Model Reactions on Roast Aroma Formation. 15. Investigations on the Formation of Pyrido[3,4-*d*]imidazoles during the Maillard Reaction

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When aqueous solutions of histidine and glucose were heated to 100, 120, 150, or 180 °C or when this mixture was roasted at 220 °C, a total of 231 volatile compounds were identified. Among them 2-acetyl- and 2-propionylpyrido[3,4-*d*]imidazole were found. Their formation pathway via a reaction of pyruvic aldehyde or 2-oxobutyraldehyde with, respectively, histidine or histamine is discussed. Experiments with ^{13}C isotope labeled glucose point out that the terminal methyl groups of both aldehydes can arise from C-1 as well as C-6 of glucose. While pyruvic aldehyde can be formed by retro aldol scission of intermediate diacetylformoin, 2-oxobutyraldehyde was obviously formed from 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. By reaction of 1-, 2-, or 3-methylhistidine with glucose seven more pyrido[3,4-*d*]imidazoles were identified, the structures of which, like the structures of the above-mentioned compounds, were unknown up to now.

Keywords: *Roast aromas; Maillard reaction; pyrido[3,4-d]imidazoles; isotope labeling*

INTRODUCTION

We have recently reported on the formation of 2-acetylpyrido[3,4-*d*]imidazole which was formed by the heating of glucose with histidine under the conditions of the Maillard reaction (Gi and Baltes, 1993). As was pointed out, this histidine-specific reaction product was formed as the main product by Strecker degradation of histidine with pyruvic aldehyde. The corresponding "Strecker aldehyde" [2-(4′-imidazolyl)acetaldehyde] was not found. In the course of further investigations we have identified more compounds of this type, among them 2-propionylpyrido[3,4-*d*]imidazole. Figure 1 demonstrates its probable formation pathway. While pyruvic aldehyde is a well-known sugar degradation product, 2-oxobutyraldehyde, which should be responsible for the new compound, was not described to be formed during the Maillard reaction up to now. Therefore, we have undertaken some investigations on the degradation of glucose labeled by ^{13}C in the 1- or 6-position with respect to the formation of pyridoimidazoles.

EXPERIMENTAL PROCEDURES

High-grade commercial L-histidine, D-glucose, 1-, 2-, and 3-methylhistidine, histamine dihydrochloride, and 2-oxobutane were used. The stated purity of 1-^{13}C and 6-^{13}C isotope labeled glucose was 99 atom % ^{13}C. Solvents for extraction were freshly distilled before use.

Sample Preparation. *(1) Formation of 2-Oxobutyraldehyde.* 2-Oxobutyraldehyde was synthesized according to the procedure given by *Organikum, Oxidation mit Selendioxide* (1965) and Riley et al. (1932). In a round-bottom flask filled with 180 mL of dioxane and equipped with a stirrer, reflux cooling, and thermometer were placed equimolar amounts of 2-oxobutane and sublimated selenium dioxide (0.25 mol). To this mixture was added dropwise 12 mL of deionized water while the temperature was held below 20 °C. Subsequently, the reaction mixture was boiled under stirring for 6 h. The separated selenium was hot filtered and washed with dioxane. After evaporation of the solvent, 2-oxobutyraldehyde was obtained via vacuum distillation at 0.2 Torr.

(2) Preparation of 2-Propionylpyrido[3,4-d]imidazole. Four milliliters of 2-oxobutyraldehyde and 0.02 mol of histamine dihydrochloride were dissolved in 30 mL of 1 M phosphate buffer (pH 5.8) and heated in a laboratory autoclave fitted with a PTFE insert magnetic stirrer for 1 h. After extraction, the ether extract was investigated via capillary GC and GC/MS. The gas chromatogram showed only one peak, the retention time (Van den Dool et al., 1963) and mass spectrum of which were identical with 2-propionylpyrido[3,4-*d*]imidazole.

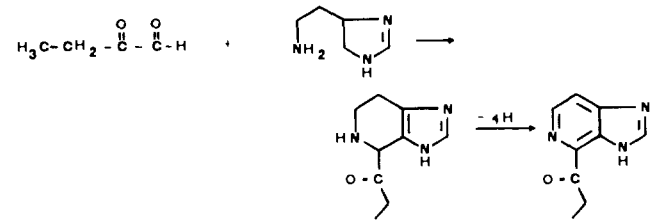

Figure 1. Formation of 2-propionylpyrido[3,4-*d*]imidazole from the reaction of 2-oxobutyraldehyde with histamine.

