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Catalysis of reduction of aldos-2-uloses ('osones') by aldose reductase: selectivity for the aldehydic carbonyl group

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Abstract

In mammalian tissues, reducing sugars and *N*-(1-deoxyfructosyl) groups of glycated proteins undergo non-enzymatic reactions to form aldos-2-uloses, or 'osones'. These compounds, which occur in relatively high concentrations in diabetic animals, are harmful in that they react with side-chain groups of proteins, adversely affecting their functions. However, there is evidence for the reduction of aldos-2-uloses in vivo, a process which would be expected to result in a lowering of reactivity, and serve as a detoxification mechanism. We report that, on incubation with aldose reductase and NADPH, D-*arabino*-hexos-2-ulose (**1**; 'glucosone'), 3-deoxy-D-*glycero*-pentos-2-ulose (**2**; '3-deoxyxylosone') and 3-deoxy-D-*erythro*-hexos-2-ulose (**3**; '3-deoxyglucosone') gave the corresponding 2-ketoses, **4**, **5**, and **6**. These results suggest that aldose reductase contributes to the conversion of **3** into **6** in vivo, thus accounting for the coexistence of both compounds in human blood and urine [K.J. Knecht et al., *Arch. Biochem. Biophys.*, 294 (1992) 130–137]. No aldoses or alditols were formed in the enzymatic reactions of **1**, **2**, and **3**, indicating that reduction had occurred exclusively at the C-1 (aldehydic) carbonyl, in contrast to the aldose reductase catalysed reduction of methylglyoxal, which was reported to occur at the C-1 (aldehydic) carbonyl, and, to a small extent, at the C-2 (ketone) carbonyl [D.L. Vander Jagt et al., *J. Biol. Chem.*, 267 (1992) 4364–4369]. The high selectivity towards the C-1 carbonyl group is discussed in the light of recent information on possible modes of binding of sugars to aldose reductase. © 1996 Elsevier Science Ltd.

Keywords: Aldosulose; Osone; Ketose; Aldose reductase

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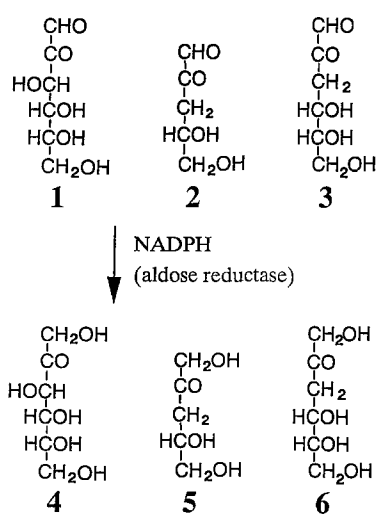
1. Introduction

Mammalian proteins undergo chemical modification as a result of exposure to endogenous reducing sugars, such as glucose, fructose, sugar phosphates and ascorbic acid. The net result is the formation of protein-attached heterocycles, or 'advanced glycosylation end-products', which crosslink proteins via amino and guanidyl groups, and adversely affect their functions [1]. These processes occur relatively quickly in subjects with diabetes, and account, in part, for the secondary effects of the disease [2]. There is mounting evidence for the intermediacy of sugar-derived α-dicarbonyl containing compounds in the generation of advanced glycosylation end-products. In particular, aminoguanidine, a compound which reacts with α-dicarbonyls, acts as a strong inhibitor of their formation in vivo and in vitro [3].

Low molecular weight α-dicarbonyls of importance in advanced glycosylation end-product formation include methylglyoxal, formed from triose phosphates, aminoacetone and acetone [4] and from glyoxal formed by oxidative cleavage of glucose [5]. However, the present study is concerned with aldos-2-uloses, or 'osones', which occur in mammalian tissues. Based upon the results of in vitro experiments, it has been suggested that D-*arabino*-hexos-2-ulose or 'glucosone' (**1**; see Scheme 1) is formed by autoxidation of glucose [6]. Alternatively, **1** may be formed by separation of 1-deoxyfructosyl groups from glycated proteins as a result of an oxygen-requiring process that is poorly understood [7]. 3-Deoxy-L-*glycero*-pentos-2-ulose or '3-deoxy-L-xylosone' (enantiomer of **2**) is formed by oxidative degradation of L-ascorbic acid [8]. However, the best biological example is that of 3-deoxy-D-*erythro*-hexos-2-ulose or '3-deoxyglucosone' (**3**) which occurs in blood and urine [9] in concentrations that are elevated in diabetes [10]. Compound **3** may be formed by elimination of a 1-deoxyfructosyl group of a glycated protein [11] or by interactions of fructose [12] or fructose 3-phosphate [13] with



Scheme 1.

proteins. In mammals, a significant proportion of **3** is reduced to form 3-deoxy-D-*erythro*-2-hexulose or '3-deoxyfructose' (**6**), which has been detected in blood and urine [9,14,15]. This conversion is likely to be biologically useful, since compound **6** would be expected to be less reactive, and therefore less damaging to tissues, than compound **3**.

