^{13}C NMR studies of isomerization of D-glucose in an aqueous solution of $Ca(OH)_2$. The effect of molecular oxygen

A. N. Simonov, O. P. Pestunova, L. G. Matvienko, and V. N. Parmon*

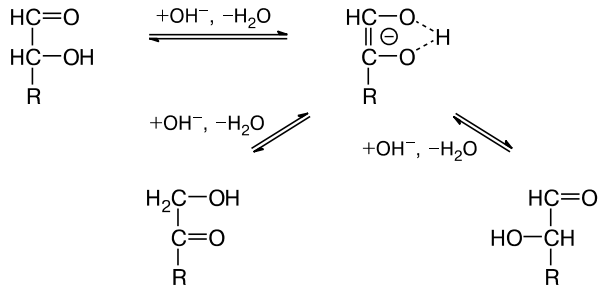
G. K. Boreskov Institute of Catalysis, Siberian Branch of the Russian Academy of Sciences, 5 prosp. Akad. Lavrent´eva, 630090 Novosibirsk, Russian Federation. Fax: +7 (383) 330 4719. E-mail: oxanap@catalysis.ru

Isomerization of D-glucose to fructose and mannose in aqueous solutions of $Ca(OH)_2$ with the initial pH 11.4 in a temperature interval of 20—90 °C was studied by ^{13}C NMR spectroscopy in the presence and absence of dissolved oxygen. In the presence of oxygen, the apparent equilibrium isomerization constant is much lower than that in the absence of oxygen. This is related to the oxidation of monosaccharides to formic and aldonic acids, a decrease in the pH of solutions, and cessation of isomerization at pH < 9.

Key words: carbohydrates, glucose, fructose, rearrangement, ^{13}C NMR spectroscopy, alkaline solution, isomerization.

Epimerization of aldoses and their rearrangement to ketoses, which occur in parallel in weakly alkaline aqueous solutions, the Lobry de Bruyn—Alberda van Ekenstein rearrangement (hereinafter LdB—AvE) were discovered in the late 1890s.[1,2] For a long time, this reaction remained to be the simplest and most convenient method for the synthesis of a series of rare monosaccharides. The isomerization is known[3,4] to proceed through the enediol form (Scheme 1).

Scheme 1



At the same time, the kinetics of transformation and accumulation of monosaccharides remains unclear even for the most studied reaction of this type, namely, isomerization of glucose to fructose and mannose.[5—8] The use of polarimetry[5,6] for this purpose is not quite correct,[9,10] because the change in the optical rotation is affected by both isomerization in the presence of $Ca(OH)_2$ as the catalyst and formation of monosaccharide complexes with calcium ions. The study of the LdB—AvE reaction by HPLC showed that the reaction is accompanied by side processes and the composition of the products depends on the nature of the starting monosaccharide.[11,12]

In the present work, we studied the transformation of glucose in an aqueous solution of calcium hydroxide by ^{13}C NMR spectroscopy. Isomerization was carried out in a wide temperature interval from 20 to 90 °C in argon, oxygen, and air.

Experimental

D-Glucose (purity grade, Reakhim), D-fructose (99%, Sigma), $Ca(OH)_2$ (analytical purity grade, Reakhim), NaOH (analytical purity grade, Reakhim), and HCl (reagent grade, Reakhim) were used. A suspension of $Ca(OH)_2$ (0.09 g) in 50 mL of water was prepared using a Bransonic 1510R-NT (USA) ultrasonic bath for 25 min.

D-Glucose (9.0 g, 0.05 mol) and the suspension of $Ca(OH)_2$ (50 mL) were placed in a three-necked reactor kept at constant temperature and equipped with a thermometer, a tube for gas supply, and a magnetic stirrer. If the reaction was carried out in argon or oxygen, the reactor was preliminarily purged with the corresponding gas, which was supplied through a temperature-controlled saturator with distilled water during the reaction. The system became homogeneous during the reaction. The temperature interval of the reaction in air was 20—90 °C, and that for argon and oxygen was 50—90 °C.

