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

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The Maillard reaction of 1-^{13}C-labeled D-glucose, D-arabinose, and D-fructose with 4-aminobutyric acid 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, 2-pyrrolidones, and some related products. The results are representative for peptide-bound lysine and systems with blocked Strecker amines. The observed distribution of the label supports 3-deoxyaldoketoses as intermediates of 2-formylpyrroles 1/3 and disqualifies 4-deoxy- and 1-deoxydiketose routes to 2-acetylpyrroles 2/4, respectively. 1,3-Dideoxy-1-amino-2,4-diketose C is postulated as a new key intermediate in the formation route to pyrroles 2/4 from glucose. Starting with [1-^{13}C]-D-fructose, a distinct tendency to a C_3/C_3-cleavage is observed. If the carbon skeleton remains intact, the reactions initiated by 2,3-enolization of the primarily formed fructosylamine are favored against those initiated by 1,2-enolization.

INTRODUCTION

The formation of pyrroles during heating of D-glucose with amines (Jurch and Tatum, 1970; Olsson et al., 1977; Njoroge et al., 1988; Beck et al., 1989) and α-amino acids (Olsson et al., 1978; Nyhammar et al., 1983; Shigematsu et al., 1971; Kato, 1967) was the subject of several studies. From *amines* the pyrroles 1 and 2 (R = alkyl) (cf. Figure 1) and from *primary amino acids* the pyrroles 3 and 4 (R = H) as well as pyridinols are generated as major compounds. (Throughout this paper structures are numbered sequentially using Arabic numerals. To differentiate individual ^{13}C-isotopomers, the isotope symbol and required locants enclosed in square brackets are added.) Labeling experiments with [1-^{13}C]glucose/glycine showed that the methyl groups in 3 and 4 (R = H) are derived from C-6 of glucose (Nyhammar et al., 1983). These results support 3-deoxyaldoketose as the reactive intermediate in the formation of 3 (R = H) but disqualify 1-deoxydiketose as the precursor of 4 (R = H). Njoroge et al. (1988) supposed 4- and 1-deoxydiketoses as intermediates of 2 and 4, respectively. Beck et al. (1989) demonstrated that pyrroles 2 are formed during heating of the corresponding Amadori rearrangement products. Up to now the formation of pyrroles of the type 1–4 from reducing sugars and amines/amino acids was not extensively investigated by labeling techniques.

Therefore, we started a series of labeling experiments with systematic variation of both the reducing sugar and the amino acid Maillard component (Tressl et al., 1993a). Among these investigations the results in the reducing sugar/4-aminobutyric acid model systems (Tressl et al., 1993b) are of special interest. With respect to the formation of pyrroles the results support 3-deoxyaldoketose as intermediate for pyrroles of type 1 and 3 and disqualify 4- and 1-deoxydiketose as intermediates for those of type 2 and 4. The labeling experiments support a β-dicarbonyl intermediate in the Maillard reaction of amines and α-amino acids with glucose.

EXPERIMENTAL PROCEDURES

Synthesis of [1-^{13}C]-D-Fructose. *Isomerization of [1-^{13}C]-D-Glucose.* To 2 g of [1-^{13}C]-D-glucose in 0.1 M phosphate buffer (20 mL, pH 7.5) was added 2 mL of glucose isomerase (Solvay, activity 12 000 TG unit^{-1} mL^{-1}). After 6 h at 50 °C, the reaction was stopped by addition of 0.5 mL of trifluoroacetic acid. The enzyme was separated by centrifugation (15 000 rpm, 10 min). After neutralization (10% NaOH) and freeze-drying, the 1:1 mixture of [1-^{13}C]-D-glucose and [1-^{13}C]-D-fructose was dissolved in 0.05 M sodium acetate (40 mL).

Enzymatic Oxidation of [1-^{13}C]-D-Glucose. Glucose oxidase (160 mg; Sigma, activity 10 000 units) and 50 mg of catalase (Sigma, activity 25 000 units) were added to the prepared 1:1 mixture at 30 ± 3 °C. After 25 min, the reaction was stopped by addition of 0.5 mL of trifluoroacetic acid. The enzymes were separated by filtration (0.7 μm) and the pH adjusted to 9–10. The glucuronic acid formed was separated by anion exchange (Dowex, 50–100 mesh). After neutralization (2 M HCl), [1-^{13}C]-D-fructose was isolated by freeze-drying. By ^{1}H NMR and ^{13}C NMR spectroscopy the isolated ketose was unequivocally identified to be pure [1-^{13}C]-D-fructose: ^{13}C NMR (in DMSO-d_6, used as internal standard δ = 39.7) δ 65.18, 64.17 (t, J = 143 Hz, C-1, 91:9 mixture of anomers).

Sample Preparation. *Reaction of 4-Aminobutyric Acid with [1-^{13}C]-D-Glucose, [1-^{13}C]-D-Fructose, and [1-^{13}C]-D-Arabinose.* Equimolar amounts (1 mmol) of 4-aminobutyric acid and anhydrous [1-^{13}C]-D-glucose, [1-^{13}C]-D-fructose, and [1-^{13}C]-D-arabinose dissolved in water (10 mL) were autoclaved for 1.5 h at 160 °C. The reaction products were extracted, separated, and investigated as described for the unlabeled compounds (Tressl et al., 1993b).

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 25 m × 0.32 mm i.d. CP-Wax fused silica capillary column Chrompack (column A, temperature was programmed from 70 to 220 °C at 2 °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).

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.

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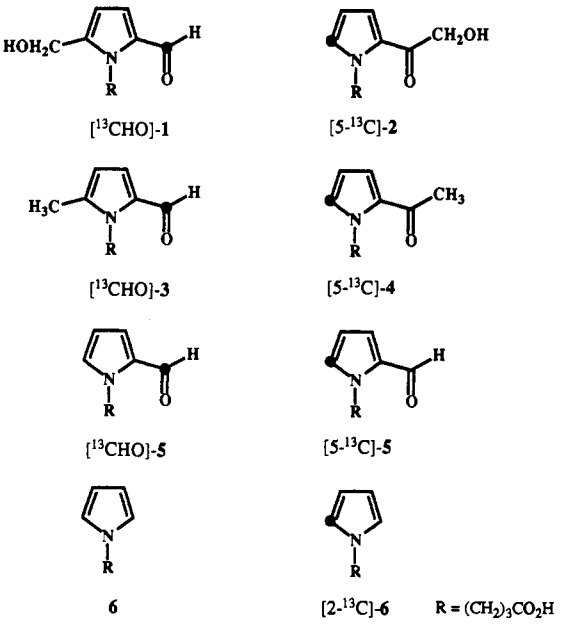

Figure 1. Selected ^{13}C-labeled pyrroles generated in [1-^{13}C]-glucose/, [1-^{13}C]fructose/, or [1-^{13}C]arabinose/4-aminobutyric acid model systems.

