Carbohydrate Research, 77 (1979) 25–36

THE STRUCTURE OF D-*threo*-2,5-HEXODIULOSONIC ACID AND DERIVATIVES IN SOLUTION

GLENN C. ANDREWS*, BRADLEY E. BACON,
Central Research, Pfizer Inc., Groton, CT 06340 (U.S.A.)
JON BORDNER, AND GLEN L. A. HENNESSEE
North Carolina State University, Raleigh, NC 27607 (U.S.A.)

(Received March 16th, 1979; accepted for publication, May 29, 1979)

ABSTRACT

The structure of D-*threo*-2,5-hexodiulosonic acid (**1**) and various derivatives in solution was determined by ^{13}C-n.m.r. spectroscopy to be a hydrated, pyranose form. The structures of the methyl ester of **1** and of its 5-(dimethyl acetal) were confirmed by chemical means and by X-ray structure analysis.

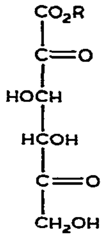
INTRODUCTION

D-*threo*-2,5-Hexodiulosonic acid (**1**), an oxidation product of D-glucose, is produced by various strains of *Acetobacter* and *Pseudomonas* bacteria[1]. It has assumed commercial importance in the synthesis of such γ-pyrone derivatives as maltol and ethylmaltol[2], as a potential intermediate in the synthesis of L-ascorbic acid[3], and as a useful intermediate for heterocyclic synthesis[4]. The unstable, diketo acid **1**, characterized chemically by Katznelson *et al.*[1a], Wakisaka[1b,5] and Bernaerts and DeLey[6], has been but little studied from the standpoint of its physical structure in solution, despite the obvious importance of structure in understanding the chemical reactivity of **1** and other hexodiulose derivatives. For these reasons, we report our findings on the chemistry and structure of **1** and its derivatives.

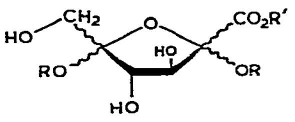
Several structures have been proposed for **1** in solution. Based primarily on (*a*) the lack of a ketone carbonyl absorption in the infrared (i.r.) spectrum of **1**, and (*b*) polarographic data, Bernaerts and DeLey suggested[6] the isomeric, furanose structures **2a**. Structure **3** was also proposed[4a], based on analogy with a proposed structure, **4**, of D-*threo*-2,5-hexodiulose[7]. The hydrated pyranose form **5a** and the hydrated, acyclic tautomer, **6**, are other possibilities. Several, analogous, structural models are mentioned in the literature. Both L-*xylo*-2-hexulosonic and D-*arabino*-2-hexulosonic acid have been shown to exist primarily in a pyranose form in aqueous solution[8]. D-*xylo*-5-Hexulosonic acid appears to exist, both in solution[8] and in the

---

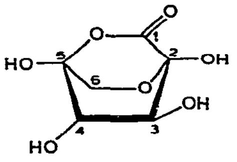
*To whom enquiries should be addressed.



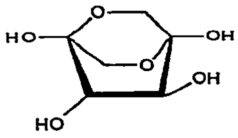
1 R = H
1a R = Ca$_{0.5}$
1b R = Na



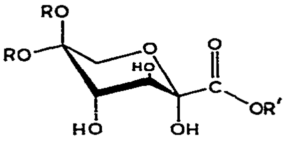
2a R = H, R′ = H
2b R = Me, R′ = Me



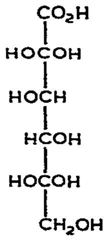
3



4



5a R = H, R′ = H
5b R = H, R′ = Me
5c R = Me, R′ = Me



6

crystalline[9] form, primarily as a furanoid structure. Thus, both pyranose and furanose forms are possible for **1**, depending on whether the 2- or the 5-ketone group exerts the dominant influence.

The calcium salt (**1a**) of **1**, isolated from shaken cultures of *Acetobacter fragum* by the method of Wakisaka[1b], was a light-yellow powder, $[\alpha]_D^{24}$ —52.5° (*c* 1, water); lit.[6] $[\alpha]_D^{24}$ —51.5° (*c* 1, water). Ion-exclusion chromatography on Dowex-50 (Ca^{2+})

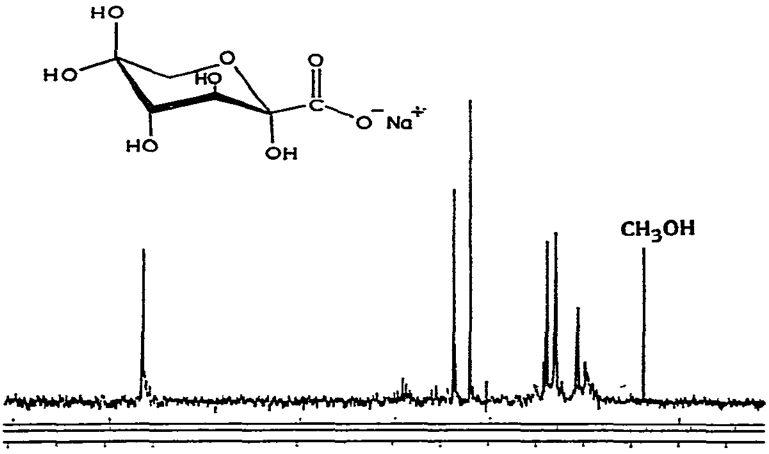


Fig. 1. ^{13}C-N.m.r. spectrum of **1b** in D$_2$O.

