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Enantioselective catalysis. Part 102 [1]. Epimerization of glucose and mannose in the presence of nickel(II) complexes of optically active ligands

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Abstract

The epimerization reaction glucose ⇌ mannose, catalyzed by nickel(II) complexes, was investigated. The achiral ligand *N,N,N',N'*-tetramethyl-1,2-diaminoethane **1** and the chiral ligands (*R,R*)- and (*S,S*)-*N,N,N',N'*-tetramethyl-1,2-diamino-1,2-diphenylethane **2** and **3** were used. The standard epimerization of glucose and mannose, respectively, was carried out in methanol at 60°C using a stoichiometric amount of nickel(II) complex. The influence of substoichiometric and overstoichiometric amounts of the catalyst and the temperature and time dependence were studied. Some of the kinetic and thermodynamic parameters turned out to be dependent on whether the (*R,R*)- or (*S,S*)-ligand **2** or **3** was used in the nickel(II) complex. The data were subjected to a Michaelis–Menten and Eyring analysis.

Keywords: Glucose; Mannose; Epimerization; Nickel(II) catalysts; Achiral and chiral ligands

1. Introduction

In 1972 Bilik et al. found that glucose and mannose can be interconverted using acidified molybdate solutions [1–3], an important process with respect to the conversion of the more abundant glucose into the less abundant mannose and its derivatives. In a series of papers Yoshikawa et al. and Tanase et al. showed that the epimerization glucose ⇌ mannose can also be brought about by nickel(II) complexes with ligands of the 1,2-diaminoethane type, the best ligand being *N,N,N',N'*-tetramethyl-1,2-diaminoethane [4–11]. The mechanism of this epimerization is a rearrangement of the carbon chain of glucose and mannose. When bound in the nickel(II) complex, carbon atom C3 of glucose and mannose, normally connected to carbon atom C2, migrates to carbon atom C1. Proton shifts complete the exchange of oxidation states between the former and the new C1 and C2 positions. Thus, the only observable change is an inversion of the configuration at carbon atom C2, resulting in a transformation of glucose into mannose and vice versa. The rearrangement of the carbon chain in this epimerization was proven by ^{13}C labeling studies [12]. In the present paper we describe our experiments to investigate whether the epimerization glucose ⇌ mannose, which normally requires stoichio-

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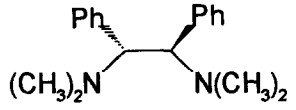
metric amounts of nickel(II)–diamine complexes, can be carried out in a truly catalytic way with substoichiometric quantities of catalyst [13]. In addition to the achiral ligand *N,N,N′,N′*-tetramethyl-1,2-diaminoethane **1**, our study includes the chiral ligand *N,N,N′,N′*-tetramethyl-1,2-diamino-1,2-diphenylethane in both optically active forms **2** and **3**. Our goal was to prove that nickel(II) complexes of the enantiomers **2** and **3** will have an influence on kinetic and thermodynamic parameters of the glucose ⇌ mannose system [13], as indicated in the preliminary study of Ref. [9].

1



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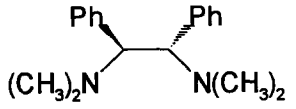

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2. The epimerization glucose ⇌ mannose

2.1. The epimerization scheme

The epimerization of glucose and mannose, respectively, catalyzed by a bis(ligand)nickel(II) complex can be represented by the following simplified Eq. (1).

$$\text{glu} + \text{NiL}_2 \rightleftharpoons \text{glu-NiL}_2 \rightleftharpoons \text{man-NiL}_2 \rightleftharpoons \text{man} + \text{NiL}_2 \quad (1)$$

glu = glucose, man = mannose, NiL_2 = bis(ligand)nickel(II) complex

In principle, such an equilibration requires that starting from glucose and mannose, respectively, the same equilibrium mixture of glucose and mannose results. However, for some of the measurements given in the literature and also for some of the experiments in the present study this is not found. The reasons for this may be threefold:

(i) As in solution glucose and mannose are present in a variety of forms, e.g. the aldehyde form, the α- and β-species of the pyranose form, and the furanose form, there are much more complexation equilibria than shown in Eq. (1). Furthermore, some of these interwoven equilibrations may be even slow reactions. Thus, part of the carbohydrates may be trapped as stable nickel(II) complexes [11] and withdrawn from the equilibria involved in the epimerization reaction.

