Maillard Reactions of Lactose and Maltose

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Maillard type reactions of disaccharides with 1,4-glycosidic linkage have been investigated. When lactose is heated with a primary amine in nearly neutral aqueous solution, the β-pyranone **5b**, the cyclopentenone **7b**, and the isomaltol derivative **8b** are obtained as main products. The disaccharide-derived degradation products **5b**, **7b**, and **8b** react further with primary amines to give pyrroles of type **10b**, furanones **12**, pyridinium betaines **9b**, pyridones **13b**, and **14**, and pyridoneimines **11**, respectively. Analogous products are obtained from maltose. Modeling side-chain reactions of proteins, these investigations were carried out with several primary amines, in particular with propylamine and α-*N*-acetyllysine.

INTRODUCTION

Reactions of several carbohydrates with amino acids, proteins, or simple amines have been thoroughly investigated. These processes, which are of importance in many heated foods, lead to the formation of flavoring ingredients, browning products, high molecular weight compounds (melanoidins), reductones and aminoreductones, and mutagenic substances and to the loss of nutritive value (Ledl and Schleicher, 1990). Many low molecular weight volatile reaction products have been identified, but our knowledge concerning the thermal degradation of sugars is far from complete.

Some investigations have shown that reaction products obtained from monosaccharides on the one hand and disaccharides on the other are different. When maltose or lactose is heated with a primary amine in nearly neutral aqueous solution, the β-pyranones **5a,b** and the cyclopentenone derivatives **7a,b** are obtained as main products. Prolonged heating leads to the formation of the stable isomaltol glycosides **8a,b** (Kramhöller et al., 1992). The β-pyranone **5b** was detected in heated milk for the first time by Ledl et al. (1986). Besides these sugar dehydration products, we were able to isolate and to identify the pyridinium betaines **9a,b** and the pyridones **14** with different amine components (Ledl et al., 1989).

All products discussed so far are typical of the degradation of carbohydrates with a 1,4-glycosidic linkage. Until now no similar compounds have been obtained from D-glucose or D-fructose. The maltose- and lactose-derived products **5a,b** and **7a,b** are unstable intermediates. In this paper we describe the reactions with primary amines.

MATERIALS AND METHODS

Apparatus. For liquid chromatography a Merck L-6200 gradient pump and a Merck programmable photodiode array detector, Model D-6500, with Merck DAD-Manager Software and a NEC pinwriter P60, were used. IR spectra were recorded in KBr disks with a Perkin-Elmer 197 spectrometer. NMR spectra (internal standard tetramethylsilane) were recorded with a JEOL 400 GSX spectrometer. Mass spectral analyses were obtained with a Varian MAT CH7 (EI, 70 eV). For UV spectra a Hitachi U 1100 spectrophotometer was used. CHN analyses were obtained with a Heraeus CHN Rapid.

Reagents. HPLC grade solvent (acetonitrile) was used without further purification. The water used for HPLC was distilled and filtered through a 0.45-μm nylon membrane. All solvents were degassed with helium. Thin-layer chromatography (TLC) was performed using 20 cm × 20 cm glass plates coated with a 0.5- or 2.0-mm thickness of silica gel 60 F_{254}.

High-Performance Liquid Chromatography (HPLC). About 75 μL of the sample was diluted with 1 mL of a solution of 1% triethylamine in methanol, filtered, and injected into the HPLC. Separation was performed on a column packed with Nucleosil 5C-18 (250 mm × 4.6 mm i.d., 5-μm particle size) (Figures 1-3), Nucleosil 120 5C-18 (250 mm × 4.6 mm i.d., 5-μm particle size) (Figure 4), and Lichrosorb RP 18 (250 mm × 4.6 mm i.d., 5-μm particle size) (Figures 5 and 6). All columns were protected with a guard cartridge (25 mm × 4.6 mm i.d.) packed with the same materials as the columns. The eluents used were water (A) and acetonitrile (B) with a gradient of 0-100% B in 25 min and a flow rate of 0.8 mL/min for Figures 1-4 and 0.05 M triethylamine acetate buffer (pH 7.0) (A) and acetonitrile (B) with a gradient of 0-50% B in 25 min and a flow rate of 1.0 mL/min (Figures 5 and 6). The substances were detected by a diode array detector from 210 to 360 nm. Identification of the samples was achieved by comparison of the retention times and UV spectra with those of the synthesized reference compounds. Compounds **10b** with R′ = propyl and **9b** and **12** with R′-NH_2 = α-*N*-acetyllysine were identified by UV spectra.

