Carbohydrate Research, 26 (1973) 41–53

THE EFFECT OF ARENEBORONIC ACIDS ON THE ALKALINE CONVERSION OF D-GLUCOSE INTO D-FRUCTOSE

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(Received April 27th, 1972; accepted for publication, June 22nd, 1972)

ABSTRACT

Benzeneboronic acid, 4-methoxybenzeneboronic acid, 3-nitrobenzeneboronic acid, and sulphonated benzeneboronic acid have been used to displace the pseudo-equilibria established in aqueous alkali between D-glucose, D-fructose, and D-mannose to give greatly increased yields of D-fructose. The effect of reaction temperature, pH, overall concentration, and molar ratio of acid:sugar on the yield of D-fructose has been investigated by using an automated assay for D-fructose.

INTRODUCTION

The Lobry de Bruyn–Alberda van Ekenstein transformation[1], whereby the interconversion of certain isomeric aldoses and ketoses is effected by general acid and base catalysis, is well known. These isomers are usually considered to be inter-related by equilibrium reactions involving enediol intermediates[1,2]. Since irreversible elimination reactions occur in the presence of alkali, the sugar isomers exist in a pseudo-equilibrium state[3]. The equilibria between the isomers may, however, be displaced if one isomer reacts to a greater extent than the others with a complexing reagent. The complexing of carbohydrates by borate has been closely studied[4,5], and Mendicino[6] has shown that the presence of borate in the reaction of D-glucose with alkali increases the yield of D-fructose. Areneboronic acids can also form complexes with carbohydrates in aqueous solution in a manner similar to that of borate[7,8], and we now report on the effect of benzeneboronic acid, 4-methoxybenzeneboronic acid, 3-nitrobenzeneboronic acid, and sulphonated benzeneboronic acid on the conversion of D-glucose into D-fructose by alkali.

DISCUSSION

A sensitive assay for D-fructose has been reported by Yaphe and Arsenault[9], and this method was automated (Fig. 1) to enable the continuous monitoring of the production of D-fructose. Fig. 2 shows the change in concentration of D-fructose when alkaline solutions (20mM) of D-glucose, D-mannose, and D-fructose were heated at 50° under nitrogen. D-Glucose gave 73% of D-fructose in a solution containing an equi-

molar amount of benzeneboronic acid and which was initially pH 12 at room temperature (Fig. 2*a*). Compared with the yields of D-fructose of less than 30% reported for the action of alkali alone on D-glucose[3,10], this result shows an increase of 250% in the yield of D-fructose by the presence of benzeneboronic acid. These conditions also give a yield of >50% of D-fructose when the starting sugar is D-mannose, whereas Sowden and Schaffer[3] obtained 20.5% of D-fructose by the action of 35mM sodium hydroxide on M D-mannose. At pH 13 (Fig. 2*b*), the yield of D-fructose from D-glucose was 66% and from D-mannose 53%, and, as would be expected, the rates of conversion were considerably greater. At pH 12 in the presence of a bimolar ratio of benzeneboronic acid, the rates of formation of D-fructose from D-glucose and D-mannose were considerably decreased and, after 5 h, the yields were 49 and 45%, respectively, and were still increasing slowly. The effect of an equimolar concentration of 4-methoxybenzeneboronic acid and 3-nitrobenzeneboronic acid on the conversion from D-glucose at pH 12 both gave maximal yields of 81% of D-fructose. These results,

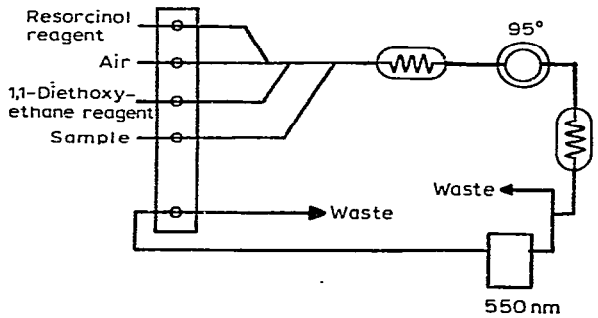

Fig. 1. Schematic diagram of automated resorcinol assay for fructose.

