8. A phenolic benzyl ether is formed with benzyl chloride and sodium alcoholate.

9. Millon's reagent gives a red color with it, a property of the hydroxyphenyl group.

10. A salt is formed when picric acid is added to its alcoholic solution.

11. It is easily darkened, dried, and hardened by means of manganese peroxide, barium peroxide, magnesium peroxide, litharge, manganese hydroxide and potassium dichromate.

12. It hardens at a temperature above 96° in the absence of its enzyme or any oxidizing agent.

13. It is slightly soluble in aqueous potassium hydroxide, but entirely soluble in alcoholic potassium hydroxide.

14. It reduced metallic salts especially silver nitrate on heating and ammoniacal silver nitrate in the cold.

15. It is precipitated by lead acetate, and forms a green precipitate with barium hydroxide.

16. On gradually adding alkali to its alcoholic solution a temporary green color is first produced which, on successive additions of alkali, turns red and brownish-red.

17. It forms a nitric compound with a violent reaction when concentrated nitric acid is added to it.

18. It is soluble in ether, chloroform, alcohol, methyl alcohol, benzin (b. p. below 60°), benzene, toluene, xylol, acetone, toluidine, pyridine, quinoline, carbon tetrachloride, amyl acetate, acetic ether, nitrobenzol, turpentine oil, glacial acetic acid, 80% solution of chloral hydrate and concentrated sodium and potassium hydroxide solutions.

19. It is precipitated from alcoholic solution by lead acetate, silver nitrate, mercurous nitrate, cupric acetate, ferric chloride, barium hydroxide, bromine, iodine, platinum chloride, gold chloride, uranium acetate, and copper nitrate.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MCGILL UNIVERSITY.]

THE PROPERTIES OF OXYNITRILASE.

BY VERNON K. KRIEBLE AND WALTER A. WIELAND.

Received August 12, 1920.

Rosenthaler, in 1908,[1] discovered that emulsin, when allowed to act on benzaldehyde and hydrocyanic acid, produced an optically active nitrile. That this reaction is catalyzed by an independent enzyme present in emulsin is very likely and the name oxynitrilase has usually been given to it. Rosenthaler in later papers showed that it is rather widely distributed, and one of us has shown that a particularly active form can

[1] Rosenthaler, *Arch. Pharm.*, **246**, 365 (1908).

easily be extracted from ordinary peach leaves.[1] Although a number of papers have appeared in connection with this enzyme, there are still several points which have not been cleared up. Armstrong and Bayliss,[2] for instance, report that they have not been able to get any asymmetric synthesis whatever, even though they have carefully followed the directions given by the original investigator. Nor has there been a satisfactory explanation for the fact noticed by Rosenthaler[3] that, as the reaction proceeds, the activity of the nitrile increases to a maximum and then declines again. This same phenomenon was noticed by Dakin[4] in the hydrolysis of inactive esters of mandelic acid by the action of lipase. He explained it by assuming that an intermediate compound was formed between the ester and the enzyme which later broke up into enzyme, mandelic acid and alcohol. But the rate of combination of the *l*-ester with the enzyme will be different from that of the *d*-ester and the enzyme. The same is true of the decomposition of the respective intermediate compounds, as they are not mirror images. Consequently the *l*- and *d*-esters are hydrolyzed at different rates and the mandelic acid liberated will be optically active during the early stages of the experiment, but later, as the reaction approaches completion, the mandelic acid will be inactive the same as the original mandelic ester.

We therefore considered it worth while to continue the study of oxynitrilase more particularly from the point of view of the rate of catalysis on hydrocyanic acid and benzaldehyde, as well as the rate of optically active nitrile produced.

Experimental.

