832. *The Mechanism of the Hydrolysis of* N-*Aryl*-D-*glucosylamines* *

By Brian Capon and Brian E. Connett

The rates of hydrolysis of a series of *N*-aryl-D-glucosylamines [(I) and (II)] have been measured at a range of acidities. All the reactions are preceded by rapid anomerisations. The hydrolyses are general-acid-catalysed and the plots of rates against acidity show maxima. The anomeric composition of the glucose formed in the hydrolysis of the *p*-hydroxyphenyl and *p*-tolyl glucosylamines has been shown to be 62 ± 5% α and 38 ± 5% β. This is the composition expected from the ring-closure of aldehydo-glucose. It is concluded that the hydrolysis proceeds by way of an intermediate Schiff-base form.

Previous work on the hydrolysis of glucosylamines has been limited by the lack of information about their structures and the difficulty in estimating free aldose or amine in the presence of unhydrolysed glucosylamine.[1] In this Paper we describe the results of our investigation of the hydrolysis of the *N*-aryl-*D*-glucosylamines, (I) and (II), the determination of the structure of which is the subject of the preceding Paper.[2] The hydrolyses of all the glucosylamines were preceded by a rapid anomerisation to a mixture of about 10%α- and 90%β-form. This was shown by following the change in optical rotation and determining the concentration of free amine spectrophotometrically. Two methods were employed; in the first, which was used with the *p*-tolyl, phenyl, and *p*-nitrophenyl compounds, the reaction solution was made alkaline and the amine was extracted and determined spectrophotometrically;[3] in the second, which was used for all the glucosylamines, the amine was determined *in situ* by making use of the small difference between the u.v. spectra of the glucosylamines and the free amines. Although the anomerisation must involve an acyclic form, this cannot be present at an appreciable concentration since the

Table 1

The rate of anomerisation of *N*-*p*-tolyl-D-glucosylamine (in pH 12·99 NaOH at 25°)

$k_1 + k_2$ starting with α-isomer: $3{\cdot}59 \times 10^{-3}$ sec.$^{-1}$
$k_1 + k_2$ starting with β-isomer: $3{\cdot}55 \times 10^{-3}$ sec.$^{-1}$

reaction shows good first-order behaviour and the measured rate constants, starting from either the α- or the β-form, are equal (see Table 1). The anomerisation shows buffer catalysis (see Table 2) and presumably involves a mechanism similar to that for the mutarotation of glucose although the exact timing of the proton transfers is unknown.

* For a preliminary account of this work see B. Capon and B. E. Connett, *Tetrahedron Letters*, 1964, 1395.

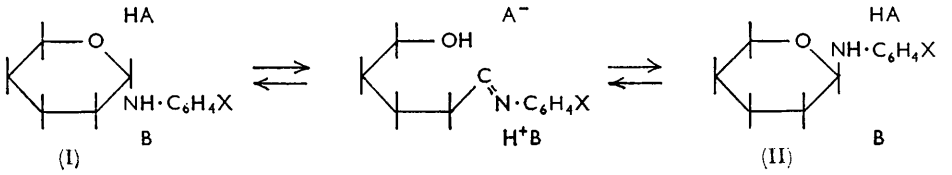
[1] See G. P. Ellis and J. Honeyman, *Adv. Carbohydrate Chem.*, 1955, **10**, 104.
[2] B. Capon and B. E. Connett, preceding Paper.
[3] Cf. C. Bamford, M.Sc. Thesis, London, 1960.

