displaced by mineral acids. The salts are insoluble in ethereal solvents and acetone, but are readily soluble in ethyl alcohol and water.

Summary.

(1) A description is given, for the first time, of a method for the isolation of tribasic phenolphthalates.

(2) Trisodium and tripotassium phenolphthalate are described.

We hope to apply this hydration method to other compounds, containing a phthalic acid group, such as the chloro and bromo derivatives of phenolphthalein, fluorescein, gallein, etc.

CONCERNING THE DECOMPOSITION OF URIC ACID BY MEANS OF DILUTE SODIUM HYDROXIDE SOLUTIONS.

By Clarence E. May.

Received September 13, 1911.

In recent years there have appeared in the literature three methods of biochemical importance, methods in which it seems to be the notion that by cutting down the dissociation of a given reagent, the activity of the reagent may be muzzled so that the desired action may be obtained without any side reactions taking place. The first of these methods to be proposed had to do with the determination of uric acid in urine, by the Folin-Shaffer method, wherein ammonium sulfate was added to prevent decomposition of the uric acid while precipitating the latter by means of ammonium hydroxide. In cutting down the dissociation of the ammonium hydroxide by having a large amount of ammonium sulfate present, the uric acid decomposition was diminished. The second method proposed was the Folin method for the determination of urea in urine. By having a large amount of magnesium chloride present along with the hydrochloric acid in the digestion mixture, it seemed that the dissociation of the acid was cut down and as a result the action of the acid on the non-urea constituents of urine was but slight.

Recently a modification of the Folin method for the ammonia estimation in urine was proposed by Steele[1] and in the modified method it was suggested that by the addition of a very large excess of sodium chloride it was possible to cut down the activity of the sodium hydroxide employed in the method to liberate the ammonia, since the dissociation of the caustic soda would be diminished by the presence of the sodium chloride. In other words, since the use of sodium hydroxide rather than sodium carbonate seemed necessary for the complete liberation of the ammonia in a mixture such as urine, the side action of the alkali on other urinary constituents would be prevented by the presence of an excess of sodium chloride.

[1] *J. Biol. Chem.*, 8, 365.

In an article published recently, the present author has been engaged jointly in an investigation of the deportment of uric acid under the influence of alkalis.[1] It was noted that after exposure to the action of alkalis, uric acid disappeared, and starting with weighed quantities of uric acid, it was never possible to recover all the original acid. This disappearance of the uric acid was more or less dependent on the amount and nature of the neutral salt present in the alkaline mixture. The salts as a rule did not seem to entirely prevent the decomposition of the uric acid; they seemed to only diminish the action. In the present work it seemed desirable to study the effect of sodium hydroxide on uric acid both with and without sodium chloride present. The main point of interest was the ammonium liberated from uric acid under conditions such as the modified Folin method required for the estimation of ammonia in urine.

In the present work, the author has used solutions of uric acid in about the concentration met in normal urine, 0.25 gram uric acid to 500 cc. solution. The alkali used was pure stick caustic soda dissolved in ammonia-free, distilled water. The two concentrations of sodium hydroxide were identical with those used by Steel, namely 1.0 gram NaOH per 25 cc. and 0.5 gram NaOH per 25 cc. H_2O. Tests were carried out using no sodium chloride, tests using a reasonable amount of sodium chloride (amounting to about one-third saturated solutions) and also tests using solutions that were thoroughly saturated with sodium chloride (Kahlbaum's). Room temperature only was employed. In some cases the aërations were started at once after dissolving the acid in the alkali. Other results were obtained on aliquot portions of the same original solutions that had been allowed to stand for a day or two. During the intervals of standing, there was no agitation.

The air current employed for pulling the ammonia from the alkaline solutions was thoroughly under control at all times and the volume of air passed through amounted to about fifty liters of ammonia-free air. A demijohn of that capacity was filled with water and a ten-foot column of water acting as a siphon gave the necessary current of air. The air bubbles passed for about six hours and at all times the bubbles were regular and at a speed that they were barely countable as they passed through the alkaline solutions and standard acid solutions, respectively. The alkali and acid used in the titrations of the ammonia recovered were checked against each other several times, using methyl orange as an indicator. The same buret was used for both acid and alkali measurements and in all titrations involved.

