Carbohydrate Research, 72 (1979) 71–78

CARBON-13-ENRICHED CARBOHYDRATES. PREPARATION OF ALDONONITRILES AND THEIR REDUCTION WITH A PALLADIUM CATALYST*

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(Received January 6th, 1978; accepted for publication in revised form, June 1st, 1978)

ABSTRACT

Cyanide condenses with aldoses at 25° in aqueous solution between pH 7.0–9.0 to produce aldononitriles in high yield. These nitriles may be reduced catalytically over palladium–barium sulfate (5%) at pH 4.2 ± 0.1 and 25° to yield the corresponding aldoses in 60–90% yield, depending on the structure of the nitrile. 1-Amino-1-deoxyalditols are produced in approximately 10% yield, and their formation is favored when hemiacetal formation is hindered in the parent aldose. Generally, the product epimeric aldoses can be separated from contaminating by-products and from each other by ion-exchange and adsorption chromatography. This procedure has been applied to the preparation of [1-^{13}C]-enriched pentoses and hexoses.

INTRODUCTION

The Kiliani–Fischer synthesis has frequently been used in the preparation of aldoses, especially those containing radioactive isotopes[1]. In this synthesis, sodium cyanide is condensed with a parent aldose to produce, after alkaline hydrolysis of the nitriles, two epimeric aldonic acid salts. The salts are separated and converted into lactones, generally in less than quantitative yields. The lactones, or their acylated derivatives, may be reduced by various reagents, including sodium amalgam[2], diborane in tetrahydrofuran[3], and disiamylborane in tetrahydrofuran[4]. Sodium amalgam and diborane yield mixtures containing product aldoses, unreacted lactone, aldonic acid salts, and alditols. Acylated aldoses are obtained in high yield by reduction with disiamylborane, but removal of the acyl groups is often accompanied by degradation of the product aldose.

Kuhn[5] has shown that 2-benzylamino-2-deoxyaldononitriles are reduced with hydrogen over palladium to the corresponding 2-amino-2-deoxyaldoses. Bayly and Turner[6] have used platinum oxide to prepare 2-deoxy-D-*erythro*-[1-^{14}C]pentose from

*Supported, in part, by a grant from the National Institute of General Medical Sciences (GM-21731) and by a program of the Stable Isotopes Resource of the Los Alamos National Laboratory, supported jointly by the Department of Energy and the National Institutes of Health (RR-00962). Michigan Agricultural Experiment Station Journal Article Number 8579.

the corresponding nitrile. In addition, Kuhn[7] prepared glucono- and mannononitriles in pyridine and reported their reduction in dilute acid to aldoses by use of hydrogen over palladium–barium sulfate. The 2-epimeric aldononitriles are the initial intermediates in the Kiliani–Fischer reaction[8,9]. During an investigation of the mechanism of this reaction using [^{13}C]cyanide[10], we observed that cyanohydrins are formed rapidly and are stabilized in aqueous solution at low pH values. These aldononitriles can be reduced with palladium and the intermediate imine[8,11] hydrolyzed readily at pH 4.2 ± 0.1 to produce the aldose. A convenient and general method for the preparation of aldononitriles, their catalytic hydrogenolysis to aldoses, and the application of these methods to the synthesis of [1-^{13}C]-enriched aldoses in high yield are the subjects of this report.

EXPERIMENTAL

Materials. — Glycolaldehyde, D-glyceraldehyde, D-arabinose, D-lyxose, D-ribose, D-xylose, D-glucose, D-mannose, and D-galactose were purchased from Sigma Chemical Company, and were used without further purification. 2,4-*O*-Ethylidene-D-erythrose was prepared according to Perlin[12]. Hydrolysis of this compound with 0.125M sulfuric acid at 90° for 35 min yielded D-erythrose (87%). The acidic solution was neutralized with barium carbonate, the mixture filtered through Celite, and the filtrate deionized with Dowex-1 ×8 (OAc^-) and Dowex-50 ×8 (H^+). D-Threose was prepared similarly from 2,4-*O*-ethylidene-D-threose[13].

