New Melanoidin-like Maillard Polymers from 2-Deoxypentoses

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In the 2-deoxy-D-ribose/methyl 4-aminobutyrate Maillard system a trapped N-substituted 2-(hydroxymethyl)pyrrole is one of the major products. However, nontrapped representatives of this type of compound were hitherto not found in other Maillard model systems, indicating their extraordinary reactivity. Model experiments with 2-deoxy-D-ribose/methylamine enabled the detection of *N*-methyl-2-(hydroxymethyl)pyrrole (**1**) and some derived linear oligomers (**2**, **3**) as minor components. Consequently, **1** was synthesized and its oligomerization studied under very mild acidic conditions. The deformylated dimeric bis(*N*-methyl-2-pyrrolyl)methane (**2**) and trimeric *N*-methyl-2,5-bis(*N*-methyl-2-pyrrolylmethyl)pyrrole (**3**) were characterized by GC/MS and NMR. Higher regular oligomers up to 6 *N*-methyl-2-pyrrolylmethyl units as well as corresponding dehydro-oligomers up to 12 units were identified by MALDI-TOF-MS. A complementary experiment starting with *N*-methyl-2-hydroxy[^{13}C]methylpyrrole ([$^{13}CH_2OH$]-**1**) confirmed the structure and the oligomerization pathway. The possible significance of this type of model oligomer for the melanoidin formation in Maillard reactions is discussed. The antioxidative activity of the isolated dimer and trimer was tested in Fe(III)−thiocyanate and DPPH assays.

Keywords: *Maillard system 2-deoxy-D-ribose/methylamine; oligomerization of N-methyl-2-hydroxymethyl(and hydroxy[^{13}C]methyl)pyrrole; antioxidative activity; model compounds for melanoidins*

INTRODUCTION

Since the first description of the amino−carbonyl reaction by Maillard (1912) most investigators in this field have focused on those products that accumulate during the course of the Maillard reaction [e.g., pyrroles (Kato, 1967; Jurch and Tatum, 1970; Olsson et al., 1977; Shigematsu et al., 1971)]. This approach, mainly motivated by great interest in the identification and formation of flavor active compounds (Ho, 1996), widely ignores the predominant (>95% p.w.) formation of macromolecular melanoidins with hitherto mostly obscure structures (Benzing-Purdie et al., 1983; Kato et al., 1986; Feather and Huang, 1986; Huang and Feather, 1988). In addition, the low molecular weight compounds, which channel the Maillard reaction irreversibly into dark colored macromolecules, have until now not been identified unequivocally. Of course, the accumulation of those reactive compounds in Maillard model reactions cannot be anticipated. Whether some low molecular weight colored condensation products isolated in Maillard model systems (Ledl and Severin, 1978; Arnoldi et al., 1997) represent some melanoidin substructures or not is questionable.

We initiated an investigation on low molecular weight Maillard products that may play an important role in the formation of melanoidins. Here we report on the formation and characterization of linear (type I) (classified as type I polymers in contrast to branched polymers of type II from pentoses, which are the subject of a following publication) polymers from *N*-methyl-2-(hydroxymethyl)pyrrole (**1**). This compound was selected because we tentatively ascribed the remarkable browning activity of nucleic acid (DNA, RNA)/amine mixtures to highly reactive *N*-alkyl-2-(hydroxymethyl)-pyrroles, a representative of which (**A**) could be trapped in 2-deoxy-D-ribose/GABA systems (Wondrak et al., 1997). The pyrrole **1** should be formed analogously in 2-deoxy-D-ribose/methylamine model systems and is easily synthesized from the corresponding formyl compound (Ryskiewicz and Silverstein, 1954). The model experiments described in this paper are of crucial importance because 2-deoxy-D-ribose is also easily formed from hexoses by C_1-C_5 fragmentations, e.g. the α-dicarbonyl cleavage of 3-deoxyhexosones (Tressl et al., 1993b). Thus, if **1** is verified to form melanoidin-like macromolecules under mild acidic conditions, the corresponding amino acid analogues could represent one type of suitable compound for the irreversible transformation of 2-deoxypentoses, and also of hexoses, into melanoidins.

EXPERIMENTAL PROCEDURES

Materials and Methods. *N*,*N*-Dimethyl[^{13}C]formamide was from Campro Scientific (Emmerich, Germany); the other reagents were from Fluka AG (Neu-Ulm, Germany). Silica gel 60 (Merck Chemical Co., Darmstadt, Germany) was used

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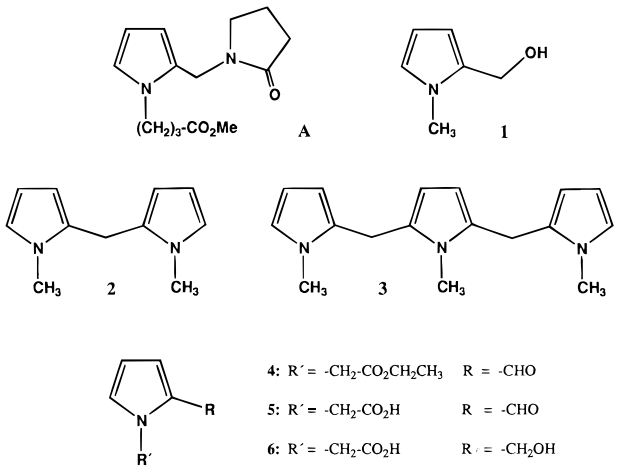
for LC. Autoclaving was done in a stainless steel laboratory autoclave (Roth, I series) equipped with a 100 mL duran glass tube and heated by an electric heater with magnetic stirrer. During autoclaving the peak temperature (120 °C) was reached after 45 min.