For workup of the carbohydrates by HPLC analysis, acid is added to the reaction mixture, the temperature is lowered to room temperature and the ligands as well as the nickel salts are removed. This procedure collects all the free carbohydrates present in solution and also the carbohydrates coordinated in labile and stable complexes, an important point if stoichiometric or close-to-stoichiometric amounts of nickel(II) are used. Thus, the concentrations of glucose and mannose given in the tables and figures of the earlier papers and the present work are the total concentrations consisting of the free and complexed forms present in the reaction mixture.

(ii) Another reason for not obtaining the same equilibrium mixtures of glucose and mannose in the epimerization, when starting from glucose and mannose, respectively, may be the deactivation of the catalyst stopping the reaction before the system is in equilibrium.

(iii) Due to the inherent instability of glucose and mannose under the conditions of the epimerization reaction there are irreversible side reactions, e.g. the formation of fructose, which

leads to a successive loss of glucose and mannose, respectively, during the epimerization reaction.

2.2. The standard epimerization procedure

The standard procedure for the epimerization of glucose and mannose, respectively, is carried out as follows. Glucose (mannose) is treated in methanol at 60°C with a stoichiometric amount of a nickel(II) complex containing two equivalents of ligand **1**. Both starting from glucose and mannose, the same equilibrium mixture is obtained after 4 min, consisting of 46.2–46.5% mannose, 48.9–49.4% glucose and 4.4–4.6% fructose (Table 1). Normally, 90 mg glucose and mannose, respectively, were used as starting material. After workup (acidification, deionization, freeze drying) the carbohydrates glucose, mannose and fructose were isolated in up to 95% yield.

3. Catalysis with achiral nickel(II) complexes

3.1. Substoichiometric amounts of catalyst

In a series of experiments it was tested whether in the epimerization of glucose and mannose, respectively, the quantity of nickel(II) catalyst can be reduced from stoichiometric to substoichiometric amounts. 50%, 30%, 10% and 1% of the stoichiometric quantity of $Ni(\mathbf{1})_2Cl_2$ were used. The reactions were carried out at 60°C in methanol solutions as described above. The results are shown in Table 1.

With half the stoichiometric quantity of the nickel(II) complex $Ni(\mathbf{1})_2Cl_2$, the time necessary to reach equilibrium was ca. 10 min. The mannose content was now down to 38.2–39.1% compared to 46.2–46.5% in the standard epimerization with stoichiometric amounts of the nickel(II) complex $Ni(\mathbf{1})_2Cl_2$. The content of glucose was 56.5–57.3% (stoichiometric amounts: 48.9–49.4%) and of fructose 4.4–4.5% (Table 1). In both cases, starting from glucose and mannose, respectively, the same equilibrium concentrations of glucose and mannose were obtained.

Table 1
Epimerization of glucose and mannose, respectively, with stoichiometric and substoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ at 60°C in methanol

$Ni(\mathbf{1})_2Cl_2$ (%)	Substrate	Man (%)	Glu (%)	Fru (%)	t (min)
100	glucose	46.5	48.9	4.6	4 [a]
100	mannose	46.2	49.4	4.4	
50	glucose	38.2	57.3	4.5	10 [a]
50	mannose	39.1	56.5	4.4	
30	glucose	33.2	64.1	2.7	35 [b]
30	mannose	54.1	42.9	3.0	
10	glucose	10.0	87.7	2.3	70 [b]
10	mannose	89.6	7.9	2.5	
1	glucose	< 1	> 99	< 1	60 [b]
1	mannose	> 99	< 1	< 1	

[a] Time after which equilibration is complete.
[b] Time after which the carbohydrate concentrations given no longer change.

For the experiments with 30% of the nickel(II) complex $Ni(\mathbf{1})_2Cl_2$ and less it was not possible to reach the equilibrium starting from glucose and mannose, even after prolonged reaction times. It turned out that after the reaction times given in Table 1 the percentages of the various carbohydrates no longer changed, except for a small increase of the fructose concentration. Obviously, the species catalytically active in the epimerization reaction were deactivated, before the systems were equilibrated.

