Reactions of saccharides catalyzed by molybdate ions XLIV.* Isolation of alditols from the mixture of saccharides

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Alditols containing at least four vicinal hydroxyl groups are firmly bound to basic ion exchanger cyclized with hexaammonium heptamolybdate. Aldopentoses, aldohexoses, and 2-ketohexoses produce less stable complexes with molybdate ions than alditols and have a smaller retention on the ion-exchanging resin. Aldopentoses and aldohexoses are eluted from the ion exchanger with water, whilst alditols with 0.1 M-NH_4OH. Oxalic, citric, and α-hydroxycarboxylic acids afford stable complexes with molybdate ions and therefore, they have to be removed from the mixture.

Alditols containing at least four vicinal hydroxyl groups were found to form molybdate complexes with ammonium molybdate in aqueous solutions [1]. Alditols, α-hydroxycarboxylic and mainly oxalic acid inhibited the epimerization of aldoses catalyzed by molybdate ions in weakly acid aqueous solutions [2]. Inhibition of the epimerization is subject to concentration lowering of the catalytically effective molybdate ions due to formation of molybdate complexes of both alditols and the above-mentioned carboxylic acids. This paper concerns the formation and stability of molybdate complexes of alditols (D-arabinitol, ribitol, galactitol, D-glucitol, D-mannitol), α-hydroxycarboxylic acids (glycolic, tartaric, malic, citric), and oxalic acid in order to utilize stability of molybdate complexes of alditols for their isolation from the mixture of saccharides.

Suitable ion exchangers (anion, cation resins or mixtures thereof) can be employed for separation of neutral saccharides from acid and basic ones. Aldoses have a considerably higher retention on a polyethylene ion exchanger in OH form than 2-ketoses and alditols. These properties made it possible to separate 2-ketoses and alditols from aldoses [3]. Now, we found that a strongly basic resin, which cyclized with hexaammonium heptamolybdate binds firmly alditols and less firmly aldoses and 2-ketoses. Alditols form more stable molybdate complexes than aldopentoses, aldohexoses, 2-ketohexoses; on the other

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hand, some saccharides (alkylglycosides, reducing and nonreducing disaccharides) do not afford molybdate complexes with molybdate ions and can be, therefore utilized for separating their mixtures. Since the retention of aldopentoses, aldohexoses, and 2-ketohexoses in molybdate form on the resin is lower, they can be eluted with water, whilst alditols have to be liberated with 0.1 M-NH_4OH. The respective aldoses or groups of aldoses revealed various retention, as shown in Fig. 1. The greatest retention was exhibited by ribose, talose, mannose, lyxose, less retained were arabinose and galactose and the least retention was shown by glucose and xylose. Methyl glycosides of aldohexopyranoses and methyl β-D-xylopyranoside, maltose, cellobiose, lactose, trehalose, and saccharose did not form complexes with molybdate ions and consequently, they were not retained on the molybdate ion-modified resin. Ketohexoses (fructose, tagatose, sorbose) could be eluted from the afore-mentioned resin with water similarly as aldopentoses and aldohexoses. D-*Manno*-heptulose, like alditols can be eluted with 0.1 M-NH_4OH. As a consequence, higher reducing saccharides (heptoses, octoses, nonoses) can be eluted quite differently with regard to the lower aldoses and ketoses.

The above-mentioned principle of isolation of alditols through formation of molybdate complexes can be processed variously. Thus, isolation of alditols

