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Effect of Borate on the Alkali-catalyzed Isomerization of Sugars[1]

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The addition of borate results in a greater conversion of aldose to ketose in alkali-catalyzed isomerizations. Borate appears to increase both the rate of formation and the maximum amount of ketose formed. The method of isomerization described may be adopted on a preparative scale, and would be especially useful when only small amounts of aldose are available or when identification of an aldose by isomerization to its ketose is desired.

Introduction

Alkali-catalyzed isomerization has frequently been employed for the synthesis of ketoses from aldoses in yields ranging from less than 10% to 50%.[3] Several observations with enzyme-catalyzed isomerizations have indicated that alkali-catalyzed epimerization employed in the preparation of ketoses from aldoses might be improved by the addition of borate. Thus Cohen has demonstrated that the equilibrium of the enzyme-catalyzed epimerization of D-arabinose to D-ribulose[4] and L-fucose to L-fuculose[5] is greatly altered in favor of the ketose when borate is added to the reaction mixture. The enzymatic conversion of D-xylose to D-xylulose is also affected by the presence of borate.[6,7] Alvarado and Sols have shown that in the presence of 0.1 *M* borate mannose 6-phosphate and glucose 6-phosphate are quantitatively converted to fructose 6-phosphate by the corresponding isomerases.[8]

Experimental

D-Fructose, fructose 6-phosphate and lactulose were estimated by the resorcinol method of Roe[9] using a sucrose standard. The volumes were reduced to one-fourth and the color was measured at 490 mμ. The concentration of reducing sugars was determined as described by Somogyi[10] and Nelson.[11] D-Xylulose was measured by the cysteine carbazole reaction[12] with the volumes reduced to one-third. The samples were allowed to stand at room temperature for 1 hour before reading at 540 mμ in a Coleman spectrophotometer in a tube of 1-cm. light path.[4,13] Bromine oxidation was carried out according to Mitsuhashi and Lampen.[6] Pentose was assayed by the orcinol reaction.[14] Sugars were chromatographed in 1-butanol–ethanol–water[15] and with a solvent composed of ethyl acetate, acetic acid and water (3:3:1).[16] Sugars were located by passing the dried papers through silver nitrate solution and then through 1 *N* alcoholic NaOH.[17]

Gottfried and Benjamin[18] have shown that alkali-catalyzed isomerization can be carried out at high temperatures (70–130°) and short times (5–15 minutes).

In most experiments described in this paper isomerization was carried out at 100° in a boiling water-bath. In experiments where a small total volume (0.2 ml.) was used the indicated amount of sugar, borate and NaOH were added to the reaction vessel and mixed at room temperature. The tube was then placed in a boiling water-bath for the specified time. Since the volume of the reaction mixture was very small, temperature equilibration was rapid. The reaction was stopped by placing the tube in an ice-bath and then adding 0.1 ml. of 1 *N* HCl. In experiments involving larger volumes the reaction mixture without sugar was brought to 90–100° by placing it in the water-bath and then the sugar dissolved in a small volume of water was added rapidly. Solutions were concentrated *in vacuo* at 40–50° in a Craig evaporator.

Results

Effect of Alkali Concentration.—One of the factors governing the rate of formation of ketose is the alkali concentration. Figure 1 shows that the amount of alkali added affects the rate of formation as well as the maximum amount of ketose formed. It may be seen that an increasing alkali concentration causes an increase in the rate of formation of ketose and also causes a slight increase in the maximum amount of ketose formed. However, increasing the alkali concentration causes an increase in the rate of destruction of ketose. Thus the amount of ketose present in 3 minutes in 0.0125 *N* NaOH is twice the amount present when 0.025 *N* NaOH is used. The maximum amount of ketose formed is about 40 to 50% of the added aldose.

