Retro-Aldol and Redox Reactions of Amadori Compounds: Mechanistic Studies with Variously Labeled D-[^{13}C]Glucose

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Oxidation−reduction reactions necessary to justify many of the products observed in Maillard model systems are usually attributed to molecular oxygen and the so-called reductons. The proline specific 1-(1′-pyrrolidinyl)-2-propanone and 1-(1′-pyrrolidinyl)-2-butanone are such compounds that require reduction steps to justify their formation. Experimental evidence using glucose separately labeled at ^{13}C1, ^{13}C2, ^{13}C3, ^{13}C4, ^{13}C5, and ^{13}C6 indicates that 1-(1′-pyrrolidinyl)-2-propanone is formed by two related pathways, initiated by a retro-aldol cleavage of proline Amadori compound at C3−C4, and 1-(1′-pyrrolidinyl)-2-butanone is formed by three pathways, one initiated by a retro-aldol reaction at C2−C3 of the 1-(prolino)-1-deoxy-4-hexosulose (an isomer of Amadori product formed by carbonyl migration) and two others by similar retro-aldol reactions at C4−C5 from both 3-deoxyglucosone and 1-(prolino)-1,4-dideoxy-2,3-hexodiulose. All of the proposed mechanisms require reduction steps for the formation of the target compounds. Model studies have indicated that reductions in Maillard systems can be effected by three pathways: through hydride transfer from formic acid; through cyclic dimerization of α-hydroxy carbonyl compounds followed by electrocyclic ring opening to produce oxidation/reduction products; and by disproportionation of enediols with α-dicarbonyl compounds through double proton transfer.

Keywords: *Amadori; decomposition mechanisms; oxidation; reduction; Maillard reaction; ^{13}C-labeled glucoses; proline; morpholine*

INTRODUCTION

Amadori products **(1)**, deoxyglucosones, and other sugar derivatives formed under Maillard reaction conditions can undergo retro-aldol reactions, especially under basic conditions, to produce more reactive C_2, C_3, C_4 and C_5 sugar fragments containing α-hydroxy carbonyl and α-dicarbonyl moieties that can react more efficiently with amines than C_6 sugars. α-Dicarbonyls are generally much more reactive toward nucleophiles than simple carbonyls. Amadori rearrangement products (ARP) under basic conditions can generate acetic acid, glyceraldehyde, pyruvaldehyde, and other lower sugars in addition to free amino acid. The process of generating more reactive C_2, C_3, and C_4 carbonyl species from Amadori products becomes important *in vivo* where less reactive amino sites such as nucleotide bases can now be modified by such species, especially in diabetic patients, to produce DNA-linked advanced glycation products (Papoulis et al., 1995). Similarly, if glycated proteins can generate more reactive sugar fragments (Zyzak et al., 1995), especially if the glycation sites are close to the basic amino acids, then, due to the proximity of these species to the target protein, the smaller fragments can further accelerate their post-translational modification. In addition, C_3 sugar fragments have been implicated in generation of free radicals, browning, and weak chemiluminescence in model systems. The reaction of pyruvaldehyde with methylamine, for example, generates free radicals and chemiluminescence (Namiki et al., 1993). Smaller sugar fragments can be formed during pre- or post-Amadori stages under Maillard reaction conditions or from sugar solutions alone in basic media. Generation of these fragments in sugar amino acid or amine mixtures has been studied extensively. Hayashi and Namiki (1980) have shown that sugar alkylamine mixtures can generate glyoxal and pyruvaldehyde as their diimine derivatives at a pre-Amadori stage if the mixtures are heated for 4 min. The formation of these C_2 and C_3 sugar fragments increased with pH, indicating a base-catalyzed retro-aldol reaction. Morita and Takagi (1986) demonstrated, by trapping α-dicarbonyl compounds with *o*-phenylenediamine (OPD), that D-glucose when incubated at 100 °C (pH 10) for 1 h can generate six α-dicarbonyl derivatives containing C_6, C_5, C_4, and C_3 fragments and almost 60% of the dicarbonyl generated was pyruvaldehyde, indicating the preference of D-glucose to undergo retro-aldol cleavage at the C3−C4 position, possibly from a 3-deoxyglucosone intermediate which was estimated to be 14% of the total α-dicarbonyls detected by OPD. Recently, Huber and Ledl (1990) were able to isolate and characterize 1-deoxy- and 3-deoxyglucosones and 1-amino-1,4-dideoxy-2,3-hexodiulose from heated Amadori products in water at 100 °C by trapping these reactive intermediates with OPD as their quinoxaline derivatives.

The use of ^{13}C-enriched sugars for the elucidation of reaction mechanisms concerning the Maillard reaction has been well documented in the literature (Tressl et al., 1993). However, in these studies, sugars enriched at positions other than C1 are seldom used. To study the mechanism of formation of short chain compounds arising from the Maillard reaction, the use of variously enriched sugars (at positions other than C1) is necessary. Weenen et al. (1994) used D-[1-^{13}C]glucose, D-[2-^{13}C]glucose, and D-[1-^{13}C]fructose in the presence of amino acid to study the decomposition reactions and the formation of pyrazines, which was proposed to proceed through dimerization of C_3 and C_4 units of α-aminocar-

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Table 1. Mass Spectrometric Data

1-(1′-pyrrolidinyl)-2-propanone (2)
from pyrolysis of Amadori proline: 127 (2), 85 (06), **84 (100)**, 82 (2), 56 (07), 55 (20), 54 (3), 43 (07), 42 (43), 41 (07)
from acetol/ pyrrolidine reaction mixture: 127 (2), 85 (07), **84 (100)**, 82 (2), 56 (07), 55 (19), 54 (3), 43 (07), 42 (42), 41 (07)
from pyruvaldehyde/proline/formic acid reaction mixture: 127 (1), 85 (06), **84 (100)**, 82 (1), 56 (06), 55 (19), 54 (2), 43 (07), 42 (43), 41 (06)
from pyrolysis of 1-(prolino)-1-deoxy-D-glyceraldehyde: 127 (2), 85 (07), **84 (100)**, 82 (2), 56 (07), 55 (18), 54 (3), 43 (07), 42 (41), 41 (08)
from glycerladehyde/pyrrolidine reaction mixture: 127 (2), 85 (06), **84 (100)**, 82 (2), 56 (06), 55 (18), 54 (3), 43 (06), 42 (39), 41 (06)
from dihydroxyacetone/pyrrolidine reaction mixture: 127 (2), 85 (06), **84 (100)**, 82 (2), 56 (06), 55 (18), 54 (3), 43 (06), 42 (39), 41 (06)
literature data:[a] 127 (2), 85 (06), **84 (100)**, 82 (2), 56 (06), 55 (18), 54 (3), 43 (07), 42 (31), 41 (04)

2-hydroxy-1-(1′-pyrrolidiyl)-1-buten-3-one (3)
from Amadori proline: 155 (72), 138 (10), 137 (12), 126 (8), 112 (96), **84 (100)**, 83 (27), 70 (74), 57 (14), 56 (30), 55 (41), 43 (66), 42 (50)
literature data:[a] 155 (67), 138 (09), 137 (11), 126 (7), 112 (97), **84 (100)**, 83 (26), 70 (64), 57 (11), 56 (24), 55 (36), 43 (52), 42 (55)

2-hydroxy-1-(*N*-morpholino)-1-buten-3-one
from Amadori morpholine: 172 (2), 171 (21), 127 (8), 126 (2), 112 (12), **100 (100)**, 98 (7), 86 (11), 85 (8), 84 (5), 83 (4), 70 (35), 57 (5), 56 (17), 55 (18), 43 (20), 42 (24), 41 (17)

3-(1′-pyrrolidinyl)-2-butanone (4)
from 3-hydroxy-2-butanone/proline reaction mixture: 141 (0.4), 99 (8), **98 (100)**, 70 (4), 69 (7), 68 (2), 56 (27), 55 (9), 54 (4), 42 (7), 41 (8)
from pyrolysis of proline Amadori: 141 (0.2), 99 (8), **98 (100)**, 70 (4), 69 (8), 68 (2), 56 (30), 55 (9), 54 (4), 42 (7), 41 (9)