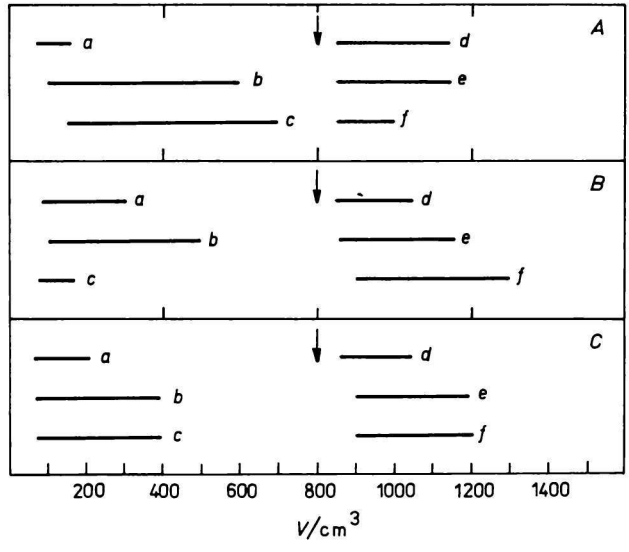

Fig. 1. Elution of saccharides with water and 0.1 M-NH_4OH (↓). Mixture *A*: D-glucose (*a*), D-galactose (*b*), D-ribose (*c*), D-glucitol (*d*), galactitol (*e*), ribitol (*f*); mixture *B*: D-xylose (*a*), D-mannose (*b*), methyl α-D-mannopyranoside (*c*), xylitol (*d*), D-mannitol (*e*), D-volemitol (*f*); mixture *C*: L-sorbose (*a*), D-fructose (*b*), D-tagatose (*c*), D-arabinitol (*d*), D-perseitol (*e*), D-*manno*-heptulose (*f*) at 100 mg each.

from the reaction mixtures after conversion of D-aldopentoses into alditols by *Rhodotorula minuta* yeast species was effected by an especially modified paper chromatography [4] on ammonium molybdate-conditioned paper and butanone—1-butanol—water as eluent. Alditols remained at the starting line and the mobility of aldoses did not differ considerably from that on the nonconditioned paper. Finally, the alditols were eluted from the paper by dilute aqueous oxalic acid.

Complexation of some alditols [5—7] and aldoses [5, 8] with molybdate ions was evidenced by potentiometric titration. The concentration change of alditols in a 4 % aqueous solution of ammonium molybdate was associated with the change of pH of the solutions (Fig. 2). The lowest pH values of solutions were achieved at a Mo(VI) : alditol amount of substance ratio 2 : 1 (Table 1). Also the specific rotations of D-arabinitol, D-glucitol, D-mannitol, and D-perseitol in 4 %

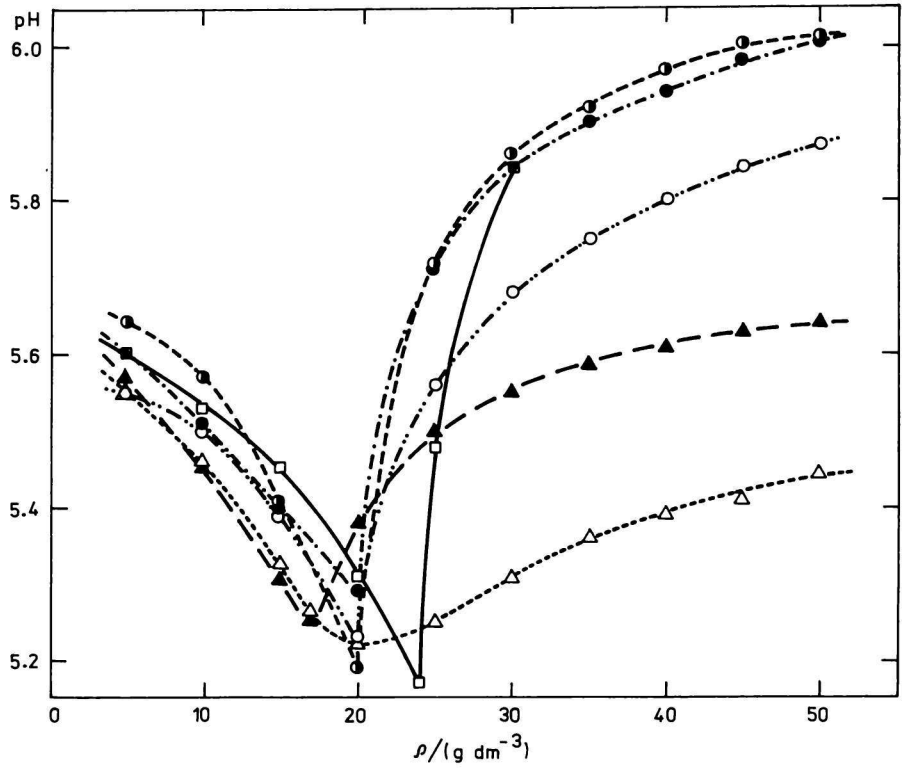

Fig. 2. Dependence of the pH value on the concentration of an alditol in 4 % aqueous ammonium molybdate.
—▲— D-Arabinitol, --△-- ribitol, —◑— galactitol, —○· D-glucitol, ● D-mannitol, —□— D-perseitol.

