Structure and Potential Cross-Linking Reactivity of a New Pentose-Specific Maillard Product

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The Maillard reaction of model compounds for peptide-bound lysine (4-aminobutyric acid, 6-aminocaproic acid, N^{α}-acetyl-L-lysine) with reducing sugars (D-ribose, D-xylose, D-arabinose, D-glucose, D-fructose, D-glyceraldehyde) was investigated under both stringent and mild conditions. With pentoses the corresponding ω-(dimethylmaleimido)carboxylic acids **1–3** were identified as substantial, hitherto unknown components by GC/MS and NMR. Their structure was confirmed by synthesis. A pathway to compounds **1–3** was derived from the results of 4-aminobutyric acid/[1-^{13}C]-D-arabinose isotopic labeling experiments. The new *N*-(dimethylmaleoyl)-ω-aminocarboxylic acid derivatives are shown to react under very mild conditions (pH 7, 32 °C) with thiols (methyl 3-mercaptopropionate, N^{α}-acetyl-L-cysteine methyl ester) to form the addition products **4–6**. This reaction demonstrates that compounds of type **1–3** can be considered as potential protein cross-linkers in Maillard reactions.

Keywords: *Maillard reaction, new pentose-specific product with ω-amino acids; N-(dimethylmaleoyl)-ω-amino acids, formation in Maillard reactions; ω-(3,4-dimethyl-2,5-dioxo-2,5-dihydropyrrol-1-yl)carboxylic acids, formation from pentoses; [1-^{13}C]-D-arabinose, Maillard reaction with 4-aminobutyric acid; pentoses, Maillard reaction with ω-amino acids; N-(dimethylmaleoyl)-ω-amino acids, potential protein cross-linking reaction with SH groups*

INTRODUCTION

Several Maillard reaction endproducts show remarkable bioactivity, i.e. reductones (Kato et al., 1985; Ninomiya et al., 1992), metal chelators of the 3-hydroxypyridone type (Kontoghiorghes et al., 1985), dietary carcinogens of the imidazoquinoline type (Sugimura, 1992), and protein cross-linkers like pyrraline (Nakayama et al., 1980). With respect to cross-linking phenomena, interest has recently focused on pentosidine (a pentose cross-link between lysine and arginine), which was isolated from biological material in age-related quantities (Sell and Monnier, 1989; Grandhee and Monnier, 1991). Due to their high reactivity (Bunn and Higgins, 1981) and natural abundance, the nonenzymatic glycosylation reactions of pentoses are of increasing interest, especially those performed under physiological conditions (Baynes et al., 1993). Therefore, in our studies on the Maillard reaction of reducing sugars with compounds resembling peptide-bound lysine (e.g. 4-aminobutyric acid; Tressl et al., 1993b,c), the investigation of pentose reactions was an important aspect. Within this scope, we described a series of 4-aminobutyric acid/D-arabinose ([1-^{13}C]-D-arabinose) experiments. Interestingly, one of the major components characterized in this system as well as in corresponding D-xylose systems could not be identified unequivocally from the GC/MS data. From these data a 1-pyrrylbutyric acid derivative was tentatively assumed (Kersten, 1991).

In this paper we describe the structure and the potential cross-linking ability of these (and analogous) until now unknown compounds. For this purpose model experiments of ω-aminocarboxylic acids (4-aminobutyric acid, 6-aminocaproic acid, N^{α}-acetyl-L-lysine) with pentoses (D-xylose, D-arabinose, D-ribose) were performed under different conditions. After separation of the acidic components by extraction and derivatization, the new compounds were characterized by GC/MS and ^{1}H/^{13}C NMR spectroscopy. From these data a clear structural assignment (**1–3**) was achieved. Surprisingly, the compounds show a very reactive dimethylmaleimide moiety. The well-known reactivity of this class of compounds (Riordan and Vallee, 1972) is exemplified by their reaction with thiols (methyl 3-mercaptopropionate, *N*-acetyl-L-cysteine methyl ester) even under very mild conditions, resulting in well-defined adducts (**4–6**). In protein/pentose systems cross-links would be generated by the same reaction sequence.

EXPERIMENTAL PROCEDURES

Materials and Methods. N^{α}-Acetyl-L-lysine was obtained from Sigma Chemical Co.; other reagents were from Fluka AG, Buchs, Switzerland. *N*-Acetyl-L-cysteine *O*-methyl ester was synthesized from *N*-acetyl-L-cysteine and methyl *N*-maleoyl-6-aminocaproate from *N*-maleoyl-6-aminocaproic acid according to standard methods (Metcalfe and Schmitz, 1961). Autoclavation 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.

