A careful examination of the problems involved in the synthesis of N-methyl-*homo*-tetrahydroquinoline by the method of v. Braun and Seemann[9] led to the discovery that γ-(*o*-aminophenyl)-butyric acid, one of the later intermediates, could be prepared more conveniently by the Beckmann rearrangement of the *p*-toluenesulfonic ester of α-tetraloneoxime.[10] This method was adopted and the subsequent steps were carried out essentially as described by v. Braun and Bartsch[11] and by v. Braun and Seemann.[9]

α-Tetralone.—The following method was used for the preparation at a reasonable cost of the relatively large quantities of α-tetralone needed for the above-mentioned synthesis. Air is blown gently for a period of one hundred hours through a suspension of finely powdered copper oxide in tetralin maintained at 90°. The suspension is then allowed to settle and the liquid decanted. Upon fractional distillation at reduced pressure 80% of the tetralin is recovered and there is obtained in 20% yield a mixture of α-tetralol and α-tetralone containing 50–75% of the latter. This product is dissolved in an equal volume of acetic acid and treated in the cold with an equal weight of chromic acid dissolved in a small quantity of water. After the addition of chromic acid is complete, the solution is allowed to warm to room temperature and is maintained at room temperature for six hours. The product is then isolated by the usual methods, yield 90% based on tetralin consumed, b. p. 134° (15 mm.).

α-Tetraloneoxime.—The oxime was prepared using a technical grade of neutral hydroxylamine sulfate, recrystallized from 50% alcohol using a little Norite; yield 90%, m. p. 102°.

Summary

Some further examples of the inhibition of the acid catalyzed hydrogen exchange reactions of dimethylaniline derivatives by ortho substituents are reported.

In support of the theory that the ortho effect is primarily a steric effect having to do with the tendency of an ortho substituent to block the formation of quinonoid structures, it is shown that the inhibition of hydrogen lability caused by an ortho fluorine atom is much less than that caused by an ortho chlorine atom. A more decisive test of the theory is provided by a study of the exchange reactions of the cyclic bases, N-methylindoline, N-methyltetrahydroquinoline, and N-methyl-*homo*-tetrahydroquinoline. The first two of these, having bicyclic structures which are coplanar or nearly so, were observed to exchange hydrogen readily, while the third, in which the heterocyclic ring must be highly puckered, exhibited the inhibition of reactivity which is characteristic of ortho substituted tertiary amines.

(10) Schroeter, *Ber.*, **63**, 1323 (1930).
(11) Von Braun and Bartsch, *ibid.*, **45**, 3376 (1912).

Inactivation of Tyrosinase in the Oxidation of Catechol

BY B. J. LUDWIG AND J. M. NELSON

When catechol is oxidized by means of an excess of the oxidase, tyrosinase, two atoms of oxygen are consumed per mole of the substrate. If, however, less than a sufficient amount of the enzyme is used, then the enzyme becomes inactivated before the catechol is completely oxidized (curves I and III, Fig. 1).

The inactivation of one unit of catecholase[1] has been found to involve a definite amount of oxygen uptake. In fact the amount of oxygen required for the inactivation may be used as a quantitative measure of the amount of catecholase present in a given preparation of the enzyme, provided the latter has been purified sufficiently. Crude tyrosinase preparations[2] are apt to contain from the

plant, serving as the source, catechol or catechol derivatives and phenols which are aerobically oxidized by the enzyme, or tend to form with the latter inactive complexes. For this reason crude tyrosinase preparations often tend to lose activity on standing. These concomitant substances are usually lost when the purification has reached the stage, in the case of tyrosinase from the common mushroom, *Psalliota campestris*, when 1 mg. dry weight is equivalent to 80 or more catecholase units (for definition of units see legend Fig. 1). Sufficiently purified tyrosinase preparations from the last-mentioned source require for complete inactivation an uptake of 100 ± 5 cu. mm. of oxygen per catecholase unit (column 4, Table I).

It has been shown by Parkinson (unpublished) in these laboratories and also by others[3,4] that in

(1) Since tyrosinase catalyzes the oxidation of both catechol and *p*-cresol, the terms catecholase and cresolase have been used for the respective enzymic activities.

(2) M. W. Onslow, "Principles of Plant Biochemistry," University Press, Cambridge, England, 1931, p. 131.

