hybrids similar to the one presently considered should clarify the relationship, if any, between genetic composition of the corn and the type of amylopectin component present in the starch.

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PEORIA, ILLINOIS

[CONTRIBUTION FROM THE STARCH AND DEXTROSE SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Kinetics of Hydrolysis of Isomaltotriose and Isomaltotriitol[2]

BY R. W. JONES, R. J. DIMLER AND C. E. RIST

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Conflicting evidence appears in the literature regarding the relation between rate of acid hydrolysis of glucosidic bonds and their position in polysaccharide molecules. The present studies were undertaken to provide information on such a relationship in the α-1,6′-glucosidically linked homologous series of oligo- and polysaccharides. Attention has been centered on the first two members of this series, isomaltose and isomaltotriose, and the alcohols obtained by reduction of these sugars. Procedures employing quantitative paper chromatography were developed for the study of the kinetics of hydrolysis of these carbohydrates. In the series isomaltose, isomaltotriose and dextran, the over-all rate constant for hydrolysis decreases with increase of chain length, the rate constant for dextran B-512 being about one-third that for isomaltose. For the individual bonds in the reduced oligosaccharide, isomaltotriitol, the bond farthest removed from the sorbitol end is cleaved twice as fast as the other linkage. It is postulated, on the basis of these data and certain assumptions concerning the effects of reduction of isomaltotriose, that the non-reducing end bond in isomaltotriose is hydrolyzed about 1.7 times as fast as the reducing end bond.

Considerable success has been attained in recent calculations of length of external branches in dextran molecules on the basis of measurements of the amounts of glucose and low molecular weight oligosaccharides formed during partial acid hydrolysis of the dextrans.[3] The interpretation of the data has been dependent in part upon the knowledge of whether the position of an α-1,6′-glucosidic linkage in the polymer chain influences its rate of hydrolysis. Since there is a lack of agreement in the literature concerning the effect of the position of a bond on its hydrolysis rate constant, K, the present studies were undertaken to provide evidence of such effects in the α-1,6′-linked glucose polymers.

A greater rate of hydrolysis of terminal compared with internal bonds in polysaccharides has been postulated by Freudenberg, Carlqvist, and others on the basis of studies of acid hydrolysis of cellulose,[4–7] starch,[4a,5,8–10] glycogen,[11] Schardinger dextrins[12] and low-molecular weight oligosaccharides.[4b,7] This hypothesis was based on the increase in K during hydrolysis of polysaccharides and the extent to which the velocity of hydrolysis increased in the order cellulose, cellohexaose, cellopentaose, cellotetraose, cellotriose, cellobiose. Swanson and Cori,[13] however, failed to detect an increase in K during hydrolysis of polysaccharides from starch. Likewise they found no difference in hydrolysis rate constant for amylose and maltose. The results obtained by Swanson and Cori may reflect the effects of differences in hydrolysis conditions[11] or in analytical methods used.

In the present studies attention was centered on the first two members of the α-1,6′-glucosidically linked, homologous series, isomaltose and isomaltotriose, which had been made available as a result of their preparation by carbon column chromatography of enzymic hydrolyzates of dextran from *Leuconostoc mesenteroides* NRRL B-512.[14] A procedure employing quantitative paper chromatography was developed for determining the effect of chain length on the over-all rates of hydrolysis (total bond cleavage) of these oligosaccharides. In addition, chromatographic techniques facilitated measurement of the rate constants for hydrolysis of the individual bonds in the reduced trisaccharide, isomaltotriitol. These data provided a basis for estimating the rates of cleavage of the individual bonds in the parent trisaccharide, isomaltotriose, although certain assumptions were necessary to allow for the difference between the over-all hy-

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(2) Presented before the Division of Carbohydrate Chemistry at the 124th National Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

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drolysis rate constants of the parent sugar and its reduced form.

Over-all Rate Constants.—In the calculation of the rate constants from hydrolysis data the equation for a first-order reaction was used throughout these studies, as has been the general practice in other investigations of polysaccharide and oligosaccharide hydrolysis. Over the range of extents of hydrolysis used the values were essentially constant (Table I), the variations being attributable at least in part to the analytical methods.

