[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Chemical Interactions of Amino Compounds and Sugars. II.[1] Methylation Experiments[2,3]

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The interaction of reducing sugars and amino acids results in a decomposition reaction in which dark-colored products are formed.[5] It has been the objective of these investigations to determine some of the underlying chemical reactions which cause this decomposition. Under alkaline conditions, as is the case with D-glucosyl-N-butylamine, the main reaction is that of reducing sugar decomposition by alkali.[1] At an acid *p*H, such as that obtaining in an aqueous equimolar mixture of D-glucose and glycine, a significant initial reaction is that of the conversion of D-glucose to 5-(hydroxymethyl)-2-furaldehyde. It is probable that the substituted furaldehyde so formed may then undergo further reactions of a complex nature in which the amino group may well play a part.

In the case of the amino acids the reactive centers may be considered to be the primary amino group and the hydrogen atoms adjacent to the carboxyl group. In the reducing sugars the potential carbonyl unit and the hydroxyl groups are considered reactive. By appropriate blocking of the reactive centers in both the amino acids and the sugars it was considered that the nature of the functional groups required for color formation could be ascertained.

For the above objective it was a first essential to establish a standard set of conditions for measuring the degree of decomposition as evidenced by color ("browning"). For this purpose 0.5 *M* aqueous solutions were heated at 90–95° for specified periods at designated *p*H values and the color formation was measured at a chosen wave length (4900 Å.) in a photoelectric colorimeter. These decompositions were very sensitive to impurities and it was necessary to subject the organic compounds employed to rigorous purification. Under such conditions the results were reproducible to *ca.* 5% of the recorded value.

The values of *p*H chosen for the work were first that normally given by the equimolar solutions of D-glucose and the amino acid. This was slightly on the acid side, *ca.* *p*H 5.5. It was then considered pertinent to repeat the experiment at a *p*H (*ca.* 7.5) slightly on the alkaline side. The *p*H (always measured at room temperature) tended to become slightly lower on heating (Tables I to IV, inclusive). In order to establish the *p*H at *ca.* 7.5 it was necessary to employ a buffer and a suitable phosphate solution was chosen.

With a method for evaluating the degree of decomposition at hand, the reactive points of the molecules concerned were blocked with suitably placed methyl groups and the effect on the decomposition reaction noted. Table I shows the results obtained on heating aqueous solutions of D-glucose with glycine and its N-methyl and α-C-methyl derivatives. In the acid range, it is at once apparent that the essential conditions for color formation are the presence of at least one hydrogen on the carbon atom adjacent to the carboxyl group (browning of glycine and *d,l*-alanine but not of α-aminoisobutyric acid) and the concomitant presence of a free amino group (no browning of N,N-dimethylglycine and negligible browning of sarcosine). An alternative explanation is that the methyl groups exert a steric hindrance in subsequent polymerization reactions.

It is to be expected that at *p*H *ca.* 7.5 some coloration will be produced on heating D-glucose alone in the phosphate buffer solution,[6] a supposition confirmed by the data of Table II, wherein the correlation between the degree of methylation and color formation is in the same order (save for 3-methyl-D-glucose) as that established between the degree of methylation and reducing power with alkaline copper reagents.[7,8] The high degree of coloration produced by 3-methyl-D-glucose is definitely anomalous. It may be due to impurities although care was exercised in its purification. Glycine, *d,l*-alanine and α-aminoisobutyric acid show coloration in the alkaline range (Table I) well above that of D-glucose alone (Table II). Sarcosine shows very nearly the same degree of coloration as D-glucose alone whereas N,N-dimethylglycine shows less. These data indicate that in the alkaline range the free amino group will produce coloration without the concomitant presence of a hydrogen atom on the carbon atom adjacent to the carboxyl group. It would further appear that N,N-dimethylglycine is acting as a color-inhibitor under these conditions.

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(3) Presented before the Division of Sugar Chemistry and Technology at the 112th Meeting of the American Chemical Society, New York, N. Y., September 16, 1947.

