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.

BIRMINGHAM, ALABAMA

[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

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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).

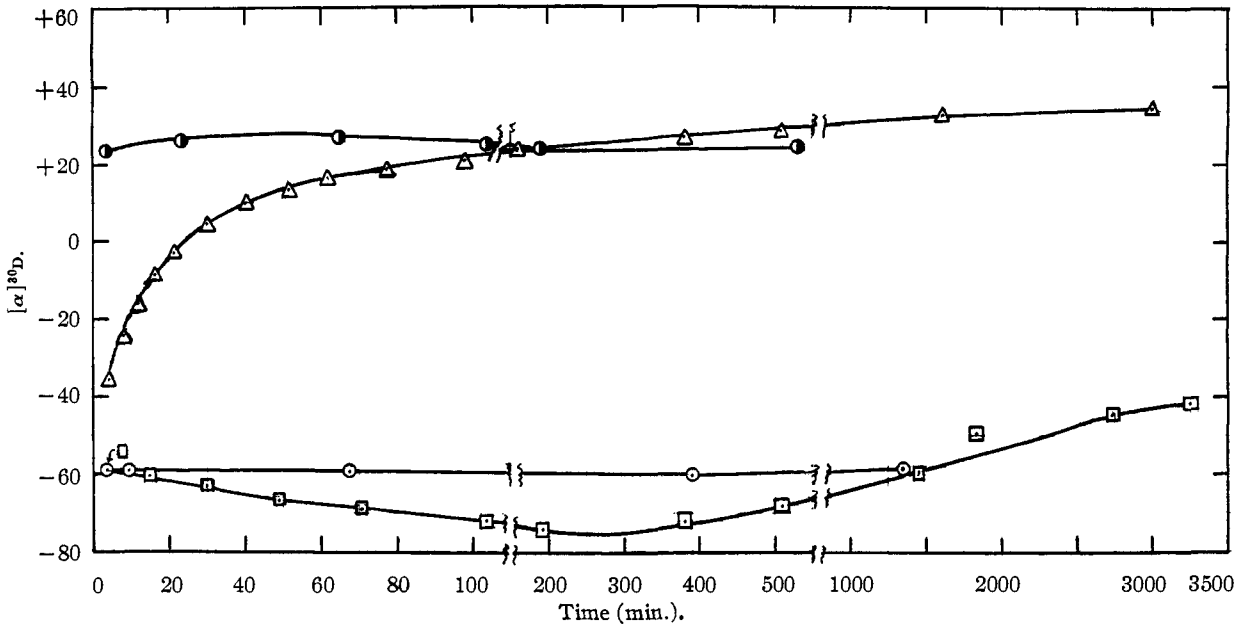


Fig. 1.—Mutarotations of 2% solutions of tetraacetyl-N-β-D-glucosylaniline at 30°: ⊙, MeOH; ⊡, MeOH–H_2O (10% phosphate buffer, *p*H 7.0); △, MeOH–HOAc (*N*); ◑, MeOH–HCl (0.121 *N*).

In methanol–acetic acid, the compound showed a rapid decrease in rotation to about +28 and then a subsequent small increase. The latter portion of the mutarotation was very similar to that for the β-isomer.

Evidently, the tetraacetates are relatively stable in methanolic solution in the absence of added acids. In the presence of acids, a pseudo-equilibrium is established between the known isomers, since both isomers have been isolated from such solutions. However, a slow secondary reaction occurs and eventually brown colored materials are formed. The presence of the acetyl groups greatly stabilizes the compounds in regard to the browning reaction which is very much slower than that of the parent N-glucosylaniline.

As pointed out by Honeyman and Tatchell,[5] these reactions and relationships are interpreted best by the assumption of a pyranoid structure for the two tetraacetates. On this assumption, the current use of α- and β- for these modifications is justified. In addition to the evidence based on the preparation of both isomers from pentaacetyl-D-glucopyranose and of the β-isomer from tetraacetyl-D-glucopyranosyl bromide, other supplemental evidence is available.