(3) Reaction of ^{13}C Isotope Labeled Glucose with Histidine. [1-^{13}C]glucose (250 mg) with 201.5 mg of histidine or [6-^{13}C]-glucose (400 mg) with 342.5 mg of histidine was dissolved in each case in 10 mL of 1 M phosphate buffer (pH 5.8). After heating in an autoclave at 150 °C for 1 h and subsequently cooling to room temperature, the mixture was extracted with 5 × 10 mL of ether. The combined ether fractions were treated with aqueous sodium bicarbonate, and the water dissolved in the ether was frozen out at −20 °C. After filtration on cotton wool, the ether extract was carefully concentrated to 0.5 mL with a Vigreux column. The amount of ^{13}C was calculated via the labeled mass peaks in comparison to unlabeled ones of molecular mass ions or characteristic fragment ions.

(4) Reaction of 2,5-Dimethyl-4-hydroxy-3(2H)-furanone with Histidine. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol) and histidine (0.02 mol each) were heated to 150 °C in 30 mL of 1 M phosphate buffer in an autoclave. Further treatment was identical to that described above (3).

(5) Reaction of 1-, 2-, or 3-Methylhistidine with Glucose. 1-, 2-, or 3-Methylhistidine and glucose (6 mmol each), dissolved in 20 mL of 1 M phosphate buffer, were heated in an autoclave at 150 °C for 1 h. After cooling to room temperature, the mixture was extracted with 5 × 20 mL of ether. Further treatment was identical to that described above (3).

Capillary Gas Chromatography (GC)/Mass Spectrometry (MS). *Column 1*: fused silica DB-Wax (J&W, Folsom, CA) 60 m; 0.25 μm film; i.d. 0.32 mm; split 1:5 or split/splitless mode (split open 90 s after injection; split ratio 1:5); temperature program, 40 °C, 5 min, 2 °C/min to 230 °C, 60 min.

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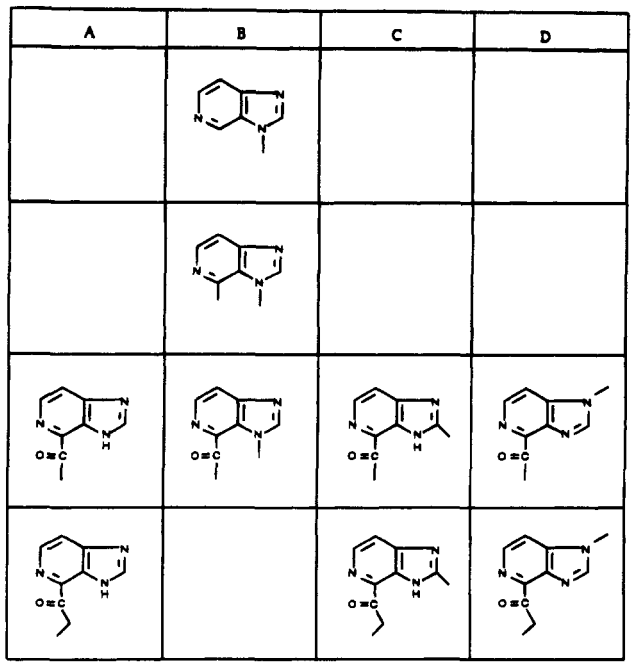

Figure 2. Pyrido[3,4-*d*]imidazoles from the reaction of glucose with histidine (A), 1-methylhistidine (B), 2-methylhistidine (C), and 3-methylhistidine (D).

Column 2: fused silica DB-1 (J&W) 60 m; 0.25 μm film; i.d. 0.32 mm; split 1:5; temperature program, 40 °C, 5 min, 3 °C/min to 260 °C, 40 min.

Gas Chromatograph 1: Carlo Erba 4130 with FID; retention indices were calculated according to the method of van den Dool and Kratz (1963) via *n*-alkane (C_8–C_{30}) standards.

Gas Chromatograph 2: Carlo Erba 5300 Mega Series with FID.

GC/MS System: Finnigan MAT 4500 with interface box 2010 and data system INCOS 2100. The technical data are as follows: closed coupling; temperature of ion source, 120 °C; ionization energy, 70 eV; cyclic scan, electron impact; mass range, 35–350 amu within 0.8 s.