The benzaldehyde and hydrocyanic acids were prepared and stored as described in previous papers. The enzyme was extracted from carefully sun-dried and preserved peach leaves. Before extraction they were ground in a coffee grinder and then in a mortar. Ten-g. samples of leaves were shaken with 240 cc. of distilled water in bottles on a shaking machine for 3 hours, after which the contents were filtered with suction through absorbent cotton. To each 100 cc. of the filtrate, 250 cc. acetone was added, which precipitated the enzyme as a flocculent precipitate. This was centrifuged for 3 minutes at 1500 revolutions per minute, which separated the enzyme. It was washed with alcohol and ether on a parchment filter paper in a Büchner funnel and dried in a vacuum over sulfuric acid to constant weight. This gave a light gray powder which was used direct without further purification.

The experiments were carried out in a glass jar of 750 cc. capacity.

[1] Krieble, THIS JOURNAL, **35**, 1643 (1913).

[2] Bayliss, "The Nature of Enzyme Action," 4th Ed., p. 75.

[3] Rosenthaler, *Biochem. Z.*, **50**, 490 (1913).

[4] Dakin, *J. Phys. Chem.*, **30**, 253 (1903).

It was fitted with a stirrer through a mercury seal and also contained a siphon through which samples could be taken by increasing the pressure inside the cell with nitrogen gas entering through another opening in the cork. This forced a certain amount of the contents into a beaker from which aliquots could be pipetted for analysis. It took less than 30 seconds to get a sample.

In order to follow the rate of total nitrile formation, we estimated the free hydrocyanic acid by a method devised by Wirth. It consists in adding a definite volume of the solution to be analyzed to standard silver nitrate solution containing nitric acid, which arrests the chemical action and precipitates silver cyanide. The excess of silver nitrate can then be titrated with ammonium thiocyanate, using a ferric salt as an indicator. In this way the amount of free hydrocyanic acid was determined.

As a certain amount of hydrocyanic acid and benzaldehyde combine spontaneously to form inactive nitrile, it is very essential, from the point of view of enzyme action, to know what per cent. of the total nitrile is optically active. It is, however, a somewhat difficult matter to estimate accurately the amount of optically active nitrile produced. It cannot be done directly, as the specific rotation of mandelonitrile is very low and not accurately known. It is therefore necessary to convert it into mandelic acid by hydrolysis. This can easily be done with hydrochloric acid. The method of carrying out this hydrolysis is important as mandelic acid is an α-hydroxy acid and easily forms an anhydride, which changes its optical activity. After trying a number of ways, we found it most satisfactory to add 15 cc. of our nitrile solution to an equal quantity of conc. hydrochloric acid in a glass-stoppered bottle. The stoppers were tied in and the bottles heated in a bath at 65°. Experiments showed that the hydrolysis was complete in 4 hours and that continued heating did not affect the optical activity of mandelic acid in the slightest. We, therefore, heated them there overnight, after which the optical activity was measured directly in a 2-dcm. tube at room temperature and recorded in all tables as α. No doubt one could determine the specific rotation of the mandelic acid better if it were extracted and dissolved in a smaller amount of water, but we have never been able to do it satisfactorily because the mandelic acid always becomes partly converted into its anhydride, so that it is impossible to get constant values in this way. With the exception of the very dilute mandelic acid solutions, our adopted method gave quite satisfactory results. Before we could calculate the per cent. of optically active mandelic acid and, therefore, of the original mandelonitrile, it was necessary to know the specific rotation of mandelic acid in the above hydrochloric acid solution. We obtained optically active mandelic acid by hydrolyzing amygdalin. After 2 recrystallizations from benzene, a 2.45% solution at 20.6° gave a specific rotation of

—150.6°; Landolt gives —153.76° under the same conditions. We next measured the specific rotations of this acid at various concentrations dissolved in a solution made up of 50% water and 50% conc. hydrochloric acid, with the following results.