TABLE 2

Buffer catalysis of the anomerisation of *N*-*p*-tolyl-D-glucosylamine (in phosphate buffers μ 0·20 at 25°)

pH 11·65	$[Na_2HPO_4]$:	0·022	0·018	0·015	pH 11·14	$[Na_2HPO_4]$:	0·042	0·034	0·025
	10^4k (sec.$^{-1}$):	3·21	3·13	3·06		10^5k (sec.$^{-1}$):	6·56	5·51	4·52

Thus in the studying of the acid-catalysed hydrolysis it is always an interconverting mixture of isomers that is being studied. This hydrolysis could be envisaged as either the



X = H, *p*-Me, *p*-NO_2, *p*-CO_2H, *p*-CF_3, *p*-OH, and *o*-CO_2H

hydrolysis of the cyclic forms (Mechanism 1) or of the acyclic Schiff-base form (Mechanism 2).[1] The hydrolyses of the more easily hydrolysed compounds were studied in buffers and were found to show general-acid catalysis (cf. Table 3). This clearly excludes Mechanism

TABLE 3

General-acid catalysis of the hydrolysis of *N*-*p*-tolyl-D-glucosylamine

Series 1. pH 2·80

$[HCO_2H]$	$10^2[HCO_2Na]$	10^2[NaCl]	10^3k (sec.$^{-1}$)
0·480	8·00	0	8·15
0·360	6·00	2·00	6·37
0·240	4·00	4·00	4·65

Slope 14·6 × 10^{-3} l. mole^{-1} sec.$^{-1}$.

Series 2. pH 3·11

$[HCO_2H]$	$10^2[HCO_2Na]$	10^2[NaCl]	10^3k (sec.$^{-1}$)
0·240	8·00	0	8·15
0·210	7·00	2·00	6·37
0·180	6·00	4·00	4·65

Slope 12·6 × 10^{-3} l. mole^{-1} sec.$^{-1}$.

1, but is consistent with Mechanism 2, since general-acid catalysis in the hydrolysis of Schiff bases is well established,[4] and has recently been shown to involve the kinetically equivalent specific-acid/general-base catalysis.[5]

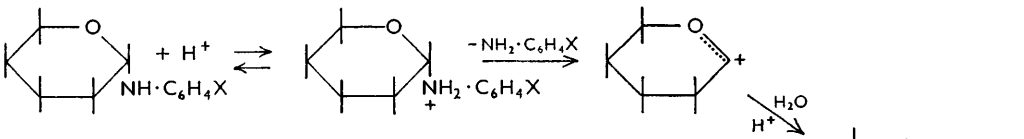
The effect of substituents on the rate also supports Mechanism 2. A small effect would be expected for Mechanism 1 since the electronic requirements of the pre-equilibrium proton transfer and the slow heterolysis are in opposite senses. With Mechanism 2, for which $k_{obs} = k$ [Schiff base], the effect of substituents on k, the rate constant for the hydrolysis of the Schiff-base form, should only be small,[4] but the concentration of the Schiff-base form should be quite strongly substituent-dependent. This is because in the cyclic glucosylamines there is a resonance interaction, due to overlap of the lone pair on the nitrogen with the π-electrons of the aryl ring, which is lost or reduced in the Schiff-base form, since now the nitrogen is doubly bonded and the loan-pair electrons, instead of occupying a *p*-orbital, occupy an sp^2-orbital which is less suitable for overlap. The effect of substituents was determined by extrapolating the observed rates of hydrolysis to pH 2, under

[4] Cf. A. V. Willi and R. E. Robertson, *Canad. J. Chem.*, 1953, **31**, 361; A. V. Willi, *Helv. Chim. Acta*, 1956, **39**, 1193.
[5] E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, 1963, **18**. 2843.

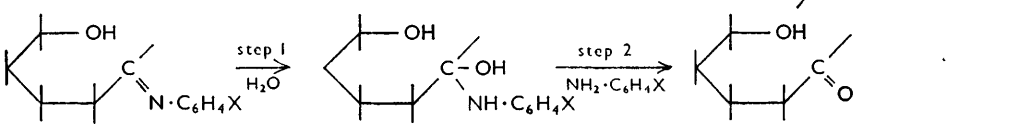
which conditions there will only be slight protonation of the nitrogen of all the glucosylamines, and calculating the ρ-constant which was −2·3. The reaction is therefore moderately sensitive to substituents, consistent with Mechanism 2.

