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SYNTHESIS AND PROPERTIES OF 3,6-DIDEOXYHEXULOSONIC ACIDS AND RELATED COMPOUNDS

A CONVENIENT PREPARATION OF 3-DEOXY-D-*erythro*-HEXULOSONIC ACID (3-DEOXY-2-KETO-D-GLUCONIC ACID)* STEREOSELECTIVITY OF NUCLEOPHILIC ADDITION TO TRIOSE CARBONYL

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ABSTRACT

Synthesis and characterization of the epimeric 3,6-dideoxy-D(and L)-hexulosonic acids are described The configuration of each epimer has been established by measurements of rate of periodate oxidation, and each assignment agrees with that determined by enzyme-specificity studies The n.m.r. spectra of the epimeric 3,6-dideoxyhexulosonic acids and that of 3-deoxy-DL-*glycero*-pentulosonic acid show that each compound exists in solution mainly as the 2,5-hemiacetal. A convenient preparation of the bacterial metabolite 3-deoxy-D-*erythro*-hexulosonic acid ("3-deoxy-2-keto-D-gluconic" acid) is reported The stereospecificity of the aldol reactions employed in these syntheses is discussed

INTRODUCTION

In the course of studies on the stereochemistry of the enzymic conversion of 3-deoxy-L-*glycero*-pentulosonate into 2,5-dioxopentanoate[1], it was found necessary to have on hand all four stereoisomers of 3,6-dideoxyhexulosonic acid Syntheses of the related compounds, *N*-acetylneuraminic acid and 3-deoxy-D-octulosonic acids, have already been performed by Cornforth *et al*[2] and Heath[3], respectively Their method, which involves aldol reaction of oxalacetic acid with the appropriate aldehyde (see Scheme I), has been extended and modified for the preparation of potassium 3-deoxy-DL-*glycero*-pentulosonate[4] at neutral pH The successful application of this modified procedure to the synthesis of the 3,6-dideoxyhexulosonic acids is now described. At the same time, it was noted that no simple method is at present available for the preparation of 3-deoxy-D-*erythro*-hexulosonic acid ("3-deoxy-2-keto-D-gluconic" acid), a well known, bacterial metabolite[5]. A convenient synthesis of this compound, by a similar method, is therefore reported here

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It was also of general interest to determine the ratios of epimers arising in these reactions, and to attempt rationalization of the results by consideration of the relevant steric and polar effects

RESULTS AND DISCUSSION

Oxalacetic acid was condensed with D-, L-, or DL-lactaldehyde under neutral, aqueous conditions[4], and the resultant mixture, containing pyruvate (arising from the decarboxylation of oxalacetate) and a pair of epimeric 3,6-dideoxyhexulosonates, was fractionated on Dowex-1 (formate) resin Under the conditions used, pyruvic acid remained adsorbed on the resin, whereas the epimeric 3,6-dideoxyhexulosonates were eluted as clearly separated peaks The pure epimers **1** and **2** were isolated as the potassium salts, and were shown to be chromatographically homogenous in four solvent systems (see Table I) Specific rotations for both pairs of enantiomorphs are recorded

TABLE I

CHROMATOGRAPHIC DATA FOR POTASSIUM 3,6-DIDEOXYHEXULOSONATES

	R_F[a]	R_F[b]	R_F[c]	R_F[d]
Isomer **1** (*erythro*)	0 25	0 53	0 72	0 78
Isomer **2** (*threo*)	0 30	0 55	0 72	0 78

[a]6 4 3 (v/v) butyl alcohol–pyridine–water [b]3 1 (v/v) propyl alcohol–0 2M NH_4OH [c]70 15 1 (v/v) *tert*-butyl alcohol–formic acid–water [d]6 3 1 (v/v) propyl alcohol–formic acid–water

TABLE II

OPTICAL ROTATORY DATA[a] FOR POTASSIUM 3,6-DIDEOXYHEXULOSONATES

Sample	*Concentration (g/100 ml)*	$[\alpha]^{22}_{437}$, *degrees*	$[\alpha]^{22}_{589}$, *degrees*
1a (*erythro*)	7 32	−21 4	−11 5
1b	5 42	+22 0	+11 3
2a (*threo*)	5 00	+41,4	+18 2
2b	4 10	+39 2	−17 1