(3) F. Kubowitz, *Biochem. Z.*, **299**, 32 (1938).
(4) D. Keilin and T. Mann, *Proc. Roy. Soc.* (London), **B125**, 187 (1938).
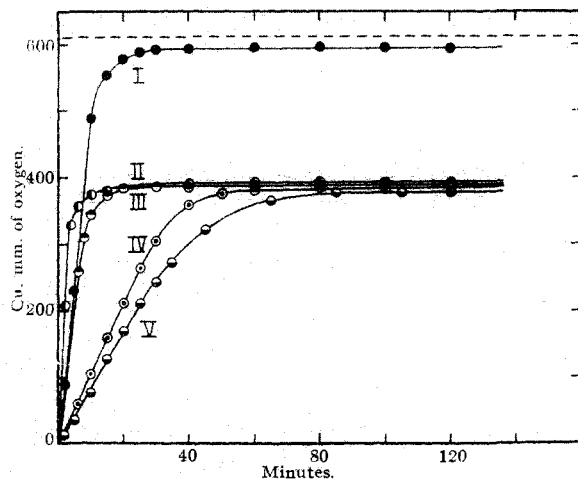
Fig. 1.—Showing oxygen uptake when catechol is oxidized aerobically in the presence of tyrosinase. Barcroft differential respirometer (ref. 5) used . Temp. 25°. Contents of control flasks differed from that in reaction flasks by substituting an equal volume of water in place of the enzyme solution. Curve I shows the complete oxidation (2 atoms of oxygen) of catechol when sufficient enzyme is used. Reaction mixture in reaction flask: 3 cc. 0.2 M phosphate buffer (pH of final reaction mixture 6.7), 1 cc. gelatin solution (5 mg.), 2 cc. tyrosinase solution (8.0 catecholase units) and sufficient water to make the final reaction mixture 8 cc.; 1 cc. catechol solution (3 mg.) was added from the side arm to the reaction mixture at zero time. Curve III shows inactivation of the tyrosinase when less enzyme (4 units) and more catechol (4 mg.) was used than in the experiment corresponding to curve I. Otherwise reaction mixture for experiment (curve III) same as that represented by curve I. After the expiration of ninety minutes 2 mg. more catechol was added to each of the two above reaction mixtures. In the case of the mixture corresponding to curve I, further oxidation took place, showing the enzyme to be still active, while in the case of the mixture (curve III) no further uptake of oxygen occurred, showing that the enzyme was no longer active. Curves II, IV and V are discussed in a later section of the text. They show that the amount of oxygen corresponding to complete inactivation is influenced neither by the length of time the enzyme is exposed to the reaction mixture nor by the rate at which the catechol is oxidized or by the concentration of the oxygen in the reaction mixture. The reaction mixtures used in these experiments were the same as that used in the experiment for curve III, but the composition of the gases in the reaction vessel differed: II 100% oxygen, III 21% O$_2$ (air), and IV 2% oxygen and 98% nitrogen. In the experiment corresponding to curve V, the air in the flask was replaced by nitrogen, the final traces of oxygen being removed by alternately evacuating and reflushing the flask with nitrogen. The catechol was then added to the main reaction flask and the latter shaken for forty minutes, after which time the nitrogen was replaced by a 2% oxygen–98% nitrogen mixture, and the oxygen uptake measured. 2% oxygen was used here, instead of air, in order to slow down the rate of the reaction and permit equilibrium conditions to be attained before appreciable oxygen consumption occurred. A comparison of the curves IV and V reveals that although a slightly different rate of oxygen consumption resulted, nevertheless, the total oxygen uptake, before inactivation of the enzyme was complete, was the same in the two experiments. The slower rate evidenced by curve V may be attributed to the initial lack of dissolved oxygen in the reaction mixture. The tyrosinase used in the above mentioned experiments was obtained from the common mushroom, *Psalliota campestris*, and contained about 320 catecholase units per γ of copper. For definition of catecholase units see paper by Adams and Nelson (ref. 6).

the case of reasonably purified preparations of tyrosinase the catecholase activity is proportional

(5) Dixon, "Manometric Methods," University Press, Cambridge, England, 1934.

