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Acyclic molybdate complexes of aldoses in the *arabino* and *xylo* series and their application to the determination of the proportion of acyclic forms in aqueous solution †

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ABSTRACT

Aldoses of the *arabino* and *xylo* series with molybdate ions in aqueous acidic medium, form tetradentate acyclic complexes which are much weaker than those of the related alditols. The formation constants (K_f) of these complexes were obtained by two independent methods, potentiometry and UV spectrophotometry, which gave values in good agreement. The structures of the complexes with aldoses were shown by ^{13}C NMR spectroscopy to involve the ligand in its acyclic form. A study of the complexes of carbohydrate derivatives proved that the magnitude of K_f was mainly dependent on the configuration of the site of chelation. The lower stabilities of the aldose complexes were due to the endergonic opening of the pyranose heterocycle in the first step of the complex formation. An application to the determination of the equilibrium constant for the ring-opening reaction of aldopyranoses is described. It ultimately allowed the calculation of the proportion of acyclic forms (aldehyde and hydrate) in aqueous solutions of the aldoses in the *arabino* and *xylo* series.

INTRODUCTION

Many carbohydrates form dinuclear anionic molybdate complexes in aqueous acidic solution, a characteristic which was applied to the separation of mixtures of sugars by paper electrophoresis[1]. Bílik's studies[2–6] have renewed the interest in the chemistry of such complexes by showing that Mo(VI) catalyzes the C-2 epimerization of aldoses, a reaction useful for the synthesis of rare sugars[7,8] which was subsequently explained by involvement of a C-1 ⇄ C-2 transposition[9]. Several papers[9–11] have pointed however out that mechanistic interpretation of this reaction is hampered by the lack of quantitative data on the formation equilibria of aldose complexes.

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A few structures have been determined by X-ray crystallography[12–14]. Alditols form stable acyclic complexes[15,16], whereas data obtained by ^{13}C, ^{1}H, and ^{95}Mo NMR studies demonstrated that aldoses are complexed in their furanose form (in the case of the *lyxo*[17] series), in their pyranose form (*ribo*[18,19] series), or in their acyclic form (*xylo*[20] and *arabino*[18,19] series).

The formation constants of the molybdate complexes were calculated from potentiometric measurements[16,21]. The finding that the sites of chelation were similar in alditols and aldoses of the *arabino* and *xylo* series raised the question of the origin of the large difference between their stabilities (alditol species are much more stable). The present work was initiated in order to relate the energetics of complex formation to the structures of the ligands (Fig. 1). The molybdate complexes of carbohydrate derivatives (diethyl dithioacetal of D-arabinose and 1-deoxy-1-methylamino-D-glucitol) were studied with regard to their structures and stabilities. The demonstration that the main reason for the differences of stability was the open chain formation from the cyclic aldoses led to a new method for the estimation of the proportion of acyclic forms in aqueous solutions of free aldoses.

RESULTS

Determination of the formation constants by the potentiometric method.—At pH > 4, all the sugars and polyols H_nL (aldoses and alditols with $n \geq 4$) react with molybdate according to the general equation:

$$2\,MoO_4^{2-} + H_nL + 2\,H^+ \rightleftarrows \left[(MoO_2)_2O(H_{n-4}L)\right]^{2-} + 3\,H_2O \qquad (1)$$

The formation constant of a molybdate complex is the equilibrium constant of reaction (*1*) and can be determined by the potentiometric method described in

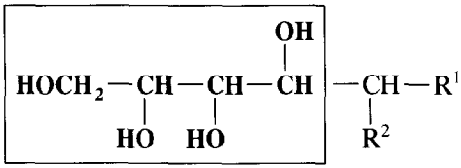
TABLE I

Formation constants [a] K_f of the molybdate complexes of carbohydrates

Sugar	log K_f [b]	log K_f [c]
D-Arabinose	13.60 [d]	13.20
D-Arabinitol	16.35	16.35
D-Arabinose diethyl dithioacetal	16.65	ND
D-Galactose	14.25 [d]	14.25
Galactitol	17.30	17.30
D-Xylose	13.70 [d]	13.60
Xylitol	16.25	16.00
D-Glucose	13.20 [d]	13.30
2-Deoxy-D-*arabino*-hexose	13.40 [d]	13.60
D-Glucitol	16.60	16.75
D-Mannitol	16.70	16.90
1-Deoxy-1-methylamino-D-glucitol	NA	16.85

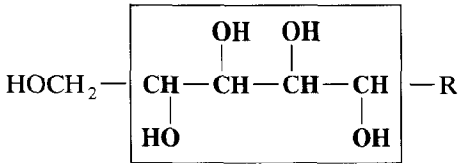
[a] Equilibrium constants K_{212} for the complexes $(2,1,2)^{2-}$. [b] By potentiometry, accuracy ±0.10. Data for alditols are taken from ref 16. [c] By spectrophotometry, accuracy ±0.10. [d] Low accuracy, ±0.30. ND: not determined. NA: not applicable.

