Reactions of saccharides catalyzed by molybdate ions XXXIV.* Molybdate complexes of furanoid structures of aldoses

V. BÍLIK, J. ALFÖLDI, and E. SOÓKYOVÁ

Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, CS-842 38 Bratislava

Received 4 March 1983

In aqueous solutions D-erythrose, D-threose, 5-deoxy-L-ribose, and 5-deoxy-L-arabinose form complexes with ammonium molybdate in the form of their α-anomers. ^{13}C NMR, ^{1}H NMR, and optical rotation data pointed out that molybdate complexes of D-erythrose and 5-deoxy-L-ribose involve interaction with the hydroxyl groups at carbon atoms C-1, C-2, and C-3, while those of D-threose and 5-deoxy-L-arabinose involve interaction with C-1 and C-3 hydroxyl groups. 5-*O*-α-D-Galactopyranosyl-D-arabinose does not complex with molybdate ions and is not epimerized under catalytic action of molybdate ions.

В водных растворах D-эритроза, D-треоза, 5-дезокси-L-рибоза и 5-дезокси-L-арабиноза в форме α-аномеров образуют комплексы с молибдатом аммония. Данные ^{13}C ЯМР, ^{1}H ЯМР и ДОВ показали, что молибдатные комплексы L-эритрозы и 5-дезокси-L-рибозы включают взаимодействие с гидроксильными группами на атомах углерода C-1, C-2 и C-3, в то время как комплексы D-треозы и 5-дезокси-L-арабинозы включают взаимодействие с гидроксильными группами на C-1 и C-3. 5-*O*-α-D-Галактопиранозил-D-арабиноза не образует комплекса с молибдат-ионами и не эпимеризуется под их каталитическим действием.

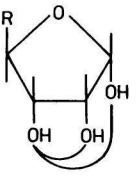
In our previous papers we have demonstrated that aldoses present in acidic aqueous solutions containing a catalytic amount of molybdate ions epimerize under formation of an equilibrium mixture of $C_{(2)}$-epimeric aldoses. This selective epimerization reaction is conditioned by the formation of active complexes between aldoses and molybdate. The existence of such complexes was established by electrophoresis [1], circular dichroism [2, 3], biologically [4], polarimetrically [5]

* For Part XXXIII see *Chem. Zvesti 38*, 491 (1984).

Table 1

^{13}C NMR chemical shifts (δ_r/ppm) of aldoses and their molybdate complexes

Atom	D-Erythrose				5-deoxy-L-Ribose*			
	I		*Ia*		*II*		*IIa*	
	α	β	α′	$\{\Delta\delta_r\}$	α	β	α′	$\{\Delta\delta_r\}$
C-1	96.7	102.3	95.2	− 1.5	97.0	102.2	95.3	− 1.7
C-2	72.3	77.4	94.0	+21.7	76.7	76.7	92.5	+15.8
C-3	70.4	71.5	81.6	+11.2	71.7	76.1	82.2	+10.5
C-4	72.7	72.3	72.5	− 0.2	79.5	79.4	81.4	+ 1.9
C-5			—	—	19.2	20.4	17.5	− 1.7
C-1′								
C-2′								
C-3′								
C-4′								
C-5′								
C-6′								
Ratio of anomers								
α:β	1 3				1:2			
α:α′	1:10				1:3			



*Antipodes measured.

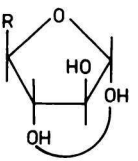
$\Delta\delta_r = \delta_{r,C(non\text{-}complex)} - \delta_{r,C(complex)}$.

and by ^1H NMR and ^{13}C NMR spectrometry [6, 7]. NMR data showed that aldopentoses, aldohexoses, and aldoheptoses are present in the molybdate complexes in the pyranoid structures [6]. Well resolved spectra of aldoses of the homomorphous series of mannose pointed to the fact that the complex formation involves interaction of the hydroxyl groups at carbon atoms C-1, C-2, and C-3 [6, 7]. Aldoses of the homomorphous series of talose also form complexes with molybdate as trident donors, however, with the hydroxyl groups at carbon atoms C-2, C-3, and C-4 [7]. In this paper we investigated molybdate complexes of aldoses which exist in solutions in cyclic furanoid structures only.

