

TABLE VIII
ACETIC ACID FROM OTHER COAL TYPES*

Sample	Ash %	Volatile %	Acetic acid %
Penna. Spore Cannel	45.5	28.6	12.6
Michigan Shaly Spore Coal	48.0	25.9	10.2
Michigan Splint under Spore Coal	4.3	37.9	8.8
Utah Cannel, Kane County	19.0	54.7	10.5
Australian Coorongite	7.6
Penna. Boghead Cannel	45.0	30.1	12.9
Colorado Oil Shale	58.9	38.9	12.8
Wyoming Oil Shale	55.2	45.9	14.4

* Samples obtained through the courtesy of Dr. James Schopf, U. S. Bureau of Mines, Pittsburgh, Pennsylvania.

the process of humification and preservation of spores and algae resulted in a product which is similar to that made from other plant materials.

Summary

The quantitative oxidation of coal to acetic acid shows that: (1) The structures in coal which yield acetic acid on oxidation are largely decomposed by carbonizing temperatures of 500°. (2) The yield of acetic acid from bituminous coals bears a direct relationship to the amount of methane produced on carbonization. (3) High rank coals give less acetic acid, the amount diminishing with the volatile matter content. (4) Woody portions of low rank coals give less acetic acid than the attrital portion. (5) High yields of acetic acid from synthetic carbohydrate-humic acids indicate that carbohydrates may have been an important factor in the formation of coals as well as lignin.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF CALIFORNIA]

Studies on the Mechanism of the Cannizzaro Reaction¹

BY ELLIOT R. ALEXANDER²

Recently the Cannizzaro reaction³ has been the subject of careful kinetic analysis.⁴ It now appears quite certain that in the case of substituted benzaldehydes at least,⁵ the reaction rate may be represented by the equation

$$\text{rate} = k(\text{ArCHO})^2(\text{OH}^-)$$

in aqueous dioxane, pure methanol and aqueous methanol.^{4,6} Since no unusual precautions were taken to exclude oxygen in carrying out the reaction, this general agreement among the different investigators suggests that the reaction proceeds by an ionic rather than radical mechanism. Also the fact that electron attracting substituents in the aromatic nucleus facilitate the reaction while electron repelling groups inhibit the reaction⁸ implies the formation of ionic intermediates. It has been proposed, however, that a chain or free radical mechanism is involved⁷ and there is indeed some evidence to support this view. Thus, some

unpublished experiments of Weiss⁸ indicate that under suitable conditions the reaction is accelerated by the presence of ferrate, silver oxide, or benzoyl peroxide and that it is inhibited by ferrous or manganous hydroxide. Furthermore, it has been shown by Kharasch and Foy⁹ and confirmed by Urushibara and Takebayashi¹⁰ that the yield of the heterogeneous reaction is lowered considerably or that the reaction is even inhibited completely by employing peroxide-free aldehyde.

Accordingly, it was the purpose of this investigation to study the Cannizzaro reaction with benzaldehyde in a homogeneous medium under peroxide-free conditions and also in the presence of added peroxides or peroxide inhibitors.

In Fig. 1 are summarized the data obtained by carrying out the reaction according to the procedure developed by Molt^{6a} with carefully purified reagents under an atmosphere of nitrogen. The experiments were conducted at 100° in 74% aqueous methanol and the course of the reaction was followed by titrating the disappearance of alkali. Under these conditions, Molt and others⁶ have shown that the transformation $2\text{ArCHO} + \text{NaOH} \rightarrow \text{ArCH}_2\text{OH} + \text{ArCOONa}$ is almost quantitative, and that the disappearance of alkali from the reaction mixture represents a measure of the extent of reaction. Runs were made with benzaldehyde alone and with benzaldehyde in the presence of one molar per cent of

(1) Presented before the Division of Organic Chemistry at the 110th meeting of the American Chemical Society, Chicago, Illinois, September, 1946.

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(3) An excellent review of the Cannizzaro reaction has been published by T. A. Geissman, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 94.

(4) Tommila, *Ann. Acad. Sci. Fennicae, Ser., A59*, No. 8, 3 (1942); *C. A.*, **38**, 6175 (1944).

(5) Paul, *J. Gen. Chem. U. S. S. R.*, **11**, 1121 (1941); *C. A.*, **37**, 3733 (1943), has found that the order of the Cannizzaro reaction with formaldehyde depends upon the concentration of the reactants employed.