Samples for analysis were withdrawn every 24 h at room temperature, and for other temperatures the intervals were the following: at 50 °C, 12 h; at 60 and 70 °C, 20—40 min in air and oxygen and 60—90 min in argon; at 80 and 90 °C, 10—20 min. The samples were cooled to room temperature and analyzed (on

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1909—1913, August, 2005.

1066-5285/05/5408-1967

the same day) by ^{13}C NMR spectroscopy on a Bruker DPX-250 pulse FT NMR spectrometer (Germany) at room temperature. Spectra were accumulated over 256 scans with a pulse delay of 3.088 s, which made it possible to obtain an acceptable signal-to-noise ratio. To reveal oxidation products of saccharides and other by-products in a reaction mixture, we recorded ^{13}C NMR spectra with a pulse delay of 6.088 s and a scan number of 8237. Acetone (δ_{Me} 30.89)[13] was used as the internal standard.

In experiments at 60 and 80 °C (in argon and air), samples were additionally analyzed by UV spectroscopy. The spectra were recorded in a 0.1-cm cell with a UVIKON 923 spectrometer (Kontron, Italy).

A solution of D-glucose (0.5 mol L^{-1}) in 0.5 *M* NaOH (pH 12.5) was stored under argon for 18 h and acidified with 2 *M* HCl to pH 11.4, 10.9, 10.5, 9.4, 8.0, and 6.2 (I-120 pH-meter, USSR), and absorption spectra were recorded.

The volume of absorbed oxygen was measured in a volumetric setup with a reactor, the temperature of which was maintained at 60 °C.

Results and Discussion

The chemical shifts of the C atoms of different isomeric forms of glucose, fructose, and mannose in the ^{13}C NMR spectra were assigned using published data.[14,15]

The relative content of each monosaccharide in a reaction mixture was determined from the integral intensities of signals corresponding to the CH_2 groups.

The intensities of signals for mannose at temperatures below 80 °C corresponded to the mannose concentration not higher than 1% of the initial concentration of glucose. Only at 90 and 80 °C, the mannose content in the mixture was 2—3% of the initial glucose concentration. Therefore, this concentration was neglected in further calculations of concentrations of glucose and fructose.

When the process was carried out in air at room temperature, the apparent equilibrium was not attained even after nine days (216 h). The calculated concentrations of monosaccharides glucose, fructose, and mannose under these conditions obtained by the extrapolation of the kinetic curves were 86, 14, and $\ll$1%, respectively. These values of the apparent equilibrium concentrations differ substantially from those determined by polarimetry under identical conditions[5,6]: 70, 28.5, and 1.5%, respectively. For the isomerization of D-glucose in a solution of KOH in an inert atmosphere,[11,12] the content of these monosaccharides was 50.0, 33.5, and 8.4%, respectively (HPLC). The mixture also contained 9.1% of other saccharides.

As should be expected, the increase in temperature is accompanied by a considerable shortening of the time of apparent equilibration. For the isomerization in argon, this time is longer than in air and oxygen (Table 1).

The concentrations of monosaccharides at the end of the reaction also differ substantially (Table 2).

We calculated the apparent equilibrium constants (K_i) of the isomerization of glucose to fructose under the conditions studied using the obtained concentrations of different forms of saccharides (see Table 2). The temperature plots of the resulting K_i values in the Arrhenius coordinates are presented in Fig. 1. These plots are very similar for the isomerization in oxygen and air (see Fig. 1). In the presence of oxygen, the increase in temperature is accompanied by an increase in K_i. On the contrary, when the isomerization occurs in argon, K_i decreases slightly with temperature. At high temperatures (80—90 °C), the K_i values for inert and oxygen-containing atmospheres are almost the same, while at 50 °C they differ approximately twofold.

Table 1. Time of apparent equilibration between glucose and fructose in air, oxygen, and argon

T/°C	Time of apparent equilibration/h	
	Air, oxygen	Argon
20	>216	—
50	48	71
60	2.5	6
70	<1	2.5
80	*	1
90	1/4	3/4

* 25 min.