Reaction of 2-Deoxy-D-ribose with Methylamine−HCl. 2-Deoxy-D-ribose (1 g) was reacted with methylamine−HCl (1.5 g) in 0.1 M phosphate buffer (20 mL, pH 7) at 120 °C for 1 h. The reaction mixture was extracted with diethyl ether (3 × 50 mL). The combined organic phases were washed with (a) 0.025 M HCl (saturated with NaCl) (20 mL) and (b) 5% $NaHCO_3$ (10 mL). After drying over Na_2SO_4 and concentration on a 20 cm Vigreux column to ~0.2 mL, the extract was directly analyzed by capillary GC/MS. *N*-Methyl-2-(hydroxymethyl)pyrrole (**1**), bis(*N*-methyl-2-pyrrolyl)methane (**2**) and



2,5-bis(*N*-methyl-2-pyrrolylmethyl)-*N*-methylpyrrole (**3**) were identified by their MS data and by comparison with authentic samples.

***N*-Methyl-2-[^{13}C]formylpyrrole.** At 4 °C 0.85 g (5.50 mmol) of $POCl_3$ was added to 0.32 g (4.32 mmol) of *N*,*N*-dimethyl[^{13}C]formamide. After 15 min of stirring at 20 °C, 5 mL of 1,2-dichloroethane and 0.35 g (4.32 mmol) of *N*-methylpyrrole in 1 mL of 1,2-dichloroethane were added at 4 °C. After warming up to room temperature, the mixture was refluxed for 15 min. Five milliliters of a solution (5.5 M) of $CH_3CO_2Na·3H_2O$ was added at 20 °C. After that, the mixture was refluxed for 15 min. The aqueous phase was extracted with diethyl ether, and the combined organic phases were washed with saturated Na_2CO_3 and dried over Na_2SO_4. Yield after evaporation and vacuum distillation (11 mmHg) of the resulting oil at 80 °C was 0.30 g (65%): ^{1}H NMR δ 3.94 (s, 3H, NCH_3), 6.19 (m, 1H, H-4), 6.86 (m, 1H, H-5), 6.90 (m, 1H, H-3), 9.53 (d, 1H, J = 173 Hz, CH=O); ^{13}C NMR δ 179.6 (s, ^{13}C=O).

***N*-Methyl-2-(hydroxymethyl)pyrrole (1).** According to the method of Ryskiewicz and Silverstein (1954) during 10 min 5.0 g (132 mmol) of $NaBH_4$ in 50 mL of distilled water was dropped into 5.54 g (5.80 mmol) of *N*-methyl-2-formylpyrrole, suspended in 100 mL of distilled water. After 3 h of stirring at 20 °C, 50 g of K_2CO_3 was added at ~10 °C. After stirring for 15 min, the mixture was extracted three times with diethyl ether (each 60 mL). The combined diethyl ether extracts were dried over Na_2SO_4, and 1.52 g of triethylamine was added. Diethyl ether (100 mL) was evaporated at 20 °C and then at 0 °C by vacuum distillation. The remaining colorless oil (6.86 g) was a 3.5:1 (w/w) mixture of pyrrole **1** and triethylamine as analyzed by ^{1}H NMR: δ 2.9 (br s, 1H, OH), 3.60 (s, 3H, NCH_3), 4.55 (s, 2H, CH_2), 6.10 (m, 2H, H-3, H-4), 6.60 (m, 1H, H-5); signals of triethylamine at 1.0 (t) and 2.55 (q); ^{13}C NMR δ 33.41 (NCH_3), 56.05 (CH_2OH), 106.4 (C-3), 108.46 (C-4), 123.11 (C-5), 131.73 (C-2); MS (EI, 70 eV), *m*/*z* 112 (4.1), 111 (55.6), 110 (10.6), 95 (7.6), 94 (100), 93 (6.7), 82 (12.9), 80 (5.7), 67 (10.4), 53 (7.9), 42 (8.5), 41 (6.0).

Several attempts to isolate the pure compound failed. After evaporation of the diethyl ether, even at low temperature, the colorless product polymerizes in an exothermic spontaneous reaction to form a colorless solid. Exposed to air, the polymer became pink and finally dark red: MS (EI, 70 eV, 160 °C), *m*/*z* 454 (2.5), 453 (7.6), 360 (8.4), 280 (5.5), 267 (23.2), 266 (8.7), 187 (9.8), 174 (43.0), 173 (51.1), 111 (53.3), 110 (1.3), 109 (20.6), 108 (21.4), 95 (11.8), 94 (100), 93 (20.8), 87 (32.5), 82 (8.3), 80 (8.8), 74 (14.1), 73 (14.1), 72 (6.8), 59, 19.3), 53 (9.4), 45 (21.3), 44 (39.9), 43 (15.8), 42 (17.4), 41 (8.1), 31 (26.8). A red polymer generated from **1** by addition of a catalytic amount of 2 N HCl (see below) shows essentially the same MS.

