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Carbohydrate Research 286 (1996) 67–76

CARBOHYDRATE RESEARCH

A multinuclear NMR spectroscopy study of the tungstate and molybdate complexes of D-fructose and L-sorbose

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Received 18 December 1995; accepted 22 January 1996

Abstract

The formation of dinuclear tungstate and molybdate complexes of D-fructose and L-sorbose was studied in aqueous solution by ^{1}H, ^{13}C, and ^{183}W NMR spectroscopy. These ketoses react only in acyclic forms, because HO-3,4 are *trans* in the cyclic forms. With tungstate, both sugars form a major complex of the same type in which the hydrated ketose is bound through the O-1,2,4 site of chelation. The ligands are tetradentate, since both O-2 atoms bridge the metal atoms. Complexes of the same type prevail in the molybdate systems, but are accompanied by one (sorbose) or two (fructose) minor complexes formed by the acyclic keto forms in which the sites of chelation are the O-3,4,5,6 systems. Multinuclear NMR data are provided for the identification of the various types of complexes. © 1996 Elsevier Science Ltd.

Keywords: Ketoses; Fructose; Sorbose; Tungstate complexes; Molybdate complexes; ^{13}C NMR; ^{183}W NMR

1. Introduction

An important difference between the chemical properties of aldoses and ketoses is that aldoses are epimerized at C-2 in the presence of molybdate ions in acidic solution, but ketoses do not react, although they can also form dimolybdate complexes. The epimerization of aldoses discovered by Bílik in 1972 [1] is a reaction useful for the synthesis of rare sugars [2]. Its mechanism involves the formation of transient dimolybdate complexes of the tetradentate (O-1,2,3,4) aldoses in which a bond shifts from C-2,3

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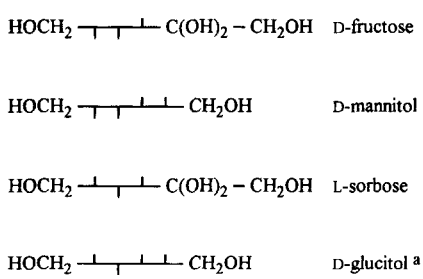
PII S0008-6215(96)00048-1

to C-1,3, corresponding to an apparent C-1/C-2 transposition [3,4]. Tungsten is closely related to molybdenum and generally gives very similar reactions [5], but tungstate is not a catalyst of the above reaction, despite its ability to form carbohydrate complexes that are often isostructural with their molybdate congeners.

Multinuclear NMR is nowadays the favourite method for the structural study of complexes of carbohydrates. The conformation of the ligand is obtained by studying the ^{1}H and more specifically the ^{13}C nuclei, since carbon atoms which belong to the site of chelation are deshielded with characteristic patterns. For molybdate complexes, ^{95}Mo NMR was used with some success for investigations on the nature of the inorganic moiety [6,7]. We have recently introduced the use of ^{183}W NMR for the study of complexes of alditols [8–11], because this technique is more informative than NMR of the quadripolar ^{95}Mo nucleus. Due to its nuclear spin 1/2, the ^{183}W isotope generates narrow signals that are sometimes coupled to protons of the ligand, allowing thus the unambiguous assignment of the sites of chelation of each tungsten atom through two-dimensional (2D) heteronuclear proton–tungsten experiments.

The formation constants of the tungstate complexes of alditols and various sugars were determined by potentiometry [12], with the result that aldoses and ketoses form 2:1 (W:L) species of comparable stabilities. Thus, it seemed interesting to investigate the structures of the complexes of ketoses, in order to find if they have specific characteristics with respect to aldoses. An earlier paper [13] claimed that all ketoses of *ribo*, *lyxo*, *xylo*, or *arabino* configuration yield the same type of molybdate complexes, in which the ligands are in pyranose form and chelate the dimolybdate group through the O-1,2,3 system. In fact, we later demonstrated that ketoses with HO-3,4 groups in *erythro* configuration, including D-psicose and D-tagatose, mainly form molybdate complexes in furanose form, in which the sugars are tridentate ligands through the O-1,3,4 system [14]. Ketoses of the *lyxo* series also form furanose complexes analogous to those obtained with related aldoses [14,15].

