4 A comparative study on the reactivity of various ketohexoses to furanics in methanol

Abstract

In this chapter, the acid-catalysed dehydration of the four 2-ketohexoses (fructose, sorbose, tagatose and psicose) to furanics was studied in methanol (65 gL⁻¹ substrate concentration, 17 and 34 mM sulphuric acid, 100 °C) using Avantium high-throughput technology. Significant differences in the reactivity of the hexoses and yields of 5-hydroxymethylfurfural (HMF) and its methyl ether MMF were observed. Psicose and tagatose were shown to be the most reactive, with psicose also giving the highest combined yield of MMF and HMF of around 55% at 96% sugar conversion. Hydroxyacetylfuran and its corresponding methylether were formed as a by-product for particularly sorbose and tagatose, with a maximum combined yield of 8% for sorbose. The formation of hydroxyacetylfuran was studied using ¹³C-NMR with labelled sorbose, providing new insights into the mechanism of its formation.
4.1 Introduction

Diminishing fossil resources and the debate on climate change require the development of sustainable processes for the production of materials and energy. Production of materials and liquid fuels present an especially interesting case, as it is clear that non-fossil sources of fixed carbon are required. Biomass is the obvious alternative to fossil resources for these applications, since it is the largest available source of fixed carbon on earth.\(^1\) Carbohydrates represent 60-80% of all biomass.\(^2\) They occur predominantly in polymeric form (cellulose, hemi-cellulose, starch) and are built up from hexoses (a.o. glucose, fructose, mannose, galactose) and pentoses (a.o. arabinose, xylose). The development of efficient catalytic processes for producing fuels and materials is the most important challenge in the conversion of biomass, especially in the case of carbohydrates. This challenge is mainly caused by the over-functionalised nature of these compounds, which have a high oxygen content. This results in both chemical and physical issues, such as reduced selectivity of potential processes and solvent incompatibilities. For most applications compounds with high carbon and low oxygen content are required, so oxygen has to be removed. The main chemical strategies to do so are dehydration,\(^3\) decarboxylation/decarbonylation\(^4\) and hydrogenation/hydrogenolysis.\(^4\) Decarboxylation and decarbonylations result in significant carbon loss in the form of CO\(_x\). Hydrogenation/hydrogenolysis requires additional hydrogen, which is expensive and is typically produced from non-renewable sources.\(^5\) As such, dehydration is an interesting approach, as it conserves all the carbon atoms in the molecule and the remaining product is characterised by having unsaturated, reactive groups (either olefinic or carbonyl) for further reactions.

The dehydration of hexoses is well known and initially leads to the formation of 5-hydroxymethylfurfural (HMF).\(^3\) This molecule is considered as a high potential platform chemical and it is mentioned in the DoE top-12 biobased chemicals.\(^6\) HMF can be converted into a number of interesting building blocks, such as 2,5-furandicarboxylic acid (FDCA, DoE top-12),\(^3,6\) and levulinic acid (LA, DoE top-12).\(^6-9\) Another interesting derivative is dimethylfuran, with applications as fuel additive, solvent, and as a precursor for green aromatics (p-xylene).\(^10\)

FDCA has been identified as a polymer precursor for polyethylenefuranoate (PEF), which is an example of a high-potential biobased polyethyleneenterptalate (PET) replacement, in which the terephtalic acid moiety is replaced by FDCA. Compared to PET,
PEF production reduces non-renewable energy use and greenhouse emissions significantly. Additionally PEF has superior physical properties compared to PET.

Besides HMF, another well known platform chemical from hexoses is levulinic acid (LA). LA may be converted to both bulk- and fine chemicals. Examples are the multistep conversion to δ-aminolevulinic acid, an active ingredient in a biodegradable herbicide; the oxidation to succinic acid, a DoE top-12 compound applicable as a monomer in polyesters; and reduction to γ-valerolactone, a precursor for methyltetrahydrofuran, which can be used as a solvent and a fuel additive.