Sample Preparation. *Polymerization of **1**.* *N*-Methyl-2-formylpyrrole (40 μL) was dissolved in 9% aqueous methanol (11 mL). After addition of $NaBH_4$ (10 mg) and stirring (1 h, 20 °C), the mixture was extracted with diethyl ether (3 × 20 mL). The organic phase was washed with (a) 0.1 N HCl (2 × 5 mL) and (b) saturated aqueous NaCl (2 × 5 mL) and dried over Na_2SO_4. The ether (an aliquot was analyzed by GC/MS) was evaporated on a 20 cm Vigreux column. The residue (25 mg) was redissolved in $CHCl_3$ (10 mL). After 2 days at room temperature (light excluded), the solvent was evaporated and the dark colored product (10 mg) was analyzed by GC/MS and MALDI-TOF-MS (see Figure 2): UV−vis (0.15 mg/mL in $CHCl_3$) λ_{max} (E) = 247 (1.20), 290 (0.76), broad shoulder 350−550 nm; fluorescence (in $CHCl_3$) λ_{ex} = 385 nm, λ_{em} = 493 nm.

Polymerization of 2-(Hydroxymethyl)furan. Furfuryl alcohol (1 mL) was dissolved in distilled water (40 mL) and stirred (12 h, 20 °C) after addition of 1 N HCl (100 μL). The brown solid was recovered by filtration, washed with water, dissolved in diethyl ether, and analyzed by GC/MS: MALDI-TOF-MS, *m*/*z* 257.3 (trimer), 281.3, 321.4, 337.3 (tetramer), 361.2, 379.2, 401.4, 417.2 (pentamer), 435.4, 459.3, 475.4, 497.3 (hexamer), 539.3, 577.4.

Isolation of Oligomers. A 10-fold amount of **1** was reacted as described for sample preparation. The product was immediately isolated after evaporation of the ether extract, redissolved in chloroform (1 mL), and fractionated by column LC (silica gel, 20 × 1 cm) with petroleum ether/ethyl acetate (5:1). The dimeric bis(*N*-methyl-2-pyrrolyl)methane (**2**) (30 mg) and the trimeric 5-bis(*N*-methyl-2-pyrrolylmethyl)-*N*-methylpyrrole (**3**) (15 mg) were isolated, and the pure compounds were characterized by TLC (R_f = 0.53 and 0.33, petroleum ether/ethyl acetate 5:1), GC/MS, and NMR. **2**: MS, *m*/*z* 174 (100), 173 (82), 94 (71), 93 (40), 42 (15); ^{1}H NMR δ 3.52 (s, 6H, N-CH_3), 3.85 (s, 2H, CH_2), 5.81 (mc, 2H, H-3 of pyrroles), 6.02 (mc, 2H, H-4 of pyrroles), 6.55 (mc, 2H, 5-H of pyrroles). **3**: MS, *m*/*z* 267 (82), 187 (18), 173 (78), 94 (100), 93 (51), 42 (20); ^{1}H NMR δ 3.37 (s, 3H, central N-CH_3), 3.515 (s, 6H, peripheral N-CH_3), 3.816 (s, 4H, CH_2), 5.69 (s, 2H, H-3,4 of central ring), 5.787 (mc, 2H, H-3 of peripheral rings), 6.01 (mc, 2H, H-4 of peripheral rings), 6.538 (mc, 2H, H-5); ^{13}C NMR δ sp^3C-H nd, 33.83 (central N$\underline{C}H_3$-group), 34.53 (peripheral N$\underline{C}H_3$-groups), 106.48, 108.61 (ring $\underline{C}$H), 121.93 (5-$\underline{C}$H of peripheral pyrroles), 131.83 (quartet $\underline{C}$ of pyrroles).

Ethyl 2-(2-Formyl-1-pyrrolyl)acetate (4). Ethyl bromoacetate (21.1 g, 127 mmol) was dropped into 4 g (42.1 mmol) of 2-formylpyrrole and 25 g of K_2CO_3 in 90 mL of *p*-dioxane. The mixture was refluxed (3 h), cooled to room temperature, and extracted with 140 mL of toluene. The filtered dark brown solution was evaporated after addition of 100 mL of water (45 °C, 20 mmHg): 6.63 g of **4** (86%) brown oil, which was used without further purification; MS, *m*/*z* 181 (70.5, M^+), 153 (19.7), 136 (15.5), 124 (15.2), 109 (9.0), 108 (100), 94 (46.9), 80 (24.5), 53 (16.3); ^{1}H NMR δ 1.30 (3H, t, CH_3), 4.20 (2H, q, OCH_2), 5.05 (2H, s, NCH_2), 6.30 (1H, dd, H-4), 6.95 (1H, m, H-3), 7.0 (1H, dd, H-5), 9.5 (1H, s, CHO).

