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FORMATION OF 3-OCTULOSES BY A SELF-ALDOL REACTION OF D-ERYTHROSE

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ABSTRACT

When kept at 105° for 2.5 h, weakly alkaline, syrupy D-erythrose was readily converted into a mixture containing mainly D-*glycero*-tetrulose, the previously unknown β-D-*altro*-L-*glycero*-3-octulofuranose (**2**), and α-D-*gluco*-L-*glycero*-3-octulopyranose, which were isolated as the corresponding acetates. Treatment of **2** with Dowex 50 (H^+) resin yielded 3,8-anhydro-β-D-*altro*-L-*glycero*-octulopyranose, identified as its acetate. Previous discrepancies in the $[\alpha]_D$ values for D-erythrose appear partly to originate in the self-aldol reaction. The dimerisation of D-erythrose 4-phosphate is also described.

INTRODUCTION

D-Erythrose exists in dilute, aqueous solution essentially as a mixture of the hydrate and the furanose forms[1–4]. Concentration of the solution promotes dimerisation, and some cyclic bis(hemiacetals) formed were isolated as their acetates[5]. Syrupy D-erythrose is readily converted into 3-octuloses by a self-aldol reaction[6]. The condensation proceeded slowly at room temperature and was readily accelerated by heating. We now report on this reaction, which explains ambiguities noted[4,7,8] for the specific optical rotation of D-erythrose and its purification.

RESULTS AND DISCUSSION

H.p.l.c. (Fig. 1) and sugar analysis by g.l.c.–m.s. of the product obtained by storing syrupy D-erythrose at 105° for 2.5 h showed that most of the tetrose had been converted into octuloses and that some had isomerised. The self-aldol reaction that occurred slowly on storage of the syrupy D-erythrose at room temperature seems to be irreversible, in contrast to the formation of bis(hemiacetals) formed by dimerisation. The reaction was probably initiated by traces of alkali. These did not originate from the glass vessel[9], but rather from the barium carbonate used in the synthesis of the erythrose[5] (through transient formation of barium hydrogencarbonate). The dimerisation[10,11] of D-erythrose 4-phosphate, which is known to produce cyclic

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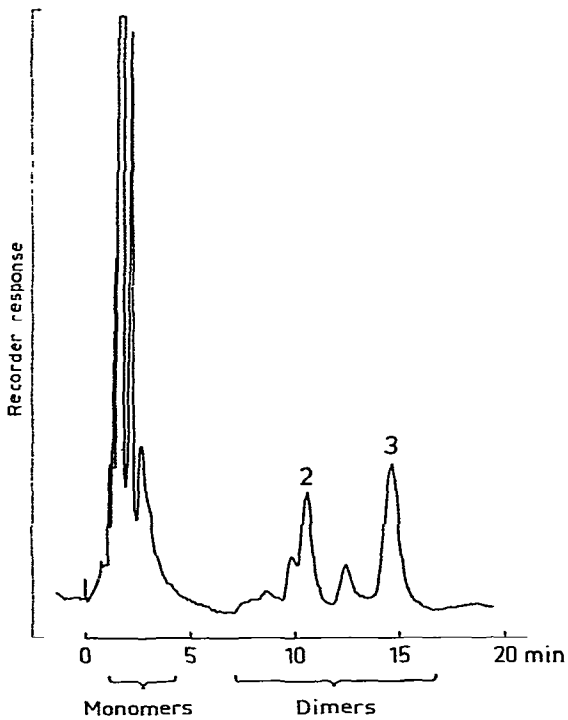
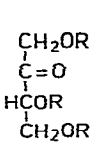


Fig. 1. H.p.l.c. analysis ("μ"-Carbohydrate column, 89:11 acetonitrile–water, 2.5 ml/min) of D-erythrose heated at 105° for 2.5 h. Compounds that were isolated are numbered.



