Carbohydrate Research, 86 (1980) 147–150
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

The isomerization of D-glucose in acidic solutions*

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(Received December 13th, 1979; accepted for publication, in revised form March 20th, 1980)

The Lobry de Bruyn–Alberda van Ekenstein transformation (the interconversion of a 2-ketose and the corresponding aldoses, epimeric at C-2) is generally thought to proceed *via* enolization, involving a 1,2-enediol intermediate, and to be subject to general acid–base catalysis[1]. The majority of kinetic studies[2] and mechanistic investigations for the transformation[3,4], however, have been performed in alkaline solution.

Much less information is available on the reaction in acidic media, although some studies have been reported, and it is generally assumed that the mechanism remains the same for acid-catalyzed processes. Hough and Pridham[5] have reported that arabinose affords ribose and *erythro*-pentulose in acid media, hexuronic acids[6] are reported to epimerize, and Ohno and Ward[7] reported that treatment of D-glucose with 2.5% sulfuric acid under conditions for hydrolysis of cellulose gives a small proportion of fructose in addition to glucose. The latter report is of interest as mannose was not reported present, although it would be expected if the foregoing interconversion mechanism were operative. As a lengthy incubation with glucose oxidase was employed to remove unreacted glucose from the mixture prior to the identification of other sugars produced, it is possible that mannose, present in small quantities, was similarly removed. This supposition is in light of the fact (as recognized by Ohno and Ward[7]) that glucose oxidase, which catalyzes the oxidation of glucose to gluconic acid, can also utilize mannose as a substrate, though more slowly, to form the corresponding aldonic acid[8]. Thus, small amounts of mannose arising from glucose via the foregoing transformation could have been readily removed from the reaction.

Because much more sensitive and precise techniques are now available for separation and analysis of individual carbohydrates by use of gas–liquid chromatography (g.l.c.), thin-layer chromatography (t.l.c.), and high-pressure liquid chromatography (l.c.), we have reinvestigated this reaction using D-glucose as the starting material. Detailed quantitative studies, using the foregoing methodologies, on the

*Journal Paper No. 8442 of the Missouri Agricultural Experiment Station.

interconversions of D-glucose, D-fructose, and D-mannose in acidic solution have not been reported.

Chromatographically pure D-glucose was treated with acid for various time-intervals under the conditions utilized by Ohno and Ward[7] in their work, and the solutions, after neutralization, were analyzed by g.l.c. to determine unreacted D-glucose. An aliquot of each solution was also subjected to l.c. to remove glucose selectively, and the fractions corresponding to fructose and mannose were collected and evaporated to dryness. A portion of each fraction was subjected to t.l.c. to demonstrate the presence of fructose and mannose. Fructose was determined in the remaining fraction by g.l.c. after conversion into the trimethylsilyl ether–oxime derivative, and mannose was determined as the aldononitrile acetate derivative[9,10]. To further verify the presence of fructose and mannose, the following techniques were employed. First, fructose was additionally derivatized to the corresponding per-*O*-acetylated-*O*-acetyl oxime, which characteristically yields *syn* and *anti* isomers separable on polar g.l.c. phases, and mannose was converted into the trimethylsilyl ether–oxime and analyzed. Secondly, utilization of two different g.l.c. columns for each derivative was employed as well as different column conditions for each. In all cases, retention times for fructose and mannose were identical to those of standards derivatized under the same conditions. Lastly, standards were also added to the mixtures and clearly demonstrated peaks in g.l.c. coincident with those of mannose and fructose.

The results of these experiments are shown in Table I. As noted, both mannose and fructose are present in the mixture after 1 h of reaction and at all time-intervals following. The concentration of mannose is seen to increase with reaction time and that of fructose appears to remain constant. Under conditions for hydrolysis of cellulose, a substantial decrease in concentration of glucose occurs, which cannot be accounted for solely by the parallel concentrations of measured mannose and fructose in solution. This reflects the fact that the system is kinetically complex, as mannose and fructose also undergo dehydration (at different rates) as they are produced. It is clear, therefore, that the results in Table I do not reflect the extent of overall formation

TABLE I

YIELDS (μg) OF SUGARS OBTAINED FROM 50 mg OF D-GLUCOSE ON TREATMENT WITH 2.5% SULFURIC ACID AT 120°

Reaction time (h)	*Glucose (mg)*	*Mannose (μg)*	*Fructose (μg)*
0	50.00	0	0
1	46.21	4.7	0.6
2.5	43.64	10.8	0.8
5.0	39.72	19.1	0.8
7.5	37.14	27.7	0.9
10.0	35.93	43.8	0.8

of either mannose or fructose under these conditions, but rather indicate the amounts generated and not subsequently degraded. In addition, other compounds are also produced *via* dehydration of sugars to form furaldehydes, pigments, other polymers, and related degradation-products, thus making it impossible to determine, at this point, the predominant pathway for the disappearance of D-glucose. It is apparent, however, that more than one pathway exists. Investigations are currently underway to determine the major products formed under these conditions, in the hope that their quantitation and measurements of their rates of degradation will allow elucidation of these pathways.

