Calculations for Triitol.—The calculation of the first-order rate constants K_1 and K_3 for hydrolysis of the individual bonds in isomaltotriitol (I, Fig. 1) is complicated by the fact that a combination of interdependent simultaneous and consecutive reactions is occurring. Equations can be derived either on the basis of certain approximations (see below) or by solution of differential equations describing exactly the kinetic course of the reaction.[31] The approximate equations are presented here and were used for calculation of the results shown in Table IV and Fig. 1 because they offer certain conveniences, the main one being that the calculation is independent of the measured amount of isomaltotriitol remaining, I_t, so that this quantity can be used as a check on the calculated values of K_1 and K_3. The kinetically exact equations are particularly strongly influenced by I_t in the early stages of hydrolysis (*e.g.*, in the present study at $t = 7$, a change of 0.2% in I_t changes K_1 about 1.4%).

The approximation equations used are

$$K_1 = \frac{1}{t} \ln \frac{(I_0 - IV'/2)}{(I_0 - IV'/2) - II'} \quad (1)$$

$$K_3 = \frac{1}{t} \ln \frac{(I_0 - II'/2)}{(I_0 - II'/2) - IV'} \quad (2)$$

in which $II' = II_t e^{K_2 t/2}$ and $IV' = IV_t e^{K_4 t/2}$; II_t and IV_t are the observed yields of isomaltitol and isomaltose, respectively, at time t; and K_2 and K_4 are the rate constants for the hydrolysis of isomaltitol and isomaltose, respectively.

In equation 1, for example, the exponential term in II' is an approximate correction of the observed yield of isomaltitol, II_t, for loss by hydrolysis during the time t, while the $IV'/2$ term is an approximate correction of the initial concentration of triitol for the portion rendered unavailable because of the alternative hydrolysis of the sorbitol end bond to give isomaltose (IV). The first correction is based on the assumption that the "average" molecules of isomaltitol (or isomaltose) are available for hydrolysis for a period of $t/2$. For the second correction, the "effective" initial concentration of, for example, the non-sorbitol end linkage (hydrolyzed with a rate constant K_1 to give isomaltitol) is assumed to be equal to the original concentration I_0 of triitol minus half of the total or corrected number of moles of isomaltose formed (or sorbitol end bonds hydrolyzed) during time t. The acceptability of these approximations,[32] which should be considered applicable only when the disappearance of triitol is below about 50%, is shown by the generally close agreement of the values in Table IV with those obtained through use of the exact equations. For the 7-hour, 24-hour and 53-hour periods and the averages thereof the latter give for K_1 9.6, 10.1, 9.6 and 9.8, respectively, and for K_3 5.6, 4.1, 5.0 and 4.9, respectively. The values of the sum $(K_1 + K_3)$ calculated[31] from the remaining triitol, I_t, compared with the results from Table IV are, respectively, at 7 hours, 0.0152 and 0.0150; at 24 hours, 0.0147 and 0.0148; and at 53 hours, 0.0147 and 0.0136. The close relationship between the approximate equations, 1 and 2, and the exact equations[31] is shown further by taking the second approximation (*i.e.*, by setting e^{ax} equal to $1 + ax + a^2x^2/2$) of the solution of equations 1 and 2 for II_t/I_0. The result is equal to the second approximation of the corresponding exact equation multiplied by the factor $4/(4 - K_1K_3t^2)$, this factor being essentially unity at the low levels of hydrolysis at which the second approximation is usable.

Acknowledgment.—The authors are indebted to Dr. F. H. Stodola for a sample of barium maltobionate, to Phyllis L. Patrick for reducing power determination, and to B. H. Alexander for chromotropic acid analyses.