(6) M. H. Adams and J. M. Nelson, This Journal, **60**, 2474 (1938).

to the copper content. It therefore follows that the amount of oxygen required for the inactivation of catecholase is proportional to the copper content (3.3×10^4 cu. mm. of oxygen per γ of copper, column 6, Table I). The first three values in column 6 are low and indicate that an appreciable amount of copper, inactive toward catechol, is present in preparations C101A, B and D. This may be due to foreign copper proteins being present in these relatively impure preparations as has been suggested by Keilin and Mann or to the presence of inactivated enzyme, removable by further purification.

The cresolase activity, determined according to the Graubard and Nelson method as modified by Adams and Nelson, appears to bear no relation to the amount of oxygen consumed in the complete inactivation of the enzyme (column 7, Table I).

In Part B, Table I, data are presented for tyrosinase preparations from sources other than *Psalliota campestris*. These data indicate that the amount of oxygen consumed for the complete inactivation of these tyrosinases is different. In the case of the wild mushroom, *Lactarius piperatus*, an oxygen uptake of 400 ± 15 cu. mm. is required for the inactivation of one unit (catecholase). Since Dalton (unpublished) has shown that the catecholase activity of the tyrosinase from this source is also proportional to the copper content (42 units per γ of copper), it follows that the total oxygen required to inactivate the amount of enzyme equivalent to one γ of copper, in this case, is about 1.7×10^4 cu. mm. This value is approximately one-half of 3.3×10^4 cu. mm., the corresponding value for the enzyme from *Psalliota campestris*.

This inactivation of tyrosinase resulting when the enzyme catalyzes the oxidation of catechol has been ascribed by several workers[7,8] to the o-benzoquinone formed in the oxidation of the catechol. The fact that the inactivation persists when the reaction is allowed to take place in the presence of sodium benzenesulfinate is an argument against this view. Bordner and Nelson[9] have shown that sodium benzenesulfinate combines almost quantitatively with o-benzoquinone, thereby rendering the latter inactive.

Further evidence that o-benzoquinone does not

(7) D. Richter, *Biochem. J.*, **28**, 901 (1934).

(8) C. R. Dawson and J. M. Nelson, This Journal, **60**, 250 (1938).

(9) C. A. Bordner and J. M. Nelson, *ibid.*, **61**, 1507 (1939).

TABLE I

Prepn.	Units enzyme per mg. dry wt.	Units enzyme per γ copper	Av. O$_2$ uptake per unit enzyme inactivated, cu. mm.	Total O$_2$ uptake per mg. dry wt., cu. mm.	Total O$_2$ uptake per γ copper, cu. mm.	Ratio: catecholase units/cresolase units
			Part A			
C101A	83	175	100	0.83×10^4	1.8×10^4	6.7
C101B	110	136	98	0.98	1.3	6.1
C101D	282	234	103	2.9	2.4	6.4
C101C	363	342	95	3.4	3.2	8.0
C101E	472	344	101	4.7	3.5	11.3
C95	500	322	95	3.2	3.1	3.1
C100	480	320	95	4.7	3.1	5.3
C102	445	365	96	4.3	3.5	8.3
C106	487	310	98	4.8	3.1	11.4
C94	...	...	185	...	...	2.2
C96	...	...	210	...	...	1.7
C97	75	...	162	1.2	...	2.6
			Part B			
Lactarius piperatus	98	42	402	3.9	1.7	0.1
Irish potato	...	...	190	...	...	.6
Sweet potato	...	...	320	...	...	...
Puff ball	...	...	750	...	...	.05
Dolichos lablab	...	...	>1500	...	...	1.2

