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THE SYNTHESIS AND ACID HYDROLYSIS OF THE METHYL 2-AMINO-2-DEOXY-D-GLUCOFURANOSIDES AND THEIR *N*-ACETYL DERIVATIVES

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ABSTRACT

Methyl 2-amino-2-deoxy-α- and β-D-glucofuranosides were isolated from the products of a Fischer glycosidation of 2-amino-2-deoxy-D-glucose. *N*-Acetylation gave crystalline methyl 2-acetamido-2-deoxy-α-D-glucofuranoside, but the β anomer was syrupy [characterised as the tris(*p*-nitrobenzoate)]. The furanose structure was confirmed by periodate oxidation. The anomeric methyl 2-acetamido-2-deoxy-D-glucofuranosides were hydrolysed at very similar rates, which were also similar to those for the methyl D-glucofuranosides and about double those for the methyl D-glucopyranosides. Comparison of the acid-catalysed hydrolysis of the methyl 2-amino-2-deoxy-D-glucofuranosides with that of the methyl D-glucofuranosides shows an inhibiting effect of the free amino group similar to that for the corresponding pyranosides. The rates of hydrolysis of the aminodeoxy- and acetamidodeoxy-glucofuranosides were greater in deuterium oxide than in water and this, together with the markedly negative entropies of activation, suggests that these compounds are hydrolysed by mechanisms similar to those put forward for the hydrolysis of aldofuranosides.

INTRODUCTION

Methyl 2-amino-2-deoxy-β-D-glucofuranoside has been synthesised[1,2] from 2-amino-2-deoxy-D-glucose hydrochloride *via* the diethyl dithioacetal derivative and is hydrolysed more rapidly by acid than the corresponding pyranoside. The same compound has been obtained[3] from 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranose, and methyl 2-deoxy-2-(2,4-dinitroanilino)-α-D-glucofuranoside was isolated from the products formed by treatment of 2-deoxy-2-(2,4-dinitroanilino)-α-D-glucose with methanolic hydrogen chloride[4]. Methyl 2-amino-2-deoxy-α- and -β-D-glucofuranosides were tentatively identified[5] among the products obtained from

reaction of 2-amino-2-deoxy-D-glucose hydrochloride with methanol in the presence of a strong cation-exchanger. A similar technique was used[6] to prepare the methyl 2-acetamido-2-deoxy-α- and β-D-mannofuranosides.

While this manuscript was in preparation, Jacquinet and Sinaÿ[7] reported the synthesis of methyl 2-acetamido-2-deoxy-β-D-glucofuranoside from 2-phenyl-4,5-(5,6-*O*-isopropylidene-D-glucofurano)-Δ^2-oxazoline[8]. Since the physical properties of their product differed from those previously described, these authors reinvestigated some of the earlier syntheses[1-3] and concluded that the earlier claims to have obtained furanose sugars were unfounded.

Although much work has been done on the kinetics and mechanism of the acid-catalysed hydrolysis of glycopyranosides[9,10], there have been far fewer studies of the glycofuranosides[11] and none on aminodeoxyglycofuranosides. However, the growing number of reports of the identification of furanose residues in glycoproteins[12] indicates a need for a more-complete knowledge of the behaviour of these substances.

EXPERIMENTAL

Melting points were recorded with a Kofler block and are uncorrected. Measurements of optical rotation (1-dm cell) were made with a Perkin–Elmer Model 141 polarimeter between 18–20°.

Preparation of the 2-amino-2-deoxy-D-glucosides. — A modification of the Fischer synthesis described by Matsushima and Miyazaki[5] was used. Amberlite CG-120(H^+) resin (Type I, 100–200 mesh) was recycled twice between the hydrogen and sodium form, then washed twice with methanol, stored overnight in anhydrous methanol, isolated, and dried at 60° in a vacuum oven.

A mixture of resin (150 g) and 2-amino-2-deoxy-D-glucose hydrochloride (15 g) in dry methanol (1.5 l) was stirred under reflux for 9 h, with exclusion of moisture. The cooled mixture was filtered and the resin was washed with methanol (400 ml). The glycosides were eluted with 0.8M methanolic ammonia, and the eluate was concentrated at 40° under reduced pressure to give a pale-buff syrup (13.7 g) which contained 10% of reducing sugar[13].