Effect of Alkali with Borate Added.—Figure 2 shows the effect of increasing concentrations of alkali on the rate of formation of ketose when a fixed amount of borate is added. At low alkali concentrations (0.025 to 0.1 *N*) the rate of formation of ketose is very slow. This may be due to the buffering action of the added borate. At higher concentrations of alkali (0.15 to 0.25 *N*) the buffering effect of the added borate is overcome. The curves at low alkali concentrations also show that the amount of ketose formed is still increasing after 10 minutes, whereas with alkali alone or at higher concentrations of alkali with added borate (0.20 to 0.25 *N* NaOH) the amount of ketose begins to decline after 1 minute. The addition of borate may thus inhibit the destruction of ketose. The

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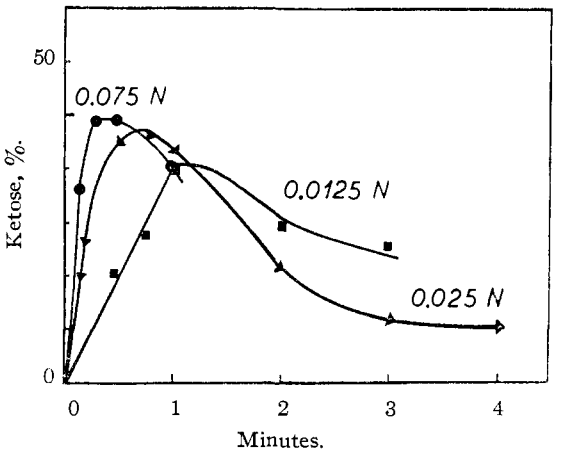

Fig. 1.—Effect of alkali concentration on the rate of formation of ketose at 100°. Each vessel contained 0.2 μmole of glucose at zero time in a total volume of 0.2 ml. The concentration of NaOH is indicated in each curve.

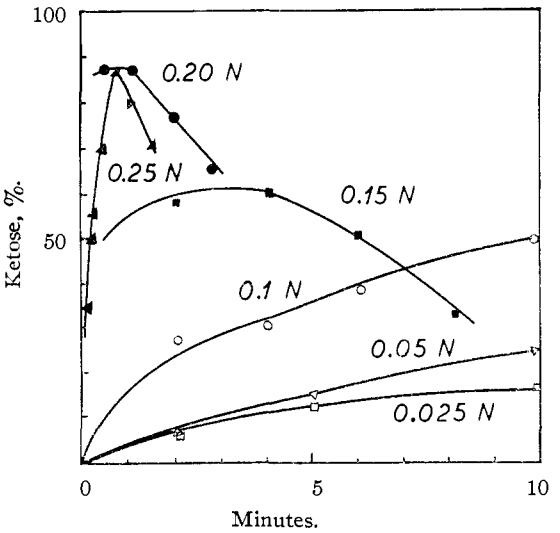

Fig. 2.—Effect of alkali concentration on the rate of formation of ketose at 100° in the presence of borate. Each vessel contained 0.2 μmole of glucose at zero time in a total volume of 0.2 ml. The concentration of borate in each case was 0.16 *N* (0.04 *M* $Na_2B_4O_7$). The concentration of NaOH is indicated in each curve.

rate of formation and destruction of ketose is directly proportional to the concentration of alkali used. The type of curve obtained with 0.2 and 0.25 *N* NaOH in the presence of borate is about the same as that obtained with alkali alone shown in Fig. 1. The amount of ketose formed in each case increases for about a minute and then declines at a rate dependent on the concentration of alkali. However, in the presence of borate the rate of formation of ketose is increased and as a consequence the maximum amount of ketose formed is greater than with alkali alone (80 to 85% in the presence of borate compared with 40 to 50% in the absence of borate). It would appear that in the presence of borate the isomerization reaction is much more rapid than those reactions which tend to remove ketose (saccharinic acid, difructose pyranose formation, etc.).

