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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-^{13}C]-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 α-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 α-dicarbonyl compound (being reduced to an α-aminocarbonyl compound, Scheme 1a) or one of its vinylogues but not, for example, an α-hydroxycarbonyl compound (Scheme 1c).

The occurrence of Strecker degradations in Maillard reactions[2,3] is shown by the formation of aldehydes, expected from naturally occurring α-amino acids according to Scheme 1, and heterocyclic nitrogen compounds, formally derived from reduced sugars and ammonia. The α-dicarbonyl compounds *1*–*3* ** or their enol forms

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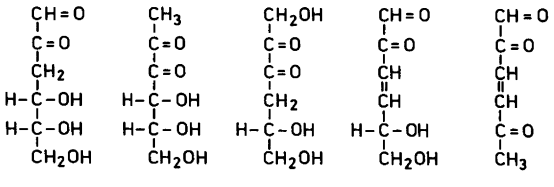
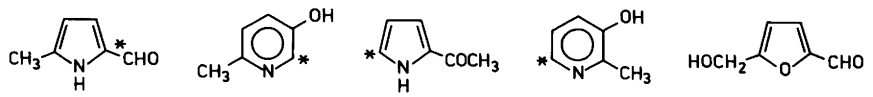
** Sugars and related compounds will here be formulated as acyclic species, even though their less reactive cyclic forms generally predominate. Geometric isomerism due to unsaturation will be neglected.



1 *2* *3* *4* *5*



6 *7* *8* *9* *10*

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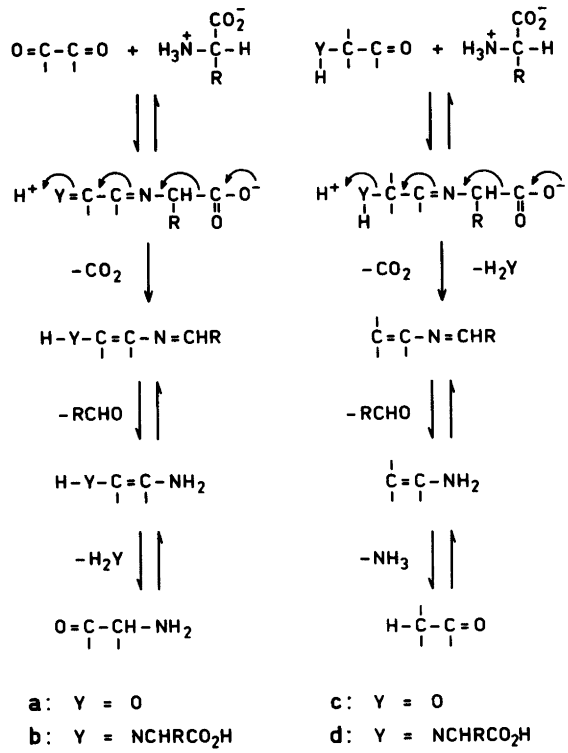


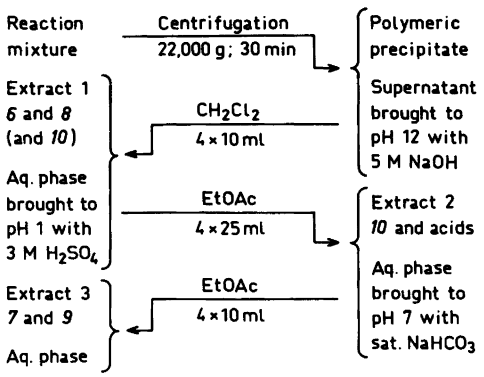
Scheme 1. Strecker degradation induced by an α-dicarbonyl (*a* and *b*), α-hydroxycarbonyl (*c*) or α-aminocarbonyl compound (*d*). In the present paper, R=H.

are important intermediates in the dehydration of D-glucose or D-fructose.[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.[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.[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-^{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.[8]

RESULTS

In order to optimize the yields of *6–9*, calculated on the expensive [1-^{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



Scheme 2. Processing of the Maillard reaction mixtures.

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.

Table 1. ^{13}C NMR chemical shifts (δ) for Strecker degradation products in CD_3OD. Shifts in italics refer to C*.

	6	*7*	*8*	*9*
CH_3	12.9	22.5	25.5	18.4
C-2	140.6[a]	*137.0*	133.2	147.7
C-3	124.2	153.6	118.8	153.7
C-4	111.3	125.3	111.2	123.2[a]
C-5	135.5[a]	125.3	*126.7*	123.5[a]
C-6/C=O	*179.4*	149.4	189.9	*139.4*

[a] Mutual assignment uncertain.

