MECHANISM OF THE CYANOHYDRIN (KILIANI–FISCHER) SYNTHESIS*

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(Received April 11th, 1972; accepted May 27th, 1972)

ABSTRACT

Polarimetry, paper chromatography, gas–liquid chromatography, and infrared and nuclear magnetic resonance spectroscopy have been used in the elucidation of the mechanism of the cyanohydrin synthesis as applied to D-arabinose. D-Arabinose was allowed to react with various proportions of potassium cyanide in aqueous sodium carbonate, and various compounds formed in the reaction mixture were identified. Polarimetry indicated that the cyanohydrin synthesis involves at least two steps: presumably, a condensation and a hydrolysis. Paper and gas–liquid chromatography showed the presence of D-gluconate, D-mannonate, D-gluconolactone, D-mannonolactone, D-gluconamide, D-mannonamide, D-gluconoimidolactone, D-mannonoimidolactone, D-gluconononitrile, D-mannononitrile, and unchanged D-arabinose. The main pathway suggested by the time course of the intermediates is: D-arabinose → D-hexononitrile (cyanohydrin) → D-hexonoimidolactone → D-hexonolactone → D-hexonic acid. The acyclic hexonamide, usually postulated as the initial hydrolysis product of the cyanohydrin, is probably only a side product that is in equilibrium with the cyclic hexonoimidolactone.

INTRODUCTION

The fundamental problem of the mechanism of the cyanohydrin synthesis (Kiliani–Fischer synthesis) has not yet been completely solved, although it is the most common method of increasing the length of the carbon chain of sugars. Numerous ^{14}C-labeled sugars have been subjected to this synthesis for use of the products in the fields of enzymology, biochemistry, and nutrition. Isbell *et al.*[1] synthesized D-glucose-*1*-^{14}C and D-mannose-*1*-^{14}C by starting with D-arabinose and radioactive sodium cyanide, but they were interested only in isolating the final products. This paper reports the use of polarimetry, paper chromatography, and gas–liquid chromatography (g.l.c.) in

*Presented at the 160th National Meeting of the American Chemical Society, Chicago, Illinois, September 14–18 (1970), Abstract No. CARB-47.

the elucidation of the mechanism of the cyanohydrin synthesis as applied to D-arabinose.

RESULTS AND DISCUSSION

Isbell *et al.*[1] conducted a series of condensations in which various proportions of cyanide were used, and they showed, by titration of the excess of cyanide, that the condensation is almost quantitative under a variety of conditions. Their studies included examination of the reaction of sodium cyanide in the presence of calcium chloride[2], ammonium chloride[3], or various proportions of sodium carbonate and sodium hydrogen carbonate, which act as buffers. Literature reports of low yields of the products isolated from the cyanohydrin synthesis were shown not to be due to a partial reversal of the condensation reaction during hydrolysis, because the cyanohydrins from D-arabinose gave an almost quantitative yield of ammonia on heating in alkaline solution. Isbell *et al.*[1] also showed that the proportions of the epimers formed in the cyanohydrin synthesis depend, in part, on the conditions under which the reaction is performed. Thus, in the condensation of D-arabinose with cyanide, the ratio of D-gluconic acid to D-mannonic acid could be varied from 18:7 to 3:7 by alteration of the experimental conditions.

Re-investigation of the mechanism of the cyanohydrin synthesis originated when a research worker in Dr. French's laboratory attempted to label the reducing ends of macrodextrins by using ^{14}C-labeled potassium cyanide, and obtained on paper