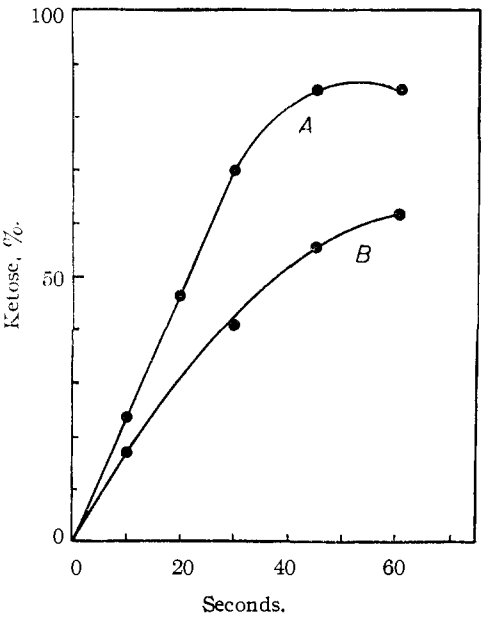

Fig. 3.—Effect of sugar-borate ratio on the rate of formation of ketose at 100°. Each vessel contained 0.2 μmole of glucose at zero time in a total volume of 0.2 ml. Curve A was obtained with 0.04 *M* $Na_2B_4O_7$–0.20 *N* NaOH, 0.025 *M* $Na_2B_4O_7$–0.125 *N* NaOH or 0.015 *M* $Na_2B_4O_7$–0.075 *N* NaOH. All gave the same relationship between time of heating and amount of ketose formed. Curve B was obtained with 0.01 *M* $Na_2B_4O_7$–0.05 *N* NaOH.

Effect of Sugar–Borate Ratio.—In order to show the effect of borate concentration on the reaction, the ratio of borate to alkali had to be kept constant since it had already been shown in Fig. 2 that this ratio affected the rate of formation of ketose. In this way, by also keeping the sugar concentration constant the rate of ketose formation and the maximum amount of ketose formed might be related to the degree of sugar–borate complexing as reflected by changes in the borate concentration. Figure 3 shows the effect of decreasing amounts of borate on the extent of conversion to ketose at a constant ratio of alkali to borate and at constant sugar concentration. Decreasing the borate concentration from 0.16 to 0.06 *N* had no effect on the rate or the extent of conversion to ketose. However, when the borate concentration was decreased to 0.04 *N* the rate and the amount of ketose formed decreased. It would appear that maximum complexing between D-fructose and borate is reached somewhere between 0.04 and 0.06 *N* borate under these conditions.

Effect of Sugar Concentration.—Table I shows the effect of increasing amounts of aldose on the extent of ketose formation. In the range of 1 μmole/ml. to 20 μmoles/ml., the starting concentration of D-glucose had no effect on the extent of conversion to ketose.

Stability of Ketose in Alkali.—Table II shows the effect of dilute alkali at 100° on the disappearance of D-glucose and D-fructose in the presence and absence of borate. It can be seen that the addition of borate decreases the destruction of fructose by alkali. Thus 85% of the fructose remains after

TABLE I

EFFECT OF D-GLUCOSE CONCENTRATION

Each vessel contained 0.08 ml. of 0.1 *M* $Na_2B_4O_7$, 0.08 ml. of 0.5 *M* NaOH and the indicated amount of glucose in a final volume of 0.2 ml. Aliquots were taken and the amount of ketose was determined.

Glucose concn., 10^{-3} *M*	Time at 90–100°, sec.	Amt. of glucose added, μmoles	Amt. of ketose formed, μmoles	Ketose, %
1	60	0.2	0.17	85
2	60	.4	.34	85
4	60	.8	.68	85
5	90	1.0	.80	80
10	90	2.0	1.7	85
20	90	4.0	3.6	90

60 seconds at 100° in the presence of borate (vessel 1) compared to only 20% remaining in the absence of borate. It may be noted (vessel 4) that the same amount of ketose is formed from D-glucose in the presence of borate. In the absence of borate (vessel 6) 40% of the glucose added is present as ketose after 60 seconds compared to only 20% (vessel 3) if the ketose is added initially. Separation of the sugars present in vessel 6 by paper chromatography showed that very little D-glucose was still present indicating that there was appreciable destruction of hexose, about 50%, in the presence of alkali alone. Table III shows that with borate present (vessel A) almost all the hexose is converted to ketose in 45 seconds. In contrast, with alkali alone (vessel B) only about 60% of the hexose present is ketose. Moreover about 25% of the hexose has been destroyed in 45 seconds in the presence of alkali alone, whereas when borate is present (vessel A) there is practically no destruction of hexose in 45 seconds. In 180 seconds with alkali alone 80% of the added hexose has been destroyed while in the presence of borate only 40% of the added hexose is destroyed.

