time, the mixture was poured into 200 ml. of cold water which decomposed the excess acetic anhydride. The inulobiose acetate was extracted from this solution with diethyl ether. The residue obtained on evaporation of the ether was dissolved in 5 ml. of hot ethyl alcohol, decolorized with carbon, and allowed to crystallize in a refrigerator. The crystalline acetate was collected on a filter and dried to constant weight; yield 0.08 g., $[\alpha]D$ −6.5° (*c* 1.5, chloroform). The acetyl groups in the derivative were determined quantitatively by saponification methods[13]; acetyl content, 51.6% (found), and 50.9% (calculated).

Acid Hydrolysis of Inulobiose.—A solution of 1.5 ml. of inulobiose (2.65%) and 1.5 ml. of 0.02 *N* hydrochloric acid was heated in a constant temperature water-bath of 70°. Aliquots of 0.2 ml. were removed at 0, 15, 30, 60 and 180 minutes and neutralized with sodium carbonate. The compounds present in the hydrolysate were resolved on paper chromatograms. The chromatogram for the 30 and 180 minute hydrolysate is reproduced in Fig. 1.

The remainder of the hydrolysate was cooled and used for qualitative and quantitative identification of the hydrolytic product of inulobiose. As shown in Fig. 1, one monosaccharide was produced on acid hydrolysis of inulobiose. This monosaccharide reacted positively with Benedict and Seliwanoff solutions, moved on paper with an R_f value identical with that of fructose, and formed an osazone derivative at the same rate as fructose. Microscopic examination of the osazone crystals showed that the two osazones were identical. The quantitative determination[14] of fructose in appropriately diluted aliquots of the hydrolysate yielded values of 0.114 and 0.118 mg. per ml.; theoretical value from the weight of inulobiose, 0.112 mg. per ml.

Rate Constants for Hydrolysis of Sucrose and Inulobiose.—Two ml. of 0.02 *N* hydrochloric acid was added to 2 ml. of 0.0388 *M* solution of sucrose or inulobiose. The test tubes containing the solutions were stoppered tightly and heated in a water-bath at 70°. 0.2-ml. samples were removed, in duplicate, at intervals of 0, 5, 10, 15, 20, 30 and 60 minutes and introduced into large test-tubes containing 4.8 ml. of water and 5 ml. of alkaline copper reagent 60. After all the samples had been collected, the reducing values of the aliquots were determined in the usual manner.[11] From the increase in reducing values the amounts of sucrose and inulobiose hydrolyzed at the various times were determined. These values and the equation for first-order kinetics were used to calculate the hydrolysis rate constants for the two compounds. At 70° and in 0.01 *N* hydrochloric acid, k for the hydrolysis of sucrose is 0.018 min.$^{-1}$ and k for the hydrolysis of inulobiose is 0.042 min.$^{-1}$.

(13) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1978 (1926).

(14) J. H. Roe, *J. Biol. Chem.*, **107**, 15 (1934).

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Reactions of Carbohydrates with Nitrogenous Substances.[1] II. Factors Affecting the Darkening of N-D-Glucosylaniline[2,3]

BY LAWRENCE ROSEN, KENNETH C. JOHNSON AND WARD PIGMAN

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The major factors involved in the darkening of methanolic solutions of N-D-glucosylaniline are the amounts of water and acid present. The rate in the early stages of darkening increases with an increase in the normality of the hydrogen chloride present at any fixed concentration of water. An important relationship at this stage is the molar ratio of hydrogen chloride to N-D-glucosylaniline. When this ratio is less than unity, there is an initial inhibition period in the development of color; when the ratio becomes greater than unity, the inhibition disappears. The darkening of N-D-glucosylaniline as a function of acid concentration at longer time periods shows an optimum at approximately a molar ratio of unity. The effect of water is to inhibit the color development at any given concentration of hydrogen chloride studied. Atmospheric oxygen, copper ion catalysis, and the darkening of the possible hydrolysis products (glucose or aniline) do not contribute significantly to the darkening of N-D-glucosylaniline.