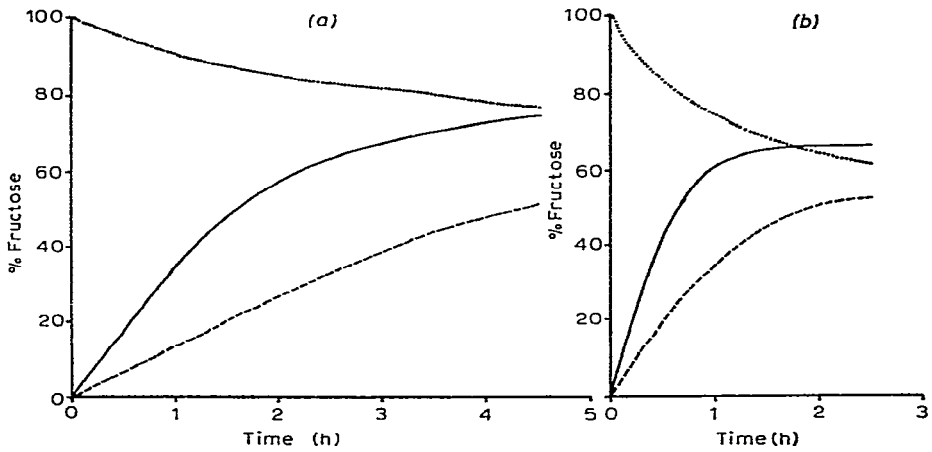

Fig. 2. Change in concentration of D-fructose when solutions (20mM) of D-glucose (——), D-mannose (– – – –), and D-fructose (.....) containing an equimolar amount of benzeneboronic acid were heated at 50° under nitrogen. Initial pH at room temperature: (*a*) 12, (*b*) 13.

which all show a displacement of the pseudo-equilibrium in favour of D-fructose, are in accordance with the results reported[8] for the complexing of benzeneboronic acid with monosaccharides and with the equilibrium constants reported by Lorand and Edwards[7].

The conversion with an equimolar amount of benzeneboronic acid at pH 12 and a higher overall concentration (125mM D-glucose) gave 72% of D-fructose after 6 h, and this value only fell to 70% over the next 18 h at 50°. With an initial pH of 11 at room temperature, the rate of conversion was much slower, and, after 20 h, the yield was 21% and was still rising slowly.

As the preparation of D-fructose is of considerable commercial importance, the conversion in more concentrated solutions was investigated. In a preliminary experiment with 25% aqueous D-glucose containing an equimolar amount of benzeneboronic acid (pH 12), a maximal yield of 52% of D-fructose was obtained after 2 h at 50°, and the yield fell during the next 2 h. It was apparent that the reaction was considerably altered when the concentration was increased by more than 13-fold, and so the transformation was repeated with D-glucose-*1*-^{14}C. After 1.67 h, the yield of D-fructose by the resorcinol assay was 49%, although this was not necessarily the optimal yield. Sugars can be separated as their complexes with borate on an anion-exchange resin[11,12]. A modified form of this technique was used to analyse the radioactive reaction mixtures, with monitoring by an automated cysteine–sulphuric acid assay[13] and by a radioactive assay (Table I). The chromatograph is shown in Fig. 3, the peak elution positions corresponding exactly for each assay. The identity of the D-fructose peak was established by radioisotope dilution. The two sets of results in Table I show that the two assays give good agreement on the yield of D-fructose and D-glucose, and the elution volume of these two components was the same as for standard compounds. However, the first peak was not easily identified as its elution volume was greater than that for D-mannose, the third component expected. Radioisotope dilution analysis of this peak and of the original reaction mixture showed a yield of D-mannose of 2.2% and 2.3%, respectively. Although the "unknown" peak contains all the D-mannose produced, only 37% of the radioactivity is thereby accounted for. In a parallel fractionation monitored with the resorcinol assay, the "unknown" peak showed a response of 6.1% of that given by the D-fructose peak, which suggests either the presence of another keto sugar or that some of the D-fructose has remained as a stable complex with benzeneboronic acid. The sugars eluted in the fractionation accounted only for 75.3% of the radioactivity, but a further 20.3% of the radioactivity was recovered by washing the resin with acid, giving an overall recovery of 95.6%. Chloride ion interfered with the assay of the latter eluate by the cysteine–sulphuric acid and resorcinol methods. The compound in the eluate behaved as a neutral compound under conditions used to separate hydroxycarboxylic acids, and it was considered possible that it contained sugars which had formed stable complexes with benzeneboronic acid. However, an acidified sample of the reaction mixture, in which such complexes should be dissociated, gave the same yield of D-fructose (46.7%) and D-glucose (24.1%) when fractionated by the borate–

anion-exchange resin method, and these yields were also given on direct fractionation. As the two analyses were otherwise also identical, it was apparent that if the absorbed component is a complex, it is not dissociated by mildly acidic conditions.