The pH–rate profiles of all the hydrolyses show sharp maxima (see Table 4) the positions

Mechanism 1



Mechanism 2



of which move to higher acidities with decreasing basicity of the amine. This is readily explicable as resulting from a change in the rate-determining step of the hydrolysis of the Schiff-base form from attack by water or hydroxide ion (step 1 in Mechanism 2) to decomposition of a carbinolamine intermediate (step 2 in Mechanism 2) (cf. ref. 5).

The effect on the rate on changing the solvent from H_2O to D_2O is shown in Table 6. With Mechanism 1, at low acidities at which the nitrogen is only slightly protonated, an isotope effect $k_{D_2O}/k_{H_2O} = 1{\cdot}8$—$2{\cdot}5$, typical of that for reactions involving a pre-equilibrium proton transfer, would be expected. The observed value of 1·05 is clearly inconsistent with this but could be explained by Mechanism 2 for which the observed rate constant is a composite of several rate and equilibrium constants, each of which will exhibit a solvent isotope effect. The solvent isotope effect observed at high acidities at which the nitrogen is completely protonated, $k_{D_2O}/k_{H_2O} = 0{\cdot}45$, may be similarly explained by Mechanism 2.

The composition of the glucose formed in the hydrolysis of the *p*-tolyl and *p*-hydroxyphenyl compounds can be determined since conditions (pH 1·2—1·3) can be found under which the rates of hydrolysis are faster than the mutarotation of glucose. For both reactions this was found to be 62 ± 5% α-D-glucose and 38 ± 5% β-D-glucose. This corresponds closely to the mixture expected from the ring-closure of *aldehydo*-glucose (64 ± 2% α and 36 ± 2% β) as calculated from the rate constants for these reactions, determined polarographically.[6] These results are clearly those to be expected if Mechanism 2 were operative but would only result with Mechamism 1 from a fortuitous set of values for the rate constants of the hydrolyses of the α- and β-forms and the percentage retention and inversion they undergo on hydrolysis.

There is thus an overwhelming body of evidence which supports the view that the hydrolyses of the aryl glucosylamines proceed *via* intermediate Schiff-base forms.

Experimental

Materials. Glucosylamines and acetylated glucosylamines were those described in the previous Paper.[2] α- and β-D-Glucose were prepared by the method of Hudson and Dale.[7] Aromatic primary amines were recrystallised commercial samples. Dioxan was purified as

[6] J. M. Los, L. B. Simpson, and K. Wiesner, *J. Amer. Chem. Soc.*, 1956, **78**, 1565.
[7] C. S. Hudson and J. K. Dale, *J. Amer. Chem. Soc.*, 1917, **39**, 320.

described by Vogel.[8] Deuterium oxide (99·8%) was the Norsk Hydro material obtained from Imperial Chemical Industries Limited.

Apparatus.—Optical rotation changes were followed in the mercury 5461 line using a Bendix–Ericsson NPL/ETL automatic polarimeter. The output current from the polarimeter was passed through a decade resistance, and the resulting potential drop was measured with a Honeywell–Brown strip chart recorder. The rotation thus appeared as a millivolt reading on the recorder scale, which had been calibrated previously against a sucrose solution.

During the early part of this work all u.v. measurements were carried out on a Hilger and Watts Uvispek spectrophotometer, but later work was performed on a Unicam S.P. 700 recording u.v. spectrophotometer. We thank Dr. M. Rosenmayer for placing his Uvispek spectrophotometer at our disposal.

TABLE 4

The dependence of the rates of hydrolysis of *N*-aryl-D-glucosylamines on acidity at 25°

The reactions were carried out in aqueous perchloric acid except where otherwise stated. The rates in buffers are extrapolated to zero buffer concentration. All rates were measured spectrophotometrically at the wave-numbers indicated.