Preparation of the Reference Compounds. 1-[3-(α-D-Glucopyranosyloxy)-2-furanyl]-1-ethanone (**8a**) was synthesized according to a method reported by Goodwin (1983). 1-[3-(β-D-Galactopyranosyloxy)-2-furanyl]-1-ethanone (**8b**) was obtained according to the method of Hodge and Nelson (1961).

4-(Glucopyranosyloxy)-2-methyl-1-propylpyridinium-3-olate (**9a**) and 4-(galactopyranosyloxy)-2-methyl-1-propylpyridinium-3-olate (**9b**) were prepared according to methods of Ledl et al. (1989).

1-[3-(α-D-Glucosyloxy)-1-propyl-2-pyrrolyl]-1-ethanone (**10a**) was obtained according to the method of Estendorfer et al. (1990).

*Preparation of the Mixture of 4-(α-D-Glucopyranosyloxy)-2-hydroxy-2-methyl-2H-pyran-3(6H)-one (**5a**) and 4,5-Dihydroxy-2-(α-D-glucopyranosyloxy)-5-methyl-2-cyclopenten-1-one (**7a**).* 1-Deoxy-1-piperidinomaltulose (4 g, 9.8 mmol), which can be obtained according to the method of Hodge and Nelson (1961), was heated at 60 °C for 3 h in 20 mL of water (phosphate buffer, pH 6.8). The water was removed under reduced pressure at 70 °C and the residue dissolved in 10 mL of methanol and filtered. The filtrate was purified by silica gel chromatography (5.5 cm × 9 cm) with ethyl acetate–methanol (9:1). Fraction 1 (200 mL) was removed from fraction 2 (350 mL), which contained **5a** and **7a**, the solvent was evaporated. The residue, in which also a small amount of **8a** occurred, was used for further investigations.

*Preparation of the Mixture of 4-(β-D-Galactopyranosyloxy)-2-hydroxy-2-methyl-2H-pyran-3(6H)-one (**5b**) and 4,5-Dihydroxy-2-(β-D-galactopyranosyloxy)-5-methyl-2-cyclopenten-1-one (**7b**).* Forty-five grams of lactose (0.12 mol), 37 g of ethanol, and 13 mL of triethylamine (0.094 mol) were heated at 75 °C until the lactose was dissolved. Then 25 mL of piperidine (0.25 mol) and 15 mL of acetic acid (0.26 mol) were added dropwise.

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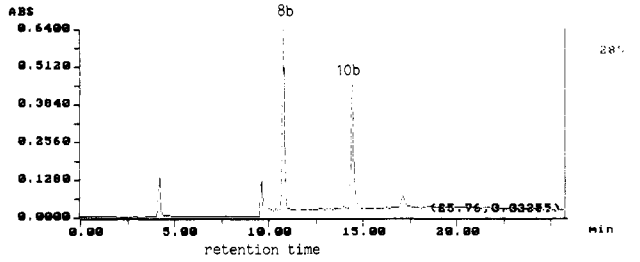

Figure 1. HPLC chromatogram for degradation of the mixture of **5b** and **7b** with propylamine in nearly neutral aqueous solution. Detection, UV 285 nm. Numbers on top of peak refer to structure in Figures 8–10 with R′ = propyl.

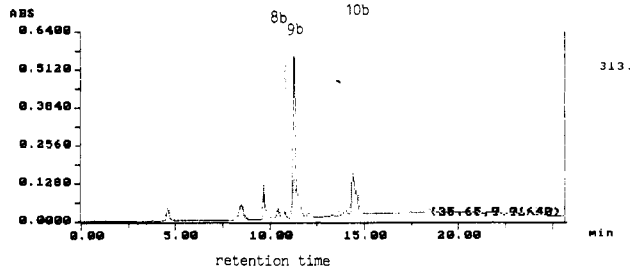

Figure 2. HPLC chromatogram for degradation of the mixture of **5b** and **7b** with propylamine in methanol. Detection, UV 285 nm. Numbers on top of peak refer to structure in Figures 8–10 with R′ = propyl.

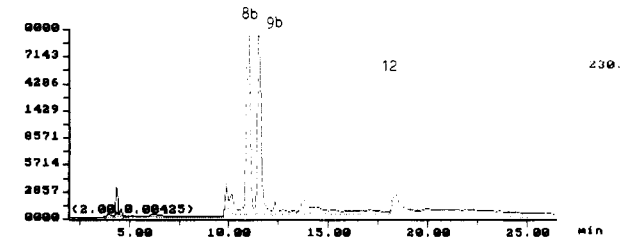

Figure 3. HPLC chromatogram for degradation of galactosylisomaltol with propylamine in nearly neutral aqueous solution. Detection, UV 230 and 346 nm. Numbers on top of peak refer to structures in Figures 9 and 10, with R′ = propyl.

