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BROWNING OF SUGAR SOLUTIONS. 3. EFFECT OF pH ON THE COLOR PRODUCED IN DILUTE GLUCOSE SOLUTIONS CONTAINING AMINO ACIDS WITH THE AMINO GROUP IN DIFFERENT POSITIONS IN THE MOLECULE[a]

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In the first paper of this series *(11)* it was reported that when dilute glucose solutions containing various types of amino acids were heated at 114° C. for 20 minutes, the presence of lysine, a diaminomonocarboxylic acid, caused the formation of more color than did alanine, a monoaminomonocarboxylic acid or glutamic acid, a monoaminodicarboxylic acid. Also, at the dilute concentration of glucose and amino acids used, over a pH range of 5 to 9, only those solutions containing lysine developed significantly more color than a solution containing only glucose. Further, in none of the solutions except those containing lysine was color produced below pH 7. The presence of lysine resulted in some color at pH 6. Generally, more color was produced at higher pH values — an observation made by many authors *(1, 2, 3, 4, 6, 8, 9, 10)*.

The general basic nature of lysine does not explain its increased activity in color production, since histidine and arginine caused no increase in color over the glucose control. Therefore a second study *(7)* was made to determine the effect of the position of the amino group in the molecule on the production of color at pH 8 in dilute glucose-amino acid solutions. The compounds used were homologs of the 2, 3, 4, 5 and 6 carbon fatty acids containing amino groups (a) on the alpha carbon, (b) on the omega carbon, and (c) on both the alpha and the omega carbon atoms. The alkaline medium was used because definite color was produced by glucose alone at this pH and differences in color development would indicate the relative effect of the various amino acids. These amino acids influenced the amount of color produced to varying degrees *(7)*. Therefore, it was deemed important to determine whether these variations are influenced by the pH of the solution. This paper deals with the effect of pH on the influence of the position of the amino groups in amino acids in the production of color in dilute glucose solutions. The pH effect in the range 5 to 7 was studied because it has been noted in the previous papers that certain amino acids caused sugar solutions to brown slightly below pH 7. As in the earlier work, dilute concentrations of glucose and the amino acids were used in order that differences in pH effect might be readily seen.

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EXPERIMENTAL

That amount of each 12 amino acids [e] was added to 0.1 molar glucose solutions to give 0.1 molar concentrations of the amino acids. Seven series of the 12 amino acid-glucose solutions were made and buffered with the appropriate phosphate buffer at pH 5, 6, 6.5, 6.8, 7, 8, and 9. Five milliliter aliquots of each of these solutions and of glucose controls were heated in sealed tubes at 114° C. as described previously (11). As earlier work had shown that the amino acids would not develop color under the conditions of the study, no amino acid controls were run in this experiment. After cooling, the absorbances of the solutions were measured in 1 cm. cells at 500 mμ in a Cary recording spectrophotometer. The data are presented in Table 1.

RESULTS AND DISCUSSION

The general effect of pH on the reaction between different amino compounds and sugars has been reported by several authors. Borsook and Wastenays (2) found that the reaction as followed by reduction of amino nitrogen was favored by an alkaline medium. Frankel and Katchalsky *(3, 4)* using titration measurements also reported that the α-amino acids and sugars react to a greater extent the higher the pH. The present study revealed the same trend, including those for solutions containing only glucose which had zero absorbance at pH 5, 6, and 6.5 and absorbances of 0.03, 0.08, 0.64 and 0.75 for pH's at 6.8, 7.0, 8.0 and 9.0, respectively. Also, the same variability reported previously *(7)* on the influence among different types of amino acids was again evident. The effect of pH on this variability is illustrated in Figure 1. Since the α-amino acids had little influence on the amount of color pro-

[e] The amino acids used in the study were glycine, DL-α-alanine, DL-β-alanine, DL-α-aminobutyric acid, DL-γ-aminobutyric acid, 2, 4-diamobutyric acid, DL-norvaline, Δ-aminovaleric acid, DL-ornithine • HCl, DL-norleucine, η-aminocaproic acid, and DL-lysine • HCl.

