Studies on Glycosides and Isopropylidene Derivatives

Synthesis by Cation Exchanger Catalysis

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Methyl glycoside formation catalyzed by acid-regenerated cation exchange resins was studied by titrimetric, polarimetric, and paper chromatographic methods. Glucose, when reacted with methanol, gave reducing power and optical rotation curves of the same shape as those obtained on mineral acid catalysis. The sequence of the reaction products formed was examined. The main product was α -methyl D-glucopyranoside (yield above 80 %). Maltose and lactose gave rise only to traces of disaccharide glycosides, methyl glycosides of the component monosaccharides constituting the main products. Fructose was rapidly degraded by the acid resin.

Furthermore, the resin catalyzed formation of isopropylidene derivatives was studied. Fructose reacted readily with acetone at 20° C to 1,2-4,5-diisopropylidene D-fructopyranose. Glucose, at 20° C reacted only slowly, but with boiling acetone a mixture of 1,2-mono- and 1,2-5,6-diisopropylidene glucoses was obtained. In the presence of borate the acetonylation was impeded.

In many instances of acid catalysis protonated cation exchangers may replace the mineral acid. Hereby several advantages are achieved, including a gentle treatment of the reactants and a ready removal of the catalyst after finished reaction. Sussman¹, apparently first in this field, in 1946 reported on the formation and solvolysis of esters and acetals and the inversion of sucrose, under the influence of ion exchange catalysts. Later the kinetics of the exchanger catalyzed ester hydrolysis was studied by Haskell and Hammett², while the corresponding sucrose inversion was quantitatively elucidated by Bodamer and Kunin³, and others. In 1952 Osman, Hobbs, and Walston⁴ applied ion exchange resins to the preparation of methyl glucuronides from glucuronolactone, and in a similar way some methyl glycosides and sugar isopropylidene derivatives were synthesized by Cadotte, Smith, and Spriestersbach⁵. The relative efficiencies of ion exchangers and of sulfuric acid in some acid catalyzed sugar reactions were examined by Wadman⁶. It was found

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Fig. 1. Resin catalyzed methyl glucoside formation, at 20° C (full circles), and at 65° C (open circles).

A. Reducing power. B. Optical rotation.

that, on a molar basis, ion exchangers (sulfonic acid type) were less effective than the mineral acid.

The experiments reported in the present paper were performed in order to study, in some detail, the materials formed in the resin-catalyzed reactions of some sugars with methanol and acetone. Besides, the resin catalyzed acetonylation was applied to the isolation of sugars subsequent to an alkali-induced aldose isomerization.

Methyl glycosides

Some mono- and disaccharides were glycosidified with methanol using as catalysts acid-regenerated cation exchangers of the sulfonated polystyrene type.

Thus, glucose was reacted with methanol at 20° C, by shaking with methanol and catalyst, and at 65° C, by percolating a boiling glucose-methanol solution through a resin column in a closed system, utilizing the apparatus shown in Fig. 4. From time to time aliquots were withdrawn for analysis. Changes in reducing power and optical rotation are presented in Fig. 1 A and B.

The curves are similar to those obtained on mineral acid catalysis, as reported by Levene *et al.*⁷ A difference in reaction velocity is apparent, however,

Fig. 2. Resin catalyzed methyl glycoside formation of mono- and disaccharides at 20° and 65° C.

A. Glucose. a. After 10 hours at 20° C. b. 15 h (20° C). c. 20 h (20° C). d. 300 h (20° C). e. 100 h (65° C). f. Mother liquor (65° C), chromatographed for 15 h. g. Same as in f, but chromatographed for 45 h.

1: Methyl glucofuranosides. 2: α -Methyl D-glucopyranoside. 3: β -Anomer of 2. 4: Glucose. 5 a, b: Disaccharide glycosides? 6: Trehalose (+ maltose?). 7: Isomaltose.

B. Maltose. a. 4 h (65° C). b. 50 h (65° C). c. Mother liquor (65° C).
1, 2, 3, 4: Same as for glucose. 5: ? 6 a, b, c: Disaccharide glycosides? 7: Trehalose + maltose. 8: Isomaltose. 9: Maltotriose? 10: Maltotetraose? 11: Maltopentaose? 12: ?