(31) The authors wish to express their thanks to Dr. L. F. McBurney, Hercules Powder Co., for the derivation of the exact equations, which are

$$I_t/I_0 = e^{-(K_1+K_3)t}$$

$$II_t/I_0 = \frac{K_1}{K_2 - (K_1 + K_3)} (e^{-(K_1+K_3)t} - e^{-K_2 t})$$

$$IV_t/I_0 = \frac{K_3}{K_4 - (K_1 + K_3)} (e^{-(K_1+K_3)t} - e^{-K_4 t})$$

The second and third of these have the same general form as the equation for the yield of the intermediate product in a series of two consecutive reactions (see, for example, F. H. MacDougall, "Physical Chemistry," The Macmillan Co., New York, N. Y., 1936, p. 425). For the calculation of the rate constants K_1 and K_3, these equations can be used in the form

$$K_1 = \frac{II_t(K_1 + K_3 - K_2)}{I_0 e^{-K_2 t} - I_t}; \quad K_3 = \frac{IV_t(K_1 + K_3 - K_4)}{I_0 e^{-K_4 t} - I_t}$$

(32) These types of correction have proven useful in our studies of the hydrolysis of polysaccharides in which the terminal linkages formed during hydrolysis were assumed to have a hydrolysis rate constant different from that of the same bond while in an inner position.

PEORIA, ILLINOIS

[CONTRIBUTION FROM MEDICAL BACTERIOLOGY DIVISION, HEADQUARTERS, CAMP DETRICK]

Xylose Isomerase from *Pasteurella pestis*, Strain A-1122[1]

BY MILTON W. SLEIN

RECEIVED AUGUST 30, 1954

An enzyme which catalyzes the isomerization of D-xylose and D-xylulose has been demonstrated in cell-free extracts of *P. pestis* which has been grown in the presence of D-xylose. It appears to require sulfhydryl groups and a metal ion for maximal activity which occurs at about *p*H 8. In the absence of borate, equilibrium is reached when about 16% xylulose is present in the reaction mixture. This value is shifted toward 60–65% xylulose in the presence of excess borate. The enzyme has half-maximal activity when the concentration of D-xylose is about 3×10^{-3} *M* at *p*H 7.5 and 30°. The isomerase is inhibited in a non-competitive manner by tris-(hydroxymethyl)-aminomethane. Crude extracts of *P. pestis* grown on D-xylose contain a xylulokinase which is able to phosphorylate D-xylulose in the presence of adenosinetriphosphate, but do not catalyze the phosphorylation of D-xylose.

Xylose isomerase catalyzes the interconversion of the free, nonphosphorylated pentoses, D-xylose and D-xylulose. It was found by Hochster and Watson[2] in extracts of *Pseudomonas hydrophila*, and by Mitsuhashi and Lampen[3] in extracts of *Lactobacillus pentosus*. As with the other organisms, *Pasteurella pestis* must be grown in the presence of xylose in order to be able to demonstrate xylose isomerase activity in cell-free extracts. Presumably, the ketose, xylulose, would also promote synthesis of the enzyme, but the substitution of glucose for xylose gives rise to cells, the extracts of which are devoid of xylose isomerase activity.

(1) Presented before the Division of Carbohydrate Chemistry at the National Meeting of the American Chemical Society at New York, N. Y., September 12–17, 1954, see Abstracts, p. 3D.

(2) R. M. Hochster and R. W. Watson, THIS JOURNAL, **75**, 3284 (1953); *Arch. Biochem. Biophys.*, **48**, 120 (1954).

(3) S. Mitsuhashi and J. O. Lampen, *J. Biol. Chem.*, **204**, 1011 (1953).

Methods

The avirulent strain A-1122 of *P. pestis* used here was originally isolated by Jawetz and Meyer.[4] Cells were inocu-

(4) E. Jawetz and K. F. Meyer, *Infectious Diseases*, **73**, 124 (1943).

lated into a liquid casein digest medium, at *p*H 7, containing 1% D-xylose. After being shaken for 24 hours at 37°, the cells were harvested, washed twice with 0.85% saline, and suspended in water to make a thin slurry which was treated in a Raytheon sonic oscillator for 30 min. at 2° at 9,000 cycles per second. The material was centrifuged at about 10,000 r.p.m., and the clear supernatant fluid was used as a source of enzyme activities.

The formation or disappearance of xylulose was followed colorimetrically by the cysteine-carbazole-sulfuric acid method of Dische and Borenfreund.[5] The reaction mixture used for enzyme assay consisted of: 0.5 ml., 0.1 *M* phosphate, *p*H 7.5; 0.1 ml., 0.02 *M* $MgCl_2$; 0.1 ml., 0.1 *M* cysteine, *p*H 7.5; 0.1 ml., D-xylose; 0.1 ml., H_2O; 0.1 ml., enzyme.

