# Formation of Isoleucine-Specific Maillard Products from [1-$^{13}$C]-D-Glucose and [1-$^{13}$C]-D-Fructose

Roland Tressl,* Christiane Nittka, and Evelyn Kersten

Technische Universität Berlin, Seestrasse 13, 13353 Berlin, Germany

Dieter Rewicki*

Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany

The Maillard reaction of 1-$^{13}$C-labeled D-glucose and D-fructose with L-isoleucine was investigated. The extent and position of the isotopic labeling (from MS data) were used to evaluate origin, reactive intermediates, and formation pathways of pyrroles and pyridinoles. The results are representative for primary α-amino acids. The observed distribution of the label supports 3-deoxyaldoketoses as intermediates of 2-formylpyrroles (**1, 3, 7**) and disqualifies 4-deoxy- and 1-deoxydiketose routes to 2-acetylpyrroles (**2, 4, 9**), respectively. Significant differences are observed in the formation of *N*-alkyl-2-acetylpyrroles and 2-acetylpyrrole in isoleucine (α-amino acid) and 4-aminobutyric acid (amine) Maillard experiments. Identically labeled pyrroles (**1, 6, 7, 9**) and pyridinoles (**8, 10**) from D-glucose and D-fructose confirm identical intermediates in the corresponding Maillard systems.

**Keywords:** *Maillard reaction; [1-$^{13}$C]-D-glucose, Maillard reaction with L-isoleucine; [1-$^{13}$C]-D-fructose, Maillard reaction with L-isoleucine; pyrroles, isoleucine-specific formation in Maillard reactions; $^{13}$C-labeled pyrroles, formation in [1-$^{13}$C]-hexose/L-isoleucine Maillard reactions*

## INTRODUCTION

Isotopic labeling experiments were introduced into studies on the Maillard reaction by Simon and Heubach (1965), Koehler et al. (1969), and Nyhammar et al. (1983). Starting in 1992 we demonstrated the high potential of the combined isotopic labeling and capillary GC/MS techniques for the evaluation of the course of the Maillard reaction (Tressl et al., 1993). In a series of labeling experiments we investigated the Maillard reaction of [1-$^{13}$C]-D-glucose, [1-$^{13}$C]-D-arabinose, and [1-$^{13}$C]-D-fructose with (a) 4-aminobutyric acid (Tressl et al., 1993c) as a Strecker inactive amino acid resembling peptide-bound L-lysine, (b) L-proline and L-hydroxyproline (Tressl et al., 1993a) as Strecker active *secondary* amino acids with a blocked transamination step, and (c) L-cysteine/L-methionine (Tressl et al., 1994a) as Strecker active primary α-amino acids. From these investigations not only could the formation pathways of a series of amino acid specific Maillard products be deduced but conclusions could also be drawn on the main routes of the Maillard reaction. These conclusions were summarized in a revised scheme for the general course of the Maillard reaction of D-glucose (as aldose prototype) (Tressl et al., 1994b) shown in Scheme 1. Compared to corresponding D-fructose Maillard systems, the observed significant changes in product formation, product distribution, and labeling characteristics, strongly influenced by the type of amino acid, are up to now only tentatively rationalized (Tressl et al., 1993c; Rewicki et al., 1994).

As shown in Scheme 1 the $^{13}$C-labeling experiments demonstrated five groups ($P_1$–$P_5$) of Maillard products with the *intact* glucose (fructose) carbon skeleton as well as unlabeled or labeled *sugar fragments* ($F_1$–$F_5$) consisting of one to five C atoms, which are possible precursors of Maillard products (as mixtures of unlabeled and singly and doubly labeled isotopomers). The products $P_1$–$P_5$ are generated along distinct routes, from which the two main routes are well established (Ledl and Schleicher, 1990), whereas their more detailed differentiation (e.g. into the β-dicarbonyl route with a 1,3-dideoxy-1-amino-2,4-diketohexose intermediate) is derived from the results of the labeling experiments.

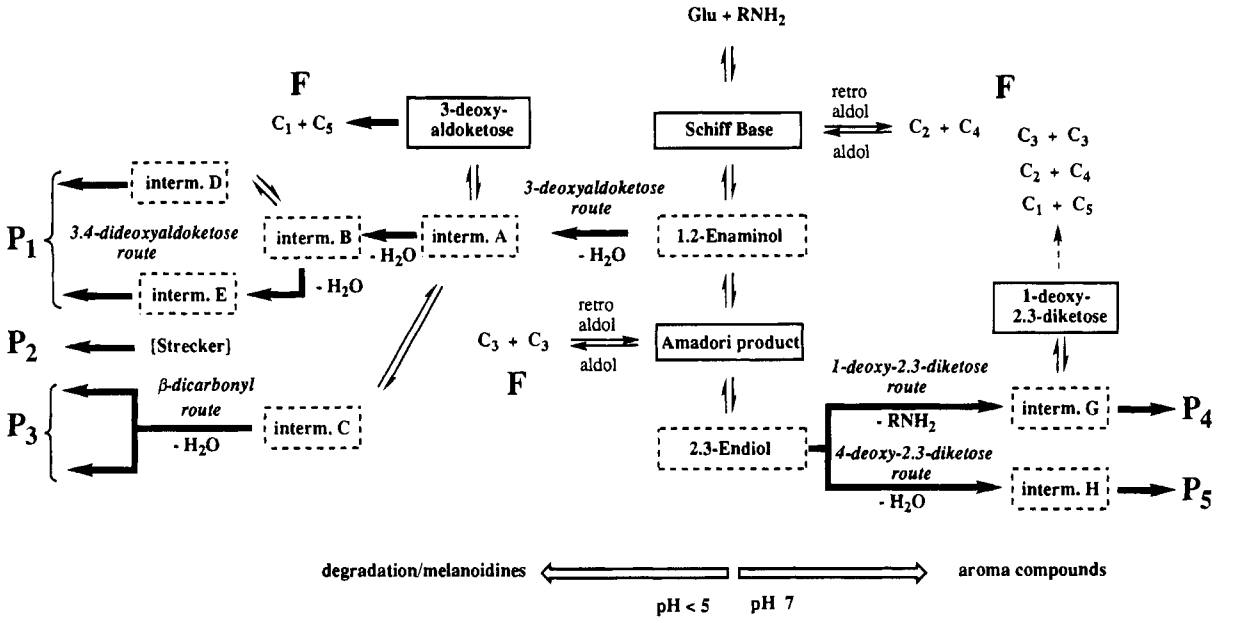
We now report in detail on the formation of pyrroles **1**–**6** (in comparison to the formation of the analogous pyrroles **11**–**16** in 4-aminobutyric acid Maillard systems) and on pyrroles (**7, 9**) and pyridinoles (**8, 10**) generated via Strecker degradation in [1-$^{13}$C]-D-glucose-([1-$^{13}$C]-D-fructose)/L-isoleucine Maillard systems. The labeled products formed in both Maillard experiments were analyzed by capillary GC/MS. From their labeling characteristics (derived from the MS data) we expected insight into reaction sequences and intermediates of the aldose and ketose Maillard systems. As we recently reported (Tressl et al., 1993c), in [1-$^{13}$C]-D-glucose/*4-aminobutyric acid* Maillard systems only trace amounts of 3-deoxyaldoketose products ($P_1$, $P_2$) were formed in the corresponding [1-$^{13}$C]-D-fructose experiments, whereas 1-deoxydiketose products ($P_4$, $P_5$) were generated in comparable amounts from D-glucose as well as from D-fructose. In addition, it must be mentioned that in D-fructose/L-isoleucine Maillard systems the most effective reaction is the formation of pyrazines ($F_3$ products) initiated by retro aldol cleavage of D-fructose into $C_3$ + $C_3$ fragments (Rewicki et al., 1994).