[a]Length = 1 dm, solvent, H_2O; pH, 7 5

in Table II Solutions of either isomer in 2M hydrochloric acid showed a slow development of a pH-dependent, u v absorption At low pH (< 7.5), the spectra showed a broad band at λ_{max} 232 nm ($\varepsilon \sim 4000$), which, at high pH (> 7.5), shifted to λ_{max} = 265 nm ($\varepsilon \sim 3600$). This behavior is attributed to the presence of the chromophore C=C(OH)C=O arising by lactonization of the original free acid, and is similar to that of 4-hydroxy-2-oxobutyro-1,4-lactone, which absorbs at 226 nm

(enol, ε 4000) and 261 nm (enolate anion, ε 2600)[6] It seems likely that the 1,4- (not the 1,5) lactone is formed, as only in the former would the presence of a 1,2-dicarbonyl grouping in the ring favor the formation at equilibrium of the conjugated enol[7]

The compounds were characterized further by preparation of the crystalline semicarbazones and, in the case of isomer **2**, of the (2,4-dinitrophenyl)hydrazone All of these derivatives gave correct analyses for a dehydrated species, assumed to be the 1,4- or 1,5-lactone that would be expected to be formed under the acidic conditions of the preparation Isomers **1** and **2** were both shown to exist in equilibrium with the 2,5-hemiacetal forms, as evidenced by their observed mutarotation and their i r. spectra (weak C=O absorption at 1705 cm^{-1}); the n m r spectra in deuterium oxide supported this conclusion In order to obtain unequivocal peak-assignments, the spectrum of 3-deoxy-DL-*glycero*-pentulosonate **3a** + **3b** (K^+ salt) was first examined The spectrum [see Fig 1(a)] showed peaks centered at τ 5 5 (1 proton), τ 6 0 (2 protons),

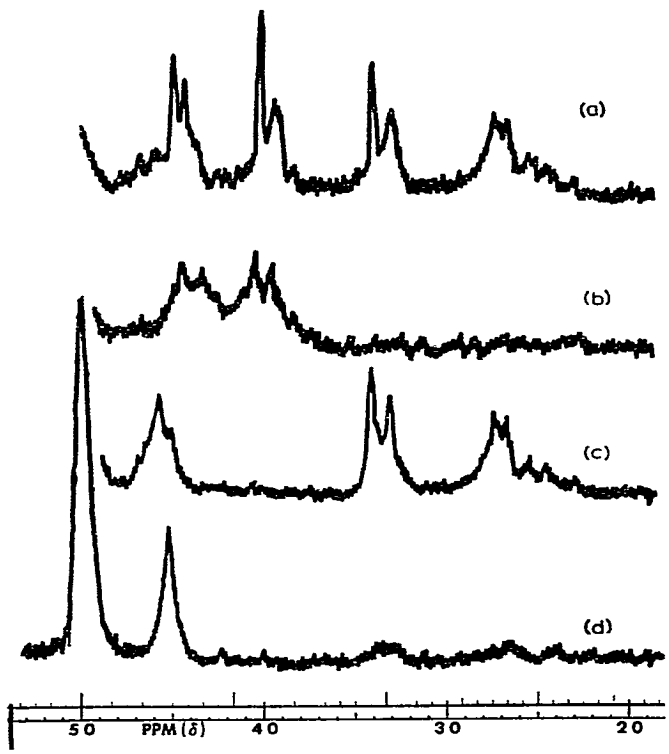


Fig 1 N m r spectra of 3-deoxy-DL-*glycero*-pentulosonate and deuterated analogs See text for explanation