(2-Formyl-1-pyrrolyl)acetic Acid (5). At room temperature 5 g of **4** was stirred for 15 min in methanolic K_2CO_3 (10% w/v, 250 mL). After addition of 15 mL of distilled water, the mixture was refluxed for 15 min. Four hundred forty milliliters of distilled water was added after the mixture had cooled to 5 °C, and the mixture was kept at 5 °C for 12 h. The aqueous phase was extracted with ethyl acetate (320 mL) and, after the pH was adjusted to 6.0, extracted again with ethyl acetate (3 × 100 mL). The combined ethyl acetate extracts

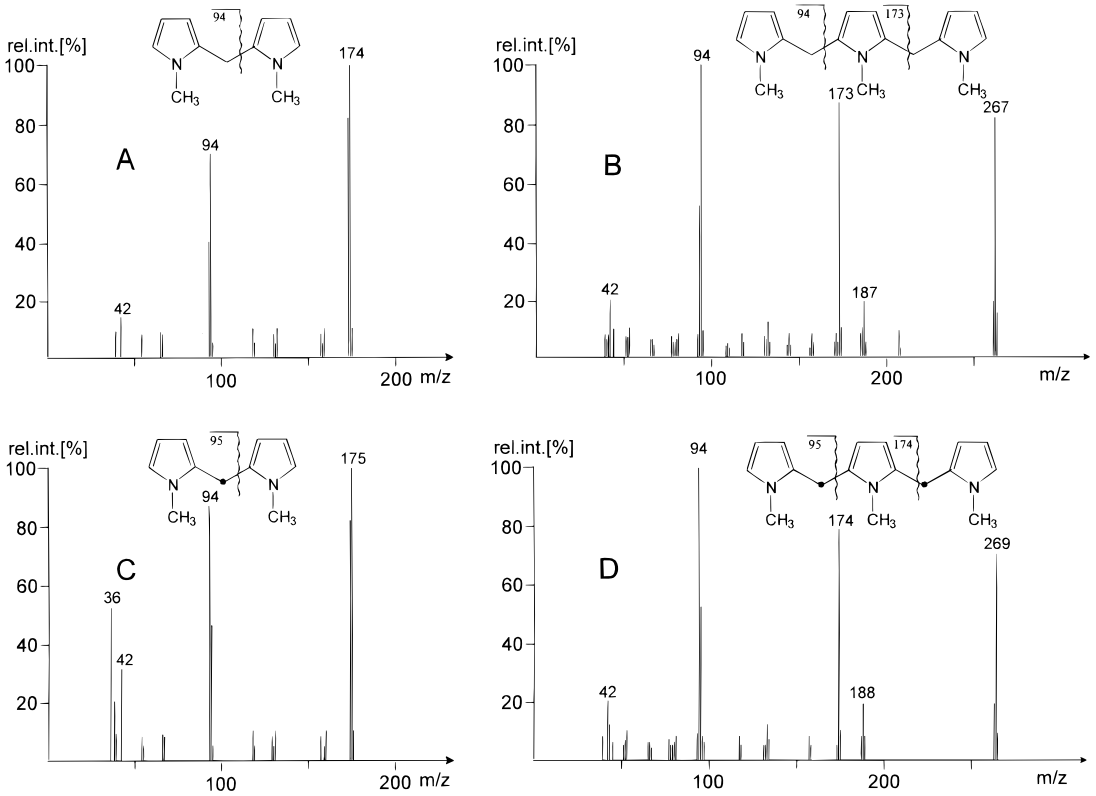


Figure 1. MS spectra (EI, 70 eV) of bis(*N*-methyl-2-pyrrolyl)methane (**2**) (A), [$^{13}CH_2$]-**2** (C), 2,5-bis(*N*-methyl-2-pyrrolylmethyl)-*N*-methylpyrrole (**3**) (B), and [$^{13}CH_2$]$_2$-**3** (D).

are almost colorless or yellow. After acidification with 1 mL of 2 N HCl, the aqueous solution turned red. From a 100 mL ethyl acetate extract (deep red) were isolated dark red crystals of **5**: MS (EI, 70 eV), *m*/*z* 154 (8.3, M^+), 153 (100), 125 (4.0), 124 (6.1), 109 (25.9), 108 (79.5), 107 (10.9), 94 (25.4), 80 (28.5), 79 (9.8), 78 (12.2), 53 (30.4), 39 (10.8).

[2-(Hydroxymethyl)-1-pyrrolyl]acetic Acid (6). At 5 °C 1.4 g (37 mmol) of $NaBH_4$ in 60 mL of distilled water was dropped into 1.29 g (8.42 mmol) of **5** in 20 mL of distilled water. The mixture was stirred at room temperature for 90 min. The pH was adjusted to pH 5 by addition of 2 N HCl, and the color changed to red-violet. Only a minor amount of organic material (<20 mg) could be extracted with 3 × 50 mL of $CHCl_3$. From the aqueous phase, after evaporation, a red solid was isolated, insoluble in organic solvents: MS (FAB, negative), *m*/*z* = 507 (2.2), 491 (3.3), 469 (2.0), 452 (1.4), 346 (1.4), 330 (5.1), 308 (5.5), 289 (4.4), 192 (17.9), 176 (29.3), 154 (95.7, M − 1), 136 (100), 107 (29.5), 91 (20.0), 90 (35.4), 89 (40.50).