Over the last decade, global research activities on the development of technoeconomically viable HMF processes have increased dramatically. The main focus has been on increasing yields via the development of selective catalysts, and the use of solvents other than water. The state of the art has shown that the maximum obtainable yield of HMF is limited by the instability of the molecule under reaction conditions. Under acidic conditions HMF reacts with water to form levulinic and formic acid (Scheme 1). In addition, insoluble black-brown by-products are formed, referred to as humins, that reduce the yield and cause processing issues such as reactor fouling. The conversion of fructose to HMF under aqueous conditions has a maximum yield of around 50%. In ionic liquids and polar aprotic solvents like DMSO yields over 90% have been reported. For glucose, an aldose, the reported yields are generally much lower (<10%), unless specific co-catalysts and advanced solvent systems are used. Avantium is currently developing a process in which hexoses are dehydrated in alcohols. This leads to the formation of HMF ethers as the predominant products (Scheme 1). Dehydration in alcohols has a number of advantages compared to HMF production in water, aprotic polar solvents and ionic liquids. The use of organic solvent increases the attainable yield of furanics by suppressing the formation of levulinic acid. Additionally, the HMF will be mainly converted to its alkyl ether. In the case of lower alcohols these ethers have a lower boiling point than HMF, which simplifies work-up by distillation. It is generally very challenging to separate HMF from aprotic polar solvents or ionic liquids, since these solvents generally have a very high affinity for HMF. Lower alcohols, on the contrary, can be readily removed by evaporation.
Despite the large volume of research on HMF formation from hexoses, the reaction mechanism is still under debate. A better understanding is of pivotal importance for the development of efficient catalytic systems for the production of HMF and derivatives. We have recently shown that aldose dehydrations follow a different reaction pathway than ketose dehydrations. This led to the conclusion that different catalyst systems are required for glucose- and fructose dehydration to HMF. Furthermore we reported that the relative position of the hydroxyl groups in the different ketoses (Scheme 2) resulted in different reactivity for the dehydration to HMF in water. Tagatose was shown to react significantly faster than sorbose and fructose. These results essentially disprove a reaction mechanism with acyclic intermediates, making a reaction mechanism with cyclic intermediates more likely.

Apart from differences in reaction rates for the ketoses, it was also shown that the maximum obtainable HMF yield in water was significantly lower for sorbose than for fructose and tagatose. It is not yet clear whether this is related to the lower rate of HMF formation relative to the rate of HMF decomposition or whether it also indicates the existence of a competing reaction pathway for sorbose that is not as accessible for the other ketoses.
The reactivity of four ketohexoses (fructose, sorbose, tagatose and psicose) in methanol using sulphuric acid as the catalyst was studied in detail in order to gain insights in sugar-furan yield relationships. For this purpose, an experimental study was performed using the Avantium high-throughput platform. In addition, a $^{13}$C-NMR experiment with labelled sorbose was performed to gain insights into the pathways leading to the formation of HAF, a by-product for sorbose.

Scheme 2: The α-furanose structures of the D-ketoses

4.2 Experimental section

4.2.1 Chemicals

D-Fructose, L-sorbose, D-tagatose (>99%), sulphuric acid (96%), 1,4-dioxane (99.8%), CDCl$_3$ (99.8%) and saccharin (98%) were purchased from Sigma-Aldrich, D-psicose and L-[6-$^{13}$C]sorbose (99.9%) were purchased from Carbosynth, 2-hydroxyacetyfuran (HAF, 95%) was purchased from Otava Ltd., acetonitrile (HPLC-S grade) from Biosolve and methanol (HPLC grade) from Fisher. Milli-Q quality water was used for all sample preparations.

4.2.2 High-throughput experimentation

All experiments were performed with a substrate concentration of 65 gL$^{-1}$ (0.36 M) and with two sulphuric acid concentrations (17 and 34 mM) at 1 mL scale in methanol under 20 bar N$_2$ at 100 °C. An Avantium Quick Catalyst Screening system was used. After the appropriate reaction time, the reactor blocks were cooled in an ice bath. After opening the reactors, 4 mL of an external standard solution containing 5.63 gL$^{-1}$ saccharin (UPLC) and 3.75 gL$^{-1}$ 1,4-dioxane (GC) in 1:1 acetonitrile/water was added to each reactor. Upon complete dissolution, the resulting mixtures were transferred to vials for storage at -20 °C. These mixtures were measured on GC and diluted in water (25 times) for UPLC analysis. For GC analysis the mixtures were neutralised with 10-30 µl aqueous NaOH solution (1 M), depending on the acid content of the sample. The level of neutralisation was checked with pH paper.
4.2.3 Synthesis of 2-methoxyacetylfuran

2-Methoxyacetylfuran (MAF) was synthesised by reacting 2-hydroxyacetylfuran (HAF) using the reaction conditions as described for the high-throughput experimentation, except that H$_2$SO$_4$ concentrations of 17, 34 and 67 mM were applied at a fixed reaction time of 60 min. The reaction mixtures were analysed using GC-MS to confirm MAF formation. These mixtures were consequently used to confirm the retention time of MAF on both the UPLC and the GC.

