NEW ASPECTS OF THE FORMATION OF 3(2H)-FURANONES THROUGH THE MAILLARD REACTION

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1 INTRODUCTION

4-Hydroxy-2,5-dimethyl-3(2H)-furanone (1, furaneol, registered trademark of Firmenich) and 2-(or 5)-ethyl-4-hydroxy-5-(or 2)-methyl-3(2H)-furanone (2, homofuraneol) are potent flavour compounds contributing to the sensory properties of many natural products and thermally processed foods. They give a caramel-like, sweet flavour and have low retronasal odour thresholds, viz 160 and 20 µg kg⁻¹ water, respectively. Homofuraneol exists in the tautomeric forms 2a and 2b in a ratio of 1:3 to 1:2. However, only 2a is odour-active.

Furaneol can be formed by thermal degradation of rhamnose, fructose and hexose-phosphates. In contrast, the formation of homofuraneol during heat-processing is not well understood. In general, the formation of furanones is thought to occur via the 2,3-enolisation leading to 1-deoxypentosones as intermediates. Analogously to the decomposition of hexoses, 4-hydroxy-5-methyl-3(2H)-furanone (3, norturaneol) is formed from pentoses. For this type of reaction, the carbon skeleton of the sugar determines the furanone formed, i.e. furaneol is produced from hexoses and norturaneol from pentoses.

Recently, we described the formation of furaneol and homofuraneol from pentose sugars in Maillard systems containing glycine and alanine. The key step of this reaction is chain elongation of the 1-deoxypentosone by the corresponding Strecker aldehydes (C₅ + C₁ and C₅ + C₂ reaction). In this paper, we support this mechanism with additional data and suggest sugar fragmentation as an alternative formation pathway of 3(2H)-furanones 1 and 2 from pentoses.

2 EXPERIMENTAL

D-Xylose, glycine and L-alanine (> 99%) were obtained from Fluka and [1-¹³C]-D-Xylose (1-Xyl), [2-¹³C]-glycine (2-Gly) and [3-¹³C]-L-alanine (3-Ala) from Cambridge Isotope Laboratories. The isotopic content of the labelled compounds was 99%. The reference
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compounds furaneol and homofuraneol were obtained from Aldrich and Givaudan-Roure, respectively.

Samples were prepared as described recently. Xylose (5 mmol) and an amino acid (5 mmol, glycine or alanine) were dissolved in phosphate buffer (Na$_2$HPO$_4$, 5 ml, 0.2 mol l$^{-1}$, pH 7.0) and heated at 90 °C for one hour. Water (100 ml) was added to the reaction mixture, saturated with NaCl (40 g) and the pH adjusted to 4.0 (aqueous HCl, 2 mol l$^{-1}$). Neutral compounds were continuously extracted with Et$_2$O (50 ml) overnight using a rotation perforator (Normag). The organic phase was dried over Na$_2$SO$_4$ at 4 °C and concentrated to 0.5 ml.

GC-MS-MS was carried out as described recently: MS Finnigan TSQ-700, GC HP-5890, autosampler HP-7673; ion source (150 °C); electron impact mode (70 eV); collision-induced dissociation (CID) of the molecular ions; collision energy: 10 eV; collision gas: argon (1.1 mTorr). Daughter spectra were recorded (20–200 Da). Chromatographic conditions were: carrier gas He (10 psi); cold ‘on-column’ injector; DB-FFAP fused silica capillary (30 m × 0.32 mm, film thickness 0.25 µm); temperature programme: 60 °C (2 min), 10 °C min$^{-1}$ to 200 °C, 30 °C min$^{-1}$ to 240 °C (10 min).

3 RESULTS AND DISCUSSION

The formation of furaneol and homofuraneol from pentose sugars has recently been described in phosphate-buffered (pH 6) Maillard reaction systems containing glycine and alanine. We report here the formation of 3(2H)-furanones at pH 7 using [1-$^{13}$C]-D-xylose (1-Xyl$^*$), [2-$^{13}$C]-glycine (2-Gly$^*$), and [3-$^{13}$C]-L-alanine (3-Ala$^*$) as precursors.

Table 1 *Relative Amounts (in %) of Norfuraneol, Furaneol and Homofuraneol Detected in Maillard Model Reactions Based on D-Xylose (Xyl), Glycine (Gly) and L-Alanine (Ala)*

<table>
<thead>
<tr>
<th>No.</th>
<th>Maillard System</th>
<th>Norfuraneoltot (%)</th>
<th>Furaneoltot (%)</th>
<th>Homofuraneoltot (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xyl</td>
<td>93</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Xyl-Gly</td>
<td>99.8</td>
<td>0.2</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Xyl-Ala</td>
<td>99.3</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>Xyl-2-Gly$^*$</td>
<td>99.8</td>
<td>0.2</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Xyl-3-Ala$^*$</td>
<td>99.4</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>1-Xyl-Gly$^*$</td>
<td>99.7</td>
<td>0.3</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>1-Xyl-Ala$^*$</td>
<td>99.3</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>1-Xyl-2-Gly$^*$</td>
<td>99.7</td>
<td>0.3</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>1-Xyl-3-Ala$^*$</td>
<td>99.2</td>
<td>0.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^*$ Reaction conditions: phosphate buffer (pH 7, 0.2 mol l$^{-1}$), 90 °C, 1 hour;

$^b$ Compound marked with + was positively identified (relative amount less than 0.1%); Indicates labelling with $^{13}$C.

