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CARBON-13-ENRICHED CARBOHYDRATES. PREPARATION OF ERYTHROSE, THREOSE, GLYCERALDEHYDE, AND GLYCOLALDEHYDE WITH ^{13}C-ENRICHMENT IN VARIOUS CARBON ATOMS

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ABSTRACT

Two-, three-, and four-carbon aldononitriles were prepared, and catalytically reduced with palladium–barium sulfate (5%) to the corresponding aldoses in high yields at pH 1.7 ±0.1 and atmospheric pressure. Carbon-13-enriched glycolaldehyde, glyceraldehyde, erythrose, and threose were prepared with enrichment in various carbon atoms, permitting unequivocal assignment of chemical shifts for all carbons and determination of the proportions of cyclic hemiacetals and linear *gem*-diol forms in solution. Carbon–carbon and carbon–hydrogen coupling-constants for the furanose ring and linear hydrates of these short-chain aldoses are reported and discussed.

INTRODUCTION

In an earlier paper[1], we described the synthesis of hexoses and pentoses in high yield by a modification of the classical cyanohydrin synthesis of Kiliani–Fischer[2]. The principal improvement involves the formation of intermediate aldononitriles at low pH in aqueous solution at 25°, and their subsequent hydrogenolysis over a palladium catalyst to produce the aldose essentially quantitatively. Formation of hexononitriles in high yield required the use of an excess of cyanide, but the pentononitriles could be prepared in yields of over 90% from stoichiometric amounts of the appropriate tetrose and cyanide. In this paper, we describe the application of this synthesis to the preparation of the tetroses, glyceraldehyde, and glycolaldehyde, with ^{13}C-enrichment in various carbon atoms, and of [^{13}C]formaldehyde. The ^{13}C-n.m.r. spectra of these compounds are interpreted in terms of the various forms present in solution. All ^{13}C chemical-shifts are assigned, and C–C and C–H coupling constants are tabulated.

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EXPERIMENTAL

Materials. — Glycolaldehyde and D-glyceraldehyde were purchased from Sigma Chemical Company and were used without further purification. Formaldehyde (37% aqueous solution) was purchased from Mallinckrodt, and assayed as described by Walker[3]. D-Erythrose was prepared from 2,4-*O*-ethylidene-D-erythrose as described by Perlin[4]. Acid hydrolysis and purification were described previously[1]. D-Threose was prepared from 2,4-*O*-ethylidene-D-threose[5] in a similar manner. D-Glyceraldehyde was prepared from D-fructose by oxidation with lead tetraacetate[6]. DL-[1-^{13}C]Glyceric acid was prepared from DL-[1-^{13}C]glyceraldehyde by oxidation with hypoiodite[7].

Sodium D-[1-^{13}C]-erythronate and -threonate were prepared by the addition of K^{13}CN (0.8 g, 12.1 mmol; containing 10^7 c.p.m. K^{14}CN) in water (50 mL) to a stirred solution of D-glyceraldehyde (1.0 g, 11.1 mmol, in water (60 mL) at 25°. After 10 days at 25°, the mixture was adjusted to pH 11–12 with 2M sodium hydroxide, concentrated at 35° *in vacuo* to about 10 mL, and incubated for 48 h at 50°. Carbon-13 n.m.r. and g.l.c.[1] confirmed the presence of the epimeric aldonic acid salts. The solution was adjusted to pH 9–10 by the addition of Dowex-50 × 8 (H^+), and 2.5 mmol of the material was applied to a column (2.2 × 60 cm) of Dowex-1 × 8 (200–400 mesh) in the acetate form. The column was developed at 4° with a linear gradient of acetic acid (3L, 0.10–0.60M) followed by 0.60M acetic acid until the acids eluted as two peaks. Fractions (4 mL) were collected at 0.4 mL per min and column peaks were detected by assaying for radioactivity[1]. Fractions containing the aldonic acids were pooled, and evaporated at 35° *in vacuo* to remove acetic acid. Gas–liquid chromatography[1] of the corresponding C-2 epimeric sodium salts and standard sodium threonate established configuration and purity (peak 1, >95% *erythro*; peak 2, >95% *threo*).

Potassium [^{13}C]cyanide (K^{13}CN) was supplied by the Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico, with 90.7 atom% ^{13}C enrichment. Potassium [^{14}C]-cyanide (K^{14}CN) was obtained from New England Nuclear and had a specific activity of 46 mCi/mmol. *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of chlorotrimethylsilane was obtained from Pierce Chemical Co. Pyridine for gas-chromatographic analyses was distilled, and stored over 4-Å molecular sieves.