3.2. Overstoichiometric amounts of catalyst

Overstoichiometric amounts of nickel(II) complexes with respect to glucose or mannose were used from a twofold excess to a tenfold excess to see whether the trend in the equilibrium concentrations of glucose and mannose found in going from stoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ to half-stoichiometric amounts continues in the other direction. This, actually, is observed (Table 2). In all the experiments the equilibrium glucose $\rightleftharpoons$ mannose was reached starting from both sides. In addition, between

Table 2
Epimerization of glucose and mannose, respectively, with overstoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ (5 min at 60°C in methanol)

$Ni(\mathbf{1})_2Cl_2$ (%)	Substrate	Man (%)	Glu (%)	Fru (%)	Σ (mg) [a]
100	glucose	46.5	48.9	4.6	85.3
100	mannose	46.2	49.4	4.4	85.0
200	glucose	55.3	40.0	4.7	78.2
200	mannose	56.1	38.9	5.0	81.7
300	glucose	62.0	31.9	6.1	59.6
300	mannose	62.7	30.6	6.7	63.2
400	glucose	71.3	21.5	7.2	56.4
400	mannose	70.9	22.1	7.0	53.4
500	glucose	80.1	12.1	7.8	45.9
500	mannose	81.3	11.2	7.5	44.3
1000	glucose	83.0	7.0	10.0	38.7
1000	mannose	85.0	5.3	9.7	40.0
[b]	glucose	1.1	95.4	3.5	38.1
[b]	mannose	93.6	1.4	5.0	40.7

[a] Starting material: 90 mg glucose and mannose, respectively; Σ = isolated carbohydrate fraction in mg.
[b] Only ligand **1** (no nickel(II) chloride) was used.

4.5 and 10% of fructose formed (Table 2). The proportion of mannose increased with increasing quantities of nickel(II) complex from 46% in the stoichiometric case to 83–85% for the tenfold excess (Table 2). However, the isolated yields dropped with increasing quantities of nickel(II) complex. This was due to losses which occurred in the separation of the nickel(II) complex from the carbohydrates. On addition of large amounts of ion exchangers to the reaction mixtures to bind the nickel excess a viscous mass formed. Therefore, to remove large quantities of nickel complexes the deionization had to be repeated with smaller amounts of ion exchangers several times. These successive separation steps resulted in carbohydrate losses.

3.3. Time dependence of the epimerization and degradation

The time dependence of the epimerization of glucose in the presence of stoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ at 60°C in methanol is shown in Fig. 1. Up to a reaction time of 1.5 min a logarithmic relation of the yield of mannose as a function of time is observed. From 1.5–2.5 min there is an almost linear increase of

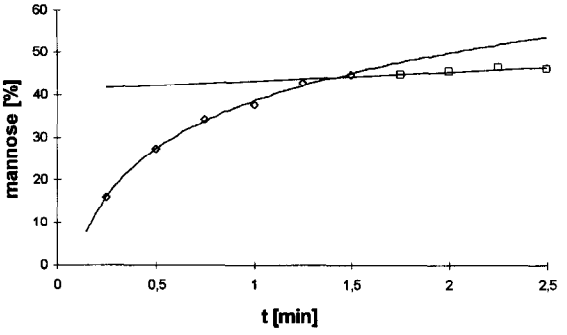

Fig. 1. Time dependence of the epimerization of glucose with stoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ at 60°C in methanol.

the mannose concentration. Thus, the epimerization glucose ⇌ mannose cannot be subjected to a rigorous kinetic analysis. After 4 min the equilibrium concentrations of glucose and mannose no longer change.

The degradation of glucose and mannose, respectively, in the presence of stoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ at 60°C in methanol as a function of time is shown in Fig. 2. Starting with the equilibrium mixture (46.2–46.5% mannose, 48.9–49.4% glucose and 4.5–4.6% fructose) the degradation sets in after ca. 10 min. The degradation of glucose is a little faster than the degradation of mannose. However, a total of only about 3% of mannose is degraded within 45 min under the conditions given.

The equilibrium concentrations of glucose and mannose are obtained more rapidly starting from glucose than from mannose (Fig. 3). Start-

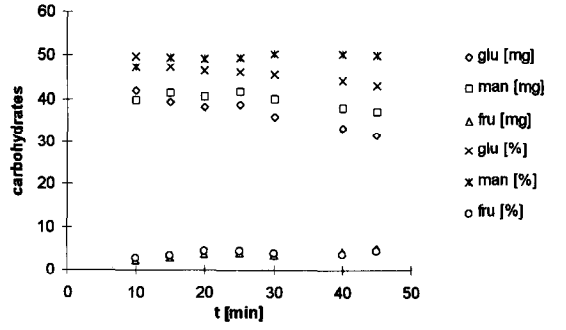

Fig. 2. Degradation of the carbohydrates as a function of time with stoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ at 60°C in methanol.