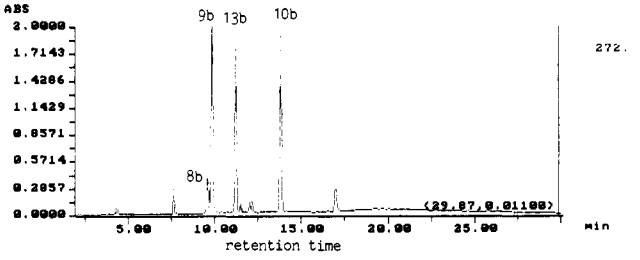

Figure 4. HPLC chromatogram for degradation of galactosylisomaltol with propylamine at slightly basic pH value. Detection, UV 272 nm. Numbers on top of peak refer to structures in Figures 8–10 with R′ = propyl.

The mixture was heated for 1 h under reflux and concentrated under reduced pressure at 45 °C. The crude syrup was used for further investigations. The syrup (4 g) was heated in 20 mL of 0.5 M phosphate buffer (pH 7.0) for 3 h at 70 °C. After removal of the solvent under reduced pressure at 70 °C, the residue was dissolved in methanol and filtered. The products were purified by silica gel chromatography (5.5 cm × 10 cm) with ethyl acetate and increasing amounts of methanol. Fraction 1 (300 mL of ethyl acetate) was removed, and from fraction 2 (400 mL of ethyl acetate–methanol 8:2), which contained **5b** and **7b**, was evaporated the solvent under reduced pressure at 45 °C. The residue was separated by TLC (2 mm) with ethyl acetate–methanol (2.5:1). From a band with an R_f value of 0.3–0.5 were eluted compounds **5b** and **7b** with hot methanol. After removal of the solvent, the residue, which contained also a small amount of **8b**, was used for further investigation.

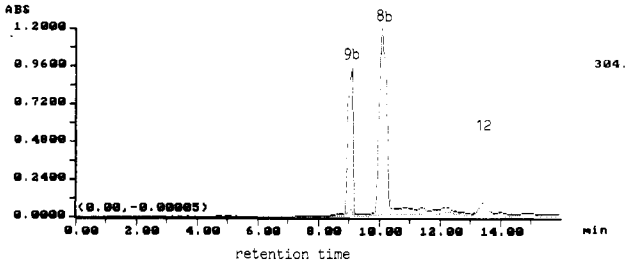

Figure 5. HPLC chromatogram for degradation of galactosylisomaltol with α-*N*-acetyllysine at slightly basic pH value. Detection, UV 304 and 343 nm. Numbers on top of peak refer to structures in Figures 8–10 with R′–NH_2 = α-*N*-acetyllysine.

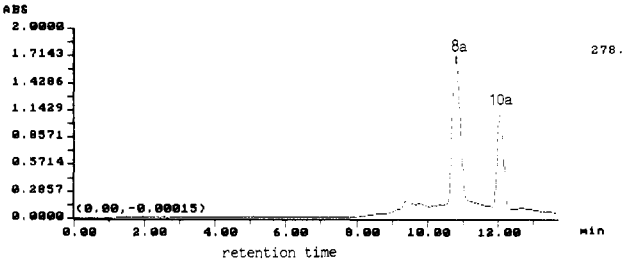

Figure 6. HPLC chromatogram for degradation of the mixture of **5a** and **7a** with α-*N*-acetyllysine in neutral aqueous solution. Detection, UV 278 nm. Numbers on top of peak refer to structures in Figures 8–10 with R′–NH_2 = α-*N*-acetyllysine.