(6) (a) Molt, *Rec. trav. chim.*, **56**, 233 (1937); (b) Blanksma and Zaaijir, *ibid.*, **57**, 727 (1938); (c) Lock and Eitel, *Monatsh.*, **72**, 392 (1939).

(7) Weiss, *Trans. Faraday Soc.*, **37**, 782 (1941).

(8) Weiss, *ibid.*, 786 (1941).

(9) Kharasch and Foy, *THIS JOURNAL*, **57**, 1510 (1935).

(10) Urushibara and Takebayashi, *Bull. Chem. Soc. Japan*, **12**, 328 (1937).

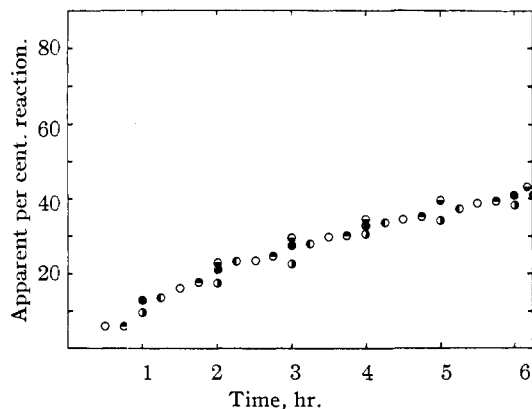


Fig. 1.—Effect of peroxides and peroxide inhibitors on the Cannizzaro reaction between benzaldehyde (0.495 *M*) and sodium hydroxide (0.250 *M*) at 100° in 74% (vol.) aqueous methanol: O, “ordinary” C_6H_5CHO ; ●, purified C_6H_5CHO ; ◐, purified C_6H_5CHO + 0.01 *M* benzoyl peroxide; ◑, purified C_6H_5CHO + 0.01 *M* hydroquinone; ◒, purified C_6H_5CHO + 0.01 *M* diphenylamine; ◓, purified C_6H_5CHO + 0.01 *M* sodium peroxide.

sodium peroxide, benzoyl peroxide, hydroquinone and diphenylamine, respectively.

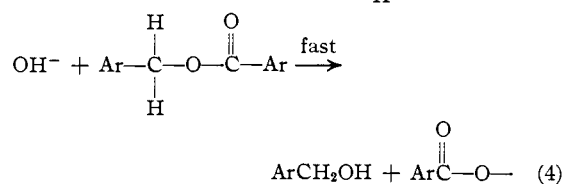
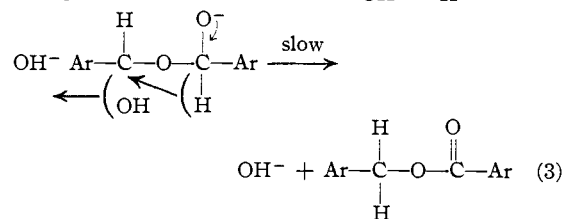
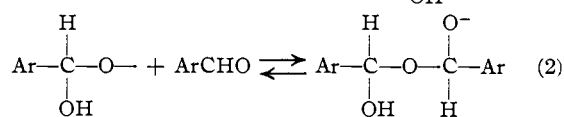
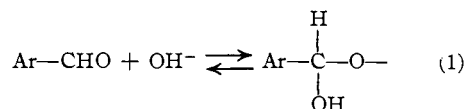
From these points, it can be seen that there is no difference between “ordinary” redistilled benzaldehyde which shows a positive peroxide test,¹¹ and benzaldehyde which has been purified, so as to give no such test. Further, within the accuracy of the method neither peroxides nor peroxide inhibitors appear to influence the reaction. In the case of the runs with benzoyl peroxide and sodium peroxide, it will be observed that the points lie respectively somewhat above and somewhat below the others. These effects are to be expected from the fact that the reactions were followed by an acid-base titration and no corrections have been made for the peroxides or inhibitors which were added. Thus, sodium peroxide represents an added basic constituent which makes the extent of reaction appear some 2% lower while the decomposition of the benzoyl peroxide generates an acidic constituent (*e. g.*, carbon dioxide or benzoic acid) the effect of which is to increase the apparent extent of reaction. In either case, however, the effects are within the expected limits and further the shape of the curves, and therefore the rate of reaction has not been significantly altered.