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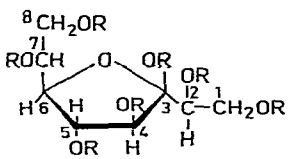
1a R=Ac



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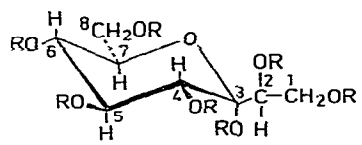
2a R=Ac



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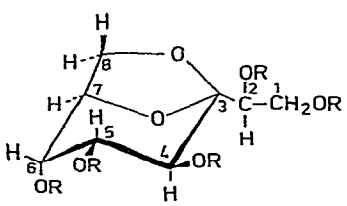
3a R=Ac



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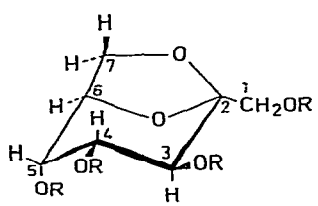
4a R=Ac



5 R=H
5a R=Ac

TABLE I

100-MHz, ^{1}H-N.M.R. CHEMICAL SHIFTS FOR COMPOUNDS **1a–5a**

Proton	*Chemical shifts (p.p.m.)*							
	1a *($CDCl_3$)*	**1a** *(C_6D_6)*	**2a** *($CDCl_3$)*	**2a** *(C_6D_6)*	**3a** *($CDCl_3$)*	**3a** *(C_6D_6)*	**4a** *(C_6D_6)*	**5a** *(C_6D_6)*
H-1	4.86s	4.61s	4.13dd	4.33dd	4.05–4.35m	4.15–4.50m	4.36dd	3.96d
H-1′	4.86s	4.61s	4.4–4.7m	4.77dd	4.60dd	4.90dd	4.79dd	4.90d
H-2/3	5.41dd	5.19dd	5.25–5.4m	5.66dd	5.10dd	5.50dd	5.95dd	5.73d
H-4	4.38dd	4.23dd	6.13d	6.59d	5.17d	5.53d	5.74d	5.44dd
H-4′	4.50dd	4.37dd						
H-5			5.50dd	5.88dd	5.48t	5.85t	5.44dd	5.27dd
H-6			4.63dd	4.93dd	5.0–5.25m	5.39m	5.17dd	4.16m
H-7			5.25–5.4m	5.6–5.75m	4.05–4.35m	4.1–4.5m	3.93dd	3.31dd
H-7′								3.39dd
H-8			4.05dd	4.08dd	4.05–4.35m	4.1–4.5m	3.14d	
H-8′			4.4–4.7m	4.62dd	4.05–4.35m	4.1–4.5m	3.14d	
OAc (s)	2.09,	1.65,	2.02 (6 H),	1.55, 1.58,	1.97,	1.74, 1.77,	1.64,	1.72,
	2.17,	1.70,	2.06, 2.07,	1.63, 1.71,	2.02 (9 H),	1.78 (6 H),	1.67, 1.71,	1.74,
	2.19	1.75	2.08, 2.11,	1.74, 1.76,	2.04, 2.08,	1.79, 1.89,	1.73, 1.80	1.75 (6 H)
			2.21	2.05	2.11	1.93		

TABLE II

^{1}H-N.M.R. COUPLING CONSTANTS FOR COMPOUNDS **1a–5a**

	Coupling constants (Hz)							
	1a *($CDCl_3$)*	**1a** *(C_6D_6)*	**2a** *($CDCl_3$)*	**2a** *(C_6D_6)*	**3a** *($CDCl_3$)*	**3a** *(C_6D_6)*	**4a** *(C_6D_6)*	**5a** *(C_6D_6)*
$J_{1,1'}$			12.5	12.0	12.5	12.5	12.0	12.2
$J_{1,2}$			6.5	8.5	7.5	8.0	9.1	
$J_{1',2}$				3.0	3.0	2.5	2.9	
$J_{3,4}$	3.6	3.5						9.0
$J_{3,4'}$	4.8	5.0						
$J_{4,4'}$	12.3	12.0						
$J_{4,5}$			7.0	7.5	9.5	9.5	9.0	4.4
$J_{5,6}$			8.5	8.5	9.5	9.5	4.5	2.3
$J_{6,7}$			5.5	5.5		9.0		4.9
$J_{6,7'}$								1.6
$J_{7,7'}$								9.2
$J_{7,8}$				6.5			3.1	
$J_{7,8'}$			4.0	3.5			3.1	
$J_{8,8'}$			12.5	12.0				

