Maillard Reaction of Free and Nucleic Acid-Bound 2-Deoxy-D-ribose and D-Ribose with ω-Amino Acids

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The Maillard reaction of free and nucleic acid-bound 2-deoxy-D-ribose and D-ribose with ω-amino acids (4-aminobutyric acid, 6-aminocaproic acid) was investigated under both stringent and mild conditions. Without (with) amines 2-deoxy-D-ribose (D-ribose) displays the strongest browning activity, and DNA is much more reactive than RNA. From stringent reaction between 2-deoxy-D-ribose (or DNA) and methyl 4-aminobutyrate, methyl 4-[2-[(oxopyrrolidinyl)methyl]-1-pyrrolyl]-butyrate (**12**) was identified by GC/MS and NMR as a new 2-deoxy-D-ribose specific key compound trapped by pyrrolidone formation. Levulinic acid-related N-substituted lactames **13**−**15** were identified as predominant products from DNA with amino acids, whereas RNA paralleled the reaction with D-ribose. α-Angelica lactone (**2**), a significant degradation product of DNA, and thiols leads under mild conditions to new addition products (e.g., **17** with glutathione). Probable reaction pathways considering activating effects of the polyphosphate backbone of nucleic acids are discussed.

Keywords: *Maillard reaction of nucleic acids/2-deoxy-D-ribose; levulinic acid; α-angelica lactone; trapping of 2-(hydroxymethyl)pyrrole; pyrroles from ω-amino acids and riboses/nucleic acids*

INTRODUCTION

Maillard reactions, i.e., nonenzymatic aminocarbonyl reactions between reducing sugars and free or peptide-bound α- and ω-amino acids, are of relevance in thermally processed food systems (Ledl and Schleicher, 1990) and have been shown to cause covalent modifications of long-lived extracellular proteins in vivo (Brownlee, 1992). Consequences of the in vivo reactions are protein glycosylation, cross-linking, and cleavage as well as formation and deposition of fluorescent age pigments with pathobiochemical implications for human diabetes and the aging process (Brownlee, 1992).

Nucleic acids, both DNA and RNA, are an abundant source of cellularly occurring polymer-bound sugars (D-ribose, 2-deoxy-D-ribose) as well as of amino group containing bases (A, G, C); e.g., about 5% of the dry weight of a yeast cell consists of nucleic acids (Reiff et al., 1962). Recently, several investigators have raised the general question of whether DNA structure and function are affected by incubation with intracellularly occurring sugars and sugar phosphates under mild conditions (Bucala et al., 1984, 1985; Lee and Cerami, 1987). It has been argued that the amino groups of DNA bases may serve as a crucial target of covalent modification by Maillard reaction. N^2-(1-Carboxyethyl)-guanine was isolated as a corresponding product (Ochs and Severin, 1994; Papoulis et al., 1995; Nissl et al., 1996).

On the basis of the facts that (1) base loss from nucleic acids is a frequent process under stringent food-processing conditions and is also operative in vivo (about 10 000 sites of base loss from DNA/day/human cell) giving rise to a polymer-bound open chain sugar phosphate (Lindahl, 1993), (2) sugar phosphates display highly accelerated Maillard reactivity compared to their nonphosphate analogues (Bunn and Higgins, 1981), and (3) nucleic acids are abundantly complexed by polyamines and histones (containing up to 30% lysine residues), we reevaluated the hypothesis of Maillard reactivity of nucleic acids (Wondrak and Tressl, 1994). Thus, we investigated the reaction between ω-amino acids and nucleic acid-bound sugars, under both stringent (160 °C) and mild (40 °C) conditions. Continuing our previous work on Maillard reaction pathways (Tressl et al., 1993a,b, 1994) we characterized the products of model compounds of peptide-bound lysine (6-aminocaproic acid, 4-aminobutyric acid) reacting with D-ribose, 2-deoxy-D-ribose, RNA, and DNA, respectively. For comparison, incubations of the nucleic acids without addition of ω-amino acids were studied.

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EXPERIMENTAL PROCEDURES

Materials and Methods. Herring sperm DNA (free acid) was from Sigma Chemical Co. 2-Deoxy-D-ribose was from Lancaster Synthesis GmbH. α-Angelica lactone was from Aldrich, and RNA (from *Torula utilis*), N^6-furfuryladenine, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), and all other reagents were from Fluka AG. Autoclaving was done in a stainless steel laboratory autoclave (Roth, I series) equipped with a 100 mL duran glass tube and heated by an electric heater with a magnetic stirrer. During autoclaving the peak temperature (160 °C) was reached after 45 min. Long term incubations at 40 °C were done in air-tight 20 mL derivatization vessels with light exclusion.

Browning Reactions. Nucleic acids (1 g) or an equivalent amount of sugars (0.40−0.45 g, corresponding to about 40% (w/w) sugar content of DNA/RNA) and methyl 6-aminocaproate (1 g) in 15 mL of phosphate buffer (0.5 M, pH 7) were incubated at 40 °C. Time dependent browning of the reaction mixture was assayed by absorbance measurements with a Uvikon spectrophotometer 922, Kontron Instruments. Turbid solutions were filtered using Sartorius Minisart SRP 15 disposable filter holders. Absorbance at 420 nm (A_{420}) was determined

