Inasmuch as we had previously determined the presence of trimethylamine, together with ammonia, in a distillate from the cotton plant, and also in the emanations from the living plant, it was presumed that the primary source of the first-mentioned alkali was choline. The correctness of this view has now been rendered evident by the isolation of both choline and betaine from the plant.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF SASKATCHEWAN]

ACTION OF ANILINE ON GLUCOSE IN ACETIC ACID SOLUTION. II

BY C. N. CAMERON

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Recently[1] it has been pointed out that alcoholic solutions of glucose, aniline and acetic acid become brown at room temperature more rapidly than comparable solutions of any of the components. It was shown that the first step in the production of the colored material consisted in the formation of glucose-anilide, a reaction which is catalyzed by the acid. The appearance of the colored material is, however, by no means confined to aniline, as *o*- and *p*-toluidine undergo similar changes in the presence of glucose and acetic acid. The effect of the acid, again, is to speed up the reaction between the amine and the carbohydrate. Further, acetic acid is not essential, as potassium dihydrogen phosphate functions as well, not only in catalyzing the reaction resulting in the formation of the anilide but also in the further changes leading to the production of the brown material.

The first step in the reaction is the formation of glucose-anilide and this substance undergoes further changes to produce the colored material. Now it has been shown[2] that this anilide has the butylene oxide structure and yet apparently the compound is somewhat unstable. True, when quite pure and dry, the anilide can be kept in contact with air for a year at least[1] without change but solutions, especially in the presence of acid, soon become colored. Likewise, it is rather rapidly hydrolyzed and reduces Fehling's solution. In addition, it is found that solutions of glucose, aniline and acetic acid, which contain glucose-anilide, show a condition of reactivity greater than that of each of the components. In general such acid solutions reduce oxidizing agents more readily than corresponding blanks. Thus, in such solutions of glucose, aniline and acetic acid, three days old, the aniline is more readily oxidized to quinone by potassium dichromate than is aniline in the presence of acetic acid. Again, these acid

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[1] Cameron, THIS JOURNAL, **48**, 2233 (1926).

[2] Irvine and Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908).

solutions give Selivanoff's reaction. This might indicate that fructose was formed but, as has been pointed out recently,[3] Selivanoff's reaction "may simply indicate the presence of a reactive sugar."

The contention is, then, that the glucose-anilide, particularly in acid solution, is changed to a more reactive form. Just what this is, of course, is open to question but Strauss[4] has shown that this anilide reacts with prussic acid, by addition to form the corresponding nitrile, so it seems reasonable to assume that the anilide can react in the aldehyde form: $HO.CH_2(CHOH)_4.CH:NC_6H_5$. This aldehyde isomer on hydrolysis would yield aniline and not the butylene oxide but the active aldehyde glucose;[5] at the same time, the condition of unsaturation between carbon and nitrogen would lend itself to oxidation.

An attempt was made to prove that the aldehyde isomer was essential to the rapid formation of the colored material by using methylaniline in place of aniline. In this case the anilide formed could not function in the aldehyde form; hence, glucose should have little effect on the formation of color. It was found that solutions of methylaniline, glucose and acetic acid become colored at relatively the same rate as comparable solutions of methyl aniline and acetic acid. Similarly, a solution of methylaniline and glucose colored at the same rate as one containing the amine only. However, the reaction between glucose and methylaniline is apparently very slow at room temperature and it could be objected that the slow formation of the intermediate compound, glucose-methylanilide, was responsible for the absence of effect. The intention is to test this point further, using aliphatic amines.

The colored material precipitated by water from alcoholic solutions of glucose, aniline and acetic acid has been found under the conditions of the reaction to contain a small amount of the oxidation products of aniline, as 2,5-dianilido-quinone has been identified. Corresponding solutions of aniline and acetic acid gave no precipitate on dilution. This would indicate that aniline was more readily oxidized in the presence of glucose. However, only a small fraction of the colored material could be identified as an oxidation product of the amine; in particular, a high-melting substance insoluble in ether and ligroin, soluble in alcohol, did not have the properties of any of the known oxidation products of aniline. Moreover, benzylamine, which is oxidized only with difficulty, gives colored products with glucose and acetic acid. Further, although it is not reported here, glucose solutions containing ammonia and a weak acid like acetic, become colored; thus, oxidation of the amine cannot be responsible entirely for the brown substance. This point is being investigated further, but it might

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[3] Hynd, *Biochem. J.*, **20**, 202 (1926).