% strength.	α.	Sp. rotation.
4	—1.31	—163.75
2	0.66	—165.00
1	0.33	—165.00

We next tried 45% water, 5% alcohol and 50% hydrochloric acid as a solvent, but the specific rotation was the same. We then heated the above solution for 3 hours, but the specific rotation did not change. These experiments were necessary, as most of our reacting solutions had 10% of alcohol present or 5% in the hydrolyzing solution. The specific rotation of our mandelic acid was, therefore, —164.58° in 18% hydrochloric. But as our acid was not quite pure according to Landolt, as it had a specific rotation of —150.6° instead of —153.76°, we therefore corrected the —164.58 by the following proportion: 150.61 : 153.76 :: 164.58 : X. This brought the specific rotation to —168°, the value used in all our calculations. The actual amount of optically active mandelic acid in the hydrolyzed solution could then be calculated by the usual formula $c = \frac{\alpha \times 100}{168 \times l}$ where c is the concentration of mandelic acid, α the angle of rotation observed and l the length of the tube in decimeters. The total amount of mandelic acid present was known from the amount of hydrogen cyanic acid combined with benzaldehyde in the original solution, consequently, the per cent. of optically active acid present, or the per cent. of optically active nitrile in the enzyme solution could easily be calculated.

Experiment 1.

Cell contents: 462 cc. of H_2O; 3 cc. of C_6H_5CHO; 60 cc. of C_2H_5OH; and 75 cc. of HCN solution. Total volume, 600 cc.; temperature, 25°.

The benzaldehyde was always measured out with the same pipet, which had been calibrated and found to deliver 3.0879 g., and the hydrogen cyanide solution was made up to such a strength that 75 cc. contained the equivalent of the benzaldehyde or 0.7867 g. The water was added first to the cell and allowed to reach the temperature of the water-bath. During this time the cell was well washed out with nitrogen. The benzaldehyde was then added, dissolved in specially purified alcohol. The reason for using the alcohol was to insure rapid and complete solution of the benzaldehyde and a separate experiment showed it had little effect on the enzyme action. In the first experiment there was no enzyme present.

Time. Min.	Combination. %	Time. Hours.	Combination. %
5	2.30	3	22.75
15	3.00	7	39.80
30	5.95	24	65.00
60	9.70	48	74.18
120	18.40	72	75.80

Experiments 2 and 3.

These experiments were carried out like Expt. 1 except that 150 mg. of oxynitrilase was added to the water at the beginning of the experiment, and the cell contents were stirred for half an hour before the benzaldehyde was added.

Time. Min.	Comb. Expt. 2. %	Comb. Expt. 3. %	Expt. 2. α.	Expt. 3. α.	Opt. Act. Expt. 2. %	Opt. Act. Expt. 3. %
2	9.14	...	—0.06°	...	52.95	...
5	17.30	20.13	—0.15°	—0.14°	69.95	56.10
7	23.75	23.89	—0.21°	—0.22°	71.33	74.26
15	39.87	38.77	—0.37°	—0.36°	74.85	74.90
30	55.46	55.25	—0.53°	—0.52°	77.10	75.93
45	62.06	62.07	—0.58°	—0.58°	75.40	75.37
Hours.						
1	66.45	65.48	—0.62°	—0.63°	75.27	77.60
2	70.00	70.77	—0.65°	—0.66°	74.90	75.23
24	71.80	74.45	—0.38°	—0.38°	42.70	41.18
48	74.20	...	—0.21°	...	22.83	...

In Fig. 1 the rates of reaction are plotted for the 3 experiments, Curve 1 representing the reaction without enzyme, and Curves 2 and 3 for Expts. 2 and 3, respectively. It is obvious that the rate is very much increased by the enzyme and the last 2 columns in the table show that