By application of the Hudson isorotation rules, the sum (B) of the partial rotations of C-2, C-3, C-4 and C-5 for the tetraacetates of N-glucosylaniline are shown to be the same as for the closely analogous phenyl tetraacetyl-D-glucopyranosides. The newly measured rotations give: 2A, 112,200 and 2B, 62,200; whereas those[7] for the tetraacetates of phenyl D-glucopyranoside are: 2A, 80,600 and 2B, 62,000 (in chloroform solutions). Frèrejacque[2b] was not able to establish such a correlation, presumably because of the impurity of the isomers and of the extrapolations required for his mutarotating solutions. For the unacetylated

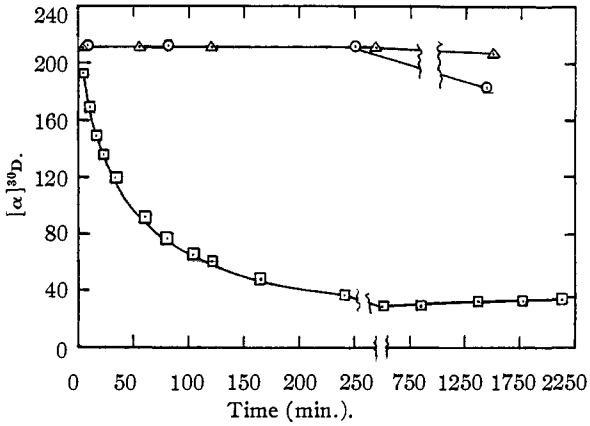


Fig. 2.—Mutarotations of 2% solutions of tetraacetyl-N-α-D-glucosylaniline at 30°: ⊙, MeOH; ⊡, MeOH–HOAc (*N*); △, MeOH–C_5H_5N (9:1).

derivatives of other glycosylamines, no similar correlation has been established.[8] The 2A value (112,200) found for the tetraacetates of N-glucosylaniline is similar to that (101,000) which can be calculated from the results of Butter, Smith and Stacey[6] for the corresponding galactose derivative.

For tetramethyl-N-D-glucopyranosylaniline, pyranose to furanose interconversions are impossible. This compound exhibits a mutarotation of $[\alpha]_D$ + 224→ + 47°, in methanol.[9] This change is very similar to that observed in the present work for the α-isomer of tetraacetyl-N-glucosylaniline in acidic methanol: $[\alpha]^{30}D$ + 212→ + 28°.

This additional evidence as well as the isolation by Frèrejacque of both isomers from acid equilibrated alcoholic solutions, indicates again that both known tetraacetates of N-glucosylaniline are α-, β-pyranoid isomers, which mutarotate in the presence of acids to an equilibrium mixture of the two

(7) W. W. Pigman and H. S. Isbell, *J. Research Natl. Bur. Standards*, **27**, 9 (1941).

(8) G. A. Howard, G. W. Kenner, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 861 (1946).

(9) J. C. Irvine and R. Gilmour, *ibid.*, **93**, 1429 (1908).

forms. However, as shown in the present work, secondary reactions occur so that the equilibrium is only an apparent one. Undoubtedly this is the reason the reactions do not follow the first-order equation.

Experimental

By the use of pyridine and acetic anhydride at 0°, glucosylaniline was acetylated to give a mixture of two products. The mixture of glucosylaniline tetraacetates was fractionated by crystallization from methanol. After recrystallization, the most insoluble (α) isomer had a m.p. of 153° and a rotation of $[\alpha]^{30}D$ +185.0° ($CHCl_3$, c 3.2).

The reaction of pentaacetyl-β-D-glucopyranose with aniline according to the method of Frèrejacque[2] gave a mixture of glucosylaniline tetraacetates from which the β-isomer was separated by use of an addition product with carbon tetrachloride. In order to remove the carbon tetrachloride, it was found necessary to carry out the final crystallizations from ethyl ether containing petroleum ether. The pure β-isomer had a m.p. of 98–98.5°, and a rotation of $[\alpha]^{28}D$ −52.8° ($CHCl_3$, c 4). No mutarotation was noted after 24 hours. Acetylation of this product with cold acetic anhydride and pyridine did not introduce additional acetyl groups. The α-isomer recovered from the mother liquors had properties identical with that obtained by the direct acetylation of glucosylaniline, and the melting points of mixtures of the two showed no depression.

By the reaction of aniline with tetraacetyl-D-glucosyl bromide according to the method of Baker,[3] only the tetraacetyl-β-D-glucosylaniline was obtained. This was shown to be identical with the product obtained above by the Frèrejacque method.