Aldos-2-uloses **1–3** are relatively good substrates for aldose reductase (EC 1.1.1.21), a monomeric, broad specificity, NADPH-dependent enzyme [16]. Of the members of the aldo–keto reductase superfamily, it is the one that has been studied most extensively. The amino acid and cDNA sequences [17], X-ray structure [18] and mechanism of action [19] of the enzyme have been examined.

Three products are formed when methylglyoxal is reduced by NADPH in the presence of aldose reductase [4]. The majority (95%) of the reduction occurs at the aldehyde group to give acetol, and the remainder takes place at the keto group, to afford D-lactaldehyde. Acetol is then reduced further, to form L-1,2-propanediol.

The sites of reduction of aldos-2-uloses, catalysed by aldose reductase, have not been studied rigorously, though Hers [20], using paper chromatography, found that fructose (**4**) was formed by reduction of D-*arabino*-hexos-2-ulose (**1**) with human placental enzyme. It should also be noted that 3-deoxy-D-*erythro*-2-hexulose (**6**) was the main product of reduction of 3-deoxy-D-*erythro*-hexos-2-ulose (**3**), in the presence of a porcine, monomeric reductase with the properties of a glycoprotein [21]. Therefore, in these instances, C-1 was the principal site of reduction. However, other possibilities should be considered. For example, by analogy with the methylglyoxal example mentioned above [4], aldose reductase would be expected to promote some reduction of the ketone carbonyl (C-2) of aldos-2-uloses **2** or **3**, to generate 3-deoxyaldoses. The latter might then undergo further reduction at C-1, to form 3-deoxyalditols. We now describe work in which we have demonstrated that recombinant, human aldose reductase catalyses the reduction of *only* the aldehydic group (C-1) of each of the aldos-2-uloses, **1**, **2**, and **3**.

2. Experimental

Materials.—Glucose 6-phosphate, glucose 6-phosphate dehydrogenase, $NADP^+$ and NADPH were products of Boehringer Mannheim. Reference sugars were obtained from Sigma. Samples of aldos-2-uloses **1–3** were prepared by established methods [8,22,23]. Minor impurities in the resulting sample of **3** were removed using a column of silica gel, eluted with 14:3:0.3 $CHCl_3$–MeOH–H_2O. D-*arabino*-[1-^{13}C]Hexos-2-ulose was prepared by converting D-[1-^{13}C]glucose (Aldrich) into its phenylosazone, and treating the latter with benzaldehyde [22]. Silylating reagents were obtained from Pierce, Rockford, IL.

Aldose reductase.—Human recombinant aldose reductase was overexpressed in *E. coli* type BL23(DE3) cells using the plasmid pET16b (Novagen, Madison, WI), with human muscle fetal aldose reductase cDNA [24] inserted at the *Nco*I/*Eco*RI site. Cultures were grown at 37 °C in LB medium containing ampicillin (50 μg/mL) until the value of A_{660} was 0.6. Isopropyl β-D-thiogalactoside (1 mM) was added, and incubation was continued for another 3 h. The cells were harvested and frozen by

submerging the centrifuge tubes in liquid nitrogen for 1 min, and then thawed by submersion in an ice–water bath for 15–20 min. The freeze–thaw process was repeated three times and the lysed cells were suspended in a solution (pH 7.0) containing sodium phosphate (10 mM), EDTA (5 mM) and 2-mercaptoethanol (5 mM) at 5 °C. The cellular debris was removed by centrifugation, and purification of the enzyme was effected as described previously [25]. Typically, a 12 L culture gave 6 mg of enzyme, with a specific activity of 1 unit/mg, where a unit of enzyme reduces DL-glyceraldehyde at 1 μmol/min under defined conditions [25]. Sodium dodecyl sulfate–polyacrylamide electrophoresis gave bands representing aldose reductase and an impurity (ca. 5% of total protein) with M_r 37,000 and 28,000, respectively.

