) may be brought about by the presence in tyrosinase preparations of a monohydric phenol dehydrogenase which uses *o*-quinone as an acceptor. This view is untenable in the light of results obtained in the following way. Homoquinone (4-methyl-*o*-benzoquinone) was formed by oxidizing homocatechol (4 methyl-catechol) by an equivalent amount of ceric sulfate. Immediately after the addition of the ceric sulfate (less than one minute) *p*-cresol and tyrosinase were added and the rate of disappearance of the quinone from the reaction mixture was followed iodometrically by the method of Dawson and Nelson.[7] The reaction mixture was kept in an atmosphere of nitrogen. Controls were also performed determining the rate of disappearance of the homoquinone in the

(6) D. Richter, *Biochem. J.*, **28**, 90 (1934).

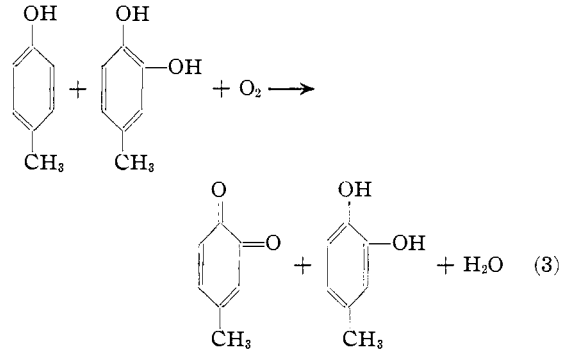
(7) C. R. Dawson and J. M. Nelson, THIS JOURNAL, **60**, 245 (1938).

absence of *p*-cresol and enzyme, as well as in the presence of enzyme only. The results obtained are shown graphically in Fig. 1. It will be noticed that the rate of disappearance of the quinone was not influenced in any way by the presence of the enzyme preparation or the *p*-cresol and enzyme.

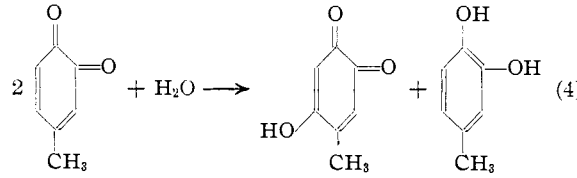
Judging from the recent literature, the question as to whether hydrogen peroxide is formed in the enzymatic oxidation of catechol and certain *o*-dihydric phenols as indicated in reaction (1) still is not settled definitely. Evans and Raper[8] offer as evidence for its formation in tyrosine–tyrosinase systems, the lowering of the ratio 3,4-dihydroxyphenylalanine produced over tyrosine oxidized when preparations of the enzyme used contained also peroxidase. If hydrogen peroxide is formed, then according to Dawson and Ludwig[5] it must be used up immediately since they were unable to detect its presence in the reaction mixture. Onslow and Robinson have offered the suggestion[9] that the hydrogen peroxide formed in reaction (1) can initiate the oxidation of the monohydric phenol to the *o*-dihydric, thus playing a role similar to that assigned to quinone in reaction (2). In support of this suggestion may be cited the observation reported by Cross, Bevan and Heiberg[10] that phenol can be converted into catechol by means of hydrogen peroxide and ferrous salts, and Raper,[11] by similar means, has succeeded in changing l-tyrosine to *l*-3,4-dihydroxyphenylalanine, phenylalanine to tyrosine, and tyramine to 3,4-dihydroxyphenylethylamine.

Experience in the present study, however, shows that in the enzymatic oxidation of monohydric phenols hydrogen peroxide is not used up in converting the latter into the *o*-dihydric form. To an aqueous solution containing *p*-cresol and hydrogen peroxide, and in an atmosphere of nitrogen, some tyrosinase was added and the rate of the disappearance of hydrogen peroxide followed iodometrically. It was found that the slow rate of disappearance of the hydrogen peroxide was the same as that of the control experiment in which neither *p*-cresol nor enzyme was present.

Since the shortening of the initial lag in the rate of oxidation of a monohydric phenol cannot be due to the action of either *o*-quinone or hydrogen peroxide as suggested by Onslow and Robinson, it becomes necessary to look for some other explanation as to how the oxidation of catechol by tyrosinase enables the enzyme to oxidize the monohydric phenol. Data obtained in the present study indicate that tyrosinase is unable to act directly on a monohydric phenol, such as *p*-cresol, unless it first is permitted to take part in the oxidation of an *o*-dihydric phenol. The data also coincide with the hypothesis that the tyrosinase must oxidize *o*-dihydric phenol before it can bring about the oxidation of the monohydric to the *o*-dihydric condition. In the light of this postulation a trace of *o*-dihydric phenol is necessary to initiate the oxidation of the monohydric phenol.



