moniacal solution obtained on hydrolysis yielded a very small quantity of a solid that dissolved readily in hydrochloric acid giving a purple color.

Reaction between Ethyl Nitrate and Phenylmagnesium Bromide.—This reaction was carried out at a very low temperature (about −80°) to determine whether nitrobenzene might be formed in accordance with the following reaction

$$C_6H_5MgBr + C_2H_5ONO_2 \longrightarrow C_6H_5NO_2 + C_2H_5O(MgBr) \qquad (V)$$

Kinkead[23] previously studied this reaction at about 0° and reported the production of a blue solution on extraction with chloroform, which had the properties of an indicator. Hepworth,[24] at −15°, obtained dimethylhydroxylamine, $(CH_3)_2NOH$, and diethylhydroxylamine from methyl- and ethylmagnesium halides, respectively.

When 0.2 mole of phenylmagnesium bromide was added dropwise and with stirring to 0.3 mole of ethyl nitrate cooled by a slush of carbon dioxide and acetone, diphenyl and phenol were obtained but no nitrobenzene.

Summary

The products obtained in the reaction between aryl nitro compounds and organomagnesium halides vary markedly with experimental conditions. Under the conditions of the experiments described here, the following general reaction takes place

$$C_6H_5NO_2 + 4C_6H_5MgBr \longrightarrow (C_6H_5)_2NH + C_6H_5OH + C_6H_5{\cdot}C_6H_5$$

With *alkyl*magnesium halides, tetra-substituted hydrazines are among the reaction products. Ethylmagnesium bromide and nitrobenzene, for example, give 1,2-diphenyl-1,2-diethylhydrazine, $(C_6H_5)(C_2H_5)NN(C_2H_5)(C_6H_5)$. The formation of alkylanilines in this reaction is due in part to the decomposition of such hydrazines.

AMES, IOWA

[CONTRIBUTION FROM THE FOOD RESEARCH DIVISION, BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE PROPERTIES AND CHEMICAL CONSTITUTION OF GLUCIC ACID

BY E. K. NELSON AND C. A. BROWNE

RECEIVED SEPTEMBER 21, 1928 PUBLISHED MARCH 6, 1929

H. Winter,[1] while working in the laboratory of Fraser Eaton and Company, Soerabaya, Java, announced in 1894 that upon warming an aqueous solution of 1% invert sugar with 0.5% of calcium oxide in the form of calcium hydroxide at 66.5°, he obtained a voluminous, white flocky precipitate which redissolved on further heating. This precipitate turned brown on exposure to the air and because of its slimy character could not be filtered readily. Winter purified the precipitate, which he supposed to be a basic calcium salt, by decanting and washing five times with lime water, the settling of the precipitate being performed in a well-closed flask, filled

---

[23] Kinkead, *Chem. News*, **122**, 4 (1921).

[24] Hepworth, *J. Chem. Soc.*, **119**, 251 (1921).

[1] Winter, *Z. Ver. Rübenzucker Ind.*, 44 (old series), 1049 (1894).

completely full in order to exclude the air. The unchanged white precipitate was then decomposed with dilute sulfuric acid and extracted with ether. Evaporation of the ether upon a watch glass left behind colorless, needle-like crystals of an organic acid in a yellow, sirupy mother liquor.

On repeating the experiment with 24 liters of invert sugar solution, Winter obtained a small amount of a yellow sirup filled with beautiful crystals 3–4 mm. long. After being placed in a desiccator over sulfuric acid the crystalline needles appeared in a few days to melt away and nothing was left in the sirup but some undeveloped crystals resembling cane sugar in appearance. After some time the sugar-like crystals also began to disappear with a darkening of color and a slight evolution of gas which ceased only after the residue had solidified to a dark resinous mass. The latter, upon distillation with water, yielded carbon dioxide and formic acid, leaving behind a residue of brown-colored flocks.

