On the Formation of 2-Acylpyrroles and 3-Pyridinols in the Maillard Reaction through Strecker Degradation

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The effects of pH, reactant ratio and reaction time on the yields of 5-methylpyrrole-2-carboxaldehyde (6), 6-methyl-3-pyridinol (7), methyl 2-pyrrolyl ketone (8) and 2-methyl-3-pyridinol (9) in the reaction of D-glucose with glycine at 100 °C in aqueous solution have been studied. The use of [1-13C]D-glucose showed that the methyl group in each of 6-9 is derived from C-6 of the glucose. The formation of 6-9 from some potential intermediates in the glucose–glycine reaction has also been investigated. The results support the previously proposed routes to 6 and 7 but disqualify those to 8 and 9. Based on the smooth formation of 8 and 9 from 2-deoxy-D-arabino-hexose (12) and ammonia, a new route to 8 and 9, through an enamine derived from 12, is proposed. This route involves a modified Strecker degradation, which was supported by the formation of 2,3-dideoxy-D-erythro-hexose from 3-deoxy-D-ribo-hexose and glycine.

In the Strecker degradation,1,2 an amino group is transferred from an a-amino acid via Schiff base intermediates to a carbonyl compound. In the process, the amino acid is oxidatively degraded to carbon dioxide and an aldehyde, while the carbonyl compound is reduced. It is generally believed that the latter reactant may be an a-dicarbonyl compound (being reduced to an a-aminocarbonyl compound, Scheme 1a) or one of its vinylogues but not, for example, an a-hydroxycarbonyl compound (Scheme 1c).

The occurrence of Strecker degradations in Maillard reactions2,3 is shown by the formation of aldehydes, expected from naturally occurring a-amino acids according to Scheme 1, and heterocyclic nitrogen compounds, formally derived from reduced sugars and ammonia. The a-dicarbonyl compounds 1-3** or their enol forms

** Sugars and related compounds will here be formulated as acyclic species, even though their less reactive cyclic forms generally predominate. Geometric isomerism due to unsaturation will be neglected.

CH₃ O CH₃ CH₃ OH CH = O CH₂ OH
CH₃ C = O CH₂ C = O CH₂ C = O CH₂ C = O
H C - OH H C - OH H C - OH H C - OH H C - OH
H C - OH H C - OH H C - OH H C - OH H C - OH
CH₂ OH CH₂ OH CH₂ OH CH₂ OH CH₃

1 2 3 4 5

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are important intermediates in the dehydration of
D-glucose or D-fructose.\textsuperscript{4,5} In Maillard reactions
of these sugars, any of 1–3 or their dehydration
products (e.g., 4 or 5) might therefore induce a
Strecker degradation.

The Strecker degradation products 6–9 were
identified in an investigation of the reaction
between D-glucose and glycine at this
Department.\textsuperscript{6} In each product the carbon chain
of the glucose is apparently retained, extending
from the methyl group to the carbon atom
marked with an asterisk (*) in the formula.
Routes were proposed to 6 and 7 via 1, and to 8
and 9 via 2 or 3, implying that C-1 of the glucose
appears as C* in 6 and 7 but as methyl in 8 and 9.
The pyrroles 6 and 8 have also been obtained from D-fructose and L-alanine.\textsuperscript{7} Routes to both
products via 1 were proposed, implying that C-1
of the fructose appears as C* in 6 and 8. Because
of these largely speculative and partly conflicting
views, the glucose–glycine reaction has now
been reinvestigated, in particular with 1-\textsuperscript{13}C-
labelled glucose. Based on the results and on the
behaviour of some potential intermediates, a new
route to 8 and 9 will be proposed, extending the
scope of the Strecker degradation. A brief
account of the present work was given at a recent
meeting.\textsuperscript{8}