τ 6 6 (1 proton), and τ 7 3 (1 0 proton) The peaks at τ 6 6 and τ 7 3 gradually diminished in intensity ($t_{1/2} \sim 3$ days at 25°), but the ratio of their intensities remained constant [see Fig 1 (b)] This loss of absorption was reversible, by equilibration of the sample with water, the peaks reappeared. Therefore, these peaks were assigned to the exchangeable protons at C-3, and the spectrum observed was rationalized in terms of an equilibrium

between the acyclic and the 2,5-hemiacetal forms On the basis of the chemical shifts observed[8], the band at τ 6 6 was assigned to H-3 of the acyclic form, and that at τ 7.3 was assigned to those of the cyclic hemiacetal As expected, the splitting patterns of both peaks are not simple The ratio of absorption intensities at τ 7 3 and τ 6 6 indicated a *ca* 1 1 ratio for the cyclic–acyclic equilibrium The peaks centered at τ 5 5 and τ 6 0 were assigned to H-4 and H-5, respectively, on the basis of their expected chemical shifts (τ secondary > τ primary) and the assumption that the chemical shift of each proton is not greatly affected by change from the acyclic to the cyclic form These assignments were confirmed by examination of the spectrum of 3-deoxy-DL-*glycero*-pentulosonate-*5-d*$_2$ [see Fig 1(c)], which showed absorption bands at τ 5 5, τ 6 6, and τ 7 3, but no absorption at τ 6 0 As noted previously, the peaks at τ 7 3 and τ 6 6 disappeared slowly [see Fig 1(d)], thus confirming the assignment of the remaining peak (at τ 5 5) to H-4.

The n m r spectrum of 3,6-dideoxy-DL-*erythro*-hexulosonate is shown in Fig 2; the spectrum of the *threo* isomer was very similar. By analogy with the spectrum of 3-deoxy-DL-*glycero*-pentulosonate, the following assignments were made τ 5 5, H-4 and H-5, τ 6 7, H-3 (acyclic, apparent doublet), τ 7 4, H-3 (cyclic form, apparent ABX system), τ 8 4, terminal methyl group. The peaks at τ 6 7 and τ 7 4 both diminished slowly by exchange with solvent deuterium, confirming their assignment to the α-ketonic protons. From the ratio of intensities of the H-3 peaks, it was estimated that the 3,6-dideoxyhexulosonic acids exist to about 85% as the cyclic hemiacetals

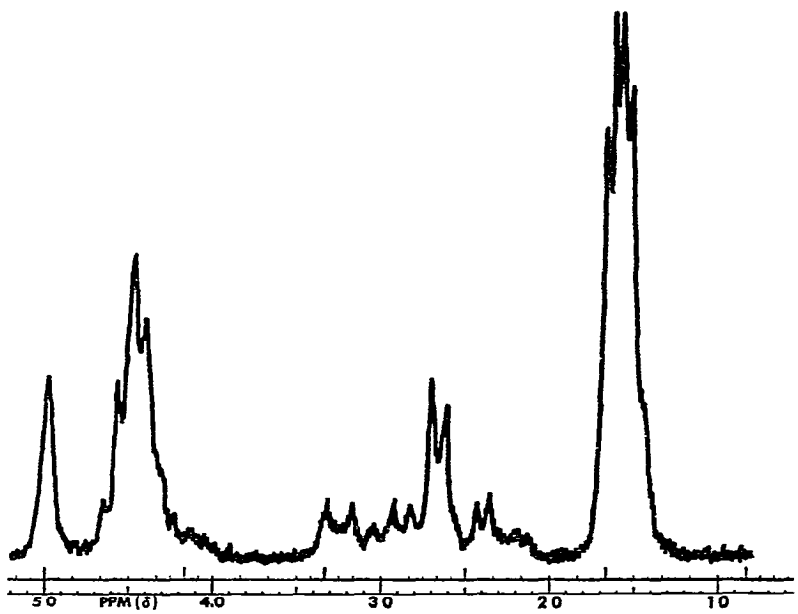


Fig 2 N m r spectrum of 3,6-dideoxy-DL-*erythro*-hexulosonate

The configurations of isomers **1** and **2** were determined from the results of periodate-oxidation studies The relative rates of periodate cleavage of a number of

pairs of diastereoisomeric 1,2-diols have been measured, and it has been clearly shown that the *threo* isomer is oxidized at a rate greater than that of the corresponding *erythro* isomer[9]. This method was therefore applied in the present study. In order to avoid any complication due to the existence of 3,6-dideoxyhexulosonic acids as cyclic hemiacetals, both isomers were oxidatively decarboxylated with hydrogen peroxide to the corresponding, acyclic 3,4-dihydroxypentanoic acids The relative rates of periodate oxidation of these diols were measured spectrophotometrically by the loss of absorption at 223 nm (periodate ion), the results are shown in Table III