Winter's efforts to purify the substance by recrystallization from water, alcohol, ether or chloroform were unsuccessful. An aqueous solution of the crystals reduced Fehling's solution in the cold and precipitated black silver immediately from silver nitrate. From these results Winter supposed that the impure crystals of his experiment might be the so-called glucic acid, which Peligot,[2] Mulder,[3] Reichardt[4] and other investigators had never succeeded in isolating in the crystalline form, and which Winter further supposed to undergo on standing a slow transition into apoglucic acid with final decomposition into carbon dioxide, formic acid and other products. As much confusion prevails in the literature concerning the substance to which Dumas first gave the name of "glucic acid," the present study was initiated in the hope of determining more exactly the nature of the unstable acid compound that was obtained by Winter as a result of the action of calcium hydroxide upon invert sugar.

Experimental

The experiment of Winter was repeated except that dextrose (commercial "Cerelose") was used in place of invert sugar. In the preliminary test fifty grams of dextrose dissolved in five liters of water was heated at 67° with 25 g. of calcium oxide which had previously been well slaked in water. The precipitate which settled out on cooling was filtered, washed with lime water, decomposed with sulfuric acid and the calcium sulfate removed by filtration. The filtrate was thoroughly extracted with ether; on evaporation of the ether a small amount of crystalline material associated with a sharp-smelling liquid remained. The crystals were separated from the liquid on a porous plate.

---

[2] Peligot, *Ann.*, **30**, 76 (1839).

[3] Mulder, *Bulletin des sciences physiques et naturelles en Neerlande*, 1–102 (1840), abstracted in *Ann.*, **36**, 243–295 (1840).

[4] Reichardt, *Z. Ver. Rübenzucker Ind.*, **20** (old series), 529 (1870).

As Winter states that the precipitated calcium compound is somewhat soluble in water, it was thought that increasing the concentration might improve the yield. This proved to be the case and the following procedure was adopted. With further improvement in technique it is possible that the yield may be still further increased.

Four hundred grams of dextrose, 5 liters of water and 100 g. of calcium oxide (slaked) were thoroughly mixed with a stream of illuminating gas in order to avoid oxidation by air and heated to 67°.

On cooling a voluminous precipitate (A) separated. This was filtered, washed with lime water and finally with alcohol and ether, care being taken to avoid the passage of air through the filter by keeping the precipitate covered with a layer of liquid.

The precipitate was dried in a vacuum desiccator, contact with air being avoided, and when dry it was kept in an atmosphere of illuminating gas.

A preparation of the calcium compound, dried in illuminating gas, rapidly rose in temperature from 20° to 48° when exposed to the air. After exposure to air overnight it yielded only a trace of crystalline substance, the resulting product appearing to be a mixture of formic acid and a liquid, oily acid. That the heating of the substance is due to absorption of oxygen was proved by the following experiment.

A quantity of the precipitate was placed in a flask attached to a train consisting of a U-tube containing liquid paraffin and a drying tube containing calcium chloride, the latter tube being connected with a supply of oxygen. The steady absorption of oxygen for many hours was noted by the bubbling through the U-tube.

The dried precipitate (A) weighed 118.6 g. and contained 30.3 g. of calcium oxide.

The calculated amount of sulfuric acid necessary to combine with the 30.3 g. of calcium oxide was diluted, cooled with ice and added to the ice-cooled suspension of the precipitate. The mixture was thoroughly agitated until the reaction was complete, when the calcium sulfate was removed by filtration and washed. The filtrate (B) was concentrated in a vacuum distillation outfit connected with a receiver cooled by ice and salt (in order to lower the temperature of distillation) and with a stream of carbon dioxide passing through a capillary tube into the distilling flask to prevent bumping and to exclude air. The distillation was conducted in a vacuum of 20–30 millimeters.

The concentrated solution was extracted with ether in an extractor designed for extracting liquids with ether, so long as crystals were obtained on evaporation of the solvent. The total yield of crystalline material (C) was 1.34 g. in this experiment. The oily product absorbed by the porous plates was recovered by extraction with ether and reserved for future investigation.