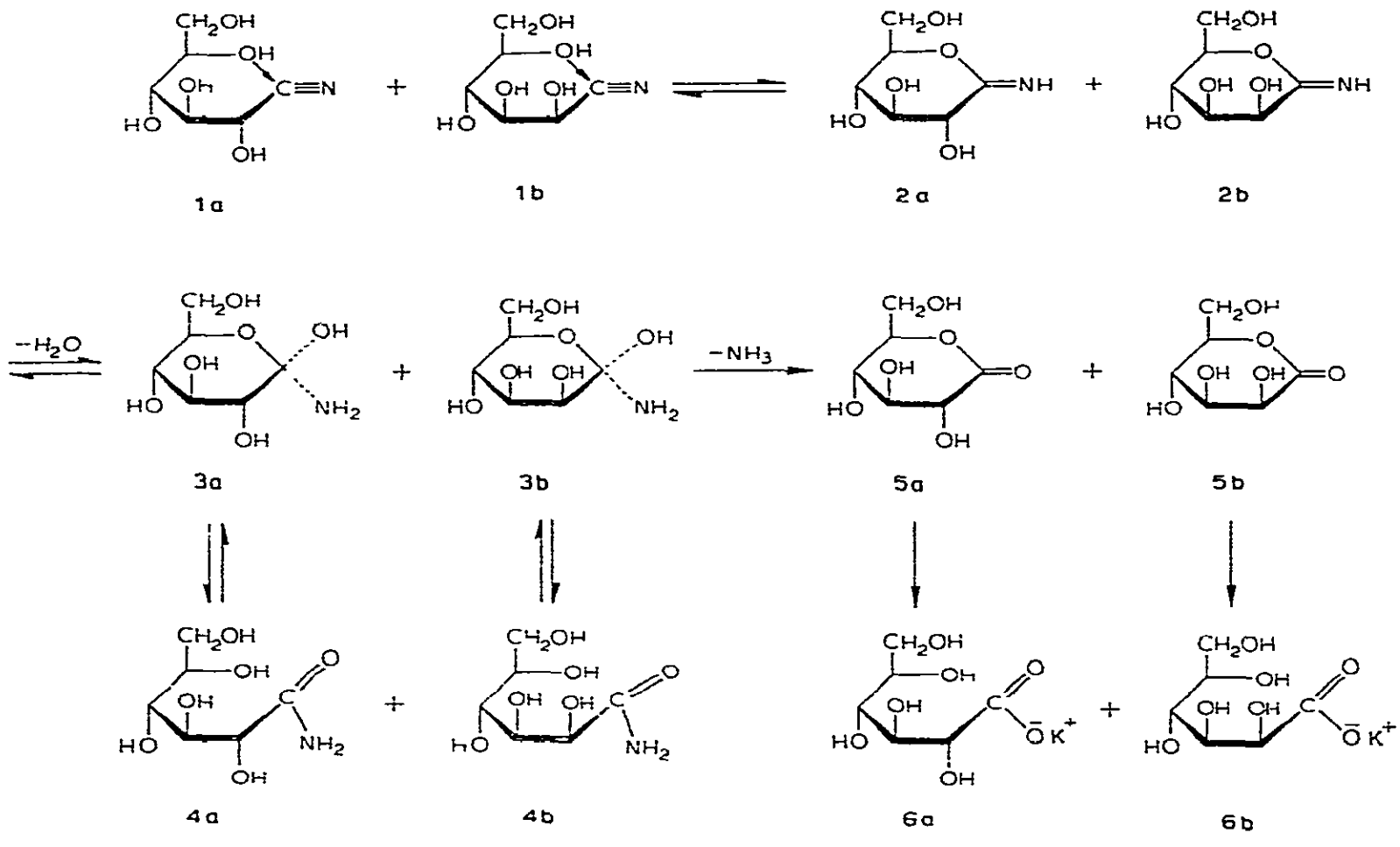

Fig. 1. Mechanism of Kiliani–Fischer cyanohydrin synthesis with α-D-arabinose.

chromatograms several spots that could not be identified. In the present study, α-D-arabinose was condensed with potassium cyanide in the presence of sodium carbonate. Various proportions of potassium cyanide were used, and the compounds present at the elapse of various intervals of time were identified by g.l.c. of the trimethylsilyl derivatives. The maximal amounts of the α- and β-cyanohydrins of D-glucose and D-mannose were formed in 20–25 min by the condensation of 66mM α-D-arabinose with 68mM potassium cyanide in the presence of anhydrous sodium carbonate (22mM). Most of the compounds that are intermediates in the production of potassium D-gluconate (**6a**) and potassium D-mannonate (**6b**) (see Fig. 1) were identified.

Polarimetry was first used to study the course of the reaction. The change, with time, in the specific rotation of aqueous reaction-mixtures in which the concentrations of D-arabinose and sodium carbonate were fixed and that of potassium cyanide was varied was determined at 25° (see Table I). There was a large change in the specific rotation of the reaction mixture during the first two to three hours, and, from then on, the change was fairly gradual; the same final value of the specific rotation was attained, regardless of the concentration of potassium cyanide employed.