C. Lactose. a. 45 h (65° C). b. 95 h (65° C). c. Mother liquor (65° C).
1: Methyl glucofuranosides. 2: Methyl galactofuranosides. 3: α -Methyl glucopyranoside. 4: β -Anomer of 3. 5: α -Methyl galactopyranoside. 6: β -Anomer of 5. 7: Glucose. 8: Galactose. 9, 10: Disaccharide glycosides? 11: Lactose. 12: ?

D. Fructose. a. 1 h (65° C). b. 40 h (65° C).
1: Methyl fructofuranosides. 2: Methyl fructopyranosides. 3: ? 4: Fructose. 5: Difructose dianhydride?

the resin-catalyzed reaction being slower by a factor of about 10, at both temperatures. It might be reasonable to attribute this inferior activity of the ion exchanger, earlier demonstrated by Wadman⁶, to the limited accessibility for solute molecules to the active groups of the resin.

The minimum in the optical rotation curve obtained at room temperature in the mineral acid experiments⁷ was found to coincide with the maximum percentage of furanose isomers in the reaction mixture. Furanoside determinations proved this to hold true also in the present case, the maximum yield of furanosides being 50 % of total sugars.

The nature and the order of appearance of reaction products were revealed by paper chromatography (Fig. 2 A). Thus, methyl glucofuranosides were the first glucosides to appear, later followed by the β -glucopyranoside, which was then gradually converted into the α -anomer. No signs of other monosaccharide derivatives, as full acetals or septanosides, were observed. On the other hand, polysaccharide synthesis manifested itself already when only minor amounts of pyranosides had been formed. Distinct spots of isomaltose and trehalose were obtained, and it is probable that small amounts of maltose were present too, although obscured by the trehalose spot. In this case higher reversion products, containing more than two monosaccharide units, were not encountered.

α -Methyl D-glucopyranoside, constituting the main reaction product, was obtained in a practical yield of 60 % after percolating for 30 hours, the yield being increased to well above 80 % by recycling the mother liquor (*cf.* Fig. 1).

The behaviour of disaccharides towards the resin was tested on maltose and lactose. When percolated as glucose they gave reducing power *versus* time curves of the same shape as in Fig. 1 A, the rate of reaction, however, being lower. In the case of lactose the extremely low velocity was attributable to the slight solubility of the sugar in methanol.

According to paper chromatograms (Fig. 2 B and C) the gradual disappearance of maltose and lactose was not attended by a considerable disaccharide glycoside formation. Instead both sugars produced essentially methyl glycosides of the component monosaccharides, indicating the glycosidification of the disaccharides to be slow compared to the methanolysis reaction. The chromatograms of the maltose series closely resembled those of glucose but contained also some new spots in the low R_F region, evidently corresponding to reducing oligosaccharides with three and more monosaccharide units. Methyl gluco- and galactosides, in all their isomeric forms, comprised the main part of the lactose series chromatograms. In addition to lactose a few disaccharide spots were observed, but reversion products containing more than two monosaccharide units seemingly were absent in this case.

Fructose, being a representative ketose, was also subjected to methanol percolation. However, owing to its sensitivity to acids, the fructose molecule was rapidly degraded when exposed to the catalyst. The solution turned brown, and degradation products were deposited in the pores of the resin, thus causing a severe inactivation of the catalyst (Table 1).

Table 1. Resin catalyzed reaction between fructose and methanol at 65° C.

Reaction time hours	Reducing power per cent
0	100
1	8.0
3	4.5
5	4.4
22 *	4.0
42	3.7

* After 22 hours the used catalyst was discarded and replaced with fresh resin.

As appears from Table 1 substitution of the catalyst by fresh material only caused a temporary improvement. Some fructoside formation took place however (Fig. 2 D), but it was not possible to isolate any crystalline fraction from the reaction mixture.

Isopropylidene derivatives

Resin catalyzed condensations of glucose and fructose with acetone were carried out. It was found that fructose reacts rather rapidly at room temperature by merely being shaken with acetone and catalyst. An unduly prolonged reaction time is to be avoided because of the danger of degradation of fructose, this process being catalyzed by the acid decomposition products. Moreover, acetone itself may undergo condensation, and the materials formed are only difficultly separable from the sugar derivatives. — Already in about six hours most of the fructose had reacted, and in less than 24 hours a 40 % yield of 1,2-4,5-diisopropylidene fructofuranose was obtained, while only small amounts remained of the monoisopropylidene derivative first formed, as demonstrated paper-chromatographically. Only traces of other acetone fructose derivatives were observed.

