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Formation of Aromatic Compounds from Carbohydrates. IX.* Reaction of D-Glucose and L-Lysine in Slightly Acidic, Aqueous Solution

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The title reaction yielded 1-deoxy-1-(N^6-lysino)-D-fructose, (±)-2-formyl-5-(hydroxymethyl)pyrrole-1-norleucine, the new 5-(3,4,5,6-tetrahydropyrid-3-ylidenemethyl)-2-furanmethanol and several compounds previously identified as products in the reaction of D-glucose with methylamine or glycine under similar conditions. The lysine slowly racemized during the reaction. Such racemization of amino acids might contribute to the nutritional loss caused by Maillard reactions in food.

The nutritional loss in cooking is partly due to the so-called Maillard reaction[2,3] between amino acids and reducing carbohydrates. At an early stage of the reaction, the amino acids are made unavailable by the practically irreversible Amadori rearrangement.[2–5] Such loss of lysine is particularly serious for two reasons. First, lysine is an essential amino acid. Second, owing to its ε-amino group, lysine may enter into the Maillard reaction when present in proteins[2–5] and not only in the free state. To balance this loss, free lysine is often added as a so-called fortifier to ready-made industrial food products.[6] The free lysine may react through either or both amino groups.

The physiological and toxicological effects of Maillard reaction products have been reviewed.[5] Although toxic effects seem to be rare,[2] mutagenic[7–9] and teratogenic[10] products have been reported to form in the reaction between glucose and lysine. The mixture produced in this reaction may also interfere with protein or carbohydrate digestion and absorption in rats.[11]

Several products in the glucose–lysine reaction have already been identified.[7,12,13] In the present reinvestigation, we have used essentially the same reaction conditions and preliminary separation methods as in studying the reaction of D-glucose with methylamine[14] or glycine.[15] It is therefore easy to compare the results obtained for the three reactions. This is of interest since methylamine and glycine may be regarded as models of the two reactive sites in lysine. Some of the present results have already been reported briefly.[16] Among the reaction products, the biologically active[9,11] amino acid *10* and the novel compound *11* have received the greatest attention.

RESULTS

A fairly dilute aqueous solution of D-glucose (Glc) and L-lysine (Lys) monohydrochloride in the molar ratio 3:2 was refluxed for 30 h, while its pH was kept at 4.5 by automatic titration with sodium hydroxide. The solution was cooled, analysed for sugars[17] and processed according to Scheme 1. The sugar analysis showed glucose in 28 % of the original amount and a 5.0 % yield of

* Part VIII: see Ref. 1.

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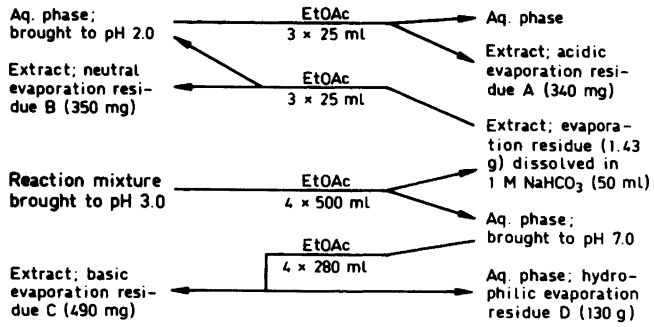


Scheme 1. Processing of the title reaction mixture, obtained at constant pH 4.5.

D-fructose as the only major components. The yield of lipophilic products (LP), *i.e.*, those extracted by ethyl acetate (residues A–C in Scheme 1), was 0.76 % based on the arbitrary eqn. (1). As shown by preliminary small-scale experiments, this yield remained constant after refluxing for 22–30 h.

$$3\,\mathrm{Glc} + 2\,\mathrm{Lys} \rightarrow \mathrm{LP} + 12\,H_2O + CO_2 \qquad (1)$$

Residues A–C were chromatographed on silica gel, resulting in the pure compounds *1–4* and the binary mixtures *5+7* and *6+8*. The pure compounds and mixtures were identical (TLC and ^{1}H NMR) with the respective authentic samples or mixtures of such samples.[18]