Gas chromatography (GC) of trimethylsilyl (TMS) derivatives of oximes.—Oximes of aldoses, ketoses and aldosuloses were prepared [26] and silylated by treatment with TMS-imidazole (0.2 mL) and *N,O*-(*bis*-TMS)trifluoroacetamide (0.2 mL) at 45 °C for 2 h. GC was conducted with an SRI model 0610 instrument, fitted with a flame-ionization detector. A CH_2Cl_2 solution (1 μL) of TMS-oxime derivatives was injected into a column (30 m × 0.53 mm i.d.) of SP-2250 (Supelco, Bellefonte, PA). Initial oven temperatures were 170, 160, and 150 °C for mixtures resulting from reaction of **1**, **2**, or **3**, respectively. Temperature program: hold initial temperature constant for 2.5 min; ramp at 10 °C/min to 200 °C; ramp at 2 °C/min to 209 °C; isothermal for 2 min; ramp at 3 °C/min to 225 °C. The TMS-oximes of each aldos-2-ulose gave two incompletely resolved peaks, representing geometrical isomers. Retention times, *T*, for **1**, **2**, and **3**, were 3.8 and 4.0, 4.8 and 5.0, and 2.6 and 2.8 min, respectively. Single, slightly broad peaks were obtained for the TMS-oximes of the enzymatic reduction products.

Combined gas chromatography and mass spectrometry (GC–MS) of deoxyalditol acetates.—Each reducing sugar sample (ca. 1 mg) was converted into deuterium-labelled alditol acetate(s) as described [9], except that 1 M HOAc was used to destroy the excess of $NaBD_4$, and formation of methyl borate was achieved by addition of a 0.1% solution of trifluoroacetic acid in MeOH.

The deoxyalditol acetates were dissolved in CH_2Cl_2 (1 mL), and a 1 μL sample was applied to a Quadrex 007-OV-17 column (25 m × 0.25 mm i.d.) in a Hewlett-Packard 5890 series chromatograph, interfaced with a Hewlett-Packard 5970 mass-selective detector. Temperature program: 120 °C for 2 min; ramp at 8 °C/min to 250 °C; hold at 250 °C for 5 min. The tetraacetates of 3-deoxy-D-*threo*-pentitol and 3-deoxy-*erythro*-pentitol (formed from five-carbon sugars with C-1 and C-2 in various states of oxidation) had retention times of 10.5 and 10.7 min, respectively. The two compounds formed from six-carbon sugars, 3-deoxy-D-*arabino*-hexitol pentaacetate and 3-deoxy-D-*ribo*-hexitol pentaacetate, had retention times of 14.05 and 14.1 min, respectively, and were not completely resolved.

Thin-layer chromatography.—TLC was conducted on Silica Gel G, in 4:5:1 1-BuOH–Me_2CO–H_2O (solvent *A*), spraying with alkaline $KMnO_4$ [27] or in 7:3:0.3 $CHCl_3$–MeOH–H_2O (solvent *B*), spraying with 9:1 MeOH–H_2SO_4.

NMR spectroscopy.—Proton-decoupled ^{13}C NMR spectroscopy was performed on solutions of sugars (1–3 mg of each) in sodium phosphate buffer (0.1 M, pH 7) made in 9:1 H_2O–D_2O. A trace of MeOH was included as a reference. Spectra were obtained at 100.6 MHz with a Bruker AM 400 instrument, using a *J*-modulated spin-echo sequence

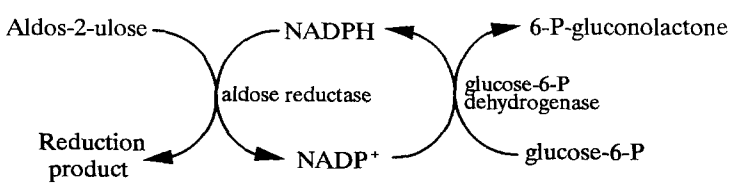
[28]. Chemical shifts are given in ppm downfield from tetramethylsilane. Signals for methine and methyl carbons are displayed as negative peaks.

Aldose reductase catalysed reduction of D-arabino-*hexos-2-ulose* (**1**) *without coenzyme recycling.*—A solution of **1** (1 mg, 1.02 mM), NADPH (Na salt, 6.4 mg, 1.4 mM) and aldose reductase (0.04 units) in 5.5 mL of sodium phosphate buffer (0.1 M, pH 7) was allowed to stand at 22 °C for 6 h. A second addition of NADPH (6.4 mg) and aldose reductase (0.04 units) was made, and the solution was allowed to stand for a further 12 h. The solution was then deproteinized with a Centriprep-10 ultrafilter (Amicon, Beverly, MA), deionized with AG 50W (H^+) and AG 1 (OAc^-) resins (BioRad, Richmond, CA), and lyophilized. Note that, although the ultrafilters were prewashed with water at least three times, some glycerol remained entrapped in the membranes; therefore each filtrate contained a trace of glycerol, which gave signals in the NMR spectrum, and a fast-flowing spot on TLC in solvent *A*.