RESULTS

In order to optimize the yields of 6–9, calculated
on the expensive [1-\textsuperscript{13}C]-glucose, aqueous
solutions of unlabelled glucose and glycine in a
molar ratio ranging from 1:2 to 1:20 were
refluxed for 72 h. The initial pH of the solutions,
which were 0.17 M in glucose, was varied from 2
to 8. Samples were withdrawn at suitable
intervals and processed according to Scheme 2. The
resulting extracts 1–3 were analyzed by GLC.
All of the relatively lipophilic 6 and 8 was found
in extract 1, and all of the weakly basic 7 and 9
in extract 3. Other products, including any 5-
(hydroxymethyl)-2-furaldehyde (10), were found

\textbf{Scheme 1.} Strecker degradation induced by an a-dicarbonyl (a and b), \(\alpha\)-hydroxycarbonyl (c) or
\(\alpha\)-amino carbonyl compound (d). In the present paper, R=H.
mainly in extract 2. A large excess of glycine was required for reasonable yields of 6–9 and for suppressing the formation of 10. Accordingly, only results obtained with glucose and glycine in the molar ratio 1:20 will be reported. Those obtained at initial pH 2, 3 or 6 are summarized in Fig. 1. In these experiments, pH remained practically constant at 2 or 3 owing to the buffer effect of the glycine but decreased gradually from 6 to about 4.5.

The experiments at pH 2, 3, and 6 were repeated with \([1^{13}C]\)-glucose (90 atom % \(^{13}C\)). The entire reaction mixtures were processed according to Scheme 2 after refluxing for 48, 13 and 24 h, respectively. The resulting extracts 1 and 3 were analyzed by \(^{13}C\) NMR spectrometry and GLC–MS, using electron impact (EI). The spectra obtained were compared with those of authentic unlabelled 6–9, recorded under the same conditions. Unless fragmentation of the glucose is involved, the labelled atom will appear in 6–9 as C* and/or methyl. To distinguish between these alternatives, unambiguous assignment of relevant NMR signals and fragment ions from the unlabelled compounds is essential.

The \(^{13}C\) NMR chemical shifts for these compounds are collected in Table 1, where the assignments were made as follows. Each of 6–9

Table 1. \(^{13}C\) NMR chemical shifts (\(\delta\)) for Strecker degradation products in CD$_3$OD. Shifts in italics refer to C*.

<table>
<thead>
<tr>
<th></th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>12.9</td>
<td>22.5</td>
<td>25.5</td>
<td>18.4</td>
</tr>
<tr>
<td>C-2</td>
<td>140.6$^a$</td>
<td>137.0</td>
<td>133.2</td>
<td>147.7</td>
</tr>
<tr>
<td>C-3</td>
<td>124.2</td>
<td>153.6</td>
<td>118.8</td>
<td>153.7</td>
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<tr>
<td>C-4</td>
<td>111.3</td>
<td>125.3</td>
<td>111.2</td>
<td>123.2$^a$</td>
</tr>
<tr>
<td>C-5</td>
<td>135.5$^a$</td>
<td>125.3</td>
<td>126.7</td>
<td>123.5$^a$</td>
</tr>
<tr>
<td>C-6/C=O</td>
<td>179.4</td>
<td>149.4</td>
<td>189.9</td>
<td>139.4</td>
</tr>
</tbody>
</table>

$^a$ Mutual assignment uncertain.

Fig. 1. GLC yields (calc. on glucose) of Strecker degradation products (6–9) from d-glucose and glycine (molar ratio 1:20) in aq. solution at 100 °C and initial pH 2.0 (\(\bigcirc\)), 3.0 (\(\triangle\)) or 6.0 (\(\nabla\)).

Table 2. Relative intensities (I for unlabelled and \( I \) for \(^{13}\)C-labelled compounds) in the EI mass spectra of Strecker degradation products obtained from \( \beta \)-glucose and glycine at pH 2 (7) or 3 (6, 8 and 9).