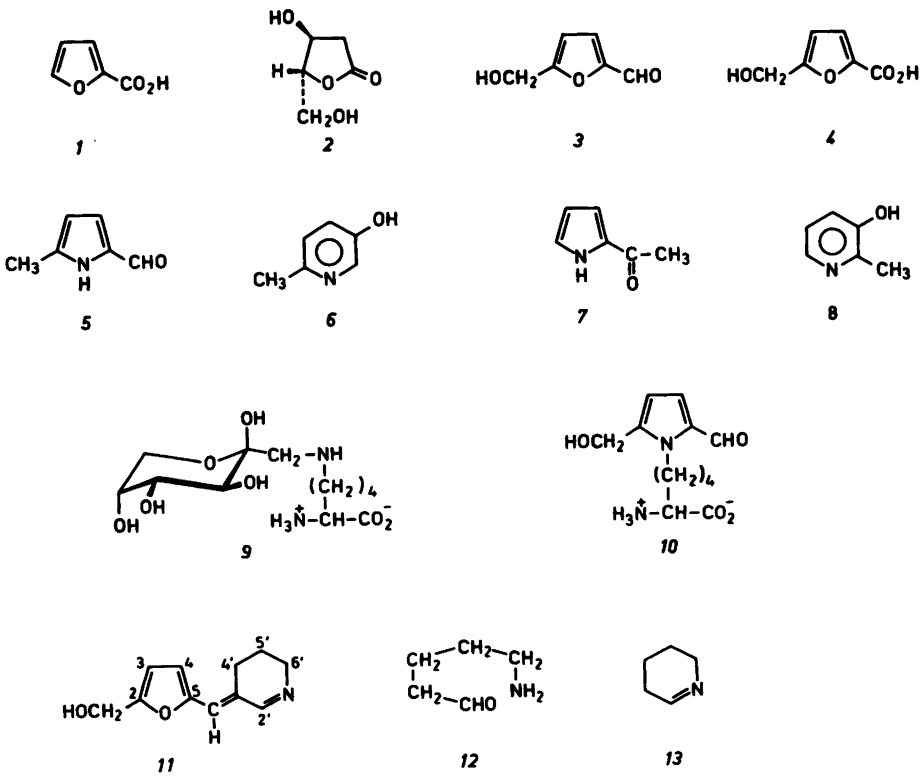
Chromatography of the hydrophilic products (residue D in Scheme 1) on Sephadex LH-20 yielded the Amadori rearrangement product *9*, while cation exchange chromatography (IEC) of residue D gave the amino acids *10* and lysine. Product *9* and the lysine were identical (TLC[19] and ^{1}H NMR) with authentic samples. Despite rechromatography on various materials, *10* did not show a correct elemental analysis, probably owing to partial decomposition on drying. However, its ^{1}H and ^{13}C NMR spectra showed no impurities and agreed with data for *10*, independently prepared (and regarded as the L-form) by Kato *et al.*[20] A recently[21] prepared synthetic sample also showed the same spectra. The yield of *10* was *ca.* 2.1 % (calc. on Lys), while lysine was recovered in *ca.* 27 % of the original amount.

Both *10* and the lysine showed practically no optical activity and were no doubt racemic. L-Lysine suffered little or no loss of optical activity when recovered by IEC from a reaction mixture which had not been refluxed. However, the racemization of lysine during the title reaction was slow; when the reaction was performed in deuterium oxide solution, the ^{13}C NMR spectrum of the reaction mixture showed <20 % exchange of the α-hydrogen in lysine after refluxing for 3 h. Nevertheless, product *9* may have been a diastereomeric mixture of the D- and L-lysino forms. In formula *9*, the β-pyranose form of the D-fructose moiety is shown in its $^2C_5$ conformation, since this is the only one present in other Amadori rearrangement products at pH≥7.[22]

When the above experiment was repeated without addition of sodium hydroxide, the pH gradually decreased from 4.9 to 3.1. In addition to previously identified compounds, a new basic product (*11*) was detected. This product was isolated most conveniently by extraction of the whole reaction mixture with 1-butanol at pH 12. Chromatography of the concentrated extract on silica gel gave pure *11* in 0.9 % yield (calc. on Lys). The product was identical (TLC, MS and ^{1}H NMR) with a synthetic sample of *11*.[21] The (*E*)-configuration tentatively assumed in formula *11* was indicated by lanthanide-induced NMR shifts for the analogue without hydroxymethyl group.[21]

DISCUSSION

Compounds *3,5,7,9*, and *10* have already been identified as title reaction products.[7,12,13] Com-



pounds *3*, *5* and *7* are also among the products in the related reaction between D-lactose and N^{α}-formyl-L-lysine.[23] Compounds *1–8* are formed in the reaction of glucose with methylamine (*1*, *3* and *4*)[14] or glycine (*2–8*),[15,18] and their formation has been discussed previously. Analogues of *9*[2,3] and *10*[14,15] are also formed in these and similar reactions of glucose along well-established routes.

