# MONOSACCHARIDES IN ALKALINE MEDIUM: ISOMERIZATION DEGRADATION OLIGOMERIZATION

# MONOSACCHARIDES IN ALKALINE MEDIUM: SOMERIZATION DEGRADATION OLIGOMERIZATION



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aan Mieke

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## CHAPTER 1

## INTRODUCTION

Insight in the alkaline transformation of monosaccharides is of importance for the industrial sucrose manufacture as the invert sugar present in diffusion juices (0.5-1.5 g/100 g sucrose) is decomposed during the juice purification by the addition of lime at high temperature (~ 80 °C). Complete degradation of invert sugar is desirable for a good process control in the sugar manufacture for practical reasons, e.g. formation of thermostable compounds, and preventing colour formation by the Maillard reaction of reducing sugars with amino acids.

Monosaccharides in aqueous alkaline medium undergo both reversible and irreversible transformations. The reversible reactions include (i) ionization, resulting in an equilibrium of neutral and ionized monosaccharides, (ii) mutarotation, resulting in an equilibrium of the different cyclic hemiacetal structures of monosaccharides, and (iii) enolization, resulting in the transformation of interconvertible monosaccharides (Fig. 1). The isomerization via the enolization reaction is accompanied by (iv) irreversible transformation of the monosaccharides into carboxylic acids, generally known as the alkaline degradation reaction. Enediol anions are generally considered as common intermediates in both the isomerization and degradation reaction.

In contrast to the well understood reversible transformations, the (mechanistic) features of the alkaline degradation of monosaccharides towards carboxylic acid products are only partly elucidated up to now. This was mainly due to difficulties in the quantitative analysis of reaction mixtures which prohibited systematic investigations with respect to the influence of reaction parameters on the degradation reactions.

A better understanding of the degradation behaviour of monosaccharides in alkaline medium became possible by combination of modern analysis techniques like HPLC, GC, GC-MS, and NMR spectroscopy. This thesis describes the results thus obtained (1982-1986) in this field. New insights in the mecha-

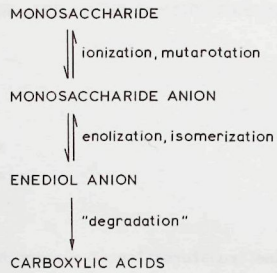



Fig. 1. Simplified overall reaction scheme of monosaccharides in alkaline medium.

nism and kinetics of the alkaline isomerization and, particularly, degradation of monosaccharides will be presented.

After a literature survey (Chapter 2, including some preliminary results of the present work) concerning the reactions of monosaccharides in aqueous alkaline solution, a systematic investigation on the influence of several reaction parameters on the final product composition follows (Chapter 3) showing valuable new information about the degradation pathways followed. In particular the formation of substantial amounts (up to 50%) of oligomeric acidic products has been most often overlooked in the literature. Their mean structural features are characterized by a combination of various analytical techniques (Chapter 4). Both the alkaline isomerization and concomitant degradation of hexoses are described by a new kinetic model which shows the importance of ketose degradation (Chapter 5). The influence of several reaction parameters on the reaction rate constants involved has been determined. On the basis of the results obtained, a general mechanistic picture of the alkaline degradation of monosaccharides is developed (Chapter 6). In particular, the role of α-dicarbonyl compounds as key-intermediates and the important retro-aldolization of ketoses are emphasized. Furthermore, it has been established that the alkaline degradation of monosaccharides and the formose reaction (the alkaline "condensation" of formaldehyde) proceed via the same mechanistic pathways. This appears from degradation experiments of D-fructose in the presence of variable amounts of formaldehyde, the so-called fructo-formose reaction (Chapter 7).



Both $^{13}C$ NMR spectroscopy (Chapter 8) and, especially, chromatographic analyses (Chapter 9) are used throughout the investigations for the identification and quantitative determination of carboxylic acid products formed by the alkaline degradation of monosaccharides.

For the sake of clearness, all transformations of monosaccharides into carboxylic acid products, including both degradation and oligomerization reactions, are generally denoted as "*alkaline degradation*".

Acknowledgements

Dr. P.W. van der Poel, Mr. N.H.M. de Visser, and Mr. M.A.M. de Schutter of Central Laboratory, CSM Suiker B.V., Breda, The Netherlands, are gratefully acknowledged for valuable discussions throughout the research described in this thesis.

# CHAPTER 2

# REACTIONS OF MONOSACCHARIDES IN AQUEOUS ALKALINE SOLUTIONS*

## Introduction

A better understanding of the reactions of monosaccharides, in particular invert sugar, in aqueous alkaline solutions is of importance in industrial sucrose manufacture. The invert sugar present in diffusion juices (in amounts of 0.5-1.5 g/100 g sucrose) originates both from the sugar beets themselves and from subsequent enzymatic hydrolysis of sucrose during the diffusion process. Complete destruction of the invert sugar by addition of lime to the diffusion juice and by raising the temperature is desirable for a good process control in the sugar industry for practical reasons:

(i) Conversion of invert sugar into thermostable components in order to prevent or limit pH drops in the evaporators.

(ii) Coloured "caramel" compounds produced upon alkaline degradation of invert sugar are unfavourable for the quality of the white sugar.

(iii) The melassigenic properties of the organic acids formed by alkaline degradation of invert sugar must be as low as possible.

(iv) Amino acids present in the juice enter into the Maillard reaction with invert sugar yielding coloured undesirable compounds (melanoidins). The Maillard reaction is only important in concentrated juices and is negligible in diluted aqueous solutions[1], e.g. in juice at the juice purification stage. Recently Waller and Feather[2] have reviewed the Maillard reaction and discussed it in detail.

Much research has been done to obtain a better understanding about the fundamental aspects of the reactions of monosaccharides in alkaline aqueous solutions. In particular, initial transformations as ionization, mutarotation, enolization and isomerization have been extensively studied with the use of modern analysis techniques, e.g. GC, UV and NMR, and are presently

* J.M. de Bruijn, A.P.G. Kieboom, H. van Bekkum, and P.W. van der Poel, Sugar Techn. Rev., 13 (1986) 21-52.

reasonably well understood. This, however, is not the case for the degradation of carbohydrates in alkaline aqueous solutions. Because of the complexity of the degradation reactions only part of the features have been elucidated up to now.

Within the scope of the present review we first report on the knowledge about the initial transformations of monosaccharides in alkaline aqueous solutions. Subsequently, as a continuation on the review of Kelly and Brown[3] the alkaline degradation of monosaccharides will be discussed with the intention to give a survey concerning the present-day knowledge of both the various reaction variables playing a role and the mechanistic insights.

## Initial transformations

### *Ionization and mutarotation*

The various cyclic hemiacetal structures in which monosaccharides occur in aqueous solutions possess a weakly acidic hydroxyl group at the anomeric carbon. Los and Simpson[4,5] were the first who determined the ionization constants of both α- and β-D-glucopyranose. They measured the pH of aqueous alkaline solutions of α-D-glucopyranose as a function of time (for various D-glucose concentrations at 0, 15 and 25 °C) and determined, by extrapolating the D-glucose concentration to zero, the $pK_a$ values of both anomers; see Table 1.

Table 1. $pK_a$ values of α- and β-D-glucopyranose in water[4,5] (extrapolated to zero D-glucose concentration).

| T (°C) | $pK_a$ α-anomer | $pK_a$ β-anomer |
|---|---|---|
| 0 | 13.08 | 12.87 |
| 15 | 12.66 | 12.41 |
| 25 | 12.48 | 12.18 |

The demonstrated difference in acid strength of about 0.2 $pK_a$ units between β- and α-D-glucopyranose was confirmed later on by $^{13}C$ NMR[6]. For a 0.01 M D-glucose at 3-5 °C $pK_a$ values of 12.78 and 12.60 were found for the α- and the β-anomer, respectively. It was also observed[6] that the acidity of a monosaccharide depends on the equatorial/axial position of both the anomeric hydroxyl group and the hydroxyl group at $C_2$. An eq/ax situation of the



anomeric hydroxyl group and the adjacent hydroxyl group results in a lower acidity than an ax-ax or eq-eq situation. From Table 2 it appears that the hexose concentration has a considerable influence on the experimental overall $pK_a$ values.

Various factors may be responsible for the differences in acid strengths, e.g. the degree of hydration, intramolecular hydrogen bonding and the so-called anomeric effects[6]. From the decrease of entropy[5,7] upon ionization of monosaccharides it is thought that better solvation of the anion occurs[9].

Table 2. $pK_a$ values of hexoses as a function of concentration.

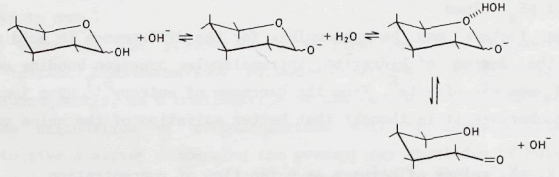
| Hexose | Concentration (M) | T (°C) | $pK_a$ | Ref. |
|---|---|---|---|---|
| D-glucose | 0.01 | 3-5 | 12.7 | 6 |
| | 0.01 | 10 | 12.72 | 7 |
| | 0.125 | 10.4 | 13.5 | 8 |
| | 0.5 | 10.4 | 13.8 | 8 |
| | 1.11 | 3-5 | 13.9 | 6 |
| | 1.13 | 3-5 | 14.0 | 6 |
| D-mannose | 0.01 | 10 | 12.45 | 7 |
| | 1.06 | 3-5 | 14.0 | 6 |
| D-fructose | 0.01 | 10 | 12.53 | 7 |
| | 1.14 | 3-5 | 14.2 | 6 |

The ionization structure of monosaccharides can be simply described as the ionization of the anomeric OH (Scheme 1).

Scheme 1. Simple ionization model of an aldopyranose.

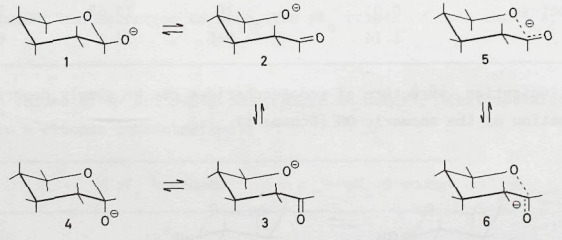
Mutarotation[10], the transition between the various hemiacetals of a monosaccharide, has been studied by using different techniques [11-20]. Isbell and Wade[11] proposed a mechanism for the mutarotation in aqueous alkaline solutions in which the fast ionization of the sugar is followed by opening of

the acetal ring with the formation of a pseudo-cyclic intermediate (Scheme 2). This intermediate can then pass into one of the anomeric forms.



Scheme 2. Mutarotation mechanism according to Isbell and Wade[11].

A more recent study[6] revealed that the ionization of a monosaccharide can not be explained by the simple ionization model of Scheme 1. From $^{13}C$ NMR measurements it appeared that ionization results in a distribution of the negative charge over both the anomeric and the ring-oxygen atom. In other words, a fast equilibrium exists between ionized cyclic and pseudo-cyclic compounds (1 ⇌ 2 of 3 ⇌ 4) or the ionized species have to be considered as a non-classical anion (5 or 6, Scheme 3). It was concluded[6] that the



Scheme 3. Ionized species and mutarotation.

rate-determining step in the mutarotation is the transition from 2 to 3 or from 5 to 6. Thus, base catalysis in the mutarotation process has its origin in the weakening of the ring C-O bond of the generated sugar anion. Both the complete disrupture of the ring C-O bond and a substantial reorganization of the water mantle upon rotation will determine the energy barrier of mutarotation. These observations are in harmony with those of other authors who

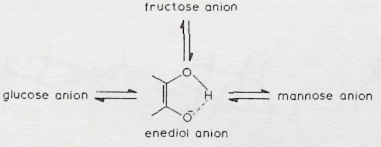


found that monosaccharides are substantially solvated in water[21,22] and that water molecules play an important role in the transition state of the mutarotation process[11,14-16].

The reaction rate of the mutarotation of monosaccharides; e.g., the first order mutarotation rate constant for D-glucose in water at 21 °C and pH ≈ 7 is[23] 0.0063 $min^{-1}$, can be markedly accelerated by raising the temperature or by increasing either the hydrogen ion or hydroxyl ion concentration.

*Enolization and isomerization*

After ionization and mutarotation, monosaccharides may undergo two subsequent rearrangements in alkaline aqueous solutions, namely enolization and isomerization. The enediol anion species is generally accepted[8,14-26] as the key intermediate in the isomerization reactions of monosaccharides. These reactions, e.g., the interconversion of D-glucose, D-fructose and D-mannose, are known as "the Lobry de Bruyn-Alberda van Ekenstein rearrangement"[27] (Scheme 4).



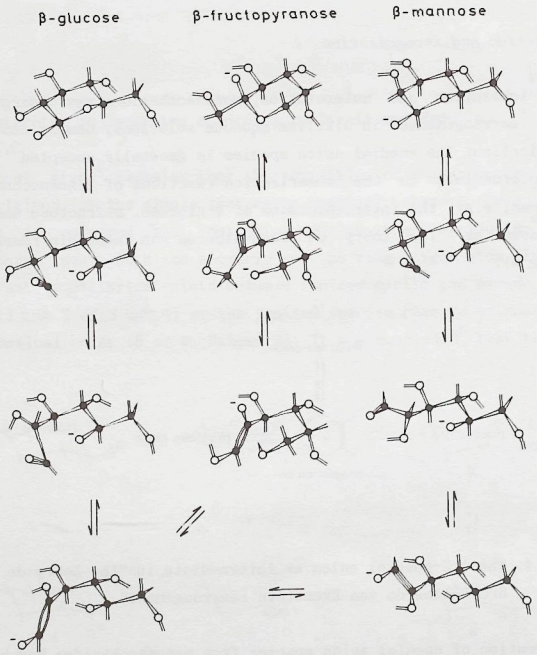

Scheme 4. The 1,2-enediol anion as intermediate in "the Lobry de Bruyn-Alberda van Ekenstein rearrangement".

The formation of enediol anion species from monosaccharides has been studied by UV spectroscopy[8,26,28] and by H/D and H/T exchange reactions[24,25,29,30]. A mechanistic model for the interconversion of D-glucose, D-fructose and D-mannose[8], which explains most of the experimental data, is based on the principle of least motion. This mechanism, depicted in Scheme 5, comprised the following features:

(i) Fast equilibrium between cyclic sugar anions and their pseudo-cyclic carbonyl structures, as evidenced by $^{13}C$ NMR spectroscopy[6].

(ii) Formation of the enediol anion by a rate-limiting, intramolecular proton shift from $C_2$ (or $C_1$ for fructose) to pseudo-cyclic (Z)-enediol anions.

(iii) Reversal of process (ii), leading, after conformational changes of the enediol anion species, to isomerization.




Scheme 5. Molecular picture of enolization and isomerization[8].

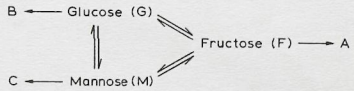


In addition to the favourable isomerization of D-fructose via the 1,2-enediol anion, D-fructose can also epimerize to D-psicose via the formation of a 2,3-enediol as intermediate species[19]. Although the latter isomerization process has generally been considered to be of minor importance, De Bruijn et al.[31,32] recently established that alkaline isomerization/degradation of D-glucose, D-fructose and D-mannose results in substantial amounts (up to 7.5%) of D-psicose.

Reaction rate constants for isomerization have been calculated on the basis of several kinetic models and at different reaction conditions[8,33-39], which complicates the comparison of these data. A survey of the relevant literature data has been given by Kooyman et al.[37]. As an illustration of the kinetic models proposed for the isomerization of monosaccharides in alkaline aqueous solutions two different types will be discussed.

First, the model of MacLaurin and Green[34] comprises both the interconversion of D-glucose, D-fructose and D-mannose and the conversion of these sugars into degradation products (Scheme 6). The reaction rate constants calculated on the basis of this scheme are given in Table 3.

In the model formulated by De Wit et al.[8] the enolization step of the monosaccharides is taken into account (Scheme 7). The reaction rate constants obtained for a series of monosaccharides are summarized in Table 4.



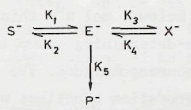

Scheme 6. Kinetic model for the isomerization/degradation reaction according to MacLaurin and Green[34]. A, B and C denote alkaline degradation products.

Table 3. Rate constants for the reaction of 0.002 M D-glucose in 1 M NaOH at 22 °C under $N_2$, according to Scheme 6[34].

| Reaction pathway | Rate constant ($10^{-4}$ $h^{-1}$) |
|---|---|
| G → F | 360 |
| G → M | 5 |
| G → B | 20 |
| F → G | 380 |
| F → M | 60 |
| F → A | 720 |
| M → G | 5 |
| M → F | 110 |
| M → C | 20 |

The latter model must be considered as the most realistic since it includes the enediol anion as the key intermediate of the isomerization reaction. Whereas the rate constant for enediol formation ($k_1$) largely differs, the overall rate constant ($k_2 + k_3 + k_5$) is essentially equal for sets of interconvertible sugars as D-glucose, D-fructose and D-mannose (0.13-0.15), D-ribose and D-arabinose (0.58 and 0.61) and D-galactose, D-talose and D-tagatose (0.48-0.54). This points to the formation of similar enediol anion species from these interconvertible sugar anions. Therefore, the mode of formation of the enediol anion from the pseudo-cyclic carbonyl intermediate[8]: i.e., 2 and 3 in Scheme 3, rather than the occurrence of (E/Z)-isomeric enediol anion structures[9,24,40] will be responsible for the differences in rate of enolization.






Scheme 7. Kinetic model for the isomerization/degradation reaction including the enediol anion according to De Wit et al.[8].

Table 4. Rate constants according to Scheme 7 (1.22 M KOH, 10.4 °C, $N_2$)[8].

| Monosaccharide | Concentration (M) | $k_1$ ($10^{-4}$ $min^{-1}$) | $k_2 + k_3 + k_5$ ($min^{-1}$) |
|---|---|---|---|
| D-ribose | 0.2 | 0.4 | 0.58 |
| D-arabinose | 0.2 | 0.6 | 0.61 |
| D-xylose | 0.1 | 0.7 | 0.25 |
| D-allose | 0.2 | 0.1 | 0.23 |
| D-glucose | 0.1 | 0.5 | 0.15 |
| D-mannose | 0.2 | 0.1 | 0.13 |
| D-galactose | 0.2 | 0.3 | 0.48 |
| D-fucose | 0.2 | 0.1 | 0.38 |
| D-talose | 0.2 | 0.2 | 0.54 |
| D-fructose | 0.0125 | 6 | 0.14 |
| L-sorbose | 0.025 | 2.5 | 0.20 |
| D-tagatose | 0.006 | 27 | 0.51 |

The amount of the intermediate enediol anion, present during the isomerization of sugar anions lies below 1 mol %[8]; this indicates that formation of this species is the rate-determining step in both the isomerization and degradation reactions of monosaccharides. The role of the enediol anion as an intermediate in the degradation reactions will be discussed in the next part of this chapter.

## Alkaline degradation reactions

### *History*

The classical paper of Lobry de Bruyn and Alberda van Ekenstein[27] in 1895 stands at the basis of the extensively studied decomposition of reducing sugars under aqueous alkaline conditions. Some years later Wohl and Neuberg[41] suggested the enediol intermediate for aldose/ketose isomerization. Although Nef[42] recognized that the isomerization of D-glucose could explain the presence of the three types of saccharinic acids formed in basic solution, it was Evans and Benoy[43] in 1926 who first suggested that 1,2- and 2,3-enediols are the key intermediates involved.

Since 1950 several review articles have been published on the alkaline degradation of carbohydrates. An enumeration thereof will be given below, not necessarily in chronological order.

A comprehensive review of the Lobry de Bruyn-Alberda van Ekenstein transformation has been given by Speck[44] emphasizing the role of enediol species as intermediates in both isomerization and degradation reactions of monosaccharides. Pigman and Anet[19] have discussed from a general point of view the reactions of sugars with acids and bases, whereas a survey of the dehydration reactions of carbohydrates is given by Feather and Harris[45]. Special attention towards the formation of saccharinic acids is given in the review of Sowden[46]. The formation, isolation and characterization of $C_6$-saccharinic acids is described by several investigators[47]; that of $C_5$-saccharinic acids has been described by Ishizu et al.[48]. Crum[49] has summarized the literature up to 1958 on the synthesis of $C_4$-saccharinic acids. The function of 3-deoxyglycosuloses in dehydration reactions has been discussed by Anet[50]; the chemistry of these compounds has been included by Theander[51] in a review on dicarbonyl sugar compounds. Aspects of the chemical conversion of glucose, including reactions in alkaline media, have recently been reviewed by Kieboom and Van Bekkum[52]. In addition, two comprehensive theses deserve attention: MacLeod[53] describes the anaerobic alkaline degradation of D-glucose, cellobiose and derivatives, and De Wit[54] deals with the behaviour of D-glucose, D-fructose and related sugars in alkaline medium. Finally, a survey of the alkaline degradation of polysaccharides has been given by Whistler and BeMiller[55].



In addition to the aspects dealt with by the above-mentioned reviews, colour formation during carbohydrate decomposition is an important factor. A survey of the literature on colour and turbidity phenomena of sugar products is given by Liggett and Deitz[56] (in particular the section concerning the chemistry of the colourant is noteworthy). Kort[57] discussed more recently the origin, chemistry and removal of colour in the cane- and beet-sugar industries. The chemistry of the browning reaction of sugars also has been investigated by Fleming et al.[58]. Ramaiah and Kumar[59] studied the kinetics of the caramelization of reducing sugars. The comprehensive thesis of Brandes[60] describes an investigation of colour-forming processes in technical sugar juices, in which the Maillard reaction plays an important role. The thermal decomposition and colour formation in aqueous sucrose solutions has recently been reviewed by Kelly and Brown[3].

Finally, the formose reaction[62], the conversion of formaldehyde with base into a mixture of sugars which is related to the alkaline degradation reaction of monosaccharides, has been extensively investigated since its discovery by Butlerow[62] over a century ago.

### *Mechanisms of alkaline degradation reactions*

The *enediol anion species*, which has to be considered as the starting intermediate in alkaline degradation reactions, enters into several reaction pathways leading to carboxylic acids as the final stable degradation products. The reactions involved have been summarized in Scheme 8[63], whereas in Scheme 9 an overall and somewhat simplified reaction scheme[54] is depicted. It clearly demonstrates that the alkaline degradation reaction must be considered as a dynamic interconversion of C-2 to C-6 monosaccharides from which the carboxylic acid products are formed in an irreversible way. It should be noted that not all the possible reaction pathways in alkaline medium are given in Scheme 9; e.g. further reaction of the aldehydes formed (by cleavage of dicarbonyl reaction intermediates) has not been taken into account.

The 1,2-enediol anion may undergo *β-elimination* (I) to afford the dicarbonyl compound 3-deoxy-erythro-hexos-2-ulose (7). In the same way the dicarbonyl compounds 4-deoxy-glycero-hexo-2,3-diulose (8) and 1-deoxy-erythro-hexo-2,3-diulose (9)[19,45] are formed from the 2,3-enediol anion by elimination of the

4- and 1-OH, respectively.

The α-dicarbonyl compounds, 7, 8 and 9 are unstable under basic reaction conditions and undergo either a *benzilic acid rearrangement* (II) yielding metasaccharinic, isosaccharinic and saccharinic acid, respectively, or a *cleavage reaction* (III) towards a carboxylic acid and an aldehyde. Upon benzilic acid rearrangement two epimeric forms of the saccharinic acids are formed, e.g. from 7 α- and β-metasaccharinic acid are formed, of which C-2 has the (R)- and the (S)-configuration, respectively. 3-O-, 4-O-, and 1-O-alkyl substituted hexoses can be successfully used to obtain selectively the dicarbonyl compounds 7, 8, and 9[55], respectively, by the preferential elimination of the alkoxy group. Subsequent benzilic acid rearrangement of each of these α-dicarbonyl intermediates results in the formation of the corresponding saccharinic acids in high yield. This rather selective alkaline degradation of O-alkyl substituted monosaccharides has been widely used for the laboratory synthesis of $C_6$-saccharinic acids[47].

The 1,2-enediol anion species can also undergo a *retro-aldol reaction* (IV) giving two triose moieties. After enolization these trioses mainly undergo β-elimination towards methylglyoxal, which is subsequently converted into lactic acid, acetic acid and formic acid.

Finally, *aldolization reactions* (V) of (small) carbonyl compounds, formed from the starting hexose, are also of importance during the alkaline degradation. For instance, isotopic labeling revealed that 40% of the 2,4-dihydroxybutyric acid is formed by recombination of C-2 fragments (4 N NaOH, 100 °C)[64].

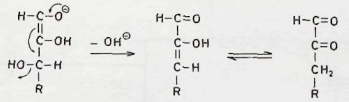
A similar recombination reaction is the already mentioned formose reaction[61]:

$$n\ CH_2O \xrightarrow[\text{trace of} \geq C_2\text{-aldehyde}]{\text{base}} (CH_2O)_n$$

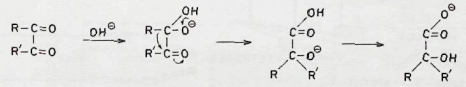
The mixture of monosaccharides thus obtained, generally named formose or formose sugars, mainly consists of both linear and branched aldo- and ketopentoses, and -hexoses. In addition, variable amounts of linear and branched alditols as well as carboxylic acids are irreversibly formed by (cross-)Cannizzaro and degradation reactions. The reaction conditions of the formose and alkaline degradation reaction are quite similar, except that often shorter reaction times are chosen for the formose reaction in order to limit subsequent alkaline degradation of the formose sugars. The formose sugars produced reach a maximum concentration at the so-called yellowing



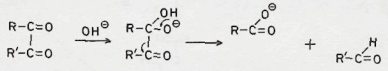
I) β-elimination



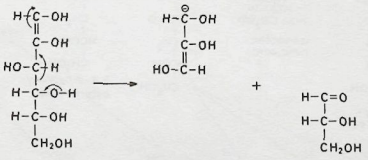
II) benzilic acid rearrangement



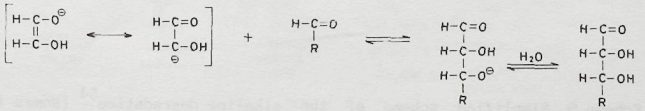
III) α-dicarbonyl cleavage



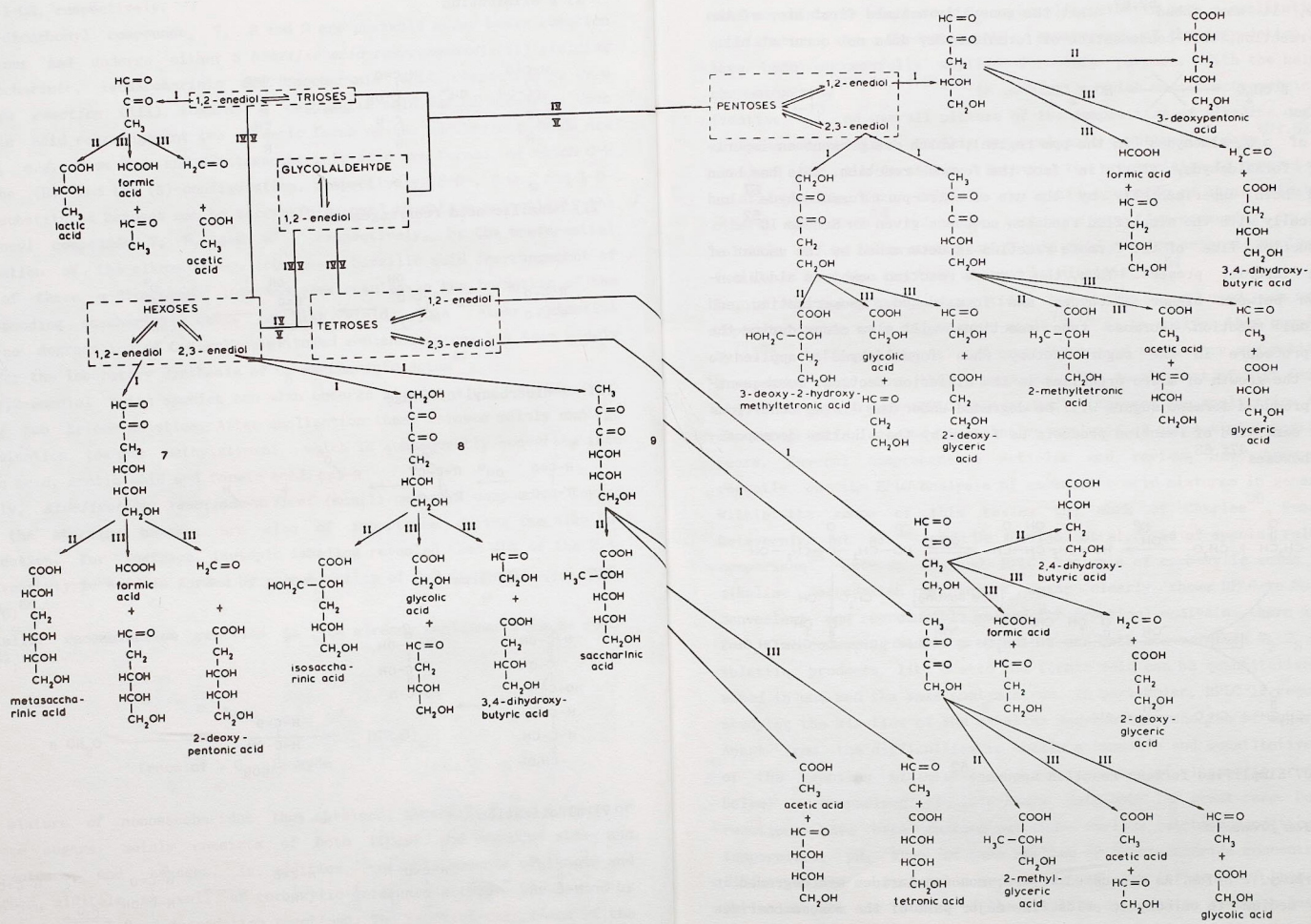
IV) retro-aldolization



V) aldolization



Scheme 8. Rearrangements in aqueous alkaline medium[63].