Sample Preparation. Reaction of ω-Aminocarboxylic Acids with Reducing Sugars. The ω-aminocarboxylic acid [4-aminobutyric acid (GABA), 6-aminocaproic acid, N^{α}-acetyl-L-lysine] (1 g) and the reducing sugar (D-ribose, D-xylose, D-arabinose, D-glucose, D-fructose, D-glyceraldehyde) (1 g) were dissolved in water (25 mL) or 0.5 M phosphate buffer (20 mL, pH 7) and incubated under the following conditions: (a) 2 h, 160 °C (autoclave); (b) 7 days, 70 °C; (c) 30 days, 50 °C. After the mixtures had cooled to 20 °C (and addition of 6-maleimidocaproic acid as internal standard for GC/MS quantification), the pH was adjusted to 2 with 1 N HCl and the compounds

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Table 1. MS Data (*m/z*, Relative Intensity) and $^1H/^{13}C$ NMR Data[a] of Compounds 1–6

1	methyl 4-(3,4-dimethyl-2,5-dioxo-2,5-dihydropyrrol-1-yl)butanoate	225 (25), 194 (19), 193 (19), 184 (4), 166 (8), 165 (8), 152 (100), 151 (19), 138 (82), 126 (5), 110 (10), 108 (5), 83 (4), 82 (8), 81 (9), 74 (12), 67 (8), 59 (5), 56 (20), 53 (20), 43 (11), 41 (24), 39 (17)
		[$^{13}C_1$ + $^{13}C_2$]–**1** (from [1-^{13}C]-D-arabinose/4-aminobutyric acid) 227 (14), 226 (15), 196 (14), 195 (24), 194 (14), 168 (7), 167 (13), 166 (9), 154 (75), 153 (100), 152 (26), 141 (6), 140 (60), 139 (67), 128 (5), 127 (7), 126 (4), 112 (8), 111 (9), 110 (6), 84 (7), 83 (12), 82 (11), 74 (15), 70 (6), 69 (10), 68 (10), 59 (7), 56 (25), 55 (24), 54 (18), 44 (8), 43 (18), 42 (11), 41 (31), 40 (13), 39 (12)
		δ 1.91 (qui, J = 7.3 Hz, 2H, 3-CH_2), 1.96 (s, 6H, C=CCH_3), 2.32 (t, J = 7.3 Hz, 2H, 2-CH_2), 3.54 (t, J = 7.3 Hz, 2H, NCH_2), 3.67 (s, 3H, CO_2CH_3)
2	methyl 6-(3,4-dimethyl-2,5-dioxo-2,5-dihydropyrrol-1-yl)hexanoate	253 (15), 222 (8), 221 (8), 193 (4), 180 (5), 177 (10), 165 (3), 164 (3), 152 (5), 139 (19), 138 (100), 127 (6), 126 (22), 110 (8), 97 (4), 96 (5), 83 (4), 82 (2), 81 (6), 74 (8), 69 (4), 68 (7), 67 (8), 59 (4), 56 (14), 55 (12), 54 (9), 53 (14), 43 (5), 42 (6), 41 (19), 39 (10)
		δH 1.31 (mc, 2H, 4-CH_2), 1.58 (qui, J = 7.6 Hz, 3[or 5]-CH_2), 1.64 (qui, J = 7.6 Hz, 5[or 3]-CH_2), 1.96 (s, 6H, C=CCH_3), 2.30 (t, J = 7.5 Hz, 2H, 2-CH_2), 3.47 (t, J = 7.2 Hz, 2H, NCH_2), 3.66 (s, 3H, CO_2CH_3)
		δC 8.70 (=CCH_3), 24.42 (*C*H_2), 26.24 (*C*H_2), 28.37 (*C*H_2), 33.48 (*C*H_2), 37.61 (*C*H_2), 51.52 (CO_2*C*H_3), 137.01 (*C*=*C*), 172.30 (ester-*C*O), 174.0 (ring-*C*O)
3	methyl 6-(3,4-dimethyl-2,5-dioxo-2,5-dihydropyrrol-1-yl)-2-(acetylamino)hexanoate	310 (4), 278 (5), 267 (7), 252 (6), 251 (41), 210 (5), 209 (41), 210 (5), 209 (42), 207 (5), 191 (10), 164 (10), 142 (8), 138 (32), 131 (13), 126 (24), 99 (12), 89 (5), 88 (15), 84 (100), 82 (10), 70 (5), 56 (14), 55 (9), 54 (8), 53 (10), 43 (51), 41 (7), 41 (10)