bis(hemiacetals), was also investigated. Syrupy D-erythrose 4-phosphate was kept at 80° for 15 min and the product was reduced with sodium borodeuteride, enzymically dephosphorylated, and acetylated. G.l.c.–m.s. showed that isomerisation had occurred, but no octuloses were formed.

Treatment of D-erythrose with alkali yielded[12] D-*gluco*-L-*glycero*-3-octulopyranose (**3**) *via* an aldol reaction between the 1,2-enediol and aldehydo forms. The formation of four octuloses is expected in such a reaction, and the two diastereomers (**2** and **3**) possessing the *threo* configuration at C-3,4 preponderate[12,13] (see Fig. 1).

When heat-treated, syrupy D-erythrose was acetylated, compounds **1a–3a** could be isolated by chromatography on silica gel. Their identification was mainly based on the ^{1}H-n.m.r. data in Tables I and II. Compound **1a** contained two methylene groups, one of which resonated as a singlet at δ 4.86; the other appeared as doublets of doublets at δ 4.38 and 4.50, forming an ABX-system with H-3 (dd, δ 5.41). That **1a** was dextrorotatory and the resolution of the signal for H-3 was not improved by means of an optically active shift-reagent[14] indicated optical purity. The D enantiomer of *glycero*-tetrulose (**1**) was obtained[15] by isomerisation of D-erythrose or D-threose in boiling pyridine. When this reaction was repeated and the product acetylated, the resulting tetrulose acetate, as expected, was identical with **1a**, thus establishing the D-*glycero* configuration. Amorphous D,L-**1a** has been synthesised[16].

Spin-decoupling experiments with **2a** (in C_6D_6) revealed two carbon chains, separated by a quaternary carbon atom and carrying three and six protons, respectively. Each chain was terminated by a methylene group coupled to H-2 or H-7, respectively. The chemical shifts observed for **2a** in $CDCl_3$ [H-4 at δ 6.13 (*cf.* δ 5.7 for the corre-

sponding proton in β-D-fructofuranose penta-acetate[17]): H-5, 5.50; H-6, 4.63; and H-7, ~5.3] are consistent with a furanosidic structure. Further evidence for this was adduced from the ^{13}C-n.m.r. spectrum; C-3 resonated at δ 107.3, typical[17–19] for the anomeric carbon atom of furanoses, and persisted as a singlet in the proton-coupled spectrum. The stereochemistry at C-2,6,7 should remain unaffected by the aldol reaction. The stereochemistry at C-3,4,5 was determined as follows.

Deacetylation of **2a** yielded the octulose **2**. A comparison of the ^{13}C-n.m.r. spectra of **2a** and **2** indicated that the latter mainly exists in the furanose form. Reduction of **2** with sodium borodeuteride, followed by acetylation and g.l.c.–m.s., showed that deuterium had been introduced at C-3, since ions *m/z* 218 and 217, but not 146, were detected. When **2** was heated with Dowex 50 (H^+) resin, the anhydro-octulose **4** was obtained, and identified by comparing the ^{1}H-n.m.r. data of its acetate **4a** with those of synthetic 1,3,4,5-tetra-*O*-acetyl-2,7-anhydro-β-D-*altro*-heptulopyranose[20] (**5a**). A comparison between the ^{13}C-n.m.r. spectrum of **4** and that reported[21] for **5** also supports the assignment of the D-*altro*-L-*glycero* configuration. Compound **2** and likewise **2a** were assigned the β configuration on the basis of a comparison with the closely related compound D-*altro*-heptulose (sedoheptulose), in which the β-furanose form is known[22] to preponderate.