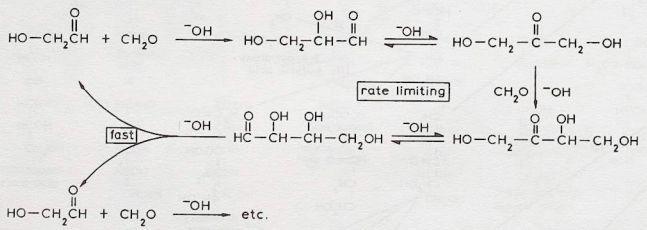



Scheme 9. Simplified scheme of the alkaline degradation[54] (Roman figures refer to reaction types of Scheme 8).

point[65,66].

Recently, it was found[52,67] that the generally assumed first step of the formose reaction, self-condensation of formaldehyde, does not occur at all.

Traces of $\geq C_2$-aldehydes (in the ppm region), which are present as impurities of formaldehyde, start in fact the formose reaction. This has been verified both experimentally by the use of ultra-pure formaldehyde[67] and theoretically with the simplified reaction sequence given in Scheme 10[52]. The induction time of the formose reaction is determined by the amount of aldehyde impurity present. Thus, the formose reaction combines aldol condensation between higher aldehydes and formaldehyde, isomerization, and retro-aldol reaction. Formose type reactions will also occur during the liming procedure in the sugar factory when formaldehyde is applied to inhibit the growth of micro-organisms in the diffusion section. Subsequently, the produced formose sugars will be degraded under the liming conditions to the same kind of reaction products as formed by the alkaline decomposition of hexoses[47f,68].




Scheme 10. Simplified formose reaction sequence[52].

*Degradation products*

*$\leq C_6$ carboxylic acids.* As discussed before, monosaccharides are degraded in alkaline medium to carboxylic acids. The major part of the monosaccharides is converted into low molecular weight carboxylic acids with the same or smaller number of carbons, which will be further denoted as $\leq C_6$ carboxylic acids. Identification of the acid mixture is of great importance in order to obtain a better insight into the degradation pattern of monosaccharides in



aqueous alkaline solutions. For this identification it is necessary to analyse mixtures of these $\leq C_6$ carboxylic acids in both a qualitative and a quantitative way. Chromatographic separations of the degradation products have been successfully applied for this purpose. With the help of gas chromatography[47f,53,54,69,70] in combination with mass-spectrometric identification[71-73] an overall picture of the composition of alkaline degradation mixtures was obtained. In this way, reliable quantitative data[47f,53,54] were obtained for the first time. However, the prerequisite to convert the non-volatile acids into volatile derivatives, mostly as their trimethylsilyl ethers and esters[74], makes this technique very time-consuming. In addition, volatile acids like acetic and formic acid require separate analysis after isolation from the reaction mixture by distillation[54].

Liquid chromatographic separations have also been used, as well as to separate acids on a preparative scale[75,76] as to analyse them quantitatively[77-82]. Different types and combinations of stationary and mobile phases have been used for these purposes. The development of High Performance Liquid Chromatography (HPLC) has markedly improved the application of liquid chromatography for the quantitative analysis of complex mixtures in recent years. Several comprehensive articles and reviews have been published recently on the HPLC analysis of carboxylic acid mixtures in general[83-89]. Within the scope of this review the work of Charles[90], Kubadinow[91], Detavernier et al.[92] and De Bruijn et al.[93] is of special relevance. A comparison[93] between GC and HPLC analysis of carboxylic acids formed by alkaline degradation of invert sugar clearly shows HPLC to be the more convenient and reproducible method for (routine) analysis: there is no need for time-consuming sample preparation and both non-volatile $C_2$-$C_6$ acids and volatile products like acetic and formic acid can be quantitatively determined in one and the same analysis run. In particular, HPLC is required when studying the kinetics of the alkaline degradation reaction of sugars.

Apart from the difficulties to obtain a complete and quantitative analysis of the reaction mixtures due to the formation of oligomeric products (see below) a comparison of literature data asks for great care. Degradation reactions have been carried out under various reaction conditions: either temperature, pH, type of base applied or monosaccharide concentration may differ while a systematic screening of the influence of these reaction variables on the course of the alkaline degradation reaction has not been performed so far. As an illustration, three examples of literature data are given in Table 5, referring to the $\leq C_6$ carboxylic acid part of degraded glucose. The present-day knowledge of the dependency of the alkaline degradation and its product composition on various reaction variables will

Table 5. $\leq C_6$ carboxylic acid composition[a] of alkaline degradation mixtures.

| Carboxylate content mol (%) | 1.22 M KOH<br>0.1 M D-glucose<br>5 °C/$N_2$ [8,54,b] | 0.01 M KOH<br>0.035 M D-glucose<br>80 °C/$N_2$ [54,b] | 0.10 M NaOH<br>0.02 M D-glucose<br>45 °C/$N_2$ [53,c] |
|---|---|---|---|
| lactate | 73 | 20 | 59 |
| glycolate | < 0.5 | 17 | - |
| 2-methylglycerate | < 0.5 | - | - |
| glycerate | 1 | 1 | 2 |
| 2,4-dihydroxybutyrate | 3 | 15 | 13 |
| 3-deoxypentonate | 1 | 4 | 2 |
| metasaccharinate | 10 | 12 | 24 |
| isosaccharinate | - | 3 | - |
| formate | -[d] | 15 | -[d] |
| acetate | -[d] | 13 | -[d] |
| total $\leq C_6$ carboxylates | 88 | 60 | -[d] |
| unidentified products | < 0.5 | 40 | -[d] |
| D-fructose | 5 | - | - |
| D-glucose | 7 | - | - |

[a] As percentage of total $\leq C_6$ carboxylic acids observed after (almost) complete conversion.
[b] pH kept constant by continuous addition of 2 M KOH.
[c] pH not kept constant.
[d] Not determined.

be discussed later on in this review.

*$> C_6$ carboxylic acids.* Generally, only the $\leq C_6$ carboxylic acid part of the alkaline degradation products is mentioned in literature. The amount of products other than $\leq C_6$ carboxylic acids may be substantial, however, and depends on the conditions of the alkaline degradation reaction. For instance, yields between 0 and 40% of these so-called $> C_6$ products have been found[31,54]. Several investigators[3,56,58-60,94-99] attempted to characterize these $> C_6$ degradation products, most often in relation to the colour formation during the alkaline degradation process. Because of the difficult separation of the complex reaction mixtures containing $> C_6$ products, a complete elucidation of the nature and structure was not achieved. Prey and



coworkers[94], however, ascertained some characteristic properties of these products, which give, supported by the results of other workers[3,56,58-60,95-99], a good indication of their structure. The oligomeric and polymeric character of the $> C_6$ products was established by gel filtration of different alkaline degradation mixtures. Compounds with mol. wt. ~ 300-400 are thought to be the precursors of polymers with mol. wt. > 5000[94]. Formation of high molecular weight substances is most probably due to (aldol) condensation of (di)carbonyl compounds present in the alkaline degradation solution. Although chemical methods (bromine consumption, Tillman's reaction) revealed the occurrence of enediol carbonyl structures[94], the mechanism for the formation of such a conjugated structure has not been clarified. Nevertheless, conjugated enol instead of enediol carbonyl structures must be preferred since enol carbonyls are easily formed by β-OH elimination (I). Enol carbonyl structures have also been suggested by Fleming et al.[58] and Ziderman et al.[97] based upon IR and UV measurements. During alkaline degradation of monosaccharides a strong absorption maximum appears at about 265 nm (see Fig. 1) measured at pH = 7, which rises to a maximum and then remains constant, while the absorption gradually extends into the visible regio[95]. A reversible hypsochromic shift and hypochromic

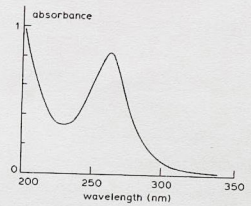



Fig. 1. UV absorption observed upon alkaline degradation of monosacharides[58].

effect of this absorption maximum was obtained by decreasing the pH below 3[32,58,97]. These UV spectral phenomena of the chromophore indicate pH-dependent keto-enol tautomerization, according to that of malonaldehyde[100]. In neutral and alkaline aqueous solutions the enol(ate) form of the chromophore predominates, while on acidification the chromophore will tautomerize into the dicarbonyl structure[97]. There is a close similarity in UV absorption between the chromophore of the alkaline degradation mixture and 5,5-dimethyl-cyclohexan-1,3-dione[58], which is almost completely enolized in aqueous solution (see Fig. 2).

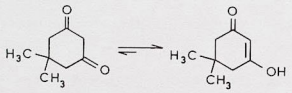


Fig. 2. Enolization of 5,5-dimethylcyclohexan-1,3-dione in water[58].

The alkaline induced chromophore discussed here may be related to the chromophores obtained by UV irradiation of monosaccharides[101,102]. The formation of the chromophore upon irradiation was markedly enhanced by the addition of alkali. The calculated $pK_a$ of these photodegradation products is 4.5[101], which demonstrates their acidic nature. Several workers[60,95,98,99,103] have also found evidence for the acidity of the polymers formed upon alkaline degradation. The carboxyl group can be either isolated from the chromophore or may be part of the chromophore. Thus, besides the well-characterized $\leq C_6$ acid product mixture, the alkaline degradation of monosaccharides results in the formation of other unidentified products up to 40% under certain conditions, which can be assigned as $> C_6$ carboxylic acids with the following properties:

(i) Molecular weights corresponding to di-, tri- up to poly-$C_6$ sugar moieties.

(ii) Conjugated enol carbonyl moieties present (UV absorption $\lambda$ = 265 nm).

(iii) Acidic character by the presence of one or more carboxylic acid groups.



*Miscellaneous products.* Other products than those described above are only formed in trace amounts (< 1%) and can be neglected in mass balance calculations. These minor products can be divided into two categories: (i) volatile non-acidic compounds[69,104,105] and (ii) cyclic unsaturated aldehydes or ketones[106-109]. Reinefeld et al.[69] were able to detect methanol, acetaldehyde, acetone and hydroxyacetone in alkaline degradation solutions of invert sugar to a total amount of 0.04 %. Lento et al.[105] detected in the condensed distillate of boiling alkaline solutions of D-glucose, D-fructose and dihydroxyacetone trace amounts of diacetyl, hydroxyacetone and methylglyoxal. Extraction of alkaline degradation mixtures with organic solvents (ether, ethyl acetate) revealed, by GC of the extracts, trace amounts of more than 20 different products[106-109]. Among them, hydroxyacetone and three hydroxybutanones were considered by Shaw et al.[106] as the precursors of cyclic diketones, which have strong caramel-like odours. Base-catalyzed intramolecular condensation of the four hydroxyketones may lead to the observed cyclic compounds (Fig. 3) as was confirmed by model experiments.

Formation of 2-hydroxy-2-cyclopenten-1-one derivatives upon alkaline degradation of monosaccharides was also reported by Forsskahl et al.[107], Enkvist et al.[108], and Koetz and Neukom[109]. In addition, trace amounts of aromatic compounds, e.g. phenols and acetophenones, have been detected[107]. The generally low yield of cyclic compounds is not surprising, since they are formed by condensation of hydroxyketones[106], which themselves are present in only minor amounts. In accordance to this, high concentrations of monosaccharides ($\geq$ 0.5 M) are required for the formation of detectable amounts of cyclic compounds.

*Kinetics of the alkaline degradation reactions*

Kinetic models of the alkaline degradation of monosaccharides[8,33-39,110,111] should take into account the reversible isomerization as well as the irreversible degradation reaction. Unfortunately, it is not possible to compare the data derived from different kinetic models since the reaction conditions differ. Two examples of such kinetic models are shown above (see Schemes 6 and 7).

As the enediol anion species is the key intermediate in both the isomerization and the degradation reaction, a complete kinetic model should take this species into account. UV spectroscopy has been applied[8] for the determination of the enediol concentration, but the lack of a reliable molar extinction coefficient prevented quantitative measurements. Therefore, most

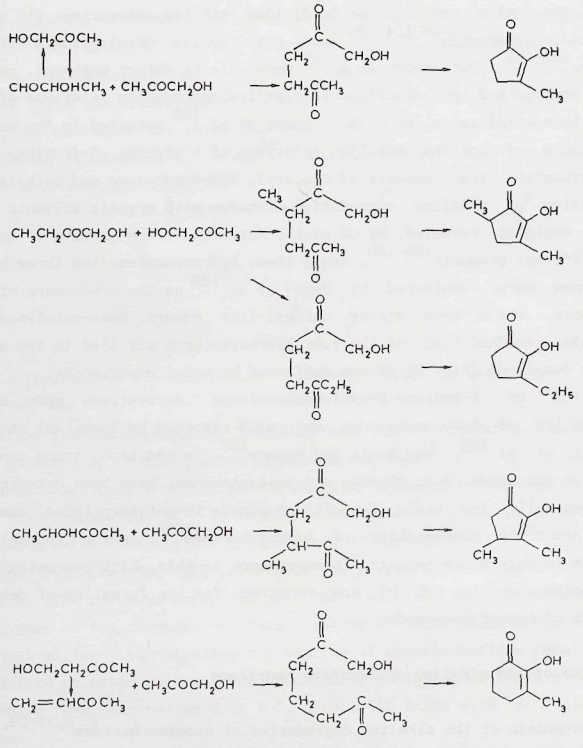


Fig. 3. Base-catalyzed formation of five cyclic diketones (hydroxycyclopentenones)[106].

kinetic data are based on the monosaccharide concentrations to which the enediol anion concentration is assumed to be proportional.



The following characteristic kinetic features can be summarized for the alkaline degradation:

(i) Without taking into account the interconversion reactions of monosaccharides, the alkaline degradation reaction can be considered as a first order reaction in the total monosaccharide concentration[33,39,110]. However, recent results show that this is only true after the isomerization equilibrium has been achieved[32].

(ii) The decomposition rate constant of hexoses is proportional to the hydroxyl ion activity[35,110,111] and depends on the temperature according to the Arrhenius equation (activation energy 107.6 kJ.mol$^{-1}$) as described by[111]

$$\log k = 16.9 - 5620/T - pOH$$

which is valid for pOH 1.5-4.0 and 50-150 °C, with k in min$^{-1}$ and T in K.

(iii) Divalent cations like calcium and magnesium accelerate the decomposition of monosaccharides[110] and influence the final products composition.

(iv) The initially fast decomposition of D-fructose with respect to D-glucose[39] and D-mannose is presumably due to the higher enolization rate of fructose[8].

(v) From degradation reactions at different hydroxyl ion concentrations, Lai[35] and Bamford et al.[39] proposed the formation of both mono- and dianion intermediates of monosaccharides.

## Influence of reaction variables on product formation

The rate and course of the alkaline degradation reaction can be influenced by variation of several reaction parameters. Variables are the hydroxyl ion concentration of the aqueous alkaline solution, the reaction temperature, the nature of base used, the concentration of monosaccharide and the nature and pressure of the gaseous atmosphere applied. Also additives may cause a change in the product composition. The role of amino acids in this context is well-known, but, for instance, the possible influence of high concentrations of sucrose (as present in industrial juices) on the alkaline degradation should also be considered. The present-day knowledge of the role of each variable in the degradation reactions is rather small, as will be shown below, but the further development of sophisticated analysis techniques

(like GC and especially HPLC) will result in more information about this area[112]. The influence of the above mentioned reaction variables on the alkaline degradation reaction as far as known, will be discussed successively below.

*Hydroxyl ion concentration*

In the past, several investigators[54,96,113] have studied the alkaline degradation as a function of the hydroxyl ion concentration. Unfortunately, some of them[96,113] have used buffer systems containing different types of cations which may highly influence the course of the degradation reaction (see below). Nevertheless, it has been ascertained[54] that the selectivity towards lactic acid increases when the hydroxyl ion concentration is raised. The total amount of formic, acetic, glycolic and glyceric acid and the total amount of saccharinic acids decreases[54]. Recently, the influence of the hydroxyl ion concentration on the final product composition has been studied in detail over a wide concentration range[31,112] with the use of HPLC analysis[93] (Fig. 4). Variation of the hydroxyl ion concentration dramatically influences both the composition of the $\leq C_6$ carboxylic acid products and the relative amounts of $\leq C_6$ and $> C_6$ products. The maximum production ($\sim$ 40%) of $> C_6$ carboxylic acids at $-\log[HO^-]$ = 2-3 indicates the formation of the high molecular weight compounds by aldol condensation between neutral sugars and ionized α-dicarbonyl compounds[114].

*Temperature*

The composition of reaction products is independent of the reaction temperature. For example, D-glucose degraded at either 5 °C (0.1 M, 1.22 M KOH) or 80 °C (0.07 M, 1.22 M KOH) gives after complete conversion the same product composition[54]. Apparently, the differences in activation energy for the formation of the various degradation and oligomerization products are negligible.



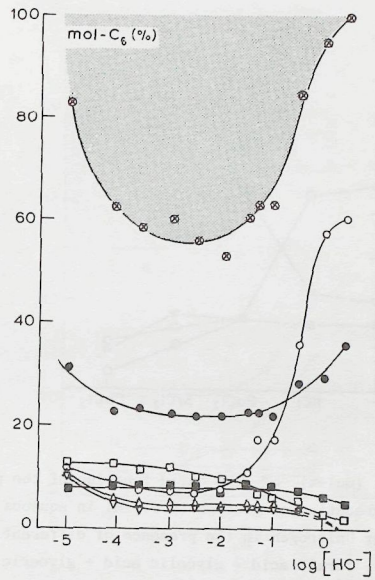



Fig. 4. Formation of carboxylic acids as a function of the hydroxyl ion concentration (0.025 M D-glucose, D-fructose or D-mannose, KOH, $H_2O$, 78 °C, $N_2$, 100% conversion)[31,112]; gray region, $> C_6$ acids; ⊗, $\leq C_6$ acids: ●, saccharinic acids; △, glycolic acid; ○, lactic acid; ▽, formic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid.

*Nature of base*

It has been found[54,94f] that the cation of the base influences the degradation pattern. In particular divalent cations favour lactic acid formation, whereas the amounts of $C_1$ to $C_3$ acids decrease[54] (Fig. 5).

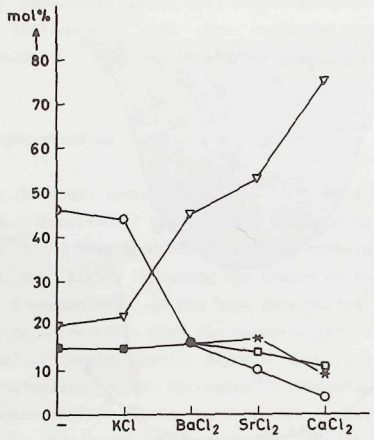



Fig. 5. Composition (mol-%) of the acid fraction of the product from the alkaline degradation of glucose (0.035 M) in aqueous KOH (0.01 M) at 80 °C under nitrogen in the presence of different metal chlorides (0.07 M); ○, formic acid + glycolic acid + glyceric acid; ▽, lactic acid; □, 2,4-dihydroxybutyric acid + 2-methylglyceric acid; *, saccharinic acids (only $\leq C_6$ acidic products have been taken into account)[54].

Probably, this is due to the complexation phenomena between divalent cations and reaction intermediates or with the hexoses.

*Concentration of monosaccharides*

Recently, it has been found that the product composition largely depends on the monosaccharide concentration[31,112], as demonstrated in Fig. 6. Alkaline degradation of very diluted monosaccharide solutions results in an almost complete conversion of the monosaccharides into $\leq C_6$ carboxylic acids. Concentrated monosaccharide solutions show the formation of substantial amounts of $> C_6$ carboxylic acids, which further supports the assumption that these acids are formed by aldol condensation reactions of monosaccharide moieties.



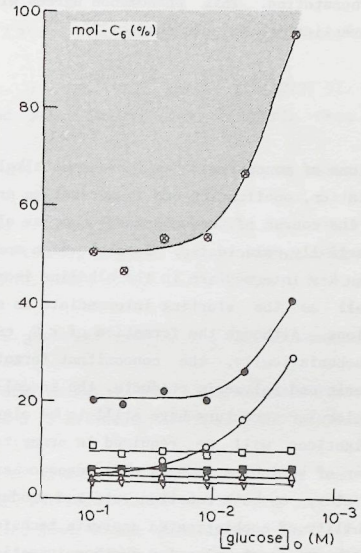



Fig. 6. Influence of D-glucose concentration on carboxylic acid formation (0.01 M KOH, $H_2O$, 78 °C, $N_2$, 100% conversion)[31,112]; gray region, $> C_6$ acids; ⊗, $\leq C_6$ acids: ●, saccharinic acids; △, glycolic acid; ○, lactic acid; ▽, formic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid.

*Nature of the gaseous atmosphere*

The alkaline degradation reactions discussed so far refer to experiments under nitrogen since oxygen was generally suspected to induce undesired oxidation reactions. When molecular oxygen is supplied at atmospheric or higher pressure the reaction becomes more selective by increased $C_1$-$C_2$ bond fission. The main products are formic acid and D-arabinonic acid[96,115,116] besides small amounts of D-erythronic, D-glyceric and glycolic acid[115,116]. Schiweck[117] reported that experiments of long duration on laboratory as well as on technical scale have shown a colour improval of thin juice if very

finely dispersed air was bubbled through the juices during the main liming or the first carbonatation. This phenomenon agrees with the findings of other industrial investigators on this subject[118-120].

## Concluding remarks

The inital reactions of monosaccharides in aqueous alkaline solution, i.e. ionization, mutarotation, enolization and isomerization are well understood. On the other hand, the course of the subsequent complex alkaline degradation has only been partially elucidated. Enediol anion species are generally accepted to be the key intermediate in the alkaline isomerization of monosaccharides as well as the starting intermediate in subsequent alkaline degradation reactions. Although the formation of $\leq C_6$ carboxylic acids has been explained mechanistically, the concomitant formation of substantial amounts of oligomeric and polymeric products, the so-called $> C_6$ carboxylic acids, and their molecular structure have still to be clarified.
Systematic investigations will be required in order to determine the influence of a number of variables such as pOH, monosaccharide concentration, nature of base, etc., on both reaction rate and product composition. The present-day availability of sophisticated analysis techniques like GC, HPLC, MS and NMR will be of great value for further investigations in order to derive a complete mechanistic and molecular picture of the alkaline degradation reaction.

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CHAPTER 3

# INFLUENCE OF REACTION PARAMETERS ON THE FINAL PRODUCT COMPOSITION OF THE ALKALINE DEGRADATION OF MONOSACCHARIDES*

## Introduction

Reactions of monosaccharides in aqueous alkaline solution have been studied extensively for almost a century since the classical paper[1] of Lobry de Bruyn and Alberda van Ekenstein, concerning the interconversion of D-glucose, D-fructose and D-mannose in alkaline medium. These reactions can be divided into two distinct groups of transformations:

(i) Initial transformations, in which the sugar moiety remains intact and undergoes reversible rearrangements like ionization, mutarotation[2-5], enolization and isomerization[6-10].

(ii) Degradation reactions[6,11-16], on the other hand, part of which are irreversible and which lead ultimately to organic acid products.

The degradation of monosaccharides (Fig. 1) starts with the elimination of a

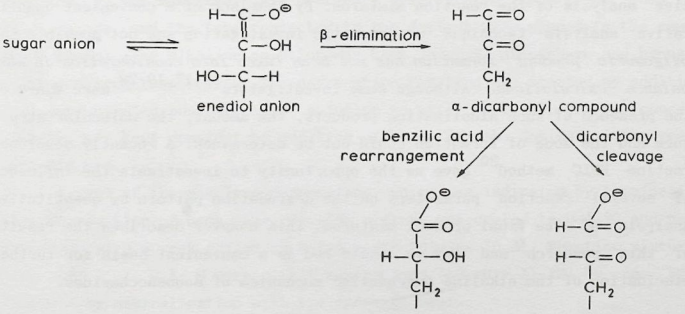


Fig. 1. Part of reaction network of monosaccharides in alkaline medium.

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β-OH group from an enediol anion species, which is also an intermediate in the isomerization reaction. The resulting α-dicarbonyl compound can undergo either a benzilic acid type of rearrangement or a cleavage of the C-C bond between the carbonyl groups (α-dicarbonyl cleavage). The products formed upon benzilic acid rearrangements are α-hydroxycarboxylic acids, e.g. lactic acid and saccharinic acids. Dicarbonyl cleavage gives both a carboxylic acid, e.g. formic acid, acetic acid, glycolic acid, and an aldehyde, e.g. formaldehyde, acetaldehyde.

Aldolization and retro-aldolization of carbonyl compounds present in the degradation mixture play also an important role in the alkaline degradation pattern of monosaccharides. These reactions result in elongation and fragmentation of the carbon chain, respectively, until one of the above-mentioned termination reactions occurs. The aldolization reaction has received little attention in mechanistic considerations of the alkaline decomposition of carbohydrates, despite the fact that it may be responsible for the formation of substantial amounts (up to 50%) of oligomeric acidic products[15,17], the so-called > $C_6$ acids.

The present-day mechanistic insights of the reactions of monosaccharides in aqueous alkaline solution have been reviewed in Chapter 2[18]. It shows that the mechanism of the initial transformations of monosaccharides in alkaline medium are well understood. The course of the subsequent complex alkaline degradation, on the other hand, has only been partly elucidated. Despite all the efforts in this area, the major challenge remained a complete quantitative analysis of the reaction mixtures. By the lack of a convenient quantitative analysis technique a systematic investigation was not possible and *oligomeric product formation has not been taken into consideration in mass balance calculations.* Although some investigators[15,17,19-29] were aware of the presence of such aldolization products, the amount, the molecular structure and the mode of formation could not be determined. A recently developed routine HPLC method[30] gave us the opportunity to investigate the influence of several reaction parameters on the degradation pattern by quantitative analysis of the final product mixtures. This chapter describes the results of this research and may be considered as a convenient basis for further elucidation of the alkaline degradation mechanism of monosaccharides.



Experimental

*Materials*

Pyruvaldehyde was obtained from Janssen Chimica as a 40 wt % aqueous solution which was freshly distilled prior to the experiments. D-Psicose was prepared[31] according to the literature. All other chemicals were obtained from Merck (analytical grade).

*Apparatus*

The alkaline degradation experiments were carried out in a 150 ml vessel equipped with a thermostat jacket, a pH-electrode connected to a pH-meter, a dosimat filled with 2 M KOH, a magnetic stirrer, a sampling tube, gas-inlet and -outlet tubes, and a revolving tubular device for adding reactants. The gas-inlet was connected to a gas burette containing nitrogen. Experiments were performed at atmospheric nitrogen pressure and constant temperature. The pH was kept constant with an impulsomat.

*Procedure*

As oxygen may induce undesired oxidation reactions, the reaction vessel, containing an aqueous solution of the desired $HO^-$ concentration, was evacuated and $N_2$ was admitted. This procedure was repeated three times. Then the pH-electrode and the tube connected to the dosimat were placed in the reaction vessel. The alkaline solution was kept under $N_2$ atmosphere and brought to the desired temperature. The degradation reaction was started by addition of a concentrated monosaccharide solution in water (1-2 ml). The $HO^-$ concentration was kept constant by addition of base from the dosimat to the reaction mixture.

At the end of the alkaline degradation, which was indicated by termination of the addition of base, the reaction mixture was cooled to 0-4 °C and neutralized with a weak cation exchange resin (BioRex 70 H). Reaction mixtures with $[HO^-]$ > 0.1 M were partly neutralized (to pH = 11-12) by conc. HCl, followed by neutralization with the exchange resin.

*Analysis method*

HPLC analyses of the carboxylic acids in the neutralized final reaction mixtures were performed as described in Chapter 9[30] (column HPX 87 H from Bio-Rad, 300 mm length, 7.8 mm i.d., column temperature 60 °C, aqueous 0.005 M $H_2SO_4$ or 0.01 M trifluoroacetic acid as the eluent, flow 0.6 ml/min, RI-detection). GC analyses[30] were carried out after freeze-drying and trimethylsilylation of the samples (capillary CP Sil 5 column, 25 m length, 0.23 mm i.d., temperature programme: 5 min at 75 °C, increase at a rate of 8 °C/min to 280 °C, 5 min at 280 °C, flame ionization detector).

The amounts of acidic products have been expressed as percentages mol-$C_6$ *produced from the starting* $C_6$*-monosaccharide according to the following* definition:

$$\text{mol } C_n = \frac{n}{6} * \text{mol-}C_6,$$

in which $C_n$ represents an organic acid with n carbon atoms. By this definition the amount of a degradation product is expressed as the fraction of the $C_6$-monosaccharide that is converted into that particular product. For example, if 100 mol D-fructose produces by alkaline degradation 6 mol formic acid, the yield of formic acid is 1 mol-$C_6$-%; 6 mol acetic acid corresponds with 2 mol-$C_6$-%, etc.

The total amount of $> C_6$ acids has been defined as the deficit on the mass balance after summation of the quantitatively analyzed $\leq C_6$ acids. The $> C_6$ acids did not produce separated peaks in HPLC but resulted in a raise of the base-line at the first part of the chromatogram due to size exclusion of these oligomeric products by the stationary phase (sulfonated styrene-divinylbenzene copolymer, $H^+$ form). There was no interference of the $> C_6$ acids with the other acidic reactions products.

## Results and discussion

*Preliminary experiments*

D-Glucose, D-fructose, D-mannose and D-psicose were treated with alkali under comparable conditions in order to verify that these monosaccharides are degraded via common enediol anions as intermediates. The almost identical final product compositions (Table 1) prove that alkaline degradation of



interconvertible monosaccharides proceeds indeed via the same 1,2- and 2,3-enediol anion species.

Table 1. Final acidic product composition of alkaline degraded monosaccharides[a].

| carboxylic acid (mol-$C_6$-%) | D-psicose | D-fructose | D-glucose | D-mannose |
|---|---|---|---|---|
| saccharinic acids | 20.3 | 22.5 | 21.5 | 23.0 |
| glycolic acid | 4.7 | 4.5 | 5.0 | 5.0 |
| lactic acid | 6.4 | 6.8 | 6.9 | 6.6 |
| formic acid | 3.0 | 3.6 | 4.0 | 3.4 |
| acetic acid | 9.8 | 10.1 | 10.5 | 9.5 |
| 2,4-dihydroxy-butyric acid | 8.6 | 8.0 | 8.5 | 7.8 |
| total $\leq C_6$ acids | 52.8 | 55.5 | 56.4 | 55.3 |
| total $> C_6$ acids | 47.2 | 44.5 | 43.6 | 44.7 |

[a] Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion.

*Effect of hydroxyl ion concentration*

In order to determine the influence of the $HO^-$ concentration on the final degradation product composition, monosaccharides were reacted over a wide $HO^-$ concentration range, i.e. from $10^{-5}$ M to 2.5 M. The results given in Fig. 2 show that in particular the amounts of lactic acid and the $> C_6$ acids are strongly dependent on the $HO^-$ concentration applied.