Arabino site of chelation



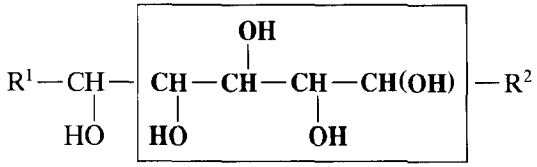
D-Arabinose, $R^1 = R^2 = OH$; D-Arabinitol, $R^1 = H$, $R^2 = OH$;
D-Arabinose diethyl dithioacetal, $R^1 = R^2 = S\text{-}C_2H_5$;
2-Deoxy-D-*arabino*-hexose, $R^1 = H$, $R^2 = CH(OH)_2$;
D-Mannitol, $R^1 = OH$, $R^2 = CH_2OH$;
D-Glucitol, $R^1 = CH_2OH$, $R^2 = OH$;
1-Deoxy-1-methylamino-D-glucitol, $R^1 = CH_2\text{–}NH\text{–}CH_3$, $R^2 = OH$.

Galacto site of chelation



D-Galactose, $R = CH(OH)_2$; Galactitol, $R = CH_2OH$.

Xylo site of chelation



D-Xylose, $R^1 = H$, $R^2 = OH$; Xylitol, $R^1 = R^2 = H$;
D-Glucose, $R^1 = CH_2OH$, $R^2 = OH$; D-Glucitol, $R^1 = CH_2OH$, $R^2 = H$.

Fig. 1. Sites of chelation in molybdate complexes, marked in **bold characters**. Formulae of aldoses are shown in acyclic hydrated form.

previous papers[21]. Because of its stoichiometry, the complex is symbolized as (2,1,2) and the formation constant is usually denoted as K_{212} (Table I). In energetic diagrams, K_{212} is abbreviated as K_f for alditols and K_f' for aldoses.

When stable molybdate complexes are formed (for alditols[16]: $14.6 < \log K_{212} < 17.6$ and sugars of the *lyxo* series[17,21]: $14.5 < \log K_{212} < 15.0$), a linear relationship is observed between the pH of half-titrated solutions of molybdate ($pH_{1/2}$) and $\log c_L$ (c_L and c_M are the concentrations of sugar and molybdate respectively):

$$pH_{1/2} = 1/2(\log K_{212} + \log c_M + \log c_L)$$

In the case of aldoses, which form weak molybdate complexes (sugars of the *arabino* and *xylo* series: $13.2 < \log K_{212} < 14.3$), the complexation of the molyb-

$$2\left(MoO_4^{2-} + C^{2-} + 2H^+ \underset{K_{112}}{\overset{(2)}{\rightleftharpoons}} [MoO_3C]^{2-} + H_2O\right)$$

$$(3)\left\uparrow\downarrow {}^{+H_nL}_{-2C^{2-},\,-2H^+}\right.$$

$$2\,MoO_4^{2-} + H_nL + 2H^+ \underset{K_{212}}{\overset{(1)}{\rightleftharpoons}} [(MoO_2)_2O(H_{n-4}L)]^{2-} + 3H_2O$$

Scheme 1. Competitive complexation of molybdate by chloranilate C^{2-} and a carbohydrate H_nL.

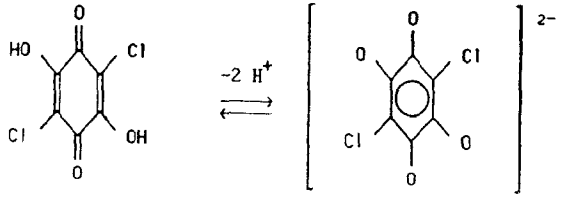
date anion competes with its polymerization to an heptamolybdate[22] which occurs at pH ≤ 5:

$$7\,MoO_4^{2-} + 8H^+ \rightleftarrows [Mo_7O_{24}]^{6-} + 4H_2O$$

This interference is revealed by a curvature of the plots of $pH_{1/2}$ vs. log c_L which indicates that the potentiometric method has a lower accuracy in the case of complexes of such aldoses. Thus, we sought another method for the determination of the formation constants that would not present the same drawback. A spectrophotometric method, based on the competitive complexation of molybdate by carbohydrates and a coloured sacrificial ligand, is described herafter and is illustrated in Scheme 1.

