[CONTRIBUTION FROM THE GENERAL MILLS RESEARCH LABORATORY]

THE EFFECT OF WATER ON THE MECHANISM OF REACTIONS OF AMINES WITH 3-C-PHENYLGLYCERALDEHYDE

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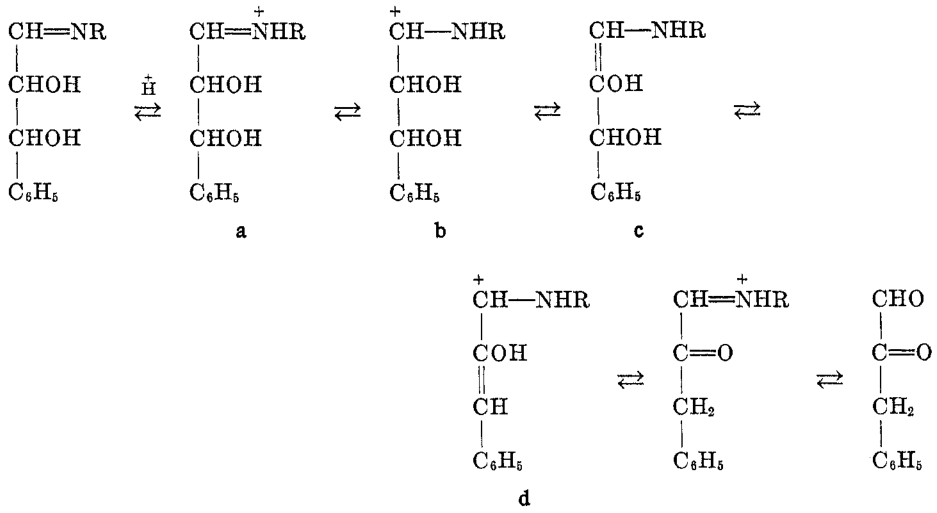
Hodge and Rist (1) recently reported that glycosylamines of certain cyclic alkyl amines and secondary aralkyl amines would undergo the Amadori rearrangement. Gottschalk (2) and Erickson (3) reported evidence for Amadori rearrangement of glycosylamines of an amino acid and of a N-alkyl amine respectively. Prior to these reports, Amadori rearrangement was confined to N-arylglycosylamines (4–6). Mitts and Hixon (7) were unsuccessful in inducing Amadori rearrangement of N-alkylglycosylamines.

Partridge and Gottschalk (8) and Hodge and Rist (1) suggested that Amadori rearrangement is a part of the mechanism of the Maillard reaction. Lea and Hannan (9) found that in a mixture of casein, glucose (1 mole per free amino group of the casein), and water, the optimum amount of water for most rapid development of a brown color was quite small, corresponding to a relative humidity of 65–70%. At concentrations of water above or below the optimum level the rate of browning was retarded. In view of the importance of the concentration of water in the Maillard reaction it occurred to the writer that the extent to which certain glycosylamines will undergo Amadori rearrangement might be largely dependent upon the amount of water in contact with them. The rearrangement of 3-C-phenylglyceraldehyde to benzylglyoxal was chosen as a model system in which to study the effects of different concentrations of water. Prior study by Smith and Anderson (10) of this rearrangement in aqueous solution at 25° showed that it occurred only in the presence of an N-arylamine and a small amount of acid; amines of other classes did not effect this rearrangement. In 0.5% sulfuric acid 3-C-phenylglyceraldehyde did not rearrange to benzylglyoxal or tautomerize to a ketotriose appreciably within two hours at 60° or 3 days at 30°. Thus rearrangement of 3-C-phenylglyceraldehyde to benzylglyoxal in weakly acidic aqueous solution at 25° in the presence of an amine may legitimately be ascribed to action of the amine. Smith and Anderson suggested for this rearrangement the following mechanism.

Smith and Anderson (10) suggested that this rearrangement might be considered an Amadori rearrangement wherein the inability of **c** to undergo ring closure leads to loss of the hydroxyl group as an anion from carbon 3.

In the present investigation the effects of certain amines on 3-C-phenylglyceraldehyde were studied in several solutions of different water content. Anhydrous solutions were not employed as it was reported by Smith and Anderson (12) that in anhydrous dioxane or ethanol, at room temperature, 3-C-phenylglyceraldehyde existed in a dimeric form and did not rearrange to benzylglyoxal in the presence of amines. Instead condensation of one mole of the amine with one mole of the dimeric form of the aldehyde occurred.



