Stereospecific Molybdic Acid-Catalyzed Isomerization of D-Fructose to Branched-Chain Aldose. The Synthesis of D-Hamamelose

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One-step, stereospecific synthesis of D-hamamelose (2-*C*-(hydroxymethyl)-D-ribose) by isomerization of D-fructose in mild acidic aqueous solution, under catalytic amount of molybdic acid, is described. The mechanism of this transformation was proposed, studied, and confirmed. The data obtained with D-(2-^{13}C)fructose indicate that the rearrangement of 2-ketose to branched-chain aldose is analogical to the mechanism of C-2-epimerization of aldoses catalyzed by molybdate ions. The structure of the branched-chain aldose, D-(2-^{13}C)hamamelose documents that the stereospecific reaction is accompanied by the carbon skeletal rearrangement. The equilibrium mixture of D-fructose and D-hamamelose is formed in the ratio of 14:1 regardless of the starting sugar, D-hamamelose or D-fructose.

The Bílik reaction, epimerization of aldoses catalyzed by molybdic acid, is an effective method for preparation of C-2 epimeric aldoses [1—4]. The reaction mechanism was analyzed by rearrangement of regiospecific ^{13}C-enriched aldoses and by monitoring the changes in ^{13}C NMR spectra of the starting compounds and the products [5]. It was found that the reaction proceeds through a complex formation between molybdate ions and aldoses where a stereospecific 1,2-shift of the carbon skeleton takes place, resulting in the inversion of configuration at C-2. The molybdate-catalyzed interconversions of 2-ketoses have been performed as well. However, the formation of branched-chain sugars was not observed [6, 7].

The possibility of extension of this approach for synthesis of rare carbohydrates with biological activity, applicable mainly in medicinal chemistry, is an attractive problem. For example, D-hamamelose, naturally occurring branched-chain saccharide with remarkable biological properties [8, 9], has been synthesized by numerous but relatively complicated chemical methods [10—12]. If one assumes, however, that 2-ketoses could form dimolybdate complexes similar to those of aldoses, the formation of 2-*C*-hydroxymethylated aldoses can be expected to occur through the rearrangement of the 2-ketose carbon skeleton. We have undertaken to verify such a possibility and prepared a 2-*C*-branched saccharide by a simple isomerization of D-fructose catalyzed by molybdic acid.

EXPERIMENTAL

300.13 MHz ^{1}H and 75.45 MHz ^{13}C NMR spectra were recorded in aqueous solution at 313 K on a Bruker DPX 300 spectrometer equipped with a 5 mm inverse broadband probe with a shielded *z*-gradient. Proton and carbon chemical shifts were expressed relatively to external TSP One-dimensional (1D) ^{1}H NMR spectra were recorded with the spectral width of 2400 Hz and 32 transients were accumulated to obtain sufficient signal/noise ratio. Presaturation of HDO resonance was achieved by low-power irradiation during part of the relaxation delay. 5 mm QNP probe was used for measurement of 1D ^{13}C NMR spectra. About 2000 transients were collected with the relaxation delay of 1.5 s (measurement of D-fructose) and 8 s (D-hamamelose), respectively. Two-dimensional (2D) correlated spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) experiments were performed using *z*-gradients; the latter experiment in phase sensitivity-enhanced pure-absorption mode [13]. Similarly, 2D heteronuclear multiple bond coherence (HMBC) was recorded with three *z*-gradient pulses, namely 12.5 G cm^{-1}, 7.5 G cm^{-1}, and 10 G cm^{-1} and 60 ms delay was set for evolution of long-range proton-carbon coupling constants. 128 transients were accumulated in each f.i.d. and the corresponding experimental time was 10 h. The spectral widths in heteronuclear experiments were 1200 Hz (^{1}H) and 5000 Hz (^{13}C), the spectra were zero-filled before Fourier transformation giving the digital

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resolution of 1.2 Hz/pt and 5.9 Hz/pt, respectively.

Specific optical rotations were measured with automatic polarimeter Perkin—Elmer, Model 141. The composition of reaction mixture was examined by paper chromatography on Whatman No. 1 sheets in the solvent system S_1: butan-1-ol—ethanol—water (volume ratio = 5:1:4), or S_2: ethyl acetate—pyridine—water (volume ratio = 8:2:1), followed by visualization with alkaline silver nitrate.

