The enzyme appears to require sulfhydryl groups for full activity, but, with the exception of the reversible inhibition produced by *p*-chloromercuribenzoate, the effect of sulfhydryl inactivators is not very striking. No inhibition by sulfhydryl inactivators was found for the xylose isomerase of *P. hydrophila*.[2]

The equilibrium value of 16% xylulose found with the isomerase from *P. pestis* is very similar to that reported by others (16%,[2] 14%[3]). However, in the presence of borate, a value of 60–65% has been obtained with the enzyme from *P. pestis*, while that from *L. pentosus* gave a value of about 45%,[3] and that from *P. hydrophila* about 81%.[2]

The relatively strong inhibition of the enzyme from *P. pestis* by Tris buffer was, apparently, not evident with the preparations from *P. hydrophila*[2] and *L. pentosus*[3] which were incubated in the presence of Tris. The inhibition appears to be of the non-competitive type, although the similarity of the arrangement of the hydroxymethyl groups of Tris to those in xylopyranose might lead one to expect a substrate competition factor. Although Tris is frequently used as a buffer for biochemical studies, relatively few instances of its behavior as an enzyme inhibitor have been published. Kimmel and Smith found it to be a strong inhibitor of the activity of crystalline papain,[15] and Novelli, *et al.*, reported its inhibition of pyruvate utilization by sonic extracts of *Escherichia coli*.[16] The latter found the inhibition to be relieved by the addition of phosphate. No significant release of Tris inhibition has been obtained by the presence of phosphate buffer in the test system for xylose isomerase.

(15) J. R. Kimmel and E. L. Smith, *J. Biol. Chem.*, **207**, 515 (1954).
(16) G. D. Novelli, H. Gest and L. O. Krampitz, *Federation Proc.*, **13**, 270 (1954).

Reisberg noted that the addition of ethylenediaminetetraacetic acid to Tris-buffered choline acetylase increased the enzyme activity 450%.[17] Treatment of the Tris buffer with a solution of diphenylthiocarbazone in CCl_4 produced no change in the deep green color of the metal-binding agent, which turns a violet color in the presence of traces of heavy metals, and did not prevent the inhibition of xylose isomerase. Furthermore, the presence of excess cysteine in the test system for xylose isomerase should prevent inhibition by heavy-metal ions which might be introduced with any of the solutions used.

Hochster and Watson reported the phosphorylation of D-xylose by ATP in the presence of extracts of *P. hydrophila*, but the interpretation was equivocal because of the presence of xylose isomerase in their extracts.[2,18] De Ley reported an adaptive xylokinase in extracts of *Aerobacter cloacae*.[19] The manometric method used would not eliminate the possibility that the D-xylose was isomerized before phosphorylation occurred. Mitsuhashi and Lampen were able to demonstrate that xylulose leads to a significantly more rapid formation of sugar phosphate ester than does xylose in the presence of ATP and an extract of *L. pentosus*.[3] Since xylose isomerase activity was also present in their preparation, the results indicated that xylose was phosphorylated less readily than xylulose, if at all. In the case of *P. pestis*, it has been possible to demonstrate a xylulokinase which catalyzes the phosphorylation of D-xylulose by ATP, and has no activity with D-xylose.

(17) R. B. Reisberg, *Biochim. Biophys. Acta*, **14**, 442 (1954).
(18) R. M. Hochster and R. W. Watson, *Nature*, **170**, 357 (1952).
(19) J. De Ley, *Enzymologia*, **16**, 99 (1953).

FREDERICK, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, RIPON COLLEGE]

Chromatographic Adsorption. IV. Cation Exchange Resins as Catalysts in Glycoside Formation[1]

BY DWIGHT FAY MOWERY, JR.

