SYNTHESES WITH STABLE ISOTOPES: D-GLUCOSE-6-^{13}C AND 1,6-ANHYDRO-β-L-IDOPYRANOSE-6-^{13}C

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SUMMARY

The synthesis of a mixture of D-glucose-6-^{13}C and 1,6-anhydro-β-L-idopyranose-6-^{13}C and their resolution by column chromatography are described. The Kiliani reaction of hydrogen cyanide-^{13}C with 5-aldo-1,2-O-isopropylidene-D-xylo-pentofuranose afforded epimeric cyanohydrins. Rapid *in situ* hydrolysis of the nitriles was effected at 50°C to obtain a mixture of the corresponding alduronic acids. The latter were reduced with $LiAlH_4$. Hydrolysis of the isopropylidene groups with trifluoroacetic acid gave a mixture of glucose and anhydroidose. After chromatographic separation, the labeled carbohydrates were obtained in yields of 48% and 25%, respectively. Results of a ^{13}C NMR study of the Kiliani cyanohydrin reaction are also reported.

Key words: D-glucose-6-^{13}C, carbon-13, ^{13}C NMR, Kiliani reaction, carbohydrates

INTRODUCTION

A method for the synthesis of D-glucose labeled with isotopic carbon at the 6-position was first described by Sowden[1]. In Sowden's procedure, the labeling pattern is established by the reaction of isotopic cyanide with 5-aldo-1,2-O-isopropylidene-D-xylo-pentofuranose (1). After hydrolysis of the epimeric nitriles obtained in the cyanohydrin reaction, Sowden isolated the isopropylidene-glucuronic acid epimer which was subsequently lactonized, reduced, and hydrolyzed to afford D-glucose-6-^{14}C. Shafizadeh and Wolfrom[2] modified Sowden's method by lactonizing the mixture of epimeric alduronic acids and separating 1,2-O-isopropylidene-D-glucurono-γ-lactone from 1,2-O-isoproplidene-L-idurono-γ-lactone by column chromatography. Modifications of Sowden's method have also been reported by Schaffer and Isbell[3]. Separation of the isopropylidene derivative

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of D-glucuronic acid was effected by crystallization of the barium salt.* It was also shown that the pH of the cyanohydrin-forming reaction affected the epimer distribution with the highest yield of the D-glucurono epimer being formed in acidic solution. Advantages in using crystalline 1 were noted.

We have employed Sowden's method for a synthesis of D-glucose-6-^{13}C and have developed other modifications which simplify and further improve the synthesis. We have also conducted a ^{13}C NMR study of the reaction of sodium cyanide-^{13}C with the aldehydo sugar 1.

RESULTS AND DISCUSSION

The Kiliani reaction of sodium cyanide with 5-aldo-1,2-O-isopropylidene-D-xylo-pentofuranose (1) proceeds with in situ hydrolysis of the initially formed cyanohydrins; the product being a mixture of epimeric isopropylidenealduronic acids having D-glucurono and L-idurono configurations. We have conducted reactions between 1 and sodium cyanide-^{13}C in NMR sample tubes and monitored the evolving reactions by ^{13}C NMR spectroscopy. Several techniques were employed to identify species present in the reactions: observation of ^{13}C chemical shifts; observation of ^{13}C-^{15}N couplings in reactions conducted with sodium cyanide-^{13}C-^{15}N; and addition of authentic compounds to reaction solutions at appropriate times. The chemical shifts of the C-6 resonances for the species identified in the reactions are given in Table I. Scheme I shows the course of the Kiliani reaction and gives the structures of the various intermediates that we have observed.

