

Reactions of saccharides catalyzed by molybdate ions. IX.* Epimerization of ketohexoses

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In acidic water solution under catalytic action of molybdate ions D-fructose epimerizes into D-sorbose, D-tagatose, and D-psicose in amounts 4.5, 1.0, and 0.5%, respectively. The epimerization of D-psicose, D-sorbose, or D-tagatose leads to conversion into corresponding ketohexoses in a similar extent. An application of this epimerization reaction to saccharose resulted in a new procedure for the preparation of D-mannose and D-sorbose.

Base catalyzed transformations of aldoses and ketoses, reviewed by *Speck* [1], involve mainly the isomerization of epimeric aldoses at C-2 into the corresponding 2-ketose or *vice versa*. In diluted mineral acids free monosaccharides undergo chiefly the intermolecular or intramolecular dehydrations. Disaccharides or glycosans can be isolated from such reaction mixtures. Further elimination reactions leading to derivatives of furan [2] and consecutive reactions giving rise to aromatic compounds [3] may also take place in acidic media. *Koizumi* and *Hashimoto* [4] reported that small amounts of arabinose, fructose, mannose, and xylose were formed on heating glucose in diluted sulfuric acid

Table 1

Fractionation of the epimerization mixture of D-fructose after fermentative removal of the starting sugar

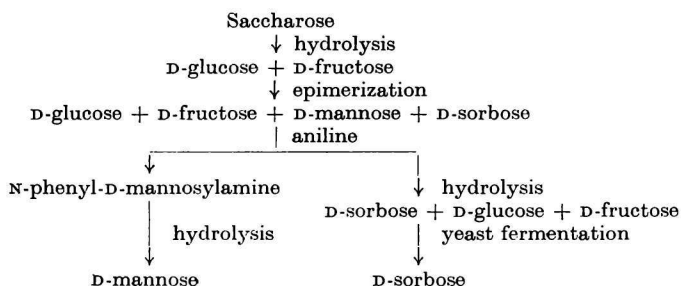
Fractionation on a Cellulose column		Refractionation by ion-exchange chromatography Amounts of ketohexoses in grams			
Fraction	ml	D-Sorbose	D-Sorbose + + D-Tagatose	D-Tagatose	D-Psicose
0	1865	—	—	—	—
1	205	—	—	0.15	0.15
2	330	0.45	—	0.25	0.2
3	1370	1.25 2.8 ^a	0.2	0.45	0.1
Sugar elution volume from the ion exchanger relative to D-sorbose (600 ml)		1.0	—	1.6	2.2

a) D-Sorbose isolated by crystallization before ion-exchange fractionation.

* For Part VIII see Ref. [11].

(≤ 1.5 N, 100°C , 2 hours). A similar treatment of fructose gave relatively higher yields of arabinose ($< 1\%$). In water solution of molybdenic acid tetroses [5], pentoses [6, 7], and hexoses [8–11] epimerize mainly at C-2 under the formation of equilibrium mixtures of epimeric aldoses; no evidence has been obtained for the formation of ketoses. Present work is devoted to molybdate catalyzed epimerization of ketohexoses.

The catalytic activity of molybdenic acid in the epimerization of ketohexoses was found to be much lower than with aldohexoses. The epimerization of D-glucose [8] or L-mannose [9] leads to the equilibrium of glucose and mannose in the ratio 3 : 1. Double reaction time is required to reach the equilibrium from glucose than from mannose. More than three times longer treatment of D-fructose, even in the presence of doubled concentration of molybdenic acid afforded only about 4.5% of D-sorbose, 1% of D-tagatose, and 0.5% of D-psicose (Table 1). Similarly, the same reaction with D-psicose, D-sorbose, or D-tagatose does not lead to a more extensive conversion into complementary ketohexoses, and also no evidence has been obtained for the formation of aldohexoses. The highest conversion into sorbose occurs in the epimerization of tagatose and fructose; it is low with psicose. The formation of psicose was observed only in the epimerization of tagatose and fructose. The epimerization of sorbose gives low yields of fructose and tagatose. The yields of these two ketoses are even lower in the case of psicose. The application of this epimerization reaction to saccharose which undergoes hydrolysis in the first step gave D-sorbose and D-mannose which can be simply separated from the reaction mixture in the form of *N*-phenyl-D-mannosylamine (Scheme 1).