RESULTS AND DISCUSSION

Recently we identified a series of pyrroles, 2-pyrrolidones, and 4-pyridones as 4-aminobutyric acid specific Maillard products (Tressl et al., 1993b). The described model reactions carried out with ^{13}C-labeled sugars led to corresponding ^{13}C-substituted products from which substantial conclusions on their formation routes can be drawn. For this aim an unambiguous determination of the distribution and position of labeling from the observed MS data (Table I) is essential. In addition, under the same aspect and for the purpose of intercorrelation, we also investigated the distribution of the isotopic label in some nonspecific, simultaneously formed Maillard products as furans, furanones, pyranones, etc. (Table II).

Identification/Origin of ^{13}C-Labeled Pyrroles (Figure 1). During heating of [1-^{13}C]glucose and 4-aminobutyric acid the pyrroles **1**–**4** are formed as almost singly labeled isotopomers and, therefore, these compounds were generated with the retained glucose carbon chain. The mass spectrometric analysis demonstrated [^{13}C]formyl groups in **1** and **3** and ^{13}C-labeled pyrrole rings in **2** and **4**. These results are comparable to those of Strecker degradation experiments of [1-^{13}C]glucose and glycine (Nyhammar et al., 1983).

The relative intensities in the mass spectra of [^{13}CHO]-**1** and [^{13}CHO]-**3** with fragment ions m/z 109, 80, and 53 (**1** and **3**: m/z 108, 80, and 53) are consistent with unlabeled pyrrole rings. Fragment ions m/z 196 in **1** and m/z 197 and 196 in [^{13}CHO]-**1** are generated by split off of the formyl as well as the hydroxymethyl group. The mass spectrum of [^{13}CHO]-**3** indicates a ^{13}C-labeled aldehyde group (M – ^{13}CO, M – ^{13}CHO).

The exact examination of the molecular mass ion intensities M^+, $(M + 1)^+$, and $(M + 2)^+$ of [^{13}CHO]-**3** showed the formation of 8–9% unlabeled and 3–4% doubly labeled **3** for [1-^{13}C]glucose. This may be explained by reversible cleavage of D-glucose to some extent during this reaction. In [1-^{13}C]glucose/proline (hydroxyproline) model reactions we observed several reversible and irreversible cleavages (e.g., $C_5 + C_1$, $C_4 + C_2$, $C_3 + C_3$) (Tressl et al., 1993a). Because of the expected strong influence of the starting hexose on the cleavage/recombination process, we investigated the formation of pyrroles, in [1-^{13}C]-fructose/4-aminobutyric acid model systems. The MS data demonstrate that [1-^{13}C]fructose is predominantly transformed into **3** by C_3/C_3-cleavage (e.g., retroaldol reaction) and recombination. The incorporation of the generated unlabeled C_3 compound is obviously more effective than that of the labeled one. In contrast, **1** from this experiment was analyzed to be [^{13}CHO]-**1** with almost intact fructose skeleton.

The mass spectrometric investigation of the compounds **2** and **4** revealed 2-acetyl-[5-^{13}C]pyrroles. The fragment ions $(M - CH_2OH)^+$ and $(M - CH_2OH - CH_3OH)^+$ of [5-^{13}C]-**2** demonstrate an unlabeled hydroxyacetyl group. The fragment ions m/z 95, 82, and 54 (in **2**: m/z 94, 81, and 53) are consistent with ^{13}C-labeling within the pyrrole ring, and as a result of its generation from [^{13}C]glucose with an almost intact carbon chain, the position of ^{13}C-substitution must be C-5. **4** is generated as a mixture of unlabeled (10%) and singly (85%) and doubly (5%) labeled isotopomers in [1-^{13}C]glucose/4-aminobutyric acid model experiments. The mass spectrum of the singly labeled isotopomer is consistent with [5-^{13}C]-**4**: m/z 195 ($M - CH_3$), 167 ($M - COCH_3$), 163 ($M - CH_3 - CH_3OH$), and 43 clearly indicate unlabeled acetyl groups. Therefore, 1-deoxydiketose must be excluded as an intermediate of [5-^{13}C]-**4** as is 4-deoxydiketose of [5-^{13}C]-**2**.

4-(2-Formyl-1-pyrrolyl)butanoic acid (**5**) was analyzed as a mixture of unlabeled and singly and doubly labeled isotopomers in the [1-^{13}C]glucose and [1-^{13}C]fructose model experiments. However, in the [1-^{13}C]arabinose/4-aminobutyric acid system, **5** is generated as a mixture of two singly labeled isotopomers with retained carbon chains (98%) and unlabeled isotopomer (2%). The mass spectrum of the [1-^{13}C]arabinose-generated **5** indicates two isotopomers with $M^+ = 196$ by the fragment ions m/z 168 (32, M – CO) and 167 (42, M – ^{13}CO). The fragment ion m/z 136 (M – 59) of the unlabeled **5** corresponds to the fragment ions m/z 137 (39, M – 59) and 136 (50, M – 60) in the ^{13}C-labeled isotopomers. This clearly demonstrates that the fragment ions at m/z 136 are formed by split off CO from the aldehyde group and OCH_3 from the methyl ester. Base peaks 122 and 123 of the unlabeled and singly labeled formyl pyrroles are formed by α-cleavage ($M - CH_2CO_2CH_3$). The mass spectrometric analysis (differentiation m/z 167/168 or 136/137) of the singly labeled pyrroles showed ratios of 60:40 for [^{13}CHO]-**5** and [5-^{13}C]-**5** (from [1-^{13}C]arabinose). This result clearly indicates two different formation pathways for **5**. In the [1-^{13}C]-glucose and [1-^{13}C]fructose model experiments the singly labeled compounds contained 60–65% [^{13}CHO]-**5** and 40–35% [5-^{13}C]-**5**, comparable to the arabinose/4-aminobutyric acid experiments.

The C_4-derived **6** is generated as mixture of unlabeled and singly and doubly labeled isotopomers in [1-^{13}C]glucose/, [1-^{13}C]fructose/, and [1-^{13}C]arabinose/4-aminobutyric acid model systems. The mass spectra showed 41% (glucose), 72% (fructose), and 68% (arabinose) unlabeled **6** from ^{13}C-labeled sugars. The position of ^{13}C-labeling cannot be determined by mass spectrometry.