In this work, the behaviour of D-fructose and L-sorbose, 2-ketohexoses which possess HO-3,4 in *threo* configuration, with disodium tungstate and molybdate, was studied in aqueous solution by multinuclear NMR. The results show conclusively that both ketoses chelate tungstate and molybdate ions in the acyclic form and not in the pyranose form. The factors that influence the stability of complexes of acyclic ligands are discussed (Scheme 1).



Scheme 1. Structures of the ketoses in acyclic hydrated form and of the related alditols. [a]D-Glucitol is represented with C-1 on the *left*-hand side, in order to show the structural relationship with L-sorbose.

2. Experimental

All chemicals were commercial products of analytical grade. The ketoses were used as supplied, after checking their purity through ^{1}H and ^{13}C NMR spectroscopy.

The ^{1}H, ^{13}C, and ^{183}W NMR spectra were obtained at 298 K on Bruker AM 360 and ARX 400 spectrometers equipped with 5- and 10-mm multinuclear probes. Experimental details have been published elsewhere [8–11,15]. The complexes were prepared by mixing the ketose (1 mmol) and disodium tungstate (or molybdate) dihydrate (2 mmol) in deuterium oxide (1 cm^3) and adding concentrated HCl (0.5–2 mmol). The pH was measured in the NMR tube with a Radiometer MI-412 combined micro glass electrode (external diameter 2 mm) and a Metrohm 632 pH meter.

2D heteronuclear ^{1}H-{^{13}C}correlation spectra were obtained using the indirect mode [16] through a HMQC pulse sequence which generates heteronuclear multiquantum coherences [17,18]. The corresponding experiments were performed with optimization for direct ($^{1}J_{H,C}$ 145 Hz) and long-range ($^{3}J_{H,C}$ 8 Hz) coupling constants. The data size was 512 points in the t_2 time ^{1}H domain. The number of experiments was 128.

3. Results

In aqueous solution, the free ketoses may exist as four forms: α-*p*, β-*p*, α-*f*, and β-*f*. The β anomers prevail for D-fructose, for which only traces of α-*p* form and not α-*f* form are observed. For free L-sorbose, the α anomers are favoured and only the α-*p* and α-*f* forms occur.

Tungstate complexes.—When two equivalents of tungstate are added to the solutions of both ketoses, with subsequent acidification to pH 7.5, the ^{13}C NMR spectra show 6 intense new signals, indicating that a single complex is formed at equilibrium. The relative proportions (complexed ligand:free ligand) are in %, for fructose 70:30, and for sorbose 60:40. The complexes were characterized after complete assignment of the carbon signals by 2D ^{1}H homonuclear and ^{1}H–^{13}C heteronuclear NMR experiments using direct and long-range couplings. The corresponding chemical shifts are reported in Table 1. A striking similarity exists between the fructose and the sorbose species, indicating very similar structures. The CIS (Coordination Induced Shift) patterns were determined, using as references "calculated" spectra for the uncomplexed acyclic ligands, obtained from the spectra of mannitol and glucitol, that are suitable models for the C-3,4,5,6 parts of the ketoses. Both CIS patterns are identical: the C-1,2,4 are clearly deshielded, contrary to C-3. For C-5 the results are not so clear, because the deshielding effect is weak ($\Delta\delta \approx 4$ ppm for sorbose), but the very low value found for fructose ($\Delta\delta \approx 1$ ppm) makes its participation in the site of chelation very unlikely. Contrary to many complexes in which the values of the direct coupling constants $^{1}J_{C,H}$ are enhanced by 2–10 Hz for carbon atoms that bear chelating oxygen atoms, the data in Table 1 do not show clear differences between the atoms of the ligand, with respect to their participation in the site of chelation.

The ^{183}W NMR spectra recorded for both complexes exhibit two singlet signals each, in agreement with their dinuclear nature (Fig. 1). The chemical shifts (Table 2) are close

Table 1
90.56-MHz ^{13}C NMR chemical shifts δ (ppm) and direct coupling constants $^{1}J_{C,H}$ (Hz) for the tungstate and molybdate complexes of D-fructose and L-sorbose at pH 7.5 [a]