Sodium [^{14}C]cyanide was purchased from New England Nuclear and had a specific activity of 54 mCi/mmol. Sodium [^{13}C]cyanide was supplied by the Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico. *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of chloromethylsilane was obtained from Pierce Chemical Company. Pyridine for gas-chromatographic analyses was distilled and stored over 4Å molecular sieves.

Palladium–barium sulfate (5%) was purchased from Sigma Chemical Company. All other chemicals were reagent grade and were used without further purification.

Instrumentation. — Carbon-13 n.m.r. spectra were obtained with a Bruker WP-60 15.08 MHz Fourier-transform spectrometer equipped with quadrature detection. Spectra were obtained at 36° with 4K 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 relative to external tetramethylsilane (Me_4Si) are accurate to ±0.1 p.p.m.

Gas-chromatographic analysis was performed on a Varian Aerograph 1200 instrument with flame-ionization detection. A column [1.8 m × 2 mm (i.d.)] containing OV-17 (3%) on High Performance Chromosorb W-AW (Applied Science) was employed, with a temperature program of 100–230° at 4°/min. The aqueous sample (6 μL) was added rapidly to a mixture of 150 μL of BSTFA containing 1% of chlorotrimethylsilane and 150 μL of dry pyridine, and the mixtures were warmed for at least 30 min at 40° prior to analysis. Retention data are given relative to D-

gluconate, and g.l.c. peak-areas were calculated from the product of peak height and peak width at one-half height.

Radioactivity was measured in a Beckman LS-100 scintillation counter using a Triton X-100 (1 L)-PPO (8 g)-POPOP (0.2 g)-toluene (2 L) cocktail. The aqueous sample (2 mL) was dissolved in 13 mL of the cocktail prior to analysis.

Catalytic reductions were carried out with a Parr pressure-apparatus and 250-mL reduction flasks.

General method for the preparation of aldononitriles. — A three-neck, 25-mL round-bottom flask was constructed as shown in Fig. 1. In a typical preparation, the flask is immersed in a water bath at 25° and charged with an aqueous solution of sodium cyanide (13 mL) prior to sealing the center neck. Port A is clamped and a 10-mL volume of air withdrawn from port B with a syringe. Port B is sealed. Acetic acid (3M) is added from syringe C until the desired initial pH of the cyanide solution is reached. Port A is opened and the solution of aldose (6 mL) is added slowly from a syringe with efficient stirring. The pH is adjusted during the reaction by addition

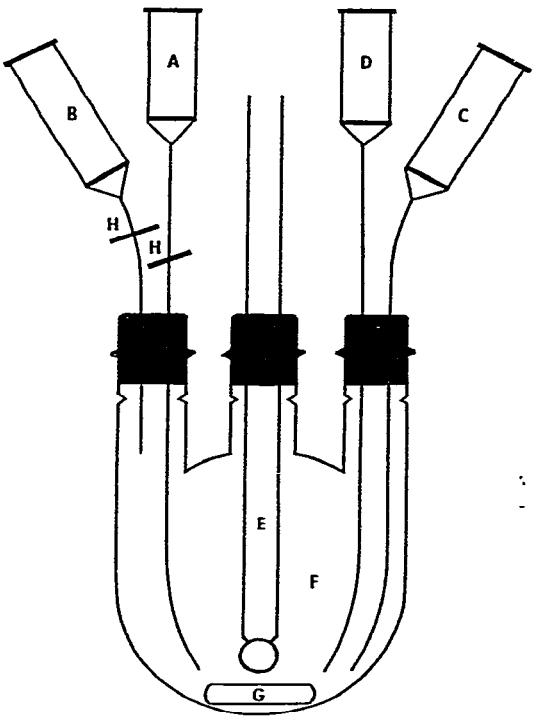

Fig. 1. Reaction vessel for the preparation of aldononitriles. The necks were sealed with rubber stoppers. Inlets for ports A and B were constructed from 18-gauge needles and PE-60 polyethylene tubing (Scientific Products). Inlets for syringes C and D were constructed from 25-gauge needles and PE-20 for finer regulation during additions. Port A contained the reactant aldose solution. Port B was used to withdraw air from the sealed flask. Syringes C and D contained acetic acid (3M) and sodium hydroxide (M) solutions. The entire assembly was immersed in a water bath at 25°, and stirring was provided magnetically. A, port A; B, port B; C, syringe C; D, syringe D; E, pH electrode; F, 25-mL flask; G, stirring bar; and H, pinch clamps.