Thus it appears that in a homogeneous system it is not necessary to suppose that the mechanism of the Cannizzaro reaction involves a chain reaction of the kind which is usually greatly influenced by the presence of peroxides or peroxide inhibitors.¹²

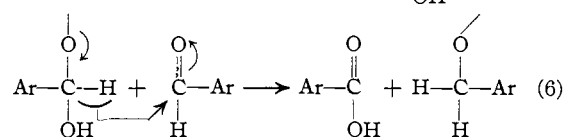
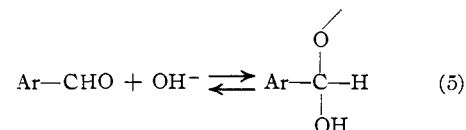
(11) Kharasch, McNab and Mayo, *THIS JOURNAL*, **55**, 2523 (1933).

(12) Geib, *Z. physik. Chem.*, **A169**, 41 (1934), reached this same conclusion with regard to the Cannizzaro reaction with furfural. His carefully performed experiments, however, indicate fourth order kinetics for this reaction.

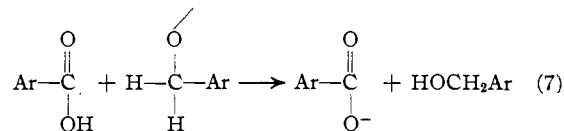
Of the ionic mechanisms which have been proposed,^{3,13,14} the first two seem to warrant rather detailed consideration. Geissman has proposed that the corresponding benzyl benzoic ester is formed as an intermediate which is rapidly saponified in the presence of the strong alkali present to produce a molecule of benzyl alcohol and benzoic acid—the products which are usually isolated. This series of reactions may be represented:



On the other hand, the hydrogen atom with its pair of electrons might be transferred by an *inter* rather than an *intra* molecular shift such as is shown in step (3). That is



followed by a rapid protolytic transfer



This mechanism is essentially the one proposed by Hammett¹³ but modified so as to fit third order kinetics.

In general either of these mechanisms is in accordance with (13) Hammett, “Physical Organic Chemistry,” McGraw-Hill Book Company, New York, N. Y., 1940, p. 350.

(14) Eistert, “Tautomerie und Mesomerie,” Ferd. Enke, Stuttgart, 1938, p. 116.

cord with much of the data,¹⁵ although in the case of the latter, it is necessary to suppose that the seemingly analogous Tischtschenko reaction¹⁶ proceeds by a different mechanism.¹⁷

There are, however, difficulties with either mechanism. Hammett¹⁷ has objected to the first scheme on the grounds that any mechanism which involves the formation and hydrolysis of benzyl benzoate is extremely improbable, because the ester is so resistant to hydrolysis. It is also to be observed that while hydride shifts appear to occur between *adjacent* carbon atoms in acidic media when carbonium ions are involved,¹⁸ the shift over a three atom system as is postulated in equation (3) appears to have no analogy with any other reaction.

The principal objection to the second of these mechanisms is that Lachman¹⁹ has been able to isolate benzyl benzoate from the Cannizzaro reaction with benzaldehyde by cooling the reaction mixture and by avoiding any excess alkali. It therefore seems reasonable to require of any mechanism which is proposed for the Cannizzaro reaction that it should at least *permit* the formation of benzyl benzoate.

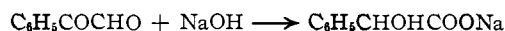
Accordingly, some of these points were investigated.

As has been mentioned earlier, Molt^{6a} has shown that for each mole of aldehyde which disappeared 0.5 mole of alkali was taken up when the reaction was carried out in aqueous methanol at 100°. Clearly this indicates that the concentration of ester (if it be an intermediate) cannot build up at most greater than the experimental error. Consequently, a study of the rate of hydrolysis of benzyl benzoate under identical conditions should permit a comparison of the rates of reactions (3) and (4) if equations (1 to 4) represent the true course of reaction.

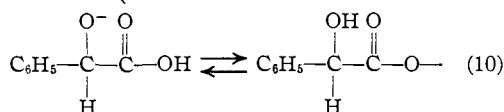
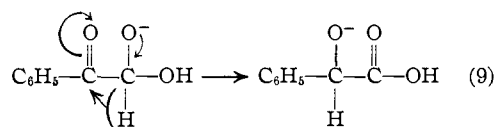
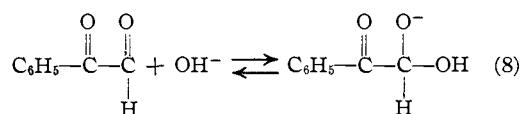
Such a study of the rate of hydrolysis of benzyl benzoate under the same conditions Molt employed was attempted. It was found, however, that the rate of hydrolysis of benzyl benzoate at these concentrations was extremely rapid *even at room temperature*, so rapid, in fact, that it could not be measured in the usual manner. Thus, after mixing the solutions, pipetting aliquots into ampules, and sealing them, 81% of the benzyl benzoate had been hydrolyzed. A comparison with Molt's data²⁰ shows that even after six hours at 100°, the Cannizzaro reaction had proceeded only to the extent of 51%. Thus, it appears that an objection to the mechanism involving the formation and hydrolysis of a benzyl benzoic ester