Table 1

The lowest pH and the highest specific rotation values as found when determining the dependence on the alditol concentration in 4 % aqueous ammonium molybdate

Alditol	Minimal pH value	Maximal $[\alpha](D, 20\ °C)/°$
	$n(Mo(VI)):n(alditol)$	
D-Arabinitol	5.25 (2:1)	+31.1 (2:1)
Ribitol	5.22 (2:1.2)	—
Galactitol	5.19 (2:1)	—
D-Glucitol	5.23 (2:1)	+22.7 (2:1)
D-Mannitol	5.29 (2:1)	+11.7 (2:1)
D-Perseitol	5.17 (2:1)	+23.5 (2:1)

aqueous ammonium molybdate were determined in relation to their concentrations (Fig. 3). The maximum rotation values of these alditols were found, similarly as the minimum pH values of their solutions, at $n(Mo(VI)):n(alditol) = 2:1$ (Table 1). The NMR study disclosed that alditols enter the binuclear molybdate complexes as tetradentate donors [1], *i.e.* also in the ratio $n(Mo(VI)):n(alditol) = 2:1$.

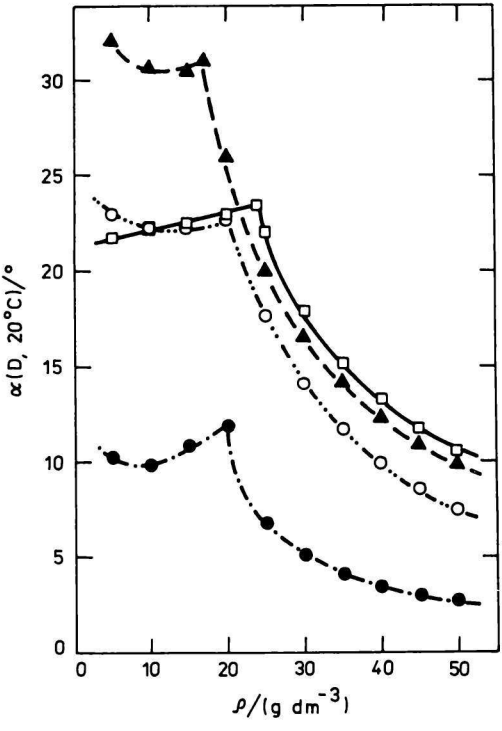

Fig. 3. Dependence of the specific rotation on the concentration of an alditol in 4 % aqueous ammonium molybdate. —▲— D-Arabinitol, —○· D-glucitol, - · ● D-mannitol, —□— D-perseitol.

Table 2

Content of alditols and carboxylic acids in the molybdate complexes at various ratios to Mo(VI) and various pH (determined by NMR spectroscopy at 25 °C)

Compound	n(Mo(VI)) : n(compound)	Content of the compound in the complex/% pH		
		2.0 ± 0.1	5.8 ± 0.1	7.5 ± 0.2
D-Arabinitol	2 : 1	100	90	—
Ribitol	2 : 1	80	70	—
Galactitol	2 : 1	100	100	40
D-Glucitol	2 : 1	100	100	20
D-Mannitol	2 : 1	100	100	30
Acid				
glycolic	1 : 2	90	—	—
	1 : 1	100	100	0
tartaric	1 : 2	—	50	—
	1 : 1	60	100	—
	2 : 1	100	100	20
malic	1 : 2	80	70	—
	1 1	100	100	10
citric	1 : 2	60	40	—
	1 : 1	80	100	30
	2 : 1	100	100	—
oxalic	1 : 2	50	50	—
	1 : 1	100	100	0