N-*p*-Hydroxyphenyl-D-glucosylamine (43,000 cm.$^{-1}$)

pH or H_0	10^3k (sec.$^{-1}$)	pH or H_0	10^3k (sec.$^{-1}$)
4·57(A)	1·10	1·56	11·6
4·45(A)	1·20	1·55	11·7
4·08(A)	1·42	1·39	12·5
3·52(A)	1·93	1·33	13·2
2·86(B)	3·35	1·07	12·8
2·77(B)	3·38	0·61	9·11
2·05	6·06	−0·49	3·19
		−0·64	1·10

N-*p*-Tolyl-D-glucosylamine (42,000 cm.$^{-1}$)

pH or H_0	10^3k (sec.$^{-1}$)	pH or H_0	10^3k (sec.$^{-1}$)
5·06(A)	0·11	0·25	9·80
4·34(A)	0·31	0·16	9·75
3·11(A)	1·03	−0·50	8·55
2·80(B)	1·21	−0·94	3·90
2·22(B)	1·65	−1·40	2·60
1·30	7·56	−1·70	1·51
0·27	10·3	−2·60	0·75

N-Phenyl-D-glucosylamine (41,000 cm.$^{-1}$)

pH or H_0	10^3k (sec.$^{-1}$)	pH or H_0	10^3k (sec.$^{-1}$)
1·03	7·04	−0·63	10·9
0·70	10·5	−0·78	10·1
−0·04	14·4	−1·06	7·70
−0·09	14·3	−1·28	6·59
−0·22	13·9	−1·73	4·70
−0·27	13·7	−2·24	2·35
−0·53	11·5		

N-*o*-Carboxyphenyl-D-glucosylamine (43,500 cm.$^{-1}$)

pH or H_0	10^3k (sec.$^{-1}$)	pH or H_0	10^3k (sec.$^{-1}$)
1·03	1·58	−1·65	93·8
0·44	6·70	−1·70	95·9
−0·12	21·3	−1·73	93·9
−0·87	65·8	−1·99	81·1
−1·06	77·2	−2·12	71·4
−1·27	83·2	−2·24	64·9
−1·30	87·8	−3·10	29·7
−1·51	93·2		

N-*p*-Carboxyphenyl-D-glucosylamine (49,000 cm.$^{-1}$)

pH or H_0	10^3k (sec.$^{-1}$)	pH or H_0	10^3k (sec.$^{-1}$)
0·69	0·87	−1·99	36·4
0·44	1·70	−2·14	37·3
−0·20	2·60	−2·56	35·7
−0·26	5·24	−2·82	31·7
−0·85	13·4	−3·23	23·9
−1·06	17·7	−3·92	12·5
−1·28	22·3	−4·14	8·74
−1·51	27·9	−4·64	3·58
−1·73	31·6		

N-*p*-Trifluoromethylphenyl-D-glucosylamine (40,500 cm.$^{-1}$)

pH or H_0	10^3k (sec.$^{-1}$)	pH or H_0	10^3k (sec.$^{-1}$)
0·55	3·00	−1·99	39·0
0·25	4·80	−2·24	35·0
−0·26	10·7	−2·56	28·2
−0·85	21·9	−3·23	14·5
−1·28	33·5	−3·75	8·00
−1·58	37·9	−4·64	2·19
−1·73	38·4		

N-*p*-Nitrophenyl-D-glucosylamine (38,500 cm.$^{-1}$)

pH or H_0	10^3k (sec.$^{-1}$)	pH or H_0	10^3k (sec.$^{-1}$)
0·43	0·33	−1·73	14·0
−0·22	2·45	−2·24	20·2
−0·80	7·00	−2·98	33·2
−1·25	9·75	−3·41	43·7
−3·53	46·4	−4·67	18·3
−3·68	47·1	−5·13	10·5
−4·30	31·7	−6·02	1·54

(A): Acetate buffer. (B): Formate buffer.

[8] A. I. Vogel, " Practical Organic Chemistry," Longmans, London, 1956, p. 171.

TABLE 5

Typical kinetic results

Method 1. Hydrolysis of *N*-*p*-nitrophenyl-β-D-glucosylamine in 0·094N-HCl at 25°. A = Absorbance

Time (min.)	0	12	24	37	51	65	79	t_∞
A_t	0·105	0·178	0·249	0·319	0·387	0·460	0·516	1·106
10^4k (sec.$^{-1}$).....		1·06	1·08	1·09	1·08	1·12	1·11	

Mean = 1·09 × 10^{-4} sec.$^{-1}$.