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 capillary column coupled with a double-focusing mass spectrometer CH 5-DF (Varian MAT), ionization voltage 70 eV, resolution 2000 (10% valley). Temperature was programmed from 80 to 280 °C at 4 °C/min.

UV−Vis and Fluorescence Spectrometry. UV−vis spectra were recorded with an Uvikon 922 spectrophotometer (Kontron Instruments) and fluorescence spectra with an RF-5000 spectrofluorometer (Shimadzu).

FAB Mass Spectrometry. The fast atom bombardment mass spectra (8 keV, xenon) were recorded on the CH5-DF spectrometer (Varian MAT) using glycerol as matrix.

MALDI-TOF Mass Spectrometry. The measurements were carried out on a Kratos Kompact MALDI III (Shimadzu). The sample solution (0.5 μL; 1 mg/mL in $CHCl_3$) and 0.5 μL of matrix solution (25 mg of 2,5-dihydroxybenzoic acid or 2,4,6-trihydroxyacetophenone) were mixed on the stainless steel sample slide, and the solvent was evaporated. Bovine insulin was used for calibration (molar mass 5734.5 g/mol). The following conditions were applied: polarity positive, flight path reflection, 20 kV acceleration voltage, nitrogen laser 337 nm; smoothed spectra.

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

Antioxidative Assays. *(a) DPPH Assay (Endo et al., 1985).* The decrease of the absorption band at λ = 516 nm (*d* = 1 cm) was measured as a function of time, using a CCl_4 solution 2 × 10^{-3} M in **2** (or **3**) and 4 × 10^{-4} M in 2,2-di-(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH). The antioxidative activities are as follows: octyl gallate, 20%; D,L-α-tocopherol, 34%; *N*-methylpyrrole, 94%; *N*-methyl-2-formylpyrrole, 97%; **2**, 61%; **3**, 59% (remaining absorption after 2 h in percent).

(b) Fe(III) Thiocyanate Assay (Inatani et al., 1983). To 2 mg of **2** (**3**, or other antioxidatives) in 2 mL of 99.5% ethanol were added 2.052 mL of linoleic acid (2.51% in 99.5% ethanol), 4 mL of phosphate buffer (0.05 M, pH 7), and 1.948 mL of distilled water. The solution was kept at 40 °C for 2 weeks. Every 24 h an aliquot (0.1 mL) was added to 9.7 mL of ethanol (75%, v/v) plus 0.1 mL of NH_4SCN (30%, w/v). Exactly 3 min after addition of 0.1 mL of $FeCl_2$ (2 × 10^{-2} M in 3.5% HCl), the absorption was measured at λ = 500 nm. The antioxidative activities are as follows: octyl gallate, 96%; L-ascorbic acid, 94%; bilirubin, 94%; *N*-methylpyrrole, 11%; *N*-methyl-2-formylpyrrole, 19%, **2**, 97%; **3**, 7% [antioxidative activity in percent = $(1 - A_{probe}/A_{blind}) \times 100$].

RESULTS AND DISCUSSION

DNA and its constituent 2-deoxy-D-ribose show remarkable browning activity during long-term incubation (40 °C) or stringent reaction (160 °C) with primary amines and primary amino acids (Wondrak et al., 1997). We assumed that this browning activity is due to *N*-alkylpyrrole analogues of furfuryl alcohol, hitherto not observed in Maillard systems because of the ex-