The first solution (solution A) contained 3-C-phenylglyceraldehyde, *m*-nitrobenzhydrazide, 5% of acetic acid, and 2% of water in dioxane. The hydrazide was added for the purpose of removing benzylglyoxal, as it formed, from the solution, so that this reactive compound could be isolated, at least in part, before reacting with itself or with amines present.

Addition of primary alkyl amines or amino acid esters to aliquots of solution A at 25° resulted in an immediate change in color of each aliquot from colorless to yellow. Subsequent browning of these solutions was rapid and in each of them a precipitate of the *m*-nitrobenzoylosazone of benzylglyoxal appeared within 72 hours.

Solution B was prepared by dilution of a portion of solution A with water to a concentration of 10% of water. Addition of primary alkyl amines or amino acid esters to aliquots of solution B resulted in an immediate change in color of each aliquot to yellow, and in rapid browning. No precipitate appeared in any of these solutions within a period of 72 hours at 25°.

It is probable that the main factor that determines whether or not rearrangement of a certain aminoglycoside shall occur is the ratio of ion **b** to ion **a** at equilibrium. Aminoglycosides of N-alkyl amines and of amino acids are hydrolyzed to a greater extent in aqueous solutions than are those of N-aryl amines (7, 11). In the presence of only a small amount of water the basicity of the amino function of easily hydrolyzable aminoglycosides may be weakened sufficiently so that the ratio of ion **b** to ion **a** at equilibrium favors rearrangement.

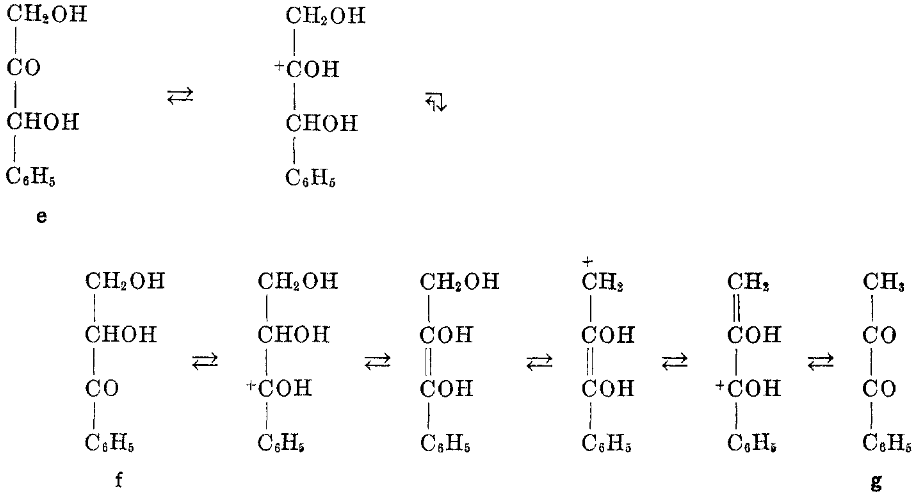
Solution C was prepared in which concentrations of solutes were the same as in solution A, but the solvent consisted of four parts of dioxane and one of water. Addition of primary alkyl amines or amino acid esters to aliquots of this solution gave results similar in each case to those obtained in aliquots of solution B.

Aliquots of solutions A, B, and C remained colorless at 25° for 72 hours; and no precipitate appeared in any of these aliquots during this period.

Addition of benzylglyoxal to aliquots of solutions A, B, and C at 25° resulted in precipitation of the osazone of benzylglyoxal and *m*-nitrobenzhydrazide in each aliquot within 24 hours.

Addition of aniline to aliquots of solutions A, B, and C resulted in precipitation of the osazone of benzylglyoxal and *m*-nitrobenzhydrazide in each aliquot within 72 hours at 25°.