2-Acetamido-6-[1-oxoethyl-3-(α-D-glucopyranosyloxy)-1-pyrrolyl]hexanoic Acid (***10a***). An aqueous solution (1 mL, 0.5 M phosphate buffer, pH 7.0) of α-*N*-acetyllysine (500 mg, 2.7 mmol) and a mixture of **5a** and **7a** (300 mg, ca. 1 mmol) were heated under reflux for 2 h. [α-*N*-Acetyllysine was synthesized according to the method of Hardy et al. (1976).] The water was removed under reduced pressure at 70 °C, and the residue was dissolved in 1 mL of methanol and filtered. The filtrate was separated and purified by TLC (2 and 0.5 mm) with methanol–ethyl acetate (7:3). From a band with an R_f value of 0.55 was eluted compound **10a** with hot methanol. ^{1}H NMR (CD_3OD) δ 1.25–1.83 (m, 6 H, $CH_2CH_2CH_2$), 1.98 (s, 3 H, CH_3CONH), 2.52 (s, 3 H, CH_3CO), 3.31–3.87 (m, 6 H, glucose), 4.18–4.22 (m, 3 H, CH_2N, HOOC-CHN), 5.47 (d, 1 H, J = 3.7 Hz, CH-1-glu), 6.06 (d, 1 H, J = 3 Hz, CH=CN), 6.87 (d, 1 H, J = 3 Hz, C=CHN); UV (CH_3OH) λ_{max} 287 nm (ϵ = 4600); MS (FAB) +FAB m/z 481 (M + Na), 459 (M + 1), 437 (M + Na, $-CO_2$).

2-[1-(Propylamino)ethylidenyl]-3-(2H)furanone (***12***). Galactosylisomaltol (400 mg, 1.4 mmol) and propylammonium acetate (480 mg, 4.0 mmol) were heated in 1 mL of water (pH 6.8, phosphate buffer) under reflux for 1 h. The water was removed under reduced pressure at 70 °C and the residue dissolved in 1 mL of methanol and filtered. The filtrate was separated by TLC (2 and 0.5 mm) with ethyl acetate–methanol (9:1). A band with an R_f value of 0.49 was eluted with methanol. After distillation, **12** was obtained as a yellow oil: bp 115 °C (9 × 10^{-2} Torr; yield 12.5%); ^{1}H NMR ($CDCl_3$) δ 1.0 (t, 3 H, CH_3-CH_2CH_2), 1.66 (m, 2 H, CH_2CH_2), 2.2 (s, 3 H, CH_3C=C), 3.2 (t, 2 H, CH_2N), 6.0 (d, 1 H, HC=CHO), 7.56 (d, 1 H, OCH=CH); ^{13}C NMR ($CDCl_3$) δ 11.1 ($CH_3CH_2CH_2$), 12.2 (CH_3C=), 22.6 (CH_2-CH_2N), 44.4 (CH_2N), 109.8 (HC=CHO), 132.2 (OC=CN), 150.3 (NC=C), 155.5 (OCH=CH), 178.6 (C=O) (the identification of the signals of the ^{1}H NMR and ^{13}C NMR was completed by a COLOC spectrum at 5 Hz); UV (CH_3OH) λ_{max} 347 nm (log ϵ = 4.29); EI-MS m/z (relative intensity) 167 (M^+, 67), 152 (M^+ – CH_3, 42), 138 (M^+ – CH_2 – CH_3, 42), 97 (100).

3-(Galactopyranosyloxy)-2-methyl-1-propyl-4(1H)-pyridone (***13b***). Betaine **9b** (30 mg, 0.09 mmol) was boiled in 2 mL of absolute dimethylformamide for 7 h. Compound **13b** was purified twice by TLC (2 and 0.5 mm) using ethyl acetate–methanol (4:6) and was obtained from a band with an R_f value of 0.46. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3 H, CH_3CH_2), 1.70 (m, 2 H, CH_2CH_2N), 2.48 (s, 3 H, CH_3C=C), 3.38–3.72 (m, 6 H, CHOH), 3.93 (m, 2 H, CH_2N), 4.50 (s, 1 H, J = 7.7 Hz, OCHO), 6.39 (d, 1 H, J = 7.7 Hz, CH=CHN), 7.68 (d, 1 H, J = 7.7 Hz, NCH=CH);

CI-MS m/z (relative intensity) 168 (M + 1 - $C_6H_{10}O_5$, 100); UV (CH_3OH) λ_{max} 278 (log ϵ = 3.95); IR 3600–3000, 2950, 1630, 1560, 1510, 1250, 1120, 1090, 1020 cm^{-1}.

Preparation of the Samples. *Degradation of the Mixture of* ***5a*** *and* ***7a*** *or* ***5b*** *and* ***7b****, Respectively, with Propylamine in Nearly Neutral Aqueous Solution.* One hundred and fifty milligrams of the mixture of **5a** and **7a** or **5b** and **7b**, respectively, 300 mg of propylamine, and 300 mg of acetic acid were dissolved in 2.5 mL of water (phosphate buffer, pH 6.8) and heated for 2 h under reflux.