4.2.4 Chromatographic analysis

The analysis of sugars and furanics was performed on a Waters Acquity UPLC (Table 1) equipped with an Acquity UPLC BEH C18 2.1x 5.0 mm, 1.7 µm column. A mobile phase (0.6 mL/min) with acetonitrile/methanol (1:1, A) and water (0.2 wt% TFA, B) was used, with a gradient from 98% A and 2% B up to 2% A and 98% B. The sugars were detected using an ELS detector (ELSD) and HMF was detected on a PDA at 230 nm. Saccharine was used as external standard, measured at 250 nm for UV and in the ELSD for sugar analysis. As the reactions were performed in alcohols, remaining sugars were mostly present in methylated form. These methylated sugars were categorised as unconverted sugars, since they are known to convert to furanics.$^{17}$

Levulinic acid and methyl levulinate were analysed on an Interscience TraceGC with an Agilent J&W FactorFour VF-WAXms, 30 m x 0.25 mm, 0.25 µm column with an FID and 1,4-dioxane as the external standard. The GC as operated at a temperature of 250 °C at a constant flow of 2 mL/min. GC-MS (Thermo Scientific traceGC, DSQ II mass detector) was used to confirm the formation of 2-hydroxyacetylfuran (HAF) and its methyl ether (MAF). Ion source and transfer line temperatures of 250 °C were used.

Calibration of the equipment was done using pure compounds. The UPLC response factor for MAF was estimated using the known response factors for HAF, HMF and MMF. The response factors of the latter two were shown to be related to the molecular weights, with the response factor ratio of the two being equal to their molecular weight ratio. The response factor of MAF was therefore calculated from that of HAF using a correction for the difference in molecular weight.
4.2.5 Experiments with L-[6-\textsuperscript{13}C]sorbose

Three experiments with L-[6-\textsuperscript{13}C]sorbose were performed at 1 mL scale with 65 gL\textsuperscript{-1} substrate and 17 mM H\textsubscript{2}SO\textsubscript{4} in methanol for 150 min at 100 °C and 20 bar N\textsubscript{2}. After reaction the samples were combined and neutralised with NaOH\textsubscript{(aq)} (8.1 mg, 50 wt%, 0.10 mmol) in 1.5 mL methanol. All volatiles were then removed under reduced pressure (5 mbar, 35 °C), leaving a brown coloured residue. 1.0 mL CDCl\textsubscript{3} was added to the residue and the resulting suspension was filtered over a syringe filter (Millex FH 0.45 µm) before measurement.

\textsuperscript{1}H and \textsuperscript{13}C (Inverse Gated Decoupled) NMR spectra were recorded in CDCl\textsubscript{3} on a Bruker Avance 500 (125.78 MHz for \textsuperscript{13}C, 2048 scans, 2.0 s delay time) digital NMR with a Cryo Platform. The residual hydrogens of CDCl\textsubscript{3} (δ 7.22 ppm) were used to reference the spectra.

4.3 Results and discussion

The acid-catalysed dehydration of the ketoses fructose, tagatose, sorbose and psicose to furanics was studied in methanol using a high-throughput batch setup with the objective to compare the reactivity of the four ketohexoses systematically under similar reaction conditions. Duplicates and triplicates of selected experiments were performed for statistical analysis regarding product yields and hexose conversions. The reactions were followed in time at a fixed temperature (100 °C) with two H\textsubscript{2}SO\textsubscript{4} concentrations (17 and 34 mM) at a constant substrate concentration (65 gL\textsuperscript{-1}). Detailed product analysis was performed using UPLC and GC, and the main soluble reaction products were HMF and its corresponding methyl ether (MMF), levulinic acid (LA) and its methyl ester (ML, see Scheme 10). In general insoluble humins were not formed, though the reaction mixtures did show brown colouring, which increased with the severity of the reaction conditions. In addition, for some of the hexoses, significant amounts of hydroxacetylfuran (HAF) and its corresponding ether (MAF) were formed. Furfural, which has been observed for the Brønsted acid catalysed conversions of hexoses to HMF and LA,\textsuperscript{3} was observed in very low quantities and yields on hexoses were always below 0.5%. In the following, the concentration versus time profiles and yield-selectivity data will be provided and discussed in detail for the four ketohexoses used in this study.
4.3.1 High-throughput experimentation

Figure 1 shows the conversion versus time profiles for experiments with all four ketohexoses. The spread in the duplicates and triplicates does not exceed 3%, showing that the experiments are highly reproducible. When comparing the conversion-time trends for the different hexoses, it is clear that tagatose and psicose show very similar reactivity. The trends for fructose and sorbose show a similar pattern, though both are less reactive than tagatose and psicose. A similar trend was observed previously in water, though a full comparison is challenging as psicose was not tested in this study. ¹⁸

![Figure 1](image.png)

**Figure 1:** The conversion vs. reaction time for fructose (▲), sorbose (★), tagatose (■) and psicose (★) at 65 gL⁻¹ substrate in methanol at 100 °C with (a) 17 mM H₂SO₄ and (b) 34 mM H₂SO₄.