Determination of the formation constants by the spectrophotometric method.— Molybdate ions form a 1 : 1 complex[23,24] with the ion of 2,5-dichloro-3,6-dihydroxy-2,5-cyclohexadiene-1,4-dione sodium salt (chloranilate C^{2-}, Fig. 2). The complex absorbs strongly in the visible and the UV (λ_{max} = 340 nm). If the concentrations and pH are adjusted so that the carbohydrate complexes are more stable than the chloranilate complex, the formation of the molybdate–carbohydrate complexes, (eq *1*), may result in the dissociation of the chloranilate complex $[MoO_3C]^{2-}$ [equilibrium (2) is displaced backwards]. The overall reaction is represented by eq *3* in Scheme 1.

Accordingly, when a sugar or polyol is added to a solution of the complex $[MoO_3C]^{2-}$, the UV spectrum gradually changed (Fig. 3), indicating the dissociation of the (1,1,2) molybdate–chloranilate complex (curve 3a) to form the colour-



a b

Fig. 2. Formulae of chloranilic acid (a) and chloranilate ion (b).

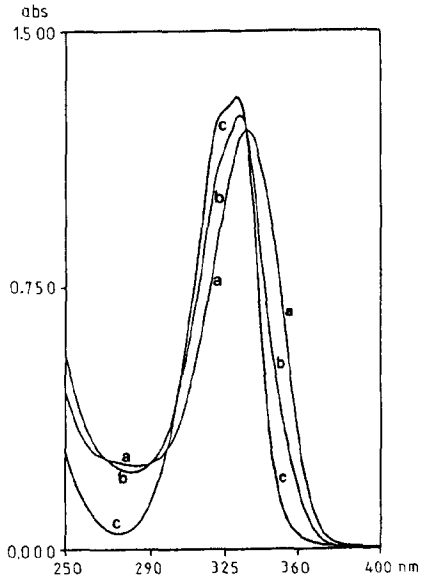


Fig. 3. Variations of the UV spectrum of the chloranilate–molybdate complex (Mo:C ≈ 2, $c_M = 10^{-3}$ M, path length $l = 1$ mm) vs. D-mannitol concentration c_L. (a) $c_L = 0$, pH 4.6; (b) $c_L = 10^{-2}$ M, pH 4.6; (c) $c_L = 10^{-2}$ M, pH 10 (complete dissociation of all complexes).

less (2,1,2) molybdate–carbohydrate complex and free chloranilate ions (curve 3c). When the reaction was not complete, intermediate spectra were obtained (curve 3b). The observation of the isosbestic point at 335 nm shows that no other species were involved. The larger variations of absorbance occurred at 347 nm (decrease) and were used for quantitative work.

The optimal conditions for the formation of the molybdate–chloranilate complex were obtained in the following way. A 2:1 molybdate–chloranilate ratio was chosen in order to increase the initial proportion of complex, since the molybdate–carbohydrate ratio is 2 in the carbohydrate complex. A comparison of the curves of variation of absorbance vs. pH for the molybdate–chloranilate complex, alone or in the presence of a model alditol (D-mannitol was used), showed that the maximum decrease of absorbance was observed at about pH 4.7. As this value is close to the pK_a of acetic acid, we eventually decided to use a 1:1 acetate buffer of pH 4.6.

Under our experimental conditions, we measured the formation constant of the molybdate–chloranilate complex: $\log K_{112} = 13.58$. This result agrees with literature data as determined by spectrophotometry[24,25] under slightly different conditions.

As only the species containing chloranilate is the only one which absorbs at $\lambda \simeq 340$ nm, the concentrations in the solution at equilibrium are related to the values of A_1 (absorbance of the chloranilate complex), A_2 (absorbance of uncomplexed chloranilate ion), and A (absorbance of the solution at equilibrium).

The total concentration of chloranilate species c_T is:

$$c_T = [C^{2-}] + [MoO_3C^{2-}]$$

The proportion α of uncomplexed ion C^{2-} is calculated from the absorbance measurements:

$$[C^{2-}] = \alpha c_T \text{ and } [MoO_3C^{2-}] = (1-\alpha)c_T$$

$$\alpha = (A_1 - A)/(A_1 - A_2)$$

The concentration of free molybdate ion is obtained, using the equilibrium constant of reaction (*2*):

$$K_{112} = \frac{[MoO_3C^{2-}]}{[MoO_4^{2-}][C^{2-}][H^+]^2}$$

$$[MoO_4^{2-}] = (1-\alpha)/\alpha K_{112}[H^+]^2$$

The concentration of the molybdate–carbohydrate complex stems from a mass balance equation, where c_M is the analytical concentration of molybdate:

$$[(MoO_2)_2O(H_{n-4}L)]^{2-} = (c_M - [MoO_3C^{2-}] - [MoO_4^{2-}])/2$$

The equilibrium concentration of the ligand is also obtained using a mass balance equation, where c_L is the analytical concentration of carbohydrate:

$$[H_nL] = c_L - [(MoO_2)_2O(H_{n-4}L)]^{2-}$$

A computer treatment, using a laboratory-produced Basic routine programme, gave the values of the formation constants K_{212} of the molybdate complexes:

$$K_{212} = \frac{[(MoO_2)_2O(H_{n-4}L)]^{2-}}{[MoO_4^{2-}]^2[H_nL][H^+]^2}$$

Interestingly, both methods of determination of formation constants of molybdate complexes yield comparable values (Table I), although based on different principles. However, for thermodynamic calculations, we favoured the values obtained by the spectrophotometric method, which are more suitable for the aldoses complexes of lower stabilities. It appears clearly that aldoses of the *arabino* and *xylo* series definitely form molybdate complexes less stable than those of the *lyxo*[21] and *ribo* ($14.3 < \log K_{212} < 14.6$) series.

Structures of the sites of chelation by ^{13}C NMR spectroscopy.—The study was limited to aldoses that are complexed in hydrated acyclic form, according to Bílik's work[18,20]. They belong to the *arabino* series (D-arabinose, D-galactose, and 2-deoxy-D-*arabino*-hexose) and *xylo* series (D-xylose and D-glucose). The site of chelation of 2-deoxy-D-*arabino*-hexose places this compound within the *arabino* series. Other compounds used as references are the corresponding alditols and derivatives substituted at C-1.

The identification of the sites of chelation of the ligands is based on the deshielding effect on carbons that bear the chelating hydroxyl groups. Free alditols and their molybdate complexes were studied in previous work[16] and relevant

TABLE II

^{13}C NMR chemical shifts for molybdate complexes of sugars and homologous alditols

Carbon		C-1	C-2	C-3	C-4	C-5	C-6
D-Arabinitol [a]							
free ligand	δ	65.3	72.4	72.6	73.1	65.1	
complex A_1	δ	65.1	84.1	84.0	92.9	71.7	
complex A_2	δ	66.1	79.9	92.4	83.7	74.1	
D-Arabinose							
complex A'_1	δ	92.0	84.5 [b]	82.1	91.5	70.3	
complex A'_2	δ	90.3	81.1[b]	91.2	82.5	72.8	
D-Arabinose diethyl dithioacetal [c]							
free ligand	δ	54.4	71.9	71.0	71.9	63.7	
complex T_1	δ	54.3	84.4	83.3	91.5	69.8	
complex T_2	δ	55.1	80.7	91.6	81.9	72.6	
2-Deoxy-D-*arabino*-hexose							
complex D_1	δ	90.8	43.0	80.0	86.4	92.2	70.7
complex D_2	δ	92.1	40.5	82.0	92.4	84.5	74.3
D-Glucitol (*erythro* complexes) [a]							
free ligand	δ	64.7	75.0	71.7	73.2	73.2	65.0
complex G_2	δ	64.8	75.2	83.8	83.7	92.9	71.3
complex G_4	δ	64.5	76.1	78.9	92.2	84.7	74.1
D-Mannitol [a]							
free ligand	δ	65.8	74.2	71.6	71.6	74.2	65.8
complex M_1	δ	65.4	73.3	83.3	83.8	93.1	71.7
complex M_2	δ	65.9	74.1	80.3	92.3	83.8	74.1
Galactitol [a]							
free ligand	δ	65.5	71.5	72.1	72.1	71.5	65.5
complex	δ	65.5	83.3	83.7	91.8	79.4	64.7
D-Galactose							
complex G_1	δ	92.0	81.6	91.4	82.9	82.5	63.8
complex G_2	δ	90.2	84.2	82.5 [b]	90.5 [b]	78.2	64.3
Xylitol [a]							
free ligand	δ	64.8	74.0	72.8	74.0	64.8	
complex	δ	77.1	84.3	83.4	86.6	64.1	
D-Xylose							
complex	δ	99.3	86.5	80.9	85.5	63.2	
D-Glucitol (*threo* complex)							
complex G_1	δ	77.2	84.2	83.1	85.7	72.1	65.3
D-Glucose							
complex G'_1 [d]	δ	99.7	86.8	80.8	85.1	71.2	64.4

[a] From ref 16. [b] Assignments of ref 18 reversed. [c] CH_3 at 14.3 ppm and CH_2 at 25–26 ppm (ref 27). [d] From ref 20.

chemical shifts of alditols are reported in Table II. The assignments of ^{13}C resonances in the spectra of complexed aldoses are generally in good agreement with literature data[18,20], except for some revised assignments that are marked (^{b}) in Table II. The low proportions of complexes of aldoses are unfavourable to 2D experiments, but assignments are made easily by comparison with the known spectra[16] of homologous alditol complexes.