Degradation of the Mixture of ***5a*** *and* ***7a*** *or* ***5b*** *and* ***7b****, Respectively, with Propylamine in Methanol.* One hundred and fifty milligrams of the mixture of **5a** and **7a** or **5b** and **7b**, respectively, 300 mg of propylamine, and 300 mg of acetic acid were dissolved in 7 mL of methanol and heated for 1 h under reflux. Heating for 2 h leads to similar results.

Degradation of Galactosylisomaltol or Glucosylisomaltol, Respectively, with Propylamine in Nearly Neutral Aqueous Solution. Galactosylisomaltol (290 mg, 1.0 mmol) or glucosylisomaltol (290 mg, 1.0 mmol), respectively, and 60 mg (1.0 mmol) of propylamine were dissolved in 2 mL of water (phosphate buffer, pH 7.3). The pH value was adjusted to pH 7.3 with acetic acid, and the solution was boiled for 2 h under reflux.

Degradation of Galactosylisomaltol or Glucosylisomaltol, Respectively, with Propylamine at Slightly Basic pH Value. Galactosylisomaltol (400 mg, 1.4 mmol) or glucosylisomaltol (400 mg, 1.4 mmol), respectively, 240 mg (4.1 mmol) of propylamine, 30 mg (0.2 mmol) of KH_2PO_4, and 43 mg (0.2 mmol) of Na_2HPO_4 were dissolved in 1 mL of water and heated for 1 h under reflux.

Degradation of the Mixture of ***5a*** *and* ***7a*** *with α-N-Acetyllysine in Neutral Aqueous Solution.* Three hundred milligrams of the mixture of **5a** and **7a** and 500 mg (2.7 mmol) of *α-N*-acetyllysine were heated in 1 mL of water (phosphate buffer, pH 7.0) at 100 °C for 30 min.

Degradation of Galactosylisomaltol with α-N-Acetyllysine at Slightly Basic pH Value. Galactosylisomaltol (300 mg, 1.0 mmol) and 500 mg (2.7 mmol) of *α-N*-acetyllysine were heated in 1 mL of water (phosphate buffer, pH 8.8) at 100 °C for 1 h.

Preparation of 1,4-Dihydro-2-methyl-1-propyl-4-(propylimino)-3-pyridinol (11). Five hundred milligrams of the mixture of **5a** and **7a** was heated with 480 mg (8.1 mmol) of propylammonium acetate in 5 mL of methanol under reflux. The solution was fractionated by column chromatography on silica gel: fraction 1, 200 mL of ethyl acetate; fraction 2, 300 mL of ethyl acetate–methanol (8:2); fraction 3, 200 mL of ethyl acetate–methanol (6:4); fraction 4, 200 mL of ethyl acetate–methanol (1:1). Fractions 3 and 4 contained **11**. The solvents were removed under reduced pressure, and the residue was purified further by TLC using ethyl acetate–methanol (6:4). Compound **11** was obtained from a band with an R_f value of 0.34. ^1H NMR ($CDCl_3$) δ 0.96 (t, 3 H, CH_3CH_2), 1.00 (t, 3 H, CH_3CH_2), 1.69 (m, 2 H, CH_2CH_2N=C), 1.79 (m, 2 H, CH_2CH_2N), 2.52 (s, 3 H, CH_3C=C), 3.20 (t, 2 H, CH_2N=C), 3.92 (t, 2 H, NCH_2CH_2), 6.20 (d, 1 H, J = 6.60 Hz, C*H*=CHCN), 6.99 (d, 1 H, J = 6.60 Hz, NC*H*=CH); ^{13}C NMR ($CDCl_3$) δ 10.9 (CH_3CH_2), 11.6 (CH_3CH_2), 12.2 (CH_3C=C), 22.4 (CH_2CH_2N=C), 24.1 (CH_2CH_2N), 44.1 (CH_2N=), 57.4 (CH_2N), 98.4 (CH=CHN), 127.2 (NCH=CH), 129.7 (Cq), 150.1 (Cq), 153.6 (Cq); IR 3400, 2920, 1610, 1600, 1570, 1490, 1230 cm^{-1}; UV (CH_3OH) λ_{max} 320 nm (log ϵ = 3.89); EI-MS m/z (relative intensity) 208 (M^+, 81), 193 ($M^+ - CH_3$, 65), 179 ($M^+ - CH_2 - CH_3$, 100), 151 (54), 137 (94), 124 (59).