The ^{1}H-n.m.r. data for **3a** (in C_6D_6) established the presence of a pyranosidic ring, for reasons given in the corresponding analysis of **4a**. The original compound **3**, obtained after deacetylation, exhibited a ^{13}C-n.m.r. spectrum identical with that of authentic D-*gluco*-L-*glycero*-3-octulopyranose. The preponderant anomeric-carbon resonance at δ 98.4 was assigned to the α anomer on steric grounds.

EXPERIMENTAL

The general procedures were essentially as previously described[5]. T.l.c. was performed on silica gel SIF (Riedel–de Haën) with ethyl acetate–light petroleum (b.p. 60–70°) mixtures *A* 1:1 and *B* 1:3, and *C* 1-propanol–acetone–water (8:10:1), with detection by charring with sulfuric acid. P.c. was performed on Whatman No. 1 paper with *D*, ethyl acetate–acetic acid–water (3:1:1), with detection by alkaline silver nitrate or resorcinol–hydrochloric acid. Sugars were analysed by g.l.c. of their alditol acetates[23] at 170–250° on a glass column containing 3% of SP 2340 on Supelcoport (100–120 mesh), using a temperature programme of 6°/min. G.l.c.–m.s. of the octitol acetates of compounds **2** and **3** was effected at 245° (OV-225, capillary column) with a Finnigan 4021 GC/MS/DATA System. Preparative h.p.l.c. was effected on a "μ"-Carbohydrate column (30 × 0.4 cm i.d., Waters) with acetonitrile–water (89:11) at 2.0 ml/min. The samples were injected as 10–20% aqueous solutions. N.m.r. spectra (^{1}H and ^{13}C) were recorded with a Jeol FX 90Q Fourier Transform spectrometer. Tris[3-(trifluoroacetyl)-(1*R*)-camphorato]europium(III) was used as the optically active shift-reagent.

D-Erythrose was obtained as a monomer[6] in aqueous solution by acid hydrolysis of 2,4-*O*-ethylidene-D-erythrose[5,24]. D-Erythrose 4-phosphate[25] was generated by

short treatment with Dowex 50 (H^+) resin of the corresponding diethyl acetal barium salt in aqueous solution, followed by hydrolysis (pH 2–3) at 40° for 6 h. The hydrolysate was then carefully neutralised with sodium hydrogencarbonate, yielding a solution of disodium D-erythrose 4-phosphate. 1,3,4,5-Tetra-*O*-acetyl-2,7-anhydro-β-D-*altro*-heptulopyranose (**5a**) was prepared by acetylation of sedoheptulosan with acetic anhydride and pyridine. The product was processed in the usual manner[5]. D-*gluco*-L-*glycero*-3-Octulose (**3**) was prepared[12] by treatment of D-erythrose (6.0 g) with aqueous calcium hydroxide. The reaction mixture was deionised with Dowex 50 (H^+) resin, filtered, and concentrated. The residue (5.25 g) was eluted from a column (50 × 4 cm) of silica gel with solvent *C*. One main fraction (1.01 g) contained a mixture of two components that migrated slightly faster and slower than D-glucose, respectively (p.c., solvent *D*). Crystallisation from methanol gave **3** (25 mg), which, after recrystallisation from methanol–water, had m.p. 173–176°, $[\alpha]_D$ +66° (*c* 1, water), and migrated slightly slower in p.c. than D-glucose; lit.[12] m.p. 164–165°, $[\alpha]_D^{26}$ +59.3° (*c* 10, water). The i.r. spectrum was indistinguishable from that of authentic D-*gluco*-L-*glycero*-3-octulose (kindly provided by Dr. R. Schaffer and Mr. A. Cohen). ^{13}C-N.m.r. data (D_2O): δ 60.6, 61.1 (C-1,8), 69.3, 70.7, 72.1, 72.6, 73.8 (C-2,4–7), and 98.4 (C-3); *cf.* δ 98.5 for C-2 of α-D-sorbopyranose[19].