After incubation at 30°, 0.2-ml. aliquots were inactivated by adding to 0.8 ml. of dilute sulfuric acid. The color reagents were added, and the samples were read in a Klett colorimeter with a #54 filter after 1 hour of development at 30°. It was not necessary to remove protein from the reaction mixture before color development since the protein remained soluble in the acid reagent and, even with crude preparations, not much color resulted from the extract itself.

Protein in the cell-free extracts was estimated colorimetrically by means of the biuret reaction of Weichselbaum,[6] or by the method of Lowry, *et al.*[7]

The ability of crude extracts to phosphorylate xylulose (xylulokinase activity) was determined in a system containing 0.05 *M* buffer, *p*H 8, 0.007 *M* $MgCl_2$, 0.0012 *M* D-xylulose,[8] 0.0043 *M* adenosinetriphosphate (ATP), and enzyme. After incubation at 30° for 30 min., phosphate compounds and protein were removed by treatment with $Ba(OH)_2$ and $ZnSO_4$.[9] Aliquots of the supernatant solutions were analyzed for residual sugar by the colorimetric reducing sugar method of Nelson[9] and by the method of Dische and Borenfreund.[5]

The presence of xylulose as the product of action of the isomerase on D-xylose was indicated by its high absorption in the cysteine-carbazole-sulfuric acid method[5]; at 540 mμ xylulose has a molecular extinction coefficient of about 3×10^7 cm.^{2} per mole as compared with a value of about 7×10^5 cm.^{2} per mole for xylose. D-Ribulose[8] has an absorption coefficient similar to that of xylulose, but the latter could be distinguished from ribulose and other pentoses by paper chromatography. A more concentrated reaction mixture was incubated in the presence of borate, which shifts the equilibrium toward the formation of xylulose.[2,3] The material was dried and borate was removed by distillation of volatile methyl borate during repeated treatment with methanol at acid *p*H *in vacuo* at about 40°. Paper chromatography of an aqueous solution of the residue showed the presence of only 2 sugars, one of which corresponded to xylose and the other to xylulose, when 4 different solvents were used. Aniline acid oxalate was used as a spray for the detection of sugar spots.[10] The results are given in Table I. Of the six D-pentoses, only the ketopentoses (ribulose and xylulose) produce the strong carbazole color reaction given after treatment of D-xylose with the enzyme preparation. A useful spray for the detection of the ketoses was based on the reagents of Dische and Borenfreund.[5] A mixture of 1 ml. of 0.12% carbazole in ethanol, 1 ml. of 1.5% cysteine·HCl, 5 ml. of ethanol and 0.2 ml. of approximately 25 *N* H_2SO_4 was sprayed onto the dried chromatograms and heated at 65–70° for 5 min. The background remained colorless, the paper dry, and not excessively fragile with the small amount of H_2SO_4 used. The omission of H_2SO_4, or its replacement with trichloroacetic acid resulted in no color reaction with the sugars. With the spray described, 3 micrograms of xylulose or ribulose was easily detected as violet spots which changed to gray after a short while at room temperature. Fifteen micrograms of xylose produced only a very faint spot, whereas, the same amount of xylulose or ribulose resulted in very black spots. The xylulose of the enzyme-catalyzed reaction was readily distinguished from ribulose by the different R_f values obtained when 76% phenol or water-saturated 2,4,6-trimethylpyridine was used as solvent. Furthermore, the fact that xylulose serves as a substrate for the enzyme, whereas ribulose does not (see below), is further support for the chromatographic evidence that the ketose formed from D-xylose is D-xylulose.

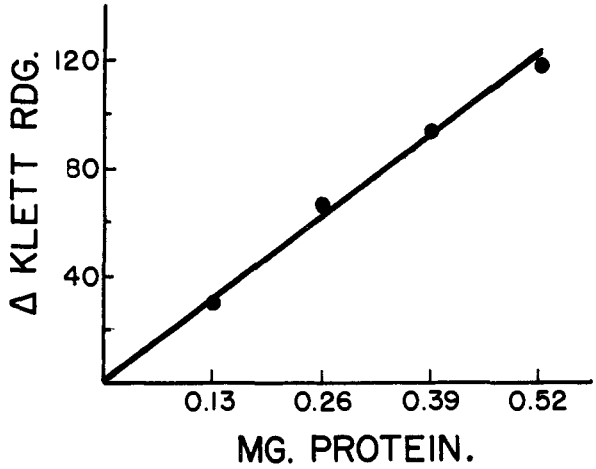

Fig. 1.—Proportionality of xylose isomerase activity to protein concentration of a protamine treated and dialyzed extract of *P. pestis;* time of incubation, 15 min.