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Table 1. MS and NMR Spectra of Selected Products, Characterized in Model Experiments of Free and Nucleic Acid-Bound Pentoses with or without ω-Aminocarboxylic Acids[a]

compound	MS data/NMR data
5-methyl-3(2*H*)-furanone (**4**)	98 (51), 69 (9), 68 (29), 43 (19), 40 (100), 39 (78)
methyl 4-[2-[(oxopyrrolidinyl)methyl]-1-pyrrolyl]butyrate (**12**)	264 (100), 181 (26), 180 (47), 163 (70), 148 (39), 120 (51), 106 (27), 101 (90), 98 (79), 94 (7), 80 (31), 59 (57), 41 (40); ^{1}H NMR 1.94 (qui, 4H, J = 7.6 Hz, 4′-CH_2, N-CH_2-CH_2-CH_2-CO_2CH_3), 2.29 (t, 2H, J = 7.6 Hz, CH_2-CO_2CH_3), 2.39 (t, 2H, J = 8.1 Hz, 3′-CH_2), 3.23 (t, 2H, J = 7.1 Hz, 5′-CH_2), 3.64 (s, 3H, -CO_2CH_3), 3.90 (t, 2H, J = 7.4 Hz, N-CH_2-CH_2-CH_2-CO_2CH_3), 4.40 (s, 2H, pyrrolyl-CH_2-N), 6.05 (mc, 2H, H-3, H-4), 6.64 (t, J = 2.2 Hz, 5-H); ^{13}C NMR 17.4 (s, N-CH_2-CH_2-CH_2-CO_2-), 26.89, 30.84, 31.17, 37.87, 45.53, 46.16, 51.74 (pr, OCH_3), 107.16/110.03 (t, C-3/4), 121.83 (t, C-5), 126.41 (q, C-2), 173.27/174.18 (C=O)
methyl 6-(5-methylene-2-oxopyrrolidin-1-yl)caproate (**13**)	225 (11), 210 (10), 194 (15), 166 (6), 152 (10), 138 (3), 124 (3), 111 (100), 98 (70), 82 (27), 55 (36), 41 (36); ^{1}H NMR 1.3−1.4 (m, 2H, 4-CH_2), 1.56 (qui, 2H, J = 7.5 Hz, 3-CH_2), 1.65 (qui, 2H, J = 7.5 Hz, 5-CH_2), 2.31 (t, 2H, J = 7.6 Hz, CH_2-CO_2CH_3), 2.49 (mc, 2H, 3′-CH_2), 2.69 (mc, 2H, 4′-CH_2), 3.45 (t, 2H, J = 7.5 Hz, N-CH_2), 3.67 (s, 3H, CO_2CH_3), 4.21 and 4.13 (each q, 1H, exo C=CH^AH^B); ^{13}C NMR 23.7, 24.3, 24.6, 26.2 (3-, 4-, 5-, 4′-CH_2), 29.0, 33.9, 39.6 (2-, 6-, 3′-CH_2), 51.5 (OCH_3), 84.0 (exo CH_2), 147.7 (5′-Cq), 174.0, 175.9 (2 × C=O)
methyl 6-(5-methyl-2-oxopyrrolin-1-yl)caproate (**14**)	225 (39), 210 (7), 194 (36), 193 (46), 165 (34), 150 (46), 136 (15), 122 (15), 111 (76), 110 (82), 98 (56), 82 (100), 69 (51), 55 (66), 41 (76); ^{1}H NMR 1.3−1.4 (m, 2H, 4-CH_2), 1.56 (qui, 2H, J = 7.5 Hz, 3-CH_2), 1.65 (qui, 2H, J = 7.5 Hz, 5-CH_2), 1.98 (dt, 3H, $J_1 = J_2$ = 2 Hz, 5′-CH_3), 2.31 (t, 2H, J = 7.6 Hz, CH_2-CO_2CH_3), 2.97 (dq, 2H, $J_1 = J_2$ = 2.5 Hz, 3′-CH_2), 3.42 (t, 2H, J = 7.5 Hz, N-CH_2), 3.67 (s, 3H, CO_2CH_3), 4.94 (mc, 1H, 4′-CH)
methyl 6-(5-methyl-2-oxopyrrolidin-1-yl)caproate (**15**)	227 (6), 196 (9), 180 (7), 154 (14), 140 (12), 126 (12), 112 (100), 84 (25), 55 (18), 41 (19); ^{1}H NMR 1.17 (d, 3H, J = 6.5 Hz, CH-CH_3), 1.29 (qui, 2H, J = 7.7 Hz, 4-CH_2), 1.40−1.70 (m, 4H, 3-CH_2 and 5-CH_2), 2.35 (mc, 2H, 3′-CH^AH^B), ≈1.52 (m, 1H, 4′-CH^AH^B), 2.15 (mc, 1H, 4′-CH^AH^B), 2.29 (t, 2H, J = 7.7 Hz, CH_2-CO_2CH_3), 3.40 and 2.89 (each mc, 1H, N-CH^AH^B), 3.64 (s, 3H, CO_2CH_3), 3.66 (mc, 1H, CH-CH_3)
4-[[2-(methoxycarbonyl)ethyl]thio]-γ-valerolactone (**16**)	218 (M^+, 0), 119 (2), 99 (100), 71 (14), 55 (11), 43 (74); ^{1}H NMR 2.16 (s, 3H, C-CH_3), 2.59 (t, 2H, J = 6.9 Hz, CH_2-CO_2H_3), 2.73−2.84 (m, 4H, ring CH_2-CH_2), 3.09 (t, 2H, J = 6.9 Hz, S-CH_2), 3.67 (s, 3H, CO_2CH_3)
4-(glutathion-*S*-yl)-γ-valerolactone (**17**)	FAB/MS 404 $(M - H)^-$, 406 $(M + H)^+$; ^{1}H NMR (∼1:1 mixture of diastereomers) 2.12 (mc, 2H, Glu-β-CH_2), 2.18 (2 × s, 3H, C-CH_3), 2.3−2.6 (m, 4H, S-CH_2, Glu-γ-CH_2), 2.85 (mc, 4H, ring CH_2-CH_2), 3.4, 3.8 (each mc, 1H, Glu-α-CH), 3.91 (mc, 2H, Gly-CH_2), 4.51, 4.58 (each mc, 1H, Cys-α-CH)
N^6-furfuryladenine[b] (**7**)	329 (84), 300 (60), 273 (22), 244 (43), 96 (19), 81 (100), 73 (100), 53 (44), 41 (20)

[a] MS: *m*/*z* (rel intensity). NMR: δ (ppm), *J* (Hz); s, singlet; d, doublet; t, triplet; q, quartet; qui, quintet; m, multiplet; mc, center of multiplet. [b] Identified as MTBSTFA derivative.

against solvent blanks as a function of time (Figures 1 and 2). Samples were diluted if A_{420} exceeded 1.