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*

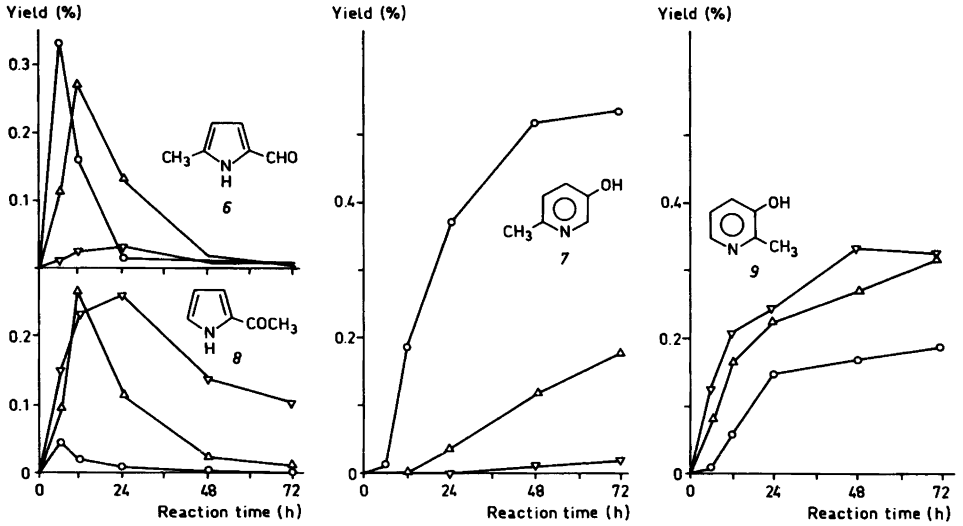


Fig. 1. GLC yields (calc. on glucose) of Strecker degradation products (*6–9*) from D-glucose and glycine (molar ratio 1:20) in aq. solution at 100 °C and initial pH 2.0 (○), 3.0 (△) or 6.0 (▽).

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 D-glucose and glycine at pH 2 (*7*) or 3 (*6*, *8* and *9*).

Compound *6*			Compound *7*			Compound *8*			Compound *9*		
m/z	I	*I*	*m/z*	I	*I*	*m/z*	I	*I*	*m/z*	I	*I*
50	6	6	50	6	6	66	54	9	50	2	1
51	11	11	51	8	9	67	5	56	51	3	3
52	13	14	52	8	8	68	0	5	52	4	4
53	40	43	53	16	16	94	100	11	53	12	13
54	3	4	54	11	14	95	5	100	54	7	9
55	0	0	55	6	10	96	0	4	55	4	6
78	5	6	56	0	5	109	77	7	56	0	1
79	3	3	80	53	8	110	5	79	80	76	8
80	52	55	81	15	58	111	0	4	81	14	73
81	3	3	82	5	11				82	2	13
82	0	0	83	1	0				83	0	1
108	85	9	108	13	0				108	3	0
109	100	91	109	100	27				109	100	14
110	6	100	110	7	100				110	5	100
111	0	5	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*.

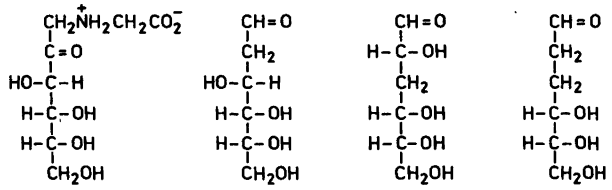
Compound	*6*	*7*	*8*	*9*
pH 2	–	2	–	6
pH 3	5	20	2	2
pH 6	25	–	5	4

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.

m/z	Main ion	*p*, %
5-Methylpyrrole-2-carboxaldehyde (*6*)		
50–55	M–C*HO·–HCN	2
78–82	M–C*HO·	0
108–111	M	102
6-Methyl-3-pyridinol (*7*)		
50–56	M–CHO·–HC*N	37
80–83	M–CHO·	100
108–111	M	98
Methyl 2-pyrrolyl ketone (*8*)		
66–68	M–CH_3CO·	96
94–96	M–CH_3·	100
109–111	M	102
2-Methyl-3-pyridinol (*9*)		
50–56	M–CHO·–HC*N	21
80–83	M–CHO·	99
108–111	M	100