Identification/Origin of ^{13}C-Labeled 2-Pyrrolidones (Figure 2). In fructose/4-aminobutyric acid model experiments **7** was tentatively identified by mass spectrometric analysis. In the [1-^{13}C]fructose experiment singly labeled [2′-^{13}C]-**7** was analyzed with a retained carbon chain. The fragmentation ions m/z 194 (70, M^+), 151 (65, $M - COCH_3$, 139 (100, M – 55), 123 (55, M – 71), 111 (20, M – 83), and 43 (90, $COCH_3$) clearly demonstrate an unlabeled acetyl group. Therefore, the 2-^{13}C of the furan

Table I. MS Data (*m/z*, Relative Intensity) of Selected Products, Characterized in 4-Aminobutyric Acid Model Experiments with [1-^{13}C]Glucose, [1-^{13}C]Fructose, and [1-^{13}C]Arabinose[a]

compd	MS data
[^{13}CHO]-**1** [4-[2-[^{13}C]formyl-5-(hydroxymethyl)-1-pyrrolyl]-butanoic acid]	
from [1-^{13}C]glucose (100% singly labeled)	226 (51), 209 (4), 197 (20), 196 (22), 168 (31), 166 (46), 165 (44), 164 (35), 149 (24), 136 (93), 125 (37), 120 (16), 109 (100), 106 (17), 101 (29), 96 (16), 94 (13), 93 (18), 80 (50), 69 (22), 68 (30), 59 (81), 53 (34), 41 (81), 39 (55)
from [1-^{13}C]fructose (100% singly labeled)	226 (48), 209 (8), 197 (35), 196 (38), 168 (46), 166 (41), 165 (41), 164 (31), 149 (23), 136 (100), 125 (32), 120 (18), 109 (80), 106 (20), 101 (26), 96 (15), 94 (12), 93 (18), 80 (53), 69 (20), 68 (38), 59 (72), 53 (35), 41 (94), 39 (76)
[5-^{13}C]-**2** [4-[2-(hydroxyacetyl)-1-[5-^{13}C]pyrrolyl]butanoic acid]	
from [1-^{13}C]glucose (100% singly labeled)	226 (25), 195 (93), 163 (52), 151 (100), 137 (80), 136 (33), 108 (31), 107 (26), 101 (15), 95 (35), 83 (20), 82 (16), 81 (41), 69 (54), 59 (93), 54 (36), 44 (81), 41 (91), 39 (45)
from [1-^{13}C]fructose (mixture of unlabeled and singly labeled isotopomer)	226 (28), 225 (10), 210 (10), 195 (60), 194 (35), 163 (46), 162 (22), 151 (78), 150 (40), 137 (62), 136 (53), 135 (23), 108 (30), 107 (33), 106 (15), 101 (33), 95 (25), 94 (22), 81 (32), 80 (28), 69 (53), 59 (80), 54 (24), 53 (28), 41 (100), 39 (62)
[^{13}CHO]-**3** [4-(2-[^{13}C]formyl-5-methyl-1-pyrrolyl)butanoic acid]	
from [1-^{13}C]glucose (mixture of unlabeled and singly and doubly labeled isotopomers)	211 (13), 210 (90), 209 (9), 195 (4), 193 (14), 181 (21), 180 (32), 151 (45), 150 (100), 137 (86), 123 (41), 120 (20), 109 (61), 101 (26), 95 (45), 94 (51), 80 (26), 69 (26), 67 (20), 59 (82), 53 (76), 41 (95), 39 (60)
from [1-^{13}C]fructose (mixture of unlabeled and singly and doubly labeled isotopomers)	211 (31), 210 (74), 209 (41), 195 (8), 193 (22), 181 (28), 180 (25), 165 (15), 151 (57), 150 (68), 137 (68), 136 (42), 123 (41), 120 (20), 109 (75), 108 (38), 101 (35), 95 (51), 94 (46), 80 (30), 69 (33), 67 (30), 59 (70), 53 (63), 41 (100), 39 (63)
[5-^{13}C]-**4** [4-(2-acetyl-1-[5-^{13}C]pyrrolyl)butanoic acid]	
from [1-^{13}C]glucose (mixture of unlabeled and singly and doubly labeled isotopomers)	211 (6), 210 (42), 209 (3), 195 (6), 167 (6), 163 (5), 151 (10), 137 (100), 123 (26), 107 (12), 101 (7), 95 (33), 94 (25), 81 (19), 69 (12), 59 (27), 53 (13), 43 (85), 39 (18)
from [1-^{13}C]fructose (mixture of unlabeled and singly and doubly labeled isotopomers)	211 (9), 210 (41), 209 (13), 195 (7), 167 (5), 163 (6), 151 (8), 150 (5), 137 (75), 136 (48), 123 (23), 122 (12), 107 (12), 106 (17), 101 (12), 95 (33), 94 (15), 81 (20), 80 (12), 69 (14), 59 (24), 53 (14), 43 (100), 41 (32), 39 (26)
[^{13}CHO]-**5**/[5-^{13}C]-**5** [4-(2-[^{13}C]formyl-1-pyrrolyl)butanoic acid/4-(2-formyl-1-[5-^{13}C]pyrrolyl)butanoic acid]	
from [1-^{13}C]glucose (mixture of unlabeled and singly and doubly labeled isotopomers)	197 (9), 196 (40), 195 (10), 169 (10), 168 (47), 167 (33), 138 (18), 137 (80), 136 (55), 123 (100), 122 (45), 109 (57), 108 (28), 95 (60), 94 (38), 81 (68), 80 (36), 69 (36), 68 (26), 67 (19), 59 (56), 53 (45), 41 (89), 39 (65)
from [1-^{13}C]fructose (mixture of unlabeled and singly and doubly labeled isotopomers)	197 (18), 196 (63), 195 (89), 169 (8), 168 (26), 167 (57), 138 (8), 137 (37), 136 (73), 123 (48), 122 (67), 109 (30), 108 (41), 95 (36), 94 (48), 81 (62), 80 (45), 69 (25), 68 (21), 67 (20), 59 (59), 53 (50), 41 (100), 39 (89)
from [1-^{13}C]arabinose (mixture of 60% [^{13}CHO]-**5** and 40% [5-^{13}C]-**5**)	196 (55), 168 (32), 167 (42), 137 (39), 136 (50), 123 (100), 122 (27), 109 (58), 95 (53), 94 (28), 82 (27), 81 (49), 80 (35), 69 (27), 68 (22), 67 (16), 59 (45), 53 (43), 41 (73), 39 (73)
[2-^{13}C]-**6** [4-(1-[2-^{13}C]pyrrolyl)butanoic acid]	
from [1-^{13}C]glucose (mixture of unlabeled and singly and doubly labeled isotopomers)	168 (20), 167 (57), 138 (4), 137 (15), 136 (37), 94 (30), 93 (20), 82 (22), 81 (100), 80 (80), 69 (13), 68 (12), 67 (17), 59 (20), 54 (20), 53 (32), 41 (33)
from [1-^{13}C]fructose (mixture of unlabeled and singly and doubly labeled isotopomers)	169 (8), 168 (31), 167 (70), 138 (5), 137 (20), 136 (40), 95 (15), 94 (40), 82 (41), 81 (100), 80 (77), 69 (18), 68 (18), 67 (17), 59 (20), 54 (20), 53 (32), 41 (33)
from [1-^{13}C]arabinose (mixture with unlabeled compound)	169 (5), 168 (28), 167 (66), 138 (4), 137 (20), 136 (50), 95 (16), 94 (41), 82 (37), 81 (100), 80 (75), 69 (17), 68 (16), 67 (16), 59 (25), 54 (20), 53 (36), 41 (38)
[2′-^{13}C]-**7** [1-(5-acetyl-3-[2-^{13}C]furyl)-2-pyrrolidone]	
from [1-^{13}C]fructose (100% singly labeled)	194 (70), 179 (8), 151 (65), 139 (100), 137 (43), 123 (55), 111 (20), 110 (17), 95 (17), 81 (15), 80 (15), 68 (22), 55 (27), 43 (90), 41 (100)
[^{13}CH$_2$]-**8** [1-(2-furoyl[^{13}C]methyl)-2-pyrrolidone]	
from [1-^{13}C]glucose (100% singly labeled)	194 (6), 111 (65), 99 (100), 95 (17), 84 (26), 71 (55), 69 (35), 44 (51), 41 (48), 39 (37)
[^{13}CH$_3$]-**9** [1-(5-[^{13}C]methyl-3-oxo-2*H*-furan-4-yl)-2-pyrrolidone]	
from [1-^{13}C]arabinose (100% singly labeled)	182 (38), 153 (15), 127 (32), 110 (15), 84 (31), 69 (27), 55 (43), 44 (81), 41 (100)
[^{13}CH$_3$,^{13}CH]-**10** [1-[2-(2-furyl[^{13}C]methylidene)-5-[^{13}C]-methyl-3-oxo-2*H*-furan-4-yl]-2-pyrrolidone]	
from [1-^{13}C]arabinose	261 (100), 206 (75), 178 (74), 176 (31), 164 (12), 148 (22), 108 (21), 84 (13), 80 (18), 79 (18), 69 (33), 55 (18), 44 (48), 42 (33), 41 (73), 39 (30)

