

## Notes

CHROM. 3647

### The anomerization of D-glucose as determined by gas chromatography

The mutarotation of D-glucose in various solvents has been studied extensively by polarimetric procedures<sup>1-7</sup>. More recently, other instruments were used to investigate this phenomenon. Nuclear magnetic resonance (NMR) was used in the study of glycopyranoses by NOBUO MORI *et al.*<sup>8</sup> and by RAO AND FOSTER<sup>9</sup>. PARKER<sup>10</sup> has investigated the behavior of carbohydrates in water by infrared spectroscopy.

SWEELY *et al.*<sup>11</sup> studied carbohydrates as their trimethylsilyl ethers by means of gas chromatography. Although since then many aspects of carbohydrate chemistry have been investigated by this method, the use of gas chromatography in the study of D-glucose anomerization has not been reported. In this investigation gas chromatographic procedures have been applied in the study of D-glucose anomerization in various solvents.

#### Experimental

A Perkin-Elmer 800 gas chromatography unit with a flame ionization detector was used in this work. The column was stainless steel, 6 ft. long by  $\frac{1}{8}$  in. O.D. The coating was 8% neopentyl glycol adipate (NPGA) on silanized Chromosorb W. The column temperature was 140° programmed to 180° at 4° per min. Injection block temperature was 230°. The nitrogen flow rate was 32 ml per min and the stream was split 80-20 in favor of the atmosphere.

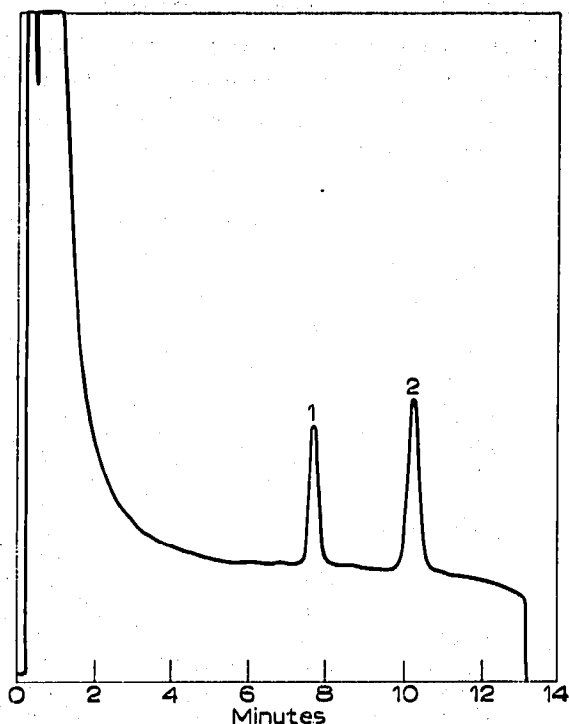


Fig. 1. Chromatographic determination of D-glucose anomers. 1 =  $\alpha$ -D-glucose; 2 =  $\beta$ -D-glucose.

TABLE I

THE ANOMERIZATION OF  $\alpha$ -D-GLUCOSE IN WATER, DEUTERIUM OXIDE, AND AQUEOUS BUFFERS

Time (min)	Water		D <sub>2</sub> O		pH 3.7		pH 7.1		pH 9.0	
	% alpha	% beta	% alpha	% beta	% alpha	% beta	% alpha	% beta	% alpha	% beta
1	90.5	9.5	95.9	4.1	95.8	4.2	92.5	7.5	94.1	5.9
15	71.0	29.0	82.0	18.0	79.5	20.5	75.0	25.0	76.0	24.0
30	60.0	40.0	76.5	23.5	60.0	40.0	64.0	36.0	68.0	32.0
45	52.5	47.5	69.5	30.5	—	—	—	—	—	—
60	49.0	51.0	67.0	33.0	51.5	49.5	49.5	50.5	59.0	41.0
75	—	—	63.0	37.0	48.5	51.5	—	—	53.0	47.0
90	—	—	60.0	40.0	47.0	53.0	46.0	54.0	50.0	50.0
105	—	—	58.5	41.5	44.5	—	—	—	47.5	52.5
120	—	—	55.5	44.5	—	—	—	—	45.0	55.0
135	—	—	54.0	46.0	40.0	60.0	—	—	—	—
150	—	—	52.0	48.0	—	—	44.0	56.0	46.0	54.0
180	—	—	—	—	41.0	59.0	—	—	42.0	58.0
240	—	—	—	—	—	—	45.0	55.0	—	—
20 h	—	—	41.0	59.0	—	—	—	—	—	—
24 h	42.0	58.0	—	—	—	—	—	—	—	—