TABLE I

POLARIMETRIC DATA FOR AQUEOUS, CYANOHYDRIN REACTION-MIXTURES[a]

A[b]		*B*[b]		*C*[b]		*D*[b]	
Time (min)	$[\alpha]_D^{25}$ *(degrees)*	*Time (min)*	$[\alpha]_D^{25}$ *(degrees)*	*Time (min)*	$[\alpha]_D^{25}$ *(degrees)*	*Time (min)*	$[\alpha]_D^{25}$ *(degrees)*
8	−19.4	8	−38.2	8	−51.8	8	−67.1
18	−16.8	30	−24.8	60	−30.4	30	−60.1
48	−3.8	60	−11.3	120	−12.8	125	−33.0
120	+5.8	90	−6.2	170	−0.055	235	−19.8
205	+9.5	185	+6.2	260	+1.1	355	−11.0
265	+11.0	310	+11.0	460	+9.0	510	−5.5
420	+11.0	355	+11.3	515	+9.9	1160	+6.4
1405	+11.0	1365	+11.3	630	+11.0	1640	+11.0
				1365	+11.0	1800	+11.0

[a]Concentration of D-arabinose, 0.5 g per 100 ml; of sodium carbonate, 1.770 g per 100 ml. [b]Grams of potassium cyanide per 100 ml: A, 1.820; B, 0.910; C, 0.405; and D, 0.225.

Paper chromatography was next used, for identification of the intermediate products and the potassium salts (**6a** and **6b**) of the two epimeric hexonic acids. A large excess of potassium cyanide and sodium carbonate could not be used, as this caused streaking on the paper chromatograms. Reasonably good separation was obtained when an equimolar proportion of sodium carbonate and a slight excess of potassium cyanide were used at 25°. Aliquots were withdrawn at different time-intervals and spotted on a paper chromatogram; this was then subjected to four ascents in 3:2:2 pyridine–butanol–water. The spots were made visible by Trevelyan's

procedure[4]. Some seven to eight spots (see Fig. 2) were obtained, and most of them were identified by comparison with standard compounds. The slowest-moving component was potassium D-gluconate (**6a**), and potassium D-mannonate (**6b**) ran very close to it. The next (elongated) spot was due to a mixture of D-gluconolactone (**5a**) and D-mannonolactone (**5b**). It could not be ascertained whether they were the 1,4- or the 1,5-lactones; use of g.l.c. did not clarify this point. However, D-gluconolactone moves slightly more slowly than D-mannonolactone. D-Gluconamide (**4a**) and D-mannonamide (**4b**) were not separable on paper. The spot above that for **4a** plus **4b**