Preparation of 6-Acetoxy-4-(α-D-2,3,4,6-tetraacetylglucopyranosyloxy)-4-hexene-2,3-dione (6a). One hundred and fifty milligrams of a mixture of **5a** and **7a** reacted with acetic anhydride/pyridine at room temperature overnight. The acetylated products were separated by TLC (0.5 mm) with toluene–ethyl acetate (1:1). From a band with an R_f value of 0.66 a mixture of α- and β-D-pentaacetylglucose and compound **6a** as peracetyl derivative were eluted with hot ethyl acetate. The mixture was separated again by TLC (0.5 mm) with diethyl ether–hexane (3:1). From a band with an R_f value of 0.34 was obtained compound **6a** (acetylated) as a bright yellow oil, which boils between 160 and 170 °C (0.1 Torr, yield 0.2%). ^1H NMR ($CDCl_3$) δ 2.05–2.12 (6 s, 18 H, CH_3C=O), 4.13 (m, J = 5.1, 10.3 Hz, 1 H, C*H*-5_{glu}), 4.32 and 4.35 (2 dd, J = 10.3, 5.1 Hz, J = 10.3, 2.2 Hz, 2 H, CH_2-6_{glu}), 4.48 (dd, J = 10.3, 3.7 Hz, 1 H, C*H*-2_{glu}), 4.80 and 4.84 (2 dd, J = 13.2, 6.6 Hz, 2 H, CH_2CH=C), 5.15 (t, J = 9.9 Hz, 1 H, C*H*-4_{glu}), 5.45 (t, J = 9.9 Hz, 1 H, CH_3-3_{glu}), 5.61 (d, J = 3.7 Hz, 1 H, α-C*H*-1_{glu}), 6.32 (t, J = 6.6 Hz, 1 H, C=C*H*CH_2); ^{13}C NMR ($CDCl_3$) δ 20.48, 20.58, 20.67, 20.74, 20.80, 21.03 (CH_3O), 57.94 (CH_2CH=C), 61.01 (*C*-6_{glu}), 66.65 (*C*-4_{glu}), 70.09 (*C*-2_{glu}), 70.46 (*C*-3_{glu}), 75.17 (*C*-5_{glu}), 91.11 (*C*-1_{glu}), 118.21 (C=*C*O_{glu}), 138.94 (*C*=C), 156.08 (C_3=O), 169.29–171.16 (CH_3C=O); UV (CH_3OH) λ_{max} 245 nm (ϵ = 6625). Anal. Calcd for $C_{22}H_{28}O_{14}$: C, 51.16; H, 5.46. Found: C, 50.56; H, 5.62.

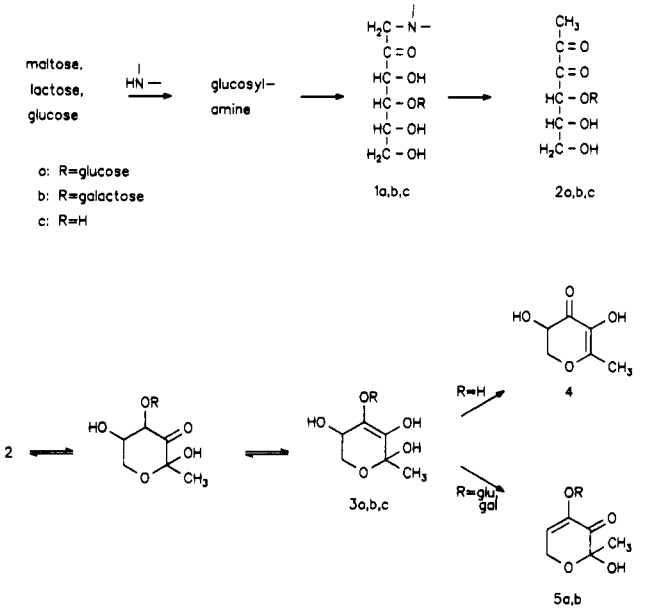

Figure 7. Initial steps of the Maillard reaction of maltose, lactose, and D-glucose.

RESULTS AND DISCUSSION

When D-glucose, maltose, or lactose is reacted with primary amines, Amadori compounds **1a–c** can be obtained in high yields. Amino sugars of this type are unstable when heated or stored. Degradation in nearly neutral solution leads to the formation of 1-deoxyhexosuloses **2a–c** as main products. The reactivity of compounds **2a–c** prevents purification, but stable quinoxaline derivatives are obtained when *o*-phenylenediamine is added to the reaction mixture (Beck et al., 1988; Nedvidek et al., 1992). For simplification, open-chain structures are written. Indeed, cyclic hemiacetals are predominant. When D-glucose is heated with amino acids, proteins, or simple amines, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**4**) can be isolated as a product typical of the Maillard reaction (Severin and Seilmeier, 1968; Mills et al., 1970; Ledl et al., 1976). In contrast, the degradation products obtained from maltose or lactose under comparable conditions are compounds with β-pyranone structure **5a,b** (Ledl et al., 1986).