For $[HO^-] > 10^{-3}$ M *these results are in agreement with earlier observations* of De Wit[15]. Although the selectivity towards lactic acid at increasing $HO^-$ concentration also confirms the data of earlier observations[32,33], a good comparison is difficult because of the use of buffer systems containing different types of cations which might effect the course of the degradation reaction (see below).

In order to determine the saccharinic acid composition we have carried out GC analyses which further confirmed the data obtained by HPLC. Apart from trace amounts of saccharinic and isosaccharinic acid, metasaccharinic acid

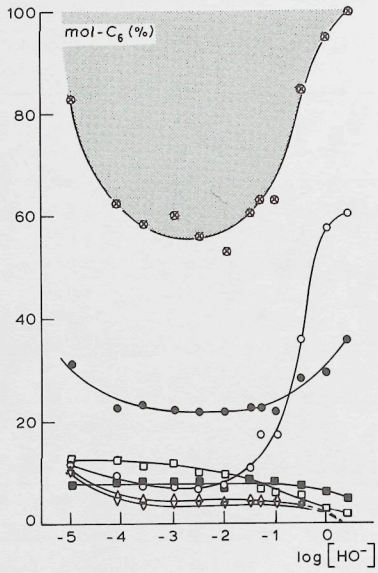


Fig. 2. Formation of carboxylic acids as a function of the $HO^-$ concentration (0.025 M D-glucose, D-fructose or D-mannose, KOH, $H_2O$, 78 °C, $N_2$, 100% conversion); gray region, > $C_6$ acids; ⊗, ≤ $C_6$ acids: ●, saccharinic acids; △, glycolic acid; ○, lactic acid; ▽, formic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid.

is the major saccharinic acid formed in the presence of the monovalent bases (KOH or NaOH) studied. In addition, minor amounts of 3-deoxypentonic acid (~ 2 mol-$C_6$-%), 2-methylglyceric and glyceric acid (< 0.5 mol-$C_6$-%) were present according to GC.



The remarkably high production of > $C_6$ acids at $HO^-$ concentrations between $10^{-1}$ M and $10^{-4}$ M indicates that the formation of these (higher molecular weight) products involves aldolization reactions between neutral and ionized (di)carbonyl compounds. As a first approximation, the > $C_6$ acid curve reflects the overall pH-dependence of the kinetic expression:
rate = constant * [anionic species] * [neutral species].

*Effect of monosaccharide concentration*

De Wit[15] has investigated the influence of the D-glucose concentration on the degradation pattern in aqueous 0.39 M NaOH at 50 °C. No change in the product composition was observed upon increasing the D-glucose concentration from 0.015 M to 0.15 M. As shown above, aldolization reactions will be of minor importance at this high $HO^-$ concentration. For that reason we have reinvestigated the influence of the monosaccharide concentration at an $HO^-$ concentration of $10^{-2}$ M where maximum > $C_6$ acid production occurs. Variation of the monosaccharide concentration between $10^{-1}$ M and $2 * 10^{-3}$ M now shows a strong dependency of the > $C_6$ acid content in the final product on the starting monosaccharide concentration (Fig. 3). Decreasing the monosaccharide concentration below $10^{-2}$ M causes a sharp decrease in the formation of > $C_6$ acids. Concomitantly, the content of saccharinic acids and lactic acid markedly increases. As α-dicarbonyl compounds are well-established precursors for saccharinic and lactic acid formation, the shift of these $C_6$ and $C_3$ acids towards > $C_6$ acids upon increasing D-glucose concentrations indicates the importance of α-dicarbonyls in the oligomerization reaction.
The effect of the sugar concentration on the product formation has been verified at two other $HO^-$ concentrations, $9 * 10^{-4}$ M and $4.4 * 10^{-2}$ M, respectively, which are also expected to create a situation for maximum > $C_6$ acid production. As illustrated in Fig. 4, the dependence of the amount of > $C_6$ acids produced on the monosaccharide concentration is comparable for the three $HO^-$ concentrations investigated.

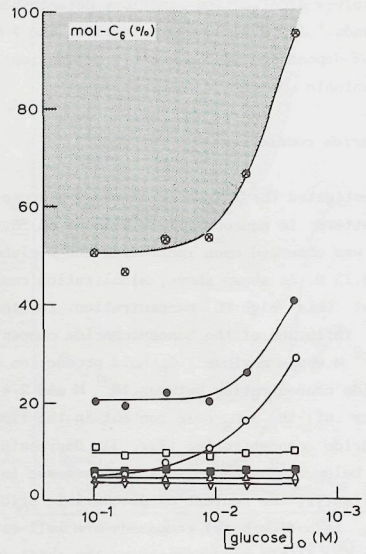


Fig. 3. Influence of D-glucose concentration on carboxylic acid formation (0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion); gray region, $> C_6$ acids; ⊗, $\leq C_6$ acids: ●, saccharinic acids; △, glycolic acid; ○, lactic acid; ▽, formic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid.



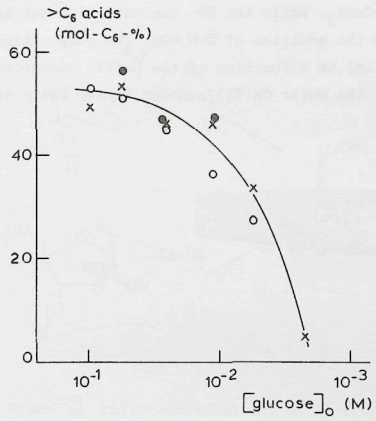


Fig. 4. Amount of $> C_6$ acids produced versus the starting monosaccharide concentration. Reaction conditions: ○, 4.4 * $10^{-2}$ M KOH; ×, $10^{-2}$ M KOH; ●, 9 * $10^{-4}$ M KOH; $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion.

*Effect of calcium(II)*

As shown before[15], the nature of base used, in particular the valence of the cation, may markedly influence the final product composition of the alkaline degradation of monosaccharides. This effect is of direct importance for the juice purification stage in the sugar manufacture which involves the alkaline degradation of invert sugar by calcium hydroxide.

At standard reaction conditions (0.025 M monosaccharide, 0.01 M KOH, $H_2O$, 78 °C, $N_2$) the influence of the Ca(II) concentration has been studied (up to 0.04 M) by the addition of $CaCl_2$, while the $HO^-$ concentration was kept constant during the reaction by the addition of 2 M KOH. The composition of the final product (HPLC analysis) as a function of the Ca(II) concentration is shown in Fig. 5. Increase of the molar Ca(II)/monosaccharide ratio increases

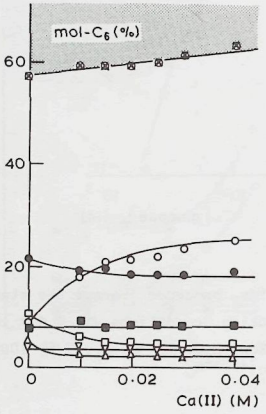


Fig. 5. Formation of carboxylic acids as a function of Ca(II) concentration (0.025 M D-fructose, 0.01 M KOH, $H_2O$, Ca(II) supplied as $CaCl_2$, 78 °C, $N_2$, 5 h, 100% conversion); gray region, $> C_6$ acids; ⊗, $\leq C_6$ acids: ●, saccharinic acids; △, glycolic acid; ○, lactic acid; ▽, formic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid.

lactic acid formation at the cost of glycolic acid, acetic acid, saccharinic acids and the $> C_6$ acids. The major effect occurs up to a 1:1 molar Ca(II)/-monosaccharide ratio. Of the various possible Ca(II)-monosaccharide complexes both the β-D-fructofuranose-Ca(II) and β-D-fructopyranose-Ca(II) complexes will occur predominantly because of their tridentate mode of coordination. As shown in Fig. 6, these complexes may explain the promoted $C_3$-$C_4$ bond fission by retro-aldolization.



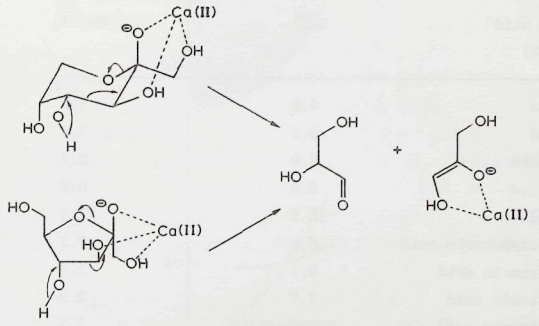


Fig. 6. Promoted retro-aldolization of D-fructose by complexation with calcium(II).

In addition, complexation of Ca(II) with reaction intermediates like enediol anion species and α-dicarbonyl compounds as well as with product α-hydroxy acids will occur. This is indicated by the increase of the benzilic acid rearrangement/dicarbonyl cleavage ratio of α-dicarbonyl intermediates upon increasing the Ca(II) concentration. The slight decrease in $> C_6$ acids shows that Ca(II) has only a small influence on the oligomerization reaction.

To obtain more detailed information about the Ca(II) effect on the composition of the reaction products, both HPLC and GC were applied (Table 2). The Ca(II)-induced formation of 2-methylglyceric, 2-methyltetronic, 3-deoxy-2-hydroxymethyltetronic, saccharinic, and isosaccharinic acid originates from benzilic acid rearrangements of 2,3-dicarbonyl compounds. Apparently, 2,3-dicarbonyls are formed in higher amounts in the presence of Ca(II), whilst the dicarbonyl cleavage reaction becomes less important as indicated by the decrease in glycolic and acetic acid.

The influence of the $HO^-$ concentration on the alkaline degradation of monosaccharides in the presence of Ca(II), see Fig. 7, resembles that in the presence of monovalent cations (Fig. 2).

Table 2. Effect of cation on the final product composition of the alkaline degradation of D-fructose[a].

| carboxylic acid (mol-$C_6$-%) | K(I) | Ca(II) |
|---|---|---|
| formic acid | 4.0 | 3.8 |
| acetic acid | 9.5 | 3.9 |
| *glycolic acid* | 5.0 | 2.4 |
| glyceric acid | 0.5 | 0.9 |
| lactic acid | 12.5 | 27.3 |
| 2,4-dihydroxybutyric acid | 8.0 | 7.1 |
| 2-methylglyceric acid | 0.1 | 2.7 |
| 3-deoxypentonic acid | 2.7 | 2.9 |
| 2-methyltetronic acid | < 0.1 | 1.2 |
| 3-deoxy-2-hydroxymethyl-tetronic acid | < 0.1 | 0.5 |
| metasaccharinic acid | 22.5 | 9.1 |
| saccharinic acid | < 0.1 | 3.8 |
| isosaccharinic acid | < 0.1 | 2.5 |
| total ⩽ $C_6$ acids | 64.8 | 68.1 |
| total > $C_6$ acids | 35.2 | 31.9 |

[a] Reaction conditions: 0.025 M D-fructose; 0.03 M KOH and 0.08 M $Ca(OH)_2$ (~ 0.03 M $HO^-$), respectively; $H_2O$, 78 °C, $N_2$, 4 h, 100% conversion.



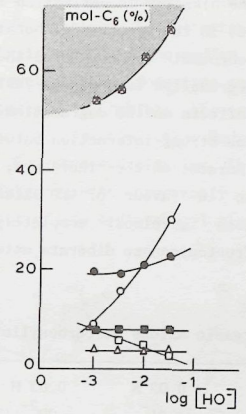


Fig. 7. Formation of carboxylic acids in the presence of Ca(II) as a function of the $HO^-$ concentration (0.025 M D-fructose, KOH, 0.015 M $CaCl_2$, $H_2O$, 78 °C, $N_2$, 100% conversion); gray region, > $C_6$ acids; ⊗, ⩽ $C_6$ acids: ●, saccharinic acids; △, glycolic acid and formic acid; ○, lactic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid.

*Effect of borate*

The possible influence of inorganic anions on the alkaline degradation of monosaccharides has been investigated for chloride, carbonate and borate. Chloride, since it is present in the reaction mixtures used to determine the influence of Ca(II) on the degradation pattern. Carbonate, because of the carbonatation of the alkaline juice at the industrial juice purification. Borate, since it is known[34-36] to shift the isomerization equilibrium of

monosaccharides by borate ester formation. For instance, an increase of the maximum D-fructose concentration from 30% to 80% is reported[34,35] upon addition of boronates to the alkaline isomerization of D-glucose, while ~ 50% D-fructose is obtained in the presence of borate[36].

The effect of chloride, carbonate and borate on the final product composition of the alkaline degradation is given in Table 3. Both carbonate and chloride show only small effects on the degradation pattern. This indicates that - as expected - no strong interaction between these anions and the reactive species occurs. Borate, on the other hand, markedly influences the final product composition in favour of the saccharinic acids. Under the conditions used, D-fructose is almost completely present as its borate esters of which the β-D-fructopyranose diborate esters A and B are the main species (Fig. 8)[37].

Table 3. Influence of inorganic anions on carboxylic acid formation[a].

| carboxylic acid (mol-$C_6$-%) | --[b] | 0.03 M $Cl^-$ | 0.03 M $CO_3^{2-}$ | 0.1 M $B(OH)_4^-$ |
|---|---|---|---|---|
| saccharinic acids | 21.5 | 21.4 | 20.0 | 42[c] |
| glycolic acid | 5.0 | 4.8 | 5.2 | 2.2 |
| lactic acid | 8.5 | 6.2 | 8.7 | 7.0 |
| formic acid | 4.0 | 4.0 | 4.5 | 1.5 |
| acetic acid | 10.5 | 8.3 | 10.1 | 3.7 |
| 2,4-dihydroxy-butyric acid | 8.0 | 7.4 | 8.7 | 6.0 |
| total ≤ $C_6$ acids | 57.5 | 52.1 | 57.2 | 62.4 |
| total > $C_6$ acids | 42.5 | 47.9 | 42.8 | 37.6 |

[a] Reaction conditions: 0.025 M D-fructose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 100% conversion; anions added as KCl, as $K_2CO_3$, and $Na_2B_4O_7.10H_2O$.

[b] Standard degradation without additives.

[c] Analyzed with GC because of coincidence of the borate and the saccharinic acid signals in the liquid chromatogram.



The differences between borate ester formation and calcium(II) complexation of monosaccharides are the more covalent coordination of borate with the monosaccharides and the proton removal of both hydroxyl groups involved. Consequently, formation of these borate ester moieties has a stabilizing influence on monosaccharides in alkaline medium, so protecting them against fast degradation[36]: borate ester formation at the anomeric site of the monosaccharide is expected to inhibit the enolization, and the second esterification of borate with two other hydroxyl groups of the monosaccharide may inhibit the retro-aldolization. In addition, borate esters of subsequent reaction intermediates apparently favour the benzilic acid rearrangement at the cost of the α-dicarbonyl cleavage.

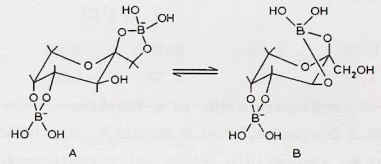


Fig. 8. β-D-Fructopyranose 1,2;4,5-diborate (A) and β-D-fructopyranose 2,3;4,5-diborate (B).

*Effect of temperature*

The influence of the reaction temperature on the final product composition has been studied at 50-90 °C under standard reaction conditions (0.025 M D-glucose, 0.01 M KOH, $H_2O$, $N_2$, 100% conversion). As shown in Fig. 9 the degradation pattern is hardly influenced by the reaction temperature which is in accordance with the data of De Wit[15]. Apparently, the differences in activation energy for the formation of the various degradation products are negligible.

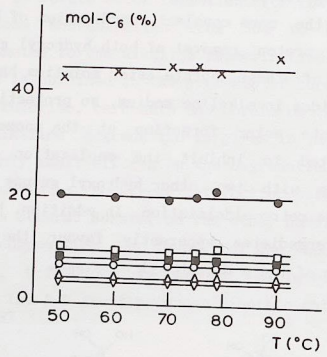


Fig. 9. Formation of carboxylic acids as a function of the reaction temperature (0.025 M D-glucose, 0.01 M KOH, $H_2O$, $N_2$, 100% conversion); ×, > $C_6$ acids; ●, saccharinic acids; △, glycolic acid; ○, lactic acid; ▽, formic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid.

*Degradation of some reaction intermediates*

Experiments have been carried out with some model reaction intermediates. Pyruvaldehyde (2-ketopropanal) was chosen as a representative of α-dicarbonyl compounds, while glyceraldehyde and 1,3-dihydroxyacetone have been used as the intermediates formed by retro-aldolization of monosaccharides. Reaction of these compounds (0.05 M) has been performed under standard conditions (0.01 M KOH, $H_2O$, 78 °C, $N_2$). As the degradation of monosaccharides continuously generates α-dicarbonyl compounds in very low concentration a degradation reaction was performed in which pyruvaldehyde was added in 50 small portions with intervals of 2 min. The results are summarized in Table 4.

The composition of the reaction mixture after the alkaline degradation of both glyceraldehyde and 1,3-dihydroxyaceton resembles that of the monosaccharides. The formation of saccharinic acid shows that substantial aldolization of $C_3$-fragments occurs prior to the alkaline degradation of these intermediates. As both $C_3$- and $C_6$-saccharides result in comparable product formation, there appear to exist a relatively fast equilibrium between $C_3$- and $C_6$-saccharides by aldolization and retro-aldolization reactions.



Table 4. Alkaline degradation of some reaction intermediates[a].

| carboxylic acid (mol-$C_6$-%) | pyruv-aldehyde[b] 0.001 M | pyruv-aldehyde 0.05 M | glycer-aldehyde 0.05 M | dihydroxy-acetone 0.05 M | D-glucose 0.025 M |
|---|---|---|---|---|---|
| saccharinic acids | < 0.1 | < 0.1 | 16.8 | 18.9 | 21.5 |
| glycolic acid | < 0.1 | < 0.1 | 5.2 | 3.7 | 5.0 |
| lactic acid | 51.7 | 15.5 | 7.6 | 6.1 | 6.9 |
| formic acid | 5.9 | 5.9 | 5.5 | 4.8 | 4.0 |
| acetic acid | 7.3 | 9.5 | 9.8 | 7.9 | 10.5 |
| 2,4-dihydroxy-butyric acid | 3.3 | 7.0 | 8.3 | 5.5 | 8.5 |
| total ≤ $C_6$ acids | 68.2 | 37.9 | 53.2 | 46.9 | 56.4 |
| total > $C_6$ acids | 31.8 | 62.1 | 46.8 | 53.1 | 43.6 |

[a] Reaction conditins: 0.01 M KOH, $H_2O$, 78 °C, $N_2$, ~ 5 h, 100% conversion.
[b] Added in 50 portions during 100 min.

The experiments with pyruvaldehyde (which is also an important intermediate in the alkaline degradation of glyceraldehyde and 1,3-dihydroxyacetone) show that dicarbonyls are important intermediates for the formation of > $C_6$ acids. The formation of > $C_6$ acids decreases upon lowering of the dicarbonyl concentration, as would be expected for a bimolecular aldolization reaction. This in favour of the unimolecular benzilic acid rearrangement of pyruvaldehyde into lactic acid. It may be noted that during the alkaline degradation of monosaccharides (including glyceraldehyde and 1,3-dihydroxyacetone) α-dicarbonyl compounds will be responsible for the formation of substantial amounts of > $C_6$ acids by mutual aldolization as well as by aldolization with other carbonyl compounds present in the reaction mixtures.

## Conclusions

The present results allow the modelling of the alkaline transformation of monosaccharides by means of the overall reaction scheme as presented in Fig. 10.

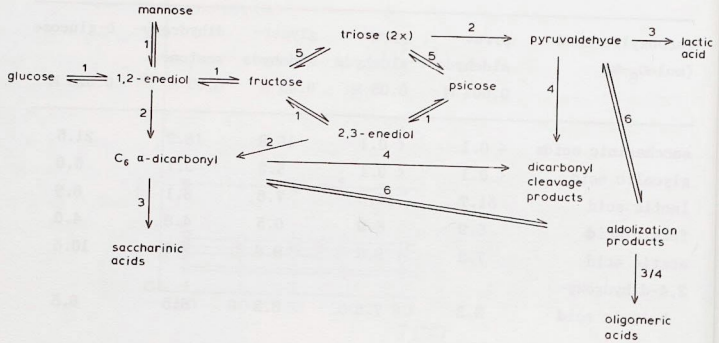


Fig. 10. Overall reaction network of monosaccharides in alkaline medium involving the following transformations:

1. enolization/isomerization,
2. β-elimination,
3. benzilic acid rearrangement,
4. α-dicarbonyl cleavage,
5. (retro-)aldolization,
6. extended (retro-)aldolization of (di)carbonyl compounds.

The main characteristics are:

(i) Enediol anion species are involved in both isomerization and degradation reactions as shown by the formation of similar product mixtures upon alkaline degradation of various interconvertible monosaccharides.

(ii) There occurs a fast equilibrium between $C_3$- and $C_6$-saccharides, involving aldolization and retro-aldolization reactions, prior to the subsequent alkaline degradation reactions.

(iii) Dicarbonyl compounds, the β-elimination products of enediol anion species, are key-intermediates which via benzilic acid rearrangement, α-dicarbonyl cleavage and aldolization lead to a variety of acid products. It may be noted that aldehydes formed by the α-dicarbonyl cleavage reaction will be also involved in aldolization reactions. The elongation of the carbon chain by aldolization of (di)carbonyls is terminated by benzilic acid rearrangement or α-dicarbonyl cleavage of oligomeric dicarbonyl intermediates.



(iv) Oligomeric acidic products, the so-called > $C_6$ acids, are formed via extensive aldolization of intermediate (di)carbonyl compounds. An intermediate $HO^-$ concentration ($10^{-3}$-$10^{-2}$ M) is responsible for the occurrence of substantial amounts of both neutral and ionized (di)carbonyl compounds, as required for optimal aldolization, while a high monosaccharide concentration (> $10^{-2}$ M) is generally favourable for the oligomerization reaction.

(v) Retro-aldolization of the ketoses is a major reaction pathway for lactic acid formation. Enhanced ionization of the ketose (high $HO^-$ concentration) or the formation of cation-ketose complexes (Ca(II) addition) increases the retro-aldolization.
Addition of borate stabilizes the monosaccharides through borate ester formation.

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# CHAPTER 4

# CHARACTERIZATION OF OLIGOMERIC PRODUCTS FORMED DURING THE ALKALINE DEGRADATION OF MONOSACCHARIDES

## Introduction

As shown in a recent literature survey[1] on the reactions of monosaccharides in aqueous alkaline solution, the mechanisms of the reversible initial transformations of monosaccharides, i.e. ionization, mutarotation, enolization and isomerization, are well understood. The mechanisms of the subsequent irreversible degradation reactions, on the other hand, are only partly elucidated. By the lack of a convenient analysis method required for the determination of all degradation products, a systematic investigation concerning the influence of reaction parameters on the degradation reactions involved, e.g. retro-aldolization of monosaccharides, β-elimination of the enediol anion species, and the subsequent benzilic acid rearrangement of α-dicarbonyl intermediates, could not be achieved so far. A recently developed HPLC analysis technique[2] for routine analyses of degradation mixtures made it possible to reinvestigate the alkaline degradation of monosaccharides in more detail. A screening[3,4] of the influence of several reaction parameters on the final product composition revealed that the amount of carboxylic acid products containing more than six carbon atoms, denoted as $> C_6$ acids, may be substantial and depends on the conditions of the alkaline degradation reaction. For instance, 40-50% of the monosaccharides are converted into these oligomeric acidic products at an $HO^-$ concentration between $10^{-3}$ and $10^{-1}$ M when the initial monosaccharide concentration exceeds $10^{-2}$ M. The formation of $> C_6$ acids has very often been overlooked by workers in this field. Only a few investigators[5-9] have been aware of the formation of products, up to 40-50%, other than the $C_1$ to $C_6$ acids. Characterization of products formed besides these $\leq C_6$ acids in the complex degradation mixtures has been attempted[10-20] most often in relation to the colour formation during the alkaline degradation process. However, a complete elucidation of the nature and structure was not achieved.

As a part of our research on the alkaline degradation of monosaccharides we have studied the nature and structure of the $> C_6$ acids by the use of several separation techniques, e.g. gel filtration and precipitation, and by structural analysis techniques, e.g. UV, IR, NMR and GC-MS. The results of this study are presented in this Chapter.

## Experimental

### *Materials*

D-Glucose, D-fructose, raffinose, and ammonium carbaminate (Merck), maltose (BDH), lead(II) acetate (Lamers & Indemans), and 2,4-dinitrophenylhydrazine (Baker) were reagent-grade and were used without further purification. 1-$^{13}$C-D-Glucose, containing 99% $^{13}C$, was obtained from C.E.A., France. Metasaccharinic acid was prepared by selective alkaline degradation of 3-O-methylglucose as described in Chapter 8. The hydrogenation catalyst 5% ruthenium on carbon (Drijfhout, Amsterdam) was activated for 2 h at 400 °C prior to use. Sephadex G-10 (40-120 μm, mol. wt. fractionation range 0-700) was supplied by Pharmacia. The cation exchange resin AG 50W-X8 ($H^+$-form, exchange capacity 1.7 mmol/ml, 100-200 Mesh) was purchased from Bio-Rad.

### *Procedures*

*Alkaline degradation experiments.* The reactions were carried out in a 500 ml thermostatted vessel under nitrogen as described elsewhere[3]. During the experiments both the temperature and the $HO^-$ concentration were kept constant. After complete conversion of the monosaccharides, as indicated by the termination of the $HO^-$ consumption, the reaction mixtures were neutralized by addition of a weak cation exchange resin (BioRex 70 H) at ~ 0 °C, which was subsequently filtered off.

*Standard alkaline degradation mixture.* Standard alkaline degradation product mixtures were prepared from 1.8 g D-fructose in 400 ml solution (0.025 M) under the following reaction conditions: 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion. This standard mixture, which was known from earlier experiments[3] to contain 42.5% oligomeric acidic products, was used throughout for the experiments described. In some cases, the reaction mixture was concentrated before use.



*Pre-separation of oligomeric products.* Two methods were used for the pre-separation of oligomeric acidic products from $\leq C_6$ carboxylic acids:

(i) Preparative liquid chromatography by size-exclusion using the cation exchange resin AG 50W-X8 (sulfonated styrene-divinylbenzene copolymer, crosslinkage 8%, $-SO_3H$ content 1.7 meq/ml, 100-200 Mesh; glass column of 400 mm length and 18 mm i.d.; eluent, aqueous 0.005 M $H_2SO_4$, flow 7 ml/h). The effluent was collected by an automatic fraction collector.

(ii) Precipitation of oligomeric products by Pb(II) acetate[15d,e] (300 g/l) at pH ~ 12, followed by centrifugation. The residue was suspended in water and Pb(II) was precipitated with ammonium carbaminate (76 g/l) as its carbonate. The dissolved $> C_6$ acids were separated from the Pb(II) carbonate by centrifugation. The supernatant was subjected to analysis.

### *Analysis methods*

*Molecular weight distribution.* The molecular weight (mol. wt.) distribution of acidic products in alkaline degradation mixtures, without pretreatment as well as after pre-separation of the oligomeric acids, was determined by gel filtration on Sephadex G-10 (glass column of 800 mm length and 26 mm i.d., water as eluent, flow 40 ml/h, RI detection). D-Glucose, maltose and raffinose were used to gauge the Sephadex column.

*Determination of unsaturated groups.* The carbonyl content of the oligomeric acidic products was determined quantitatively by the 2,4-dinitrophenylhydrazine precipitation method.[21]. The sum of both olefinic and carbonyl groups present in the $> C_6$ acids was determined by measuring the hydrogen uptake upon hydrogenation of 10% of the standard alkaline degradation mixture over 200 mg 5% Ru/C in 150 ml water (pH = 7) at 30 °C and 1 atm $H_2$ in an automatic hydrogenation apparatus[22].

*UV and IR spectroscopy.* UV Spectra of aqueous alkaline degradation mixtures were recorded at 20 °C, pH = 7 on a Pye Unicam SP 8-250 UV/VIS spectrophotometer. KBr-Tablets of dry samples, obtained by freeze-drying of degradation mixtures, were prepared in order to record IR spectra on a Beckman IR 4210 infrared spectrophotometer.

*$^{13}C$ NMR Spectroscopy.* 1-$^{13}$C-D-Glucose (450 mg) in 100 ml solution (0.025 M) was degraded under standard reaction conditions (see above) and the distri-

bution of the $^{13}C$ label in the various products was determined quantitatively by $^{13}C$ NMR. The $^{13}C$ NMR spectra of concentrated reaction mixtures in $D_2O$ were recorded on a Nicolet NT-200 WB spectrometer (50 MHz). Reliable quantitative data were obtained using gated $^{1}H$-decoupling, a pulse width of 12.0 µs (45° flip angle), and a pulse delay of 100.0 s. The identification of the signals in the spectrum was performed as described in Chapter 8[23].

*HPLC and GC-MS analysis.* HPLC analysis of carboxylic acid products was performed as described in Chapter 9[2] (column HPX 87 in the $H^+$ form from Bio-Rad, column temperature 60 °C, eluent 0.01 M aqueous trifluoroacetic acid, flow 0.6 ml/min, RI detection). For GC analysis[2] (capillary CP Sil 5 column of 48.5 m length and 0.52 mm i.d., flame ionization detector, temperature programme: 5 min at 75 °C, followed by increasing the temperature to 280 °C at a rate of 8 °C/min, 5 min at 280 °C) the acidic products were converted into their trimethylsilyl derivatives. Identification of a number of trimethylsilylated carboxylic acids was performed with a Varian GC-MS system Mat 44 in which the GC part was identical with that described above. Two types of ionization techniques were used: (i) electron impact (80 eV) in which serious fragmentation of the molecules occurred and (ii) chemical ionization (electrons of 200 eV energy were used to generate ions from the methane reagent gas) in order to obtain the molecular weight of the acid compounds.