11 *12* *13* *14*

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_2H_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 $C_4H_6^{\cdot+}$ and $C_3H_4N^+$. Their intensity ratio was about 2:1. At *m/z* 55, $C_3H_3O^+$ and $C_3H_5N^{\cdot+}$ predominated. Such ions may also

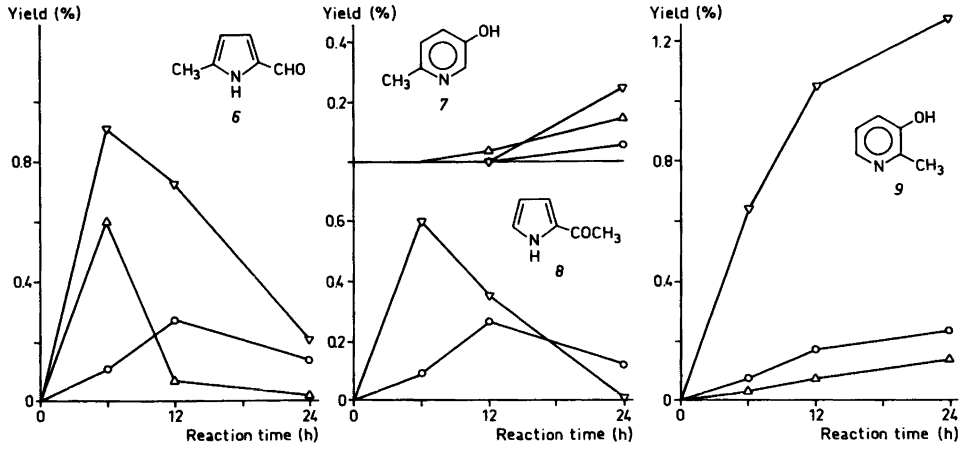


Fig. 2. GLC yields (calc. on glucose, *1* or *11*) of Strecker degradation products (*6*–*9*) in the reaction of D-glucose (○), *1* (△) or *11* (▽) with glycine (molar ratio 1:20) in aq. solution at 100 °C and initial pH 3.0. The yield of *8* from *1* and glycine was negligible.

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 pyrroles *6* and *8*, the clean loss of the acyl group from M led to *p* values close to 0 or 100 %, confirming the ^{13}C 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-glycino-D-fructose (*11*), 2-deoxy-D-*arabino*-hexose (*12*), and 3-deoxy-D-*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 *1* or *11* 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 pyrroles *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*-acetylaldononitriles.[11] Among the several GLC peaks, two were due to the nitrile derivatives of *13* and 2,3-dideoxy-D-*erythro*-hexose (*14*). The latter derivative was identified by prominent $M+H^+$ and $M+H^++NH_3$ 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.

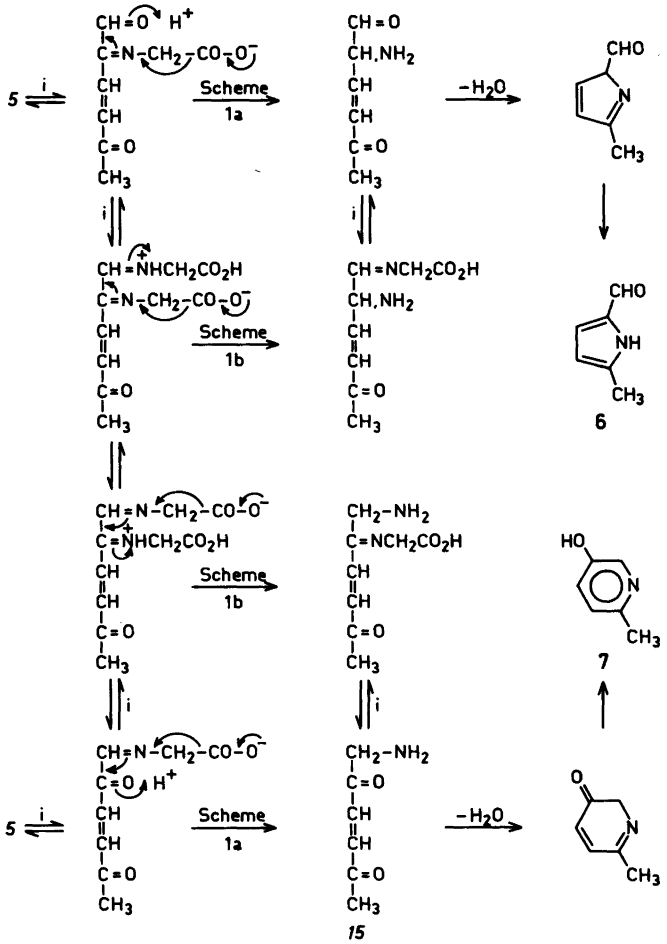
DISCUSSION

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[6] for the glucose–glycine reaction, since *1* 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.[5] However, this interpretation is incorrect, since the routes to *8* and *9* are incompatible with the ^{13}C-tracer results (see below). These results also indicate that little or no *6–9* is formed through fragmentation and recombination of the C_6 chain.