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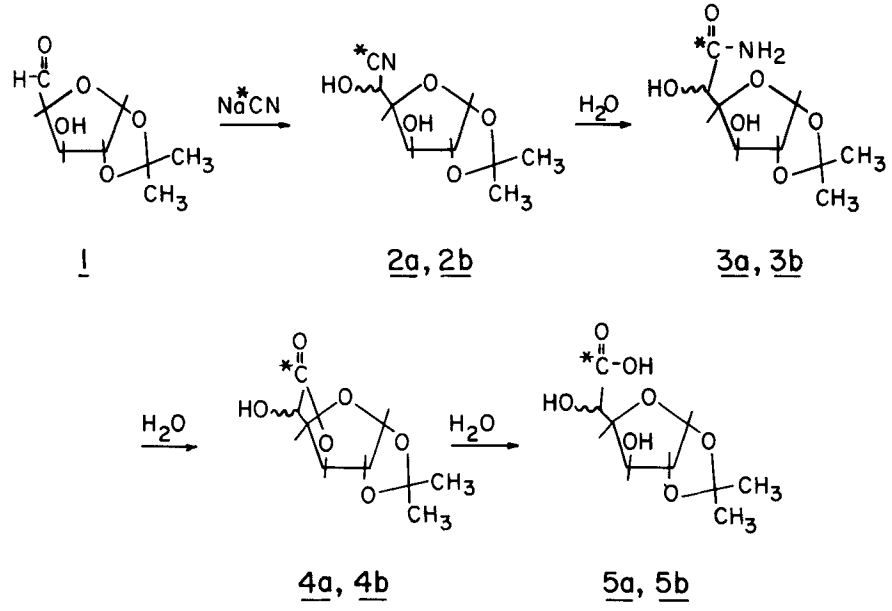
*In a subsequent paper [Schaffer R. and Isbell H. S. - J. Am. Chem. Soc. 79: 3867 (1957)], it was shown that the L-idurono epimer could best be purified as the calcium salt.

Table I. ^{13}C Chemical Shifts and ^{13}C-^{15}N Coupling Constants for C-6 Resonances Observed in the Kiliani Reaction of 1 and $Na^{13}CN$ or $Na^{13}C^{15}N$.

Species[a]	Chemical Shift (ppm)	$^1J_{CN}$(Hz)
1,2-IP-D-glucurononitrile (2a)	120.2	16.6
1,2-IP-L-iduronononitrile (2b)	119.1	16.1
1,2-IP-D-glucuronamide (3a)	178.0	17.6
1,2-IP-L-iduronamide (3b)	177.2	16.9
1,2-IP-D-glucurono-3,6-lactone (4a)	177.5	-
1,2-IP-L-idurono-3,6-lactone (4b)	177.5	-
Sodium 1,2-IP-D-glucuronate	179.0[b]	-
Sodium 1,2-IP-L-iduronate	178.5[b]	-

[a] 1,2-IP = 1,2-O-isopropylidene [b] pH = 4.5-11.0

SCHEME I



In their study of the cyanohydrin reaction, Schaffer and Isbell[3] found that the optimum yield (58%) of 1,2-O-isopropylidene-D-glucuronate was obtained in an acetic acid-sodium acetate buffer (pH 4.5). Unfortunately, the reaction was slow at this pH and required 25 days at 25-30°C to reach completion. When the reaction was conducted in a sodium bicarbonate-sodium carbonate buffer the reaction was faster (5 days at 7-9°C and 3 hours at 100°C); however, the yield of the glucuronate fell to 27%. Our ^{13}C NMR study of the Kiliani reaction began with a reaction conducted at room temperature in a pH 4.5 acetate buffer (Table II, experiment #1). Under these conditions cyanide addition was rapid, being 96%

Table II. Effect of Temperature and pH on Rate of Reaction and Ratio of Products in Kiliani Reaction of 1 with $Na^{13}CN$.

Exp. No.	Cyanohydrin Reaction pH[a]	Hydrolysis Conditions				Product Ratio[c]
		Initial pH[b]	Final pH	Temp.	Time	
1	4.7	-	4.5	RT	35 d	60:40[d]
2	4.7	7.9	5.9	RT	64 d	58:42[e]
3	4.5	9.8	9.2	RT	8 d	48:52
4	4.5	11.0	10.6	RT	23 d	46:54
5	4.5	7.5	6.3	50-51°C	105h	52:48[f]
6	4.5	-	4.5	48-50°C	76 h	60:40[g]