EXPERIMENTAL

Materials and methods. — G.l.c. analyses were performed with a Perkin–Elmer Sigma III gas chromatograph equipped with the following columns: 2% of DEGA (stabilized) and 2.5% of OV-225/3.0% tetramethylcyclobutanediol succinate on Chromosorb W-HP (100–120 mesh) for analysis of acetylated derivatives, and 1.5% of SE-52 on Chromosorb W-HP (100–120 mesh) and 3% of SP-2250 on Supelcoport (100–120 mesh) for the analysis of trimethylsilylated derivatives. Oximation and derivatization of carbohydrates were performed as previously described[10,11]. For quantitative g.l.c. analyses, the following internal standards were used: *myo*-inositol (for Me_3Si derivatives), and *myo*-inositol plus methyl α-D-glucopyranoside (for acetylated derivatives). Standards were added to samples after neutralization of acid solutions. A Waters Associates l.c. unit equipped with a differential refractometer was used. The column (maintained at 80°) was a jacketed column (91.4 cm × 9.5 mm) packed with Aminex Q-155 cation-exchange resin in the calcium form. Water was used as the eluant at a flow rate of 0.8 mL per min. Relative to D-glucose (0 min), D-mannose and D-fructose had retention times of 2.85 and 4.63 min, respectively. T.l.c. of carbohydrates was accomplished on glass plates coated with a 250-μm thickness of silica gel G containing 0.15M potassium dihydrogenphosphate, with (4:5:1 (v/v/v) 1-butanol–acetone–water as the developing irrigant.

Acid treatment of D-*glucose.* — Chromatographically pure samples (50 mg each) of D-glucose were placed in Teflon-sealed, glass tubes with 2.0 mL of 2.5% sulfuric acid and heated at 120° in a steam sterilizer. At 1-, 2.5-, 5-, 7.5-, and 10-h intervals, tubes were removed, their contents diluted to 4.0 mL with distilled water, made neutral with barium carbonate, and centrifuged. A 100-μL aliquot was removed from each tube, evaporated to dryness, and assayed for D-glucose[9,11]. The remaining sample was passed through Dowex 50W-X8 (H^+) resin, evaporated to dryness, and made to 2.0 mL with distilled water; 100 μL of this solution was injected into the l.c. for the removal of D-glucose and the preparative isolation of mannose and fructose. The appropriate effluent fractions were evaporated to dryness, derivatized, and assayed by g.l.c. as already described.

REFERENCES

1 J. C. SPECK, JR., *Adv. Carbohydr. Chem.*, 13 (1958) 63–99, and references cited therein.
2 D. J. MACLAURIN AND J. W. GREEN, *Can. J. Chem.*, 47 (1969) 3947–3955.
3 J. C. SOWDEN AND R. SCHAFFER, *J. Am. Chem. Soc.*, 74 (1952) 505–507.
4 Y. J. TOPPER AND D. STETTEN, JR., *J. Biol. Chem.*, 189 (1951) 191–202.
5 L. HOUGH AND J. B. PRIDHAM, *Chem. Ind. (London)*, (1957) 1178–1179.
6 B. CARLSSON AND O. SAMUELSON, *Carbohydr. Res.*, 11 (1969) 347–354.
7 Y. OHNO AND K. WARD, *J. Org. Chem.*, 26 (1961) 3928–3931.
8 A. SOLS AND G. DE LA FUENTE, *Biochim. Biophys. Acta*, 24 (1957) 206–207.
9 R. VARMA, R. S. VARMA, AND A. H. WARDI, *J. Chromatogr.*, 77 (1973) 222–227.
10 T. P. MAWHINNEY, M. S. FEATHER, J. R. MARTINEZ, AND G. J. BARBERO, *Carbohydr. Res.*, 75 (1979) c21–c23.
11 T. P. MAWHINNEY, M. S. FEATHER, J. R. MARTINEZ, AND G. J. BARBERO, *Anal. Biochem.*, 101 (1980) 112–117.