In Table II, the spectra of the D-arabinose and D-galactose complexes are compared to those of D-arabinitol and galactitol. Both aldoses are hydrated and the anomeric carbon is not involved in chelation, as the C-1 signals appear at δ 91 ± 1 ppm. Complexation with molybdate induces variations $\Delta\delta$ of the chemical shifts of the carbons involved in the site of chelation, that present characteristic deshielding patterns. The existence of two isomeric complexes of D-arabinitol in equilibrium was discussed elsewhere[16]. Within a pair of complexes, the opposite patterns were attributed to the chelation of molybdate by the ligand at the same site in reversed orientations.

D-Arabinose gives a mixture of two complexes in low yields (A_1 5% and A_2 5%) of *erythro* type at the *arabino* site[18] (HO-2,3,4,5). Revised assignments are given in Table II, taking into account the analogy with the site of chelation of D-arabinitol. For ligands involving an *arabino* site, the major complex (subscript 1) is that in which the more deshielded carbon atom is C-4 (pentoses) or C-5 (hexoses). For the minor complex (subscript 2), the more deshielded carbon atom is respectively C-3 or C-4.

D-Galactose also affords a mixture of two *erythro* type complexes (G_1 7% and G_2 4%). The presence of two unshifted C-6 signals at, respectively, 63.8 and 64.3 ppm shows that the CH_2OH group is not chelated. Hence the site of chelation in D-galactose is the same (HO-2,3,4,5) as in galactitol. The existence of two complexes of galactose in equilibrium is due to the molecular asymmetry which does not exist in galactitol. As in other *erythro* complexes, the deshielding patterns show a very deshielded carbon ($\Delta\delta \simeq 20$ ppm) that is not the same in both isomers: C-3 in G_1, C-4 in G_2. Accordingly, we reversed literature assignments[18] for C-3 and C-4 in complex G_2.

For 2-deoxy-D-*arabino*-hexose, two complexes were detected. Data in Table II show that they are analogous to the foregoing *arabino* complexes. The site of chelation was assigned to HO-3,4,5,6. A recent paper[11] reported a circular dichroism study that concluded either to complexation in the pyranose or the acyclic form. The present data strongly supports the conclusion that 2-deoxy-D-*arabino*-hexose is indeed complexed in its acyclic form.

A model compound was examined in order to check the structural factors that might influence the stabilities of the complexes. We chose the diethyl dithioacetal of D-arabinose, that possesses the same *arabino* site of chelation (HO-2,3,4,5) as D-arabinose and D-arabinitol. Data in Table II show that the expected pair of *arabino* complexes were obtained, on the basis of their characteristic deshielding patterns.

In the *xylo* series, only one complex was detected for D-xylose. By comparison with the spectrum of the xylitol complex which was recently entirely assigned[16], we could assign unambiguously the spectrum of the D-xylose complex. Our data agree with the recent assignment[20] of the site of chelation to the *threo* HO-1,2,3,4 system including one hydroxyl group of the hydrated carbonyl function. The major complex of D-glucose is homologous[20] to the D-xylose complex and both species are expected to possess the same structure, in agreement with their comparable formation constants.

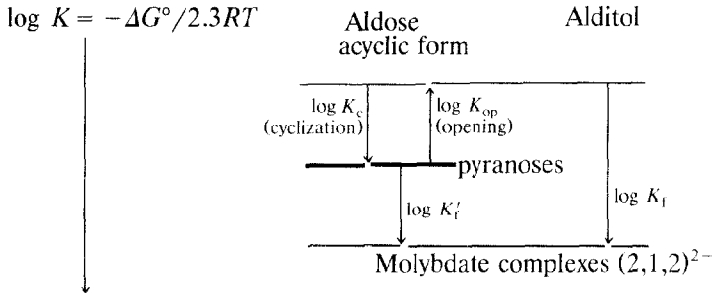
DISCUSSION

The relationship between the configurations of the sites of chelation and the stabilities of the complexes.—In the *arabino* series, considering first acyclic ligands, NMR data prove without doubt that the sites of chelation are homologous *arabino* systems in the diethyl dithioacetal of D-arabinose (log $K_{212} = 16.65$), D-arabinitol (log $K_{212} = 16.35$), and D-mannitol (log $K_{212} = 16.70$). The higher stability for mannitol vs. arabinitol may be attributed to the existence of two equivalent sites (HO-1,2,3,4 or HO-3,4,5,6) in D-mannitol. The more important result is that the values of the formation constants are very close for alditols and the acyclic diethyl dithioacetal. It confirms that carbohydrates with similar structures form complexes of equivalent stabilities, regardless of the nature of substituents surrounding the site of chelation.

