SUGAR ANALYSIS

Ultraviolet Spectra of Sugars in Alkaline Solution

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The ultraviolet absorption spectra which are produced in alkaline solutions of some of the simple sugars have been measured. Investigation of these spectra shows that they are not due to the presence of reductone or pyruvaldehyde, as has previously been supposed. The chromogen responsible for the spectra, which was isolated by partition chromatography, is also responsible for the brown color which develops in alkaline sugar solutions. It is suggested that this chromogen is the result of condensation of compounds produced by the fragmentation of sugars in alkaline solution.

THE ULTRAVIOLET SPECTRA OF SEVERAL SIMPLE SUGARS in alkaline solution have been examined in the hope of gaining information about the early stages of the decomposition of sugars under these conditions. The action of alkalies on sugars causes isomerization and fragmentation, followed by stabilization of the fission products. Under severe reaction conditions, formic, acetic, and lactic acids are produced (*8*), while milder conditions allow the isolation of less stable compounds. Pyruvaldehyde is generally postulated as the precursor of lactic acid (*4*). However, the evidence for the presence of pyruvaldehyde (the Ariyama test with arsenophosphotungstic acid) has been criticized as being ambiguous because of its inability to distinguish pyruvaldehyde from acetol (*16*). Better evidence has been obtained for the formation of acetol and reductone (glucic acid) in alkaline sugar solutions. The former may be steam distilled from neutral or alkaline sugar solutions (*7*) and uniquely identified by conversion to 3-hydroxyquinaldine, while the latter may be precipitated as the lead salt from heated alkaline glucose solutions (*6*).

As is pointed out by Sattler and Zerban (*15*), heteropolar fission of the C_3-C_4 linkage in the glucose molecule could lead to the formation of one molecule of reductone and one of acetol, so that the intermediate formation of the trioses or of pyruvaldehyde need not be postulated. A somewhat similar mechanism has been proposed by Montgomery (*13*) by which one molecule of reductone, two molecules of pyruvaldehyde, and one molecule of hydroxypyruvaldehyde (or dihydroxyacetone) would be produced from two molecules of glucose.

Color formation in acid sugar solutions ("browning") has been extensively investigated (*17*), but that due to the action of alkalies on sugars has received less attention. Like its acid counterpart, however, the alkaline browning reaction is of importance in industrial processes, such as the conversion of sugars to lactic acid (*14*).

The sensitivity of ultraviolet absorption spectrophotometry makes possible the detection of reaction intermediates, and it was hoped that this technique would yield some information about the mechanism of formation of acetol, reductone, or pyruvaldehyde in alkaline sugar solutions. The results which were obtained do not furnish this information; the ultraviolet spectra of alkaline sugar solutions are apparently due not to reaction intermediates, but to stable products of sugar degradation. However, the observations permit the establishment of some conclusions about the mechanism of alkaline sugar fragmentation, and may be of interest in connection with color formation in alkaline sugar solutions.

Experimental Work

All spectra were obtained with the Beckman Model DU spectrophotometer, using 1-cm. matched silica cells. A 3% solution of C.P. D-glucose in distilled water shows no absorption in the region covered by the instrument—i.e., to 200 mμ. The addition of sodium hydroxide to give a 0.01*N* solution causes the immediate development of end adsorption beginning at about 240 mμ (Figure 1). On the immediate neutralization of the solution, the end adsorption disappears, and reappears unchanged on subsequent addition of alkali.

If the solution is allowed to remain alkaline, however, an adsorption band with a maximum at 295 mμ develops slowly, as is shown in Figure 1. The formation of this spectrum is accompanied by the production of a yellow or brown color. Acidification of the solution after the appearance of the absorption band at 295 mμ does not cause its disappearance, but produces a shift in the position of the maximum to 265 mμ (Figure 2). The spectral shift is reversible and occurs at a pH of approximately 7.5.

The same absorption spectrum was found to be produced by several hexoses and pentoses, as well as by the trioses. The rate of production of the spectrum depends on the sugar used. In Table I are given absorbances at 295 mμ of 1.0% solutions of various sugars in 0.10*N* sodium hydroxide, after 24 hours at room temperature. The absorbances were calculated from measured absorbances and the dilution factors used in their measurement. The rate of development of the spectrum depends on the hydroxide ion concentration.