1-(*N*-morpholino)-2-propanone (6)
from pyrolysis of Amadori morpholine: 143 (2), 115 (4), 101 (6), **100 (100)**, 86 (2), 85 (2), 70 (11), 57 (6), 56 (33), 55 (2), 54 (2), 43 (11), 42 (22), 41 (1)
from morpholine/acetol reaction mixture: 143 (2), 115 (1), 101 (7), **100 (100)**, 86 (1), 85 (2), 70 (12), 57 (3), 56 (32), 55 (2), 54 (2), 43 (11), 42 (20), 41 (8)

di-TMS of 1-(prolino)-1-deoxy-D-glyceraldehyde (7)
from pyrolysis of Amadori proline: 332 (1), 331 (3), 316 (6), 288 (2), 258 (1), 216 (3), 215 (10), 214 (56), 202 (5), 201 (16), **200 (100)**, 189 (2), 186 (8), 174 (1), 173 (2), 172 (13), 170 (1), 156 (1), 147 (1), 142 (11), 130 (3), 129 (17), 103 (18), 96 (8), 83 (7), 82 (8), 75 (11), 74 (4), 73 (40), 70 (4), 68 (2), 61 (2), 59 (4), 58 (2), 55 (14), 54 (3), 45 (7), 43 (3), 42 (4)

1-(*N*-morpholino)-1-deoxy-D-glyceraldehyde
from pyrolysis of Amadori morpholine: 159 (0.5), 129 (0.2), 114 (2), 101 (6), **100 (100)**, 87 (2), 86 (2), 85 (2), 72 (3), 70 (8), 57 (6), 56 (22), 55 (3), 44 (5), 43 (13), 42 (16)

1, 2-(1′,1′-dipyrrolidinyl)-1-propene (10)
from pyrolysis of glyceraldehyde/proline mixture: 181 (14), **180 (100),** 179 (2), 165 (3), 152 (8), 151 (16), 150 (3), 139 (9), 138 (11), 137 (47), 136 (8), 135 (2), 134 (3), 125 (9), 124 (20), 123 (70), 122 (23), 121 (3), 120 (3), 112 (5), 111 (61), 110 (68), 109 (47), 108 (20), 107 (2), 106 (2), 98 (11), 97 (29), 96 (56), 95 (15), 94 (8), 93 (2), 85 (3), 84 (40), 83 (70), 82 (27), 81 (20), 80 (10), 79 (3), 71 (4), 70 (38), 69 (25), 68 (40), 67 (9), 66 (3), 56 (17), 55 (33), 54 (19), 53 (7), 52 (2), 44 (3), 43 (9), 42 (42), 41 (43)
from acetol/2× pyrrolidin reaction mixture: 181 (13), **180 (100),** 179 (7), 165 (4), 152 (7), 151 (15), 150 (4), 139 (8), 138 (9), 137 (42), 136 (7), 135 (2), 134 (2), 125 (8), 124 (18), 123 (63), 122 (22), 121 (2), 120 (3), 112 (5), 111 (61), 110 (64), 109 (46), 108 (20), 107 (2), 106 (2), 98 (10), 97 (27), 96 (54), 95 (15), 94 (9), 93 (2), 85 (3), 84 (43), 83 (72), 82 (26), 81 (20), 80 (10), 79 (3), 71 (5), 70 (39), 69 (25), 68 (40), 67 (9), 66 (3), 56 (17), 55 (35), 54 (21), 53 (8), 52 (3), 44 (3), 43 (10), 42 (47), 41 (48)

1,2-(*N*,*N*-dimorpholino)-1-propene
from Amadori morpholine: 213 (13), **212 (100)**, 167 (3), 155 (5), 154 (35), 153 (32), 140 (7), 139 (36), 138 (7), 129 (7), 128 (6), 127 (45), 126 (26), 125 (13), 124 (7), 123 (8), 114 (5), 113 (5), 112 (27), 111 (4), 110 (11), 109 (10), 108 (4), 100 (35), 98 (9), 97 (18), 96 (66), 95 (32), 94 (7), 86 (14), 85 (11), 84 (19), 83 (16), 82 (23), 81 (6), 80 (6), 70 (18), 69 (25), 68 (22), 67 (9), 58 (3), 57 (9), 56 (28), 55 (30), 54 (19), 45 (9), 44 (8), 43 (19), 42 (41), 41 (36)
from acetol/2× morpholine reaction mixture: 213 (13), **212 (100)**, 167 (2), 155 (5), 154 (36), 153 (33), 140 (8), 139 (38), 138 (6), 129 (7), 128 (6), 127 (48), 126 (27), 125 (13), 124 (6), 123 (8), 114 (7), 113 (5), 112 (29), 111 (4), 110 (11), 109 (10), 108 (4), 100 (38), 98 (11), 97 (20), 96 (70), 95 (33), 94 (8), 86 (15), 85 (11), 84 (21), 83 (17), 82 (26), 81 (6), 80 (6), 70 (19), 69 (27), 68 (24), 67 (9), 58 (4), 57 (10), 56 (32), 55 (33), 54 (19), 45 (10), 44 (12), 43 (20), 42 (47), 41 (40)

[a] Tressl et al. (1993).

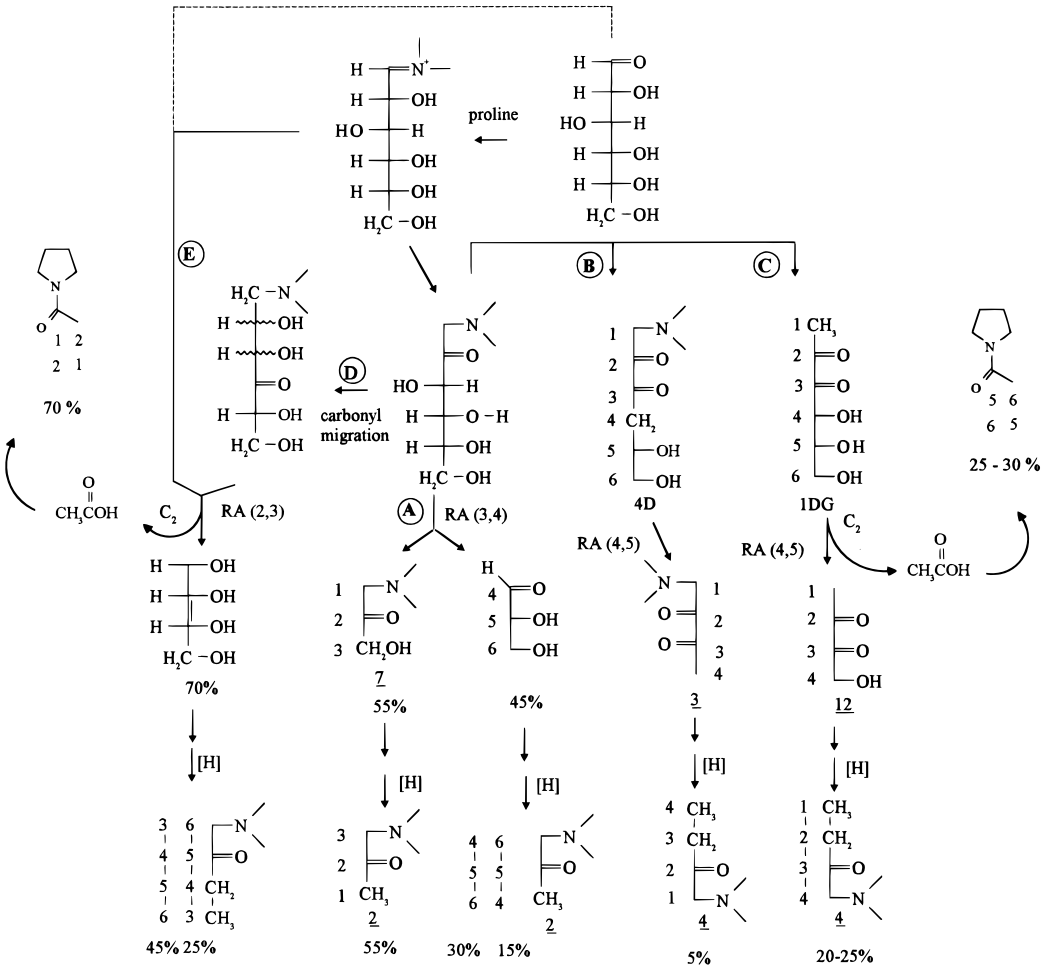
bonyl compounds arising from the interaction of α-dicarbonyls with amino acids through Strecker degradation (Weenen et al., 1994). These authors proposed a mechanism consistent with the ^{13}C distribution, according to which the sugars first underwent β-elimination to form various deoxyosones which then, through retroaldol cleavages at C3−C4, C2−C3, or C5−C4, formed pyruvaldehyde and 1-hydroxy-2,3-butanedione. To understand and confirm the mechanism of formation of C_3 and C_4 units in the Maillard reaction and to circumvent their dimerization to form pyrazines, proline was used as the model amino acid, which is known to form volatile short chain pyrrolidine derivatives related to pyruvaldehyde and 1-hydroxy-2,3-butanedione. Furthermore, D-glucoses ^{13}C-enriched at C1, C2, C3, C4, C5, and C6 positions were systematically reacted with proline to confirm the origin of C_3 and C_4 units and to monitor the occurrence, if any, of chain lengthening through aldol condensations that could be detected by observing the scrambling of labels. However, it should be recognized that more than one mechanism can be compatible with the observed distribution patterns of the labeled products.