RECEIVED AUGUST 26, 1954

Strongly acidic ion exchange resins in two mesh sizes and six different degrees of crosslinking were used to catalyze the reaction of D-galactose with boiling methyl alcohol. The rapid disappearance of reducing sugar and the slower conversion of the initial methyl β-D-galactosides (furanoside and pyranoside) to methyl α-D-galactosides were followed, the latter by means of a chromatographic method. It was found that the rates of both reactions increased with decreased crosslinking of the resin, closely approaching in each case the rate of the corresponding reaction catalyzed by dissolved benzenesulfonic acid. Evidence was obtained which indicated penetration of the resins by galactose and methyl β-D-galactoside molecules, less crosslinked resins producing faster reactions, presumably by allowing greater mobility of the sugar molecules within the resin. The only difference in distribution of isomers obtained from the resin and the dissolved acid-catalyzed reactions, respectively, appeared to be a tendency for the resin to produce slightly higher yields of the α-furanoside.

Several investigators[2–5] have reported the successful use of acidic ion exchange resins as catalysts in methyl glycoside formation by the Fischer method. While it has been well established that methyl glycosides are formed, little is known concerning the effect of particle size or degree of crosslinking of the resin upon the rate of the reaction. Also unknown is the effect of the resin upon isomer distribution, in particular whether this distribution is the same or fundamentally different from that produced by dissolved acid. It appeared that an answer to these questions could be obtained by utilization of the chromatographic separation of methyl α- and β-galactosides reported previously.

(1) Presented at the 126th National Meeting of the American Chemical Society in New York, September 17, 1954.
(2) G. R. Dean and R. E. Pyle, U. S. Patent 2,606,186 (1952); British Patent 670,480 (1952).
(3) E. M. Osman, K. C. Hobbs and W. E. Walston, THIS JOURNAL, **73**, 2726 (1951).
(4) J. E. Cadotte, F. Smith and D. Spriestersbach, *ibid.*, **74**, 1501 (1952).
(5) W. H. Wadman, *J. Chem. Soc.*, 3051 (1952)
(6) D. F. Mowery, Jr., and G. R. Ferrante, THIS JOURNAL, **76**, 4103 (1954).

By this means it had been shown, for instance, that the hydrochloric acid-catalyzed reaction proceeded by the rapid initial formation of principally methyl β-D-galactosides followed by the much slower change of these to methyl α-D-galactosides, mainly methyl α-D-galactopyranoside.

Experimental

Resin Preparation.—The ion exchange resins used were sulfonated polystyrene resins of 1, 2, 4, 8, 12 and 16% crosslinking manufactured by the Dow Chemical Company under the trade name of Dowex 50 by incorporation of corresponding percentages of divinylbenzene before polymerization. 50–100 and 200–400 mesh sizes were investigated and for the sake of brevity these mesh sizes will be designated in this paper as coarse and fine, respectively. The resins, supplied moist in the free sulfonic acid form, were prepared for use by six 24-hour treatments with methanol followed by partial drying on a büchner funnel. The alcohol content, determined by drying two hours at 110°, was found to be 82, 70, 57, 43, 39 and 32%, respectively, for the 1, 2, 4, 8, 12 and 16% crosslinked materials. These resins were stated by the manufacturer to have capacities of 5.2, 5.2, 5.2, 5.1, 5.0 and 4.9 meq. per g. of dry resin, respectively. For each resin and mesh size two reactions were run, one using 4.10 g. and the other 3.85 meq. per 100 ml. of methanol.