Sample Preparation. *Reaction of* D-*Ribose (2-Deoxy-*D-*ribose, RNA, DNA) with ω-Amino Acid Methyl Esters.* D-Ribose (2-Deoxy-D-ribose, RNA, DNA) (1 g) was reacted with methyl 6-aminocaproate (1 g) under the following conditions: (a) in 0.5 M phosphate buffer (20 mL, pH 7) at 160 °C for 2 h and (b) in 0.5 M phosphate buffer (20 mL, pH 7) at 40 °C for 4 weeks. Under the same conditions, 2-deoxy-D-ribose and DNA were reacted with methyl 4-aminobutyrate. For comparison, D-ribose (2-deoxy-D-ribose, RNA, DNA) (1 g) was incubated without addition of an ω-amino acid methyl ester under the following conditions: (a) in distilled water (20 mL) at 160 °C for 15 min and (b) in 0.5 M phosphate buffer (20 mL, pH 7) at 40 °C for 4 weeks. After addition of 3-hexanol and 6-maleimidocaproic acid as internal standards for quantification, the reaction mixtures were adjusted to pH 2 with 1 N HCl and extracted with diethyl ether (3 × 30 mL). Acidic compounds were separated from the combined ether extracts by extraction with 5% $NaHCO_3$ (2 × 5 mL). The ether extract was dried over anhydrous sodium sulfate and concentrated to about 0.5 mL on a 20 cm Vigreux column. The pH of the combined aqueous phases was readjusted to 2 with 1 N HCl, and the acids were extracted with diethyl ether (3 × 30 mL) and concentrated as described before. The neutral extract was directly analyzed by capillary GC/MS (column D), whereas the acid-containing extract was analyzed by capillary GC/MS (column C) after derivatization with BF_3 (10% in methanol) as described elsewhere (Metcalfe and Schmitz, 1961).

Reaction of Levulinic Acid with Methyl 6-Aminocaproate. Levulinic acid (1 g) and methyl 6-aminocaproate (1 g) were dissolved in 0.5 M phosphate buffer (20 mL) and reacted under the following conditions: (a) 2 h, 160 °C; (b) 12 h, 40 °C; (c) 4 weeks, 40 °C. Neutral ether extracts were prepared as described above.

Isolation and Derivatization of N^6-Furfuryladenine. DNA (1 g) was incubated under the following conditions: (a) in distilled water (20 mL) at 160 °C for 15 min and (b) in 0.5 M phosphate buffer (20 mL, pH 7) at 40 °C for 4 weeks. At room temperature and pH 7, the mixture was extracted with freshly distilled ether (3 × 100 mL). The combined organic phases were extracted with 0.05 N HCl (2 × 10 mL). The aqueous extract was readjusted to pH 7 and again extracted with ether (2 × 75 mL). This ether extract was dried over anhydrous sodium sulfate, and the ether was totally evaporated on a 20 cm Vigreux column. The residue was redissolved in 1 mL of freshly distilled and dried pyridine. For GC/MS analysis a 25 μL aliquot was derivatized (Woo and Chang, 1993). After transfer to a Teflon-capped derivatization vessel, 15 μL of MTBSTFA and 2 μL of triethylamine were added. The mixture was heated at 80 °C (30 min); 2 μL of this mixture was analyzed by capillary GC/MS (column C). Authentic N^6-

furfuryladenine was used as a standard for identification and quantification (by external standardization). For MS data, see Table 1.

Isolation of 5-Methyl-3(2H)-furanone (4). DNA (1 g) in water (20 mL) was autoclaved for 15 min at 160 °C. The ether extract (3 × 30 mL) was fractionated by preparative GC (column A) yielding 1 mg of the pure compound. For MS and NMR data, see Table 1.

Isolation of Methyl 4-[2-[(Oxopyrrolidinyl)methyl]-1-pyrrolyl]-butyrate (12). Methyl 4-aminobutyrate (6 g) and 2-deoxy-D-ribose (6 g) were dissolved in 0.5 M phosphate buffer (100 mL, pH 7) and refluxed for 14 h. After extraction with ethyl acetate (3 × 100 mL) the combined organic phase was washed with 0.1 N HCl (2 × 20 mL) and saturated aqueous NaCl (+5% $NaHCO_3$; 2 × 30 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was separated by column chromatography on silica gel 60 (Merck Chemical Co.; activity IV, column 20 × 1 cm) into five fractions with pentane (F1, 40 mL), diethyl ether (F2, 40 mL), and ethyl acetate (F3−F5, 20 mL each). Fractions F4 and F5 were combined, evaporated, and redissolved in methanol. The combined methanol extract of four 6 g batches was subjected to preparative HPLC as described below; yield: 2.5 mg. For MS and NMR data, see Table 1.