^1H NMR and ^{13}C NMR spectra and optical rotations of D-erythrose (*I*), 5-deoxy-L-ribose (*II*), D-threose (*III*), 5-deoxy-L-arabinose (*IV*), and 5-*O*-α-D-galactopyranosyl-D-arabinose (*V*) were measured in water and aqueous solution of ammonium molybdate. ^{13}C NMR signals of the tested aldoses (Table 1)

Table 1 (Continued)

D-Threose				5-deoxy-L-Arabinose*				5-*O*-α-D-Galacto-pyranosyl-D-arabinose	
III		*IIIa*		*IV*		*IVa*		*V*	
α	β	α′	{Δδ$_r$}	α	β	α′	{Δδ$_r$}	α	β
103.2	97.7	99.6	−3.6	101.8	95.8	99.8	− 2.0	102.5	96.7
81.8	77.3	85.9	+4.1	77.9	80.1	80.9	+ 3.0	82.5	77.3
76.2	75.9	84.8	+8.6	79.7	79.7	91.0	+11.3	77.3	75.5
74.1	71.6	78.3	+4.2	83.2	82.0	80.9	+ 2.3	72.1	70.6
—	—	—	—	18.9	20.3	16.7	− 2.2	69.5	68.2
								99.8	
								69.5	
								70.6	
								70.5	
								72.2	
								62.4	



R				
H	*I*	*Ia*	*III*	*IIIa*
CH_3	*II*	*IIa*	*IV*	*IVa*
CH_2-*O*-α-D-galactopyranosyl			*V*	

1.5:1	1.5:1	1.5:1
2:1	3:1	—

were assigned by using the rules valid for chemical shifts [8] and, in the case of D-erythrose and D-threose, also according to the literature [9]. The saccharides *I*, *II*, *III*, and *IV* exhibit considerably different specific rotations in water and in 4 % aqueous solution of ammonium molybdate (Table 2). Compound *V* is an exception. From the ^{13}C NMR spectra it follows that in aqueous solutions *I* and *II* occur preferably in the β-anomeric form, and *III*, *IV*, and *V* in the α-anomeric form (Table 1, α:β). The differences in the values of specific rotation of D-erythrose in water obtained at various concentrations (Table 2) are possibly a consequence of the presence of the sugar dimer. Specific rotation of D-threose did not show differences in the same concentration range. ^{13}C NMR spectra of D-erythrose contain more signals than those of D-threose, and the additional signals can also be ascribed to the presence of D-erythrose dimers in aqueous solutions. Comparison of the values of specific rotation of *I*, *II*, *III*, and *IV* in water and in the molybdate

Table 2

Specific rotation of aldoses in water and in 4 % aqueous solution of ammonium molybdate

	Saccharide	Water	Ammonium molybdate solution
		α(D, 23 °C)/°	
I	D-Erythrose	+ 9 → −32	+ 7
		+ 9 → −29[a]	
		+ 9 → −24[b]	
III	D-Threose	−11 → −12	+ 9
		−11 → −12[c]	
IV	5-deoxy-L-Arabinose	− 6	−38
II	5-deoxy-L-Ribose	−12	−52
V	5-*O*-α-D-Galactopyranosyl-D-arabinose	+75.5	+75.5

Saccharide mass fractions: *a*) 4.8 %, *b*) 9.1 %, *c*) 9.3 % and in all other cases 1—2 %.

solution indicates a shift in favour of α-anomers in the presence of molybdate (Table 2). Based on the ^{13}C NMR data one may infer that the extent of structural adjustment of aldose molecule to form the molybdate complex in the form of α-anomer is conditioned by the magnitude of the substituent (R) at carbon atom C-4 of the furanoid structure of aldose (Table 1, $\alpha:\alpha'$). Under the same reaction conditions about 30 % of D-threose and 20 % of 5-deoxy-L-arabinose are bound in the molybdate complex. 5-*O*-α-D-Galactopyranosyl-D-arabinose does not seem to interact with molybdate ions. This is apparently a result of the fact that the bulky α-galactopyranosyl substituent prevents conformational changes of the furanoid structure of D-arabinose moiety, prerequisite for the rise of the molybdate complex.

The largest differences in the chemical shifts of carbon atoms (Table 1, $\Delta\delta_r$) in the absence and in the presence of molybdate were observed in the case of *I* and *II* at carbon atoms C-2 and C-3 and in the case of *III* and *IV* at carbon atom C-3. ^{13}C NMR spectrometry of molybdate complexes of pyranoid structures of aldohexoses and aldopentoses [7] showed that the greatest changes of chemical shifts occurred with those carbon atoms which carry the hydroxyl groups entering the complexes. The differences in the chemical shifts of carbon atoms C-1 are not very much pronounced, however, the formation of α-anomers of *I*, *II*, *III*, and *IV* in the complexes serves as an evidence that the hemiacetal group is also involved in the complex formation.