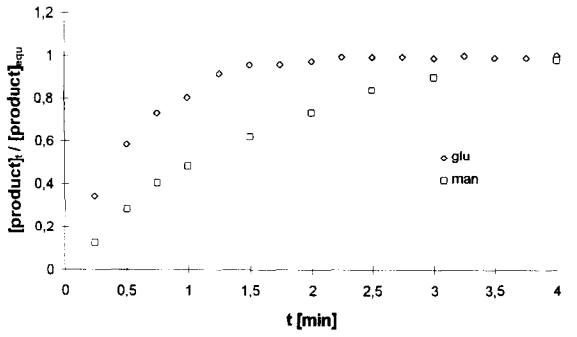

Fig. 3. Epimerization of glucose and mannose, respectively, with stoichiometric amount of Ni(**1**)$_2$Cl$_2$ at 60°C in methanol, normalized with respect to the equilibrium concentrations.

ing from glucose the equilibrium is reached after 1.5 min, whereas starting from mannose equilibration takes about 4 min.

4. Catalysis with chiral nickel(II) complexes

4.1. Influence of the temperature

Glucose and mannose, respectively, were epimerized in methanol solution with stoichiometric quantities of the complexes Ni(**2**)$_2$Cl$_2$ and Ni(**3**)$_2$Cl$_2$. At 60°C the equilibrium concentrations of 49.8 and 49.7% mannose starting from glucose and mannose, respectively, were measured using the *R,R*-catalyst Ni(**2**)$_2$Cl$_2$ (Table 3). The pertinent values for the *S,S*-catalyst Ni(**3**)$_2$Cl$_2$ (49.2 and 49.6% mannose) were a little lower, indicating a trend that the *R,R*-ligand **2** gives a higher mannose equilibrium concentration than the *S,S*-ligand **3**, which was corroborated and amplified at lower temperatures. Each of the values at 60°C was deter-

Table 3
Epimerization of glucose and mannose, respectively, at different temperatures with stoichiometric amounts of Ni(**2**)$_2$Cl$_2$ and Ni(**3**)$_2$Cl$_2$ in methanol

T (°C)	Substrate	Ligand	Man (%)	Glu (%)	Fru (%)	Σ (mg) [a]	t (min)
60	glucose	(*R,R*) **2**	49.8	47.3	2.9	80.0	5 [b]
		(*S,S*) **3**	49.2	47.5	3.3	82.4	5 [b]
60	mannose	(*R,R*) **2**	49.7	47.7	2.6	78.9	5 [b]
		(*S,S*) **3**	49.6	47.2	3.2	83.4	5 [b]
50	glucose	(*R,R*) **2**	51.2	45.7	3.1	75.3	8 [b]
		(*S,S*) **3**	48.0	49.0	3.0	70.1	8 [b]
50	mannose	(*R,R*) **2**	50.5	47.8	2.8	77.3	8 [b]
		(*S,S*) **3**	48.7	47.9	3.4	75.7	8 [b]
40	glucose	(*R,R*) **2**	52.0	45.0	3.0	81.2	25 [b]
		(*S,S*) **3**	46.9	50.1	3.0	78.5	25 [b]
40	mannose	(*R,R*) **2**	52.7	44.7	2.6	73.3	25 [b]
		(*S,S*) **3**	47.4	49.8	2.8	79.4	25 [b]
30	glucose	(*R,R*) **2**	52.0	46.1	1.9	68.4	60 [b]
		(*S,S*) **3**	44.1	53.2	2.7	70.3	60 [b]
30	mannose	(*R,R*) **2**	54.3	43.4	2.3	73.5	90 [b]
		(*S,S*) **3**	46.0	51.5	2.5	71.7	90 [b]
20	glucose	(*R,R*) **2**	53.4	44.8	1.8	78.0	300 [b]
		(*S,S*) **3**	41.7	55.9	2.4	80.9	300 [c]
20	mannose	(*R,R*) **2**	54.8	43.7	1.5	79.2	480 [b]
		(*S,S*) **3**	45.0	52.8	2.2	83.3	480 [c]
0	glucose	(*R,R*) **2**	54.4	44.8	0.8	79.0	230 [c,d]
		(*S,S*) **3**	34.2	64.3	1.5	66.4	230 [c,d]
0	mannose	(*R,R*) **2**	57.5	41.6	0.9	70.3	230 [c,d]
		(*S,S*) **3**	42.7	56.1	1.2	74.8	230 [c,d]

[a] Starting material: 90 mg glucose and mannose, respectively; Σ = isolated carbohydrate fraction in mg.
[b] Time after which equilibration is complete.
[c] Time after which the carbohydrate concentrations given no longer change.
[d] Time given in hours.

mined four times (averages given), whereas the values at lower temperatures are single measurement values.