## Results and discussion

### *Isolation and molecular weight distribution*

Quantitative analyses of the $\leq C_6$ acids in reaction mixtures of the alkaline degradation of monosaccharides revealed substantial deficits in the mass balance (up to 50%)[3]. GC Analysis of the head-space of the reaction vessel during the alkaline degradation showed minor amounts of volatile compounds. This agrees with a carbon analysis of the freeze-dried standard alkaline degradation mixture containing 57.5% $\leq C_6$ acids, which emphasized that essentially all the carbon originating from the monosaccharide is still



present. The unknown products (42.5%) could not be detected by GC, whereas by HPLC a raise of the base line is observed immediately after the void peak which extends to the first peak of the $\leq C_6$ acids, i.e. metasaccharinic acid (Fig. 1). In view of the properties of the cation exchange resin column applied for this HPLC analysis (see Experimental) the chromatogram points to size-exclusion of a complex mixture of products with molecular weights higher than those for the $\leq C_6$ acids. Determination of the molecular weight

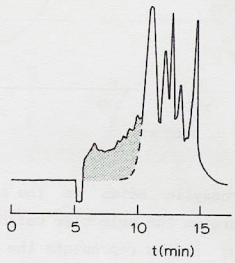


Fig. 1. HPLC separation of the carboxylic acid products in the standard degradation mixture of D-fructose (see Experimental). The gray region represents the oligomeric reaction products.

distribution of this alkaline degradation mixture was carried out by gel filtration on Sephadex G-10 (Fig. 2). In addition, HPLC analysis of each fraction of the gel filtration established that the gray region in Fig. 2 represents degradation products other than the $\leq C_6$ acids.
The analytical HPLC results (size exclusion) induced us to perform a preparative separation of 11.2 g of a freeze-dried standard degradation mixture using the cation exchange resin AG 50W-X8 in the $H^+$ form. Three fractions (A, 240 mg; B, 405 mg; C, 645 mg of dry matter) were collected containing only oligomeric products as demonstrated by HPLC analysis (Fig. 3).

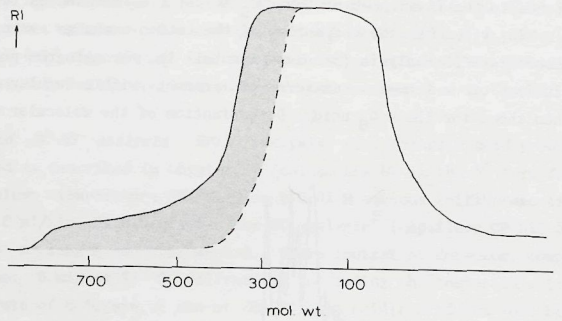


Fig. 2. Gel filtration of carboxylic acids in the standard degradation mixture in order to determine the molecular weight distribution (see Experimental). The gray region represents the oligomeric reaction products.

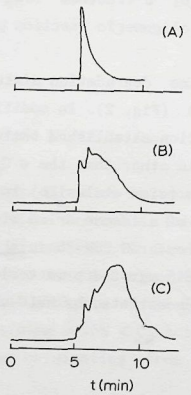


Fig. 3. HPLC analysis of fractions A, B, and C of oligomeric reaction products obtained by preparative separation on AG 50W-X8 ($H^+$ form).



The molecular weight maxima regions present in these three fractions according to gel filtration appeared to be ~ 350, ~ 500, and ⩾ 700 (Fig. 4), which points to products of dimerization, trimerization, etc., i.e. oligomerization of monosaccharide moieties.

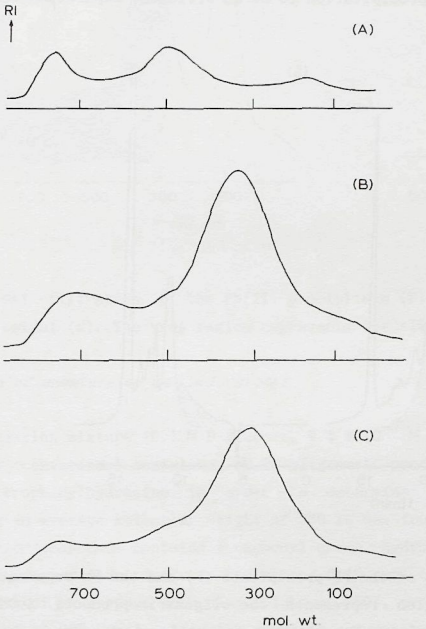


Fig. 4. Gel filtration of fractions A, B, and C of oligomeric products obtained by preparative separation on AG 50W-X8 ($H^+$ form).

The other method applied for the pre-separation of the oligomeric products was precipitation with Pb(II). In this way the precipitate of 5.6 g of a freeze-dried standard degradation mixture yielded after removal of Pb(II) 1.0 g of dry product. This product contained mainly oligomeric products as shown by HPLC analysis (Fig. 5P). The supernatant of the Pb(II) precipita-

tion, on the other hand, contained mainly $\leq C_6$ acids (Fig. 5S). The molecular weight distribution of the oligomeric products (gray region in Fig. 6P) shows a close similarity with those obtained by the preparative chromatographic separation on the cation exchange resin. The supernatant (Fig. 6S) only contains a small amount of oligomeric products with lower molecular weight showing Pb(II) precipitation to be an efficient separation technique.

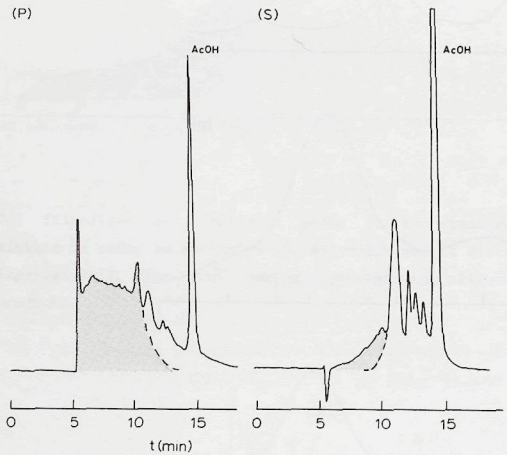


Fig. 5. HPLC Analysis of the Pb-precipitate (P) and the Pb-supernatant (S). The gray region represents the oligomeric products. AcOH results from the $Pb(OAc)_2$ used.

Fractions of the oligomeric products obtained by the two methods described have been used for the elucidation of their molecular structure.



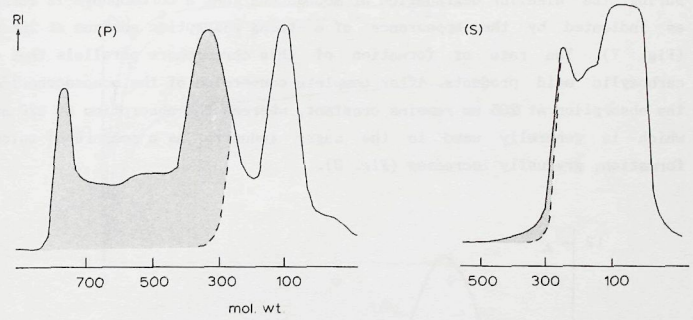


Fig. 6. Gel filtration of the Pb(II)-precipitate (P) and the Pb(II)-supernatant (S). The gray region represents the oligomeric products.

*Presence of unsaturated and acid groups*

A degradation mixture (0.1 M D-glucose, $4.4 * 10^{-2}$ M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion) containing 53.0% oligomeric products[3] was treated with 2,4-dinitrophenylhydrazine in order to determine its carbonyl content. Assuming an average molecular weight of 350 it was found that 8 mol-% of the oligomeric compounds contains a carbonyl group. Hydrogenation of 10% of the standard degradation mixture, containing 0.45 mmol oligomeric products (on the basis of an average molecular weight of 350), over Ru/C gave a hydrogen uptake of 0.086 mmol. Assuming complete saturation, the total content of unsaturated groups amounts to 19 mol-%, i.e. the oligomeric products contain comparable amounts of carbonyl (8 mol-%) and olefinic (11 mol-%) groups.
From the hydroxyl ion consumption during the standard alkaline degradation of D-fructose and the known $\leq C_6$ acid composition a value of 1.37 acid groups per $C_6$ moiety of the oligomeric product is obtained. Therefore, we will denote these products as $> C_6$ acids.

*UV Spectroscopy*

During the alkaline degradation of monosaccharides a chromophore is formed as indicated by the appearance of a strong absorption maximum at 265 nm (Fig. 7). The rate of formation of this chromophore parallels that of carboxylic acid products. After complete conversion of the monosaccharides the absorption at 265 nm remains constant, whereas the absorption at 420 nm, which is generally used in the sugar industry as a measure of colour formation, gradually increases (Fig. 8).

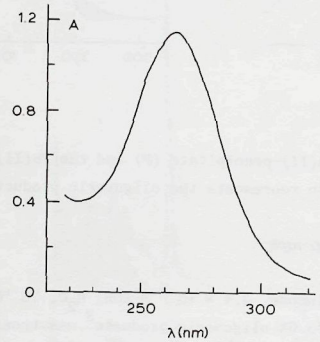


Fig. 7. UV Absorption at $pH^{20}$ = 7 of the standard alkaline degradation mixture of D-fructose (0.025 M) after 30 x dilution.



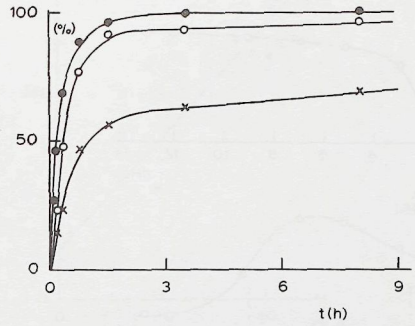


Fig. 8. Acidic product formation (●) and UV/Vis absorption at $pH^{20}$ = 7 (as percentage of the ultimate absorption at 265 nm (○) and at 420 nm (×)) as a function of time for the degradation of D-glucose (0.025 M) in 0.01 M aqueous KOH at 78 °C under nitrogen.

The absorption maximum of the chromophore in aqueous solution at $pH^{20}$ = 7 shifted towards lower wavelength (hypsochromic shift) upon decreasing the pH which is accompanied by a decrease of the absorbance (hypochromic effect). On the other hand, an increase of the pH only lowers the absorbance. A possible structure for the chromophore which would explain these phenomena have been included in Fig. 9. Such β-dicarbonyl structures are known to exhibit pH-dependent keto-enol tautomerization phenomena as described for malonaldehyde[24] and 2,5-dimethylcyclohexan-1,3-dione[12] in water. The

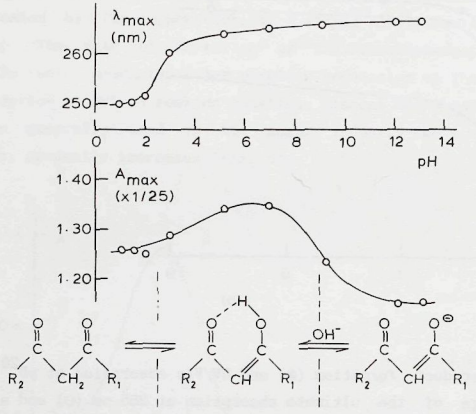


Fig. 9. UV Absorption of the chromophore formed by alkaline degradation of D-fructose (0.025 M D-fructose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion) as function of the pH.

hypochromic effect by an increase of the pH around $pH^{20}$ = 9 indicates an ionization of the enol into the enolate structure which agrees with the ionization constants[25] (water, 25 °C) of pentan-2,4-dione (pK = 8.24), hexan-2,4-dione (pK = 8.49), and 3-methylpentan-2,4-dione (pK = 9.16).

In conclusion, the β-dicarbonyl chromophore might be considered as a structural moiety of the oligomeric acid products (cf. Table 2). As the absorption at 265 nm is a measure of the total content of this chromophore, a linear relation between this absorption and the total amount of oligomeric products is expected to exist if the percentage of chromophore is constant. This, indeed, is the case as demonstrated by Fig. 10. The intercept on the absorbance axis is caused by substantial absorption of carboxylate groups (at 190-200 nm) which partly overlaps with the absorption of the chromophore.



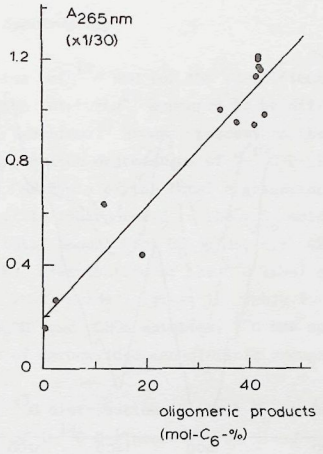


Fig. 10. Absorbance at 265 nm (after 30 x dilution, $pH^{20}$ = 7) as a function of the percentage of oligomeric products formed by the alkaline degradation of D-glucose or D-fructose (0.025 M) at different KOH concentrations[3] ($H_2O$, 78 °C, $N_2$, 100% conversion). The total amount of degradation products is expressed as the percentage of monosaccharide that is converted (denoted as mol-$C_6$-%).

*IR Spectroscopy*

For the identification of functional groups present in the oligomeric products infrared spectra have been recorded of the standard alkaline degradation mixture, of oligomeric products with mol. wt. ≥ 700 and 700 > mol. wt. > 450 as indicated by gel filtration of the Pb(II) precipitate (cf. Fig. 6), and of metasaccharinic acid as a representative of $< C_6$ acids. The IR spectra of the two oligomeric products were identical. From the spectra given in Fig. 11 the following characteristic absorption bands[26] appear for the oligomeric products.

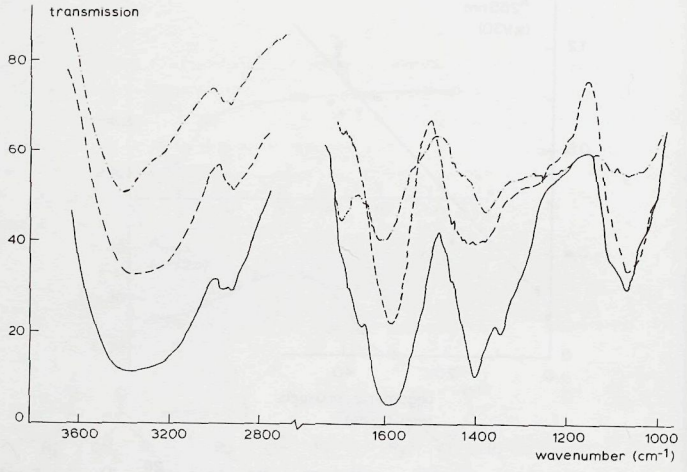


Fig. 11. IR spectra (KBr) of alkaline degradation products as their $K^+$ salts.
——— standard alkaline degradation mixture,
– – – metasaccharinic acid,
–·–·– oligomeric products with mol. wt. ≥ 700, or with 700 > mol. wt. > 450.

(i) A carbonyl band at 1700 $cm^{-1}$ which is in accordance with the β-dicarbonyl moiety indicated by UV spectroscopy.

(ii) Carboxylate bands at 1600 $cm^{-1}$ and 1400 $cm^{-1}$ which support the (poly)carboxylate nature of the oligomeric products, in accord with earlier results[14,16,19,20,27].

(iii) Hydroxyl bands at 3400 $cm^{-1}$ and 1050 $cm^{-1}$ which indicate the presence of alcohol groups along the carbon chain of the oligomeric products.



*$^{13}C$ NMR Spectroscopy*

Application of $^{13}C$ NMR for the identification of the > $C_6$ acids in alkaline degradation mixtures[23] appeared to be difficult. Some information, however, of the functional groups present in the oligomeric products was obtained from the alkaline degradation of 1-$^{13}C$-D-glucose using $^{13}C$ NMR. Quantitative $^{13}C$ NMR analysis of the total degradation mixture revealed that 32% of the $^{13}C$ label is incorporated in the > $C_6$ acids, which is of the same magnitude as the total amount of > $C_6$ acids, i.e. 42.5 mol-$C_6$-%, present in that mixture. The distribution of the $^{13}C$ label over the various functional groups in the > $C_6$ acids is given in Table 1. In addition to the expected $CH_3$, $CH_2$, $CH_2OH$ and CHOH moieties, $^{13}C$ NMR spectroscopy further emphasizes the presence of carboxylate and olefinic groups in the > $C_6$ acids.

Table 1. $^{13}C$ distribution in the > $C_6$ acids obtained by alkaline degradation of 1-$^{13}C$-D-glucose[a].

| Functional group | $^{13}C$ label (%) |
|---|---|
| $CH_3$, $CH_2$ | 16 |
| $CH_2OH$, CHOH | 6 |
| C=C | 7 |
| COOH | 3 |
| total | 32 |

[a] Reaction conditions: 450 mg 1-$^{13}C$-D-glucose in 100 ml $H_2O$ (0.025 M), 0.01 M KOH, 78 °C, $N_2$, 7 h, 100% conversion.

*GC-MS Analysis*

For GC-MS analysis the > $C_6$ acids were precipitated with Pb(II) and, subsequently, the precipitate was freed from lead and fractionated by gel filtration (Fig. 6P) as described in the Experimental Part. The oligomeric products with mol. wt. < 350 were collected, freeze-dried, trimethylsilylated, and analyzed by GC (Fig. 12). The gas chromatogram of this low molecular weight Pb(II) precipitate fraction (B) as compared with that of the standard alkaline degradation mixture (A) shows the occurrence of extra acidic products (1-6), which have been characterized by mass spectrometry (Table 2). The mass spectra of the trimethylsilylated > $C_6$ acids 4, 5, and 6, respectively, are given in Fig. 13 together with the most important fragmentations using data of trimethylsilylated aldonic and deoxyaldonic acids[28,29].

Table 2. Acidic products[a], identified by GC-MS, in the Pb(II) precipitate fraction with mol. wt. < 350 of the standard alkaline degradation of D-fructose[b].

| | |
|---|---|
| 1 | $CH_3$-CO-$CH_2$-CHOH-COOH |
| 2 | $CH_3$-CHOH-$CH_2$-CHOH-COOH |
| 3 | $(CH_3$-CHOH$)_2$-CH-COOH (and isomers) |
| 4 | $(CH_3$-CHOH$)_2$-CH-CHOH-COOH (and isomers) |
| 5 | $CH_3$-CO-C($CH_2$OH)=C(OH)-$CH_2$-CHOH-COOH |
| 6 | $CH_3$-CO-C(CHOH$CH_3$)=C(OH)-$CH_2$-CHOH-COOH |

[a] The numbering corresponds with the peaks in the gas chromatogram of Fig. 12B.

[b] 0.025 M D-fructose, 0.01 M KOH, 78 °C, $N_2$, 7 h, 100% conversion.



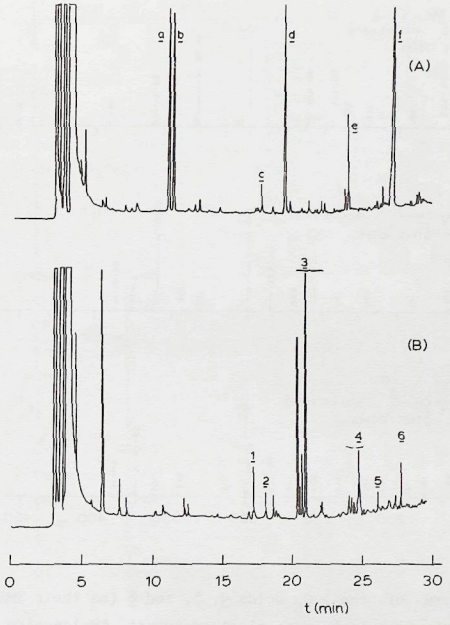


Fig. 12. GC Analysis of carboxylic acids (as their TMS-derivatives) as present in the standard alkaline degradation mixture (A) and in the Pb(II) precipitate fraction with mol. wt. < 350 (B); a, lactic acid; b, glycolic acid; c, 2-methylglyceric acid; d, 2,4-dihydroxybutyric acid; e, 3-deoxypentonic acid; f, metasaccharinic acid. The structures of 1-6 are given in Table 2.
GC conditions: see Experimental.

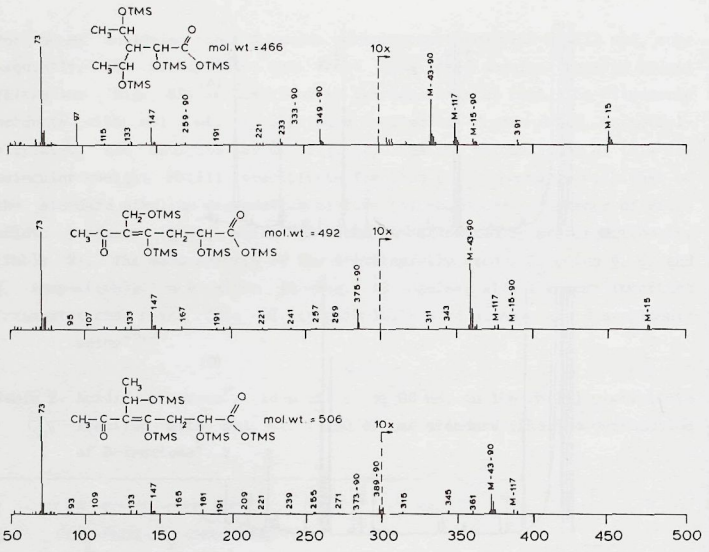


Fig. 13. Mass spectra of the > $C_6$ acids 4, 5, and 6 (as their TMS-derivatives). Ionization technique: electron impact. Explanation of fragmentation ions[28,29]: M-15: loss of $CH_3$ from $SiMe_3$; M-15-28: subsequent loss of CO; M-n*90: loss of n*$Me_3SiOH$; M-117: loss of $COOSiMe_3$; m/e = 73 is base peak.

A possible explanation for the formation of the carboxylic acids 1-6 (Table 2) is presented in Fig. 14. Starting from several molecules known to be formed in the alkaline degradation, the pathways indicate the importance of the aldolization of (di)carbonyl compounds during the alkaline degradation of monosaccharides. By β-elimination, benzilic acid rearrangement and dicarbonyl cleavage reactions[3], the chain elongation by aldolization is terminated and the final products are formed.



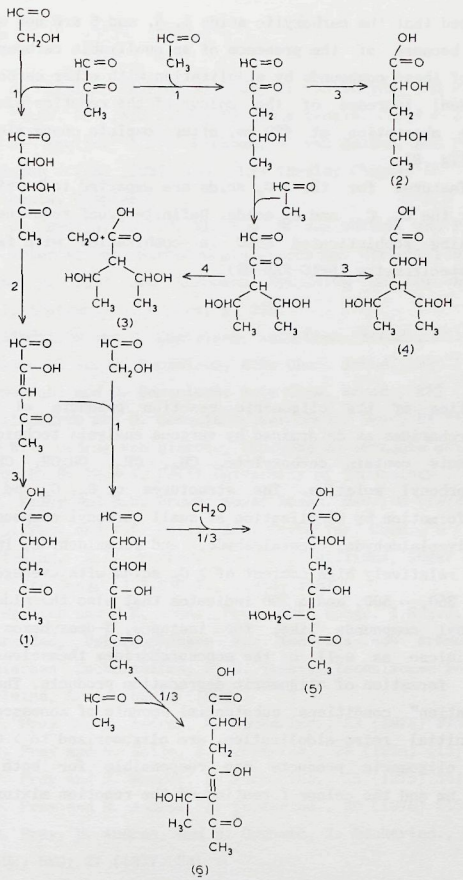


Fig. 14. Possible pathways for the formation of the acidic degradation products 1-6 via aldolization of (di)carbonyl compounds.
1. aldolization,
2. β-elimination,
3. benzilic acid rearrangement,
4. α-dicarbonyl cleavage.

It may be mentioned that the carboxylic acids 1, 5, and 6 are not stable in alkaline medium because of the presence of an enolizable carbonyl group. Further reaction of these compounds by aldolization with other carbonyls may explain the gradual increase of the colour of the reaction mixture, as indicated by the absorption at 420 nm, after complete conversion of the monosaccharides (Fig. 8).

The structural features for the $> C_6$ acids are expected to be similar to those obtained for the $C_7$, $C_8$, and $C_9$ acids. Definite proof requires further investigations using sophisticated HPLC in combination with fast-atom-bombardment mass spectrometry (HPLC-FAB-MS).

## Conclusions

The characteristics of the oligomeric reaction products of alkaline decomposed monosaccharides as determined by various analysis techniques show that these products contain carboxylate, $CH_3$, $CH_2$, $CH_2OH$, CHOH, and (enolized) β-dicarbonyl moieties. The structures of $C_7$, $C_8$ and $C_9$ acids point to their formation by aldolization of small carbonyl compounds, e.g. pyruvaldehyde, glycolaldehyde, acetaldehyde, and formaldehyde, in various combinations. The relatively high content of $> C_6$ acids with average molecular weights of ~ 350, ~ 500, and ≥ 700 indicates that also the aldolization of $C_6$ (di)carbonyl compounds like, for instance, 3-deoxyhexos-2-ulose, 4-deoxyhexo-2,3-diulose as well as the monosaccharides themselves will be involved in the formation of oligomeric degradation products. Thus, under alkaline "degradation" conditions substantial amounts of monosaccharides, partially via initial retro-aldolization, are oligomerized to $> C_6$ acidic products. These oligomeric products are responsible for both the UV absorption at 265 nm and the colour formation of the reaction mixture.

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# CHAPTER 5

# KINETICS OF THE ALKALINE ISOMERIZATION AND DEGRADATION OF MONOSACCHARIDES

## Introduction

In aqueous alkaline solutions enolization of sugar anions causes the isomerization of (mono)saccharides, known as "the Lobry de Bruyn-Alberda van Ekenstein rearrangement"[1]. The intermediate enediol anion species have been generally accepted[2-5] as the starting intermediates in the alkaline degradation of monosaccharides. The first step in the degradation reaction is the elimination of a hydroxyl group in β-position to the ionized enol group. The resulting product of this so-called β-elimination reaction is an α-dicarbonyl compound which may be considered[6] as key-intermediate in the subsequent alkaline degradation reactions, i.e. benzilic acid rearrangement, α-dicarbonyl cleavage and aldolization. The enediol anion and α-dicarbonyl intermediates reach only low concentrations. Enediol anion species, for instance, are detected in alkaline medium in amounts up to 0.6% of the monosaccharide concentration as determined by UV-spectroscopy[2].

The kinetics of the isomerization and concomitant degradation of monosaccharides, e.g. D-fructose and D-glucose, in aqueous alkaline solutions have been studied by many investigators[2,7-12]. A survey of the relevant kinetic data has been given by Kooyman et al.[13], which together with some recent results[2,14] represents the present-day knowledge on the kinetic behaviour of monosaccharides in alkaline medium.

It may be noted that formation of D-psicose has not been taken into consideration[2,7-14], while D-mannose formation has been often left out in the kinetic treatment of the alkaline degradation[7,8,11-14].

As we established substantial D-psicose and D-mannose formation during the alkaline degradation of both D-glucose and D-fructose, we have reinvestigated the kinetics of the alkaline degradation reaction. With the aid of HPLC accurate data of the reaction course as a function of time were obtained. The kinetic parameters thus derived require a kinetic model which differs from models proposed before in the literature.

## Experimental

### *Materials*

D-psicose was prepared[15] according to the literature. All other chemicals were obtained from Merck (analytical grade).

### *Apparatus*

The alkaline degradation reactions were carried out in a 500 ml thermostatted vessel under nitrogen as described elsewhere[6]. During the experiments both the temperature and the $HO^-$ concentration were kept constant. Samples taken during the reaction were quenched by cooling in acetone-dry ice. The samples were neutralized by addition of a weak cation exchange resin (BioRex 70 H) at ~ 0 °C and filtered off.

### *Analysis method*

Analysis of monosaccharides present in the neutralized reaction mixtures was performed with HPLC[15] (carbohydrate column HPX 87 C from Bio-Rad, column temperature 60 °C, eluent $H_2O$, flow 0.4 ml/min, RI-detection). The total amount of carboxylic acid degradation products was taken as 100%-% total sugars. The carboxylic acid composition was also determined by HPLC analysis[16] (organic acid column HPX 87 H from Bio-Rad, column temperature 60 °C, eluent 0.01 M trifluoroacetic acid aqueous solution, flow 0.6 ml/min, RI-detection). Mutual interference of monosaccharides and acidic products allowed an accurate analysis of the monosaccharides only up to a conversion of ~ 40%.

## Results and discussion

### *Analysis of the reaction course as function of time*

In preliminary experiments the course of the alkaline degradation of D-fructose was followed in order to determine the applicability of HPLC as analysis technique for kinetic measurements. For this purpose the isomerization[15]



and acidic degradation products[16] were analyzed on different columns as described in the experimental part. Fig. 1A and 1B present typical examples of liquid chromatograms obtained on the carbohydrate and the organic acid column, respectively. The reaction course as function of time thus obtained

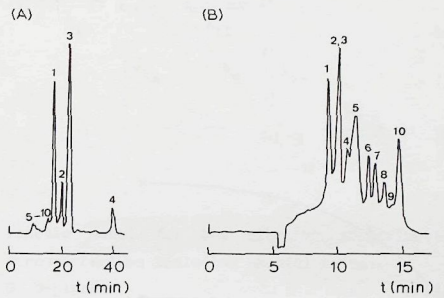


Fig. 1. HPLC analysis of products formed during the alkaline degradation of D-fructose (0.025 M D-fructose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$). Chromatographic conditions: see experimental.

A. Separation of monosaccharides, sample taken after 10 min reaction time.

B. Separation of carboxylic acids, sample taken after 1 h reaction time.

1. D-glucose
2. D-mannose
3. D-fructose
4. D-psicose
5. saccharinic acids
6. glycolic acid
7. lactic acid
8. formic acid
9. 2,4-dihydroxybutyric acid
10. acetic acid

is depicted in Fig. 2. Since the relative amounts of the acidic products did not change mutually during the degradation reaction use has been made of the total amount of acidic products, defined as 100%-% sugars, as a measure of conversion. In consequence, the analysis of just the monosaccharides as a function of time suffices for a kinetic picture of isomerization and degradation.

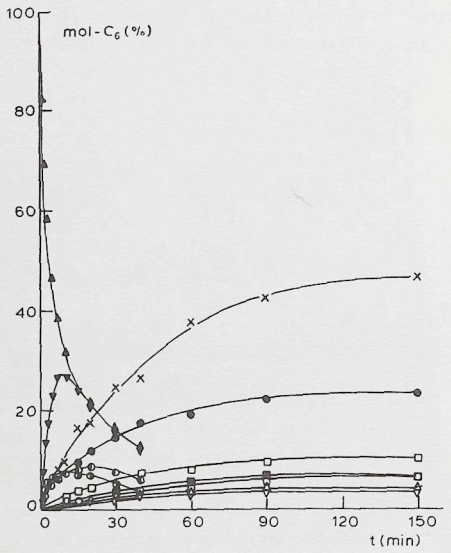


Fig. 2. Reaction course of D-fructose in alkaline medium (0.025 M D-fructose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$); ▲, D-fructose; ▼, D-glucose; ◐, D-mannose; ◑, D-psicose; ●, saccharinic acids; △, glycolic acid; ○, lactic acid; ▽, formic acid; □, acetic acid; ■, 2,4-dihydroxybutyric acid; ×, > $C_6$ acids.