Solid mixtures and solutions of amino acids and sugars or their derivatives spontaneously form dark odorous products on storage. This type of reaction (non-enzymic browning) may be responsible for some of the dark colors, odors and changes in protein solubility that take place during the drying and subsequent storage of foods.[4] Maillard[5] was the first to investigate systematically the browning reaction by means of model systems. Such model systems represent a fundamental approach to the complex browning problem because they offer a much more readily controlled set of reactions. The use of such model systems has become increasingly apparent.[6–9]

The compound N-D-glucosylaniline (N-phenyl-D-glucosylamine) undergoes darkening in acid solution.[7,10] Cameron[10b] believed that N-D-glucosylaniline in acetic acid solution underwent hydrolysis to *aldehydo*-glucose and aniline. This open chain form of glucose was thought to be especially reactive and to undergo changes to the darkened material. Cameron[10b] isolated 2,5-dianilidoquinone from the dark material. However, this aniline oxidation product formed but a small part of the total dark material.

(1) For Paper I of this series, see THIS JOURNAL, **73**, 1976 (1951).

(2) This paper is taken in part from the thesis of Lawrence Rosen submitted to the University of Alabama in partial fulfillment for the degree of Master of Science, June, 1952.

(3) Presented in part before the Division of Sugar Chemistry of the American Chemical Society at the 122nd National Meeting, September 16, 1952, in Atlantic City.

(4) For reviews see: J. P. Danehy and W. W. Pigman, "Advances in Food Research," Vol. III, Academic Press Inc., New York, N. Y., 1951, pp. 241–290; E. R. Stadtman, *ibid.*, Vol. I, 1948, pp. 325–372.

(5) (a) L. C. Maillard, *Compt. rend.*, **154**, 66 (1912); (b) *Compt. rend. soc. biol.*, **72**, 599 (1912).

(6) M. L. Wolfrom and associates, (a) THIS JOURNAL, **68**, 2022 (1946); (b) **69**, 2411 (1947); (c) **70**, 514 (1948); (d) **71**, 3518 (1949).

(7) H. H. Beacham and M. F. Dull, *Food Research*, **16**, 439 (1951).

(8) B. Singh, G. R. Dean and S. M. Cantor, THIS JOURNAL, **70**, 517 (1948).

(9) C. H. Lea and associates, *Biochem. J.*, **47**, 626 (1950); *Biochim. Biophys. Acta*, **3**, 313 (1949); **4**, 518, 532 (1950); **5**, 433 (1950); **7**, 366 (1951); **9**, 56 (1952).

(10) (a) C. N. Cameron, THIS JOURNAL, **48**, 2233 (1926); (b) *ibid.*, 2737 (1926).

In the present work, the relative effects of water and hydrogen chloride in promoting color formation from N-D-glucosylaniline have been studied systematically. Methanol solutions were used so that the effect of small amounts of water could be studied without the complications of heterogeneous reactions in semi-solid systems as used by earlier workers. It should be noted that N-D-glucosylaniline undergoes partial hydrolysis in aqueous solutions.[1,11]

Experimental

N-D-Glucosylaniline.—The method of Weygand[12] was modified as follows: A mixture of 16 ml. of freshly redistilled aniline (b.p. 181°), 20 g. of anhydrous D-glucose and 6 ml. of water was heated on a water-bath with mechanical stirring. The heating was continued four minutes after the appearance of a light brown homogeneous solution. The solution was then poured into 40 ml. of hot alcohol and allowed to stand at 20°. The white gelatinous mass which appeared was filtered with suction, washed with methanol, methanol-ether and finally with ether. The material was recrystallized twice from boiling alcohol as approximately a 5–10% solution and washed as above. The material was then dried *in vacuo* for 2–4 hours, ground finely, and the final drying was *in vacuo* for 30 minutes at 63°. A further crop was obtained from the mother liquors of the recrystallization processes. This crop was combined with the above product before the final drying.

The dried material was stored in a desiccator over calcium chloride and sodium hydroxide at 0° until used. Samples prepared in the above manner showed the following specific rotations: $[\alpha]^{30}D$ +7.21° (7 min.) → −49.0° (2100 min.) (*c* 2.0, anhydrous methanol); +4.75° (5 min.) → −50.6° (1980 min.) (*c* 2.0, 90% methanol–10% pyridine).

Anhydrous methanol was prepared by refluxing and distilling C.P. methanol in contact with lime and soda lime. The methanol was then stored under anhydrous conditions.

Methanolic hydrogen chloride was prepared by dropping C.P. sulfuric acid on sodium chloride and collecting the evolved hydrogen chloride in the previously prepared anhydrous methanol under anhydrous conditions. The material was stored under anhydrous conditions and was standardized before each use.