While *1–4* are derived entirely from the glucose, *5–8* also contain the α-amino group of an amino acid, which has suffered Strecker degradation.[18] In this reaction, the amino group of an α-amino acid is transferred to a carbonyl compound *via* Schiff base intermediates. In the process, the amino acid is oxidatively degraded to carbon dioxide and an aldehyde, while the carbonyl compound is reduced. In Maillard reactions, the latter is derived from a sugar. Dehydration and cyclization of the resulting amino sugar leads to heterocyclic nitrogen compounds, such as *5–8*, formally derived from deoxy sugars and ammonia. In Maillard reactions of hexoses, *5–8* may therefore arise from any α-amino acid, including lysine, but not from simple alkylamines, such as methylamine.[14]

The Strecker degradation of lysine, here indicated by the observation of *5–8*, should give rise to the aldehyde *12* (or a related Schiff base), which probably cyclizes rapidly to 2,3,4,5-tetrahydropyridine (*13*).[24] Compound *11* has been synthesized from *3* and *13* through aldol condensation and dehydration[21] and is probably formed in that way in the title reaction. If so, the failure to observe *11* in the first experiment may be due to the lower yield of *3* at the higher pH (4.5). An aldol condensation involving *12* or a precursor of *3* with subsequent cyclization to *11* cannot be ruled out, however.

The above results show that both amino groups of lysine are involved in the Maillard reaction. Thus, reactions of the α-amino group are responsible for products *5–8*, whereas only the ε-amino group has reacted in *9* and *10*. In *11*,

both amino groups have reacted. However, it is harder to express the relative importance of the two amino groups in quantitative terms, and this applies also to their catalytic effect on the formation of *1–4*.

In acetic acid at 100 °C, the racemization of L-lysine and other optically active α-amino acids is strongly catalysed by aldehydes.[25] The reaction is believed to proceed through a Schiff base. Although no such catalysis was observed in acetate buffer at pH 4 (after 1 h),[25] the slow (30 h) racemization of L-lysine in the title reaction probably follows a similar course. However, the catalyst is perhaps not an aldehyde but rather an α-dicarbonyl compound formed by sugar dehydration. Schiff bases derived from such a compound and an α-amino acid are important intermediates in the Strecker degradation.[18] The electron-withdrawing effect of their free carbonyl group should promote exchange of the α-hydrogen in the amino acid moiety.

Compared with the present experiments, Maillard reactions in food are often completed within a much shorter time. On the other hand, the water content may be much lower and the temperature higher, for example, in roasting or frying. In such cases, partial or even complete racemization of L-lysine and other free amino acids seems possible and should then contribute to the nutritional loss known to accompany Maillard reactions. For determining the nutritional value of some foods, methods which discriminate between the D- and L-amino acids may therefore be preferable.

EXPERIMENTAL

General

Materials. Reference samples of *2*,[26] *4*,[14] *5*,[27] *8*,[28] *9*,[19] *10* and *11*[21] were prepared according to the literature. Other chemicals were commercial samples. Solvents were freshly distilled before use. All solvent mixtures were prepared on a volume basis (v/v).

Chromatography. Sugar analyses were performed as described,[17] using a Varian 1840-1 gas chromatograph with OV-1 as the stationary phase. Unless otherwise stated, column chromatography (CC) was performed on silica gel (Merck 60, 230–400 mesh). All separations were monitored by TLC on silica gel (Riedel-de Haën, SIF) with the same eluent as in CC. After the TLC plates had been inspected in UV light, ethanolic *p*-anisaldehyde–sulfuric acid, phloroglucinol–hydrochloric acid,[29] aq. sodium carbonate–diazotized sulfanilic acid or ethanolic ninhydrin was used as spray reagent.

Spectrometry. The mass spectrum of *11* was recorded at 70 eV with a Finnigan 4021 instrument, using direct insertion and electron impact. The IR spectrum of *11* was recorded with a Perkin-Elmer 337 instrument. NMR spectra were recorded at 89.60 (^{1}H) or 22.53 MHz(^{13}C) with a Jeol FX-90Q instrument.

Other methods. Evaporations were performed at reduced pressure below 40 °C. pH was kept constant with a Metrohm TA 2–20 automatic titrator and changed by manual titration with 3 M hydrochloric acid or sodium hydroxide. Optical rotations (α_D) were measured in aqueous solution with a Perkin-Elmer 141 polarimeter. The melting point of *11* was determined under a Kofler hot-stage microscope and is corrected.