| Compound 6 \( m/z \) | I | I | | Compound 7 \( m/z \) | I | I | | Compound 8 \( m/z \) | I | I | | Compound 9 \( m/z \) | I | I |
|----------------------|---|---|---|----------------------|---|---|---|----------------------|---|---|---|----------------------|---|---|---|
| 50                   | 6 | 6 | 6 | 50                   | 6 | 6 | 6 | 66                   | 54| 9 | 50 | 2 1                     |
| 51                   | 11| 11| 11| 51                   | 8 | 9 | 9 | 67                   | 5 | 56| 51 | 3 3                     |
| 52                   | 13| 14| 14| 52                   | 8 | 8 | 8 | 68                   | 5 | 0 | 52 | 4 4                     |
| 53                   | 40| 43| 43| 53                   | 16| 16| 16| 94                   | 100| 11| 53 | 12 13                   |
| 54                   | 3 | 4 | 4 | 54                   | 11| 14| 14| 95                   | 5 | 100| 54 | 7 9                     |
| 55                   | 0 | 0 | 0 | 55                   | 6 | 10| 10| 96                   | 0 | 4 | 55 | 4 6                     |
| 78                   | 5 | 6 | 6 | 56                   | 0 | 5 | 5 | 109                  | 77| 7 | 56 | 0 1                     |
| 79                   | 3 | 3 | 3 | 80                   | 53| 8 | 8 | 110                  | 5 | 79| 80 | 76 8                    |
| 80                   | 52| 55| 55| 81                   | 15| 58| 58| 111                  | 0 | 4 | 81 | 14 73                   |
| 81                   | 3 | 3 | 3 | 82                   | 5 | 11| 11| 82                   | 2 | 13| 83 | 2 13                    |
| 82                   | 0 | 0 | 0 | 83                   | 1 | 0 | 1 | 83                   | 0 | 1 | 83 | 0 1                     |
| 108                  | 85| 9 | 9 | 108                  | 13| 0 | 0 | 109                  | 100| 27| 109| 100 14                  |
| 110                  | 6 100 | 100 | 110 | 7 | 700 | 110 | 111 | 0 | 7 | 111 | 0 5                     |

shows three doublets in the "off-resonance" spectrum. Being linked to oxygen or nitrogen, C* is responsible for the doublet at lowest field. The methyl group, of course, corresponds to the signal at highest field. Table 1 shows that the C* and methyl signals from 6–9 may be clearly distinguished from each other and from the other signals without separation of 6 from 8 or 7 from 9. After changing the solvent to methanol-\( d_4 \), the proton-decoupled \(^{13}\)C NMR spectra of extracts 1 and 3, obtained from labelled glucose, showed only the solvent signal and the C* signals from 6–9. From the signal-to-noise ratios in these spectra, from the intensity ratios of the C* and methyl signals in the spectra of unlabelled 6–9, and from the \(^{13}\)C content (90 atom %) at C-1 of the glucose, the following maximum values were estimated for the percentage of the label located at the methyl group in the labelled 6–9.

Similar values were estimated in the same way for the internal carbon atoms of the C\(_6\) chain present in each of 6–9. The values 20 and 25 % simply reflect poor yields with consequent low signal-to-noise ratios and neither rule out nor imply minor labelling at sites other than C*. However, the other values show clearly that the \(^{13}\)C label appears almost exclusively at C* in 6–9, when each is formed at optimum pH.

Although a confirmation of this conclusion

Table 3. Extent (\( p \)) of labelling in ions formed on electron impact from \(^{13}\)C-labelled 6–9. The \( p \) values were calculated from the data in Table 2.

<table>
<thead>
<tr>
<th>( m/z )</th>
<th>Main ion</th>
<th>( p, % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Methylpyrrole-2-carboxaldehyde (6)</td>
<td>50–55</td>
<td>M–C(^{13})O(^{13})H(^{13})O(^{13})–HCN 2 2</td>
</tr>
<tr>
<td>78–82</td>
<td>M–C(^{13})O(^{13})</td>
<td>0 0</td>
</tr>
<tr>
<td>108–111</td>
<td>M</td>
<td>102 102</td>
</tr>
<tr>
<td>6-Methyl-3-pyridinol (7)</td>
<td>50–56</td>
<td>M–CHO–HC(^{13})N 37 37</td>
</tr>
<tr>
<td>80–83</td>
<td>M–CHO 100 100</td>
<td></td>
</tr>
<tr>
<td>108–111</td>
<td>M</td>
<td>98 98</td>
</tr>
</tbody>
</table>