*Kinetic model: pseudo first order rate constants*

A kinetic model for the alkaline isomerization/degradation of D-glucose and D-fructose, which includes D-mannose and D-psicose as well as overall degradation rate constants, is given in Fig. 3. This kinetic model does not include sugar anions since the sugar anion concentrations at constant $HO^-$



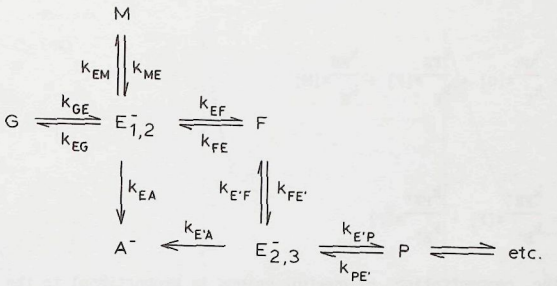


Fig. 3. Kinetic model for the alkaline isomerization and degradation of monosaccharides including enediol anions.

| | | | |
|---|---|---|---|
| G | D-glucose | $A^-$ | acidic products |
| F | D-fructose | $E^-_{1,2}$ | 1,2-enediol anion |
| M | D-mannose | $E^-_{2,3}$ | 2,3-enediol anion |
| P | D-psicose | $k_{mn}$ | denote the various reaction rate constants |

concentration are proportional to the total sugar concentrations. The reactive enediol anion intermediates will have pseudo-steady state concentrations $[E^-_{1,2}]$ and $[E^-_{2,3}]$, i.e.

$$\frac{d[E^-_{1,2}]}{dt} = 0 = k_{GE}*[G] + k_{FE}*[F] + k_{ME}*[M] - (k_{EG} + k_{EF} + k_{EM} + k_{EA})*[E^-_{1,2}] \qquad (1a)$$

and

$$\frac{d[E^-_{2,3}]}{dt} = 0 = k_{FE'}*[F] + k_{PE'}*[P] - (k_{E'F} + k_{E'P} + k_{E'A})*[E^-_{2,3}] \qquad (1b)$$

This means that, with $k_{EG} + k_{EF} + k_{EM} + k_{EA} = k_E$ and $k_{E'F} + k_{E'P} + k_{E'A} = k_{E'}$,

$$[E^-_{1,2}] = \frac{k_{GE}}{k_E}*[G] + \frac{k_{FE}}{k_E}*[F] + \frac{k_{ME}}{k_E}*[M] \qquad (2a)$$

and

$$[E^-_{2,3}] = \frac{k_{FE'}}{k_{E'}}*[F] + \frac{k_{PE'}}{k_{E'}}*[P] \qquad (2b)$$

Thus, the concentration of enediol anions is proportional to the monosaccharide concentrations and, therefore, can be eliminated in the kinetic model. This allows the simplified kinetic model as presented in Fig. 4 which has been used throughout in our kinetic considerations. The various *pseudo first order rate constants*, which include $HO^-$ concentration-dependent enolization rate constants, have been determined by computer simulations of

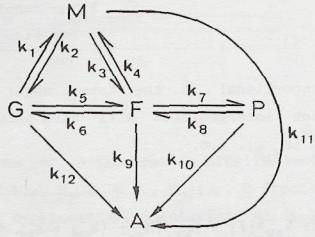


Fig. 4. Simplified kinetic model for the alkaline isomerization and degradation of D-glucose (G), D-mannose (M), D-fructose (F), and D-psicose (P) into acidic products (A). Pseudo first order rate constants ($k_i$) include different enolization and subsequent rearrangement steps.

the experimental data on the basis of this simplified kinetic model. As an example, in Fig. 5 the computer simulations are given for the reaction course of D-fructose, D-glucose and D-mannose in alkaline medium. It may be



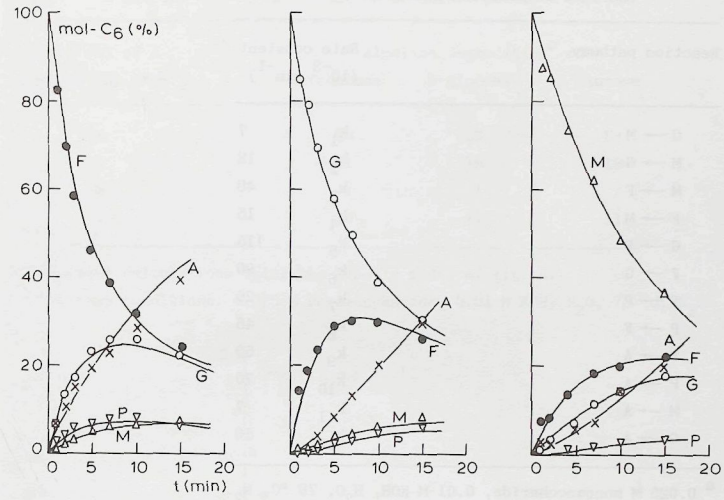


Fig. 5. Alkaline isomerization and degradation of D-fructose, D-glucose and D-mannose (0.025 M monosaccharide, 0.01 M KOH, $H_2O$, 78 °C, $N_2$). experimental data: ● (F) D-fructose; ○ (G) D-glucose; △ (M) D-mannose; ▽ (P) D-psicose; × (A) acidic products. The curves are obtained by computer simulation using the kinetic model of Fig. 4.

noted that the experimental data of the three degradations have been simulated by one and the same set of $k_i$. The various isomerization ($k_1$-$k_8$) and degradation ($k_9$-$k_{12}$) pseudo first order rate constants obtained by the computer simulations are summarized in Table 1. The data show that the isomerization of both D-glucose and D-fructose to D-mannose, the isomerization of D-fructose to D-psicose, and the subsequent degradation of both D-mannose and D-psicose cannot be neglected in kinetic considerations. Especially, the degradation rate constant of D-psicose is noteworthy since it exceeds that of D-fructose.

Table 1. Pseudo first order rate constants for the reactions of monosaccharides in alkaline medium[a].

| Reaction pathway | | Rate constant ($10^{-3}$ $min^{-1}$) |
|---|---|---|
| G ⟶ M | $k_1$ | 7 |
| M ⟶ G | $k_2$ | 18 |
| M ⟶ F | $k_3$ | 49 |
| F ⟶ M | $k_4$ | 15 |
| G ⟶ F | $k_5$ | 115 |
| F ⟶ G | $k_6$ | 90 |
| F ⟶ P | $k_7$ | 25 |
| P ⟶ F | $k_8$ | 45 |
| F ⟶ A | $k_9$ | 50 |
| P ⟶ A | $k_{10}$ | 70 |
| M ⟶ A | $k_{11}$ | 7 |
| G ⟶ A | $k_{12}$ | 10 |

[a] 0.025 M monosaccharide, 0.01 M KOH, $H_2O$, 78 °C, $N_2$.

The part of the acidic products that originates from D-fructose, D-psicose, D-mannose, and D-glucose, respectively, has been obtained by computer calculations using the kinetic model of Fig. 4. The results given in Table 2 show that *whatever monosaccharide is degraded about 20% of the products originates from D-psicose whereas 63-68% of the products is formed via D-fructose.* The high reactivity, i.e. enolization rate, of these ketoses is responsible for this phenomenon.

In addition to the alkaline isomerization/degradation of D-fructose, D-glucose, and D-mannose that of D-psicose (Fig. 6) emphasizes that the isomerization (and consequently the degradation) of aldoses and 2-ketoses proceeds preferentially via 1,2- and 2,3-enediol anions as intermediates.



Table 2. The share[a] of D-fructose (F), D-psicose (P), D-mannose (M) and D-glucose (G) in the formation of acidic products (A).

| Part (%) of A originating from: | Alkaline degradation[b] of D-fructose | D-glucose | D-mannose |
|---|---|---|---|
| F | 68 | 63 | 63 |
| P | 21 | 19 | 19 |
| M | 1 | 1 | 7 |
| G | 10 | 17 | 11 |

[a] Computer calculations using the kinetic model of Fig. 4.

[b] Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH, $H_2O$, 78 °C, $N_2$.

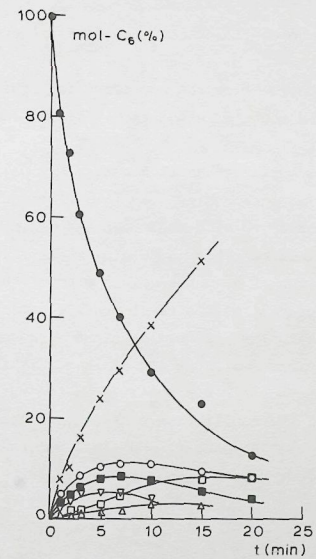


Fig. 6. Reaction course of D-psicose in alkaline medium (0.025 M D-psicose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$); ● , D-psicose; ○ , D-fructose; □ , D-glucose; ■ , D-altrose; ▽ , D-allose; △ , D-sorbose; × , acidic products.

Only the formation of D-sorbose, up to 3%, during the alkaline degradation of D-psicose points to 3,4-enolization. However, the formation of D-sorbose as well as D-altrose and D-allose as isomerization products of D-psicose will be less than 1% in the alkaline degradation of D-fructose, D-glucose and D-mannose.

*Influence of reaction parameters*

*Monosaccharide concentration.* At a first approximation, the alkaline degradation rate of monosaccharides might be considered to be proportional to the total monosaccharide concentration, i.e.

$$\frac{-d\Sigma[S_t]}{dt} = k_{overall} * \Sigma[S_t] \qquad (3)$$

in which $k_{overall}$ denotes the overall pseudo first order degradation rate constant, thus including the $HO^-$ dependence, and $\Sigma[S_t]$ the total concentration of monosaccharides. Using the concentration profiles of the monosaccharides as obtained by computer simulation (Fig. 5), equation (3) leads to the curves given in Fig. 7.

Clearly, overall pseudo first order kinetics are only valid after a reasonable period of time, depending on the starting monosaccharide. The alkaline degradation started with D-glucose and D-fructose follows overall pseudo first order kinetics after 15-20 min, whereas it takes more than 40 min for the degradation of D-mannose. This phenomenon can be explained by the fact that only overall pseudo first order kinetics may be expected if the isomerization equilibrium has been attained[7]. As demonstrated by Fig. 5 the isomerization equilibrium is indeed achieved within 20 min for D-fructose and D-glucose, but not for D-mannose. From the rate constants of Table 1 the composition of the equilibrium mixture is calculated as 36% D-fructose, 35% D-glucose, 18% D-mannose, and 11% D-psicose, which explains the overall pseudo first order degradation rate constant $k_{overall} = 0.030\ min^{-1}$.



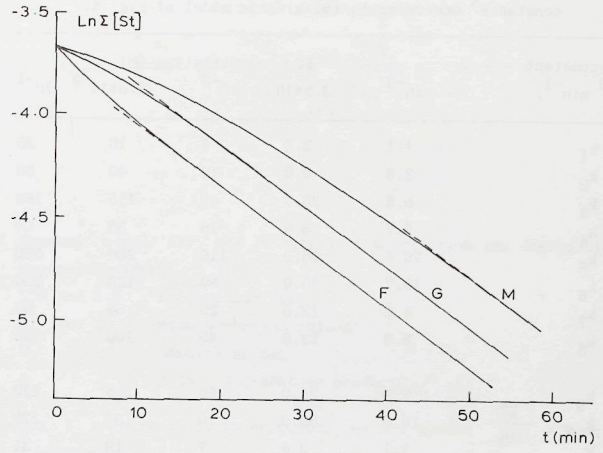


Fig. 7. Logarithmic plot of the total concentration of monosaccharides versus time for the alkaline degradation of D-mannose (M), D-glucose (G), and D-fructose (F): deviations from overall pseudo first order kinetics are due to isomerization reactions.
Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH, $H_2O$, 78 °C, $N_2$.

*Hydroxyl ion concentration.* As already mentioned, the reaction rate constants of the simplified kinetic model (Fig. 4) include the $HO^-$ concentration. The influence of the $HO^-$ concentration on the reaction rate constants is shown in Table 3. The dependence observed can be understood using the general model of Fig. 8, including both the ionization and enediol anion formation from the different monosaccharides. As the ionization of monosac-

Table 3. Influence of the $HO^-$ concentration on the pseudo first order rate constants[a] according to the kinetic model of Fig. 4.

| rate constant ($10^{-3}$ $min^{-1}$) | $HO^-$ concentration (M) $10^{-3}$ | $3.5*10^{-3}$ | $10^{-2}$ | $3.5*10^{-2}$ | $10^{-1}$ |
|---|---|---|---|---|---|
| $k_1$ | 1.1 | 3.0 | 7 | 15 | 25 |
| $k_2$ | 2.8 | 8.0 | 18 | 40 | 60 |
| $k_3$ | 8.8 | 26.0 | 49 | 115 | 160 |
| $k_4$ | 2.7 | 8.0 | 15 | 35 | 60 |
| $k_5$ | 20.0 | 55.0 | 115 | 260 | 360 |
| $k_6$ | 15.7 | 45.0 | 90 | 220 | 330 |
| $k_7$ | 4.2 | 12.0 | 25 | 60 | 90 |
| $k_8$ | 8.0 | 23.0 | 45 | 100 | 140 |
| $k_9$ | 7.9 | 24.0 | 50 | 135 | 220 |
| $k_{10}$ | 12.0 | 36.0 | 70 | 190 | 320 |
| $k_{11}$ | 1.1 | 3.0 | 7 | 19 | 31 |
| $k_{12}$ | 1.6 | 5.0 | 10 | 27 | 46 |

[a] Reaction conditions: 0.025 M monosaccharide, KOH, $H_2O$, 78 °C, $N_2$.

charides in alkaline medium is fast with respect to subsequent enediol anion formation we may write

$$\frac{-d[S^-]}{dt} = \frac{-d[S_t]}{dt} = k_{1s}*[S^-] - k_{2s}*[E^-] \qquad (4)$$

with the total monosaccharide concentration $[S_t] = [SH] + [S^-]$. The conversion rate of the enediol anion can be expressed by

$$\frac{-d[E^-]}{dt} = (\Sigma k_{2s} + k_d)*[E^-] - \Sigma k_{1s}*[S^-] \qquad (5)$$



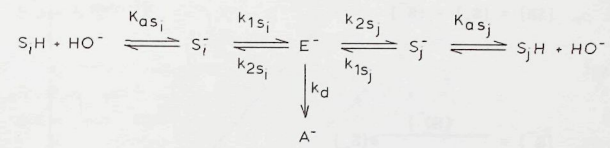


Fig. 8. General model for the alkaline isomerization and degradation of monosaccharides.

$S_iH$ and $S_jH$ monosaccharides;
$S_i^-$ and $S_j^-$ monosaccharide anions;
$E^-$ enediol anion;
$A^-$ acidic degradation products.

Considering $E^-$ as a reactive intermediate, we may assume $\frac{-d[E^-]}{dt} = 0$ so that $[E^-]$ will be proportinal to the various sugar concentrations:

$$[E^-] = \frac{\Sigma k_{1s}}{\Sigma k_{2s}+k_d}*[S^-] \qquad (6)$$

Substitution of (6) in equation (4) gives

$$\frac{-d[S_t]}{dt} = k_{1s}*[S^-] - \frac{k_{2s}\Sigma k_{1s}}{\Sigma k_{2s}+k_d}*[S^-] \qquad (7)$$

With

$$K_{as} = \frac{[S^-]*[H^+]}{[SH]} = \frac{[S^-]*K_w}{[SH]*[HO^-]} \qquad (8)$$

and

$$[SH] = [S_t] - [S^-]$$

we get

$$[S^-] = \frac{[HO^-]}{K_w/K_{as}+[HO^-]} * [S_t] \qquad (9)$$

Combination of (7) and (9) then results in

$$\frac{-d[S_t]}{dt} = \frac{A*[HO^-]}{B+[HO^-]} * [S_t] = \Sigma k_i [S_t] \qquad (10)$$

with $A = k_{1s} - \frac{k_{2s}\Sigma k_{1s}}{\Sigma k_{2s}+kd}$, $B = K_w/K_{as}$, and $\Sigma k_i$ the sum of the $HO^-$ dependent pseudo first order rate constants $k_i$ of the simplified kinetic model (Fig. 4). *For D-fructose, the kinetic data presented in Table 3 have been plotted versus* $\frac{[HO^-]}{B+[HO^-]}$ (Fig. 9).

As would be expected from relation (10), a linear relationship is obtained for the pseudo first order isomerization rate constants $k_4$, $k_6$ and $k_7$. However, some deviation occurs for the degradation reaction ($k_9$) at $[HO^-] > 10^{-2}$ M which indicates the importance of a second ionization of D-fructose at high alkalinity in degradation product formation. Although some investigators[8,12] reported first order kinetics with respect to the monosaccharide as well as the $HO^-$ concentration, Lai[11,17] and Bamford et al.[7] also found apparent deviations from simple first order kinetics. These authors proposed that at high $HO^-$ concentrations, $[HO^-] > 10^{-2}$ M, also di-anionic sugar species will be involved. Bamford et al.[7] assumed that ionization of a hydroxyl group, presumably on $C_4$, occurs. If so, then an increased $C_3$-$C_4$ bond fission (retro-aldolization) into two trioses has to be expected as depicted in Fig. 10. This extra triose formation results in higher amounts of lactic acid, which is in accordance with the observed sharp increase of lactic acid production at higher $HO^-$ concentrations[6].



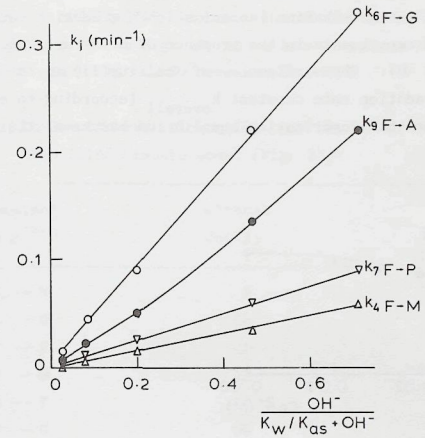


Fig. 9. Pseudo first order rate constants $k_i$, according to the simplified kinetic model of Fig. 4, as a function of the $HO^-$ concentration ($pK_w$ = 14.1 and $pK_{as}$ = 12.7). Reaction conditions: 0.025 M monosaccharide, KOH, $H_2O$, 78 °C, $N_2$.

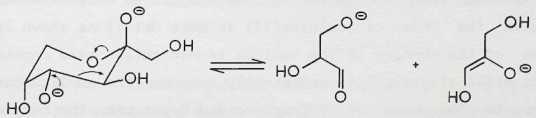


Fig. 10. Direct retro-aldolization of the β-D-fructopyranose di-anion at high $HO^-$ concentration.

*Calcium(II) ion concentration.* Vukov[8] and Mottard[12] observed that calcium(II) ions, in comparison with monovalent cations like $K^+$ and $Na^+$,

accelerate the alkaline degradation of monosaccharides. In order to identify the role of calcium(II) in the alkaline isomerization/degradation pattern we have performed kinetic experiments in the presence of different calcium(II) concentrations (0-0.06 M). The influence of calcium(II) on the overall pseudo first order degradation rate constant $k_{overall}$ (according to equation (3)), determined after the isomerization equilibrium has been attained, is depicted in Fig. 11.

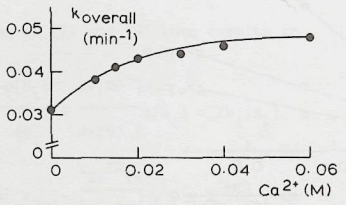


Fig. 11. The overall pseudo first order degradation rate constant as a function of the calcium(II) concentration.
Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH, $CaCl_2$, $H_2O$, 78 °C, $N_2$.

Clearly, the formation of Ca(II)-monosaccharide complexes will be responsible for the increase of the degradation rate. The influence of calcium(II) on the various rate constants of the simplified kinetic model (Fig. 4) demonstrates the role of calcium(II) in more detail as shown in Table 4. Comparison of the changes in the various isomerization rate constants shows that calcium(II) slightly but consistently enhances the enolization rate, in particular in the case of D-fructose and D-psicose. The complexation of calcium(II) is known to involve the anomeric hydroxyl which apparently favours enediol anion formation and thus isomerization of the monosaccharides. The average increase in rate of isomerization of D-glucose ($k_1$, $k_5$), D-mannose ($k_2$, $k_3$), D-fructose ($k_4$, $k_6$, $k_7$) and D-psicose ($k_8$) is about 5 times lower than the increase of its degradation rate constants. Thus, besides a general increase of all reaction rates due to a higher rate of enolization, calcium(II) has an additional favourable effect on the rate of formation of degradation products. In this respect, it may be noted that the enhanced production of lactic acid observed upon addition of



calcium(II)[6] is also found at high $HO^-$ concentrations without calcium(II). Presumably, calcium(II) induces the second ionization of another hydroxyl group as depicted in Fig. 10, which leads to extra degradation product formation at relatively low $HO^-$ concentrations.

Table 4. Influence of calcium(II) on the rate constants[a] applying to the simplified kinetic model (Fig. 4).

| rate constant ($10^{-3}$ min$^{-1}$) | | without Ca(II) | 0.06 M $CaCl_2$ |
|---|---|---|---|
| $k_1$ | G → M | 7 | 7 |
| $k_2$ | M → G | 18 | 19 |
| $k_3$ | M → F | 49 | 52 |
| $k_4$ | F → M | 15 | 18 |
| $k_5$ | G → F | 115 | 120 |
| $k_6$ | F → G | 90 | 100 |
| $k_7$ | F → P | 25 | 30 |
| $k_8$ | P → F | 45 | 50 |
| $k_9$ | F → A | 50 | 90 |
| $k_{10}$ | P → A | 70 | 110 |
| $k_{11}$ | M → A | 7 | 9 |
| $k_{12}$ | G → A | 10 | 13 |

[a] Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH, $CaCl_2$, $H_2O$, 78 °C, $N_2$.

*Borate concentration.* Alkali catalyzed isomerization may be employed for the synthesis of ketoses from aldoses: e.g. D-fructose from D-glucose[18-22] and lactulose from lactose[23]. Fig. 5, for instance, shows that D-fructose is formed in concentrations of about 30%. Addition of borate[24] or boronates[25,26] to the reaction mixture is known to improve the equilibrium concentration of ketose to 50-80%. As borate forms esters with polyhydroxy compounds[27,28] like carbohydrates[29] in aqueous alkaline solutions, differences in the stability constants of the various monosaccharide borate esters involved are responsible for the observed shift in the isomerization equilibrium. The influence of borate on both the isomerization and degradation rate of the different monosaccharides can be determined by the use of the

kinetic model of Fig. 4, which then gives the opportunity to establish which monosaccharides are preferentially esterified by borate.

The work of Makkee et al.[29] on the interaction of borate with D-glucose and D-fructose has revealed that at molar borate/monosaccharide ratio > 1 D-glucose and D-fructose are esterified with two borates in 5-membered rings (Fig. 12). At lower molar borate/monosaccharide ratios esters of one borate with two monosaccharide units predominate (Fig. 13). In both monosaccharide

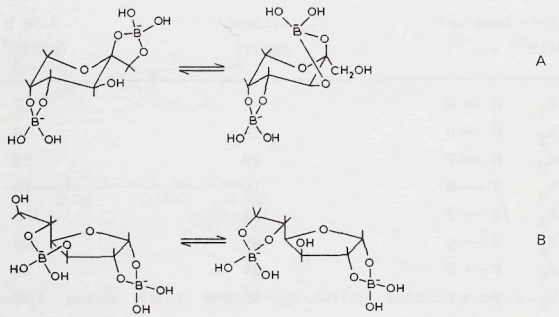


Fig. 12. D-fructose (A) and D-glucose (B) diborate esters[29].

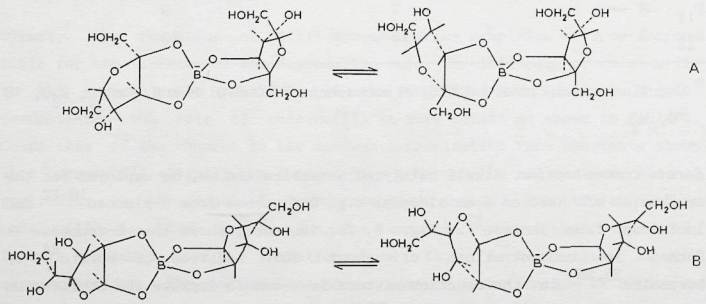


Fig. 13. Bis(D-fructose) (A) and bis(D-glucose) (B) borate esters[29].



diborate and bis(monosaccharide) borate esters the anomeric hydroxyl is esterified by borate which is expected to retard enolization and so the subsequent isomerization and degradation reactions. Kinetic experiments at low (0.4) and high (4) molar borate/monosaccharide ratios are shown in Fig. 14 for D-glucose and D-fructose, from which the kinetic data have been obtained by computer simulation (Table 5). Because the signals of D-mannose and borate interfere in the liquid chromatogram, the kinetic data for the isomerization and degradation of D-mannose have been estimated assuming that the influence of borate on D-mannose conversion is comparable to that of D-glucose. On this basis, in Fig. 14 the D-mannose concentration and, consequently, the concentration of acidic products as a function of time have been calculated using the simplified kinetic model of Fig. 4.

When the monosaccharide concentration exceeds that of borate, only the isomerization and degradation rate constants of D-fructose and D-psicose decrease substantially, whereas those of D-glucose and D-mannose are not influenced. On the other hand, if the borate concentration exceeds the monosaccharide concentration all rate constants decrease substantially.

From the kinetic data obtained, the following conclusions can be drawn:

(i) With an excess of monosaccharide the borate esters of D-fructose and D-psicose occur predominantly. As a consequence, only the isomerization and degradation rates of these monosaccharides decrease markedly and the isomerization equilibrium shifts towards these sugars.

(ii) An excess of borate, on the other hand, causes the formation of (di)borate esters of all monosaccharides and thus largely reduces all isomerization and degradation rates. When the reaction is started with D-glucose, the formation of the relatively stable D-fructose diborate esters (A in Fig. 12) is responsible for the increase of the maximum D-fructose concentration to 53%.

degradation reactions. In addition, direct retro-aldolization of monosaccharides, in particular D-fructose, becomes of importance at $HO^-$ concentrations exceeding $10^{-2}$ M. This is presumably due to the formation of di-anion species. Under these conditions, a second order dependence of the degradation rate constants on the $HO^-$ concentration becomes apparent.

Calcium(II) increases the enolization rate of monosaccharides, especially that of D-fructose. Furthermore, the influence of calcium(II) on the retro-aldolization reaction and so on the degradation rates is comparable with the influence of $HO^-$ at high concentrations. In both cases the increased retro-aldolization is reflected by the enhanced production of lactic acid.

In addition to the borate induced shift of the isomerization equilibrium towards D-fructose and D-psicose, due to the formation of various monosaccharide (di)borate esters and bismonosaccharide borate esters with different stability constants, there is a remarkable decrease in the degradation rate of the monosaccharides. Borate ester formation of monosaccharides involving the anomeric hydroxyl group, therefore, has a stabilizing effect on monosaccharides in alkaline medium.

The rate-enhancing effect by Ca(II) versus the rate-retarding effect by borate will find its origin in the different character of the O-Ca and O-B bonds formed, i.e. ionic versus covalent oxygen bonding, respectively.

Acknowledgements

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# CHAPTER 6

## MECHANISTIC PICTURE OF THE ALKALINE DEGRADATION OF MONOSACCHARIDES

### Introduction

It has been known for almost a century[1,2] that monosaccharides are unstable in alkaline medium and undergo, besides isomerization, degradation reactions which irreversibly lead to carboxylic acid products. The mechanism of these degradation reactions, however, has been only partly elucidated up to now[3] because a complete and quantitative analysis of the reaction mixture was not possible. Recently, a routine HPLC analysis method[4] was developed which, in some cases combined with GC analysis, allows a quantitative determination of the carboxylic acid composition of the final alkaline degradation mixtures of monosaccharides. In this way, a systematic investigation on the influence of several reaction parameters on the degradation pattern was performed[5-7] from which the following features became apparent.

(i) Formation of oligomeric acidic products, the so-called $> C_6$ acids, takes place at the cost of the well-known $C_1$ to $C_6$ acidic products (like lactic acid and saccharinic acids) and is maximal (up to 50%) when the $HO^-$ concentration lies between $10^{-3}$ M and $10^{-2}$ M.

(ii) Initial monosaccharide concentrations lower than $10^{-2}$ M result in an increased formation of saccharinic acids and lactic acid at the cost of the $> C_6$ acids. This implies that the precursors of saccharinic acids and lactic acid, i.e. α-dicarbonyl intermediates, are also involved in the aldolization reactions leading to the $> C_6$ acidic products.

(iii) An $HO^-$ concentration higher than $10^{-1}$ M results in a sharp increase in lactic acid production together with a somewhat higher formation of saccharinic acids. This at the cost of the $> C_6$ acids and of acetic acid, glycolic acid, and formic acid. In strongly alkaline medium the aldolization reaction is of minor importance and the α-dicarbonyl intermediates almost completely undergo benzilic acid rearrangement.

In addition, the increased amount of lactic acid is partly due to an enhanced direct retro-aldolization of ketoses.

(iv) Coordination of monosaccharides by divalent cations, as proven for calcium in the case of D-fructose, may favour the retro-aldolization reaction. Apart from the resulting enhanced production of lactic acid, the interaction of calcium(II) with α-dicarbonyls affects the benzilic acid rearrangement/dicarbonyl cleavage ratio of these intermediates as demonstrated by the formation of branched saccharinic acids at the cost of acetic acid and glycolic acid.

(v) Alkaline degradation experiments with pyruvaldehyde, glyceraldehyde, and 1,3-dihydroxyaceton as the starting compounds, all assumed to be reaction intermediates, indicate that aldolization of (di)carbonyl compounds causes the formation of substantial amounts of $> C_6$ acids.

(vi) The nature and structure of these $> C_6$ acids, having average molecular weights of ~ 350, ~ 500, and ≥ 700, further point to formation via aldolization of (di)carbonyls like pyruvaldehyde, 3-deoxyhexos-2-ulose, formaldehyde, acetaldehyde, glycolaldehyde and monosaccharides.