TABLE I

ANALYSIS[a] OF RADIOACTIVE REACTION MIXTURES AFTER FRACTIONATION

	Unknown peak	D-*Fructose*	D-*Glucose*	*Acid wash*
Radioactive assay	5.9	42.8	26.6	20.3
Cysteine–H_2SO_4 assay	11.7 (based on D-mannose)	43.7	24.5	—

[a]Yields expressed as % based on original weight of D-glucose.

The investigation of the mixture of radioactive transformation products by these various techniques showed that the resorcinol assay gave values for D-fructose ~4% higher than those obtained by the chromatographic and radioactive dilution methods when applied to concentrated reaction mixtures. Most of this difference is accounted for by a component in the first peak to be eluted in the chromatography. However, the resorcinol assay is a convenient way of rapidly monitoring the formation of D-fructose, and it was therefore used in subsequent experiments. In all such analyses of the reaction mixtures (sp. gr. ~1.2, ~1.67M D-glucose), samples were weighed because volumetric sampling was not reliable.

The effect of various parameters on the conversion of D-glucose into D-fructose in concentrated solutions was then investigated; Table II summarizes the results. The yield of D-fructose usually rose with time to a maximum and then fell. As it is the maximal yield that is of interest, this value has been given for each set of conditions and also a value for a typical analysis to show the decrease in D-fructose with time after the maximum. The effects of air and nitrogen on the maximal yield (Table II, *A*) are similar within experimental error. The effect of temperature (Table II, *B*) suggests that 37° may be slightly more advantageous than 50.5° or 61.5°, but the increase in yield is not large.

The rate of attainment of this maximal yield is, as would be expected, considerably greater at the higher temperatures. The effect of the concentration of sodium hydroxide, and hence pH, on the rates of reaction at 50° (Table II, *C*) is again what might be expected by increasing the concentration of a catalyst. At pH 10.95, the reaction is slow but the maximal yield of D-fructose is much less (34%) than at higher pH values, suggesting that hydroxide is not acting solely as a catalyst. The reaction at pH 11.97 gave a yield of 55% which was constant from 2.5–3.5 h, at which point the experiment was terminated. A much higher concentration of alkali (10.25% w/w) gave a maximal yield of 53% in only 21 min. The effect of altering the ratio of benzeneboronic acid to D-glucose from 1:1, which was used in the above experiments, to other values was investigated (Table II, *D*). In the absence of benzeneboronic acid,

TABLE II

EFFECT OF ARENEBORONIC ACIDS ON THE CONVERSION OF D-GLUCOSE INTO D-FRUCTOSE UNDER VARIOUS CONDITIONS

			Molar ratio of arene-boronic acid-D-glucose	*Concentration of NaOH (% w/w)*	*pH of initial solution*	*Temperature of pH measurement (degrees)*	*Initial concentration of D-glucose (% w/w)*	*Temperature of reaction (degrees)*	*Maximal yield (%) of D-fructose by resorcinol assay*	*Time to maximal yield (min)*	*Later analyses*	
											Fructose (%)	*Time (min)*
Benzeneboronic acid												
A	1	(air)	1:1	6.79	12.05	20	25.1	50.0	59	120	50	265
	2	(nitrogen)	1:1	6.79	12.05	20	25.1	50.0	57	120	51	265
B	1	(N_2)	1:1	7.3	12.16	21–23	25.7	37.0	55	340	30	4100
	2	(N_2)	1:1	7.3	12.16	21–23	25.7	50.5	50	90	31	400
	3	(N_2)	1:1	7.3	12.16	21–23	25.7	61.5	50	15	39	47
C	1	(N_2)	1:1	6.17	10.95	20	26.5	50.0	34	720	30	3200
	2	(N_2)	1:1	10.25	—	—	24.9	50.0	53	21	28	90
	3	(N_2)	1:1	6.50	11.97*	25	24.8	50.0	55	150	55	220
	4	(N_2)	1:1	6.75	12.08*	25	24.9	50.0	52	110	50	220
D	1	(N_2)	2:1	—	12.03	22–24	20.0	50.0	(>7.5)	(280)	—	—
	2	(N_2)	2:1	14	—	—	17.8	50.0	19	60	10	360
	3	(N_2)	1:2	3.48	12.00	20–21	27.9	50.0	55	95	42	260
	4	(N_2)	0:1	3.70	12.03	20–21	30.2	50.0	40	90	33	150
Sulphonated benzeneboronic acid			1:1	—	12.00	25	26.4	50.0	46	140	36	280
4-Methoxybenzeneboronic acid			1:1	6.16	12.14	22	25.1	50.0	55	120	53	180
3-Nitrobenzeneboronic acid			1:1	6.89	12.04	27	24.6	50.0	18	90	18	390