The distillate obtained in concentrating the acids in the filtrate (B) required 189.5 cc. of $N/10$ barium hydroxide for its neutralization, which yielded upon evaporation 2.1802 g. of barium salt. Since 189.5 cc. of $N/10$ barium hydroxide is equivalent to 2.1542 g. of barium formate, the volatile acid obtained by decomposition of the calcium precipitate (A) with sulfuric acid is almost entirely formic acid. The fact that no alcohol-soluble lead salt was found after conversion of the barium compound into the lead derivative indicated the absence of acetic acid.

The crystalline acid (C), Winter's "glucic" acid, is easily soluble in water and alcohol, but only moderately soluble in ether, chloroform or ethyl acetate.

The substance was freed from liquid impurities by wetting with a little ethyl acetate and spreading on a porous plate. If left in contact with the liquid acid which accompanies it, the substance is very unstable. When the crystals wet with the liquid acid were left overnight in a desiccator, the solid substance almost disappeared and a brown, pasty mass was left. This confirms Winter's observations. An optical examination[5] gave the following results.

In ordinary light: The material consisted of plates. Its indices of refraction were $N\alpha = 1.475$, $N\gamma = 1.734$, both ±0.002. Birefringence was extremely strong. Between crossed Nicols it showed anomalous blue and purple polarization colors due to peculiar dispersion. This may be of use in recognizing the substance. An aqueous solution of the compound does not rotate the plane of polarized light.

The substance partially sublimes in beautiful crystals at the temperature of the water-bath. The unsublimed portion appears to have polymerized, as it no longer crystallizes from water.

The compound has no definite melting point. It turns brown and polymerizes at temperatures over 110°. Heated to 170° there was no evidence of melting in the dark colored transformation product.

An aqueous solution of the compound reduces silver nitrate, Fehling's solution and mercuric chloride at room temperature, the reductions taking place suddenly after an instant of time. The solution does not give a normal aldehyde reaction with Schiff's reagent. After treatment with this reagent, a solution of the substance gradually develops a violet-blue color. When a solution of ferric chloride is added to a solution of the compound, the ferric chloride is reduced and a faint blue color is developed which instantly disappears. The evanescent blue color is more marked if a solution of the substance is carefully added to a solution of ferric chloride. A very delicate and beautiful reaction is given by treating the substance in aqueous solution with a few drops of a solution of sodium nitroprusside, a drop of 10% solution of sodium hydroxide and finally a drop or two of acetic acid. An intense greenish-blue to blue color results and, on standing, a blue lake is deposited, leaving a nearly colorless supernatant liquid.

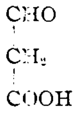
The substance is an acid and gives a sharp end-point on titration: 0.7333 g. = 16.27 cc. of $N/2$ NaOH. The neutralizing equivalent is therefore 90.

An analysis was made for carbon and hydrogen and the molecular weight was determined by the freezing-point method, using water as the solvent. The results obtained are as follows.

Anal. Calcd. for $C_3H_4O_3$: C, 40.89; H, 4.58; mol. wt., 88. Found: C, 40.53, 40.50, 40.36; H, 4.93, 4.73, 4.58; mol. wt., 88.4, 91.5, 94.9. The molecular weight appears to increase with the concentration, suggesting molecular aggregation.

A solution of the substance bleaches bromine water instantly; iodine absorption, calculated for one double bond, 288.3; found, 289, 285.

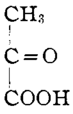
There are five possible constitutional formulas for an acid of the formula $C_3H_4O_3$.



$$\begin{array}{c} CHO \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

I

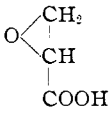
Semi malonic aldehyde



$$\begin{array}{c} CH_3 \\ | \\ C=O \\ | \\ COOH \end{array}$$

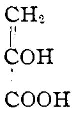
II

Pyruvic acid



III

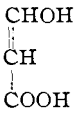
Glycidic acid



$$\begin{array}{c} CH_2 \\ \| \\ COH \\ | \\ COOH \end{array}$$

IV

α-Hydroxy-acrylic acid



$$\begin{array}{c} CHOH \\ \| \\ CH \\ | \\ COOH \end{array}$$

V

β-Hydroxy-acrylic acid

The semialdehyde of malonic acid (I) should give an aldehyde reaction with Schiff's reagent, and should be oxidized easily into malonic acid. Pyruvic acid (II), a liquid, has properties altogether different from those of