Table I (Continued)

compd	MS data
[$^{13}CH_2$]-11 [1-(2-furyl[^{13}C]methyl)-2-pyrrolidone]	
from [1-^{13}C]glucose (mixture of unlabeled and singly labeled isotopomers)	166 (57), 165 (44), 137 (16), 136 (8), 110 (20), 109 (20), 95 (24), 94 (12), 84 (30), 82 (100), 81 (93), 69 (26), 54 (48), 53 (46), 44 (88), 41 (56)
from [1-^{13}C]fructose (mixture of unlabeled and singly labeled isotopomers)	166 (100), 165 (66), 137 (26), 110 (31), 109 (26), 95 (34), 94 (18), 82 (30), 81 (23), 53 (10), 41 (15)
from [1-^{13}C]arabinose (100% singly labeled)	166 (41), 137 (8), 110 (13), 109 (7), 95 (14), 84 (14), 82 (60), 81 (12), 54 (15), 41 (24)
12 [1-(3-furyl)-2-pyrrolidone]	
from [1-^{13}C]glucose (50% singly labeled)	152 (49), 151 (45), 123 (7), 122 (4), 97 (91), 96 (100), 67 (12), 55 (10), 41 (42)
from [1-^{13}C]fructose (50% singly labeled)	152 (61), 151 (45), 123 (10), 122 (5), 97 (100), 96 (75), 69 (8), 68 (8), 55 (6), 41 (40), 39 (29)
[$^{13}CH_3$]-13 [1-(2-oxo-[4-^{13}C]butyl)-2-pyrrolidone]	
from [1-^{13}C]glucose (mixture of unlabeled and singly labeled isotopomers)	156 (10), 155 (41), 99 (35), 98 (100), 84 (10), 70 (48), 69 (23), 43 (30), 41 (35)
[$^{13}CH_3$]-14 [1-(2-oxo-[3-^{13}C]propyl)-2-pyrrolidone]	
from [1-^{13}C]glucose (mixture of unlabeled and singly labeled isotopomers)	142 (27), 141 (46), 99 (27), 98 (100), 84 (17), 70 (65), 69 (35), 44 (16), 43 (40), 41 (60)
from [1-^{13}C]fructose (30% singly labeled)	142 (20), 141 (50), 99 (37), 98 (100), 84 (20), 70 (63), 69 (12), 41 (55)
from [1-^{13}C]arabinose (77% singly labeled)	142 (50), 141 (11), 99 (27), 98 (100), 70 (62), 69 (31), 41 (51)

[a] The pyrroles 1–6 were analyzed as methyl butanoates.

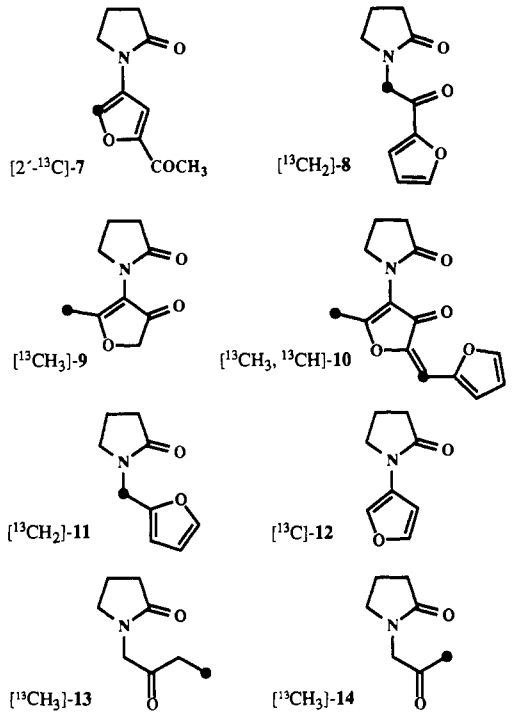

Figure 2. Selected ^{13}C-labeled 2-pyrrolidones generated in [1-^{13}C]glucose/, [1-^{13}C]fructose/, or [1-^{13}C]arabinose/4-aminobutyric acid model systems.

ring system is derived from C-1 of fructose. In pyrrolidine (proline)/glucose (maltose) model experiments a structurally related 3-pyrrolyl-2-acetylfuran was identified (Tressl et al., 1985a).