All the tyrosinase preparations mentioned in Part A were obtained from the common mushroom, *Psalliota campestris* The fresh mushrooms were ground in a meat grinder, and the ground pulp frozen. The frozen material was then placed in an equal volume of water and allowed to stand overnight to thaw out. The material was then filtered, the residue pressed dry in a hydraulic press and the pressed juice and filtrate combined. The latter was treated with an equal volume of cold acetone, the precipitate filtered off, dissolved in water and made 0.6 saturated with ammonium sulfate. The precipitate from the ammonium sulfate treatment was redissolved in water and made *p*H 4.8 by adding 2% trichloroacetic acid. The precipitate thus obtained was centrifuged off, discarded and the supernatant liquid remaining was brought to *p*H 7 by means of dilute ammonium hydroxide. The solution thus obtained was treated with just enough alumina to adsorb about 15% of the enzyme together with some of the accompanying impurities. After filtering off the alumina, the filtrate remaining was made 0.6 saturated with ammonium sulfate, the precipitate formed filtered off and redissolved in a small amount of water and dialyzed against glass-distilled water. The dialyzed solution contained preparation C101A. The greater portion of the solution containing preparation C101A was treated with enough alumina to adsorb the enzyme completely. The enzyme was eluted from the alumina by means of disodium phosphate, filtered and the solution concentrated by making it 0.6 saturated with ammonium sulfate, filtering off the precipitate, redissolving in a small amount of water and dialyzing against glass-distilled water. The solution thus prepared contained preparation C101B. The major portion of the latter solution was adjusted to *p*H 7.4 by 0.1 *M* ammonium hydroxide, treated with a small amount of recently prepared tricalcium phosphate and filtered. The filtrate, which contained most of the enzyme, was made 0.4 saturated with ammonium sulfate, the precipitate obtained dissolved in water and the *p*H adjusted to 6.5 by 0.1 *M* ammonium hydroxide. The enzyme in the latter solution was adsorbed to alumina, eluted with disodium phosphate and dialyzed. The solution thus obtained contained preparation C101C. The tyrosinase in a portion of the solution containing the preparation C101C was adsorbed to alumina, eluted with disodium phosphate and the solution obtained made 0.3 saturated with ammonium sulfate. The precipitate obtained was dissolved in water and dialyzed. This solution contained preparation C101D. The filtrate remaining after the treatment of the solution from which preparation C101D was obtained, with 0.3 saturated ammonium sulfate still contained considerable enzyme which was not precipitated under those conditions. This portion of the tyrosinase was precipitated by making the above mentioned filtrate 0.4 saturated with ammonium sulfate. The precipitate thus obtained was dissolved in water, dialyzed and the resulting solution contained preparation C101E. Preparations C95, C100, C102 and C106 were obtained from different lots of mushrooms (*Psalliota campestris*), and were purified by procedures similar to those described above. Preparations C94, C96 and C97 were also from *Psalliota campestris* but only partly purified.

exert a destructive effect on tyrosinase is shown by the data represented graphically in Fig. 2. In one of these experiments (curve II) the enzyme was allowed to react with only a fraction of the amount of catechol necessary to inactivate it completely. The active enzyme remaining after the oxidation of this initial addition of catechol was shaken in the Warburg apparatus for forty minutes in the presence of the *o*-benzoquinone previously formed, and then an excess of catechol was added to the reaction vessel, whereupon further consumption of oxygen occurred. The total

oxygen uptake was found to be the same as in the case of the control (curve I) in which the enzyme was allowed to react on an excess of catechol at the start.

The inactivation of tyrosinase in the presence of an excess of catechol is not influenced by the rate at which the catechol is oxidized, hence the length of time at which the enzyme is exposed to the reaction mixture is without effect on its activity. This fact is brought out by comparing the oxygen uptakes necessary for inactivation when the oxidation took place rapidly in the pres-
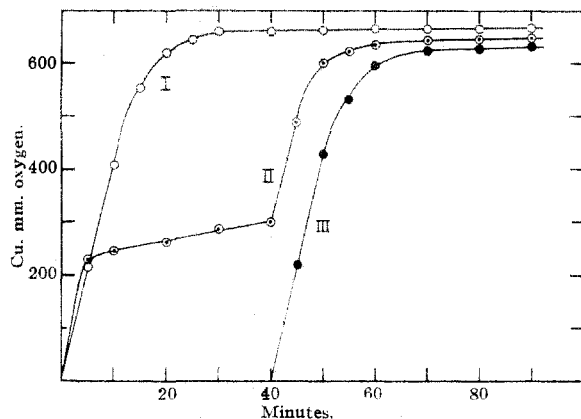
Fig. 2.—Showing that the products of the enzymatic oxidation of catechol are without destructive effect on the enzyme. Warburg respirometer, 25°. Reaction mixture: 2 cc. of tyrosinase solution (8 units), 3 cc. of 0.2 M phosphate buffer (final pH 4.3), 1 cc, of 0.5% gelatin (5 mg.) solution and 1.5 cc. of water. Final volume was 8 cc. The catechol (7.5 mg.) was added from a side-arm in experiment (curve I) at zero time; in experiment (curve III) the same amount of catechol was added after the rest of the reaction mixture had been shaken in the Warburg flask for forty minutes; in experiment (curve II) the catechol was added in two portions, one portion (2.25 mg.) was introduced at zero time by means of a "dangling tube" (Dixon[3]) and the remainder (5.25 mg.) from the side-arm after the expiration of forty minutes. At completion of the experiments further addition of catechol brought about no more oxygen uptake, showing the tyrosinase to be inactive. Tyrosinase from *Psalliota campestris* was used in the above experiment. A low pH was used because *o*-benzoquinone is less fugitive in acid solutions.[10]