		δ 1.25, 1.34 (each mc, 1H, 4-C*HH'*), 1.57 (mc, 2H, 5-C*HH'*), 1.71, 1.84 (each mc, each 1H, 3-C*HH'*), 1.95 (s, 6H, C=CCH_3), 2.04 (s, 3H, COCH_3), 3.47 (t, J = 7 Hz, 2H, N-CH_2), 3.74 (s, 3H, CO_2CH_3), 4.56 (dt, J = 7 Hz, 7.6 Hz, 1H, *C*HNH), 6.27 (d, J = 7.6 Hz, 1H, N*H*)
4	methyl 6-[3,4-dimethyl-3-[[2-(methoxycarbonyl)ethyl]-thio]-2,5-dioxopyrrolidin-1-yl]hexanoate (about 12:1 mixture of diastereomers)	371 (M^+, 0), 342 (7), 310 (10), 256 (15), 255 (100), 224 (10), 223 (70), 208 (5), 196 (9), 195 (64), 180 (23), 152 (7), 140 (11), 139 (13), 138 (21), 127 (17), 126 (9), 124 (16), 112 (10), 97 (10), 88 (10), 87 (18), 83 (53), 69 (12), 59 (36), 56 (12), 55 (92), 53 (14), 45 (7), 43 (10), 41 (82), 39 (10)
		δ 1.28, 1.33 (each d, J = 7.4 Hz, 2H = 1:12, CHCH_3), 1.34 (mc, 2H, 4-CH_2), 1.59 (qui, J = 7.6 Hz, 2H, 3[or 5]-CH_2), 1.65 (qui, J = 7.6 Hz, 2H, 5[or-3]-CH_2), 1.65 (s, 3H, Cq-CH_3), 2.31 (t, J = 7.5 Hz, 2H, 2-CH_3), 2.59 (mc, 2H, 2′-CH_2), 2.79 (q, J = 7.4 Hz, 1H, C*H*CH$_3$), 2.97 (mc, 2H, 3′-CH_2), 3.49 (t, J = 7.9 Hz, 2H, NCH_2), 3.66, 3.69 (each s, 6H, CO_2CH_3)
5	methyl 6-[3,4-dimethyl-3-[[2-(methoxycarbonyl)-2-(acetylamino)ethyl]thio]-2,5-dioxo-pyrrolidin-1-yl]-hexanoate(mixture of four diastereomers)	δ 1.20–1.40 (m, 2H, 4-C*HH'*), 1.324, 1.321, 1.277, 1.275 (each d, J = 7.4 Hz, 3H, CHCH_3), 1.62, 1.61, 1.47, 1.48 (each s, 3H, Cq-CH_3), 1.55–1.68 (m, 4H, 3-C*HH'*, 5-C*HH'*), 2.041, 2.058, 2.059, 2.087 (each s, 3H, COCH_3), 2.316, 2.317 (each t, J = 7.3 Hz, 2H, CH_2CO_2CH$_3$), 2.68, 2.682, 2.80 (each q, J = 7.4 Hz, 1H, C*H*CH$_3$), 3.00–3.29 (m, 2H, 3′-C*HH'*), 3.49 (mc, 2H, NCH_2), 3.67 (s, 3H, CO_2CH_3), 3.760, 3.770, 3.782, 3.784 (each s, 3H, $CO_2CH'_3$), 4.85, 4.91 (each mc, 1H, *C*HNHCOCH$_3$), 6.335, 6.350, 6.418, 6.618 (each d, J = 7.6 Hz, 1H, N*H*)
6	methyl 6-[3,4-dimethyl-3-[[2-(methoxycarbonyl)-2-(acetylamino)ethyl]thio]-2,5-dioxopyrrolidin-1-yl]-2-(acetylamino)hexanoate (mixture of four diastereomers)	δ 1.20–1.41 (m, 2H, 4-C*HH'*), 1.266, 1.270, 1.314 (each d, J = 7.3 Hz, 3H, CHCH_3), 1.408, 1.420, 1.551, 1.562 (each s, 3H, Cq-CH_3), 1.51–1.75 (m, 2H, 5-C*HH'*), 1.80 (mc, 2H, 3-C*HH'*), 1.972, 1.984, 1.988, 2.030 (each s, 3H, COCH_3), 2.599, 2.619, 2.740, 2.755 (each q, J = 7.3–7.5 Hz, 1H, C*H*CH$_3$), 3.04–3.28 (m, 2H, S-C*HH'*), 3.43 (mc, 2H, NC*HH'*), 3.684, 3.712 (each br s, each 3H, CO_2CH_3), 4.53 (mc, 1H, *C*HNH–Lys), 4.75 (mc, 1H, *C*HNH–Cys), 6.264, 6.294, 6.312, 6.322 (each d, J = 7.9 Hz, 1H, NH–Lys), 6.500, 6.546, 6.680, 6.744 (each d, J = 7.7 Hz, 1H, N*H*–Cys)