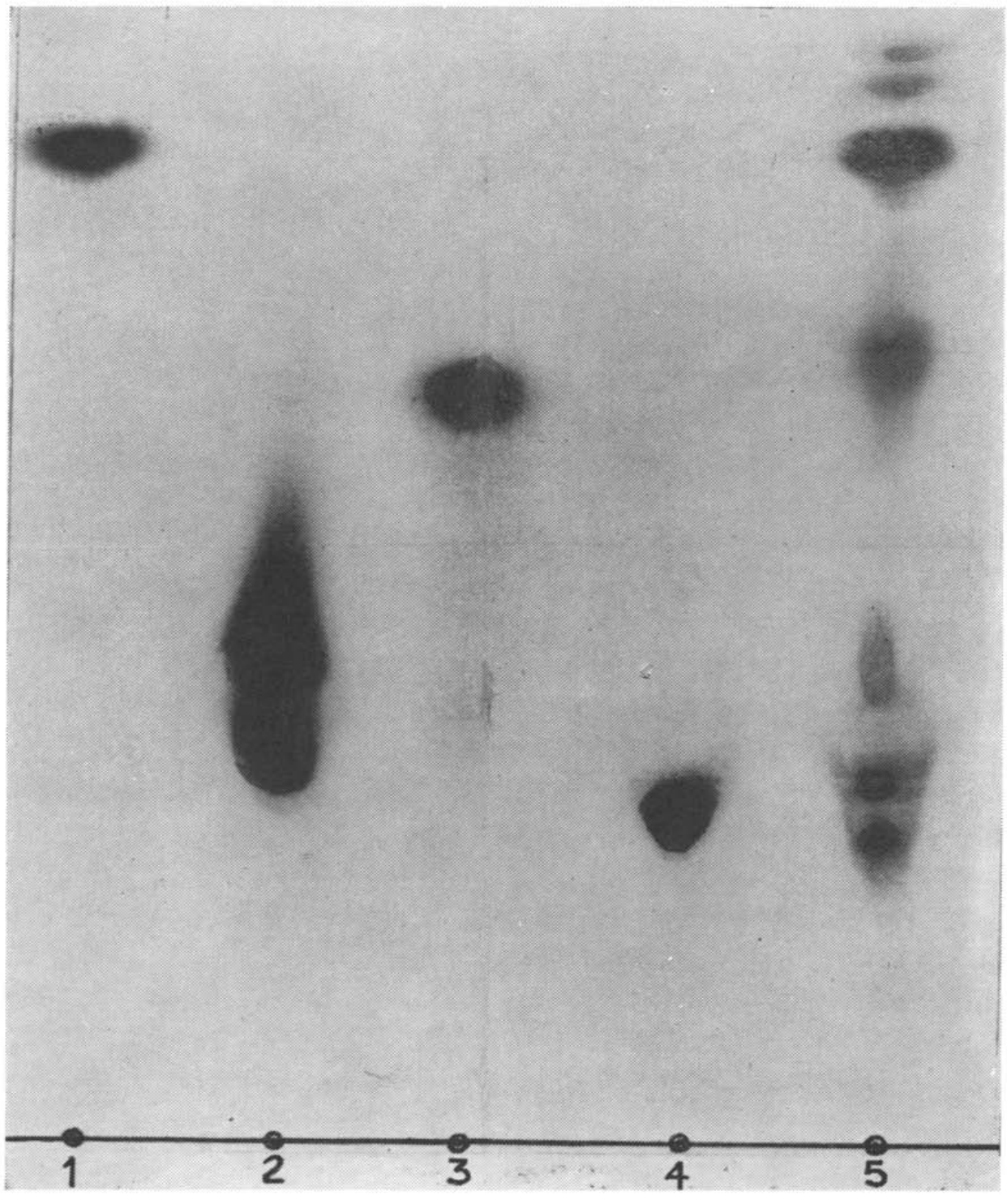

Fig. 2. Paper chromatogram of the reference compounds (lanes 1, 2, 3, and 4) and the cyanohydrin reaction-mixture after 25 min (lane 5). Spots (from top to bottom): lane 1, D-arabinose; lane 2, D-mannono-1,5-lactone, D-glucono-1,5-lactone; lane 3, D-gluconamide; lane 4, sodium D-gluconate; lane 5, D-mannono- and D-glucono-imidolactones, D-mannono- and D-glucono-nitriles, D-arabinose, D-mannonamide and D-gluconamide, D-mannono- and D-glucono-lactones, potassium D-mannonate, potassium D-gluconate.

was due to unchanged D-arabinose. An attempt was made to identify the next two spots, although no standard compounds were available; the faster-moving of these spots may contain a mixture of D-gluconoimidolactone (**2a**) and D-mannonoimidolactone (**2b**). The spot just behind it may be due to a mixture of D-glucononitrile (**1a**) and D-mannononitrile (**1b**). Attempts were made to quench the reaction at fixed time-intervals by using a mixed-bed, ion-exchange resin (Amberlite MB-3); however, this also removed the D-gluconic acid and D-mannonic acid. When Amberlite IR-120 (H^+) ion-exchange resin was used, the cyanohydrins were hydrolyzed to the corresponding two epimeric acids. Spotting on paper, and drying by evaporation under diminished pressure, were the only two satisfactory methods of stopping the reaction at any chosen time.