Fractionation of the 2-amino-2-deoxy-D-glucosides. — Half of a solution of the foregoing glycoside mixture in water (50 ml) was applied to a column (5 × 20 cm) of Deacidite FF(HO^-) resin which was then eluted with deionised, distilled, carbon dioxide-free water at 44–46 ml/h. Fractions (30 ml) were collected and those which showed optical rotation were combined (total volume 1800 ml), and concentrated to give a pale straw-coloured syrup that was free from reducing sugar.

A solution of a portion (3 g) of the syrup in water was applied to, and eluted from, a column of Dowex-1(HO^-) resin (2% cross-linked, 200–400 mesh), as described by Neuberger and Wilson[14]. Fractions (10 ml) were collected, and monitored by measurement of optical rotation at 365 nm (Fig. 1). Fractions containing the separated isomers were combined and concentrated at 40° under reduced pressure or by freeze-drying to give colourless syrups. With each manipulation, the furanoside

syrups became progressively darker in colour, as also happened on storage of solutions at 4° for periods longer than 21 days. However, these compounds were successfully stored as syrups for several months at −20°.

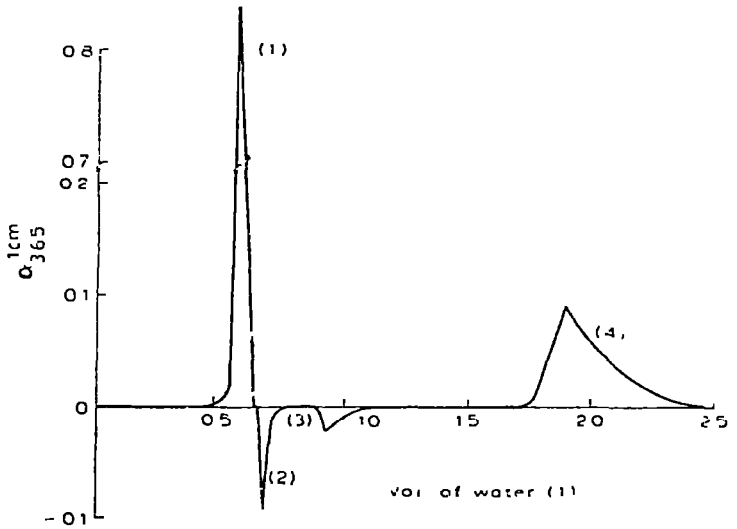

Fig. 1. Elution pattern on Dowex-1(OH⁻) resin of the four isomeric methyl 2-amino-2-deoxy-glucosides (elution volumes in parentheses). Peak 1, methyl 2-amino-2-deoxy-α-D-glucopyranoside (570 ml); 2, methyl 2-amino-2-deoxy-β-D-glucopyranoside (670 ml); 3, methyl 2-amino-2-deoxy-β-D-glucofuranoside (940 ml); 4, methyl 2-amino-2-deoxy-α-D-glucofuranoside (1950 ml).

Descending p.c. of the separated glycosides gave compact, single spots (Table I). Analysis of each fraction on the 23-cm column of a Locarte Mini amino acid analyser [citrate buffer (pH 5.28), 0.35M, 50°; see Moore and Stein[16]] gave a single peak for each substance. Elution times (min): α-pyranoside 123, β-pyranoside 107, α-furanoside 173, β-furanoside 132 (*cf.*, 100 for 2-amino-2-deoxy-D-glucose).

TABLE I

DESCENDING P.C. OF THE METHYL AMINODEOXYGLUCOSIDES AND THEIR *N*-ACETYLATED DERIVATIVES

Solvent[a]	*α-Furanoside*	*β-Furanoside*	*α-Pyranoside*	*β-Pyranoside*
Methyl 2-amino-2-deoxy-D-glucosides				
A	1.38[b]	1.24	1.30	1.26
B	2.23	1.81	1.95	1.84
Methyl 2-acetamido-2-deoxy-D-glucosides				
A	1.42[c]	1.41	1.18	1.28

[a] *A*, 1-butanol–pyridine–acetic acid–water (10:45:4:30); *B*, 1-butanol–acetic acid–water (4:1:1); Whatman 3mm paper, detection with ninhydrin in acetone or alkaline silver nitrate[15]. [b] $R_{Glc\cdot NH_2}$ values. [c] $R_{Glc\cdot NAc}$.