TABLE I

HYDROLYSIS, 80°, H_2SO_4, *p*H 1.0 (0.18*N*)

($K \times 10^3$, first-order reaction, ln, hr.$^{-1}$)

Time, hr. (approx.)[a]	5	24	55
Hydrolysis, % (approx.)[a]	5	20	39
Isomaltose	13.2	12.1	11.5
Average 12.3	12.4	12.2	11.6
	12.4	12.7	
Isomaltotriose	9.2	9.3	
Average 9.2	8.7	9.1	9.6
Isomaltitol	9.4	8.9	
Average 9.1	9.5	8.4	9.0
Isomaltotriitol	8.0[b]	7.6[b]	7.7[b]
Average 7.8		7.4[c]	8.6[c]
Dextran B-512			
Average 3.9[d]			

[a] The experimental values in each run were used to calculate K, using the first-order equation $1 - \alpha = e^{-Kt}$, where α is the hydrolyzed fraction of the total number of bonds. [b] Average K based on these results, obtained from two separate chromatograms (developed with butanol–pyridine–water and phenol–water, respectively) and considered more accurate than by other methods used. [c] Based on separation of all components on a single chromatogram. [d] Determined by reducing power, Somogyi reagent, instead of by paper chromatography; average of results at four points between 0.4 and 2% hydrolysis.

Preliminary studies indicated that the accuracy and precision of the analytical methods could have a large effect on the rate-constant determinations. In particular, analyses of known mixtures of isomaltose and D-glucose by reducing power, using the Somogyi copper–phosphate reagent,[15] gave apparent compositions in error as much as 20%. Such errors apparently arose from the sensitivity of the calculation to variations in the experimental determination of total carbohydrate concentration and in the reducing power factors for the constituent saccharides. This was particularly true for mixtures corresponding to relatively small extents of disaccharide hydrolysis, such as 10%.

More reliable results, with accuracy within 2 to 5%, were obtained in analyses of the known mixtures by the quantitative paper chromatography procedure described by Dimler, Schaefer, Wise and Rist.[16] This method involves the determination of the number of bonds broken in an oligosaccharide on the basis of the measured amount of each fragment, rather than from an average reducing power value for the entire mixture. Quantitative paper chromatography was adopted, therefore, for the determination of the over-all rate constants for the oligosaccharides, thereby avoiding the complicating influences of changes in molecular reducing power with degree of polymerization (*e.g.*, isomaltose = 116% of D-glucose with the Somogyi reagent), as well as the magnified influence of errors in the determinations of total carbohydrate and of reducing power.

The hydrolysis rate constants for isomaltose and isomaltotriose thus obtained are given in Table I for hydrolysis with sulfuric acid at *p*H 1.0 (0.18 *N*) and 80°. For comparison the constant for dextran ($K = 3.9 \times 10^{-3}$) also is listed.[17] While this is not an exact value, it represents an approximation to the limiting rate constant for the α-1,6′-glucosidically linked, homologous series of polymers. The dextran NRRL B-512 preparation used here is Dextran A, as described by Jeanes, Wilham and Miers,[18] and has been shown to contain 95% α-1,6′-bonds.[19] The rate constant was determined at four points between 0.4 and 2% hydrolysis in order to decrease the influence of the higher hydrolysis constants of the low molecular weight oligosaccharides which would become predominant at greater extents of hydrolysis. The necessity of using reducing power to follow the course of hydrolysis, together with the possible influence of the 5% non-1,6′-linkages on the rate of hydrolysis, introduce some uncertainty into the interpretation of the results. However, any corrections for these factors probably would lower the calculated rate constant and, therefore, have no effect on the conclusions drawn from these data.

The results in Table I show that K of α-1,6′-glucosidically linked polymers varies with chain length. Such a characteristic had been noted for the β-1,4′-linked series previously by Freudenberg[4b] and Wolfrom.[7] It may be concluded, therefore, that the hydrolysis rate constant of the α-1,6′-linkage is dependent on its position in the polymer molecule and/or the degree of polymerization of the saccharide in a homologous series.

The differences in K for saccharides of various degrees of polymerization could be explained in one of five ways, each of which has been indicated at some time in the literature: 1. Both terminal bonds (at reducing and non-reducing ends) are broken at the same rate (aK) with all other bonds being broken at a slower rate K. 2. The non-reducing end bond is broken at the rate aK while all other bonds are cleaved at rate K. 3. The reducing end bond is broken at rate aK and all others at rate K. 4. There is a progressive decrease in K as one moves from either or both of the terminal bonds toward bonds in the interior of the molecule. 5. All bonds in a given oligosaccharide are broken at the same rate, with this rate being dependent upon the degree of polymerization of the molecule.