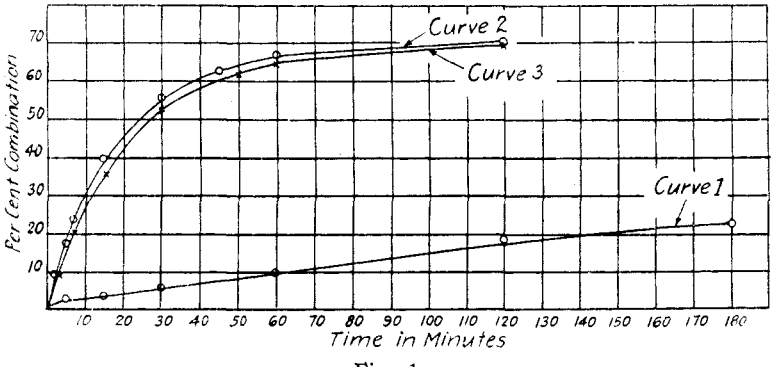

Fig. 1.

a very large part of the nitrile synthesized is optically active. It would appear from our results that the enzyme is not as efficient at the beginning of the experiment as later on. This is doubtful as the amount of optically active material is so small that it cannot be determined with

any great degree of accuracy. It is also possible that a certain amount of the hydrogen cyanide is combined with the enzyme which does not come off with silver nitrate in nitric acid. Consequently, the amount combined with benzaldehyde would be represented as too high and the per cent. of optically active nitrile calculated would, therefore, be lower than the amount actually present. We will frequently refer later on to Expts. 2 and 3 as the standard experiments.

Experiments 4 and 5.

In Expt. 4 the enzyme concentration was doubled and in Expt. 5 the amounts of benzaldehyde and hydrocyanic acid were doubled; the other conditions remained the same.

Time. Min.	Comb. Expt. 4. %.	Comb. Expt. 5. %.	Expt. 4. α.	Expt. 4. α.	Opt. Act. Nitrile. Expt. 4. %.	Opt. Act. Nitrile. Expt. 5. %.
2	18.3	6.10	—0.17°	—0.06°	74.9	39.7
5	37.4	10.25	—0.35°	—0.17°	75.5	66.9
10	52.0	17.70	—0.50°	—0.36°	77.56	82.1
15	59.7	25.30	—0.59°	—0.56°	79.7	89.3
30	68.1	42.55	—0.69°	—0.86°	81.73	81.5
45	70.5	52.50	—0.67°	—1.05°	76.67	80.9
90	72.85	68.00	—0.64°	—1.36°	70.9	80.7
Hours.						
3	73.77	75.50	—0.58°	—1.52°	63.4	81.2
24	73.37	79.00	—0.24°	—1.12°	26.4	57.2
48.5	...	79.25	...	—0.81°	...	41.2

As would be expected, the rate of reaction is nearly double during the first few minutes in Expt. 4 compared with Expts. 2 and 3. The same is true of α, although the per cent. of optically active nitrile is just about the same as in Expts. 2 and 3. Expt. 5 shows that oxynitrilase behaves like most enzymes in acting upon a definite amount of the substrate irrespective of its concentration, as α is the same as in Expts. 2 and 3 for the first few minutes. After that, however, α increases rapidly as compared to Expts. 2 and 3. This illustrates that the enzyme in Expts. 2 and 3 was working at its maximum capacity for only a few minutes. This was brought out better by Fig. 3 where Curves 1, 2 and 3

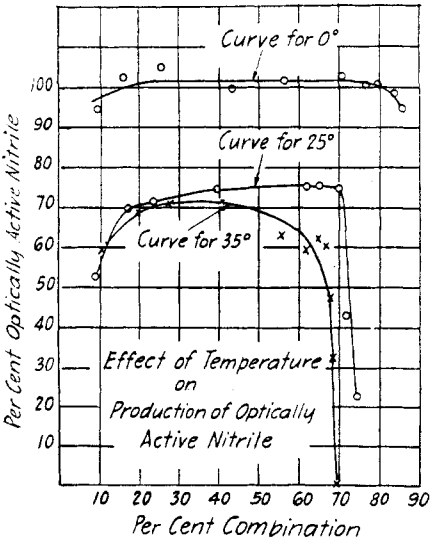

Fig. 2.

represent α plotted against time for Expts. 2, 4 and 5. We would have preferred to use this more concentrated substrate in our later experiments had it not been for the fact that it did not make a homogeneous solution, as there was benzaldehyde undissolved 15 minutes after the experiment was begun. We, therefore, continued our work with the lower concentration of benzaldehyde and hydrocyanic given in Expt. 2.