the maximal yield of D-fructose was 40%, but with a half-molar ratio of acid the yield (55%) was the same as with an equimolar ratio. When a bimolar ratio of benzeneboronic acid was used, the rate at pH 12.03 was very slow; the yield after 4.7 h was only 7.5% and was still rising. When the concentration of alkali was increased to ~14%, a maximal yield of 19% was attained after 1 h. In summary, a maximal yield of D-fructose of 50–59% may be attained under air or nitrogen, with a half- or equimolar ratio of benzeneboronic acid at pH 12 with reaction temperatures in the range 37–61.5°. To investigate the effect of small changes in pH in the region of pH 12, two special electrodes were used; the glass electrode was designed for use in highly alkaline solutions and the reference electrode was a sleeve-type which is more convenient to use in viscous solutions. An extensive series of experiments was conducted by using an equimolar ratio of benzeneboronic acid and an approximately 25% concentration of D-glucose with a reaction temperature of 50°. The concentration of sodium hydroxide was varied to give pH values of the initial solution in the range 11.8–12.5 at room temperature.

The results, not given here, were all in the range reported above for maximal yield of D-fructose, except for one run (pH 12.19 at 28°, 7.05% of NaOH) when a yield of 66% of D-fructose was obtained after 1.75 h. However, this result could not be repeated. It was noticeable that, in this series of runs, there was no observable correlation between concentration of sodium hydroxide, pH, and maximal yield of D-fructose. There was no significant difference when (*a*) all the alkali was added to the mixture of benzeneboronic anhydride and water before the D-glucose; and (*b*) the alkali was added to the mixture of D-glucose, benzeneboronic anhydride, and water. The reason for this variation in yield with pH and concentration of sodium hydroxide is not clear and requires further investigation.

Two transformations were carried out (50°, N_2, equimolar amount of benzeneboronic acid) with intermediate, overall concentrations, following the general procedure used with the highly concentrated solutions. A solution containing originally 7.1% of D-glucose (pH 11.96 at 22°) gave 65% of D-fructose after 5 h, whereas a solution containing 14.1% of D-glucose (pH 11.98 at 22°) gave 63% of D-fructose after 180 min and 60% after 4 h. These two results, together with the previous results obtained from dilute and concentrated solutions, show that the yield of D-fructose falls as the overall concentration is increased.

The pH was monitored (Fig. 3) during two typical conversions of D-glucose into D-fructose in concentrated solutions containing a molar equivalent of benzeneboronic acid. Also shown is the change in pH when the starting sugar was D-fructose. The shape of the pH–time curve for the two runs with D-glucose is similar to that for D-fructose. Fig. 4 shows the change in pH as the initial solutions were heated and as one of the solutions, initially containing D-glucose, cooled after being heated for 3.1 h at 50°. The complexes of D-glucose and D-fructose in solution both give a decrease in pH with increase in temperature. The effect of substituted benzeneboronic acids on the conversion of D-glucose into D-fructose in concentrated solutions was also investigated (Table II). 4-Methoxybenzeneboronic acid gave 55% of D-fructose

which is of the same order as that obtained with benzeneboronic acid, but 3-nitrobenzeneboronic acid gave a much lower yield (18%) which did not decrease in the following 5 h. It has been shown[8] that 3-nitrobenzeneboronic acid complexes strongly with D-glucose, D-fructose, and D-mannose, and possibly the low concentration of free sugars in the reaction mixture contributed to the low yield, but this does not explain why, at low concentrations, the yield is 81%.

