Isomerization of pentoses and 2-pentuloses by inorganic phosphates

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2-Pentuloses isomerize to the corresponding pentoses in the presence of potassium hydrogen phosphate. The yield and the purity of the final products are influenced by temperature and concentration of the salt. Similarly, pentoses isomerize to the corresponding 2-pentuloses at the same conditions. These isomerization reactions were studied with respect to the preparation of 2-pentuloses.

2-Пентулозы в среде гидрофосфата калия изомеризуются в соответствующие пентозы в зависимости от температуры и концентрации соли, что влияет на их выход и чистоту. Аналогично, пентозы изомеризуются в этих условиях в соответствующие 2-пентулозы. Указанные реакции изомеризации были изучены с точки зрения приготовления 2-пентулоз.

Biochemical dehydrogenation of pentitols by strains of *Acetobacter* is the most advantageous method for preparation of 2-pentuloses [1]. This method gives 2-pentuloses in high yields. However, the final product contains also pentoses which are removed by separation on cellulose column. Due to the similar R_f values of the mentioned compounds, this separation is rather difficult and results in lower yields of 2-pentuloses. The separation of *erythro*-pentulose from ribose is especially difficult.

We supposed that the appearance of pentoses in the reaction medium during the enzymic preparation of 2-pentuloses was not a result of biochemical dehydrogenation of pentitols but of isomerization of 2-pentuloses by the action of inorganic salts present in the culture medium of *Acetobacter*.

For enzymic preparation of L-*erythro*-pentulose and D-*threo*-pentulose media containing hydrogen phosphate as well as magnesium sulfate are used. We investigated the effect of potassium dihydrogen phosphate, potassium hydrogen phosphate, and potassium phosphate on isomerization of the above-mentioned compounds in dependence on temperature and concentration of phosphates. Simultaneously, we studied the isomerization of D-ribose and D-xylose to D-*erythro*-pentulose and D-*threo*-pentulose, respectively. The isomerization of 2-pentuloses and pentoses was followed by measuring the amounts of 2-pentuloses in the reaction mixtures polarographically as reduction waves of α-hydroxy groups

to carbonyl group in amine buffer [2]. The products were identified by gas—liquid chromatography in the form of trimethylsilyl derivatives of oximes [3] as well as by paper chromatography.

Experimental

Instruments

Polarographic measurements were performed on a Radelkis polarograph, type OH 102 (Budapest) in a thermostatic polarographic vial with separate reference saturated calomel electrode. The temperature was maintained with the accuracy of ±0.1°C using a U 10 (Prüfgeräte Mederigen, Dresden) thermostat. The transformation reactions were carried out also in this thermostat. The pH values were measured by a Radiometer titrator, type TTT 2 (Copenhagen) with glass electrode EA 10921 and calomel electrode EA 404 (Methrom, Herisaex).

Gas—liquid chromatography was carried out isothermally at 150°C on a Hewlett—Packard Research Gas Chromatograph 5750 using capillary column (45 m) coated with OV-17 as a stationary phase.

Optical rotations were measured on a Perkin—Elmer 141 polarimeter.

Chemicals

L-*erythro*-Pentulose and D-*threo*-pentulose were prepared by biochemical dehydrogenation of ribitol with *Acetobacter suboxydans B.S. 2356* and of D-arabitol with *Acetobacter pasteurianus B.S. 1775* (Czechoslovak collection of microorganisms), respectively [4]. The purity of the above-mentioned saccharides was controlled by paper chromatography and optical rotation. D-Ribose and D-xylose were commercial products; their purity was controlled by paper chromatography and melting point.

Isobutylamine, used in polarographic determinations of 2-pentuloses, was a product of Fluka, Buchs. Phosphate buffer of pH 7.0 of the series "Titrisol" (E. Merck, Darmstadt) was used as standard for pH measurements.