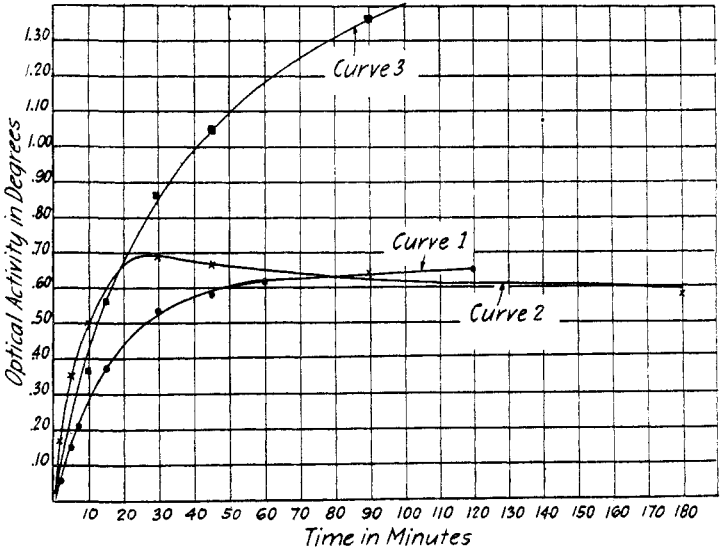

Fig. 3.

We next tried the effect of temperature on the reaction. The details were the same as in Expts. 2 and 3 except that the temperature was 35°.

Experiment 6.

Time. Min.	Combination. %.	α.	Optically Active Nitrile. %.
2	10.9	—0.08°	59.2
5	19.8	—0.17°	69.2
7	27.2	—0.24°	71.15
10	33.8	—0.31°	74.0
15	41.85	—0.37°	71.3
30	56.15	—0.44°	63.2
45	62.00	—0.46°	59.8
Hours.			
1	64.8	—0.49°	62.55
1.5	66.6	—0.50°	60.5
6.3	67.85	—0.41°	48.7
6.5	68.85	—0.26°	32.8
24	69.55	0.00°	0.00
48	...	0.00°	0.00

It is surprising that the rate of combination is about the same as in the standard experiment although the temperature is 10° higher. The

optical activity represented by α is about the same as in Expt. 2 for the first 3 points, after which it gradually falls behind. The percentage of optically active nitrile never reaches the same maximum as in all the other experiments and, at the same time, starts much earlier in the reaction. It is evident that at this temperature compared with 25° we were getting some differentiation either between the spontaneous combination and that catalyzed by the enzyme or else between the reactions caused by different enzymes.

Expt. 7 was tried at 0° without any enzyme; the other conditions were the same as for the standard experiment. The rate was very slow: at 2 hours 2.5% of hydrocyanic acid had combined, and at 24 hours 10.6%.

The following experiment differed from Expt. 7 in having 150 mg. of enzyme added to it.

Experiment 8.

Time. Hours.	Combination. %.	α.	Optically Active Nitrile. %.
0.25	9.4	—0.11°	94.4
0.5	16.5	—0.21°	102.6
1	31.4	—0.38°	97.6
2	46.2	—0.58°	101.2
3	56.5	—0.71°	101.4
6	70.8	—0.90°	102.5
9	76.8	—0.96°	100.8
12	79.5	—0.99°	100.4
24	83.5	—1.02°	98.3
48	85.22	—1.00°	94.6