[a]Cyanohydrin reaction run for 24h at room temperature (RT) [b]Adjusted with NaOH [c]Ratio of D-glucurono to L-idurono species, determined from ^{13}C NMR peak areas, expressed as 1,2-O-isopropylidene-D-glucuronate to 1,2-O-isopropylidene-L-iduronate unless otherwise noted [d]Includes 6% 1,2-IP-D-glucuronolactone [e]Includes 10% 1,2-IP-D-glucuronamide [f]Includes 9% 1,2-IP-D-glucuronamide [g]Includes 10% 1,2-IP-D-glucuronolactone.

complete in 6 hours. The two cyanohydrins 2a and 2b (in a ratio of 63:37) and a small lactone resonance (probably of the idurono epimer) were present at this time. As the hydrolysis proceeded at room temperature and pH 4.5, resonances from the two alduronic acids 5a and 5b appeared and the lactone resonance

persisted. The coincidence of both lactones, 4a and 4b, in the 177.5 ppm resonance was shown by addition of authentic compounds. About 4% of the glucurononitrile 2a remained after 20 days, but was not observed after 26 days. A small amount of the glucuronolactone 4a did not diminish after 29 days. The final epimer ratio of 60:40 agrees with the Schaffer and Isbell result.

Since our first experiment showed that cyanohydrin formation was rapid and hydrolysis of the nitriles was slow, we conducted experiments (entries 2,3, and 4 in Table II) in which the pH was raised after cyanide had been consumed in order to accelerate the rate of hydrolysis. Under slightly alkaline conditions (experiment 2), the hydrolysis was still slow, but raising the pH did increase the rate of hydrolysis dramatically (experiments 3 and 4). Unfortunately, in the alkaline hydrolysis the ratio of glucurono epimer to idurono epimer in the product alduronates decreased from the ratio established in the cyanohydrin-forming reaction. The alteration of the epimer ratio probably results from several competing reactions. In their study of the hydrolysis of D-glucono-nitrile-1-^{13}C, Blazer and Whaley[4] found reversibility of the cyanohydrin-forming reaction over a pH range of 5.1 to 11.6 as well as deprotonation (and thus possible epimerization) of most of the hydrolysis intermediates over the pH range of 6.9 to 11.6.

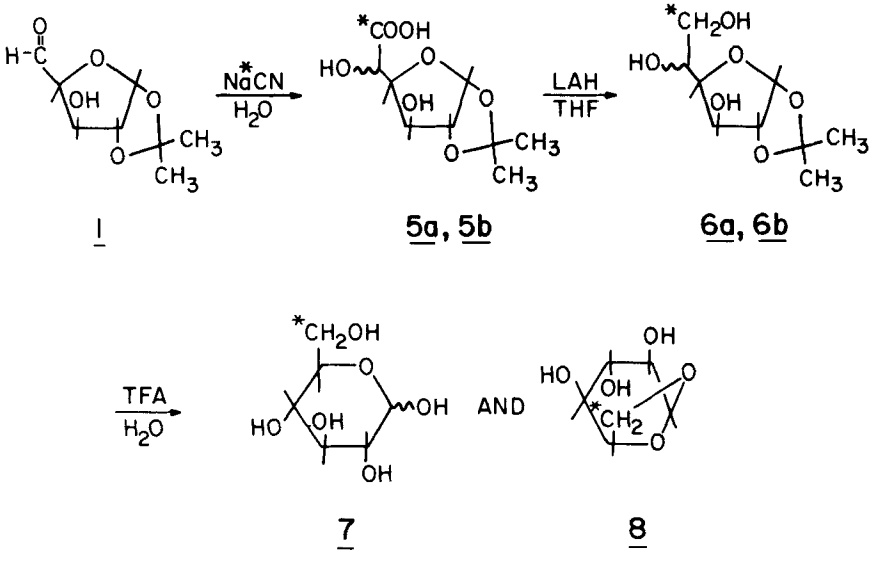
The course of the hydrolysis of the nitriles 2a and 2b in basic solution differed from that observed in acid medium. In contrast to the acid hydrolysis where the nitriles persist, the basic hydrolysis of 2a and 2b was rapid. For example, at pH 7.9 and 25°C both nitriles had disappeared in less than 4 hours. In the basic hydrolysis of the nitriles, the amides 3a and 3b were seen as prominant intermediates. Amides were not observed in the course of the acid hydrolysis. Basic hydrolysis of the amides was the rate-limiting step in the overall process.