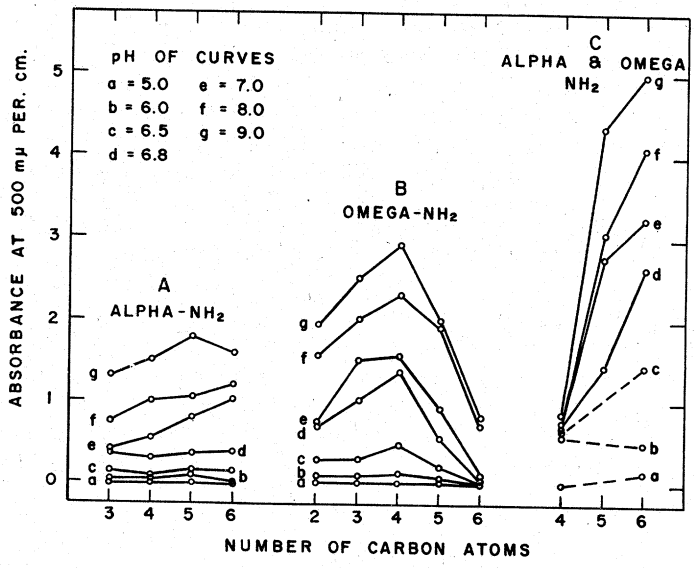

Figure 1. Effect of pH on vanability of reaction between amino compounds and mgars.

duced at pH 8, a change of pH was expected to produce only a small change in their influence. This is confirmed in Figure 1A. In the two series, containing the ω-monoamino and the α, ω-diamino acids, where the position of the amino group and chain length of the acids caused a significant difference in the amount of color produced, the effect of the amino group was accentuated by increasing the pH (Fig. 1B and 1C). In Figure 1B the color producing effect of the chain length of the amino acids containing only omega positioned amino groups is compared at different pH values. Here the amount of color produced at pH 5 and 6 by all the compounds was so small that differences due to chain length were negligible. At pH 6.8 an appreciable amount of color was produced and this differed from that developed at higher pH values only in amount. It should be noted, therefore, that the positive effect of these acids on color is not limited to pH values above 7, but under the conditions of this study continue down to at least pH 6.5. Further, the difference in the amount of color produced appears to be due to an accentuated effect of the amino group as the pH was raised.

Figure 1C shows that pH has an even greater influence on the browning effect of the diamino acids.

These data indicate that the amount of color produced in glucose-amino acid solutions is a function of (a) the pH, (b) the chain length of the acid, and (c) the number and position of the amino groups. The greatest effect of the pH was with solutions containing diamino acids and the least effect was with the α-amino acids. Even though the greatest effect on color was demonstrated by the diamino acids the pronounced increase with increased pH cannot be attributed only to the doubling of the number of amino groups. Were this true, the solutions of diamino acids of a given number of carbon atoms would have (a) browned only twice as much as the monoamino acid solution or (b) had a color value equal to the algebraic sum of the amounts of color formed by both the α-amino and the ω-amino acids of the equivalent chain lengths. Neither of these obtains.

CONCLUSION

This study shows the effect of pH on the amount of color formed in heated dilute glucose solutions containing a straight chain amino acid of three homologous series containing either an alpha or an omega amino group or both, and a chain length of two to six carbons. The pattern of the pH effect is different for the three homologous series but similar for the acids within each series.

In the α-amino acid series little color was produced, but there was a significant increase from pH 7 to 9.

In the ω-amino acid series there was no definite effect at pH 5 and 6, but from 6.5 color production increased progressively with increase of pH up to 9.0. The characteristic item of this series was the maximum effect at a chain length of four carbons.

In the α, ω-amino acid series little color was produced at any pH by the 4 carbon acids, but with the 5- and 6-carbon acids color was greatly increased, especially at the highest pH values. At pH 5 and 6 there was no increase in color with increase in chain length.

Considering the pH effect, especially in the critical range between 5 and 7, the following is observed in the three series of amino acid-glucose solutions:

(a) At pH 5 no browning occurred.

(b) At pH 6 only the α, ω-diamino acid series produced appreciable browning.

(c) At pH 6.5 the ω-amino acid of 4 carbons as well as the α, ω-acids produced browning.

(d) At pH 6.8 both the ω (up to 4 carbon atoms) and the α, ω-acids produced significant browning.

(e) At pH 7 and above all of the series produced browning.

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