The yields of HMF in time are shown in Figure 2. Again the reproducibility was shown to be very high. The HMF yield shows a clear optimum in all cases; for both acid concentrations, the HMF yield is the highest for psicose, followed by tagatose, fructose and sorbose, respectively. Contrary to sugar dehydration in water,¹⁸ the HMF yield decreases in time at low conversions. This is mainly caused by the acid-catalysed methylation of HMF to methoxymethylfurural (MMF)¹⁹ and at this early stage of the reaction not to the subsequent conversion to LA and ML. The MMF yield (Figure 3) is also consistently the highest for
psicose, followed by tagatose and fructose with approximately equal values, and sorbose. The highest MMF yield was around 55%, obtained from psicose. For fructose and tagatose the maximum MMF yield was around 43% and for sorbose this was only around 25%.

Figure 2: The yield of HMF vs. reaction time for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 gL\(^{-1}\) substrate in methanol at 100 °C with (a) 17 mM H\(_2\)SO\(_4\) and (b) 34 mM H\(_2\)SO\(_4\)
Figure 3: The yield of MMF vs. reaction time for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 gL⁻¹ substrate in methanol at 100 °C with (a) 17 mM H₂SO₄ and (b) 34 mM H₂SO₄.

The combined furan yield (HMF and MMF, abbreviated as RMF) is of particular importance for this study and the results are given in Figure 4. The RMF yield versus time data show an optimum when working at the highest acid concentration and reach up to 55% for psicose at around 80 min reaction time. The subsequent decrease in yield is due to the successive reaction to LA and ML (vide infra).

When the sugar conversion (Figure 1) and RMF yield versus time profiles (Figure 4) are compared, it is clear that psicose and tagatose have comparable conversion rates, but psicose forms more RMF. This implies that psicose is converted to RMF with a higher selectivity than tagatose. A comparable trend is observed for fructose and sorbose, where fructose converts more selectively to RMF.

Additional information is obtained by plotting the yields of RMF for all hexoses versus the conversion for both acid concentrations (Figure 5). A clear trend is visible for each hexose, irrespective of the acid concentration. This is an important observation, as it reveals that the acid concentration does not have a measurable influence on the selectivity to RMF. When comparing the results for the four hexoses, it is clear that the selectivity to RMF is
consistently higher for fructose and psicose than for sorbose and tagatose. Due to their higher reactivity, the amount of data points below 70% conversion for tagatose and psicose is limited. As such, it is difficult to distinguish between psicose and fructose or between sorbose and tagatose at lower conversions. The data available for psicose below 80% conversion appear to follow the same trend as the fructose data, which suggests comparable selectivity for fructose and psicose in this range. The tagatose data at conversions below 65% appear to fit with the trend of the sorbose data, which points to comparable selectivity at low conversions for tagatose and sorbose. At higher conversions, though, a clear separation is visible in both cases. At conversions over 80%, the RMF yield from psicose is consistently higher than for fructose. This results in a maximum RMF yield around 55% from psicose, whereas the maximum yield from fructose is around 45%. These results clearly show that from a yield and selectivity point of view, psicose is the favoured substrate for the formation of HMF and MMF. Sorbose is clearly the worst substrate, with a maximum yield that is only about half of the yield obtained from psicose. The differences between fructose and tagatose are smaller, though significant, as fructose converts slower but more selectively to HMF and MMF than tagatose.

The observed differences in selectivity between the four ketoses could be caused by the differences in the rate of RMF formation and competing side-reactions. A known side-reaction for the dehydration of sugars is the formation of 2-hydroxyacetylfuran (HAF, Scheme 1).\textsuperscript{20-23} As such, attempts were made to identify and quantify HAF in the product samples. Improved analytics showed an additional peak in the UPLC chromatogram next to HMF with a similar UV spectrum. Spiking experiments with pure HAF on the UPLC and GC-MS confirmed the presence of HAF. Besides HAF, its methyl ether (2-methoxacetylfuran, MAF), was also observed on the UPLC. Etherification experiments with pure HAF, in combination with GC-MS analysis, confirmed MAF was formed under the reaction conditions. Subsequent spiking experiments on the UPLC provided the retention time of MAF. The formation of MAF had not been previously reported in sugar dehydration reactions, though it is a known compound.\textsuperscript{24}
Figure 4: The yield of RMF vs. reaction time for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 gL⁻¹ substrate in methanol at 100 °C with (a) 17 mM H₂SO₄ and (b) 34 mM H₂SO₄.