Method 1. Hydrolysis of *N*-*p*-tolyl-β-D-glucosylamine in pH 4·68 acetate buffer at 25°

Time (min.)	0	5	10	15	20	25	30	t_∞
A_t	0·436	0·668	0·852	1·000	1·132	1·194	1·253	1·471
10^4k (sec.$^{-1}$).....		8·48	8·56	8·71	8·85	8·79	8·66	

Mean = 8·66 × 10^{-4} sec.$^{-1}$.

Method 2. Hydrolysis of *N*-*p*-tolyl-β-D-glucosylamine in pH 4·34 acetate buffer at 25°. "α" indicates the number of divisions on a Honeywell–Brown strip chart and is proportional to the optical rotation of the sample.

Time (min.)	0	2	4	6	8	10	12	14	t_∞
α	53·3	47·3	42·1	37·6	33·5	29·7	26·1	23·2	−1·4
10^3k (sec.$^{-1}$).....		0·96	0·96	0·94	0·94	0·94	0·95	0·95	

Mean = 0·95 × 10^{-3} sec.$^{-1}$.

Method 2. Hydrolysis of *N*-*p*-tolyl-β-D-glucosylamine in pH 5·27 acetate buffer at 25°

Time (min.)	0	20	30	40	50	60	70	80	t_∞
α	80·0	72·7	69·6	66·9	64·6	62·3	60·5	58·7	44·5
10^4k (sec.$^{-1}$).....		1·92	1·93	1·92	1·89	1·92	1·90	1·91	

Mean = 1·91 × 10^{-4} sec.$^{-1}$.

Method 3. Hydrolysis of *N*-*p*-carboxyphenyl-β-D-glucosylamine in 3·11M-$HClO_4$ at 25°

Time (sec.)	0	7·5	15	22·5	30	37·5	45	t_∞
A_t	1·824	1·745	1·678	1·620	1·576	1·532	1·501	1·311
10^2k (sec.$^{-1}$).....		2·23	2·23	2·25	2·20	2·25	2·21	

Mean = 2·23 × 10^{-2} sec.$^{-1}$.

Method 3. Hydrolysis of *N*-*p*-nitrophenyl-β-D-glucosylamine in 1·00M-$HClO_4$ at 25°

Time (min.)	0	1·5	3	4·5	6	7·5	9	t_∞
A_t	1·556	1·387	1·251	1·142	1·058	0·988	0·936	0·706
10^3k (sec.$^{-1}$).....		2·47	2·47	2·47	2·45	2·45	2·43	

Mean = 2·45 × 10^{-3} sec.$^{-1}$.

TABLE 6

Solvent deuterium-isotope effect on the hydrolysis of *N*-*p*-tolyl-D-glucosylamine (μ = 0·04 pH = pD = 4·44)

Aqueous buffers

[HOAc]	0·06M	0·05M	0·04M	zero *
10^4k sec.$^{-1}$	7·09	6·35	5·61	2·65

Deutero buffers

[DOAc]	0·16M	0·12M	0·08M	zero *
10^4k sec.$^{-1}$	13·4	10·7	8·1	2·79

k_{D_2O}/k_{H_2O} = 2·79/2·65 = 1·05

Hydrolysis in 7·15M-HCl. k = 3·66 × 10^{-4} sec.$^{-1}$.
Hydrolysis in 7·15M-DCl. k = 1·64 × 10^{-4} sec.$^{-1}$.

k_{D_2O}/k_{H_2O} = 0·45

* By extrapolation.

Kinetic Procedure.—Method 1. This method was introduced by Miss C. Bamford [3] and was used for *N*-*p*-tolyl-α- and -β-D-glucosylamine and for *N*-*p*-nitrophenyl-β-D-glucosylamine. The glucosylamine was dissolved quickly in 25 ml. of the hydrolysing solvent at thermostat temperature and at known time intervals 2-ml. aliquot portions were withdrawn and further reaction was stopped by quenching in sodium hydroxide solution. The liberated *p*-toluidine

was extracted with chloroform (2 × 5 ml.) and the *p*-nitroaniline with benzene (4 × 5 ml.) and the extracts were diluted to 50 ml. with 95% ethanol. Absorbances were then measured at 288 mμ for *p*-toluidine and at 374 mμ for *p*-nitroaniline.