TABLE I

R_f VALUES OF PENTOSES IN FOUR SOLVENT SYSTEMS

Chromatograms were developed for 2 hours with the aqueous acetone solvent, and for 16 hours in the other solvents.

Pentose	Acetone[a]	Phenol[b]	Collidine[c]	Alcohol[d]
D-Arabinose	0.47	0.51	0.45	0.21
D-Lyxose	.55	.48	.56	.28
D-Ribose	.58	.57	.62	.32
D-Ribulose	.62	.62	.63	.33
D-Xylulose	.64	.57	.67	.34
Sugars from enzyme reaction	.66 .54	.57 .46	.66 .55	.34 .25
D-Xylose	.55	.46	.53	.24

[a] 77% (v./v.) acetone in water. [b] 76% (v./v.) phenol in water. [c] 2,4,6-trimethylpyridine saturated with water. [d] 1-Butanol:ethanol:water (4 vol.:1 vol.:5 vol.).

No systematic study of purification of the isomerase has been made, but it was found that treatment with protamine and dialysis gave a cleaner preparation with a doubling of specific activity and a recovery of about 70% of the activity. A solution of crude extract containing about 10 mg. protein per ml. was treated in an ice-bath with 0.25 volume of 2% protamine sulfate. The clear supernatant solution obtained after centrifugation was dialyzed in the cold against 0.02 *M* phosphate buffer, *p*H 7.5 for 30 hours. A small amount of precipitate, which formed during dialysis, was removed by centrifugation. When ultraviolet absorption spectra were plotted, it was found that dialysis alone only lowered the 260:280 mμ ratio from 1.8 to 1.7. Protamine treatment plus dialysis removed most of the substances responsible for the strong absorption at 260 mμ. The resultant spectrum was more nearly typical for protein solutions and had a 260:280 ratio of only 0.8.

Experimental

Proportionality.—Proportionality of enzyme activity to protein concentration is shown in Fig. 1. No corrections were needed except for the initial value due to xylose, since no color was produced by the amounts of partially purified enzyme used. In order to obtain proportionality, it is necessary to prevent the reaction from progressing beyond 30–40% toward equilibrium. This allows a working range of about 200 Klett colorimeter scale units which may be extended somewhat by the addition of borate to the assay system.

(5) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

(6) T. E. Weichselbaum, *Am. J. Clin. Pathol.*, **10**, 40 (1946).

(7) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(8) A sample kindly supplied by Dr. B. L. Horecker of the National Institutes of Health, Bethesda, Maryland.

(9) N. Nelson, *J. Biol. Chem.*, **153**, 375 (1944).

(10) S. M. Partridge, *Biochem. Soc. Symposia, Cambridge, Engl.*, **3**, 52 (1950).

Effect of *p*H.—Optimal xylose isomerase activity is obtained at about *p*H 8 as may be seen in Fig. 2.

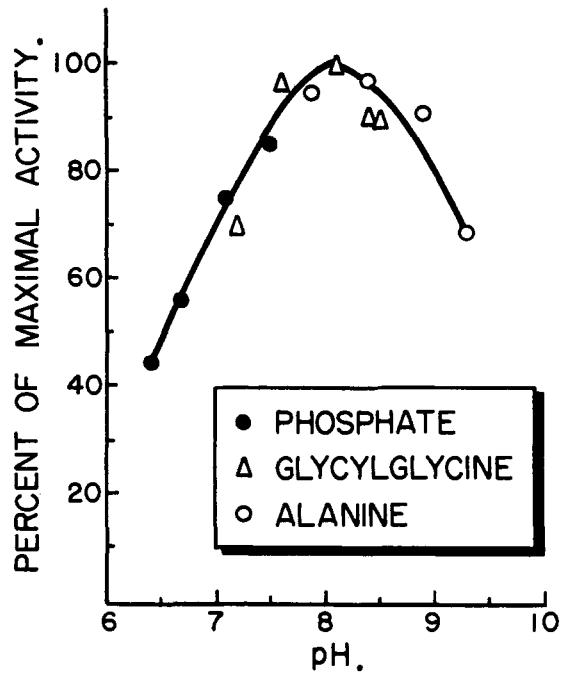

Fig. 2.—Effect of *p*H on the activity of xylose isomerase; time of incubation, 30 min.