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(5) L.-C. Maillard, *Compt. rend.*, **154**, 66 (1912); *Ann. chim.*, [9] **5**, 258 (1916).

(6) *Cf.* H. A. Spoehr and P. C. Wilbur, *J. Biol. Chem.*, **69**, 421 (1926).

(7) G. Zemplén and G. Braun, *Ber.*, **58B**, 2566 (1925).

(8) H. Sobotka, *J. Biol. Chem.*, **69**, 267 (1926).

TABLE I

RELATION BETWEEN DEGREE OF METHYLATION OF GLYCINE AND COLOR DEVELOPMENT IN THE PRESENCE OF D-GLUCOSE

Solutions (0.5 *M*) of amino acids of high purity heated in the presence of highly purified D-glucose at 90–95° for the specified periods at the designated *p*H (measured at room temperature).

		Acid range					Alkaline range				
		*p*H Hours			% Absorption[a] at 4900 Å. Hours		*p*H (phosphate buffer) Hours			% Absorption[a] at 4900 Å. Hours	
Amino acid	Formula	0	4	6	4	6	0	0.5	1	0.5	1
Glycine	$H_2NCH_2CO_2H$	5.3	4.8	4.6	7.3	24.8	7.4	6.6	6.0	87.4	99.8
Sarcosine	$(CH_3)HNCH_2CO_2H$	5.7	4.5	4.4	0	5.3	7.6	7.3	7.0	7.9	19.1
N,N-Dimethylglycine[b]	$(CH_3)_2NCH_2CO_2H$	5.1	4.6	4.5	0	0	7.8	7.8	7.7	0	6
d,l-Alanine	$H_2NCH(CH_3)CO_2H$	5.7	5.1	4.8	12.0	36.0	7.3	6.8	6.4	52.2	96.2
α-Aminoisobutyric acid	$H_2NC(CH_3)_2CO_2H$	5.9	5.1	4.8	0	2.3	7.5	7.2	6.7	22.0	85.5

[a] Measured in a photoelectric calorimeter (Lumetron) after cooling rapidly to room temperature. [b] Added to the buffer as the sodium salt and the *p*H adjusted to 7.8 with hydrochloric acid.

TABLE II

RELATION BETWEEN DEGREE OF METHYLATION OF D-GLUCOSE AND COLOR DEVELOPMENT IN THE ABSENCE OF AMINO ACIDS

Solutions (0.5 *M*) of the highly purified sugars heated in phosphate buffers for the specified periods at 90–95°.

	*p*H[b] Hours			% Absorption[a] at 4900 Å. Hours	
Sugar	0	0.5	1	0.5	1
D-Glucose	7.8	7.6	7.2	2.4	23.0
3-Methyl-D-glucose	7.5	7.2	7.0	26.3	44.4
3,5,6-Trimethyl-D-glucose	7.7	7.3	7.3	14.0	19.5
2,3-Dimethyl-D-glucose[c]	7.5	7.3	7.3	6.7	14.8
2,3,4,6-Tetramethyl-D-glucose	7.8	7.7	7.7	4.8	8.5

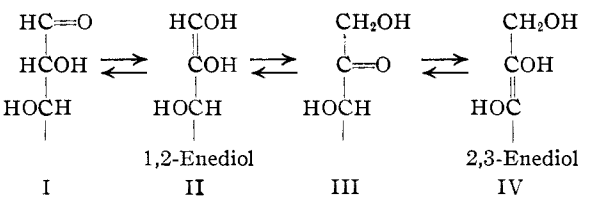
[a] *Cf.* footnote *a*, Table I. [b] Measured at room temperature. [c] In the preparation of this substance according to the procedure of P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **65**, 535 (1925), Drierite (soluble anhydrite) was substituted for sodium sulfate in the preparation of methyl 4,6-benzylidene-α-D-glucopyranoside. This resulted in an improved yield and a more readily purified product.