[4] Strauss, *Ber.*, **27**, 1284 (1894).

[5] Irvine, *Ind. Eng. Chem.*, **15**, 1162 (1923).

be mentioned that as solutions of glucose, aniline and acetic acid after some time fail to give Fehling's reaction, then both glucose and glucose-anilide must disappear as such; however, in the filtrate from the colored material neither such oxidation products as gluconic acid nor reduction products such as sorbitol could be detected in appreciable amounts. It seems feasible to assume, then, that the bulk of the colored material is a condensation product of aniline and some decomposition product (methylglyoxal?) of glucose.

Experimental Part

***o*- and *p*-Toluidine, Glucose and Acetic Acid.**—Glucose solutions containing *o*- and *p*-toluidine were made up as shown in Table I and at the same time comparable solutions of the toluidines but without glucose. The color development was noted in all cases.

It was found that at room temperature a solution of *p*-toluidine, glucose and acetic acid had acquired a tinge of yellow in five hours, and was brown in 12 days; a comparable solution of *p*-toluidine and glucose was brown in 72 days; one of *p*-toluidine and acetic acid was orange in 240 days, whereas a solution of *p*-toluidine was a deep yellow in 240 days. Similarly, a solution of *o*-toluidine, glucose and acetic acid was a faint yellow in two days and brown in 28 days; one of *o*-toluidine and glucose was brown in 202 days; one of *o*-toluidine and acetic acid and *o*-toluidine alone were both light yellow in 160 days.

The effect of acetic acid on the reaction between the toluidines and glucose was determined by following the change in rotation. As it was shown in the case of aniline, glucose and acetic acid that glucose-anilide is formed in the acid solution, it was assumed in this case that the same compound was formed by the toluidines in the presence and absence of acid. The solutions were at room temperature, roughly 21°; time was computed from the addition of the amine.

TABLE I

EFFECT OF ACETIC ACID ON THE REACTION BETWEEN GLUCOSE AND *p*-TOLUIDINE; GLUCOSE AND *o*-TOLUIDINE

	Glucose, 1.5 g.; *p*-toluidine, 0.89 g. Alcohol to 50 cc. Tube, 1 dcm.		Glucose, 1.5 g.; *o*-toluidine, 0.89 g. Alcohol to 50 cc. Tube, 1 dcm.	
Acetic acid	0.5 g.	None	0.5 g.	None
Time	α	α	α	α
5 min.	+1.59	+1.68	+1.65	+1.68
2 hours	+0.15	+1.69	+0.91	+1.69
6 hours	− .56	+1.65	+ .08	+1.66
1 day	− .61[a]	+1.46	− .52	+1.57
6 days	?	+0.03	− .63[a]	+0.64
18 days		− .82[a]		− .18
60 days		− .78		− .64[a]

[a] Maximum values observed.

Sodium Dihydrogen Phosphate, Glucose and Aniline.—To show that the production of color in solutions of glucose, aniline and acetic acid was not due to any property peculiar to the acetic acid, the effect of sodium dihydrogen phosphate was tried. Of necessity the solvent used was water. The solutions were made up as in Table II at room temperature (23–24°); the colored material is insoluble in water and in this case precipitates. Compound formation is shown by the change in rotation only, as the reaction is being investigated further.

TABLE II

THE EFFECT OF SODIUM DIHYDROGEN PHOSPHATE ON GLUCOSE AND ANILINE COMPARED WITH THAT OF ACETIC ACID

Glucose, 1.5 g. Aniline, 0.775 g. (1 mol. prop.) Tube, 1 dcm. Water to 50 cc.

	Sodium dihyd. phosphate 1.15 g. (1 mol. prop.)		Acetic acid, 0.5 g. (1 mol. prop.)	
Time	α	Color	α	Color
5 min.	+1.44	Colorless	+1.34	Colorless
3 hours	−0.32	Colorless	0.00	Tinge of yellow
6 hours	− .56	Slight yellow	− .02	Light yellow
2 days	− .56	Yellow; pptn. starts	− .01	Yellow; pptn. starts
12 days	− .49	Yellow; marked pptn.	.00	Yellow; marked pptn.