Both HAF and MAF were only formed in significant amounts from sorbose and tagatose (Figure 6). The yields of both are consistently higher for sorbose than for tagatose. HAF is by far the major component, thus the methylation of HAF to MAF appears relatively slow on the timescale of the experiments. The sum of the HAF and MAF yields is at maximum around 8% for sorbose at 150 min with 17 mM H₂SO₄ (Figure 6a) or at 75 min with 34 mM H₂SO₄ (Figure 6b).
Figure 5: The yield of RMF vs. sugar conversion for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 g L⁻¹ substrate in methanol at 100 °C for all reaction times with 17 or 34 mM H₂SO₄.

In Figure 7 the combined yield of all identified furanics (HMF, MMF, HAF and MAF) vs. the conversion is shown for the four ketohexoses. The total furanics yield is consistently higher for psicose and fructose than for tagatose and sorbose. Especially at conversions over 85% large differences in furanics yield are observed, with psicose yielding around 55% furanics and sorbose yielding only around 32% furanics at best. This means that the formation of HAF and MAF in the case of tagatose and especially sorbose does not compensate entirely for the lower RMF yields observed in Figure 5.
Figure 6: The yield of HAF (grey) and MAF (black) vs. reaction time for sorbose (●) and tagatose (■) at 65 gL\(^{-1}\) substrate in methanol at 100 °C with (a) 17 mM H\(_2\)SO\(_4\) and (b) 34 mM H\(_2\)SO\(_4\).

Figure 7: The combined yield of HMF, MMF, HAF and MAF vs. sugar conversion for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 gL\(^{-1}\) substrate in methanol at 100 °C for all reaction times with 17 or 34 mM H\(_2\)SO\(_4\).
In sugar dehydration to furanics, the yield is known to be limited by two important side-reactions: the hydration of HMF to levulinic acid (LA) and the formation of humins. Brown solid materials were not present after reaction, so any humins formed should be soluble, which hampers quantification. As the reactions were performed in methanol under acidic conditions, LA was mainly present as its methyl ester (ML). In Figure 8 the yield of ML versus time is shown. The ML yield is a clear function of the time and, as expected, is formed particularly at longer reaction times (Figure 8). The yields of LA were typically below 1%, showing that almost all LA is esterified to ML under the prevailing reaction conditions. In Figure 9 the yield of ML is plotted against the RMF yield, which shows clearly different trends for the different ketoses. At the same RMF yield, psicose shows clearly the lowest yield of methyl levulinate (ML), followed by fructose and tagatose with comparable values, and sorbose with by far the highest yield in ML relative to the RMF yield. This relates the maximum obtainable RMF yield to the formation of levulnates. From Figure 4 it was observed that the RMF formation rate is the highest for psicose, followed by fructose and tagatose with approximately equal values, and sorbose with clearly the lowest formation rate of RMF. When this information is combined with the observations from Figure 9, it shows that a high rate of RMF formation is favoured for high RMF yields. This can be explained by a simplified reaction pathway, considering a two-step consecutive reaction (Scheme 3) involving the dehydration of the sugar to MMF (step 1) and the formation of ML (step 2). The experimental data reveal that $k_1$ is a function of the sugar substrate. Based on this scheme, $k_2$ should not be a function of the sugar substrate. As such, a higher reactivity of the sugar (higher $k_1$) is expected to lead to higher RMF yields, which was also shown experimentally in this research.

**Scheme 3:** Simplified reaction network for C6 sugar conversion to MMF and ML
Figure 8: The yield of methyl levulinate vs. time for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 gL\(^{-1}\) substrate in methanol at 100 °C with (a) 17 mM H\(_2\)SO\(_4\) and (b) 34 mM H\(_2\)SO\(_4\).
Figure 9: The yield of methyl levulinate vs. the combined yield of HMF and MMF for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 gL⁻¹ substrate in methanol at 100 °C for all reaction times with 17 or 34 mM H₂SO₄