The different behavior of mono- and disaccharides can be derived from the intermediates **3**, shown in the reaction scheme (Figure 7). Elimination of water from **3c** (R = H) leads to the formation of the dihydro-γ-pyrone **4**, whereas in disaccharide-derived intermediates **3a,b** (R = glu or gal) elimination of the OH group at C5 is in accordance with expectation.

Formation of **5a,b**, **7a,b**, and **8a,b** from maltose or lactose, respectively, has already been studied in detail. The results are summarized as follows: When disaccharides or the Amadori compounds **2** are heated for a short time (for instance, 70 °C, 15 min), the β-pyranones **5a,b** are the main products and minor amounts of **7a,b** can be detected. With continued heating the amounts of **7a,b**

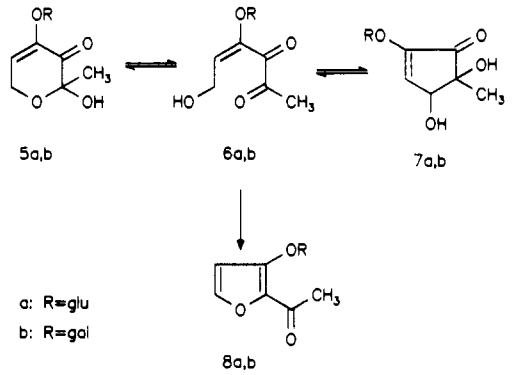

Figure 8. Continuing degradation of maltose and lactose.

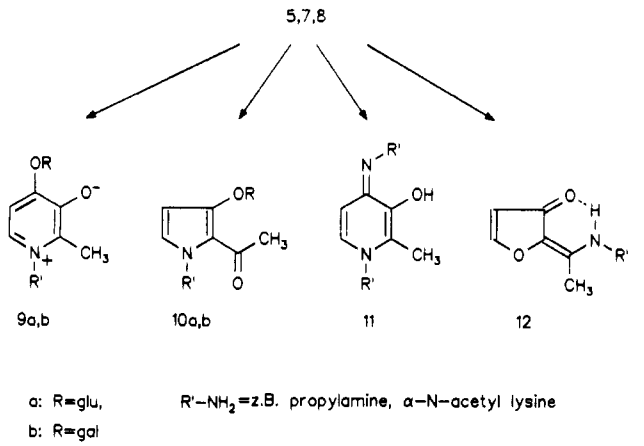

Figure 9. Reaction of **5**, **7**, and **8** with primary amines.

increase. Only after prolonged heating do the stable isomaltol glycosides **8a,b** dominate (Kramhöller et al., 1992) (Figure 8).

In addition to these results, we were able to isolate the open-chain intermediate **6a** as peracetyl derivative (see Materials and Methods), but the yield was low.

The disaccharide degradation products can be separated from unreacted sugars or Amadori compounds by chromatography on silica gel, but they are labile intermediates which react further with primary amines to give some N-containing heterocycles. Heating a mixture of **5a,b** and **7a,b** with propylamine in nearly neutral aqueous solution leads to the formation of the pyrrole derivatives **10a,b** (Figure 9) and the isomaltol derivatives **8a,b** as main products. When the same reaction is performed in boiling methanol, besides **8a,b** and **10a,b**, appreciable amounts of the pyridinium betaines **9a,b** (Figure 9) can be detected. The corresponding HPLC chromatograms for the separation of the galactosyl derivatives are shown in Figures 1 and 2.

Compared to **5** and **7** the isomaltol glycosides **8** are less reactive. However, when **8b** is heated with propylamine at 100 °C (2 h, neutral solution), the pyridinium betaine **9b** and the furanone derivative **12** (Figure 9) are obtained as main products (Figure 3). When the same reaction is performed under slightly basic conditions, **9b** isomerizes to a considerable extent to give the galactosyloxypyridone **13b** (Figures 4 and 10), and besides these compounds the pyrrole derivative **10b** can be detected. Recently the synthesis of 2-acetyl-3-hydroxy-1-*n*-propylpyrrole from isomaltol was reported (Bartulin et al., 1992). The structures of **12** and **13b** are established by spectroscopic data (see Materials and Methods).