Scheme 1

Psicose can be efficiently isolated from the mixture of ketohexoses by ion-exchange chromatography on Dowex 50W in the Ba^{2+} cycle (Table 1). The diphenylamine reagent [12] was very suitable for identification of ketohexoses on paper chromatograms since it colourly distinguishes sorbose from tagatose and these two ketoses from fructose and psicose (Table 2). Other detection reagents gave identical colour with all ketohexoses, e.g. anilinium oxalate brown, urea hydrochloride blue, orcinol yellow and vaniline blue [13].

The epimerization of ketohexoses in acidic water solutions of molybdate ions may be ascribed to the formation of labile anhydro derivatives at carbon atoms C-2 and C-3, and also at C-3 and C-4, which give upon hydrolysis the epimeric ketohexoses. The complexing of psicose, fructose, and tagatose with molybdate was reflected in significant changes in their specific rotations in water (Table 2).

Table 2
Characterization of ketohexoses

Ketohexose	$[\alpha]_D^{24}$		$R_{\text{sorb.}}$	Colour reaction
	a	b	c	d
D-Psicose	3.1	-12.5	1.40	Wine red
D-Fructose	-90.4	-77.7	1.08	Wine red
D-Sorbose	41.1	39.4	1.00	Blue green
D-Tagatose	0.2	13.5	1.20	Brick red

a) 2% solutions in water.

b) 1% solutions in 4% aqueous solution of molybdenic acid.

c) Chromatography on Whatman No. 1 paper in *n*-butanol-ethanol-water (5 : 1 : 4 v/v) for 70 hours.

d) Coloured with the diphenylamine reagent.

Ref. [15] gives for D-psicose $[\alpha]_D^{20}$ 3.1° (c 1.619, water); ref. [16] for β-D-fructose $[\alpha]_D^{20}$ -133° → -92° (water), for D-sorbose $[\alpha]_D^{23}$ 43° (c 1, water) and for D-tagatose $[\alpha]_D^{25}$ -1° (c 5, water); ref. [17] for D-tagatose $[\alpha]_D$ -2.3° (c 2.19, water).

Experimental

Epimerization of D-fructose

A solution of D-fructose (100 g) and molybdenic acid (2 g) in water (500 ml) was heated at 95°C for 20 hours. The reaction mixture was then treated with charcoal and deionized on an ion-exchange column (4.5 × 80 cm) of Wofatit SBW in the OH⁻ cycle. The water eluate (7 l) was concentrated *in vacuo* to 1.5 l, the same volume of tap water and baker's yeast (20 g) were added to remove fermentable sugars for 4 days. The mixture was filtered and the filtrate, after evaporation to sirup, was dissolved in methanol (500 ml), purified with charcoal and evaporated again. The residue was chromatographed on a Whatman CF 12 Cellulose column (4 × 130 cm) in *n*-butanol-ethanol-water (5 : 1 : 4 v/v) and four fractions were collected (Table 1) and concentrated *in vacuo*. The residue of fraction 3 was crystallized from aqueous methanol to give D-sorbose (2.8 g). Other fractions were refractionated on a Dowex 50WX8 (100-200 mesh, Ba²⁺) column (3.5 × 120 cm) using elution with water at a rate 45 ml/hour (Table 1). The fractions containing D-tagatose and D-psicose were further purified by paper chromatography on Whatman No. 3 paper in *n*-butanol-ethanol-water (as above). The characterization of isolated ketohexoses and starting D-fructose is presented in Table 2.

Epimerization of D-psicose, D-fructose, D-sorbose, and D-tagatose

A solution of one of the ketohexoses (1.0 g) in 0.4% water solution of molybdenic acid (5.0 ml) was heated at 95°C for 20 hours. After adding water (50 ml) the mixture was deionized on a Wofatit SBW (OH⁻). The ion exchanger was filtered off and washed with water (3 × 20 ml). The filtrate was evaporated under reduced pressure to sirup which was then dissolved in water (5.0 ml). After addition of methanol (5.0 ml), the mixtures were resolved by two-dimensional chromatography on Whatman No. 1 paper in *n*-butanol-ethanol-water in both directions (one for 30, the second for 70 hours). The chromatograms were detected with the diphenylamine reagent and evaluated on the basis of colour and intensity of spots.