Eight series of determinations gave the following results as tabulated. The standard acid used was 1.0695 0.2 *N* sulfuric acid and the alkali was 0.8405 0.2 *N* sodium hydroxide. The table explains itself.

[1] *J. Am. Chem. Soc.*, 33, 364.

Series.	Grams NaOH per ½ liter.	Grams NaCl per ½ liter.	Hours standing.	Hours aëration.	Cc. H_2SO_4 soln. to collect NH_3.	Cc. NaOH soln. required for neutralization.	Cc. NaOH soln. required by theory.	
I–A.....	20	None	18	6	5.0	6.0	6.36	duplicates
A′.....	20	None	18	6	5.0	6.0	6.36	
B.....	20	40	17.5	6	5.0	6.0	6.36	duplicates
B′.....	20	40	17.5	6	5.0	6.0	6.36	
C.....	20	200	65	6	9.35	11.5	11.90	triplicates
C′.....	20	200	65	6	9.35	11.5	11.90	
C″.....	20	200	65	6	9.35	11.65	11.90	
II–A.....	20	None	17	5.5	9.35	11.70	11.90	
B.....	20	50	17	5.5	9.35	11.65	11.90	
C.....	20	150	17	5.5	9.35	11.65	11.90	
III–A.....	20	None	None	6.25	9.35	11.65	11.90	duplicates
A′.....	20	None	None	6.25	9.35	11.70	11.90	
B.....	20	45	None	6.25	9.35	11.65	11.90	
C.....	20	150	None	6.25	9.35	11.65	11.90	
IV–A.....	20	None	42	6	9.35	11.70	11.90	duplicates
A′.....	20	None	42	6	9.35	11.70	11.90	
B.....	20	45	42	6	9.35	11.60	11.90	
C.....	20	150	42	6	9.35	11.60	11.90	
V–A.....	10	None	None	6	9.35	11.70	11.90	duplicates
A′.....	10	None	None	6	9.35	11.70	11.90	
B.....	10	40[1]	None	6	9.35	11.60	11.90	
C.....	10	150[1]	None	6	9.35	11.60	11.90	
VI–A.....	10	None	22	5.75	9.35	11.70	11.90	duplicates
A′.....	10	None	22	5.75	9.35	11.80	11.90	
B.....	10	40[1]	22	5.75	9.35	11.90	11.90	
C.....	10	150[1]	22	5.75	10.28	13.0	13.09	
VII–A.....	10	None	None	6.5	9.35	11.75	11.90	duplicates
A′.....	10	None	None	6.5	9.35	11.75	11.90	
B.....	10	40[1]	None	6.5	9.35	11.75	11.90	
C.....	10	150	None	6.5	9.35	11.75	11.90	
VIII–A.....	10	None	42.5	6.5	9.35	11.70	11.90	duplicates
A′.....	10	None	42.5	6.5	9.35	11.70	11.90	
B.....	10	40[1]	42.5	6.5	9.35	11.70	11.90	
C.....	10	150[1]	42.5	6.5	9.35	11.65	11.90	

In the above work it is evident that there is a slight liberation of ammonia. The quantity is small but uniformly constant. With prolonged exposure to the caustic soda there is a slight increase in the amount of

[1] Part of the sodium chloride was placed in the beaker along with the dry uric acid, then covered with the dilute caustic soda solution. By this means some sodium chloride was present in the alkaline urate solution at all times, although the major portion of the sodium chloride had already been placed in the graduated flask before dilution to the mark.

ammonia liberated. This amount seemed to be independent of the amount of sodium chloride present, for the same increase in acidity was obtained where no sodium chloride was present as where the solution was saturated with salt. The author is inclined to believe that the liberation of ammonia is not the main action of this particular decomposition of uric acid, since the quantity of ammonia evolved seems too small to justify any such conclusions.

