Reactions of saccharides catalyzed by molybdate ions. XIX.* Molybdate complexes and epimerization of aldoses as a function of pH

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In the presence of molybdate ions aldoses exhibit extremum values of specific rotation in pH range 2.6—6.8. Thirty of the tested aldoses showed extremum values at pH 5.5—6 and some of them also at pH 3—4. The rate of epimerization of aldoses catalyzed by molybdate ions achieved maximum at pH 2.5—3.5 and decreased with increasing pH. The rate of epimerization of D-glucose at pH 5.9 was five times slower than at pH 2.9 and the rate of D-mannose epimerization decreased as far as twenty times.

Альдозы с молибдатными ионами в области pH 2,6—6,8 показывают предельные величины удельного вращения. Тридцать испытаемых альдоз дают предельные величины при pH 5,5—6 и некоторые также в области pH 3—4. Скорость эпимеризации альдоз, катализированной молибдатными ионами, является оптимальной при pH 2,5—3,5 и с повышением pH падает. Скорость эпимеризации D-глюкозы при pH 5,9 является в пять раз меньше чем при pH 2,9 и при эпимеризации D-маннозы скорость реакции понизится в двадцать раз.

Aldopentoses and aldohexoses in the imagination of pyranoid structures having *cis-cis* arrangement of the hydroxyl groups at carbon atoms 1—3, form at pH 5 complexes with molybdate ions which are mobile on paper electrophoresis (ribose, lyxose, mannose, talose). Molybdate complexes of aldoses which do not fulfil the above requirement are immobile (arabinose, xylose, glucose, galactose). On the other hand, tetroses (erythrose, threose) and all of the so far tested aldoheptoses (D-*glycero*-L-gluco-, -D-galacto-, -L-galacto-, -D-ido-, -D-gulo-, -D-manno-, and -D-allo-) are mobile during electrophoresis [1, 2]. Measurements of the specific rotation of molybdate solutions of aldoses as a function of pH showed extremum values of specific rotation at pH 5.5 in the case of D-ribose,

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D-lyxose, and D-mannose, but not in the case of D-glucose, D-galactose, D-arabinose, and D-xylose [3]. The molybdate complexes of aldoses which are immobile on electrophoresis and which exhibit at pH 5.5 only negligible changes of specific rotation of aldoses in comparison with the values recorded in water, show two Cotton effects in the circular dichroism measurements (242—247 and 272—280 nm). Aldopentoses and aldohexoses having *cis-cis* arrangement of hydroxyl groups exhibit three Cotton effects (230—237, 263—270, and 294—317 nm) [4, 5]. Alditols show in the presence of molybdate ions extremum values of specific rotation at pH 2—3. This fact is, for instance, used for a determination of D-glucitol and D-mannitol based on measurements of their specific rotation either at two different pH values [6] or at two wavelengths [7]. In both cases, however, the presence of other saccharides interferes.

The optimum pH of the epimerization of aldoses catalyzed by molybdate ions lies between pH 2.5 and 3.5. The optimum of the formation of aldose-molybdate complexes occurs at pH 5.5—6; in this region the epimerization reaction is considerably slackened, however. The results in Table 1 demonstrate that the

Table 1

Effect of pH on the rate of epimerization of D-glucose and D-mannose catalyzed by molybdate ions at 80°C

pH	% of epimeric aldose						
	Time h	*1*	*2*	*3*	*4*	*5*	k h^{-1}
Epimerization of D-glucose							
2.9		25	27.5	27.5			0.68
4.7		14	21	24			0.19
5.9		8.5	11	12			0.12
Epimerization of D-mannose							
2.9		17	35	47	56	62.5	0.22
4.7		4.5	8	11	13.5	15	0.05
5.9		1	2	3	4	5	0.01

epimerization of D-glucose at pH 5.9 is more than five times slower than at pH 2.9; the epimerization of D-mannose under the same conditions was slowed down as far as twenty times. These observations pointed out that in the pH region optimal for the complexing of aldoses, a different complex is formed which is unsuitable or at least less suitable for the catalysis of the epimerization reaction. Our previous study of epimerization of D-glucose-1-^{3}H and D-mannose-2-^{3}H (at pH 2.9) has proved that the epimerization reaction involves an intramolecular migration of the