3.1 Relative Amounts of 3(2H)-Furanones Formed

Norfuraneol was the major furanone compound in all samples analysed (Table 1), particularly in the presence of amino acids (systems 2–9), i.e. more than 99% of the total 3(2H)-furanones formed. In system 1, where only xylose was reacted, the total amount of
3(2H)-furanones was more than 100 times lower, thus indicating that the 1-deoxyosone of xylose was preferentially generated via the Amadori compound (Figure 1).

As shown in Table 1, both furaneol and homofuraneol were detected in all samples. However, the amount of these furanones corresponds to less than 1% of the total 3(2H)-furanones formed. Homofuraneol was preferentially generated in the presence of alanine (systems 3, 5, 7 and 9) compared to the reaction systems containing glycine. Conversely, the formation of furaneol was less dependent on the amino acid used. These data suggest different formation mechanisms of furaneol and homofuraneol from pentoses.

Even though the amount of norfuraneol was much higher than that of furaneol and homofuraneol, the last two dominated from the sensory point of view. This is most likely due to the high retronasal odour threshold of norfuraneol (8300 μg kg⁻¹ water).

![Chemical structure](image1)

### Figure 1
Schematic formation of 3(2H)-furanones detected in Maillard reaction systems based on D-xylose and glycine or L-alanine, i.e. norfuraneol ($X = H$), furaneol ($X = CH_3$) and homofuranone ($X = C_2H_5$). Step a represents the early stage of the Maillard reaction including Amadori rearrangement; b the degradation of the Amadori compounds ($R = H$ from glycine and $R = CH_3$ from alanine) via 2,3-enolisation; c cyclisation, giving rise to norfuraneol, or Strecker-assisted chain elongation ($C_2 + C_1$ and $C_2 + C_2$ reaction) and recombination of sugar fragmentation products yielding furaneol and homofuraneol.

### 3.2 Formation of Labelled 3(2H)-Furanones

Formation and distribution of 3(2H)-furanones was investigated by GC-MS-MS in Maillard reaction systems containing the labelled precursors 1-Xyl, 2-Gly and 3-Ala. Data were obtained by selecting molecular ions of unlabelled and labelled norfuraneol ($m/z$ 114–117), furaneol ($m/z$ 128–131) and homofuraneol ($m/z$ 142–145) followed by monitoring the daughter ion spectra after CID.

### 3.2.1 Reaction of Xylose with Labelled Amino Acids (Table 2)
Systems 4 and 5 resulted in unlabelled and singly labelled furaneol (Fur⁺) and homofuraneol (Hom⁺), respectively. Furaneol detected in the Maillard system Xyl-3-Ala was unlabelled. These results indicate that furanones 1 and 2 are not exclusively formed by the reactions $C_5 + C_1$ and $C_5 + C_2$, as recently described, but also via recombination of sugar degradation products. This was particularly characteristic for furaneol of which about 55% was
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generated by sugar fragmentation. Norfuraneol was unlabelled, showing that Strecker aldehydes are not incorporated into the molecule.

Table 2  Relative Amounts (in %)\(^a\) of Norfuraneol (Nor), Furaneol (Fur) and Homofuraneol (Hom) formed in Maillard Model Reactions Based on Xylose (Xyl), Glycine (Gly) and Alanine (Ala)\(^b\)

<table>
<thead>
<tr>
<th>No.</th>
<th>Maillard System</th>
<th>Nor</th>
<th>Nor(^*)</th>
<th>Fur</th>
<th>Fur(^*)</th>
<th>Fur(^**)</th>
<th>Hom</th>
<th>Hom(^*)</th>
<th>Hom(^**)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Xyl(^\prime)–Gly(^\prime)</td>
<td>100</td>
<td>55</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Xyl(^\prime)–Gly(^\prime)</td>
<td>100</td>
<td>10</td>
<td>65</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1-Xyl(^\prime)–Gly</td>
<td>100</td>
<td>10</td>
<td>55</td>
<td>35</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1-Xyl(^\prime)–Ala</td>
<td>100</td>
<td>10</td>
<td>55</td>
<td>35</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1-Xyl(^\prime)–2-Gly(^\prime)</td>
<td>100</td>
<td>5</td>
<td>45</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1-Xyl(^\prime)–3-Ala(^\prime)</td>
<td>100</td>
<td>5</td>
<td>60</td>
<td>35</td>
<td>15</td>
<td>10</td>
<td>60(^c)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Relative amounts of less than 1% of a compound are not reported in the table;

\(^b\) Reaction conditions: phosphate buffer (pH 7, 0.2 mol I\(^-\)), 90 °C, 1 hour;

\(^c\) About 15% of the triply labelled homofuraneol was found in 1-Xyl\(^\prime\)–3-Ala\(^\prime\);

\(^*\) Labelling with one \(^13\)C atom;

\(^**\) Labelling with two \(^13\)C atoms.