Methyl 2-pyrrolyl ketone (8)

<table>
<thead>
<tr>
<th>( m/z )</th>
<th>Main ion</th>
<th>( p, % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>66–68</td>
<td>M–CH(_3)CO(^{13})</td>
<td>96 96</td>
</tr>
<tr>
<td>94–96</td>
<td>M–CH(_3) 100 100</td>
<td></td>
</tr>
<tr>
<td>109–111</td>
<td>M</td>
<td>102 102</td>
</tr>
</tbody>
</table>

2-Methyl-3-pyridinol (9)

<table>
<thead>
<tr>
<th>( m/z )</th>
<th>Main ion</th>
<th>( p, % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>50–56</td>
<td>M–CHO–HC(^{13})N 21 21</td>
<td></td>
</tr>
<tr>
<td>80–83</td>
<td>M–CHO 99 99</td>
<td></td>
</tr>
<tr>
<td>108–111</td>
<td>M</td>
<td>100 100</td>
</tr>
</tbody>
</table>

appeared superfluous, we wished to establish whether it was possible to arrive at the same conclusion from the mass spectra. If so, future tracer experiments might be performed on a much smaller scale. Relevant mass spectral data for unlabelled 6–9 and for the labelled compounds obtained at pH 2 (7) or 3 (6, 8 and 9) are collected in Table 2. The extent (p) of labelling in each molecular ion (M) and diagnostic fragment from the labelled compounds was calculated from these data and from the $^{13}$C content (90 atom %) at C-1 of the labelled glucose, as outlined in the experimental part. The results are listed in Table 3, where the fragment symbols are based on previous investigations of 2-acylpyrroles$^9$ and 3-pyridinols.$^{10}$

As seen from Table 2, the mass spectra showed clusters of peaks, including those of interest, at consecutive $m/z$ values, resulting in extensive overlap. To simplify the calculation of $p$, we had therefore to assume that the $^{13}$C label was located at one carbon atom, and that the ions forming each cluster differed only in hydrogen content and/or isotopic composition and not in the origin of their carbon atoms. The first assumption appears reasonable in view of the $^{13}$C NMR results, but the second one is more doubtful.

Thus, a minor part of the cluster at $m/z$ 80–83 in the spectra of 7 is due to M–HC*N.$^{10}$ Although this has been neglected in Table 3, $p$ is close to 100 %. At lower $m/z$, however, such neglected fragments may be more abundant and lead to meaningless $p$ values. This is exemplified by the high $p$ values for the clusters at $m/z$ 50–56 in the spectra of 7 and 9. As seen from Table 2, the clusters shown by corresponding labelled and unlabelled compounds differed mainly at $m/z$ 54–56, indicating the presence of ions which had lost unlabelled C$_2$H$_2$ instead of HC*N. Accordingly, a high resolution mass spectrum of unlabelled 7 showed five peaks at $m/z$ 54. The major peaks were due to C$_6$H$_6$ + and C$_5$H$_4$N$^+$. Their intensity ratio was about 2:1. At $m/z$ 55, C$_3$H$_5$O$^+$ and C$_3$H$_5$N$^+$ predominated. Such ions may also

![Graphs showing GLC yields](image-url)

**Fig. 2.** GLC yields (calc. on glucose, I or II) of Strecker degradation products (6–9) in the reaction of D-glucose (O), I (∆) or II (▽) with glycine (molar ratio 1:20) in aq. solution at 100 °C and initial pH 3.0. The yield of 8 from I and glycine was negligible.

contribute to the cluster at m/z 50–55 shown by 6, but since C* has already been lost from all ions in the cluster, the p value is not affected.

For the pyroles 6 and 8, the clean loss of the acyl group from M led to p values close to 0 or 100%, confirming the 13C NMR results. As exemplified by the pyridinols 7 and 9, however, detailed knowledge of the fragmentation is imperative in more complex cases. Even so, it may be hard to obtain more than qualitative results.