In a duplicate experiment we got practically the same results. As was to be expected, the rate was much faster than in Expt. 7 but slower than in Expts. 2 and 3. From van't Hoff's rule the rate should be $^1/_6$ of the rate at 25°. It is more nearly $^1/_4$ to $^1/_3$, and it is of interest to note that it is roughly $^2/_5$ of that found at 25°. The second interesting point is that α instead of reaching a maximum in an hour or two (usually attained at 65 to 70% combination) and then declining, increases **for** 24 hours and drops very little even in 48. The most interesting point, however, is that the nitrile produced is 100% *levo* nitrile and remains so for 24 hours while in Expt. 6 the amount of *levo* nitrile never exceeded 75% and at the end of 24 hours it was completely inactive. This proves conclusively that we are dealing with but one oxynitrilase, namely, *dextro* oxynitrilase which synthesizes only *d*-mandelonitrile, which on hydrolysis yields *l*-mandelic acid. It also gave us the first clue as to the cause of the drop in optical activity in all the previous experiments as well as those recorded earlier by Rosenthaler.

When benzaldehyde and hydrocyanic acid combine spontaneously to form mandelonitrile they form an equal number of *levo* and *dextro* nitrile molecules. When, however, they combine under the influence of *d*-oxy-

nitrilase, they form only *dextro* molecules. As we are dealing with a reversible reaction, the dissociation of the nitrile back to benzaldehyde and hydrogen cyanide must also be considered. If the enzyme catalyzes only *dextro* nitrile molecules, it will catalyze the dissociation of *dextro* nitrile molecules. Spontaneously, however, *levo* or *dextro* molecules will be dissociated in proportion to the amounts of each present. If 75% of the nitrile is *dextro* rotatory 3 molecules of *dextro* for each molecule of *levo* will be dissociated spontaneously. Consequently as the reverse reaction becomes more marked the excess of *dextro* nitrile decomposed becomes greater and greater and the optical activity gradually declines.

At chemical equilibrium the enzyme combines and dissociates an equal number of *dextro* nitrile molecules.

$$\text{Catalyzed by enzyme} \begin{cases} \text{Combination} & C_6H_5CHO + HCN = C_6H_5CHOHCN\ \textit{dextro} \\ \text{Dissociation} & C_6H_5CHOHCN\ (\textit{dextro}) = C_6H_5CHO + HCN. \end{cases}$$

The spontaneous action, however, forms equal numbers of *dextro* and *levo* nitrile molecules but decomposes more *dextro* than *levo* because there are more present.

$$\text{Action without enzyme} \begin{cases} C_6H_5CHO + HCN = C_6H_5CHOHCN \text{ equal numbers of } \textit{dextro} \text{ and } \textit{levo} \\ \textit{dextro} \text{ and } \textit{levo} \text{ in ratio to number present} \end{cases} C_6H_5CHOHCN = C_6H_5CHO + HCN.$$

The rate at which the nitrile becomes inactive after chemical equilibrium has been established between benzaldehyde and hydrocyanic acid depends upon the relative rates of enzyme action to the spontaneous action. At 35°, where the spontaneous combination is very marked, the percentage of optically active nitrile is never very high and it decreases very rapidly, while at 0°, where the spontaneous action is almost negligible, the nitrile is practically all formed by the enzyme and, therefore, the drop in the per cent. of optically active nitrile is correspondingly slow, although not entirely eliminated. In the duplicate experiment to No. 8, the per cent. of optically active nitrile had dropped from 100 to 93.4 in 72 hours. As we have already stated, Dakin, in explaining a similar phenomenon for the action of lipase on mandelic ester, came to the conclusion that it was due to 2 enzymes, one acting on the *dextro* ester and the other on the *levo*, but at unequal rates. This explanation is untenable in our case because the decrease in activity of the nitrile continues after chemical equilibrium between hydrocyanic acid and benzaldehyde is established, which would be impossible if it was caused by enzymes.

The assumption that the drop in activity is caused by the enzyme being slowly destroyed is disproved by the following experiment.

Experiment 9.