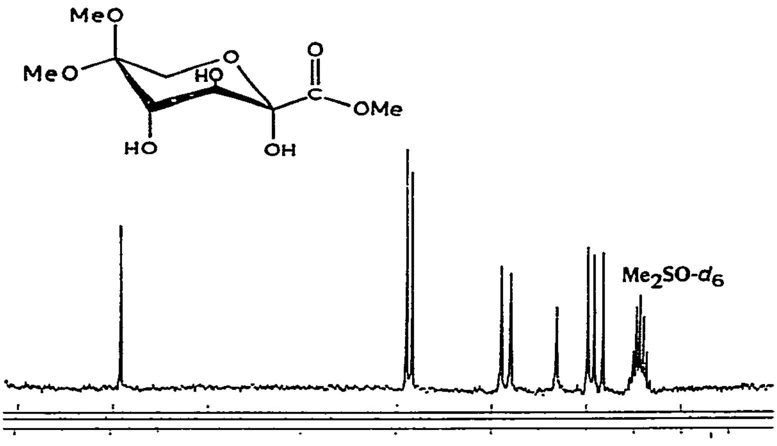


Fig. 2. ^{13}C-N.m.r. spectrum of **5c** in Me_2SO-d_6.

ion-exchange resin, with elution with water, afforded a homogeneous fraction that, on lyophilization, generated a white, amorphous calcium salt whose combustion analysis was consistent with the formula $(C_6H_7O_7)_2Ca \cdot 3\ H_2O$. Infrared analysis in aqueous solution and in the solid state showed no evidence for ketonic carbonyl. The ^{1}H-n.m.r. spectrum of **1a** in D_2O is a complex multiplet centered at 4.0 p.p.m. The ^{13}C-n.m.r. spectrum of **1b** in D_2O, shown in Fig. 1, suggested the presence of two anomeric centers (singlets at 97.84 and 97.45 p.p.m.) and three absorptions, for C-3, C-4, and C-6, at 74.36 (s), 72.19 (s), and 66.69 (d) p.p.m. The chemical shifts of C-3 and C-4 (74.36 and 72.19 p.p.m.) are at higher field than would be expected for a furanose structure (*e.g.*, *versus* 77.41 and 77.29 for C-3 and C-4 of D-*xylo*-5-hexulofuranosonic acid[8]), and are thus more consistent with the pyranose structure for **1b**.

When **1** in methanol is boiled under reflux for 48 h in the presence of Dowex-50 (H^+), a sulfonic acid, ion-exchange resin, the intermediate methyl ester **5b** initially formed is converted in 76% yield into the methyl ester dimethyl acetal **5c** having m.p. 120° (dec.), $[\alpha]_D^{23}$ —37.97° (*c* 1, water). Combustion analysis suggested the formula $C_9H_{16}O_8$ for **5c**. ^{1}H-N.m.r. spectroscopy of **5c** in $(CD_3)_2SO$ showed the presence of three methoxyl groups and three exchangeable protons (hydroxyl) including two doublets at 4.62 and a singlet at 6.55 p.p.m. (indicative of a hydrogen-bonded, tertiary hydroxyl group α to carbonyl[10]). An AB quartet centered at 3.48 p.p.m. (J = 9.8 Hz) is consistent with the *trans*-H-3,H-4 orientation for a pyranose system[11]. The i.r. spectrum contained a band for a single, unstrained, ester carbonyl (ν_{max}^{KBr} 1754 cm^{-1}). The ^{13}C-n.m.r. spectrum of **5c** (see Fig. 2 and Table I) shows a close similarity to that of **1b**, and suggests that they have similar structures in solution. Present are two anomeric carbon atoms, at 97.73 and 96.57 p.p.m. Isotope-shift experiments with H_2O–D_2O in Me_2SO-d_6 indicated a 35% increase in the width of the peak (at half-height) of the carbon atom at 96.57 p.p.m. relative to the absorption at 97.58, suggesting that the upfield, anomeric carbon atom bears a hydroxyl group,

TABLE I

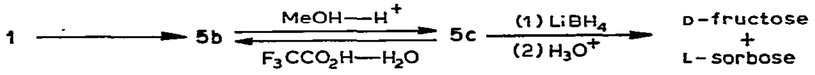
^{13}C-N.M.R. CHEMICAL SHIFTS[a] OF D-*threo*-2,5-HEXODIULOSONIC ACID AND DERIVATIVES