ence of 100% oxygen and when the rate was slow in the presence of 2% oxygen and 98% nitrogen (curves II and IV, Fig. 1). Permitting the tyrosinase to remain in contact with the catechol and the other components of the reaction mixture, but in the absence of oxygen, had no inactivating effect on the enzyme. This fact is shown by Curve V, Fig. 1, which represents an experiment in which the enzyme was introduced into the reaction mixture in the absence of oxygen. After the expiration of forty minutes a mixture of 2% oxygen and 98% nitrogen was introduced and the oxygen uptake measured.

The data given in Table II show that using the amount of oxygen consumed in the inactivation of a given amount of enzyme as a measure, the inactivation is independent of the hydrogen-ion concentration between pH 5 and 7.5.

The temperature at which the inactivation is permitted to take place does, however, influence the amount of oxygen uptake required to bring about complete inactivation. At 25° the oxygen uptake, as already stated, is 100 ± 5 cu. mm. per unit for tyrosinase from *Psalliota campestris*. At 35° this value drops to about 80 cu. mm. of oxygen.

(10) C. R. Dawson and B. J. Ludwig. THIS JOURNAL, 60, 1617 (1938).

TABLE II

SHOWING THE INFLUENCE OF pH ON THE TOTAL OXYGEN CONSUMPTION PER UNIT OF CATECHOLASE (*Psalliota campestris*) INACTIVATED, WHEN THE LATTER WAS PERMITTED TO ACT ON AN EXCESS OF CATECHOL

Warburg respirometer; temp. 25°; reaction mixture: 1 cc. tyrosinase solution (4 units); 1 cc. gelatin solution (5 mg.); 3 cc. 0.2 M phosphate buffer solution (except for the first two in which phosphate–citrate was used); sufficient water to make final volume 8 cc.; 1 cc. catechol solution (4 mg.) added from the side-arm.

pH of reacn. mixt.	4.0	4.3	4.8	5.2	5.8
Cu. mm. O_2 per unit of enzyme inactivated	67	86	90	95	95
pH of reacn. mixt.	6.2	6.6	7.6	8.1	8.6
Cu. mm. O_2 per unit of enzyme inactivated	95	95	93	82	78

The oxidation of a definite amount of catechol is necessary for the complete inactivation of a given quantity of tyrosinase, irrespective of the concentration of the catechol in the reaction mixture. This was shown by oxidizing various amounts of catechol in the presence of an excess of a suitable reducing agent, such as ascorbic acid. Such a reducing agent converts the quinone as fast as it is formed to catechol, thereby maintaining a constant supply of catechol during the course of the reaction. Under these conditions it was found that although the rate of oxygen uptake was influenced by the amount of catechol present, the total oxygen consumed per unit of catecholase inactivated was independent of the concentration of catechol used, and was the same as when the enzyme was allowed to act in the presence of an excess of catechol (curves II and III, Fig. 3).

Tyrosinase from *Psalliota campestris* is incapable of catalyzing the oxidation of hydroquinone, but the latter like ascorbic acid reduces *o*-benzoquinone. Hence, through the medium of catechol, tyrosinase oxidizes ascorbic acid to dehydroascorbic acid and hydroquinone to *p*-benzoquinone. It is therefore possible to maintain a low concentration of catechol constant in the reaction mixture by means of hydroquinone, just as when ascorbic acid is used as the reductant. However, the presence of hydroquinone allows the reaction to continue for a longer time, and as a result, the total oxygen consumed before the enzyme is inactivated is much greater than that consumed when the enzyme is allowed to act in the presence of an excess of catechol (curves I and II, Fig. 3). This difference in behavior when these substances are used with catechol as the substrate makes it apparent that whereas both

function as reductants of *o*-benzoquinone and tend to keep the supply of catechol constant, hydroquinone, in addition, exerts a protective influence on the tyrosinase.