The MS data of [$^{13}CH_2$]-8 from the [1-^{13}C]glucose/4-aminobutyric acid experiment are in agreement with an intact carbon chain and an unlabeled furoyl moiety, indicated by m/z 95 and 99 (base peak, α-cleavage). The fragment ion 111 (M − 83), which is formed by a McLafferty rearrangenent, clearly indicates a [^{13}C]methylene group derived from the C-1 of glucose. The corresponding [$^{13}CH_2$]-18 was also characterized with retained carbon chain and unlabeled furoyl moiety.

Compounds **9**–**11** were identified in the [1-^{13}C]arabinose/4-aminobutyric acid model experiments as major products with intact carbon skeleton by the observation of 100% labeling. The mass spectrometric fragmentation m/z 182 (38, M^+), 127 (32, M − 55) and 44 (81, $^{13}COCH_3$) of [$^{13}CH_3$]-**9** clearly demonstrates the labeled position. The mass spectrum of [$^{13}CH_3$, ^{13}CH]-**10** is in agreement with a doubly labeled compound, which is generated from [$^{13}CH_3$]-**9** by condensation with ^{13}CHO-labeled 2-furfuraldehyde. [$^{13}CH_2$]-**11** is formed as 100% singly labeled compound from [1-^{13}C]arabinose and as a mixture of unlabeled and singly labeled isotopomers in [1-^{13}C]glucose/ and [1-^{13}C]fructose/4-aminobutyric acid model experiments.

12 and **13** are minor compounds in both the glucose and fructose model systems. The C_4 fragments were analyzed as mixtures of unlabeled and singly labeled isotopomers. From the mass spectrometric fragmentation of [^{13}C]-**12** (M^+ and M − 55) the position of the ^{13}C-label in the furan ring cannot be deduced. M^+ peaks at m/z 156 and 155 and base peaks at m/z 98 in **13** and [$^{13}CH_3$]-**13** clearly demonstrate partially labeled CO–CH_2–$^{13}CH_3$ groups in the latter isotopomer.

14 is generated as a mixture of singly and unlabeled isotopomers from [1-^{13}C]glucose/, [1-^{13}C]arabinose/, and [1-^{13}C]fructose/4-aminobutyric acid model experiments. The mass spectra of [$^{13}CH_3$]-**14** possess labeled acetyl groups and nearly unlabeled base peaks at m/z 98 (M − $CO^{13}CH_3$). The distribution of the ^{13}C-labeling of **14** corresponds to that of acetol [$^{13}CH_3$]-**24** (Table II), which is obviously a possible precursor.

Identification /Origin of ^{13}C-Labeled Furans, Furanones, and Pyranones (Figure 3). The mass spectrometric investigation of furans, furanones, and pyranones (Table II) showed comparable distributions of the ^{13}C-labeling in the [^{13}C]glucose/4-aminobutyric acid and proline model experiments (Tressl et al., 1993a). [^{13}CHO]-**15** and [^{13}CHO]-**16** are generated as 100% singly labeled isotopomers indicated in their mass spectra by (M − ^{13}CO). Only unlabeled **17** is generated in [1-^{13}C]glucose/4-aminobutyric acid systems as observed in proline/hydroxyproline systems. From [1-^{13}C]arabinose **17** is formed as 100% singly labeled compound, but the position of ^{13}C-labeling ([5-^{13}C] or [$^{13}CH_2$]) cannot be derived by mass spectrometric analysis. From [1-^{13}C]fructose/4-aminobutyric acid model experiments **17** was examined as a mixture of singly labeled (19%) and unlabeled (81%) isotopomers.

Table II. MS Data (m/z, Relative Intensity) of Selected Furans, Furanones, and Pyranones, Characterized in 4-Aminobutyric Acid Model Experiments with [1-^{13}C]Glucose, [1-^{13}C]Fructose, and [1-^{13}C]Arabinose