Acetyl determinations on the α- and β-isomers gave the following results. Alkaline saponification for 8 hours at 0°: β-isomer; found, 40.1% acetyl; α-isomer; found, 40.4%. Acid deacetylation (p-toluenesulfonic acid in ethanol): β-isomer; found, 40.3% acetyl; α-isomer, 42.3%. Theory for tetraacetate, 40.7% acetyl; theory for pentaacetate, 46.2%.

BIRMINGHAM, ALABAMA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

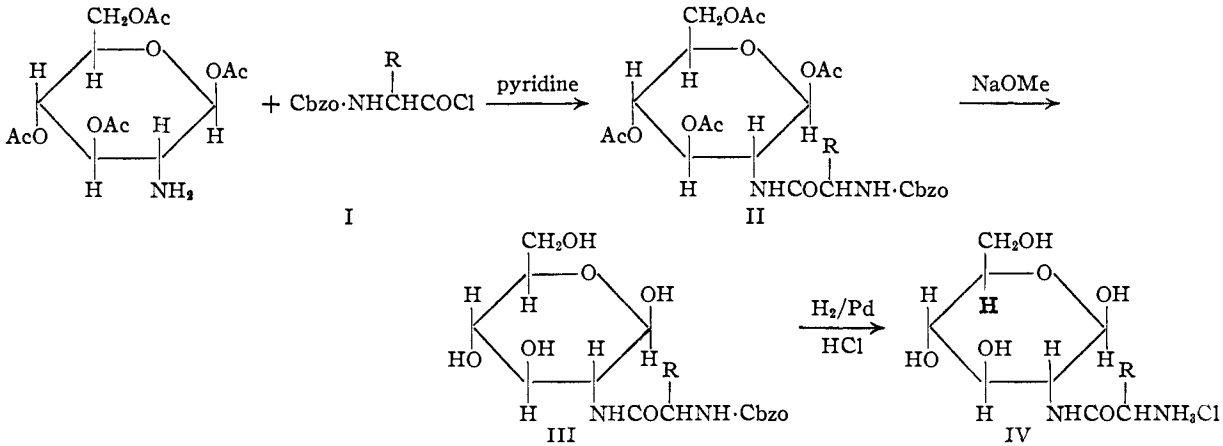
Amino Acid Derivatives of D-Glucosamine[1]

BY DAVID G. DOHERTY,[2] EDWIN A. POPENOE AND KARL PAUL LINK

RECEIVED FEBRUARY 28, 1953

A series of derivatives of D-glucosamine substituted on the nitrogen atom by acyl amino acid residues has been prepared. By coupling 1,3,4,6-tetraacetyl-β-D-glucosamine with an acylamino acid chloride in the presence of pyridine in an anhydrous solvent, the following compounds have been prepared: N-hippuryl-1,3,4,6-tetraacetyl-β-D-glucosamine, dicarbobenzoxy-L-cystyl-di-(1,3,4,6-tetraacetyl-β-D-glucosamine), N-(dicarbobenzoxy-L-lysyl)-1,3,4,6-tetraacetyl-β-D-glucosamine, N-(carbobenzoxy-L-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine and N-(carbobenzoxy-D-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine. With carbobenzoxy-L-glutamic anhydride N-(carbobenzoxy-L-α-glutamyl)-1,3,4,6-tetraacetyl-β-D-glucosamine was obtained. Of these "glucopeptide" acetates, only the first and last gave crystalline products on alkaline deacetylation.

The carbobenzoxy derivatives of amino acids were originally used by Bergmann and Zervas[3] for coupling with 1,3,4,6-tetraacetyl-β-D-glucosamine in the first definitive synthesis of so-called "glucopeptides." For example, carbobenzoxyglycyl chloride (Ia) and 1,3,4,6-tetraacetyl-β-D-glucosamine were coupled in the presence of pyridine to give N-(carbobenzoxyglycyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IIa), followed by deacetylation and hydrogenolysis of the carbobenzoxy group to give N-glycyl-D-glucosamine (IVa). In this way N-glycyl-D-glucosamine and N-alanyl-D-glucosamine (IVb) were obtained.



I, II: (a) R = H
(b) R = CH_3
(c) R = $CH_2CH_2CH_2CH_2NH$·Cbzo
(d) R = CH_2CH_2—S—CH_3
Cbzo = $COOCH_2C_6H_5$

III, IV: (a) R = H
(b) R = CH_3

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(2) Junior Fellow, National Institutes of Health, 1946–1948.

(3) M. Bergmann and L. Zervas, *Ber.*, **65**, 1201 (1932).