D-(2-^{13}C)Fructose was purchased from Omicron Biochemicals. All other materials and reagents were commercial products and were used without further purification.

Preparation of D-Hamamelose

A solution of D-fructose (40 g) in 0.2 % aqueous solution of molybdic acid (400 cm^3) was heated at 80 °C for 4 h. After cooling to room temperature, the mixture was deionized with Amberlite IRA 400 in HCO_3^- form (200 cm^3). The resin was filtered off and washed with water (3 × 50 cm^3). The combined filtrate was concentrated, the residue was dissolved in tap water (2 dm^3), baker's yeast (2 g) was added, and the mixture was kept for 3 days to ferment D-fructose. The mixture was filtered, concentrated to *ca.* 200 cm^3, and the equal volume of methanol was added. The mixture was treated with charcoal and, after filtration, concentrated. The mixture was treated with Amberlite IR 120 in H^+ form (25 cm^3) for 15 min, filtered and the resin was washed with water (3 × 10 cm^3). Amberlite IRA 400 in HCO_3^- form (100 cm^3) was added to the combined filtrates and, after 15 min, the mixture was filtered and the resin was washed with water (3 × 20 cm^3). The deionized solution was concentrated to a sirup which was fractionated on a column (95 cm × 1.6 cm) of Dowex 50W X-8 (37—75 mm) in Ba^{2+} form, using water as the eluant, at the flow-rate 5 cm^3 h^{-1}. Fraction *1* (collected in the volume 60—120 cm^3) contained D-sorbose and fraction *2* (collected in the volume 125—240 cm^3) contained chromatographically pure D-hamamelose. The first fraction was concentrated, and crystallization from methanol gave D-sorbose (1.0 g, 2.5 %); m.p. = 164 °C, [α](D, 20 °C, ρ = 10 g dm^{-3}, water) = + 42.7°, R_{Fru} = 0.91 (S_1); R_{Fru} = 0.96 (S_2). Ref. [14] gives m.p. = 165 °C, [α](D, 20 °C, ρ = 25 g dm^{-3}, water) = + 42.9°; ^{13}C chemical shifts of the compound were in agreement with the data published [15].

Concentration of the second fraction gave sirupy D-hamamelose (2.6 g, 6.5 %), [α](D, 20 °C, ρ = 10 g dm^{-3}, water) = −7.1°, R_{Fru} = 1.26 (S_1); R_{Fru} = 1.88 (S_2). Ref. [11] gives m.p. = 110—111 °C, [α](D, 21 °C, ρ = 10 g dm^{-3}, water) = + 7.7 → −7.0°

Isomerization of D-(2-^{13}C)Fructose

A solution of D-(2-^{13}C)fructose (250 mg) in 0.2 % aqueous molybdic acid (2.5 cm^3) was heated at 80 °C for 5 h. After cooling, the reaction mixture was stirred with Amberlite IRA-400 in HCO_3^- form (25 cm^3) for 15 min, filtered and the resin was washed with water (3 × 30 cm^3). The filtrate was concentrated under diminished pressure to give a light-yellow sirup (245 mg). ^{1}H and ^{13}C NMR spectra revealed the presence of three components, namely D-(2-^{13}C)sorbose, D-(2-^{13}C)fructose, and D-(2-^{13}C)hamamelose. Separation of this mixture on a column (95 cm × 1.6 cm) of Dowex 50W X-8 in Ba^{2+} form, using water as the eluant, at the flow-rate 5 cm^3 h^{-1}, afforded three fractions. Fraction *1*, collected in volume 70—80 cm^3, contained D-(2-^{13}C)sorbose (6.2 mg, 2.5 %), [α](D, 20 °C, ρ = 10 g dm^{-3}, water) = + 42.8° Fraction *2*, collected in volume 90—120 cm^3, contained recovered D-(2-^{13}C)fructose (222 mg, 89 %). Fraction *3*, collected in volume 135—180 cm^3, contained D-(2-^{13}C)hamamelose (16.3 mg, 6.5 %), [α](D, 20 °C, ρ = 10 g dm^{-3}, water) = −7.1°