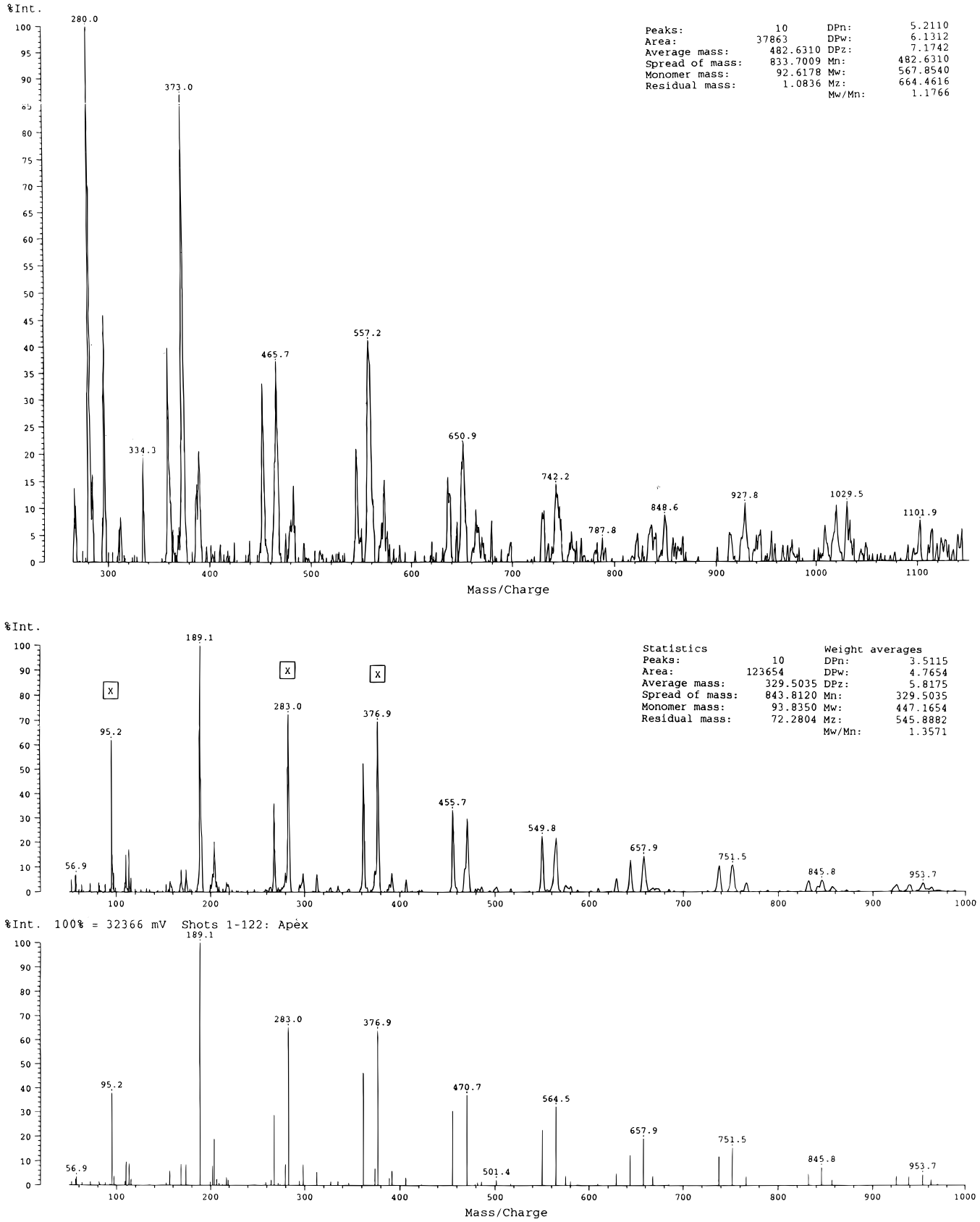
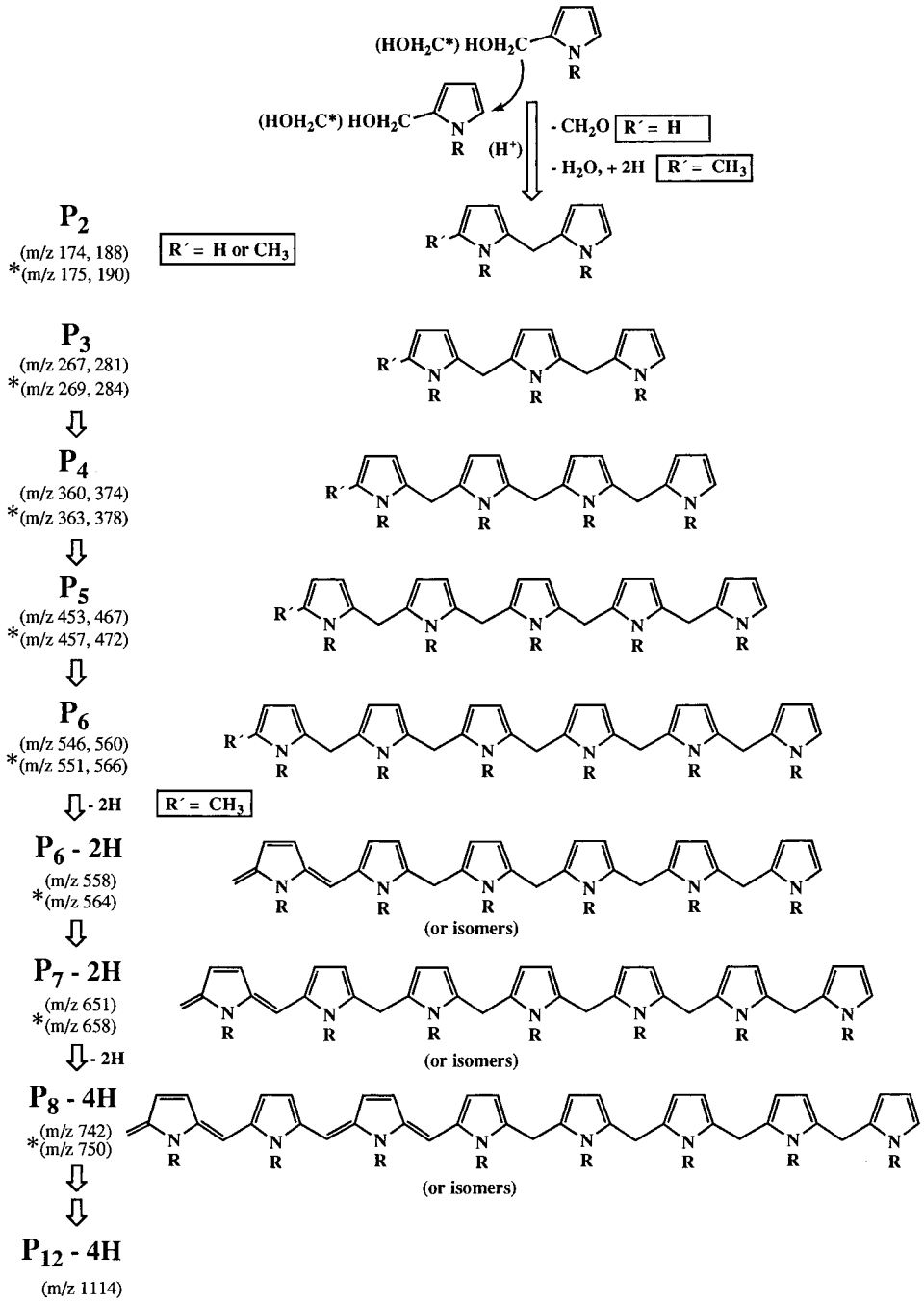