Equilibrium.—As is indicated in Fig. 3, equilibrium is reached when about 16% xylulose is present in the reaction mixture. This value was obtained when either D-xylose or D-xylulose was present initially. The presence of borate shifts the equilibrium position toward the formation of 60–65% xylulose. This is shown in Fig. 4. No difference in the equilibrium value was found when the ratio of borate: sugar was varied from 10:1 to 40:1.

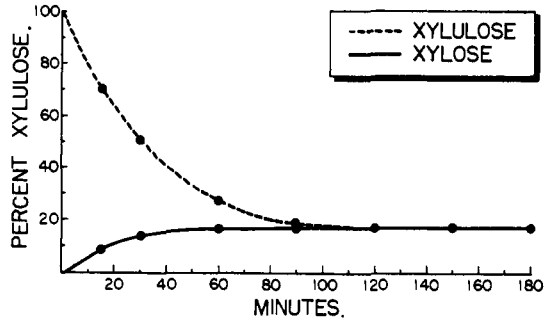

Fig. 3.—Equilibrium between D-xylose and D-xylulose catalyzed by xylose isomerase in the absence of borate; concentration of sugars, 10^{-3} *M*.

Substrate Specificity and Affinity.—No activity was found with any D-sugars tested in the absence of borate except with D-xylose and D-xylulose. Other pentoses tested were: arabinose, ribose, lyxose and ribulose. Negative results were also obtained with glucose and mannose.

The plot of reciprocal velocity *versus* reciprocal D-xylose concentration is included in Fig. 7. The dissociation constant (K_m) calculated from the lower straight line shows that the enzyme has half-maximal activity when the xylose concentration is 3.7×10^{-3} *M*. Replicate determinations gave an average K_m of about 3×10^{-3} *M*.

Metal Requirement.—Xylose isomerase is almost completely inactivated by dialysis against cold distilled water for 16 hours, and its activity cannot be restored by the addition of metal ions. Dialysis against 0.02 *M* phosphate, *p*H 7.5, for 30 hours also inactivates the enzyme, but the activity

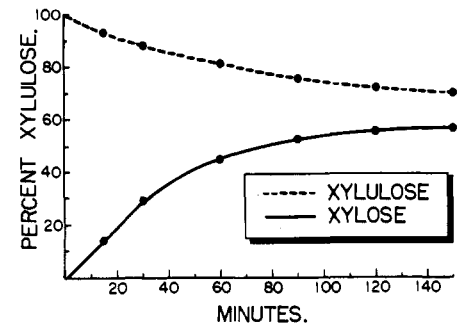

Fig. 4.—Equilibrium between D-xylose and D-xylulose catalyzed by xylose isomerase in the presence of borate; concentration of sugars, 10^{-3} *M*; concentration of borate, 2×10^{-2} *M*.

is completely restored by the addition of Mg^{++} or Mn^{++}. Figure 5 shows that full activity is attained with about 10^{-4} *M* Mn^{++}, whereas 10^{-3} *M* Mg^{++} is required. The dissociation constants calculated from these data are: 1.1×10^{-5} *M* for Mn^{++} and 2.8×10^{-4} *M* for Mg^{++}, indicating that Mn^{++} is about 25 times more strongly bound by the enzyme than is Mg^{++}. Furthermore, the specific activity of the isomerase was found to be 20–50% higher with an optimal concentration of Mn^{++} than with Mg^{++}.