Hydroxylammonium chloride of anal. grade (Lachema, Brno), used for the preparation of oximes, was dissolved in pyridine which was refluxed over potassium hydroxide and distilled. TRI-SIL Concentrate (Pierce, Rockford, Il.) was used as silylation agent.

The used phosphates were commercial products.

Descending chromatography was performed in the systems: *a*) ethyl methyl ketone—butanol—water (7:2:1); *b*) chloroform—acetic acid (7:2+1.4 ml of water-/100 ml of solvent).

Procedures

The isomerization of L-*erythro*-pentulose and D-*threo*-pentulose by potassium hydrogen phosphate was followed in dependence on temperature (50—90°C) and concentration of the salt (0.004, 0.02, 0.1, and 0.5 M). The increase of the amount of D-*erythro*-pentulose and D-*threo*-pentulose from D-ribose and D-xylose, respectively, was followed at similar conditions (time and concentration of potassium hydrogen phosphate).

Polarographic analysis of the sample (0.05 ml; 0.1 mole) was accomplished in 0.15 M isobutylamine buffer (10 ml) (0.15 M isobutylamine and 0.15 M isobutylammonium chloride of $pH = pK = 10.4$). The polarographic waves were registered in a vessel maintained at 20°C. The solution for polarographic analysis was 5×10^{-4} M with respect to concentration of the starting sugar.

Preparation of the sample for gas—liquid chromatography

The sample (6 ml), after the reaction adequate to *ca.* 10 mg of the original sugar, was distilled off at 35°C *in vacuo* and the solution (0.7 ml) of hydroxylammonium chloride in anhydrous pyridine (15 mg/ml) was added. The mixture was allowed to react in a closed vessel for 1 h at laboratory temperature and to assure quantitative reaction, for 1 h at 70°C. After cooling to room temperature, the obtained oximes were silylated in the known manner.

Results and discussion

It is known that isomerization of monosaccharides in alkaline medium is a general base-catalyzed reaction [5] while the effect of specific catalysis is incomparably greater. Therefore, in the presence of potassium phosphate the transformation reactions proceeded very fast at high pH values. Though at preliminary experiments the amounts of the original pentoses decreased rapidly in the reaction mixture, the formation of pentuloses was negligible. Due to irreversible dehydration and subsequent oxidation-reduction disproportionation of the dehydration products, all sugars were transformed to saccharinic acids and pH of the reaction medium decreased. The experiments with potassium dihydrogen phosphate did not lead to satisfactory results. In this medium the concentration of OH^- is very low and the transformation reaction is catalyzed only by the ions of

Table 1

Transformation (%) of 0.1 M L-*erythro*-pentulose in 0.1 M-K_2HPO_4 at different temperature with time

t, h	*t*, °C				
	50	60	70	80	90
0.25	94.0	92.0	89.5	87.0	80.0
0.5	91.5	88.0	85.0	72.5	70.0
1	89.5	85.0	82.0	65.0	55.5
2	88.0	81.0	79.5	57.5	43.0
4	85.5	76.0	74.0	47.5	32.5
6	82.0	72.5	68.5	40.0	29.0
8	79.5	70.0	64.5	35.0	27.0
10	78.0	68.5	60.0	32.0	24.5

dihydrogen phosphate (according to general catalysis). Consequently, the transformation reactions proceeded very slowly and only trace amounts of pentuloses were formed.

The effect of potassium hydrogen phosphate on isomerization was studied in detail. The following optimum conditions were concluded: 0.1—0.5 M potassium hydrogen phosphate, 80—90°C, and pH of 8—9.