In order for the reaction to be autocatalytic the amount of the *o*-dihydric phenol present must gradually increase. This gradual increase of the *o*-dihydric phenol is just what would be expected since Wagreich and Nelson[12] have shown that *o*-benzoquinone undergoes a reaction in which half of the quinone is reduced back to *o*-dihydric condition, while the other half becomes oxidized to a hydroxy quinone which polymerizes.



Considering reactions (3) and (4) together it becomes evident that an ever-increasing amount of *o*-dihydric phenol is oxidized per unit of time, thus enabling the tyrosinase to act upon an ever-increasing amount of the monohydric phenol. As the rate of oxidation of the monohydric phenol increases autocatalytically, a maximum rate is finally reached when the enzyme is working at full capacity after which the rate remains prac-

(8) W. C. Evans and H. S. Raper, *Biochem. J.*, **31**, 2155 (1938).

(9) M. W. Onslow and M. E. Robinson, *Biochem. J.*, **19**, 420 (1925).

(10) C. F. Cross, E. J. Bevan and T. Heiberg, *Ber.*, **33**, 2015 (1900).

(11) H. S. Raper, *Biochem. J.*, **26**, 2000 (1932).

(12) H. Wagreich and J. M. Nelson, THIS JOURNAL, **60**, 1545 (1938).

tically constant for a considerable length of time, as noted by Califano and Kertesz and also by workers in these Laboratories.

According to the above view concerning the mechanism involved in the enzymatic oxidation of a monohydric phenol such as *p*-cresol, reaction (4) becomes the controlling factor in the early stages of the reaction. It should therefore be possible, by imposing conditions which would tend to vary the rate of reaction (4) or the rate at which *o*-dihydric phenol is furnished to reaction (3), to influence the magnitude of the initial lag period observed in the enzymatic oxidation of the monohydric phenol. Any conditions which tend to increase the rate of reaction (4) should furnish more *o*-dihydric phenol and thereby increase the rate of oxidation of the monohydric phenol in reaction (3). Such a condition would be expected to prevail as the reaction mixture is made more alkaline since Dawson and Nelson[7] have shown that under such conditions *o*-benzoquinone disappears more rapidly. The data in Fig. 2 show the decrease in the initial lag period as the oxidation of *p*-cresol is carried out under more alkaline conditions.

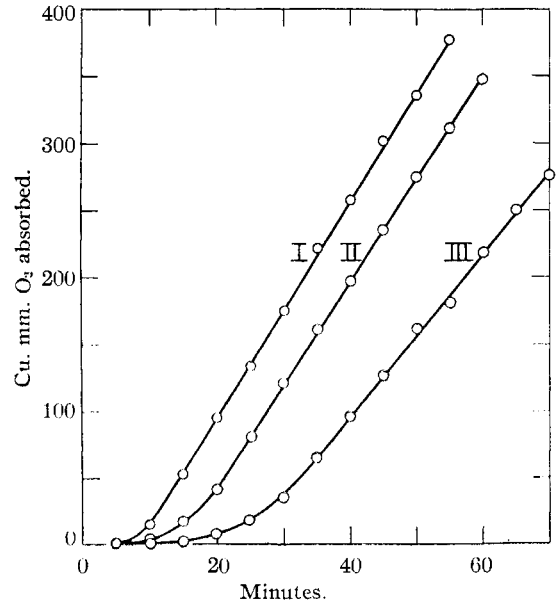


Fig. 2.—Showing the effect of varying hydrogen-ion concentration on the length of the lag period in the initial rate of oxidation of *p*-cresol by tyrosinase. Reaction mixtures used in the Warburg respirometer were the same as that corresponding to curve II in the legend for Fig. 3, except for *p*H of buffer and 0.8 cresolase units instead of 1.4. Values for *p*H corresponding to curves I, II and III were 7.6, 6.6 and 5.7, respectively.