A chocolate-brown precipitate was gradually formed both in a water solution of glucose, aniline and acetic acid and in a similar solution of glucose, aniline and sodium dihydrogen phosphate. In each case, the melting point of the precipitate was vague; the substance started to shrink at 145° and at about 170° melted with decomposition. With both the acetic acid and the sodium dihydrogen phosphate the rotation of the solution became negative, although more definitely so in the case of the latter, indicating the likely formation of the compound glucose-anilide.

Glucose, Aniline and Acetic Acid.—The following experiments indicate that solutions containing glucose, aniline and acetic acid show a greater activity than comparable solutions without all three components present.

Five solutions of 50 cc. were made up as follows: a water (1) and an alcoholic (2) solution of 1.5 g. of glucose, 0.5 g. of acetic acid and 0.775 g. of aniline; a comparable alcoholic solution of glucose and aniline (3); an alcoholic solution of aniline and acetic acid (4) and an alcoholic solution of glucose and acetic acid (5).

Solution 2, when one day old, became pink as soon as it was warmed with hydrochloric acid and resorcinol and a deep red on being heated for one minute. When Solution 3 was heated under the same conditions, it became decidedly pink, whereas Solution 5 gave no color. When Solution 1 was three days old, it became pink on the addition of the acid only but this color disappeared on warming. It was thought that the strong acid acted on the active glucose-anilide to form oxymethylfurfural and this with the aniline gave the red coloration.

To three separate test-tubes containing 5 cc. of approximately 0.4 *N* potassium dichromate solution, 2 cc. of dil. sulfuric acid and 10 cc. of ether, was added 2 cc. of Solutions 1, 2 and 4 (three days old) and the tubes were shaken. Solutions 1 and 2 became an opaque green at once; the yellow color of quinone was apparent as soon as the ether had separated—a matter of a few seconds. Solution 4 was green in 30 seconds and gradually became darker but it was not until four minutes had passed that the yellow of quinone could be detected in the ether.

Other tests were tried, such as with methylene blue, acid and alkaline permanganate and, in general, under suitable conditions, Solutions 1 and 2 reduced the reagent more rapidly than did the corresponding blanks. However, owing to the fact that alcohol, aniline and glucose (alkaline) are readily oxidized, these tests are being repeated under more suitable conditions.

Methylaniline and Glucose.—Two alcoholic solutions of 1.5 g. of glucose were allowed to stand for 24 hours. To one (1) was added 0.5 g. of acetic acid and 0.89 g. (1 mol. prop.) of freshly distilled methylaniline; to the other (2) 0.89 g. of the methylaniline only. The rotation was followed as shown in Table III to determine whether compound formation had occurred. At the same time the color was noted and compared with that of similar solutions containing (3) comparable quantities of methylaniline and acetic acid and (4) methylaniline only in 50 cc. of alcohol. The solutions were at room temperature, 20–22°.

Table III

Reaction of Glucose and Methylaniline

Glucose, 1.5 g. Methylaniline, 0.89 g. Alcohol to 50 cc. Tube, 1 dcm.

	1	2
Acetic acid	0.5 g.	None
Time	α	α
5 min.	+1.71	+1.72
6 hours	+1.26	+1.70
1 day	+1.15	+1.67
30 days	+0.82	+0.76

As a result of this experiment it was found that both Solutions 1 and 3 showed the first slight tinge of color in 12 days; the color gradually deepened until after 85 days they were both red. Both Solutions 2 and 4 showed a slight tinge in 70 days; thereafter, the color very slowly deepened but was apparently the same in both cases.

The Colored Material.—The colored material produced in alcoholic solutions of glucose, aniline and acetic acid can be precipitated by dilution with water. The precipitate is a mixture of which only a part is discussed, as definite separation of other pure fractions has not been accomplished.

To an alcoholic solution of 7.5 g. of glucose was added three molecular proportions of aniline and acetic acid. The solution was then made up to 250 cc. with alcohol, at 23–24°, and shaken every second day. In 34 days when Fehling's reaction was negative, the solution was diluted to 1300 cc.