Table I. Absorbances of Alkaline Sugar Solutions

Sugar	D_{295m}
D-Glucose	3.0
D-Mannose	1.2
D-Fructose	7.4
L-Sorbose	8.9
D-Arabinose	4.7
D,L-Glyceraldehyde	11.9
Dihydroxyacetone	55.3

The rate of formation of the spectrum in a 1.0% solution of D,L-glyceraldehyde in 0.010*N* sodium hydroxide at 25° C. is shown in Figure 3. Also shown in this figure is the curve for the formation of ketohexoses from D,L-glyceraldehyde under the same conditions, as measured with the anthrone reagent (*2*). The rate of chromogen production from dihydroxyacetone under the same conditions was found to be approximately the same, but the amount of chromogen produced was much greater (cf. Table I).

The compound producing the characteristic alkaline sugar spectrum (the

"sugar chromogen") appears to be stable in either acid or alkaline solution.

In order to determine whether the sugar chromogen is identical with known compounds, the ultraviolet spectra of several keto and hydroxyaldehydes and acids were obtained in aqueous solution in various pH ranges. Pyruvaldehyde solutions [prepared by the distillation of acidified glyceraldehyde solutions (*18*)] have a single absorption maximum at 280 mμ at all pH values from 2 to 10. Dihydroxyacetone solutions absorb weakly at 270 mμ, while D,L-glyceraldehyde solutions show no ultraviolet absorption, except for the slow development of the sugar chromogen spectrum.

Reductone (prepared via the lead salt) and its tautomer, hydroxypyruvaldehyde (*7*), have identical spectra, with a maximum at 265 mμ at pH 2 to 3.5, which is shifted to 290 mμ at pH 6.5 to 8. Both peaks disappear rather rapidly when the solutions are allowed to stand at room temperature, since these substances are not stable in solution. Acetol solutions show a maximum at 270 mμ at pH 4 to 11, a small shift to 275 mμ being observed at pH 11.5. The prolonged action of alkali on acetol causes the development of a different spectrum (that of the "acetol chromogen"), which is shown in Figure 4. The curve with a maximum at 290 mμ was produced by a 0.1% solution of acetol in 0.01*N* sodium hydroxide after 18 hours at room temperature. Acidification causes a shift of the maximum to 255 mμ, the shift occurring at about pH 8.

In Figure 5 are shown plots of λ_{max} against pH for the sugar and acetol chromogens, and reductone.

A preliminary examination of the spectra of several other compounds (pyruvic acid, hydroxypyruvic acid, glucosone, 2-ketogluconic acid, saccharinic acids) showed that their absorption spectra did not at all resemble that of the sugar chromogen.

To obtain further insight into the nature of the sugar chromogen, separations by ion exchange and chromatography were undertaken. Passage of sugar solutions containing the chromogen over an anion exchange resin (Amberlite IRA-400) removed the chromogen (as shown by the disappearance of the ultraviolet absorption spectrum and the decolorization of the solution), but the chromogen could not be recovered from the resin by regeneration with acid. The brown solution obtained had an absorption spectrum with maxima at 270 mμ in acid and 280 mμ in alkaline solution.

Partition chromatography of sugar-chromogen mixtures on a powdered cellulose column (*3*) gave a successful separation of sugar and chromogen. A solution of 2 grams of fructose in 100 ml. of 0.10*N* sodium hydroxide was allowed to stand at room temperature for 18 hours. It was then acidified to pH 6 and evaporated to a sirup under reduced pressure. The sirup was poured onto the top of the cellulose column and chromatographed with butanol-water at 60° C., portions of the effluent being examined for absorbance at 265 mμ and for reducing sugar content by cerimetry (*12*). The results are shown in Figure 6. Evaporation under reduced pressure of the portion of the effluent containing chromogen gave a brown sirup which, on being dissolved in water, showed an ultraviolet absorption practically identical with that of the original sugar-chromogen solution. The positions of the maxima were at 265 mμ in acid and 295 mμ in alkaline solution.

On standing for several weeks, the water solution of the chromogen slowly deposited a brown insoluble material. This observation was taken to indicate that the chromogen may itself be an intermediate stage in the formation of a more stable product, which results from further condensation and dehydration of the chromogen.