Compound	*C-1*	*C-2*[b]	*C-3*[c]	*C-4*[c]	*C-5*[b]	*C-6*
1a[d]	175.4	93.6	72.2	74.7	97.7	66.7
5b[d]	170.35	92.84	71.50	73.2	96.86	65.99
5c[e]	169.87	96.57	71.91	74.19	97.73	60.43
7a[f]	169.11	95.39	68.91	71.65	96.21	62.54
7b[f]	169.63	96.56	72.29	75.67	97.26	65.38
7c[f]	169.67	97.73	72.58	74.65	99.40	64.40
7c[f]	169.67	96.53	73.71	75.10	97.03	62.30

[a]In p.p.m. [b]Assignments based on those for **5c**. [c]Assignments may be interchanged. [d]Solvent, D_2O; internal standard, MeOH, adjusted to 49.3 p.p.m. [e]Solvent and standard Me_2SO-d_6 adjusted to 39.6 p.p.m. [f]Solvent and standard $CDCl_3$ adjusted to 76.9 p.p.m.

and, therefore, that the lower-field carbon atom is engaged as a methyl acetal[12]. Only structure **5c**, or its anomer, is consistent with these data.

Chemical verification of structure **5c** was achieved by acetylating the compound with acetic anhydride–pyridine, to give triacetate **7a** (m.p. 130–130.5°). The observed, downfield shift of only two proton resonances in the p.m.r. spectrum of **7a** excluded furanose structure **2b**, whose triacetate would afford a downfield shift of four proton resonances. In addition, the coupling constant (J = 10.0 Hz) for H-3,H-4 is similar to that of H-3,H-4 for D-fructopyranose[11]. Furthermore, reduction of **5c** with lithium borohydride, followed by acid hydrolysis, afforded a 51:49 mixture of D-fructose and L-sorbose (see Scheme 1). Hydrolysis of **5c** in 19:1 trifluoroacetic acid–water afforded an unstable, white, amorphous material, identical by analysis by high-performance liquid chromatography (h.p.l.c.) with both the intermediate observed in the formation of **5c** and the compound obtained by treatment of **1** with ethereal diazomethane. The ^{13}C-n.m.r. data (see Table I) were consistent with formulation as methyl ester **5b**; neither the unhydrated ketone nor the acyclic tautomer was detectable. Thus, esterification of **1** to afford **5b** is followed by formation of the dimethyl acetal **5c**, all three of which appear to exist exclusively in a pyranose form.



Scheme 1

The chemical and physical data for **5c** were confirmed by a single-crystal, X-ray structure analysis (see Table II). A stereoview of the molecule is given in Fig. 3. As with β-D-fructopyranose[13], L-sorbose[14], and L-*xylo*-2-hexulosonic acid[15] in their respective crystalline states, the anomeric hydroxyl group adopts the axial orientation.

TABLE II

CRYSTAL AND REFINEMENT PARAMETERS FOR COMPOUND **5c**

Crystal parameters		*Refinement parameters*	
Formula	$C_9H_{16}O_8$ (252.2)	Number of reflections	655
Crystal size	0.1 × 0.2 × 0.3 mm	Non-zero reflections	627
Cell dimensions	a = 681.5 (4) pm	R index	
	b = 688.6 (6)	($R = \Sigma\|\|F_0\| - \|F_0\|\|/\Sigma\|F_0\|$)	0.043
	c = 1199.6 (8)		
	β = 100.82°(5)		
	V = 553.00 (7)	Weighted R index	
Space group	$P2_1$	($R' = \Sigma w(F_0^2 - F_c^2)^2/\Sigma w F_0^4$)	0.0079
Molecules per unit cell	2	Final shifts	$<0.01\sigma$
Density observed	1.50 g/cm^3		
Density calculated	1.514 g/cm^3		
Linear absorption coefficient (μ)	1.45 cm^{-1}		

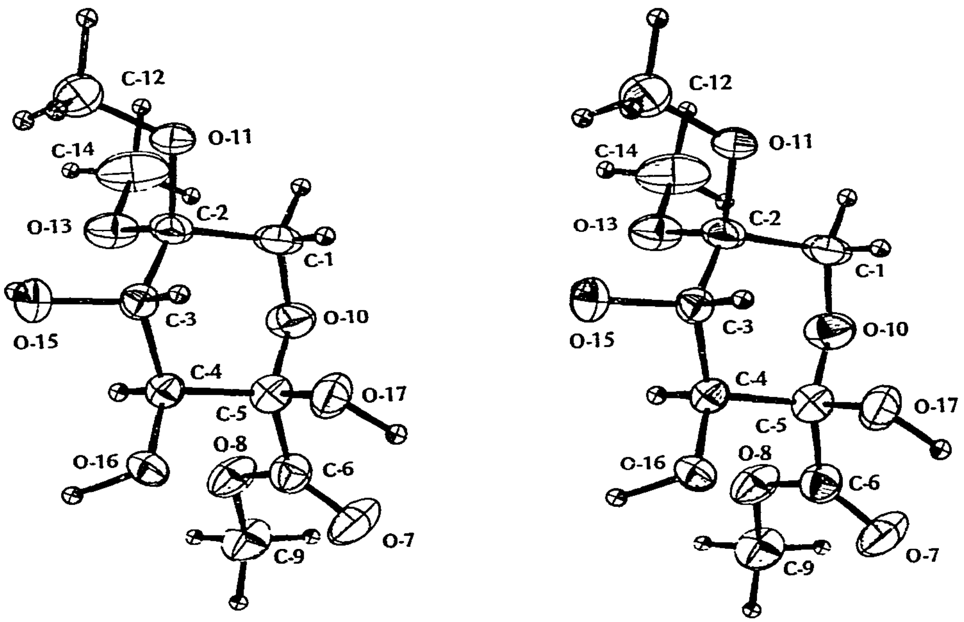


Fig. 3. Stereoview of a molecule of **5c**.