Isomerisation and dimerisation of D-*erythrose*. — (*a*) An aqueous solution of monomeric D-erythrose was concentrated to a syrup, which was dried in a vacuum desiccator overnight. Analysis of this syrup by p.c. (solvent *D*), with authentic **2** as reference, demonstrated that minor formation of octuloses had occurred. A similar syrup was stored in a glass vessel at 105° for 2.5 h, dissolved in water, and, at equilibrium (72 h), subjected to h.p.l.c. (Fig. 1) and to g.l.c.–m.s. (3% of OV-1, 185→200°, 2°/min), after conversion into alditol acetates, revealing that most of the D-erythrose had isomerised or been converted into octuloses, and that **4a** was not present.

(*b*) 2,4-*O*-Ethylidene-D-erythrose (0.12 g, m.p. 146–148°) was hydrolysed[5] in dilute sulphuric acid. After neutralisation with barium carbonate and filtration, samples of the filtrate (pH 7.2) were (*1*) analysed, without evaporation, for sugars as alditol acetates[23] by g.l.c.–m.s.; (*2*) concentrated to dryness, and heated at 105° for 2.5 h, and the product analysed as in (*1*); (*3*) treated with Dowex 50 (H^+) resin and filtered, and the resulting solution (pH 3.6) processed as in (*2*). The results showed that isomerisation and aldol condensation of D-erythrose to octuloses occurred in (*2*), but not in (*1*). Procedure (*3*) resulted in isomerisation (~10%) of D-erythrose, but also gave a number of minor components all having similar mass spectra. The preponderant component of the latter mixture, probably dimeric, tetrose-reversion products[2], exhibited (g.l.c.–m.s., OV-225, capillary column, 180→250°, 6°/min) peaks at *m/z*: 301 (0.04%), 289 (0.13), 231 (12), 217 (19), 187 (16), 158 (2), 145 (1), 129 (5), 127 (9), 116 (3), 115 (10), 103 (2), 86 (2), 85 (16), 69 (7), 61 (1), 55 (1), and 43 (100).

Syrupy D-erythrose, prepared as in procedure (*3*), but not heated, was kept at room temperature for 5 days and then analysed as in (*1*). No isomerisation, aldol condensation, or reversion of D-erythrose had occurred.

(*c*) An aqueous solution of monomeric D-erythrose, prepared as in (*a*), was concentrated to dryness, and the resulting syrup (0.6 g) was kept at 105° for 2.5 h, and then acetylated and processed essentially as in ref. 5. The resulting oil (1.05 g, 85%) was eluted from a column (90 × 2.5 cm) of silica gel with solvent *A*. Seven main fractions were collected (E = tri-*O*-acetyl-D-erythrofuranose).

Fraction	*Wt. (mg)*	*Major (minor) components*
I	120	β-E
II	60	**1a** (α-E)
III	60	**2a** (**1a**)
IV	140	**2a**
V	60	**2a** (**3a**)
VI	130	**3a**
VII	230	Not investigated

Further chromatography of II with solvent *B* gave **1a**, and **2a** was obtained likewise from IV. Crystallisation of VI from chloroform–light petroleum (b.p. 60–70°) gave **3a**. The triacetates of D-erythrose were identified by comparison (t.l.c.) with authentic samples[5].

1,3,4-Tri-*O*-acetyl-D-*glycero*-tetrulose (**1a**) was amorphous, $[\alpha]_{578}$ +27° (*c* 1.4, chloroform), R_F 0.55 (t.l.c., solvent *A*). Mass spectrum: *m/z* 187 (M^+ — OAc, 5%), 145 (9), 127 (6), 103 (15), 102 (4), 101 (5), 85 (14), 73 (3), and 43 (100).

Anal. Calc. for $C_8H_{11}O_5$ (M^+ — OAc): *m/z* 187.061. Found: *m/z* 187.061.