Discussion

The absorption spectrum shown in Figure 1 is the simplest which can be obtained in alkaline sugar solutions. Reaction conditions which are more rigorous (higher temperature or higher hydroxide ion concentrations) lead to more complex spectra which are correspondingly more difficult to interpret (*9*, *11*).

The data that were obtained do not allow a final conclusion to be drawn concerning the composition of the chromogen responsible for the absorption spectrum produced under mild conditions. It is apparent that the observed spectrum is not, as has previously been supposed, due to one of the common hexose fragmentation products which may be isolated from alkaline sugar solutions.

Pyruvaldehyde has been postulated as responsible for the spectra of alkaline sugar solutions (*10*), but the absorption maximum of the pyruvaldehyde spectrum was found to be independent of pH. Reductone has also been considered as the source of the alkaline sugar spectrum (*5*). Reductone and the sugar chromogen have approximately the same spectra in both acid and alkaline solution, but the spectral shift for reductone occurs at pH 5—i.e., at the pK value for reductone. Furthermore, the sugar chromogen is much more stable in either acid or alkaline medium than is reductone.

The absorption spectrum of acetol

Figure 1. Development of alkaline sugar spectrum in 3% solution of D-glucose in 0.01N sodium hydroxide

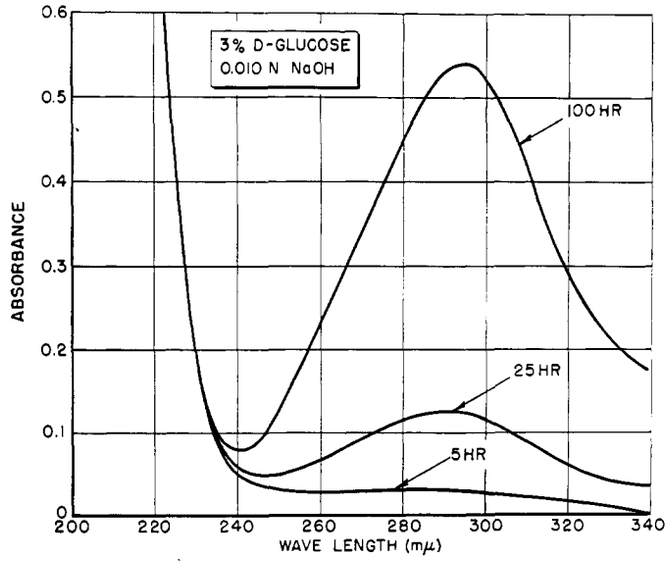
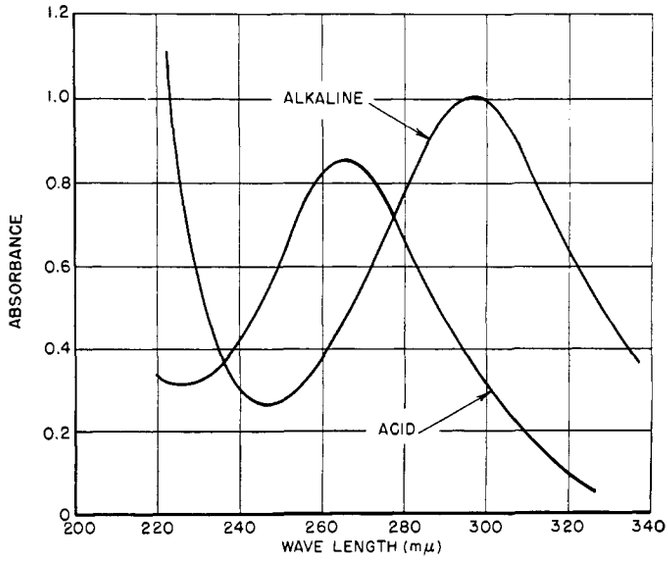