Kinetic Analysis

A solution of D-fructose or D-hamamelose (100 mg) in 0.2 % aqueous molybdic acid (5 cm^3) was kept at 80 °C. Samples (0.5 cm^3) were periodically withdrawn, molybdic acid was removed with Amberlite IRA-400 in the HCO_3^- form (3 cm^3), and selected resonances in ^{1}H NMR spectra were integrated to determine the mole ratio of D-fructose and D-hamamelose, until the equilibria were reached. Concentrations of the products *vs.* time are given in Fig. 1. To compare the rates of the interconversions, the ratios of the concentra-

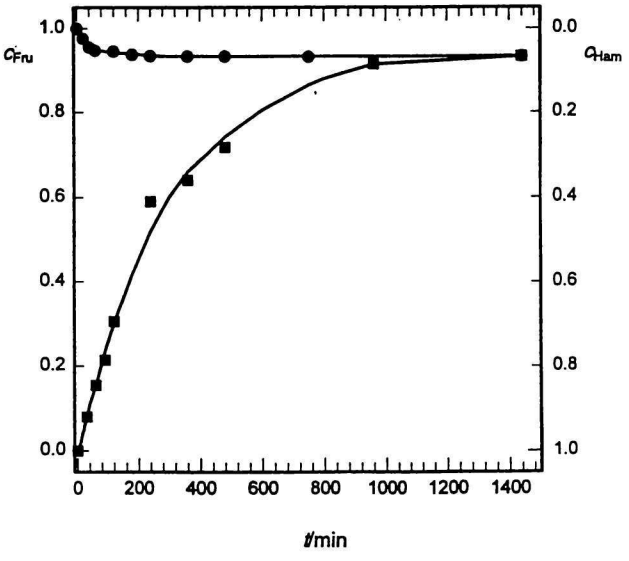


Fig. 1. Time dependence of the concentration of D-fructose (●) and D-hamamelose (■) during the conversion to their mutual equilibrium in 0.2 % molybdic acid at 80 °C.

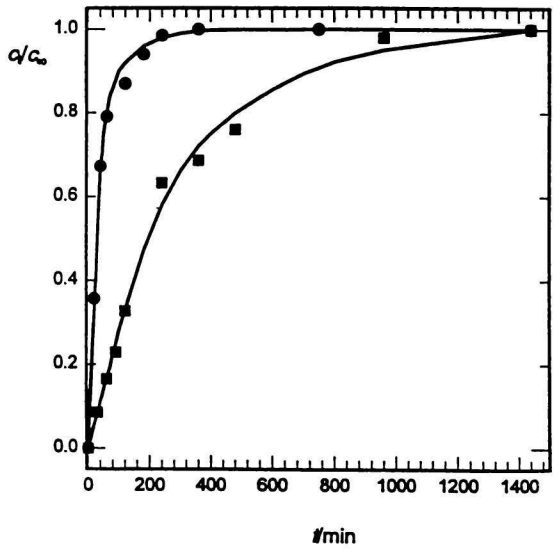


Fig. 2. Comparison of the rates of molybdic acid-catalyzed interconversions of D-fructose and D-hamamelose; conversion D-fructose → D-hamamelose (●), conversion D-hamamelose → D-fructose (■).

tion at each time (c_t) and at the equilibrium (c_∞) are shown in Fig. 2.

RESULTS AND DISCUSSION

Preliminary ^{1}H NMR investigation of reaction mixtures obtained from treatment of D-fructose with a catalytic amount of molybdic acid at increased temperatures revealed the presence of four singlets in the anomeric region of the spectra. A comparison of these singlets with published data [15] suggested that they correspond to four tautomeric forms of D-hamamelose. In order to perform a detailed study of the interconversion, a multi-gram quantity of D-hamamelose was prepared first. Thus, a 4 h heating at 80 °C of a 10 % aqueous solution of D-fructose containing 0.2 % of molybdic acid, followed by removal of the catalyst, fermentation of unreacted starting ketose and a chromatographic separation, afforded the requested branched-chain aldose in 6.5 % yield. D-Hamamelose thus obtained is of a high purity due to its significant retention on a strongly acidic cation-exchange resin in the Ba^{2+} form. Thus the sugar, similarly as riboses and taloses [16, 17], can be easily separated from complex mixtures by this chemisorption process. In spite of this high purity, as shown by ^{1}H NMR spectroscopy and elemental analysis, we have not succeeded to obtain D-hamamelose in the crystalline state.