All the present results support the previously proposed routes to *6*[6,7] and *7*[6] *via 1*. 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*,[7] 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 *1, 4* and *5*.

In the first place, *6* and *7* formed faster from *1* than from glucose (Fig. 2), but this does not necessarily imply that *1* is an intermediate in the latter reaction. An obvious alternative is that *1* is converted faster than glucose to the true intermediate. Indeed, this is probably not *1* but rather its enol.[4,5] Similarly, this enol is probably not formed through *11*[2] or fructose[5] but rather through their 1,2-enols, despite the smooth formation of *6* and *7* from *11* and glycine (Fig. 2) and the reported[7] 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−H_2O.

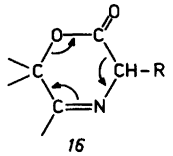
the parts played by the less reactive keto and enol forms in the absence of amines. In our experiments, *1*, *4*, *5* and their enols are therefore less probable intermediates than the corresponding Schiff bases and enamines, particularly in view of the large excess of glycine generally employed.

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 B_6 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*.[4] It also shows

that the cyclization *4*→*10* is essentially irreversible under the reaction conditions and that *10*, being a vinylogous α-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* 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



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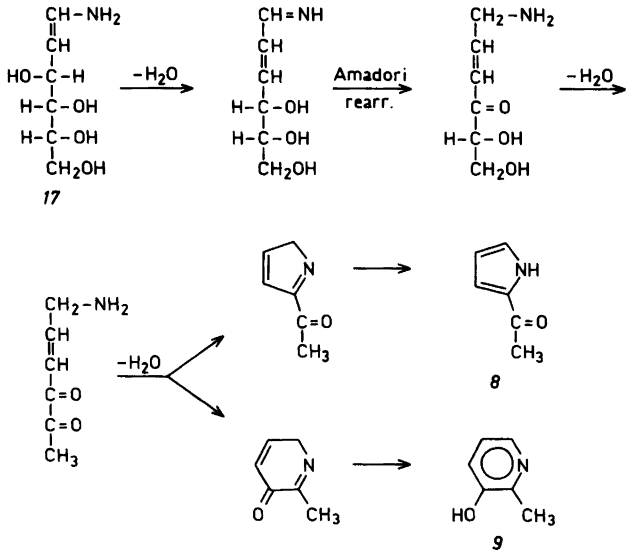
amine derivatives as intermediates in the formation of *8* or *9* is to account for the necessary elimination of the hetero atom at the bifunctional C-2 atom.

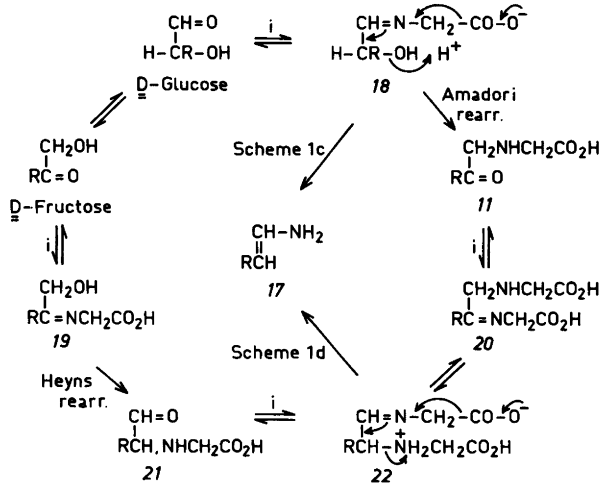
This difficulty is avoided, if elimination of the hydroxyl group at C-2 of the glucose according to Scheme 1c is assumed. Such elimination might be promoted by initial lactonization, as indicated in formula *16*. Several early samples from glucose–glycine reaction mixtures were analyzed for the 2-deoxy 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 β-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 β-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. 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=+glycine−H_2O, R=*arabino*-H(CHOH)$_4$.