---

[5] Made by G. L. Keenan, Food, Drug and Insecticide Administration, U. S. Department of Agriculture.

the acid under discussion. Moreover, when pyruvic acid is treated with sodium nitroprusside and alkali, a wine-red color is developed which is *discharged* on the addition of acetic acid. Glycidic acid (III) is a liquid, miscible with water, alcohol and ether. Its calcium salt readily takes up water, forming calcium glycerate, so that it could not be formed under the conditions of our experiments.

The ready absorption of bromine suggests the presence of a double bond as in IV and V, while the remarkable reducing properties indicate an aldehyde or an enolic form readily convertible into an aldehyde. These conditions seem to be best met by Formula V (β-hydroxy-acrylic acid), which is the enolic form of the semialdehyde of malonic acid.

Such a compound has been described by Pinner[6] under the name of "Acrolactic Acid," which was prepared by the action of barium hydroxide on monochloro-ethyl acrylate. The free acid was obtained as a sirup which showed a tendency to crystallize. On account of scarcity of material Pinner was unable to make an analysis. However, he states that acrolactic acid is extremely unstable, and, though not mentioning any direct experiments on its reducing properties, observes that its silver salt darkened at once.

The acid which we obtained appears to be fairly stable, if well separated from the liquid acid extracted with it and kept in a closed container in a cool place.

If the substance is acrolactic acid, it should yield formic and oxalic acids on gentle oxidation.

An excess of sodium hydroxide was added to an aqueous solution of 1 g. of the crystalline compound and the solution boiled for a short time. Sulfuric acid was added in slight excess and the solution was distilled. The distillate was neutralized with barium hydroxide and evaporated to dryness.

The barium salts weighed 0.6862 g., corresponding to 0.2781 g. of formic acid or 27.8% of the substance.

No acetic acid was found and optical examination showed the salt to consist entirely of barium formate.

One-half gram of the substance was oxidized with hydrogen peroxide in excess. After the addition of ammonia, ammonium chloride and calcium chloride, the solution was boiled. A copious precipitate resulted which weighed 0.3309 g., equivalent to 0.204 g. of oxalic acid, or 40.8% of the sample.

The precipitate was shown by optical methods to be calcium oxalate.

A preparation of Winter's glucic acid was hydrogenated, platinum dioxide and 0.0001 mole of ferric chloride being used. The absorption of hydrogen ceased after less than one-fifth of the calculated amount of gas necessary for completely hydrogenating a compound of the type of Formula

---

[6] Pinner, *Ber.*, **7**, 250 (1874); *Ann.*, **179**, 91 (1875).

V had been taken up. The substance recovered after hydrogenation was no longer crystalline and had the appearance of the polymerized product. It was thought that the ferric chloride might have caused polymerization and thus interfered with a normal hydrogen absorption.

The experiment was repeated, colloidal palladium being used as a catalyst: 0.3764 g. of substance absorbed 166 cc. of hydrogen at 25° and 764.7 mm.; reduced to 0° and 760 mm. = 148.3 cc. or 0.01328 g. Calculated for two atoms of hydrogen absorbed by 0.3764 g. of mol. wt. 88, 0.00855 g.; and for three atoms, 0.01283 g. The product recovered after the reaction did not have an acid taste. It was highly soluble in water, very hygroscopic and scarcely soluble in ether. When dissolved in alcohol and precipitated with ether, an amorphous, very hygroscopic solid was obtained. It is evident again that a normal hydrogenation has not taken place, the product having probably suffered molecular change and polymerization.