Compound **1a** was also prepared by adding acetic anhydride (15 ml) to the cooled reaction mixture obtained[15] by boiling, under reflux, a solution of D-erythrose (120 mg) in dry pyridine (15 ml) for 3 h under nitrogen. The resulting[5] syrup (108 mg) was eluted from a column (18 × 1 cm) of silica gel with solvent *B*, to give **1a** (12 mg) in a fraction that showed $[\alpha]_{578}$ +16° (*c* 1, chloroform) and also contained traces of α- and β-D-erythrose. An attempt to deacetylate[16] **1a** with saturated, aqueous barium hydroxide failed, since, on re-acetylation of the product, only acetylated, polymeric material was detected by ^{1}H-n.m.r. analysis.

1,2,3,4,5,7,8-Hepta-*O*-acetyl-β-D-*altro*-L-*glycero*-3-octulofuranose (**2a**) was amorphous, $[\alpha]_{578}$ +56° (*c* 2.1, chloroform), R_F 0.41 (solvent *A*). ^{13}C-N.m.r. data ($CDCl_3$): δ 61.5, 61.8 (C-1,8), 69.5, 70.1, 74.7, 77.6, 78.3 (C-2,4–7), and 107.3 (C-3). Mass spectrum: *m/z* 475 (M^+ — OAc, 4%, observed at 20 eV), 389 (3), 347 (6), 288 (4), 287 (30), 245 (4), 173 (9), 157 (4), 145 (7), 131 (7), 115 (7), 103 (10), 98 (5), 97 (4), and 43 (100).

Anal. Calc. for $C_{20}H_{27}O_{13}$ (M^+ — OAc): *m/z* 475.145. Found: *m/z* 475.146.

1,2,3,4,5,6,8-Hepta-*O*-acetyl-α-D-*gluco*-L-*glycero*-3-octulopyranose (**3a**) was hy-

groscopic and had m.p. 128–131° [from chloroform–light petroleum (b.p. 60–70°)], $[\alpha]_{578}$ +69° (*c* 0.41, chloroform), R_F 0.29 (solvent *A*). ^{13}C-N.m.r. data ($CDCl_3$): δ 62.0, 62.1 (C-1,8), 68.4, 68.9, 70.9, 71.5 (C-2,4–7), and 96.9 (C-3). Mass spectrum: *m/z* 475 (M^+ — OAc, 0.9%, observed at 20 eV), 347 (1), 287 (3), 173 (7), 157 (7), 131 (6), 115 (5), 103 (5), 98 (7), 97 (13), 46 (8), and 43 (100).

Anal. Calc. for $C_{22}H_{30}O_{15}$: C, 49.4; H, 5.6. Found: C, 49.2: H, 5.7.

D-altro-L-glycero-*3-Octulofuranose* (**2**). — Compound **2a** (100 mg) was treated with a 1:1 mixture (7 ml) of methanol and saturated, aqueous barium hydroxide at 4° for 1 h, and then deionised with Dowex 50 (H^+) resin. Preparative h.p.l.c. of the filtrate gave **2** (eluted 13 min after injection), which had $[\alpha]_{578}$ +8° (*c* 1, water). ^{13}C-N.m.r. data (D_2O): δ 61.7, 62.3 (C-1,8), 72.7, 74.2, 75.5, 75.8 (C-2,4–6), 80.3 (C-7), and 102.0 (C-3): *cf.* δ 102.6 for C-2 of β-D-fructofuranose[19]. G.l.c.–m.s. of **2** (after reduction with sodium borodeuteride and acetylation) showed (for the first-eluted octitol octa-acetate) peaks at *m/z* 506 (M^+ — CH_2OAc, 0.06%), 434 (2), 404 (1), 362 (1), 361 (1), 332 (3), 290 (1), 289 (1), 260 (2), 259 (2), 218 (4), 217 (4), 188 (2), 187 (2), 170 (7), 169 (3), 158 (6), 157 (5), 145 (9), 140 (7), 139 (5), 129 (2), 128 (3), 127 (2), 116 (7), 115 (7), 104 (1), 103 (6), 98 (3), 97 (2), 86 (2), 85 (2), 74 (1), 73 (3), and 43 (100).