The effect of temperature on the rate of hydrolysis was also investigated. At a slightly alkaline pH, the hydrolysis was much more rapid at 50°C than at room temperature (cf. 105 h vs. 64 d in Table II). A slight decrease in the glucurono to idurono epimer ratio was still observed. Elevated temperatures were

also found to increase the rate of hydrolysis in acid solution (experiment 6), and in this case the epimer ratio was not adversely affected. A similar set of conditions was used in the synthesis of D-glucose-6-^{13}C.

The series of reactions employed for the synthesis of D-glucose-6-^{13}C is shown in the Scheme II. Our strategy was to conduct the entire sequence of reactions without purification or separation of intermediate products and then to separate the glucose and idose mixture thus obtained. Our previous experience[5,6] in using the chromatographic system of Jones and Wall[7] for preparative-scale separations of carbohydrates suggested this to be a reasonable approach.

SCHEME II



The starting material, 5-aldo-1,2-O-isopropylidene-D-xylo-pentofuranose (1) was prepared by the metaperiodate oxidation[3,8] of 1,2-O-isopropylidene-α-D-glucofuranose. Schaffer and Isbell[3] have shown that it is desirable to purify 1 by crystallization in order to eliminate formaldehyde which consumes cyanide in the cyanohydrin reaction. We have found that partial purification of 1 (which for many years was known only as a syrup) by column chromatography

(Dowex 50, Ba^{++}) greatly facilitates its crystallization from water. Schaffer and Isbell have shown[9] that crystalline 1 exists as a dimeric 5,5':3',5-cyclic acetal. We have observed the ^{13}C-NMR spectrum of 1 in solution. In anhydrous DMSO, only the dimer is present while in an aqueous solution of 1 only the monomer is observed.

The cyanohydrin reaction of 1 and $K^{13}CN$ was conducted at pH 4.5 and 25°C. After 16 hours ^{13}C NMR indicated that all of the $K^{13}CN$ had been utilized. Small amounts of iduronolactone 4b and iduronic acid 5b were present at this time. Hydrolysis was effected by heating the reaction at 50°C for 31 hours, at which time glucuronic acid 5a, iduronic acid 5b, and glucuronolactone 4a were present. Removal of cations and evaporation of solvent gave a product (presumably an alduronic acid-alduronolactone mixture) that could be reduced directly with $LiAlH_4$ in THF to afford a mixture of 1,2-O-isopropylidene-D-glucofuranose-6-^{13}C (6a) and 1,2-O-isopropylidene-L-idofuranose-6-^{13}C (6b); lactonization[1-3,10] prior to reduction is unnecessary. Hydrolysis of the isopropylidene groups in the 6a-6b mixture was accomplished with aqueous trifluoroacetic acid to yield a mixture of D-glucose-6-^{13}C (7, as a mixture of α and β anomers) and 1,6-anhydro-β-L-idopyranose-6-^{13}C (8). This product mixture was separated into 7 and 8 by column chromatography (Dowex 50, Ba^{++}). The yields of 7 and 8 were 48% and 25%, respectively. Presumably, the hydrolysis of the isopropylideneidose 6b to the anhydro sugar proceeds through the intermediacy of L-idose-6-^{13}C. Other workers[11,12] have reported that the hydrolysis of isopropylideneidofuranose in dilute sulfuric acid gives idose, but the product is known only as a syrup. Our ^{13}C NMR observations of the hydrolysis of 6b with trifluoroacetic acid showed a decrease in the 63.4 ppm resonance (6b) with a concomitant appearance and increase in a 65.6 ppm resonance (8). No major intermediate peaks were observed.