In order to gain further information concerning the nature of the protective action on the enzyme exhibited by hydroquinone under these conditions, a study was made of the effect of varying the ratio of hydroquinone to catechol on the rate of oxygen uptake and on the total amount of oxygen consumed during the inactivation of the enzyme. Using a constant concentration of catechol in the reaction mixture, it was found that the rate of oxygen uptake was practically independent of the amount of hydroquinone present. On the other hand, the total volume of oxygen consumed per unit of catecholase inactivated was found to increase with increasing amounts of hydroquinone.

Additional evidence has been obtained which suggests that the protective action shown by the hydroquinone might be due to a tendency of this substance to combine with the enzyme. It was found that benzoic acid, when added to the reaction mixture in concentrations equal approximately to that of the catechol used, produced a marked inhibitory effect on the rate of oxygen uptake. Yet the total oxygen uptake consumed per unit of catecholase inactivated was unchanged. This inhibition due to benzoic acid can be seen by comparing curves II and III with curves IV and V, respectively, in Fig. 3. Furthermore, upon adding to the reaction mixture a sufficient quantity of benzoic acid (*p*H adjusted to 7) to inhibit completely the enzymic action, and subsequently subjecting the reaction mixture to dialysis, it was found that the enzyme, dialyzed free of benzoic acid, had regained all of its original activity.

The behavior of the benzoic acid toward the tyrosinase–catechol reaction suggests that tyrosinase exerts its catalytic action by first combining with the substrate to form a complex, this combination being of such a nature that it is reactive toward molecular oxygen. In such a mechanism, benzoic acid acts as an inhibitor simply by competing with the substrate for the space available at the active enzyme center.

Only compounds of certain molecular configuration are capable of combining with the enzyme, the tendency being dependent on the molecular structure. Of those substances which can combine with the enzyme, only a few are capable of becoming activated sufficiently so that they are
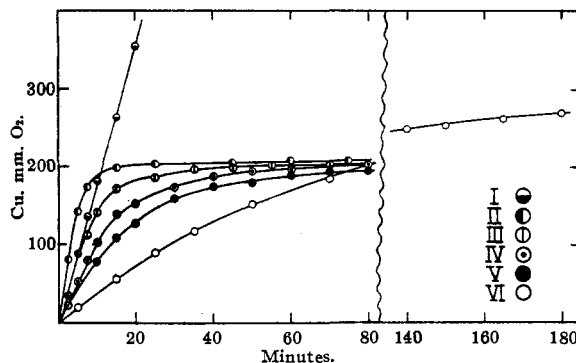

Fig. 3.—Showing the effects of ascorbic acid, hydroquinone, *p*-cresol and benzoic acid on the inactivation of tyrosinase. Tyrosinase prepared from *Psalliota campestris*. Temp. 25°, Barcroft respirometer (Dixon[9]). Curve II: inactivation of enzyme in the presence of an excess of catechol. Reaction mixture was 3 cc. of 0.2 M phosphate buffer (final *p*H 7.0), 1 cc. of gelatin (5 mg.), 1 cc. of tyrosinase solution (2 catecholase units), sufficient water to make final volume 8 cc. and 1 cc. of catechol solution (4 mg.) in side-arm. Curve III, showing that ascorbic acid only reduces the *o*-benzoquinone back to catechol without influencing the amount of oxygen uptake required for inactivation. Reaction mixture was same as for expt. II except a 1.5 cc. of solution containing 0.1 mg. of catechol and 5 mg. of ascorbic acid in side-arm. Curve I, showing the difference in effect on the reaction when hydroquinone is used in place of ascorbic acid as in expt. III. Reaction mixture was same as for expt. III except 5 mg. of hydroquinone in place of the 5 mg. of ascorbic acid. Curve IV shows the inhibiting influence of benzoic acid on the oxidation of catechol by tyrosinase. Reaction mixture was same as for expt. II except that the reaction flask contained in addition 1 cc. of 0.1 M sodium benzoate. Curve V, showing the inhibiting influence of benzoic acid on the oxidation of catechol in the presence of ascorbic acid. Reaction mixture was the same as for expt. III except that reaction flask contained in addition 1 cc. of 0.002 M sodium benzoate. Curve VI, showing the protective action against the inactivation of the enzyme by *p*-cresol. Reaction mixture was same as for expt. II except 1 cc. of a solution containing 4 mg. *p*-cresol and 0.1 mg. catechol in side-arm. In all experiments the contents of the side arm were transferred to the main reaction vessel at zero time, when the mixture had attained the temperature of the thermostat. The control flasks in each experiment contained the same reaction mixture except that water replaced the enzyme solution.