does not seem to be justified on the grounds that the rate of hydrolysis is too slow.²¹

The problem of determining whether the hydrogen transfer occurs as is shown in equation (3) is an extremely difficult one. One possible indirect approach to the question of whether such an intramolecular hydride shift *ever* occurs would be to study the kinetics of the transformation of phenylglyoxal to mandelic acid. If this reaction proceeds by an intramolecular hydrogen shift,



the mechanism (8-10) which is similar to (1-3) would require second order kinetics, while an intermolecular oxidation-reduction would require kinetics of at least third order.



Obviously the analogy of this transformation to the Cannizzaro reaction is rather strained since the shift here involves addition to a carbonyl group rather than the displacement of a hydroxyl group (*cf.* equations 9 and 3). A study of the kinetics, however, should indicate clearly whether an intramolecular hydrogen shift (such as is postulated in equation 3) *ever does* occur under alkaline conditions.

In Table I are shown the kinetic data obtained from a study of the transformation of phenylglyoxal to mandelic acid which was brought about by alkali in 50% methanol at 25°. From these data the lines shown in Fig. 2 were obtained by plotting the value

$$\frac{2.303}{b-a} \log \left(\frac{b-x}{a-x} \right)$$

against time in minutes. In this expression, *b* is the initial concentration of hydroxide ion, *a* is the initial concentration of aldehyde, and *x* is the change in concentration of aldehyde or hydroxyl ion after time *t*. From these lines which were obtained at various concentrations of aldehyde and base, it is evident that the reaction is of second order and that the rate may be expressed by the equation

$$\text{rate} = 0.069 [\text{OH}^-][\text{C}_6\text{H}_5\text{COCHO}]$$

(21) Unknown to the author, Dr. Geissman had come to the same conclusion as early as 1941. By carrying out the reaction in the presence of added benzyl benzoate, he found that the ester was completely hydrolyzed when all of the aldehyde had reacted; T. A. Geissman, private communication.

(15) Geissman, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 96-97.

(16) Tischtschenko, *Chem. Zentr.*, **77**, II, 1309, 1552 (1906).

(17) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Company, New York, N. Y., 1940, p. 352.

(18) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940, p. 320.

(19) Lachman, *THIS JOURNAL*, **45**, 2356 (1923).

(20) Molt, *Rec. trav. chim.*, **56**, 239 (1937).

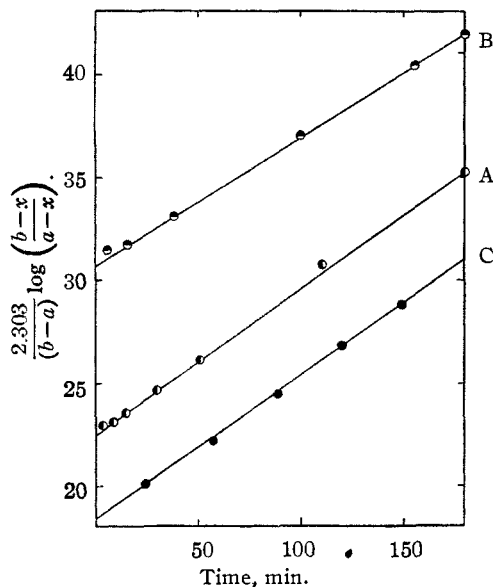


Fig. 2.—Plot showing that the conversion of phenylglyoxal to mandelic acid follows second order kinetics: A, $a = 0.0435 M$, $b = 0.0500 M$, $KCl = 1.490 g.$, $\lambda = 0.071$; B, $a = 0.02177 M$, $b = 0.0500 M$, $KCl = 1.490 g.$, $\lambda = 0.066$; C, $a = 0.04354 M$, $b = 0.07208 M$, $\lambda = 0.069$.