(vii) The kinetics of the alkaline isomerization and degradation of monosaccharides show that the alkaline isomerization/degradation of D-glucose, D-mannose, and D-fructose also includes the isomerization to and the degradation of D-psicose. Irrespective of the starting monosaccharide, substantial amounts of acidic products, i.e. ~ 65% and ~ 20%, are formed via D-fructose and D-psicose, respectively.

On the basis of the foregoing new insights in the alkaline degradation of monosaccharides this Chapter will discuss the mechanism of the various degradation reactions, the effect of reaction parameters on the degradation pattern, and the role of α-dicarbonyl compounds as key-intermediates. Additional information from $^{13}C$ NMR spectroscopic[8] measurements of alkaline degraded 1-$^{13}$C-D-glucose will be presented and discussed.

## Materials and methods

1-$^{13}$C-D-glucose, containing 99% $^{13}C$, was obtained from C.E.A., France. Under $N_2$ at 78 °C and at constant $HO^-$ concentration 340 mg 1-$^{13}$C-D-glucose (0.025 M) was degraded in 75 ml aqueous 0.01 M KOH as described elsewhere[5]. After



complete conversion of the monosaccharide (7 h) the neutralized reaction mixture was freeze-dried. $^{13}C$ NMR spectra of this reaction mixture in $D_2O$ were recorded on a Nicolet NT-200 WB spectrometer (50 MHz) using dioxane as external standard (δ = 66.6). Assignment of the signals was performed by comparison of the chemical shifts with those of authentic compounds[8]. Quantitative $^{13}C$ spectra were obtained by using gated $^1$H-decoupling, a pulse width of 12.0 μs (45° flip angle), and a pulse delay of 100.0 s.

## Results and discussion

### *Pattern of $^{13}C$ label upon alkaline degradation of 1-$^{13}$C-D-glucose*

The $^{13}C$ NMR spectrum of alkaline degraded 1-$^{13}$C-D-glucose[8] has given the distribution of $^{13}C$, originating from this labeled compound, over the different functional groups in each of the $C_1$-$C_6$ acids (Table 1). This $^{13}C$ distribution in the $\leq C_6$ acids offers further insight into their way of formation from D-glucose (Fig. 1).

Table 1. $^{13}C$ Distribution over the functional groups in $^{13}C$ labeled $C_1$-$C_6$ acids, originating from alkaline degraded 1-$^{13}$C-D-glucose[a].

| Carboxylate | Distribution of $^{13}C$ label (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | $COO^-$ | CHOH | $CH_2$ | CHOH | CHOH | $CH_2OH$ | $CH_3$ |
| metasaccharinate | 87 | 0 | 0 | 9 | 3 | 1 | |
| glycolate | 27 | | | | | 73 | |
| lactate | 41 | 4 | | | | | 55 |
| formate | 100 | | | | | | |
| acetate | 17 | | | | | | 83 |
| 2,4-dihydroxybutyrate | 21 | 0 | 10 | | | 69 | |

[a] Reaction conditions: 0.025 M 1-$^{13}$C-D-glucose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion.

In addition, the increased amount of lactic acid is partly due to an enhanced direct retro-aldolization of ketoses.

(iv) Coordination of monosaccharides by divalent cations, as proven for calcium in the case of D-fructose, may favour the retro-aldolization reaction. Apart from the resulting enhanced production of lactic acid, the interaction of calcium(II) with α-dicarbonyls affects the benzilic acid rearrangement/dicarbonyl cleavage ratio of these intermediates as demonstrated by the formation of branched saccharinic acids at the cost of acetic acid and glycolic acid.

(v) Alkaline degradation experiments with pyruvaldehyde, glyceraldehyde, and 1,3-dihydroxyaceton as the starting compounds, all assumed to be reaction intermediates, indicate that aldolization of (di)carbonyl compounds causes the formation of substantial amounts of $> C_6$ acids.

(vi) The nature and structure of these $> C_6$ acids, having average molecular weights of ~ 350, ~ 500, and ⩾ 700, further point to formation via aldolization of (di)carbonyls like pyruvaldehyde, 3-deoxyhexos-2-ulose, formaldehyde, acetaldehyde, glycolaldehyde and monosaccharides.

(vii) The kinetics of the alkaline isomerization and degradation of monosaccharides show that the alkaline isomerization/degradation of D-glucose, D-mannose, and D-fructose also includes the isomerization to and the degradation of D-psicose. Irrespective of the starting monosaccharide, substantial amounts of acidic products, i.e. ~ 65% and ~ 20%, are formed via D-fructose and D-psicose, respectively.

On the basis of the foregoing new insights in the alkaline degradation of monosaccharides this Chapter will discuss the mechanism of the various degradation reactions, the effect of reaction parameters on the degradation pattern, and the role of α-dicarbonyl compounds as key-intermediates. Additional information from $^{13}C$ NMR spectroscopic[8] measurements of alkaline degraded 1-$^{13}$C-D-glucose will be presented and discussed.

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| Carboxylate | Distribution of $^{13}C$ label (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | $COO^-$ | CHOH | $CH_2$ | CHOH | CHOH | $CH_2OH$ | $CH_3$ |
| metasaccharinate | 87 | 0 | 0 | 9 | 3 | 1 | |
| glycolate | 27 | | | | | 73 | |
| lactate | 41 | 4 | | | | | 55 |
| formate | 100 | | | | | | |
| acetate | 17 | | | | | | 83 |
| 2,4-dihydroxybutyrate | 21 | 0 | 10 | | | 69 | |

[a] Reaction conditions: 0.025 M 1-$^{13}$C-D-glucose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion.

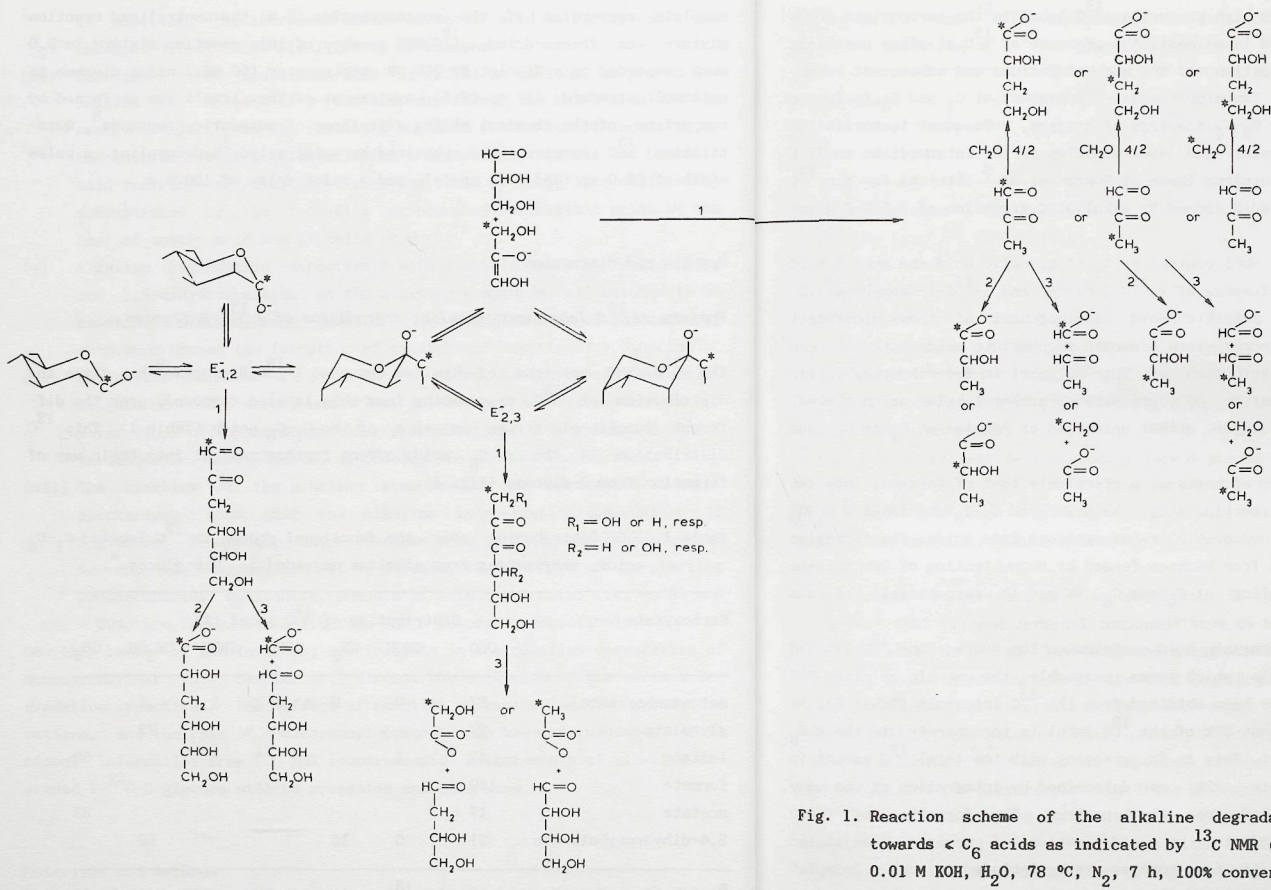


Fig. 1. Reaction scheme of the alkaline degradation of monosaccharides towards $\leq C_6$ acids as indicated by $^{13}C$ NMR (0.025 M 1-$^{13}C$-D-glucose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion); * denotes the $^{13}C$ label.

Numbering of reaction types:

1. β-elimination,
2. benzilic acid rearrangement,
3. α-dicarbonyl cleavage,
4. aldolization.

Although the relatively high proportion $^{13}C$ label in the carboxylate group of metasaccharinic acid is evident, the presence of $^{13}C$ at other positions points to retro-aldolization of the monosaccharides and subsequent recombination of fragments. The almost equal $^{13}C$ content at $C_1$ and $C_3$ in lactic acid can be explained by $C_3$–$C_4$ cleavage of hexoses, subsequent isomerization of the resulting trioses, and β-elimination of the intermediate enediol anion species. The data confirm those of Sowden et al.[9] obtained for the $^{14}C$ distribution in lactic acid formed by alkaline degradation of 3-$^{14}$C-D-glyceraldehyde.

The relatively high $^{13}C$ label content of $C_2$ of glycolic acid and acetic acid is due to dicarbonyl cleavage of 1-$^{13}$C-4-deoxy- and 1-$^{13}$C-1-deoxyhexo-2,3-diulose, respectively. Benzilic acid rearrangement of these dicarbonyl intermediates does not occur since branched saccharinic acids have not been detected. Finally, distribution of the $^{13}C$ label in 2,4-dihydroxybutyric acid indicates aldolization of formaldehyde, either labeled or unlabeled, with a triose or pyruvaldehyde, either unlabeled or labeled at $C_1$ or $C_3$ (see above).

As the retro-aldolization of hexoses, particularly that of ketoses, into two trioses is an important reaction step, the unlabeled $C_4$–$C_6$ and labeled $C_1$–$C_3$ moieties partly undergo comparable transformations into acids. The formation of metasaccharinic acid from hexoses formed by recombination of two trioses explains the $^{13}C$ labeling at $C_4$ and $C_6$, 9% and 1%, respectively, of this acid product.

Assuming that metasaccharinic acid contains on the average one $^{13}C$ labeled carbon atom per molecule, which seems reasonable, the amounts of other $^{13}C$ labeled $\leq C_6$ acids have been obtained from the $^{13}C$ integrals (Table 2). In this way, it appears that 67% of the $^{13}C$ label is incorporated in the $\leq C_6$ acid part of the product. This is in agreement with the total $^{13}C$ amount in the $> C_6$ acidic products, 32%, as determined by integration of the many small signals distributed over the spectrum. The formation of these oligomeric acidic products by aldolization of various (un)labeled (di)carbonyl compounds is too complex to be included in such a "simple" reaction sequence as depicted in Fig. 1, but the coupling and rearrangement reactions are considered to be similar to the $\leq C_6$ region network.



Table 2. HPLC and $^{13}C$ NMR analysis of the $\leq C_6$ acids formed by alkaline degradation of 1-$^{13}$C-D-glucose[a].

| Carboxylate | HPLC Total (mol-%)[b] | $^{13}C$ NMR $^{13}C$ labeled (mol-%)[b] | $^{13}C$ label per molecule |
|---|---|---|---|
| metasaccharinate | 21.5 | 21.5 | 1.0 |
| glycolate | 15.0 | 6.3 | 0.42 |
| lactate | 17.0 | 10.9 | 0.64 |
| formate | 24.0 | 5.4 | 0.23 |
| acetate | 31.5 | 16.8 | 0.53 |
| 2,4-dihydroxybutyrate | 12.0 | 6.0 | 0.50 |

[a] Reaction conditions: 0.025 M 1-$^{13}$C-D-glucose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion.

[b] mol-% = mol of degradation product formed per mol of hexose, multiplied by 100%.

*Simplified overall reaction scheme*

From recent investigations, as mentioned in the introduction, it became apparent that (i) α-dicarbonyl compounds have to be considered as important intermediates in the alkaline degradation of monosaccharides[5] since conversion of these compounds by benzilic acid rearrangement, α-dicarbonyl cleavage and aldolization reactions largely determines the final product composition, and (ii) the ketoses D-fructose and D-psicose may undergo substantial retro-aldolization[5,7] towards the important triose intermediates, i.e. glyceraldehyde and 1,3-dihydroxyaceton. These mechanistic insights together with the present results from 1-$^{13}$C-D-glucose (Fig. 1) have been used to develop a simplified overall reaction model, as given in Fig. 2, including the major pathways of monosaccharides in alkaline medium. This simplified model is useful to indicate which reaction paths are affected by variation of reaction parameters. For instance, the mutual importance of routes 2 and 4a is dependent on the $HO^-$ concentration and the nature of the cation present. So,

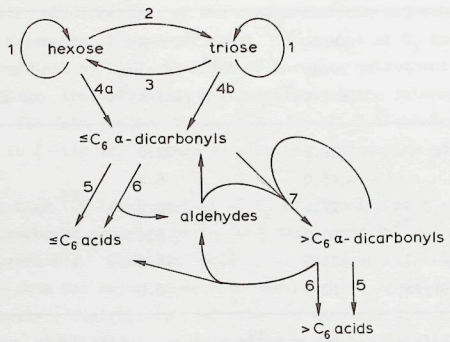


Fig. 2. Main reaction pathways of the alkaline degradation of monosaccharides.

1. isomerization via enediol anion species,
2. retro-aldolization of, in particular, ketoses,
3. aldolization of trioses,
4. enolization and β-elimination into (a) $C_6$ α-dicarbonyls and (b) pyruvaldehyde,
5. benzilic acid rearrangement,
6. α-dicarbonyl cleavage, leading to an acid and an aldehyde,
7. (retro-)aldolization of (di)carbonyl compounds, which is terminated by benzilic acid rearrangement (5) or α-dicarbonyl cleavage (6) of subsequent > $C_6$ α-dicarbonyl intermediates.

an increase of the $HO^-$ concentration or the use of Ca(II) instead of K(I) or Na(I) largely favours route 2 resulting in an enhanced formation of lactic acid[5].



Also the ratio of benzilic acid rearrangement (5), α-dicarbonyl cleavage (6) and aldolization (7), the three reactions by which all α-dicarbonyls are converted, can be influenced by variation of the reaction conditions, i.e. initial monosaccharide concentration, $HO^-$ concentration, valency of cation. Direct benzilic acid rearrangement and α-dicarbonyl cleavage of the ≤ $C_6$ α-dicarbonyls result in the formation of the ≤ $C_6$ acidic products. In the latter case, the reaction provides a final product (acid) as well as a still reactive aldehyde which further will undergo aldolization. The share of both benzilic acid rearrangement and α-dicarbonyl cleavage in the ≤ $C_6$ acid formation is nearly constant up to $[HO^-] = 10^{-2}$ M. At higher $HO^-$ concentrations, however, benzilic acid rearrangement occurs preferentially and lactic acid and saccharinic acids preponderate[5].

The precursors for > $C_6$ acids are assumed to be formed by aldolization of α-dicarbonyls with other (di)carbonyls present in the reaction mixture. This oligomerization is terminated by benzilic acid rearrangement or α-dicarbonyl cleavage of subsequent > $C_6$ α-dicarbonyl intermediates into > $C_6$ acids. Obviously, the latter reaction contributes to the formation of ≤ $C_6$ acid products as well. In Fig. 3 an example is given for the possible formation of a dimeric acidic product by such a reaction sequence. The structure as well as the molecular weight of this dimeric product corresponds to the description of oligomeric acidic products, formed by alkaline "degradation" of monosaccharides, as given in Chapter 4. Up to 50% of > $C_6$ acids may be formed at favourable aldolization reaction conditions[5], i.e. moderate $HO^-$ concentration ($10^{-3}$-$10^{-2}$ M) in combination with high monosaccharide concentrations (> $10^{-2}$ M).

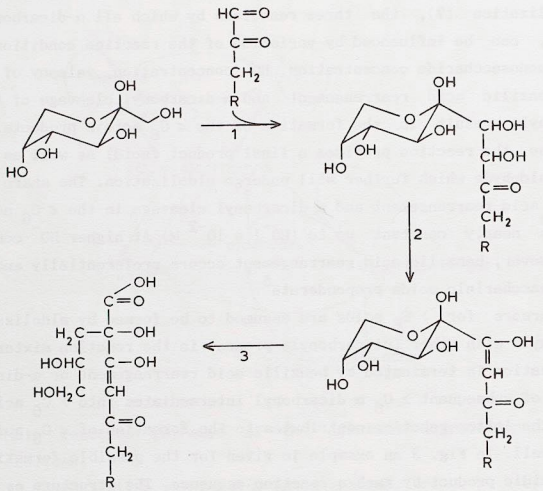

Fig. 3. Proposed mechanism for the formation of a dimeric acidic product ($R = (CHOH)_2CH_2OH$).
1. aldolization,
2. enolization and β-elimination,
3. enolization, β-elimination, and benzilic acid rearrangement.

*Remarks on the degradation behaviour of α-dicarbonyl compounds*

Apart from enediol anion species, α-dicarbonyl compounds appear to be important intermediates in the alkaline degradation of monosaccharides. Knowledge of their molecular structure is necessary to explain the observed degradation behaviour of these compounds. In this respect, Anet[10] established that 3-deoxyhexos-2-ulose exists in at least three forms, presumably hydrated and/or hemiacetal structures. In aqueous solution the extent of hydration of carbonyls is sensitive to the inductive effect of the substituent at the adjacent carbon atom[11,12], the length (and nature) of the carbon



chain[13], the temperature[12,13], and the concentration[11]. In particular aldehydes are considerably hydrated into the aldehydrol, i.e. gem-diol, form. For example, in water at ambient temperature, formaldehyde is for > 99% present as methylene glycol[11] and the monomers of glycolaldehyde[14] and glyceraldehyde[13] are hydrated to an extent of 95%. On the other hand, ketones are just slightly hydrated, if at all[11,15], depending on the substituents at the carbon atoms vicinal to the carbonyl groups[12]. Besides the aldehyde/aldehydrol equilibrium, short chain sugar aldehydes, like all α- and β-hydroxyaldehydes, are also in equilibrium with dimeric forms[13] which content increases at increasing concentration. Aldoses with a secondary 4-OH are almost completely present in cyclic hemiacetal forms[13]. In conclusion, on the analogy of hexoses, the $C_6$ α-dicarbonyls involved in the alkaline degradation of monosaccharides will be mainly present in (hydrated) acyclic and hemiacetal structures. Furthermore, the aldehydrol content of 1,2-dicarbonyls is supposed to exceed by far the degree of hydration of the keto groups in 2,3-dicarbonyls.

Based on these realistic structural properties of the α-dicarbonyl compounds some remarks will be made on their degradation behaviour, as determined by the analysis of final alkaline degradation product mixtures[5,16-19]. As no data are available on the relative amounts and reactivity of the different α-dicarbonyl structures, one must realize that these remarks will have a somewhat speculative character. Nevertheless, the key-role of α-dicarbonyl intermediates in the alkaline degradation of monosaccharides deserves this attention and, consequently, may induce further research in order to elucidate the striking behaviour of these compounds in aqueous alkaline solution.

As an example the structures of 3-deoxy-D-hexos-2-ulose, on the analogy of D-fructose, are depicted in Fig. 4. Stereoselective benzilic acid rearrangement of this 1,2-dicarbonyl into β-metasaccharinic ($\beta/\alpha > 4$)[16-19], having the (2S)-configuration, occurred using up to 0.1 M NaOH or KOH. This stereospecificity cannot be easily understood by enantiomeric induction in the acyclic form on the hydride shift from $C_1$ to $C_2$. The hemiacetal structures, on the other hand, contain a chiral centre at (the anomeric) $C_2$ to which the hydride is added in the reaction. An $S_Ni$-type hydride transfer *with inversion* of configuration at $C_2$ for both the β-furanose and the β-pyranose forms, which may be expected to occur predominantly on the analogy of the

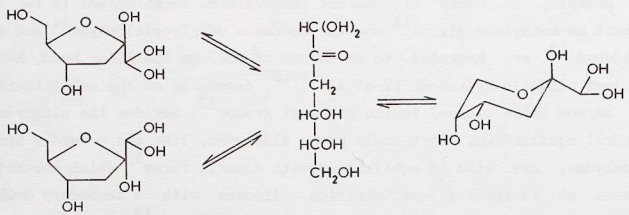

Fig. 4. Most probable acyclic and cyclic structures of 3-deoxy-D-hexos-2-ulose (β-pyranose > β-furanose > α-furanose on the analogy of D-fructose).

favourable β-conformations of D-fructose, explains the observed diastereoselective formation of β-metasaccharinic acid (Fig. 5). It will be evident that in this way the α-furanose form, present in minor amount, rearranges into α-metasaccharinic acid.

The alkaline degradation of 2,3-dicarbonyls is quite different from that of the 1,2-dicarbonyl compounds as illustrated by our alkaline degradation experiment with 1-$^{13}$C-D-glucose. As shown by $^{13}$C NMR spectroscopy no isosaccharinic acid and saccharinic acid were formed by degradation of 0.025 M 1-$^{13}$C-D-glucose in 0.01 M KOH ($H_2O$, 78 °C, $N_2$, 7 h, 100% conversion), but only typical α-dicarbonyl cleavage products of the 2,3-dicarbonyl intermediates, i.e. glycolic acid and acetic acid, could be determined in the final reaction mixture. On the other hand, upon addition of calcium(II) benzilic acid rearrangement of 2,3-dicarbonyls was established by the observation of substantial amounts of isosaccharinic acid and saccharinic acid as well as branched $C_4$ and $C_5$ saccharinic acids[5]. The different degradation behaviour of 2,3-dicarbonyls in comparison with that of 1,2-dicarbonyls might be due to the lower degree of hydration of the keto groups and the fact that a shift of a $CH_3$ or $CH_2OH$ carban ion instead of a hydride is involved in the benzilic acid rearrangement.



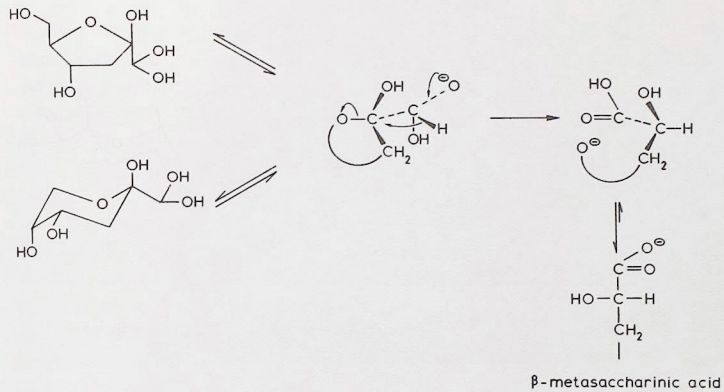


Fig. 5. Stereoselective benzilic acid rearrangement of the β-furanose and β-pyranose form of 3-deoxy-D-hexos-2-ulose into β-metasaccharinic acid.

The effect of the $HO^-$ concentration on the benzilic acid rearrangement/α-dicarbonyl cleavage/aldolization ratio of α-dicarbonyl intermediates can be understood considering their ionization behaviour. The α-dicarbonyls will have a $pK_a$ of 10-11 as estimated from $pK_a$ = 11.0 for pyruvaldehyde and $pK_a$ = 10.3 for 1,2-cyclohexanedione[20]. Furthermore, a second ionization may occur at $[HO^-] > 10^{-1}$ M as expected from the $pK_a$'s of 1,2-dihydroxybenzene ($pK_1$ = 9.45, $pK_2$ = 12.8)[20] and of hydrated carbonyl compounds like formaldehyde ($pK_a$ = 13.27)[20], acetaldehyde ($pK_a$ = 13.57)[20] and monosaccharides ($pK_a$ ~ 13)[3]. Using $pK_1$ = 10 and $pK_2$ = 13, in Fig. 6 the neutral and ionized forms of α-dicarbonyls are depicted as a function of the $HO^-$ concentration. The neutral species will undergo benzilic acid rearrangement and α-dicarbonyl cleavage. As the enolate, required for aldolization, is not present only a

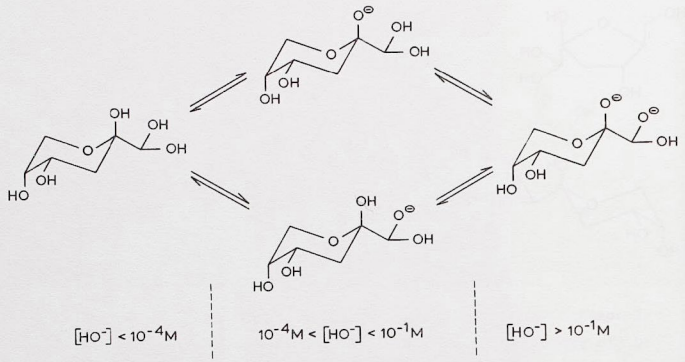


Fig. 6. Ionization of the β-pyranose form of 3-deoxy-D-hexos-2-ulose as a function of the $HO^-$ concentration.

small amount of oligomeric products is formed. The ionized (enol) species ($10^{-4}$ M < $[HO^-]$ < $10^{-1}$ M), on the other hand, is responsible for a substantial formation of oligomeric products. The benzilic acid rearrangement of α-dicarbonyls[21], in contrast to their hydrolytic cleavage, depends on the $HO^-$ concentration. Ionization markedly increases the benzilic acid rearrangement at the cost of the cleavage reaction, especially at $[HO^-] > 10^{-1}$ M, because of the promoting effect of ionization on the shift of $R_1$ to the other carbonyl group. As the formation of oligomeric products involves aldolization reactions between neutral and ionized (di)carbonyl compounds[5], it will be obvious that at high alkalinity ($[HO^-] > 10^{-1}$ M), when almost all carbonyls are ionized, this reaction is of little importance in the product formation.



## Conclusions

Enediol anions are the well-known intermediates in the isomerization of monosaccharides as well as the starting intermediates in the subsequent alkaline degradation.

Monosaccharides themselves, in particular ketoses like D-fructose and D-psicose, may be considered too as intermediates in the alkaline degradation reaction by their retro-aldolization into small saccharides (and vice versa). The extent of retro-aldolization of monosaccharides appears to depend strongly on the $HO^-$ concentration.

Considering α-dicarbonyl compounds, i.e. the β-elimination products of enediol anion species, as key-intermediates completes the mechanistic picture of the alkaline degradation of monosaccharides. The influence of reaction parameters on the benzilic acid rearrangement/α-dicarbonyl cleavage/aldolization ratio of these intermediates, as determined by the change of the final product composition, can be understood by the observed effects of these parameters on the reactivity of the various (hydrated) acyclic and hemiacetal α-dicarbonyl structures involved.

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## CHAPTER 7

## A NOTE ON THE FRUCTO-FORMOSE REACTION: ALKALINE DEGRADATION OF D-FRUCTOSE IN THE PRESENCE OF FORMALDEHYDE

The formose reaction[1], i.e. the oligomerization of formaldehyde in aqueous alkaline solution, results in a mixture of monosaccharides, called formose or formose sugars. The product mixture largely consists of both linear and branched aldo- and ketopentoses, and the respective hexoses. In addition, variable amounts of linear and branched alditols as well as hydroxycarboxylic acids are irreversibly formed by (cross-)Cannizzaro reactions. Much research has been devoted to the improvement of the selectivity of the reaction. It has been reported that a proper choice of reaction conditions, catalysts, and additives leads to a preferential conversion of formaldehyde into ethylene glycol[2], 2-C-hydroxymethylglycerol[3], D,L-arabinitol[4], or 2,4-di-C-hydroxymethyl-3-pentulose[5], respectively.

The reaction conditions of the formose reaction are quite similar to those of the alkaline degradation of monosaccharides[6], i.e. $[HO^-] > 10^{-3}$ M, T = 40-90 °C, and 0.01-1 M reactant. It should be noted that in comparison with the fast conversion of formaldehyde, the alkaline degradation of formose sugars or monosaccharides is slow. For example, at 80 °C in aqueous saturated $Ca(OH)_2$, formaldehyde is converted within a few minutes, apart from a variable time of induction (see below), whereas the alkaline degradation of D-fructose takes about 2 hours. Thus, by the use of short reaction times subsequent degradation reactions can be largely prevented. Maximum conversion of formaldehyde together with a maximum yield of formose sugars has experimentally found to be attained at the so-called "yellowing point", based on colour development in the reaction mixture as an indication that irreversible alkaline degradation of the formose sugars starts to occur. The composition of the reaction mixture after complete alkaline conversion of the formose sugars is close to that of the product mixture of alkaline degradation of monosaccharides[7,8]. Furthermore, the formose reaction requires the initial presence of trace amounts of $\geq C_2$ aldehydes[9,10], which means that the processes taking place are "normal" aldolization and retro-aldolization reactions.

It is expected that monosaccharides, besides initiating the formose reaction, also might influence the selectivity of the reaction when present in higher concentrations. In addition, knowledge of the final reaction products of formaldehyde and monosaccharides in alkaline solution is of importance for the sugar manufacture. The formaldehyde used for controlling microbiological activity in the diffusion section is decomposed in the presence of invert sugar during the main liming. For these reasons, we have investigated the influence of monosaccharides, in particular D-fructose, on the formose reaction at various monosaccharide/formaldehyde ratios using HPLC and GC analysis[11] of the final carboxylic acid products.

