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Note

Formation of 1-amino-1,4-dideoxy-2,3-hexodiuloses and 2-aminoacetylfurans in the Maillard reaction

Bernhard Huber
Institut für Pharmazie und Lebensmittelchemie der Universität München, Sophienstr. 10, D-8000 München (F.R.G.)

and Franz Ledl*
Institut für Lebensmittelchemie und Analytische Chemie der Universität Stuttgart, Pfaffenwaldring 55, D-7000 Stuttgart (F.R.G.)

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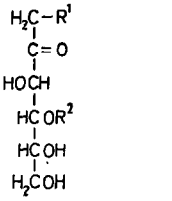
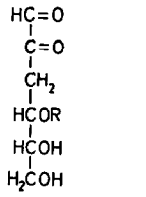
The reaction of D-glucose and maltose with piperidine and propylamine yields Amadori compounds (1-amino-1-deoxyketoses, **1**) that exist as cyclic hemiacetals. Amadori compounds have been detected in many heated and stored foods[2], and in the human body[3]. The degradation of **1a** and **1b** in heated aqueous solutions (pH 7) yields the 3-deoxyhexos-2-ulose **2a** and the 1-deoxy-2,3-hexodiulose[4] **3a**, respectively; from **1c**, the glucosyl-deoxyosones **2b** and **3b** and the 1,4-dideoxy-1-piperidino-2,3-hexodiulose **4a** were obtained[5]. Compounds **2–4** were trapped with *o*-phenylenediamine to give the quinoxalines **5ab**, **6ab**, and **7a**, which were quantified by g.l.c. and h.p.l.c. as the corresponding acetyl derivatives **5cd**, **6cd**, and **7c**.

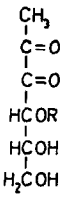
Furthermore, the aminoacetylfuran **8a** was separated[5] from a heated neutral solution of **1c**. Hitherto, substances of structure **8** have been obtained only after treatment of Amadori compounds with strong acids (a well-known example[6] is the



1 a R^1 = N⟨⟩, R^2 = H
b R^1 = NHPr, R^2 = H
c R^1 = N⟨⟩, R^2 = α-D-Glc
d R^1 = NHPr, R^2 = α-D-Glc



2 a R = H
b R = α-D-Glc



3 a R = H
b R = α-D-Glc

* Author for correspondence.

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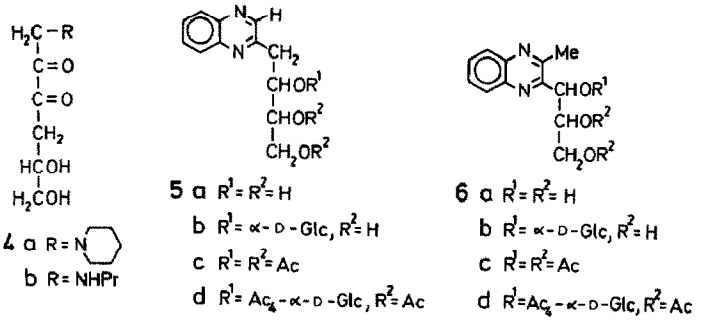


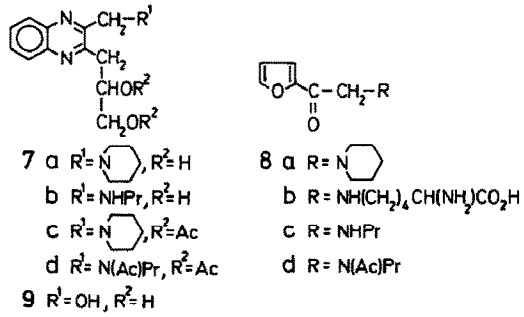
formation of furosine **8b**). Compounds of type **8** can be assumed to be degradation products of the osones **4**.

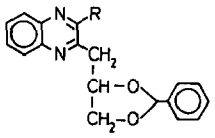
From these results, it may be inferred that, in the Maillard reaction, **4** and **8** are formed only when disaccharides and secondary amines are involved. Apart from corn and milk products, where proline, maltose, and lactose are important precursors of the Maillard reaction, in foods, and in the human body, primary amino acids or the ε-amino groups of lysine react with glucose and fructose. Therefore, the degradation pattern of the Amadori compounds **1b** and **1d** has been studied with regard to the formation of **4b** and **8c**.

In order to prove the formation of **4b**, *o*-phenylenediamine was used as a trapping reagent to give **7b**. Authentic **7b** was synthesized from the quinoxaline **9** derived from an alkaline mixture of maltose and *o*-phenylenediamine[7]. Benzylidenation of **9** gave **10a**, the formyl derivative (**10b**) of which was converted into **7b** by reduction of the imine. Compound **7b** was not stable, but the triacetate **7d** was amenable to g.l.c.