MATERIALS AND METHODS

All reagents and chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). [1-^{13}C]-D-Glucose, [2-^{13}C]-D-glucose, [6-^{13}C]-D-glucose, and [2-^{13}C]-D-ribose were also purchased from Aldrich. [3-^{13}C]-D-Glucose, [4-^{13}C]-D-glucose, and [5-^{13}C]-D-glucose were purchased from ICON Services Inc. (Summit, NJ). Melting points were determined on a Fischer melting point apparatus and are uncorrected. ^{13}C NMR spectra were recorded at 125.8 MHz using dioxane as external standard (δ 67.4) on a Bruker AMX500 instrument. Infrared spectra were recorded in CaF_2 IR cells on a Nicolet 8210 Fourier-transform spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector. The synthesis of Amadori-proline was performed according to published procedures (Vernin et al., 1992).

Synthesis of 1-(Prolino)-1-deoxy-D-glyceraldehyde (7). D-Glyceraldehyde (2.0 g, 0.02 mol) and l-proline (9.2 g, 0.04 mol) were mixed in methanol (40.0 mL), stirred at room temperature for 3 h, and stored at 4 °C for 15 h. The resulting precipitate was filtered and crystallized from water/methanol (1:1 v/v): yield 17.5% (0.67 g, 0.0035 mol); mp 111−112 °C; ν_{max} 270, 210 nm; FTIR (methanol) 1743 (C═O), 1622 cm^{-1} (COO−); ^{13}C NMR (D_2O) δ 206.84 (C═O), 176.33 (COO−), 72.87 (C-2′ proline), 68.51 (C-5′ proline), 62.76 (CH_2OH), 58.77 (−CH_2N), 31.22 (C-3′ proline), 25.69 (C-4′ proline); EIMS, di-TMS derivative, m/z (relative intensity) 332 (1), 331 (3), 316

Scheme 1. Pathways of Retro-Aldol Reactions of Proline Amadori Compound and Percent Distribution of Products in Each Pathway[a]



[a] [H] = reductions; RA [*x*,*y*] = retro-aldol cleavage at C*x*−C*y*. Carbon numbering indicates original D-glucose carbon locations.

(6), 288 (2), 258 (1), 216 (3), 215 (10), 214 (56), 202 (5), 201 (16), 200 (100), 189 (2), 186 (8), 174 (1), 173 (2), 172 (13), 170 (1), 156 (1), 147 (1), 142 (11), 130 (3), 129 (17), 103 (18), 96 (8), 83 (7), 82 (8), 75 (11), 74 (4), 73 (40), 70 (4), 68 (2), 61 (2), 59 (4), 58 (2), 55 (14), 54 (3), 45 (7), 43 (3), 42 (4).

Pyrolysis/GC/MS Analysis. A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) interfaced to a CDS Pyroprobe 2000 unit was used for the Py/GC/MS analysis. Two modes of sample introduction were used: (a) a 1 mg equivalent of sample dissolved in methanol/water (70:30) was applied on the platinum filament with a total heating time (THT) of 10 s or (b) 1−5 mg solid samples (^{13}C-labeled or unlabeled) were introduced inside the quartz tube (0.3 mm thickness) and plugged with quartz wool and inserted inside the coil probe with a THT of 20 s. The GC column flow rate was 0.8 mL/min for a split ratio of 92:1 and a septum purge of 3 mL/min. The Pyroprobe interface was set at the temperature at which the sample was to be pyrolyzed, and the Pyroprobe was set at the desired temperature at a rate of 50 °C/ms. Capillary direct MS interface temperature was 180 °C; ion source temperature was 280 °C. The ionization voltage was 70 eV, and the electron multiplier was 1494 V. The mass range analyzed was 20−350 amu. The column was a fused silica DB-5 column (30 m length × 0.25 mm i.d.; 0.25 μm film thickness; Supelco, Inc.). The column initial temperature was −5 °C for 3 min and was increased to 50 °C at a rate of 30 °C/min; immediately the temperature was further increased to 270 °C at a rate of 8 °C/min and kept at 270 °C for 5 min. Products that were not found in the mass spectral libraries were tentatively identified by comparison with literature mass spectral data and/or by comparison of the mass spectra produced from their heated precursors as listed in Table 1.

RESULTS AND DISCUSSION

A. Retro-Aldol Reactions and Formation of C_2, C_3, and C_4 Fragments. 1-(1′-Pyrrolidinyl)-2-propanone **(2)**, 2-hydroxy-1-(1′-pyrrolidiyl)-1-buten-3-one **(3)**, *N*-acetylpyrrolidine, and 1-(1′-pyrrolidinyl)-2-butanone **(4)** are C_2, C_3, and C_4 sugar adducts of pyrrolidine that can be detected in l-proline/D-glucose heated mixtures (Tressl et al., 1993) and also in the pyrolysis products of l-proline/D-glucose and proline Amadori compound (**1**) (Huyghues-Despointes et al., 1994), in relatively good yields. Understanding the mechanism of their formation can elucidate the post- and pre-Amadori fragmentation pathways of sugar moieties into smaller fragments and their chemical reactivity. Tressl et al. (1993) using D-[^{13}C1]glucose and proline demonstrated by GC/MS that 60% of the 1-(1′-pyrrolidinyl)-2-propanone formed was unlabeled and 40% was labeled at the C3 position of the propanone moiety, indicating at least two different pathways of formation. Huyghues-Despointes et al. (1994) studied the effect of pyrolysis temperature on the yield of 1-(1′-pyrrolidinyl)-2-propanone **(2)** from the proline Amadori product and found that the temperature vs yield relation had two maxima, one at 200 °C and another at 300 °C, which confirms the presence of more than one mechanism of formation, each having different energy requirements. In addition, comparison of the relative yields of 1-(1′-pyrrolidinyl)-2-propanone from the Amadori product at the same temperature (250 °C) but under different

Table 2. Percent Chromatographic Peak Areas of Compounds Identified by Py/GC/MS from Morpholine Amadori Compound Pyrolyzed for 20 s at 250 °C

t_R (min)	compound	area %
7.1	2,3-butanedione	0.5
8.9	acetol	0.7
9.8	acetic acid	21.6
11.7	morpholine	32.6
15.1	2,5-dimethyl-4-hydroxy-2,3-dihydro-3-furanone	0.2
16.4	1-(*N*-morpholino)-2-propanone[a] **(5)**	10.1
16.8	2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one	1.9
19.3	1-(*N*-morpholino)-1-deoxy-D-glyceraldehyde[b]	0.3
22.9	2-hydroxy-1-(*N*-morpholino)-1-buten-3-one[b] **(6)**	10.1
23.8	1,2-(*N,N*-dimorpholino)-1-propene[a]	1.9
total		79.8

[a] Suggested structures based on mass spectrometric fragmentation patterns and analysis of reaction mixture of morpholine with acetol which produces products having the same fragmenation as the corresponding peaks in the morpholine Amadori compound. [b] Suggested structures based on mass spectrometric fragmentation patterns only.

pyrolysis conditions (one in a quartz tube which favors bimolecular interactions and the other on a ribbon probe) have indicated that the formation of 1-(1′-pyrrolidinyl)-2-propanone **(2)** mainly occurs through bimolecular interactions of fragments cleaved from Amadori product (Huyghues-Despointes et al., 1994).