The results of isomerization of L-*erythro*-pentulose and D-*threo*-pentulose in dependence on temperature (Table 1, Fig. 1) and concentration of hydrogen phosphate (Table 2, Fig. 2) indicate that the increase of both the temperature of

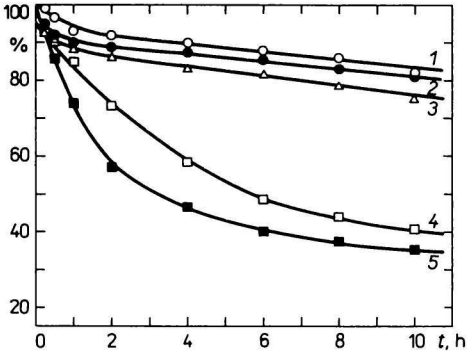

Fig. 1. Transformation (%) of 0.1 M D-*threo*-pentulose in 0.1 M-K_2HPO_4 in dependence on temperature.
1. $t = 50°C$; *2.* $t = 60°C$; *3.* $t = 70°C$; *4.* $t = 80°C$; *5.* $t = 90°C$.

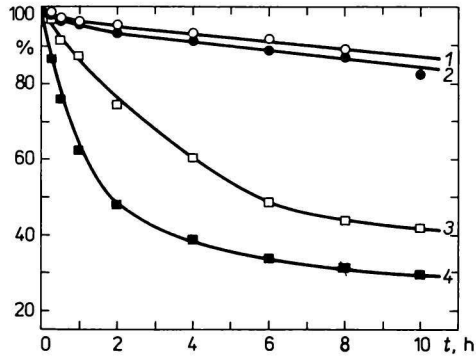

Fig. 2. Transformation (%) of 0.1 M L-*erythro*-pentulose at 80°C in dependence on concentration of K_2HPO_4.
1. 0.004 M; *2.* 0.02 M; *3.* 0.1 M; *4.* 0.5 M.

Table 2

Transformation (%) of 0.1 M D-*threo*-pentulose at 80°C and different concentration of K_2HPO_4 with time

t, h	conc, M			
	0.004	0.02	0.1	0.5
0.25	99.0	98.0	96.0	86.0
0.5	97.5	96.5	91.5	76.0
1	96.5	95.5	87.5	62.5
2	95.5	93.3	74.5	48.0
4	93.5	91.5	60.5	39.0
6	92.0	89.0	48.7	34.0
8	89.0	87.0	44.0	31.5
10	—	82.5	42.0	30.0

the reaction mixture and concentration of the salt bring about gradual decrease of the amount of 2-pentuloses in the reaction medium. This decrease is greater in the case of L-*erythro*-pentulose. The biochemical preparation of 2-pentuloses should be therefore carried out at temperature not exceeding 40°C and the reaction medium should be deionized before evaporation so that the concentration of salts in the solution did not increase.

From the amounts of D-*erythro*-pentulose and D-*threo*-pentulose formed by isomerization of D-ribose and D-xylose, respectively, in dependence on temperature (Table 3, Fig. 3) and concentration of potassium hydrogen phosphate (Table 4, Fig. 4) it is evident that ribose underwent transformations incomparably easier than xylose. This statement is supported by great amount of *threo*-pentulose beside *erythro*-pentulose determined by gas chromatography.

Table 3

Time dependence of D-*erythro*-pentulose formation (conc in %) from 0.1 M D-ribose in 0.1 M-K_2HPO_4 at different temperature

t, h	t, °C				
	50	60	70	80	90
0.25	—	—	—	5.7	11.5
0.5	0.8	3.0	4.6	9.1	15.8
1	1.7	3.7	7.7	12.1	21.1
2	2.3	6.0	11.4	17.0	21.6
4	2.6	9.1	13.9	20.6	19.8
6	3.6	10.5	16.2	21.7	16.8
8	4.0	11.1	18.9	20.8	14.2
10	5.0	11.8	20.2	20.0	11.6