In order to develop a method for quantification, **8c** was isolated from a solution of **1b** heated with 6M hydrochloric acid[8]. The aminoacetylfuran **8c** is not stable in neutral aqueous solution, and the main degradation products are **11** and **12c** formed by oxidation and condensation, respectively. The structure of **12c** indicates that proteins can be cross-linked when aminoacetylfurans, formed from lysyl side-chains, undergo a condensation reaction. Cross-linked proteins have altered biological functions and thus cause complications in the human body[9].

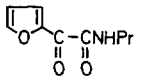
Since acetylated **7b** and **8c** are amenable to g.l.c., the degradation of the Amadori compounds **1b** and **1d** in heated aqueous solutions buffered to pH 5 was studied. This





10 a R = CH_2OH

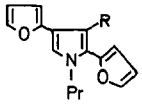
b R = CHO



11

pH was chosen because the stability of **7b** and **8c** decreases at higher pH. The propylaminoacetylfuran **8c** was formed from **1b** and **1d** in amounts comparable to that of other typical Maillard products. When *o*-phenylenediamine was added to the solutions of **1b** and **1d** before heating, **7b** was formed from **1d** and possibly from **1b**.

On the assumption that the enediol **13** is an intermediate in the degradation of **1** leading to **3** and **4**, it seems that the amine moiety at C-1 is eliminated from **13a** to produce the 1-deoxyosone **3a** in amounts higher that that of **4b**. In the enediol **13b**, the α-D-glucopyranosyl residue is a better leaving group than HO-4 in **13a** and therefore the deoxyosone **4b** is formed in higher amounts. Due to the lower stability of **7b** and **8c** in heated aqueous solutions at pH > 5, interpretations of the results based on the concentrations of **7b** and **8c** are only tentative. Further studies are necessary in order to assess the importance of the deoxyosones **4** and the aminoacetylfurans **8** in the Maillard reaction.

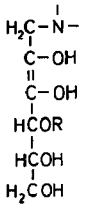


12 a R = OH

b R = OAc

c R = NHPr

d R = N(Ac)Pr



13 a R = H

b R = α-D-Glc

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi 510 apparatus and are uncorrected. I.r. spectra were recorded for KBr discs with a Perkin–Elmer 197 spectrometer. N.m.r. spectra (internal Me_4Si) were recorded with a Jeol 400 spectrometer. Mass spectra were produced with a Kratos MS-80 spectrometer equipped with a probe inlet. Silical Gel 60 F_{254} (Merck, 5554 and 5717) was used for t.l.c., and Silica Gel 60 (Merck, 9385) for column chromatography. Solvents were generally removed under diminished pressure. G.l.c. was performed with a Perkin–Elmer 8320 instrument

equipped with a flame-ionisation detector and a quartz capillary column (25 m × 0.25 mm i.d., permaphase, Perkin–Elmer 698345) coated with dimethylsilicone; the injection and detection ports were at 280° and the temperature programme was 100° → 200° at 6°. min.$^{-1}$ G.l.c.–m.s. was performed with a Carlo Erba 2110 instrument connected to a Varian MAT CH7 A spectrometer; the conditions and column were as described for the Perkin–Elmer instrument.

2-(2,3-Benzylidenedioxypropyl)-3-hydroxymethylquinoxaline (**10a**). — To a suspension of **9** (ref. 7) (469 mg, 2 mmol) in chloroform (5 mL) were added benzaldehyde (318 mg, 3 mmol) and *p*-toluenesulfonic acid (10 mg in 0.5 mL of 1,4-dioxane). The mixture was boiled under reflux for 3 h, triethylamine (0.5 mL) was then added, the solvent was removed, and the residue was dissolved in ethyl acetate (5 mL). Column chromatography (1:1 ethyl acetate–hexane) gave **10a** (325 mg, 50%) as a 1:1 mixture of the diastereomers, m.p. 102°; ν_{max} 3420, 2900, 1410, 1070, 770, and 700 cm^{-1}. ^{1}H-N.m.r. data ($CDCl_3$): δ 3.16 and 3.38 (2 dd, 2 H), 3.89 and 4.02 (2 dd, 1 H), 4.40 and 4.56 (2 dd, 1 H), 4.54 (s, OH), 4.91 and 5.03 (2 m, 1 H), 4.93 (s, 2 H), 5.81 and 6.00 (2 s, 1 H), 7.36 (m, 3 H), 7.45 (m, 2 H), 7.72 (m, 2 H), 8.04 (m, 2 H). Mass spectrum: *m/z* 322 (3%, M^+), 216 (84), 201 (45), 174 (100), 91 (94), 77 (68).

Anal. Calc. for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.88; H, 5.47; N, 8.73.