The methyl ester carbonyl and the anomeric hydroxyl groups are almost coplanar, the torsional angle of O-17–C-5–C-6–O-7 in Fig. 3 being ~6.6°. The α-anomeric, C–OH bond-length is 138 pm, and the combined effect of this and the adjacent ester carbonyl gives rise to a difference of 8 pm in the C–O bond-lengths in the pyranoid ring (C-5–O-10 = 143.4 pm, *versus* C-1–O-10 = 144.2 pm, see Table III). This difference is smaller than that observed for L-*xylo*-2-hexulosonic acid (a difference

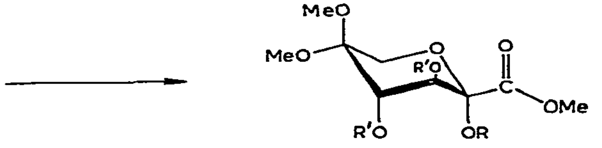
TABLE III

ATOMIC DISTANCES (pm) AND ANGLES (DEGREES) FOR **5c**

C–C	*Distances (pm)*	*C–C–C*	*Angles (degrees)*	*C–C–C*	*Angles (degrees)*
1– 2	153.0(9)	10–1– 2	110.0(5)	6– 5– 4	112.5(6)
1–10	144.2(8)	3–2– 1	109.2(7)	10– 5– 4	109.0(8)
2– 3	152.3(9)	11–2– 1	101.1(6)	17– 5– 4	108.2(6)
2–11	143.9(8)	13–2– 1	114.0(6)	10– 5– 6	104.4(5)
2–13	138.4(10)	11–2– 3	113.4(6)	17– 5– 6	111.0(8)
3– 4	154.0(10)	13–2– 3	107.8(6)	17– 5–10	111.9(6)
3–15	142.4(12)	13–2–11	111.4(7)	7– 6– 5	122.4(8)
4– 5	152.7(13)	4–3– 2	108.6(5)	8– 6– 5	112.5(9)
5– 6	152.9(11)	15–3– 4	107.7(5)	9– 8– 6	116.9(8)
5–10	143.4(8)	5–4– 3	111.7(6)	5–10– 1	111.9(5)
5–17	138.0(10)	16–4– 3	110.2(5)	12–11– 2	118.4(6)
6– 7	118.6(14)	16–4– 5	106.2(7)	14–13– 2	116.4(6)
6– 8	131.5(11)				
8– 9	144.1(10)				
11–12	144.0(14)				
13–14	141.8(9)				

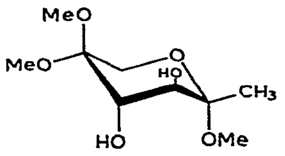
of 2.2 pm in the C–O bond-lengths in the pyranoid ring[15]) and fails to support the view of Hvoslef and Bergen[15] that the effect of carbonyl substitution at C-2 does not obey the empirical rule[16] that such a difference in bond lengths occurs only with substituted, anomeric hydroxyl groups. Interestingly, the triacetate **7a** and the 3,4-di-*O*-(trimethylsilyl) ether **7b** appear to be single anomers at C-2. However, the 3,4-di-*O*-(*tert*-butyldimethylsilyl) ether **7c** exists as a 2:3 mixture of anomers, as evidenced by ^{13}C-n.m.r. spectroscopy. It would appear that the equatorially attached methyl ester group is favored, except in the case of severe steric-interactions.

5c

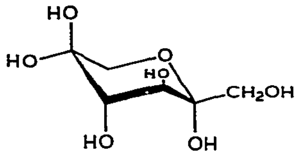


7a R = OAc , R' = OAc
7b R = H, R' = Me_3Si
7c R = H, R' = Me_3CMe_2Si

The structure of **1** in solution, being exclusively in the pyranose form, differs considerably from the reported structure of 1-deoxy-D-*threo*-2,5-hexodiulose (**8**), which exists in solution in water as a 1:1 mixture of acyclic and cyclic forms[17]. The methyl glycoside 5-(dimethyl acetal) of **8** is reported to adopt the α-D-pyranoid structure **9**, having an axial glycosidic methoxyl group[17]. Also, the structure of D-*threo*-2,5-hexodiulose is reported[18] to be the pyranose **10**. Thus, the series of known 2,5-hexodiuloses all appear to exist to a great extent in the ${}^5C_2$ conformation, with the C-2 substituent (containing C-1) in the equatorial disposition. An understanding of the structure of this series of hexodiuloses in solution is fundamental to prediction and exploitation of the chemical reactivity of these compounds.