If the carboxyl group be removed from the amino acid molecule, there is left only the amino group which acts essentially to bring about an alkaline sugar decomposition as was established in the first communication[1] of this series. That the carboxyl group is not in itself an essential factor in the decomposition reaction is to be noted by the lack of browning of N,N-dimethylglycine (at both the acid and alkaline reaction) and of α-aminoisobutyric acid (in the acid range). The data of Table I also show enhanced coloration with an increase in *p*H, in harmony with the results of other workers.[9]

Our next objective was to study the effect on color formation of suitably substituted derivatives of D-glucose. These derivatives were heated with glycine (Table III) and with (*dextro*)-glutamic acid (Table IV). The data show that the reducing group is essential for the color formation (non-browning of sorbitol and methyl α-D-glucopyranoside), a result in harmony with the reports of other workers.[6,9]

With the reducing group present, the data on the acid side (*p*H *ca.* 5.5 for glycine and 4.5–55. for (*dextro*)-glutamic acid) show no correlation between the degree of etherification of the sugar and color formation. It is known that methylation does not block furaldehyde formation from pentoses on heating with mineral acids.[10] It is possible that even under these mild conditions of acidity, the powerful forces tending to form 5-(hydroxymethyl)-2-furaldehyde may be exerting an incipient effect which in turn is not blocked by the ether group.

The situation is different, however, on the alkaline side (*p*H 7.5–8.0). Here a definite correlation can be noted. The solutions containing the 2,3-dimethyl or the 2,3,4,6-tetramethyl ethers of D-glucose (Tables III and IV) showed nearly the same degree of color formation as did those containing no amino acid (Table II). D-Glucose, 3-methyl-D-glucose and 3,5,6-trimethyl-D-glucose, however, exhibited a marked increase in color formation when the amino acids were present. This constitutes definite evidence that the coloration at this *p*H is concerned with enolization down the sugar chain (I → IV). Such enolization must proceed through the 2,3-enediol (IV), since



it has been demonstrated[11] that etherification of D-glucose on carbon two will prevent the shift of the carbonyl group down the chain with the consequent prevention of 2,3-enolization. When such enolization does occur an eventual scission of the sugar molecule results with the formation of the very reactive methylglyoxal (pyruvic aldehyde).[12]

The data of Table III (for glycine) show an en-

(9) *Cf.* C. Enders, *Biochem. Z.*, **312**, 339 (1942).

(10) H. T. Neher with W. L. Lewis, THIS JOURNAL, **53**, 4411 (1931); H. G. Bott and E. L. Hirst, *J. Chem. Soc.*, 2621 (1932).

(11) M. L. Wolfrom with W. Lee Lewis, THIS JOURNAL, **50**, 837 (1928); R. D. Greene with W. Lee Lewis, *ibid.*, 2813; D. J. Loder with W. Lee Lewis, *ibid.*, **54**, 1040 (1932).

(12) W. L. Evans, Rachel H. Edgar and G. P. Hoff, *ibid.*, **48** 2665 (1926); W. L. Evans, *Chem. Revs.*, **31**, 537 (1942).

TABLE III

RELATION BETWEEN DEGREE OF METHYLATION OF D-GLUCOSE AND COLOR DEVELOPMENT IN THE PRESENCE OF GLYCINE

Solutions (0.5 *M*) of sugars of high purity heated in the presence of highly purified glycine (0.5 *M*) at 90–95° for the specified periods at the designated *p*H (measured at room temperature).