Aldose reductase catalysed reduction of aldos-2-uloses, with coenzyme recycling.—A solution of **1**, **2**, or **3** (5.62 μmol, 1.02 mM), $NADP^+$ (Na salt, 0.45 mg, 0.11 mM), glucose 6-phosphate (disodium salt, 2.0 mg, 1.2 mM), yeast glucose 6-phosphate dehydrogenase (1.8 units) and aldose reductase (0.04 units) in 5.5 mL of sodium phosphate buffer (0.1 M, pH 7), was kept at 22 °C for 6 h. It was then supplemented with $NADP^+$ (0.45 mg), glucose 6-phosphate dehydrogenase (0.9 units) and aldose reductase (0.04 units), and allowed to stand for a further 12 h. The solution was then deproteinized and deionized as described above. Although the resulting mixture of sugars always included a small quantity of the original substrate, purification of the product was not attempted.

3. Results

The product of enzymatic reduction of D-arabino-*hexos-2-ulose* (**1**).—Incubation with NADPH and aldose reductase resulted in a 5% conversion of (**1**) into a product, **p**, which was difficult to identify due to its small mass. A higher yield was expected on thermodynamic grounds, since aldose reductase catalysed reductions have large equilibrium constants [29]. Hence it was possible that the low yield was attributable to inhibition of the enzyme by $NADP^+$ formed in the reaction [30]. Therefore, in subsequent experiments a low $NADP^+$ concentration was maintained by coupling the reduction of each aldos-2-ulose to the enzyme-catalysed oxidation of glucose 6-phosphate (see Scheme 2). Use of this 'coenzyme recycling' method [31] gave 95%



Scheme 2.

Table 1
R_f values for TLC on Silica Gel G

Compound	Solvent	R_f
1	*A*	0.36 [a]
p = 4	*A*	0.46
D-Glucose	*A*	0.43
D-Mannose	*A*	0.50
D-Glucitol	*A*	0.34
D-Mannitol	*A*	0.37
2	*B*	0.64
3	*B*	0.52
q = 5	*B*	0.47
r = 6	*B*	0.46

[a] Elongated spot.

conversion into compound **p**. Its behavior on TLC (Table 1) and on GC ($T = 3.05$ min for the TMS-oxime) corresponded to that of D-fructose (**4**). In addition, compound **p** was identified as D-fructose, using the Boehringer Mannheim enzymatic assay system. This confirmed the finding of Hers [20] that fructose was the main reaction product.

Further information was obtained by using ^{1}H-decoupled ^{13}C NMR spectroscopy to identify the sugars in a solution obtained from an enzymatic reduction of a sample of D-*arabino*-[1-^{13}C]hexos-2-ulose (see Fig. 1A and B). This was feasible because, for each form of **1** and its possible reduction products, C-1 resonates at a different frequency (see Table 2). ^{13}C NMR data (assignments made by reference to Table 2): [1-^{13}C]fructose: δ 62.8 (C-1 of β-furanose), 63.05 (C-1 of α-furanose), 64.0 (C-1 of β-pyranose); D-*arabino*-[1-^{13}C]hexos-2-ulose: δ 89.5, 91.0, 94.7, 95.1, 99.6, 100.0 (C-1 of six forms); unlabelled glycerol from ultrafilter: δ 62.6 (C-1,3), 72.1 (C-2).

Hence NMR spectroscopy demonstrated that the solution contained fructose, a small quantity of the substrate (**1**), and traces of unidentified compounds producing weak signals at 100.3 and 103.0 ppm. Signals representing C-1 of glucose, mannose or mannitol were absent. The intensities of the 62.6 and 72.1 ppm signals of glycerol were in the expected ratio of 2:1, respectively. Therefore it is unlikely that any of the 62.6 ppm signal was attributable to C-1 of D-[1-^{13}C]glucitol. The conclusion reached from the NMR and chromatographic analyses was that the enzymatic reduction of **1** had occurred only at C-1, to give D-fructose (**3**).

The product of enzymatic reduction of 3-deoxy-D-glycero-*pentos-2-ulose* (**2**).—In the presence of NADPH and aldose reductase, compound **2** underwent an 83% conversion into a new compound, **q**, which moved more slowly than **2** on TLC (Table 1), and gave a TMS-oxime of $T = 4.05$ min. No other product was detected by TLC or GC.