Palladium–barium sulfate (5%) was purchased from Sigma Chemical Company. Silver carbonate was freshly prepared from silver nitrate and sodium carbonate as described by McCloskey and Coleman[8].

Glycerol kinase (EC 2.7.1.30) from *E. coli*, D-fructose 1,6-bisphosphate aldolase (EC 4.1.2.13) from rabbit muscle, and disodium adenosine 5′-triphosphate (ATP) were obtained from Sigma Chemical Company. 1,3-Dihydroxy-2-propanone phosphate was prepared from the dicyclohexylammonium dimethyl acetal (obtained from Sigma) as described previously[9].

Chemicals were reagent-grade or better, and were used without further purification.

Instrumentation. — Carbon-13 n.m.r. spectra were obtained by using a Bruker WP-60 15.08 MHz Fourier-transform spectrometer equipped with quadrature detection. All spectra were obtained at 36° with 4 K spectral points, a spectral width of 3000 Hz, and a filter setting of 2400 Hz. The spectrometer was locked to the resonance of D_2O in a capillary. Chemical shifts are given relative to external tetramethylsilane (Me_4Si) and are accurate to within ±0.1 p.p.m.

Methods for gas-chromatographic analyses and retention data were described previously[1].

Catalytic hydrogenolysis was conducted at atmospheric pressure and 25° in an apparatus described by Vogel[10]. The reaction vessel consisted of a side-arm flask equipped with an addition funnel tightly secured with a rubber stopper, and stirring was provided magnetically.

Preparation of aldononitriles. — Two-, three-, and four-carbon aldononitriles were prepared by using the apparatus and procedures described previously[1]. The apparatus consists of a vessel equipped to measure pH in the mixture and to make additions of acid or base to maintain a given pH. Provisions are made for stirring and to remove samples for analysis. The flask is charged with 0.15M $K^{13}CN$, sealed, and the pH adjusted to 8.5 ±0.1 with 2.0M acetic acid. The starting aldose is added so that the final concentration of both $K^{13}CN$ and aldose is 0.1M. The pH is maintained at 8.5 ±0.1 by addition of 2.0M acetic acid or M sodium hydroxide as required. Samples are taken for analysis of the aldononitriles by g.l.c.[1] or ^{13}C-n.m.r. at 10-min intervals. All condensations were complete (>95%) in 20–30 min and virtually no hydrolysis had occurred. The pH was adjusted to 4.0 with 6.0M acetic acid and then to 1.7 ±0.1 with 6.0M hydrochloric acid. At this pH, the aldononitriles are stable for several weeks at 4°. The preparation and purification of glycolonitrile have been described previously[11]. In the present study, this compound and the other aldononitriles were used without purification.

[^{13}C]Formaldehyde was prepared from a solution (0.1M) of $K^{13}CN$ at 5° after adjusting the pH as already described, prior to reduction. Caution should be exercised in the preparation of formaldehyde and glycolaldehyde, as hydrogen chloride and formaldehyde on contact can produce the potent carcinogen, bis(chloromethyl) ether[12].

Reduction of short-chain aldononitriles. — Palladium–barium sulfate (5%, 62 mg per mmol of nitrile) was weighed into a side-arm flask, 5–10 mL of water was added, and the suspension reduced with hydrogen for 15–20 min at atmospheric pressure and 25° with efficient stirring. During this period, the suspension changed from brown to a whitish-gray tint. The solution of aldononitriles at pH 1.7 ±0.1 was added from an addition funnel, and the reduction vessel was evacuated 3 times prior to a final charging with hydrogen. Reduction times varied with the aldononitrile: hydrogen cyanide, 8 h; glycolonitrile, 18 h; glyceronitrile, 8–10 h; erythrono- and threononitriles, 18 h. Completion of the reaction was determined by ^{13}C-n.m.r. and gas

chromatography. G.l.c. retention-times[1] for the Me_3Si derivatives of the short-chain aldoses relative to that of the D-gluconate derivative are: glycolaldehyde, 0.25, 0.26, 0.27; DL-glyceraldehyde, 0.36, 0.83, 0.85, 0.87, 0.90; D-erythrose, 0.37; D-threose, 0.33, 0.36. After reduction, the mixture was freed of catalyst by filtration through Celite.

For the preparation of [^{13}C]formaldehyde, the reduction flask was cooled to 5° prior to the addition of hydrogen cyanide, and the reduction was started immediately after addition, omitting evacuation. The reaction vessel was allowed to warm to room temperature slowly during the reduction.