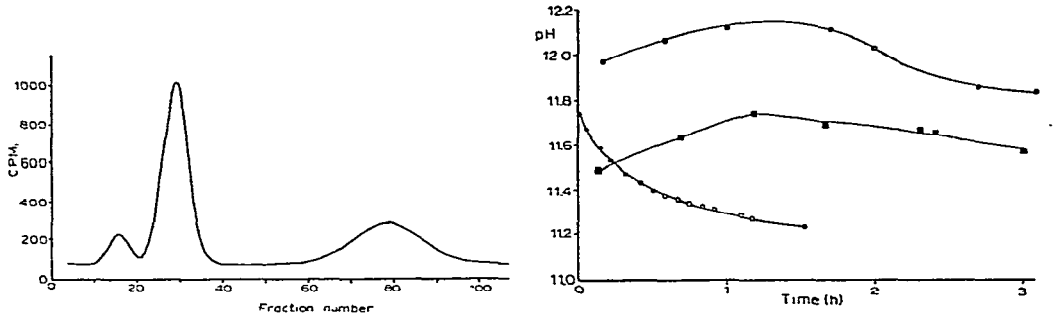

Fig. 3. Fractionation of the reaction mixture obtained from the action of alkali on D-glucose-^{14}C in the presence of benzeneboronic acid.

Fig. 4. Change in pH on heating solutions of D-glucose and D-fructose at 50° with an equimolar amount of benzeneboronic acid. •—•, 25.7% of D-glucose, pH 12.42 at 24°; ■—■, 25.8% of D-glucose, pH 12.27 at 20°; O—O, 24.6% of D-fructose, pH 12.27 at 25°.

Research into the nature of the reaction in concentrated solutions will continue as it is clear that this is very different from the reactions in dilute solutions. It was shown earlier[8] that the preferential complexing of benzeneboronic acid with D-fructose compared with D-glucose is decreased when the overall concentration of the solution is increased, but this effect does not seem large enough to explain the decreased yields. It is possible that, in concentrated solutions, areneboronic acids form polymers of a type similar to those known to occur with borate, and thus less of the free acid is available for complex formation.

EXPERIMENTAL

Materials. — Benzeneboronic anhydride was prepared by the method of Washburn and Levens[15] and recrystallised twice from benzene. Sulphonated benzeneboronic acid was prepared as its potassium salt by the method of Garegg and Lindberg[16]. 3-Nitrobenzeneboronic acid was prepared by the method of Seaman and Johnson[17]. 4-Methoxybenzeneboronic acid was prepared as its anhydride, by the same method as benzeneboronic acid.

Radioactively labelled compounds were supplied by The Radiochemical Centre (Amersham, Bucks).

Measurements of pH were made by using a standard, combined glass-reference

electrode, except where specified, when a 205 HA glass electrode (for highly alkaline solutions) and a 305W sleeve reference electrode (for viscous solutions) were used. All electrodes were supplied by Pye-Unicam (Cambridge). Borasorb resin for the removal of borate anions from solution was supplied by Calbiochem Ltd. (Los Angeles).

Analytical methods. — The automated analytical techniques used Technicon autoanalyzer modular equipment. Hexoses were determined by an automated spectrophotometric cysteine–sulphuric acid assay[13]. Formaldehyde, obtained by the periodate oxidation of carbohydrates, was determined by an automated, spectrofluorometric periodate–pentane-2,4-dione assay[18].

Assay for fructose by an automated resorcinol method. — The automated assay is based on the manual method of Yaphe and Arsenault[9]. Reagents: *A*, resorcinol (recrystallised from benzene and stored in the dark, 0.5 g) in conc. hydrochloric acid (sp. gr. 1.18, 1 litre) and water (100 ml). *B*, 1,1-diethoxyethane (0.25 ml) in water (500 ml). Fig. 1 shows the assay schematically.

An aqueous solution of D-fructose (0–80 μg/ml) was sampled (0.1 ml/min) and mixed with air (0.42 ml/min), reagent *A* (0.53 ml/min), and reagent *B* (0.03 ml/min). The reaction flow-stream was heated for 3 min at 95° and cooled, and its absorbance measured at 550 nm. Calibration of the assay showed that it gave a linear response against concentration of D-fructose, and that interference by benzeneboronic acid, sodium benzeneboronate, D-glucose, and D-mannose in equimolar amounts was $<1.5\%$.