A different strategy was used to determine the position of the carbonyl group in **q**, since most of the reference compounds, representing possible enzymatic reduction products, were not available. The first approach was to apply ^{13}C NMR spectroscopy to the 83:17 mixture of **q** and **2** formed by reduction of unlabelled **2**. Owing to the availability of only a small mass of material, a detailed analysis of the spectrum (Fig. 2A) was not undertaken. However, the data were consistent with the coexistence of

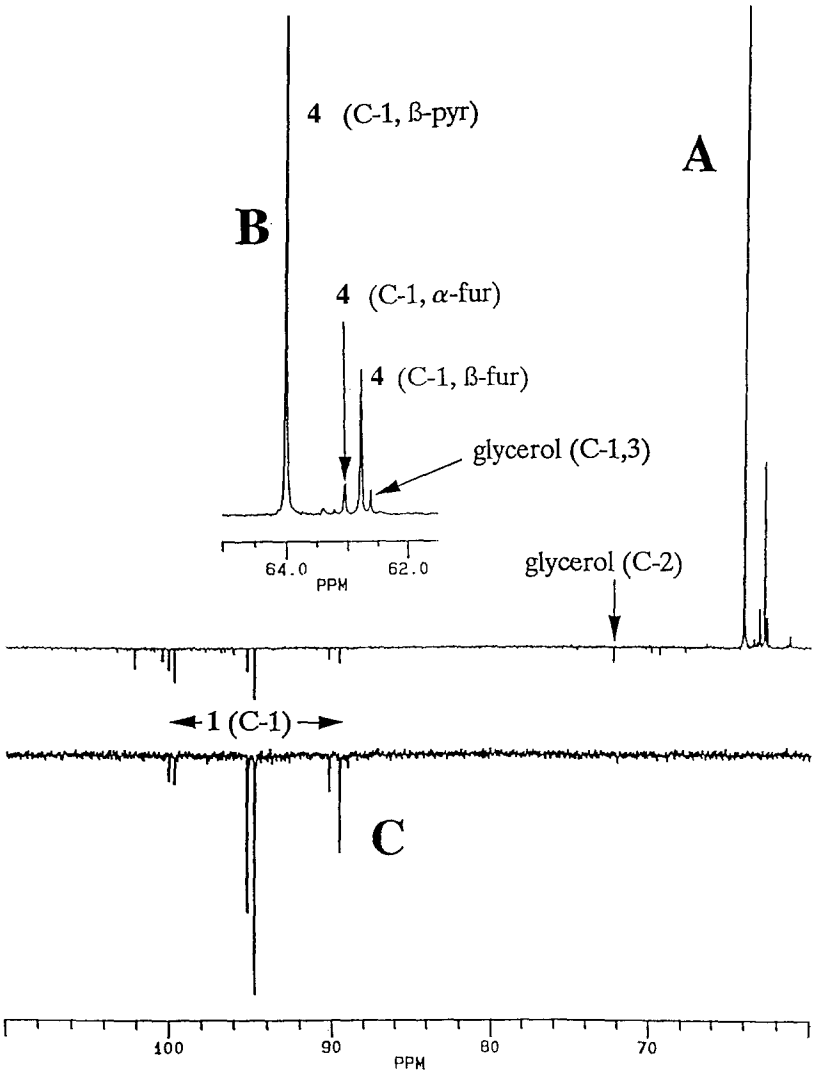


Fig. 1. Proton-decoupled ^{13}C NMR spectra of: (A) mixture obtained by aldose reductase/NADPH treatment of D-*arabino*-[1-^{13}C]hexos-2-ulose; (B) partial spectrum shown in (A), expanded; (C) D-*arabino*-[1-^{13}C]hexos-2-ulose.

several tautomers of a 3-deoxy-2-pentulose. The four strong signals were assigned as follows: δ 41.5 (C-3), 65.1 (C-1 or C-5), 67.8 (C-4), 67.9 (C-1 or C-5). Signals at δ 104.7 (C-2 hemiketal) and 211.6 (C-2 carbonyl) showed that cyclic and acyclic forms were present, a situation resembling that of D-*erythro*-2-pentulose [33]. The relatively weak signals (six positive and two negative) in the 40–80 ppm region formed a pattern expected from C-1, C-3, C-4, and C-5 of two minor forms of **5**. This pattern could not arise from a 3-deoxypentose, a 3-deoxypentitol, or a mixture of them. It was assumed