The sum of all measured products (HMF, MMF, HAF, MAF, furfural, ML and LA) is plotted against the hexose conversion in Figure 10. In this figure the maximum attainable yield line is given (100% mass balance). Interestingly, below 80% conversion the trend for the sum of measured products vs. conversion for each sugar is approximately parallel with the 100% mass balance line. However, the difference between the measured yields and the 100% mass balance closure line is significant, meaning that other, unidentified, products are formed as well. For instance, the fructose trend is consistently 20% below the 100% mass balance line. However, at higher conversions the difference between the actual total identified yield line and the 100% mass balance closure line is much lower. This is an important observation as it suggests the presence of unidentified intermediates in the early stage of the reaction that in a later stage are converted to RMF and ML, rather than to humin by-products. A possible explanation is the formation of sugar dimers or oligomers in the initial stage of the reaction when the sugar concentration is relatively high. It is possible that these compounds are not detectable by UPLC or that they overlap with the known sugars, but have different
response factors, thereby underestimating the total amount of sugars present. Fructose is known to form dimers, so-called difructose dianhydrides which makes it likely that the other ketoses are also prone to dimerisation.\textsuperscript{25} As the reactions are performed in methanol, the initial water concentration is very low, favouring dimerisation reactions. These oligomers are known to be in equilibrium with the monomeric sugars and as such the equilibrium will shift to the monomers at higher monomer conversions and accompanying water formation from the dehydration, in line with the experimental data. It also suggest that humin formation, a major by-product in water, is suppressed considerably in methanol and that the amounts of humins are at maximum 20-30 \% at high sugar conversion (assuming that all unknowns are humins and not unidentified methanol solubles). For example for psicose, the most reactive hexose with the highest attainable RMF yield, the amount of unidentified products is about 10-15\% at psicose conversions higher than 90\%, indicating that humin formation is less than 10\%.

Additionally, the results in Figure 10 show that the trend in the total product yield for psicose and fructose is comparable. For psicose and fructose the combined yields of furanics and levulinates follow approximately the same trend vs. the conversion. This shows that the lower furanics yield at higher conversion from fructose (Figure 7) is completely compensated by the additional amount of ML formed. As levulinates are generally considered to be formed from HMF/MMF species, they should be considered as a product in the RMF reaction pathway.\textsuperscript{3} The fact that only the ratio of RMF and ML differs, and not their combined yield, indicates that the selectivity of psicose and fructose towards the RMF pathway is approximately identical. Therefore the conversion rate of these two sugars to RMF has no measurable influence on the selectivity towards the RMF pathway (Scheme 1) relative to other available sugar conversion routes, such as humin formation.

The total product yields for tagatose and sorbose follow a similar trend, though the total yield of identified products is consistently lower than for psicose and fructose. Both tagatose and sorbose form significant amounts of HAF and MAF, whereas for psicose and fructose only traces of HAF/MAF were observed. This indicates the existence of a parallel reaction pathway to HAF/MAF for tagatose and sorbose. As such, the lower amount of total identified products for tagatose and sorbose compared to tagatose and fructose could be (partly) due to additional side-reactions in the HAF formation pathway.
A comparative study on the reactivity of various ketohexoses to furanics in methanol

Figure 10: The sum of the yields of HMF, MMF, HAF, MAF, furfural, LA and ML vs. the sugar conversion for fructose (▲), sorbose (●), tagatose (■) and psicose (★) at 65 gL⁻¹ substrate in methanol at 100 °C for all reaction times with 17 or 34 mM H₂SO₄. The dashed line represents 100% mass balance.

It is of interest to compare the results from the current study to those published recently by our group for the dehydration of three of the four ketoses in water catalysed by sulphuric acid.¹⁸ The reactivity of the ketoses is clearly higher in methanol than in water. In methanol significantly less severe conditions were applied (100 °C, 17, 34 mM H₂SO₄) to obtain comparable conversion levels to those in the previous study in water (120°C, 100 mM H₂SO₄). In water, the experiments were carried out with tagatose, fructose and sorbose. Tagatose was by far more reactive than fructose and sorbose, which is in line with the data reported here in methanol. Thus the reactivity patterns for the three ketohexoses in methanol are in line with the results obtained earlier in water. The study in water was combined with DFT calculations relating the structure of the ketoses to their reactivity. The DFT calculations were in line with the experimental reactivity data for tagatose, fructose and sorbose, and predicted psicose to be the least reactive ketose. These conclusions were based on the differences in calculated stability of proposed intermediate carbocations. The current experimental study in methanol shows that psicose is not the least reactive but as reactive as
Tagatose. Thus, additional experiments in water with psicose will be reported to experimentally verify the DFT calculations.

An important difference between the reactions in water and methanol can be found in the rate of RMF formation from fructose relative to tagatose. In water, the HMF yields at the same reaction time were higher for tagatose than for fructose whereas in methanol the values for RMF yield in time are comparable. This could be caused by the difference of the orientation of the hydroxyl group on C4, which is the key difference (Scheme 2). The formation of HMF is slower in water than in methanol.\(^{18}\) This suggests that the interaction of the sugar with water has a stronger negative effect on the HMF formation rate if the hydroxyl group on C4 is pointed down than when it is pointed up in Scheme 2. The same relative drop in rate would be expected for psicose relative to tagatose in the case of water. Water has a higher capacity to form hydrogen bonds than methanol and is therefore more likely to disrupt intramolecular hydrogen bonds within the sugars. This makes it plausible that for the formation of HMF an intramolecular hydrogen bond with the C4 hydroxyl group is of importance.