In the presence of a strongly basic amine in aqueous solution, 3-C-phenylglyceraldehyde may tautomerize to either phenyldihydroxyacetone **e** or α,β-dihydroxypropiophenone (f). Either of these ketones may rearrange to acetylbenzoyl (g) (10).



$$\underset{\mathbf{e}}{\begin{array}{c} CH_2OH \\ | \\ CO \\ | \\ CHOH \\ | \\ C_6H_5 \end{array}} \rightleftarrows \begin{array}{c} CH_2OH \\ | \\ {}^{+}COH \\ | \\ CHOH \\ | \\ C_6H_5 \end{array} \;\;\rightleftarrows$$

$$\underset{\mathbf{f}}{\begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ CO \\ | \\ C_6H_5 \end{array}} \rightleftarrows \begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ {}^{+}COH \\ | \\ C_6H_5 \end{array} \rightleftarrows \begin{array}{c} CH_2OH \\ | \\ COH \\ \| \\ COH \\ | \\ C_6H_5 \end{array} \rightleftarrows \begin{array}{c} \overset{+}{C}H_2 \\ | \\ COH \\ \| \\ COH \\ | \\ C_6H_5 \end{array} \rightleftarrows \begin{array}{c} CH_2 \\ \| \\ COH \\ | \\ {}^{+}COH \\ | \\ C_6H_5 \end{array} \rightleftarrows \underset{\mathbf{g}}{\begin{array}{c} CH_3 \\ | \\ CO \\ | \\ CO \\ | \\ C_6H_5 \end{array}}$$

The *m*-nitrobenzoylosazone of benzylglyoxal is only sparingly soluble in hot dioxane, whereas the *m*-nitrobenzoylosazone of acetylbenzoyl is readily soluble in cold dioxane and is slightly soluble in dioxane-water (1-1). This may account in part for browning of aqueous solutions of 3-C-phenylglyceraldehyde and strongly basic amines under conditions employed in this investigation without precipitation of any osazone of a dicarbonyl compound, for removal of acetylbenzoyl as it is formed may be precluded by the solubility of the osazone.

The efficacy of amino acids in causing rearrangement of 3-C-phenylglyceraldehyde to benzylglyoxal was difficult to assess because of the insolubility of amino acids in dioxane. However, in solutions of amino acids, *m*-nitrobenzhydrazide, acetic acid, and 3-C-phenylglyceraldehyde in water at 25°, development of yellow color was extremely slow and no osazone was obtained. Two solutions, each of which contained an amino acid and 3-C-phenylglyceraldehyde in water were heated to boiling and were distilled under a vacuum. As the solutions were heated they turned yellow and became brown by the time the boiling point was reached. An appreciable amount of acetylbenzoyl was found in the distillate in each case. Presumably tautomerization of 3-C-phenylglyceraldehyde to a ketotriose and subsequent dehydration of the ketotriose resulted in acetylbenzoyl. Similar heating and distillation of the aldehyde in water did not produce an appreciable amount of acetylbenzoyl.

It appears probable that browning of 3-C-phenylglyceraldehyde in solutions which contain water may occur by any one or a combination of several different mechanisms. In the presence of N-aryl amines at room temperature, browning occurs principally by subsequent reaction of benzylglyoxal which is formed by rearrangement of the aldehyde. In the presence of N-alkyl amines or amino acid esters, 3-C-phenylglyceraldehyde may undergo browning by at least two different mechanisms, only one of which, rearrangement to benzylglyoxal, is known. Rearrangement of the aldehyde to benzylglyoxal in the presence of N-alkyl amines is inhibited by increasing the amount of water present. In the presence of amino acids in water, 3-C-phenylglyceraldehyde browns very slowly. If heat is applied in order to accelerate the reaction, at least part of the brown product formed may be attributed to further reaction of acetylbenzoyl which has been formed by tautomerization of the aldehyde to a ketotriose and subsequent dehydration of the ketotriose.

EXPERIMENTAL PART

3-C-Phenylglyceraldehyde (I) was prepared by the method of Smith and Anderson (10).

Osazone of m-nitrobenzhydrazide (II) *and benzylglyoxal* (III). Solution A contained water (2.0 cc.), I (2.0 g.), II (6 g.), acetic acid (5.0 cc.), and dioxane (93 cc.).

Solution B was prepared by addition of water (9 cc.) to 100 cc. of solution A.

Solution C was prepared by addition of water (19 cc.) to a solution of I (2.0 g.), II (6 g.), and acetic acid (5.0 cc.) in dioxane (76 cc.).

Benzylglyoxal (III) prepared by the method of Dakin (13), was added (0.1 g.) to an aliquot (10 cc.) of each of solutions A, B, and C. These aliquots and control aliquots (10 cc.) of each solution were kept at 25° for 72 hours. During this time precipitates appeared in the aliquots to which III had been added, but the controls remained clear. After recrystallization from dioxane, the osazones were white; yields were 0.15 g. (50%), 0.14 g. (46%), and 0.17 g. (58%) from solutions A, B, and C respectively. Each osazone melted at 189–192° and had $\lambda_{\text{max.}}^{\text{EtOH or dioxane}}$ 300 mμ in agreement with values obtained on an authentic specimen (10) of the *m*-nitrobenzoylosazone of benzylglyoxal.