Likewise, glucosylisomaltol reacts with propylamine in neutral aqueous solution to give **9a** as main product, whereas only a comparatively small amount of **12** is

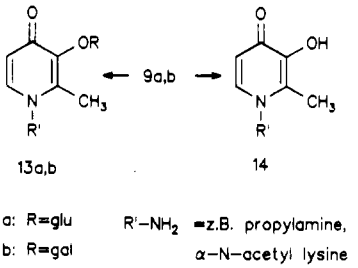

Figure 10. Reaction of **9a,b** to give γ-pyridone derivatives.

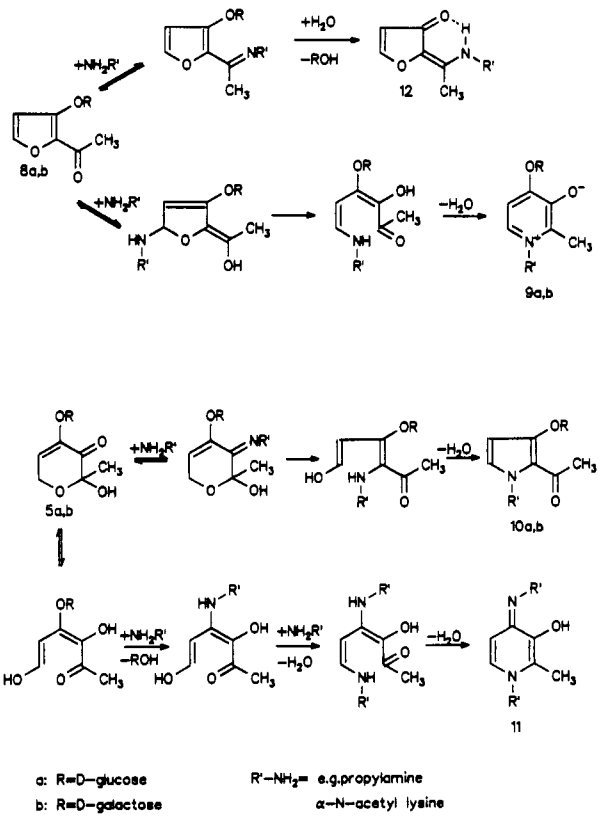

Figure 11. Proposed reaction mechanisms.

obtained. Under slightly basic conditions **10a** arises besides the main product **9a**.

After prolonged heating, in particular at slightly acid pH value, **9a** and **9b** readily eliminate the sugar residue to give rise to the pyridones **14** (Figure 10), which are strong complexing agents for several metal ions (Severin and Loidl, 1976; Ledl et al., 1989; Kontoghiorghes, 1985).

When **5a** and **7a** are heated with propylamine in concentrated solution, besides **9a** and **10a**, the pyridoneimine **11** can be isolated by chromatography on silica gel. The pyridoneimine **11** is a basic compound and forms stable complexes with several metal ions (Figure 9).

A reaction mechanism for the formation of the products **9a,b**, **10a,b**, **11**, and **12** that arise from the β-pyranones **5a,b** or the isomaltol glycosides **8a,b**, respectively, is proposed in Figure 11.

The investigations have been extended to α-*N*-acetyllysine as a protein model, and the results are as expected. Heating of α-*N*-acetyllysine with a mixture of **5a** and **7a** leads to the formation of the lysine-derived pyrrole derivative **10a**. This substance was synthesized for the first time, and the structure was established unequivocally by spectral data (see Materials and Methods). Certainly pyrroles such as **10** with galactose as sugar portion and a protein-bound lysine residue are formed in heated milk. Galactosylisomaltol reacts with α-*N*-acetyllysine to give

galactosyl betaine **9b** and furanone derivative **12** (see Figures 5 and 6).

If it is assumed that proteins react in the same way, the formation of the pyridoneimines of type **11** means a cross-linking of proteins. Recently it has been reported that lactose is effective in the cross-linking of proteins, but the molecular base is still unknown.

It must be emphasized that the degradations of maltose and lactose discussed above are reactions prevailing under neutral conditions. Degradation of the Amadori compounds **1** under slightly acidic conditions leads to the formation of a mixture of **2** and 3-deoxyhexosuloses. The aldohexosuloses give rise to several other reaction products, and further investigations will be carried out in this direction.

ACKNOWLEDGMENT

This work was supported by grants from Fond der Chemischen Industrie and Deutsche Forschungsgemeinschaft.

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Received for review September 18, 1992. Accepted December 7, 1992.