RESULTS AND DISCUSSION

Among 231 compounds identified in the mixture of volatiles from the reaction of glucose with histidine, we found also a compound with the mole mass *m/z* 175, the mass spectrum of which was very similar to that of 2-acetylpyrido[3,4-*d*]imidazole (*m/z* 161). Besides a suggestion that we had found the homologous 2-propionyl compound with the radical cation of *m/z* 57, we also took into consideration a possible methyl derivative of 2-acetylpyrido[3,4-*d*]imidazole. Methylations of ring systems by formic aldehyde (Velisek et al., 1976) are well-known to occur in the course of the Maillard reaction because formic aldehyde is easily formed by sugar degradation.

To investigate this question, we have reacted glucose with 1-, 2-, and 3-methylhistidine in the autoclave at 150 °C. 1-Methylhistidine occurs in the dipeptide anserine (β-alanyl-1-methylhistidine), which is found in goose muscle and beef extracts (Baltes, 1968a). 3-Methylhistidine is part of the dipeptide balenine (β-alanyl-3-methylhistidine), which occurs in whale muscle (Baltes, 1968a,b). Among the volatiles we identified seven compounds that were methylated at the imidazole ring. They are shown in Figure 2. They contain preferably acetyl as well as propionyl residues at the pyridine ring. From the reaction of 1-methylhistidine with D-glucose, among others, two methylated compounds without carbonyl groups at the pyridine ring could also be identified. They might have been formed by reaction of 1-methylhistamine (from 1-methylhistidine) with formic or acetic aldehyde. All compounds mentioned in Figure 2 are referred to for the first time. Their mass spectra showed fragment ions of *m/z* 133/132 instead of *m/z* 119/118, which we observed exclusively for pyrido[3,4-*d*]imidazoles with an unmethylated imidazole residue. The masses *m/z* 133/132, like *m/z* 119/118, represent the base peaks which were obviously formed by cleavage of the substituents bound at the pyridine ring. Other fragments resulted from a CO elimination from the acetyl or propionyl derivatives. The fragmentation of 2-propionylpyrido[3,4-*d*]imidazole proceeds analogously to that of the homologous 2-acetylated compound. Figure 3 shows the mass spectra of 2-propionylpyrido[3,4-*d*]imidazole and of the compounds methylated at the imidazole ring in the 2′- or 3′-position.

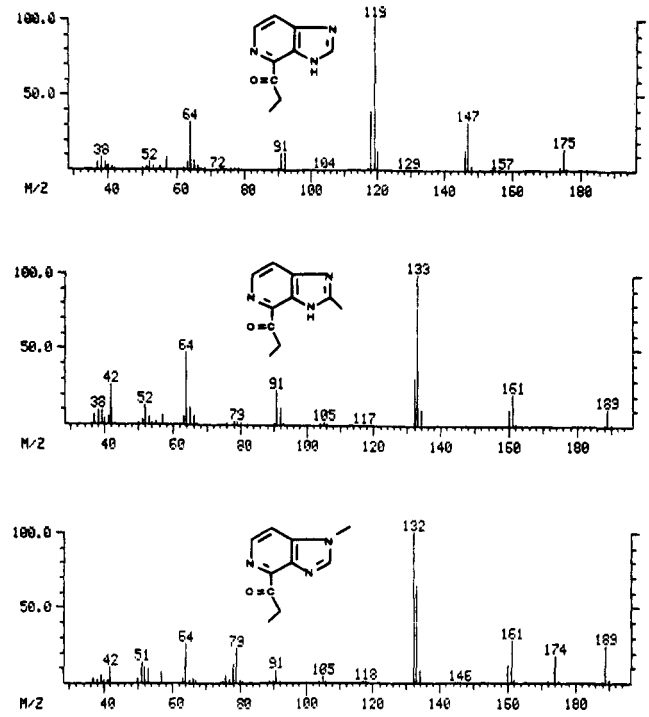

Figure 3. Mass spectra of 2-propionylpyrido[3,4-*d*]imidazole and its derivatives methylated at the 2′- or 3′-position.