The total concentration of carbohydrates, expressed as mol-$C_6$/L (e.g. 6 mol formaldehyde/L corresponds to 1 mol-$C_6$/L) was chosen between 0.025 mol-$C_6$/L and 0.050 mol-$C_6$/L in order to eliminate any concentration effects[12]. The reactions were performed in a thermostatted (78 °C) vessel (150 ml) under $N_2$. An excess of solid calcium hydroxide (590 mg in 100 ml water) was applied as the base, resulting in a constant $HO^-$ concentration (~ 0.03 M) during the reaction. The composition of the final reaction mixtures as a function of the starting molar D-fructose/formaldehyde ratio is depicted in Fig. 1. Going from the D-fructose alkaline degradation (left) to the formose reaction (right) there is a gradual change in the composition except for lactic acid and the > $C_6$ acids, i.e. the oligomeric acidic products[13], which show a minimum and a maximum, respectively, at D-fructose/formaldehyde = 1/6. In addition, more branched $C_4$ and $C_5$ saccharinic acids are formed at higher formaldehyde concentrations.

Aldolization of formaldehyde with saccharides and their intermediate alkaline degradation compounds explains the observed change in composition of the final carboxylic acid products upon addition of formaldehyde (Fig. 2). Comparison of the degradation of D-fructose towards lactic acid (route A) with that including the interference by formaldehyde (routes A + B) clearly demonstrates the possible effects of formaldehyde on the degradation pattern. At a first approximation, there exists a direct correlation between the amounts of > $C_6$ acids and lactic acid formed (Fig. 1). Apparently, formaldehyde functions as a scavenger for $C_3$ enediol (anion) species - precursors of lactic acid - by the formation of the non-enolizable species I



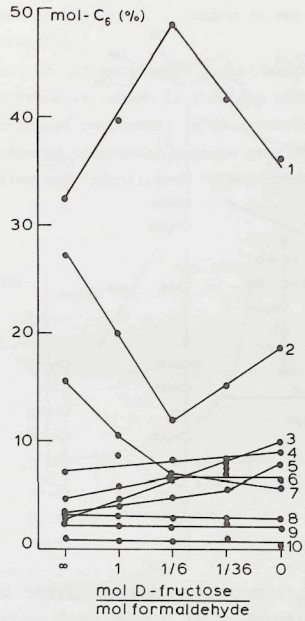


Fig. 1. Carboxylic acid products as a function of the molar D-fructose/formaldehyde ratio. Reaction conditions: 0.025 M D-fructose (except at the ratios 1/36 (0.0042 M) and 0 (0.15 M formaldehyde)); 590 mg $Ca(OH)_2$ in 100 ml $H_2O$, 78 °C, $N_2$, 100% conversion.

1. > $C_6$ acids
2. lactic acid
3. 2-methylglyceric acid
4. 2,4-dihydroxybutyric acid
5. formic acid
6. $C_5$-saccharinic acids
7. $C_6$-saccharinic acids
8. acetic acid
9. glycolic acid
10. glyceric acid

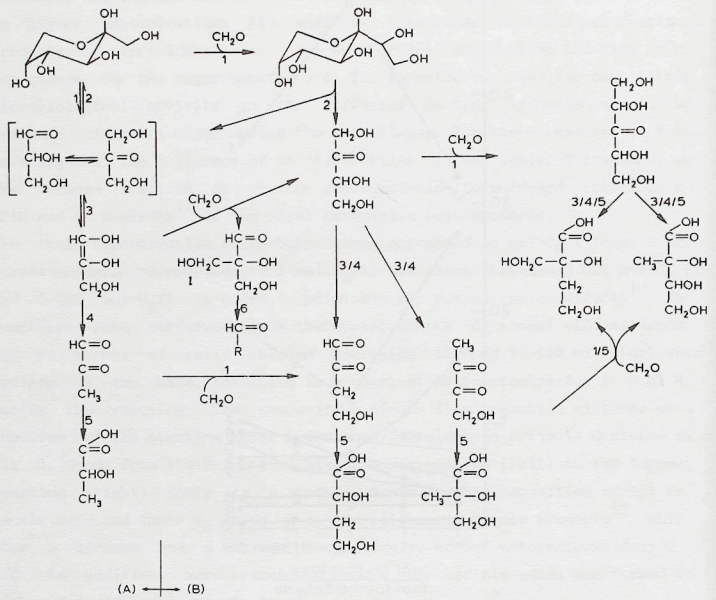


Fig. 2. Simplified reaction sequence for the alkaline degradation of D-fructose into lactic acid without (A) and in the presence of formaldehyde (A + B).
Numbering of reaction types:
1. aldolization,
2. retro-aldolization,
3. enolization,
4. β-elimination,
5. benzilic acid rearrangement,
6. aldolization with other enolizable carbonyl compounds.



(Fig. 2). As a result, lactic acid formation decreases, whereas aldolization of I with other enolizable carbonyl compounds may be responsible for the enhanced > $C_6$ acid formation.
Furthermore, aldolization of enols with formaldehyde will compete with the β-elimination to dicarbonyls, which in turn may aldolize with formaldehyde in stead of other carbonyl compounds. As dicarbonyls are assumed to be the main precursors of coloured compounds[13], these reactions explain the retardment of colour formation upon addition of formaldehyde (Fig. 3). The rate of

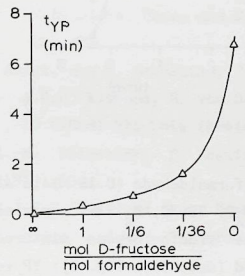


Fig. 3. Time elapsed until the yellowing point ($t_{yp}$) as a function of the molar D-fructose/formaldehyde ratio. Reaction conditions: see Fig. 1.

colour formation is indicated by the time elapsed until the yellowing point ($t_{yp}$). Up to the yellowing point, the formation of acidic products can be neglected and, consequently, only formaldehyde and saccharides derived thereof will be present in the reaction mixture[1]. At the yellowing point formaldehyde is almost completely converted (Fig. 4) and concomitantly a maximum concentration of saccharides is attained.
The various fructo-formose reactions carried out can be also considered as normal formose reactions since the initial composition of these mixtures reflects more or less that of the formose reaction mixture between t = 0 and $t_{yp}$. Indeed, the $t_{yp}$ of the various fructo-formose reactions versus their initial formaldehyde-saccharide compositions, as indicated by Δ in Fig. 4, correlate quite well with the conversion curve of formaldehyde in the formose reaction. The results also confirm the earlier conclusion[9,10] that

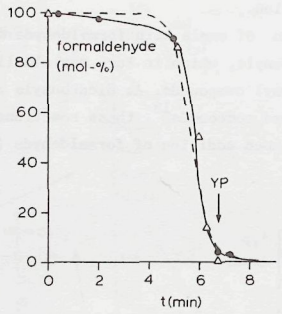


Fig. 4. Reaction course of formaldehyde (0.15 M) in alkaline medium (590 mg $Ca(OH)_2$ in 100 ml $H_2O$, 78 °C, $N_2$): ●, experimental data; △, initial compositions of the fructo-formose mixtures versus their corresponding $t_{yp}$ observed (data from Fig. 3); YP yellowing point; dotted line is the theoretical conversion curve of formaldehyde calculated according to the kinetic relation for an autocatalytic reaction sequence (see text).

the formose reaction has to be considered as an autocatalytic reaction sequence consisting of aldolization and retro-aldolization steps and that the first step of the formose reaction ($C_1 + C_1 \longrightarrow C_2$) does not occur at all. Using the derived relation[10]

$$[CH_2O] = \frac{\alpha}{1+\beta e^{kt}},$$

in which $\alpha$ is the initial $CH_2O$ concentration and $\beta = \frac{[C_n]}{[CH_2O]}$ with $C_n \geq C_2$ carbonyl species, the experimental data in Fig. 4 correlate with a pseudo first order reaction rate constant $k = 2.7\ min^{-1}$ and $\beta = 2 * 10^{-7}$ (see dotted line in Fig. 4).

The results clearly show that (retro-)aldolization reactions are of major importance for both the formose reaction and the alkaline degradation of



monosaccharides. In other words, there is no essential mechanistic difference between these two reactions.

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# CHAPTER 8

# $^{13}C$ NMR SPECTROSCOPY OF ALKALINE DEGRADATION PRODUCTS OF MONOSACCHARIDES

## Introduction

It is well known[1] that in aqueous alkaline solution monosaccharides are converted into a complex mixture of carboxylic acids. The reaction mechanism, however, is only partly elucidated. The influence of several reaction parameters on the degradation pattern has been investigated recently[2] by the quantitative analysis of degradation products in the final reaction mixtures by HPLC and GC[3]. In this way, the formation of substantial (up to 50%) amounts of oligomeric carboxylic acids (mol. wt. up to 1000) was established. The general structure of these oligomeric products could be characterized using UV, IR, MS, and gel chromatography as the analysis techniques[4]. $^{1}H$ NMR spectroscopy has been applied before[5] for the identification of alkaline degradation products, but only the presence of three acidic degradation products, i.e. formic, acetic, and lactic acid, could be ascertained, as shown in Fig. 1. Mutual interference of signals from the other products did not allow their analysis by $^{1}H$ NMR. Therefore, we have studied the use of $^{13}C$ NMR spectroscopy for the identification of alkaline degradation products of monosaccharides. In this chapter, $^{13}C$ NMR data for the most important $C_1$ to $C_6$ acidic degradation products, i.e. $\leq C_6$ acids, are presented as well as the identification of functional groups incorporated in the structures of the oligomeric acids obtained from 1-$^{13}C$-D-glucose.

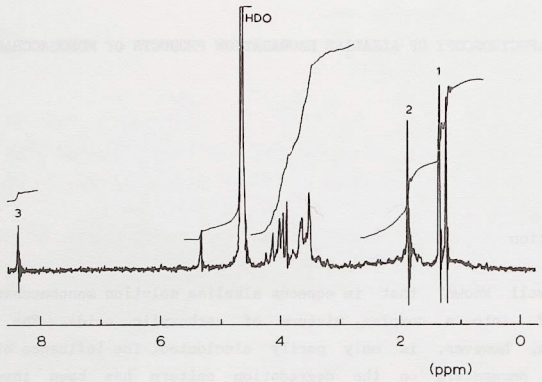


Fig. 1. $^{1}H$ NMR spectrum of the alkaline degradation product mixture of D-glucose[5] (0.035 M D-glucose, 0.01 M KOH, $H_2O$, 80 °C, $N_2$, 100% conversion);
1. lactic acid ($CH_3$);
2. acetic acid ($CH_3$);
3. formic acid (CH).

Experimental

*Materials*

1-$^{13}$C-D-glucose, containing 99% $^{13}C$, was obtained from C.E.A., France. Formic acid, sodium acetate.3 aq, glycolic acid, and calcium(II) lactate.5 aq, were reagent-grade. Metasaccharinic acid[6,7] (3-deoxyhexonic acid) was obtained by alkaline degradation of 3.0 g (15 mmol) of 3-O-methyl-D-glucose (Aldrich) with 1.6 g (22 mmol) of $Ca(OH)_2$ in 800 ml $H_2O$, under $N_2$ at room temperature. After 9 days the reaction mixture was saturated with $CO_2$ and heated for 1 h at 100 °C. After filtration the reaction mixture was freeze-



dried. Crystallization from water gave 0.3 g (10%) of pure calcium(II) metasaccharinate.

Isosaccharinic acid[8-10] (3-deoxy-2-(hydroxymethyl)pentonic acid) was prepared by alkaline degradation of 33 g (92 mmol) of lactose.1 aq with 9 g (122 mmol) of $Ca(OH)_2$ in 300 ml $H_2O$ under $N_2$ at room temperature. After 3 days the reaction mixture was heated for 10 h at 100 °C, saturated with $CO_2$ and the $CaCO_3$ filtered off. Subsequently, the solvent was evaporated in vacuo and 2.5 g of crude calcium(II) isosaccharinate was precipitated by addition of water/acetone (1:2) to the remaining dark coloured syrup. Finally, crystallization from water gave 2.0 g (11%) of pure calcium(II) isosaccharinate. The sodium salt was obtained by cationic exchange with an excess of zeolite NaA in water.

2,4-Dihydroxybutyric acid was prepared in quantitative yield from its corresponding lacton[11-13] by heating in saturated aqueous $Ca(OH)_2$ during 2 h at 100 °C. Subsequently, the $Ca(OH)_2$ precipitate was filtered off and the sodium salt of 2,4-dihydroxybutyric acid was obtained by cationic exchange with an excess of zeolite NaA in water.

2-Methylglyceric acid was prepared by dihydroxylation of 8.6 g (0.1 mol) of methyl acrylate[14,15] with 9 ml (0.5 mol) of 30% $H_2O_2$ and 86 ml (2.3 mol) of formic acid at 55-60 °C for 2 h, followed by heating at 90 °C for 25 min. After concentration of the reaction mixture by evaporation in vacuo, 175 ml of 2.5 M NaOH was added and the mixture heated for 30 min at 100 °C. The mixture was cooled, acidified by addition of concentrated HCl and the solvent distilled off in vacuo. The resulting solid was extracted in a Soxhlet apparatus with ethyl acetate. Concentration of the ethyl acetate solution yielded 1.5 g (13%) of 2-methylglyceric acid.

*$^{13}C$ NMR analysis*

The $^{13}C$ NMR spectra of the salts of carboxylic acids and neutralized alkaline degradation mixtures of monosaccharides in $D_2O$ at 30 °C were recorded on a Nicolet NT-200 WB spectrometer (50 MHz) or a Varian CFT-20 spectrometer (20 MHz) using 1,4-dioxane as external standard ($\delta$ = 66.6). Quantitative $^{13}C$ NMR spectra were obtained by using gated $^{1}H$-decoupling, a pulse width of 12.0 µs (45° flip angle), and a pulse delay of 100.0 s.

Results and discussion

The assignments of the $^{13}C$ chemical shifts of a number of carboxylic acids formed during the alkaline degradation of monosaccharides are summarized in Table 1. In some cases proton-coupled $^{13}C$ NMR spectra were required to identify all $^{13}C$ signals, as shown for isosaccharinate in Fig. 2.
*On the basis of these $^{13}C$ NMR data it was possible to identify the low-molecular weight products in alkaline degradation mixtures of monosaccharides as shown in Fig. 3.*

Table 1. $^{13}C$ chemical shifts of salts of carboxylic acids[a].

| carboxylate | chemical shift (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $COO^-$ | CHOH | COH | $CH_2$ | CHOH | CHOH | $CH_2OH$ | $CH_3$ |
| formate | 171.0 | | | | | | | |
| acetate | 181.3 | | | | | | | 23.4 |
| glycolate | 179.8 | | | | | | 61.4 | |
| lactate | 182.4 | 68.6 | | | | | | 20.2 |
| 2-methylglycerate | 182.7 | | 77.9 | | | | 69.2 | 23.0 |
| 2,4-dihydroxybutyrate | 181.4 | 70.3 | | 36.5 | | | 58.5 | |
| isosaccharinate | 181.1 | | 79.1 | 39.4 | 69.9 | | 67.5 | |
| | | | | | | | 69.2 | |
| metasaccharinate | 181.8 | 74.9 | | 37.1 | 62.4 | 69.8 | 68.7 | |

[a] Concentration of the Na(I) salts, or Ca(II) salt in the case o metasaccharinate, 0.1-0.5 M in $D_2O$, pD ~ 7, 30 °C.

The qualitative composition thus obtained agrees with the composition of the $\leq C_6$ acid part of the mixture as determined by HPLC and GC[2]. However, no separate $^{13}C$ signals are detected for the ~ 50% of oligomeric products, the so-called $> C_6$ acids, which are present in this reaction mixture. Apparently, the composition of this part of degradation products is very complex in nature, the many rather small signals disappearing in the noise of the NMR spectrum. To verify this we carried out an alkaline degradation reaction of



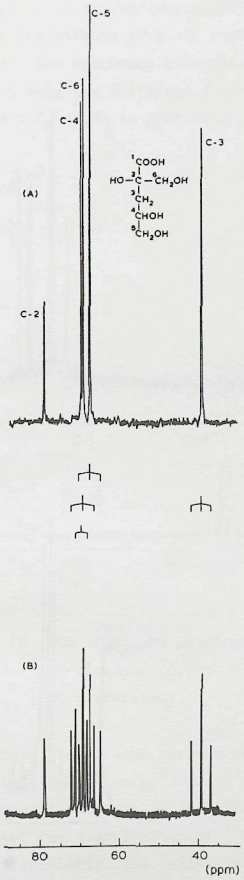


Fig. 2. Part of the proton-decoupled (A) and the proton-coupled (B) 50 MHz $^{13}C$ NMR spectra of sodium isosaccharinate (0.5 M in $D_2O$, 30 °C).

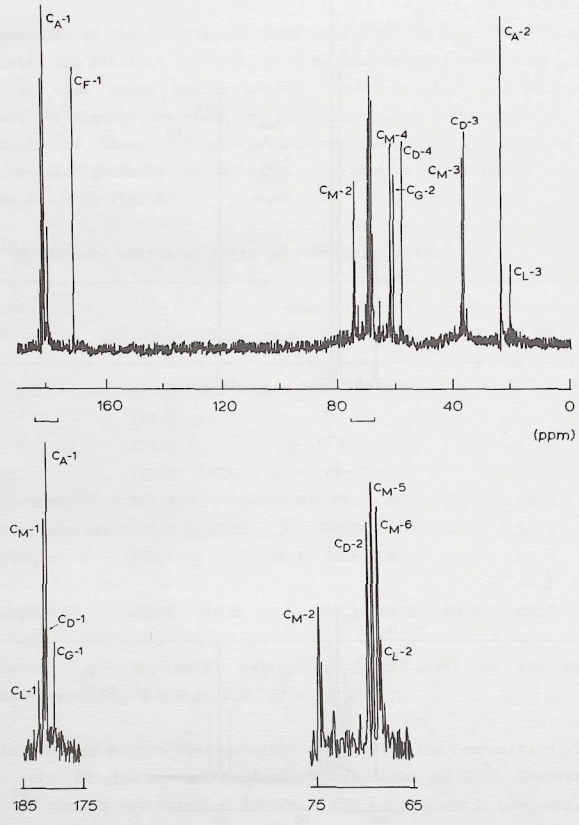


Fig. 3. 20 MHz $^{13}C$ NMR spectrum of an alkaline degradation mixture of D-glucose (0.1 M D-glucose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 7 h, 100% conversion).
F = formate; A = acetate; G = glycolate; L = lactate; D = 2,4-dihydroxybutyric acid; M = metasaccharinate.



1-$^{13}$C-D-glucose. Apart from the various signals of $\leq C_6$ acids, the $^{13}C$ NMR spectrum of this reaction mixture (Fig. 4) contains a large number of small signals distributed over the spectrum, belonging to the $> C_6$ acids.
The distribution of $^{13}C$ over the different functional groups present in both the $\leq C_6$ acids and the $> C_6$ acids is presented in Table 2.

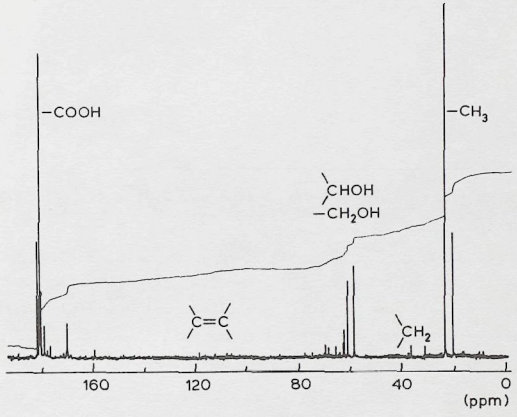


Fig. 4. Quantitative 50 MHz $^{13}C$ NMR spectrum of the alkaline degradation mixture of 1-$^{13}$C-D-glucose (0.025 M 1-$^{13}$C-D-glucose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 100% conversion).

The $^{13}C$ distribution over the various carbons in each of the $\leq C_6$ acids will be dealt with in detail elsewhere[16], as it contains valuable information concerning the mechanistic pathways of the alkaline degradation of monosaccharides. Comparison of the distribution of $^{13}C$ in both $\leq C_6$ acids and $> C_6$ acids shows that particularly the relative amount of $^{13}C$ in carboxylate groups differs. Aldolization reactions of the $^{13}C$ labeled aldehyde group in 1-$^{13}$C-D-glucose or in its subsequent reaction intermediates, which are responsible for substantial formation of oligomeric products, explain the relatively small content of $^{13}C$ in the carboxylate groups of these $> C_6$ acids in favour of that in the other functional groups. The α-dicarbonyl cleavage reaction of 1-$^{13}$C-4-deoxy- and 1-$^{13}$C-1-deoxyhexo-2,3-diulose

Table 2. $^{13}C$ NMR determination of $^{13}C$ in the different functional groups of both $\leq C_6$ acids and $> C_6$ acids obtained by alkaline degradation of 1-$^{13}$C-D-glucose[a].

| Functional group | $^{13}C$ (%) | |
|---|---|---|
| | $\leq C_6$ acids | $> C_6$ acids |
| $COO^-$ | 35 | 3 |
| $CH_2OH$, CHOH | 12 | 6 |
| $CH_3$, $CH_2$ | 21 | 16 |
| C=C | | 7 |
| total | 68 | 32 |

[a] Reaction conditions: 0.025 M 1-$^{13}$C-D-glucose, 0.01 M KOH, $H_2O$, 78 °C, $N_2$, 100% conversion.

intermediates[16], which produces $^{13}C$ labeled glycolic and acetic acid, respectively, as well as a still reactive unlabeled (2-deoxy)tetrose, explains the relatively low $^{13}C$ label content in the $> C_6$ acidic products, i.e. only 32% $^{13}C$ label present in the ~ 50% $> C_6$ acids.

## Conclusions

In contrast with $^1H$ NMR, $^{13}C$ NMR spectroscopy proves to be a convenient analysis technique for the determination of the complex mixture of carboxylic acids formed by the alkaline degradation of monosaccharides. The $\leq C_6$ acidic products can easily be determined, while valuable structural and mechanistic information is obtained on the various carbons present in the $> C_6$ acidic products.



## Acknowledgements

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CHAPTER 9

# ANALYSIS OF CARBOXYLIC ACIDS FORMED BY ALKALINE DEGRADATION OF INVERT SUGAR: A COMPARISON BETWEEN LIQUID AND GAS CHROMATOGRAPHY*

## Introduction

Invert sugar is present in diffusion juices in amounts between 0.5 and 1.5 g/100 g sucrose. Apart from its presence in sugar beets, invert sugar also finds its origin from enzymatic hydrolysis of sucrose during the diffusion process. One of the aims of the main liming is destruction of invert sugar into thermostable components, thus preventing or limiting pH drops in the evaporators. This destruction of invert sugar under alkaline conditions has been studied extensively[1-8], but is not completely elucidated yet with respect to the deficit on the mass balance[4], and mechanistic and kinetic features. Two types of degradation products can be distinguished: $C_1$-$C_6$ acids[1-4] and products of higher molecular weight[5-8], which are either coloured or considered as precursors for colour formation.

As a part of the Delft research program on carbohydrate chemistry, we have been engaged in the study of the various reactions of monosaccharides in aqueous alkaline media[4]. In this respect ionization[9], mutarotation[9], enediol anion formation[10], isomerization[11] and alkaline degradation[4,11] have been investigated in order to obtain further insight into the mechanisms of these transformations. At present, a common investigation of the degradation reaction of monosaccharides is being performed[12] in order (i) to study the influence of reaction variables on product formation and (ii) to compare results from laboratory experiments with those from procedures on a technical scale. A rapid and quantitative analysis technique is a prerequisite for this investigation, in particular to follow the course of the reaction as a function of time.

The quantitative analysis of the carboxylic acid products formed is known to

* J.M. de Bruijn, A.P.G. Kieboom, H. van Bekkum, and P.W. van der Poel, Int. Sugar J., 86 (1984) 195-199.

be difficult as the composition of the reaction mixture after alkaline degradation of invert sugar is rather complex. The mixture contains more than ten different $C_1$-$C_6$ acids; among these lactic acid, glycolic acid, formic acid, acetic acid, $C_6$-saccharinic acids (saccharinic, metasaccharinic, and isosaccharinic acid), and 2,4-dihydroxybutyric acid are the most abundant. Paper and thin-layer chromatography are not very suitable, since these analysis methods give only a qualitative or semi-quantitative picture of the composition of the reaction mixture. Therefore, quantitative analysis of the acidic products requires gas chromatography (GC), as studied by Oldfield et al.[13] and Reinefeld et al.[14,15], and/or high performance liquid chromatography (HPLC), as studied by Charles[16] and Kubadinow[17].

In this paper the scope and limitations of both GC and HPLC analysis techniques will be discussed on the basis of industrial juice samples as well as reaction mixtures from the alkaline degradation of invert sugar in laboratory experiments.

## Experimental

### *GC analysis*

Sucrose and non-acidic degradation products in juice samples were removed by the aid of an ion exchange clean-up procedure as described by Oldfield et al.[13]. Samples from model experiments were neutralized in the cold (< 4 °C) with a weak cation exchange resin (BioRex 70 H), delivered by Bio-Rad) in order to prevent lactonization of the saccharinic acids.

After freeze-drying of the samples, the carboxylic acids were dissolved in pyridine and converted into their trimethylsilyl (TMS) derivatives by the method of Petersson[18].

The samples thus obtained were analyzed with a Varian Model 3700 gas chromatograph, equipped with a capillary CP Sil 5 column (25 m length, 0.23 mm i.d.), a splitter (splitter ratio 100:1), and a flame ionization detector (FID).

The temperature of the column oven was programmed as follows: 5 min at 75 °C, increasing to 280 °C at a rate of 8 °C/min, and another stationary period of 5 min at 280 °C. The temperature of injector and FID was 230 °C. Identification of the trimethylsilylated carboxylic acids was performed by mass spectrometry using a Varian GC-MS system Mat 44 in which the GC part was identical with that described above.



### *HPLC analysis*

The samples either freed from sugars and neutral products or neutralized as described above were analysed on an 'organic acid analysis' column from Bio-Rad (HPX 87, 300 mm length, 7.8 mm i.d.). This stainless steel column contains a sulfonated styrenedivinylbenzene copolymer as the strong cation exchange resin (crosslinkage 8%; particle size 7-11 µm; $-SO_3H$ content 1.7 mmol/ml).

A micro guard column (delivered by Bio-Rad), containing the same material as the analytical column, was used to prevent column-fouling.

The resin was always applied in the hydrogen form, which was shown to give the best results[16,17,19,20] at 60 °C using an 0.005 M $H_2SO_4$ aqueous solution (0.6 ml/min) as the eluent.

The equipment used was as follows: Waters Associates Chromatography Pump M-6000 A, Differential Refractometer RI 401 and Differential Refractometer Electronics unit, Rheodyne injector with a 100 µl sample loop.

## Results and discussion

### *Gas chromatography*

Gas chromatography analyses together with mass spectrometric identification[21-23] of a juice sample, taken from the evaporation unit of the sugar factory of CSM Breda (The Netherlands), allowed the assignment of 17 peaks (Fig. 1).

The gas chromatogram shows an excellent separation of the various acidic products. Citric, malic and oxalic acid are already present in the sugar beet and have not been eliminated completely in the juice purification.

Pyrrolidonecarboxylic acid originates from saponification of glutamine. The other acids are originating from alkaline degradation of invert sugar during the main liming process or from microbial destruction of sugar. The 1,4-lactones of the $C_6$-saccharinic acids are the result of the ion exchange clean-up procedure of the juice sample before derivatization, as mentioned in the experimental part. Performing this procedure, first of all the samples pass a strong cation exchange column, resulting in a sharp pH drop to pH ~ 2. This causes at room temperature partly lactonization of the $C_6$-saccharinic acids before they enter the second column containing a strong anion

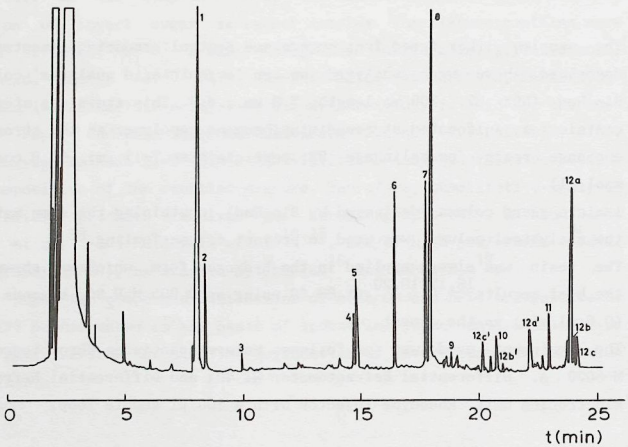


Fig. 1. Gas chromatogram of carboxylic acids (as their TMS-derivatives) of a thick juice sample from the evaporation unit.

1. lactic acid
2. glycolic acid
3. oxalic acid
4. 2-C-Me-glyceric acid
5. glyceric acid
6. 2,4-dihydroxybutyric acid
7. malic acid
8. pyrrolidonecarboxylic acid
9. tetronic acid
10. 3-deoxypentonic acid
11. citric acid
12. $C_6$-saccharinic acids
    a. metasaccharinic acid
    a'. metasaccharinic acid-1,4-lactone
    b. isosaccharinic acid
    b'. isosaccharinic acid-1,4-lactone
    c. saccharinic acid
    c'. saccharinic acid-1,4-lactone

Chromatographic conditions: see experimental part.

exchange resin. For comparison, Fig. 2 shows a typical example of a gas chromatogram of a reaction mixture from a laboratory experiment, in which glucose is completely degraded. In this chromatogram no $C_6$-saccharinic acid lactones are present, because for neutralization the reaction mixture is treated in the cold with a weak cation exchange resin, as described in the experimental part, thus preventing lactonization.



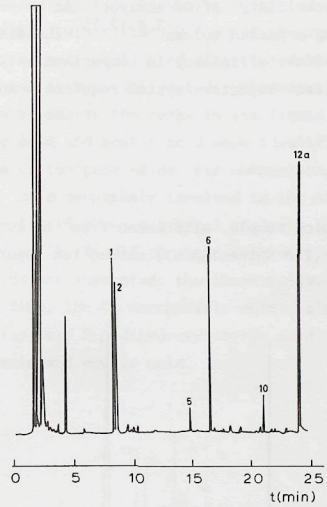


Fig. 2. Gas chromatogram of acidic products (as their TMS-derivatives) from a laboratory experiment, in which glucose is degraded in alkaline medium.

Reaction conditions: aqueous solution of 0.03 M glucose and 0.01 M KOH, 80 °C, $N_2$, 7 h. During the reaction the pH was kept constant by the addition of 2 M KOH.