## EXPERIMENTAL PROCEDURES

**Sample Preparation.** Equimolar amounts (3 mmol) of L-isoleucine and anhydrous [1-$^{13}$C]-D-glucose (or [1-$^{13}$C]-D-fructose) dissolved in water (10 mL) were autoclaved for 1.5 h at 150 °C. After the mixture cooled to room temperature, the pH was adjusted to 4 with 0.1 N HCl. The mixture was extracted three times with 30 mL of freshly distilled diethyl ether. The aqueous layer was adjusted to pH 9 by addition of 0.1 N NaOH and extracted as described by Tressl et al. (1993b) to give the basic ether extract. To separate the free acids, the pH 4 ether extract was extracted three times with 20 mL of saturated $NaHCO_3$ solution. The aqueous layer was acidified

0021-8561/95/1443-1163$09.00/0 

**Scheme 1. Pathways and Products ($P_1$–$P_5$ = with the Intact Carbon Skeleton, F = One to Five Sugar Carbon Fragments) of the Maillard Reaction of D-Glucose (Schematic)**




to pH 2–3 with 6 N HCl and extracted with diethyl ether (acidic ether extract). The basic, neutral, and acidic ether extracts were dried over anhydrous sodium sulfate and concentrated to about 1 mL on a 20 cm Vigreux column. After methylation of the acidic ether extract according to the procedure of Tressl et al. (1970), aliquot amounts of the extracts were investigated by capillary GC/MS.

**Gas Chromatography (GC)/Mass Spectrometry (MS).** The extracts prepared according to the described techniques (Tressl et al., 1993b) were analyzed by GC/MS using a 50 m × 0.32 mm i.d. fused silica capillary column coated with Carbowax 20M (column A, temperature was programmed from 70 to 220 °C at 4 °C/min) and a 50 m × 0.32 mm i.d. CP Sil fused silica capillary column Chrompack (column B, temperature was programmed from 80 to 280 °C at 4 °C/min). The columns were coupled with a double-focusing mass spectrometer CH 5-DF (Varian MAT), ionization voltage 70 eV, resolution 2000 (10% valley).

**Synthesis of [1-$^{13}$C]-D-Fructose.** [1-$^{13}$C]-D-Fructose was synthesized by isomerization of [1-$^{13}$C]-D-glucose according to the method of Tressl et al. (1993c).

**Isolation of L-Isoleucine-Specific Pyrroles (1, 2, 4, 5).** Equimolar amounts of L-isoleucine and D-glucose (L-rhamnose, D-xylose) were autoclaved and extracted as described by Tressl et al. (1993c). Individual compounds were separated from the methylated ether extracts by thin layer chromatography.

**MS Data Interpretation of Labeled Compounds.** Isotopic labeling distributions were determined by calculating the ratio of molecular mass ion intensities $M^+$, $(M + 1)^+$, and $(M + 2)^+$ of the analyzed products, which were corrected to their natural content of $^{13}$C isotopes and, if necessary, to any (M – 1) fragmentation. Labeling positions were estimated by interpretation of characteristic mass fragmentation.

**Thin Layer Chromatography (TLC).** Thin layer chromatography was performed with Merck silica gel 60 F-254 plates (1 mm) using toluene/ethyl acetate (9:1) as solvent. Compounds **1** and **3–5** were isolated from the ether extract by repeated TLC and eluted with ethyl acetate from bands with $R_f$ values 0.60 (**5**), 0.57 (**4**), 0.40 (**3**), and 0.36 (**1**). The purified compounds were investigated by MS, IR, and $^1$H NMR spectroscopy (Table 1).

**$^1$H NMR and IR Spectroscopy.** $^1$H NMR spectra were recorded at 270 MHz on a Bruker WH 270 NMR spectrometer in $CDCl_3$ solution; chemical shifts are referenced to tetramethylsilane in parts per million and $J$ values are given in hertz. Infrared spectra were obtained from $CCl_4$ solutions with a Perkin-Elmer Model 275 instrument.

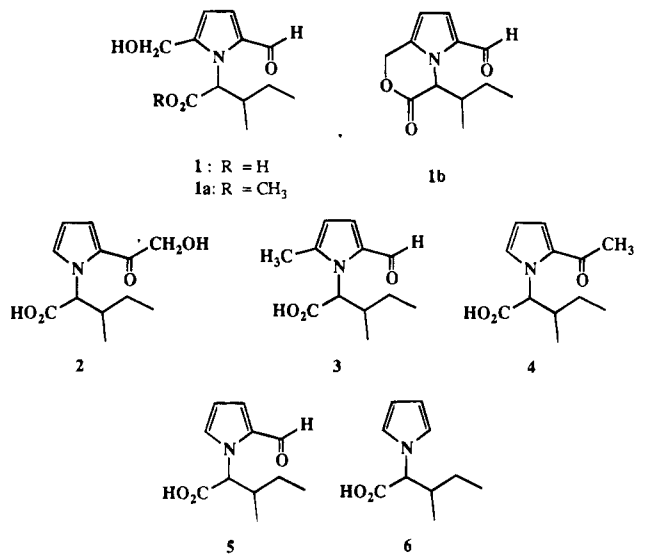
## RESULTS AND DISCUSSION

During heating of L-isoleucine with [1-$^{13}$C]-D-glucose (or [1-$^{13}$C]-D-fructose) six N-substituted pyrroles (**1–6**), structurally related to those (**11–16**, Table 3) generated in 4-aminobutyric acid Maillard experiments (Tressl et al., 1993c), were formed as isoleucine-specific Maillard products. In addition, two further pyrroles (**7, 9**) and two pyridinoles (**8, 10**) were generated with the retained glucose (or fructose) skeleton. The distribution of the isotopic label in some nonspecific Maillard products as furans, furanones, pyranones, etc. was comparable to that in the 4-aminobutyric acid Maillard experiments (Tressl et al., 1993c). The distribution and the position of the isotopic label in the selected pyrroles and pyridinoles (Table 1) were analyzed by mass spectrometry. For this purpose, the unlabeled components were at first isolated and identified by MS, IR, and $^1$H NMR spectroscopy from Maillard systems using unlabeled starting compounds. After that, the mass spectrometric fragmentations of unlabeled and labeled individual pyrroles were compared in detail. The mass spectrometric investigation of $^{13}$C-labeled 2-pyrrolyl-3-methylpentanoic acids revealed α-cleavage and hydrogen transfer into labeled pyrroles and unlabeled 3-methyl-2-pentenoic acid as specific fragmentation. Therefore, the position of the $^{13}$C label in selected pyrroles could be identified. These results give insight into formation pathways of Maillard products from aldose and ketose systems with Strecker active primary amines.