2-(2,3-Benzylidenedioxypropyl)-3-formylquinoxaline (**10b**). — To a solution of **10a** (3.22 mg, 1 mmol) in dichloromethane (3 mL) were added anhydrous sodium acetate (65 mg, 0.8 mmol) and pyridinium chlorochromate (345 mg, 1.6 mmol). The mixture was stirred for 3 h at room temperature, ether (15 mL) was added, and the mixture was filtered and concentrated. A solution of the residue in ethyl acetate (3 mL) was used for preparative t.l.c. (4:6 ethyl acetate–hexane) to give **10b** (130 mg, 41%) as a 1:1 mixture of the diastereomers (R_f 0.6), b.p. 125°/0.1 Torr; ν_{max} 3050, 2900, 1715, 1100, 770, and 700 cm^{-1}. ^{1}H-N.m.r. data ($CDCl_3$): δ 3.60 and 3.73 (2 dd, 2 H), 3.90 and 4.06 (2 dd, 1 H), 4.02 and 4.11 (2 dd, 1 H), 4.35 and 4.51 (2 dd, 1 H), 5.82 and 5.97 (2 s, 1 H), 7.32 (m, 3 H), 7.44 (m, 2 H), 7.76 (m, 2 H), 8.10 (m, 2 H), 10.28 and 10.31 (2 s, 1 H). Mass spectrum: *m/z* 320 (2%, M^+), 214 (100), 197 (63), 185 (44), 149 (40), 91 (85), 77 (33).

Anal. Calc. for $C_{19}H_{16}N_2O_3$: C, 71.24; H, 5.03; N, 8.75. Found: C, 71.19; H, 5.28; N, 8.50.

2-(2,3-Diacetoxypropyl)-3-(N-propylacetamidomethyl)quinoxaline (**7d**). — A solution of **10b** (96 mg, 0.3 mmol), propylamine (hydrochloride, 286 mg, 3 mmol), and sodium cyanoborhydride (12.5 mg, 0.2 mmol) in anhydrous methanol (3 mL) was stirred for 24 h at room temperature. Acetic acid (0.05 mL) was added, the solvent was removed, and a solution of the residue in methanol (1 mL) was adjusted to pH 12 with 2M sodium hydroxide. After removal of water and methanol, the residue was acetylated in chloroform (3 mL) with acetic anhydride (0.5 mL) and sodium acetate (20 mg) by boiling under reflux for 3 h. After filtration and removal of the solvent and unreacted acetic anhydride, the residue was dissolved in methanol (2 mL) and aqueous 6% sulfuric acid (2 mL). The mixture was stirred for 24 h at room temperature, then neutralized with 2M sodium hydroxide and concentrated. A solution of the residue in methanol (3 mL)

was filtered and concentrated, and the residue was acetylated as described above. Preparative t.l.c. (ethyl acetate) of the product gave **7d** (30 mg, 25%), R_F 0.5, b.p. 145°/0.1 Torr; ν_{max} 2930, 1740, 1640, 1225, 1040, and 760 cm^{-1}. ^{1}H-N.m.r. data ($CDCl_3$): δ 0.94 (t, 3 H), 1.66 (m, 2 H), 1.99 (s, 3 H), 2.10 (s, 3 H), 2.21 (s, 3 H), 3.36 (dd, 2 H), 3.39 (t, 2 H), 4.29 (dd, 1 H), 4.48 (dd, 1 H), 4.95 (d, 2 H), 5.75 (m, 1 H), 7.70 (m, 2 H), 8.00 (m, 2 H). Mass spectrum: *m/z* 401 (1%, M^+), 358 (4), 302 (100), 260 (78), 200 (40), 183 (82), 169 (76), 159 (38), 72 (34).

Anal. Calc. for $C_{21}H_{27}N_3O_5$: C, 62.83; H, 6.78; N, 10.47. Found: C, 62.71; H, 6.85; N, 10.47.

N-*(2-Furoylmethyl)*-N-*propylacetamide* (**8d**). — A suspension of **8c** (ref. 8) (oxalate, 257 mg, 1 mmol) in chloroform (5 mL), acetic anhydride (2 mL), and sodium acetate (100 mg) was boiled for 3 h under reflux, then filtered, and concentrated. Column chromatography of the residue (ethyl acetate) gave **8d** (180 mg, 86%), b.p. 100°/0.1 Torr; ν_{max} 3120, 2950, 1640, 1470, 1250, and 770 cm^{-1}. ^{1}H-N.m.r. data ($CDCl_3$): δ 0.94 (t, 3 H), 1.62 (m, 2 H), 2.20 (s, 3 H), 3.34 (t, 2 H), 4.64 (s, 2 H), 6.55 (dd, 1 H), 7.27 (dd, 1 H), 7.59 (dd, 1 H). Mass spectrum: *m/z* 209 (2%, M^+), 150 (7), 138 (7), 114 (31), 110 (16), 100 (53), 95 (17), 81 (13), 72 (100).