Isolation of Methyl 6-(5-Methyl-2-oxopyrrolidin-1-yl)caproate (15). DNA (6 g) and 6-aminocaproic acid (2 g) were dissolved in distilled water (30 mL) and autoclaved at 160 °C for 2 h. The pH was adjusted to 2 with 1 N HCl. The mixture was extracted with ethyl acetate (3 × 30 mL), and the carboxylic acids were separated from the ethyl acetate phase by extraction with 5% $NaHCO_3$ (3 × 5 mL). After the pH was readjusted to 2 with 1 N HCl, the acids were extracted with ethyl acetate as described before. The organic phase was dried, concentrated, and derivatized with BF_3 (10% in methanol) as already described. The methyl esters were subjected to preparative TLC (silica gel 60, 0.5 mm, ethyl acetate/ethanol, 10:1). A broad band at $R_f = 0,65$ was eluted with ethyl acetate, and after evaporation and dissolution in methanol subjected to HPLC, as described below, 2 mg of pure **15** was obtained. For MS and NMR data, see Table 1.

Isolation of Methyl 6-(5-Methylene-2-oxopyrrolidin-1-yl)-caproate (13) and Methyl 6-(5-Methyl-2-oxopyrrolin-1-yl)caproate (14). Levulinic acid (2 g) and 6-aminocaproic acid (1 g) in distilled water (20 mL) were autoclaved at 160 °C for 2 h. The reaction mixture was extracted and derivatized as described for compound **15** and subjected to preparative GC (column B) as described below; 2 mg of a pure mixture of **13**/**14** was isolated. For MS and NMR data, see Table 1.

Model Reaction of α-Angelica Lactone (2) with Methyl 3-Mercaptopropionate, Glutathione, and Methyl 6-Aminocaproate. α-Angelica lactone (10 μL) and methyl 6-aminocaproate (10 mg) were solubilized in 50 mM phosphate buffer (pH 7, 0.9 mL) + methanol (0.2 mL). After 14 h at 40 °C the mixture was extracted with diethyl ether (2 × 1 mL). The ether extract was dried, concentrated (see above), and subjected to capillary GC/MS analysis (column C). With methyl-3-mercaptopropionate (100 μL) by the same procedure (100 μL of α-angelica lactone), 3.5 mg of 4-[[2-(methoxycarbonyl)ethyl]-thio]-γ-valerolactone (**16**) was isolated by preparative GC (column B). For MS and NMR data see Table 1. Glutathione (100 mg) and α-angelica lactone (500 μL) in water/methanol (5:1) (6 mL) were incubated for 14 h at 40 °C. The mixture was extracted with ether (4 × 5 mL) to remove the unreacted α-angelica lactone. An aliquot (400 μL) of the aqueous phase was evaporated by a stream of nitrogen. 4-(Glutathion-*S*-yl)-γ-valerolactone (**17**) was isolated in quantitative yield. For FAB/MS and ^1H NMR spectra, see Table 1.

Preparative GC. Two packed glass columns (3 m) were used. Column A: 15% Carbowax 20M on Chromosorb W-AW/DMCS (80−90 mesh), temperature was programmed from 70 to 220 °C at 4 °C/min. Column B: 5% SE 30 on Chromosorb W-AW/DMCS (100−110 mesh), temperature was programmed from 180 to 230 °C at 4 °C/min.

Preparative HPLC. The components of the methanol extracts were separated on a Waters model 510 HPLC system with a Spherisorb ODS II, 5 μm, 250 mm × 8 mm column (Bischoff, Germany). The isocratic eluent was methanol/water (3:2) (flow rate, 1.5 mL/min; UV detection at 230 nm). Combined fractions were evaporated, redissolved in water free methanol, and identified by GC/MS.

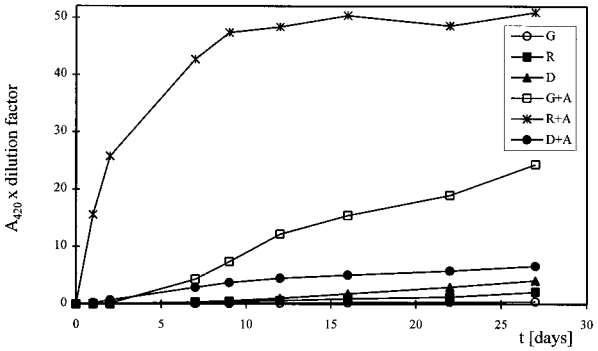


Figure 1. Browning during long term incubation (40 °C, pH 7, 0.5 M phosphate buffer) of sugars (G = D-glucose, R = D-ribose, D = 2-deoxy-D-ribose) and sugar/amine mixtures (A = methyl 6-aminocaproate).

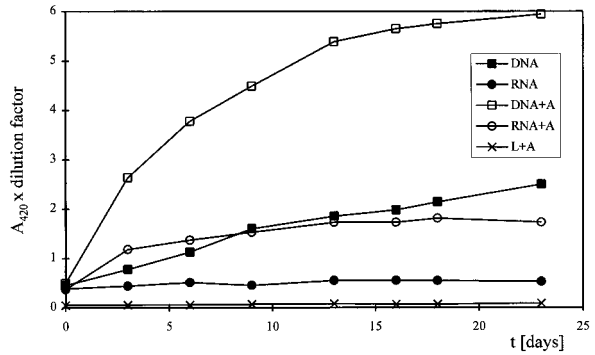


Figure 2. Browning during long term incubation (40 °C, pH 7, 0.5 M phosphate buffer) of nucleic acids and nucleic acid/amine mixtures (A = methyl 6-aminocaproate, L = levulinic acid).