G.l.c. of the per(trimethylsilyl) derivatives of the compounds in the reaction mixture was performed at 180°. The concentration of the reaction mixture was the same as that used for paper chromatography. A suitable aliquot of reaction solution was evaporated to dryness under diminished pressure at 25° after a suitable time-interval. The per(trimethylsilyl) derivatives of the various samples were prepared as described in the Experimental section. The per(trimethylsilyl) ether of *myo*-inositol was used as the internal standard. Nine peaks (see Fig. 3) were observed after the

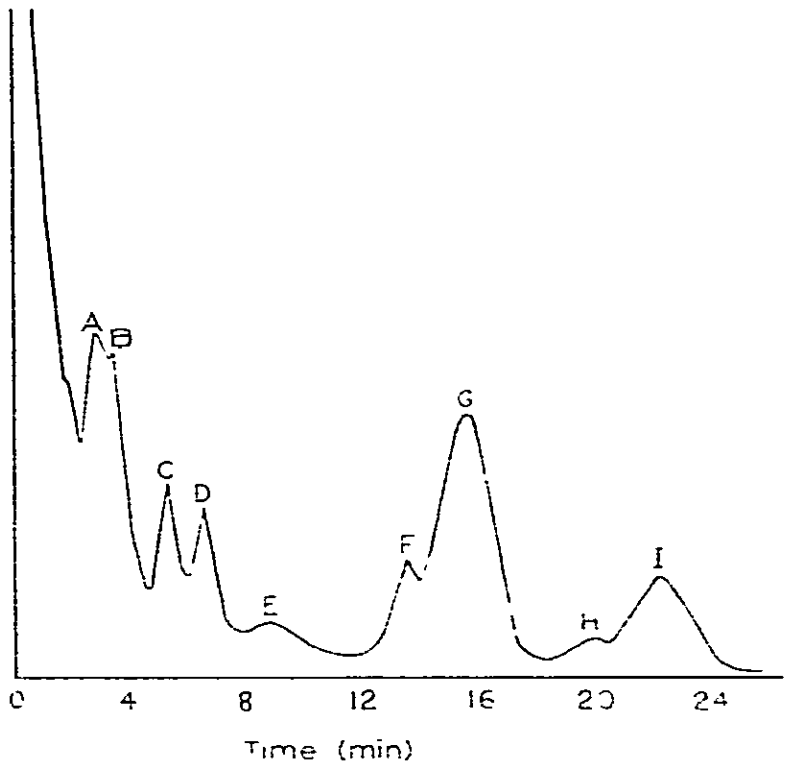

Fig. 3. Gas chromatogram of the per(trimethylsilyl) derivatives of the compounds in the cyanohydrin reaction-mixture after 25 min. The peaks were identified as follows: A, α-D-arabinose; B, β-D-arabinose; C, D-mannono- and D-glucono-imidolactones; D, D-mannono- and D-glucono-nitriles; E, D-mannono- and D-glucono-lactones; F, potassium D-mannonate; G, potassium D-gluconate; H, D-mannonamide and D-gluconamide; I, *myo*-inositol (internal standard).

solvent peak. The first two peaks were due to the per(trimethylsilyl)ated α- and β-D-arabinose, respectively. The third peak may be due to a mixture of the per-(trimethylsilyl) ethers of D-mannonoimidolactone (**2b**) and D-gluconoimidolactone (**2a**). The fourth peak was due to a mixture of the per(trimethylsilyl) ethers of D-

mannononitrile (**1b**) and D-gluconononitrile (**1a**). The synthesis of D-gluconononitrile was performed by the method of Wohl and Wollenberg[5]; the nitrile was used as the standard for the identification of the compounds constituting the fourth peak. The fifth peak was due to the per(trimethylsilyl) derivatives of D-mannono-1,4(or 1,5)-lactone (**5b**) and D-glucono-1,4(or 1,5)-lactone (**5a**). The sixth and seventh peaks, due to the per(trimethylsilyl) derivatives of potassium D-mannonate (**6b**) and potassium D-gluconate (**6a**), were not well resolved; the ratio of **6a** to **6b** was ~3:1, in agreement with the results of Isbell and co-workers[1]. The eighth peak consisted of a mixture of the per(trimethylsilyl) derivatives of D-mannonamide (**4b**) and D-gluconamide (**4a**), which have the same retention time. The last peak was due to the hexakis-*O*-(trimethylsilyl)-*myo*-inositol used as the internal standard.