At 50°C the mannose concentrations are 51.2 and 50.5% starting from glucose and mannose, respectively, for the catalyst $Ni(\mathbf{2})_2Cl_2$ (Table 3). The corresponding values for $Ni(\mathbf{3})_2Cl_2$ are 48.0 and 48.7%. The differences between catalysts $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ at 40°C increased to the average values 52.4 and 47.2%, at 30°C to 53.2 and 45.0%, and at 20°C to 54.1 and 43.4% (Table 3). At 0°C the approach to equilibrium for the catalyst $Ni(\mathbf{2})_2Cl_2$ is almost complete, whereas the values obtained with $Ni(\mathbf{3})_2Cl_2$ are further away from equilibrium (vide infra).

The glucose contents are complementary to the mannose contents, taking into account the small fructose concentrations formed (Table 3). Starting with 90 mg of glucose and mannose, respectively, the carbohydrate quantity isolated after workup is between 70 and 80 mg (Table 3, column 7). The reaction times necessary to reach equilibrium increase from 5 min at 60°C to 300 min and 480 min, respectively, at 20°C (Table 3, column 8). As mentioned above approach to equilibrium is not complete after 230 h at 0°C.

4.2. Time dependence

The time dependence of the epimerization of glucose and mannose, respectively, with

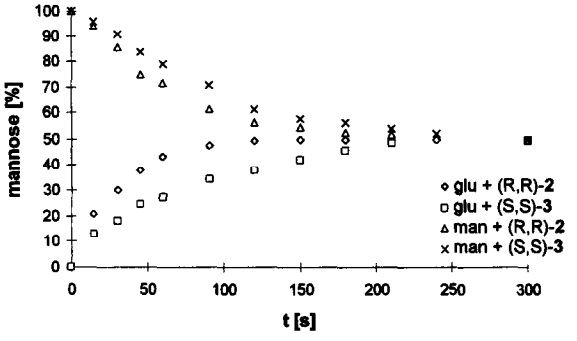

Fig. 4. Time dependence of the epimerization of glucose and mannose, respectively, with stoichiometric amounts of $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ at 60°C in methanol.

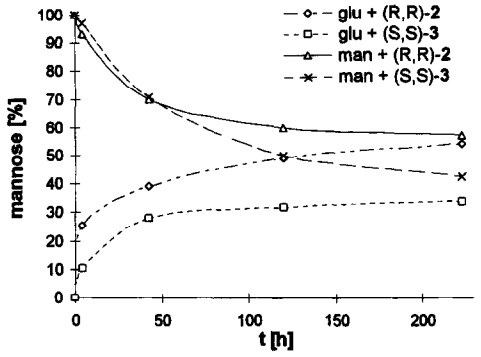

Fig. 5. Time dependence of the epimerization of glucose and mannose, respectively, with stoichiometric amounts of $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ at 0°C in methanol.

$Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ at 60°C is given in Fig. 4. The equilibrium concentration of 49.5% mannose is reached after 5 min starting from both carbohydrates and using both catalysts. However, the approach to equilibrium is appreciably faster with $Ni(\mathbf{2})_2Cl_2$ than with $Ni(\mathbf{3})_2Cl_2$ and faster for glucose than for mannose.

The epimerization reactions at 0°C are shown in Fig. 5. After 230 h the reactions with $Ni(\mathbf{2})_2Cl_2$ starting from both glucose and mannose are almost at equilibrium. On the other hand for $Ni(\mathbf{3})_2Cl_2$ equilibration is not complete (vide supra).

4.3. Substoichiometric amounts of catalyst

Using nickel complexes of ligands **2** and **3** in substoichiometric quantities, the equilibrium concentrations are no longer reached. For 50% catalyst the values obtained are relatively close to each other, whereas for 10% catalyst the values are far from equilibrium (Table 4). Obviously, the catalytically active species are deactivated during epimerization.

5. Michaelis–Menten and Eyring analysis

It has already been mentioned that the kinetic data of the catalytic epimerization glucose ⇌ mannose cannot be analyzed rigorously. Based

Table 4
Epimerization of glucose and mannose, respectively, with substoichiometric amounts of $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ in methanol at 60°C

Complex (%)	Substrate	Ligand	Man (%)	Glu (%)	Fru (%)	Σ (mg) [a]	t (min) [b]
50	glucose	(*R*,*R*) **2**	43.2	51.8	5.0	83.5	10
50	glucose	(*S*,*S*) **3**	40.5	54.3	5.2	81.3	10
50	mannose	(*R*,*R*) **2**	50.2	45.0	4.8	80.0	10
50	mannose	(*S*,*S*) **3**	49.8	45.9	5.2	82.8	10
10	glucose	(*R*,*R*) **2**	11.6	84.9	3.5	78.7	30
10	glucose	(*S*,*S*) **3**	9.8	86.4	3.8	75.3	30
10	mannose	(*R*,*R*) **2**	87.3	9.4	3.3	80.1	30
10	mannose	(*S*,*S*) **3**	85.9	10.1	4.0	77.9	30