3.2.2 Reaction of Labelled Xylose with Unlabelled Amino Acids (Table 2). In samples 6 and 7, norfuraneol was singly labelled. The mass spectrum with m/z 115 (M\(^+\)) and m/z 44 ([\(^13\)CH\(_3\)CO]) indicates that the methyl group is derived from C-1 of xylose.

The presence of unlabelled, singly and doubly labelled furaneol in both samples confirms its formation via a C\(_5\)\(^+\) + C\(_1\) reaction and sugar fragmentation. The mass spectrum of 4-hydroxy-2-methyl-5-\([\(^13\)C\]methyl-3(2H)-furanone, formed as the major furaneol compound in the model reaction 1-Xyl\(^\prime\)–Gly, is shown in Figure 2A. The fragmentation pattern is almost identical with that of the 2-\([\(^13\)CH\(_3\)]\) isotopomer detected in Xyl\(^\prime\)–2-Gly\(^\prime\) (system 4). Most probably, this is due to the symmetry of the molecule.

Homofuraneol was mainly produced by a C\(_5\)\(^+\) + C\(_2\) reaction in the Maillard system 1-Xyl\(^\prime\)–Ala. The fragment m/z 44 in the mass spectrum of the singly labelled tautomer 2b suggests the presence of a \([\(^13\)CH\(_3\)CO] \) substructure (Figure 2B), i.e. 2-ethyl-4-hydroxy-5-\([\(^13\)C\]methyl-3(2H)-furanone. This is in good agreement with the recently described formation mechanism considering 1-Xyl\(^\prime\) as sugar precursor (see also Figure 3).

3.2.3 Reaction of Labelled Xylose with Labelled Amino Acids (Table 2). Similar to samples 6 and 7, norfuraneol was singly labelled (Nor\(^*\)), i.e. 4-hydroxy-5-\([\(^13\)C\]methyl-3(2H)-furanone (samples 8 and 9). It can be concluded that norfuraneol is directly formed from pentoses, without recombination of sugar fragmentation products.

Furaneol was generated by a C\(_5\)\(^+\) + C\(_1\) reaction (Figure 3), resulting in doubly labelled Fur\(^**\)\(^\prime\) and by sugar fragmentation forming singly labelled Fur\(^*\). The mass spectra of Fur\(^*\) and Fur\(^**\)\(^\prime\) found in systems 1-Xyl\(^\prime\)–Ala and 1-Xyl\(^\prime\)–3-Ala\(^\prime\) were identical, thus suggesting the same formation mechanism. In agreement with this, similar distribution of furaneol compounds was found in samples 7 and 9. The relatively high level of Fur\(^*\) in sample 8 (45%) is due to the pH (= 7). Under these conditions, sugar fragmentation via the 1-deoxyosone pathway is favoured.\(^10\) At pH 6, the amount of Fur\(^*\) was lower (12%).\(^1\)
The predominant homofuraneol compound in system 1-Xyl"-3-Ala (sample 9) was 2- (or 5-)[2-13C]ethyl-4-hydroxy-5-(or 2-)[13C]methyl-3(2H)-furanone (Hom') formed by a $\text{C}_3^* + \text{C}_2^*$ reaction (Figure 3). Formation involving sugar fragmentation is indicated by the presence of Hom, Hom', and Hom''.

4 CONCLUSION

The formation of norfuraneol, furaneol and homofuraneol was studied in Maillard systems by reacting D-xylene with glycine or L-alanine in a pH 7 phosphate-buffered aqueous solution at 90 °C for one hour. Norfuraneol was found to be the major component. The relative amounts of furaneol and homofuraneol were less than 1%. Experiments using the $^{13}$C-labelled precursors suggest incorporation of the Strecker degradation products formaldehyde and acetaldehyde into the pentose moiety forming furaneol and homofuraneol, respectively (Figure 3). However, both furanones were partly generated by sugar fragmentation, particularly furaneol. On the contrary, homofuraneol was preferably formed by a $\text{C}_3 + \text{C}_2$ reaction in the presence of alanine.
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![Chemical structure diagram]

**Figure 3** Schematic formation of differently labelled furaneol and homofuraneol via a $C_5^*$ + $C_1^*$ and a $C_5^*$ + $C_2^*$ reaction, respectively. The symbols indicate the origin of the carbon atoms.

**REFERENCES**