of 3M acetic acid or M sodium hydroxide from syringes C and D as required. Samples are withdrawn from port A at 10-min time intervals and the extent of reaction determined by g.l.c. After the reaction is complete, the pH is lowered to 4.2 $\pm$0.1 and the contents of the flask are reduced over palladium–barium sulfate.

Concentrations of sodium cyanide and starting aldose, pH requirements, yields and ratios of the epimeric aldononitriles are listed in Table I for specific aldoses. G.l.c. retention-times for the Me_3Si derivatives of aldononitriles relative to that of the D-gluconate derivative are: DL-*glycero* 0.17, D-*erythro* and D-*threo* 0.41, D-*ribo* 0.66, D-*arabino* 0.63, D-*xylo* and D-*lyxo* 0.65, 3,5-*O*-ethylidene-D-*ribo* and *arabino* 0.63, D-*gluco* 0.92, D-*manno* 0.89, D-*galacto* 0.91, D-*talo* 0.87, D-*allo* and D-*altro* 0.89 and 0.90 (not identified), D-*gulo* 0.90, and D-*ido* 0.92.

Catalytic reduction of aldononitriles. — Palladium–barium sulfate (5%, 62 mg per mmol of nitrile) was weighed into a 250-mL reduction flask, 10 mL of water was added, and the suspension was reduced with hydrogen at 4.2 kg/cm^2 (60 lb.in.$^{-2}$) for 10 min with shaking. The solution of the C-2 epimeric aldononitriles at pH 4.2 $\pm$0.1 was transferred to the reduction vessel, which was evacuated twice and then filled with hydrogen to an initial pressure of 4.2 kg/cm^2 (60 lb.in.$^{-2}$). The reduction was allowed to proceed for 2 h at 25° with vigorous agitation, after which time a decrease in hydrogen pressure of 0.14 kg/cm^2 (2 lb.in.$^{-2}$) per mmol of nitrile was observed. After completion of the reaction, the solution was filtered through Celite to remove the catalyst. The pH of the filtrate was 4.8 $\pm$0.2.

Purification of the reduction mixture. — The filtrate containing the reduction products was acidified to pH 2.8 $\pm$0.2 with batchwise addition of Dowex-50 X8 (H^+) to remove amine byproducts. The resin was recovered by filtration and washed with dilute acetic acid, and the filtrate and washings were concentrated to a syrup. Hydrolysis products formed during the preparation of the nitriles were removed by dissolving this syrup in water and adjusting the solution to pH 9.5 with dilute sodium hydroxide. After 30 min at room temperature, the alkaline solution was applied to a column of Dowex-1 X8 (OAc^-) that was eluted with water. The effluent was collected in a flask containing an excess of Dowex-50 X8 (H^+). This solution contained the product aldoses and unreacted starting aldose, which are separable by chromatography on ion-exchange resins[14,15]. Aldonic acid byproducts were recovered from the resin by elution with M acetic acid.

Neutral products were analyzed by ^{13}C-n.m.r. spectroscopy, and reduced with sodium borohydride for g.l.c. analysis of the alditols.

RESULTS AND DISCUSSION

The ^{13}C-n.m.r. spectra obtained at various stages during the synthesis of C_5 aldoses using [^{13}C]cyanide are shown in Fig. 2. The spectra show the composition of a typical reaction-mixture, the products obtained after hydrogenolysis, and the purified products. As only the enriched carbon atom gives an observed resonance, the spectra are simple, and the various products are readily identified on the basis