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A knowledge of which of these possibilities is the correct one is particularly important in attempts to correlate the hydrolytic yields of mono- and oligosaccharides with the structure of branched polysaccharides. Early in the hydrolysis many non-reducing end groups will contribute a disproportionately large amount of monosaccharide if the non-reducing terminal linkage is hydrolyzed more rapidly than the internal linkages. A significant step toward the selection of the correct possibility for the α-1,6'-glucosidically linked polymers now has been made through the determination of the hydrolysis rate constants for each of the two bonds in the reduced trisaccharide, isomaltotriitol (I), and the application of the data to an estimation of the rate constants for the bonds in the parent sugar, isomaltotriose (III).

Rate Constants of Isomaltotriitol Bonds.—In order to determine the relative rates of cleavage of the two types of bonds in isomaltotriose (III), it was necessary to label one end of the molecule, since breaking either of the linkages of the free sugar would produce glucose and isomaltose. For trisaccharides, reduction[20–22] of the aldehyde group to the alcohol or oxidation[23] to the acid has provided a satisfactory means of determining the source of a fragment formed during hydrolysis. In the present studies, the reduced rather than the oxidized triose was used because the alcohols produced well-defined spots on the chromatogram in contrast to the streaks given by the acids. The reduced saccharides travel on the paper at the same rate as the reducing sugars when butanol–pyridine–water (3:2:1.5)[24] is used as a developing agent. With phenol–water (3:1) solvent, the alcohols move much faster than the corresponding sugars, isomaltitol having essentially the same R_f as glucose and isomaltotriitol the R_f of isomaltose. By very careful control of the time of development and weight of hydrolyzate applied, it was possible to separate the components into five separate fractions by using phenol–water solvent followed by butanol–pyridine–water in the same direction on the same chromatogram.

Through the use of quantitative chromatography employing the two solvent combinations on two different aliquots of the hydrolyzate and the anthrone method of carbohydrate determination, it was possible to calculate the first-order rate constant (see also Experimental) for the hydrolysis of each of the two bonds of isomaltotriitol (I). The values obtained are shown in Fig. 1. The bond farthest removed from the sorbitol end has a rate constant (K_1) twice as high as that of the linkage on the sorbitol end group (K_3).

Rate Constants of Isomaltotriose Bonds.—For the original trisaccharide, isomaltotriose (III), the hydrolysis rate constants of the individual bonds (K_5 and K_6) were calculated from the data on isomaltotriitol (Fig. 1) together with the over-all rate constants for the di- and trisaccharides (K_{IV} and K_{III}) and their reduced forms (K_{II} and K_I). Ideally, the hydrolysis rate constant of the di- and trisaccharide would be unchanged by reduction of the aldose end group. However, as shown in Fig. 1, changing the aglycone from an aldose (probably in ring form) to the open-chain sorbitol structure lowered the rate constant. That the direction and magnitude of such change is dependent on the structure of the saccharide was indicated by the observation that the corresponding rate constants for maltose and maltitol were 51×10^{-3} and 58×10^{-3}. A small change of rate constant also accompanied oxidation of maltose, K for maltobionic acid being 56×10^{-3} under the same conditions.

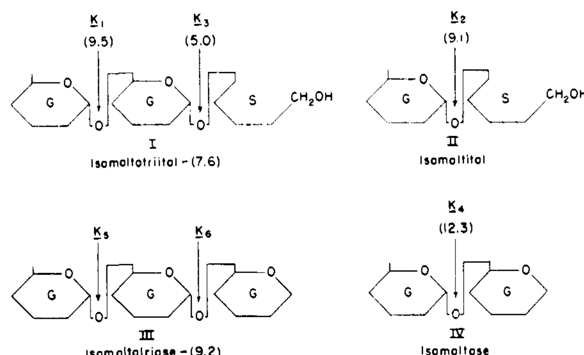

Fig. 1.—Numerical values = $K \times 10^3$, first-order reaction, ln hr.$^{-1}$, for hydrolysis at pH 1.0 (H_2SO_4), 80°; G = glucose unit; S = sorbitol unit.