Gas Chromatography (GC)/Mass Spectrometry (MS). The extracts prepared were analyzed by GC/MS using a 60 m × 0.32 mm i.d. DB-1 fused silica gel capillary column (column C) or a 50 m × 0.32 mm i.d. fused silica gel capillary column coated with Carbowax 20M (column D) coupled with a double-focusing mass spectrometer CH5-DF (Varian MAT), ionization voltage 70 eV, resolution 2000 (10% valley). Temperature was programmed from 80 to 280 °C at 4 °C/min (for MTBSTFA-derivatized samples starting from 165 °C) or from 80 to 230 °C at 4 °C/min, respectively.

FAB/Mass Spectrometry. The fast atom bombardment mass spectra (8 keV, xenon) of the glutathione derivative **17** were recorded on the CH5-DF spectrometer using glycerol as matrix.

$^1H/^{13}C$ NMR Spectroscopy. ^1H NMR spectra were recorded at 270 (500 MHz) on Bruker WH 270 and AMX 500 NMR spectrometers in $CDCl_3$ and D_2O solution, respectively. Chemical shifts are referenced to tetramethylsilane (TMS) as internal standard. Coupling constants (*J*) are in hertz.

RESULTS AND DISCUSSION

Naturally occurring sugars (D-glucose, D-ribose, 2-deoxy-D-ribose) as well as nucleic acids (DNA, RNA) show browning reactions during long term incubation (40 °C) with or without primary amines. In Figures 1 and 2 the increase of A_{420} over a period of 4 weeks is shown for sugars and nucleic acids, respectively.

The most remarkable browning phenomena are the following: (1) With amines D-ribose is the most active compound. Generally, amines induce a much stronger browning. (2) Surprisingly, even in the presence of amines, DNA shows stronger browning than RNA.

Table 2. Selected Compounds Characterized from Sugar and Nucleic Acid Incubations without Amines (Data Represent Concentrations in ppm)

compound	D-ribose, 160 °C[a]	2-deoxy-D-ribose, 160 °C[a]	RNA 160 °C[a]	RNA 40 °C[b]	DNA 160 °C[a]	DNA 40 °C[b]
levulinic acid (**1**)		29	1680		89200	55
α-angelica lactone (**2**)		46			130	35
β-angelica lactone (**3**)		2			480	47
5-methyl-3(2*H*)-furanone (**4**)					1370	71
2-(hydroxymethyl)furan (**5**)		4300			380	278
2-formylfuran (**6**)	1120	10	34800	89		30
N^6-furfuryladenine (**7**)	nd	nd			1430	57

[a] 15 min, distilled water. [b] 4 weeks, phosphate buffer (0.5 M, pH 7).

Table 3. Selected Compounds Characterized from Sugar and Nucleic Acid Incubations with Methyl 4-Aminobutyric Acid and Methyl 6-Aminocaproic Acid, Respectively (Data Represent Concentrations in ppm)

compound	D-ribose, 160 °C[a]	2-deoxy-D-ribose, 160 °C[a]	RNA 160 °C[a]	RNA 40 °C[b]	DNA 160 °C[a]	DNA 40 °C[b]	levulinic acid 160 °C[a]	levulinic acid 40 °C[b]
methyl 6-(2-formyl-1-pyrrolyl)hexanoate (**8**)	2000	420	300	25				
methyl 6-(1-pyrrolyl)hexanoate (**9**)	300	910	85		220			
methyl 6-(2-acetyl-1-pyrrolyl)hexanoate (**10**)	300	125						
methyl 6-(3,4-dimethyl-2,5-dioxo-2,5-dihydropyrrol-1-yl)hexanoate (**11**)	3500		(+)[c]					
methyl 4-[2-[(oxopyrrolidinyl)methyl]-1-pyrrolyl]butyrate[d] (**12**)		500			(+)[c]			
methyl 6-(5-methylene-2-oxopyrrolidin-1-yl)hexanoate (**13**)					2130	1750	31700	2900
methyl 6-(5-methyl-2-pyrrolin-1-yl)hexanoate (**14**)					430	350	6300	600
methyl 6-(5-methyl-2-oxopyrrolidin-1-yl)caproate (**15**)					1740			

[a] 15 min, distilled water. [b] 4 weeks, phosphate buffer (0.5 M, pH 7). [c] Trace (<10 ppm). [d] Methyl 4-aminobutyrate was used instead of the corresponding hexanoate.

Without amines a comparable browning is observed with sugars as well as with nucleic acids, but with amines the browning level is much lower in the latter case.

To understand the observed browning phenomena, we tried to gain insight into the operative reaction pathways. Thus, the characteristic sugar-derived reaction products (Table 2) as well as the characteristic amino acid-derived products (Table 3) were analyzed during incubations of D-ribose, 2-deoxy-D-ribose, DNA, and RNA under stringent conditions. The results were compared to those of long term incubations of DNA and RNA under mild conditions.