Second order kinetics for this oxidation-reduction is in agreement with the kinetics of the benzilic acid rearrangement.²² It indicates that in this case at least, the hydrogen transfer takes place intramolecularly.

Acknowledgment.—The author wishes to express his gratitude to Professor Melvin Calvin and Dean Wendell M. Latimer for their helpful interest in this work. He is further indebted to the trustees of the Frank B. Jewett Fellowships for a fellowship which made the investigation possible.

Experimental²³

Materials

Benzaldehyde.—The benzaldehyde which was used in the experiments designed to determine the effect of peroxides and peroxide inhibitors was purified immediately before each run. A mixture of 50 ml. of benzaldehyde and 50 ml. of benzene was shaken with 50 ml. of 10% aqueous ferrous sulfate, containing 1 ml. of concentrated hydrochloric acid. The benzene solution was then washed twice with water, the water was drained as completely as possible from the mixture, and the solution was poured into a 125-ml. Claisen flask equipped with a thermometer, condenser, adapter and receiver. A gas outlet tube stuffed with cotton was attached to the adapter. A vigorous stream of nitrogen was then introduced into the contents by a glass tube leading through the capillary neck almost to the bottom of the flask. When the apparatus had been thoroughly flushed with nitrogen, the flow of gas was decreased somewhat. Heating was commenced and the benzene together with traces of water was removed cautiously. After the benzene had been removed, heating was increased until benzaldehyde began to distill. When the temperature of the distilling benzaldehyde appeared to remain constant (about 177°), heating was decreased

(22) Westheimer, *THIS JOURNAL*, **58**, 2209 (1936).

(23) All boiling points and melting points are uncorrected.

TABLE I

DETERMINATION OF THE ORDER OF THE REACTION
 $C_6H_5COCHO + NaOH \rightarrow C_6H_5CHOHCOONa$

Time, min.	$y^{a,e}$	$\frac{(b-x)}{\times 10^{-3b}}$	$\frac{(a-x)}{\times 10^{-3a}}$	$\frac{2.303}{(b-a)} \log \frac{b-x}{a-x}$
Run A: $a = 0.04354 M$; $b = 0.05000 M^d$; $KCl = 1.490 g.$				
3.5	2.95	47.05	40.59	22.87
8.5	3.29	46.71	40.25	23.04
15.0	4.12	45.88	39.42	23.49
30.0	6.06	43.94	37.48	24.62
52.0	8.35	41.65	35.19	26.10
111.0	13.90	36.10	29.64	30.77
180.0	18.28	31.72	25.26	35.26
Run B: $a = 0.02177 M$; $b = 0.05000 M^f$; $KCl = 1.490 g.$				
6.0	1.99	48.01	19.78	31.42
16.0	2.14	47.86	19.63	31.58
38.5	3.46	46.54	18.31	33.05
101.0	6.46	43.54	15.31	37.03
157.0	8.52	41.48	13.25	40.43
180.0	9.29	40.71	12.48	41.88
Run C: $a = 0.04354 M$; $b = 0.07208^g$				
24.5	8.43 ^h	65.57	37.03	20.03
57.5	14.05 ^h	60.95	32.41	22.13
89.5	18.20 ^h	56.80	28.26	24.47
121.0	21.61 ^h	53.39	24.85	26.80
150.0	23.94 ^h	51.06	22.52	28.79

^a Ml. of 0.1000 *N* sodium hydroxide solution required for back titration. ^b The initial concentration of hydroxide ion. ^c The initial concentration of phenylglyoxal. ^d Made up from 100 ml. of 0.4354 *M* phenylglyoxal solution and 50 ml. of 1.000 *N* sodium hydroxide. ^e Back titrated against 50.00 ml. of 0.1000 *N* hydrochloric acid. ^f Made up from 50 ml. of 0.4354 *M* phenylglyoxal solution and 50 ml. of 1.000 *N* sodium hydroxide. ^g Made up from 100 ml. of 0.4354 *M* phenylglyoxal solution and 72.08 ml. of 1.000 *N* sodium hydroxide solution. ^h Back titrated against 75.00 ml. of 0.1000 *N* hydrochloric acid.

until distillation stopped and a second clean, dry, 125-ml. Claisen flask, (already fitted with a thermometer) was substituted for the receiver. This exchange was effected by attaching a nitrogen source to the side arm of the flask, flushing it thoroughly, and making the exchange while a steady stream of nitrogen was flowing both through the receiver and through the distilling apparatus. The stream of nitrogen through the side arm of the receiver was then shut off, heating was increased under the distilling flask, and a fraction of benzaldehyde boiling from 177–180° was collected. Before opening the apparatus, it was cooled and nitrogen again allowed to flow through the side arm of the receiver.