with water. The precipitate that separated was removed by filtration, washed with water and dried in a vacuum. A comparable solution of aniline and acetic acid gave no precipitate on dilution in 34 days. The precipitate weighed 4.63 g., was chocolate-brown in color and, on heating, darkened at about 90° and melted at 125°. The separation of the mixture is omitted for the present as it resulted in a large number of fractions. Suffice it to say that the whole precipitate was soluble in alcohol but part was very slightly soluble in absolute ether and in ligroin. Two fractions in particular were separated; one, a brown substance, non-crystalline, insoluble in ether and ligroin, soluble in ethyl and methyl alcohols. It dissolves in concd. sulfuric acid with a brown color but separates out again on dilution. It dissolves to some extent in glacial acetic acid to give a brown solution; it does not melt below 300°. The amount finally obtained in a pure condition was not sufficient for analysis. There was also isolated a substance of melting point 202°, agreeing in properties with 2,5-dianilido-quinone of melting point 202–203°.[6] The amount secured was not sufficient for analysis but a mixed-melting-point determination with the quinone-anil, made in the usual manner, was the same as that of the pure substance alone.

A precipitate obtained from 50 cc. of a similar solution on steam-distillation yielded neither quinone nor azobenzene.

The filtrate from the colored material was neutralized with barium hydroxide solution and evaporated under diminished pressure. A small amount of colored material separated during distillation and this was filtered off. The white residue was dissolved in water and the solution divided into two parts. From the one, barium was removed by the addition of sulfuric acid; the filtrate from the barium sulfate was evaporated. A small amount of sirup was thus obtained but on examination there was no evidence that sorbitol was present. The other part of the filtrate was heated with phenylhydrazine and acetic acid. There was obtained 0.89 g. of a red-brown precipitate; m. p., 122–125°. It was separated into a very small fraction of what was probably the hydrazide of gluconic acid (m. p., 200° with decomposition); when this was mixed with known hydrazide of gluconic acid the melting point was unchanged. A very small fraction of a brown material melting at 186–187° was also obtained; this was not identified. The larger part of the precipitate was found difficult to purify; thus, after ten recrystallizations from benzene, it was colorless but the melting point was 126–128°. The amount left was too small for further purification.

Benzylamine, Glucose and Acetic Acid.—If the color developed in solutions of glucose, aniline and acetic acid were entirely due to oxidation of the amine, then with benzylamine in place of aniline little color should

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[6] Schunck and Marchlewski, *Ber.*, **25**, 3574 (1892).

appear. However, solutions of benzylamine, glucose and acetic acid become colored more rapidly than corresponding blanks.

Four alcoholic solutions were made up to contain in 50 cc.: (1) 1.5 g. of glucose, 0.89 g. of benzylamine and 0.5 g. of acetic acid, (2) a comparable solution of glucose and benzylamine, (3) of benzylamine and acetic acid, (4) of benzylamine alone. They were left at room temperature, 24–25°. Solution 1 had a tinge of yellow in one day, orange in four and a red-brown in ten days; Solution 2 acquired a slight tinge of yellow in ten days, but in 125 days it was still yellow. In 245 days Solution 3 was a faint yellow but Solution 4 was still colorless.

After 75 days the colored material of Solution 1 was precipitated by dilution with water to 300 cc. The precipitate was washed with water and dried in a vacuum. It weighed 0.10 g., was a sandy-brown in color, blackened at 98° and melted at about 135°. Except to discover that the precipitate was soluble in alcohol and a portion was insoluble in ether, the investigation was left until later.

Summary

The coloration produced in solutions of glucose, aniline and acetic acid is not due to any peculiar property of the amine, as *o*- and *p*-toluidine behave in a similar manner, nor is it due to acetic acid as such, for potassium dihydrogen phosphate can be used as the acid component. As solutions of glucose, aniline and acetic acid show a reactive condition and as glucose has little effect on the coloration of methylaniline solutions, it is held that the glucose-anilide is changed to a more reactive form, probably the aldehyde isomer. The coloration may, in part, be due to oxidation of the aniline in presence of glucose, but only in part, as benzylamine, which is difficult to oxidize, in the presence of glucose and acetic acid rapidly becomes colored.

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[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY OF YALE UNIVERSITY]

SOME ACETOPHENONYL DERIVATIVES OF BARBITURIC ACID[1]

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It is well known that the ketone carbonyl group confers hypnotic properties on many organic compounds, a notable case being that of acetophenone, or "Hypnone." In view of this fact it would seem quite probable that the presence of a ketonyl group, particularly acetophenonyl, together with a suitable alkyl group, on the 5-position of the barbituric acid nucleus, would produce a compound having desirable hypnotic properties.

[1] This investigation has been conducted in coöperation with the National Research Council, Sub-Committee on Hypnotics.