The comparison with D-glucitol is not so obvious. This alditol possesses several possible sites of chelation and accordingly forms four complexes[16]: two of the "xylitol or *threo* type", G_1 and G_3, and two of "arabinitol or *erythro* type", G_2 and G_4. Their stabilities must be analogous because of their comparable proportions (G_1 32%, G_2 26%, G_3 and G_4 21% each) at equilibrium. Thus, it is estimated that the *erythro* complexes of D-glucitol (HO-3,4,5,6) are probably almost as stable as those of D-mannitol. The slightly lower value found for the overall formation constant of the D-glucitol complexes (log $K_{212} = 16.60$) is probably due to the presence of *threo* complexes that are weaker, as is the xylitol complex (log $K_{212} = 16.25$).

Although the nature of the site of chelation for 1-deoxy-1-methylamino-D-glucitol could not be proven by NMR, the prevailing complexes probably involve the *arabino* system (HO-3,4,5,6) analogous to those of D-glucitol, D-arabinitol, and D-mannitol. Accordingly, its formation constant has a comparable value (log $K_{212} = 16.85$).

Therefore, one can assume that the formation constant of the molybdate complex of any acyclic carbohydrate chelating at an *arabino* site cannot noticeably differ from 16.7, the mean value of log K_{212} for the above compounds. Since the sites of chelation are of the same type for D-arabinose and 2-deoxy-D-*arabino*-hexose, the lower log K_{212} values found for the complexes of these aldoses obviously reflect the low proportion of acyclic form in the solutions of the uncomplexed sugars.



Scheme 2. Energy diagram.

In the *xylo* series, the aldoses D-xylose and D-glucose form complexes involving the same *xylo* site of chelation, HO-1,2,3,4, as for xylitol or D-glucitol (in complex G_1). The same relationship holds in the *galacto* series for D-galactose and galactitol. In both cases, the lower stabilities of the aldose complexes (K'_f) compared to the alditol complexes (K_f) cannot be attributed to a peculiar type of chelation, but to the fact that the sugars are mainly in their pyranose form when the complexes formation occurs.

Determination of the proportion of the acyclic form of aldoses.—Because free aldoses exist mainly in pyranose (α and β) forms[28], they must open to an acyclic form before chelating the dimolybdate group, and the energetic cost of this unfavourable step decreases the overall formation constant of their complexes (K'_f is lower than K_f).

The proposed two-step mechanism is illustrated in Scheme 2. The energy levels were expressed in log K_f units, as the variations of free energy $\Delta G°$ are related to the equilibrium constants K_f by:

$$\Delta G° = -2.3RT \log K_f$$

We made the reasonable assumption that comparable variations of free energy were involved in the complexation of the acyclic form of an aldose and in that of a related acyclic molecule (alditol or derivative). The variation of free energy in the complexation of an aldopyranose is the sum of the free energy changes in both steps, first the opening reaction, and secondly the complexation of the acyclic ligand.

Once transformed in equations relating the log K values, the experimental formation constant K'_f for an aldose can be expressed by the equations:

$$\log K'_f = \log K_f + \log K_{op} \text{ or } \log K'_f = \log K_f - \log K_c$$

in which K_{op} and K_c are the opposite equilibrium constants for the reaction:

$$\text{cyclic form(s)} \underset{K_c}{\overset{K_{op}}{\rightleftharpoons}} \text{acyclic form(s)}$$

Values of the free energy of cyclization $\Delta G°$ (Table III) were calculated by:

$$\Delta G°_c = -2.3RT \log K_c$$

TABLE III

Cyclization constants [a] K_c and proportion of acyclic form of aldoses in aqueous solution

Aldose	$\Delta \log K_f$	K_c	ΔG_c° (kJ mol^{-1})	Acyclic form (%)	Aldehyde form (%)
D-Arabinose	3.15	1412	−18.0	0.07	0.03 [b]
D-Galactose	3.05	1122	−17.4	0.09	0.02 [b]
2-Deoxy-D-*arabino*-hexose	2.75	562	−15.7	0.18	0.008 [c]
D-Xylose	2.40	251	−13.7	0.40	0.02 [b]
D-Glucose	3.45	2818	−19.7	0.04	0.002 [b]

[a] Equilibrium constants for the reaction: pyranoses ⇄ acyclic form(s) (T, 25°C; KCl, 0.1 M). [b] at 31°C, from ref 29. [c] at 44°C, from ref 29. The reference compound (100% acyclic form) for each aldose is the corresponding alditol, except for 2-deoxy-D-*arabino*-hexose for which D-arabinitol was used.