Although material prepared in this way was never found to show a positive peroxide test,¹¹ it was always redistilled in the manner just described. Benzaldehyde, which had been twice distilled and purified in this way, was found to be a water white liquid, b. p. 177–179°, which showed no tendency to form benzoic acid crystals as long as it was kept in the receiver under nitrogen as described. Even pouring it from the receiver into a clean bottle flushed with nitrogen, however, caused some benzoic acid to be deposited and the liquid to become faintly yellow after standing a few hours. It was, therefore, used at once for the experiments described later.

“Ordinary” redistilled benzaldehyde was not treated in the above manner or handled under nitrogen. It was simply redistilled (b. p. 177–179°), and used at once.

Methanol.—Commercial, anhydrous methanol was used. It was found to show no test for peroxides.¹¹

Sodium Hydroxide Solution.—The sodium hydroxide solution used in the Cannizzaro reaction was an analytical

standard solution adjusted to 1.000 $N \approx 0.3\%$; on acidification it showed no peroxide test.¹¹

Benzoyl Peroxide.—Eastman Kodak Co., white label.

Sodium Peroxide.—Baker and Adamson, reagent.

Hydroquinone.—Eastman, m. p. 169–170°.

Diphenylamine.—Eastman, m. p. 151–152°.

Benzyl Benzoate.—Eastman, redistilled, b. p. 133–135° (0.5 mm.), n_D^{20} 1.5672.

Phenylglyoxal Solution.—Phenylglyoxal was prepared by the method of Riley and Gray²⁴; b. p. 99–102° (20 mm.). It was twice distilled before use and made up at once into a solution containing methyl alcohol and water. Thus 29.1950 g. (0.2177 mole) of phenylglyoxal was dissolved in about 250 ml. of anhydrous methanol contained in a 500-ml. volumetric flask. Water, 50 ml., was added and the solution made up to the mark with more methanol. A solution prepared in this way showed no tendency to polymerize.²⁴

Methods

Pipetting Solutions and Reagents.—In order to maintain the solutions and purified reagents under an atmosphere of nitrogen at all times, the transfer and measurement of the materials was carried out volumetrically by a rather simple apparatus. A standardized pipet was attached in a vertical position to the center tube of a "T" tube by a short length of rubber tubing. One of the two arms of the tube was connected by rubber tubing to a source of nitrogen at slightly more than atmospheric pressure and the other to a weak vacuum line. These lines were closed by screw clamps. In order to withdraw samples under an atmosphere of nitrogen, it was only necessary to flush the pipet by loosening the clamp attached to the nitrogen tank, to close it again, and by loosening the clamp on the vacuum line, liquid could be sucked up to the mark on the pipet and held there by tightening it again. By interchanging the vessel from which the sample had been removed with another, both under an atmosphere of nitrogen, an aliquot could be introduced by opening the clamp to the nitrogen source. This was the technique used to make all transfers and standardizations.

Determinations of the Effect of Peroxides and Peroxide Inhibitors.—The technique used to follow the Cannizzaro reaction with benzaldehyde was essentially the method developed by Molt^{6a} with precautions taken to maintain the solutions at all times under an atmosphere of nitrogen. However, since this feature was the crucial part of the experiments, they will be described in some detail.

In order to make up the required solutions and to remove aliquots, the apparatus described above for pipetting was supplemented with an additional piece of slender glass tubing attached to a long piece of rubber tubing which was allowed to hang vertically through a clamp tightened enough to allow the jet to be raised and lowered but not to slip after adjustment. A stream of nitrogen was allowed to flow through this tube continuously and the tip was always kept inside the mouth of the volumetric flask containing the reaction mixture so as to maintain it under nitrogen at all times.