Purification of the reduction mixture. — The filtrate containing the reduction products was treated with an excess of Dowex-50 × 8 (H^+) resin to remove amine by-products. This step should be completed immediately after reduction, to minimize reactions between by-product amines and the desired aldose. The resin was removed by filtration and the acidic solution treated with an excess of silver carbonate with stirring until the pH was approximately 6.5. The precipitate (AgCl and Ag_2CO_3) was removed by filtration through Celite and the filtrate treated with an excess of Dowex-50 × 8 (H^+) resin with stirring for 60 min at 25°. After removal of the resin, the clear solution was concentrated at 30° *in vacuo* to 30 mL and treated again with Dowex-50 × 8 (H^+) for 30 min. The mixture was filtered through Celite, concentrated at 30° to 30 mL, and treated with an excess of Dowex-1 × 8 (200–400 mesh) resin in the acetate form for 10 min. The solution was filtered, concentrated to 3 mL at 30°, and analyzed by ^{13}C-n.m.r. and g.l.c. Erythrose and threose were separated at 25° on a column (2.2 × 92 cm) of Dowex-50 × 8 (200–400 mesh) resin in the barium form[13]; 4-mL fractions were collected at 0.4 mL per min. Threose was eluted between fractions 55 and 65, and erythrose between fractions 105 and 122. Unreacted C_4 nitriles and glyceraldehyde eluted with threose. The column capacity exceeded 4 mmol of tetrose.

Concentration of acidic solutions (pH < 2) of erythrose and threose produced oligomeric mixtures of these sugars. Oligomerization may be reversed by heating a 50–70mM solution of oligomers for 30 min at 90° with the addition of dilute sulfuric acid to a final concentration of ~0.02M. The acidic solution, after being neutralized with barium carbonate, and deionized with Dowex-1 (OAc^-) and Dowex-50 (H^+) resins, contains only monomeric tetroses, as evaluated by ^{13}C-n.m.r. spectroscopy.

Summary of ^{13}C-enriched products. — The following ^{13}C-enriched compounds were prepared: [^{13}C]formaldehyde, [1-^{13}C]glycolaldehyde, DL-[1-^{13}C]-glyceraldehyde, DL-[2-^{13}C]glyceraldehyde, D-[1-^{13}C]erythrose, DL-[2-^{13}C]erythrose, DL-[3-^{13}C]erythrose, D-[1-^{13}C]threose, DL-[2-^{13}C]threose, DL-[3-^{13}C]threose, DL-[1,2-^{13}C]erythrose, DL-[1,2-^{13}C]threose, [1-^{13}C]glycolonitrile, DL-[1-^{13}C]glycero-nitrile, DL-[2-^{13}C]glyceronitrile, D-[1-^{13}C]erythrononitrile, DL-[2-^{13}C]erythrono-nitrile, DL-[3-^{13}C]erythrononitrile, D-[1-^{13}C]threononitrile, DL-[2-^{13}C]threono-nitrile, DL-[3-^{13}C]threononitrile, DL-[1,2-^{13}C]erythrononitrile, DL-[1,2-^{13}C]threo-nonitrile, DL-[1-^{13}C]glyceric acid, sodium D-[1-^{13}C]erythronate, sodium D-[1-^{13}C]-threonate, L-[2-^{13}C]glyceraldehyde 3-phosphate, D-[5-^{13}C]fructose 1-phosphate, and L-[5-^{13}C]sorbose 1-phosphate.

RESULTS AND DISCUSSION

The preparation and hydrogenolysis of aldononitriles described here and in a previous report[1] is a modification of the traditional cyanohydrin synthesis of Kiliani–Fischer[2] that provides a direct and convenient route for incorporation of carbon-13 into C-1 of aldoses in approximately 85% yield. The traditional cyanohydrin synthesis of aldoses involves the reduction of lactones or acetylated lactones with various reducing agents, most commonly sodium amalgam[1]. The preparation of tetroses and lower-carbon aldoses with isotopic enrichment (^{13}C or ^{14}C) has not been accomplished satisfactorily by these methods. For example, Glattfeld and Kribben[14] were able to prepare DL-erythrose from the aldonic acid by reduction of the acylated acyl chloride with palladium, having been unable to obtain erythrose from the lactone by reduction with sodium amalgam. The overall yield was 5%. Thus, for the tetroses and lower-carbon aldoses, the synthesis reported here is the first chemical process for the production of enriched compounds in satisfactory yields. These yields permit serial application of the synthesis to produce multiply-enriched compounds.