The addition of reducing agents to a reaction mixture consisting of water, buffer, *p*-cresol, tyrosinase and gelatin[13,14] was found to reduce the initial lag period. This is to be expected since reducing agents would tend to reduce the *o*-quinone as it is formed in reaction (3) and thereby make available more homocatechol for inducing the oxidation of the *p*-cresol than would result from reaction (4). The influence of potassium ferrocyanide as the reducing agent is shown in Fig. 3. Shortening of the initial lag period was also observed when other reducing agents were used such as alanine, hydrogen peroxide and hydroquinone, all of which are known to reduce *o*-benzoquinone or homoquinone under these conditions.

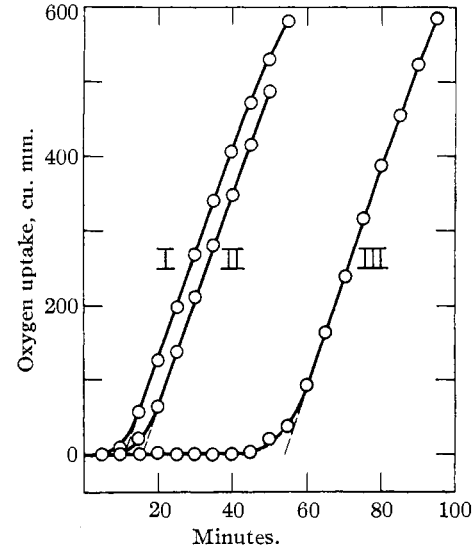


Fig. 3.—The effect of oxidizing and reducing agents on the length of the lag in the initial rate of the oxidation of *p*-cresol by tyrosinase. The rate of oxidation was determined by following the rate of oxygen uptake in a Warburg respirometer; temp. 25.°

Curve II. Reaction vessel contained: 1 cc. enzyme preparation (1.4 cresolase units), and sufficient water and citrate–phosphate buffer to make the final volume 9 cc. and approximately 0.1 molar with respect to buffer, *p*H 6.6. One cc. of *p*-cresol solution (4 mg.) was added from the side-arm at zero time. Since *p*-cresol is slightly volatile, care was taken to place its solution in the side-arm as late as possible.

Curve I. Reaction mixture was the same as for curve II, except that it contained 1 cc. of a freshly prepared solution of potassium ferrocyanide (0.0015 mmol.) in place of 1 cc. of water.

Curve III. Reaction mixture same as for curve I, except 1 cc. of a solution of potassium ferricyanide (0.0015 mmol.) in place of 1 cc. of potassium ferrocyanide solution.

The amounts of potassium ferri- and ferrocyanide used were equivalent to 8.4 cu. mm. of oxygen, and therefore could cause no appreciable effect on the oxygen absorption during the course of the reaction.

On the other hand, when agents capable of oxidizing catechol or homocatechol were added to the tyrosinase–*p*-cresol system the initial lag

(13) Gelatin or egg albumin was added to all reaction mixtures in order to stabilize the enzyme.

(14) M. H. Adams and J. M. Nelson, THIS JOURNAL, **60**, 2472 (1938).

period was lengthened. This effect again is what would be expected since the amount of homocatechol present in the reaction mixture would be decreased and thus retard reaction (3). The effect of potassium ferricyanide as the oxidizing agent is shown in Fig. 3; similar results were obtained with a suspension of manganese dioxide, and also by adding to the reaction mixture an oxidase (often termed laccase in the literature), which is known to oxidize under these conditions *o*-dihydric phenols, but has only a slight action on monohydric phenols, such as *p*-cresol.

The lengthening of the initial lag period in the oxidation of *p*-cresol by tyrosinase noted by Graubard and Nelson[15] when sodium borate was used as the buffer in the more alkaline range, *p*H 7–8, is what should be expected. It is known that borates tend to combine with *o*-dihydric phenols in slightly alkaline solution, *p*H 7–9, and therefore would tend to hold back the homocatechol, formed in reaction (4), from taking part in reaction (3).

Benzenesulfinic acid is known to combine with *o*-benzoquinone yielding an *o*-dihydroxydiphenylsulfone.[16] The presence of this reagent in a solution of *p*-cresol undergoing oxidation by means of tyrosinase should therefore not only tend to lengthen the initial lag period, due to preventing the quinone from forming *o*-dihydric phenol according to reaction (4), but also tend to limit the total oxygen uptake to two atoms per mole of *p*-cresol instead of three since the third atom of oxygen uptake is due to the quinone being oxidized to hydroxyquinone in reaction (4). Curves I and IV in Fig. 4 represent the rates as well as total oxygen consumed in the oxidation of 2 mg. of *p*-cresol by tyrosinase in the absence and presence of sodium benzenesulfinate, and show how the presence of the latter increases the lag period and limits the oxygen uptake to two atoms.