oxidized by the oxygen which also takes part in the complex. On this basis the difference in effect manifested by ascorbic acid, *p*-cresol, benzoic acid and hydroquinone on the tyrosinase–catechol reaction becomes clear. Ascorbic acid does not have a suitable configuration to permit it to combine with the enzyme molecule; hence when it is added to the tyrosinase–catechol reaction mixture, it behaves simply as a reductant of the *o*-quinone. On the other hand, according to some observations of Gregg (unpublished) *p*-cresol combines more readily with tyrosinase (*Psalliota campestris*) than does catechol. When the latter is added to a reaction mixture containing the enzyme and an equal amount of the cresol, the course of the reaction is practically the same as if no catechol[11] had been added. Benzoic acid

(11) Except the trace necessary to remove the initial lag period occurring in the enzyme oxidation of the *p*-cresol.

seems to combine with the enzyme to about the same extent as catechol, but is not sufficiently activated by the enzyme to become oxidized. Hydroquinone also combines with tyrosinase and like benzoic acid is not activated sufficiently, when combined with the enzyme, to become oxidized. Unlike benzoic acid, however, it resembles ascorbic acid, as has been mentioned above, in that it acts as a reductant toward o-benzoquinone. The protective action of the hydroquinone on the activity of tyrosinase may also be due to its tendency to combine with the enzyme. p-Cresol also tends to protect tyrosinase, as is shown by curve VI in Fig. 3, and it, too, as has been pointed out, seems to have a strong tendency to combine with the enzyme. The inactivation of the enzyme takes place in the process of the reaction and is not due to the reactants nor to the reaction products present in the reaction.

One substance which might be suspected of inactivating the enzyme in the tyrosinase–catechol reaction is hydrogen peroxide. The latter is known to inactivate many enzymes. Dawson and Ludwig,[10] however, were unable to detect any formation of peroxide in this reaction, and reached the conclusion that if it is formed it must be used up in performing some intermediary function at a rate faster than its rate of formation. Therefore, if hydrogen peroxide, or some activated form of oxygen, is responsible for the inactivation, the inactivation must occur during the process of the oxidation of the catechol. As a matter of fact, in the light of our present knowledge, the cause of the inactivation of tyrosinase, when it catalyzes the oxidation of catechol, is still unknown.

Summary

It has been found when tyrosinase, from *Psalliota campestris*, is inactivated in the oxidation of catechol, that:

1. The inactivation, at 25°, involves a constant uptake of oxygen: 100 ± 5 cu. mm. per unit of enzyme, or 3.3×10^4 cu. mm. per γ of copper.

2. This amount of oxygen is independent of: (a) the rate of oxidation of the catechol; (b) the concentration of oxygen; (c) the hydrogen-ion concentration between pH 5 and 7.5; (d) the ratio of catecholase activity to cresolase activity; (e) but is dependent on temperature.

3. The inactivation is not due to any products, known to be formed, in the oxidation of the catechol, but occurs at the time the catechol is oxidized.

4. Tyrosinase from different sources consumes different amounts of oxygen per unit of catecholase inactivated. The total oxygen consumed in the inactivation of purified preparations from *Lactarius piperatus*, at 25°, is 400 ± 15 cu. mm. per unit or 1.7×10^4 cu. mm. per γ of copper.

5. Hydroquinone, when used in excess with low concentration of catechol, besides serving as a reductant of o-benzoquinone, exerts a protective influence against inactivation of the enzyme. Ascorbic acid, also a reductant toward o-benzoquinone, exerts no protective influence.

6. Benzoic acid exerts a retarding influence on the activity of the enzyme toward catechol, but has no protective action.