There are significant differences in the behavior of the short-chain derivatives in both steps of this synthesis as compared with the behavior of the intermediates in the synthesis of the pentoses and hexoses[1]. In the condensation step, the formation of nitrile is strongly favored by the inability of the starting aldose to exist as a pyranose. This results in the condensation proceeding to completion almost instantaneously without the addition of excess cyanide. This effect was also observed in the preparation of the pentoses[1] in which the starting aldoses can exist only in furanose or acyclic forms.

The reduction of the intermediate nitriles also proceeds differently. Apparently, in the synthesis of the pentoses and hexoses, the intermediate five- and six-carbon imines can cyclize, facilitating formation of the aldose, presumably through an intermediate glycosylamine[15]. In the synthesis of shorter-chain homologues, cyclized intermediates can form only from the four-carbon nitriles. It appears that this process does not occur readily, however, as the reduction of erythrono- and threono-nitriles at pH 4.2 $\pm$0.1 and 60 lb.in.$^{-2}$ over palladium–barium sulfate at 25° affords 1-amino-1-deoxyalditols as the principal products[1]. These conditions generally give excellent yields of the pentoses and hexoses, where pyranosyl intermediates can form. The ease with which aldononitriles can be hydrogenolyzed to aldoses at high pressure parallels exactly their susceptibility to alkaline hydrolysis. In both processes, cyclization has been implicated[15,16]. We have found, however, that the aldononitriles which yield imines that cannot cyclize can be hydrogenolyzed smoothly and almost quantitatively at 25° to the corresponding aldehydes at pH 1.7 $\pm$0.1 and atmospheric pressure over palladium–barium sulfate (Table I). Presumably, these conditions permit the intermediate imine to dissociate from the catalyst and to hydrolyze without the intramolecular participation that appears to occur during the preparation of the hexoses and pentoses.

TABLE I

YIELDS OF ALDOSES FROM HYDROGENOLYSIS OF TWO-, THREE-, AND FOUR-CARBON ALDONONITRILES[a]

Compound	*Conditions*			
	Pd–$BaSO_4$, atm. press. pH 1.7	*PtO_2, 60 lb. in.$^{-2}$ pH 0.8*	*PtO_2, 30 lb. in.$^{-2}$ pH 0.8*	*Pd–$BaSO_4$, 60 lb. in.$^{-2}$ pH 4.2*
Glycolaldehyde	90 (80)[b]			
DL-Glyceraldehyde	85 (75)[b]		trace[c]	<15
D-Threose and D-erythrose	85 (70)[d]	trace[c]		<10

[a]Based on ^{13}C-n.m.r. peak areas. Spectra were obtained by using a 55° pulse and 10-sec delay time to minimize relaxation-effects. Spectra were obtained of reduction mixtures. Integration was performed by computer. [b]Percent yield based on weight of products as gums. [c]Determined by gas chromatography. [d]Percent yield based on weight of products as gums after separation by chromatography on Dowex-50 × 8 (200–400 mesh) (Ba^{2+}).

Glycolaldehyde, DL-glyceraldehyde, and purified erythrose prepared by this method are more than 95% pure by ^{13}C-n.m.r. and g.l.c., and compare favorably to compounds prepared by standard methods. Preparations of DL-glyceraldehyde and unpurified erythrose and threose occasionally contained small quantities (<5%) of the keto isomers. Threose typically contains small proportions of unreacted glyceraldehyde and tetrononitriles.

The various forms of the aldoses in solution may be determined with ease from ^{13}C-n.m.r. spectra of the [1-^{13}C]-enriched compounds (Figs 1A, 2A, and 3B). Formaldehyde[17], glycolaldehyde[18], and glyceraldehyde[19] (Fig. 3B) appear to exist predominantly as *gem*-diols in dilute, aqueous solution. This conclusion is based on the observation of major carbon-13 resonances for the [1-^{13}C]-enriched compounds at approximately 90 p.p.m. downfield from tetramethylsilane. This chemical shift is not typical of aldehydes or hemiacetals, and has been shown to be characteristic of *gem*-diols of aldohexose derivatives[20]. Interestingly, a significant proportion of *gem*-diol exists in solutions of erythrose (~12%) and threose (~12%). The remainder of the tetroses is present in aqueous solution as β-furanose (37% threose, 63% erythrose) and α-furanose forms (51% threose, 25% erythrose). Assignments of the carbon-13 resonances to α- and β-furanoses were made by analogy with those of the methyl α- and β-glycosides[21].

The formation of dimers and higher polymers of glycolaldehyde, DL-glyceraldehyde, and D-erythrose has been noted in concentrated solutions or in solutions freshly prepared from syrups (Fig. 1D, Fig. 3). In the case of glycolaldehyde, these products are hydrolyzed rapidly to the *gem*-diol form[18]. The depolymerization of glyceraldehyde is slower, but eventually yields monomer[22]. Concentrated, neutral solutions of D-[1-^{13}C]erythrose were found to contain dimers or higher polymers having C-1 chemical shifts at 98.5, 97.9, 92.5, and 92.1 p.p.m.