The conditions were the same as in Expt. 7. The rate of combination as well as the optically active nitrile were determined as usual. After 18 hours chemical equilibrium had been established and the solution was practically inactive. The residue in the cell, 385 cc., was cooled to 0°

and 2 cc. of benzaldehyde and its equivalent in hydrogen cyanide in 13 cc. of water was added. In one hour α was —0.28; in 6 hours, —0.72; and in 23 hours, —1.13. It is obvious that the enzyme is still a very active one and that the decrease in activity during the first part of the experiment was not due to the destruction of the enzyme. When the solution was allowed to warm up again, the activity slowly dropped again.

Effect of the Hydrogen Ion on the Rate of Reaction.

After the above series of experiments was completed, our stock of benzaldehyde, which had been carefully preserved over nitrogen gas, gave out, so that a new stock had to be prepared. The old stock, when tested for acids by dissolving one cc. in carefully purified alcohol, required 0.6 cc. of 0.01 *N* alkali to neutralize it, using phenolphthalein as indicator. The new stock was quite neutral to phenolphthalein when tested in the same way. This difference at first sight seemed negligible, but the following 2 experiments show how sensitive this particular chemical reaction is to hydrogen ions.

Experiment 10.

The cell contents were the same as in Expt. 1, except that the new or neutral benzaldehyde was used. The temperature was 25°.

Time. Min.	Combination. %.
5	7.35
7	23.80
15	41.70
30	54.65
45	63.65
Hours.	
1	67.53
2	74.90
20	77.20

Experiment 11.

The cell contents were the same as above, except that 150 mg. of enzyme was added. The temperature was 25°.

Time. Min.	Combination. %.	α.	Optically Active Nitrile. %.
2	39.20	—0.10°	20.55
5	63.20	—0.17°	21.70
7	69.45	—0.18°	26.30
15	76.60	—0.19°	20.00
30	78.35	—0.15°	15.45
45	79.20	—0.11°	11.20
Hours.			
1	79.00	—0.10°	10.20
2	78.35	—0.05°	5.30
3	78.60	—0.03°	3.10
$6^2/_3$	78.85	0.00°	0.00

When the above experiments are compared with Expts. 1, 2 and 3, it is at once obvious that the rate for the chemical reaction has very much

increased and consequently the enzyme in the experiment has very little chance, so that the amount of optical activity is never very high and rapidly falls to zero.

Experiments 12 to 14.

Three more experiments were carried out with varying concentration of hydrogen ions. The amounts of benzaldehyde, hydrogen cyanide alcohol and water were the same in all these experiments as in the standard. The amounts of hydrogen ions present were varied by adding different amounts of acetic acid to the cell.

In Expt. 12 the hydrogen-ion concentration was between $10^{-6.7}$ and $10^{-6.1}$; in Expt. 13 between $10^{-5.2}$ and $10^{-6.1}$; and in Expt. 14 about 10^{-4}

Time.	Combination. %.			α.			Optically Active Nitrile. %.		
Mins.	Expt. 12.	13.	14.	12.	13.	14.	12.	13.	14.
3	38.9	..	..	—0.16	..	..	33.2	..	..
5	48.8	25.4	17.6	—0.20	—0.20	—0.14	33.0	63.5	64.2
7	59.7	..	..	—0.27	..	..	36.6	..	..
10	..	37.0	24.4	..	—0.36	—0.27	..	78.5	89.2
15	71.6	46.9	33.0	—0.34	—0.46	—0.36	38.3	79.1	88.0
30	77.2	61.8	47.5	—0.33	—0.60	—0.54	34.3	78.4	91.0
Hours.									
1	77.5	69.8	61.1	—0.28	—0.66	—0.68	29.2	76.3	89.8
2	77.5	72.4	..	—0.25	—0.66	..	26.0	73.6	..
3	77.5	72.7	67.3	—0.16	—0.64	—0.77	16.7	71.1	92.3
8	79.0	74.8	..	—0.09	—0.50	..	9.20	..	..
24	79.0	..	71.4	—0.00	..	—0.55	0.0	54.0	62.2