Two approaches for allowing for the effect of hydrogenation on the rate constant merit consideration in estimating the rate constants K_5 and K_6 for the individual bonds in isomaltotriose (III). 1. Reduction of isomaltotriose may lower rate constant K_6 (Fig. 1) by the same proportion as rate constant K_4 of isomaltose (IV) was lowered by hydrogenation. Then $12.3/9.1 = K_6/5.0$ or $K_6 = 6.8 \times 10^{-3}$ ln hr.$^{-1}$. For small extents of hydrolysis K_5 is calculated from the equation[25] $K_5 + K_6 = 2K_{III}$, and $K_5 = 11.6 \times 10^{-3}$ ln hr.$^{-1}$. 2. It might be argued that reduction of isomaltotriose (III) to isomaltotriitol (I) should produce a change in rate constant K_6 but would not affect K_5 since this bond is some distance from the sorbitol end. In this case $K_5 = 9.5 \times 10^{-3}$ and $K_6 = 8.9 \times 10^{-3}$. Since these two values are very close to the over-all rate constant (K_{III}) for the trisaccharide, it might be assumed that both of the triose bonds have the same rate constant, 9.2×10^{-3}.

The results obtained by the two methods are brought together in Table II. The correct values of K_5 and K_6 most likely are between the two extremes given. Further research obviously is required to prove the validity of our hypothesis that the non-reducing end bond in isomaltotriose is hydrolyzed more rapidly (K_5) than the other bond (K_6) and to establish the true ratio between the two rate constants. A source of such proof would be hydrolysis studies on C^{14}-labeled isomaltotriose in which only one of the two end units is radioactive, while the middle unit preferably is not labeled.

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Table II

Isomaltotriose Hydrolysis, pH 1.0, 80°

$K \times 10^3$ calcd. from data in Fig. 1

Method of calcn.	1	2
$K_{over\text{-}all}$ or K_{III}	9.2	9.2
$K_{non\text{-}reducing}$ or K_5	11.6	9.2
$K_{reducing}$ or K_6	6.8	9.2
Ratio $K_{non\text{-}reducing}/K_{reducing}$	1.7	1.0

If the non-reducing end group of isomaltotriose is split off faster than the reducing end, only the second and fourth of the five possibilities listed above remain under consideration. That is, either the non-reducing bond is hydrolyzed at the rate K and all other bonds at a slower rate, or there is a progressive decrease in K as one proceeds from one or both terminal bonds toward bonds in the interior of the chain. Further investigation must be carried out to prove which of these possibilities is the correct one. One method of attacking the problem would be the hydrolysis of reduced isomaltotetraose. A second would be following, by paper chromatography, the hydrolysis of polysaccharides containing many long external branches of α-1,6'-linked units. The second would perhaps be the better, since the other would involve a rather difficult calculation. For the α-1,4'-linked glucose polymers, limited studies of the hydrolysis of amylopectin indicate that the non-reducing end bonds of this polymer have a hydrolysis rate constant about twice as large as other bonds of the external branches in the molecule and about the same magnitude as that of maltose. These results, which will be published later, suggest by analogy that the second of the five possibilities is preferable.

Experimental

Preparation of Samples.—The isomaltose and isomaltotriose were prepared by Jeanes and co-workers[14] by fractionation of the enzymic hydrolyzate of dextran from *Leuconostoc mesenteroides* NRRL B-512 on carbon–Celite columns. The saccharides were deionized by passage through Dowex 50 and Duolite A-4 ion-exchange columns.[26] The identity and high purity of the sugars were established by paper chromatography, periodate oxidation, molecular weight, and C, H and O analyses.[14]

The sugars were reduced by catalytic hydrogenation at 125° and 2,400 p.s.i. using Raney nickel catalyst.[27] The alcohols were isolated as amorphous powders. They gave only one spot on a paper chromatogram and had reducing power equivalent to less than 1% unreacted sugar.

The maltobionic acid solution was obtained by acidifying electrolytically prepared barium maltobionate[28] with sulfuric acid and removing the insoluble $BaSO_4$ by centrifugation.

Hydrolysis Conditions.—The hydrolyses were carried out in sealed glass tubes held in a water-bath at 80 ± 0.5°. The pH of the solution was determined at room temperature before the tubes were sealed. The tubes were removed from the bath and cooled. The H_2SO_4 was neutralized with $BaCO_3$, the $BaSO_4$ being removed by centrifugation. Aliquots were analyzed at reaction times of 5, 24 and 54 hours. The degrees of hydrolysis of the isomaltotriose bonds were about 4, 19 and 39%.

Chromatography.—For a detailed description of the method used at this Laboratory for quantitative determination of glucose and its low molecular weight oligosaccharides by paper chromatography see Dimler, Schaefer, Wise and Rist.[16] The fractions obtained are listed in Table III.