**Identification/Origin of $^{13}$C-Labeled N-Substituted Pyrroles 1–6.** During heating of [1-$^{13}$C]-D-glucose and L-isoleucine pyrroles **1–3, 7,** and **9** and pyridinoles **8** and **10** are formed as almost singly labeled isotopomers and, therefore, these compounds were generated with the retained glucose carbon chain. In the corresponding [1-$^{13}$C]-D-fructose/L-isoleucine experiments only pyrrole **1** and the Strecker degradation products **7–10** were identified with the retained ketose skeleton.

Pyrrole **1** is formed as title compound during heating of L-isoleucine with D-glucose (and D-fructose) and was identified as methyl 2-[5-(hydroxymethyl)-2-formyl-1-pyrrolyl]-3-methylpentanoate (**1a**) and the correspond-

ing pyrrole lactone **1b**, respectively. Analogous compounds were identified during roasting of alanine (valine) with D-glucose (Kato et al., 1977). The $^1$H NMR data of compound **1b** are consistent with the presented structure. The mass spectrometric fragmentations of **1a** and **1b** were investigated in detail for determination of the $^{13}$C labeling in the [1-$^{13}$C]-D-glucose and [1-$^{13}$C]-D-fructose Maillard experiments. The mass spectrometric fragmentations of the pyrrole lactone **1b** with *m/z* 221 (21, $M^+$), 165 (17, M − $CH_2CHCH_2CH_3$, McLafferty rearrangement), 137 (6, M − $CH_2CHCH_2CH_3$ − CO), 136 (5, M − $CH_2CHCH_2CH_3$ − CHO), and base peak 120 are consistent with a pyrrole lactone. The fragment ions *m/z* 108, 80, and 53 result from rearrangements and hydrogen transfers. During heating of [1-$^{13}$C]-D-glucose (or [1-$^{13}$C]-D-fructose) with L-isoleucine, pyrrole **1** is generated with the retained glucose (fructose) chain. The mass spectrometric analysis demonstrated the pyrrole lactone isotopomer [$^{13}$CHO]-**1b**. The relative intensities of the fragment ions *m/z* 166 (21, M − $CH_2CHCH_2CH_3$), 137 (7, M − $CH_2CHCH_2CH_3$ − $^{13}$CO), 136 (3, M − $CH_2CHCH_2CH_3$ − $^{13}$CHO), and base peak at 121 (100, M − $CO_2$ − $CH_3CHCH_2CH_3$) are consistent with a $^{13}$C-labeled formyl group. The fragment ions *m/z* 109, 80, and 53 confirm an unlabeled pyrrole ring.




In addition, the mass spectrometric fragmentations of the methyl ester **1a** and of the $^{13}$C-labeled isotopomers formed in [1-$^{13}$C]-D-glucose and [1-$^{13}$C]-D-fructose Maillard experiments confirm the position of the $^{13}$C label of lactone **1b**. The mass spectrometric fragmentations *m/z* 253 (20, $M^+$), 222 (5, M − $CH_3O$), 221 (10, M − $CH_3OH$), 194 (12, M − $CO_2CH_3$), 125 [40, M − $CH_3O_2CCHCH(CH_3)CH_2CH_3$], 124 [20, M − H − $CH_3O_2CCHCH(CH_3)CH_2CH_3$], and 120 (60, M − 133) are in agreement with an *N*-alkyl-5-(hydroxymethyl)-2-formylpyrrole. The fragment ions *m/z* 125 correspond to 5-(hydroxymethyl)-2-formylpyrrole (α-cleavage/H-transfer) and are further degraded into fragments 108, 80, and 53. In the [1-$^{13}$C]-D-fructose/L-isoleucine Maillard experiments the pyrrole ester **1a** is analyzed as 100% singly labeled [$^{13}$CHO]-**1a**. The mass spectrometric fragmentations *m/z* 126, 109, 80, and 53 clearly indicate a $^{13}$C-labeled formyl group and an unlabeled pyrrole ring.

According to the mass spectrometric fragmentation and chromatographic separations, pyrrole **2** was tentatively identified as 2-[2-[(hydroxymethyl)carbonyl]-1-pyrrolyl]-3-methylpentanoic acid. The mass spectrometric fragmentations of the methyl ester of this minor constituent *m/z* 253 (15, $M^+$), 222 (32, M − $CH_2OH$), and 94 [53, M − $CH_2OH$ − $CH_3O_2CCH(CH_3)CH_2CH_3$, α-cleavage/hydrogen transfer] are consistent with the presented structure. Major fragment ions result by α-cleavage into base peak 194 (100, M − $CO_2CH_3$) and further degradations into 138 (95, M − $CO_2CH_3$ − $CH_2CHCH_2CH_3$), 80, and 53, respectively. The corresponding 2-[(hydroxymethyl)carbonyl]pyrrole from [1-$^{13}$C]-D-glucose/L-isoleucine Maillard experiments was analyzed as a mixture of two 100% singly labeled isotopomers. The mass spectrometric fragmentations of the $^{13}$C-labeled isotopomers *m/z* 254 (12, M + 1), 223 (18, M − $CH_2OH$), 222 (10, M − $^{13}CH_2OH$), 195 (58, M − $CO_2CH_3$), 194 (20, M − $CO^{13}CH_2OH$), 95 (28), and 94 (22) indicate 64% [5-$^{13}$C]-**2** and 36% [$^{13}CH_2OH$]-**2**, respectively. Pyrrole **2** is not generated in the [1-$^{13}$C]-D-fructose/L-isoleucine Maillard experiments.