compd	MS data
[^{13}CHO]-**15** [2-[^{13}C]formyl-5-(hydroxymethyl)furan]	
from [1-^{13}C]glucose (100% singly labeled)	127 (45), 110 (7), 97 (74), 69 (27), 53 (16), 41 (100), 39 (46)
from [1-^{13}C]fructose (100% singly labeled)	127 (57), 110 (12), 97 (92), 69 (38), 53 (18), 41 (100), 39 (61)
[^{13}CHO]-**16** [2-[^{13}C]formyl-5-methylfuran]	
from [1-^{13}C]glucose (100% singly labeled)	111 (100), 110 (93), 81 (7), 53 (74)
[^{13}C]-**17** [[^{13}C]furfurylalcohol]	
from [1-^{13}C]glucose (unlabeled)	98 (94), 97 (48), 81 (55), 70 (34), 69 (36), 53 (57), 41 (100), 39 (84)
from [1-^{13}C]fructose (mixture of unlabeled and singly labeled isotopomers)	99 (30), 98 (100), 97 (45), 82 (13), 81 (38), 70 (28), 69 (26), 53 (37), 41 (63), 39 (50)
from [1-^{13}C]arabinose (100% singly labeled)	99 (100), 98 (63), 82 (47), 70 (30), 69 (21), 54 (39), 41 (76), 39 (69)
[$^{13}CH_2$]-**18** [2-[2-^{13}C]hydroxyacetyl)furan]	
from [1-^{13}C]glucose (100% singly labeled)	127 (55), 95 (100), 39 (24)
from [1-^{13}C]fructose (mixture of unlabeled and singly and doubly labeled isotopomers)	128 (3), 127 (16), 126 (29), 97 (8), 96 (57), 95 (100), 39 (24)
[$^{13}CH_3$]-**19** [2,3-dihydro-3,5-dihydroxy-6-[^{13}C]methyl-4*H*-pyran-4-one]	
from [1-^{13}C]glucose (100% singly labeled)	145 (60), 102 (55), 74 (23), 73 (48), 56 (25), 44 (100)
from [1-^{13}C]fructose (100% singly labeled)	145 (93), 102 (66), 74 (38), 73 (55), 56 (28), 44 (100)
[5-$^{13}CH_3$]-**20**/[2-$^{13}CH_3$]-**20** [4-hydroxy-2-methyl-5-[^{13}C]methyl-3(2*H*)-furanone/4-hydroxy-2-[^{13}C]methyl-5-methyl-3(2*H*)-furanone (furaneol)]	
from [1-^{13}C]glucose (100% singly labeled)	129 (55), 86 (10), 85 (11), 73 (5), 58 (45), 57 (52), 44 (93), 43 (100)
from [1-^{13}C]fructose (mixture of unlabeled and singly labeled isotopomers)	129 (55), 128 (5), 86 (11), 85 (12), 73 (7), 58 (42), 57 (51), 44 (82), 43 (100)
[$^{13}CH_3$]-**21**/[4-^{13}C]-**21** [2-hydroxy-3-[^{13}C]methyl-2-cyclopentenone/2-hydroxy-3-methyl-2-[4-^{13}C]cyclopentenone (cyclotene)]	
from [1-^{13}C]glucose (100% singly labeled)	113 (100), 97 (5), 85 (17), 84 (13), 70 (38), 69 (20), 57 (22), 56 (33), 55 (39), 44 (35), 43 (50), 41 (60)
from [1-^{13}C]fructose (mixture of unlabeled and singly and doubly labeled isotopomers)	114 (22), 113 (100), 112 (46), 85 (25), 84 (35), 70 (50), 69 (43), 58 (30), 57 (30), 56 (55), 55 (45), 44 (36), 43 (71), 42 (55), 41 (93)
from [1-^{13}C]arabinose (mixture of unlabeled and singly and doubly labeled isotopomers)	114 (51), 113 (88), 112 (71), 85 (27), 84 (33), 83 (18), 70 (51), 69 (46), 57 (37), 56 (55), 55 (45), 44 (73), 43 (100), 42 (70), 41 (88)
[$^{13}CH_3$]-**22** [4-hydroxy-5-[^{13}C]methyl-3(2*H*)-furanone (norfuraneol)]	
from [1-^{13}C]glucose (100% singly labeled)	115 (95), 71 (10), 56 (37), 55 (29), 44 (86), 43 (100)
from [1-^{13}C]fructose (mixture of unlabeled and singly labeled isotopomers)	115 (7), 114 (27), 71 (3), 55 (10), 44 (20), 43 (100)
from [1-^{13}C]arabinose (100% singly labeled)	115 (65), 71 (5), 56 (18), 44 (100), 43 (25)
[^{13}CHO]-**23** [2-formylfuran]	
from [1-^{13}C]glucose (mixture of unlabeled and singly labeled isotopomers)	97 (89), 96 (100), 95 (32), 67 (7), 39 (90)
from [1-^{13}C]fructose (mixture of unlabeled and singly and doubly labeled isotopomers)	98 (5), 97 (25), 96 (90), 95 (68), 69 (4), 68 (6), 67 (12), 39 (100)
from [1-^{13}C]arabinose (100% singly labeled)	97 (98), 96 (100), 68 (4), 67 (8), 39 (87)
[^{13}C]-**24** [1-hydroxy-[3-^{13}C]propan-2-one (acetol)]	
from [1-^{13}C]glucose (mixture of unlabeled and singly labeled isotopomers)	75 (13), 74 (50), 44 (34), 43 (100)
from [1-^{13}C]fructose (mixture of unlabeled and singly labeled isotopomers)	75 (18), 74 (38), 44 (37), 43 (100)
from [1-^{13}C]arabinose (mixture of unlabeled and singly labeled isotopomers)	75 (68), 74 (19), 44 (100), 43 (25)
[2-^{13}C]-**25** [[2-^{13}C]acetic acid]	
from [1-^{13}C]glucose (mixture of unlabeled and singly labeled isotopomers)	61 (38), 60 (15), 45 (100), 44 (75), 43 (48)
from [1-^{13}C]fructose (mixture of unlabeled and singly labeled isotopomers)	61 (5), 60 (50), 45 (94), 44 (9), 43 (100)

These results clearly indicate different routes to **17** in glucose, fructose, and arabinose Maillard model systems.

[$^{13}CH_2$]-**18** is generated as 100% singly labeled compound in [1-^{13}C]glucose/4-aminobutyric acid model experiments. α-Cleavage (M – $^{13}CH_2OH$) clearly indicates an unlabeled furoyl moiety. The formation of 100% singly labeled [$^{13}CH_3$]-**19** from [1-^{13}C]glucose/ and [1-^{13}C]fructose/4-aminobutyric acid model experiments clearly demonstrates intact carbon chains. Fragment ions m/z 145 (60, M^+), 102 (55, M – $COCH_3$), and 44 (100, $CO^{13}CH_3$ indicate that the $^{13}CH_3$ group of the pyranone is derived from C-1 of glucose and fructose, respectively. **19** is not formed from arabinose.

20 and **21** are generated as 100% singly labeled isotopomers from [1-^{13}C]glucose comparable to proline/hydroxyproline model experiments. Furaneol (**20**) is formed as a mixture of two singly labeled isotopomers, [2-$^{13}CH_3$]-**20** and [5-$^{13}CH_3$]-**20**, from [1-^{13}C]glucose. The mass spectrometric fragmentation m/z 129 (55, M^+), 86 (10, M – $COCH_3$), 85 (11, M – $CO^{13}CH_3$), 44 (93, $CO^{13}CH_3$), and 43 (100, $COCH_3$) clearly demonstrates two isotopomers in a ratio of 48:52. From [1-^{13}C]fructose **20** was

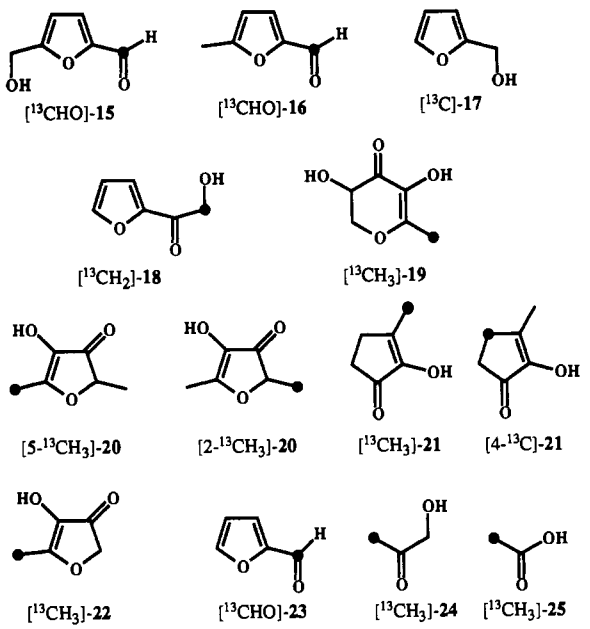

Figure 3. Selected ^{13}C-labeled furans, furanones, and pyranones generated in [1-^{13}C]glucose/, [1-^{13}C]fructose/, or [1-^{13}C]arabinose/4-aminobutyric acid model systems.

analyzed as a mixture of 100% singly labeled isotopomers (90%) and unlabeled isotopomer (10%).