TABLE II

STABILITY OF KETOSE IN ALKALI

Reaction carried out at 90–100°; conditions as described in the methods. The vessels were heated at 100° for 1 minute.

Substance added	Vessel 1	2	3	4	5	6
Fructose, μmole	0.2	0.2	0.2	....	...	....
Glucose, μmole	....	....	....	0.2	0.2	0.2
Sodium borate, *M*	0.06	0.06	....	0.06	0.06	....
NaOH, *N*	0.075	....	0.075	0.075	...	0.075
Ketose present, %	85	100	20	85	10	40

TABLE III

DESTRUCTION OF HEXOSE AT 100°

Each vessel contained 2 μmoles of D-glucose at zero time; vessel A contained 0.4 ml. of 0.1 *M* $Na_2B_4O_7$ and 0.4 ml. of 0.5 *N* NaOH in a total volume of 2 ml.; vessel B contained 0.1 ml. of 0.5 *N* NaOH in a total volume of 2 ml.; aliquots were taken at the indicated times.

Vessel time at 100°, sec.	A —With borate— Ketose present, μmoles	Hexose present, μmoles	B —Without borate— Ketose present, μmole	Hexose present, μmoles
45	1.8	1.9	0.9	1.5
180	1.1	1.2	0.4	0.4

Preparation of D-Fructose.—A reaction mixture containing 100 ml. of 0.1 *M* $Na_2B_4O_7$, 100 ml. of 0.5 *N* NaOH and 100 ml. of water was heated to 90–100°. Then 3 ml. of 1.12 *M* glucose was added rapidly. The heating was continued for another 70 seconds at 90–100°. The solution was then poured into a beaker containing 100 g. of ice and stirred. The solution contained 2700 μmoles of ketose. The yield was 80%.

The solution was passed through a resin column containing 20 g. of Dowex-50 in the acid form to remove Na^+. The column was washed with an equal volume of distilled water. The *p*H was 3 to 4. The solution was evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml. of methanol and again evaporated to dryness to remove boric acid as methyl borate. This was repeated. The sirup was dissolved in a small volume of water and Celite was added. The Celite was dried *in vacuo*. The 2,3-4,5-diacetone derivative of D-fructose was prepared by the procedure of Bell.[19] The m.p. of the crystals obtained after evaporation of the chloroform was 92–94° (cor.). The overall yield was 60%. The diacetone derivative was decomposed in acid and the solution was deionized. The behavior of the isolated sugar in paper chromatography in two solvents was identical with that of D-fructose.

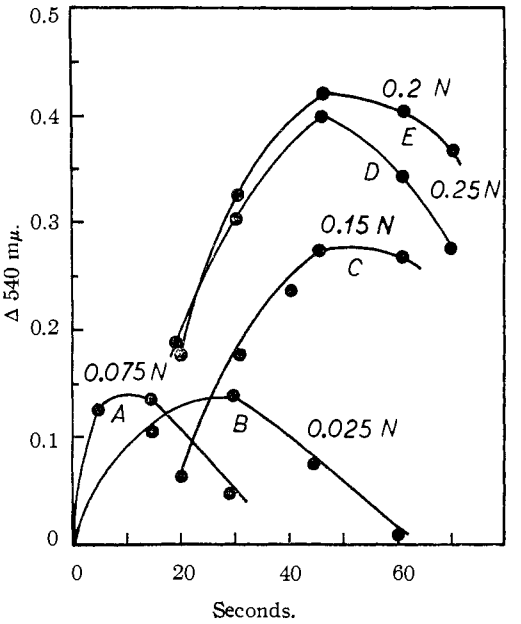

Fig. 4.—Effect of borate on the conversion of xylose to ketopentose at 100°. Each vessel contained 0.2 μmole of xylose at zero time and the total volume was 0.2 ml. The concentration of alkali is indicated in each case. The concentration of borate in curves C, D and E was 0.16 *N* (0.04 *M* $Na_2B_4O_7$).