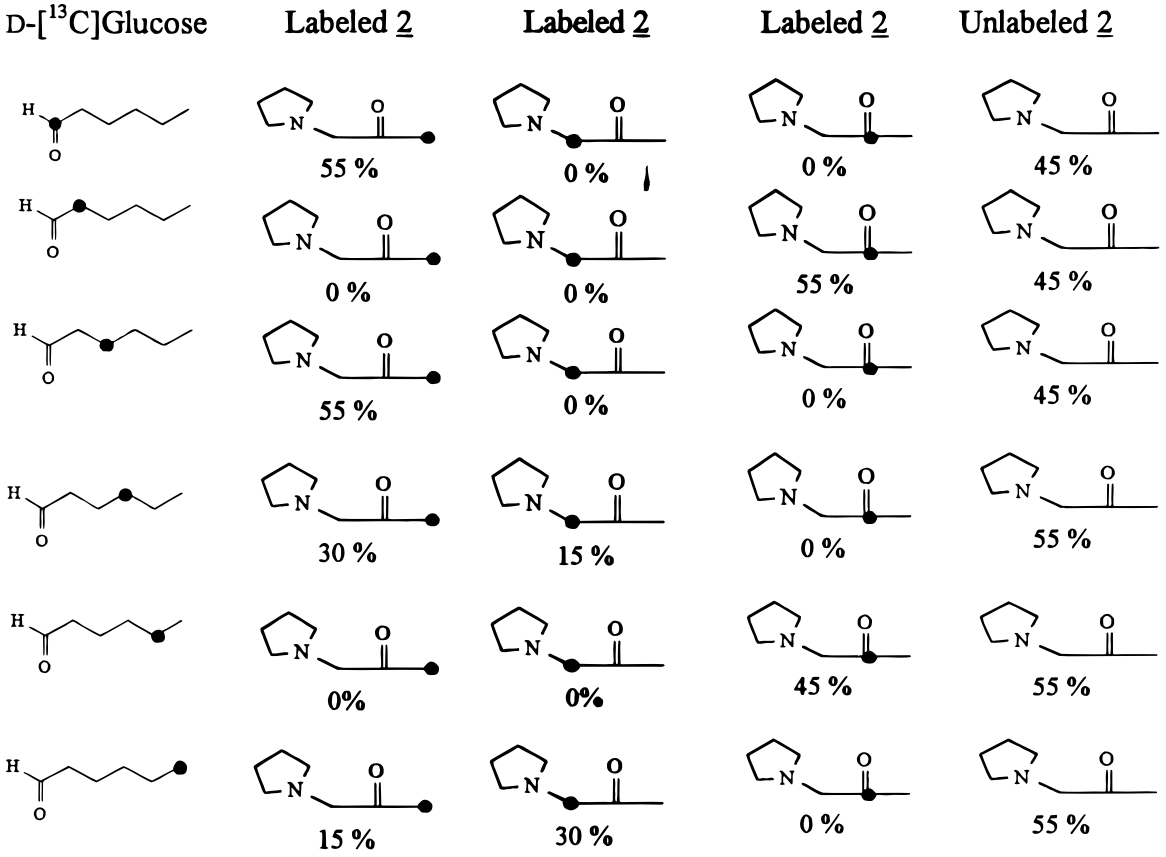
Detailed studies to elucidate the different pathways and mechanism of formation of C_2, C_3, and C_4 sugar fragments and their derivatives in model systems require the use of D-glucose labeled at different carbons to accurately assign the position in the final products. Quartz tube Py/GC/MS is especially suited to perform small scale reactions without the need to isolate or extract the reaction mixture that leads to loss of valuable isotopically labeled products. In the present study, Py/GC/MS was used to investigate the distribution of ^{13}C labels in 1-(1′-pyrrolidinyl)-2-propanone **(2)**, 2-hydroxy-1-(1′-pyrrolidiyl)-1-buten-3-one **(3)**, *N*-acetylpyrrolidine, and 1-(1′-pyrrolidinyl)-2-butanone **(4)** generated from the pyrolysis of proline with D-glucoses separately ^{13}C-labeled at C1, C2, C3, C4, C5, and C6. Careful analysis of the mass spectra of **2**, **3**, **4**, and *N*-acetylpyrrolidine from all ^{13}C-labeling experiments has indicated that C_3 fragments are produced by a retro-aldol reaction at C3−C4 (designated RA [3,4]) of Amadori compound (pathway A, Scheme 1) and C_2 and C_4 fragments are produced simultaneously by retro-aldol cleavage at C4−C5 (RA [4,5]) of both 1-prolino-1,4-dideoxy-2,3-hexodiulose (4D) and 1-deoxyglucosone (1DG) (pathways B and C, Scheme 1). However, the major retro-aldol cleavage to produce C_2 and C_4 fragments is initiated at C2−C3 (RA [2,3]) from the Amadori compound, after a carbonyl migration (pathway D, Scheme 1). In principle, similar retro-aldol cleavage (RA [2,3]) at a pre-Amadori stage can occur from the free D-glucose and/or glycosylamine (pathway E, Scheme 1). Higher relative yields of C_4 products obtained from the pyrolysis of pure Amadori compounds relative to D-glucose/L-proline mixtures indicates the importance of pathway D relative to pathway E.

Mechanism of Formation of C_3 Fragments—Pathway A. Under the basic conditions of Amadori products derived from secondary amines, retro-aldol reactions become important relative to Amadori products derived from primary amines. 1-(1′-Pyrrolidinyl)-2-propanone (**2**) and 2-hydroxy-1-(1′-pyrrolidinyl)-1-buten-3-one (**3**) could be considered products of such reactions in proline Amadori compound **1**. Corresponding morpholino (secondary amine) derivatives **5** and **6** (see Tables 1 and 2) are similarly observed in the pyrolysis products of morpholine Amadori compound as major peaks. In addition, **5** was formed from a reaction mixture of acetol and morpholine as the only major product. Retro-aldol reactions leading to these products and carbon−nitrogen cleavage reaction to form proline and 2,3-dihydro-3,5-

Table 3. Mass Spectrometric Data of Selected Products of the Reaction of ^{13}C-Enriched D-Glucose with Proline

	N-Acetylpyrrolidine
D-glucose	114 (4.4), 113 (54.3), 112 (3.2), 86 (1.2), 85 (19.6), 72 (1.8), 71 (8.0), 70 (77.0), 69 (2.0), 68 (5.1), 56 (6.7), 55 (7.6), 44 (3.6), **43 (100)**, 42 (13.7), 41 (12.9)
D-[1-^{13}C]glucose	114 (53.5), 113 (19.3), 112 (0.4), 86 (18.4), 85 (6.4), 72 (1.1), 71 (9.9), 70 (99.1), 69 (2.5), 68 (6.2), 56 (8.6), 55 (8.8), 44 (33.0), **43 (100)**, 42 (14.1), 41 (16.3)
D-[2-^{13}C]glucose	114 (55.1), 113 (20.2), 112 (3.1), 86 (19.4), 85 (6.1), 72 (1.2), 71 (10.5), 70 (97.8), 69 (2.1), 68 (6.0), 56 (4.1), 55 (8.7), 44 (31.7), **43 (100)**, 42 (14.6), 41 (16.0)
D-[3-^{13}C]glucose	114 (6.2), 113 (51.0), 112 (3.1), 86 (1.9), 85 (18.6), 72 (1.7), 71 (7.9), 70 (75.4), 69 (1.8), 68 (5.0), 56 (6.6), 55 (7.4), 44 (4.5), **43 (100)**, 42 (13.2), 41 (13.1)
D-[4-^{13}C]glucose	114 (5.8), 113 (47.3), 112 (2.2), 86 (1.7), 85 (17.8), 72 (1.2), 71 (7.5), 70 (74.4), 69 (1.5), 68 (4.7), 56 (6.2), 55 (7.3), 44 (4.1), **43 (100)**, 42 (13.3), 41 (13.3)
D-[5-^{13}C]glucose	114 (12.0), 113 (42.3), 112 (2.8), 86 (4.1), 85 (15.5), 72 (1.5), 71 (7.7), 70 (75.4), 69 (1.8), 68 (5.1), 56 (6.0), 55 (7.8), 44 (9.1), **43 (100)**, 42 (13.6), 41 (13.5)
D-[6-^{13}C]glucose	114 (14.3), 113 (50.0), 112 (3.4), 86 (4.6), 85 (17.5), 72 (1.6), 71 (8.1), 70 (81.2), 69 (1.6), 68 (5.3), 56 (6.3), 55 (7.2), 44 (9.6), **43 (100)**, 42 (13.4), 41 (12.9)
	1-(1′-Pyrrolidinyl)-2-propanone (2)
D-glucose	128 (0.2), 127 (2.0), 85 (06.4), **84 (100)**, 82 (2.2), 56 (6.0), 55 (17.8), 54 (2.4), 44 (0.4), 43 (6.3), 42 (38.2), 41 (5.5)
D-[1-^{13}C]glucose	128 (1.3), 127 (1.4), 85 (13.6), **84 (100)**, 82 (2.2), 56 (7.6), 55 (19.6), 54 (2.6), 44 (2.6), 43 (7.8), 42 (42.1), 41 (6.0)
D-[2-^{13}C]glucose	128 (1.4), 127 (1.3), 85 (06.4), **84 (100)**, 82 (2.1), 56 (6.0), 55 (16.8), 54 (2.3), 44 (2.8), 43 (4.0), 42 (34.8), 41 (5.0)
D-[3-^{13}C]glucose	128 (2.6), 127 (2.2), **85 (100)**, 84 (99.2), 82 (2.2), 56 (14.8), 55 (28.2), 54 (2.9), 44 (2.1), 43 (43.3), 42 (44.1), 41 (9.0)
D-[4-^{13}C]glucose	128 (0.1), 127 (1.7), 85 (22.2), **84 (100)**, 82 (2.1), 56 (7.9), 55 (19.9), 54 (2.7), 44 (2.0), 43 (11.7), 42 (40.7), 41 (6.6)
D-[5-^{13}C]glucose	128 (1.1), 127 (1.3), 85 (09.1), **84 (100)**, 82 (2.2), 56 (6.6), 55 (18.4), 54 (2.4), 44 (2.3), 43 (5.9), 42 (39.6), 41 (6.1)
D-[6-^{13}C]glucose	128 (1.4), 127 (2.0), 85 (43.7), **84 (100)**, 82 (2.2), 56 (9.5), 55 (21.4), 54 (2.5), 44 (1.6), 43 (19.3), 42 (39.6), 41 (6.9)
	1-(1′-Pyrrolidinyl)-2-butanone (4)
D-glucose	142 (0.0), 141 (1.6), 85 (5.7), **84 (100)**, 82 (1.5), 56 (3.5), 55 (10.7), 54 (1.5), 44 (0.2), 43 (1.5), 42 (21.0), 41 (3.6)
D-[1-^{13}C]glucose	142 (0.5), 141 (1.3), 85 (7.2), **84 (100)**, 82 (1.4), 56 (3.8), 55 (11.3), 54 (1.7), 44 (0.2), 43 (1.8), 42 (22.4), 41 (3.9)
D-[2-^{13}C]glucose	142 (0.5), 141 (1.2), 85 (7.3), **84 (100)**, 82 (1.4), 56 (3.7), 55 (10.9), 54 (1.7), 44 (0.1), 43 (1.8), 42 (21.8), 41 (3.8)
D-[3-^{13}C]glucose	142 (2.6), 141 (0.7), 85 (92.6), **84 (100)**, 82 (1.5), 56 (9.7), 55 (17.5), 54 (2.3), 44 (1.5), 43 (21.8), 42 (27.6), 41 (6.4)
D-[4-^{13}C]glucose	142 (1.8), 141 (0.4), 85 (33.3), **84 (100)**, 82 (1.3), 56 (5.5), 55 (12.8), 54 (1.9), 44 (0.8), 43 (7.8), 42 (24.6), 41 (4.6)
D-[5-^{13}C]glucose	142 (1.1), 141 (0.7), 85 (7.0), **84 (100)**, 82 (1.4), 56 (4.0), 55 (11.4), 54 (2.1), 44 (0.5), 43 (2.0), 42 (24.5), 41 (4.0)
D-[6-^{13}C]glucose	142 (1.7), 141 (1.0), 85 (45.2), **84 (100)**, 82 (1.4), 56 (5.7), 55 (12.7), 54 (1.7), 44 (0.5), 43 (9.4), 42 (22.5), 41 (4.1)