Detailed examination of the ^{1}H NMR spectra showed that the conversion D-fructose → D-hamamelose in 0.2 % aqueous molybdic acid at 80 °C reached its equilibrium x_τ = 14:1 in 4 h (Fig. 1). The ratio of the two sugars was determined by integration of all four anomeric singlets of D-hamamelose and three signals of the furanose form of D-fructose, namely H-3β, H-4β, and H-3α. When the conversion was performed, at otherwise identical reaction conditions, using D-hamamelose as the starting compound, the ratio of sugars formed was 3 2 after 4 h (Fig. 1). In this case, the same equilibrium of the sugars, x_τ = 14:1, was reached after 24 h. The significant difference in the rates of conversion D-fructose → D-hamamelose and the reverse conversion is more obvious from the time dependence of c_t/c_∞ (Fig. 2), and can be explained by the different extent of the formation of unreactive complexes of the sugars with molybdic acid. Unlike D-fructose, D-hamamelose apparently forms similar, catalytically ineffective complexes as D-ribose [18]. Thus, in the early stage of the D-hamamelose → D-fructose conversion, the effective concentration of catalyst is lower than its total concentration so that the rate of the conversion is lower as well. Due to these significant differences in the reaction rates of the reversible reaction, it was impossible to further analyze the kinetics of the interconversion D-fructose ⇌ D-hamamelose applying kinetic equations valid for a simple reversible reaction A ⇌ B.

In order to analyze the reaction mechanism in detail and to confirm the structure of the final compounds, a regiospecifically ^{13}C-substituted D-fructose was used for the isomerization study. ^{1}H NMR spectrum of D-(2-^{13}C)hamamelose, obtained from D-(2-^{13}C)fructose, is shown in Fig. 3*b*. The spectrum, in comparison to ^{13}C-natural abundance ^{1}H NMR spectrum of D-hamamelose (Fig. 3*a*), is more complex due to splittings of signals for anomeric protons and some of ring protons. The presence of two-bond proton-carbon coupling constants ($^2J_{C-H}$), visible mainly in the anomeric region (inset in Fig. 3*b*), can be considered as evidence of the presence of ^{13}C-2 in the structure. Since the value of $^2J_{C-H}$ depends, *inter alia*, upon geometry of the segment C-2—C-1—H-1, various magnitudes of $^2J_{C-H}$ in the furanose and the pyranose forms (both α and β) were observed, ranging from $^2J_{C-H}$ = 5.14 Hz (α-pyranose) to $^2J_{C-H}$ < 1.0 Hz (β-furanose). The position of the ^{13}C atom in carbon skeleton could also be monitored by the changes in ^{1}H-decoupled ^{13}C NMR spectra of the starting compound (Fig. 4*a*) and of the product (Fig. 4*b*). As shown in the spectra, 100 % abundance of ^{13}C-2 in D-(2-^{13}C)fructose resulted in appearance of strong signals in the spectrum in anomeric region (Fig. 4*a*) with mutual ratio corresponding to the equilibrium of individual forms (α, β, furanose, pyranose) of fructose in aqueous solution. Although these resonances were found more intense than those originating from ^{13}C natural abundance carbons, the relative intensities (*i.e.* ^{13}C-2 with respect to other carbons) did not correspond quantitatively to their isotopic abundances in the compound owing to a relatively short relaxation delay used during the acquisition of ^{13}C NMR spectra. Such a delay did not allow to fully