[a] 1H NMR data: s, singlet; d, doublet; t, triplet; qui, quintet, m, multiplet; mc, center of a multiplet; br s, broad singlet; br t, broad triplet; br q, broad quartet; dd, doublet of doublets.

were extracted three times with freshly distilled diethyl ether (30 mL). The carboxylic acids were separated from the combined ether extracts by extraction with 5% $NaHCO_3$ (three times, 5 mL). The pH of the combined aqueous phases was readjusted to 2 with 1 N HCl, and the acids were extracted with diethyl ether as described before. The combined ether extracts were dried over anhydrous sodium sulfate and concentrated to about 1 mL on a 20 cm Vigreux column. Aliquots of this solution were derivatized and investigated by capillary GC/MS.

Derivatization. Aliquots (100 mg) were subjected to derivatization with BF_3 (10% in methanol) according to the standard method of Metcalfe and Schmitz (1961): After addition of 3 mL of BF_3/CH_3OH and incubation at 70 °C for 10 min, the methylated compounds were dissolved in 50 mL of diethyl ether, washed with 20 mL of 5% aqueous $NaHCO_3$ and 20 mL of saturated aqueous NaCl, dried over anhydrous sodium sulfate, and concentrated to a volume of 1 mL on a 20 cm Vigreux column. In the case of N^{α}-acetyl-L-lysine, ethyl acetate was used instead of diethyl ether. As a control for

artifact formation during derivatization, one other method was tested; methylation by diazomethane according to the procedure of Tressl et al. (1970). With respect to the compounds of interest the results were found to be in coincidence.

Isolation of Methyl *N*-(Dimethylmaleoyl)-6-aminocaproate (2). After derivatization, the extract of the 6-aminocaproic acid/D-ribose experiment (1 g of each component) was separated by liquid–solid chromatography on silica gel 60 (Merck Chemical Co., activity IV, column 20 × 1 cm) into six fractions with pentane (F1), pentane–dichloromethane 4:1 (F2), 1:1 (F3), and 1:4 (F4), pentane–diethyl ether 1:1 (F5), and diethyl ether (F6) in 40 mL portions. Fraction F5 was rechromatographed by thin layer chromatography on silica gel 60 F_{254} (Merck, 1 mm) using toluene–ethyl acetate (3:1) as solvent. A band with $R_f = 0.71$ was separated and eluted with diethyl ether: 1 mg of **2** was obtained as a yellow oil. For MS and NMR data see Table 1.

Syntheses of Methyl *N*-(Dimethylmaleoyl)-4-aminobutyrate (1), Methyl *N*-(Dimethylmaleoyl)-6-aminocaproate (2), and N^{ϵ}-(Dimethylmaleoyl)-N^{α}-acetyl-L-lysine Methyl Ester (3). Compounds **1–3** were prepared according to the method of Keller and Rudinger (1975), but their procedure was simplified: To the ω-aminocarboxylic acid (1 g) in water (20 mL) was added an excess of dimethylmaleic anhydride (2.5 g) in small portions. The solution was stirred at room temperature for 30 min. During this time the pH was continuously readjusted to 9 by addition of 5 N NaOH. Then the mixture was autoclaved for 2 h at 160 °C. After cooling to room temperature, the pH was adjusted to 2 by adding 1 N HCl. The solution was extracted three times with freshly distilled diethyl ether (**3**: ethyl acetate). The combined organic extracts were dried over anhydrous sodium sulfate, evaporated, and methylated as described before. After distillation (2 mbar, 110 °C, 6 min), the residue was purified by preparative TLC (silica gel 60; 1 mm; eluent, diethyl ether or ethyl acetate).

1: yield 150 mg (7%), yellow oil, $R_f = 0.44$ (ethyl acetate–cyclohexane 1:2). For MS and NMR data see Table 1.

2: yield 150 mg (8%), yellow oil, $R_f = 0.50$ (ethyl acetate–cyclohexane 1:2). For MS and NMR data see Table 1.

3: yield 70 mg (4%), yellow oil, $R_f = 0.30$ (ethyl acetate–cyclohexane 4:1). For MS and NMR data see Table 1.

Model Reactions of *N*-(Dimethylmaleoyl)amino Acid Methyl Esters (2, 3) with Thiol Compounds (Methyl 3-Mercaptopropionate, N^{α}-Acetylcysteine Methyl Ester). The dimethylmaleimidocarboxylic acid methyl ester (5 mg) and the thiol compound (100 mg) were dissolved in 0.2 M phosphate puffer (pH 7, 4 mL) plus methanol (3 mL) by short ultrasonification and incubated for 11 h at 32 °C. After addition of saturated aqueous NaCl (2 mL), the solutions were extracted three times with diethyl ether (in the case of **4**) or ethyl acetate (in the case of **5** and **6**). After evaporation of the organic phase (60 °C), the residue was dissolved in methanol (1 mL) and subjected to TLC as described before (0.5 mm). The addition compounds were eluted with ethyl acetate.