The condensation of glucose with acetone requires a higher temperature to become of preparative value, and hence a modified technique involving soxhlet extraction with acetone of a glucose-resin-drierite mixture was employed. Since the acetone glucose derivatives are removed from the catalyst immediately after their formation, and since monoacetone glucose is formed more readily than the diacetonlated compounds the extraction technique enhances the relative proportion of the monoacetone derivative. The mixture resulting from a 24 hours extraction contained besides acetone-glucoses appreciable amounts of acetone autocondensation products. Resolution of the mixture by differential extraction yielded 1,2-mono- and 1,2-5,6-diisopropylidene glucofuranoses in roughly equal amounts.

The experiences gained with the ion exchange resins employed as catalysts indicate this technique to be a convenient method for the preparation of many sugar derivatives. Particularly methyl aldoses and acetone ketose derivatives may be advantageously produced in this way. Apparently the resin functions analogously to the strong acid in the Fischer type syntheses of glycosides and acetone derivatives.

Aldose isomerization

In the alkali induced isomerization of aldoses the equilibrium may be displaced towards the ketose by adding borate, as has been pointed out by Myrbäck², the operative effect being the high stability of the ketose-borate complexes involved.

This principle was applied to glucose, the isomerization being effected by alkaline borate buffers. When the transformation, as indicated by the optical rotation of the solution (Table 2), had come to an end, the mixture was examined paper-chromatographically (Fig. 3). Glucose and fructose in ample amounts — and their borate complexes — were present, but mannose, which ought to be formed according to the enediol mechanism of the Lobry de Bruyn transformation was not observed.

Most of the electrolytes were removed from the solution by ethanol precipitation, and after concentration of the filtrate the acetonlation technique was attempted to the isola-

Table 2. Isomerization of glucose at 20° C by means of alkaline borate. Composition of solution: glucose 0.56 M, boric acid 0.63 M and potassium hydroxide 0.88 M.

Time min	$[\alpha]_{20}^D$	Time min	$[\alpha]_{20}^D$
7	+ 20.4°	240	+ 15.0°
14	21.7	420	6.9
20	21.9	1 260	— 12.5
30	22.5	1 500	15.2
36	22.8	1 860	18.2
46	22.9	2 700	20.4
60	22.5	3 180	20.9
90	21.8	4 140	20.6
130	20.2		

tion of fructose. The process proved to be very slow, however, shaking for 95 hours with acetone and resin catalyst producing only minor amounts of isopropylidene fructose derivatives (Fig. 3). The acetonylation of pure fructose, on the other hand, proceeds rather rapidly, as shown above. Calcium hydroxide precipitation⁸ following acid hydrolysis of the acetone derivatives was then tried but with negative result. Since the impeded reactivity of the fructose was regarded as a borate interference, the residual boric acid in the mixture was removed by complexing with catechol and subsequent anion exchange. As a matter of fact, when all boric acid was eliminated the fructose in the isomerization mixture was readily available to calcium hydroxide precipitation and to acetonylation (Fig. 3).

Fig. 3. Alkaline isomerization of glucose.

a. Isomerization mixture after 69 hours (chromatographed for 4 hours.) b. After acetonylation. c. After acid hydrolysis of acetonylation mixture. d. Fructose precipitated from c. with calcium hydroxide. e. Mother liquor from d. freed from borate. f. Fructose precipitated from e. with calcium hydroxide. g. Same as in a, but chromatographed for 20 hours. h. Fructose, mannose, and glucose.

1, 2, 3: Isopropylidene fructoses. 4, 5, 6: Isopropylidene glucoses. 7: ? 8: Fructose. 9: Glucose. 10 a, b: Fructose-borate complex. 11: Glucose-borate complex. 12: Difructose dianhydride? M: Mannose.

Fig. 4. Percolation apparatus. A. Boiling tube. B. Catalyst tube. C. Resin column. D. Sintered glass disc. E. Funnel supporting catalyst tube. F. Centering device for catalyst tube. G. Reflux condenser. Scale 1:4.