Method 2. It was found that when either isomer of *N*-*p*-tolyl-D-glucosylamine was dissolved in a buffer solution of pH 4 or lower, the initial values of the optical rotations were identical. The following change in rotation could then be attributed entirely to hydrolysis. That this was in fact so, was demonstrated by following a parallel hydrolysis by method 1. Rate constants obtained by the two methods never differed by more than 3%.

A quantity of glucosylamine, not necessarily a weighed amount, was dissolved in the required solvent, which had previously been brought to 25° in a thermostat-controlled bath. The solution was injected into the thermostated cell-holder of the recording polarimeter. A graph was obtained showing change of rotation against time. From the graph values of the optical rotation at known time intervals could be calculated. These values were then used to calculate a rate constant for the reaction.

Method 3. The rate of hydrolysis of all the glucosylamines could be followed by observing the change in u.v. absorption of the reaction mixture, at a predetermined wavelength, without having to perform any prior extractions. Solvent (2·5 ml.) was pipetted into a 1-cm. silica cell, equipped with ground-glass stopper, in the thermostated cell-holder of a recording u.v. spectrophotometer. A suitable quantity of glucosylamine was dissolved in an appropriate solvent and a known amount (20—40 μl.) was injected into this cell using a Hamilton microlitre syringe. The reference cell contained solvent of an identical composition, but excluding the glucosylamine. A graph was obtained showing the change in percentage transmission with time. After conversion to absorbance values the rate constant could be calculated in the normal manner.

Rate constants were calculated using the integrated first-order equation. Typical runs are shown in Table 5.

Solvent Deuterium-isotope Effect.—A series of hydrolyses was carried out with *N*-*p*-tolyl-β-D-glucosylamine in aqueous buffers and in buffer made up in deuterium oxide. *p*D was made equal to pH and measured in the standard manner using calomel and glass electrodes; pD = pH (meter reading) + 0·40.[9]

*The Anomeric Composition of the Glucose formed in the Hydrolysis.**—The half-life for the hydrolysis of *N*-*p*-hydroxyphenyl-β-D-glucosylamine at pH 1·31 was about 50 sec. whilst that for the mutarotation of glucose under the same conditions was about 10 min. Hence it is possible to determine the anomeric composition of the glucose produced in the reaction. *N*-*p*-Hydroxyphenyl-β-D-glucosylamine was dissolved in pH 1·31 perchloric acid and zero time was taken at the instant the perchloric acid contacted the glucosylamine. The subsequent reaction was followed polarimetrically and spectrophotometrically and hydrolysis was complete after 6 min. The optical rotation was noted at this time. The rotation then drifted to a smaller positive value and this final reading was noted also. This part of the graph was extrapolated back to zero time to obtain an estimate of the optical rotation the liberated glucose would have had, if no mutarotation had taken place. The results obtained were: final value $[M]_{5461}^{25}$ = +11,200 (calc. +11,000), extrapolated value $[M]_{5461}^{25}$ = +15,400. This corresponds to a mixture containing 62 ± 5% α-D-glucose and 38 ± 5% β-D-glucose.

A similar series of experiments was carried out with the *N*-*p*-tolyl-D-glucosylamines at pH 1·20 to yield results 60 ± 5% α-D-glucose, 40 ± 5% β-D-glucose.

We thank Professor W. G. Overend for his interest and encouragement.

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* It has recently been claimed (H. Simom and D. Palm, *Chem. Ber.*, 1965, **98**, 443) that the hydrolyses of the glucosylamines are too slow compared with the mutarotation of glucose for this composition to be determined. However, with the two compounds studied here, under the conditions given, this is not so.

[9] P. K. Glascoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.