EXPERIMENTAL

Materials and Methods--1,2-O-Isopropylidene-α-D-glucofuranose was prepared from α-D-glucose by a published procedure[13]. Sodium cyanide-^{13}C[14] and sodium cyanide-^{13}C-^{15}N[15] were prepared in this laboratory. ^{13}C NMR spectra were recorded using a Varian Model CFT-20 spectrometer. Chemical shifts were referenced to solvent DMSO-d_6 (39.6 ppm) or dioxane (10 vol.% in D_2O, coaxial tube, 67.4 ppm) and are reported relative to TMS. Optical rotations were obtained with a Rudolph Research Autopol III polarimeter. Melting points were observed on a Fischer-Johns apparatus and are uncorrected.

Preparation of Authentic Samples for NMR Studies--A cyanohydrin reaction between 1 and $Na^{13}CN$ was conducted and barium 1,2-O-isopropylidene-D-glucuronate-6-^{13}C and calcium 1,2-O-isopropylidene-L-iduronate-6-^{13}C were isolated as described by Schaffer and Isbell[16]. The acids 5a and 5b were obtained by treatment of the corresponding salts with Dowex 50-X4(H^+). Acid 5a was crystallized from EtOAc; mp 146-147°C (reported[17] mp 147°C). Acid 5b was crystallized from EtOH; mp 136-137°C (slow heating, lactonizes prior to melting), mp 150-151°C (stage preheated to 150°C). The lactones 4a and 4b were prepared from the acids 5a and 5b by heating in toluene. Lactone 4a was crystallized from EtOH-C_6H_6; mp 119-120°C (reported[18] 120°C); $[\alpha]_D^{25}$ = +68° (c 1.0, MeOH) [(reported[18] $[\alpha]_D^{25}$ = +70° (c 1.0, MeOH)]. Lactone 4b was crystallized from C_6H_6; mp 135-136°C (reported[8] 137-138°C); $[\alpha]_D^{25}$ = +99.6° (c 0.98, MeOH) [reported[8] $[\alpha]_D^{18}$ = +100° (c 2, acetone)]. Amides 3a and 3b were prepared from the lactones 4a and 4b by treatment with NH_3-EtOH. Amide 3a was crystallized from EtOH; mp 164°C (dec.) [reported[18] 164° (dec.)]; $[\alpha]_D^{27}$ = -11.8° (c 1.1, H_2O) (reported[18] $[\alpha]_D^{18}$ = -14° (c 0.9, H_2O). Amide 3b was crystallized from EtOH; mp 137-138°C (dec.); $[\alpha]_D^{25}$ = + 9.2 (c 1.0, MeOH); $C_9H_{15}NO_6$ (93 mol% ^{13}C at C-6) requires C 46.56 H 6.46, N 5.98, found C 46.81, H 6.71, N 5.73.

5-Aldo-1,2-O-isopropylidene-D-xylo-pentofuranose (1)--1,2-O-Isopropyldene-α-D-glucofuranose (152.9g, 0.694 mol) was oxidized with $NaIO_4$ (152.9 g, 0.715 mol) according to the procedure of Schaffer and Isbell[3]. The crude product was obtained as a syrup. Partial purification of the product was accomplished by chromatography on a Dowex 50-X8 (Ba^{++}, 200-400 mesh) column (4.4 x 150 cm). Aliquots of an aqueous solution of the crude product (ca. 10 g in 28 mL), were applied to the column and eluted with H_2O (flow rate 0.7 mL/min., ca. 17 mL fractions). The following fractions were collected: I 49-64; II 65-78; III 79-89; IV 90-104; V 105-115; VI 116-170. Inorganic ions were present in fraction I and ethylene glycol was present in IV. ^{13}C NMR spectra of III, IV, V, and VI were quite similar with 1 being the major component. Material from III crystallized readily from H_2O (2.4 mL/g) as a hydrate, mp 173-175°C. A total of 90.6 g (68%) of 1 was obtained from fractions III-VI from all column runs. Recrystallization from 3-pentanone gave 1 as an anhydrous dimer; mp 196-197°C (reported[3] 182-184°C); ^{13}C NMR (DMSO-d_6):111.1, 110.8, 104.8, 89.3, 88.8, 84.5, 83.0, 80.2, 75.9, 74.4, 73.3, 26.7, 26.5, 26.1 ppm; ^{13}C NMR (D_2O):113.6, 105.5, 89.0, 85.5, 83.6, 74.4, 26.5, 26.0 ppm.

