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Note

An ^{18}O-labelling study of the deamination of 2-amino-2-deoxy-D-mannose

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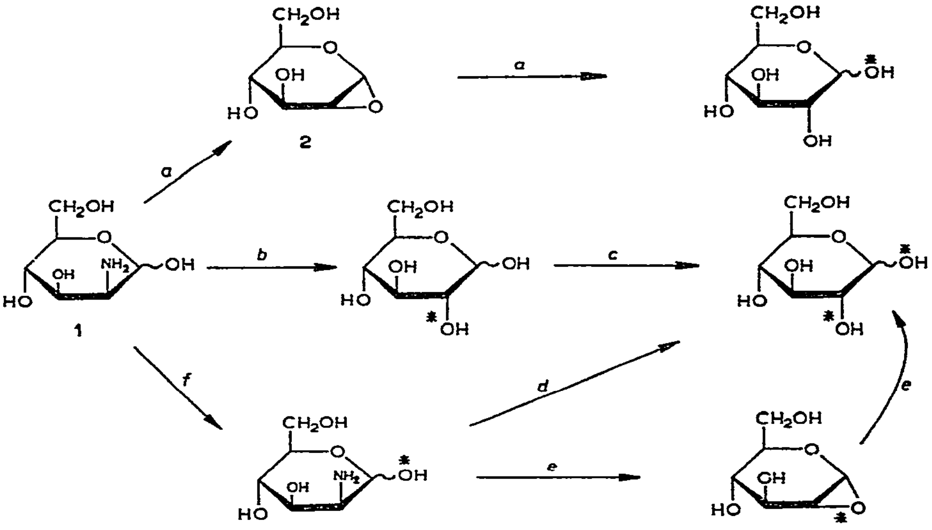
In an earlier paper[1], we suggested that the stereospecific formation of D-glucose in high yield on deamination of 2-amino-2-deoxy-D-mannose (**1**) might be due to participation of the anomeric hydroxyl group in the α-anomer to give 1,2-anhydro-D-glucose (**2**). Horton and Philips claimed[2] that this pathway was inconsistent with the preponderance of the β-anomer of 2-amino-2-deoxy-D-mannose hydrochloride in aqueous solution, and they suggested that nucleophilic attack of water at C-2 of the diazonium ion occurred. We subsequently presented[3] a detailed argument in support of the epoxide pathway, and we now report the results of an ^{18}O-labelling study which provides evidence for the epoxide intermediate.



Scheme 1. Labelling resulting from deamination in ^{18}O-enriched water; ^{18}O-exchange (routes *c* and *f*) can result in the formation of D-glucose labelled at both C-1 and C-2.

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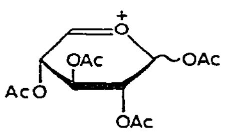
The epoxide and substitution pathways (*a* and *b*) can be distinguished by carrying out the deamination in ^{18}O-labelled water, ^{18}O being incorporated at C-1 and C-2, respectively (Scheme 1). The deamination was carried out in water containing (nominally) 10% of ^{18}O, and the crude product obtained after lyophilisation was acetylated under mild conditions (pyridine and acetic anhydride at room temperature). The syrupy product crystallised on standing and was shown to be $\alpha\beta$-D-glucose penta-acetate by g.l.c. analysis. The ^{18}O-content of this product was determined by mass spectrometry, the ion (3) at *m/e* 317 providing the total ^{18}O-content, and the ions (4 and 5) at *m/e* 331 and 242 providing the ^{18}O-content at C-2.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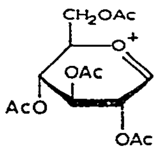
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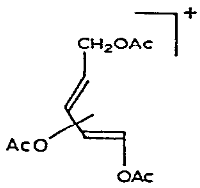
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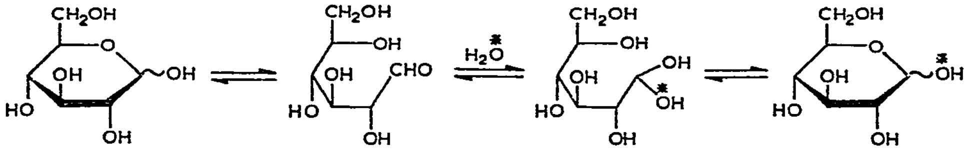
The D-glucose penta-acetate contained 13.35% of ^{18}O, the excess of 3.35% being due to ^{18}O-exchange *via* the acyclic form of D-glucose (Scheme 2) after deamination, and of the amine prior to deamination. The extent of this exchange was estimated in control experiments in which D-glucose was exposed to the same deamination conditions and work-up. 2-Amino-2-deoxy-D-mannose was similarly treated except that sodium nitrite was omitted. The ^{18}O-content of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannose was determined by using the molecular ion in the field-ionisation spectrum and, less accurately, by using the ion at *m/e* 346 in the electron-impact spectrum. The ^{18}O-content of the water was determined (see Experimental) and all results were normalised for 10% of ^{18}O.



Scheme 2.