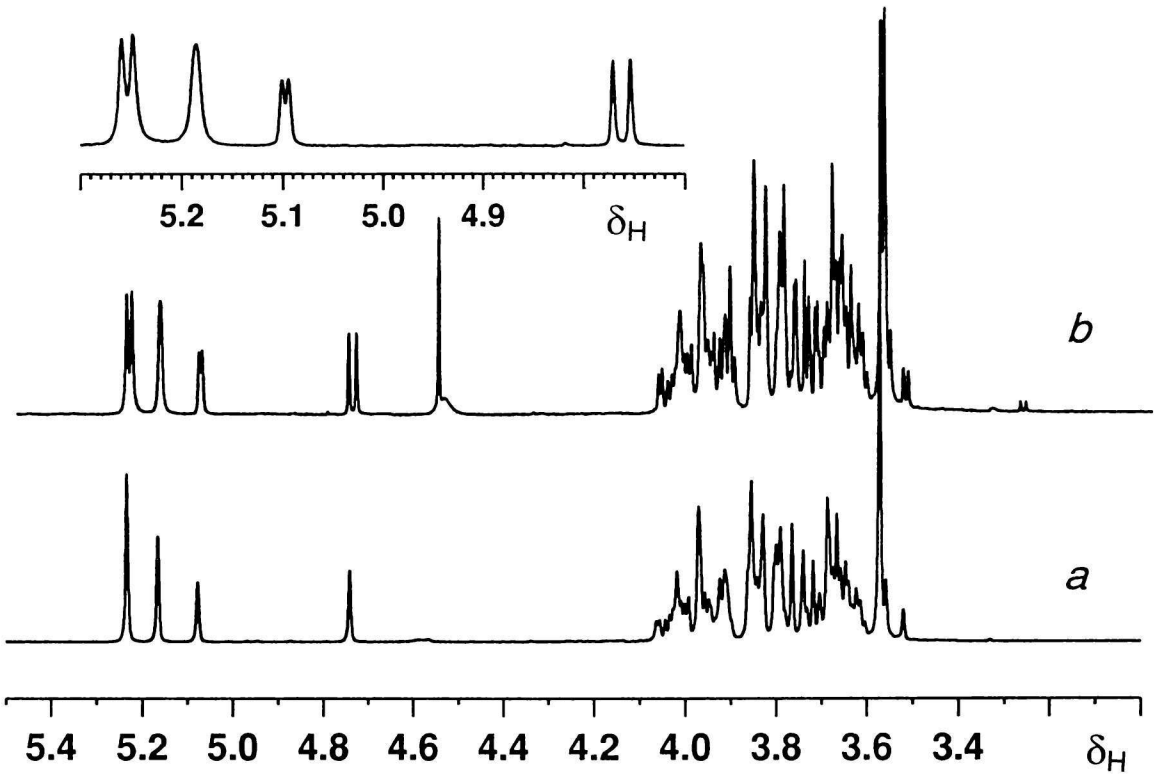


Fig. 3. 300 MHz ^{1}H NMR spectrum of D-hamamelose in aqueous solution at 40 °C. Conventional spectrum of natural abundance compound (*a*) and the compound obtained by epimerization reaction from D-(2-^{13}C)fructose in the presence of molybdate ions (*b*). Splittings, visible mainly in the anomeric proton resonances (inset), originated from two-bond proton-carbon coupling constants between H-1 and ^{13}C-2 in the synthesized compound, 2-*C*-(hydroxymethyl)-D-ribose (D-hamamelose).