### 4.3.2 Mechanistic considerations and \(^{13}\)C labelling experiments with sorbose

The observed differences in reactivity of the four ketoses to furanics in methanol are remarkable. The ketoses differ only in the orientation of the hydroxyl group orientations on C3 and C4 (Scheme 2). When the hydroxyl groups are cis-oriented in the Fischer projection, and therefore also in the furanose structures, the conversion rate is higher than when these hydroxyl groups are trans-oriented. This implies that the relative orientation of the hydroxyl groups on C3 and C4 is a key factor with regard to the conversion rate of the sugars and indicates that it plays a major role in the rate-determining step of the reaction sequence. This strongly suggests that the C3-C4 lacks free rotation in the rate-determining step. As such, reaction mechanisms with cyclic intermediates (Scheme 4) are more likely than reaction mechanisms with acyclic intermediates, as the relative orientation of the latter is of less importance due to free rotation of the carbon-carbon bonds. This cyclic mechanism was also considered the most appropriate to explain the differences in the reactivity of ketohexoses and aldohexoses in water with sulphuric acid as the catalyst.\(^{18}\)
The formation of HAF and MAF was only observed in significant amounts for tagatose and sorbose, both having the same orientation of the hydroxyl group at C4 (Scheme 2). This suggests that the hydroxyl group orientation at C4 plays a major role in the formation of HAF and MAF.

**Scheme 4**: The dehydration of fructose through cyclic intermediates as proposed in literature.\(^3\)

An experiment with L-[6-\(^{13}\)C]sorbose was performed to gain insights in the reaction pathways from sorbose to HAF and HMF/MMF. Sorbose was selected as the amount of HAF/MAF is the highest for all ketohexoses tested. The experiments were performed with 17 mM H\(_2\)SO\(_4\) for 150 min at 100 °C, as these conditions gave the highest HAF yields (7%, MAF 2% and MMF around 25%), which was favourable for the analysis by NMR. A \(^{13}\)C-NMR measurement of the reaction mixture after work-up is shown in Figure 11. The largest peak, at δ 66.3 ppm, is attributed to MMF, the major product of hexose dehydration in methanol under these conditions. As expected from the proposed dehydration mechanisms in literature,\(^3\) the label at C6 in sorbose ends up in the CH\(_2\) unit of the methoxymethyl group of MMF. The significant peak at δ 29.8 ppm corresponds to the methyl group next to the carbonyl moiety of LA. Thus, the carbonyl group of HMF/MMF is converted into formic acid. Both observations are in agreement with NMR studies by Zhang and Weitz for fructose in water and DMSO and suggests that the formation of HMF from sorbose in methanol and fructose in water follows a similar reaction pathway.\(^26\) The smaller peak at δ 66.6 ppm is assigned to the hydroxymethyl group of HAF (see also Figure 12a), which shows that the label at C6 on sorbose ends up in the hydroxymethyl group of HAF. Besides HAF, MAF is also formed in the reaction. The C6 peak of a representative sample of MAF is present at δ 76 ppm (see Figure 12b). Indeed, it is also clearly present in the NMR spectrum of the reaction mixture and indicates that the label is also present at the etherified hydroxymethyl group of MAF (Figure 11).
At around $\delta$ 147 ppm HAF resonances are expected in case the labelling is present in the furan ring. Here no significant peak is observed, only a cluster of small peaks is present, excluding the possibility that these originate from HAF. It is more likely that the peaks at around $\delta$ 147 ppm originate from minor furan-like products, like furfural,$^{27}$ furan dimers and trimers, and/or soluble humin by-products.$^{28}$

The group of very small peaks in the $\delta$ 60-64 ppm area are expected to arise from sorbose and methyl sorbosides.$^{29,30}$

**Figure 11:** $^{13}$C-NMR of the reaction mixture of the acid catalysed dehydration of L-[6-$^{13}$C]sorbose in methanol
Figure 12: $^{13}$C-NMR spectrum of (a) pure 2-hydroxyacetylfuran (HAF) and (b) 2-methoxyacetylfuran (MAF) in CDCl₃

The interpretation of the NMR results regarding HAF formation pathways is not straightforward. The reaction mechanism for HAF formation proposed in the literature involves an isomerisation to a 2,3-enediol, which would have placed the $^{13}$C label in the furan ring (Scheme 5).²¹,³¹