The hydrogen-ion concentrations given in these experiments are only approximately correct as they were measured by indicators, namely *p*-nitrophenol, phenolphthalein and methyl orange. Expts. 12 to 14, however, illustrate very strikingly the effect of small variations of hydrogen ions on this interesting reaction. It is rather hard to draw any conclusions as to the optimum hydrogen-ion concentration for the enzyme, as in the experiments where the solution was nearly neutral, the equilibrium point between benzaldehyde and hydrogen cyanide was reached so quickly that we have only a point or two which give us any information. There is no doubt that an increase in hydrogen ions retards the spontaneous or chemical action more than the enzyme action and apparently from our results the optimum concentration is around 10^{-5} to 10^{-6} as the values for α for the first 3 points in Expt. 13 are higher than those of any other experiment. These experiments should be repeated at a low temperature where the chemical reaction is much slower. We believe, however, that these experiments fall in line with our explanation of the fall in optical activity. The effect of hydrogen ions on optical activity is very well brought out by Fig. 4 where α is plotted against time. Curve 1 represents α when the hydrogen-ion concentration was $10^{-5.2}$ to $10^{-6.21}$,

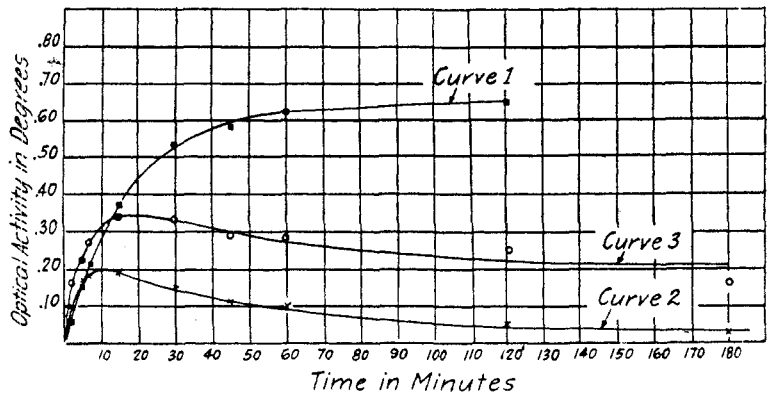

Fig. 4.

Curve 2 when the solution was practically neutral, and Curve 3 when the hydrogen ion was about $10^{-6.7}$ to $10^{-6.1}$.

Summary.

A method has been devized to follow the rate of enzyme action when oxynitrilase acts on benzaldehyde and hydrogen cyanide.

The enzyme does not have the same temperature coefficient as the spontaneous action. At 0° the enzyme is more efficient, at 35° the spontaneous action.

A new explanation has been offered for the phenomenon noticed by Rosenthaler, that the optical activity of the nitrile rises to a maximum and then falls to zero.

The hydrogen-ion concentration has a very marked effect on the ratio of spontaneous to enzyme action. When the solutions are neutral, practically all the benzaldehyde and hydrogen cyanide combine spontaneously and almost instantaneously. As the hydrogen-ion concentration is increased, the spontaneous action is repressed, and at 10^{-5} to 10^{-6} the enzyme exhibits its maximum activity.

HARTFORD, CONN.

4-METHYL-BENZOPHENONE CHLORIDE AND ITS CONDENSATION WITH PHENOL.

BY F. C. HAHN.

Received October 5, 1920.

The reaction between 4,4′-dimethyl-benzophenone chloride and phenol has been studied by Gomberg and Todd,[1] and the existence of the corresponding tautomeric carbinols has been indicated. In this work the condensation of 4-methyl-benzophenone chloride with phenol has been followed, and evidence obtained that the corresponding carbinol exists in 2 tautomeric forms.

[1] Gomberg and Todd, THIS JOURNAL, 39, 2392 (1917).