Effect of Borate on the Isomerization of D-Xylose. Figure 4 shows the effect of borate on the isomerization of D-xylose in alkali. In the presence of alkali alone, curves A and B, it may be seen that increasing the alkali concentration increases the rate of formation of ketopentose, but not the maximum amount formed. When borate is added there is a 3-fold increase in the maximum amount of ketopentose formed (0.13 compared to 0.42). The borate to alkali ratio giving a maximum conversion is the same as that found with D-glucose (Fig. 2). When less alkali is added the maximum amount of ketopentose formed decreases (curve C). When more alkali is added the maximum amount of ketopentose formed is about the same but the rate of destruction of ketopentose increases (curve E compared to curve D). It may also be noted that

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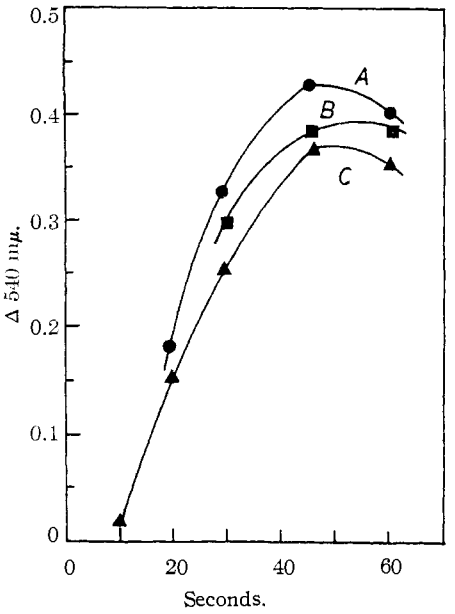

Fig. 5.—Effect of xylose–borate ratio on the formation of ketopentose. Each vessel contained 0.2 μmole of xylose at zero time: curve A was obtained with 0.04 *M* $Na_2B_4O_7$–0.2 *N* NaOH, curve B with 0.03 *M* $Na_2B_4O_7$–0.15 *N* NaOH and curve C with 0.02 *M* $Na_2B_4O_7$–0.1 *N* NaOH. An unheated control containing 0.2 μmole of xylose was subtracted in each case.

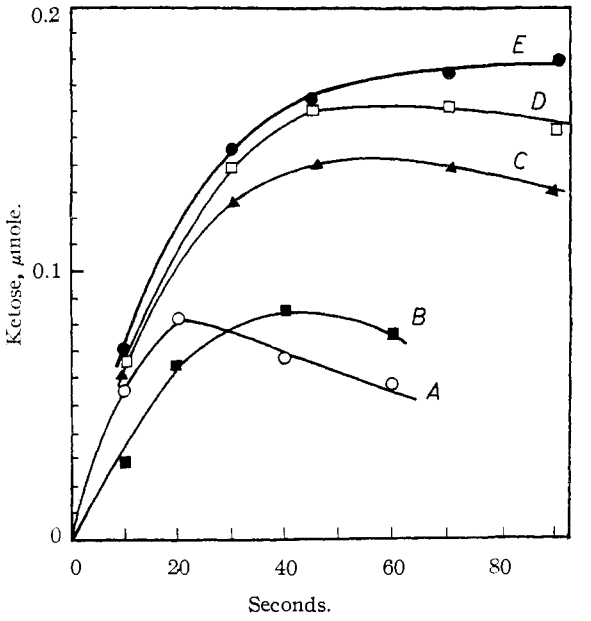

Fig. 6.—Isomerization of lactose. Each vessel contained 0.2 μmole of lactose in a total volume of 0.2 ml. at zero time: curve A was obtained with 0.075 *N* NaOH, curve B with 0.025 *N* NaOH, curve C with 0.04 *M* $Na_2B_4O_7$–0.25 *N* NaOH, curve D with 0.04 *M* $Na_2B_4O_7$–0.1 *N* NaOH and curve E with 0.4 *M* $Na_2B_4O_7$–0.2 *N* NaOH.

the maximum conversion in the presence of borate is reached in 45 seconds.