To reveal a reason for that strong differences in the isomerization parameters in the presence and absence of oxygen at low temperatures, we studied the isomerization

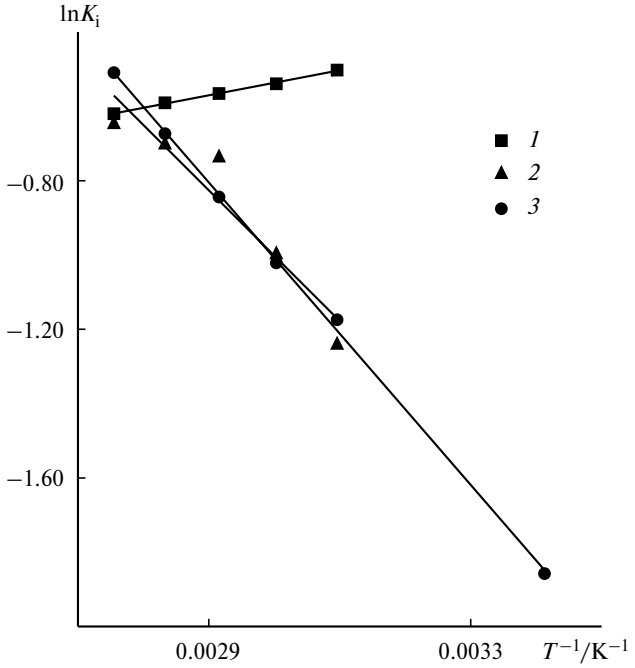


Fig. 1. Temperature plots of the obtained apparent constants K_i in the Arrhenius coordinates for D-glucose isomerization in argon (*1*), oxygen (*2*), and air (*3*).

Table 2. Calculated apparent equilibrium isomerization constants (K_i) and concentrations (C) of monosaccharides at the end of the reaction under different conditions

T/°C	Air			Oxygen			Argon		
	C/mol L^{-1}		K_i	C/mol L^{-1}		K_i	C/mol L^{-1}		K_i
	Glc	Fru		Glc	Fru		Glc	Fru	
20	0.86	0.14	0.16	—	—	—	—	—	—
50	0.76	0.24	0.31	0.78	0.22	0.29	0.62	0.38	0.61
60	0.73	0.27	0.37	0.73	0.27	0.37	0.63	0.37	0.58
70	0.70	0.30	0.43	0.68	0.32	0.48	0.64	0.36	0.57
80	0.67	0.33	0.50	0.67	0.33	0.50	0.65	0.35	0.55
90	0.63	0.375	0.60	0.66	0.34	0.53	0.65	0.35	0.54

of glucose at 60 and 80 °C in more detail, including the kinetics of oxygen absorption from air by the reaction mixture at 60 °C (Fig. 2). In addition, changes in the absorption spectra in the UV region (Fig. 3) and in the pH of solutions (Fig. 4) at 60 and 80 °C in air and argon were studied in parallel. The effect of pH is very important, because the rate of the LdB—AvE reaction was shown[11,12] to depend strongly on the pH of solutions, no interaction occurring at pH < 9.

Oxygen absorption by the reaction mixture is described by the zero-order kinetics with respect to oxygen and stops after ~3 h (see Fig. 2). A small induction period in the initial region can be explained by the time necessary for complete dissolution of glucose. When the solution that absorbed oxygen is acidified to pH 7, some amount of oxygen is evolved, which is, most likely, reversibly bound with monosaccharides. The amount of irreversibly absorbed oxygen was approximately 2.2% of the amount of glucose. After completion of the reaction in the presence of oxygen, the ^{13}C NMR spectra also contained signals at δ 170—220 corresponding to the carbonyl groups. Figure 5 shows the spectrum of the reaction mixture after completion of the reaction in oxygen at 80 °C. The spectrum was detected with a pulse delay of 6.088 s and a scan number of 8192 and exhibits signals for by-products that