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

In the first place, α-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 *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 alanine[7] is also easier to rationalize by means of the well-known[14] 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 dehydration of 2-amino-2-deoxy-D-glucose[5] 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 ^{13}C-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 N_2/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_3OD). The chemical shifts (δ) 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×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, CEH_k^+, 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 CEH_k^+. The possible contribution of other ions to the cluster was neglected. Since the mass distribution of EH_k due to its natural isotopic composition has been tabulated,[16] the relative intensity of each peak (j) 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 CEH_k^+. 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-^{13}C]-D-glucose (Prochem, London; 90 atom % ^{13}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 4. Experiments with unlabelled materials at 100 °C in aq. solution 0.17 M in reactant 1. Glu=D-glucose.

Reactants		Molar ratio	Initial pH
1	2		
Glu	Glycine	1:20	2.0
Glu	Glycine	1:20	3.0
Glu	Glycine	1:20	6.0
1	Glycine	1:20	3.0
6	Glycine	1:20	3.0
6	NH_4OAc	1:20	3.0
8	Glycine	1:20	3.0
8	NH_4OAc	1:20	3.0
10	Glycine	1:20	3.0
11	Glycine	1:20	3.0
11	—	—	3.0
12	NH_4OAc	1:20	3.0
12	NH_4OAc	1:20	6.0
13	Glycine	1:5	3.0
13	Glycine	1:5	6.0

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 somtimes analyzed for sugars according to Ref. 11 and/or 12.

[1-^{13}C]-D-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-d_4 and the ^{13}C NMR spectrum of the solution recorded.

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REFERENCES

1. Baddar, F. G. *J. Chem. Soc.* (1949) S 163; Schönberg, A. and Moubacher, R. *Chem. Rev. 50* (1952) 261.
2. Mauron, J. *Prog. Food Nutr. Sci. 5* (1981) 5.
3. Baltes, W. *Food Chem. 9* (1982) 59.
4. Feather, M. S. and Harris, J. F. *Adv. Carbohydr. Chem. Biochem. 28* (1973) 161.
5. Feather, M. S. *Prog. Food Nutr. Sci. 5* (1981) 37.
6. Olsson, K., Pernemalm, P.-Å. and Theander, O. *Acta Chem. Scand. B 32* (1978) 249.
7. Shaw, P. E. and Berry, R. E. *J. Agric. Food Chem. 25* (1977) 641.
8. Nyhammar, T., Olsson, K. and Pernemalm, P.-Å. *ACS Symp. Ser. 215* (1983) 71.
9. Budzikiewicz, H., Djerassi, C. and Williams, D. H. *Mass Spectrometry of Organic Compounds,* Holden-Day, San Francisco 1967, p. 602.
10. Undheim, K. and Hurum, T. *Acta Chem. Scand. 26* (1972) 2075.
11. Seymour, F. R., Chen, E. C. M. and Bishop, S. H. *Carbohydr. Res. 73* (1979) 19; see also Li, B. W., Cochran, T. W. and Vercellotti, J. R. *Ibid. 59* (1977) 567; Mawhinney, T. P., Feather, M. S., Barbero, G. J. and Martinez, J. R. *Anal. Biochem. 101* (1980) 112.
12. Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W. *J. Am. Chem. Soc. 85* (1963) 2497; Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. *Anal. Chem. 37* (1965) 1602.
13. Baltes, W. and Franke, K. *Z. Lebensm. Unters. Forsch. 167* (1978) 403.
14. Anet, E. F. L. J. *Aust. J. Chem. 10* (1957) 193; Heyns, K., Breuer, H. and Paulsen, H. *Chem. Ber. 90* (1957) 1374.
15. Carson, J. F. *J. Am. Chem. Soc. 77* (1955) 1881; Heyns, K., Eichstedt, R. and Meinecke, K.-H. *Chem. Ber. 88* (1955) 1551.
16. Beynon, J. H. and Williams, A. E. *Mass and Abundance Tables for Use in Mass Spectrometry,* Elsevier, Amsterdam 1963.
17. El Khadem, H., Horton, D., Meshreki, M. H. and Nashed, M. A. *Carbohydr. Res. 17* (1971) 183.
18. Silverstein, R. M., Ryskiewicz, E. E. and Willard, C. *Org. Synth. Coll. Vol. 4* (1963) 831.
19. Rapoport, H. and Volcheck, E. J., Jr. *J. Am. Chem. Soc. 78* (1956) 2451.
20. Hodge, J. E. and Fisher, B. E. *Methods Carbohydr. Chem. 2* (1963) 99.
21. Garegg, P. J. and Samuelsson, B. *J. Chem. Soc. Perkin Trans. 1* (1980) 2866.

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