9



10

EXPERIMENTAL

General methods. — The ^{13}C-n.m.r. spectra were recorded in the fast, Fourier-transform (fFt) mode with a Varian XL-100 A-15 (25.2 MHz) spectrometer equipped with a Nicolet technology 1080 data system. Complete proton-decoupling was provided by square wave-modulation[19] of the Varian gyrocode, heteronuclear decoupler. The spectra were obtained with a tip angle of ~30° and an acquisition time of 1.4 s, using quadrature phase-detection. The upfield frequency-lock was maintained by the deuterium resonance of the solvent in a 5-mm (o.d.) sample-tube. Methanol was used as the internal standard, adjusted[20] to 49.3 p.p.m. to provide chemical-shift values relative to tetramethylsilane.

The ^{1}H-n.m.r. spectra were recorded with a Varian Associates Model EM-360-L spectrometer. Chemical shifts are reported on the δ scale, relative to an internal standard of tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-1-sulfonate. Infrared spectra were recorded with a Perkin–Elmer model 727B spectrometer. Mass-spectral data were obtained with an MS-30 instrument having a PS-50 data system.

H.p.l.c. was performed in a column (4 mm i.d. × 1 m) packed with Aminex A-25 resin (formate) thermostated at 55° (refractive index detector), at 2000 lb.in.$^{-2}$. The mobile phase used was 0.5M ammonium formate at pH 5.5. Melting points are uncorrected.

Calcium D-threo-*2,5-hexodiulosonate* (**1a**). — Fermentations based on the work of Oga and co-workers[1c] using *Acetobacter fragum* on D-glucose were used, to obtain 10–15% (w/v) broths of **1** in 85–87% yield, based on D-glucose. The broths were clarified and freeze-dried, and the freeze-dried solids were subjected to ion-exclusion chromatography on Dowex-50 X-8 (Ca^{2+}) resin. Elution with water afforded fractions homogeneous by h.p.l.c. analysis. The purified, aqueous fractions were freeze-dried, to afford a white amorphous material; $[\alpha]_D^{23}$ −52.5° (*c* 1, water); lit.[1b] $[\alpha]_D^{23}$ −51.5° (*c* 1, water); ν_{max}^{KBr} 3330 (s, OH), 1623 (s, CO_2^-), and 1418 cm^{-1} (m, C-O).

Anal. Calc. for $(C_6H_7O_7)_2Ca \cdot 3\ H_2O$: C, 30.25; H, 4.24. Found: C, 30.18; H, 4.25.

Methyl D-threo-*2,5-hexodiulosonate 5-(dimethyl acetal)* (**5c**). — To a 1-L, 3-necked, round-bottomed flask equipped with a mechanical stirrer and an ice–water cooling-bath were added 81.2 g (0.30 mol) of sodium D-*threo*-2,5-hexodiulosonate, anhydrous methanol (500 mL), and concentrated sulfuric acid (1.23 mL). After being stirred for 1 h, sodium sulfate was removed by filtration, and the filtrate was transferred to a 2-L, 3-necked, round-bottomed flask equipped with a reflux condenser, a distillation head, and a mechanical stirrer and containing 2,2-dimethoxypropane[21] (100 mL). The mixture was boiled under reflux, with slow distillation of solvent, for 36 h. During this time, a total of 500 mL of distillate was removed at ~64° (head temperature), and 4:1 (v/v) methanol–2,2-dimethoxypropane (500 mL) was introduced into the reaction vessel. At this time, h.p.l.c. analysis indicated the presence of three products, namely, **5b** (26 min), **5c** (14 min), and an unknown product (20 min) in the ratios of 78:9:13. The mixture was cooled, the acid neutralized by the addition of sodium hydrogencarbonate (37.2 g), and the precipitated sodium sulfate removed by filtration, affording an orange filtrate (650 mL) which was passed through Amberlyst A-21 tertiary amine resin (250 mL). The eluate was evaporated under diminished pressure, to afford an oil that crystallized; yield 24.8 g (32.5%) of **5c**; m.p. 170–172°, $[\alpha]_D^{23}$ +37.97° (*c* 1, water); ν_{max}^{KBr} 1754 (s, C=O) and 333 cm^{-1} (s, OH); ^{1}H-n.m.r. (Me_2SO-d_6): δ 6.55 (s, 1 H, O*H*), 4.64 (2 d, 2 H, O*H*), 3.68 (s, 3 H, C*H*$_3$-O-CO), 3.33 and 3.22 [2 s, 6 H, $(CH_3O)_2$-], and 3.52 (AB q, *J* 9.8 Hz, -C*H*OH -C*H*OH-); *m/e* (70 eV): 203, 191, 175, 161, 157, 143, and 133.

Anal. Calc. for $C_9H_{16}O_8$: C, 42.86; H, 6.39. Found: C, 43.13; H, 6.09.

Second and third crops from the mother liquor afforded an additional 23.1 g of **5c**; total, 47.9 g (62.9%).