Next, a combination of paper chromatography and g.l.c. was used for the qualitative analysis of the intermediates formed during the cyanohydrin synthesis with α-D-arabinose; this condensation was conducted for 20–25 min at room temperature, and the solution was evaporated to dryness under diminished pressure. The components of the solid residue were separated on paper. The different bands were located, cut out of the paper chromatogram, and separately eluted overnight with water. The separate extracts were evaporated to dryness and investigated by g.l.c. of their per(trimethylsilyl) derivatives. The slowest band gave a single peak for potassium D-gluconate (**6a**). The next gave one peak, for potassium D-mannonate (**6b**). The third band was cut out, horizontally, in three equally wide (lengthwise) bands, consisting of the lowest, the middle, and the uppermost band. Each one of these three bands was separately eluted with water. G.l.c. of the per(trimethylsilyl) derivative of the lowest band indicated that it was a mixture of **5a** and D-gluconic acid (**6a**) (formed by hydrolysis during elution overnight). The uppermost band was found to consist of **5b** plus D-mannonic acid (**6b**). The middle band consisted of a mixture of the two lactones and their two acids; 1,5(or 1,4)-D-gluconolactone (**5a**) runs more slowly than 1,5 (or 1,4)-D-mannonolactone (**5b**) in 3:2:2 pyridine–butanol–water. The next band was found to consist of a mixture of D-gluconamide (**4a**) and D-mannonamide (**4b**), which also had undergone hydrolysis during overnight elution. Only one peak was obtained for the per(trimethylsilyl) ethers of the two amides at 180°. The next peak was a well separated band for the per(trimethylsilyl) ethers of the unchanged α- and β-D-arabinose. The next two bands were not well separated from each other. The second-fastest band was shown to contain a large proportion of D-gluconononitrile (**1a**) and D-mannononitrile (**1b**) (their trimethylsilyl derivatives have the same retention time, and thus give a single peak) and a small proportion of the two imidolactones (**2a** and **2b**). G.l.c. of the per(trimethylsilyl) derivatives of the fastest-moving band gave two peaks; the larger one was the same as that obtained for the imidolactones (**2a** and **2b**), and the smaller peak was due to the two nitriles (**1a** and **1b**). Confirmation. of the qualitative g.l.c. analysis of D-gluconononitrile (**1a**) and D-mannononitrile (**1b**) was obtained by using standard D-gluconononitrile prepared by the method of Wohl and Wollenberg[5]. The mechanism shown in Fig. 1 is proposed for the cyanohydrin synthesis starting with α-D-arabinose.

An attempt was made to elucidate the conformation of D-gluconamide (**4a**) by i.r. and n.m.r. spectroscopy. The i.r. spectrum of D-gluconamide shows a strong absorption at 1680 cm^{-1} characteristic of a primary amide group, thus indicating that it is an open-chain compound. The n.m.r. spectrum (see Fig. 4) of this compound