Cyclotene (**21**) was examined as a mixture of two 100% singly labeled isotopomers from [1-^{13}C]glucose/4-aminobutyric acid comparable to proline/pyrrolidine model experiments. From [1-^{13}C]fructose compound **21** is generated as mixture of unlabeled (31%) and singly (60%) and doubly (9%) labeled isotopomers. These results demonstrate different pathways to **21** (which is an important precursor of flavor compounds) in glucose and fructose Maillard systems.

The C_5-derived compound **22** was generated as 100% singly labeled isotopomer in [1-^{13}C]arabinose/4-aminobutyric acid systems and identified as [$^{13}CH_3$]-**22**. The mass spectrum with m/z 115 (M^+) and 44 ($CO^{13}CH_3$) (**22**: m/z 114, 43) clearly indicates that the methyl group of **22** is derived from C-1 of arabinose. From [1-^{13}C]glucose [$^{13}CH_3$]-**22** is also generated as 100% singly labeled compound, but from [1-^{13}C]fructose a mixture of unlabeled (84%) and singly labeled (16%) isotopomers is observed. 2-Formylfuran (**23**) is formed as a mixture of unlabeled and singly labeled isotopomers from [1-^{13}C]glucose, while [1-^{13}C]fructose leads to a mixture of unlabeled and singly and doubly labeled **23**. Only in the case of [1-^{13}C]arabinose is the 100% labeled [^{13}CHO]-**23** generated. Acetol (**24**) is found to be a 64:36 and a 71:29 mixture of unlabeled and singly labeled isotopomers from glucose and fructose, respectively. The label is located in the methyl group, indicated by m/z 44.

Formation Pathways of Pyrroles and Selected Compounds. The results of the [1-^{13}C]glucose experiments support 3-deoxyaldoketoses as intermediates of [^{13}CHO]-**1**, [^{13}CHO]-**3**, and [^{13}CHO]-**5** and disqualify 1-amino-1,4-dideoxydiketose and 1-deoxydiketose as intermediates of [5-^{13}C]-**2**, [5-^{13}C]-**4**, and [5-^{13}C]-**5**, respectively. As outlined in Figure 4 the formation of the title compounds **1**/**3** and **2**/**4** is initiated by an allylic dehydration of the 1,2-enaminol to the intermediate **A**. A further dehydration step to the corresponding 3,4-dideoxyaldo(imino)ketose **B**, which is a known intermediate in the formation of 5-(hydroxymethyl)furfuraldehyde (Anet, 1963), and a subsequent vinylogous Amadori rearrangement of the bis(imine) (to **D**), cyclization, and

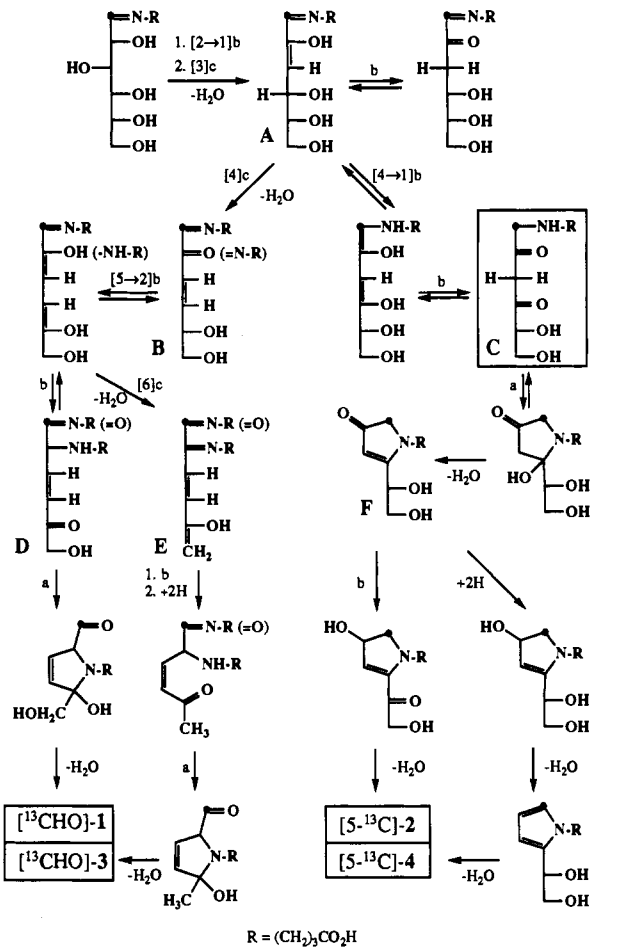

Figure 4. Formation of pyrroles 1–4 from [1-^{13}C]glucose and 4-aminobutyric acid; a, intramolecular ring closure by nucleophilic addition; b, keto/enol or vinylogous keto/enol tautomerization; c, allylic dehydration.

dehydration form **1**. The pathway to **3** from intermediate **B** via dehydration and reduction corresponds to the formation of **3** (R = H) described by Nyhammar et al. (1983) with the exception that the reduction step in the Maillard reaction of 4-aminobutyric acid is unknown (blocked Strecker degradation).

As key intermediate in the formation of **2** we postulate the 1-amino-1,3-dideoxy-2,4-diketose **C**, which is derived from enol **A** via vinylogous Amadori rearrangement. Cyclization, dehydration, and keto–enol tautomerization generate the end products **2** and, by simultaneous reduction, **4**. Of course, in the corresponding [1-^{13}C]arabinose maillard system both the 3-deoxyaldoketose and the β-dicarbonyl routes generate **5**. The two pathways were examined by ^{13}C-labeling experiments at a ratio of 60:40.

In the [1-^{13}C]fructose/4-aminobutyric acid Maillard system only the compounds **1**, **7**, **15**, **19** indicate an intact carbon chain by 100% labeling. In contrast to the [1-^{13}C]-glucose experiments the pyrroles **2**–**6** as well as the compounds **18** and **20**–**23** were analyzed as mixtures of unlabeled and singly (or doubly) labeled isotopomers. These results demonstrate a cleavage of [1-^{13}C]fructose into labeled and unlabeled C_3 components during the early stage of the Maillard reaction. Under the same conditions glucose is easily split into C_2 + C_4 fragments (Namiki et al., 1983; Tressl et al., 1993a).