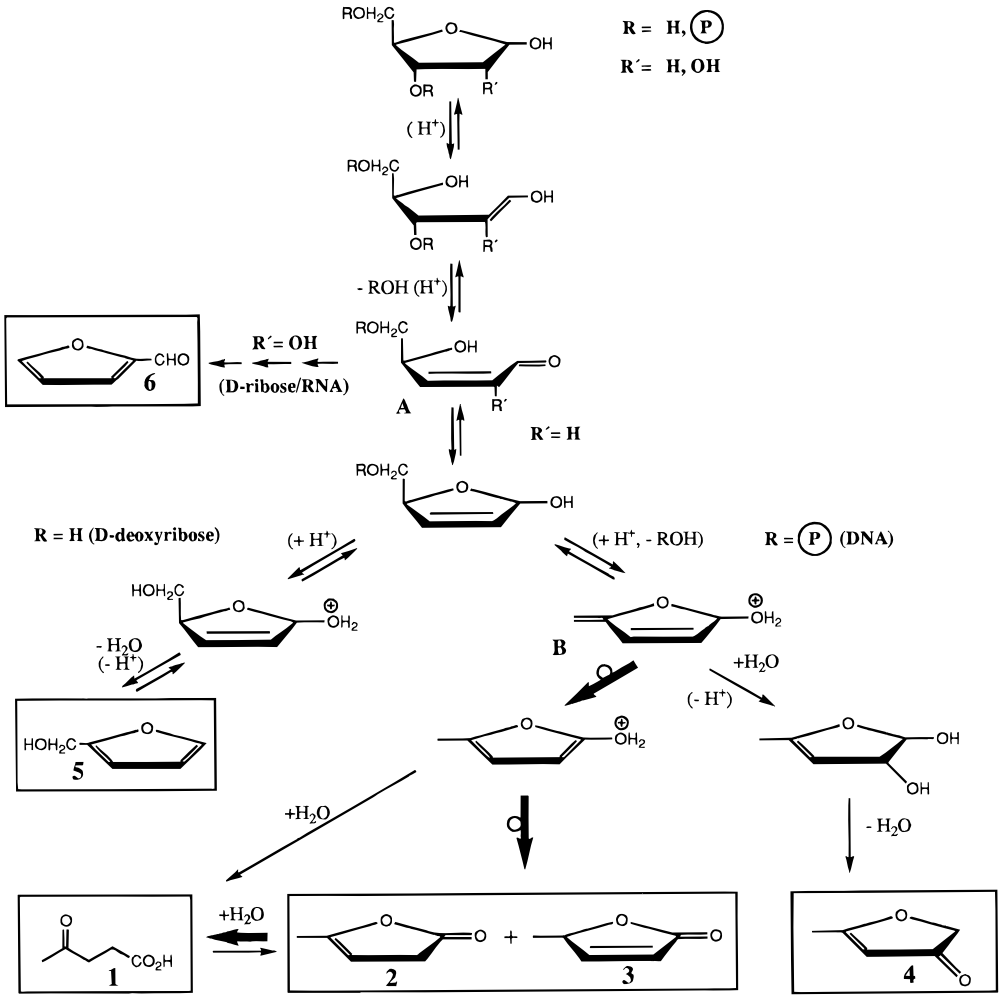
Reaction of Sugars and Nucleic Acids without Added Amine. As expected, *under stringent conditions* furfural (**6**) and furfuryl alcohol (**5**) were identified as the main degradation products of ribose and deoxyribose, respectively. Levulinic acid (**1**) was detected only in trace amounts. Interestingly, from RNA the yield of **6** was dramatically increased, whereas from DNA not the expected **5** but **1** was the main product (about one-third of the sugar is transformed into **1**). In addition, several furanoid compounds (**2**−**5**, not detectable in the RNA system) were formed in remarkable quantities from DNA: The predominant 5-methyl-3(2*H*)-furanone (**4**) was purified by preparative GC and identified by GC/MS and NMR; α- and *β*-angelica lactone (**2** and **3**) as well as **5** were determined by GC/MS. The furanone **4** has previously been shown to arise from 2-deoxyribose by an acid-catalyzed reaction (1 N HCl, 80 °C) (Seydel et al., 1967). Of course, *under mild conditions* RNA and DNA show strongly reduced reactivity. The most significant result of the DNA experiment under mild conditions is the drastic decrease of levulinic acid formation. The amount of the furanoid compounds is diminished with the exception of **5**.

The dramatic increase of formation of **6** from RNA as compared to D-ribose may be due to the specific route of RNA 3′,5′-phosphodiester cleavage after base loss: The formed ribose-3-phosphate represents an activated precursor of 3-deoxy-D-ribosone easily leading to **6** (Scheme 1). Compared to RNA, base loss from DNA is favored. Furthermore, hydrolytic 3′,5′-phosphodiester cleavage will occur more slowly (due to the absence of 2′-OH group) and in an unspecific manner resulting in 3- as well as 5-phosphorylated 2-deoxyribose derivatives. As shown in Scheme 1 elimination of the 3′-substituent leads to the 2,3-unsaturated 5-phosphate **A** as a key intermediate. Obviously, under stringent conditions 4,5-elimination from **A** is favored against 5′-hydrolysis. The latter pathway finally leads to the furan **5** as in the 2-deoxy-D-ribose reaction. The elimination pathway generates the intermediate **B**, already postulated for the acid-catalyzed transformation of compound **5** to **1** (Birkofer and Dutz, 1962), from which compound **1** as the main product as well as **2** and **3** are formed. The formation of the side reaction product **4** requires hydration of the postulated α,*β*-unsaturated carbonyl intermediate **B** as shown in Scheme 1.

In view of these pathways the browning phenomena can be interpreted as follows: (1) Increased browning in the 2-deoxy-D-ribose experiment compared to D-ribose might be the result of the superior polymerizing activity of **5** as compared to **6** (Tressl et al., unpublished). (2) The remarkably enhanced browning activity of DNA compared to RNA may be the result of the increased rate (10^2−10^3) of hydrolytic cleavage of the *N*-glycosidic bond as the initial step of the browning process (Lindahl, 1993).