hydrogen atom from carbon C-1 to C-2 and *vice versa*, resulting in simultaneous change of the configuration at the same positions [8]. This intramolecular rearrangement is mediated by a complex formed between molybdate ion and C-1 and C-2 hydroxyl groups of aldose. The reaction also requires *trans*-axial arrangement of the hydrogen atoms linked to carbon atoms C-1 and C-2. In the imagination of pyranoid structures, this condition is fulfilled by one group of aldoses in the α-anomeric form (*II—VIII, XI, XX—XXIV, XXX;* Table 2) and by one group of aldoses in the β-anomeric form (*I, XII—XIX, XXV—XXIX;* epimers of the aldoses of the former group). Aldoses which cannot exist in the pyranoid structures, such as erythrose, threose, 5-deoxyribose, and 5-deoxyarabinose, also undergo the epimerization reaction; erythrose and 5-deoxyribose obviously as β-furanoids and threose and 5-deoxyarabinose, as α-furanoids. Under the conditions of the epimerization of aldoses, pentitols (arabitol, ribitol, xylitol) do not exhibit transformation changes. In aldofuranoses, similarly as in aldopyranoses, the molybdate complex formed with hydroxyl groups at C-1 and C-2 causes a deformation of the bond angles associated with a rehybridization at the corresponding carbon atoms. This factor enables the epimerization reaction when the C-1 and C-2 hydrogens are arranged *trans*-axially. Contrary to the cyclic structures, in alditols the rest of the molecule can be spatially suitably modified, so that the complex formed does not bring about a significant deformation of bond angles; as a consequence, the epimerization does not take place.

The different rate of epimerization of individual aldoses is conditioned by a number of factors involved in the conformation and anomeric equilibria. The higher rate of D-glucose than D-mannose epimerization (Table 1) may be due to the fact that the state of D-glucose satisfies better the epimerization conditions (conformation C1, equilibrium shifted in the favour of the β-anomer). On the contrary, D-mannose epimerization requires conformational change to unfavourable 1C conformer, which reflects negatively in the rate of epimerization. However, the reaction rate does not influence the final thermodynamic equilibrium of epimeric aldoses which is in all cases shifted in the favour of the aldose having a more stable conformation.

Specific rotations of aldoses in aqueous solutions of molybdate measured in pH region 2.6—6.8 (Table 2; Figs. 1 and 2) show extremum values around pH 5.9 and in the case of some aldoses also around pH 3.6. In comparison with specific rotations in water, aldoses of the homomorphous series of L-lyxose (*XXI—XXIV*) exhibit around pH 5.9 higher values of specific rotation which draw closer to the values of specific rotation of their β-anomers. On the other hand, aldoses of the homomorphous series of L-xylose (*XXV—XXVIII*) exhibit at pH 5.9 lower values of specific rotation, which indicates that their equilibria are shifted to the site of their α-anomers. Aldoses of the homomorphous series of L-talose (*II—VI*), with the exception of talooctoses (*VII, VIII*), also show lower values of specific rotation

Table 2

Extremum values of specific rotation of saccharides in aqueous solutions of molybdate and the effect of some aldoses on the rate of growth regeneration of *Saccharomyces cerevisiae* inhibited by molybdate ions as a measure of complex formation

	Saccharide	$[\alpha]_D^{23}$			v_{rel}
		Water	Aqueous solution of molybdate at pH 5.9	pH 3.6	
I	D-Ribose	− 18.7	− 77.5	− 26.0	27*
II	L-Talose	− 21.2*	− 61.2*	+ 8.5*	38*
III	6-Deoxy-L-talose	− 17.5	− 26.2	+ 6.8	27*
IV	L-*glycero*-L-Taloheptose	− 15.0	− 46.2	− 13.0	43
V	7-Deoxy-L-*glycero*-L-taloheptose	− 1.9	− 29.9	− 3.5	34
VI	D-*glycero*-L-Taloheptose	− 15.6	− 28.1	+ 72.5	46
VII	D-*threo*-L-Talooctose	− 14.4	+ 23.1	− 21.5	58
VIII	D-*erythro*-L-Talooctose	− 4.4	+ 90.6	+159.0	73
IX	5-Deoxy-D-ribose	+ 11.9*	+ 52.6*	+ 23.8*	
X	5-Deoxy-D-arabinose	+ 6.7*	+ 32.0*	+ 6.6*	
XI	D-Arabinose	−100.0	− 83.8	−100.0	14*
XII	L-Galactose	− 76.8	− 48.7	− 78.0	17
XIII	6-Deoxy-L-galactose	− 72.9	− 52.4	− 65.0	
XIV	L-*glycero*-L-Galactoheptose	− 61.8	− 36.5	− 25.5	
XV	7-Deoxy-L-*glycero*-L-galactoheptose	− 61.8	− 37.5	− 70.5	
XVI	D-*glycero*-L-Galactoheptose	− 63.1	− 28.1	− 62.3	
XVII	D-*threo*-L-Galactooctose	− 56.8	+ 2.5	+ 39.7	
XVIII	D-*erythro*-L-Galactooctose	− 43.7	+ 58.7	+ 78.5	
XIX	D-Allose	+ 14.5	− 62.0	− 3.5	
XX	D-Altrose	+ 31.5	+ 40.0	+ 39.0	
XXI	L-Lyxose	+ 14.0	+ 59.0	0.0	25*
XXII	L-Mannose	− 14.5	+ 35.0	− 13.5	31
XXIII	6-Deoxy-L-mannose	+ 8.0	+ 24.5	+ 1.5	25