Concentrated D-glucose solutions were made up in the various solvents and kept for the indicated time intervals. Aliquots of these solutions were then mixed with Tri-sil reagent (Pierce Chemical Co., Ill.) left at room temperature for 3 min and the trimethylsilyl ethers formed examined gas chromatographically. A Honeywell 1 mV single pen recorder was used. Peak height was taken as a measure of anomer concentration in the examined sample. A typical chromatogram is shown in Fig. 1.

The non-aqueous solvents were examined for water content prior to use in D-glucose anomerization studies. A Beckman GC-5 with a thermal conductivity detector was used. The column support was Porapak-Q and the column temperature was 130°. All other conditions were as recommended by Waters Associates, Inc., Framingham, Mass., manufacturers of Porapak-Q.

TABLE II

THE ANOMERIZATION OF  $\beta$ -D-GLUCOSE IN WATER, DEUTERIUM OXIDE, AND pH 9.0 BUFFER

Time (min)	Water		D <sub>2</sub> O		pH 9.0	
	% alpha	% beta	% alpha	% beta	% alpha	% beta
1	14.0	86.0	3.5	96.5	15.0	85.0
15	22.0	78.0	17.0	83.0	24.0	76.0
30	27.0	73.0	24.0	76.0	32.0	68.0
45	32.0	68.0	29.5	70.5	—	—
60	37.0	63.0	32.5	67.5	39.0	61.0
75	39.0	61.0	—	—	—	—
90	41.0	59.0	35.0	65.0	44.0	56.0
105	40.5	59.5	—	—	—	—
120	42.0	58.0	37.0	63.0	41.0	59.0
180	40.0	60.0	—	—	—	—
210	—	—	—	—	45.0	55.0
1320	—	—	—	—	43.0	57.0
3 days	—	—	40.5	59.5	—	—

*Results and discussion*

The ratio of D-glucose anomers in water, deuterium oxide, and in aqueous buffers at various time intervals, is shown in Table I. The figures in Table I show that the anomerization of  $\alpha$ -D-glucose proceeds at a faster rate in water than in deuterium oxide. The hydrogen ion concentration seems to have no effect upon the anomerization rate. D-glucose in water, deuterium oxide, and pH 9.0 buffer was also investigated with

TABLE III

ANOMERIZATION OF  $\alpha$ -D-GLUCOSE IN FORMAMIDE AND DIMETHYLFORMAMIDE

Time (min)	Formamide		Dimethylformamide	
	% <i>alpha</i>	% <i>beta</i>	% <i>alpha</i>	% <i>beta</i>
1	96.7	3.3	98.7	1.3
15	82.0	18.0	98.3	1.7
30	68.0	32.0	98.6	1.4
45	59.0	41.0	—	—
60	55.0	45.0	—	—
150	46.0	54.0	—	—
210	—	—	97.6	2.4
245	44.0	56.0	—	—

$\beta$ -D-glucose as the starting material. The results obtained are shown in Table II. A comparison of the figures in Tables I and II shows that the same equilibrium is reached whether the starting material is  $\alpha$ - or  $\beta$ -D-glucose. However, the rate of anomerization of the  $\alpha$  form to the  $\beta$  form seems to be faster than the reverse process.