Scheme 5: Reaction mechanism for HAF formation from fructose with acyclic intermediates as proposed in literature²¹

The labelling experiments presented here do not agree with this mechanism, as the label ends up in the CH₂OR side chain. Based on the observation that the orientation of the hydroxyl group on C4 of the ketose plays a key role in the selectivity towards HAF formation, it is hypothesised that the pathway to HAF proceeds through a 1,4-anhydroketose (Scheme 6).
**Scheme 6:** Proposed formation of HAF with the involvement of a 1,4-anhydrosorbose

The hydroxyl groups on C1 and C4 can be oriented in such a way that they are in close proximity to allow the formation of a second five-membered ring through etherification, particularly in the pyranose (six-membered ring) form, but also in the α-furanose form. The hemiacetal bond in the pyranose ring could then potentially open up due to the ring strain to form 1,4-anhydrosorbose. This would place the labelled carbon (C6) in the proper position to end up in the CH$_2$OR side chain of HAF. A mechanism that explains the conversion of 1,4-anhydrosorbose to HAF is not straightforward. In Scheme 7 four potential pathways are described in order to form HAF from sorbose: two through monocyclic and one through bicyclic intermediates.
Both proposed pathways through monocyclic 1,4-anhydrofuranose require the migration of double bonds through the ring to C5 in order to form a carbonyl at the proper position. The main difference is that in one an enediol between C2 and C3 is proposed, whereas in the other an enol is formed between C1 and C2. The first pathway would require migration of the double bond, followed by dehydration at C2. The second pathway would lead to an initial dehydration at C3. The challenge in both cases is the required migration of a double bond to C5, followed by an additional dehydration on the ring to form HAF, since both intermediates are already conjugated hydroxyfuran rings.

Scheme 7: Possible mechanisms to form HAF through a bicyclic anhydrofuranose
The alternative bicyclic pathways suggest dehydration at the C2 position upon formation of the 1,4-anhydrosorbose to form a bicyclic carbocation. This step is comparable to the initial dehydration suggested in the conversion of fructose to HMF (Scheme 4). Steric constraints make it less likely that a carbocation is formed at the expected C2 position, leading to a hydride shift from C1 to C2 to form a carbocation on C1. Even if a carbocation would be formed at C2, then a hydride shift from C1 to C2 is required in order to break the C2-O bond in the following step to form a C1-C2 double bond. For the furanose ring this is then followed by deprotonation on C5, opening the five-membered ring and forming a ketone on C5 and a double bond between C1 and C2. A regular β-dehydration on C3 then leads to HAF. For the pyranose form the steps are comparable, but an additional isomerisation of the C6 aldehyde to the C5 ketone is required to form HAF. The isomerisation of the aldehyde to the ketone should take place through an enediol and should be favoured because of the formation of a conjugated furfural system.

4.4 Conclusions

In this research, the Brønsted acid catalysed conversion of four different 2-ketohexoses (fructose, sorbose, tagatose and psicose) was investigated in methanol with the objective to identify the most suitable precursor for HMF/MMF formation, and to establish hexose structure-yield relations. Significant differences in the reactivity and selectivity were observed. Psicose and tagatose were the most reactive and psicose gave the highest selectivity towards HMF and MMF (appr. 55% combined yield at around 96% conversion). As such, psicose would be the ideal monosaccharide for the production of HMF and its derivatives. However, psicose is a rare sugar, present in small amounts in wheat, _Itea_ plants, processed sugar cane and beet molasses.\footnote{32-35} For a techno-economically viable process, either a psicose producing organism or an isomerisation process to obtain psicose from readily accessible sugars would need to be developed.\footnote{36}

The significant differences in reactivity between the four ketohexoses were related to their molecular structure. The hexoses differ only in the orientation of the vicinal hydroxyl groups on C3 and C4, which showed that these structural differences are essential in the dehydration to both HMF/MMF and HAF. It was shown that the reactivity of the sugar is determined by the relative orientation of these hydroxyl groups, with _cis_-orientation leading
to faster conversion than *trans*-orientation. This is best explained by a dehydration mechanism with cyclic intermediates.

The formation of HAF was shown to depend on the orientation of the hydroxyl group on C4 of the ketohexoses, which consequently also affected the selectivity for the HMF/MMF formation. The reaction mechanism of HAF formation was proposed to involve a 1,4-anhydroketose as an intermediate. This would have all the carbons in the right position to form HAF, as evidenced by performing dehydration experiments with $^{13}$C-labelled sorbose.
4.5 References