Carbon-13 chemical-shifts obtained from specific ^{13}C-enrichment of the

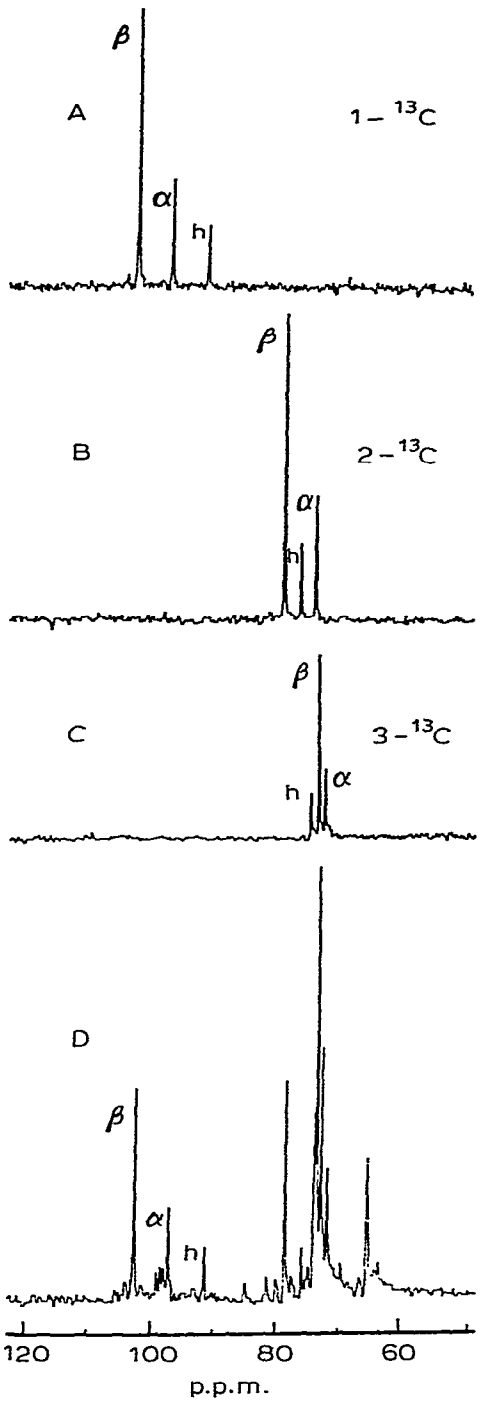

Fig. 1. The proton-decoupled, ^{13}C-n.m.r. spectra of the enriched region of (A) D-[1-^{13}C]erythrose, (B) DL-[2-^{13}C]erythrose, and (C) DL-[3-^{13}C]erythrose. Spectrum (D) is the natural-abundance carbon-13 n.m.r. spectrum of D-erythrose, which was prepared according to Perlin[4]. Minor resonances are due to dimers and/or oligomers. The symbol (h) denotes the linear hydrate form of D-erythrose.