[a] Starting material: 90 mg glucose and mannose, respectively; Σ = isolated carbohydrate fraction in mg.
[b] Time after which the carbohydrate concentrations given no longer change.

on the analogy of the formation of an enzyme/substrate complex and a nickel(II)/carbohydrate complex (Eq. (1)), a Michaelis–Menten analysis was performed. According to Eq. (1) the Michaelis–Menten equation for the initial rate v of the epimerization of glucose is

$$v = \frac{v_{max} \cdot [glu]}{K_M + [glu]}$$

K_M is the Michaelis constant and v_{max} is the maximal rate. To test whether the measured data fit the model, the Michaelis–Menten equation is converted into a Lineweaver–Burk correlation.

$$\frac{1}{v} = \frac{K_M}{v_{max}} \cdot \frac{1}{[glu]} + \frac{1}{v_{max}}$$

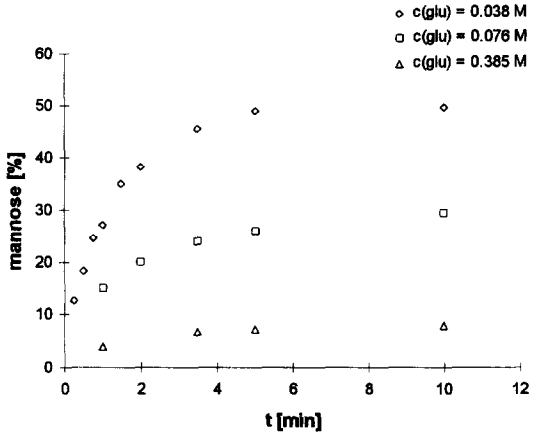

Fig. 6. Epimerization of glucose with $Ni(\mathbf{3})_2Cl_2$ (concentration 0.038 M) for different substrate concentrations at 60° in methanol.

If the Michaelis–Menten equation is valid, there should be a straight line which allows the determination of K_M and v_{max}.

The epimerization of glucose with a catalyst concentration of 38 mM of $Ni(\mathbf{3})_2Cl_2$ in methanol at 60°C was measured with substrate concentrations of 38, 76 and 385 mM (Fig. 6). The percentages of the mannose content of Fig. 6 have to be converted into concentrations. Only 95% of these concentrations are used for the calculations; 5% are substracted (by-product formation).

The rate at the beginning of the epimerization is obtained by differentiation of the function product concentration versus time at $t = 0$. This function was approximated by a cubic polymon based on the first three measurements and derived using the method of Lagrange [13,14]. This treatment gives the rates at the beginning of the epimerization of glucose and mannose with the catalysts $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ at different substrate concentrations as shown in Table 5.

Table 5
Initial rates v_0 ($M\,s^{-1}$)·10^{-4} of the epimerization of glucose and mannose, respectively, at different substrate concentrations ($Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ = 0.038 M)

c_0 (substr.) (M)	Substrate: glucose		Substrate: mannose	
	$Ni(\mathbf{2})_2Cl_2$	$Ni(\mathbf{3})_2Cl_2$	$Ni(\mathbf{2})_2Cl_2$	$Ni(\mathbf{3})_2Cl_2$
0.038	4.84	2.40	2.35	0.83
0.076	6.75	2.75	2.47	0.92
0.385	8.32	3.53	3.03	0.93

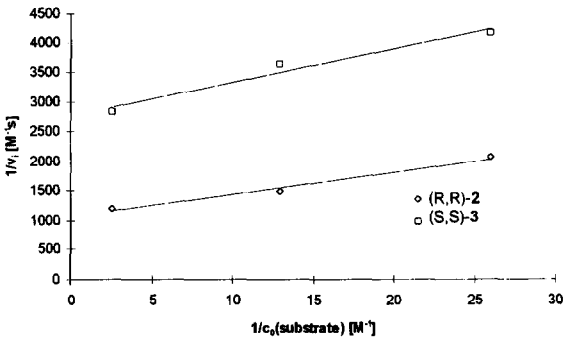

Fig. 7. Lineweaver-Burk plot of the epimerization of glucose with $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$.