Carbon atom	C-1	C-2	C-3	C-4	C-5	C-6
Fructose, u, ac, hydr, δ [b]	66	100	72	72	74	66
Sorbose, u, ac, hydr, δ [c]	65	100	72	73	73	65
Tungstate complexes						
D-Fructose, δ	**74.7**	**110.6**	70.6	**79.5**	73.5	67.1
D-Fructose, $^{1}J_{C,H}$	**146**	–	148	**148**	145	148
L-Sorbose, δ	**74.8**	**110.0**	70.5	**82.5**	77.6	65.5
D-Sorbose, $^{1}J_{C,H}$	**146**	–	147	**147**	149	149
Molybdate complexes						
D-Fructose, F_1, δ	**74.5**	**106.6**	69.9	**78.7**	72.7	65.9
D-Fructose, F_2, δ	66.9	217.0	**82.4** [d]	**82.8** [d]	**92.4**	**73.0**
D-Fructose, F_3, δ	64.1	215.0	**82.1**	**91.5**	**84.6**	**72.4**
D-Mannitol, M_1, δ [e]	65.4	73.1	**83.3**	**83.8**	**93.1**	**71.7**
D-Mannitol, M_2, δ [e]	65.9	74.1	**80.3**	**92.3**	**83.8**	**74.1**
L-Sorbose, S_1, δ	**74.2**	**106.1**	69.6	**81.8**	76.6	64.2
L-Sorbose, S_2, δ	67.6	214.6	**87.9**	**82.1**	**83.9**	**75.9**
Carbon atom [f]	C-6	C-5	C-4	C-3	C-2	C-1
D-Glucitol, G_1, δ [e]	65.3	72.1	**85.7**	**83.1**	**84.2**	**77.2**
Carbon atom		C-1	C-2	C-3	C-4	C-5
Xylitol, δ [e]		64.1	**86.6**	**83.4**	**84.3**	**77.1**

[a] u, Uncomplexed; ac, acyclic; hydr, hydrate. Accuracy $\delta \pm 0.1$ ppm; $^{1}J_{C,H} \pm 1$ Hz. Assignments of the tungstate complexes were made by 2D homo- and hetero-nuclear experiments. Data for carbon atoms that bear chelating oxygen atoms are boldface.
[b] Estimated from chemical shifts for mannitol.
[c] Estimated from chemical shifts for glucitol.
[d] These assignments may be reversed.
[e] Ref. [7].
[f] The numbering of carbon atoms of D-glucitol and xylitol are explicitely given, in order to locate matching carbon atoms in the same columns.

for both species, confirming their similarity deduced from the ^{13}C NMR data. A comparison with the ranges generally observed for the chemical shifts of tungsten atoms in the various types of complexes [8–11] shows that the complexes of ketoses belong to a new type. However, the values of the chemical shifts, δ −95 and −110 ± 5 ppm, are not far from those measured for complexes of alditols in which the ligands are tetradentate with four vicinal hydroxyl groups, and the metal atoms are located at the centres of two octahedra sharing a face.

Data for the ^{1}H chemical shifts for the tungstate complexes of ketoses were also obtained (Table 3). Again, the chemical identity of the complexes is obvious. Atoms H-1,4 borne by carbon atoms of the site of chelation are clearly deshielded, whereas H-3,5 are not deshielded, confirming in particular that the O-5 do not belong to the site of chelation. Nevertheless, the variations of chemical shifts of protons induced by

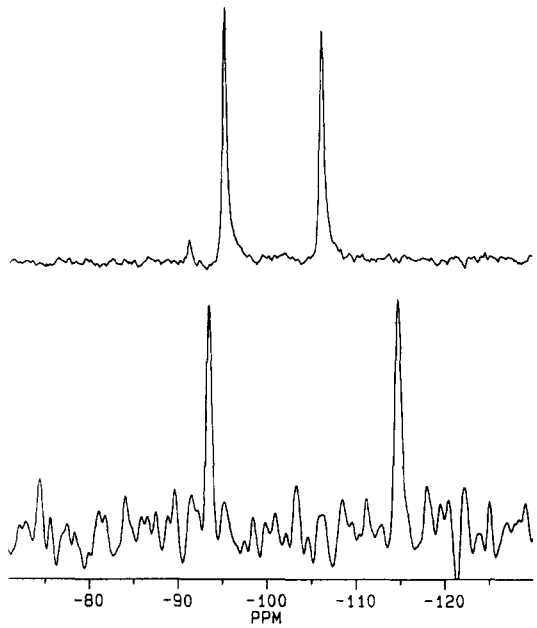


Fig. 1. 15.005-MHz ^{183}W NMR spectra of the tungstate complexes of D-fructose (top, 11 h, 11360 scans) and L-sorbose (bottom, 2 h, 2000 scans) at pH 7.5.

chelation do not display such characteristic CIS patterns as those for the corresponding carbon atoms.