The fact that curve IV, representing the oxidation of *p*-cresol in the presence of benzenesulfinate shows a gradual increase in the rate of oxygen uptake for a considerable time before a constant rate is attained can be accounted for most logically by assuming a distribution of the homoquinone, formed in reaction (3), between water (reaction 4) and the benzenesulfinic acid. In other words, enough quinone changes over to the *o*-dihydric condition to increase gradually the rate of oxidation of the *p*-cresol in reaction (3) until the rate reaches the constant maximum value dependent on the capacity of the enzyme.

That a sharing of the homoquinone, formed in reaction (3), between water and the benzenesulfinic acid occurs is shown by the data represented by curves II, III and IV in Fig. 4. Thus curve II representing the oxidation of 2 mg. of *p*-cresol in the presence of one equivalent of the sulfinate (3.03 mg.) shows that this amount is not sufficient to limit the oxygen uptake to 2 atoms per mole of *p*-cresol, and that some of the homoqui-

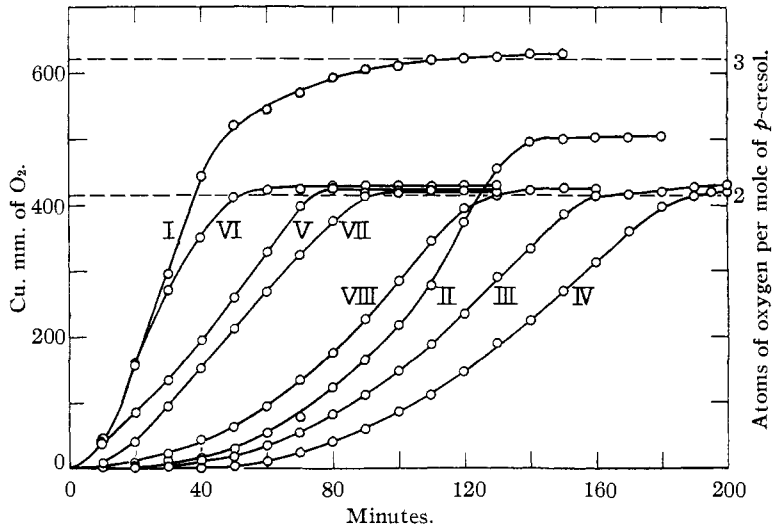


Fig. 4.—The effect of sodium benzenesulfinate on the oxidation of *p*-cresol by tyrosinase. Oxygen uptake determined by means of Warburg respirometer; temp. 25°. Curve I, reaction mixture: 2 cc. of citrate–phosphate buffer, 1 cc. of gelatin solution (5 mg. per cc.), 1 cc. of tyrosinase preparation containing 1.4 cresolase units, and sufficient water to make final volume in reaction vessel 9.5 cc. One-half cc. of *p*-cresol solution (4 mg. per cc.) was placed in the side-arm of reaction vessel and mixed with the reaction mixture as described in legend of Fig. 3. Reaction mixtures corresponding to curves II, III and IV contained in place of an equal volume of water 0.25, 0.5 and 1 cc., respectively, of a solution of sodium benzenesulfinate (12.14 mg. per cc.) equivalent to 1, 2 and 4 times the homoquinone produced. Curves V and VI correspond to reaction mixtures the same as for curve IV except the sulfinate was added four and eleven minutes, respectively, after the beginning of the reaction (when the *p*-cresol was transferred from the side-arm into the reaction mixture). Results represented by curve VII obtained by replacing 1 cc. of water in a reaction mixture, like that for curve IV, by 1 cc. of a freshly prepared solution of potassium ferrocyanide (0.0015 mmol.). In the case of curve VIII 0.25 cc. of a solution of hydrogen peroxide (0.00083 mmol.) in place of 0.25 cc. of water in a reaction mixture like that for curve IV. Hickinbottom's method ("Reactions of Organic Compounds," 1936, p. 349) for the preparation of benzenesulfinic acid was modified so that the sodium salt could be obtained directly from the iron salt by the addition of sodium carbonate to a suspension of the iron salt in water. The sodium benzenesulfinate was purified by crystallizing from alcohol.