Complexation of selected alditols at a ratio n(Mo(VI)) : n(alditol) = 2 : 1 was investigated by ^{13}C NMR spectroscopy (Table 2). As found, hexitols form molybdate complexes in the pH range 2—8.5 at 25 °C; at pH 2 and 5.8, hexitols are bound completely in the complex, at pH 7.5 and 8.5, the amount of alditol in the complex was 20—40 % and about 5 %, respectively. The complexation of pentitols at pH = 5.8 proceeded worse than that of hexitols; at pH = 2, the complexation of D-arabinitol and ribitol was found to be 100 % and 80 %, respectively. Complexations of D-mannitol and ribitol dropped at pH = 5.8 and 50 °C to 90 % and 80 %, respectively and at pH = 2 the decrease of ribitol to 70 % was observed. These results showed that alditols can be retained quantitatively during their isolation from the mixture of other types of saccharides on an ion exchanger cyclized with hexaammonium heptamolybdate within pH = 2—7 and θ = 20—30 °C.

In addition to the isolation of alditols, of interest is also the separation of α-hydroxycarboxylic acids and oxalic acid. The X-ray analysis of molybdate—oxalic acid complexes disclosed that each central molybdenum atom

binds one oxalic acid molecule [9, p. 132]. Our NMR measurements proved a quantitative binding of oxalic acid in the complex at the n(Mo(VI)) : n(oxalic acid) = 1 1 and pH 2 and 5.8; at the ratio 2 : 1, only 50 % of oxalic acid was bound at the given pH values. A concurrent formation of complexes with oxalic acid or galactitol was seen when measuring the ^{13}C and ^{95}Mo NMR spectra of the mixture consisting of oxalic acid, galactitol, and Mo(VI) in a 1 1 1 amount of substance ratio. Thus, oxalic acid entered quantitatively into the complex at pH = 2, whilst galactitol remained totally free. On the other hand, 50 % of galactitol was bound in the binuclear molybdate complex at pH = 5.8 and oxalic acid remained unbound. To be completely bound, galactitol has to be present in the ratio n(Mo(VI)) : n(galactitol) = 2 : 1.

The ^{13}C NMR study of molybdate complexes of lactic acid in the pH range 3—8 showed this acid to enter the complex at a 2 : 1 ratio in favour of Mo(VI) [10], like glycolic and malic acids. As found, glycolic and lactic acids are virtually not effective inhibitors for epimerization of aldoses. The inhibitory effect of malic acid was observed at high concentrations only at n(Mo(VI)) : n(malic acid) = 1 : 6 [2]. Tartaric acid entered quantitatively into the molybdate complex at pH = 5.8 even at the ratio n(Mo(VI)) : n(tartaric acid) = 1 1, and at pH = 2 the ratio was 2 : 1. Consequently, it acted at pH 2 as a tetradentate donor in the binuclear complex like alditols.

Potentiometric and calorimetric studies revealed that complexes of amount of substance ratio Mo(VI) : citric acid 2 : 1 and 1 1 were formed in the pH range 2—7.5. At the 1 1 ratio, carboxyl and α-hydroxyl groups entered the complex. Carboxyl, α-hydroxyl, and further carboxyl groups were involved in the complexes with 2 : 1 ratio in solutions at lower pH values [11]. Results of our measurements were in line with these findings. The X-ray analyses of molybdate complexes with malic and citric acids disclosed the involvement of carboxyl, α-hydroxyl, and the second carboxyl groups [9, p. 144]. Galactitol in the mixture with citric acid and Mo(VI) in a 1 1 1 ratio and at pH = 2 did not enter the complex and due to the fact that citric acid forms molybdate complexes in the 1 1 and 2 : 1 ratios a small amount of citric acid remained free in the solution. Composition of the reaction mixture containing citric acid and galactitol at pH = 5.8 is more complex than that of the mixture containing oxalic acid and galactitol because a part of both citric acid and galactitol was simultaneously bound in the complex.

Due to concurrent formation of molybdate complexes oxalic, citric, and further α-hydroxycarboxylic acids have to be separated from the mixture (*e. g.* by passing through a basic ion resin) prior to isolation of alditols on the heptamolybdate form of the basic ion exchanger.