Table 2
^{13}C NMR parameters for C-1 of reference compounds [a]

Compound	Structure [b]	Chemical shift (ppm)
1 [c]	unknown	89.5, 91.0, 94.7, 95.1, 99.6, 100.0
D-Glucose	α-pyranose	92.1
	β-pyranose	95.9
D-Mannose	β-pyranose	93.7
	α-pyranose	94.0
D-Fructose	β-furanose	62.7
	α-furanose	63.0
	β-pyranose	63.9
D-Glucitol	acyclic	62.5
D-Mannitol	acyclic	63.3

[a] For solvent used, and conditions, see Experimental.
[b] Assignments are based upon ref. [32].
[c] From spectrum of 1-^{13}C labelled form of **1** (Fig. 1C).

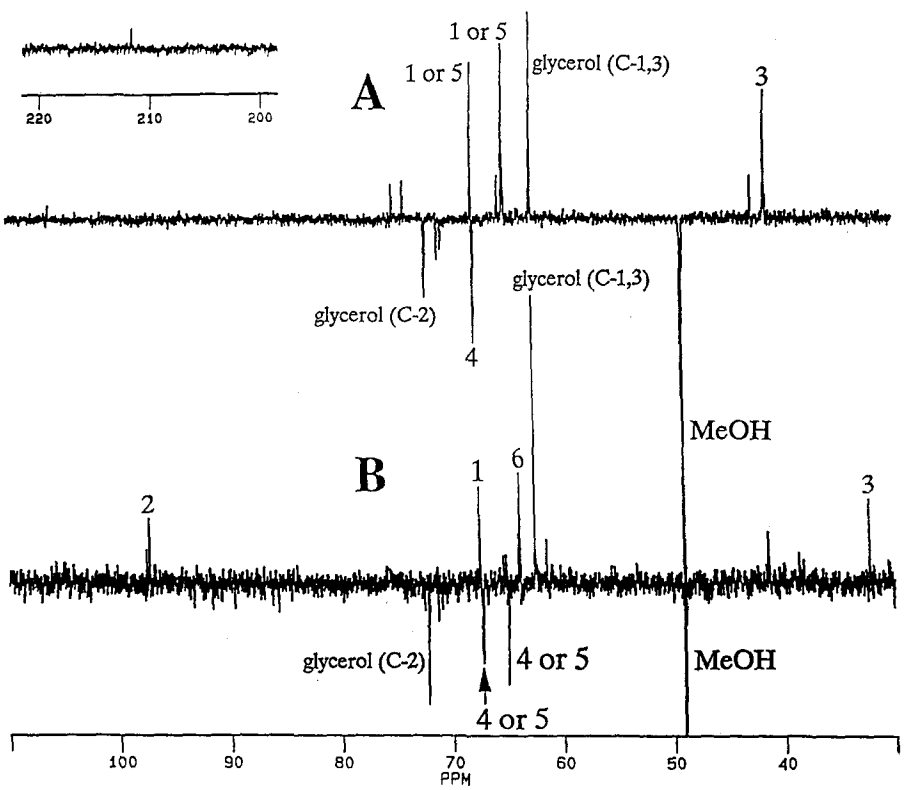
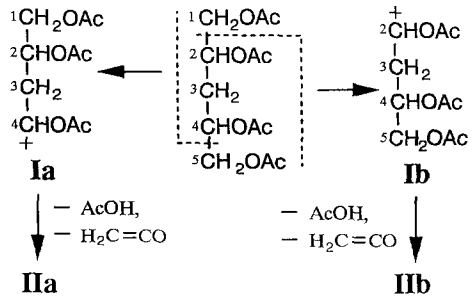


Fig. 2. Proton-decoupled ^{13}C NMR spectrum of: (A) mixture obtained by exposure of 3-deoxy-D-*glycero*-pentos-2-ulose (**2**) to aldose reductase and NADPH, in which the main component, **q**, was shown to be (**5**) (signal assignments refer to the most abundant form); (B) mixture obtained by aldose reductase/NADPH treatment of 3-deoxy-D-*erythro*-hexos-2-ulose (**3**), in which the main component, **r**, was shown to be **6**.



Scheme 3.

that the apparent absence of signals for the relatively small quantity of unreacted **2** was due to the multiplicity of tautomers, each giving rise to signals that were too weak to detect. Hence the NMR data indicated that only the aldehydic group of **2** had been reduced. Compound **q** was therefore 3-deoxy-D-*glycero*-2-pentulose or '3-deoxyxylulose' (**5**).