(15) Mark Graubard and J. M. Nelson, *J. Biol. Chem.*, **111**, 757 (1935).

(16) O. Hinsberg and A. Himmelschein, *Ber.*, **29**, 2028 (1896).

none formed in reaction 3 is oxidized further to the hydroxyquinone via reaction (4). Curves III and IV, however, show that 2 and 4 equivalents, respectively, of the sulfinate cut down the amount of quinone undergoing the Cannizzaro reaction (4) to such an extent that no appreciable oxygen uptake beyond the 2 atoms occurs. It was pointed out in the earlier part of this paper that a trace of *o*-dihydric phenol is sufficient to overcome the initial lag in the oxidation of the *p*-cresol. Therefore, the autocatalytic nature of the oxidation of the *p*-cresol in the presence of an excess of the benzenesulfinate (curves III and IV) is most likely due to a trace of the quinone escaping the action of the sulfinic acid and forming a trace of homocatechol via reaction (4). In other words, in the oxidation of *p*-cresol the benzenesulfinate does not eliminate reaction (4) completely.

Since sodium benzenesulfinate retards the oxidation of *p*-cresol by removing the homoquinone, the addition of reducing agents such as hydrogen peroxide or potassium ferrocyanide should tend to compete with the sulfinic acid for the quinone by reducing the latter and thus making more homocatechol available for inducing the oxidation of the *p*-cresol in reaction (3). In other words, reducing agents should tend to counteract the retarding influence of the benzenesulfinic acid in the enzymatic oxidation of the *p*-cresol. Curves VII and VIII in Fig. 4 show this neutralizing effect by reducing agents on the retarding influence of the benzenesulfinic acid.

Pugh and Raper[17] reported that they were unable to oxidize phenol by tyrosinase when benzenesulfinic acid was present. The same experience has been encountered in these Laboratories. Recalling, however, that reducing agents, such as hydrogen peroxide, tend to offset the retarding action of the sulfinic acid in the oxidation of *p*-cresol by tyrosinase, it was decided to see whether the phenol could be oxidized by tyrosinase when benzenesulfinate was present if some hydrogen peroxide were added to the reaction mixture. It was found that the oxidation does take place under these conditions.

The most logical conclusion to be drawn from the fact that the enzymatic oxidation of phenol in the presence of benzenesulfinate requires the presence of a reducing agent while the oxidation of *p*-cresol under similar conditions does not, seems to be that the sulfinic acid tends to remove more completely the *o*-benzoquinone resulting from the oxidation of the phenol (reaction 3) than it does the homoquinone from the oxidation of the *p*-cresol. The exclusion of reaction (4) by the complete removal of the *o*-benzoquinone formed in reaction (3) by the benzenesulfinate prevents the formation of the extra catechol necessary for overcoming the initial lag period in the oxidation of phenol.

In connection with the influence of benzenesulfinic acid mention also should be made of the fact that the addition of sulfinate to the reaction mixture (water, phenol, tyrosinase and buffer *p*H 6.6) after the oxidation of the phenol has passed the initial lag period, stops the reaction within the lapse of a few minutes. This interruption of the oxidation of the phenol indicates that the *o*-dihydric phenol formed in reaction (3) is not sufficient to maintain the oxidation of the monohydric phenol, and that additional *o*-dihydric phenol must be supplied continuously to reaction (3) by means of reaction (4). In other words, the one molecule of *o*-dihydric phenol formed in reaction (3) when one molecule of monohydric phenol is oxidized to the *o*-dihydric condition is not quite sufficient to permit the reaction to continue.

Data have already been given (curves IV, Fig. 4) indicating that the benzenesulfinate does not completely exclude reaction (4) in the oxidation of *p*-cresol and that in this case sufficient homocatechol is formed via reaction (4) to accelerate the initial rate of oxygen uptake until the capacity of the enzyme is attained. This less drastic effect of the sulfinate in the case of the oxidation of *p*-cresol as compared to its influence on the oxidation of phenol is also borne out by the fact that the addition of the sulfinate to the reaction mixture in the case of *p*-cresol after the reaction has passed the initial lag period does not stop the reaction but permits it to continue practically at the same rate as if no sulfinate were present (Curves V and VI, Fig. 4). It also shows that excess of *o*-dihydric phenol, beyond that formed in reaction (3), necessary for the reaction to continue, at the rate corresponding to the capacity of the enzyme, must be very small.