Assay of radioactive materials. — Aqueous samples (1 ml) of sugars-^{14}C (0–0.2 nCi) were emulsified with a phosphor (10 ml) of Butyl-PBD [2-(4′-*tert*-butylphenyl)-5-(4″-biphenylyl)-1,3,4-oxadiazole; 15 g], toluene (scintillation grade, 666 ml), and Triton X-100 (333 ml). The solutions were counted in phials (low potassium, Hewlett–Packard) in a ABAC SL40 Intertechnique scintillation spectrophotometer for 10 min. Calibration with D-glucose-*1*-^{14}C (~5 μCi/g) showed that the counts/min (c.p.m.) value was directly proportional to the concentration of sugar, both when the sample was dissolved in water and in the borate buffer used in the chromatography.

Analysis of sugars by fractionation on an ion-exchange resin. — The method used was based on that described by Wood and Cousins[11]. AG Dowex-1 x8 (borate) resin (200–400 mesh) was packed into a jacketed column to give a bed (~76 × 0.6 cm) at 50°. The column was pumped (1.2 ml/min) with a borate-buffer solution (16mM $Na_2B_4O_7$, 0.2M H_3BO_3) and the eluate, after suitable dilution where necessary, was assayed for hexoses by the automated cysteine–sulphuric acid method. The system was calibrated by fractionating samples (0.5–1 ml) containing D-mannose, D-glucose, and D-fructose (2–4 mg each) and benzeneboronic acid–sodium benzeneboronate (~1:1, in total an equimolar amount with respect to all the sugars present). The analysis was the same in the absence of benzeneboronic acid.

Transformation of radioactively labelled glucose. — A sample of dry, anhydrous D-glucose (10 g) containing D-glucose-*1*-^{14}C (~50 μCi) was prepared. To a solution of sodium hydroxide (0.264 g) and benzeneboronic anhydride (1.152 g) in water

(4.017 g) was added a sample (2.000 g) of the radioactive D-glucose. A clear solution (pH 12.17 at 24.5°) was obtained on the addition of a concentrated solution of sodium hydroxide (50.0% w/w; 0.39 ml). The solution was sealed under an atmosphere of nitrogen in a glass phial and heated at 50° for 1.67 h. The reaction mixture was stored at −15° until analysis.

The syrupy transformation mixture was analysed for fructose. A sample (~100 μl) was accurately weighed and a known weight of water (9.97 g) added from a dispenser. After mixing, the diluted sample was further diluted by adding an aliquot (500 μl) to a known weight of water (9.97 g), and then analysed by the automated resorcinol assay which had been calibrated with a standard solution of D-fructose; the yield of D-fructose was 49.4%.

An aliquot (42.27 mg) of the transformation mixture was diluted with water (~0.5 ml) and fractionated on a column of anion-exchange resin in the borate form in the manner described previously. Another aliquot (38.14 mg) was similarly diluted and then slightly acidified with dilute sulphuric acid. The precipitate which formed almost completely redissolved on warming to 50°, and the sample was then fractionated in a similar manner to the previous one. Both fractionations showed a yield of D-fructose of 46.7%, and that 24.1% of the original D-glucose remained. The elution position of the third peak was ~20% greater than that of standard D-mannose.

The fractionation of an aliquot (79.37 mg) of the transformation mixture was monitored with both the automated resorcinol and cysteine–sulphuric acid assays. The unknown peak gave an area on the resorcinol assay 6.1% that of the D-fructose peak, corresponding to a yield of 2.8% for the unknown product, based on the assumption that it gave the same molar response as D-fructose. The D-glucose peak gave no response on the resorcinol assay.

Radioactive assay of transformation reaction products. — An aliquot (57.10 mg) of the transformation mixture was chromatographed on a fresh batch of the resin in the borate form, and the remainder of the eluate (after having been sampled for the assay) was collected in fractions (8.7 ml) and each sample was assayed for radioactivity. Fig. 3 shows the chromatogram obtained, the three radioactive peaks corresponding exactly to the peaks in the cysteine–sulphuric acid assay. When the last (D-glucose) peak had been eluted, an attempt was made to wash the column with M hydrochloric acid, but the precipitated boric acid blocked the outlet. The resin was removed and washed with the acid (100 ml) in a sintered-glass funnel, and the washings were assayed for radioactivity; Table I shows the results. The sum of radioactivity recovered accounted for 95.7% of that calculated to be present in the original sample. The fractions containing the fructose peak were combined, a sample (5.00 g) of D-fructose was added, and the resulting solution, after neutralisation, was passed down a column of Borasorb resin to remove the boric acid. The eluate was evaporated under reduced pressure to give a syrup, and dry methanol was added. After a few days, the crystals which had formed were filtered off, crushed, and dried at 61° over phosphorus pentaoxide. The radioactive assay gave 93.5% recovery of D-fructose, based on the amount of fructose calculated to be present in the fractions.