Experimental

Changes in the pH values of solutions (Fig. 2) were measured on a Standard PHM-82 (Radiometer, Copenhagen) apparatus; specific rotations (Fig. 3) were recorded with an automatic polarimeter, model 241 (Perkin—Elmer) and the influence of 5—50 g dm^{-3} mass concentration of the alditol was investigated in 4 % aqueous solution of ammonium molybdate.

The ^{13}C NMR chemical shifts of molybdate complexes of alditols, α-hydroxycarboxylic and oxalic acids measured with a FT spectrometer AM 300 (Bruker) operating at the frequency of 75.46 MHz, at 298 K and 323 K, pulse width corresponding to 45° flip angle, acquisition time 3 s, digital resolution 1.6 Hz per point are relative to methanol ($\delta = 50.15$). Samples of alditols and carboxylic acids (30—60 g dm^{-3}) were dissolved in deuterium oxide in a ratio n(Mo(VI)) : n(alditol) = 2 : 1, and n(Mo(VI)) : n(carboxylic acid) = 2 : 1, 1 1, and 1 : 2. The pH values of solution were adjusted to 2.0 ± 0.1, 5.8 ± 0.1, and 7.5 ± 0.2 by addition of aqueous solutions of HCl or NaOH. Content of the substance free or bound to the molybdate complex was estimated as follows: with alditols, mainly from intensities of carbon atoms with primary or possibly also secondary hydroxyl groups; with carboxylic acids, from α-carbons bearing the hydroxyl group (Table 2).

The ^{95}Mo NMR spectra of the mixture of oxalic or citric acids, galactitol, and Mo(VI) were recorded with the same spectrometer at 298 K, 25000 Hz spectral width, pulse width corresponding to 45° flip angle, acquisition time 0.04 s. Digital resolution 12.2 Hz per point was achieved by zero filling of 2 K data point to 4 K. Chemical shift data are relative to the external reference (aqueous solution of 2 M-Na_2MoO_4, $\delta = 0$). To avoid acoustic probe ringing 150 μs pre-acquisition delay was applied. The ^{95}Mo NMR chemical shifts and halfwidths of signals (Hz) for ammonium molybdate were 6 (800), at pH = 2, 35 (360) and − 2 (160) at pH = 5.8; for galactitol in the molybdate complex 38 (450) and 18 (280) at pH = 2, 32 (260) and 26 (360) at pH = 5.8; for oxalic acid in the molybdate complex − 35 (240) at pH = 2, 3 (170) at pH = 5.8.

Isolation of alditols from the mixture of saccharides

Alditols (erythritol, xylitol, ribitol, D-arabinitol, D-mannitol, D-glucitol, galactitol, perseitol, volemitol) were separated from aldoses (D-ribose, D-arabinose, D-lyxose, D-xylose, D-allose, D-altrose, D-mannose, D-glucose, D-talose, D-galactose), 2-ketohexoses (D-fructose, D-tagatose, L-sorbose), methyl glycosides (β-D-xylopyranoside, α-D-glucopyranoside, α-D-galactopyranoside), disaccharides (maltose, cellobiose, lactose, trehalose, saccharose) on a column packed with a strong basic ion exchanger in the molybdate form.

The column (55 cm × 1.5 cm) was packed with Ostion AT 0809 (Spolek pro chemickou a hutní výrobu, Ústí nad Labem, CSFR; equivalent to IRA 400) in OH form and cyclized with ammonium molybdate (5 %, 150 cm^3). The maximal retention capacity for galactitol, D-mannitol or glucitol is 1.5—1.8 g.

The aqueous solution of the mixture of saccharides (900 mg, containing the single saccharides in 50—150 mg amounts) was filled in the column and eluted with water (30—40 $cm^3 h^{-1}$ flow rate). The first effluent (50—800 cm^3) contained the above-mentioned saccharides, but no alditols, which were afterwards eluted with 0.1 M-NH_4OH (200—300 cm^3; Fig. 1).

The elution of saccharides was monitored by paper chromatography on Whatman No. 1 paper in the solvent systems 1-butanol—ethanol—water ($\varphi_r = 5:1:4$), or butanone—1-butanol—water ($\varphi_r = 16:2:1$); detection with anilinium hydrogen phthalate, diphenylamine or silver nitrate.

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