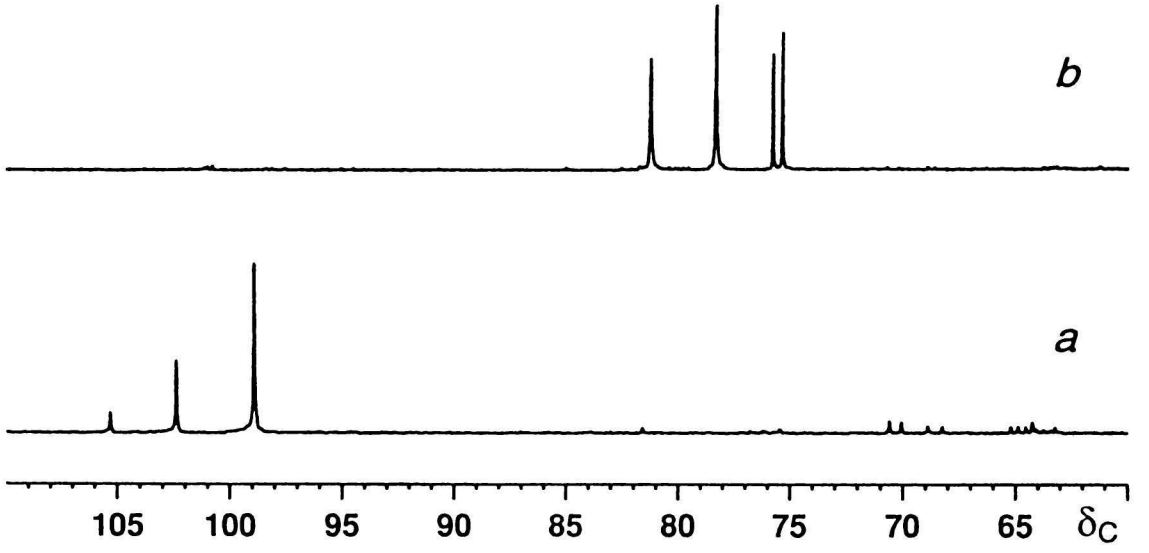


Fig. 4. 75.5 MHz ^{1}H-decoupled ^{13}C NMR spectra, in aqueous solution at 40 °C, of the starting compound, D-(2-^{13}C)fructose (*a*), and the product, D-(2-^{13}C)hamamelose (*b*) obtained from rearrangement reaction.

relax the carbonyl carbon atoms due to their relatively long longitudinal relaxation time in comparison to those of the CH or CH_2 carbon atoms. In the product, D-(2-^{13}C)hamamelose, four signals for quaternary C-2 were present in ^{13}C NMR spectrum. They corresponded to four cyclic forms; β-furanose (δ = 81.2), α-furanose (δ = 78.3), α-pyranose (δ = 75.7), and β-pyranose (δ = 75.3) and well agreed with the data published [15]. Another evidence for the presence of ^{13}C-2 in D-(2-^{13}C)hamamelose was based on two-

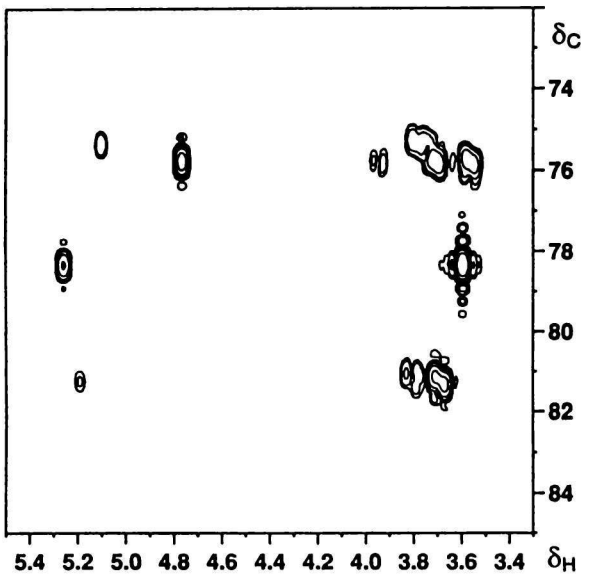


Fig. 5. Part of two-dimensional HMBC spectrum of D-hamamelose. The cross-peaks correspond to scalar interactions, among ^{13}C-2 and H-3, H-4, H-2′, across two and three bonds.