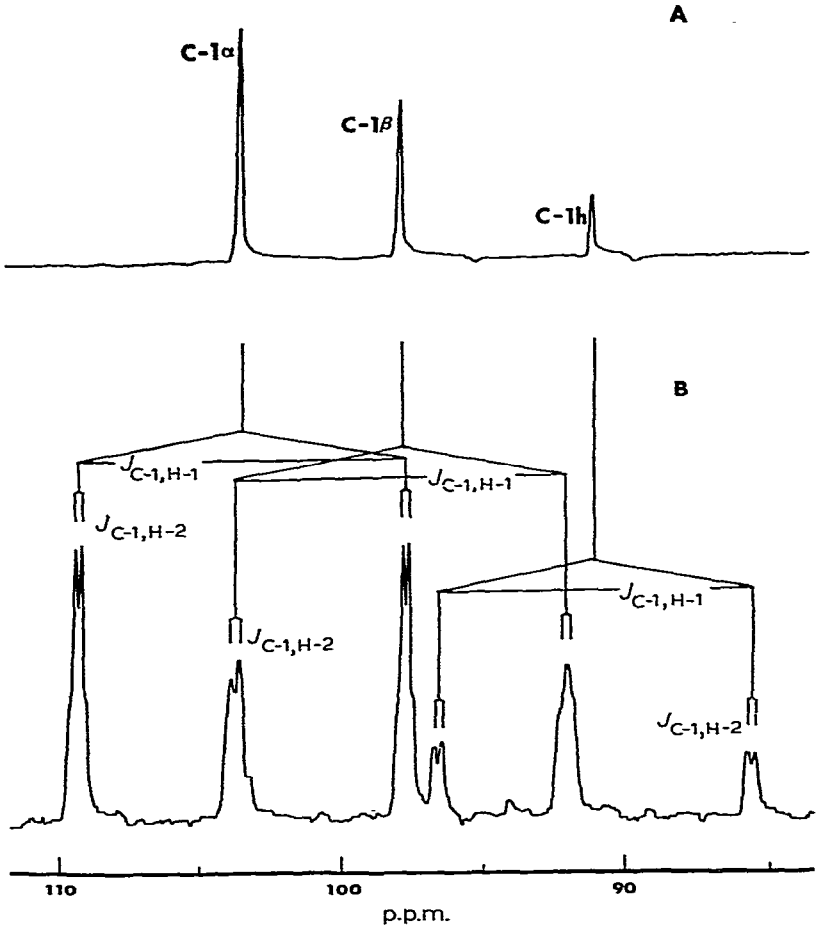

Fig. 2. The proton-decoupled (A) and coupled (B) ^{13}C-n.m.r. spectra of the C-1 region of α- and β-D-[1-^{13}C]threose and D-[1-^{13}C]threose hydrate, showing C-1–H-1 and C-1–H-2 couplings for the three conformations in aqueous solution.

short-chain aldoses are presented in Table II. As demonstrated for erythrose, ^{13}C-enrichment permits unequivocal assignment of chemical shifts of all carbon atoms (Fig. 1, Table II) and of direct C–C couplings (Table III). As expected[23], the magnitude of direct coupling between C-1 and C-2 increases as the s-character of the C-1–C-2 bond increases, being greater in the aldononitriles and aldonic acid salts than in the hemiacetal and *gem*-diol forms. Vicinal C–C coupling[24,25] is observed only in the case of *threo* derivatives; the nitrile and aldonic acid salts have $^{3}J_{C-1,C-4}$ values of 4.0 ±1.5 Hz and 3.7 ±0.7 Hz, respectively. This result is surprising, as, in the acyclic forms of both *erythro* and *threo* derivatives, a *trans* relationship should exist between C-1 and C-4, and be somewhat more stable in the *erythro* isomers. The observation of coupling only in the *threo* forms indicates that the arrangement of the hydroxyl substituents along the coupling pathway is important.

Direct C-1 to H-1 coupling in the 1-^{13}C-enriched tetroses varies with confi-

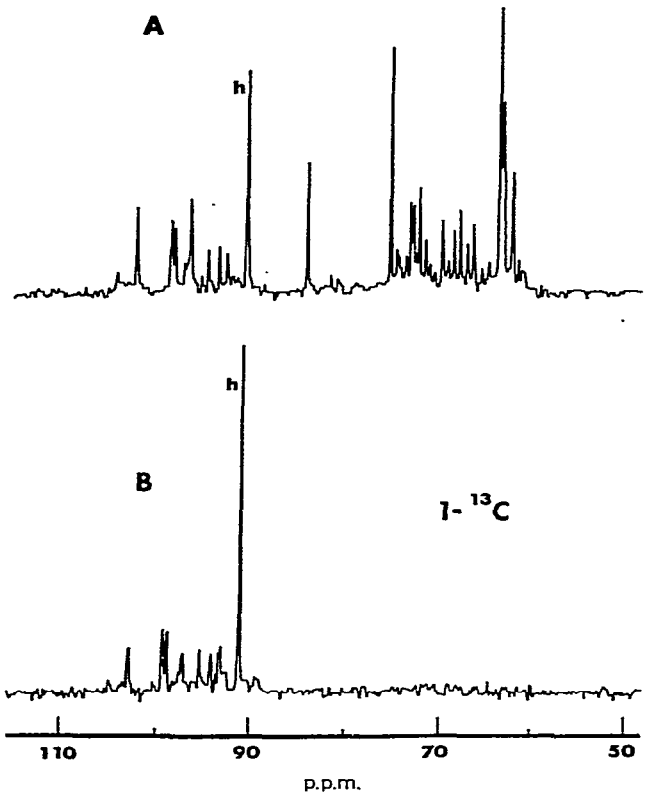

Fig. 3. The natural-abundance ^{13}C-n.m.r. spectrum of polymeric D-glyceraldehyde (A) prepared according to Perlin[6], and the ^{13}C-n.m.r. spectrum of DL-[1-^{13}C]glyceraldehyde (B) showing only C-1 resonances. Peak (h) is the linear, *gem*-diol carbon at C-1; the remaining downfield peaks arise from the C-1 resonances of dimers and higher polymers.