Figure 2. Shift of sugar chromogen spectrum with change of pH



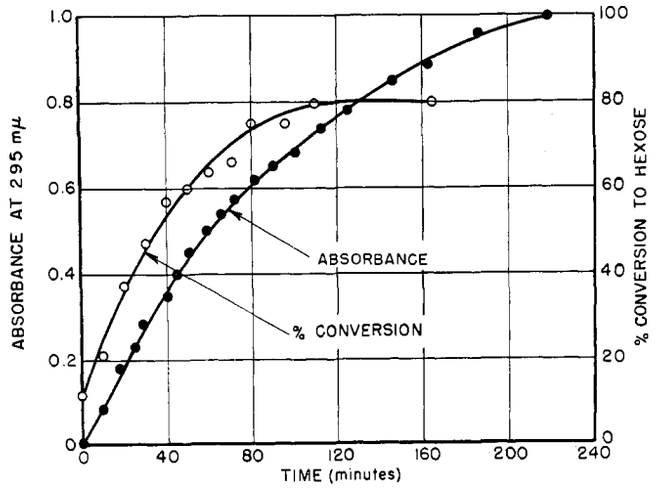


Figure 3. Rate of formation of sugar chromogen and keto-hexoses from D,L-glyceraldehyde

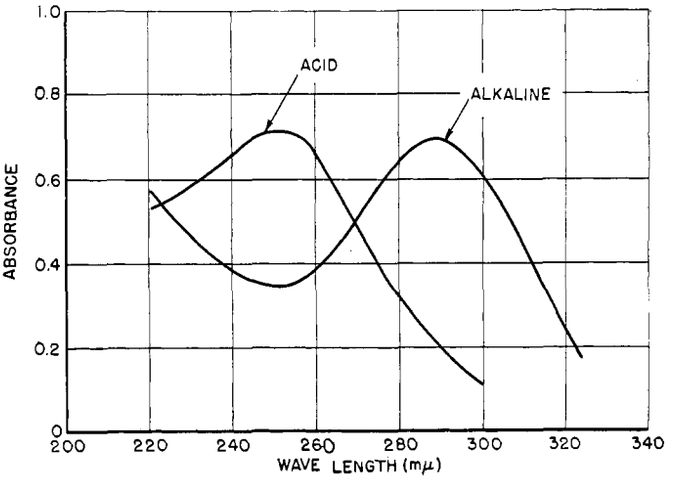


Figure 4. Ultraviolet absorption spectrum of acetol chromogen

also shows that it cannot be the sugar chromogen; however, it may be significant that the action of dilute alkali on acetol gives rise to an acetol chromogen having an absorption spectrum distinct from that of acetol itself. The production of a brown color in alkaline acetol solutions is well known.

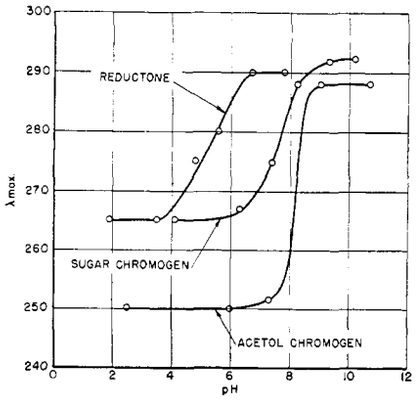


Figure 5. Variation of λ_{max} with pH

The rate of formation of the sugar chromogen in alkaline glyceraldehyde solutions is of interest because it gives some indication of the mechanism of chromogen formation. This rate is slightly lower than that of hexose formation from glyceraldehyde, and many times faster than the rate at which the chromogen is formed from fructose or sorbose, which are the main products of triose condensation (*12*). These facts, together with the observation that dihydroxyacetone gives an especially large amount of the chromogen, suggest that three carbon compounds are intermediates in chromogen formation from the hexoses or pentose. Furthermore, the similarities between the sugar chromogen spectrum and those of reductone and the acetol chromogen indicate structural similarities. It is possible, therefore, that the sugar chromogen is formed by the condensation of sugar fragmentation products, such as acetol, followed by dehydration.

The brown color of the sugar chromogen shows that it is responsible for the coloration of sugar solutions under alkaline conditions. The chromogen appears to be similar to certain humic substances encountered in sugar manufacture (*19*). The separation of the chromogen by partition chromatography will allow the study of its undoubtedly very complex structure.

Acknowledgment

The authors wish to express their appreciation to E. J. Frazza and R. M. Moyerman for their very valuable assistance in carrying out this work.

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Received for review August 11, 1953. Accepted November 5, 1953. Presented before the Division of Sugar Chemistry at the 122nd Meeting of the AMERICAN CHEMICAL SOCIETY, *Atlantic City, N. J. Work supported by the U. S. Navy Bureau of Ordnance under Contract NOrd-7386.*

Figure 6. Separation of sugar and chromogen by partition chromatography on powdered cellulose