TABLE III

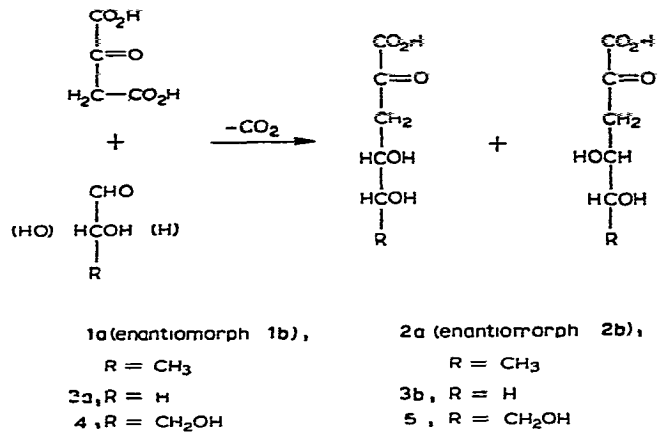
PERIODATE OXIDATION OF 3,6-DIDEOXYHEXULOSONATES

Isomer	*Molarity*	*pH*	*Initial rate**	*Initial rate* **2** / *Initial rate* **1**
1	0.417×10^{-4}	4.0	0.231	1.9
2		4.0	0.445	
1	2.085×10^{-4}	4.0	0.123	2.6
2		4.0	0.325	
1	4.170×10^{-4}	4.0	0.488	2.4
2		4.0	1.150	
1	0.417×10^{-4}	5.5	0.249	2.1
2		5.5	0.532	
1	2.085×10^{-4}	6.5	0.194	2.4
2		6.5	0.466	

*Units are arbitrary, being dependent, in each pair of measurements, on the speed of the recorder chart

It may be seen that the diol derived from isomer **2** was oxidized 1.9–3.2 times faster than the diol derived from isomer **1** under several conditions of pH and concentration of substrate It was concluded, therefore, that isomer **1** has the *erythro* configuration, whereas isomer **2** has the *threo* configuration This assignment of configuration is in agreement with enzyme-specificity studies Of the four 3,6-dideoxyhexulosonates, only isomer **1b** (derived from L-lactaldehyde) is active towards 3-deoxy-L-*glycero*-pentulosonate dehydrase[1]. Because this enzyme is specific for the "C-4-L" configuration[4], the reactive isomer **1b** must have the L-*glycero* configuration at C-4, and must therefore be the L-*erythro* isomer. The corresponding isomer **2b** must possess the *threo* configuration.

Synthesis of the 3-deoxy-D-hexulosonic acids **4** and **5** was effected by condensation of oxalacetic acid with D-glyceraldehyde by the procedure used for the 3,6-dideoxyhexulosonic acids Paper electrophoresis showed the product mixture to contain, in addition to pyruvate and unchanged D-glyceraldehyde, two other ketonic acids, **4** and **5**, in the approximate ratio of 10:1 By fractionation on Dowex-1 (formate) resin, it was possible to isolate approximately half of the total yield of α-keto acid as isomer **4**, but it was not practicable to isolate appreciable quantities of isomer **5**



SCHEME I

Isomer **4** was isolated as the syrupy potassium salt, which was chromatographically and electrophoretically homogenous, having R_F values in agreement with those previously reported for 3-deoxy-D-*erythro*-hexulosonic acid (see Table IV) (Isomer **5** has about the same R_F values as isomer **4**, as expected from their very similar structures) It was thus concluded that compounds **4** and **5** are the epimeric 3-deoxy-D-hexulosonic acids, and their configurations were assigned by comparison of the observed and previously reported optical rotations Isomer **4** had $[\alpha]_D^{22}$ −31.6°, which compares well with the value of $[\alpha]_D^{22}$ −29.2° observed by Merrick and Roseman for the D-*erythro* isomer[10] A roughly equimolar mixture of isomers **4** and **5** (as determined by paper electrophoresis) had $[\alpha]_D^{22}$ −7 8°, in agreement with the value of −7 1° calculated by using the rotation at $[\alpha]_D^{22}$ reported by Kuhn *et al*[11] for the D-*threo* epimer Thus, it was concluded that isomer **4** is 3-deoxy-D-*erythro*-hexulosonate, and isomer **5** is the *threo* epimer. Compound **4** was further characterized by its (2,4-dinitrophenyl)hydrazone, apparently the first crystalline derivative to be reported, except for the calcium salt[10]