Both complexes have therefore a common structure that could be unambiguously deduced from the above NMR data and by the consideration of molecular models. First, by assuming a tridentate site of chelation at O-1,2,4, no structure could be imagined in which the tungsten atoms would have environments similar enough to justify the close values of their chemical shifts. On the other hand, the unusual finding that O-3 is not a donating atom, while located between O-2,4 that are undoubtedly chelating atoms, could be understood by observing that if *both* O-2 atoms are bound to each metal atom and HO-3 remains free, then O-1 and O-4 can each bind a tungsten atom. In this case, the complex has a rather symmetrical structure, in which W-1 is bound to O-1,2,2′ and W-2 to O-4,2,2′. If one ignores the substituents at C-3 and C-4, the site of chelation would

Table 2
15.005-MHz ^{183}W NMR chemical shifts δ (ppm) for the tungstate complexes of D-fructose and L-sorbose at pH 7.5 [a]

Tungsten atom	W-1	W-2
Fructose complex, δ	−95.2	−106.5
Sorbose complex, δ	−93.5	−114.5

[a] Reference: Na_2WO_4 in alkaline D_2O. Accuracy $\delta \pm 0.1$ ppm. The signals were of equal intensities in each complex.

Table 3
400.13-MHz ^{1}H NMR chemical shifts δ (ppm) for the tungstate complexes of D-fructose and L-sorbose [a]

Hydrogen atom	H-1	H-1′	H-2	H-3	H-4	H-5	H-6	H-6′
D-Fructose	3.73	**4.49**	–	3.98	**4.60**	3.74	3.77	4.00
L-Sorbose	3.72	**4.43**	–	3.57	**4.30**	3.55	3.64	3.73

[a] Data for protons borne by carbon atoms of the site of chelation are boldface. The assignments were made by 2D homo- and hetero-nuclear experiments (COSY and HMQC).

possess a plane of symmetry defined by O-1;C-1;C-2;C-3;C-4;O-4. The corresponding structure of the complexes is represented in Fig. 2. Since D-fructose and L-sorbose only differ by the configuration at C-5, it suggests the assignment of the variable tungsten signal at -110 ± 5 ppm to W-2 (bound to O-4) and of the signal at -95 ppm to W-1. The absence of coupling between W-1 and H-1, and W-2 and H-4 agrees with the dihedral angles observed in this structure.

Molybdate complexes.—In the presence of molybdate, both ketoses form mixtures of complexes, the relative amounts of which were estimated from the relative intensities of the carbon signals in the ^{13}C NMR spectrum, within 1 h after mixing the reagents. Variations of these proportions occurred with the pH. The overall proportion of complexes decreased in acidic medium (below pH 5). In alkaline medium (pH 8–9), the major species of fructose, F_1, and sorbose, S_1, were obviously analogous to the tungsten complexes characterized above, on the basis of their similar ^{13}C NMR spectra (Table 1).

The proportions of the minor molybdate species are larger below pH 7. Fructose affords two species in similar proportions, F_2 and F_3, for which the C-2 signals lie at δ 216 ± 1 ppm, indicating that the ligand is in the keto form. The signals were assigned to the corresponding complexes by studying solutions acidified under slightly different conditions, which contained F_2 as the major complex. Both species F_2 and F_3 give rise to a characteristic signal near δ -92 ppm, which is always present in the spectra of complexes of carbohydrates which have tetradentate sites in sickle conformation, as is

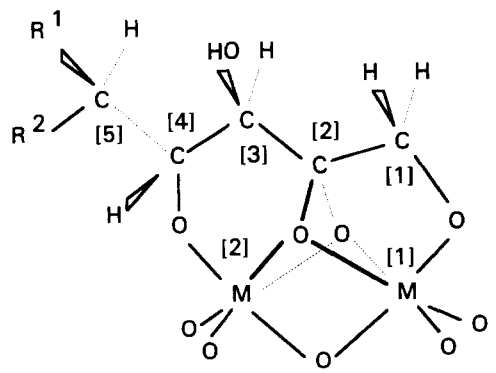


Fig. 2. Proposed structures for the tungstate and molybdate complexes of the ketoses in acyclic hydrated form (M = W or Mo). D-Fructose, $R^1 = HO$, $R^2 = CH_2OH$. L-Sorbose, $R^1 = CH_2OH$, $R^2 = HO$.