Some (unlabelled) potential intermediates in the glucose–glycine reaction, including 1-deoxy-1-glycido-p-fructose (11), 2-deoxy-p-arabino-hexose (12), and 3-deoxy-p-ribo-hexose (13), were treated for 24 h at 100 °C and initial pH 3 as already described for the glucose–glycine experiments, see Table 4. Thus, 1, 6, 8, 10 or 11 was treated with glycine in the molar ratio 1:20. The experiment with 11 was repeated without glycine. Compound 6, 8 or 12 was treated with ammonium acetate (molar ratio 1:20), and 13 with glycine (molar ratio 1:5). The experiments with 12 and 13 were repeated at initial pH 6.

The yields of 6–9 from I or II after reaction with glycine are compared in Fig. 2 with those obtained from glucose (Fig. 1, pH 3). When treated with glycine or ammonium acetate, the pyroles 6 and 8 disappeared gradually without forming any pyridinol 7 or 9. Compound 10 also disappeared, when treated with glycine, yielding only traces of 6 and 7. From 11 alone, no 6–9 was obtained. The maximum yields 0.9% of 8 and 28% of 9 from 12 and ammonium acetate were obtained after only about 3 h reaction at initial pH 6. In the experiments with 13, the sugars in the final aqueous phase (Scheme 2) were analyzed by GLC–MS as their per-O-acetyladononitrides. Among the several GLC peaks, two were due to the nitrile derivatives of 13 and 2,3-dideoxy-p-erythro-hexose (14). The latter derivative was identified by prominent M+H+ and M+H+NH3 peaks in the chemical ionization (CI) spectrum, recorded with ammonia as reaction gas. The maximum yield of 14 was obtained after about 6 h reaction at initial pH 6 and was 5%, if the difference in GLC response factor between the nitrile derivatives of 13 and 14 is neglected.

DISCUSSION

As seen from Fig. 1, the yields of 7 and 9 rose steadily with increasing reaction time up to a certain limit, whereas those of 6 and 8 reached a maximum after 6–24 h and then declined. This was not due to the conversions 6–7 and 8–9, as shown by the experiments where 6 or 8 was treated with glycine or ammonium acetate. Higher acidity seemed to favour the further reactions of 6 and 8, since the maximum yields were obtained earlier. Higher acidity also favoured the formation of 6 and 7 but disfavoured that of 9, and perhaps also that of 8. These results seemed to support the routes to 6–9 proposed for the glucose–glycine reaction, since I is formed through the 1,2-enol but 2 and 3 through the 2,3-enol of fructose; the relative importance of the 2,3-enol is believed to increase with pH. However, this interpretation is incorrect, since the routes to 8 and 9 are incompatible with the 13C-tracer results (see below). These results also indicate that little or no 6–9 is formed through fragmentation and recombination of the C6 chain.

All the present results support the previously proposed routes to 6,7 and 7 via I. The routes to 6 differ only as to the stage at which the Strecker degradation takes place. We now prefer the route through 4 and 5, since the necessary dehydration steps are more readily rationalized before than after the Strecker degradation. However, the results are equally compatible with several closely related routes, some of which are more likely than that through I, 4 and 5.

In the first place, 6 and 7 formed faster from I than from glucose (Fig. 2), but this does not necessarily imply that I is an intermediate in the latter reaction. An obvious alternative is that I is converted faster than glucose to the true intermediate. Indeed, this is probably not I but rather its enol.4,5 Similarly, this enol is probably not formed through 112 or fructose5 but rather through their 1,2-enols, despite the smooth formation of 6 and 7 from 11 and glycine (Fig. 2) and the reported7 formation of 6 from fructose and alanine.

Secondly, the well-known amine catalysis of sugar dehydration in Maillard reactions is due to partial conversion of the various carbonyl intermediates into Schiff bases and enamines. In a nearly neutral medium, these largely take over
Scheme 3. Alternative routes to products 6 and 7 from Schiff bases derived from compound 5.
i = +glycine–H₂O.

the parts played by the less reactive keto and enol forms in the absence of amines. In our experiments, 1, 4, 5 and their enols are therefore less probable intermediates than the corresponding Schiff bases and enamines, particularly in view of the large excess of glycine generally employed.