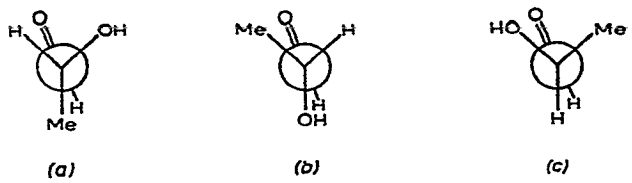
TABLE IV

CHROMATOGRAPHIC AND ELECTROPHORETIC DATA FOR POTASSIUM 3-DEOXY-D-HEXULOSONATES

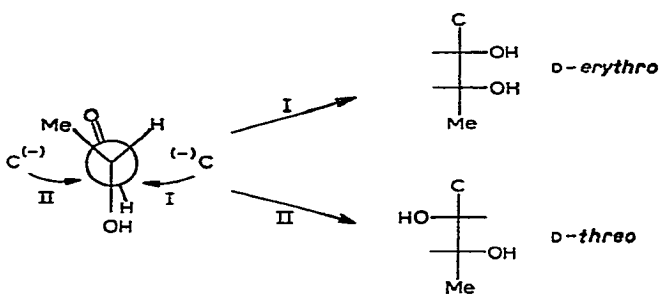
	R_F[a]	R_F[b]	R_F[c]	R_F[d]	M_G[e]
Isomer **4** (*erythro*-)	0 73 (0 89[f])	0 63 (0 68[f])	0 51 (0 59[f])	0 20 (0 35[g])	1 4
Isomer **5** (*threo*-)	0 73	0 68	0 51		1 6

[a] 6 1 3 methanol–0 88 NH_4OH–water [b] 80 15 5 methanol–formic acid–water [c] 6 3 1 propyl alcohol–formic acid–water [d] 4 1 1 butyl alcohol–acetic acid–water. [e] $M_G = \frac{\text{mobility of compound}}{\text{mobility of D-glyceraldehyde}}$ [f] See ref 10 [g] See ref 14

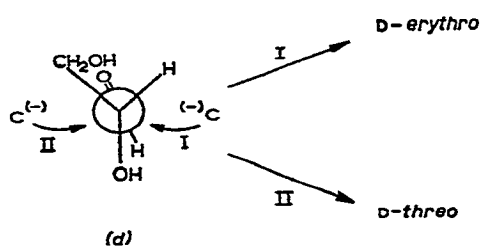
The stereoselectivity of the above condensations is significant, and may be rationalized by the following considerations Free D-lactaldehyde may exist in solution as three rotamers.



Conformer (*c*) is probably least favored, and, although there is little steric favoring for either (*a*) or (*b*), the latter is probably favored because of a minimization of dipolar interactions Nucleophilic addition to the carbonyl group of conformer (*b*) of lactaldehyde is likely to occur by mode I



that is, by the least sterically hindered approach (Cram's rule[16]) Thus, the resultant diol is more likely to be the *erythro* isomer than the *threo* Actually, when $C^{(-)}$ is the pyruvate carbanion, the *erythro–threo* ratio is about 2 1 For addition of the same carbanion to D-glyceraldehyde, whose most favored rotamer is probably (*d*)



(*d*)

the preference for mode I of attack should be greater, since the hydroxymethyl group should hinder attack by mode II more effectively than would a methyl group; this conclusion is borne out, since the *erythro–threo* ratio for this reaction was found to be greater than 10 1.