Table 2 (Continued)

	Saccharide	$[\alpha]_D^{23}$			v_{rel}
		Water	Aqueous solution of molybdate at pH 5.9	pH 3.6	
XXIV	D-*glycero*-L-Mannoheptose	− 14.0	+ 0.5	− 21.5	39
XXV	L-Xylose	− 19.0	− 36.0	− 20.5	11
XXVI	L-Glucose	− 52.0	− 56.0	− 52.5	12
XXVII	6-Deoxy-L-glucose	− 30.0	− 34.0	− 31.3	
XXVIII	D-*glycero*-L-Glucoheptose	− 53.0	− 55.5	− 58.5	
XXIX	D-*glycero*-D-Guloheptose	− 20.0	+ 72.5	+ 2.3	42
XXX	D-*glycero*-D-Idoheptose	0.0	+104.4	+ 45.5	34
XXXI	D-Arabitol	− 1.3	+ 19.7	+112.0	
XXXII	1-Deoxy-D-lyxitol	− 10.6*	+222.4*	+ 69.3*	
XXXIII	1-Deoxy-L-ribitol	− 13.2*	+172.2*	− 18.0*	

* The values obtained by measuring specific rotations of optical antipodes.

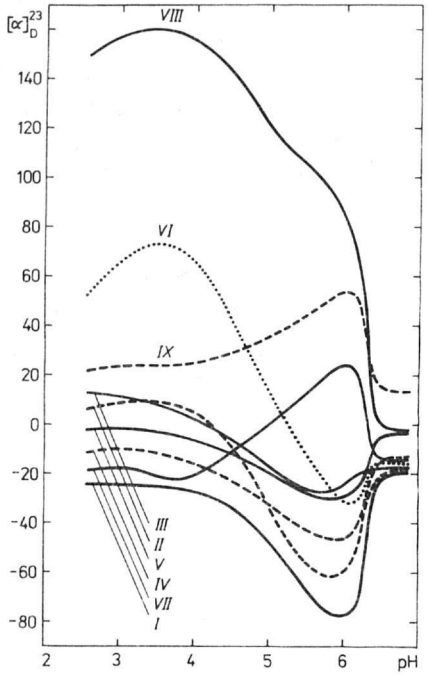

Fig. 1. Specific rotation of aldoses in aqueous solutions of molybdate as a function of pH. *I.* D-Ribose; *II.* L-talose; *III.* 6-deoxy-L-talose; *IV.* L-*glycero*-L-taloheptose; *V.* 7-deoxy-L-*glycero*-L-taloheptose; *VI.* D-*glycero*-L-taloheptose; *VII.* D-*threo*-L-talooctose; *VIII.* D-*erythro*-L-talooctose; *IX.* 5-deoxy-D-ribose.

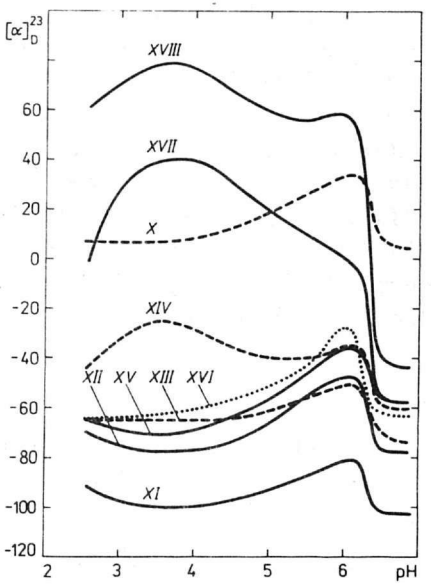

Fig. 2. Specific rotation of aldoses in aqueous solutions of molybdate as a function of pH. *X.* 5-Deoxy-D-arabinose; *XI.* D-arabinose; *XII.* L-galactose; *XIII.* 6-deoxy-L-galactose; *XIV.* L-*glycero*-L-galactoheptose; *XV.* 7-deoxy-L-*glycero*-L-galactoheptose; *XVI.* D-*glycero*-L-galactoheptose; *XVII.* D-*threo*-L-galactooctose; *XVIII.* D-*erythro*-L-galactooctose.