To a mixture of 50.00 ml. of 1.000 N sodium hydroxide solution and 100 ml. of anhydrous methanol contained in a 200-ml. volumetric flask was added under an atmosphere of nitrogen 10 ml. (10.4381 g., 0.09839 mole) of purified benzaldehyde. The solution was mixed thoroughly by swirling and made up to the mark with anhydrous methanol. The nitrogen jet was then removed, the stopper inserted quickly, and the solution mixed thoroughly by shaking. When mixing was complete, the stopper was removed, the nitrogen jet again replaced and aliquot portions removed in the manner already described by raising the volumetric flask and the nitrogen jet simultaneously until the tip of the pipet was immersed in the reaction mixture. Liquid was sucked up to the mark, held there by closing the clamp on the vacuum line tightly and the aliquot allowed to drain into a Pyrex glass ampule which had been previously flushed with nitrogen. These

ampules had a volume of about 30 ml. and the necks were drawn down to about 4–6 mm. in diameter. They were sealed at once. In this way, 7 samples of 25 ml. were made up and six of them immersed in a steam thermostat to be removed at various time intervals. The seventh was opened and analyzed at once.

After time t (Fig. 1) had elapsed, an ampule was removed, cooled rapidly to room temperature, opened and rinsed into 75.00 ml. of 0.1000 N hydrochloric acid. The alkali which had been absorbed during the reaction was determined by back titrating the excess hydrochloric acid with 0.1000 N sodium hydroxide solution using phenolphthalein as an indicator.

Hydrolysis of Benzyl Benzoate.—Benzyl benzoate (8.879 g., 0.04184 mole) and 100 ml. of anhydrous methanol were mixed in a 200-ml. volumetric flask. To this mixture was added 41.82 ml. (0.04182 mole) of 1.000 N sodium hydroxide solution. Toward the end of the addition of the sodium hydroxide solution, a faint turbidity appeared. This was intensified upon making the solution up to the mark with distilled water and small droplets of ester separated. The mixture warmed slightly and after shaking thoroughly for a few minutes became homogeneous. Aliquots, 25 ml., were pipetted (by mouth) into ampules which were sealed and introduced into the thermostat in the usual manner. One ampule was opened at once and rinsed into 75.00 ml. of sodium hydroxide solution. Back titration of the excess hydrochloric acid required 60.80 ml. of 0.1000 N sodium hydroxide which indicated that 81.2% of the benzyl benzoate had been hydrolyzed. After fifteen minutes at 100°, hydrolysis was to be complete.

Determination of the Order of the Conversion of Phenylglyoxal to Mandelic Acid.—A mixture of the appropriate amount of the phenylglyoxal solution, which has already been described, and enough anhydrous methyl alcohol calculated to bring the total volume of methyl alcohol to 500 ml. was diluted to about 900 ml. in a 1-liter volumetric flask. It was mixed thoroughly and allowed to stand one hour in a 25° thermostat. The reaction was started by adding the appropriate amount of 1.000 N sodium hydroxide solution, making the solution up to the mark with distilled water, and mixing thoroughly. It was followed by withdrawing 100-ml. portions into a known excess of 0.1000 N hydrochloric acid and back-titrating with 0.1000 N sodium hydroxide solution using phenolphthalein as an indicator.

As the reaction proceeded, the solutions became yellow and somewhat turbid (particularly in the case of run C where a larger excess of alkali was used). This by-product appears to be a dimer of phenylglyoxal²⁵ but under these conditions, only small amounts were formed. Thus, after two days when presumably the reaction had gone to completion, analysis showed that 95.3% of the calculated amount of alkali had been taken up.

Summary

1. The homogeneous Cannizzaro reaction does not appear to be influenced by the presence of peroxides, such as benzoyl peroxide or sodium peroxide nor peroxide inhibitors, such as hydroquinone or diphenylamine.
2. The rate of hydrolysis of benzyl benzoate is extremely fast as compared to the rate of the Cannizzaro reaction with benzaldehyde. Thus benzyl benzoate is 81% hydrolyzed in aqueous methanol after about fifteen minutes at room temperature, while at the same concentration but at 100°, the literature shows that the Cannizzaro reaction has proceeded to the extent of only 51% after six hours.
3. The alkaline internal oxidation-reduction of

(24) Riley and Gray, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 509.

(25) Söderbaum, *Ber.*, **24**, 1386 (1891).

phenylglyoxal to mandelic acid has been found to follow the equation, $\text{rate} = 0.069[\text{C}_6\text{H}_5\text{COCHO}][\text{OH}^-]$ in aqueous methanol at 25° . This implies that the reaction goes by way of an intramolecular shift of a hydrogen atom.

These facts are in agreement with an ionic mechanism for the Cannizzaro reaction based upon the intermediate formation of benzyl benzoate.