According to the above discussion, it should be possible to predict the steric outcome of the cyanohydrin synthesis, which involves a similar carbonyl-addition

reaction However, previous results[15] show that the ratio of epimers produced by the addition of cyanide ion to most of the reducing monosaccharides changes greatly according to the reaction conditions, especially the pH. Three factors may account for this Firstly, the attacking nucleophile, namely, cyanide, is much smaller than the pyruvate carbanion, and its favoring of a particular mode of attack may not be critically dependent on the steric factors just discussed Secondly, most additions of cyanide to reducing monosaccharides previously reported involve substrates that are more complex than either lactaldehyde or glyceraldehyde, and the conformation of their acyclic form is not yet known with certainty Finally, the ratio of epimeric adducts may be thermodynamically, not kinetically, controlled, since the establishing of equilibrium may be rapid under certain conditions In such cases, Cram's rule is not applicable[16] For this reason, the ratios of epimers produced in the synthesis of neuraminic acid and 3-deoxy-D-*manno*-octulosonic acid, already mentioned, could not have been predicted by the simplified considerations discussed earlier, because these reactions were conducted at pH 11, where the rate of reverse aldolization is probably significant

Thus, an essential factor for successful prediction of the stereospecificity of nucleophilic additions to carbonyl groups of reducing sugars is the choice of conditions for kinetic control of the products, this is exemplified by the syntheses here described

EXPERIMENTAL

General. — N m r. spectra were recorded with a Varian A-60A n m r spectrometer Chemical shifts in deuterium oxide were referred to the residual hydroxyl (HOD) proton arbitrarily assigned to τ 5 0 on the Varian chart scale* Hydroxylic protons of samples were pre-exchanged with deuterium by freeze-drying with 99.7% deuterium oxide at least twice. Paper chromatography (ascending) was performed with Whatman No 1 paper and alkaline silver nitrate or semicarbazide sprays for detection I r. (KBr disc) and u v. spectra (in ethanol) were obtained with a Perkin–Elmer SP-621 and a Unicam SM-800 spectrometer, respectively 3-Deoxy-DL-*glycero*-pentulosonate and 3-deoxy-DL-*glycero*-pentulosonate-*5*-d_2 were prepared as previously described[4], but with the simplified purification procedure now described for the 3,6-dideoxyhexulosonates D- and L-Lactaldehyde were prepared from L- and D-threonine by oxidation with ninhydrin[12] DL-Lactaldehyde was a gift from Dr Perry A Frey

3,6-Dideoxyhexulosonic acids. — A typical procedure is as follows Oxalacetic acid (2 4 g, 18 mmoles) was added slowly to a solution of L-lactaldehyde (9 mmoles) in 90 ml of 0.05M potassium phosphate buffer (pH 7 5), the pH being kept between 6 and 8 by addition of 40% potassium hydroxide After 15 h at room temperature, the pH was adjusted to 4, and the solution was degassed The resultant solution was

*Under the conditions employed, the HOD proton signal appears at τ = 4 7 relative to the methyl protons of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS)

admitted to a column (3 8 × 50 cm) of Dowex-1 (formate), 200–400 mesh, and eluted with 0 23M formic acid (2 liters), followed by 0 46M formic acid Fractions (22 ml) were collected, and assayed quantitatively for α-keto acid by the semicarbazide method[13] Fractions 5–15 contained unreacted L-lactaldehyde (*ca* 5 mmoles), which was retained for further use. Fractions 112–128 and 135–154, containing isomers **1** and **2**, respectively, were pooled individually, intermediate fractions were discarded The purified solutions of isomers **1** and **2** so obtained were freed of formic acid by several evaporations under diminished pressure, care being taken that the flask temperature did not rise above 15° Each isomer was obtained as a colorless syrup, which was dissolved in a small volume (5–10 ml) of water and brought to pH 7 5 with 40% potassium hydroxide The compounds could be stored indefinitely in solution at pH 7 5 and at 5° without significant deterioration, or could be isolated as the partly crystalline potassium salts by freeze-drying The yield of isomers **1** and **2** thus obtained was 1.9 and 0 9 mmoles, respectively, as estimated by semicarbazide assay The D and DL compounds were prepared similarly from D- and DL-lactaldehyde, respectively All compounds prepared by the above method were homogenous by paper chromatography, and had the R_F values recorded in Table I Specific rotations are given in Table II The optically pure isomers exhibited rapid mutarotation towards zero The i r spectra of isomers **1** and **2**, obtained with amorphous, freeze-dried potassium salts, were grossly similar, but showed clear differences in the region of 9–10 μm Bands due to hydroxyl groups (2–9 μm), carboxylate anion (6 2 μm), and ketonic carbonyl (5 9 μm, weak), were present