EXPERIMENTAL *

Materials. D-Glucose, anhydrous; maltose, monohydrate; lactose, monohydrate; D-fructose, all of *p. a.* quality. Methanol, absolute, purissimum, water content below 0.03 %. Acetone, purissimum, dried with drierite. Cation exchange resins, IR-120 and Dowex 50, in the hydrogen cycle.

Methods. For methyl glycoside synthesis the following standard procedure was employed:

In the boiling tube of the apparatus (borosilicate glass; Fig. 4) dissolve 25 g of the sugar in 400 ml of methanol by refluxing. Meanwhile prepare the catalyst column by placing a thin layer of glass wool in the bottom of the tube B and over that the resin (25 g) covered by another layer of glass wool. The stem of the supporting funnel E should protrude about an inch above the surface of the column. When the sugar is dissolved insert the prepared column, remount the condenser, and heat the boiling tube on a water bath. By boiling the solution is forced up through the stem of the funnel, then it percolates through the resin and finally drains back into the boiling tube through the sintered glass disc D. Bumping is avoided by placing broken quartz in the boiling tube. Percolation is continued, until the reducing power of the solution has attained a minimum value. The ensuing solution is crystallized fractionally. During the reaction aliquots are withdrawn for paper chromatographic examination and for the determination of reducing power and optical rotation.

The reducing power was determined by the Somogyi iodimetric micro method¹⁰.

Paper chromatography was carried out on Whatman No. 54 paper, using ethyl acetate, acetic acid, water (60:17:17.5), and *n*-butanol, pyridine, water (3:2:1.5) mixtures as solvents. The silver reagent of Trevelyan *et al.*¹¹ was capable of detecting, in addition to reducing sugars, glycosides and other nonreducing materials, provided sufficient time was allowed for the reaction. By means of sodium sulfide black silver sulfide spots were eventually obtained. Alternatively, the naphthoresorcinol reagent¹² was used for the detection.

* All melting points were determined on the Kofler block.

Although not explicitly stated in every instance, running of parallel chromatograms with authentic specimens was used as a general method of characterization of reaction products.

Methyl glycosides

Glucose. Glucose (10 g) was shaken at 20° C with cation exchanger (10 g) and methanol (160 ml). Reducing power and optical rotation are given in Fig. 1 A and B, and paper chromatograms in Fig. 2 A.

At 65° C glucose (25 g) was reacted according to the standard procedure (Figs. 1 A and B and 2 A). In about 60 hours the reducing power had decreased to a constant value of about 0.5 % of the original value. In 30 hours 15 g of crude α -methyl glucoside were obtained, and recycling of the mother liquor for at least 30 hours yielded another 6 g of glucoside. Processing of the crystalline fractions and the mother liquors according to Patterson and Robertson¹³ yielded in all 22 g (82 %) of α -methyl-D-glucopyranoside, m. p. 166° (from methanol), $[\alpha]_D^{20} + 160^\circ$ (c 0.5, in water), and 0.9 g of β -methyl glucoside, m. p. 103–7° (from methanol), $[\alpha]_D^{20} - 32^\circ$ (c 1.1, in water). The authenticity of the glucosides was confirmed by infra-red absorptiometry.

Maltose. Maltose (25 g) was percolated for 50 hours according to the standard procedure. Chromatograms are given in Fig. 2 B. In 50 hours the reducing power had decreased to 1.7 %. Working up of the solution yielded one crystalline fraction, m. p. 154–62° (from methanol), $[\alpha]_D^{20} + 155^\circ$ (c 0.5, in water), identified as α -methyl D-glucopyranoside (mixed melting point, paper chromatography, IR spectrum). Yield 14 g (48 %).

Lactose. By percolating a suspension of lactose (25 g) and methanol (400 ml) through the resin 15 g lactose could be brought into solution after 45 hours. After removal of undissolved lactose the solution was percolated further for 50 hours as above. Chromatograms are given in Fig. 2 C. The end value of the reducing power was 0.5 %. Upon crystallization α -methyl D-glucopyranoside, m. p. 161–5° (from methanol), and α -methyl D-galactopyranoside were obtained.

Fructose. The standard procedure was applied to fructose (25 g). The solution rapidly turned yellow, and then brown, and the catalyst became impregnated with black condensation products. The decrease of the reducing power finally stopped at about 4 % (Table 1), but the percolation was continued after substituting the resin by fresh material. After percolation the solution was decolorized by charcoal and concentrated to 25 ml. All attempts to crystallize the syrup failed, however. Chromatograms are given in Fig. 2 D.