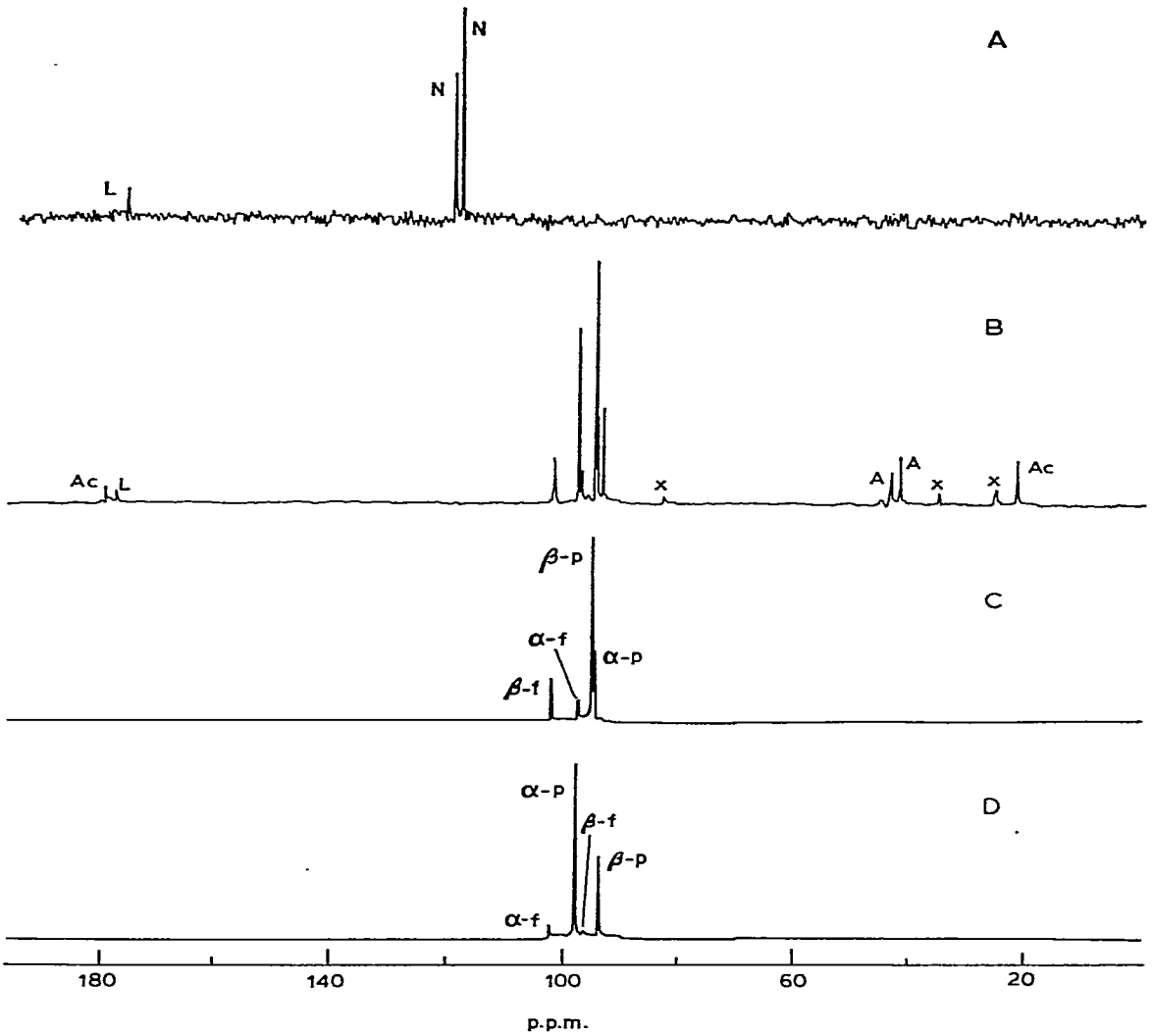

Fig. 2. Proton-decoupled ^{13}C-n.m.r. spectra of the intermediate aldononitriles (A), the reduction products (B), and the purified product aldoses (C and D) in the synthesis of D-[1-^{13}C]ribose and arabinose from D-erythrose and 89.5%-enriched sodium [^{13}C]cyanide. All spectra show only the resonances of the enriched nuclei, unless otherwise indicated. A, Cyanide condensation reaction mixture showing D-[1-^{13}C]ribono- and arabinono-nitriles (N), and D-[1-^{13}C]arabinonolactone (L). B, Products from the hydrogenolysis of the mixture shown in A: Ac = natural abundance resonances of acetic acid, A = [1-^{13}C]aminoalditols, L = [1-^{13}C]arabinonolactone, X = unidentified contaminants; the resonances between 95–103 p.p.m. are due to product [1-^{13}C]aldoses. C and D, product aldoses after chromatography on Dowex-50 ×8 (200–400 mesh) in the barium form[14]: C = D-[1-^{13}C]ribose, D = D-[1-^{13}C]arabinose. Examination of the enriched aldoses by g.l.c. shows these products to be free of D-erythrose. The overall yield of the purified [1-^{13}C]-enriched pentoses, using a 1:1 ratio of reactants, was 78%. Conditions for obtaining ^{13}C-n.m.r. spectra are described in the Experimental section.

TABLE I

SPECIFIC REACTION-CONDITIONS AND RESULTS FOR THE PREPARATION OF ALDONONITRILES[a]

Sugar reactant	*[NaCN]*	*[Sugar]*	*pH*[b]	*Reaction time (min)*	*Percent of nitrile*	*Percent of unreacted sugar*	*Ratio of nitriles*
Glycolaldehyde			8.5	30	95	5	1:1 (N)
D-Glyceraldehyde			8.5	20	95	5	1:1 (N)
D-Erythrose	0.10M	0.10M	9.0	4	95	5	1.4:1 Ara
D-Threose			9.0	7	95	5	1.6:1 Lyx
2,4-*O*-Ethylidene-D-erythrose			8.5	30	95	5	1:1 (N)
D-Arabinose			pH_i = 8.5	25	63	15	1.7:1 Man
			6.3	45			
D-Lyxose			pH_i = 8.4	4			