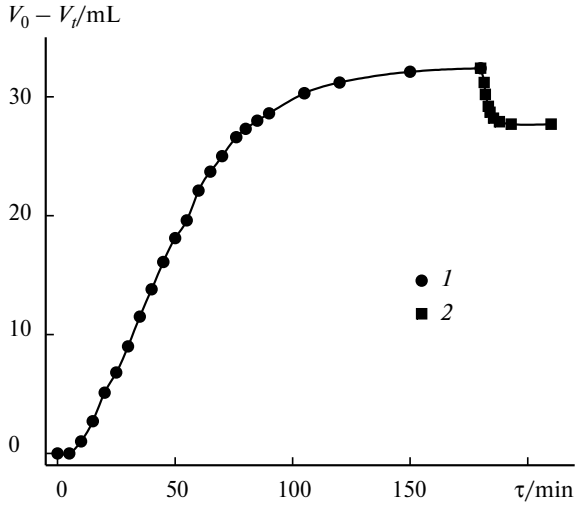


Fig. 2. Kinetics of oxygen absorption by an aqueous solution of glucose at 60 °C under atmospheric pressure (*1*) and oxygen evolution upon acidification of the final solution to pH 7 (*2*).

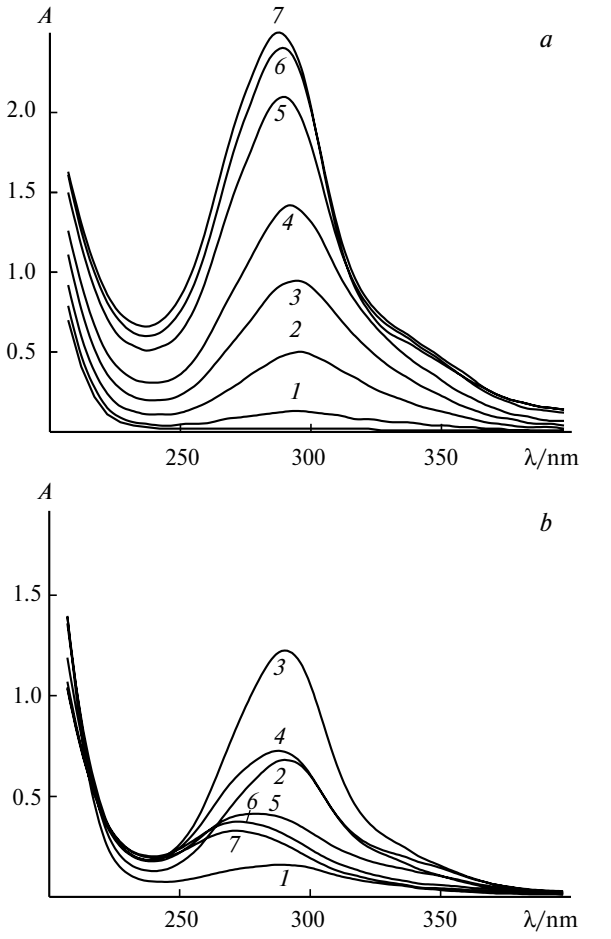


Fig. 3. Changes in the electronic absorption spectra of aqueous solutions of glucose during isomerization at 60 °C in argon (*a*) and in air (*b*); *a*: t = 30 (*1*), 60 (*2*), 90 (*3*), 120 (*4*), 240 (*5*), 300 (*6*), and 360 min (*7*); *b*: t = 30 (*1*), 60 (*2*), 90 (*3*), 120 (*4*), 150 (*5*), 180 (*6*), and 210 min (*7*).