Methyl 2-amino-2-deoxy-D-*glucofuranosides*. — Attempts to crystallize the methyl 2-amino-2-deoxy-D-glucofuranosides, as the free amino compounds, hydrochlorides, or sulphates, were unsuccessful and were complicated by the ease with which these compounds decomposed. They were quantified by titration to pH 3.5 with hydrochloric acid. Optical rotations were determined on syrups which had been dried over phosphorus pentaoxide at 78.5°: β anomer (**1**), $[\alpha]_D^{20}$ −86°, $[\alpha]_{365}^{20}$ −252° (*c* 1, water); lit.[7] m.p. 125–127°, $[\alpha]_D^{20}$ −65° (*c* 1, methanol); α anomer (**2**), $[\alpha]_D^{20}$ +105°, $[\alpha]_{365}^{20}$ +303° (*c* 1, water).

Acetic anhydride (0.3 ml) was added to a stirred mixture of **2** in water (15 ml), methanol (1.5 ml), and Dowex-1(CO_3^{2-}) resin (5 g, 2% cross-linked, 200–400 mesh) at 0°. After 90 min, the mixture was poured into a column (2 × 14 cm), and the resin was washed with water (3 × 20 ml). The filtrate and washings were combined and concentrated, and the resulting, colourless syrup was crystallised from methanol–ethyl acetate (1:2) to give methyl 2-acetamido-2-deoxy-α-D-glucofuranoside (**3**), m.p. 139–141°, $[\alpha]_D^{18}$ +122°, $[\alpha]_{365}^{18}$ +318° (*c* 1, water). Dissolution of **3** (10 μmol) in 0.1M sodium metaperiodate (2 ml) at 0° liberated 9.6 μmol of formaldehyde in 15 min[17].

Anal. Calc. for $C_9H_{17}NO_6$: C, 45.95; H, 7.28; N, 5.95. Found: C, 46.01; H, 7.41; N, 6.03.

N-Acetylation of **1**, as described above, gave methyl 2-acetamido-2-deoxy-β-D-glucofuranoside (**4**) as a syrup, $[\alpha]_D^{18}$ −37°, $[\alpha]_{365}^{18}$ −101° (*c* 1, water); lit.[7] $[\alpha]_D^{20}$ −52° (*c* 1, water); which gave 0.98 mol. of formaldehyde on periodate oxidation. Treatment[7] of **4** with *p*-nitrobenzoyl chloride gave a tris(*p*-nitrobenzoate), m.p. 113.5–114.5°, $[\alpha]_D^{20}$ −160°, $[\alpha]_{436}^{20}$ −390° (*c* 0.91, chloroform); lit.[7] m.p. 115–116°, $[\alpha]_D^{20}$ −142.5° (*c* 1, chloroform).

Both **3** and **4** gave compact, single spots on descending p.c. (Table I); and could be separated on Dowex-1 (elution volumes, 2680 and 880 ml, respectively).

Kinetic measurements. — (*a*) *Methyl 2-amino-2-deoxy*-D-*glucofuranosides*. To aliquots (1 ml) of M hydrochloric acid frozen at −70° in glass tubes, solutions of the glycoside (10–50 μl) containing 20 μmol were added. The tubes were sealed and then thermostated at the desired temperature (±0.2°). At intervals, tubes were cooled and opened, and 0.5M sodium carbonate (1 ml) was added. Reducing sugar was then estimated by the Hanes[18] modification of the Hagedorn–Jensen[19] method. First-order rate constants were calculated according to Guggenheim[20], or from the integrated first-order rate equation, by the method of least squares, from a smooth curve drawn through the experimental points.

(*b*) *Methyl 2-acetamido-2-deoxy*-D-*glucofuranosides*. The hydrolyses were followed polarimetrically; no hydrolysis of the acetamido group occurred under the conditions used, as measured by the ninhydrin reaction[21].

Solutions of the glycoside (100 μl) containing 20 μmol were mixed (time zero) with portions (1 ml) of acid at the reaction temperature and transferred rapidly to the polarimeter cell. Maximum deflexion was reached in 30–45 sec and the subsequent procedure was the same as that described elsewhere[22].

Determination of the ionisation constants of the methyl 2-amino-2-deoxy-D-

glucofuranosides. — A solution of each glycoside in deionised, distilled, carbon dioxide-free water (1 ml), containing 300 μmol, was titrated with M hydrochloric acid at 25 ±0.1° under nitrogen. The acid was added in 10-μl portions, the solution was stirred for 30 sec, and the pH was measured with a Beckman Research Model pH meter (sensitivity, 0.001 pH unit) equipped with a combination electrode. The dissociation constants, calculated according to the procedure of Albert and Serjeant[23], and also from a plot of log ([HA]/[A⁻]) against H^+, are given in Table II.