Methyl D-threo-*2,5-hexodiulosonate* (**5b**). — To a 25-mL, round-bottomed flask containing 19:1 (v/v) trifluoroacetic acid–water (10.5 mL) was added **5c** (500 mg, 1.98 mmol). The solution was stirred under a nitrogen atmosphere for 5 min at room temperature, and the solvent was then evaporated under vacuum, to afford 443 mg (99%) of a white solid, homogenous by h.p.l.c. analysis, and identical to the intermediate initially formed in the esterification of **1** already described: ν_{max}^{KBr} 3330 (OH) and 1736 cm^{-1} (s, Me ester); ^{1}H-n.m.r. (D_2O): δ 3.21 (s, 3 H).

*Methyl 2,3,4-tri-O-acetyl-*D-threo-*2,5-hexodiulosonate 5-(dimethyl acetal)* (**7a**). — To a solution of **5c** (1.0 g, 3.97 mmol) in pyridine (30 mL) at room temperature was added acetic anhydride (0.8 mL). The solution was kept overnight, and evaporated to dryness *in vacuo*; the residue was dissolved in ether, and the solution was successively washed with 10%, aqueous $Cu(OAc)_2$ solution and brine, dried (anhydrous, granular sodium sulfate), and evaporated *in vacuo*, to afford an oil that was chromatographed by preparative, thin-layer chromatography on neutral alumina (7:3 ethyl acetate–hexane), to afford 610 mg (1.61 mmol, 41%) of crystalline **7a**; m.p. 128–130°; ν_{max}^{KBr} 1742 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 5.29 (AB q, *J* 12.5 Hz, 2 H), 3.66 (s, 3 H,

TABLE IV

NON-HYDROGEN ATOM PARAMETERS[a] AND THEIR STANDARD DEVIATIONS[b] FOR **5c**

	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C-1	4671(9)	9025(0)	2485(6)	21(4)	46(5)	57(5)	3(4)	9(4)	−16(4)
C-2	3935(9)	7004(13)	2070(6)	24(4)	37(5)	37(4)	0(4)	12(3)	−10(4)
C-3	1899(9)	7185(14)	1295(5)	32(4)	24(5)	28(4)	−2(4)	12(3)	0(3)
C-4	487(9)	8288(14)	1935(5)	27(4)	24(4)	30(4)	2(4)	6(3)	2(4)
C-5	1368(9)	10237(15)	2387(5)	35(4)	31(5)	29(4)	1(4)	6(4)	−1(4)
C-6	132(10)	11224(16)	3165(6)	41(5)	34(5)	37(5)	0(4)	9(4)	2(4)
O-7	−433(8)	12850(13)	3026(5)	76(4)	33(4)	64(4)	19(3)	36(3)	10(3)
O-8	−227(7)	10069(13)	3976(4)	64(3)	36(3)	36(3)	13(3)	22(3)	−1(3)
C-9	−1337(10)	10880(16)	4776(6)	63(5)	49(6)	43(4)	7(4)	30(4)	−5(4)
O-10	3277(6)	9908(13)	3099(4)	30(3)	45(4)	49(3)	4(3)	−4(2)	−17(3)
O-11	5493(7)	6404(12)	1485(4)	31(3)	34(3)	56(3)	2(3)	25(3)	−5(3)
C-12	5449(11)	4449(17)	1055(7)	49(5)	46(6)	66(6)	−4(4)	26(4)	−25(5)
O-13	3743(6)	5725(13)	2933(4)	37(3)	50(4)	34(3)	12(3)	7(2)	14(3)
C-14	5466(11)	5440(19)	3785(6)	50(5)	99(8)	42(5)	34(6)	−2(4)	10(6)
O-15	1027(6)	5339(12)	980(3)	40(3)	26(3)	29(3)	−6(3)	3(2)	−2(3)
O-16	−1337(6)	8718(11)	1191(3)	21(3)	33(3)	43(3)	−3(2)	1(2)	2(3)
O-17	1516(7)	11402(12)	1471(4)	51(3)	25(3)	48(3)	2(3)	22(3)	2(3)

[a] The values have been multiplied by 10^4. [b] The temperature factor is in the form: $T = \exp\{-2\pi^2(U_{11}h^2a^{*2} + \ldots + 2U_{23}klb^*c^*)\}$.

TABLE V

HYDROGEN COORDINATES[a] FOR **5c**

Atom	x/a	y/b	z/c	*Atom bearing H*
H-18	6159	8924	3008	1
H-19	4805	9920	1739	1
H-20	2103	7958	536	3
H-21	270	7336	2624	4
H-22	802	5108	40	15
H-23	−2441	7580	1272	16
H-24	1333	12879	1772	17
H-25	−613	12128	5206	9
H-26	−1578	9753	5409	9
H-27	−2855	11299	4357	9
H-28	4645	4412	209	12
H-29	4773	3491	1607	12
H-30	7020	3982	1079	12
H-31	6755	5109	3453	14
H-32	5203	4081	4306	14
H-33	5660	6662	4407	14

[a] The values have been multiplied by 10^4. The temperature factors were fixed.

methyl ester), 3.35 and 3.24 (2 s, 6 H), 2.14, 2.03, 1.97 (3 s, 9 H); mass spectrum (70 eV): *m/e* 347.0991 (Calc. for parent — CH_3O: 347.0992).