It may be stated, finally, that the present investigation has not altogether confirmed the conclusion of Winter that his crystalline glucic acid undergoes first a transformation into apoglucic acid and then a further decomposition into carbon dioxide, formic acid and other products. No evidence was obtained in these experiments of the spontaneous decomposition of Winter's glucic acid into any such product as the apoglucic acid described by Mulder or into carbon dioxide, although abundant quantities of formic acid and of unidentified polymerization products were obtained.

The readiness with which glucic acid decomposes into formic acid leads to the conclusion that it may be a parent substance of the formic acid which occurs in the sirups and molasses of cane sugar factories, where the calcium hydroxide employed in clarification reacts with the reducing sugars of the juice. The instability of this compound in the presence of atmospheric oxygen and in contact with accompanying organic substances is no doubt the reason of its having escaped the attention of so many investigators.

The problem of the formation and decomposition of glucic acid under varying conditions is being subjected in this Laboratory to a further study in the hope that additional information may be secured upon some of the theoretical and practical questions involved.

Summary

1. The unstable calcium salt of an acid, originally described by Winter under the name of glucic acid, was prepared by the action of calcium hydroxide upon dextrose in aqueous solution at 67° in the absence of air. The precipitated calcium compound absorbed oxygen rapidly from the air with decomposition and evolution of heat.

2. The unstable calcium salt, after decomposing with sulfuric acid and extracting with ether, yielded a small amount of crystallizable unsaturated acid, corresponding to the formula $C_3H_4O_3$, which from its strong reducing

power, iodine absorption and other properties is in all probability the enolic form of the semialdehyde of malonic acid. The crystalline acid can be partially sublimed without decomposition but undergoes upon heating a considerable degree of polymerization. It yields formic and oxalic acids upon oxidation and is exceedingly unstable in the presence of organic impurities.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, MEDICAL SCHOOL, UNIVERSITY OF CALIFORNIA]

THE RATE OF HYDROLYSIS OF CASEIN IN ACID SOLUTIONS AS MEASURED BY THE FORMATION OF AMINO NITROGEN

BY E. S. NASSET AND DAVID M. GREENBERG

RECEIVED OCTOBER 3, 1928 PUBLISHED MARCH 6, 1929

Introduction

Greenberg and Burk[1] from their own measurements and such data as were available, calculated the order of the reaction of the hydrolysis of the proteins in solutions of acids. The hydrolysis for gelatin, silk fibroin and gliadin was found to conform to the equation of a second order reaction. A single series of measurements on casein by Dunn[2] apparently fitted a first order reaction best. This was in agreement with the findings of Carpenter,[3] who worked, however, under radically different conditions. It seemed worth while to check the order of the reaction of the hydrolysis of casein, since if a universal structural basis is common to all proteins, individual proteins would not be expected to show different orders of hydrolysis under the same fixed conditions. From the results given in the following, it is to be seen that casein conforms to the proteins already measured and that the hydrolysis reaction is of the second order under the conditions employed. Also, as was found for the other proteins, the effect of acids in accelerating the rate of hydrolysis is shown to be proportional to the hydrogen-ion activity of the acid solutions.

Experimental

The measurements were carried out at the temperatures of 127.5, 117.5 and 105.5°, employing the acids, hydrochloric, sulfuric and phosphoric. The hydrolyses were carried out in an autoclave with a steam line the pressure of which could be regulated by a reducing valve. The casein used was prepared according to the method, somewhat modified, of Van Slyke and Baker.[4] One-gram samples of thoroughly dried casein were placed in 50-cc. pyrex Erlenmeyer flasks, 40 cc. of the desired acid was added and

---

[1] D. M. Greenberg and N. F. Burk, THIS JOURNAL, **49**, 275 (1927).

[2] M. S. Dunn, *ibid.*, **47**, 2564 (1925).

[3] D. C. Carpenter, *J. Biol. Chem.*, **67**, 647 (1926).

[4] L. L. Van Slyke and J. C. Baker, *ibid.*, **35**, 128 (1918); D. M. Greenberg and C. L. A. Schmidt, *J. Gen. Physiol.*, **7**, 287 (1924).