Pyrrole **3** was isolated from L-rhamnose/L-isoleucine model experiments, where it is generated as a major product. The $^1$H NMR, IR, and MS spectrometric data of the methyl ester of pyrrole **3** are consistent with methyl 2-(5-methyl-2-formyl-1-pyrrolyl)-3-methylpentanoate. The MS data show fragments at *m/z* 237 (33, $M^+$), 208 (8, M − CHO and M − $CH_2CH_3$), 180 [11, M − $CH(CH_3)CH_2CH_3$], and 178 (11, M − $CO_2CH_3$). The base peak *m/z* 109 results by α-cleavage/hydrogen transfer into 5-methyl-2-formylpyrrole (and methyl 2-methylpentenoate), which is further degraded into 108, 80, and 53. In the [1-$^{13}$C]-D-glucose/L-isoleucine Maillard experiment pyrrole **3** was analyzed as 100% singly labeled [$^{13}$CHO]-**3**, indicated by the following MS data of the methyl ester: *m/z* 238 (35, $M^+$), 209 (6, M − $CH_2CH_3$), 208 (4, M − $^{13}$CHO), 181 [6, M − $CH(CH_3)CH_2CH_3$], 180 (11, M − $^{13}$CO − $CH_2CH_3$), 179 (10, M − $CO_2CH_3$), and base peak 110. The fragment ions *m/z* 109, 80, and 53 confirm a $^{13}$C-labeled formyl group and an unlabeled pyrrole ring. In the corresponding [1-$^{13}$C]-D-fructose/L-isoleucine Maillard experiment pyrrole **3** was generated as a mixture of 86% unlabeled and 12% singly and 2% doubly labeled isotopomers.

Pyrrole **4** is generated as a minor constituent in D-glucose (D-fructose)/L-isoleucine Maillard experiments and was isolated and identified from the corresponding L-rhamnose/L-isoleucine experiments. The $^1$H NMR, IR, and MS spectroscopic data confirm 2-(2-acetyl-1-pyrrolyl)-3-methylpentanoic acid. The mass spectrometric fragmentations of the methyl ester derivative *m/z* 237 (12, $M^+$), 194 (22, M − $COCH_3$), 109 [56, M − $CH_3O_2CCH(CH_3)CH_2CH_3$], 94, 69, and base peak 43 (100, $COCH_3$) indicate an α-cleavage/hydrogen transfer into 2-acetylpyrrole and methyl 2-methylpentenoate as typical degradation. In the [1-$^{13}$C[-D-glucose/L-isoleucine Maillard experiments pyrrole **4** is generated as a 100% singly labeled mixture of [5-$^{13}$C]-**4** and [$^{13}CH_3$-CO[-**4**, whereas from [1-$^{13}$C]-D-fructose/L-isoleucine 70% singly and 30% doubly labeled isotopomers are generated, indicating retro aldol cleavage during its formation.

Pyrrole **5** was isolated from D-xylose/L-isoleucine Maillard experiments and identified by $^1$H NMR, IR, and MS spectroscopy as 2-(2-formyl-1-pyrrolyl)-3-methylpentanoic acid. The mass spectrometric fragmentations of the methyl ester derivative are analogous to those of the structurally related compounds **1** and **3**. α-Cleavage and hydrogen transfers into 2-formylpyrroles (*m/z* 96, 95, 94) and methyl 2-methylpentenoate

**Table 1. MS, IR, and $^1$H NMR Spectra of Selected Products, Characterized in L-Isoleucine Model Experiments with [1-$^3$C]-D-Glucose and [1-$^{13}$C]-D-Fructose**[a]