^1H NMR spectra of *I*, *II*, *III*, and *IV* measured in aqueous solutions contain besides the signals of anomeric protons also signals of higher orders which are not

useful for the ascertainment of aldose conformation. Anomeric signals of D-erythrose and D-threose were assigned according to *Angyal* and *Wheen* [10]. The interpretation of the spectra of aldotetroses measured in the presence of molybdate ions is also restricted to the anomeric signals. The signal of D-erythrose α-anomer lies at $\delta_r = 5.23$ ppm ($J_{1,2} = 5.15$ Hz) and at $\delta_r = 5.4$ ppm (singlet) in the complex. Analogous δ_r values for D-threose are 5.21 ppm ($J_{1,2} = 1.0$ Hz) and 5.22 ppm (singlet) and for 5-deoxy-L-arabinose 5.23 ppm ($J_{1,2} = 1.3$ Hz) and 5.33 ppm (singlet). The differences in the interaction constants before and after complexation demonstrate the change in the population of conformers in the furanoid structures.

Based on the above results one may conclude that *I* and *II* enter the molybdate complex as α-anomers and trident donors through the hydroxyl groups at carbon atoms C-1, C-2, and C-3. Aldoses *III* and *IV* also enter the complex in the α-anomeric form but as bident donors through the hydroxyl groups at carbon atoms C-1 and C-3. Saccharide *V* does not form a molybdate complex. This fact can be ascribed to conformational rigidity of the arabinofuranosyl moiety due to the bulky substituent at carbon atom C-4.

The different behaviour of *I*, *II*, *III*, and *IV* in comparison to *V* is also reflected in their molybdate-catalyzed epimerization. The epimerization of any aldotetrose leads to an equilibrium mixture of threose and erythrose in the ratio 4:3 [11]. The epimerization of 5-deoxy-L-arabinose and 5-deoxy-L-ribose gives an equilibrium mixture of both aldoses in the ratio 3:1 [12]. Under similar conditions 5-*O*-α-D-galactopyranosyl-D-arabinose does not undergo any changes while under more violent reaction conditions only the cleavage of the α-glycosidic linkage in nonepimerized disaccharide occurs. 5-*O*-α-D-Galactopyranosyl-D-arabinose was prepared by oxidative degradation of melibiose 4-nitrophenylhydrazone. Finally, we have to note that 1→4-linked disaccharides such as lactose, maltose, cellobiose, epilactose, and epimaltose also do not epimerize [13]. In the case of melibiose as an 1→6-linked dissaccharide, the epimerization reaction proceeds and leads to epimelibiose which can be obtained in this way in 15—20 % yield [14].

Experimental

^{13}C NMR spectra of saccharides were measured on a FT-NMR spectrometer Jeol-FX-60 in D_2O solutions and in D_2O solutions of ammonium molybdate (100 mg of saccharide and 200 mg of ammonium molybdate in D_2O) at 25 °C using methanol as an internal standard in a proton decoupling mode. Chemical shifts are referred to that of TMS (δ_r(TMS) = 50.1 ppm). Other parameters used were: pulse interval 1 s, pulse width 4 μs (flip angle 5°), frequency width 4000 Hz and 8 K real data points. The results are summarized in Table 1. ^1H NMR spectra were measured on a FT-NMR spectrometer Jeol-FX-100 in D_2O and D_2O solutions of ammonium molybdate at 25 °C using DSS as an internal standard.

Literature procedures were used for preparation of D-erythrose [15], D-threose, 5-deoxy-L-arabinose [12], and 5-deoxy-L-ribose [12]. The aldoses were characterized by specific rotations in water and 4 % aqueous solution of ammonium molybdate measured with a Perkin—Elmer polarimeter, type 141 (Table 2). Purity of aldoses was examined by chromatography in ethyl acetate—acetic acid—saturated solution of boric acid (volume ratio = 9:1:1) on Whatman No. 1 paper for 17—20 h at 20—24 °C. The mobilities of saccharides relative to D-arabinose (1.0) were 1.5 for erythrose, 0.8 for threose, 1.7 for 5-deoxy-arabinose, and 1.9 for 5-deoxy-ribose.

Preparation of 5-O-α-D-galactopyranosyl-D-arabinose and melibiose 4-nitrophenylhydrazone

Melibiose (5 g) was dissolved in water (5 cm^3), mixed with 4-nitrophenylhydrazine (2.5 g), methanol (30 cm^3) and heated at 60 °C for 7 h. In the 3rd and the 5th hour the mixture was supplied with 30 cm^3 portions of methanol and finally left to stand at room temperature for 20 h. Crystalline melibiose 4-nitrophenylhydrazone (6 g, 77 % yield) was filtered off, washed with methanol (α(D, 23 °C, 1 h, 3 g dm^{-3}, pyridine) = +81°) and subjected to oxidative degradation.

Melibiose 4-nitrophenylhydrazone recrystallized from a mixture methanol—water (volume ratio = 1:1) showed m.p. = 148—150 °C (Kofler) and α(D, 23 °C, 1 h, 7 g dm^{-3}, pyridine) = +81.5 °. For $C_{18}H_{27}N_3O_{12} \cdot 2H_2O$ w_i(calculated) = 42.2 % C, 6.08 % H, 8.18 % N; w_i(found) = 42.07 % C, 6.21 % H, 8.14 % N.