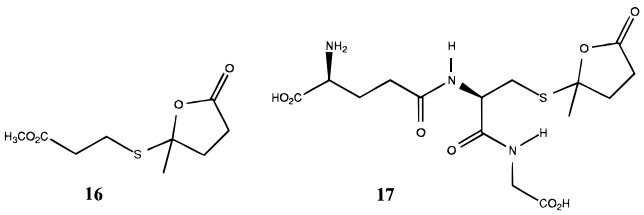
Due to their chemical reactivity the detected furanoid compounds may be assumed to be of toxicological relevance in cellular systems: (1) **6** in submillimolar concentrations has been shown to induce DNA strand fragmentation in vitro (Uddin and Hadi, 1995). (2) **4** and **5** are strong polymerizing compounds under mild conditions in the presence of trace amounts of acids (Feather and Harries, 1973). (3) **3** may undergo Michael addition with nucleophilic groups of proteins. (4) **2**

Scheme 1. Formation of Compounds 1−6 in Sugar and Nucleic Acid Reactions without Added Amine



strongly increases GSH *S*-transferase activity and inhibits benzo[*a*]pyrene-induced neoplasia of the forestomach of the mouse (Sparnins et al., 1982).

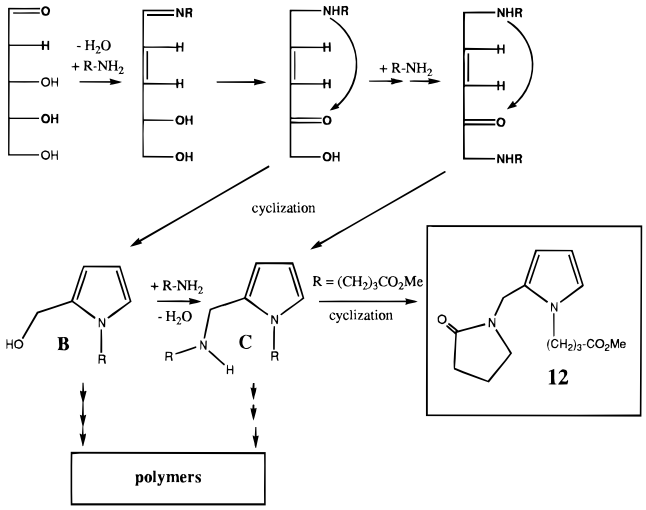
To check for the nonenzymatic reactivity of **2** according to point 4, we incubated (pH 7, 37 °C, 12 h) equimolar amounts of **2** and methyl 3-mercaptopropionate (or reduced glutathione) as a model of peptide-bound cysteine. In both cases addition takes place in high yield. The products were separated by preparative GC and identified by GC/MS, FAB/MS, and NMR (Table 1) as **16** and **17**. These data support a potential reactivity of **2** toward thiol group-containing biomolecules.



16 **17**

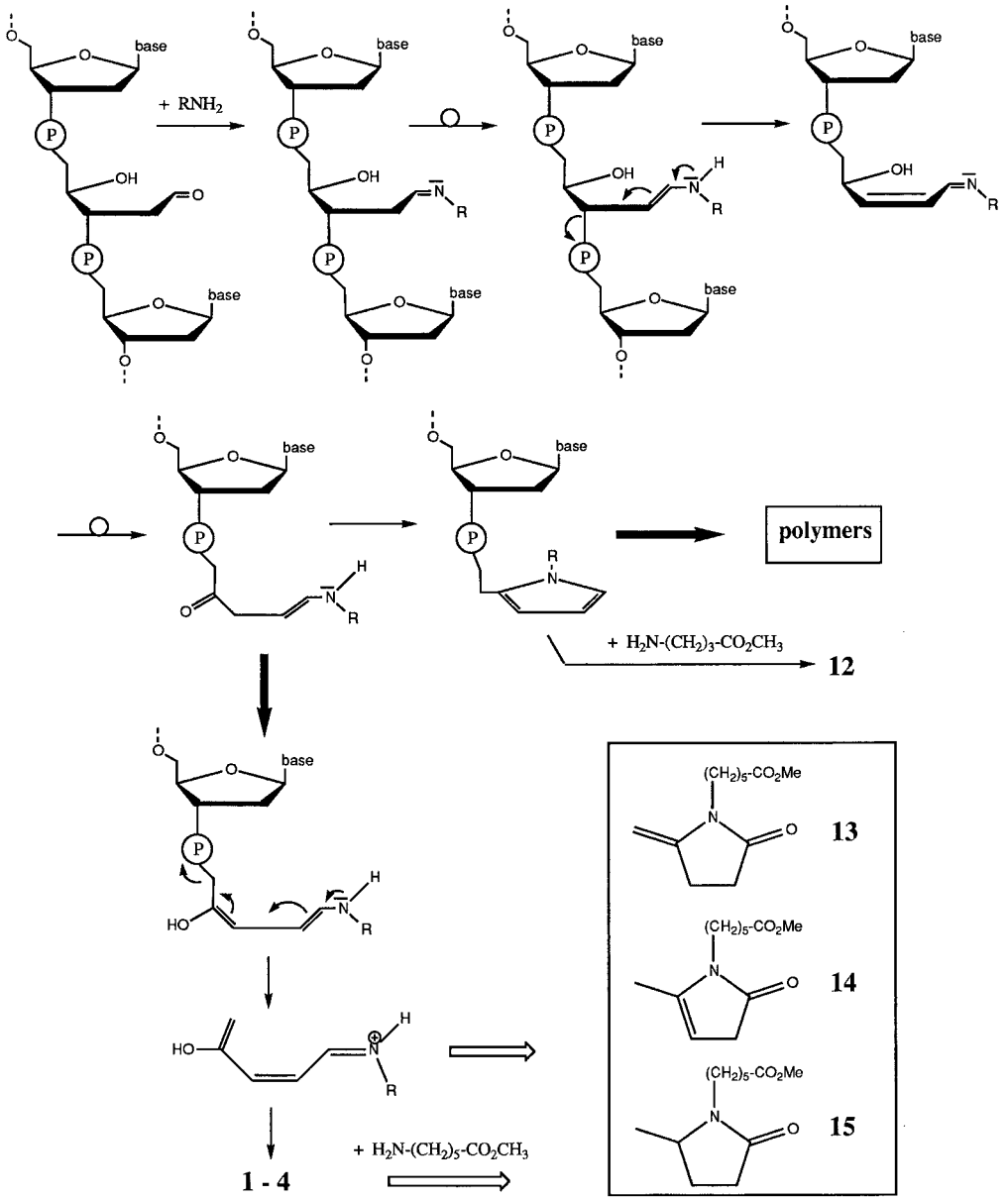
From autoclaving as well as from mild long term incubation of DNA, a further component was extracted and identified by GC/MS as N^6-furfuryladenine (**7**), the parent compound of the so-called "kinetin" family of plant hormones. The formation of kinetin from DNA by autoclaving was originally described by Miller et al. (1955). Our experiments indicate the formation of **7** from DNA even under mild conditions. Complementary, N^6-furfuryladenine could not be detected from RNA under stringent or mild conditions.