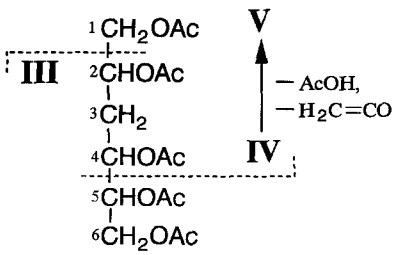
The sugars resulting from aldose reductase treatment of **2** were then converted into deuterated deoxypentitol acetates, which were studied by GC–MS. Preliminary tests on 3-deoxypentitol acetates formed by successive treatment of 3-deoxy-D-*erythro*-pentose or **2** with $NaBD_4$ and acetic anhydride showed that their mass spectra could be interpreted in terms of the fragmentation pattern of Scheme 3. Equal quantities of ions **Ia** and **Ib** were formed, together with secondary ions **IIa** and **IIb**. Therefore the oxidation state of C-1 and C-2 of five-carbon sugars resulting from enzymatic conversions could be examined by comparing the MS data for derived tetraacetates with values compiled in Table 3.

The mixture of sugars resulting from treatment of **2** with aldose reductase and NADPH gave approximately equal quantities of the tetraacetates of 3-deoxy-*erythro*-pentitol and 3-deoxy-D-*threo*-pentitol (shown by GC; see Experimental). MS data for 3-deoxy-*erythro*-pentitol tetraacetate (relative abundances in parentheses): *m/z* 130 (100), 131 (17), 232 (17), 233 (5). The spectrum of 3-deoxy-D-*threo*-pentitol tetraacetate was similar. By reference to the data in Table 3, it was inferred that the original mixture contained a 3-deoxy-2-pentulose (presumably **5**) with a relatively small quantity of a

Table 3
Important MS ions expected from 3-deoxypentitol acetates produced by successive treatment of five-carbon sugars with $NaBD_4$ and acetic anhydride

3-Deoxysugar	Position(s) of carbonyl(s)	Mass of fragment [a]			
		Ia	**Ib**	**IIa**	**IIb**
3-Deoxypentos-2-ulose	1 and 2	233	232	131	130
3-Deoxypentose	1 only	232	231	130	129
3-Deoxy-2-pentulose	2 only	232	232	130	130
3-Deoxypentitol	None	231	231	129	129

[a] See Scheme 3 for a fragmentation diagram.



Scheme 4.

3-deoxypentos-2-ulose (presumably **2**), thus confirming the conclusion reached in the GC and NMR analyses. In summary, enzymatic reduction of **2** occurred only at C-1 to afford 3-deoxy-D-*glycero*-2-pentulose (**5**).

The product of enzymatic reduction of 3-deoxy-D-erythro-*hexos-2-ulose* (**3**).—Encouraged by the progress made in the study of the enzymatic reduction of **2**, we decided to apply similar methods to the identification of the site(s) of reduction of compound **3**. In this case a single product, **r**, was formed, with a smaller R_f value than that of **3** (Table 1), and with a value of $T = 2.2$ min for the TMS-oxime. The extent of conversion of **3** into **r** was estimated to be 87%, based on analysis by GC.

The ^{13}C NMR spectrum (Fig. 2B) of the mixture resulting from the enzymatic reduction of **3** was almost identical to the published spectrum [14] of 3-deoxy-D-*erythro*-2-hexulose, or '3-deoxyfructose' (**6**). There was an absence of sets of signals that could be ascribed to 3-deoxyhexoses or 3-deoxyhexitols. Assignments, based upon ref. [14]: compound **6** (pyranose form): δ 32.3 (C-3), 64.0 (C-6), 65.0 (C-4 or C-5), 67.3 (C-4 or C-5), 67.6 (C-1), 97.4 (C-2); glycerol from ultrafilter: δ 62.6 (C1,3), 72.2 (C-2).