URBANA, ILLINOIS

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, TEMPLE UNIVERSITY SCHOOL OF MEDICINE]

The Mechanism of Urease Inhibition by Urea

BY CLARA L. DEASY

Howell and Sumner¹ have shown that urease activity increases with increasing urea concentrations only if the *pH* is below 6. At *pH* values less acid than 6, inhibition of urease activity occurs with higher urea concentrations, the concentration of urea required to inhibit urease activity decreasing with increasing *pH*.

The mechanism of this inhibition is the object of this study.

Experimental

Materials.—Merck reagent urea and Baker reagent sodium acetate were used in preparing the solutions.² The urease, which was obtained from jack bean meal, was purified by precipitations of the crude water-soluble material, first with alcohol, and then with acetone; the final solution, which precipitated characteristic crystals of urease in the cold, was diluted for the hydrolysis experiments.

Methods. Non-Enzymatic Hydrolysis.—In expts. 1–4 (Table I) a mixture of 0.5 ml. of urea solution and 0.5 ml. of 3% sodium acetate solution was heated one hour at 100° without reflux; in expts. 5 and 6 the hydrolysis was carried out with refluxing. In expts. 7–10 a mixture of 1 ml. urea solution and 1 ml. of 2% sodium acetate solution was heated five hours at 60° in stoppered tubes. Analyses for the ammonia formed were made both by direct nesslerization of the solution and by nesslerization after the solution had been acidified and allowed to stand at room temperature.

Enzymatic Hydrolysis.—A mixture of 0.5 ml. of urea solution, 0.5 ml. of buffer solution, and 0.3 ml. of diluted urease solution was heated at 30° for five minutes. The reaction was stopped either by cooling the solution or by

the addition of sulfuric acid. Ammonia analyses were carried out as in the non-enzymatic hydrolysis.

Discussion

Ammonium cyanate has been shown to be an intermediate in the non-enzymatic hydrolysis of urea.³ Since the cyanate ion is hydrolyzed to ammonia in acid solution, the amount of ammonia obtained on nesslerization after acidification of an alkaline hydrolysis mixture would be expected to be, in the limit, twice that obtained on direct nesslerization. This expectation was realized approximately when urea was hydrolyzed at 60° for five hours in a stoppered tube (expts. 7–10, Table I), but the production on acidification of much larger amounts of ammonia than the expected two-fold increase was encountered when the hydrolysis was carried out for one hour at 100° without reflux (expts. 1–4, Table I). This extra ammonia production was almost negligible if the solution was refluxed at 100° (expts. 5 and 6, Table I), but became evident again if the refluxed solution was evaporated before analysis (a 2.3-fold increase in ammonia with the 10% urea solution on acidification without evaporation, but a 3.4-fold increase (from 0.10 to 0.34 mg. ammonia nitrogen) after evaporation).

The anomalous production of the extra ammonia can be accounted for by the occurrence in the alkaline solution of ammonia in a "bound" form, as the compound $\text{NH}_3\cdot\text{CO}(\text{NH}_2)_2$.⁴ The fact that the conditions under which the extra ammonia production is encountered (higher urea concentrations, increased alkalinity, and decreased amounts of water) would be expected to favor the formation of the ammonia-urea compound, is evidence in favor of this assumption.

Direct evidence that the production of the extra ammonia on acidification was due to the presence of ammonia in a "bound" form in an alkaline solution rather than to the presence of an acid-labile intermediate was derived from experiments with solutions of ammonium hydroxide containing added urea. It was found that the color produced with such solutions was deeper on nesslerization after acidification than on direct nesslerization. The effect was intensified when the

TABLE I
NON-ENZYMATIC HYDROLYSIS

Expt.	Concn. of added urea soln., %	Ammonia nitrogen direct, mg.	Ammonia nitrogen after acidification, mg.
1	1	0.01	0.04
2	3	.03	.08
3	5	.02	.19
4	10	.02	.29
5	1	.05	.10
6	10	.21	.48
7	1	.02	.02
8	3	.03	.06
9	5	.04	.07
10	10	.06	.14

(1) S. F. Howell and J. B. Sumner, *J. Biol. Chem.*, **104**, 619 (1934).

(2) All solutions were made up on a weight-volume percentage basis.

(3) See, for example, R. C. Warner, *J. Biol. Chem.*, **142**, 705 (1942).

(4) E. Jänecke and E. Rahlfs, *Z. Elektrochem.*, **36**, 645 (1930).