	0.50M	0.50M	7.6	11	74	11	1.3:1 Tal
			5.6	45			
D-Ribose			pH_i = 7.0	10	77	16	2.9:1 All[c]
			7.6	20			
D-Xylose			pH_i = 7.0	5	80	20	1.5:1 Gul
			7.5	75			
D-Lyxose			7.6	20	88	3	1:1
D-Xylose	1.50M	0.50M	8.0	4	95	5	1.5:1 Gul
D-Ribose			8.0	5	80	5	2:1 All
D-Arabinose			8.0	10	95	5	1.8:1 Man

[a]Percentages and ratios were determined from g.l.c. peak-areas unless the symbol (N) appears with the value. In these cases, determinations were made by ^{13}C-n.m.r. spectral analysis. [b]pH_i = initial pH of NaCN solution; pH adjustments were made as indicated. [c]Assignment is tentative.

of their characteristic chemical-shifts. Although quantitation by ^{13}C-n.m.r. spectroscopy is difficult, these spectra, which were obtained with long delays between pulses to improve quantitation, clearly show that the condensation of cyanide with aldose is essentially complete, and that the crude product is predominantly the desired aldoses. The absence of the starting aldose was shown by g.l.c. analysis of the purified products.

Preparation of aldononitriles. — The addition of cyanide to C_4, C_3, and C_2 aldoses proceeds almost quantitatively at low pH with a 1:1 ratio of reactants, as shown in Table I. Aqueous solutions of the short-chain cyanohydrins at pH 4.3 are relatively stable and can be stored at low temperature (−15°) for extended periods of time.

The complete conversion of C_5 aldoses into aldononitriles by using stoichiometric amounts of cyanide is hindered by an unfavorable equilibrium. With 1:1 ratios of cyanide to aldose, at least 10% of the starting aldose remains unreacted. Use of a threefold excess of cyanide at pH 7.5–8.0 in these reactions produces the aldononitriles in better than 90% yields, and little hydrolysis to the aldonic acids is