TABLE II

VALUES OF p*K* FOR SOME AMINODEOXY SUGARS

Sugar	p*K*
Methyl 2-amino-2-deoxy-α-D-glucofuranoside	7.65[a]
Methyl 2-amino-2-deoxy-β-D-glucofuranoside	6.75[a]
Methyl 2-amino-2-deoxy-α-D-glucopyranoside	7.54[31], 7.55
Methyl 2-amino-2-deoxy-β-D-glucopyranoside	7.23[31], 7.14[40]
2-Amino-2-deoxy-α-D-glucopyranose	7.71[39]
2-Amino-2-deoxy-β-D-glucopyranose	7.27[39]

[a]Measurements on anomeric pairs were made at the same ionic strength. Data from Ref. 31 were measured at 22°, all others at 25°.

DISCUSSION

The order of elution from Dowex-1 (HO^-) resin of the four methyl 2-amino-2-deoxy-D-glucosides (Fig. 1) reflects their relative acidities[24]. If [25] the separation of pyranosides is largely dependent on the extent of ionisation of the ring hydroxyl groups, then the ionisation of these in the furanosides must be enhanced since the furanosides were eluted after the pyranosides. Furanoid derivatives are more flexible than the corresponding 6-membered rings[26], and for D-ribofuranose derivatives, the envelope forms ^2E or ^3E were generally preferred[27,28]. This departure from planarity is of the order[29] of 0.5–0.6 Å; together with the fact that in the 2-aminofuranosides HO-3 is the only hydroxyl group attached to a ring carbon atom, this may account for the slightly greater acidity of the β-furanoside compared with the β-pyranoside. The markedly greater acidity of the α-furanoside is probably due to interaction between the dipoles associated with the C–O–3 and C–O–1 bonds, which in a furanoside would tend to reinforce rather than oppose each other.

The values obtained for the acid dissociation constants of **1** and **2** are given in Table II, together with values reported for the corresponding pyranosides. The order of magnitude of a dissociation constant can be calculated as described by Clarke and Perrin[30]. With cyclohexylamine as the reference compound, Inouye[31] calculated the p*K* values of a number of aminodeoxy sugars. In a similar manner, we have calculated the p*K* value of **1** by successive subtraction of 2.2 units for the effect of MeO-1 and the ring oxygen, and 0.1 unit for the effect of the ring oxygen, from the value of 9.28 for the p*K* of *trans*-1-amino-2-hydroxycyclopentane[32]. This procedure gave a theoretical value of 6.98, which compares favourably with that of 6.75 obtained by

experiment (Table II). The higher value of 7.65 found for the pK of the α anomer is probably due to hydrogen bonding or other similar interaction between the glycosidic oxygen and the amino group, since the strongest non-bonded interaction in a furanose ring would be expected to be between vicinal *cis* substituents[33] on C-1 and C-2 in **2**.

The anomers **1** and **2** were hydrolysed at very similar rates (Table III), in contrast to the aminodeoxypyranosides where the rate for the β anomer is 2–5 times that for the α anomer. For the methyl D-glucofuranosides, the α anomer is hydrolysed approximately three times faster than the β anomer[38] (Table IV), whereas for **3** and **4**, the β anomer is hydrolysed at about twice the rate of the α anomer. Comparison of the acid-catalysed hydrolysis of the amino- and acetamido-furanosides (Table III) with that of the methyl D-glucofuranosides shows an inhibiting effect of the free amino group on the reaction rate similar to that[34] (Table IV) for the corresponding pyranosides. From the data in Table IV, it appears that **3** and **4** are hydrolysed at rates very similar to those of the methyl D-glucofuranosides, whereas the rates of hydrolysis of **1** and **2** are about double those of the methyl D-glucopyranosides. However, the rate constants for the glucofuranosides were obtained with perchloric acid as catalyst, and caution must be exercised when comparing results obtained with one acid with those obtained with a different acid[35].

TABLE III

THE KINETICS OF HYDROLYSIS OF THE METHYL AMINODEOXY-D-GLUCOFURANOSIDES IN M HYDROCHLORIC ACID

Methyl furanoside	*Temperature (degrees)*	$10^4\ k.s^{-1}$	$\Delta H^{\ddagger}$ $(kJ.mol^{-1}.K^{-1})$	$\Delta S^{\ddagger}$ $(J.mol^{-1}.K^{-1})$
2-Amino-2-deoxy-α-	70	0.66	69.5	−128 8
	80	1.33		
2-Amino-2-deoxy-β-	70	0.73	61.2	−146 7
	80	1.38		
2-Acetamido-2-deoxy-α-	35	5.05	81 7	−41.5
	45	14.3		
2-Acetamido-2-deoxy-β-	35	11.15	84.5	−26.9
	45	32.5		