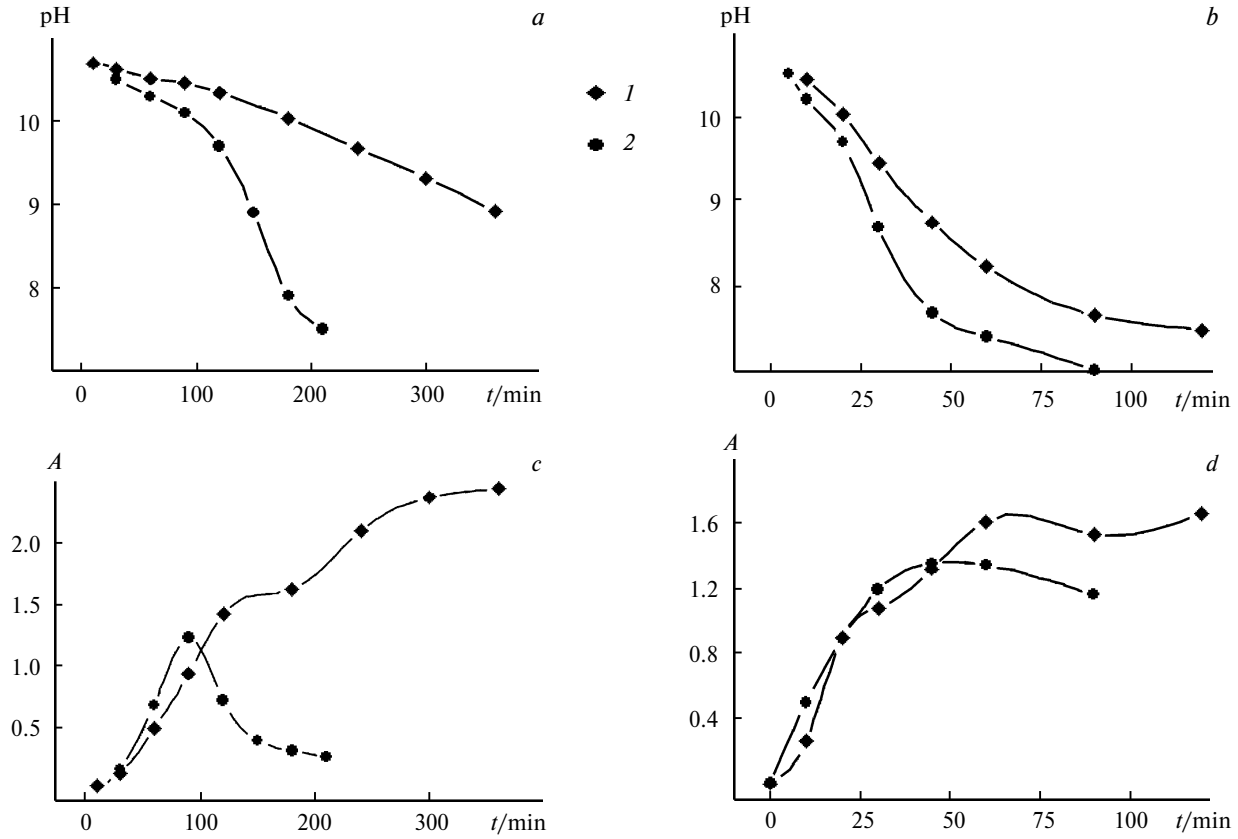


Fig. 4. Changes in pH (*a*, *b*) and absorbance (*c*, *d*) of aqueous solutions of glucose at 290 nm in an inert atmosphere (*1*) and in air (*2*) during isomerization at 60 (*a*, *c*) and 80 °C (*b*, *d*).

are present in the reaction mixture. Their amount is about 2—3%.

Oxygen absorption by solutions of monosaccharides can be explained[16] by the oxidation of the latter to acids. In this case, oxygen attacks the same enediol form of monosaccharides, which is the intermediate in the monosaccharide rearrangement. The formation of acids explains completely the observed decrease in the pH of the solution by more than three units (from 10.7 to 7.5, see Fig. 4, *a*).

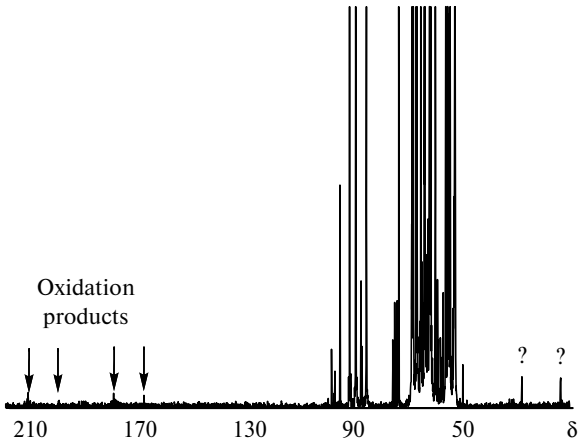


Fig. 5. ^{13}C NMR spectrum of the reaction mixture after the end of D-glucose isomerization in $Ca(OH)_2$ solutions in an oxygen atmosphere at 80 °C.