4 [methyl 6-[3,4-dimethyl-3-[[2-(methoxycarbonyl)ethyl]thio]succinimido]caproate]: yield 6 mg (81%), colorless oil, $R_f = 0.32$ (cyclohexane–ethyl acetate 2:1). For MS and NMR data see Table 1.

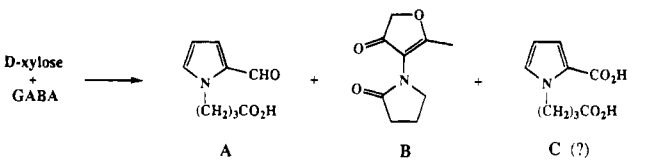
5 [methyl 6-[3,4-dimethyl-3-[[2-(methoxycarbonyl)2-(acetylamino)ethyl]thio]succinimido]caproate]: yield 2 mg (24%), colorless oil, $R_f = 0.32$ (cyclohexane–ethyl acetate 1:4). For NMR data see Table 1.

6 [*N*,*N*-[3,4-dimethyl-3-[[2-(methoxycarbonyl)-2-(acetylamino)ethyl]thio]succinyl]lysine methyl ester]: yield 2 mg (25 %), colorless oil, $R_f = 0.06$ (cyclohexane–ethyl acetate 1:4), 0.3 (ethyl acetate–methanol 100:8). For NMR data see Table 1.

Gas Chromatography (GC)/Mass Spectrometry (MS). The extracts prepared according to the described techniques were analyzed by GC/MS using a 60 m × 0.32 mm i.d. DB-1 fused silica capillary column (temperature was programmed from 80 to 300 °C at 4 °C/min) coupled with a double-focusing mass spectrometer CH 5-DF (Varian MAT), ionization voltage 70 eV, resolution 2000 (10% valley).

^{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 $CDCl_3$ solution; chemical shifts are referenced to tetramethylsilane (TMS) as internal standard. Coupling constants (J) are in hertz.

Scheme 1



RESULTS AND DISCUSSION

Recently we described the specific Maillard compounds formed in model experiments of reducing sugars with the Strecker inactive 4-aminobutyric acid (Tressl et al., 1993b). Moreover, we established the formation pathways of most of the characteristic components by isotopic labeling techniques (Tressl et al., 1993c). If the results of corresponding experiments with Strecker active amino acids (Tressl et al., 1993a, 1994) are taken into account, the Maillard reaction sequences starting with *hexoses* can be generalized in a new detailed scheme.

In *pentose*/4-aminobutyric acid Maillard systems the anticipated C_5 components are generated, e.g. 1-(2-formyl-1-pyrrolyl)butanoic acid (**A**) (Scheme 1) and 1-(5-methyl-3-oxo-2*H*-furan-4-yl)-2-pyrrolidone (**B**) as major compounds from D-xylose. From D-xylose (but also from other pentoses) a third predominant component (**C**) was detected (**A**:**B**:**C** = 1870:600:375). Its structure was only tentatively assigned as 2-carboxy-1-pyrrylbutyric acid (Kersten, 1991).

The MS spectrum of **C**, generated from [1-^{13}C]-D-arabinose, was at variance with the primarily postulated structure: Instead of a 100% singly labeled isotopomer (if **C** would be generated from precursors with the intact sugar skeleton) or a mixture of unlabeled and singly and doubly labeled isotopomers (if **C** would be generated from both labeled and unlabeled sugar fragments) an extraordinary 1:1 mixture of singly and doubly labeled isotopomers was observed. This is demonstrated in Figure 1, where the MS spectra of the unlabeled and labeled components are compared.

Therefore, the formation of compounds of type **C** was reinvestigated, starting with several pentoses and hexoses and different Strecker inactive amino acids (4-aminobutyric acid, 6-aminocaproic acid, N^{α}-acetyl-L-lysine). Selected results are summarized in Table 2. Compounds of type **C** (**1–3**), identified by their characteristic MS fragmentation patterns, are generated in each case. Obviously, they are formed in high yields only from pentoses (D-xylose, D-arabinose, D-ribose), even under mild conditions. Their minor formation from hexoses is correlated to the well-known ($C_1 + C_5$)-cleavage reactions of hexoses during the Maillard reaction. Compounds of type **C** were not observed in glyceraldehyde (methylglyoxal)/ω-amino acid systems, and, therefore, these very common C_3 fragments can be excluded as their precursors.