General Procedure.—In a typical reaction 5.25 g. of D-galactose (Pfanstiehl C.P.) and 350 ml. of methanol were refluxed in a 500-ml. flask with good agitation. When the alcohol had reached boiling and part of the galactose had dissolved, the resin catalyst or methanol solution of benzenesulfonic acid was added and the time noted. One-ml. samples were withdrawn at intervals and titrated for reducing sugar.[7] The reaction mixtures were worked up by simply filtering off the resin, dividing the filtrate, including the washings, into two equal parts and evaporating the alcohol under vacuum at 50°. The weight of sirup obtained, usually slightly under 3 g., was determined to the nearest hundreth of a gram. The sirup was transferred quantitatively with methanol to the top of a 4.8 × 122 cm. air-blown Florex XXX column.[8] Methanol was then run through the column under about one atmosphere pressure and the column effluent, which was analyzed by passage through a 4-dcm. polarimeter tube, was separated into the positively rotating α-galactoside and negatively rotating β-galactoside fractions. These fractions were evaporated under vacuum and the weight of α- and β-isomers produced by the resin was thus determined. In some cases duplicate runs were performed and found to differ by no more than about 5% in the weights of α- and β-isomers obtained. Thus both the initial rapid formation of non-reducing methyl D-galactosides, principally methyl β-D-galactosides, and the subsequent slower formation of α- from β-isomers could be followed and compared with the corresponding reaction using dissolved benzenesulfonic acid as catalyst. Polarimetric elution curves were plotted and areas measured so that furanoside and pyranoside distributions in each of the chromatographic fractions could be calculated in the usual way.[6] The benzenesulfonic acid used as catalyst in the comparison cases was removed after the reaction by passing the solution through a column of a weakly basic ion exchange resin. An anhydrous solution of benzenesulfonic acid in methanol was prepared most easily by passage of a methanol solution of anhydrous sodium benzenesulfonate through an alcohol-treated Dowex 50 column.

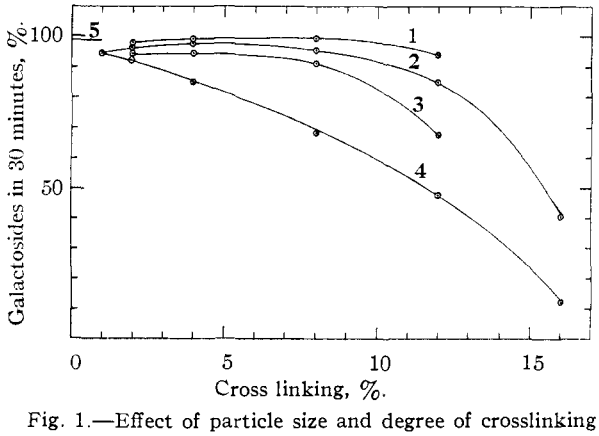

Fig. 1.—Effect of particle size and degree of crosslinking of resins upon initial rate of methyl galactoside formation: 1, equal wts. of fine; 2, equal wts. coarse; 3, equiv. wts. fine; 4, equiv. wts. coarse; 5, equiv. wt. dissolved acid.

(7) L. J. Heidt and F. W. Southam, THIS JOURNAL, **72**, 590 (1950).
(8) D. F. Mowery, Jr., *ibid.*, **73**, 5047; **73**, 5049 (1951).

Results Obtained

The initial rapid reaction of galactose with boiling methanol was found to be approximately a first-order reaction with the following rate constants for equivalent weights of 50–100 mesh 1, 2, 4, 8, 12 and 16% crosslinked ion exchange resins, respectively: 6.6, 5.1, 4.0, 2.3, 1.4 and 0.26 hr.$^{-1}$. An equivalent amount of benzenesulfonic acid produced the fastest reaction with a rate constant of 9.9 hr.$^{-1}$. The 200–400 mesh sizes produced somewhat faster reactions, the rate constants for the 2, 4, 8 and 12% crosslinked resins being about 6.6, 6.6, 5.1 and 2.3 hr.$^{-1}$, respectively. The increase in reaction rate produced by decreasing the particle size is not nearly the increase expected if the reaction is limited to the resin surface, thus indicating essentially complete, although somewhat hindered, penetration of the galactose molecules into the resin particles.

The curves of Fig. 1, calculated from the reducing sugar analysis after 30 minutes of reaction, assume that no appreciable quantities of non-reducing materials other than the four methyl galactosides are produced. Numbers 3 and 4 were obtained using *equivalent* weights of resins of various degrees of crosslinking and two mesh sizes. The value for an equivalent weight of benzenesulfonic acid also is included in the figure as the short line numbered 5. These curves, like the reaction rate constants, indicate the approach of the rate of the resin-catalyzed reaction to that of the dissolved acid-catalyzed reaction as the degree of crosslinking is decreased. The effect of decreased particle size is also evident. Curves 1 and 2, obtained for *equal* weights of resins, indicate a maximum reaction rate for the 8% crosslinked resin. This is explained by the fact that on an equal weight basis the capacity of the alcohol moist resins used for this work increases with the increased crosslinking.