Figure 5 shows the effect of the D-xylose to borate ratio on the rate of formation of ketopentose. The alkali to borate ratio and the sugar concentration was kept constant. The rate of formation of ketopentose increases with increasing borate–xylose ratios. It would appear that the ketopentose requires a high borate concentration for maximum complexing. Thus 0.015 *N* $Na_2B_4O_7$–0.075 *N* NaOH gave a maximum rate of conversion in the D-glucose to D-fructose isomerization (Fig. 3). At the highest concentration of borate used (0.04 *N* $Na_2B_4O_7$–0.2 *N* NaOH) for the isomerization of D-xylose the rate of formation of ketopentose was still increasing. The maximum conversion with D-xylose was obtained in 45 seconds regardless of what concentration of borate was used.

Preparation of D-Xylulose.—The following reaction mixture was prepared: To 600 ml. of 0.1 *M* $Na_2B_4O_7$, 157.8 ml. of 1.9 *N* NaOH and water were added to a final volume of 900 ml. The solution was heated to 90–100°. Then 10 ml. of a solution containing 13 mmoles of D-xylose was added rapidly and the heating was continued another 45 seconds. The solution was poured into a beaker containing 100 g. of ice and stirred. The solution was passed through a column containing 20 g. of Dowex-50 in the acid form to remove Na^+. The column was washed with water. The solution made 0.01 *M* with respect to sodium tetraborate. D-Xylulose was separated from D-xylose by chromatography on a Dowex-1 (borate) column according to the procedure of Khym and Zill[20] as adapted for D-xylose and D-xylulose.[13] The D-xylulose absorbed on the Dowex-1 (borate) column (8.7 sq. cm. × 20 cm.) was eluted with 0.02 *M* sodium tetraborate. The eluate was passed through a Dowex-50 H^+ column and evaporated to dryness *in vacuo*. The residue was dissolved in methanol and evaporated to dryness to remove borate. This was repeated twice. The over-all yield was 20%.

The isolated sugar was identified as D-xylulose on the basis of its stability to bromine oxidation, its reaction with the orcinol and cysteine–carbazole reagents and its behavior on paper chromatography in two solvents. The sugar on paper chromatography in two solvents migrated at the same rate as D-xylulose.

Isomerization of Lactose.—The isomerization of lactose to ketose in alkali in the presence and absence of borate is shown in Fig. 6. Lactose behaves in much the same way as glucose except that it is much less affected by changes in alkali or borate concentration (curves C to F). The product is more stable to alkali than fructose in the presence of borate. In 90 seconds there is little or no decrease in the amount of ketose formed at several different borate and alkali concentrations. The principal product of the reaction isolated by paper chromatography was identified as a disaccharide containing a free keto group.

Isomerization of Glucose 6-Phosphate.—Glucose 6-phosphate was also converted to a ketohexose phosphate when treated with NaOH and borate giving about the same conversions as free D-glucose. Table IV shows the extent of conversion to ketose phosphate as several different times. Hydrolysis of this sugar phosphate with alkaline phosphatase resulted in a free sugar which was identified as D-fructose by paper chromatography. Glucose 1-phosphate did not give any ketose phosphate when heated under the same conditions as glucose-6-phosphate.

Isomerization at 37°.—The extent of conversion to the ketose is independent of the temperature employed. Thus in Table V it may be observed that the maximum amount of ketose formed in the

(20) J. X. Khym and L. P. Zill, THIS JOURNAL, **74**, 2090 (1952).

TABLE IV

CONVERSION OF GLUCOSE 6-PHOSPHATE TO KETOSE PHOSPHATE

Each vessel contained 0.2 μmoles of glucose 6-phosphate 12 μmoles of sodium borate and 15 μmoles of NaOH in a total volume of 0.2 ml.

Time at 90–100°, sec.	0	30	45	60	90	120
Ketose phosphate present, %	0	30	55	75	80	70

absence and presence of borate is about the same as the maximum conversion at 100°. The rate of formation of ketose in each case is much slower at 37°.

TABLE V

ISOMERIZATION AT 37°

Each vessel contained 2 μmoles of each of the sugars indicated; vessel A contained 50 μmoles of NaOH; vessel B contained 400 μmoles of NaOH and 80 μmoles of $Na_2B_4O_7$; the final volume in each case was 2.0 ml.; aliquots were taken at the indicated times.