Semicarbazones. — *A Isomer* **1** (DL) A solution of semicarbazide hydrochloride (133 mg, 1 14 mmoles) and sodium acetate (140 mg, 1 71 mmoles) in 1 ml of water was added to a solution of isomer **1** (0 57 mmole) in 1 ml of water, and the mixture was kept overnight After acidification of the mixture to pH 1 7 with conc hydrochloric acid, yellowish crystals (109 mg, m p 234–236° dec) were formed, these were removed by filtration after 24 h After two recrystallizations from water, the pale-yellow prisms had m p 231–233° (dec , changed in crystalline form at ~180°), λ_{max} 250 nm (ε 11,000) (Found C, 41 82; H, 5 63, N, 20 48 $C_7H_{11}N_3O_4$ (lactone) calc C, 41.88, H, 5 5, N, 20 8%) The i r spectrum was consistent with the expected structure

B Isomer **2b**. A solution of semicarbazide hydrochloride (56 mg, 0 5 mmole) and sodium acetate (62 mg, 0 75 mmole) in 1 ml of water was added to a solution of isomer **2b** (0 5 mmole) in 1 ml of water, and the mixture was kept overnight at room temperature After acidification of the solution to pH 1.5 with conc hydrochloric acid, white crystals were formed (20 mg, m p 225–230°, dec.), and these were removed by filtration After recrystallization from water, the white needles had m p 229–230°, dec ; λ_{max} 250 nm (ε 11,000) (Found C, 41 91, H, 5 52, N, 21 00 $C_7H_{11}N_3O_4$ (lactone) calc C, 41 88, H, 5 5; N, 20 8%) The i r spectrum was grossly similar to, but not identical with, that of the previous semicarbazone, its major bands were in the same regions

(2,4-Dinitrophenyl)hydrazones — *A Isomer* **1** (DL) (2,4-Dinitrophenyl)-

hydrazine (100 mg, 0 5 mmole) was dissolved in 3 ml of conc. hydrochloric acid by warming, and the solution was diluted to 20 ml with water, and added to a warm solution of isomer **1** (0 5 mmole) in 10 ml of water The mixture was kept for 5 min at 50°, and kept overnight at room temperature The resulting, yellow precipitate (105 mg, m p. 205–208°, dec) was removed by filtration, washed with a little ethanol, and dried. Two recrystallizations from methanol gave the pure derivative, m p 217–218°, dec (Found C, 44 59; H, 3 84, N, 17 11; $C_{12}H_{12}N_4O_7$ (lactone) calc C, 45 0, H, 3 7; N, 17 28%) The i r spectrum was consistent with the expected structure

B Isomer **2** (DL) The (2,4-dinitrophenyl)hydrazone was prepared in a manner similar to the preceding preparation, but attempts to recrystallize the crude material failed. The i r spectrum was, however, grossly similar to that of the previous derivative

Periodate-oxidation studies — A. Oxidative decarboxylation of isomers **1** *and* **2**, Solutions I and II, each containing 50 μmoles of isomer **1a** or isomer **2a** and 500 μmoles of hydrogen peroxide in a total volume of 1 2 ml of water, were prepared, and kept for 15 h at 5° (pH 5 5) Semicarbazide assays[13] at this stage showed the reaction to be complete, since there was no remaining absorption for α-keto acid semicarbazone at 250 nm Excess of hydrogen peroxide was decomposed by treatment with 1 mg of catalase at room temperature for 24 h (A negative peroxide test by starch–KI was obtained after less than 1 h) The solutions of the resultant diols were used without further purification in the experiments next described.