Assay for D-*fructose by isotope dilution.* — An aliquot (0.1555 g) of the transformation syrup and D-fructose (5.2093 g) was dissolved in water (10 ml). The neutralised solution was left overnight at room temperature and then evaporated under reduced pressure to give a syrup. The di-*O*-isopropylidene derivatives of the hexoses present were made by the method of Bell[19]. After the treatment with 50mM sulphuric acid, the di-*O*-isopropylidene derivative of D-fructose was removed from the mono-*O*-isopropylidene derivatives of D-glucose and D-mannose by extraction with chloroform. 1,2:4,5-Di-*O*-isopropylidene-*β*-D-fructopyranose, recrystallised twice from light petroleum (b.p. 60–80°), had m.p. 95–96°, $[\alpha]_{589}^{20}$ −34.5° (*c* 0.9, water). The radioactive assay of this sample corresponded to a yield of 44.5% of D-fructose.

Assay for D-*mannose by isotope dilution.* — To an aliquot (0.1237 g) of the transformation mixture, D-mannose (2.0333 g) and water (20 ml) were added. The resulting solution was neutralised and, after 2 h, concentrated to a syrup under reduced pressure. 2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose was prepared by the method of Bell[19] and recrystallised to constant specific radioactivity from light petroleum (b.p. 60–80°). The radioactive assay showed that 2.3% of the D-glucose had been converted into D-mannose.

The fractions corresponding to the unknown peak collected from two fractionations of the transformation mixture were combined. A known amount was added to D-mannose and treated in a similar way to that described above, removing borate with the Borasorb resin. The radioactive assay showed that 36.8% of this peak was D-mannose, corresponding to an overall conversion of 2.2% of D-glucose into D-mannose.

Investigation of unknown product absorbed on the resin. — The radioactive product that had been washed from the ion-exchange resin by dilute hydrochloric acid was analysed for the presence of acidic compounds. A sample was basified with aqueous sodium hydroxide and left for 1 h to allow lactone hydrolysis. The sample was then added to a column (132 × 0.6 cm) of Dowex-AG1 x8 resin (200–400 mesh) which was maintained at 30°. The column was eluted with 0.5M acetic acid (0.8 ml/min) and the eluate was monitored with the automated, spectrofluorimetric periodate–pentane-2,4-dione assay. The only products detected were eluted rapidly from the column, in a position corresponding to that of neutral compounds.

Investigation of parameters affecting the conversion of D-*glucose into* D-*fructose in the presence of benzeneboronic acid in concentrated solutions.* — (*a*) *General procedure.* An accurately weighed amount of benzeneboronic anhydride was added to water containing a known weight of sodium hydroxide. To the resulting solution or suspension, D-glucose (2, 1, or 0.5 molar equivalents with respect to benzeneboronic acid) was added, and a clear solution was obtained by stirring. The pH at room temperature was measured and adjusted, when necessary, with a small, known volume of a concentrated, standard solution of sodium hydroxide. Aliquots (0.5–1.0 ml) of the solution were put in small glass phials and, where applicable, deaerated with nitrogen. The sealed phials were placed in a constant temperature environment, and, at known times, removed and stored at 0° or −15° until analysis. The samples were

analysed with the automated resorcinol assay for fructose; a weighed amount of the syrup was diluted twice with a known weight of water to give a solution containing up to 80 μg of D-fructose per ml.

The effects of nitrogen and air (*A*), the temperature of the reaction (*B*), the concentration of sodium hydroxide (*C*), and the ratio of benzeneboronic acid to D-glucose (*D*) on the maximal yield of D-fructose were investigated, and the results are given in Table II.

(*b*) *Effect of substituted benzeneboronic acids.* The previous experiment was repeated by using the potassium salt of sulphonated benzeneboronic acid, 3-nitrobenzeneboronic acid, and 4-methoxybenzeneboronic acid. The results are also given in Table II.