Thus, the routes of 4-aminobutyric acid specific Maillard products from glucose and fructose show characteristic differences. This is also obvious from Table III, where the amounts of 4-aminobutyric acid specific Maillard

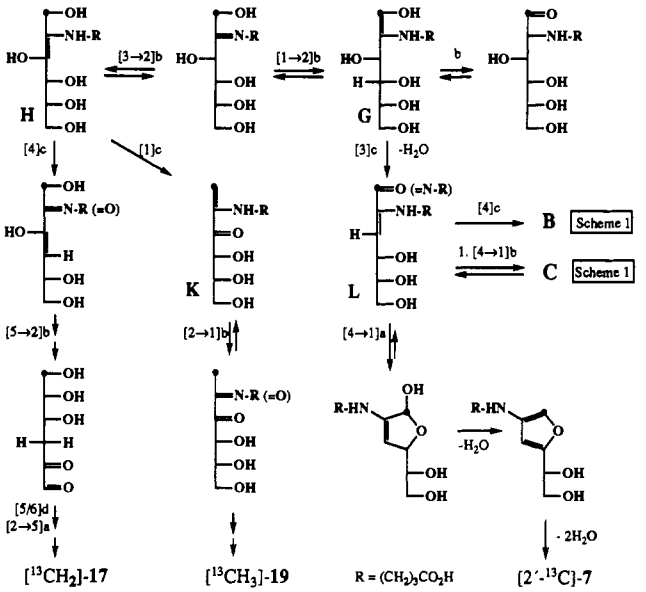

Figure 5. Formation of 4-aminobutyric acid specific Maillard products from [1-^{13}C]fructose: a, intramolecular ring closure by nucleophilic addition; b, keto/enol or vinylogous keto/enol tautomerization; c, allylic dehydration; d, C,C-cleavage.

Table III. Formation of 4-Aminobutyric Acid Specific Maillard Products (1.5 h, 160 °C; Values Represent Concentrations in Parts per Million)

	formation with		
component	Glu	Fru	Rha
1	3000	20	–
2	380	+	–
3	280	+	8200
4	1250	+	2300
5	450	50	–
6	220	20	50
7	–	50	–
8	50	–	–
9	100	–	750
15	+	4	–
16	60	+	2800
17	135	37	305
18	+	–	–
19	215	200	–
20	310	30	31200
21	55	+	–
24	65	5	122
25	+	+	–

products formed from glucose and fructose, respectively, are compared. According to Heyns et al. (1968) 4-aminobutyric acid undergoes a complete ketosyl rearrangement to the corresponding 2-amino-2-deoxyaldose (Heyns rearrangement product). Up to now (Ledl and Schleicher, 1990) degradation of the 2-amino-2-deoxyaldoses via a 3-deoxyaldoketose route was supposed. The results in Table III disagree with this view and clearly show that the 1-deoxydiketose route is predominant in the fructose/4-aminobutyric acid system generating **19** as title compound. The formation of the pyrroles **1–6** is a minor pathway compared to glucose. In Figure 5 the alternative routes from [^{13}C]fructose to labeled 4-aminobutyric acid specific Maillard products are shown. The ketosylamine generated first is transformed into the 1,2-enaminol (**G**) and the 2,3-enaminol (**H**), respectively, which undergo allylic dehydrations to intermediates (**L**, **K**) with an enamine structure. The 3-deoxy derivative **L** is a precursor of the intermediates **B** and **C** (Figure 4) and, therefore, from fructose and glucose identical end products were generated to a certain extent. Table III demonstrates ratios of 3000:20 for the formation of **1** in glucose and fructose model systems. The generation of **7** (with intact carbon skeleton) in the fructose, but not in the glucose model experiment is coincident with the same precursor (**L**). In contrast, compound **8** (also with intact carbon skeleton) was identified only in the glucose/4-aminobutyric acid system and, therefore, this compound represents a typical aldohexose Maillard product generated via 1-amino-1,4-dideoxydiketose as reactive intermediate.

The results in Table III clearly indicate the formation of 1-deoxydiketose products in the fructose/4-aminobutyric acid systems. Title compound [$^{13}CH_3$]-**10** from [1-^{13}C]glucose/ and [1-^{13}C]fructose/4-aminobutyric acid corresponds to 1-deoxy-[1-^{13}C]diketose as intermediate. Thus, the 2,3-enaminol **H**, which can be easily transformed into 1-deoxydiketose products (Figure 5), is a reactive precursor in the Maillard reaction of fructose.

In contrast to the [1-^{13}C]glucose/4-aminobutyric acid experiments resulting in 100% singly labeled products, in the corresponding [1-^{13}C]fructose systems furaneol (**20**) is generated as a mixture of unlabeled (9%) and two singly labeled isotopomers (90%) and cyclotene (**21**) as a mixture of unlabeled (32%) and singly (60%) and doubly (9%) labeled isotopomers. This demonstrates that **20** and **21** are generated from [1-^{13}C]glucose/4-aminobutyric acid via acetylformoin, whereas in the corresponding fructose system **20** (partly) and **21** (predominantly) were generated via fragmentation and recombination. With respect to the formation of **21** from glucose in different Maillard systems, the generation of a *mixture* of 100% labeled isotopomers instead of individual isotopomers (Tressl et al., 1993a) induced by 4-aminobutyric acid shows that the course of the reaction is specifically influenced by the amino acid.

As already mentioned, the results of the labeling experiments demonstrate three different routes to **17** in glucose, fructose, and arabinose systems. In the latter system **17** is formed as 100% singly labeled compound from the corresponding 3-deoxyaldoketose simply by cyclization, dehydration, and reduction. From [1-^{13}C]-glucose the route to the observed unlabeled **17** must involve a complete 1,2-cleavage of the 3-deoxyaldoketose. In the fructose/4-aminobutyric acid system the formation of both the singly labeled (19%) and unlabeled (81%) isotopomer shows that **17** is formed by two pathways: Unlabeled **17** is generated from a 3-deoxyaldoketose intermediate by 1,2-cleavage as described for glucose, whereas the formation of [^{13}C]-**17** is explained by a complete inversion of the functional groups along the chain (Figure 5) with subsequent 5,6-cleavage, ring closure, dehydration, and reduction. This route was proved to be true by analogous experiments starting with [6-^{13}C]-D-fructose, which lead to the expected complementary results (Tressl et al., unpublished results).

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