Table 4. Distribution of Labels in 2

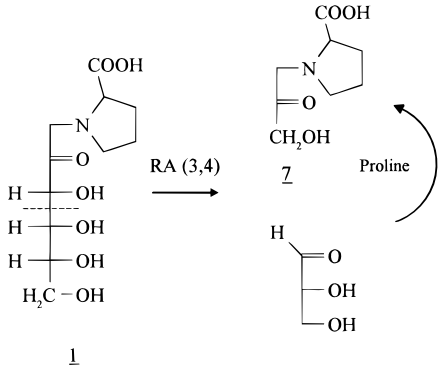


dihydroxy-6-methyl-4(*H*)-pyran-4-one seem to be the principal degradation steps in Amadori compounds derived from secondary amines under ribbon pyrolysis condition, which, as demonstrated earlier (Huyghues-Despointes et al., 1994), releases the initial degradation products of an analyte.

To verify the pathways of formation of C_3 fragments, model studies with D-[^{13}C]glucose labeled at different carbons were carried out with proline using Py/GC/MS as described earlier (Huyghues-Despointes et al., 1994). The distribution of the ^{13}C labels in the C_3 fragmentation indicator compound **2** was analyzed. The results are summarized in Tables 3 and 4. According to Table 4, 55% of **2** is formed from the first three carbon atoms of D-glucose and 45% from the last three carbon atoms. This distribution could be rationalized by the formation of 1-(prolino)-1-deoxy-D-glyceraldehyde and glyceraldehyde from proline Amadori product by a retro-aldol reaction at C3−C4 (pathway A, Scheme 1) as described in detail below.

Formation of 1-(Prolino)-1-deoxy-D-glyceraldehyde and Glyceraldehyde from Proline Amadori Product. Amadori products can undergo a retro-aldol reaction by cleavage at the C3−C4 bond as shown in Scheme 2 to produce 1-(prolino)-1-deoxy-D-glyceraldehyde (**7**) and a free glyceraldehyde molecule. The latter, being in equilibrium with the dihydroxacetone form, can react with free proline, from either end of the molecule, to produce an isotopomeric mixture of compound **7** with labeled D-glucoses at the last three carbon atoms by Amadori rearrangement mechanism. It seems that carbonyl migration occurs quite efficiently in glyceraldehyde at 250 °C through an enediol mechanism. 1-(Prolino)-1-deoxy-D-glyceraldehyde (**7**) was not detected in the pyrolysis products of the proline Amadori

Scheme 2. Retro-Aldol Fragmentation at C3−C4 of Proline Amadori Product (Pathway A)[a]



1

[a] RA [*x*,*y*] = retro-aldol cleavage at C*x*−C*y*.

compound but was detected as its trimethylsilyl derivative in trace amounts after treatment of the reaction mixture with trimethylsilyl chloride. However, the corresponding 1-(morpholino)-1-deoxy-D-glyceraldehyde was detected in the pyrolysate of morpholine Amadori compound without silylation. The structure was tentatively assigned on the basis of the mass spectrometric fragmentation pattern in comparison with the proline analog (Tables 1 and 2). Other products observed in the pyrogram of morpholine Amadori compound are listed in Table 2.

1-(Prolino)-1-deoxy-D-glyceraldehyde (**7**) was synthesized to examine its stability and potential of conversion into 1-(1′-pyrrolidinyl)-2-propanone under pyrolysis conditions. Figure 1 shows the pyrogram of compound **7** generated at 250 °C. The main product formed under pyrolysis condition was 1-(1′-pyrrolidinyl)-2-propanone, indicating the facile formation of **2** from **7**. However,

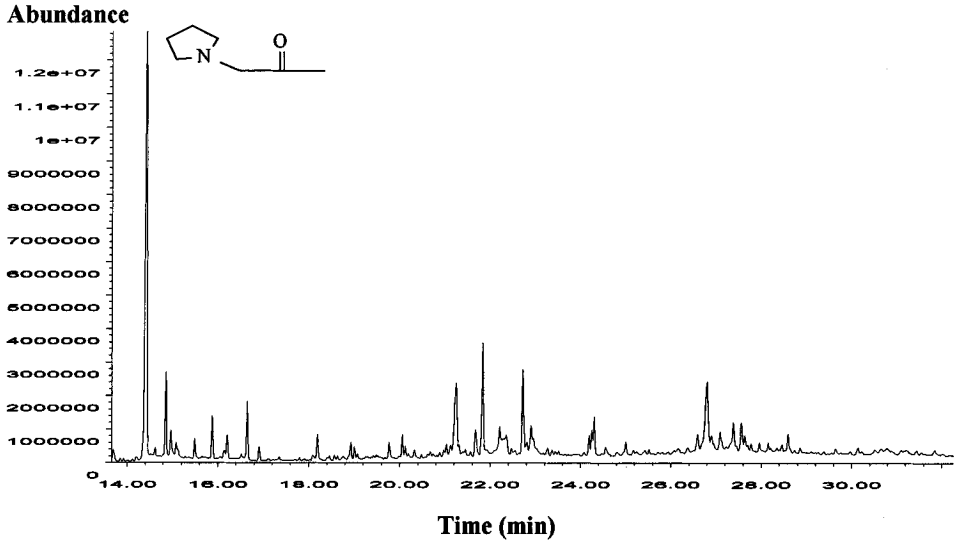
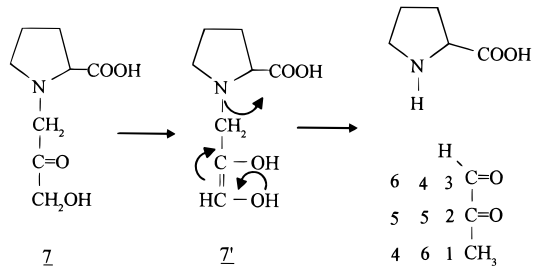


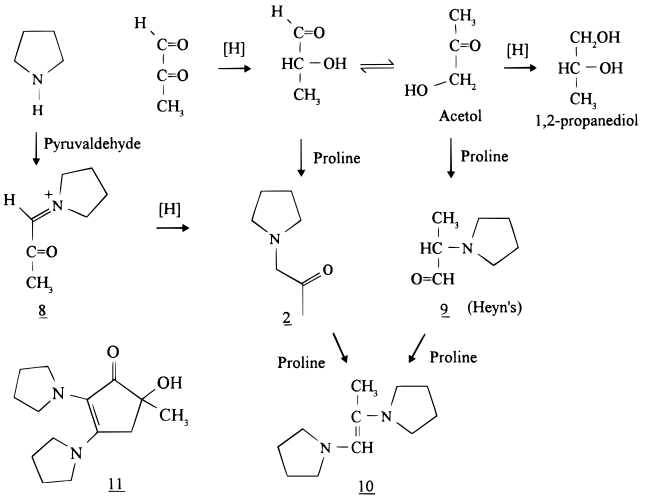
Figure 1. Pyrogram of 1-(prolino)-1-deoxy-D-glyceraldehyde (**7**).