| compd | MS (IR, NMR) data |
|---|---|
| 2-[2-formyl-5-(hydroxymethyl)-1-pyrrolyl]-3-methylpentanoic acid[b] (**1**) (unlabeled) | 253 (20), 222 (5), 221 (10), 206 (9), 196 (4), 194 (12), 178 (6), 176 (30), 148 (7), 136 (12), 125 (40), 124 (20), 120 (60), 108 (40), 101 (18), 97 (20), 96 (30), 80 (20), 69 (30), 59 (15), 53 (20), 41 (100) |
| 2-[2-[$^{13}$C]formyl-5-(hydroxymethyl)-1-pyrrolyl]-3-methylpentanoic acid[b] ([$^{13}$CHO]-**1**) from [1-$^{13}$C]-D-glucose (100% labeled) | 254 (22), 223 (5), 222 (15), 207 (7), 196 (8), 195 (7), 194 (11), 177 (22), 166 (12), 137 (12), 126 (35), 125 (22), 121 (85), 109 (40), 101 (18), 97 (37), 96 (18), 80 (20), 69 (30), 59 (15), 53 (25), 41 (100) |
| 2-[2-formyl-5-(hydroxymethyl)-1-pyrrolyl]-3-methylpentanoic acid lactone (**1b**) (unlabeled) | 222 (3), 221 (21), 193 (4), 192 (3), 177 (2), 165 (17), 160 (4), 137 (6), 136 (5), 120 (100), 108 (20), 97 (10), 93 (8), 80 (6), 65 (15), 57 (12), 53 (15), 41 (45), 39 (35)<br>IR 2960 (s), 2800 (m), 2720 (w), 1760 (s), 1670 (s), 1540 (m)<br>$^1$H NMR 0.98 (t, 3H, $J$ = 7.3 Hz, $CH_2CH_3$), 0.96 (d, 3H, $J$ = 7 Hz, $CHCH_3$), 1.3−1.7 (m, 2H, $CH_2CH_3$), 2.08 (mc, 1H, $J$ = 6.5 Hz, $CHCH_3$), 5.48, 5.34 (AB, each 1H, $J$ = 15.5 Hz, $OCH_2$), 5.67 (d, 1H, $J$ = 6.5 Hz, NC*H*−), 6.28 (d, 1H, $J$ = 4 Hz, 4-H), 6.99 (d, 1H, $J$ = 4 Hz, 3-H), 9.50 (s, 1H, CHO) |
| 2-[2-[$^{13}$C]formyl-5-(hydroxymethyl)-1-pyrrolyl]-3-methylpentanoic acid lactone ([$^{13}$CHO]-**1b**) from [1-$^{13}$C]-D-glucose (100% labeled) | 223 (2), 222 (23), 194 (4), 193 (2), 178 (2), 166 (21), 161 (4), 137 (7), 136 (3), 121 (100), 109 (18), 97 (10), 94 (6), 93 (5), 80 (5), 65 (12), 57 (12), 53 (13), 41 (42), 39 (28) |
| from [1-$^{13}$C]-D-fructose (100% labeled) | 223 (5), 222 (33), 194 (8), 193 (3), 178 (2), 166 (19), 137 (9), 136 (5), 121 (100), 109 (15), 108 (5), 97 (11), 94 (6), 93 (6), 80 (5), 65 (10), 57 (11), 53 (12), 41 (37), 39 (21) |
| 2-[2-[(hydroxymethyl)carbonyl]-1-pyrrolyl]-3-methylpentanoic acid[b] (**2**) (unlabeled) | 253 (15), 222 (32), 194 (100), 162 (25), 138 (95), 134 (25), 126 (32), 101 (10), 94 (53), 80 (18), 79 (10), 69 (75), 59 (15), 53 (18), 41 (95) |
| 2-[2-[(hydroxymethyl)carbonyl]-1-[5-$^{13}$C]pyrrolyl]-3-methylpentanoic acid[b] ([5-$^{13}$C]-**2**)/2-[2-[(hydroxy[$^{13}$C]-methyl)carbonyl]-1-pyrrolyl]-3-methylpentanoic acid[b] ([$^{13}CH_2$OH]-**2**) from [1-$^{13}$C]-D-glucose (mixture of two 100% singly labeled isotopomers) | 254 (12), 223 (18), 222 (10), 196 (8), 195 (58), 194 (20), 163 (17), 162 (10), 139 (55), 138 (30), 135 (12), 134 (10), 127 (15), 126 (10), 101 (12), 95 (28), 94 (22), 81 (17), 80 (10), 79 (7), 69 (78), 59 (18), 54 (15), 53 (15), 41 (100) |
| 2-(2-formyl-5-methyl-1-pyrrolyl)-3-methylpentanoic acid[b] (**3**) (unlabeled) | 237 (33), 208 (8), 180 (11), 178 (11), 176 (5), 148 (15), 129 (8), 110 (21), 109 (100), 108 (38), 97 (15), 94 (18), 81 (5), 80 (12), 69 (28), 67 (11), 59 (18), 53 (24), 41 (80), 39 (31)<br>IR 2960 (s), 2800 (w), 2720 (m), 1740 (s), 1655 (s), 1540 (w)<br>$^1$H NMR 0.99 (t, 3H, $J$ = 7.4 Hz, $CH_2CH_3$), 1.17 (d, 3H, $J$ = 6.2 Hz, $CHCH_3$), 1.2−1.25 (m, 2H, $CH_2CH_3$), 2.26 (br s, 1 H, pyrrol-5-$CH_3$), 2.45 (mc, 1H, $CHCH_3$), 3.69 (s, 3H, $OCH_3$), 5.7 (br s, 1H, NC*H*), 6.06 (d, 1H, $J$ = 4 Hz, 4-H), 6.88 (d, 1H, $J$ = 4 Hz, 3-H), 9.4 (s, 1H, CHO) |
| 2-([$^{13}$C]formyl-5-methyl-1-pyrrolyl)-3-methylpentanoic acid[b] ([$^{13}$CHO]-**3**) from [1-$^{13}$C]-D-glucose (100% labeled) | 238 (35), 209 (4), 208 (4), 181 (11), 180 (6), 179 (11), 176 (6), 149 (9), 148 (7), 129 (11), 111 (22), 110 (100), 109 (38), 97 (19), 94 (22), 81 (8), 80 (11), 69 (20), 67 (10), 59 (16), 53 (17), 41 (59), 39 (26) |
| from [1-$^{13}$C]fructose (mixture in 86% unlabeled, 12% singly and 2% doubly labeled isotopomers) | 239 (2), 238 (13), 237 (46), 210 (2), 209 (4), 208 (12), 181 (5), 180 (19), 178 (14), 176 (7), 153 (11), 148 (15), 129 (7), 122 (17), 121 (15), 120 (19), 111 (6), 110 (32), 109 (100), 97 (14), 95 (14), 94 (21), 80 (14), 69 (18), 67 (9), 59 (11), 53 (21), 41 (50), 39 (26) |
| 2-(2-acetyl-1-pyrrolyl)-3-methylpentanoic acid[b] (**4**) (unlabeled) | 237 (12), 209 (4), 194 (22), 178 (14), 176 (20), 166 (8), 148 (10), 134 (17), 122 (31), 110 (16), 109 (52), 94 (31), 80 (13), 69 (24), 59 (13), 43 (100), 41 (53), 39 (22)<br>IR 2960 (s), 2800 (w), 2720 (m), 1740 (s), 1655 (s), 1540 (w)<br>$^1$H NMR 0.99 (t, 3H, $J$ = 7.4 Hz, $CH_2CH_3$), 1.17 (d, 3H, $J$ = 6.2 Hz, $CHCH_3$), 1.2−1.5 (m, 2H, $CH_2CH_3$), 2.44 (s, 3H, $COCH_3$), 2.45 (mc, 1H, $CHCH_3$), 3.72 (s, 1H, $OCH_3$), 5.75 (br s, 1H, NC*H*), 6.22 (dd, 1H, $J_1$ = 3.8 Hz, $J_2$ = 2 Hz, 4-H), 6.99 (dd, 1H, $J_1$ = 3.8 Hz, $J_2$ = 1.3 Hz, 3-H), 7.3 (mc, 5-H) |