in molybdate solutions at pH 5.9 than in water. This points again to the equilibrium shift to the favour of their α-anomers. A shift of the equilibria in the favour of β-anomers can be observed with aldoses of the homomorphous series of L-galactose (*XII—XVIII*); their specific rotations in aqueous solutions of molybdate (pH 5.9) have more positive values. D-Ribose and D-allose behave similarly as aldoses of the homomorphous series of L-talose (specific rotations in molybdate solutions at pH 5.9 are lower than in water), with a difference however; there is a simultaneous change in the designation of the anomer regarding the change in belonging to D- and L-series, respectively. The same is true for D-arabinose and D-altrose in comparison with aldoses of the homomorphous series of L-galactose, as well as for D-*glycero*-D-guloheptose in comparison with aldoses of the

homomorphous series of L-lyxose. The change of specific rotation of 5-deoxy-D-ribose (*IX*), which can exist in the cyclic structure exclusively as a furanoid form, is in an aqueous solution of molybdate at pH 5.9 similar to that of talooctoses (Fig. 1). From this one may infer that the furanoid structures of talooctoses are preferred in the pH range of maximum complex formation. Based on the comparison of the changes of specific rotations of aldoses *X*, *XVII*, and *XVIII*, one cannot exclude eventual existence of galactooctoses around pH 5.9 in the furanoid structures (Fig. 2). Some higher aldoses (*VI—VIII*, *XIV*, *XVII*, *XVIII*), similarly as alditols [4, 6, 7, 9], exhibit extremum values of specific rotation also around pH 3.6 (Figs. 1 and 2). However, the extremum of 1-deoxyalditols *XXXII* and *XXXIII* occurs at pH 5.9 (Fig. 3). The acyclic part of the molecules of higher aldoses (substituent of aldopyranose and aldofuranose at carbon atom C-5 and C-4, respectively) apparently preserves alditol character in solutions of molybdate.

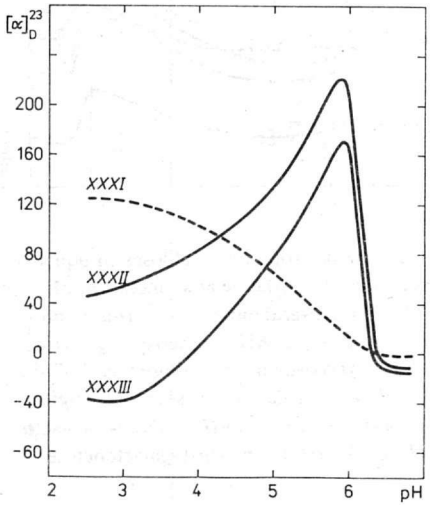

Fig. 3. Specific rotation of pentitols in aqueous solutions of molybdate as a function of pH. *XXXI*. D-Arabitol; *XXXII*. 1-deoxy-D-lyxitol; *XXXIII*. 1-deoxy-L-ribitol.

Aldoses of the homomorphous series of xylose which are distinguished by the highest conformational stability, exhibit the lowest ability to complex with molybdate ions. This has been proved by the determination of the complex formation measuring the rate of regenerated growth of the yeast *Saccharomyces cerevisiae*. The determination was based on the fact that a nonfermentable aldose due to the formation of a complex with molybdate eliminates the inhibitory effect of molybdate ions resuming thus the yeast growth. The growth rate (v_{rel}) in this case corresponds to the conditions under which a part of molybdate ions during cultivation is bound in the complex with a nonfermentable aldose. Aldoses having *cis* arrangement of the hydroxyl groups at carbon atoms C-2 and C-3 have a higher ability to complex with molybdate than aldoses having *trans* arrangement of the

hydroxyl groups. In the imagination of pyranoid forms of aldoses, the complex formation also seems to be dependent on the configuration of the C-4 hydroxyl group. The ability of aldoses to complex with molybdate as a function of the arrangement of the hydroxyl groups at carbon atoms C-2, C-3, and C-4 (in this line further indicated as *cis* or *trans*) decreases in the order *cis-cis*, *cis-trans*, *trans-cis*, *trans-trans* (Table 2). Within a homomorphous series higher aldoses also show higher ability of complex formation.