It has already been shown that the location(s) of carbonyl group(s) at C-1 and C-2 of six-carbon, 3-deoxy sugars can be determined by examining their derived, deuterium-labelled, 3-deoxyhexitol acetates by GC–MS [9]. Diagnostic ions **III**, **IV** and **V** are produced according to Scheme 4, Fig. and Fig. , and their predicted masses are shown in Table 4. When this method was applied to the mixture of sugars obtained by enzymatic reduction of **3**, approximately equal quantities of the acetates of 3-deoxy-D-*arabino*-hexitol and 3-deoxy-D-*ribo*-hexitol were formed. MS data for 3-deoxy-D-*arabino*-hexitol

Table 4
Important MS ions expected from 3-deoxyhexitol acetates produced by successive treatment of six-carbon sugars with $NaBD_4$ and acetic anhydride

3-Deoxysugar	Position(s) of carbonyl(s)	Mass of fragment [a]		
		III	**IV**	**V**
3-Deoxyhexos-2-ulose	1 and 2	304	233	131
3-Deoxyhexose	1 only	303	232	130
3-Deoxy-2-hexulose	2 only	304	232	130
3-Deoxyhexitol	none	303	231	129

[a] See Scheme 4 for a fragmentation diagram.

pentaacetate (relative abundances in parentheses): *m/z* 130 (100), 131 (12), 232 (56), 233 (8), 304 (16), 305 (3). The data for the *ribo* epimer were similar. Using Table 4 to interpret the spectra, it was concluded that the main species contained a single deuterium atom located at C-2, which must have arisen from a 3-deoxy-2-hexulose. This supported the conclusion, reached by interpretation of the NMR spectrum (above), that the enzymatic product, **r**, was **6**. The low-intensity fragments of *m/z* 131 and 233 represented the unreacted **3** that had been detected by GC of its TMS-oxime (see above). The absence of ions of *m/z* 129, 231, and 303 showed that 3-deoxyhexoses or 3-deoxyhexitols were not formed in the enzymatic reaction.

4. Discussion

Aldose reductase is an efficient detoxification catalyst [34]. Since it occurs in many mammalian tissues, it is considered to be one of several enzymes responsible for the reduction of 2-ketoaldehydes [16]. The data presented here suggest that this type of reaction is likely to result in the conversion of various endogenous aldos-2-uloses into 2-ketoses in mammalian tissues. In particular, aldose reductase could be partly responsible for the transformation of 3-deoxy-D-*erythro*-hexos-2-ulose (**3**) into 3-deoxy-D-*erythro*-2-hexulose (**6**) in vivo, and account for the coexistence of these compounds in blood and urine [9]. Presumably this type of reaction results in a lowering of the capacity to cross-link proteins [12], and is an example of the protective action of the aldose reductase. Experiments designed to test this hypothesis are now in progress.

In the presence of aldose reductase, methylglyoxal is reduced rapidly at C-1, and relatively slowly at C-2 (see Introduction). For each of the aldos-2-uloses, **2** and **3**, the structure of the C-1 to C-3 portion of the molecule is the same as that of methylglyoxal. Therefore, the complete lack of reduction of the keto group of the aldos-2-uloses seemed surprising. There are two main reasons why it is difficult to provide a complete explanation of the selectivity towards C-1 of aldos-2-uloses: (a) The nature and proportions of the tautomeric and hydrated-carbonyl forms of each aldos-2-ulose are not known, though, judging from an NMR study of pentos-2-uloses, relatively large numbers of such forms must exist [35]; (b) crystal structures of ternary complexes of aldose reductase, NADPH, and a substrate are not yet available.

Computer modelling studies indicate that side-chain groups of Tyr48, His110, and Trp111 of aldose reductase, and the nicotinamide amide of bound NADPH, are involved in anchoring acyclic forms of aldoses to the active site by forming hydrogen bonds with oxygen atoms attached to C-1 and C-2 [36]. The aldehydic carbonyl can then be protonated by one of the side-chain groups, and receive the 4-*pro-R* hydrogen from the reduced nicotinamide ring of bound NADPH [37].

In a substrate specificity study of a variety of hydrophilic compounds, Vander Jagt et al. noted that relatively low K_m values are obtained for the reduction of 2-ketoaldehydes [38]. They concluded that a carbonyl group at C-2 "may be an especially important determinant of substrate specificity". Therefore, when an aldos-2-ulose is bound to the holoenzyme, its aldehydic and ketone carbonyls may be anchored to the enzyme in a similar manner to the aldehydic and 2-hydroxyl groups of an aldose. The aldehydic

carbonyl, but not the ketone carbonyl, would then be in a suitable position for hydride attack.

The hydroxyl groups of an aldos-2-ulose may impose restrictions on the number of ways in which binding to the enzyme can occur, precluding reduction at C-2. Such restrictions would not apply to methylglyoxal, permitting reduction to occur at C-2.

Presumably the 2-ketoses, **4**, **5**, and **6**, cannot bind to the enzyme in a productive manner, and are therefore not converted into alditols. The results presented here reinforce the view that aldose reductase usually acts on aldehydes, but not ketones [39]. Reduction of the keto group of methylglyoxal [4] appears to be an exceptional case.

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