the case for *arabino* sites. A typical example is found in the complexes of D-mannitol [7], which exist as a pair of isomers because the site may be occupied in reversed orientations. It strongly suggested that F_2 and F_3 are a similar pair of isomers in which the site of chelation is the *arabino* system O-3,4,5,6. The NMR data given in Table 1 demonstrate the analogies between the pairs of fructose and the mannitol species, which present very similar CIS patterns.

Sorbose affords, at pH 6–7, a single minor complex S_2, which also involves the keto form (C-2 signal at δ 215 ppm). By analogy with the case of fructose, we examined if the O-3,4,5,6 system, of *xylo* configuration, could be the site of chelation. In other carbohydrates, such sites always form a single complex, with a typical CIS pattern, in which the tetradentate ligand adopts a zigzag conformation. The data in Table 1 show that the CIS pattern for S_2 is exactly the same as those for the isostructural xylitol and glucitol (G_1) complexes [7].

Our results mainly disagree with a previous study of the molybdate complexes of ketoses in which the major complexes were claimed to involve the ligands pyranose forms with the O-1,2,3 site of chelation [13]. This hypothesis must now be excluded, since the new assignments made by 2D experiments show that the CIS for C-3 are almost nil. For all complexes, and particularly the minor ones, the chemical shifts reported in Table 1 differ substantially from those in ref. [13], in which signals that belong to different species were apparently confused, owing to the similar proportions of the complexes. However, the data for C-2, which cannot be mistaken, were in agreement with our findings about the number of isomers. In this respect, the NMR spectra of the molybdate complexes of D-*gluco*-2-heptulose were reported [13] to display 2 signals for C-2 at δ 106.8 and 215.1 ppm, indicating the existence of two species probably analogous with the sorbose complexes.

4. Discussion

This study demonstrates that both ketohexoses form a prevailing type of tungstate or molybdate complexes, in which the ligands adopt the acyclic hydrated form. No other significant complexes were detected with tungstate, but minor species of the acyclic keto form were observed with molybdate.

Table 4
Formation constants log K_{212} for the tungstate and molybdate complexes of 2-ketohexoses [a]

Ketose	Psicose	Tagatose	Fructose	Sorbose
Tungstate complex	nd [b]	19.10	16.90 [c]	16.40 [c]
Molybdate complex	16.30	16.35	14.45	14.15

[a] By potentiometry, accuracy: log $K \pm 0.05$. K_{212} is the equilibrium constant for reaction (M = W or Mo): $2MO_4^{2-} + H_nL + 2H^+ = [(MO_2)_2O(H_{n-4}L)]^{2-} + 3H_2O$.
[b] nd, not determined.
[c] Ref. [12].

Comparison of the stabilities of the complexes.—The formation constants of the tungstate and molybdate complexes of the four ketohexoses were obtained by potentiometry, following the published procedures [7,12] and are compared in Table 4. Tungstate complexes are stronger than molybdate complexes by approximately 3 orders of magnitude. Such a difference is common for carbohydrate complexes with similar dinuclear structures and is probably related to the known greater tendency of tungsten(VI) towards condensation [19].