In so far as the decomposition of uric acid under the influence of sodium hydroxide in the presence or absence of sodium chloride is concerned there seems but little room for objecting to the modified Steel-Folin method for determining ammonia in urine. In the results obtained above, one hundred cubic centimeters of the alkaline urate were used in every case. This volume was supposed to contain 0.05 gram of uric acid. In the actual determination as carried out with urine only one-fourth of this weight of uric acid is usually present, so the error introduced would be correspondingly less. The author feels that in the proposed modification of the Folin method there are compensating errors. In this laboratory it was found impossible to dissolve at room temperature as much as half the sodium chloride recommended by Steel. In this work it was found that 150 grams of sodium chloride would not completely dissolve in 500 cc. of 4 per cent. sodium hydroxide solution containing 0.25 gram of uric acid. By Steel's figures there should have been added to this volume of alkali solution 320 grams sodium chloride instead of the weight actually used. With the addition of the amount of sodium chloride recommended more than half remained undissolved. On passing air through this suspension, about one-third of the solution did not get thorough aëration because the undissolved sodium chloride settled to the bottom of the jar and refused to remain stirred up into the moving solution. With lack of stirring there would naturally be an incomplete removal of ammonia, even were it liberated. Since sodium hydroxide was used as the alkalinizing agent, perhaps more ammonia was liberated than should have been freed. With two oppositly directed errors present these may account for the closely concordant results of Steel.

The main interest in this paper has been in the quantity of ammonia that uric acid yielded from the action of dilute sodium hydroxide solutions. It seems that in so far as ammonia is given up by the uric acid, there is very little difference whether the sodium chloride is added or not. It may develop that constituents of urine other than uric acid are protected by the addition of sodium chloride in large amounts, but the author fails to see any justification for the use of the unduly large amounts of sodium chloride. At the present time, for the correction of the error due to the presence of ammonium magnesium phosphate, which seems

to be present only in abnormal urines, according to Folin, it seems desirable to use sodium hydroxide rather than the sodium carbonate to free the ammonia. In general, it looks as though one is correcting an occasional appreciable error by introducing a frequent slightly smaller error.

UNIVERSITY OF INDIANA, BLOOMINGTON, IND.

THE SYNTHESIS OF FATS BY THE ACTION OF ENZYMES.

By F. L. DUNLAP AND L. O. GILBERT.

Received September 1, 1911.

As a result of the various studies on the subject of lipolysis, it has been recognized that the reaction is never a complete one, the degree of hydrolysis depending on the conditions under which the experiments have been conducted. An hydrolysis of over 90 per cent. is not unusual, while on the other hand it may be considerably less, as for example, in the case of tributyrin when hydrolyzed by the lipase of the castor oil bean. Here Connstein, Hoyer and Wartenberg found but 9.5 per cent. hydrolysis after 24 hours.[1] In the latter case it is probable that no equilibrium is reached between the glyceride and the water and the products of hydrolysis, but rather that the acid produced either inhibits the lipolytic action when it reaches a certain concentration or the enzyme is destroyed.[2] The inhibition of fermentative processes by the normal products of such fermentation is well recognized, as in the case of lactic acid and alcoholic fermentation. That the lipolytic ferment, such as exists in the liver, pancreas, castor oil bean, etc., would not produce complete hydrolysis led to various successful attempts to synthesize fats through their action on mixtures of glycerol and the various fatty acids. Not only has the synthetic power of lipase been indicated, but of other enzymes as well.

With one exception experimental studies of synthesis due to lipase have been confined to this enzyme obtained from animal sources when acting on glycerol and the acids normal to fats. It is also worthy of note that many of these studies have been made with acids of exceptional occurrence in fats. This is seen, for example, in the work of Kastle and Loevenhart,[3] who studied pancreatic lipase and its synthetic effect on isobutyric acid and ethyl alcohol. Again, Hanriot[4] has produced monobutyrin by the action of glycerol on butyric acid by means of pancreatic

[1] *Ber.*, 35, 3988 (1902).

[2] Bradley [(*J. Biol. Chem.*, 8, 251 (1910)] is of the opinion that by increasing sufficiently the mass of the lipase, complete hydrolysis will ensue, but this is hardly in keeping with the views ordinarily expressed. For example, see Taylor [Univ. of California Publications, *Pathology*, 1, 35 (1904)].

[3] *Am. Chem. J.*, 24, 491 (1900).

[4] *Compt. rend.*, 132, 212 (1901).