Scheme 3. β-Elimination Reaction of 1-(Prolino)-1-deoxy-D-glyceraldehyde (Pathway A)[a]



[a] Carbon numbering indicates original D-glucose carbon locations.

Scheme 4. Formation Pathways of 1-(1′-Pyrrolidinyl)-2-propanone (2) from Pyrrolidine and Pyruvaldehyde (Pathway A)[a]



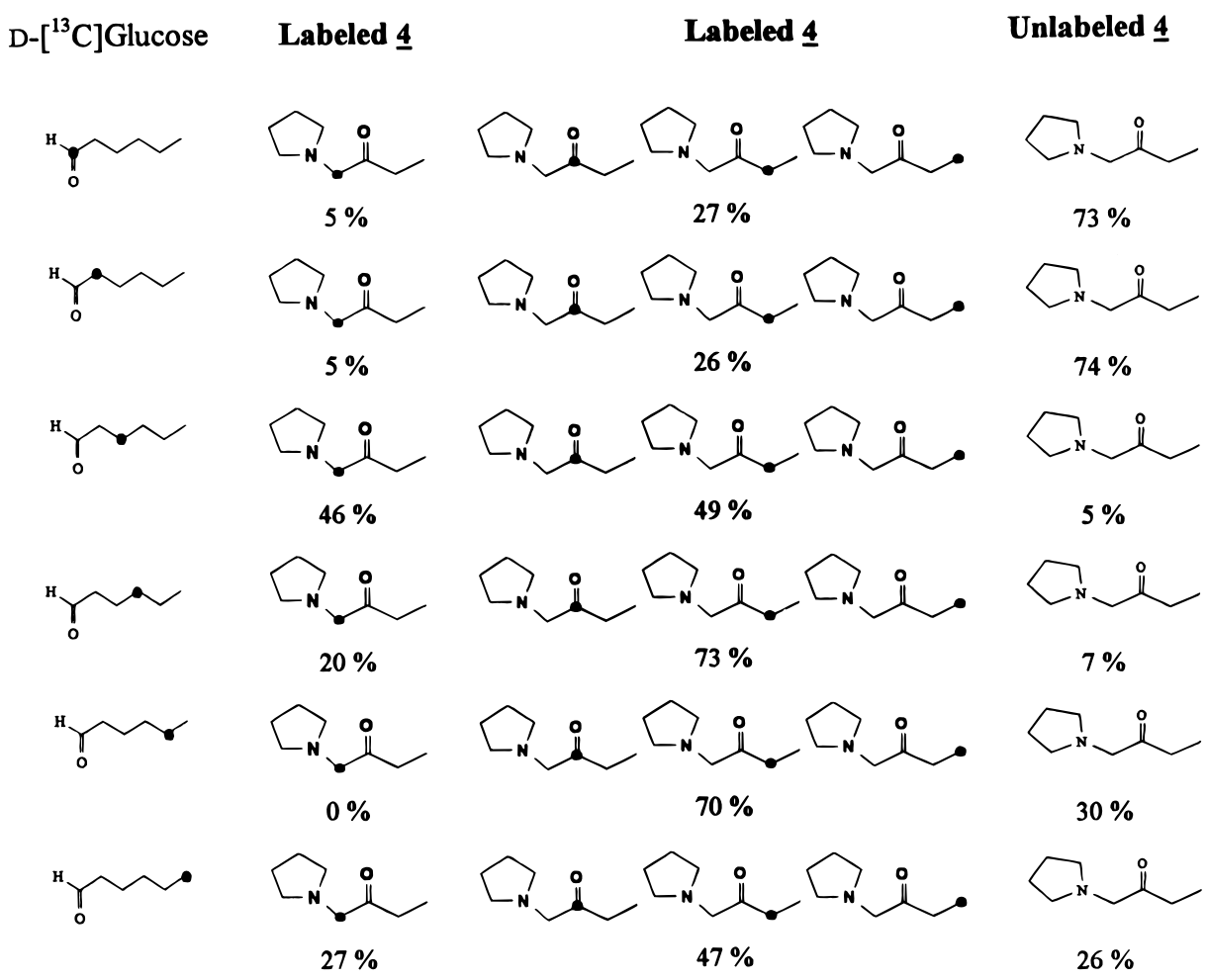
[a] [H] = reductions.

the pyrolysis of a mixture of proline and glyceraldehyde produces several major peaks, among them 1-(1′-pyrrolidinyl)-2-propanone. Although **7** is stable enough to be formed from Amadori products, it degrades quickly to form 1-(1′-pyrrolidinyl)-2-propanone as described below.

Mechanism of Conversion of 1-(Prolino)-1-deoxy-D-glyceraldehyde into 1-(1′-Pyrrolidinyl)-2-propanone. 1-(Prolino)-1-deoxy-D-glyceraldehyde (**7**), similar to other Amadori products, can undergo β-elimination reaction initiated from the 2,3-enediol form (**7′**) as shown in Scheme 3 to produce a free proline and a pyruvaldehyde molecule. To assess the potential of pyruvaldehyde/proline to produce 1-(1′-pyrrolidinyl)-2-propanone, the mixture was analyzed by Py/GC/MS. The major products identified were 1-(1′-pyrrolidinyl)-2-propanone, acetol, 1,2-propanediol, and acetic acid among others, including several aldol condensation products. Gi and Baltes (1994) also detected 1,2-propanediol and acetol in the heated mixtures of histamine and pyruvaldehyde. Pyruvaldehyde, therefore, can produce 1-(1′-pyrrolidinyl)-2-propanone in the presence of free proline or pyrrolidine. Two possible mechanisms can be considered for this transformation: either the pyruvaldehyde reacts with pyrrolidine and forms the imminium ion (**8,** Scheme 4), which is then reduced to **2**, or pyruvaldehyde is reduced first into 2-hydroxypropionaldehyde, which can exist in equilibrium with acetol, and then undergoes Amadori rearrangement to **2** as shown in Scheme 4. Acetol has been detected in heated mixtures of pyruvaldehyde/amino acids and in heated Amadori product mixtures. In addition, it can react with proline (or pyrrolidine) to produce the Heyn's product **9** as shown in Scheme 4. To test this hypothesis, acetol was reacted with pyrrolidine at 100 °C for 10 min and the ether extract was analyzed by GC/MS. When equimolar amounts of reactants were used, surprisingly, the major product was **2**, not **9**, and only trace amounts of Heyn's product (**9**) was detected in addition to 1,2-dipyrrolidinyl-1-propene (**10**). However, when acetol was treated with a 2-fold excess of pyrrolidine, the area of the peak assigned to **10** increased 6 times relative to the acetol/pyrrolidine equimolar mixture. Compound **10** can be detected in the pyrolysis mixtures of pyruvaldehyde/proline, glycerladehyde/proline, proline Amadori, and acetol/pyrrolidine and in 1-(prolino)-1-deoxy-D-glyceraldehyde. A related compound **(11)** was isolated by Tressl et al. (1993) in a heated D-glucose/L-proline mixture, which in principle could be formed from 1-deoxyglucosone through a similar reaction sequence followed by cyclization.