Anal. Calc. for $C_{15}H_{22}O_{11}$: C, 47.60; H, 5.86. Found: C, 47.87; H, 5.79.

Methyl D-threo-*3,4-di-*O-*(trimethylsilyl)-2,5-hexodiulosonate 5-(dimethyl acetal)* (**7b**). — To a stirred solution of **5c** (1.0 g, 3.97 mmol) in pyridine (15 mL) at 0° were added hexamethyldisilazane (2 mL) and chlorotrimethylsilane (1 mL). After being stirred for 1 h, the mixture was poured into water (100 mL), and extracted with ether (50 mL). The extract was successively washed with 5% aqueous copper sulfate, water, and brine, dried (anhydrous K_2CO_3), and evaporated *in vacuo*, to afford 1.17 g (91%) of crystalline **7b**: m.p. 133–134°; ν_{max}^{film} 1752 (s) and 3490 cm^{-1} (s, OH); ^{1}H-n.m.r. ($CDCl_3$): δ 3.83 (s, 3 H, methyl ester), 3.49 and 3.21 (2 s, 6 H), 0.12 and 0.09 (2 s, 18 H); mass spectrum (70 eV): *m/e* 381.1425 (parent — CH_3; calc., 381.1427), 365.1474 (parent — CH_3O; calc., 365.1476), and 335.1391 (parent — $C_2H_3O_2$; calc., 335.1396).

Methyl D-threo-*3,4-di-*O-(tert-*butyldimethylsilyl)-2,5-hexodiulosonate 5-(dimethyl acetal)* (**7c**). — To **5c** (1.0 g, 3.97 mmol) in dry *N,N*-dimethylformamide (3 mL) were added *tert*-butylchlorodimethylsilane (3.59 g, 23.8 mmol) and imidazole[22] (3.24 g, 47.6 mmol). After an additional 1 mL of *N,N*-dimethylformamide had been added, the mixture was warmed under a nitrogen atmosphere, overnight at 37°. The mixture was cooled to room temperature, and extracted with ether (30 mL), and the extract was successively washed with water and brine, dried (anhydrous K_2CO_3), and evaporated *in vacuo*, to afford 2.05 g of an oil, 1.2 g of which was chromatographed on 50 g of SiO_2. Elution with 1:9 ether–benzene yielded a single compound (homogeneous by t.l.c. in the same system), which solidified on evaporation of the eluate. Molecular distillation of the residue gave 426 mg (39%) of a waxy oil, b.p. 140°/0.01 mm Hg; ν_{max}^{film} 3598 (s, OH), 1768, and 1747 cm^{-1} (s, C=O); ^{1}H-n.m.r. ($CDCl_3$): δ 3.62 and 3.56 (2 s, 3 H, ratio of 47:53, methyl esters), 3.37 and 3.35 (2 s, 3 H, ratio of 47:53, axial methyl acetal), and 3.21 and 3.24 (2 s, 3 H, equatorial methyl acetal); mass spectrum (70 eV): *m/e* 465.2253 (parent — CH_3; calc. 465.2340), 423.1901 (parent — C_4H_9; calc. 423.1870), and 421.2372 (parent — CO_2CH_3; calc. 421.2376). ^{13}C-N.m.r. spectroscopy suggested a 2:3 mixture of the anomers of **7c**.

Anal. Calc. for $C_{21}H_{44}O_2Si_2$: C, 52.47; H, 9.23. Found C, 52.31; H, 9.09.

X-Ray analysis of **5c**. — Suitable crystals of **5c** were grown from water. The crystals were surveyed, and a 150-pm intensity data set (maximum sin θ/λ = 0.5) was obtained with a Syntex P$\bar{1}$ diffractomer, using molybdenum radiation (λ = 71.069 pm) at room temperature. The crystal density was measured in aqueous KI by the flotation technique. Final, unit-cell dimensions were obtained by using 15 high-angle reflections ($2\theta > 20°$). The diffractometer was equipped with a graphite, incident-beam monochromator mounted in the perpendicular mode. During data collection, a θ — 2 θ scan-technique was employed, the scan rate was 2° per min in 2 θ, the scan range was 1.2° below Kα_1 and 1.2° above Kα_2, and the background was counted for half the scan time on each side of the peak. A single, check reflection was monitored every 30 reflections, and indicated no crystal damage or slippage, because it was reproducible within counting statistics.

The diffractometer output was processed by using subprograms of the CRYM crystallographic computer system[23]. The processing included corrections for background, Lorentz, and polarization effects. The polarization effect due to the graphite monochromator was included in these corrections[24]. No corrections were made for absorption. The data processing also included calculation, for each reflection, of the F^2 value and its standard deviation. The standard deviations were assigned on the basis of the equation: $\sigma^2(\mathrm{I}) + \mathrm{S} + \alpha^2(B_1 + B_2) + (dS)^2$, where S is the scan count, B_1 and B_2 are the background counts, d is an empirical constant equal to 0.02, and α is the ratio of the scan time to the total, background time. All intensities less than twice their standard deviation were set equal to zero, with zero weight. The data set was placed on an approximately absolute scale by means of Wilson statistics. Crystal and refinement parameters are summarized in Table II.