guration at C-1 and with sugar conformation[26,27], with values ranging from 172 to 174 Hz for cyclic forms and from 162 to 164 Hz for acyclic hydrates (Table III). Direct C-2–H-2 coupling in the [2-^{13}C]-enriched tetroses ranges from 150–153 Hz for cyclic forms and 141–143 Hz for the hydrates. Direct C-3 to H-3 coupling in the [3-^{13}C]-enriched tetroses ranges from 152–154 Hz for cyclic forms and 145–146 Hz for acyclic hydrates.

Two-bond C-1 to H-2 coupling appears to be present in the coupled ^{13}C-n.m.r. spectra of (1-^{13}C)-enriched compounds (Fig. 2) and differences appear to exist between *erythro* and *threo* furanoses (Table III). Although these differences probably reflect the conformational preferences of the various forms[26–30], analysis of the ^{1}H-n.m.r. spectra will be required to establish the magnitude of $^{2}J_{C\text{-}1,H\text{-}2}$ more precisely before conformational inferences are made. Differences in C-1–H-2 couplings are also observed between the *erythro* and *threo* hydrates. The *threo* isomer has $^{2}J_{C\text{-}1,H\text{-}2} = 4.4$ Hz, whereas the *erythro* isomer gives a single broad resonance. Again, these differences probably reflect the conformational preferences of the acyclic forms,

TABLE II

CARBON-13 CHEMICAL-SHIFTS OF SHORT-CHAIN CARBOHYDRATES AND DERIVATIVES

Compound	*Carbon position, p.p.m.*[a]			
	C-1	*C-2*	*C-3*	*C-4*
Formaldehyde, hydrate	83.3			
Glycolaldehyde, hydrate	91.2	66.0		
DL-Glyceraldehyde, hydrate	91.2	75.5	63.4	
DL-Erythrose				
α-furanose	96.8	72.4	70.6	72.9
β-furanose	102.4	77.7	71.7	72.4
hydrate	90.8	74.9	73.0	64.0
DL-Threose				
α-furanose	103.4	82.0	76.4	74.3
β-furanose	97.9	77.5	76.2	71.8
hydrate	91.1	74.6	72.2	64.4
Glycolonitrile	120.6	49.2		
DL-Glyceronitrile	121.0	63.2	64.4	
DL-Erythrono- and	120.9[b]	63.2[b]	73.2[b]	62.9[b]
-threono-nitriles	120.9[b]	63.6[b]	73.4[b]	63.0[b]
DL-Glyceric acid	177.2	72.8	64.8	
D-Erythronate, sodium	179.7	75.1	74.9	63.5
D-Threonate, sodium	180.2	73.7	74.2	64.5

[a]All chemical shifts are relative to Me_4Si as external standard. [b]Tentative assignment.

TABLE III

CARBON-13 COUPLING CONSTANTS OF ENRICHED CARBOHYDRATES AND DERIVATIVES

Compound	*Coupling constant, Hz*[a]					
	C-1–C-2	*C-1–H-1*	*C-2–H-2*	*C-3–H-3*	*C-1–H-2*	*C-1–C-4*
Glycolaldehyde, hydrate	A	163.9	A		[b]	
Glyceraldehyde, hydrate	A	162.1	143.3	A	[b]	
Erythrose, hydrate	48.4	164.2	141.5	145.9	[b]	A
Threose, hydrate	49.0	162.8	141.5	145.0	4.4	A
α-Erythrofuranose	43.3	172.3	150.3	154.0	[b]	A
α-Threofuranose	45.9	172.3	152.5	152.8	~1.9	A
β-Erythrofuranose	46.9	172.3	150.3	152.1	~3.4	A
β-Threofuranose	42.3	173.8	151.8	152.8	4.4	A
Glyceronitrile	59.4		A	A	[d]	
Erythrononitrile	60.8		153.0	A	[d]	[c]
Threononitrile	60.8		153.1	A	[d]	~4.0
Glyceric acid	59.4		A	A	A	
Erythronate, sodium	52.8		A	A	2.9	[c]
Threonate, sodium	54.2		A	A	3.7	3.7

[a]Coupling constants are accurate to within ±0.7 Hz. The letter (A) indicates that no experiments were performed to evaluate coupling between the designated atoms. [b]Broadened peaks. [c]No coupling observed. [d]Coupling observed, but could not be reliably measured.

but additional data will be required to confirm and interpret them. Complex, carbon–hydrogen, geminal and vicinal couplings were observed in the [1-^{13}C]-enriched three- and four-carbon aldononitriles. The availability of the [^{13}C]-enriched compounds described here may permit a fuller elucidation of the relationships of coupling to conformation for these compounds.