Recently, Kubowitz[2b] has shown tyrosinase to be a copper protein, and claims that in the oxidation of catechol by the enzyme the copper is reduced by the catechol and then reoxidized by oxygen. The data given in the present paper do

(17) C. E. M. Pugh and H. S. Raper, *Biochem. J.*, **21**, 1370 (1927).

not appear to conflict with his theory, and if the theory is correct then it would appear that in the oxidation of monohydric phenols, the latter must first combine or form a complex with the cuprous copper before it is oxidized to the *o*-dihydric condition. It also follows that the lengthening of the initial lag period by oxidizing agents, in the oxidation of monohydric phenols, might be attributed to the oxidation of the cuprous copper instead of the *o*-dihydric phenol as suggested in the earlier part of this paper. The oxidation of the cuprous copper would tend to prevent the formation of the cuprous complex necessary for the oxidation of the monohydric phenol.

Summary

1. It has been shown that *o*-benzoquinone does not oxidize spontaneously phenol or *p*-cresol to their respective *o*-dihydric phenols.

2. Tyrosinase cannot catalyze the insertion of a hydroxyl group in *p*-cresol by hydrogen peroxide.

3. Tyrosinase cannot catalyze the oxidation of *p*-cresol by homoquinone.

4. The length of the initial lag in the rate of aerobic oxidation of *p*-cresol by tyrosinase was found to be: (a) shortened by reducing agents such as potassium ferrocyanide, alanine, hydroquinone and hydrogen peroxide, all of which reduce *o*-quinones; (b) prolonged by oxidizing agents capable of oxidizing homocatechol to homoquinone, such as potassium ferricyanide, a suspension of manganese dioxide and laccase; (c) prolonged by the addition of sodium benzenesulfinate; (d) shortened as the solution becomes more alkaline.

5. A mechanism has been offered concerning some of the reactions involved in the aerobic oxidation of monohydric phenols when catalyzed by tyrosinase.

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Relative Reactivities of Organometallic Compounds. XXVII. Triphenylthallium

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Introduction

The few simple organothallium compounds now known were made available in recent years,[1] and there is no report of their reaction with an organic functional group like carbonyl. On the basis of other evidence, the prediction was made that one of the R groups in R_3Tl compounds might be more reactive than the R group in a symmetrical R_2Hg compound.[2] This finds support in experiments reported at this time. For example, triphenylthallium reacts after the manner of moderately reactive organometallic compounds with compounds like benzaldehyde, phenyl isocyanate and benzoyl chloride. The essential absence of reaction, under corresponding conditions, with ethyl benzoate and benzonitrile suggests that the Entemann–Johnson series[3] of relative reactivities of some functional groups applies not only to phenylmagnesium bromide but also to triphenylthallium. However, there may not be a rigorous correlation. In the series established with phenylmagnesium bromide, benzophenone and benzoyl chloride are of approximately equal reactivity, but we observed with triphenylthallium a prompt reaction with benzoyl chloride and practically no reaction with benzophenone. Recent studies[4a] have shown that the order of reactivity of benzoyl halides with RM compounds that are less reactive than Grignard reagents is unlike the order established with phenylmagnesium bromide.

In reactions of Group III organometallic compounds with organic functional groups, the three R groups of R_3Al compounds participate; but only two of the R groups in R_3B compounds are involved under corresponding conditions, one of the products in the latter case being a monosubstituted boric acid,[4b] $RB(OH)_2$. With triphenylthallium, only one R group enters into reaction, and an invariable product is a diphenylthallium salt. We have now shown that triphenylaluminum[4c] and tri-*p*-tolylaluminum react much more readily with benzaldehyde than does triphenylthallium.

(1) (a) Groll, THIS JOURNAL, **52**, 2998 (1930); (b) Menzies and Cope, *J. Chem. Soc.*, 2862 (1932); (c) Birch, *ibid.*, 1132 (1934).

(2) See p. 437 of Gilman, "Organic Chemistry," John Wiley and Sons, Inc., New York, N.Y, 1938.

(3) Entemann and Johnson, THIS JOURNAL, **55**, 2900 (1933).

(4) (a) Gilman and Nelson, *ibid.*, **61**, 743 (1939); (b) Gilman and Marple, *Rec. trav. chim.*, **55**, 76 (1936); (c) **55**, 133 (1936).