It is even possible that the Strecker degradation proceeds via a diimine ("double" Schiff base) according to Scheme 1b rather than by the accepted route in Scheme 1a. These alternative routes to 6 and 7 from Schiff bases derived from 5 are shown in Scheme 3. In a weakly acidic medium, where the carboxyl group of the amino acid being degraded may ionize sufficiently, a carbonyl oxygen is protonated (if at all) to a much smaller extent that a Schiff base nitrogen. The electrophilic centre is therefore expected to be more powerful in Scheme 1b than in Scheme 1a. Until experimental evidence for the existence and equilibrium concentration of the diimines has been obtained, it is however impossible to choose between the alternative routes. It may be noted here that metabolic transaminations, racemizations and decarboxylations of α-amino acids take place through a vinylogous diimine derived from the B₆ vitamin pyridoxal, but in this case one imino group forms part of an aromatic ring.

The negligible formation of 6 and 7 from 10 and glycine supports the view that the dehydration 4→5 does not proceed via 10. It also shows

that the cyclization $4 \rightarrow 10$ is essentially irreversible under the reaction conditions and that $10$, being a vinylogous $\alpha$-hydroxycarbonyl compound, cannot induce a Strecker degradation. (According to Scheme 1c, such a degradation, followed by hydrolytic ring opening, might yield $15$ in Scheme 3.)

Since $C^\ast$ in each of $6-9$ has now been shown to originate mainly or exclusively from C-1 of the glucose, the proposed $^6$ routes to $8$ and $9$ via $2$ or $3$ are unimportant or incorrect. The negligible formation of $8$ from $1$ and glycine (Fig. 2) disqualifies the route proposed $^7$ to $8$ via $1$. A difficulty with all of $1-5$, their enols and their amine derivatives as intermediates in the formation of $8$ or $9$ is to account for the necessary elimination of the hetero atom at the bifunctional C-2 atom.

This difficulty is avoided, if elimination of the hydroxyl group at C-2 of the glucose according to Scheme 1c is assumed. Such elimination might be promoted by initial lactonization, as indicated in formula $16$. Several early samples from glucose–glycine reaction mixtures were analyzed for the 2-deoxy sugar $12$, expected according to Scheme 1c. Thus, the final aqueous phase obtained by processing each sample according to Scheme 2 was analyzed for sugars by three different methods, $^{11,12}$ but in no case was any $12$ detected. However, this might be because dehydration of the enamine $17$ through the $\beta$-elimination shown in Scheme 4 is much faster than its hydrolysis to $12$ according to the last step in Scheme 1c.

That this may be the case was indicated by the high yields of $8$ and $9$ readily obtained from $12$ and ammonium acetate—presumably via $17$—compared with the optimum yields of $0.3\%$ obtained from glucose and glycine after $12-48$ h (Fig. 1). Further support was offered by the formation of the dideoxy sugar $14$ in about $5\%$ yield from $13$ and glycine within $6$ h. In this case, Scheme 1c may be followed all the way, since there is no $\beta$-elimination competing with the last step. Possible routes to $8$ and $9$ from $17$ are suggested in Scheme 4, where the "vinylogous" Amadori rearrangement following the dehydration of $17$ may be noted.

Although the evidence in favour of $17$ as an intermediate in the formation of $8$ and $9$ from glucose and glycine seems convincing, there are several objections against the simple route to $17$ according to Scheme 1c. A related but less direct route to $17$ involving Scheme 1d will therefore

![Scheme 4](image)

*Scheme 4.* Routes to products $8$ and $9$ from the precursor $17$.
Scheme 5. Alternative routes to the postulated precursor 17 of products 8 and 9. \( i = +\text{glycine-H}_2\text{O}, \ R=\text{arabino-H(CHOH)}_4 \).

also be discussed. The alternative routes are outlined in Scheme 5.