The rates, determined chromatographically,[6] of the slower conversions of methyl β-D-galactosides to methyl α-D-galactosides drop below those expected of first-order reactions as the reactions continue, the faster reactions showing a greater drop than the slower ones. The reactions obtained using equivalent weights of benzene sulfonic acid and the 1% crosslinked resin have identical rates, each producing about 82% α-galactosides in 72 hours. An equivalent weight of the 12% crosslinked resin produces about 75% and an equal weight of this resin about 89% α-galactosides in the same length of time.

Curves 3 and 4 of Fig. 2 show how the 24-hour conversion of β-galactosides to α-galactosides drops off with increased crosslinking from that produced by an equivalent amount of

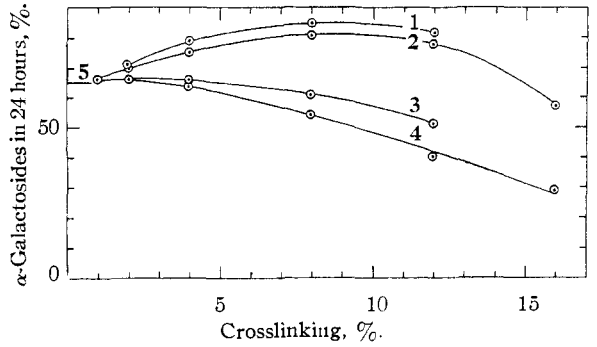

Fig. 2.—Effect of particle size and degree of crosslinking of resins upon the conversion of β- to α-galactosides: 1, equal wts. of fine; 2, equal wts. coarse; 3, equiv. wts. fine; 4, equiv. wts. coarse; 5, equiv. wt. dissolved acid.

benzenesulfonic acid, represented by the short line numbered 5. The rate for coarser resins drops off slightly faster than for the finer size. Curves 1 and 2, like curves 1 and 2 of Fig. 1, show a maximum reaction rate for equal weights of resins. As far as the reaction rates of the initial methyl galactoside formation and the conversion of β- to α-isomers are concerned, therefore, the resin-catalyzed reaction becomes more and more like the dissolved acid-catalyzed reaction as crosslinking is decreased and is almost identical for a 1% crosslinked resin.

In order to determine whether there was any fundamental difference in furanoside and pyranoside distribution in the α- and β-isomer fractions produced by dissolved benzenesulfonic acid on the one hand and the ion exchange resins on the other, the percentage furanoside, calculated according to the method previously[6] described, was plotted against percentage α-isomers for each of the chromatographic fractions. The circles in Fig. 3 represent the points obtained for benzenesulfonic acid reactions and the dots points obtained for the resin reactions. The maximum errors expected due to drying and weighing the sirups are indicated, an allowance of 0.1 g. in either direction being made. Curve 2, representing percentage furanoside in the β-isomer fractions, indicates an insignificant difference between furanoside distribution in the resin and the benzenesulfonic acid catalyzed reactions. Curve 1, however, representing % furanoside in the α-isomer fractions, indicates a tendency for the resins to produce a slightly higher % furanoside than the dissolved acid. No relationship was observed between % furanoside produced and the degree of crosslinking or subdivision of the resins. The fact that values of % furanoside greater than 100 have been obtained in some of the resin reactions can be explained on the basis of not quite complete separation of the α- and β-isomer fractions by the chromatographic column. Preliminary evidence for this has been obtained by subjecting the column effluent to anthrone analysis for total carbohydrate. Rough calculations of the effect of this incomplete separation have indicated that it will cause in all probability less than 5% error in the % of α- or β-isomers or % furanoside in the β-isomer fractions but may cause the calculated % furanoside in the α-isomer fractions to be quite high. Since this matter affects both this and the previous paper of this series, it will be considered further in a future communication. Paper chromatograms, which are capable of separating D-galactose and all four of the methyl galactosides except the two α-isomers, give no indication of the presence of a fifth substance of low optical activity which could account for the high α-furanoside values.