**Table 1 (Continued)**

| compd | MS (IR, NMR) data |
|---|---|
| 2-(2-acetyl-1-[5-$^{13}$C]pyrrolyl)-3-methylpentanoic acid ([5-$^{13}$C]-**4**)/2-([2-$^{13}$C]acetyl-1-pyrrolyl)-3-methylpentanoic acid ([$^{13}CH_3CO$]-**4**) | |
| from [1-$^{13}$C]-D-glucose (100% labeled) | 239 (3), 238 (8), 196 (8), 195 (7), 194 (10), 179 (15), 178 (7), 177 (18), 176 (3), 166 (4), 150 (6), 149 (8), 140 (9), 139 (12), 138 (6), 124 (18), 123 (25), 122 (16), 111 (28), 110 (58), 109 (15), 96 (23), 95 (24), 94 (24), 82 (5), 81 (10), 80 (10), 70 (6), 69 (54), 44 (100), 43 (76), 41 (92), 39 (20) |
| from [1-$^{13}$C]-D-fructose (70:30 mixture of singly and doubly labeled isotopomers | 239 (4), 238 (10), 196 (10), 195 (9), 194 (15), 181 (5), 180 (13), 179 (19), 178 (10), 167 (2), 166 (5), 136 (12), 135 (10), 134 (14), 124 (4), 123 (6), 122 (4), 112 (15), 111 (35), 110 (72), 109 (25), 96 (28), 95 (32), 94 (28), 82 (5), 81 (12), 80 (10), 69 (50), 59 (15), 44 (35), 43 (83), 41 (100), 39 (18) |
| 2-(2-formyl-1-pyrrolyl)-3-methylpentanoic acid[b] (**5**) (unlabeled) | 223 (38), 194 (27), 166 (15), 164 (28), 162 (15), 134 (22), 129 (15), 108 (60), 107 (30), 106 (25), 97 (17), 96 (37), 95 (67), 94 (40), 80 (32), 69 (36), 53 (21), 41 (100) |
| | IR 2960 (s), 2810 (m), 2780 (w), 2720 (m), 1740 (s), 1670 (s), 1530 (m), 1250 (s) |
| | $^1$H NMR 0.83 (t, 3H, $J$ = 7.3 Hz, $CH_2CH_3$), 0.98 (d, 3H, $J$ = 6.6 Hz, $CHCH_3$), 1.03 (m, 2H, $CH_2CH_3$), 2.17 (m, 1H, $CHCH_3$), 3.74 (s, 3H, $OCH_3$), 6.05 (d, 1H, $J$ = 9.7 Hz, CHCH), 6.31 (dd, 1H, $J_1$ = 3.68 Hz, $J_2$ = 2.9 Hz, H-4), 6.93 (dd, 1H, $J_1$ = 3.9 Hz, $J_2$ = 1.5 Hz, 3-H), 7.4 (br s, 1H, 5-H), 9.53 (s, 1H, CHO) |
| 2-(2-[$^{13}$C]formyl-1-pyrrolyl)-3-methylpentanoic acid[b] ([$^{13}$CHO]-**5**)/2-(2-formyl-1-[5-$^{13}$C]pyrrolyl)-3-methylpentanoic acid[b] ([5-$^{13}$C]-**5**) | |
| from [1-$^{13}$C]-D-glucose (mixture of unlabeled, singly, and doubly labeled isotopomers) | 225 (10), 224 (25), 223 (5), 196 (7), 195 (15), 194 (13), 166 (15), 165 (18), 164 (9), 140 (5), 135 (15), 134 (11), 110 (16), 109 (49), 108 (31), 97 (52), 96 (65), 95 (39), 81 (16), 80 (27), 69 (48), 54 (10), 53 (23), 41 (100) |
| from [1-$^{13}$C]-D-fructose (mixture of unlabeled, singly, and doubly labeled isotopomers) | 225 (5), 224 (26), 223 (64), 196 (3), 195 (14), 194 (44), 166 (28), 165 (14), 164 (14), 140 (3), 139 (18), 136 (14), 135 (17), 134 (34), 110 (7), 109 (24), 108 (69), 97 (34), 96 (63), 95 (100), 81 (13), 80 (38), 69 (44), 68 (26), 67 (7), 53 (28), 41 (88), 39 (43) |
| 2-(1-pyrrolyl)-3-methylpentanoic acid[b] (**6**) (unlabeled) | 195 (30), 139 (60), 138 (18), 136 (94), 124 (31), 108 (19), 107 (53), 81 (10), 80 (100), 79 (22), 69 (34), 68 (66), 67 (26), 59 (25), 53 (28), 52 (10), 41 (88), 39 (36) |
| 2-(1-[2-$^{13}$C]pyrrolyl-3-methylpentanoic acid[b] ([2-$^{13}$C]-**6**) | |
| from [1-$^{13}$C]glucose (mixture of unlabeled and singly labeled isotopomers) | 197 (2), 196 (8), 195 (26), 143 (13), 139 (48), 137 (24), 136 (76), 125 (7), 124 (25), 108 (9), 107 (53), 81 (34), 80 (100), 79 (24), 69 (49), 68 (62), 67 (22), 59 (25), 54 (8), 53 (28), 52 (13), 41 (86), 39 (29) |
| from [1-$^{13}$C]fructose (mixture of unlabeled and singly labeled isotopomers) | 197 (2), 196 (11), 195 (41), 139 (71), 137 (24), 136 (98), 125 (7), 124 (27), 108 (19), 107 (58), 81 (29), 80 (100), 69 (39), 68 (64), 67 (23), 59 (22), 54 (7), 53 (20), 41 (71), 39 (32) |

[a] MS: *m/z* (relative intensity). IR: $cm^{-1}$; s, strong; m, middle; w, weak. [H] NMR: $\delta$ (ppm), $J$ (Hz); s, singlet; d, doublet; t, triplet; m, multiplet; mc, center of multiplet, br s, broad singlet; dd, doublet of doublets. [b] Identified as methyl pentanoate.

(*m/z* 97, 69, 41) and α-cleavage into *m/z* 164 (28, M − $CO_2CH_3$), 108 (60, M − $CO_2CH_3$ − $CH_2CHCH_2CH_3$), 80, and 53 are in agreement with the presented structure. In the [1-$^{13}$C]-D-glucose/L-isoleucine Maillard experiments pyrrole **5** is generated as a mixture of 14% unlabeled and 67% singly and 19% doubly labeled isotopomers, comparable to the distribution observed in **15** (Table 3) in 4-aminobutyric acid Maillard systems (Tressl et al., 1993c). The distribution of 74% unlabeled and 23% singly and 3% doubly labeled isotopomers from [1-$^{13}$C]-D-fructose/L-isoleucine Maillard experiments indicates differences in the fragmentations of the carbon chains of D-glucose and D-fructose, respectively.

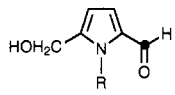
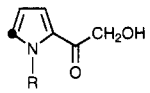
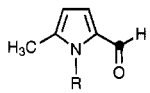
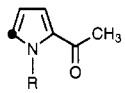
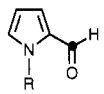
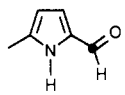
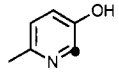
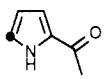
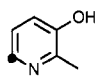
Pyrrole **6** was identified by mass spectrometric fragmentations of the methyl ester derivative as 2-(1-pyrrolyl)-3-methylpentanoic acid. α-Cleavages into *m/z* 138 [18, M − $CH(CH_3)CH_2CH_3$] and 136 (94, M − $CO_2CH_3$) and α-cleavage/hydrogen transfer into pyrrole and methyl 2-methylpentanoate are in agreement with the presented structure. The base peak at *m/z* 80 is generated by two subsequent α-cleavages and hydrogen transfer, and the fragment is further degraded to 53. In the corresponding [1-$^{13}$C]-D-glucose/L-isoleucine ([1-

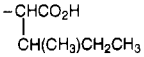
**Table 2. Labeling Characteristics of Selected Pyrroles and Pyridinoles from [1-$^{13}$C]-D-Glucose and [1-$^{13}$C]-D-Fructose, Respectively**

| | | distribution (unlabeled/singly/doubly) | |
|---|---|---|---|
| compound | route[a] | from D-glucose | from D-fructose |
| [$^{13}$CHO]-**1** | $P_1$ | 0/100/0 | 0/100/0 |
| [$^{13}$CHO]-**3** | $P_1$ | 0/100/0 | 86/12/2 |
| **2** | $P_3$ | 0/100/0 | (−) |
| **4** | $P_3$ | 0/100/0 | 0/70/30 |
| **5** | F | 14/67/19 | 74/23/3 |
| **6** | F | 81/19/0 | 85/15/0 |
| [$^{13}$CHO]-**7**[b] | $P_2$ | 2/98/0 | 10/90/0 |
| [2-$^{13}$C]-**8**[b] | $P_2$ | 2.5/97.5/0 | 2.5/97.5/0 |
| [5-$^{13}$C]-**9**[b] | $P_2$ | 1.6/98.4/0 | 4/96/0 |
| [6-$^{13}$C]-**9**[b] | $P_2$ | 3/97/0 | 7.5/92.5/0 |

[a] $P_1$ = 3,4-dideoxyaldoketose route; $P_2$ = 3-deoxyaldoketose route + Strecker degradation; $P_3$ = β-dicarbonyl route; F = fragmentation route. [b] Compounds **7**−**10** from α-amino acids/D-glucose and from glycine/D-fructose Maillard experiments.