An important feature of the synthesis of aldoses via reduction of aldononitriles lies in the ease with which it can be applied serially to produce monosaccharides having enrichment at positions other than C-1, or having multiple enrichment. For example, the [2-^{13}C]- and [3-^{13}C]-enriched tetroses were produced in about 50 and 40% overall yield, respectively, from glycolaldehyde by two cycles of the reaction, and from formaldehyde by three cycles of the reaction.

Our principal interest in the enriched compounds described in this report lies

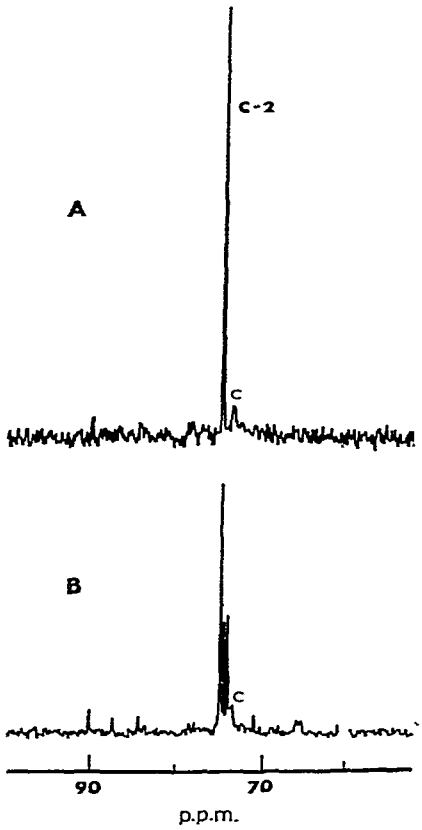

Fig. 4. The ^{13}C-n.m.r. spectra of DL-[2-^{13}C]glyceraldehyde (A), 35mM in water, and the mixture (B) produced by treatment with 1.5 molar equivalents of Mg^{2+}-ATP and glycerol kinase at pH 7.5 for 3 h at 36°. Approximately 45% of the starting glyceraldehyde is converted to the L-3 phosphate, and generates a doublet at 74.9 p.p.m. having $^3J_{C-P} = 6.6$ Hz. The [2-^{13}C]glyceraldehyde remaining after phosphorylation was found to be 91% D enantiomer (see Results and Discussion). Peak (C) is an unidentified contaminant. The resonance at 91 p.p.m. is [1-^{13}C]glycolaldehyde remaining from the preparation of DL-[2-^{13}C]glyceraldehyde.

in their value as substrates or substrate precursors for various enzymes for carbohydrate metabolism, and as tracers in biological systems. In this connection, we have examined DL-[2-^{13}C]glyceraldehyde as a substrate for glycerol kinase and for D-fructose 1,6-biphosphate aldolase. When DL-[2-^{13}C]glyceraldehyde is incubated at 36° with 1.5 molar equivalents of Mg^{2+}-ATP and a catalytic amount of glycerol kinase from *E. coli* at pH 7.5, L-[2-^{13}C]glyceraldehyde 3-phosphate is rapidly formed (Fig. 4)[31,32]. This product can be adsorbed at pH 4.5 onto a DEAE-Sephadex (OAc^-) A-25 column equilibrated with 0.05M acetate buffer, pH 4.5, at 4°. After washing with the same buffer to remove unreacted glyceraldehyde, the phosphate may be recovered by elution with 0.3M acetic acid, pH 4.5, at 4°. L-Glyceraldehyde 3-phosphate may also be purified on Dowex-1 × 8 (formate)[33]. Unreacted glyceraldehyde was shown to be 91% D-isomer by reaction with 1,3-dihydroxy-2-propanone phosphate and D-fructose 1,6-bisphosphate aldolase to produce 91% D-[5-^{13}C]-fructose 1-phosphate (70.5 and 81.7 p.p.m.) and 9% L-[5-^{13}C]sorbose 1-phosphate (70.9 p.p.m.). Rabbit-muscle aldolase can act on both D- and L-glyceraldehyde, and gives an equimolar mixture of the ketose 1-phosphates when the original mixture of DL-[2-^{13}C]glyceraldehyde is used. The synthesis of other [^{13}C]-enriched aldose phosphates by hydrogenolysis of aldononitrile phosphates over palladium–barium sulfate is at present under investigation.

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