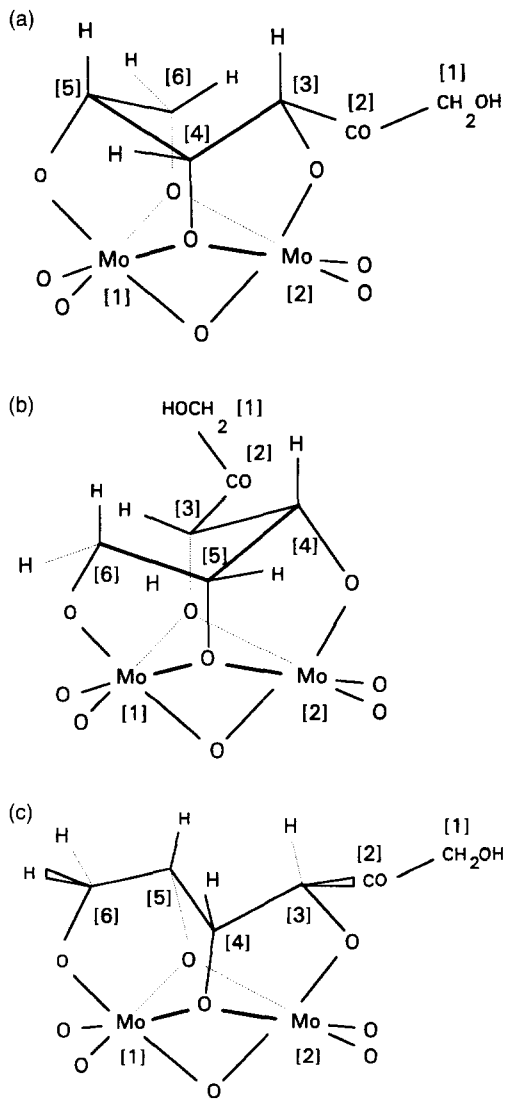


Fig. 3. Proposed structures for the molybdate complexes of the ketoses in acyclic keto form. (a) D-Fructose, F_2; (b) D-fructose, F_3; (c) L-sorbose, S_2.

Ketoses that form complexes as cyclic ligands (psicose, tagatose) give stronger complexes than ketoses that form acyclic complexes (fructose, sorbose). The origin of this effect, which is also observed with aldoses, is due to the endoenergetic reaction of opening of the pyranose forms as a preliminary step in the complex-forming reaction pathway [20].

Molybdate complexes of the hydrated ligands (F_1 and S_1) are stronger than those involving the keto forms (F_2, F_3, and S_2). A possible reason is that hydration of the carbonyl group is expected to increase the stability of the acyclic sugar. The prevalence of complexes of the hydrated form should probably be related to a higher chelating ability of the $C(OH)_2$ group. A proof of this effect is found in the case of xylose, which forms acyclic molybdate complexes at the O-1,2,3,4 site and not at the O-2,3,4,5 site [2], showing that chelation by a $CH(OH)_2$ group yields stronger chelates than chelation by a CH_2OH group. Various tetroses are also complexed in acyclic hydrated forms with participation of the $CH(OH)_2$ group [21].

A surprising difference was noticed between the molybdate complexes of fructose and arabinose [20,22]. Arabinose in the acyclic form yields a pair of complexes in which the site of chelation is the *arabino* O-2,3,4,5 system, and the non-chelating aldehyde group at C-1 is hydrated, contrasting with the occurrence of the keto form of fructose in complexes F_2 and F_3.

Structures of the complexes.—In the complexes of the acyclic hydrated ligands, each tungsten atom is bound to three donating oxygen atoms, which are of two different types. Both O-1 and O-4 are bound to a single tungsten atom, whereas the O-2,2′ bridge the tungsten atoms and are therefore triply bonded. The molybdate complexes are isostructural (Fig. 2).

It was observed that the configuration of C-5 may influence the stability of the complexes of the hydrated form, depending on the orientation of the free HO-5 group with respect to the donating O-4 atom. If one assumes that the smaller substituent at C-5 (H-5) is oriented towards C-3 (Fig. 2), then the whole carbon chain of the ligand is in a zigzag conformation. Thus, in fructose, HO-5 is *trans* to O-4. On the contrary, in sorbose, HO-5 is *cis* to O-4 with possible steric repulsion. It may be related to the variations in the CIS of C-5 in the complexes: the CIS is almost nil for fructose, but is increased to 4 ppm in sorbose.

The minor molybdate complexes of fructose and sorbose are, respectively, similar to the complexes of mannitol and glucitol [7] and are represented in Fig. 3.

5. Conclusions

Ketoses in which HO-3,4 are in *threo* configuration, namely D-fructose and L-sorbose, form a major, common type of tungstate and molybdate complexes, involving the acyclic hydrated ketoses with the tetradentate O-1,2,2′,4 site of chelation. Minor molybdate species, similar to those formed by the corresponding alditols, are formed by the keto forms, in which the tetradentate sites of chelation are the O-3,4,5,6 systems. The results of this work are dramatically different from those for ketoses of the *ribo* and *lyxo* series in which HO-3,4 are in *erythro* configuration, which chelate molybdate and tungstate in their cyclic forms.

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