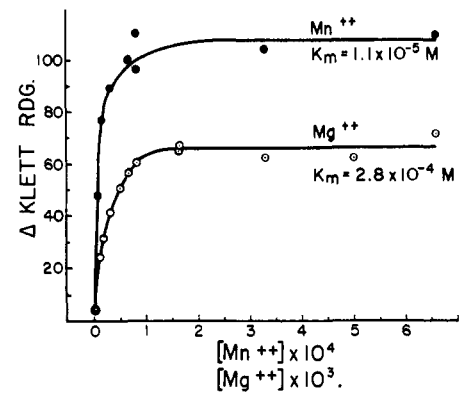

Fig. 5.—Effect of metal ions on the xylose isomerase activity of a protamine treated extract of *P. pestis* after dialysis for 30 hours against 0.02 *M* phosphate, *p*H 7.5. Incubated for 15 min. with 0.52 mg. protein. The dissociation constants (K_m) for the metal–enzyme complexes were calculated from the straight lines obtained by plotting reciprocal velocity *versus* reciprocal metal ion concentration.

Sulfhydryl Properties.—Xylose isomerase appears to require sulfhydryl groups for full activity. The activity is stimulated 2- to 3-fold by the presence of cysteine. Glutathione is less effective than cysteine which produces maximal activity at a concentration of about 0.005 *M*. Sulfhydryl-inactivators are not very effective against the enzyme; N-ethylmaleimide inhibits about 40% at a concentration of 0.001 *M*, while iodoacetic acid and iodoacetamide are even less inhibitory. These inhibitions are not reversed by cysteine whereas that produced by *p*-chloromercuribenzoate (PCMB) is completely reversible, as is shown in Table II. The enzyme preparation was incubated in the presence and absence of approximately 0.0025 *M* PCMB for 8 min. at 30°, and then for 8 min. with and without an excess of cysteine before measuring activity. It may be seen that in the absence of PCMB, the enzyme activity was doubled by the addition of cysteine.

Other Inhibitors.—While studying the effect of *p*H on activity of xylose isomerase, it was found that tris-(hydroxymethyl)-aminomethane,[11] which was being used as buffer,[12]

(11) To be referred to as Tris.

(12) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **62**, 33 (1946).

TABLE II

REVERSIBILITY OF *p*-CHLOROMERCURIBENZOATE (PCMB) INHIBITION OF XYLOSE ISOMERASE

Protamine treated and dialyzed enzyme (0.26 mg.) was incubated at 30° with or without 1 micromole of PCMB for 8 min. in a volume of 0.4 ml., and then for 8 min. in the presence or absence of 10 micromoles of cysteine before the addition of D-xylose. Activity is expressed as change in Klett colorimeter scale units during incubation for 30 min. with D-xylose.

PCMB	Cysteine	Activity
−	−	43
−	+	85
+	−	0
+	+	86

strongly inhibited the enzyme. Figure 6 shows the inhibition of enzyme activity with various concentrations of Tris in the presence of phosphate buffer. The inhibition is not

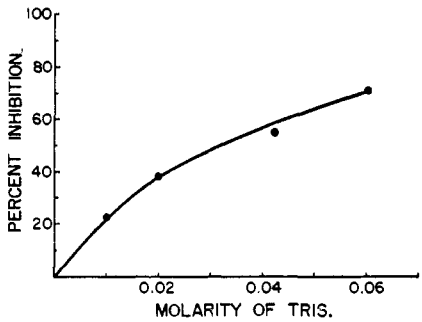

Fig. 6.—Effect of Tris on the activity of xylose isomerase. Incubation of 0.26 mg. protein in the presence of 0.05 *M* phosphate, *p*H 7.5, for 30 min. at 30°.

caused by a sequestering action of Tris on Mg^{++}, since the inactivation is not relieved by increasing the Mg^{++} concentration over a 25-fold range. The data plotted in Fig. 7 indicate that the inhibition is non-competitive.[13] The in-

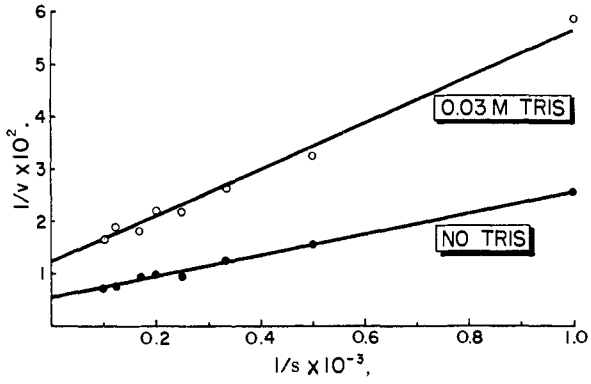

Fig. 7.—Non-competitive inhibition of xylose isomerase by 0.03 *M* Tris. Incubation of 0.26 mg. protein for 45 min. in the presence of 0.05 *M* phosphate, *p*H 7.5.