TABLE II

ALDOSE YIELDS BASED ON WEIGHTS, BOROHYDRIDE-REDUCTION PRODUCTS, AND RADIOACTIVITY

Starting aldose (10 mmol)	*Ratio of CN^- to sugar*	*Weight after Dowex-50 (g) (theory 1.8 g)*	*Weight after Dowex-1 (g) (theory 1.8 g)*	*Ratio of product aldoses*	*Product sugars in Dowex-1[a] residue (percent)*	*Overall Yield based on total CN^- (percent)*	*Overall Yield based on reacted CN^- (percent)*
D-Arabinose	1:1	1.6 (73)[b]	1.2	2.3:1 Man	70	47	56
D-Lyxose	1:1	1.5	0.97	1.3:1 Tal	75	40	50
D-Ribose	1:1	1.7 (75)[b]	1.4 (68)[b]	2.3:1 All[c]	70	54	65
D-Xylose	1:1	1.5	1.4	1.5:1 Gul	75	58	70
D-Lyxose	3:1	1.7	1.5	1:1	97		85

[a]Values were obtained by reaction of the residue [after Dowex-1 ×8 (OAc^-) treatment] with sodium borohydride. The resulting alditols were determined by g.l.c. [b]Yield of material based on incorporation of [^{14}C]cyanide. [c]Assignment is tentative.

observed under these conditions. When cyanide is the limiting reactant, for example, in the preparation of [^{13}C]-enriched compounds, an excess of cyanide can be used and the unreacted reagent recovered efficiently and almost quantitatively by aerating the acidic mixture (pH 4.2) with nitrogen and trapping the hydrogen cyanide released in 8M alcoholic potassium hydroxide. The nitriles are stable during removal of cyanide.

Reduction of aldononitriles to reducing sugars. — The catalytic hydrogenolysis of C_5 and C_6 aldononitriles proceeds readily to yield the aldoses. Typical yields, determined from product weights and ^{14}C incorporation into products, are presented in Table II.

In reactions using 1:1 ratios of cyanide to C_5 aldose, 10% of the nitriles are converted into aminoalditols, and the extent of nitrile hydrolysis varies between 15 and 20%. The final mixture of compounds, after treatment with Dowex-1 X8 (OAc^-) contains 70–75% product aldose, with an overall yield based on the starting aldose of about 55%. Total incorporation of cyanide is 85% or better; that is, 55% in product aldoses, 20% in aldonic acids, and 10% in aminoalditols, all of which may be readily separated by ion-exchange and recovered.

The overall yield of hexoses from pentoses is greatly improved when a three-fold excess of cyanide is used in the condensation reaction (Table I). Any residual cyanide is reduced to methylamine or formaldehyde, both of which are separated readily from the desired products. D-Galactose and D-talose have been prepared in 85% yield, based on D-lyxose, and the excess of cyanide was recovered almost quantitatively. This method has been used for the preparation of [1-^{13}C]-enriched hexoses, whereas [1-^{13}C]-enriched C_5 (Fig. 2), C_4, and C_3 aldoses were prepared with 1:1 reactant ratios. [1-^{13}C]-Enriched aldoses prepared by these methods include

D-glucose, D-mannose, D-galactose, D-talose, D-allose, D-altrose, D-idose, D-gulose, D-lyxose, D-xylose, D-ribose, D-arabinose, D-erythrose, D-threose, and DL-glyceraldehyde. D-[2-^{13}C]Glucose and D-[2-^{13}C]mannose have been prepared by the serial application of the method. These and related enriched compounds will be used to evaluate chemical shifts and coupling constants and their relationship to carbohydrate conformation in aqueous solution[16].

Blocking groups that restrict the formation of cyclic intermediates may determine the extent of reduction to aminoalditols. The hydrogenolysis of 3,5-*O*-ethylidene-D-ribono- and arabinononitriles have yielded, in addition to 3,5-*O*-ethylidene-D-ribose and D-arabinose, larger percentages (>40%) of amines than the unblocked homologues. The C_3 and C_4 nitriles, in which cyclization is prevented or slow[10], are reduced at pH 4.2 $\pm$0.1 to the corresponding aldoses, although the extent of amine formation in these cases is greater than 50%. Further studies of the factors regulating aminoalditol formation during hydrogenolysis are in progress[17].

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