Acetylation of **2** regenerated **2a**.

D-gluco-L-glycero-*3-Octulopyranose* (**3**). — Deacetylation of **3a**, as described for **2a**, yielded a chromatographically pure, but amorphous, compound that exhibited ^{13}C-n.m.r. data and h.p.l.c. retention in agreement with those of authentic **3**. G.l.c.–m.s. of the two diastereomeric octitol octa-acetates derived from **3** gave, for the first-eluted, *m/z* 519 (M^+ — OAc, 0.03%), 505 (1), 433 (1), 403 (2), 361 (1), 331 (1), 289 (9), 259 (4), 217 (5), 187 (18), 170 (7), 169 (6), 158 (2), 157 (11), 145 (7), 139 (11), 128 (7), 127 (6), 115 (14), 103 (5), 73 (3), and 43 (100).

Acetylation of **3** regenerated **3a**.

3,8-Anhydro-β-D-altro-L-glycero-*3-octulopyranose* (**4**). — A solution of **2** in water (5 ml) was heated with Dowex 50 (H^+) resin (2 ml) at 96° for 1 h, filtered, and analysed by h.p.l.c. Compound **4** (~70%) was eluted 11.6 min after injection and obtained by preparative h.p.l.c. as a chromatographically homogeneous syrup, $[\alpha]_{578}$ −88° (*c* 0.6, water). ^{13}C-N.m.r. data (D_2O): δ 61.6 (C-1), 66.1 (C-8), 69.7, 70.1, 70.5, 72.4 (C-2, 4, 6), 78.2 (C-7), and 107.6 (C-3).

Acetylation of **4** afforded amorphous 1,2,4,5,6-penta-*O*-acetyl-3,8-anhydro-β-D-*altro*-L-*glycero*-octulopyranose (**4a**), $[\alpha]_{578}$ −32° (*c* 0.55, chloroform), R_F 0.36 (solvent *A*). ^{13}C-N.m.r. data ($CDCl_3$): δ 62.1 (C-1), 66.8 (C-8), 68.5, 69.3, 70.8 (C-2, 4–6), 75.6 (C-7), and 105.8 (C-3). Mass spectrum: *m/z* 373 (M^+ — OAc, 0.9%), 271 (2), 217 (6), 173 (9), 145 (2), 141 (6), 131 (7), 115 (5), 103 (6), 69 (5), and 43 (100).

Anal. Calc. for $C_{16}H_{21}O_{11}$ (M^+ — OAc): *m/z* 373.113. Found: *m/z* 373.108.

Isomerisation of D-*erythrose 4-phosphate.* — Aqueous solutions of disodium D-erythrose 4-phosphate were (*1*) reduced with sodium borodeuteride (10 mg) overnight at room temperature; (*2*) concentrated to dryness, and a solution of the residue in water was reduced as in (*1*); (*3*) concentrated to dryness, and the residue was

heated at 80° for 15 min and then reduced as in (*1*). The excess of borohydride in each product was decomposed with acetic acid, and the boric acid present was removed as volatile methyl borate[26]. A solution of the residue in water (3 ml) was acidified (pH 5) with 0.2M hydrochloric acid. Acid, potato phosphatase (2 mg) was added[27] and the solution was kept at 37° overnight. The enzyme was then denatured by heating to 80°, the precipitate was removed, and the solvent was evaporated. The residue was reduced with sodium borodeuteride, and then acetylated and subjected to g.l.c.–m.s. The results revealed that D-erythrose 4-phosphate in (*3*) had isomerised significantly, as threitol tetra-acetate (40%) labelled with deuterium at C-1 or C-2 was identified by comparison with an unlabelled sample. No octitol octa-acetates were detected.

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