Mechanism of Formation of C_2 and C_4 Fragments. Detection of 2-hydroxy-1-(1′-pyrrolidiyl)-1-buten-3-one **(3)**, 1-(1′-pyrrolidinyl)-2-butanone **(4)**, and *N*-acetylpyrrolidine in the reaction mixture of L-proline/D-glucose or in the pyrolysis products of proline Amadori compound indicates the occurrence of fragmentation of

Table 5. Distribution of Labels in 4

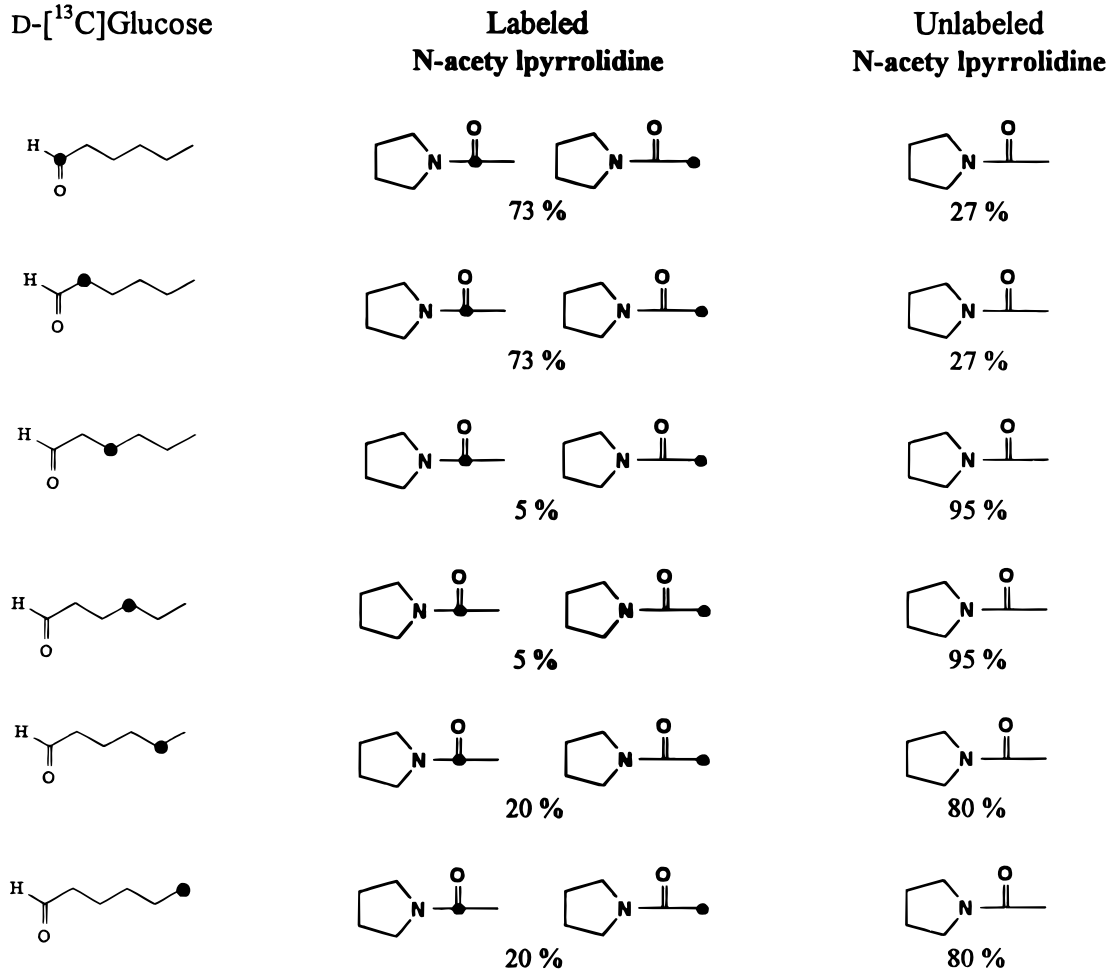


D-glucose and/or Amadori compounds to produce C_2 and C_4 fragments. Analysis of the data from labeling experiments has indicated that both 2-hydroxy-1-(1′-pyrrolidinyl)-1-buten-3-one **(3)** and 1-(1′-pyrrolidinyl)-2-butanone **(4)** have similar distributions of labels; however, due to the purity of the peak associated with 2-hydroxy-1-(1′-pyrrolidiyl)-1-buten-3-one, percentage distributions of labels are shown only for 1-(1′-pyrrolidinyl)-2-butanone (Tables 3 and 5) and *N*-acetylpyrrolidine (Tables 3 and 6). Careful analysis of the data from all of the labeling experiments indicates at least three pathways of formation of 1-(1′-pyrrolidinyl)-2-butanone, designated B, C, and D (Scheme 1). Tables 3, 5, and 6 summarize the distribution of labels in *N*-acetylpyrrolidine and **4**. According to the labeling experiments, 70% of **3, 4**, and *N*-acetylpyrrolidine is produced through retro-aldol cleavage at C2−C3 (pathway D) and 30% through similar retro-aldol cleavage at C4−C5 (pathways B and C) (Scheme 1). In pathways B, C, and D acetic acid (which eventually reacts with pyrrolidine to form *N*-acetylpyrrolidine) is assumed to be formed from oxidation of the C_2 fragment glycoladehyde, due to the similar percentage of distribution of expected labels in *N*-acetylpyrrolidine and compounds **3** and **4**.

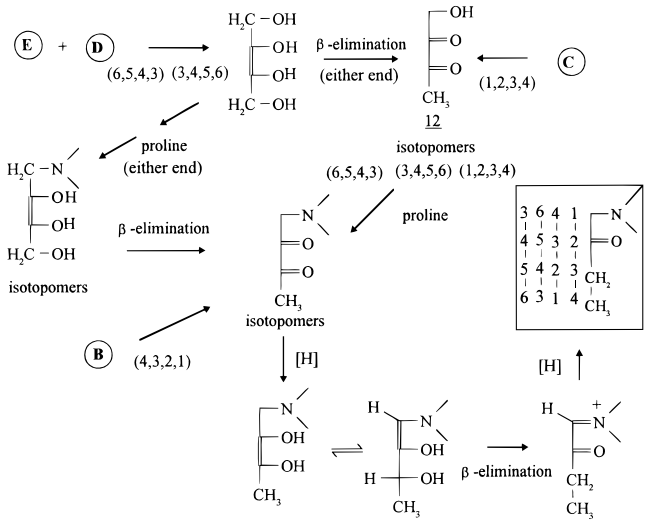
Formation of C_2/C_4 Fragments from Pathway B. The formation of 1-(amino acid)-1,4-dideoxy-2,3-hexodiuloses (4D, Scheme 1) in Maillard mixtures has been established (Huber et al., 1990). 1-(Prolino)-1,4-dideoxy-2,3-hexodiulose formed from the Amadori compound can undergo a retro-aldol reaction at C4−C5 (RA [4,5]) to produce 2-hydroxy-1-(1′-pyrrolidinyl)-1-buten-3-one and glycoldehyde, and both products can undergo thermal oxidation−reduction to produce 1-(1′-pyrrolidinyl)-2-butanone and acetic acid, respectively (see Schemes 1 and 5 and section B for the mechanism of oxidation−reduction). This pathway, however, represents only around 5% of the total yield of C_2/C_4 products. This pathway leads to the formation of acetic acid containing C5 and C6 of the D-glucose and to 1-(1′-pyrrolidinyl)-2-butanone containing C1, C2, C3, and C4 of D-glucose; C1 of D-glucose is attached to pyrrolidine, as shown in Schemes 1 and 5.

Formation of C_2/C_4 Fragments from Pathway C. 1-Deoxyglucosones (1DG) can be formed through β-elimination of Amadori compounds under basic conditions, which also promotes retro-aldol reactions from the acyclic forms of 1DG. Such retro-aldol reaction at C4−C5 (RA[4,5]), similar to pathway B, can generate a glycoladehyde (eventually acetic acid) and 1-hydroxy-2,3-butandione **(12)**. The latter can react with pyrrolidine through Amadori rearrangement to produce 2-hydroxy-1-(1′-pyrrolidinyl)-1-buten-3-one and ultimately 1-(1′-pyrrolidinyl)-2-butanone as described above. Pathway C represents around 25% of the total yield of C_2/C_4 products and leads to the formation of acetic acid consisting of C5 and C6 of the D-glucose and 1-(1′-pyrrolidinyl)-2-butanone containing C1, C2, C3, and C4 of D-glucose, but in this case, contrary to pathway B, C4 of D-glucose is attached to the pyrrolidine, as shown in Scheme 1.

Table 6. Distribution of Labels in *N*-Acetylpyrrolidine



Scheme 5. Formation Pathways of 1-(1′-Pyrrolidinyl)-2-butanone (4) (Pathways B−E)[a]

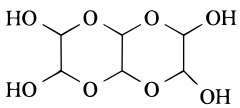


[a] [H] = reductions, Carbon numbering indicates original D-glucose carbon locations.

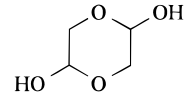
Formation of C_2/C_4 Fragments from Pathway D. Pathway D, the major pathway for the formation of C_2/C_4 fragments (70%), is based on the migration of the carbonyl group of the Amadori product into C4 of the D-glucose moiety, forming 1-(prolino)-1-deoxy-4-hexosulose. Migration of carbonyl groups has been verified in carbohydrates by NMR (Cervantes-Laurean et al., 1993) and by FTIR (Yaylayan and Ismail, 1995; Yaylayan et al., 1994). A retro-aldol reaction at C2−C3 (RA[2,3]) of 1-(prolino)-1-deoxy-4-hexosulose can produce a tetrulose moiety in equilibrium with tetrose and consisting of C3, C4, C5, and C6 atoms of the glucose. The symmetrical enediol form of the tetrulose can undergo a β-elimination from either end of the molecule to produce 1-hydroxy-2,3-butandione **(12)**, a common intermediate with pathway C. Alternatively, the tetrose form can undergo an Amadori rearrangement, again from either end of the molecule; the resulting isotopomers of the Amadori product can β-eliminate and produce 2-hydroxy-1-(1′-pyrrolidinyl)-1-buten-3-one, the common intermediate of pathways B−E. Similar to glyceraldehyde, carbonyl migration occurs quite efficiently also in tetrose moieties at 250 °C.