General Procedure.—To prepare a test system containing 2% glucosylaniline in a solvent composed of 90% methanol and 10% water at 0.08 *N* hydrogen chloride, the following method was employed.

A freshly prepared 4% solution of N-D-glucosylaniline in anhydrous methanol was added to equal volumes of 0.16 *N* hydrochloric acid in a mixture of 80% methanol and 20% water contained in a Klett tube. The tube was then stoppered and the contents mixed by swirling. The final composition of the system was 2% N-D-glucosylaniline in 90% methanol–10% water at 0.08 *N* hydrochloric acid. The time of addition of the acid to the glucosylaniline was taken as zero time.

The solution was placed in a water-bath at 30 ± 0.3°. Readings were taken periodically on a Klett–Summerson photoelectric colorimeter,[13] and when the color development was greater than 450 Klett units, a dilution was made with a diluting fluid identical to the system undergoing study with respect to methanol, water and hydrogen chloride content. Generally, the dilution was such that the readings on the Klett colorimeter were between 250 and 450 units.

A detailed series of experiments[14] which were undertaken to investigate the effect of dilution on darkened N-D-glucosylaniline solutions indicated that the range of 250 to 450 Klett units was satisfactory when the samples were corrected for dilution. These experiments also showed that when an aliquot was diluted with anhydrous methanol, it exhibited significantly greater color intensity than when the diluent contained water. Variation of the water content of the diluent from 5 to 20% by volume did not cause a significant change in color intensity. The inclusion of acid in the diluent also caused significantly greater color intensity to be exhibited by the diluted aliquot than in the absence of acid. A fourfold increase in the hydrogen chloride content of the diluent did not cause a significant change in the color intensity shown by diluted samples.

For the above reasons, dilutions were made with a diluent identical in water, hydrogen chloride and methanol content to the solution undergoing dilution.

Oxygen Experiments.—In the first of these series of experiments, the surface to volume ratios were varied (Table II). The glucosylaniline systems were placed in unstoppered vessels of varying diameter, and the vessels were then placed in a closed container.

In the second series of experiments (Table III), the solutions were prepared in the usual manner and placed in gas washing bottles. Within 20 seconds after zero time, oxygen was slowly bubbled through the solutions. The oxygen was previously passed successively through soda lime, lime and a solution identical in composition to the test solution. This latter treatment was necessary to saturate the oxygen in order to prevent evaporation of the solvent in the test solution. A portion of the test solution was treated in the usual manner as a control. After 90 minutes, the color was measured on an aliquot. Subsequently, oxygen was bubbled only intermittently through the system, and the amount of color was determined at 24 hours.

Identical solutions, exposed similarly to nitrogen, were treated in a similar fashion.

Results

For all moisture contents studied (0, 5, 10 and 20% by volume) the color development up to periods of 90 minutes increases with an increase in the hydrogen chloride concentration (Table I, Fig. 1). At any particular normality of hydrogen chloride, the amount of color formed at 90 minutes lessens as the water content is increased.

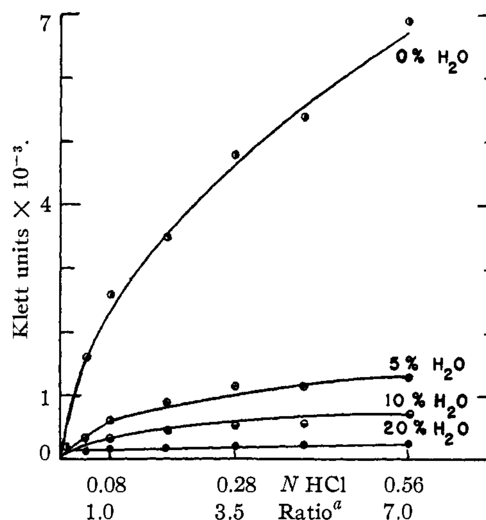

Fig. 1.—Color development of 2% N-D-glucosylaniline solutions in aqueous methanol after 90 minutes at 30°. The abscissa is given as both HCl concentration and as the molar ratio of the HCl to N-D-glucosylaniline.

An important aspect of the color development is the molar ratio of hydrogen chloride to N-D-glucosylaniline. Figure 2 shows that if the molar ratio is less than unity, there is profound initial inhibition in the formation of color. When the molar ratio is increased above unity, the initial inhibitory period is lost (Fig. 3).

The development of color for time periods over 90 min. shows a more subtle dependency on acid

(11) J. C. Irvine and R. Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908).