In the first place, \( \alpha \)-hydroxycarbonyl compounds like glucose and other sugars are not expected to induce Strecker degradations. This may be due to insufficient proton assistance to the hydroxyl group in the Schiff base 18 (this may be less critical for the more electrophilic carbonyl group in Scheme 1a). This problem does not occur in the corresponding Scheme 1d intermediate 22.

Secondly, Scheme 1c does not explain why 8 and 9 are formed faster from 11 than from glucose in the presence of glycine (Fig. 2), unless the improbable reversal of the Amadori rearrangement 18→11 is postulated. On the other hand, the sequence 11→20→22 has already been carried out with 2-p-chloroaniline as the amine,13 and the cyclic N-glycoside forms of products analogous to 20 and 22 have been isolated. The formation of 8 from fructose and alanine7 is also easier to rationalize by means of the well-known14 Heyns rearrangement 19→21 than by invoking glucose, as required by Scheme 1c. Some aliphatic amines may even convert fructose to analogues of 22.15 In this connection, the catalyzing effect of glycine on the dehydrogenation of 2-amino-2-deoxy-d-glucose5 may be recalled (cf. 21).

Elimination of the 1-glycino group from 20 according to Scheme 1d might also occur but should be less favourable than elimination from a secondary atom like C-2 of 22. No attempt was made to identify the products of any such elimination from C-1 of 20. In these products, C-1 of glucose should appear as a methyl group. In view of the 13C-tracer results, 6–9 are therefore probably not among the products.

For the reasons given above, and because no 8 or 9 was obtained from 11 without glycine, we tend to prefer Scheme 1d to Scheme 1c. However, before making a final choice, we wish to complete a study on reactions of 11 and amino sugars related to 20–22.

**EXPERIMENTAL**

**Chromatography**

Separate GLC analyses were performed at 30 ml N₂/min with a Varian 1840-1 instrument, fitted with dual flame-ionization detectors and 1.8 m × 2 mm i.d. glass columns. Sugars were analyzed as described in Ref. 11 or 12, and 6–9 on 100–120 mesh Varaport 30 coated with 3% NPGS ("neopentyl glycol succinate"). The pyrroles (6 and 8, extract 1) were analyzed at 165 °C with biphenyl as internal standard, and the pyridinols (7 and 9, extract 3) at 200 °C with fluorene as internal standard. Peak areas were measured with a Varian CDS 111C instrument.

Spectrometry

\(^{13}\)C NMR spectra (Table 1) were recorded at 22.53 MHz and ca. 35 °C with a Jeol FX-90Q instrument, using 5 mm o.d. NMR-tubes. The lock signal was provided by deuterium of the solvent (CD\textsubscript{3}OD). The chemical shifts (\(\delta\)) were related to internal tetramethylsilane.

The high resolution EI mass spectrum of unlabelled 7 was recorded at 100 eV with a V.G. ZAB instrument at the Institute of Medical Chemistry, University of Gothenburg. Low resolution EI mass spectra (Table 2) were recorded at 70 eV with a Finnigan 4021 GC/MS/Data System. The samples were introduced through a 20 m x 0.25 mm i.d. capillary GLC column coated with OV-225. The helium flow rate was 25 cm/s (ca. 0.7 ml/min) and the column temperature was programmed from 80 to 150 °C at 6 °C/min. The background was subtracted from all spectra, which were recorded under as similar conditions as possible. Ammonia CI mass spectra were recorded under the same conditions, but the capillary column was coated with CP Sil 5 and its temperature programmed from 100 to 250 °C at 10 °C/min.

The extent (\(p\), Table 3) of labelling in the molecular ion or in a diagnostic fragment ion from any of the \(^{13}\)C-labelled Strecker degradation products (6–9) was calculated by comparing the spectrum with that of the respective unlabelled reference sample. This was done as follows, assuming any label to be located at one carbon atom. Each ion belonged to a series of \(n\) ions, \(\text{CEH}_{k}^2\), where \(C\) is the potentially labelled atom, \(E\) the other atoms common to the ions, H hydrogen and \(k=0, 1, \ldots, n-1\). In the mass spectrum, these ions form a cluster of \(r\) peaks, where \(r > n\). The average \(^{13}\)C content (\(c\)) of \(C\) was assumed to be the same for all the ions \(\text{CEH}_{k}^2\). The possible contribution of other ions to the cluster was neglected. Since the mass distribution of \(\text{EH}_{k}^2\) due to its natural isotopic composition has been tabulated,\(^{16}\) the relative intensity of each peak (\(I\)) in the cluster is given by