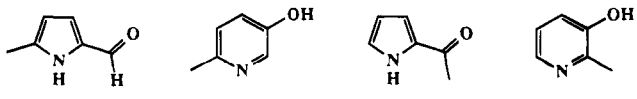
**Table 3. Formation of Pyrroles in Reducing Sugars/4-Aminobutyric Acid (Isoleucine) Maillard Systems**

| no. | | | 4-aminobutyric acid | | isoleucine | |
|---|---|---|---|---|---|---|
| $R_1$[a] | $R_2$[a] | component | D-glucose | D-fructose | D-glucose | D-fructose |
| 1 | 11 |  | 3000 | 20 | 4500 | 1080 |
| 2 | 12 |  | 380 | + | + | − |
| 3 | 13 |  | 280 | + | 150 | 40 |
| 4 | 14 |  | 1250 | + | 8 | 10 |
| 5 | 15 |  | 215 | 50 | 55 | 50 |
| 6 | 16 |  | 225 | 20 | 17 | 22 |
| 7 | |  | + | − | 800 | 240 |
| 8 | |  | − | − | 370 | 80 |
| 9 | |  | − | − | 390 | 400 |
| 10 | |  | − | − | 140 | + |

[a] $R_1$ =  $R_2$ = $-(CH_2)_3CO_2H$. Compounds **1**–**10** ($R = R_1$) from isoleucine/Glu(Fru); compounds **11**–**16** ($R = R_2$) from 4-aminobutyric acid/Glu(Fru). [b] Concentration (ppm); +, <1 ppm. −, not detected.

$^{13}$C]-D-fructose/L-isoleucine) Maillard experiments pyrrole **6** was examined as a mixture of 81% unlabeled and 19% singly labeled (85% unlabeled and 15% singly labeled) isotopomers. These results demonstrate similar fragmentations during the generation of pyrrole **6** from D-glucose and D-fructose.

**Identification/Origin of $^{13}$C-Labeled Pyrroles and Pyridinoles Generated by Strecker Degradation.** Compounds **7**–**10** were identified as 100% singly labeled isotopomers by Nyhammar et al. (1983) in [1-$^{13}$C]-D-glucose/glycine Maillard experiments. These



**7** **8** **9** **10**

pyrroles and pyridinoles are generated during Strecker degradation of [1-$^{13}$C]-D-glucose and [1-$^{13}$C]-D-fructose with primary α-amino acids with the retained carbon skeletons. The mass spectrometric fragmentations of pyrrole **7** *m/z* 109 (100, $M^+$), 108 (80, M − H), 80 (38, M − H − CO), and 53 (42, M − H − CO − HCN) are changed into *m/z* 110 (100, $M^+$), 109 (78, M − H), 80 (42, M − H − $^{13}$CO) and 53 (50, M − H − $^{13}$CO − HCN) in the [1-$^{13}$C]-D-glucose/L-isoleucine Maillard experiments. These results are in agreement with the formation of [$^{13}$CHO]-**7**. The ratio **7**/[$^{13}$CHO]-**7** is changed from 2/98 ([1-$^{13}$C]-D-glucose) to 10/90 (1[1-$^{13}$C]-D-fructose).

Pyridinole **8** is generated as a mixture of 97.5% singly labeled and 2.5% unlabeled isotopomers from [1-$^{13}$C]-D-glucose as well as [1-$^{13}$C]-D-fructose. The mass spectrometric fragmentations *m/z* 109 (100, $M^+$), 108 (15, M − H), 94 (2, M − $CH_3$), 82 (4, M − HCN), and 81 (12, M − CO) indicate a methyl group and a hydroxyl group in 6-methyl-3-pyridinol. In the $^{13}$C-labeled **8** an unlabeled methyl group is analyzed by *m/z* 95 (4, M − $CH_3$) and the 2-$^{13}$C labeling is indicated by *m/z* 82 (8, M − H$^{13}$CN). The fragment ions *m/z* 110, 109, 81, 54, 53, 40, and 39 are consistent with [2-$^{13}$C]-**8**.

2-Acetylpyrrole (**9**) is generated mainly as [5-$^{13}$C]-**9** with the retained carbon chain from both [1-$^{13}$C]-D-glucose and [1-$^{13}$C]-D-fructose. The mass spectrometric fragmentations of the unlabeled compound **9** with *m/z* 109 (90, $M^+$), 94 (100, M − $CH_3$), 66 (52, M − $COCH_3$), 43 (15, $CH_3CO^+$), and 39 (38, $C_3H_3^+$) are changed in the $^{13}$C-labeled experiments into *m/z* 110 (80, $M^+$), 95 (100, M − $CH_3$), 67 (57, M − $COCH_3$), 43 (18, $CH_3CO^+$), and 40 (48, $C_2{}^{13}CH_3^+$), clearly indicating an unlabeled acetyl group and a $^{13}$C-labeled pyrrole ring.

In contrast to **7**–**9** the location of the $^{13}$C-label in the 100% singly labeled pyridinole **10** is not clearly detectable by mass spectrometry. There is no fragment ion (M − $CH_3$) in **10**, as was observed in case of isomer **8**. The fragment ions *m/z* 80 (45, M − CHO), 53 (13, $C_4H_5^+$), and 39 (45, $C_3H_3^+$) are shifted to *m/z* 81, 54, and 40, whereas *m/z* 41 (6, $CH_3CN^+$) is unchanged in the $^{13}$C-labeled **10**, confirming an unlabeled methyl group. Therefore, **10** is characterized as [6-$^{13}$C]-**10**, comparable to the results of [1-$^{13}$C]-D-glucose/glycine experiments (Nyhammar et al., 1983). The $^{13}$C-labeled isotopomers of **7**–**10** from [1-$^{13}$C]-D-glucose and [1-$^{13}$C]-D-fructose Maillard experiments are comparable, indicating identical intermediates in the formation pathways of aldohexose and ketohexose Maillard systems.