hibitions produced in the presence of 0.05 *M* solutions of substances related to Tris are given in Table III. 2-Amino-2-methyl-1,3-propanediol, which also has been recommended as a buffer,[12] inhibits xylose isomerase about half as strongly as Tris, whereas 2-amino-2-methyl-1-propanol and *t*-butylamine have no significant effects on the enzyme at this concentration.

Xylulokinase.—Crude sonic extracts of *P. pestis*, strain A-1122, grown in the presence of D-xylose were tested for their ability to phosphorylate glucose, xylose and xylulose in the absence of cysteine and in the presence of 0.05 *M* Tris buffer, conditions unfavorable for the action of xylose isomerase. The results are presented in Table IV. In the absence of ATP no glucose was utilized, while, in its presence, 90% of the glucose was phosphorylated. No reducing power disappeared when xylose was incubated with the extract in the presence or absence of ATP. The method used[9] would not detect a small conversion of xylose into xylulose. However, in the case of xylulose, approximately 0.1 and 0.4 micromole disappeared in the absence of ATP because of residual isomerase activity, which was about 70% lower in the presence of Tris than in the presence of glycylglycine buffer. Even less than 0.1 micromole of xylose must have been isomerized in the presence of Tris, since the rate of conversion of xylose is less than that for xylulose (see Fig. 3). The very low concentration of xylulose formed from xylose under these conditions would not be sufficient to result in measurable xylulokinase activity. In the presence of ATP, a marked decrease in xylulose occurred as a result of phosphorylation. After correcting for the xylose isomerase activity, the amounts of xylulose phosphorylated were found to be 0.71 and 0.61 micromole in the presence of Tris and glycylglycine buffer, respectively.

TABLE III

INHIBITION OF XYLOSE ISOMERASE BY SUBSTANCES RELATED TO TRIS-(HYDROXYMETHYL)-AMINOMETHANE

The isomerase reaction (0.26 mg. protein) was measured in the presence of 0.05 *M* test substance and 0.05 *M* phosphate, *p*H 7.5. Incubation time, 30 min.

Substance	Inhibition (%)	Substance	Inhibition (%)
$HOH_2C-C(CH_2OH)_2-NH_2$	68	$H_3C-C(CH_2OH)_2-NH_2$	3
$H_3C-C(CH_2OH)_2-NH_2$	36	$H_3C-C(CH_3)_2-NH_2$	0

TABLE IV

XYLULOKINASE AND XYLOSE ISOMERASE ACTIVITIES IN A CELL-FREE EXTRACT OF *P. pestis*

See Methods. Glucose and xylose were determined as reducing sugars[9]; xylulose was measured by the method of Dische and Borenfreund.[5] 1.4 micromoles sugar present initially.

Sugar	D-Glucose		D-Xylose		D-Xylulose			
Buffer	Tris		Tris		Tris		Glycylglycine	
ATP	−	+	−	+	−	+	−	+
Sugar utilized, μmoles	0	1.28	0	0	0.11	0.82	0.38	0.99
Sugar phosphorylated, μmoles	1.28		0		0.71		0.61	

Discussion

Xylose isomerase of *P. pestis*, strain A-1122, has properties similar to that obtained by others from other microörganisms.[2,3] It appears to be less stable to dialysis against water than that found by Hochster and Watson.[2] Mitsuhashi and Lampen, on the other hand, were unable to inactivate the isomerase from *L. pentosus* by dialysis against water, and had to dialyze against Versene in order to demonstrate metal ion effects.[14] As with the enzyme from *P. pestis*, they found optimal activity with about 10^{-4} *M* Mn^{++}, but, unlike the *P. pestis* enzyme, that from *L. pentosus* had only 10% as much activity when tested with Mg^{++} as with Mn^{++}.[14]

(13) H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).

(14) J. O. Lampen, private communication.

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).

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

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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).