Table 4

Time dependence of D-*threo*-pentulose formation (conc in %) from 0.1 M D-xylose at 80°C and different concentration of K_2HPO_4

t, h	conc, M			
	0.004	0.02	0.1	0.5
0.25	0.65	—	1.6	2.4
0.5	1.1	1.7	3.6	4.5
1	2.2	3.8	5.6	7.7
2	3.8	6.3	9.5	11.3
4	4.9	7.9	10.8	12.7
6	5.2	8.6	11.3	12.5
8	5.4	8.6	11.5	12.4
10	5.7	8.8	11.3	12.0

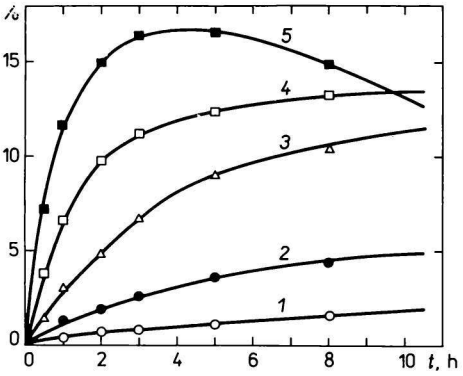

Fig. 3. Formation (%) of D-*threo*-pentulose from 0.1 M D-xylose in 0.1 M-K_2HPO_4 in dependence on temperature.
1. $t = 50°C$; *2.* $t = 60°C$; *3.* $t = 70°C$; *4.* $t = 80°C$; *5.* $t = 90°C$.

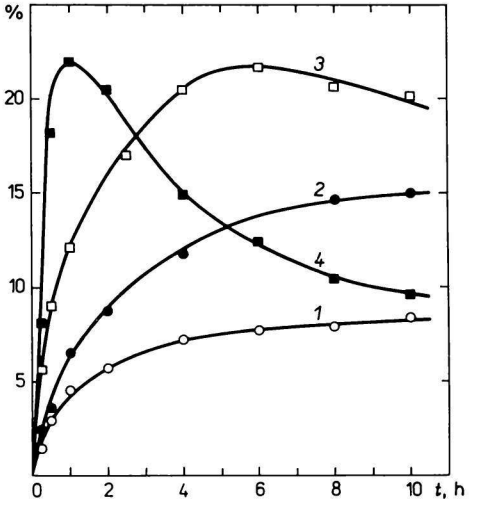

Fig. 4. Formation (%) of D-*erythro*-pentulose from 0.1 M D-ribose at 80°C in dependence on concentration of K_2HPO_4.
1. 0.004 M; *2.* 0.02 M; *3.* 0.1 M; *4.* 0.5 M.

The results of isomerization of 2-pentuloses obtained by gas chromatography are presented in Table 5. In addition to the main products of isomerization, also arabinose and lyxose were formed in amounts not exceeding 1%. More by-products were formed from *erythro*-pentulose than from *threo*-pentulose. At the above-mentioned conditions of gas chromatography, trimethylsilyl derivatives of oximes of the determined compounds revealed two peaks corresponding to *E* and *Z* isomers of cyclic oximes. The method of separation of these compounds by gas chromatography will be the subject of a next communication.

The isomerization was polarographically followed only by determination of concentration changes of the starting compounds and reaction products, respec-

Table 5

Products of transformation of 2-pentuloses followed by gas—liquid chromatography for 1 h (0.1 M-K_2HPO_4; 80°C)

Starting compound	Composition of reaction mixtures in %			
	erythro-Pentulose	*threo*-Pentulose	Ribose	Xylose
L-*erythro*-Pentulose	30	35	7—11	25—28
D-*threo*-Pentulose	80—85	5	10—15	1

tively. It is to be considered that is was possible to determine only the total content of 2-pentuloses. Thereupon the results with L-*erythro*-pentulose, mainly in the initial phase of the reaction, were laded with a certain error that could not be neglected. This fact was affirmed also by the results of gas chromatography. We did not study the kinetics of isomerization because the simultaneous epimerization reaction made the system very complicated. The results of isomerization of L-*erythro*-pentulose obtained by gas chromatography could not be used for approximative solution.

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