The proportions P_{op} of acyclic form(s) of free aldoses, in %, were calculated from the values of K_c (Table III):

$$K_c = [\text{cyclic form(s)}]/[\text{acyclic form(s)}]$$

$$P_{op} = 100/(1 + K_c)$$

Comparison of our results with literature data requires some comments. Few methods allow the determination of the proportion of acyclic forms of carbohydrates which could be useful for mechanistic studies of the mutarotation reaction. A review[28] covering literature up to 1983 pointed out that no method was completely satisfactory, and that they all relied on questionable assumptions. At this time, the most reliable technique[29] estimated the carbonyl content of numerous sugars by using the circular dichroism band at 280 nm. The corresponding data are displayed in Table III for comparison.

The polarographic method was used for the determination of the aldehyde forms of D-glucose, 0.0026% (ref 30) and D-xylose, 0.04% (ref 31). Other experiments in unbuffered solution indicated 0.3% for D-xylose and 0.04% for D-glucose[31], in close agreement with our results. A reason for the discrepancies is probably that our method (like that of Ikeda et al.[31]) allowed the calculation of the overall proportion of the acyclic form (aldehyde and hydrate), whereas other techniques[29,30] only detected the carbonyl species.

Recently, the equilibrium tautomeric composition of several sugars has been determined with high accuracy, using new, powerful ^{13}C NMR techniques applied to [1-^{13}C]-enriched aldoses. The C-1 signals are usually found at $\delta \simeq 205$ ppm for the aldehyde and $\delta \simeq 91$ ppm for the hydrate. Applied to D-glucose, this technique indicated[32] 0.0077% of hydrate and 0.0024% of aldehyde at 37°C. In the case of D-ribose, the proportion of aldehyde was estimated[33] to be close to 0.05%. For D-talose[34], another sugar of the *ribo* series, the proportions of acyclic forms (hydrate and aldehyde) were 0.03% each. In the case of D-idose[35], the proportions of hydrate and aldehyde are, respectively, 0.5 and 0.1%. The corresponding value of K_c is 166. It seems that D-idose, like D-xylose, gives rather large proportions of

acyclic forms.

The present method is limited to aldoses that do not complex with molybdate ions in the cyclic form, and thus cannot be employed for aldoses of the *lyxo* series, that are complexed in the furanose form[17], or of the *ribo* series, probably complexed in their pyranose form[18]. Owing to its simplicity (no ^{13}C enrichment of sugars), it gives results in good agreement with other methods. Nevertheless, contrary to the NMR methods that yield specific values for each acyclic form, it affords average values (for the mixture of forms at equilibrium) that are of interest when the overall properties of a solution of sugar are investigated.

In conclusion, it is demonstrated that the difference in stabilities of aldose and alditol complexes is mainly related to the chain-opening of the aldopyranoses. Thermodynamic calculations were performed and showed that the proportions of acyclic forms obtained following this assumption were in agreement with those obtained by more specific techniques.

EXPERIMENTAL

All chemicals were of analytical-reagent grade. Water was de-ionised in a Millipore apparatus.

Potentiometric method.—Formation constants were determined by potentiometry[21], based on the determination of the half-equivalence $pH_{1/2}$ values in acidimetric (HCl) titrations of disodium molybdate solutions ($c_{Mo} = 5 \times 10^{-3}$ M) that contained various amounts of ligand (aldose, alditol or derivatives species). Measurements were made at constant ionic strength (KCl, 0.1 M; T, 25°C) with a Hanna pHmeter and a combined glass electrode.

Spectrophotometric method.—A stock solution of the molybdate–chloranilate complex in acetate buffer (pH ≃ 4.6) was prepared by mixing in the following order: NaOH (0.1 M), chloranilic acid (Fluka, puriss, 5.0×10^{-4} M), AcOH (0.2 M), disodium molybdate dihydrate (U.C.B. reagent, 10^{-3} M) and diluting with purified water. The solution could be kept protected from light for one month at room temperature.

Aldoses were added in solid form (200-mg increments) to 50 mL of the above solution. Alditols were added to 100 mL as aliquots (0.1 mL) of aqueous solutions with a Gilson micropipette.

After waiting 5 min for temperature equilibration, absorbance was measured at $\lambda = 347$ nm using a Kontron Uvikon 860 spectrophotometer equipped with 1-mm quartz cells. The temperature was fixed at 25.0 ± 0.3°C by a water bath and a pump.