The kinetics of changes in pH of solutions in an inert atmosphere and in oxygen differ significantly (see Fig. 4). In the presence of oxygen, pH decreases much more rapidly, and the sharp decrease in pH (see Fig. 4, *a*) occurs simultaneously with the retardation of oxygen absorption (see Fig. 2). In an inert atmosphere, pH of solutions also decreases, but much more slowly (see Fig. 4). In this case, the decrease in pH is related, most likely, to rearrangements of carbohydrates due to which saccharinic acids are formed. A comparison of the kinetic curves of pH changes (see Fig. 4) and the times of apparent equilibration of the system (see Table 1) shows that the isomerization ceases approximately when the pH of the solutions becomes lower than 9.

Changes in the concentration of the active intermediate enediol form of monosaccharides during the reaction can be monitored by UV spectroscopy.[17,18] As can be seen from the data in Figs 3, *a* and *b*, the absorption band appears at $\lambda = 290$ nm in the presence and absence of oxygen. The absorbance of this band in argon increases

and reaches a plateau to the moment of equilibration. In the presence of oxygen, the absorbance of this band first increases and then decreases but a new band with a maximum at λ = 270 nm appears. The absorbance maximum at λ = 290 nm corresponds to the transition from the slow region of the kinetic curve of pH change to the fast region.

The behavior of monosaccharides in aqueous-alkaline media (pH ≥ 12.5) was studied by UV spectroscopy.[17,18] It was shown[17] that in an inert atmosphere the spectrum of glucose contains an absorption band with λ = 310 nm, which shifts to 340 nm in the presence of $CaCl_2$. After saturation of an alkaline aqueous solution of a sugar with oxygen, an intense band appears at λ = 265 nm. This band can be ascribed[17] to oxidation products of carbohydrates. A comparison of the kinetics of changes in the absorbance of bands in the UV spectra with the kinetics of H/D exchange and degradation of sugars suggests that the band with λ = 310 nm is caused by the absorbance of the enediol anion, the band at λ = 340 nm is attributed to calcium complexes with the enediol form of carbohydrate, and that with λ = 265 nm corresponds to the oxidation products of monosaccharides. Similar conclusions about the nature of bands with λ = 310 and 340 nm are reported.[18]

The spectra observed in the present work somewhat differ from those described in literature. In an oxygen-containing atmosphere, they contain two intense bands with λ = 270 and 290 nm and a weak band at λ = 340 nm. Low intensity of the latter can be explained by low concentration of calcium as compared with that of glucose. The shift of the band with λ = 310 nm, which corresponds to the enediol anion, to λ = 290 nm is related to lower pH values of the solutions under study. In fact, the UV spectrum of a 0.5 *M* solution of D-glucose in 0.5 *M* NaOH (pH 12.5) stored under argon at room temperature for 18 h contains a single band with λ_{max} = 310 nm. When this solution is acidified to pH 11.4, 10.5, 9.4, and 8.0, the position of the band maximum shifts to 290 nm and the absorbance decreases slightly, which can be related, probably, to the transformation of the enediol anion into the protonated form.

Differences in the absorption spectra and dynamics of pH changes in aqueous solutions of glucose in an inert and oxygen-containing atmosphere are much more pronounced at 60 °C than at higher (80 °C) temperature (see Figs 3 and 4). At the same time, the concentrations of glucose and fructose at the end of the reaction in an inert and oxygen-containing atmosphere at 80 and 90 °C are almost the same, which can be explained as follows. At least two reactions contribute to a decrease in pH of solutions in the presence of oxygen. These are, first, oxidation of saccharides with atmospheric oxygen to form acids and, second, rearrangement of carbohydrates to form saccharinic acids. Since the first reaction is of zero order with respect to the oxygen pressure over the solution, we believe that its rate is mainly determined by dissolution of oxygen in water. At high temperatures, the solubility of oxygen in water decreases and, hence, this reaction rate should decrease with temperature. At the same time, rearrangements of monosaccharides in the aqueous phase involve no gaseous oxygen and, therefore, their rates increase and become higher than the oxidation rate. As a result, the pH of the solution at an elevated temperature has time to decrease to a critical value of 9 more rapidly than noticeable oxidation of monosaccharides with oxygen occurs.