(*c*) *Effect of changes of pH.* A solution was prepared containing D-glucose (25.7% w/w), an equimolar amount of benzeneboronic acid, and sodium hydroxide (7.02% w/w). This solution (pH 12.42 at 24°) was put in a closed, jacketed container and heated to 50°. The electrodes were introduced intermittently and the pH measured at 48 ± 1°. A similar experiment was performed, starting with D-fructose (24.6% w/w), an equimolar amount of benzeneboronic acid, and sodium hydroxide (6.15% w/w); the solution initially had pH 12.27 at 25°. A third solution contained D-glucose (25.8% w/w), an equimolar amount of benzeneboronic acid, and sodium hydroxide (7.13% w/w), and had pH 12.27 at 20°. The results are shown in Fig. 4.

In these experiments, the pH was also monitored during the period (~10 min)

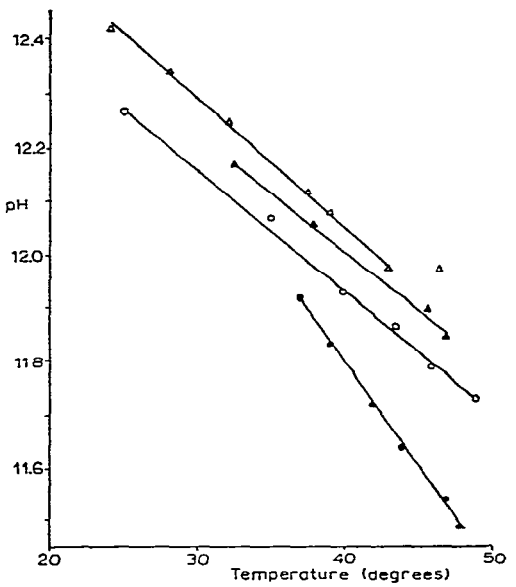

Fig. 5. Change in pH with temperature of solutions of sugars containing an equimolar amount of benzeneboronic acid. (*a*) △—△, 25.7% of D-glucose, initially pH 12.42 at 24°; (*b*) •—•, 25.8% of D-glucose, initially pH 12.27 at 20°; (*c*) O—O 24.6% of D-fructose, initially pH 12.27 at 25°; (*d*) ▲—▲, solution (*a*), cooled after heating for 3.1 h at 50°.

that the solutions took to attain a steady temperature. The pH of the first solution was also monitored during the cooling period after 3.1 h at the reaction temperature. Fig. 5 shows the results.

Transformations in dilute solutions in the presence of benzeneboronic acid. — An aliquot (20 ml) of an alkaline solution of benzeneboronic acid (20 or 40mM, pH 12 or 13, NaOH) was added to an anhydrous sample (72 mg) of the sugar (D-glucose, D-mannose, or D-fructose), and the resulting solution was adjusted to the required pH (12 or 13) at 20° with M sodium hydroxide (~0.1 ml). The solution was briefly deaerated with nitrogen and then maintained at 50° in a closed system, with continous monitoring for D-fructose by an automated resorcinol assay which incorporated a suitable dilution-stage. Fig. 2 shows graphically the percentage of D-fructose present (based on amount of original sugar) against time when the reactions were carried out at pH 12 and 13 with an equimolar concentration of benzeneboronic acid, and at pH 12 with a bimolar ratio of benzeneboronic acid to sugar.

A similar experiment performed with a more-concentrated solution of benzeneboronic acid (0.125M) and D-glucose (0.125M) gave comparable results. Use of a solution which initially had pH 12.0 at 20° gave a yield of 72% of D-fructose after 6 h at 50°, and this value fell by 2% during the next 18 h. The pH fell by 0.13 unit in the first 6 h and a further 1.17 units during the next 18 h. A reaction mixture of the same composition, but at pH 11.0 at 20°, gave a yield of 21% of D-fructose after 20 h, and during this period the pH had fallen by 1.5 units.

Transformations in dilute solutions in the presence of substituted benzeneboronic acids. — Transformations were also carried out with aqueous solutions of D-glucose (20mM), at pH ~12, which contained one molar equivalent of (*a*) 4-methoxybenzeneboronic acid and (*b*) 3-nitrobenzeneboronic acid. The reaction conditions and method of analysis were the same as for the experiment with benzeneboronic acid.

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey, C.B.E., F.R.S., for the opportunity to pursue this research and for his interest therein. They also thank Boehringer Mannheim GmbH, Germany, for a Research Fellowship (to B.W.H.).

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