Isopropylidene derivatives

Fructose. A mixture of fructose (8.0 g) and ion exchanger (8 g) was shaken at room temperature with acetone (150 ml), until the optical rotation of the solution became constant (24 hours). The solution was filtered, a little calcium carbonate was added, and the solvent was driven off at a reduced pressure. Extraction of the residue with an 1:20 ether-light petroleum (b. p. 40–60° C) mixture followed by crystallization yielded 4.4 g (38 %) of ("a")-1,2,4,5-diisopropylidene D-fructopyranose, m. p. 118° (from light petroleum), $[\alpha]_D^{20} - 159^\circ$ (c 0.5, in water). Trace amounts of the monoisopropylidene derivative were also present.

Glucose. A mixture of glucose (4.0 g), drierite (4 g) and resin catalyst (8 g) was extracted with boiling acetone (75 ml) for 25 hours in a soxhlet apparatus. After separation of unchanged glucose and acetone autocondensation products (2.5 g) the filtrate was evaporated to dryness at a diminished pressure. Most of the residual acetone condensation products were eliminated by treating the residue with water (50 ml) at room temperature and filtering through talc. The water was driven off from the filtrate at a reduced pressure and the residue on benzene extraction at room temperature and decolorization with charcoal yielded 1.6 g of 1,2,5,6-diisopropylidene D-glucofuranose, m. p. 105–7° (from *n*-heptan). The benzene-insoluble on room temperature extraction with acetone yielded 0.8 g of 1,2-monoisopropylidene glucose, m. p. 143–5° (from ethanol-light petroleum).

Aldose isomerization

10.0 g of glucose (56 millimoles) were dissolved in 25.0 ml of a borate buffer (2.50 *M* in boric acid and 3.50 *M* in potassium hydroxide) and the volume was adjusted to 100 ml with water. The solution was deaerated with nitrogen gas and was kept under nitrogen at 20° C. Part of the solution was filled in a polarimeter tube, and readings were taken, till the optical rotation became constant (70 hours, Table 2). Paper chromatographic examination of the solution then proved the presence of glucose and fructose as sole sugars. The pH of the faintly yellow solution was adjusted to 4 by means of sulfuric acid, and electrolytes were precipitated with 95 % ethanol (250 ml). The precipitate was washed on the filter with some ethanol and the filtrates were concentrated at a reduced pressure to a thin syrup whereby a great part of the residual boric acid was deposited. After filtration the syrup was concentrated *in vacuo* to dryness, the water being driven off as the ternary azeotrope with ethanol and benzene.

The dried and powdered reaction product (8.0 g) was shaken at room temperature with ion exchanger (10 g) and acetone (150 ml) for 95 hours. After 40 hours drierite (5 g) was added. The reaction mixture contained, in addition to glucose and fructose and their borate complexes, small amounts of fructose isopropylidene derivatives (Fig. 3). The solution was filtered from the catalyst, a little calcium carbonate was added, and the solvent was driven off at a reduced pressure. The residue was leached with water (100 ml) at room temperature, the mixture was filtered through charcoal, and the dissolved isopropylidene derivatives were hydrolyzed by 0.01 *N* hydrochloric acid at 85° for 18 hours. The hydrolysate was precipitated at +5° C with powdered calcium hydroxide (6 g), and the precipitate was washed on the filter with water (3 × 10 ml) and then suspended in 100 ml of water and decomposed with 2.5 *M* sulfuric acid (22 ml). After removal of the calcium sulfate, the pH of the solution was adjusted to 4, and the water was evaporated at a reduced pressure. The residue (0.5 g) contained only traces of fructose (Fig. 3).

The filtrate from the above calcium hydroxide precipitation gave a distinct borate reaction. Catechol (6 g) and potassium carbonate (2 g) were added, and the solution was filtered and passed through a column of anion exchanger (IR-4B, in the hydroxyl cycle). The eluate was acidified with hydrochloric acid, and excess catechol was removed by ether extraction.

Two thirds of the borate-free eluate were subjected to the calcium hydroxide precipitation, and decomposition of the precipitate yielded 2.5 g of fructose. The remainder of the eluate was brought to pH 4 and, after evaporation to dryness at a reduced pressure, was acetonylated as above. Diisopropylidene fructose was readily formed.

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