For definite structure determination of type **C** compounds the reaction product of 6-aminocaproic acid/D-ribose (2 h, 160 °C), separated (after derivatization) by column chromatography and subsequent thin layer chromatography, was investigated by ^{1}H and ^{13}C NMR spectroscopy. Surprisingly, the observed spectra clearly reveal a symmetrical structure for **C**: besides the characteristic signals for the ω-aminohexanoic acid

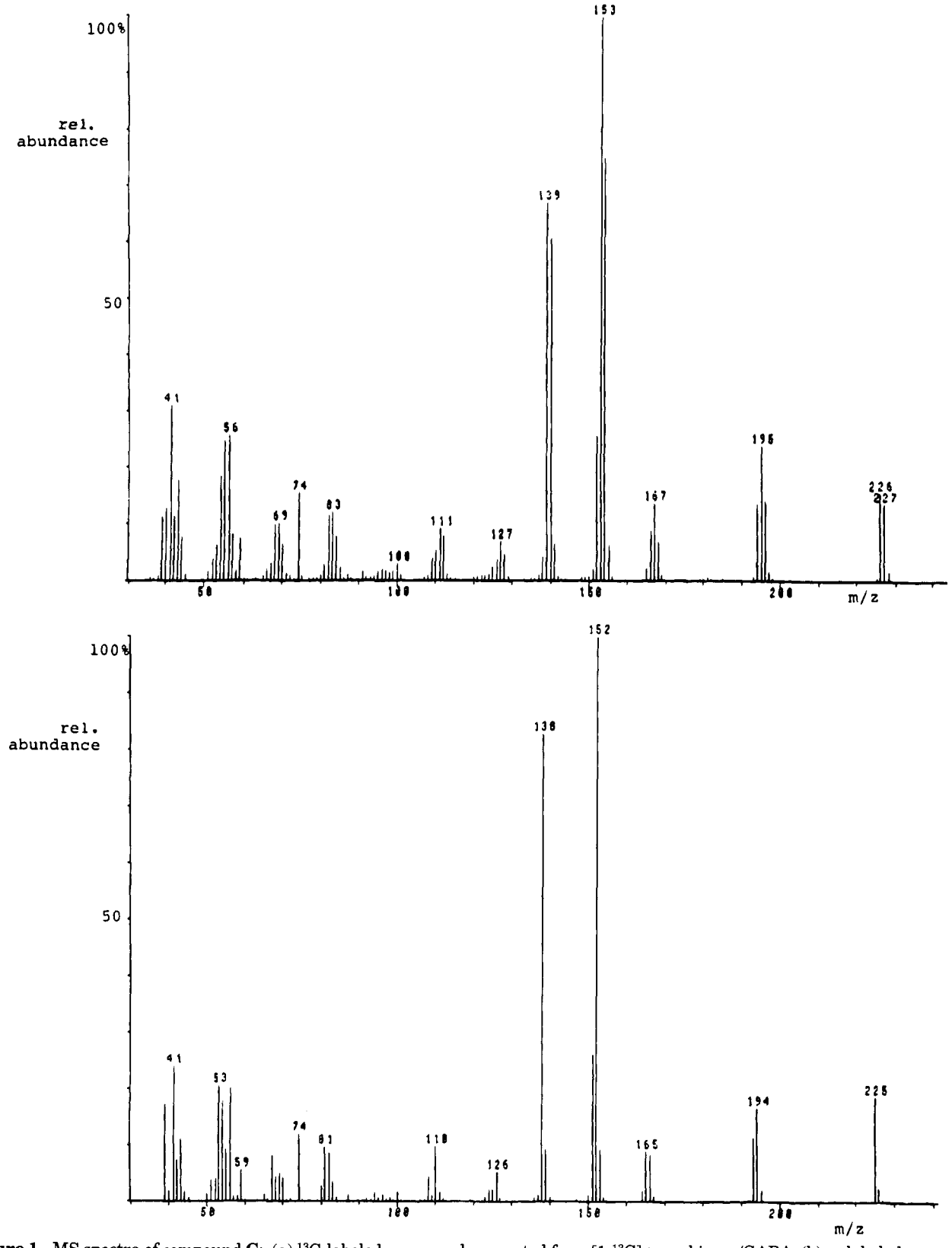


Figure 1. MS spectra of compound **C**: (a) ^{13}C-labeled compound generated from [1-^{13}C]-D-arabinose/GABA; (b) unlabeled compound generated from D-xylose/GABA.

moiety the ^{1}H NMR shows only one singlet for two equivalent methyl groups, and the ^{13}C NMR shows two equivalent primary, two equivalent quaternary C=C, and two equivalent quaternary C=O atoms. Their chemical shifts fit those of the well-known dimethylmaleimide unit. The MS fragmentation is in agreement with this assignment. Besides the α-, β-, and γ-cleavages of the alkyl chain into fragment ions *m/z* 194/193, 166/165, 152, and 138, the degradation into fragment ions *m/z* 110, 82, and 67 indicates the dimethylmaleimide structure of compound **1**.