(12) F. Weygand, *Ber.*, **72B**, 1663 (1939).

(13) The colorimeter was fitted with a #42 filter which transmits 85% of light between 4000–4500 Å. The round cells were 12 mm. inside diameter.

(14) L. Rosen, Thesis, University of Alabama, June 1952.

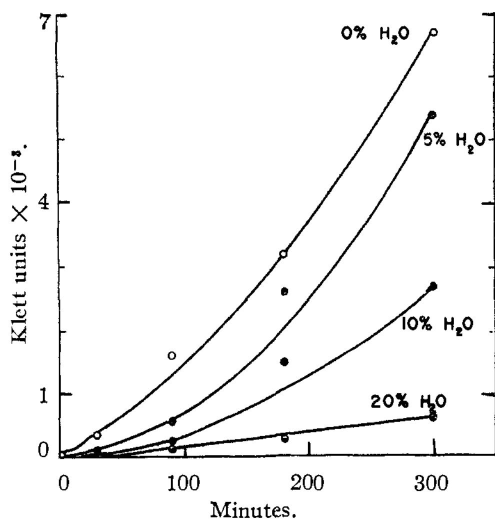

Fig. 2.—Color development of 2% N-D-glucosylaniline solutions in aqueous methanol in the presence of 0.04 N hydrogen chloride at 30°.

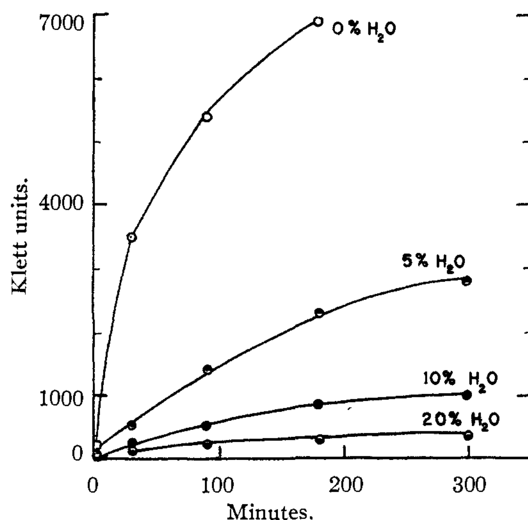

Fig. 3.—Color development of 2% N-D-glucosylaniline solutions in aqueous methanol in the presence of 0.39 N hydrogen chloride at 30°.

concentration than in the early phase. An example of this different dependency is shown in Fig. 4, for solutions containing 10% of water. The rate of color development of the N-D-glucosylaniline solutions containing 0.04 and 0.08 N hydrogen chloride show lesser initial color development than the solutions at 0.39 and 0.56 N acid concentrations. However, shortly after 90 minutes, the rate curves intersect and the solutions at the lower acid concentrations darken more rapidly than those containing more acid.

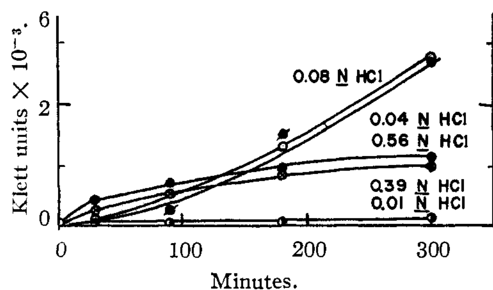

Fig. 4.—Color development of acidic 2% N-D-glucosylaniline solutions in 90% methanol–10% water at 30°.

TABLE I

BROWNING[a] OF 2% N-D-GLUCOSYLANILINE SOLUTIONS UNDER VARIOUS CONDITIONS OF MOISTURE AND ACIDITY

N HCl →	0.01	0.04	0.08	0.17	0.28	0.39	0.56
Ratio[b] →	0.13	0.51	1.03	2.13	3.59	4.88	7.18
Time min.	Color development (Klett units)						
	100% Methanol						
30	120	350	500	1800	3000	3500	4000
90	200	1600	2600	3500	4800	5400	6900
300	1000	6700	9500	7000	6900	8200	8300
24 hr.	3000	16500	16000	12500	16000	19000	22500
	95% Methanol–5% Water (by vol.)						
30	25	100	190	500	550	550	410
90	50	320	610	900	1150	1150	1300
300	500	5400	4600	3200	3900	2800	1900
24 hr.	1700	9600	12000	5000	5400	3600	3700
	90% Methanol–10% Water (by vol.)						
30	20	45	110	170	190	280	450
90	40	270	310	450	530	540	700
300	150	2700	2760	1450	1000	1000	1130
24 hr.	640	4200	7700	2000	1370	1300	1430
	80% Methanol–20% Water (by vol.)						
30	20	40	45	85	105	120	160
90	30	130	160	190	200	230	270
300	60	640	1000	560	390	360	470
24 hr.	155	1500	2700	1000	720	650	640