Anal. Calc. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.70. Found: C, 62.85; H, 7.50; N, 6.77.

*Isolation of 2-(*N-*phenyloxamoyl)furan* (**11**). — The pH of a solution of **8c** (ref. 8) (oxalate, 257 mg, 1 mmol) in water (4 mL) was adjusted to pH 7–8 with sodium hydroxide and the solution was stirred at room temperature for 2 h. Sulfuric acid (6 mL) was added, the solution was extracted with ethyl acetate (3 × 10 mL), and the combined extracts were concentrated. Preparative t.l.c. (4:6 ethyl acetate–hexane) of the residue gave **11**, R_F 0.7, isolated as colourless oil (9 mg, 5%), b.p. 95°/0.1 Torr. ^{1}H-N.m.r. data ($CDCl_3$): δ 0.91 (t, 3 H), 1.57 (m, 2 H), 3.27 (m, 2 H), 6.56 (dd, 2 H), 7.30 (s, NH), 7.69 (dd, 1 H), 8.13 (dd, 1 H). Mass spectrum: *m/z* 181 (80%, M^+), 124 (15), 96 (70), 95 (100), 86 (85), 68 (66).

Isolation of 3-acetoxy-2,4-bis(2-furyl)-1-propylpyrrole (**12b**) and *2,4-bis(2-furyl)-1-propyl-3-(*N-*propylacetamido)pyrrole* (**12d**). — The pH of a solution of **8c** (ref. 8) (oxalate, 257 mg, 1 mmol) in water (4 mL, buffered with 500 mg of potassium dihydrogen phosphate) was adjusted to 7.5 by the addition of 2M sodium hydroxide. The solution was heated for 1 h at 100° and then extracted with ethyl acetate (3 × 5 mL), the combined extracts were concentrated, and the residue was acetylated as described above. Preparative t.l.c. (4:6 ethyl acetate–hexane) of the product gave **12b** (2 mg, 1,5%), R_F 0.75, isolated as a colourless oil. ^{1}H-N.m.r. data ($CDCl_3$): δ 0.91 (t, 3 H), 1.73 (m, 2 H), 2.30 (s, 3 H), 3.97 (t, 2 H), 6.20 (dd, 1 H), 6.37 (dd, 1 H), 6.39 (dd, 1 H), 6.46 (dd, 1 H), 6.93 (s, 1 H), 7.33 (dd, 1 H), 7.47 (dd, 1 H). Mass spectrum: *m/z* 299 (28%, M^+), 257 (80), 228 (100), 214 (17), 186 (24), 158 (22), 92 (39), 63 (23).

Also isolated was **12d** (8 mg, 5.5%) as a slightly yellow, syrupy oil, R_F 0.65. ^{1}H-N.m.r. data ($CDCl_3$). δ 0.70 (t, 3 H), 0.89 (t, 3 H), 1.30 (m, 2 H), 1.75 (m, 2 H), 1.95 (s, 3 H), 3.94 (t, 2 H), 4.06 (t, 2 H), 6.19 (dd, 1 H), 6.37 (dd, 1 H), 6.39 (dd, 1 H), 6.47 (dd, 1 H), 7.03 (dd, 1 H), 7.49 (dd, 1 H). Mass spectrum: *m/z* 340 (84%, M^+), 298 (24), 269 (41), 256 (21), 241 (42), 198 (17), 169 (17), 84 (100).

Detection of **8c** *in heated aqueous solutions of* **1b** *and* **1c**. — A solution of the Amadori compound **1b** (ref.10) (oxalate, 311 mg, 1 mmol) and dipotassium hydrogenphosphate (200 mg) in water (7 mL; pH adjusted to 5 with 2M sodium hydroxide) and 1,4-dioxane (7 mL) was boiled for 3 h under reflux, then concentrated. A solution of the residue in anhydrous methanol was filtered and concentrated, and the residue was acetylated. G.l.c. of the product revealed **8d** (T 20.05), the identity of which was confirmed by g.l.c.–m.s. (M^+ at m/z 209).

After treatment of **1d** (ref. 5) (oxalate, 474 mg, 1 mmol) in a similar manner, **8c** was detectable in comparable amounts.

Detection of **7b** *in heated aqueous solutions of* **1b** *and* **1d**. — The reaction was performed as described for the detection of **8c**, with the exception that *o*-phenylenediamine (216 mg, 2 mmol) was added and the heating time was 8 h. In the solution from **1d**, **7d** was detected (T 35.76) and identified by g.l.c.–m.s. (M^+ at m/z 401). In the solution from **1b**, a considerably smaller peak with T 35.76 was obtained and the identification by g.l.c.–m.s. was not definite.

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