Determination of structure, and refinement. — A trial structure was obtained by using the MULTAN Program of Germain and co-workers[25]. This trial structure refined routinely to an acceptable R index (see Table II). The later stages of the refinement included a full-matrix, least-squares treatment of all non-hydrogen coordinates, anisotropic temperature-factors, and scale factor in one matrix. Methylene and methine hydrogen positions were calculated. Methyl and hydroxyl hydrogen positions were located by difference-Fourier techniques.

Although hydrogen parameters were added to the calculations of structure factors in the final stages of refinement, they were not refined. The quantity minimized in the least-squares procedures was $\Sigma w(F_o^2 - F_c^2)^2$, where $w = 1/\sigma^2(F_o^2)$. A final, difference-Fourier treatment revealed no missing, or misplaced, electron density. A stereoplot of the molecule is given in Fig. 3. Other, pertinent, crystallographic parameters may be obtained from one of the authors (Bordner).

REFERENCES

1 (a) H. KATZNELSON, S. W. TANENBAUM, AND E. L. TATUM, *J. Biol. Chem.*, 240 (1953) 43–59; (b) Y. WAKISAKA, *Agric. Biol. Chem.*, 28 (1964) 819–827; (c) S. OGA, K. SATO, K. IMADA, AND K. ASANO, Jpn. Pat. 45-24391 (1970); (d) K. ASANO, K. IMADA, AND K. SATO, U.S. Pat. 3,654,316 (1972).

2 K. HAGASHI, M. KAWASE, AND H. UEDA, Jpn. Pat. 11,586 (1967); I. ICHIMOTO, K. FUJII, AND C. PATSUMI, *Agric. Biol. Chem.*, 29 (1965) 325–330.

3 T. SONOYEMA, H. TANI, B. KAGEYAMA, K. KOBAYASHI, T. HONJO, AND S. YAGI, U.S. Pat. 3,998,697 (1976); M. KUHLANEK, *Adv. Appl. Microbiol.*, 12 (1976) 11–33.

4 (a) K. IMADA AND K. ASANO, *Chem. Pharm. Bull.*, 21 (1973) 796; (b) K. IMADA, *ibid.*, 22 (1974) 1732–1738; *J. Chem. Soc., Chem. Commun.*, (1973) 796–797.

5 Y. WAKISAKA, *Agric. Biol. Chem.*, 28 (1964) 369–374.

6 M. BERNAERTS AND J. DELEY, *Antonie van Leeuwenhoek J. Microbiol. Serol.*, 37 (1971) 185–195.

7 S. ENGLARD, G. AVIGAD, AND L. PROSKY, *J. Biol. Chem.*, 240 (1965) 2302–2307.

8 G. C. ANDREWS AND T. C. CRAWFORD, unpublished results.

9 A. A. BALCHIN AND C. H. CARLISLE, *Acta Crystallogr.*, 19 (1965) 103–111.

10 O. L. CHAPMAN AND R. W. KING, *J. Am. Chem. Soc.*, 86 (1964) 1256–1258.

11 S. J. ANGYAL, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, Vol. 1A, Academic Press, New York, 1972, p. 200.

12 D. Gagnaire and M. Vincendon, *J. Chem. Soc., Chem. Commun.*, (1977) 509.

13 R. D. Rosenstein, *Abstr. Am. Crystallogr. Assoc. Meet.*, Buffalo, N.Y., (1968) 92. [Taken from *Adv. Carbohydr. Chem. Biochem.*, 31 (1975) 365.]

14 S. H. Kim and R. D. Rosenstein, *Acta Crystallogr.*, 22 (1967) 648–656.

15 J. Hvoslef and B. Bergen, *Acta Crystallogr. Sect. B*, 31 (1975) 697–702.

16 J. H. Robertson and B. Sheldrick, *Acta Crystallogr.*, 19 (1965) 820–826; S. S. Chu and G. A. Jeffrey, *ibid.*, 23 (1967) 1038–1040; S. H. Kim and G. A. Jeffrey, *ibid.*, 22 (1967) 537–545.

17 M. Dizdaroglu, J. Leitch, and C. von Sonntag, *Carbohydr. Res.*, 47 (1976) 15–23.

18 G. C. Andrews and B. E. Bacon, unpublished results.

19 J. B. Grutzner and R. E. Santini, *J. Magn. Reson.*, 19 (1975) 173–187.

20 G. C. Levy and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*, Wiley–Interscience, New York, 1972.

21 N. B. Lorette and W. L. Howard, *J. Org. Chem.*, 25 (1960) 521–525.

22 E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, 94 (1972) 6190–6191.

23 D. J. Duchamp, *Am. Crystallogr. Assoc. Meet.*, Bozeman, Montana, (1964) Paper B-14, p. 29.

24 L. V. Azaroff, *Acta Crystallogr.*, 8 (1955) 701–704.

25 G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr. Sect. A*, 27 (1971) 368–376.