	Acid range					Alkaline range				
	*p*H Hours			% Absorption[a] at 4900 Å. Hours		*p*H (phosphate buffer) Hours			% Absorption[a] at 4900 Å. Hours	
Sugar	0	4	6	4	6	0	0.5	1	0.5	1
D-Glucose	5.3	4.8	4.6	7.3	24.8	7.4	6.6	6.0	87.4	99.8
3-Methyl-D-glucose	4.5	4.6	4.4	24.5	47.6	7.5	7.2	7.0	7.7	86.0
3,5,6-Trimethyl-D-glucose	4.6	4.5	4.4	57.4	56.8	7.6	7.4	7.3	35.0	67.0
2,3-Dimethyl-D-glucose	5.1	5.4	4.9	24.0	23.0	7.5	7.2	7.1	8.0	15.7
2,3,4,6-Tetramethyl-D-glucose	5.3	4.7	4.6	9.5	19.2	7.5	7.3	7.3	3.3	6.8
Methyl α-D-glucopyranoside	5.7	5.3	5.1	0	4.0	7.4	7.4	7.3	0	3.0
Sorbitol (D-glucitol)	5.6	5.6	5.4	0	0	7.5	7.4	7.3	0	7.4

[a] *Cf.* footnote *a*, Table I.

TABLE IV

RELATION BETWEEN DEGREE OF METHYLATION OF D-GLUCOSE AND COLOR DEVELOPMENT IN THE PRESENCE OF (*dextro*)-GLUTAMIC ACID

Solutions (0.5 *M*) of sugars of high purity heated in the presence of highly purified (*dextro*)-glutamic acid at 90–95° for the specified periods at the designated *p*H (measured at room temperature).

	Acid range					Alkaline range				
	*p*H Hours			% Absorption[a] at 4900 Å. Hours		*p*H (phosphate buffer) Hours			% Absorption[a] at 4900 Å. Hours	
Sugar	0	4	6	4	6	0	0.5	1	0.5	1
D-Glucose	5.3	5.2	5.1	43.7	75.2	8.2	7.0	6.8	47.6	71.2
3-Methyl-D-glucose	5.2	5.1	5.0	60.0	93.0	7.7	7.3	6.9	22.8	83.1
3,5,6-Trimethyl-D-glucose	5.7	5.4	5.4	62.3	67.5	7.7	7.5	7.3	28.1	44.7
2,3-Dimethyl-D-glucose	5.8	5.8	5.7	28.0	44.1	7.7	7.5	7.4	1.4	17.0
2,3,4,6-Tetramethyl-D-glucose	5.3	5.2	5.2	67.0	93.1	7.7	7.7	7.7	0	9.2
Methyl α-D-glycopyranoside	5.4	5.2	5.2	0	0	8.3	8.1	8.1	0	4.2
Sorbitol (D-glucitol)	5.6	5.5	5.4	0	0	7.7	7.7	7.5	0	1.8

[a] *Cf.* footnote *a*, Table I.

hancement of coloration with an increase in *p*H for the first three members in the table. These are the ones exhibiting a high degree of browning. With (*dextro*)-glutamic acid (Table IV) the reverse relation seems to hold quite well throughout, a result that is probably concerned with the higher basicity of this dicarboxylic acid. The degree of browning on the acid side is also markedly higher than with glycine (Table II) under the same conditions.

We may mention here the results obtained by Bate-Smith and Hawthorne,[13] which bear some relation to the work herein reported. These workers removed the reducing sugars present in dried egg white by fermentation and replaced them with various methyl ethers of D-glucose. They then subjected the product to a heating (browning) process and measured the degree of insolubilization produced. Herein they noted that etherification (carbon one was not etherified) decreased but did not prevent insolubilization and that the degree of insolubilization was roughly proportional to reducing value.

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Summary

By means of appropriately methylated derivatives it is demonstrated that: (1) sugar enolization is involved in the decomposition resulting in the formation of colored substances when aqueous solutions of D-glucose and glycine (or (*dextro*)-glutamic acid) are heated at a slightly alkaline reaction; (2) that the hydrogen of the amino group and the hydrogen on the carbon adjacent to the carboxyl group of glycine are together involved in this decomposition at an acid reaction (*p*H *ca.* 5.5), or alternatively, that the substituting methyl groups exert a steric hindrance effect; (3) the free amino group only is required for the decomposition at a slightly alkaline reaction (*p*H *ca.* 7.5).

(13) E. C. Bate-Smith and J. R. Hawthorne, *J. Soc. Chem. Ind.*, **64**, 297 (1945).

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