\[ I_j = \sum_k \lambda_{j,k} A_k \]

where each coefficient \(\lambda_{j,k}\) is a function of \(c\) only, and \(A_k\) is the relative abundance of \(\text{CEH}_{k}^2\). The spectrum of the unlabelled compound yielded \(r\) such equations, and that of the labelled compound \(r\) more equations. In the former equations, \(\lambda_{j,k}\) may, of course, be evaluated, since \(c=1\%\). In the latter equations, \(c\) was varied. For each \(c\) value, \(\lambda_{j,k}\) were evaluated; the remaining unknowns, \(A_k\), were then calculated from all the \(2r\) equations by the method of least squares. By minimization of the resulting deviation, the preferred \(c\) value was obtained. The extent of labelling is then given by

\[ p = 100(c-1)/(90-1) = 1.124(c-1) \]

if \(c\) and \(p\) are expressed in percent. The calculations were performed with a Commodore PET computer, using a program written in BASIC. The program is available on request.

Materials

Compounds 1,\(^{17}\) 6,\(^{18}\) 9,\(^{19}\) 11\(^{20}\) and 13\(^{21}\) were prepared according to the literature for use as starting materials or reference samples. Other reagents were commercial samples, including \([1,\text{\(^{13}\)}\text{C}]\text{-d-glucose}\) (Prochem, London; 90 atom \% \(\text{\(^{13}\)}\text{C}\)). Solvents were freshly distilled before use.

Maillard reaction procedure

The experiments with unlabelled materials are listed in Table 4. Reactant 1 (15.0 mmol) and the appropriate amount of reactant 2 were dissolved in water (60 ml) by gentle heating. The generally supersaturated solution was brought to the desired pH at about 25 °C by addition of conc. hydrochloric acid or 2 M sodium hydroxide, diluted to 90 ml and refluxed for 24–72 h. At

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Molar ratio</th>
<th>Initial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu</td>
<td>Glycine</td>
<td>1:20</td>
</tr>
<tr>
<td>Glu</td>
<td>Glycine</td>
<td>1:20</td>
</tr>
<tr>
<td>Glu</td>
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<tr>
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<td>1:20</td>
</tr>
<tr>
<td>6</td>
<td>Glycine</td>
<td>1:20</td>
</tr>
<tr>
<td>8</td>
<td>Glycine</td>
<td>1:20</td>
</tr>
<tr>
<td>10</td>
<td>Glycine</td>
<td>1:20</td>
</tr>
<tr>
<td>11</td>
<td>Glycine</td>
<td>1:20</td>
</tr>
<tr>
<td>12</td>
<td>NH(_4)OAc</td>
<td>1:20</td>
</tr>
<tr>
<td>12</td>
<td>NH(_4)OAc</td>
<td>1:20</td>
</tr>
<tr>
<td>13</td>
<td>Glycine</td>
<td>1:5</td>
</tr>
<tr>
<td>13</td>
<td>Glycine</td>
<td>1:5</td>
</tr>
</tbody>
</table>

suitable intervals, 15 ml aliquots were withdrawn and processed according to Scheme 2. The resulting extracts 1 and 3 were analyzed by GLC. The final aqueous phase was sometimes analyzed for sugars according to Ref. 11 and/or 12.

[1-13C]-p-Glucose was treated as unlabelled glucose but on a 10 times smaller scale. The entire reaction mixture was processed after 48, 13 or 24 h according to whether the initial pH was 2.0, 3.0 or 6.0. The resulting Extracts 1 and 3 were analyzed by GLC–MS, dried with sodium sulfate and evaporated at reduced pressure below 40 °C. Each residue was dissolved in methanol-d4 and the 13C NMR spectrum of the solution recorded.

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REFERENCES


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