Oxidative degradation of melibiose 4-nitrophenylhydrazone

A mixture of melibiose 4-nitrophenylhydrazone (5 g), water (50 cm^3), methanol (30 cm^3), 26 % aqueous solution of ammonia (3 cm^3), and 30 % H_2O_2 (5 cm^3) was stirred at room temperature for 8 h. After addition of further 30 % H_2O_2 (5 cm^3) the mixture was left to stand at room temperature for another 20 h. Finally the mixture was diluted with water (100 cm^3), purified with activated charcoal and then treated with 5 % Pd/C for 24 h. The resulting solution was concentrated and fractionated on a column (2.5 cm × 100 cm) of Dowex 50 W ion exchanger (X-8, Ba cycle, 75—150 μm), eluted with water at a rate of 15 cm^3 h^{-1}. Fraction *1* (elution volume 200—230 cm^3) contained melibiose, fraction *2* (elution volume 230—260 cm^3) 5-*O*-α-D-galactopyranosyl-D-arabinose contaminated with melibiose and D-galactose, fraction *3* (elution volume 260—350 cm^3) D-galactose and D-arabinose. Fraction *2* was rechromatographed on Whatman No. 1 paper (5 sheets) to give chromatographically homogeneous 5-*O*-α-D-galactopyranosyl-D-arabinose (730 mg, 22 % yield). The compound dried over P_2O_5 is amorphous and its specific rotation is given in Table 2.

5-*O*-α-D-Galactopyranosyl-D-arabinose (50 mg) is quantitatively decomposed to D-galactose and D-arabinose during hydrolysis in 1 M-hydrochloric acid (1 cm^3) (90 °C, 1 h).

A heating of 5-*O*-α-D-galactopyranosyl-D-arabinose (50 mg) in 2 % aqueous solution of molybdic acid (1 cm^3) at 90 °C for 3 h does not cause any changes. A prolonged heating (8 h) results in partial hydrolysis of the disaccharide and in the epimerization of the liberated aldoses.

Purity of saccharides and composition of the reaction mixtures was examined by paper chromatography in 1-butanol—ethanol—water (volume ratio = 5:1:4; 20 h at θ = 20—24 °C). Mobility of saccharides to that of galactose (1.0) is 0.4 for melibiose, 0.7 for 5-*O*-α-D-galactopyranosyl-D-arabinose, 1.2 for glucose, 1.3 for arabinose, 2.1 for melibiose 4-nitrophenylhydrazone, and 3.3 for 4-nitrophenylhydrazone. Chromatography in the solvent system ethyl acetate—acetic acid—saturated aqueous solution of boric acid was also employed to follow the epimerization of the disaccharide.

References

1. Weigel, H., *Advan. Carbohyd. Chem. 18*, 61 (1963).
2. Voelter, W., Kuhfittig, G., Oster, O., and Bayer, E., *Chem. Ber. 104*, 1234 (1971).
3. Voelter, W., Kuhfittig, G., Schneider, G., and Bayer, E., *Justus Liebigs Ann. Chem. 734*, 126 (1970).
4. Zemek, J., Bílik, V., and Zákutná, L., *Folia Microbiol. 20*, 467 (1975).
5. Bílik, V., Petruš, L., and Zemek, J., *Chem. Zvesti 32*, 242 (1978).
6. Alföldi, J., Petruš, L., and Bílik, V., *Collect. Czechoslov. Chem. Commun. 43*, 1476 (1978).
7. Alföldi, J., Bílik, V., and Petruš, L., *Collect. Czechoslov. Chem. Commun. 45*, 123 (1980).
8. Shashkov, A. S. and Chizov, O. S., *Bioorg. Khim. 2*, 437 (1976).
9. Serianni, A. S., Clark, E. L., and Barker, R., *Carbohyd. Res. 72*, 79 (1979).
10. Angyal, S. J. and Wheen, R. G., *Aust. J. Chem. 33*, 1001 (1980).
11. Bílik, V. and Stankovič, Ľ., *Chem. Zvesti 27*, 544 (1973).
12. Bílik, V., Petruš, L., Stankovič, Ľ., and Linek, K., *Chem. Zvesti 32*, 372 (1978).
13. Bílik, V., Jurčová, E., and Sutoris, V., *Chem. Zvesti 32*, 252 (1978).
14. Bílik, V., Jurčová, E., and Sutoris, V., *Listy cukrovarnické 94*, 88 (1978).
15. Perlin, A. S. and Brice, C., *Can. J. Chem. 33*, 1216 (1955).

Translated by P. Biely