Of all the tested aldoses, the complexing is most pronounced with aldoses of the homomorphous series of talose. This is evident from both the maximum changes of specific rotations in aqueous solutions of molybdate and the measurements of the stability of complexes. This obviously holds good for conformationally unstable D-*glycero*-D-idoheptose (*XXX*), an exception among the aldoses of the homomorphous series of xylose. From this it follows that the more unstable is the conformation of aldose, the greater is its ability to complex with molybdate ions. The complex formed obviously possesses a lower content of free energy and, consequently, a higher conformation stability than the original noncomplexed aldose. If this condition is not fulfilled, the complex formation is not significant. This is characteristic of conformationally stable aldoses.

Experimental

Specific rotation of saccharides was measured with a Perkin—Elmer polarimeter, type 141. The effect of pH on specific rotation of saccharides in the presence of molybdate ions was examined in 4% aqueous solution of molybdenic acid, ammonium molybdate or sodium molybdate, or in solutions prepared by mixing the above given basic molybdate solutions to give the desired pH, at 2% concentration of saccharides (Table 2; Figs. 1—3).

Effect of pH on the rate of epimerization of D-*glucose and* D-*mannose*

D-Glucose or D-mannose (4 g) was dissolved in 0.3% aqueous solution of ammonium molybdate (20 ml), the pH of which was adjusted to a desired value with hydrochloric acid or ammonium hydroxide (Table 1). The solutions were incubated at 80°C and at time intervals aliquots (0.8 ml) were taken to determine the ratio of epimeric aldoses in the samples by measuring specific rotation and using a calibration curve constructed on the basis of the measured solutions containing different known ratios of D-glucose and D-mannose. The solutions were buffered to pH 5.9 with ammonium molybdate to enhance the accuracy of the polarimetric determination as a consequence of the changes in specific rotation of D-mannose (Table 2).

Procedure

A sample (0.8 ml) was diluted with water (3.2 ml), mixed with 8% aqueous solution of ammonium molybdate (4 ml) and used for measurement of specific rotation. The ratio of

aldoses in the epimerization mixture at a given time was ascertained by means of the calibration curve.

The rate constants of D-glucose and D-mannose epimerization were calculated from a system of two reversible reactions of the first order approaching to an equilibrium. When the starting aldose concentration is marked by a, the amount of aldose which had reacted after time t by x, and the amount which had reacted in the equilibrium state by x_r, one gets for the rate constant of aldose epimerization k

$$k = \frac{x_r}{a\,t} \ln \frac{x_r}{x_r - x}$$

The results are presented in Table 1.

Stability of pentitols under epimerization conditions

A solution of alditol (1 g, D-arabitol, ribitol or xylitol) and molybdenic acid (0.2 g) in water (10 ml) was heated at 90°C for 16 h. After deionization (Wofatit SBW in acetate form) the mixture was chromatographed on Whatman No. 1 paper in the solvent system cyclohexanol—pyridine—water (saturated by boric acid) 6:5:2. The mobilities of arabitol and ribitol related to those of xylitol (R_{xyl} 1.00) were 1.22 and 1.55, respectively. Chromatographic examination of the reaction mixtures showed only the presence of the starting alditol.

Estimation of the ability of aldoses to complex with molybdate ions

Modification of the procedure [10]

Saccharomyces cerevisiae, strain CCY [11] was grown at 28°C in a medium containing D-glucose as a sole carbon source. The starting culture was grown in a medium (25 ml) containing D-glucose (1%) and yeast nitrogen base (YNB, Difco Laboratories, USA) (0.160 mg). The pH of the medium was adjusted to pH 5.5 with hydrochloric acid, always before sterilization. Aqueous solutions of all tested aldoses as well as the solution of sodium molybdate (pH 5.5) were sterilized separately. Sterile growth medium (5 ml, containing 0.032 mg of YNB, pH 5.5) was supplied with equimolar amounts of sodium molybdate (1/3 mmole) and D-glucose (1/3 mmole), and inoculated by exponential phase yeast culture in D-glucose medium (0.2 ml). After 20 h of cultivation, nonfermentable aldose (1/3 mmole) was added to the growth medium. The growth rate was followed by measuring the optical density at 420 nm (0.5 cm cells) of the yeast suspension on a PYE UNICAM (England) spectrophotometer and checked by counting the yeast cells in a Bürker chamber.

The ability of individual aldoses to complex with molybdate ions was estimated on the basis of the rate of resumed growth of *S. cerevisiae* inhibited by molybdate ions from the relation

$$v_{rel} = \frac{100\,v}{v_0}$$

where

$$v = \frac{\Delta \log_2 x}{\Delta t}$$

is the average rate of the exponential growth of the culture in the presence of molybdate ions expressed by the above relation, x — optical density or number of cells, and v_0 — the growth rate of *S. cerevisiae* in D-glucose medium in the absence of molybdate ions.

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