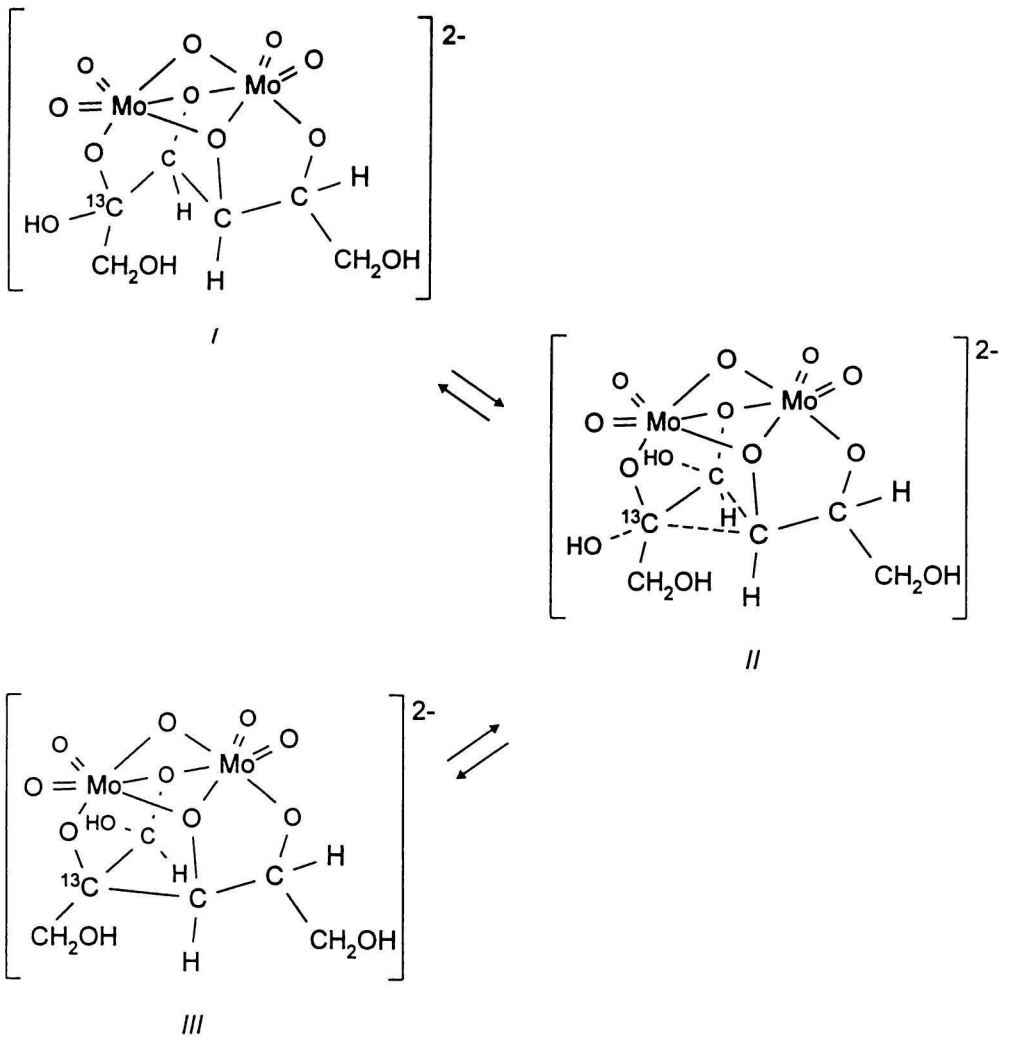
dimensional HMBC spectrum (Fig. 5). Two sets of scalar interactions were detected in the spectrum. The first one corresponded to the interaction between the anomeric protons (at $\delta = 5.25$ (α-furanose), $\delta = 5.18$ (β-furanose), $\delta = 5.09$ (β-pyranose), and $\delta = 4.76$ (α-pyranose)) and the quaternary ^{13}C atoms (at $\delta = 81.2$, $\delta = 78.3$, $\delta = 75.7$, and $\delta = 75.3$). The other cross-peaks represented scalar interactions among ^{13}C-2 nuclei and H-3, H-4, and H-2′ protons, respectively. The intensity of the cross-peaks (not shown) varied according to the magnitudes of $^2J_{C-H}$ and reflected the mentioned structural factors in the molecule.