[a] Measured with a Klett Summerson Photoelectric Colorimeter equipped with a #42 filter. Cell, 12 mm. inside diameter. Klett unit = optical density × 500. [b] Molar ratio of HCl to N-D-glucosylaniline.

This difference in color development as a function of acid concentration during the early and later phases leads to the establishment of a definite pattern at 300 minutes at any given water concentration (Table I), and this pattern is maintained and well defined at 24 hours (Fig. 5). As the molar ratio of acid to glucosylaniline increases to a value near unity, the amount of color also increases. This increase is then followed by a decrease in total color as the molar ratio increases above unity. Under the anhydrous conditions there is a new rise in color development as the molar ratio is increased

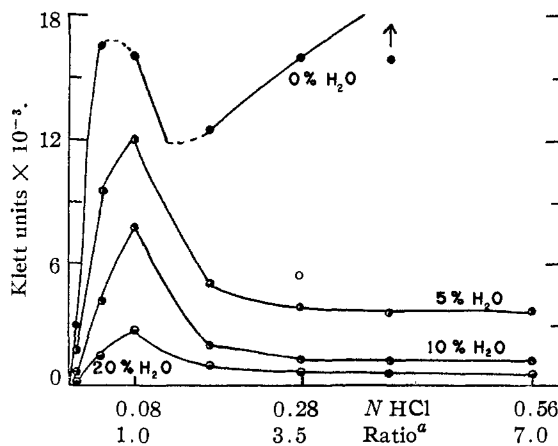

Fig. 5.—Color development of 2% N-D-glucosylaniline solutions in aqueous methanol after 24 hours at 30°. The abscissa is given as both HCl concentration and as the molar ratio of HCl to N-D-glucosylaniline.

still further. As was also found true at the early stages of darkening, water continues to exert an inhibitory effect on color development at 24 hours.

Similar results were obtained for 1% glucosylaniline solutions under anhydrous conditions. These experiments indicated that the maximum darkening at 24 hours takes place for solutions 0.03 to 0.04 *N* in hydrogen chloride, again at a molar ratio near unity. For 2% glucosylaniline solutions in methanol containing 10% water, the action of sulfuric acid was studied. With this system, maximum darkening at 24 hours occurred for solutions containing 0.14 to 0.16 *N* sulfuric acid. This acid concentration corresponds to a molar ratio of approximately unity, although the equivalent ratio of sulfuric acid to glucosylaniline is nearly two.

To determine the possible effect of atmospheric oxygen on the darkening of glucosylaniline, a series of experiments were run in which the surface to volume ratios were varied for glucosylaniline solutions exposed to air. The results (Table II) show that atmospheric oxygen has no detectable effect on color development. These experiments were done under anhydrous conditions and with 10% water.

TABLE II

THE EFFECT OF ATMOSPHERIC OXYGEN ON THE COLOR DEVELOPMENT OF 2% METHANOLIC N-D-GLUCOSYLANILINE SOLUTIONS

Volume, ml.	Surface area, cm.²	Surface ratio volume, cm.²/ml.	Color at 24 hr., Klett units
0.16 *N* HCl 100% Methanol			
4	0.50	0.13	7000
4	1.78	0.45	7000
4	4.20	1.05	7000
4	11.30	2.82	7000
0.24 *N* HCl 90% Methanol–10% Water			
8	0.95	0.12	1520
4	.64	.16	1270
4	.95	.24	1360
4	1.54	.39	1340
2	0.95	.48	1300
4	4.52	1.13	1530
4	6.16	1.54	1430

A comparison of the color development of glucosylaniline solutions in the presence of oxygen or nitrogen with identical systems run simultaneously in the usual manner showed no significant difference (Table III).

TABLE III

THE EFFECT OF OXYGEN AND NITROGEN ON COLOR DEVELOPMENT OF 2% METHANOLIC SOLUTIONS OF N-D-GLUCOSYLANILINE CONTAINING 0.08 *N* HYDROGEN CHLORIDE

Time	Color (Klett units) Gas saturated system	Control system[a]
	Oxygen	Air
90 min.	2440	2480
24 hr.	16600	15400
	Nitrogen	Air
90 min.	2540	2560
24 hr.	18300	18400

[a] Part of the original solution kept under the usual conditions.