The non-aqueous solvents contained less than 0.03% water except for the

TABLE IV

ANOMERIZATION OF  $\alpha$ -D-GLUCOSE IN PYRIDINE AND PYRIDINE-WATER MIXTURES

Time (min)	100% pyridine		90% pyridine		80% pyridine		50% pyridine	
	% <i>alpha</i>	% <i>beta</i>	% <i>alpha</i>	% <i>beta</i>	% <i>alpha</i>	% <i>beta</i>	% <i>alpha</i>	% <i>beta</i>
1	—	—	97.1	2.9	95.0	5.0	79.0	21.0
15	—	—	88.5	11.5	63.0	37.0	40.0	60.0
20	95.3	4.7	—	—	—	—	—	—
30	—	—	83.0	17.0	51.0	49.0	42.0	58.0
45	93.0	7.0	—	—	46.5	53.5	—	—
60	92.2	7.8	69.0	31.0	44.5	55.5	—	—
75	—	—	65.0	35.0	43.0	57.0	—	—
90	—	—	—	—	43.5	56.5	—	—
105	—	—	57.5	42.5	—	—	—	—
120	86.5	13.5	54.5	45.5	—	—	—	—
170	82.5	17.5	48.5	51.5	—	—	—	—
195	—	—	46.0	54.0	—	—	—	—
240	75.5	24.5	45.0	55.0	—	—	—	—
300	71.0	29.0	—	—	—	—	—	—
24 h	47.0	53.0	43.0	57.0	—	—	—	—
29 h	46.0	54.0	—	—	—	—	—	—
120 h	45.5	54.5	—	—	—	—	—	—

TABLE V

ANOMERIZATION OF  $\alpha$ -D-GLUCOSE IN DIMETHYL SULFOXIDE (DMSO) AND DMSO-WATER MIXTURES

Time	DMSO		80% DMSO		50% DMSO	
	% <i>alpha</i>	% <i>beta</i>	% <i>alpha</i>	% <i>beta</i>	% <i>alpha</i>	% <i>beta</i>
1 min	98.3	1.7	98.7	1.3	—	—
15 min	98.3	1.7	97.3	2.7	94.0	6.0
30 min	96.2	3.8	97.2	2.8	92.3	7.7
60 min	96.5	3.5	—	—	—	—
105 min	—	—	—	—	88.0	12.0
135 min	—	—	96.7	3.3	—	—
150 min	—	—	—	—	80.0	20.0
270 min	—	—	—	—	45.0	55.0
300 min	—	—	95.7	4.3	—	—
16 h	96.2	3.8	—	—	—	—
24 h	—	—	—	—	47.0	53.0
84 h	—	—	77.0	23.0	—	—
100 h	95.6	4.4	—	—	—	—
105 h	—	—	67.0	33.0	—	—
225 h	—	—	53.0	47.0	—	—
300 h	—	—	52.0	48.0	—	—
336 h	92.0	8.0	—	—	—	—

formamide, which contained 0.17% water. The results obtained with formamide and dimethylformamide (DMF) are shown in Table III. The figures show that no anomerization occurred in the DMF while in the formamide there was anomerization and an equilibrium was reached within 3-4 h.

The behavior of  $\alpha$ -D-glucose in pyridine and pyridine-water mixtures is shown in Table IV. The equilibrium between  $\alpha$  and  $\beta$  anomers is established at faster rates in the mixtures containing larger amounts of water, however anomerization does occur even in the 100% pyridine.

In dimethyl sulfoxide (DMSO) the  $\alpha$ -D-glucose remained unchanged even after 300 h. The results of this experiment and of tests conducted in DMSO-water mixtures

TABLE VI

ANOMERIZATION OF  $\alpha$ -D-GLUCOSE IN 50% DIOXANE

Time (min)	50% dioxane	
	% <i>alpha</i>	% <i>beta</i>
1	100	—
15	94	6.0
30	90	10.0
60	83.5	16.5
90	77.8	22.2
120	73.2	26.8
150	70.0	30.0
180	66.0	34.0
210	62.2	37.8
240	58.0	42.0
270	59.6	40.4
22 h	45.0	55.0
46 h	44.0	56.0

are shown in Table V. It can be seen from the figures in the table that the anomerization of  $\alpha$ -D-glucose proceeds very slowly in DMSO-water mixtures. The  $\alpha$ -D-glucose anomerization in 50% pyridine is about 48 times faster than in 50% DMSO.

The behavior of  $\alpha$ -D-glucose in 50% dioxane is shown in Table VI. The anomerization is again comparatively slow. It proceeds at a rate similar to the one found for 50% DMSO.

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