Epimerization of components of saccharose

A solution of saccharose (200 g) and molybdenic acid (4 g) in water (1000 ml) was processed as it is described in the case of D-fructose epimerization. The deionized solution was evaporated to sirup which was dissolved in water (100 ml). After addition of aniline (100 ml) and ethanol (100 ml), the mixture was left to stand at room temperature for 20 hours. Separated crystalline N-phenyl-D-mannosylamine (15.5 g) was filtered off and washed with cold 50% aqueous ethanol. The filtrate was diluted with water (ca. 100 ml) and distilled with steam until complete removal of aniline (volume of distillate in the receiver 1200–1300 ml). The solution was then treated with charcoal and evaporated *in vacuo*. The sirupy residue was dissolved in tap water (4 l) and baker's yeast was added to remove the fermentable sugars in the course of 5 days. The mixture was then filtered, concentrated and fractionated on a Cellulose column as it is described at the epimerization of D-fructose. The fraction containing D-sorbose was concentrated and crystallized from aqueous methanol. D-Sorbose was isolated in amount of 2.4 g; m.p. 160–162°C (Kofler), $[\alpha]_D^{24} + 41.5^\circ$ (c 3, water).

Release of D-mannose

N-Phenyl-D-mannosylamine (15.5 g, $[\alpha]_D^{24} - 125^\circ$ (5 min) $\rightarrow -65^\circ$ (3 hours) $\rightarrow -65^\circ$ (70 hours), (c 0.2, methanol); ref. [14] gives $[\alpha]_D^{17} - 101.4^\circ \rightarrow -45.0^\circ$ (70 hours), (c 0.2, methanol)) was suspended in water (400 ml) and subjected to steam distillation (volume of distillate 600–800 ml). Water solution of released D-mannose was treated with charcoal and evaporated *in vacuo*. The sirupy residue was dissolved in methanol (8 ml), ethanol (8 ml) was added under heating and the mixture was left to crystallize at room temperature (2 days) to give crystalline D-mannose (6.4 g) having $[\alpha]_D^{24} + 14.0^\circ$ (c 3, water); ref. [16] $[\alpha]_D^{20} + 14^\circ$ (c 4, water).

References

1. Speck, J. C., Jr., *Advan. Carbohydr. Chem.* **13**, 63 (1958).
2. Anet, E. F. L., *Advan. Carbohydr. Chem.* **19**, 203 (1964).
3. Popoff, T. and Theander, O., *Carbohydr. Res.* **22**, 135 (1972).
4. Koizumi, K. and Hashimoto, K., *Yakugaku Zasshi* **92**, 1133 (1972).
5. Bílik, V. and Stankovič, L., *Chem. zvesti* **27**, 544 (1973).
6. Bílik, V., *Chem. Zvesti* **26**, 372 (1972).
7. Bílik, V. and Čaplovič, J. *Chem. Zvesti* **27**, 547 (1973).
8. Bílik, V., *Chem. Zvesti* **26**, 183 (1972).
9. Bílik, V., *Chem. Zvesti* **26**, 187 (1972).
10. Bílik, V., Voelter, W., and Bayer, E., *Justus Liebigs Ann. Chem.* **759**, 189 (1972).
11. Bílik, V., Voelter, W., and Bayer, E., *Justus Liebigs Ann. Chem.*, in press.
12. Hais, I. M. and Macek, K., *Handbuch der Papierchromatographie*, Bd. I, p. 741. Fischer Verlag, Jena, 1958.
13. Strecker, G. and Montreuil, J., *International Symposium V, Chromatographie-Electrophorese*, p. 340. Bruxelles, 1968. Presses Académiques Européennes éd., Bruxelles, 1969.
14. Ellis, G. P. and Honeyman, J., *J. Chem. Soc.* **1952**, 1490.
15. Steiger, M. and Reichstein, T., *Helv. Chim. Acta* **19**, 184 (1936).
16. Whistler, R. L. and Wolfrom, M. L., *Methods in Carbohydrate Chemistry*, Vol. I, p. 116–157. Academic Press, New York, 1962.
17. Reichstein, T. and Bosshard, W., *Helv. Chim. Acta* **17**, 753 (1934).

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