A 2% solution of N-D-glucosylaniline in anhydrous methanol at 0.08 *N* hydrogen chloride and containing 0.1% cupric acetate did not show any significant difference in color development at 90 minutes or 24 hours when compared with like controls containing no cupric acetate.

Hydrolysis of N-D-glucosylaniline in acidic solutions occurs when water is present.[1,11] Conceivably the color could arise from the hydrolysis products.[6c,8,10b,15,16] Accordingly solutions containing either 0.08 *M* glucose or aniline (2% N-D-glucosylaniline, 0.08 *M*) were tested under representative conditions. The conditions chosen were such that the molar ratio of hydrogen chloride to glucose or aniline was varied from 0.5 to 3.5, in both anhydrous methanol and in 90% methanol–10% water. In all cases the color developed by these solutions was insignificant when compared to corresponding N-D-glucosylaniline solutions. The maximum color developed by these solutions was 390 Klett units at 24 hours, for 0.08 *M* aniline in anhydrous methanol in the presence of 0.04 *N* hydrogen chloride.

Discussion

The inhibitory effect of water on color development in methanolic solutions of N-D-glucosylaniline at any given normality of hydrogen chloride is apparent (Table I, Figs. 1, 5). This inhibition may be due to the hydrolysis of N-D-glucosylaniline in an aqueous solvent[1,11] particularly under acidic conditions. This hydrolysis would decrease the concentration of N-D-glucosylaniline which is apparently the initial chromogenic precursor. The darkening of either glucose or aniline, the hydrolysis products, is much slower than that of N-D-glucosylaniline. In agreement with Cameron,[10b] aniline by itself contributes only a small portion of the coloration. 5-Hydroxymethylfurfural (HMF) a degradation product of glucose has been suggested as being an important precursor of colored material in many systems.[8,15,16] The darkening of furfural, an analog of HMF, has been shown to be oxygen dependent.[17] The present work indicates that the darkening of N-D-glucosylaniline under the conditions of this work is not oxygen dependent (Tables II, III) but this does not mean that HMF may not contribute in some other manner to chromogen formation. However, HMF may be formed in such small amounts that the oxygen dependence of the system may be too small to be detected by the experimental methods employed.

The evidently complicated nature of the browning reaction of N-D-glucosylaniline makes it impossible to interpret the results at this stage, and further work is in progress. The maximal darkening rate for solutions with a molar ratio of hydrogen chloride to N-D-glucosylaniline of unity and the decrease in rate at higher ratios indicates that the active color precursor is the free N-D-glucosylaniline and not its hydrochloride. If this behavior is representative of other glycosylamines and natural browning reactions, it would mean that

(15) V. A. Beckley, *J. Agr. Sci.*, **11**, 69 (1921).

(16) B. L. Scallet and J. H. Gardner, THIS JOURNAL, **67**, 1934 (1945).

(17) A. P. Dunlop, P. R. Stout, and S. Swadesh, *Ind. Eng. Chem.*, **38**, 705 (1946).

browning reactions might be very extensive in narrow ranges of acid concentration. Although there is no assurance that these factors affecting the darkening of N-D-glucosylaniline have any general validity, the lack of oxygen dependence and the influence of water are comparable with natural browning reactions. It should be noted, however, that solutions such as used in this study may differ from solid reactions in the regions of low water concentration, where diffusion factors may become predominant for the solid reactions.

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[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT OF THE UNIVERSITY OF ALABAMA, MEDICAL COLLEGE AND DENTAL SCHOOL]

Reactions of Carbohydrates with Nitrogenous Substances.[1] III. The Tetraacetates of N-D-Glucosylaniline

BY WARD PIGMAN AND K. C. JOHNSON

RECEIVED JANUARY 5, 1953

The two acetates of N-D-glucosylaniline are shown to have four O-acetyl groups. Both isomers are produced from pentaacetyl-β-D-glucopyranose and one from tetraacetyl-α-D-glucopyranosyl bromide. The results are in agreement with the simultaneously performed experiments of Honeyman and Tatchell. In the presence of acids, methanolic solutions exhibit mutarotations which are apparently an equilibration of the anomeric isomers of the pyranoid form, followed by a slow secondary reaction of an unknown type. Browning of the solutions in the presence of acids is much slower than for N-D-glucosylaniline but occurs eventually.