^{13}C NMR spectrometry.—All spectra were recorded with a Bruker AM 360 spectrometer equipped with a 5-mm multinuclear probe. Solutions containing the carbohydrate (0.5 mmol) and disodium molybdate dihydrate (1.5 mmol) in D_2O (0.5 mL) were acidified with concd HCl (0.75 mmol).

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REFERENCES

1 E.J. Bourne, D.H. Hutson, and H. Weigel, *J. Chem. Soc.*, (1960) 4252–4256; (1961) 35–38.
2 V. Bílik, *Chem. Zvesti*, 26 (1972) 76–81; 183–186; 187–189; 372–375.
3 V. Bílik, L. Petruš, and V. Farkaš, *Chem. Zvesti*, 29 (1975) 690–696.
4 V. Bílik, L. Petruš, and J. Zemek, *Chem. Zvesti*, 32 (1978) 242–251.
5 V. Bílik, L. Petruš, and L. Kuniak, *Chem. Zvesti*, 33 (1979) 114–118.
6 V. Bílik, *Chem. Listy*, 77 (1983) 496–505.
7 Patents cited in, *Chem. Abstr.*, 81 (1974) P 78189 k; 82 (1975) P 86557 q; 83 (1975) P 164510 f; 88 (1978) P 74522 k; 94 (1981) P 175440 m.
8 E.L. Clark Jr, M.L. Hayes, and R. Barker, *Carbohydr. Res.*, 153 (1986) 263–270.
9 M.L. Hayes, N.J. Pennings, A.S. Serianni, and R. Barker, *J. Am. Chem. Soc.*, 104 (1982) 6764–6769.
10 M. Sankovíc, S. Emini, S. Rusman, and V. Šunjíc, *J. Mol. Catal.*, 61 (1990) 247–258.
11 G. Snatzke, J. Guo, Z. Raza, and V. Šunjíc, *Croat. Chim. Acta*, 64 (1991) 501–517.
12 B. Hedman, *Acta Cryst. Sect. B*, 33 (1977) 3077–3083.
13 G.E. Taylor and J.M. Waters, *Tetrahedron Lett.*, 22 (1981) 1277–1278.
14 L. Ma, S. Liu, and J. Zubieta, *Polyhedron*, 8 (1989) 1571–1573.
15 M. Matulová, V. Bílik, and J. Alföldi, *Chem. Pap.*, 43 (1989) 403–414.
16 S. Chapelle, J.F. Verchère, and J.P. Sauvage, *Polyhedron*, 9 (1990) 1225–1234.
17 J.-P. Sauvage, S. Chapelle, and J.-F. Verchère, *Carbohydr. Res.*, 237 (1992) 23–32.
18 M. Matulová and V. Bílik, *Chem. Pap.*, 44 (1990) 77–87.
19 M. Matulová and V. Bílik, *Chem. Pap.*, 44 (1990) 705–709.
20 V. Bílik and M. Matulová, *Chem. Pap.*, 44 (1990) 257–265.
21 J.-F. Verchère and S. Chapelle, *Polyhedron*, 8 (1989) 333–340.
22 J.J. Cruywagen and J.B.B. Heyns, *Inorg. Chem.*, 26 (1987) 2569–2572.
23 W.F. Lee, N.K. Shastri, and E.S. Amis, *Talanta*, 11 (1964) 685–690.
24 J.-F. Verchère, *J. Chem. Res.*, (S) 178, (M) (1978) 2216–2240.
25 J.-F. Verchère and J.M. Poirier, *J. Inorg. Nucl. Chem.*, 42 (1980) 1514–1515.
26 K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–65.
27 G.W. Schnarr, D.M. Vyas, and W.A. Szarek, *J. Chem. Soc., Perkin Trans. 1*, (1979) 496–503.
28 S.J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 15–68.
29 L.D. Hayward and S.J. Angyal, *Carbohydr. Res.*, 53 (1977) 13–20.
30 J.M. Los, L.B. Simpson, and K. Wiesner, *J. Am. Chem. Soc.*, 78 (1956) 1564–1568.
31 T. Ikeda and M. Senda, *Bull. Chem. Soc. Jpn*, 46 (1973) 1650–1656, 2107–2111.
32 S.R. Maple and A. Allerhand, *J. Am. Chem. Soc.*, 109 (1987) 3168–3169.
33 M.J. King-Morris and A.S. Serianni, *J. Am. Chem. Soc.*, 109 (1987) 3501–3508.
34 J.R. Snyder, E.R. Johnston, and A.S. Serianni, *J. Am. Chem. Soc.*, 111 (1989) 2681–2687.
35 J.R. Snyder and A.S. Serianni, *J. Org. Chem.*, 51 (1986) 2694–2702.