D-Glucose-6-^{13}C (7) and 1,6-Anhydro-β-L-idopyranose-6-^{13}C (8)--A solution of 1 (6.21 g, 33.0 mmol) in H_2O (198 mL), 1N acetic acid (120 mL), 1N NaOH (30 mL), and $K^{13}CN$ (1.995 g, 99.3% KCN, 90.7 mol% ^{13}C, 30.0 mmol) were added to a flask containing a magnetic stirring bar. The flask was tightly stoppered and the solution was stirred at 25°C for 16 h at which time ^{13}C NMR indicated that the cyanide had been consumed. To effect hydrolysis, the solution was heated at 50°C for 31 h. Cations were removed by passing the solution through a Dowex 50-X4 (H^+) column (3.4 x 33 cm, ca. 300 meq). The column was washed with deionized H_2O until a total of 1 L of eluate had been collected. This solution was rotary evaporated to a syrup which was dissolved in absolute alcohol (100 mL), filtered, and evaporated. Dissolution in absolute alcohol and evaporation was repeated twice. Reduced pressure was maintained until the residue became a friable, dry froth which weighed 6.95 g.

This mixture of alduronic acids was dissolved in THF (100 mL) and the solution was filtered through a bed of Celite to remove a fine precipitate. The filtrate was

evaporated and redissolved in THF (100 mL). This solution was added dropwise at room temperature over a period of 1.3 h to a stirred suspension of $LiAlH_4$ (4.55 g, 0.12 mol) in THF (200 mL) under an atmosphere of dry N_2. After the addition was complete, stirring at room temperature was continued for 6 h. With stirring and cooling in an ice bath, the following were cautiously added: H_2O (5 mL) in THF (10 mL), 15% NaOH (4.6 mL) and H_2O (15 mL). The mixture was allowed to warm to room temperature and stirring was continued until H_2 evolution ceased. The mixture was filtered through a bed of Celite in a fritted funnel. All of the precipitated material was transfered to the funnel by washing with MeOH. The precipitate in the funnel was thoroughly washed by repeated trituration with H_2O. Rotary evaporation of MeOH and THF from the filtrate gave a pH 11.4 aqueous solution of the isopropylidenealdoses. The pH of this solution was adjusted to 3.3 by the addition of Dowex 50-X4 (H^+). After filtering and thoroughly washing the resin, the filtrate was evaporated; the residue, after drying at 50°C under reduced pressure, weighed 5.83 g. ^{13}C NMR peak areas indicated the ratio of 6a (64.4 ppm) to 6b (63.4 ppm) to be 58:42.

The above mixture of 6a and 6b was dissolved in 0.5N aqueous CF_3COOH (100 mL) and heated at reflux for 3.5 h. After cooling, the solution was evaporated to a syrup. The syrup was dissolved in water and filtered. The filtrate was reduced in volume to about 12 mL. This solution was applied to a Dowex 50-X8 (Ba^{++}, 200-400 mesh) column (4.4 x 150 cm) and products were eluted with deionized H_2O (flow rate 0.75-0.8 mL/min, ca. 15-16 mL fractions). D-Glucose-6-^{13}C was collected in fractions 77-91; yield 2.61 g (48% from $K^{13}CN$); after crystallization from H_2O-EtOH, mp 150-151°C, $[\alpha]_D^{25}$ = +51.6° (c 10, H_2O) [reported[19] mp 146°C, $[\alpha]_D^{25}$ = +52.7°]; ^{13}C NMR (H_2O) 61.6 and 61.8 ppm. 1,6-Anhydro-β-L-idopyranose-6-^{13}C was collected in fractions 99-111; yield 1.21 g (25% from $K^{13}CN$); after crystallization from 3-pentanone, mp 127-128°C, $[\alpha]_D^{25}$ = +114° (c 1.18, acetone) [reported[20] mp 128-129°C, $[\alpha]_D^{23}$ = +113° (c 1.2, acetone); ^{13}C NMR (H_2O) 65.6 ppm.

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