The stereospecific transformation of D-fructose to D-hamamelose and *vice versa* observed, proved also by regiospecifically ^{13}C-substituted sugars, is in accordance with the known mechanism of the Bílik reaction [5]. The mechanistic model, however, has to be modified according to the strict stereochemical rules of molybdenum(VI) complexes, valid also for dimolybdate complexes of D-lyxose [19], D-mannitol [20], and erythritol [21], that have been adopted as models for the explanation of the stereospecific molecular rearrangement. In agreement with the rules, two molybdenum atoms are surrounded by nine in-the-complex-shared oxygen atoms which form two distorted octahedrons joined together *via* three common oxygen atoms. In such a complex, five oxygen atoms come from dimolybdate anion and form four Mo=O terminal bonds and a Mo—O—Mo bridge. The other four oxygen atoms, two of them shared by both molybdenum atoms, come from four adjacent sugar hydroxyls and are linked in the ester-type bonds. The two shared sugar oxygen atoms are located opposite to the terminal oxygen atoms, while two unshared ones occupy the octahedral vertices opposite to the bridge oxygen atom. It is very probable that the catalytically efficient molybdate complexes of reducing sugars (aldoses or 2-ketoses) obey these rules as well. It means that the reducing sugar enters the complex, not *via* its carbonyl oxygen atom but *via* one of its *gem*-diol oxygen atoms of its hydrated form. This assumption is well supported by the observation of high populations of such molybdate complexes in ^{1}H NMR spectroscopy [22]. In special cases, such as dimolybdate complexes of aldotetroses and 5-deoxyaldopentoses, aldoses form complexes solely in their acyclic hydrated forms, in the zig-zag (D-threose, 5-deoxy-L-arabinose) or sickle conformations (D-erythrose, 5-deoxy-L-ribose) [23]. Aldooctoses having two quartets of complexing hydroxyl groups behave very similarly [24].

The mechanism of the rearrangement is shown in Scheme 1. D-(2-^{13}C)Fructose in the catalytically efficient dimolybdate complex *I* is linked *via* its four hydroxyl oxygen atoms at C-2, C-3, C-4, and C-5 of its hydrated form. Hence, the rearrangement can occur in the transition state *II*. Bond formation between carbon atoms C-2 and C-4 and simultaneous cleavage of the C-3—C-4 bond gives rise to the dimolybdate complex of D-(2-^{13}C)hamamelose *III*, while the opposite process regenerates D-(2-^{13}C)fructose. During the process, zig-zag conformation of the complexing site of the starting D-fructose is changing into the sickle conformation of the complexing site of the product D-hamamelose, which is in accordance with the ^{1}H NMR observations made for model sugars.

As already mentioned, the interconversion afforded an equilibrium of D-fructose and D-hamamelose x_r = 14:1, independently of the starting sugar. Since only a truly catalytic amount of molybdic acid was used (expressed as the mole ratio of dimolybdate to sugar y_r = 1:100), the ratio reached can be considered as the thermodynamic equilibrium of the two sugars. The recently described analogous interconversion of D-fructose and D-hamamelose catalyzed by nickel(II)—diamine complexes, claiming 56 % of the latter sugar in its equilibrium [25, 26], is very similar in its nature. In spite of such a high conversion of D-fructose to the naturally occurring branched-chain sugar, the authors have not given a preparative example. In addition, as the amounts of the D-fructose and the catalyst used were equimolar, the Ni(II)—diamine-catalyzed process did not give the two sugars in the thermodynamically equilibrium ratio but in that of their complexes.

Finally, the formation of a significant amount (2.5 %) of the by-product D-sorbose is noteworthy. As clearly shown in the interconversion study with D-(2-^{13}C)fructose, at the relatively mild reaction conditions applied, D-(2-^{13}C)sorbose was the only by-product. The most simple explanation of its formation can be drawn from the general, acid-catalyzed



Scheme 1

isomerization of D-fructose. In such a case, however, in spite of sorbose being the most stable 2-hexulose, other 2-hexuloses should also be present in the reaction mixture. Therefore it is very likely that D-sorbose is formed from D-fructose by a molybdic acid-catalyzed secondary process, *e.g.* in analogy with the formation of D-xylose from D-arabinose and *vice versa* [5, 27]. The corresponding 2-*C*-(hydroxymethyl)-D-lyxose, possibly formed from D-sorbose by the molybdic acid-catalyzed primary process, was not detected in the reaction mixture due to its low concentration. The molybdic acid-catalyzed conversion of sorbose and other 2-ketoses to the corresponding 2-*C*-(hydroxymethyl)aldoses will be published elsewhere.

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