Two acetates of N-glucosylaniline (N-phenyl-N-D-glucosylamine) are known.[2-4] Their composition is somewhat in question since Frèrejacque described one as a pentaacetate and the other as a tetraacetate. Later, without explanation, both were described as tetraacetates.[2b] The present work was carried out in order to clarify this problem and to provide information of help in the elucidation of the structure and reactions of the parent N-D-glucosylaniline. After the completion of this work, Honeyman and Tatchell[5] published results which, where comparable, are in complete agreement with the present work. The tetraacetates of N-D-galactosylaniline have been studied in a similar fashion by Butter, Smith and Stacey.[6]

Direct acetylation of N-glucosylaniline with acetic anhydride and pyridine at 0° gave a mixture of the two acetates which were separated and purified by fractional crystallization from methanolic solutions. The same isomers were also obtained when pentaacetyl-β-D-glucopyranose was treated in alcoholic solution with aniline and acetic acid according to the method of Frèrejacque. This resulting mixture was separated by crystallization from carbon tetrachloride which forms a rather stable addition product with the β-isomer. The β-isomer was the sole product isolated from the products of reaction of tetraacetyl-D-glucosyl bromide and aniline according to the method of Baker.[3] Honeyman and Tatchell[5] carried through a similar reaction sequence with identical results.

The two isomers when highly purified had the following properties: β-tetraacetate, m.p. 98–98.5°, $[\alpha]^{30}D$ −52.8° ($CHCl_3$; *c* 4); α-tetraacetate, m.p. 153°, $[\alpha]^{30}D$ +185° ($CHCl_3$; *c* 3.2). The values are similar to those reported earlier (Frèrejacque and Honeyman), but indicate a somewhat higher purity for the α-isomer. No mutarotation in chloroform solution was observed. Frèrejacque's observed mutarotation may have arisen from the presence of traces of acid.

Quantitative acetyl determinations by alkaline saponification and by the acid transesterification procedure of Freudenberg and Harder proved the presence of four acetyl groups in both compounds, and indicated that no N-acetyl groups were present. Previous compositions had been based on C, H and N determinations, which are rather insensitive to differences in acetyl compositions. Hence, it is now established that the compounds are isomeric tetra-O-acetyl-N-glucosylanilines.

The mutarotations of these isomers in anhydrous methanol solution and in methanol containing pyridine or acetic acid are shown in Figs. 1 and 2.

The tetraacetyl-β-glucosylaniline (Fig. 1) showed a rotation $[\alpha]^{30}D$ −61° in anhydrous methanol or in methanol–pyridine, with no mutarotation. In methanol which contained acetic acid, the initially negative rotation became rapidly positive and appeared to attain a temporary equilibrium at $[\alpha]^{30}_D$ +25°, but thereafter the rotation slowly increased with the final development of a brown color. In methanol–HCl, the first observed values were close to $[\alpha]^{30}D$ +25°, but the rotation slowly increased and then decreased eventually with the development of color. In methanol containing 10% aqueous buffer (*p*H 7.0), an increase in levorotation to −74° from the initial value of −61° was followed by a period of slow decrease in rotation to about −38° in two days, and thereafter there was a slow increase. None of these curves fitted the first-order equation.

For tetraacetyl-α-glucosylaniline (Fig. 2), the rotation in methanol–pyridine was $[\alpha]^{30}D$ +212° with no mutarotation. A very slow decrease in rotation was observed in anhydrous methanol.

(1) Part I. THIS JOURNAL, **73**, 1976 (1951). Part II, *ibid.*, **75**, 3460 (1953). This work was presented before the Alabama Academy of Science, March 17, 1950, and an abstract has appeared in *J. Alabama Acad. Sci.*, **22**, 102 (1952).

(2) (a) M. Frèrejacque, *Compt. rend.*, **202**, 1190 (1936); (b) *ibid.*, **204**, 1480 (1937).

(3) J. W. Baker, *J. Chem. Soc.*, 1583 (1928).

(4) F. Weygand, *Ber.*, **72**, 1663 (1939).

(5) J. Honeyman and A. R. Tatchell, *J. Chem. Soc.*, 967 (1950).

(6) K. Butter, F. Smith and M. Stacey, *ibid.*, 3371 (1949).