Scheme 2. Formation of N-Substituted 2-[Hydroxy(or amino)methyl]pyrroles from 2-Deoxy-D-ribose and Amines



Reaction of Pentoses with Amines. In contrast to the strong Maillard activity of D-ribose, a much lower activity of 2-deoxy-D-ribose might be expected. The absence of an α-hydroxy group blocks enaminol formation and Amadori rearrangement. The observed remarkable browning activity of 2-deoxy-D-ribose induced us to study methyl 4-aminobutyrate/2-deoxy-D-ribose

Scheme 3. Formation of Compounds 12−15 in DNA/Amine Reactions



model systems. For D-ribose and D-arabinose corresponding results have already been published (Tressl et al., 1993a). As can be seen from Table 3, the product spectrum of the 2-deoxypentose system corresponds to that of the pentose system, but the yield of the selected compounds **8**−**10** (derived from C_3, C_4, and C_5 fragments) is lower. Unambigous assignment of relevant pathways by isotopic labeling experiments as described for D-ribose/D-arabinose (Tressl et al., 1993b) is now under investigation.

The only 2-deoxy-D-ribose specific, hitherto undescribed compound, was pyrrole **12**. This key compound was separated by preparative HPLC and identified by GC/MS and NMR (Table 1). This is in fact a N-substituted 2-(hydroxymethyl)pyrrole derivative, stabilized by formation of a pyrrolidone ring system. This trapping depends on methyl 4-aminobutyrate as a pyrrolidone precursor. Thus, no analogous compounds are detectable with other amino compounds (e.g., methyl 6-aminocaproate).

Scheme 2 shows, in analogy to the formation of **5**, a reasonable reaction pathway to **12** and the related N-substituted 2-[hydroxy(or amino)methyl]pyrrole intermediates **B** or **C** by 2,3-dehydration, vinylogous Amadori rearrangement, and subsequent cyclization. Pyrrols of type **B** or **C** display a unique polymerizing activity under very mild conditions and readily undergo polymerization to methylene-bridged polypyrroles (Tressl et al., unpublished), which could account for the remarkable browning activity of 2-deoxy-D-ribose.

Reaction of Nucleic Acids with Amines. The loss of purines and pyrimidines from nucleic acids is a prerequisite for any Maillard reactivity of nucleic acids. Under physiological conditions hydrolytic cleavage of the *N*-glycosidic bond is more likely to occur in DNA than in RNA, and purine base loss is dominant (Lindahl, 1993). The apurinic sites induce DNA strand break by β-elimination of the 3′-*O*-phosphodiester bond which is further promoted by mono- and polyamines (Male et al., 1982). These early observations together with the remarkable browning activity of nucleic acid/amine systems led us to analyze the product spectrum of the Maillard reaction of DNA and RNA with methyl 4-aminobutyrate (6-aminocaproate) under stringent as well as mild conditions. Reaction products and yields are listed in Table 3.

In contrast to RNA, which parallels the ribose reactivity (formation of **8**, **9**, **11**), from incubations of DNA with methyl 6-aminocaproate three new title compounds (**13**−**15**) were isolated by preparative GC/HPLC and identified by MS/NMR. The isomers **13** and **14** were formed in a 5:1 ratio. **13**−**15** are obviously related to **1** as the main product. In fact, the isomers **13** and **14** could be generated in high yields under stringent and mild conditions from levulinic acid and methyl 6-aminocaproate. Interestingly, this reaction takes place without any browning (Figure 2). Compound **15** is not observed; its formation in the case of DNA probably depends on the parallel formation of strongly reducing Maillard compounds. **13** and **14** could also be synthesized under very mild conditions in high yields from the more reactive **2** and methyl 6-aminocaproate. Therefore, α-angelicalactone may be regarded as an activated levulinic acid.

In Scheme 3 reasonable pathways to the different observed products are postulated. First, in contrast to the corresponding reactions with 2-deoxy-D-ribose, the phosphorylation of the 3′-position in DNA favors β-elimination with subsequent vinylogous Amadori rearrangement. Second, the remaining excellent leaving group in the 5′-position promotes the formation of polymers as well as the formation of levulinic acid derivatives as probable precursors of **13**−**15**. The observed complete lack of browning during the corresponding levulinic acid reactions led us to favor the pyrrole-type polymerization as the source of browning as already mentioned above. Of course, the described pathways must be substantiated by isotopic labeling experiments using ^{13}C-labeled compounds.

Our study indicates that Maillard reactions of nucleic acid-bound pentoses with primary amino compounds occur under stringent and mild conditions in vitro. Interestingly, in vitro glycation of histones by glucose has recently been observed (De Bellis and Horowitz, 1987). Therefore, aminocarbonyl reactions of basic nuclear proteins or polyamines with nucleic acid-bound sugars should be considered to occur in vitro as well as in vivo.

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