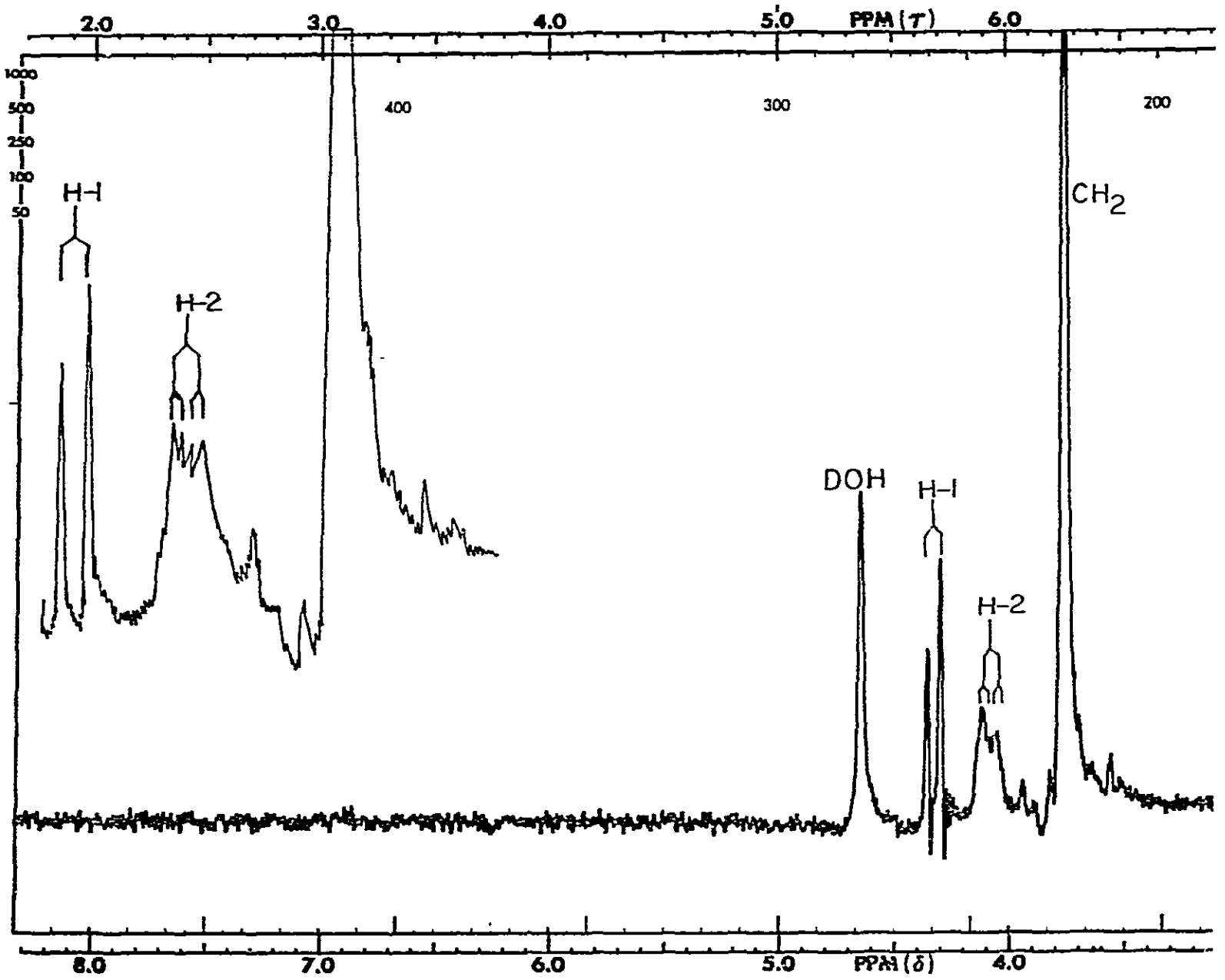

Fig. 4. N.m.r. spectrum, at 60 Hz, of D-gluconamide in deuterium oxide at 25°.

in deuterium oxide showed a sharp doublet for H-2 at 4.32 p.p.m. ($J_{2,3}$ 4 Hz). A sharp pair of doublets appeared for H-3 at 4.03 p.p.m., one of the coupling constants of which was 4 Hz, and the other was 1.5 Hz. There is a possibility that hydrogen bon-

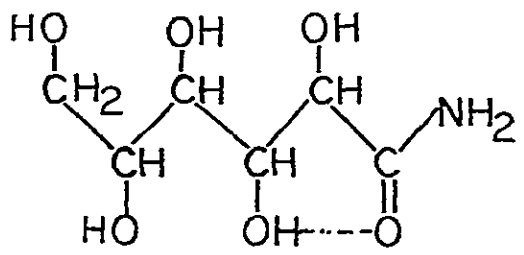

Fig. 5. Hydrogen bonding between the carbonyl oxygen atom at C-1 and the hydrogen atom of the 3-hydroxyl group, forming a distorted, six-membered ring.

ding may exist (see Fig. 5) between the carbonyl oxygen atom of C-1 and the hydrogen atom of the 3-hydroxyl group, thus forming a distorted, six-membered ring. The formation of the ring would fix the stereochemistry of H-2 and H-3; thus, it accounts for the sharp doublet given by H-2 and the sharp pair of doublets given by H-3. If there were no ring formation due to hydrogen bonding, H-2 and H-3 would have free rotation about the C-2–C-3 bond and would not be capable of giving sharp signals in the n.m.r. spectrum.