The rates of hydrolysis of **1**–**4** were greater in D_2O than in H_2O (Table V). These data suggest that a rapid, initial, proton transfer occurs to form a conjugate acid of the furanoside in which either the glycosidic oxygen or the ring oxygen is protonated, and which is followed by a slow exchange of the proton finally removed[36]. If the reaction mechanism is written as

$$S + HA \rightleftharpoons SH^+ + A^-$$

$$SH^+ + H_2O \xrightarrow{\text{slow}} \text{products},$$

then the solvent isotope ratios are of the order to be expected when water is a reactant

TABLE IV

KINETIC DATA ON THE ACID-CATALYSED HYDROLYSIS OF SOME METHYL GLYCOSIDES[a]

Methyl glycoside	*Acid*	*T/K*	$10^5 k\ s^{-1}$	$\Delta H^{\ddagger}$ ($kJ\ mol^{-1}\ K^{-1}$)	$\Delta S^{\ddagger}$ ($J\ mol^{-1}\ K^{-1}$)	*Ref.*
α-D-Glucopyranoside	2M HCl	334.2	0,086	141.9	+61.9	41
		344.2	0.404			
		352.6	1,24			
	1.23M HCl	338	0.062	142.7	+73.6	35
		348.7	0,298			
		373	9.04			
	0.94M $HClO_4$	345.9	0.213			42
β-D-Glucopyranoside	2M HCl	332.1	0,107	142.7	+69.0	41
		344.7	0,738			
		352.7	2.24			
	1.23M HCl	338	0.117	136.4	+59.8	35
		348.7	0.490			
		373	12.3			
	0.93M $HClO_4$	345.9	0.429			42
α-D-Glucofuranoside	M $HClO_4$	297.92	5.97	78.3	−46.0	38
		308.12	17,5			
β-D-Glucofuranoside	M $HClO_4$	298.0	2.10	83.7	−37.7	38
		307.0	6.43			
2-Amino-2-deoxy-α-D-glucopyranoside	2.5M HCl	373	0.0403			43
	0.5M H_2SO_4	373	0.0022			44
2-Amino-2-deoxy-β-D-glucopyranoside	M HCl	373	0.0405	142	+109	34
		383.8	0.183			
	2.5M HCl	353	0,0158	177	+125	34
		383.8	0,795			
	0.5M H_2SO_4	373	0.041			44
2-Acetamido-2-deoxy-α-D-glucopyranoside	M HCl	334.5	0,125	177	+32.5	34
		353	1,21			
	0.12M HCl	351.2	0,614			45
	0.5M H_2SO_4	373	1,85			44
2-Acetamido-2-deoxy-β-D-glucopyranoside	0.12M HCl	351.2	1,88			45
	0.5M H_2SO_4	373	29.9			44

[a]Selected values from the literature; to provide a basis for comparison, thermodynamic data have been converted to SI units; some values have been re-calculated from the original data.

in the slow step[37]. The large negative entropies of activation for the hydrolysis of these compounds (Table III) indicate an A2 mechanism, which, by definition, is one in which a pre-equilibrium protonation by hydronium ion is followed by a rate-determining attack of water on the transition state. Hence, it appears that the methyl aminodeoxyfuranosides are hydrolysed by mechanisms similar to those put forward by Capon and Thacker[38] for the hydrolysis of non-amino aldofuranosides. Three possible reaction mechanisms have been suggested[10,38], namely, a simple A2 mechanism, or two involving ring opening, with either the reversible formation of a carbonium ion or a concerted process. At present, the available evidence is insufficient to distinguish between these possibilities.

TABLE V

SOLVENT ISOTOPE EFFECT FOR THE ACID-CATALYSED HYDROLYSIS OF THE METHYL AMINODEOXYGLUCOFURANOSIDES IN M DEUTERATED HYDROCHLORIC ACID[a]

Methyl glucofuranoside	*Temperature (degrees)*	10^4 k s^{-1}	$k_{D_3O^+}/k_{H_3O^+}$
2-Amino-2-deoxy-α-D	80	4.52	3.39
2-Amino-2-deoxy-β-D	80	3.52	2.55
2-Acetamido-2-deoxy-α-D	35	9.1	1.80
2-Acetamido-2-deoxy-β-D	35	13.95	1.25

[a]For rate constants in M HCl, see Table III.

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