Using the values of Table 5, Lineweaver–Burk correlations give straight lines from the axial sections and inclinations of which the Michaelis constants K_M and the maximal rates v_{max} of the epimerizations are obtained [13]. Fig. 7 shows the Lineweaver–Burk plots of the epimerization of glucose with $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$. Table 6 compiles all the data. The Michaelis constants reveal that nickel complexes are less efficient than most of the enzymes [13]. The higher K_M values in the epimerization of glucose indicate a lower affinity to the nickel–diamine complex compared with mannose which also explains the smaller epimerization rate of mannose versus glucose. Similarly, nickel complexes of the *S,S*-ligand **3** form more stable carbohydrate adducts but are slower epimerization catalysts than nickel complexes of the *R,R*-ligand **2**.

To determine the Eyring parameters the epimerizations of glucose and mannose were carried out at 60, 50, 40, 30 and 20°C with both catalysts $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$. The Eyring plot of the epimerization of mannose with $Ni(\mathbf{2})_2Cl_2$ is shown in Fig. 8. All the Eyring parameters are given in Table 7.

Table 6
Michaelis–Menten parameters of the epimerization of glucose and mannose at 60°C in methanol with $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$ = 0.04 M

Complex	Glu → man		Man → glu	
	$Ni(\mathbf{2})_2Cl_2$	$Ni(\mathbf{3})_2Cl_2$	$Ni(\mathbf{2})_2Cl_2$	$Ni(\mathbf{3})_2Cl_2$
K_M (M)·10^{-2}	3.49	2.04	1.19	0.54
v_{max} ($M\,s^{-1}$)·10^{-4}	9.38	3.62	3.01	0.96

Table 7
Eyring parameters of the epimerization of glucose and mannose, respectively, catalyzed by $Ni(\mathbf{2})_2Cl_2$ and $Ni(\mathbf{3})_2Cl_2$

Complex	Glu → man		Man → glu	
	$Ni(\mathbf{2})_2Cl_2$	$Ni(\mathbf{3})_2Cl_2$	$Ni(\mathbf{2})_2Cl_2$	$Ni(\mathbf{3})_2Cl_2$
$\Delta G^{\neq}$ (kJ/mol)	90.6	91.4	92.4	92.0
$\Delta H^{\neq}$ (kJ/mol)	121	107	120	115
$\Delta S^{\neq}$ (J/K·mol)	91.2	48.0	82.7	68.8

6. Discussion

Starting from 1986, in a series of papers the epimerization of glucose and mannose, respectively, with nickel(II) complexes was presented [4–11]. Although normally stoichiometric amounts of nickel(II) complexes were used, occasionally the epimerization was carried out with substoichiometric amounts. Therefore, it seemed possible to conduct the reaction catalytically with small amounts of nickel(II) complexes only. In the present paper we checked this point. However, it turned out that the epimerization glucose ⇌ mannose in methanol at 60°C cannot be rendered truly catalytic. The amount of the catalyst $Ni(\mathbf{1})_2Cl_2$ can only be reduced from stoichiometric to half-stoichiometric. If it is reduced to 0.3 equivalents, there is no longer complete equilibration (Table 1).

An interesting point is the glucose/mannose ratio after equilibration in methanol at 60°C. As pointed out in Section 2.1 the nickel(II) complexes take part in the epimerization reaction by forming carbohydrate complexes. This implies that the equilibrium composition depends on the amount of nickel(II) catalyst present. For a tenfold excess of $Ni(\mathbf{1})_2Cl_2$ the mannose concentration at equilibrium is as high as 83.0–85.0%, for stoichiometric amounts of $Ni(\mathbf{1})_2Cl_2$ it is 46.2–46.5% and for half-stoichiometric amounts

it is only 38.2–39.1%. Of course it is the other way round with the glucose concentration, the fructose content being between 2.3 and 10.0% (Tables 1 and 2).

From data in the literature the impression arises that the epimerization glucose ⇌ mannose catalyzed by nickel(II) complexes is a clean reaction. Sometimes the formation of fructose is mentioned but sometimes it is explicitly stated that fructose is absent in the reaction mixture [9]. In our experiments in methanol both with the achiral catalyst Ni(**1**)$_2$Cl$_2$ and with the chiral catalysts Ni(**2**)$_2$Cl$_2$ and Ni(**3**)$_2$Cl$_2$ fructose formation was universally observed in accord with a recent report of Sunjic et al. [15] which showed that in the metal catalyzed epimerization glucose ⇌ mannose fructose formation may be considerable.