Figure 2. MALDI-TOF-MS spectra of the oligomerization/polymerization product generated from **1** (top) and [$^{13}CH_2OH$]-**1** (bottom). Usually, the $(M - 1)^+$ peak is observed.

pected extraordinary reactivity of such compounds. Substantial support for this assumption comes from the isolation of the trapped *N*-alkyl-2-(hydroxymethyl)-pyrrole **A** as a main product in 2-deoxy-D-ribose/methyl 4-aminobutyrate Maillard systems. Obviously, the formation of this product parallels the well-known route from 2-deoxy-D-ribose to furfuryl alcohol.

We started 2-deoxy-D-ribose/methylamine model experiments expecting that the generated *N*-methyl-2-(hydroxymethyl)pyrrole (**1**) and derived compounds

Scheme 1. Formation and Structure of Linear Polymers of Type I from 1 and [$^{13}CH_2OH$]-1, Respectively



* indicates *m/z* values of the labeled compounds.

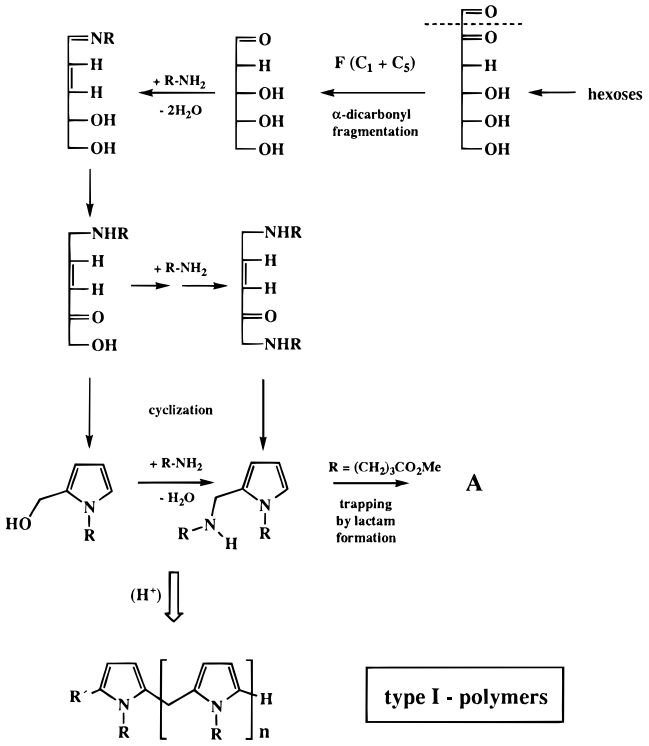
should be much more suitable for separation and identification by GC/MS and NMR than the polar analogues generated from amino acids. After the formation of **1** under Maillard conditions is established, its further transformations can be easily studied with the synthetic compound.

In fact, the pyrrole **1** and the deformylated dimer **2** and trimer **3** could be identified by GC/MS by comparison with authentic samples (see below). The MS spectra of **2** and **3** are shown in Figure 1 together with the spectra of their ^{13}C isotopomers. Besides furfuryl alcohol (>2000 ppm) the pyrroles **1**−**3** were detected in this Maillard system as main products, but only in very low yield (100−300 ppm, each), pointing to the generation of higher homologous compounds, not detectable by GC/MS.

Verifying this hypothesis, we analyzed the material generated by acid-catalyzed polymerization of synthetic **1** ([$^{13}CH_2OH$]−**1**). These pyrroles were synthesized by $NaBH_4$ reduction of the corresponding formyl compounds according to Ryskiewicz and Silverstein (1954). The very labile **1** can be stabilized by addition of $NaBH_4$ or triethylamine. In the presence of catalytic amounts of acid in solution or as bulk substance, **1** polymerizes spontaneously in an exothermic reaction within minutes at room temperature. The resulting colorless solid, exposed to air, became dark red to black by unknown oxidative processes.

As the most powerful tool to investigate the structure of the formed material, the MALDI-TOF-MS method (Siuzdak, 1994) was selected. This new method has been proven to be most valuable in the analysis of

Scheme 2. Generation of Linear Polymers of Type I from Hexoses and 2-Deoxypentoses



mixtures of regular synthetic polymers (Bahr et al., 1992; Krüger, 1995): Molar masses of the distinct species are indicated without fragmentation and, therefore, the monomeric unit is easily revealed. The MALDI-TOF-MS spectra of the oligomers/polymers generated from **1** with catalytic amounts of acid are shown in Figure 2.