	Sugar added					
	A Glucose B		A Lactose B		A Xylose B	
Time, hr.	Alkali alone	With borate	Alkali alone	With borate	Alkali alone	With borate
	Ketose formed, μmoles		Ketose, formed, μmoles		Δ 540 mμ	
8	0.7	1.2	0.7	1.3	1.2	2.9
18	.8	1.5	.7	1.6	0	3.9
25	.8	1.6	.7	1.5	0	3.7
42	.4	0.75	.5	1.0	0	1.9

Discussion

The Lobry de Bruyn–Alberda van Ekenstein transformation usually gives mixtures of aldoses and ketoses as well as products from various side reactions. Organic acids are the principal products of the irreversible reactions along with small amounts of saccharinic acids. Another side reaction, the formation of color, is directly related to the time of heating, molarity of base and the aldose concentration.[18] Increasing amounts of alkali cause an increase in the conversion of aldose to ketose but also a corresponding increase in the formation of acids from the ketose. Thus a maximum yield of ketose is obtained when the rate of reactions forming ketose equals the rate of reactions removing ketose.

The present study indicates that by using dilute alkali and sugar concentrations and by the addition of borate to the reaction mixture the yield of ketose is increased. It appears that the additions of borate causes the alkali-catalyzed isomerization reaction to be altered in favor of ketose. Secondary irreversible reactions arising from further transformations of the ketose are greatly reduced in the presence of borate ion.

Cohen[4] observed that the degradation of ribulose and ribulose-5-P at neutral or slightly alkaline *p*H's is greatly inhibited by the addition of borate at *p*H 8. In the present study it would appear that D-fructose and D-xylulose are also protected from degradation in alkali when borate is added to the solution. The reaction was allowed to proceed only one minute. At longer times or at higher sugar concentrations, the yield of ketose decreases and the amount of color formed increases. The percentage of ketose formed also appears to be a function of the sugar to borate ratio. It is probable that this ratio would be different for different aldoses. The ratio of sugar to borate for optimum conversion to the ketose has been shown to vary greatly in enzyme-catalyzed isomerizations.[4,5,7]

When the starting aldose is available in only small quantities or when identification of an aldose by conversion to the corresponding ketose is desired a procedure for bringing about the formation of ketose in high yield by alkali isomerization might prove to be quite useful. Amounts from 0.2 μmole to 3000 μmoles of aldose were used in the experiments described.

Acknowledgments.—The author wishes to express his gratitude to Dr. Luis Leloir and Dr. Enrico Cabib for their inspiring guidance and advice throughout his stay in this Laboratory. The author acknowledges with deep appreciation the assistance and helpful criticism of the members of the "Instituto de Investigaciones Bioquimicas."

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, GEORGETOWN UNIVERSITY, WASHINGTON 7, D. C.]

2-Deoxy Sugars. II. 3β-(2,6-Dideoxy-α-D-*ribo*-hexopyranosyl)-14β-hydroxy-5β-card-20(22)-enolide. A Direct Method of Synthesis of 2-Deoxyglycosides Involving a Crystalline 2-Deoxy-acylglycosyl Halide[1]

BY W. WERNER ZORBACH AND THOMAS A. PAYNE

RECEIVED FEBRUARY 11, 1960

The partial synthesis of a 2'-deoxycardenolide is described. The coupling of a crystalline digitoxosyl chloride with digitoxigenin gave, after saponification, a monodigitoxoside of digitoxigenin. The anomeric configurations of the new monoside and of two crystalline digitoxosyl halides have been assigned. The synthetic cardenolide shows a definite digitalis-like action.

In a previous communication[2] we reported the successful coupling of 3β,14β-dihydroxy-5β-card-20(22) - enolide (digitoxigenin) (VI) with 2,6 - dideoxy-D-*ribo*-hexose (digitoxose) (I), which are, respectively, the steroidal and carbohydrate components of the natural cardenolide, digitoxin. The latter is very important clinically as a cardiotonic agent and is the U.S.P. reference standard for related drugs.

At the outset it was hoped that a successful synthesis would yield a monodigitoxoside identical

(1) This work was supported largely by National Science Foundation Grant G-7351.

(2) W. W. Zorbach and T. A. Payne, THIS JOURNAL, **81**, 1519 (1959).