Natural glucose and mannose are optically active materials belonging to the D configuration. Their epimerization with the optically active catalysts Ni(**2**)$_2$Cl$_2$ and Ni(**3**)$_2$Cl$_2$ having *R,R* and *S,S* configuration, respectively, should lead to diastereomeric interactions showing up in kinetic and thermodynamic parameters of the system glucose ⇌ mannose. Using stoichiometric amounts of the optically active catalysts in methanol at 60°C, the equilibrium concentrations of mannose were around 49% and of glucose around 47% ee (Table 3). The values were only slightly different for the chiral catalysts Ni(**2**)$_2$Cl$_2$ and Ni(**3**)$_2$Cl$_2$, and they were also similar to those obtained with the achiral catalyst Ni(**1**)$_2$Cl$_2$. Interestingly, lowering the temperature revealed increasing differences for the chiral catalysts. Thus, at 20°C the mannose concentration was as high as 53.4% for the *R,R*-catalyst Ni(**2**)$_2$Cl$_2$ and as low as 41.7% for the *S,S*-catalyst Ni(**3**)$_2$Cl$_2$ (vice versa for the glucose concentrations). Similar trends have been reported for other optically active nickel catalysts in Ref. [9]. The diastereomeric interactions in the epimerization of glucose and mannose with the chiral catalysts Ni(**2**)$_2$Cl$_2$ and Ni(**3**)$_2$Cl$_2$ also showed up in the kinetic parameters of Figs. 4 and 5 and in the Michaelis–Menten and Eyring analysis of Figs. 6–8 and Tables 6 and 7.

As the central point of the present paper was the substoichiometric aspect of the catalyst in the epimerization glucose ⇌ mannose, the chiral catalysts Ni(**2**)$_2$Cl$_2$ and Ni(**3**)$_2$Cl$_2$ were used in catalytic amounts only. For 0.5 equivalents of the catalysts the epimerizations starting from glucose and mannose almost went to equilibrium, for 0.1 equivalents of the catalysts, however, the reactions stopped far away from equilibrium (Table 4).

7. Experimental

N,N,N′,N′-tetramethyl-1,2-diamino-1,2-diphenylethane was synthesized and resolved according to Refs. [16–18].

7.1. Epimerization procedure

0.5 mmol (120 mg) $NiCl_2 \cdot 6H_2O$ and 1.0 mmol ligand are heated to 60°C in 3 ml of methanol for 1 min. Then, the solution is brought to the temperature at which the epimerization is to be carried out. A solution of 0.5 mmol (90 mg) glucose and mannose, respectively, in 10 ml of methanol of the same temperature is added to the solution of the nickel complex. The reaction mixture is stirred for the given time interval. Then, 25 ml of water are added which contain 15 drops of 0.5 M sulphuric acid to stop the reaction. The solution is brought to pH 6.5 using 0.5 M sulphuric acid and stirred at room temperature for 1 h. The solution is deionized by the successive addition of an excess of the ion exchangers DOWEX 50W X8 (H^+) and DOWEX 1 X2 (HCO_3^-), respectively. After 45 min the solution is filtered and the respective ion exchanger is washed with several ml of water. The ultimate filtrate should be almost neutral and it should contain no nickel(II) ions. If a test with diacetyldioxime shows the presence of nickel ions, the deionization is repeated. Then, the reaction mixture is frozen. Methanol

and water are removed by freeze drying (17 h at −50°C, pressure 0.1 mbar). The colorless crystalline residue which is obtained does no longer contain any methanol (vide infra).

7.2. HPLC analysis

For the separation of sucrose, glucose, mannose and fructose a HPLC column HPX-87 P (BioRad) is used. It consists of a sulfonated divinylbenzene/styrene copolymer of 9 μm average particle size loaded with lead ions. In addition, a BioRad microguard pre-column is used.

The residue obtained in Section 7.1 is dissolved in distilled water, sucrose (20 mg) is added as an internal standard and the solution is filled up to a volume of 10 ml. The HPLC measurements are performed at 60°C with a flux of 1 ml/min. Water is used as the eluent. Detection is by UV absorption at 190 nm. The retention times of sucrose, glucose, mannose and fructose are 6.8, 8.3, 11.1 and 12.0 min, respectively. The peaks of mannose and fructose were not completely baseline separated. They were analyzed with the program DISPLAY 3.2 (Spectra Physics).

To correlate the areas in the HPLC diagrams and the amounts of substance used, a calibration with respect to sucrose, glucose, mannose and fructose was carried out. These measurements showed that the relative limits of error are 2%.

It turned out that traces of methanol present in the product to be analyzed appear below the mannose peak. However, it could be shown that the samples obtained according to Section 7.1 by freeze drying did not contain residual methanol.

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