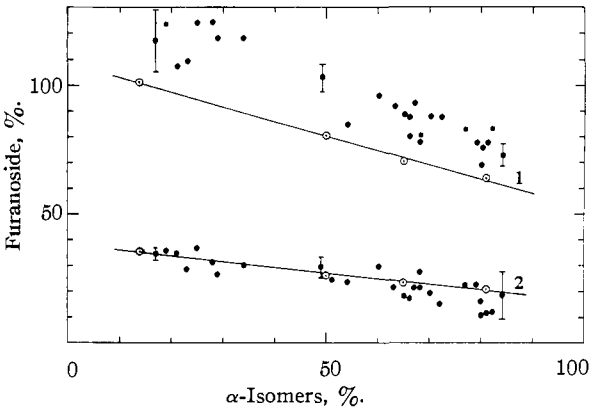

Fig. 3.—Methyl galactofuranoside distribution in α- and β-isomer fractions produced by ion exchange resins (●) and dissolved acid (⊙): 1, α-isomer fractions; 2, β-isomer fractions.

Acknowledgment.—The author wishes to express his gratitude to the Research Corporation of New York for the grant in support of this investigation and his thanks to Mr. Gerald A. Stelter for his conscientious performance of much of the routine work.

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NOTES

Vinyl Ether–Alcohol Interchange[1]

BY ROBERT L. ADELMAN

RECEIVED NOVEMBER 11, 1954

Vinyl ethers are known to react rapidly with compounds containing primary hydroxyl groups near 0° in the presence of strong acids as catalysts to form acetals.[2,3] We have observed that under considerably milder conditions of temperature and acidity than those mentioned above, and in the presence of mercuric sulfate as catalyst, the rate of acetal formation may be greatly reduced, and vinyl ethers and certain aliphatic compounds containing primary hydroxyl groups undergo an interchange reaction to form the vinyl ether of the hydroxy compound and the corresponding alcohol.

$$\xrightarrow{HgSO_4} \quad \begin{matrix} R{-}O{-}H + R'{-}O{-}CH{=}CH_2 \\ R{-}O{-}CH{=}CH_2 + R'{-}O{-}H \end{matrix}$$

Successful interchanges were accomplished using vinyl butyl ether with ethanol (expt. I), and vinyl ethyl ether with tetrahydrofurfuryl alcohol (expts. II, III). Successful interchanges in scouting runs were indicated for vinyl ethyl ether with ethylene glycol, and vinyl ethyl ether with methyl glycolate. In the case of vinyl ethyl ether with tetrahydrofurfuryl alcohol, interchange conditions sufficiently mild were found (−78°/2 hours) that no acetal formation was observed.

Mercuric sulfate appears to be a specific catalyst for the reaction as the extent of interchange is negligible in the presence of mercuric acetate or sulfuric acid (expts. IV, V). A vinyl ether appears to be a necessary reactant as no interchange of diallyl ether with ethanol occurred. Also, it appears that vinyl ethers do not undergo interchange with other ethers, as no reaction was observed in a vinyl ethyl ether–diallyl ether–mercuric sulfate mixture.

Discussion

Hill has suggested that an equilibrium exists between vinyl ethers, alcohols and acetals in the presence of acid catalysts.[2]

(1) From work presented at the New York City Meeting of the American Chemical Society, September, 1954. See also U. S. Patent 2 579,412.

(2) H. S. Hill, THIS JOURNAL, **50**, 2727 (1928).

(3) W. J. Croxall, F. J. Glavis and H. T. Neher, *ibid.*, **70**, 2805 (1948).