Experiment at constant pH 4.5

Anhydrous D-glucose (240 g, 1.33 mol) and L-lysine monohydrochloride (162 g, 0.89 mol) were dissolved in water (2.00 l). The solution was refluxed under nitrogen for 30 h, while its pH was kept at 4.5 by automatic titration with 3 M sodium hydroxide. After cooling, the solution was analysed for sugars[17] (see text) and processed according to Scheme 1. The resulting residues A–C and part of residue D were chromatographed as follows.

CC (MeCOEt, saturated with H_2O) of residue A yielded *1* (57 mg). Continued elution with methanol yielded *4* (150 mg). CC ($CHCl_3$–EtOAc–95 % EtOH, 15:2:2) of residue B yielded a mixture (15 mg) of *5* and *7*. CC ($CHCl_3$–EtOAc–95 % EtOH, 5:5:2) of residue C yielded *3* (24 mg), then a mixture (15 mg) of *6* and *8*, and finally *2* (23 mg). CC (H_2O) of residue D (5.0 g) on Sephadex LH-20 yielded *9* (28 mg). The isolated amounts of *1–9* may have been much smaller than those actually formed. IEC of residue D (6.5 g) was performed on a column of Dowex 50 WX 8 (Serva, 50–100 mesh, H^+ form) with water (600 ml) as eluent. Continued elution with 1 M pyridine (100 ml) yielded crude *10*. CC (MeCN–H_2O–95 % EtOH, 4:1:1) of this product by the "flash" technique[30] yielded pure *10* (dry wt. *ca.* 240 mg, 2.1 %), $|[\alpha]_D^{25}|<0.2°$ (*c* 4). The fractions obtained on continued elution of the Dowex column with 0.1 M sodium hydroxide (1.0 l) were pooled, brought to pH 5.5

(with HCl) and concentrated. CC (57 % EtOH) of the residue by the "flash" technique [30] yielded lysine monohydrochloride (*ca.* 2.2 g, 27 %), $[\alpha]_D^{25} \sim -0.85°$ ($c \sim 22$). The slight negative rotation is probably due to traces of *9*.

An aqueous solution of the L-lysine monohydrochloride used as starting material showed pH 5.5 and $[\alpha]_D^{25} +8.7°$ (*c* 22). Refluxing was omitted in a blank experiment. The L-lysine was recovered by IEC and converted to its monohydrochloride as described above. This showed $[\alpha]_D^{25} \geq +8°$ (c~22).

Experiment at pH 4.9–3.1

The following modified procedure is convenient for isolation of *5-(3,4,5,6-tetrahydropyrid-3-ylidenemethyl)-2-furanmethanol* (*11*).

Anhydrous D-glucose (32.4 g, 180 mmol) and L-lysine monohydrochloride (22.0 g, 120 mmol) were dissolved in water (270 ml). The solution was refluxed under nitrogen for 30 h and cooled. Sometimes, a very small, apparently polymeric precipitate had formed and was removed by filtration. The solution was brought to pH 12 and extracted with 1-butanol (3×100 ml). The extract was evaporated. CC (EtOAc–95 % EtOH, 1:1) of the residue by the "flash" technique [30] yielded a crude product (420 mg), which was rechromatographed in the same way. Pure *11* (210 mg, 0.9 %) was obtained, m.p. 104–105 °C (after recryst. from toluene). Anal. $C_{11}H_{13}NO_2$: C, H, N. MS, *m/e* (rel. int.): 191 (100, M), 174 (72), 77 (36), 51 (33), 42 (32), 41 (29), 160 (24), 79 (21), 105 (20), 91 (20). IR (KBr), $\tilde{\nu}_{max}$: 1515 (m), 1610 (s), 3100 (m) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.75 (5′-H_2, quintet), 2.5 (OH, broad s), 2.74 (4′-H_2, td), 3.64 (6′-H_2, td), 4.63 (2-CH_2, s), 6.40 (3-H, d), 6.42 (5-CH, broad s, overlap), 6.50 (4-H, d), 7.92 (2′-H, broad t); $|J|$ 1.9 (2′,6′), 1.9 (4′,5), 3.5 (3,4), 5.5 (5′,6′), 6.5 (4′,5′) Hz. The NMR data and assignments were confirmed by the appropriate spin decoupling experiments.

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