The derived structure of an ω-(dimethylmaleimido)-carboxylic acid was proven by synthesis according to a known method reported by Keller and Rudinger (1975) starting with dimethylmaleic anhydride. The GC/MS data and the NMR spectral data of the synthesized

Table 2. Yield of Selected Products from Various ω-Amino Acid/Reducing Sugar Model Experiments (Concentration in Parts per Million)

compound	amino acid[a]	D-ribose 2 h/160 °C	D-ribose 7 days/70 °C[b]	D-ribose 28 days/50 °C[b]	D-glucose 2 h/160 °C	D-fructose 2 h/160 °C
1	4-ABA	2300				
2	6-ACA	3500[c]	130	180	150	100
3	N^{α}-AL	3900				
6-(2-formyl-1-pyrrolyl)caproic acid	6-ACA	2000[c]	7	260		<1
6-(1-pyrrolyl)caproic acid	6-ACA	300[c]	30	110	<1	
6-(2-acetyl-1-pyrrolyl)caproic acid	6-ACA	300[c]	10	80	1600	300

[a] 4-ABA, 4-aminobutyric acid; 6-ACA, 6-aminocaproic acid; N^{α}-AL, N^{α}-acetyl-L-lysine. [b] 0.2 M phosphate buffer, pH 7. [c] Also formed from D-arabinose or D-xylose.

compounds (**1**–**3**) are in full agreement with those of the compounds characterized in the Maillard experiments.

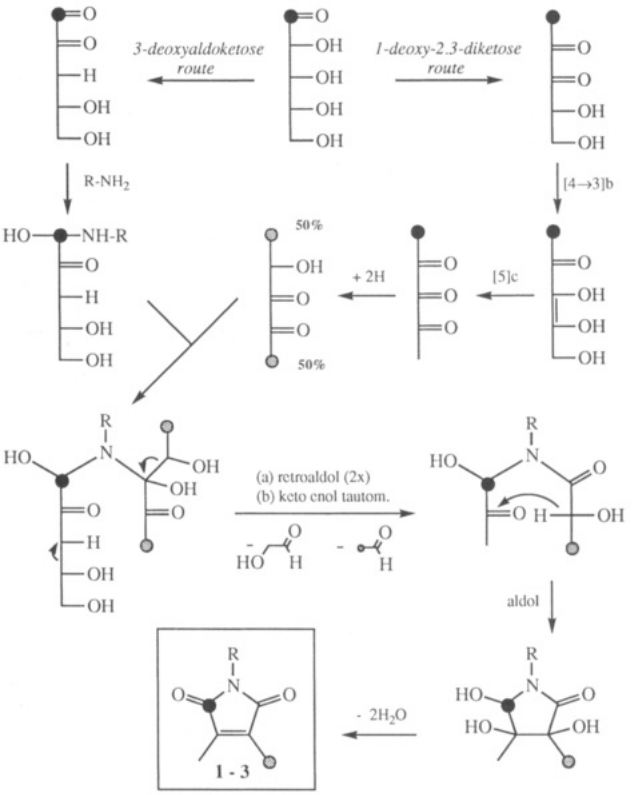
There is no trivial route from the pentoses to the six-carbon skeleton in type **C** compounds. At first sight, a cyclizing condensation reaction of two C_3 sugar fragments with the starting ω-amino acid seems to be reasonable. However, this assumption is incompatible with both the observed pentose specificity of the reaction and the definite 1:1 distribution solely to singly and doubly labeled isotopomers if [1-^{13}C]pentoses are used as starting compounds. From the latter fact it must be concluded that the formation route to type **C** compounds involves at least two essential steps: one accompanied by a 100% labeling, the other by a 50% labeling of the resulting intermediate. The second step can be accomplished either with participation of 1:1 mixtures of labeled and unlabeled precursors or, more probably, with sugar-derived precursors provided with two equivalent sites of reactivity (Rewicki et al., 1993a).

A formation route compatible with all of the experimental facts is shown in Scheme 2. From the primarily formed 3-deoxyaldoketose and 1-deoxydiketose, respectively, two intermediates, a hemiaminal by reaction with the ω-aminocarboxylic acid and a triketone with two equivalent sites of reactivity, are formed. These may combine (after partial reduction of the triketone) to a bishemiaminal as the key intermediate. Degradation by two retroaldol cleavages, cyclization, and dehydration lead to the compounds **1**–**3**. The resulting distribution of the label to the CH_3 (50%) and C=O positions (100%) cannot be quantified from the MS data, but the MS fragmentation (Figure 1) is in agreement with this location of the ^{13}C labels.