B. Redox Reactions of α-Hydroxy Carbonyl and α-Dicarbonyl Compounds. Most of the reduction steps needed in Maillard systems to justify the structures of observed products are attributed to the so-called reductones, cyclic or acyclic enediols. The mechanisms of these reduction reactions are not explored yet in Maillard systems. Three distinct mechanisms of oxidation−reductions were identified that involve common Maillard intermediates such as α-hydroxy carbonyls, α-dicarbonyls, and formic acid.

Reduction of Imminium Ions by Formic Acid. Formic acid is a common degradation product of carbohydrates, and it is an effective and specific reagent for reductive amination, known as the Wallach reaction (Moore,



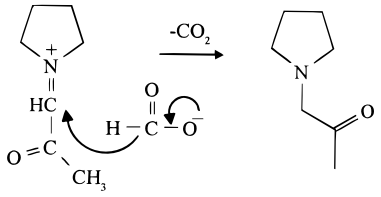
Trimeric glyoxal dihydrate



Glycoaldehyde dimer

Figure 2. Structures of trimeric glyoxal dihydrate and glycoaldehyde dimer.

Scheme 6. Reduction Mechanism of Imminium Ions and Carbonyl Compounds by Formic Acid

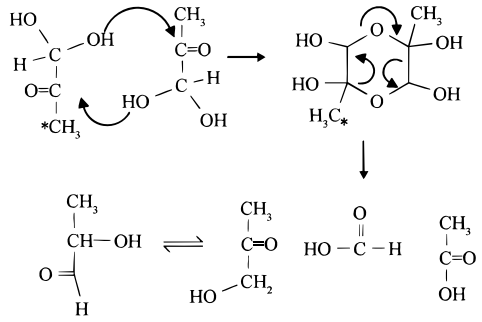


2

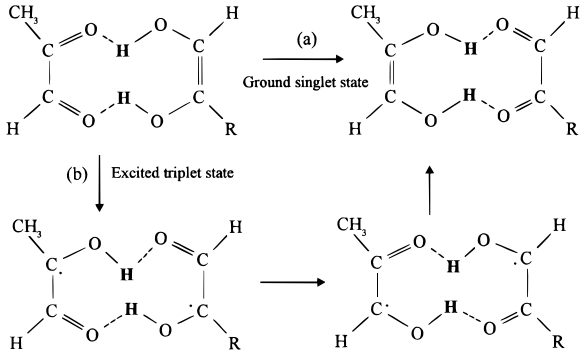
1949). If formamide or formate salts are used instead of formic acid, the reaction is called the Leuckart reaction (Moore, 1949). The mechanism of this reaction has been elucidated by deuterium labeling studies (Leonard and Sauers, 1957) and is illustrated in Scheme 6 for the reduction of **8** to **2**. The mechanism involves a hydride transfer to the unsaturated site and conversion of formic acid into carbon dioxide. When benzaldehyde in the presence of pyrrolidine was treated with formic acid or sodium cyanoborohydride, a specific reductive amination reagent, both reaction mixtures produced high yields of benzylpyrrolidine. Similarly, when pyruvaldehyde was treated with formic acid in the presence of pyrrolidine, compound **2** was produced as the major product.

Cyclic Dimer Formation of α-Hydroxy Carbonyl Compounds followed by Thermally Allowed Electrocyclic Ring Opening—Formation of Acetol from Pyruvaldehyde. α-Hydroxy carbonyls and hydrated α-keto aldehydes are known to undergo spontaneous dimerization reactions to produce various cyclic 1,4-dioxane derivatives. Glyceraldehyde and dihydroxyacetone are known to exist in different cyclic dimeric forms that can be crystallized separately (Kobayashi et al., 1976); glyoxal and glycoaldehyde can exist in different dimeric and trimeric forms which are available commercially (Figure 2). These 1,4-dioxane derivatives when heated can undergo electrocyclic ring opening reactions to produce oxidation and reduction products. When trimeric glyoxal diyhydrate was pyrolyzed at 250 °C, in addition to monomeric and dimeric units, formic acid and carbon dioxide were the major products identified. Similarly, when pyruvaldehyde was heated at 100 °C for 8 min and analyzed by GC/MS, 40 peaks were produced but the 3 major products were 1,2-propanediol (18%), acetic acid (30%), acetol (7%), and 2,4-dimethyl-1,3-dioxolane (7%) (formaldehyde protected 1,2-propanediol). Scheme 7 shows the dimerization of the hydrated pyruvaldehyde and subsequent electrocylic ring opening reaction to produce the observed main products and formic acid. This process can explain the formation of acetol from pyruvaldehyde and its subsequent reaction with pyrrolidine to produce compound **2**. The further reduction of acetol

Scheme 7. Dimerization of Pyruvaldehyde Hydrate and Subsequent Thermally Allowed Electrocyclic Ring Opening



Scheme 8. Disproportionation of α-Hydroxy Carbonyls and α-Dicarbonyl Compounds[a]

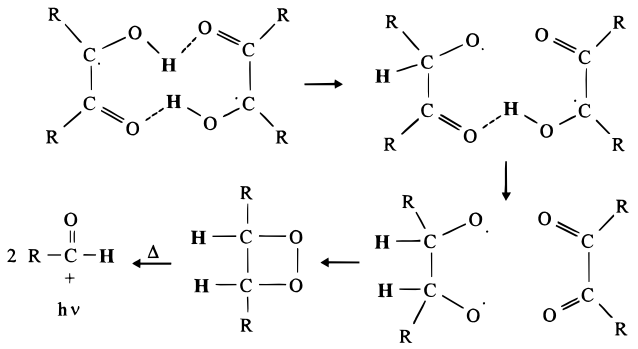


[a] Pathway a is the concerted mechanism. Pathway b is the biradical mechanism.

through its isomeric 2-hydroxypropionaldehyde into 1,2-propanediol can be explained by the reducing action of formic acid (one of the predicted but not observed products) on aldehydes similar to imminium ions (Scheme 6). As shown before, after reduction, formic acid is converted into carbon dioxide and hence not detected in the reaction mixture.

Disproportionation of α-Dicarbonyl and α-Hydroxy Carbonyl Compounds through Enediol Intermediate—Reduction of α-Dicarbonyls to Enediols. α-Dicarbonyl and α-hydroxy carbonyl compounds play a major role in the Maillard reaction. The enediol forms of α-hydroxy carbonyl compounds are known as reductones. One application of the chemistry of reductones that has been studied outside the Maillard reaction context is their use as dynamic molecular memory devices (Aviram et al., 1982). In this context, an enediol/α-dicarbonyl complex undergoes a double proton transfer (see Scheme 8) to convert the starting enediol into its corresponding dicarbonyl and the starting dicarbonyl into its enediol, thus affecting a redox reaction. These interconversions can proceed either by a stepwise biradical mechanism from the excited triplet state or by a concerted double proton transfer from the ground state (Scheme 8). For the model dihydroxyethylene/glyoxal complex the energy requirement for the biradical pathway was calculated to be 113.8 kcal mol^{-1} as opposed to 149.7 kcal mol^{-1} for the concerted mechanism (Tachibana et al., 1989). The significance of these calculations for the Maillard reaction lies in the fact that it can provide a theoretical explanation for the importance of an α-diketone/enediol redox couple in generating oxidation—reduction products and for the detection of free radicals in Maillard systems. Reduction of 2-hydroxy-1-(1′-pyrrolidiyl)-1-buten-3-one into its enediol form shown in Scheme 5 could proceed through a similar mecha-

Scheme 9. Proposed Origin of Chemiluminescence Species



nism. The origin of chemiluminescent species (Namiki et al., 1993) might be explained by the rearrangement of carbon-centered free radicals of a α-diketone/enediol complex shown in Scheme 8 to produce 1,2-dioxetane structures (Scheme 9) which are known to emit photons upon thermal decomposition (Waldemar and Katsumasa, 1979).

Conclusion. Pyrolysis/GC/MS is a convenient analytical tool to perform small scale chemical reactions with labeled starting materials. Labeling experiments using pyrolysis/GC/MS indicate the importance of retro-aldol reactions in model systems containing secondary amino acids.

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