b Measurements of rate of periodate oxidation Each of the above solutions (1, 5, or 10 μl), derived from isomer **1a** or isomer **2b**, was added to 1-cm, quartz cuvettes containing 1 ml of 0 1mM sodium periodate in 10mM sodium formate buffer (pH 4 0) Under these conditions, oxidation was complete in less than 1 h, and the rate of loss of periodate-ion absorption at 223 nm was followed spectrophotometrically with a Unicam SP-800 spectrometer The measurements were repeated at pH 5 5 and pH 6 5, the results are recorded in Table III The initial rates of oxidation shown are expressed in arbitrary units based on the initial slope of the curve of absorbance at 223 nm against time Since the initial concentration of periodate ion and diol are the same in each pair of measurements, the ratio of the arbitrary, initial rates gives the ratio of the rate constants for oxidation of the pair of diols

Small samples of solutions containing, separately, the diol derived from isomer **1a** and isomer **2a**, at pH 1, were heated for 15 min at 90° to effect lactonization Aliquots of each of the resulting solutions were added to the buffered periodate solution as previously described No loss of periodate-ion absorption at 223 nm occurred, supporting the conclusion that the loss of periodate absorption previously observed was caused by diol cleavage and not by oxidation of impurities

*3-Deoxy-*D*-hexulosonic acids* — Oxalacetic acid (2 64 g, 20 mmoles) was slowly added to a solution of D-glyceraldehyde (276 mg, 8 mmoles) in 50 ml of 50 mM potassium phosphate buffer pH 7 5, while the pH was kept between 6 and 8 by addition of 40% potassium hydroxide solution. After 15 h at room temperature, the pH was adjusted to 4, and the solution was degassed. The composition of the product mixture

was determined by paper electrophoresis of aliquots removed at this stage Electrophoretograms (on Whatman No 3 paper) in 50mM borate buffer showed two, clearly separated, keto acid spots, **4** and **5**, in addition to pyruvate and unchanged D-glyceraldehyde The compounds **4** and **5** were shown to be present in the ratio of *ca* 10 1, as determined by elution of the spots with water, and assay by the semicarbazide method (The u v. spectra of the resultant semicarbazone solutions were typical for α-keto acids, showing a broad maximum at 250 nm.) The resultant solution was admitted to a column (3 8 × 75 cm) of Dowex-1 (formate), 200–400 mesh, and eluted with 230 to 460 mM formic acid (linear gradient, 2 5-liter reservoirs). Fractions (20–25 ml) were collected, and assayed for α-keto acid with semicarbazide Fractions eluted after the void volume contained unreacted D-glyceraldehyde, and fractions 110–140 contained the 3-deoxyhexulosonic acids as partly overlapping zones Fractions 110–121, containing electrophoretically pure isomer **4**, and fractions 122–140, containing isomers **4** and **5**, were pooled individually After removal of formic acid by several evaporations under diminished pressure, the solutions were brought to pH 7 with 40% potassium hydroxide solution The potassium salt of isomer **4** was obtained as a syrup by freeze-drying From a total yield of 1 5 mmole of α-keto acid obtained in this way, it was possible to isolate up to 1 mmole of pure isomer **4** Isomer **4** had $[\alpha]_{437}^{22}$ −55.8°, $[\alpha]_{589}^{22}$ −31 6° (*c*, 1 67, water at pH 6) A mixture of **4** and **5**, shown by electrophoresis to be about equimolar in both isomers, had $[\alpha]_{437}^{22}$ −24 2°, $[\alpha]_{589}^{22}$ −7 8° (*c*, 0 97, water at pH 6) The i r spectrum of isomer **4** (freeze-dried potassium salt) showed bands at 2 9 (OH) and 6 2 μm (CO_2^-), but no ketonic carbonyl (5 9 μm) The (2,4-dinitrophenyl)hydrazone of isomer **4** was prepared by a method similar to that described for the hydrazones of the 3,6-dideoxyhexulosonic acids After recrystallization from ethyl acetate–heptane, the (2,4-dinitrophenyl)-hydrazone of **4** had m p 189–191° (dec) (Found C, 42 32, H, 3 67, N, 16 25 $C_{12}H_{12}N_4O_8$ (lactone) calc C, 42 37, H, 3 56, N, 16 47%)

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