EXPERIMENTAL

Reagents and standards. — Sodium D-gluconate (Eastman, Rochester, New York) and *myo*-inositol (Matheson, Coleman and Bell, Norwood, Ohio) were used as reference compounds. D-Gluconamide[6] and D-gluconononitrile[5] were synthesized, and used as reference compounds. Hexamethyldisilazane (purum 98%) and chlorotrimethylsilane (puries 99% Fluka, A. G.) were used without further purification. Pyridine (reagent grade) was redistilled, and stored over pellets of potassium hydroxide.

Preparation of trimethylsilyl derivatives. — The per(trimethylsilyl) derivatives were prepared by mixing the dry sample (10 mg) with dry pyridine (1 ml), hexamethyldisilazane (0.2 ml), and chlorotrimethylsilane (0.1 ml) in a conical centrifuge-tube. The solution was gently shaken, and kept for 30 min at room temperature. The excess of trimethylsilylation reagents was evaporated off under diminished pressure, and the per(trimethylsilyl) derivatives were extracted from the precipitate with dry ether.

Gas–liquid chromatography. — A Varian Aerograph, Model 1520, equipped with a flame-ionization detector was used. A coiled-metal column (5 ft × 1/8 in.) containing 15% (w/w) of SE-30 on siliconized Gas-Chrom P (100–140 mesh, acid-washed) was used at 180°. The rate of nitrogen flow was 60 ml per min. Samples were injected into the chromatograph with a 10-μl syringe. α-D-Arabinose (250 mg), anhydrous sodium carbonate (59 mg), and potassium cyanide (112.2 mg) were dissolved in water, and the solution was made to 25 ml with water. Aliquots (1 ml) were evaporated to dryness under diminished pressure every 10 minutes for 24 h. The per(trimethylsilyl) derivatives of the solid residues obtained by evaporation were prepared for g.l.c. It was concluded from the size of the peaks that the maximal amount of cyanohydrins was formed after 20–25 min of reaction.

Paper chromatography. — Chromatographic separations of the components of the cyanohydrin reaction were made at 25° on Whatman No. 3 MM filter paper (16 × 20 cm) with 3:2:2 (v/v) pyridine–butanol–water. Four "ascents" were given to the chromatogram. The dried chromatogram was dipped in a 1% solution of silver nitrate in acetone, dried, dipped in 0.5% sodium hydroxide in ethanol, dried, and then washed with 4% aqueous sodium thiosulfate. The locations of the components on the paper were determined by spraying guide strips cut from the edges and center of each paper chromatogram. The bands were excised, and eluted overnight with several

portions of water. The extracts were separately evaporated to dryness at room temperature under diminished pressure.

α-D-Arabinose (50 mg), anhydrous sodium carbonate (11.8 mg), and potassium cyanide (22.5 mg) were made to 5 ml with water. Aliquots (0.1 ml) were spotted on a paper chromatogram every ten minutes during 24 h. A qualitative determination of the progress of the reaction was made from the intensity of the spots.

Chromatographic separation was also conducted on a preparative scale. α-D-Arabinose (30 mg), anhydrous sodium carbonate (7.1 mg), and potassium cyanide (13.5 mg) were made to 5 ml with water. The reaction was allowed to proceed for 20–25 min, to obtain the maximal amount of the cyanohydrins, and evaporated to a small volume. The components of the reaction mixture were separated on paper.

ACKNOWLEDGMENT

The authors thank Mr. Carl Wolf for photographic assistance.

REFERENCES

1 H. S. ISBELL, J. V. KARABINOS, H. L. FRUSH, N. B. HOLT, A. SCHWEBEL, AND T. T. GALKOWSKI, *J. Res. Nat. Bur. Stand.*, 48 (1952) 163.
2 C. S. HUDSON, O. HARTLEY, AND C. B. PURVES, *J. Amer. Chem. Soc.*, 56 (1934) 1248.
3 E. ZISSIS, N. K. RICHTMYER, AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 72 (1950) 3882.
4 W. E. TREVELYAN, D. P. PROCTOR, AND J. S. HARRISON, *Nature*, 166 (1950) 444.
5 A. WOHL AND O. WOLLENBERG, *Ann.*, 500 (1933) 281.
6 J. W. W. MORGAN AND M. L. WOLFROM, *J. Amer. Chem. Soc.*, 78 (1956) 1897.