Our study of isomerization shows that the data obtained in the early XX century on the equilibrium (as it was believed) concentrations of monosaccharides formed by the LdB—AvE rearrangement should be corrected substantially using the modern direct methods of investigation. The ratio of concentrations of isomeric monosaccharides at the end of isomerization in aqueous solutions is substantially affected by both temperature and pH. In addition, it should be taken into account that the presence of oxygen in a solution and, as a consequence, the formation of an even insignificant amount of oxidation products of monosaccharides can result in a substantial and usually uncontrolled decrease in pH of the solution. As a result, the isomerization can be retarded strongly followed by the apparent equilibration, which does not correspond to the real thermodynamic properties of saccharides. Therefore, the data on isomerization constants of saccharides can be considered as correct only when the reaction occurs in pre-deaerated solutions under pH-static conditions.

The authors thank A. V. Golovin for help in registration of NMR spectra.

This work was financially supported by the Division of Chemistry and Materials Science of the Russian Academy of Sciences (Grant 9.3), Program "Integration Projects of the Siberian Branch of the Russian Academy of Sciences" (Grant 148), and Council on Grants of the President of the Russian Federation (Program of State Support for Leading Scientific Schools of the Russian Federation, Grant NSh 1484.2003.3).

References

1. C. A. Lobry de Bruyn and W. A. Alberda van Ekenstein, *Recl. Trav. Chim. Pays-Bas*, 1895, **14**, 195.
2. C. A. Lobry de Bruyn and W. A. Alberda van Ekenstein, *Recl. Trav. Chim. Pays-Bas*, 1897, **16**, 256.
3. J. C. Speck, Jr., *Adv. Carbohydr. Chem.*, 1958, **13**, 63.
4. S. J. Angyal, in *Glycoscience: Epimerization, Isomerization, and Rearrangement Reactions of Carbohydrates*,

Ed. A. F. Stütz, Springer-Verlag, Berlin—Heidelberg, 2001, p. 1.
5. J. C. Sowden and R. Schaffer, *J. Am. Chem. Soc.*, 1952, **74**, 505.
6. M. L. Wolform and W. L. Lewis, *J. Am. Chem. Soc.*, 1928, **50**, 837.
7. A. M. Kuzin, *Zh. Obshch. Khim.*, 1935, **5**, 1373.
8. R. Yanagihara, K. Saeda, S. Osana, and S. Yshikawa, *Bull. Chem. Soc. Jpn*, 1997, **66**, 2268.
9. K. Fujino, J. Kobayashi, and I. Higuchi, *Nippon Kagaku Kaishi*, 1972, 2287.
10. J. A. Rendelman, Jr., *Adv. Carbohydr. Chem.*, 1966, **21**, 209.
11. H. S. El Khadem, S. Ennifar, and H. S. Isbell, *Carbohydr. Res.*, 1987, **169**, 13.
12. H. S. El Khadem, S. Ennifar, and H. S. Isbell, *Carbohydr. Res.*, 1989, **185**, 51.
13. H. E. Gottlieb, V. Kotlyar, and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512.
14. *The Sadtler Standard Spectra: C-13 Nuclear Magnetic Resonance Spectra*, Sadtler Research Lab., Philadelphia, 1978, No. 822c; No. 4681c.
15. K. Bock and R. Thoegersen, *Ann. Repts NMR Spectroscopy*, 1982, **13**, 1.
16. W. B. Gleason and R. Barker, *Can. J. Chem.*, 1971, **49**, 1425; 1433.
17. G. de Wit, A. P. G. Kieboom, and H. van Bekkum, *Carbohydr. Res.*, 1979, **74**, 157.
18. T. I. Khomenko and O. V. Krylov, *Kinet. Katal.*, 1974, **15**, 625 [*Kinet. Catal.*, 1974, **15** (Engl. Transl.)].

Received January 30, 2004;
in revised form January 19, 2005