Rearrangement of I *to* III *in solution A*. Each of a number of N-alkyl amines and amino acid esters was added (0.4 g.) to a separate aliquot (10 cc.) of solution A. During a period of 72 hours at 25°, each of the solutions became brown and in each a precipitate appeared. Amines used in this experiment were ethanolamine, ethylamine, hexylamine, octadecylamine, and the ethyl esters of glycine, alanine, and phenylalanine. The osazones obtained were recrystallized from dioxane; each melted within the range of 188–191° and had $\lambda_{\text{max.}}^{\text{EtOH or dioxane}}$ 300 mμ. The melting point of each osazone was not depressed by mixing the osazone with an authentic specimen of the *m*-nitrobenzoylosazone of benzylglyoxal. Weights of the osazones were 0.08 g. (14%), 0.07 g. (12%), 0.10 g. (17%), 0.21 g. (36%), 0.08 g. (14%), 0.12 g. (20%), and 0.15 g. (25%) from solutions of the ethyl esters of phenylalanine, alanine, and glycine; and of ethanolamine, ethylamine, hexylamine, and octadecylamine respectively.

m-Nitrobenzoylosazone of acetylbenzoyl (IV). Acetylbenzoyl, prepared by the method of Hartmann and Roll (14) was added to a solution of II and acetic acid (5%) in dioxane-water. The osazone was recrystallized from dioxane-water, m.p. 186–187°, $\lambda_{\text{max.}}^{\text{EtOH or dioxane}}$ 262 mμ. Thus, the osazone of IV was not the osazone obtained upon rearrangement of I in any aliquot of solution A.

Action of amines in solutions B and C. The same amines that were added to aliquots of solution A were added in the same amounts to aliquots (10 cc.) of solution B and to aliquots (10 cc.) of solution C. All aliquots became brown in color during a period of 72 hours at 25°, but no precipitate appeared in any of them.

Rearrangement of I *to* III *induced by aniline in solutions A, B, and C.* Aniline (0.4 g.) was added to an aliquot (10 cc.) of each of solutions A, B, and C. Within 72 hours at 25° all three aliquots turned brown and a precipitate appeared in each one. After recrystallization from dioxane, the osazone from each solution melted within the range of 188 of 191°, $\lambda_{\text{max.}}^{\text{EtOH or dioxane}}$ 300 mμ; yields were 0.19 g. (33%), 0.20 g. (35%), and 0.25 g. (42%) from solutions A, B, and C respectively.

Rearrangement of I *to acetylbenzoyl* (IV). A solution of I (0.5 g.) in water (20 cc.) was mixed with a suspension of phenylalanine (1 g.) and several drops of acetic acid in water (20 cc.). The mixture was distilled at atmospheric pressure until 30 cc. of distillate had been collected. The distillate was shaken with ether. From the ether extract, a yellow oil was obtained (0.22 g.) which was identical with an authentic specimen of IV; b.p. 124–126°/37 mm., n_D^{30} 1.5280.

The experiment was repeated, using glycine instead of phenylalanine; IV (0.14 g.) was isolated from the distillate.

The experiment was repeated omitting the amino acid; no IV could be isolated from the distillate.

SUMMARY

Addition of primary aliphatic amines or amino acid esters to solutions of 3-C-phenylglyceraldehyde in a mixture of dioxane and water at room temperature resulted in an immediate formation of a yellow color and rapid browning in the solutions. Rearrangement of the aldehyde in these solutions to benzylglyoxal occurred on addition of primary aliphatic amines or amino acid esters only if the amount of water present was small. No osazone of benzylglyoxal could be isolated from solutions in which water was present to the extent of 10% or more. Solutions of amino acids and 3-C-phenylglyceraldehyde in water became yellow very slowly at room temperature, and no benzylglyoxal could be isolated from these solutions. Several solutions of amino acids and 3-C-phenylglyceraldehyde in water were heated to boiling and distilled under a vacuum. Each solution became brown during heating, and an appreciable amount of acetylbenzoyl was obtained from each distillate.

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