Obviously, the monomeric unit of the formed detectable oligomers $\mathbf{P_2}-\mathbf{P_{12}}$ is *N*-methylmethylenepyrrole ($\Delta m = 93$ and 94, respectively). Up to $\mathbf{P_6}$ the oligomers are either deformylated or reduced to the stage of methyl-substituted oligomers. The higher oligomers $\mathbf{P_6}-\mathbf{P_{12}}$ are always of the latter type and occur as di- or tetradehydro derivatives. Thus, delocalized systems over two or four pyrrole rings are favored in this series.

These oligomers were formed by successive electrophilic substitutions of the electron-rich pyrrole system by pyrrolylmethyl cations, a well-known principle in the chemistry of porphyrinogen synthesis (Franck and Nonn, 1995). This is illustrated in Scheme 1. The regular constitution of the oligomers is demonstrated by the ^{13}C-labeling experiment: Up to $\mathbf{P_6}$ the observed molecular mass increases step by step, as expected, by one to six mass units. In higher oligomers this trend is retained, although deviations by one mass unit are registered.

Compared to the outstanding polymerizing potential of *N*-alkyl-2-(hydroxymethyl)pyrrole, its furan analogue, furfuryl alcohol, is of minor reactivity. The MALDI-TOF-MS analysis of the product, generated under the same conditions, indicates the formation of oligomers only up to six 2-furylmethylene units ($\Delta m = 80$) even after 12 h. The oligomers are either hydroxymethyl-substituted furylmethanes or their ether-bridged isomers, indistinguishable by the technique used. The formation of ether bridges may be reponsible for the extensive formation of Na^+ and K^+ complexes registered in the MALDI-TOF-MS spectra. Clearly, the observed differences are due to the lower electron density in the furan compared to the N-substituted pyrrole. The polycondensation of furfuryl alcohol was the subject of several studies (Barr and Wallon, 1971; Wewerka et al., 1971; Vogel et al., 1989).

The described oligomers of *N*-methylpyrroles represent only melanoidin model compounds. Will *N*-(carboxyalkyl)(hydroxymethyl)pyrroles, the appropriate intermediates in amino acid/2-deoxypentose Maillard systems, show a similar polymerizing potential? To address this question, we tried to isolate the glycine analogue of **1**. Starting with 2-formylpyrrole, we synthesized the ethyl acetate **4**, which was, after saponification to **5**, reduced by $NaBH_4$ to the *N*-carboxymethyl compound **6**. Upon neutralization (for isolation of **6**) the reaction mixture immediately turned red to violet. With organic solvents no organic material could be extracted. From the aqueous phase a red-violet solid was prepared. FAB-MS analysis indicated the presence of the expected pyrrole **6** ($m/z = 154$, M − 1). Obviously, the initially formed **6** readily polymerizes, but the polar water-soluble material could be neither isolated nor characterized by our methods. Nevertheless, the high polymerizing potential of compound **6** is illustrated by this experiment.

To evaluate the melanoidin character of our model compounds, we examined whether some of their functional qualities correspond to those of native melanoidins. The brown to black color of synthetic type I polymers after exposure to air and the strong yellow fluorescence in chloroform resemble those of native melanoidins. The color will be due either to the chromophore of the methine-bridged polypyrroles (Scheme 1) or to derived delocalized radicals. In principle, the oligomer/polymer mixture should also show antioxidative activity well-known from native melanoidins (Hayase et al., 1989). Therefore, we tested this property by two antioxidative assays [DPPH assay, Fe(III) thiocyanate assay]. In the case of the oligomers **2** and **3** a moderate to strong antioxidative activity could be demonstrated. Compound **2** proved to be especially efficient as standard antioxidants (ascorbic acid, octyl gallate) in the inhibition of the linoleic acid autoxidation. Unfortunately, the unfractionated polymer mixture could not be tested because of its very low solubility.

Our results offer a new possible route to melanoidins, which is, remarkably, not restricted to the Maillard reaction of 2-deoxypentoses. It is well-known that in Maillard systems at pH <7 fragmentations occur resulting in several C_2-C_5 moieties (Tressl et al., 1993b). Thus, 2-deoxypentose is the direct scission product of 3-deoxyhexosones by α-dicarbonyl cleavage (Hayase and Kato, 1985; Ledl and Schleicher, 1990). Moreover, several labeling experiments, starting with either [1-^{13}C]- or [6-^{13}C]-D-glucose (Tressl et al., 1993b, and unpublished results), have demonstrated a substantial generation of 2-deoxy-D-ribose related C_5 products from hexoses by this route established by complementary labeling experiments. Scheme 2 summarizes the suggested pathway from hexoses and 2-deoxypentoses to polymers of type I. Surprisingly, the browning phenomenon observed in the reaction of lipid peroxidation products with amines was ascribed to the formation of *N*-alkyl-2-(hydroxyalkyl)pyrroles, which also form linear oligomers of the (*N*-alkylmethylenepyrrole) type (Hidalgo et al., 1993).

It has to be mentioned that in addition to the described type I compounds, further melanoidin-like structures (type II) were easily formed from Maillard-type pyrroles and furans, which will be the topic of the following paper.

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