In the control experiment, D-glucose incorporated 1.2% of ^{18}O (see Table I) in 4 h, and this represents the maximum ^{18}O-exchange in product D-glucose. In 1.5 h, 2-amino-2-deoxy-D-mannose incorporated* 4.04% of ^{18}O, and this represents

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*The greater exchange in the amine than in D-glucose is consistent with the much more rapid anomerisation of the amine[4].

the maximum exchange prior to deamination since most of the amine had reacted after 15 min[*].

TABLE I

^{18}O-CONTENT (%) FROM MASS-SPECTRAL DATA

Compound	*Total ^{18}O From ratio 319/317*		*^{18}O at C-2*	
			From ratio 333/331	*From ratio 244/242*
D-Glucose penta-acetate (from deamination)	13.35 (14.1)[a]		5.0 (5.0)[a]	5.2 (4.95)[a]
D-Glucose penta-acetate (control)	1.2		0.25[b]	0.35[b]
	From ratio 391/389	*From ratio 348/346*		
Amine penta-acetate (control)	4.04[c]	(3.1)[a]		

[a] Values in parentheses were calculated from peak-height ratios. [b] These values should be zero. [c] Calculated from peak-area ratios in field-ionization spectra.

The ^{18}O-content at C-1 is 8.25% (13.35 minus 5.1). If exchange in D-glucose is allowed for, the ^{18}O-content at C-1 should be written as >7.05% (8.25 minus 1.2). Exchange in the amine prior to deamination is at least 2.15% (3.35 minus 1.2), but no allowance can be made for this without a knowledge of the relative importance of the two routes, *d* and *e*. Table II shows the expected labelling for the two reaction pathways, together with the values found.

TABLE II

^{18}O-CONTENTS FOR SUBSTITUTION AND EPOXIDE PATHWAYS

Reaction pathway	*^{18}O-Content (%)*	
	At C-1	*At C-2*
Substitution	>2.15	10
via Epoxide	10	>2.15
Found	>7.05	5.1

Calculation shows that at least 62% of the D-glucose was formed *via* the epoxide. The results therefore show that the epoxide pathway is of major importance, and that substitution also occurs. Substitution has been found to occur to the extents of at least 32% and 11% in the deamination of two other pyranoses containing an

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[*] Determined in a separate experiment by removing aliquots and analysing for amine, after destruction of unreacted nitrous acid by excess of sulfamic acid, using an amino acid analyser.

axial amino group, namely, methyl 3-amino-3-deoxy-β-D-allopyranoside and methyl 4-amino-4-deoxy-α-D-galactopyranoside[1].

EXPERIMENTAL

Deamination. — Sodium nitrite (28 mg) was dissolved in ^{18}O-enriched water (0.5 ml) in a pear-shaped (25 ml) two-necked flask fitted with a cold finger and stopper. Glacial acetic acid (0.03 ml) was added and the solution was allowed to equilibrate for 10 min. The solution was cooled in an ice-bath, and 2-amino-2-deoxy-D-mannose hydrochloride (21.6 mg) was added. The stoppered flask was kept in an ice-bath for 4 h, and the solvent was then transferred to the cold finger by lyophilisation. Pyridine (0.5 ml) and acetic anhydride (0.25 ml) were added to the residue, and the suspension was shaken at room temperature for 48 h. The usual work-up by chloroform extraction gave a syrup which crystallised on standing. G.l.c. analysis, using a 10% butane-1,4-diol succinate column, showed two components corresponding to α- and β-D-glucopyranose penta-acetates, and a third (<5% by peak area measurement, flame-ionisation detector) having a much lower retention time.

The solvent which had been collected on the cold finger was allowed to melt in a stoppered flask. Benzoyl chloride (50μl) was then added, and the stoppered flask was shaken overnight. The resulting benzoic acid (45 mg) was filtered off, dried, and analysed by mass spectrometry.

Control experiments. — (*a*) The above experiment was carried out with D-glucose (18 mg) substituted for 2-amino-2-deoxy-D-mannose hydrochloride.

(*b*) The deamination in water was repeated with the omission of the sodium nitrite, and the time was reduced to 1.5 h. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-αβ-D-mannose was obtained as a syrup.

Mass spectrometry. — Attempts to measure the molecular ion of D-glucose penta-acetate by field ionization and field desorption on a Varian mass spectrometer were unsuccessful, and the relative abundances were measured on an AEI MS-9 spectrometer (using the direct-insertion probe) in two ways: (*a*) by measuring, in rapid succession, the collector currents for the P and P+2 ions while maintaining the total ion monitor current constant (at a resolution of 1000), and (*b*) by measuring peak-height ratios on the chart. The latter measurements were less precise.

The field-ionization spectrum of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-mannose contained a molecular ion at *m/e* 389, and the abundance ratio (391/389) was calculated from peak areas that were measured on a chart obtained by slow scanning. The abundance ratio (348/346) was obtained from peak-height measurements of the electron-impact spectrum.

The (M+2)/M abundance ratio for benzoic acid was obtained from peak-height measurements on the electron-impact spectrum. The ^{18}O-content was obtained by comparing the (P+2)/P ratios for the labelled and unlabelled compounds. The benzoic acid isolated from the deamination, D-glucose control, and amine control experiments contained 10.1, 11.2, and 9.4% of ^{18}O, respectively.

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