To explain the pentose specificity of the reaction, the corresponding reaction sequence in the hexose series should be considered shortly: Instead of the noncyclizing triketone (Scheme 2), the very reactive diacetylformoin, easily transformable into different products, is generated on the 1-deoxydiketose route. Thus, the pentose specificity of the formation of type **C** compounds may be partly due to the accumulation of the triketone species in pentose Maillard systems and its effective transformation into the dihydro derivative by transamination with the ω-amino acid.

Altogether, our investigation results in the identification of a hitherto not observed class of pentose-specific Maillard products. Remarkably, other representatives of this class are very common tools used as sulfhydryl reagents in protein chemistry: *N*-ethylmaleimide (NEM) is commonly employed as a SH label for the modification and identification of cysteine residues in peptides and proteins (Colman and Chu, 1970). *N*-Polymethylenecarboxymaleimides, among them 6-maleimidocaproic acid, have been developed as a new class of probes for membrane SH groups (Griffith et al., 1981).

Scheme 2. Formation Pathway of ω-(Dimethylmaleimido)carboxylic Acids (1–3) in 1-^{13}C-Labeled Pentose/ω-Amino Acid Maillard Systems

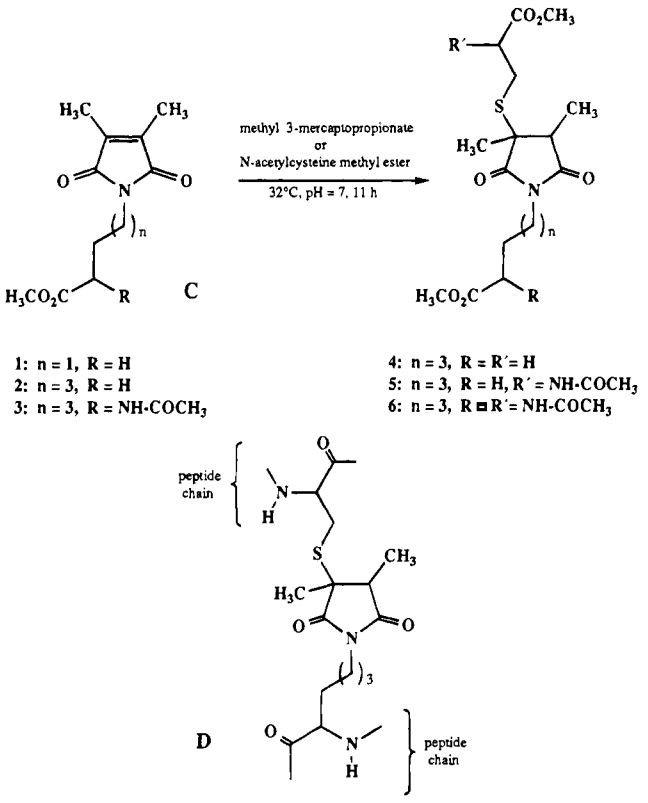


In analogy, the Maillard compounds **1**–**3** show similar reactivity toward SH compounds under very mild conditions. Even at pH 7 (32 °C, 11 h, in water–methanol 4:3) their Michael addition products (**4**–**6**) with methyl 3-mercaptopropionate or N^{α}-acetylcysteine methyl ester are easily formed (Scheme 3).

The structure of the addition products was definitely proven by their MS (in the case of **4**, see Table 1) and their ^{1}H NMR spectra (**4**–**6**). The NMR spectra clearly show the expected number of diastereomers (two in the case of **4**, four in the case of **5** and **6**). The singlet of two equivalent CH_3 groups in **1**–**3** (1.95–1.96 ppm) has disappeared, and, instead, new signals for the now nonequivalent CH_3 groups ($CHCH_3$, 1.26–1.37 ppm, d, J = 7.4 Hz; C_q-CH_3, 1.41–1.68 ppm, s) as well as for the methine CH (2.60–2.79 ppm, q, J = 7.4 Hz) are detected. Of course, the signals of the thiol component are additionally observed in each case.

The reactions described above may be of relevance also in biological systems. Peptide-bound lysine and pentoses may form maleimido compounds of type **C**, which are able to add peptide-bound cysteine with the effect of cross-linking. In this sense compound **6** represents a new potential protein cross-link (structure

Scheme 3



D). Further investigations are necessary to establish the in vitro and in vivo relevance of this process.

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