Table III

Sugar hydrolyzed	Developing[a] agent used	Fractions obtained
Maltose	A or B	1, glucose; 2, maltose
Isomaltose	A or B	1, glucose; 2, isomaltose
Isomaltotriose	A or B	1, glucose; 2, isomaltose; 3, isomaltotriose
Maltitol	A	1, glucose–sorbitol; 2, maltitol
Isomaltitol	A	1, glucose–sorbitol; 2, isomaltitol
Maltobionic acid	A	1, glucose; 2, maltobionic acid–gluconic acid
Isomaltotriitol	A	1, glucose–sorbitol; 2, isomaltose–isomaltitol; 3, isomaltotriitol
	C	4, sorbitol; 5, glucose–isomaltitol; 6, isomaltose–isomaltotriitol

[a] A = butanol-pyridine-water (3:2:1.5)[24]; B = butanol-methyl cellosolve-water (2:1:1); C = phenol-water (3:1).

Measurement of Yields of Fragments.—From the hydrolysis of maltose, isomaltose and isomaltotriose: The weight of each fraction, as glucose, was determined by the anthrone method,[16] the exact weight of each component being calculated from these data. From the hydrolysis of maltitol, isomaltitol, maltobionic acid: Since neither sorbitol nor gluconic acid interferes with anthrone analysis, the moles of glucose, maltitol, isomaltitol and maltobionic acid can be determined by this method. The moles of sorbitol and gluconic acid present in a hydrolyzate are equal to the moles of glucose found. From the hydrolysis of isomaltotriitol: The yields of components were obtained as follows: glucose, from fraction 1; isomaltotriitol, from fraction 3; isomaltose, from fraction 6 minus fraction 3; and isomaltitol, from fraction 5 minus fraction 1. Results obtained by this method are shown in Table IV for hydrolysis of isomaltotriitol.

Table IV

Hydrolysis of Isomaltotriitol, 80°, H_2SO_4, pH 1.0 (0.18 N)

	Yield of products (moles/100 moles isomaltotriitol)		
Time, hr	7	24	53
Sorbitol	4.12	11.5	26.4
Glucose	7.41	24.7	54.0
Isomaltitol	6.14	18.2	27.1
Isomaltose	3.50	8.4	13.0
Isomaltotriitol	89.93	70.3	45.9

	Calcd. rate constants ($K \times 10^3$, in hr.$^{-1}$)			
Hydrolysis, %	5.46	16.6	33.5	Average
$K_{over\text{-}all}$	8.0	7.6	7.7	7.8
K_1	9.5	10.0	9.0	9.5
K_3	5.5	4.8	4.6	5.0

A second method of determining the yield of fragments from isomaltotriitol hydrolyzate also was attempted, but was not used because of the extremely high paper blanks. This procedure involved the determination of weights of material by periodate oxidation followed by chromotropic acid analyses for the formaldehyde formed.[29,30]

It is possible to separate the five components of triitol hydrolyzate by developing a chromatogram with solvent C followed by solvent A. This procedure, however, is not recommended, because the weight of sugar that may be used on a chromatogram is small and the developing time must be carefully controlled.

(26) Mention of trade names should not be construed as a recommendation or endorsement by the Department of Agriculture over those not mentioned.

(27) A. A. Pavlic and H. Adkins, This Journal, 68, 1471 (1946).

(28) H. S. Isbell and H. L. Frush, *J. Research Natl. Bur. Standards*, 6, 1145 (1931).

(29) Marguerite Lambert and A. C. Neish, *Can. J. Research*, B28, 83 (1950).

(30) B. H. Alexander and J. Sloan, unpublished work.

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.

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(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

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

$$II_t/I_0 = \frac{K_1}{K_2 - (K_1 + K_3)} (e^{-(K_1+K_3)t} - e^{-K_2t})$$

$$IV_t/I_0 = \frac{K_3}{K_4 - (K_1 + K_3)} (e^{-(K_1+K_3)t} - e^{-K_4t})$$

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

$$K_1 = \frac{II_t(K_1 + K_3 - K_2)}{I_0e^{-K_2t} - I_t}; \quad K_3 = \frac{IV_t(K_1 + K_3 - K_4)}{I_0e^{-K_4t} - I_t}$$

(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.

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

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

BY MILTON W. SLEIN

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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 pH 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 pH 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.

Methods

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

(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).

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