Numbering of the peaks according to Fig. 1.

Chromatographic conditions: see experimental part.

Formic acid and acetic acid, normally formed upon alkaline degradation of monosaccharides, cannot be found in the gas chromatogram, because their TMS-derivatives are too volatile and have the same retention as an unretained compound. For quantitative analysis of the acids, the response factors can be calculated by the method of Verhaar and De Wilt[24]. However, the wide range of volatilities of the acid derivatives in combination with the splitting of injected samples make the quantitative analysis of acids unre-

producible. This uncertainty of GC analyses can be prevented by on-column injection, or by using a packed column[3,4,13-15]. The disadvantage of using a packed column is the lower efficiency in comparison with a capillary column, whereas a splitterless injector system requires a more tedious injection procedure.

*Liquid chromatography*

HPLC analysis of a juice sample, also taken from the evaporator of the sugar factory of CSM Breda (The Netherlands), showed ten separated peaks (Fig. 3).

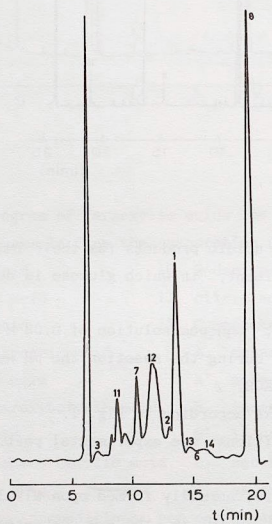


Fig. 3. HPLC separation of carboxylic acids of a thick juice sample from the evaporation unit.
Numbering of the peaks according to Fig. 1.
13. formic acid 14. acetic acid
Chromatographic conditions: see experimental part.



In order to identify these peaks we first carried out a preparative HPLC separation by injecting twenty times 0.6 mg of the juice sample on the same column. 12 Fractions were collected after the detector, neutralized (2 M KOH), freeze-dried, and trimethylsilylated. Subsequent GC-MS analysis of the fractions allowed us to assign the peaks in the liquid chromatogram.
The peaks of formic acid and acetic acid were identified by comparison with the retention times of the pure acids. For comparison Fig. 4 shows the HPLC separation of the main components involved in the alkaline degradation of invert sugar, while Fig. 5 shows a typical analysis of a laboratory experiment. As can be seen in the liquid chromatograms of the Figures 3, 4, and 5, not all of the acids are separated: the three $C_6$-saccharinic acids possess the same retention time, the $C_6$-saccharinic acids, glycolic acid and lactic acid show some overlap, and 2,4-dihydroxybutyric acid is not completely separated from formic acid and acetic acid.

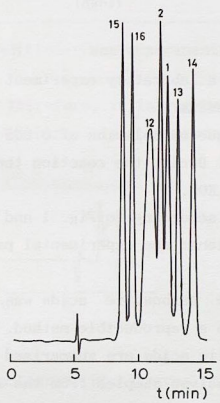


Fig. 4. HPLC separation of some compounds involved in the alkaline degradation of invert sugar.
Numbering of the peaks according to Fig. 1 and Fig. 3.
15. D-glucose 16. D-fructose
Chromatographic conditions: see experimental part.

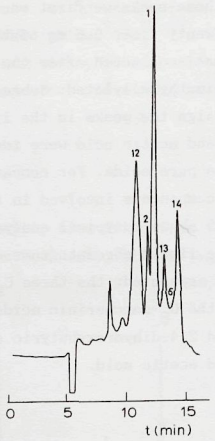


Fig. 5. Liquid chromatogram of a laboratory experiment in which D-glucose is degraded in alkaline medium.
Reaction conditions: aqueous solution of 0.005 M D-glucose and 0.009 M KOH, 80 °C, $N_2$, 7 h. During the reaction the pH was kept constant by the addition of 2 M KOH.
Numbering of the peaks according to Fig. 1 and Fig. 3.
Chromatographic conditions: see experimental part.

Quantitative analysis of the carboxylic acids was derived from the peak heights, which proved to be a reproducible method. The relative response factors of a number of carboxylic acids are summarized in Table 1.
With HPLC it is possible to analyse samples from the laboratory experiments in a straight-forward manner: neutralization of the samples, as described in the experimental part, is the only prerequisite necessary. If these samples still contain invert sugar, analysis of both D-glucose, D-fructose and the carboxylic acids can be performed simultaneously, as shown by Fig. 4.
On the other hand, sucrose, present in juice samples of the sugar factory, cannot be directly analyzed under the separation conditions described, because sucrose is partly hydrolyzed in the column into invert sugar. This



Table 1. Relative response factors of carboxylic acids, as derived from peak heights/weight in liquid chromatograms[a].

| carboxylic acid | relative response factor |
|---|---|
| 2,4-dihydroxybutyric acid | 0.22 |
| metasaccharinic acid | 0.42 |
| formic acid | 0.51 |
| isosaccharinic acid | 0.53 |
| acetic acid | 0.61 |
| pyrrolidonecarboxylic acid | 0.96 |
| glycolic acid | 0.97 |
| lactic acid | 1.00 |
| glyceric acid | 1.42 |
| malic acid | 1.44 |
| citric acid | 1.69 |

[a] Chromatographic conditions: see experimental part.

is shown in Fig. 6. Therefore, it is recommended to remove the large amount of sucrose by an ion exchange clean-up procedure[13].

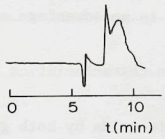


Fig. 6. Liquid chromatogram of sucrose showing hydrolysis in the column during elution.
Chromatographic conditions: see experimental part.

Comparison of GC and HPLC separation

The efficiency N of a chromatographic column may be defined by:

$$N = 5.54 \left(\frac{t_r}{w_{1/2h}}\right)^2$$

(in which $t_r$ is the retention time of a compound and $w_{1/2h}$ is the peak width at half peak height. The GC capillary CP Sil 5 column has a much higher efficiency ($N \sim 1.5 * 10^6$) than the HPLC HPX 87 column ($N \sim 1.1 * 10^4$) as is demonstrated by the great difference in peak widths.
The capacity factor k' of a compound may be defined by

$$k' = \frac{t_r - t_o}{t_o}$$

in which $t_r - t_o$ is the difference in retention time between a retained and an unretained compound. As shown in Table 2 the range in k'-values of the carboxylic acids is much higher for GC than for HPLC, so with GC the carboxylic acids can be separated over a relative larger range of retention times.
Both measures of separation quality N and k' show that GC gives by far the best separation results. On the other hand, the possibility to analyse both formic and acetic acid with HPLC is an advantage of this technique with respect to GC.
Table 3 summarizes and compares some characteristics of GC and HPLC analyses.
The capacity factors of some carboxylic acids by both gas and liquid chromatographic separation are graphically represented in Fig. 7, in which the location of the different peaks of the carboxylic acids is indicated with the aid of simulated gas and liquid chromatograms.



Table 2. k'-values of carboxylic acids for both GC and HPLC separation[a].

| carboxylic acid | $k'_{GC}$ | $k'_{HPLC}$ |
|---|---|---|
| formic acid | 0 | 1.43 |
| acetic acid | 0 | 1.64 |
| glycolic acid | 1.79 | 1.19 |
| lactic acid | 1.71 | 1.28 |
| glyceric acid | 3.40 | 1.01 |
| 2,4-dihydroxybutyric acid | 3.81 | 1.51 |
| 2-C-Me-glyceric acid | 3.35 | 0.98 |
| tetronic acid | 4.50 | 0.97 |
| malic acid | 3.61 | 0.74 |
| pyrrolidonecarboxylic acid | 3.65 | 2.22 |
| 3-deoxypentonic acid | 4.98 | 1.29 |
| metasaccharinic acid | 5.80 | 1.01 |
| isosaccharinic acid | 5.84 | 1.04 |
| saccharinic acid | 5.87 | 1.01 |
| citric acid | 5.57 | 0.45 |

[a] Chromatographic conditions: see experimental part.

Table 3. Comparison of GC and HPLC analysis of carboxylic acids[a].

| property | GC | HPLC |
|---|---|---|
| sample preparation time | ~ 1 day | 5 min (60 min)[b] |
| analysis time | 30 min | 20 min |
| separation quality | ++ | + |
| direct analysis of formic acid and acetic acid | - | + |
| quantification | - | + |

[a] Chromatographic conditions: see experimental part.
[b] Removal of sucrose in the case of an industrial sugar juice.

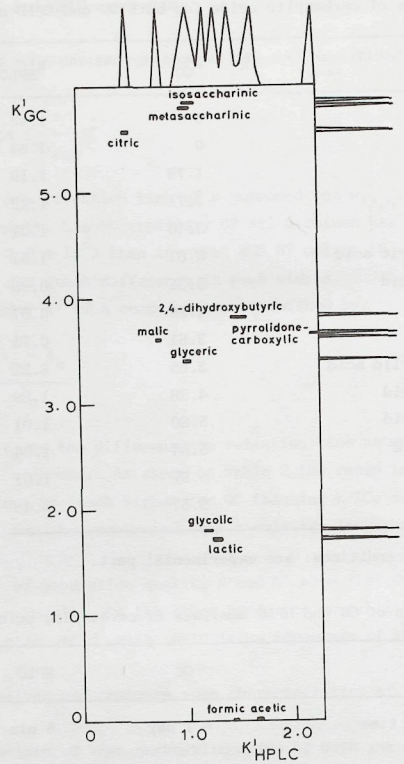


Fig. 7. Correlation chart of GC and HPLC for acidic (degradation) products. Chromatographic conditions: see experimental part.

With the help of this correlation chart and the comparison of both analytic techniques discussed in this paper we may conclude the following:
The major advantage of HPLC is that it requires no time consuming freeze-drying and derivatization of the samples. Thus, despite its lower separation capacity, HPLC is a very rapid, convenient, and reproducible method for the



quantitative analysis of acidic products from alkaline degradation reactions. In addition, GC analysis may be preferred for special purposes. For instance, when the ratio of isosaccharinic acid and metasaccharinic acid has to be investigated, it is necessary to perform also GC analysis of the samples.

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## SUMMARY

This thesis deals with the behaviour of monosaccharides in aqueous alkaline solutions with respect to isomerization and degradation reactions. For the sake of clearness, all transformations of monosaccharides into carboxylic acid products, including both degradation and oligomerization reactions, are generally denoted as alkaline degradation.

Chapter 1 gives a short introduction including the scope of the research performed and the subdivision of this Thesis.

In Chapter 2 a survey is given of the literature data concerning reactions of monosaccharides in aqueous alkaline solutions, which can be devided in two categories, namely (i) initial reversible reactions without skeletal rearrangement of the sugar moiety, and (ii) irreversible degradation reactions involving C-O and C-C bond-breaking and -making. The first category consists of ionization, mutarotation, enolization, and isomerization reactions. The mechanisms of these reactions are briefly discussed on the basis of some models proposed in the literature. On the other hand, the rather complex mechanism of the subsequent alkaline degradation reactions towards the formation of carboxylic acid products is only partly elucidated up to now. From this literature survey it became apparent that further research is required, especially on the influence of reaction parameters on both the degradation pattern and the kinetics, in order to obtain a better insight into the way of formation, the composition and the structure of the products, particularly of the oligomeric products, formed during the alkaline degradation of monosaccharides. Use of sophisticated analysis methods as NMR spectroscopy, MS, GC, and HPLC is a prerequisite for such studies.

Chapter 3 describes a systematic investigation on the influence of several parameters on the alkaline degradation of monosaccharides. The final product compositions of the reaction mixtures were determined by HPLC analysis and, in some cases, by GC analysis. The $HO^-$ and the monosaccharide concentrations markedly influence the final product composition with respect to both the

amount of $C_1$ to $C_6$ acids and the formation of oligomeric acidic products, the so-called $> C_6$ acids. Maximum yields of these $> C_6$ acids, up to 50 mol-$C_6$-%, are obtained at an $HO^-$ concentration between $10^{-3}$ M and $10^{-2}$ M if the monosaccharide concentration exceeds $10^{-2}$ M. The presence of divalent calcium increases the retro-aldolization of monosaccharides in alkaline medium, as illustrated by the enhanced production of lactic acid, by complexation with, for instance, D-fructose. Borate partly protects monosaccharides against alkaline degradation through their borate esters, whilst the amount of saccharinic acids in the degradation product mixture is doubled. On the other hand, neither chloride and carbonate nor the reaction temperature influences the final product composition. Alkaline degradation experiments with pyruvaldehyde, glyceraldehyde and 1,3-dihydroxyacetone, all assumed to be reaction intermediates, indicate that aldolization of (di)carbonyl compounds cause the formation of substantial amounts of $> C_6$ acids.

In Chapter 4 the characterization of oligomeric products formed during the alkaline degradation of monosaccharides is discussed. The nature and structure of these products have been studied by UV, IR, $^{13}C$ NMR spectroscopy and GC-MS analysis. Separation of the reaction mixture into $C_1$-$C_6$ acids and oligomeric products was performed by gel filtration and by selective precipitation with lead(II) salts. It appeared that the oligomeric products comprise a complex mixture of acidic compounds containing similar structural moieties, i.e. carboxylate, β-dicarbonyl, $CH_3$, $CH_2$, $CH_2OH$ and CHOH groups. These $> C_6$ acids with average molecular weights ~ 350, ~ 500, and ≥ 700 are considered to be formed via aldolization of (di)carbonyl compounds which are important intermediates of the alkaline degradation of monosaccharides. Such (di)carbonyls are, for instance, pyruvaldehyde, 3-deoxyhexos-2-ulose as well as the monosaccharides themselves. The structures of a $C_7$, a $C_8$, and a $C_9$ acid have been elucidated by GC-MS analysis. The oligomerization reaction is directly correlated with the UV absorbance at 265 nm during the alkaline degradation of monosaccharides.

Chapter 5 deals with the kinetics of the alkaline isomerization and degradation of monosaccharides. A new kinetic model is presented which includes both the interconversion and degradation of D-glucose, D-fructose, D-mannose and D-psicose. Computer simulations using this model fit the experimental data and allow the determination of all relevant reaction rate constants. Additionally, it has been established that for the alkaline degradation of either D-fructose, D-glucose or D-mannose substantial amounts of acidic



products, i.e. ~ 65% and ~ 20%, are formed via D-fructose and D-psicose respectively.

The influence of some reaction parameters on the pseudo first order rate constants involved in the kinetic model has been investigated. The enolization of monosaccharides appears to be the rate-limiting step in both isomerization and degradation reactions. The enolization rate is markedly enhanced at higher $HO^-$ concentration or by the addition of calcium(II). Direct retro-aldolization of D-fructose and D-psicose occurs at $[HO^-] > 10^{-2}$ M or in the presence of calcium(II), leading to an additional increase of the degradation rate towards lactic acid. The shift of the isomerization equilibrium by borate towards D-fructose and D-psicose is accompanied by a decrease of the degradation rate constants, due to stabilization of the monosaccharides as their borate esters.

In Chapter 6 recent data on the alkaline degradation of monosaccharides have been used to develop a mechanistic picture of the alkaline degradation of monosaccharides. It appears that the degradation of both monosaccharides themselves, particularly ketoses, is directed by the reaction conditions. So, the extent of retro-aldolization of ketoses largely determines the amount of lactic acid formed whereas the benzilic acid rearrangement/α-dicarbonyl cleavage/aldolization ratio of α-dicarbonyls further influences the composition of the $C_1$-$C_6$ acid part of products as well as the total amount of oligomeric products.

Chapter 7 describes the fructo-formose reaction, i.e. the alkaline degradation of D-fructose in the presence of formaldehyde. The final product compositions of the alkaline (calcium hydroxide) degradation of D-fructose in the presence of formaldehyde at several D-fructose/formaldehyde ratios, and of the formose reaction were determined by HPLC and GC analysis. At decreasing D-fructose/formaldehyde ratio a gradual change of the composition was observed except for lactic acid and the oligomeric acidic products, which show a minimum and a maximum, respectively. A mechanistic explanation is given by the aldolizatidon of formaldehyde both with saccharides and with their subsequent α-dicarbonyl intermediates, which favours the formation of oligomeric products at the cost of lactic acid. It is established both experimentally and theoretically that there is no essential mechanistic difference between the formose reaction and the alkaline degradation of monosaccharides.

Chapter 8 describes the analysis of alkaline degradation products of monosaccharides by $^{13}C$ NMR spectroscopy. The $^{13}C$ chemical shifts of several carboxylic acids produced by alkaline degradation of monosaccharides have

been determined. The application of these data to the identification and quantification of products in an alkaline degradation mixture of D-glucose is presented. Carboxylic acid products up to 6 carbon atoms have easily been identified in such complex reaction mixtures. Furthermore, information on the different functional groups present in the oligomeric reaction products has been obtained from the alkaline degradation of 1-$^{13}$C-D-glucose.
In Chapter 9 a comparison is made between GC and HPLC analysis of carboxylic acids, formed by alkaline degradation of invert sugar. GC analyses of the trimethylsilylated carboxylic acids have been performed on a capillary column with temperature programming. HPLC analyses of the carboxylic acids have been carried out on a strong cation exchange resin in the $H^+$ form. Despite the higher resolution of GC, HPLC has proved to be a convenient and a reproducible method for (routine) analyses of carboxylic acids: there is no need for time consuming freeze-drying and derivatization procedures and volatile products like acetic acid and formic acid are quantitatively detected.



## SAMENVATTING

Dit proefschrift handelt over het gedrag van monosacchariden in waterig alkalisch milieu met betrekking tot isomerisatie- en afbraakreacties. Om misverstanden te voorkomen, dient opgemerkt te worden dat wanneer in dit proefschrift gesproken wordt over de alkalische afbraak van monosacchariden eveneens de vorming van oligomere producten bedoeld wordt.
In Hoofdstuk 1 wordt de reden van het onderzoek kort uiteengezet en de indeling van dit proefschrift besproken.
Hoofdstuk 2 geeft een literatuuroverzicht betreffende de reacties van monosacchariden in waterige alkalische oplossingen. Daarbij kunnen twee reactietypen worden onderscheiden: (i) initiële reversibele reacties, zonder aantasting van het suikermolecuul, en (ii) irreversibele afbraakreacties, waarbij zowel C-O en C-C bindingen doorbreken als gevormd worden. Het eerste reactietype omvat ionisatie-, mutarotatie-, enolisatie- en isomerisatiereacties. De mechanismen van deze reacties worden besproken aan de hand van enkele in de literatuur voorgestelde modellen. Het gecompliceerde mechanisme van de afbraakreacties die leiden tot carbonzure producten, daarentegen, is slechts gedeeltelijk opgehelderd. Uit dit literatuuroverzicht kan geconcludeerd worden dat verder onderzoek noodzakelijk is, met name naar de invloed van reactieparameters op het afbraakpatroon en de kinetiek. Gebruik van geavanceerde analysetechnieken als NMR spectroscopie, MS, HPLC en GC is voor een dergelijk onderzoek een vereiste.
Hoofdstuk 3 beschrijft een systematisch onderzoek naar de invloed van enkele reactieparameters op de alkalische afbraak van monosacchariden. De eindsamenstelling van de reactiemengsels werd bepaald met HPLC en, in enkele gevallen, met GC. De $HO^-$- en monosaccharide-concentraties bepalen in belangrijke mate deze eindsamenstelling, zowel wat betreft de onderlinge samenstelling van de $C_1$-$C_6$ zuren als de hoeveelheid gevormde oligomere zuren, de zogenaamde > $C_6$ zuren. Een maximale hoeveelheid van deze > $C_6$ zuren, tot 50 mol-$C_6$-%, wordt gevormd bij een $HO^-$-concentratie tussen $10^{-3}$ M en $10^{-2}$ M als de monosaccharide-concentratie groter is dan $10^{-2}$ M. In aanwezigheid van het

divalente calcium neemt de retro-aldolisatie van monosacchariden toe, geïllustreerd door de toegenomen melkzuurvorming, als gevolg van complexering met bijvoorbeeld D-fructose. Boraat beschermt monosacchariden gedeeltelijk tegen alkalische afbraak door de vorming van boraatesters, terwijl de hoeveelheid saccharinezuren in het afbraakmengsel verdubbelt. Chloride, carbonaat noch de reactietemperatuur, daarentegen, beïnvloeden de eindsamenstelling van het product. Alkalische afbraakexperimenten met methylglyoxal, glyceraldehyd en 1,3-dihydroxyaceton, die beschouwd kunnen worden als reactie-intermediairen, geven aan dat aldolisatie van (di)carbonylverbindingen verantwoordelijk is voor de vorming van flinke hoeveelheden > $C_6$ zuren.

In Hoofdstuk 4 wordt de karakterisering van de oligomere producten besproken. Met behulp van UV, IR, $^{13}C$ NMR spectroscopie en GC-MS analyse werden de herkomst en de structuur van deze producten onderzocht. Scheiding van reactiemengsels in $C_1$-$C_6$ zuren en oligomere producten werd bereikt met behulp van gelfiltratie of selectieve precipitatie met lood(II). De oligomere producten bestaan uit een complex mengsel van zure verbindingen die zijn opgebouwd uit dezelfde structuureenheden, namelijk carbonzuur, β-dicarbonyl, $CH_3$, $CH_2$, $CH_2OH$ en CHOH groepen. Deze > $C_6$ zuren, met gemiddelde molecuulgewichten van ~ 350, ~ 500 en ≥ 700, worden verondersteld te zijn gevormd door aldolisatie van (di)carbonylverbindingen, belangrijke intermediairen tijdens de alkalische afbraak van monosacchariden. Enkele belangrijke (di)carbonylen zijn methylglyoxal, 3-deoxyhexos-2-ulose en de monosacchariden zelf. De structuren van een $C_7$, een $C_8$ en een $C_9$ carbonzuur zijn opgehelderd met behulp van GC-MS analyse. De oligomerisatiereactie is direct gecorreleerd aan de UV absorptie bij 265 nm gedurende de alkalische afbraakreactie.

Hoofdstuk 5 behandelt de kinetiek van de alkalische isomerisatie en afbraak van monosacchariden. Een nieuw kinetisch model wordt gepresenteerd dat de interconversie en afbraak beschrijft van D-glucose, D-fructose, D-mannose en D-psicose. Computersimulaties volgens dit model stemmen overeen met de experimentele gegevens en staan de bepaling toe van alle relevante reactiesnelheidsconstanten. Tevens is gebleken dat, onverschillig of het de alkalische afbraak van D-glucose, D-fructose of D-mannose betreft, ~ 65% van de zure producten gevormd wordt via D-fructose en 20% via D-psicose. De invloed van enkele reactieparameters op de verschillende pseudo eerste orde reactiesnelheidsconstanten uit het kinetische model werd bestudeerd. De enolisatie van monosaccharide blijkt de snelheidsbepalende stap te zijn in zowel de isomerisatie- als de afbraakreacties. De enolisatiesnelheid neemt toe bij hogere



$HO^-$-concentraties of door de toevoeging van calcium(II). Directe retro-aldolisatie van D-fructose en D-psicose vindt vooral plaats als $[HO^-] > 10^{-2}$ M of in de aanwezigheid van calcium(II), hetgeen een extra toename van de afbraaksnelheid (naar melkzuur) veroorzaakt. De verschuiving van het isomerisatie-evenwicht naar D-fructose en D-psicose door boraat gaat gepaard met een afname van de afbraaksnelheid ten gevolge van stabilisatie van de monosacchariden door hun verestering met boraat.

In Hoofdstuk 6 wordt met behulp van de verkregen resultaten een mechanistisch beeld geschetst van de alkalische afbraak van monosacchariden. Het blijkt dat de afbraak van monosacchariden zelf, in het bijzonder van ketoses, en van de daaruit gevormde α-dicarbonylintermediairen gedirigeerd wordt door de reactiecondities. Zo bepaalt de mate van directe retro-aldolisatie van ketoses grotendeels de hoeveelheid melkzuur dat gevormd wordt, terwijl de verhouding benzylzure omlegging/α-dicarbonylsplitsing/aldolisatie van α-dicarbonylen de samenstelling van het $C_1$-$C_6$ zurenpakket evenals de hoeveelheid > $C_6$ zuren beïnvloedt.

Hoofdstuk 7 beschrijft de fructo-formose-reactie, ofwel de alkalische afbraak van D-fructose in aanwezigheid van formaldehyde. De eindproductsamenstellingen van de alkalische (calciumhydroxide) afbraak in aanwezigheid van formaldehyd by verschillende D-fructose/formaldehyd verhoudingen en van de formose-reactie werden bepaald met HPLC en GC analyse. Bij een afnemende D-fructose/formaldehyd verhouding vindt een geleidelijke verandering van het productenpakket plaats, uitgezonderd melkzuur en de oligomere zuren die respectievelijk een minimum en een maximum vertonen. In de aldolisatie van formaldehyd met saccharide en de daaruit gevormde α-dicarbonylintermediairen is een mechanistische verklaring gevonden voor de extra vorming van oligomere producten ten koste van melkzuur. Op basis van experimentele en theoretische gegevens kan geconcludeerd worden dat er geen essentieel verschil is tussen het mechanisme van de formose-reactie en de alkalische afbraak van monosacchariden.

Hoofdstuk 8 beschrijft de analyse van alkalische afbraakproducten van monosacchariden met behulp van $^{13}C$ NMR spectroscopie. De $^{13}C$ chemische verschuivingen van diverse $C_1$-$C_6$ carbonzuren, gevormd door alkalische afbraak van monosacchariden, werden bepaald. Toepassing van deze gegevens in de identificatie en kwantificering van producten in een alkalisch afbraakmengsel van D-glucose wordt besproken. Zure producten met 6 of minder koolstofatomen kunnen op eenvoudige wijze worden geïdentificeerd in zo'n complex reactiemengsel. Verder is belangrijke informatie verkregen over de verschillende

functionele groepen in de oligomere reactieproducten (> $C_6$ zuren) uit de alkalische afbraak van 1-$^{13}$C-D-glucose.

In Hoofdstuk 9 wordt een vergelijking gemaakt tussen GC en HPLC analyse van $C_1$-$C_6$ carbonzuren, gevormd door alkalische afbraak van invertsuiker. GC analyses van getrimethylsilyleerde carbonzuren werden uitgevoerd op een capillaire kolom met temperatuurprogrammering. HPLC analyse van carbonzuren werd uitgevoerd op een sterk zure ionenwisselaar in de $H^+$-vorm. Ondanks de betere resolutie bij GC, blijkt HPLC een geschiktere techniek te zijn voor reproduceerbare (routine) analyses van carbonzuren: het vereist geen vriesdroogen derivatiseringsprocedures en vluchtige producten als azijnzuur en mierezuur worden kwantitatief gedetecteerd.



## CURRICULUM VITAE

Jan Maarten de Bruijn werd op 16 mei 1956 te Rotterdam geboren. Na aldaar de lagere school doorlopen te hebben, genoot hij als eerste mammoeter voorbereidend wetenschappelijk onderwijs aan de Libanon Scholengemeenschap, eveneens te Rotterdam. In 1974 werd het Atheneum B examen met goed gevolg afgelegd.

Ondanks de getoonde behendigheid in het oplossen van wiskundige vraagstukken motiveerden de eerste chemische experimenten hem tot een andere studiekeuze: Scheikundige Technologie aan de Technische Hogeschool te Delft. Na het nemen van alle studiehindernissen bereikte hij op 12 juni 1981 de finish, het ingenieursdiploma.

De tijdens het afstudeerwerk, onder het toeziend oog van prof.dr. A. Fuchs, gewekte belangstelling voor de koolhydraatchemie was aanleiding het verblijf aan de TH Delft met 4 jaar te verlengen. Aldus werd op 1 december 1981 aangevangen met een promotieonderzoek bij de vakgroep Organische Chemie, onder de stimulerende leiding van prof.dr.ir. H. van Bekkum en dr.ir. A.P.G. Kieboom, waarvan het tastbare resultaat thans voor u ligt.

Sinds 1 december 1985 verbonden aan het Centraal Laboratorium van de CSM Suiker B.V. te Breda, kan eindelijk de verworven kennis in praktijk gebracht worden.

STELLINGEN

1. De conclusie van Shukla c.s. dat zeolieten de "hydrolyse" van disacchariden katalyseren, is niet juist.

   *R. Shukla, X.E. Verykios en R. Mutharasan, Carbohydr. Res., 143 (1985) 97-106.*

2. Cortes beweert ten onrechte dat de invloed van de pH van het eluens op de retentietijden van anionen veroorzaakt wordt door de mate van protonering van de amino-groepen van de stationaire fase.

   *H.J. Cortes, J. Chromatogr., 234 (1982) 517-520.*

3. Het feit dat de loogverbruikcijfers bij de door Moulik c.s. bestudeerde alkalische afbraak van monosacchariden betrekking hebben op verschillende conversiegraden, is in de discussie ten onrechte buiten beschouwing gelaten.

   *S.P. Moulik, D. Basu en P.K. Bhattacharya, Carbohydr. Res., 63 (1978) 165-172.*

4. Het verdient aanbeveling om bij het vermelden van gemeten pH waarden de daarbij behorende temperatuur op te geven.

5. De door Niemelä en Sjöström voorgestelde vorming van 2',4-anhydro-3-deoxy-2-C-(hydroxymethyl)pentaarzuur door alkalische afbraak van pectinezuur is onwaarschijnlijk.

   *K. Niemelä en E. Sjöström, Carbohydr. Res., 144 (1985) 87-92.*

6. De mechanistische beschouwing van Matsumoto en Inoue over de vorming van 2-C-(hydroxymethyl)glycerol uit D-fructose en formaldehyd is voor verbetering vatbaar.

   *T. Matsumoto en S. Inoue, J. Chem. Soc. Perkin Trans., I (1982) 1975-1979.*

7. Het lezen van toneelstukken voor een literatuurlijst bestemd voor het eindexamen MAVO, HAVO of VWO is als het lezen van een kookboek om de honger te stillen.

8. De bediening van een apparaat is veelal eenvoudiger dan het begrijpen van de bijbehorende gebruiksaanwijzing.

9. In plaats van de huidige facultatieve mistachterlichten bij personenwagens zouden alle motorvoertuigen voorzien dienen te zijn van achterlichten met voldoende en vergelijkbare lichtopbrengst.

10. Het afwisselend weergeven van tijd en temperatuur langs auto(snel)wegen komt de verkeersveiligheid niet ten goede.

11. Het nut van stellingen zal toenemen als betrokkenen en belanghebbenden geïnformeerd worden over de inhoud ervan.

J.M. de Bruijn

13 mei 1986