**Formation Pathways of Pyrroles and Pyridinoles in [1-$^{13}$C]-D-Glucose/[1-$^{13}$C]-D-Fructose)/L-Isoleucine Maillard Systems.** The results of the $^{13}$C-labeling experiments support analogous reaction sequences to pyrroles in the isoleucine Maillard systems as demonstrated for 4-aminobutyric acid (and amines) (Tressl et al., 1993c). Tables 2 and 3 clearly indicate the differences between amine and α-amino acid Maillard systems. Both systems generate [$^{13}$CHO]-**1**, [$^{13}$CHO]-**3** via 3-deoxyaldoketose from [1-$^{13}$C]-D-glucose with the retained carbon chain and in equal amounts. In the corresponding [1-$^{13}$C]-D-fructose Maillard systems only isoleucine generates [$^{13}$CHO]-**1**, comparable to the aldose experiment. The $^{13}$C-labeling of the [$^{13}$CHO]-**3** (86% unlabeled and 12% singly and 2% doubly labeled) indicates retro aldol reactions during generation of compound **3** from D-fructose. *N*-Alkyl-2-[(hydroxymethyl)carbonyl]- and *N*-alkyl-2-acetylpyrroles (**12, 14**), which are major products (containing the intact carbon chain) in D-glucose/4-aminobutyric acid Maillard experiments, are observed as minor compounds (**2, 4**) in isoleucine systems, whereas components **7**–**10** ($P_2$) are exclusively formed in Maillard experiments of *primary* α-amino acids. The $C_5$ and $C_4$ compounds, which result by distinct fragmentations of the sugar skeletons, are comparable in 4-aminobutyric acid (amine) and L-

isoleucine (α-amino acid) Maillard systems. The distribution and extent of $^{13}$C-labeling of [$^{13}$CHO]-**1**, [$^{13}$CHO]-**7**, [2-$^{13}$C]-**8**, [5-$^{13}$C]-**9**, and [6-$^{13}$C]-**10** and their amounts generated in [1-$^{13}$C]-D-glucose/ and [1-$^{13}$C]-D-fructose/L-isoleucine Maillard experiments indicate identical intermediates in aldohexose and ketohexose Maillard systems. In addition, the labeling characteristics confirm the importance of 3-deoxyaldoketose/β-dicarbonyls in the Maillard reaction of α-amino acids into reactive pyrroles, comparable to 4-aminobutyric acid (and amine) Maillard systems. Significant differences are observed in the formation of *N*-alkyl-2-[(hydroxymethyl)carbonyl]- and *N*-alkyl-2-acetylpyrroles from amine (4-aminobutyric acid) and amino acid (L-isoleucine) Maillard experiments. Components [5-$^{13}$C]-**12** and [5-$^{13}$C]-**14** are formed as major products by the β-dicarbonyl route in 4-aminobutyric acid/D-glucose Maillard experiments. In the corresponding isoleucine experiments compound **2** is analyzed as a singly labeled isotopomer, whereas compound **4** is obtained as a mixture of unlabeled and singly and doubly labeled isotopomers, indicating retro aldol cleavages on the formation route. The formation pathway to [5-$^{13}$C[-**9** and [6-$^{13}$C]-**10** from [1-$^{13}$C]-D-glucose/ and [1-$^{13}$C]-D-fructose/amino acid Maillard experiments, which was explained by Strecker degradation via Schiff base (Nyhammar et al., 1983), will be reported separately.

ACKNOWLEDGMENT

We are grateful to H. Köppler for assistance in mass spectrometric analysis.

LITERATURE CITED

Koehler, P. E.; Mason, M. E.; Newell, J. A. Formation of Pyrazine Compounds in Sugar-Amino Acid Model Systems. *J. Agric. Food Chem.* **1969**, *17*, 393.

Ledl, F.; Schleicher, E. New Aspects of the Maillard Reaction in Foods and in the Human Body. *Angew. Chem.* **1990**, *102*, 597–626; *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 656–694.

Nyhammar, T.; Olsson, K.; Pernemalm, P.-A. On the Formation of 2-Acylpyrroles and 3-Pyridinoles in the Maillard Reaction through Strecker Degradation. *Acta Chem. Scand., Ser. B* **1983**, *37*, 879–889.

Rewicki, D.; Kersten, E.; Helak, B.; Nittka, C.; Tressl, R. Mechanistic Studies on the Formation of Maillard Products from [1-$^{13}$C]-D-Fructose. In *Maillard Reactions in Chemistry, Food, and Health*; Labuza, T. P., Reineccius, G. A., Monnier, V. M., O'Brien, J., Baynes, J. W., Eds.; Proceedings of the Fifth International Symposium on the Maillard Reaction, Minneapolis, 1993; The Royal Society of Chemistry: Cambridge, U.K., 1994; pp 61–74.

Shigematsu, H.; Kurata, T.; Kato, H.; Fujimaki, M. Formation of 2-(5-Hydroxymethyl-2-formylpyrrol-1-yl)alkyl Acid Lactones on Roasting Alkyl-α-amino Acids from Glucose and Rhamnose. *Agric. Biol. Chem.* **1977**, *35*, 2097–2105.

Simon, H.; Heubach, G. *Chem. Ber.* **1965**, *98*, 3703–3710.

Tressl, R.; Drawert, F.; Heimann, W. *Z. Lebensm. Unters. Forsch.* **1970**, *142*, 393–403.

Tressl, R.; Helak, B.; Kersten, E.; Nittka, C. In *Recent Developments in Flavor and Fragrance Chemistry*; Hopp, R., Mori, K., Eds.; Proceedings of the 3rd International Haarmann & Reimer Symposium, Kyoto, 1992; Verlag Chemie: Weinheim, Germany, 1993; pp 165–181.

Tressl, R.; Helak, B.; Kersten, E.; Rewicki, D. Formation of Proline- and Hydroxyproline-Specific Maillard Products from [1-$^{13}$C]Glucose. *J. Agric. Food Chem.* **1993a**, *41*, 547–553.

Tressl, R.; Kersten, E.; Rewicki, D. Formation of Pyrroles, 2-Pyrrolidones and Pyridones by Heating of 4-Aminobutyric Acid and Reducing Sugars. *J. Agric. Food Chem.* **1993b**, *41*, 2125–2130.

Tressl, R.; Kersten, E.; Rewicki, D. Formation of 4-Aminobutyric Acid Specific Maillard Products from [1-$^{13}$C]-D-Glucose, [1-$^{13}$C]-D-Arabinose, and [1-$^{13}$C]-D-Fructose. *J. Agric. Food Chem.* **1993c**, *41*, 2278–2285.

Tressl, R.; Kersten, E.; Nittka, C.; Rewicki, D. Formation of Sulfur Containing Flavor Compounds from [1-$^{13}$C]-labeled Sugars, Cysteine and Methionine. In *Sulfur Compounds in Foods*; Mussinan, C. J., Keelan, M. E., Eds.; ACS Symposium Series 564; American Chemical Society: Washington, DC, 1994a; pp 224–235.

Tressl, R.; Kersten, E.; Nittka, C.; Rewicki, D. Mechanistic Studies on the Formation of Pyrroles and Pyridines from [1-$^{13}$C]-D-Glucose and [1-$^{13}$C]-Arabinose. In *Maillard Reactions in Chemistry, Food, and Health*; Labuza, T. P., Reineccius, G. A., Monnier, V. M., O'Brien, J., Baynes, J. W., Eds.; Proceedings of the Fifth International Symposium on the Maillard Reaction, Minneapolis, 1993; The Royal Society of Chemistry: Cambridge, U.K., 1994b; pp 51–60.

Received for review July 7, 1994. Revised manuscript received December 8, 1994. Accepted February 6, 1995.[⊗] This work was supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, and by the Arbeitsgemeinschaft Industrieller Forschungsvereinigungen e.V., Köln.

JF9403696

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1995.