To establish the structure of 2-propionylpyrido[3,4-*d*]imidazole, this compound was synthesized by reaction of 2-oxobutyraldehyde with histamine. 2-Oxobutyraldehyde, which is not commercially available, was synthesized from 2-oxobutane by oxidation with SeO_2. After reaction in an autoclave at 150 °C, 2-propionylpyrido[3,4-*d*]imidazole was obtained as the only product, which was identical in its behavior to the compound formed by reaction of histidine with glucose.

While pyruvic aldehyde is a well-known sugar degradation product, 2-oxobutyraldehyde is unknown in the Maillard reaction up to now. Therefore, some tracer experiments with ^{13}C isotope labeled glucose in the 1- or 6-position were carried out to determine the formation pathway of 2-oxobutyraldehyde during sugar degradation. 1-^{13}C or 6-^{13}C isotope labeled glucose was reacted in two different experiments with histidine under the same conditions as were reported for the other model reactions.

Figure 4 shows the mass spectra of 2-acetylpyrido[3,4-*d*]imidazole from the reaction of histidine with unlabeled and 1-^{13}C and 6-^{13}C isotope labeled glucose. It can be recognized that there are different amounts

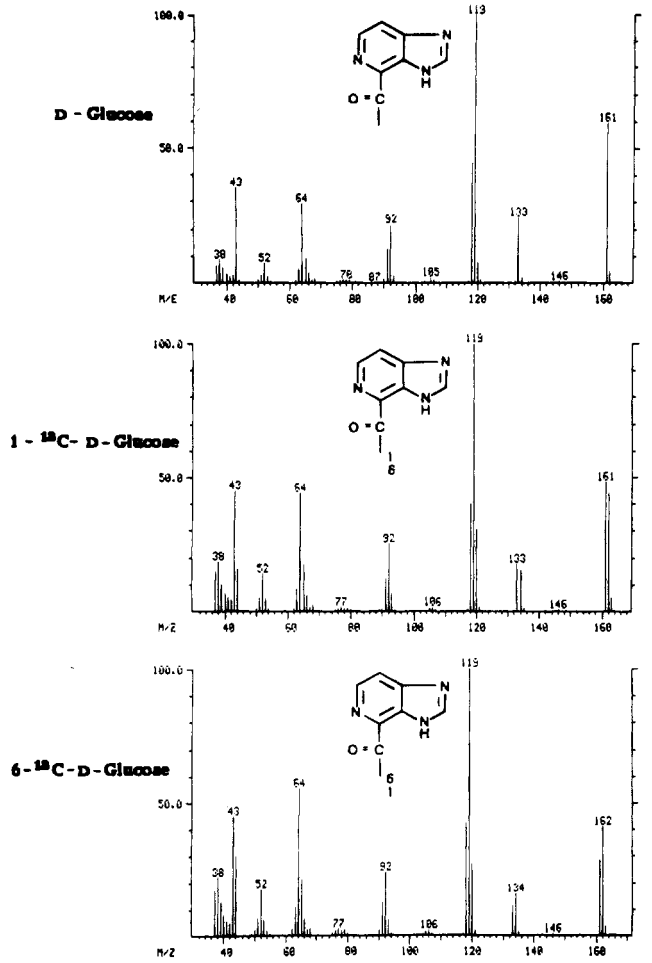

Figure 4. Mass spectra of 2-acetylpyrido[3,4-*d*]imidazole from the reaction of histidine with unlabeled and 1-^{13}C and 6-^{13}C isotope labeled glucose.

of isotope labeling at *m/z* 43/44, 133/134, and 161/162. In the case of 1-^{13}C isotope labeled glucose, the mole peak *m/z* 162 appears with about 45% ^{13}C labeling, while the product formed from [6-^{13}C]glucose showed about 55% ^{13}C labeling. On the other hand, the base peak *m/z* 119 is formed in all three spectra without any additional isotope labeling. Because the peak group of *m/z* 119, 118, and 120 is formed after elimination of the acetyl group, it can be concluded that the ^{13}C isotope labeling is located in the acetyl group. Contrary to the base peak, the fragment ion *m/z* 133 shows a ^{12}C/^{13}C ratio similar to that of the mole peak. Because of the similar ^{12}C/^{13}C ratio at *m/z* 133/134 compared with the mole peak, it can be assumed that the terminal methyl group is ^{13}C isotope labeled by the C-1 atom of glucose as well as the C-6 atom. Also, *m/z* 44 is enhanced after use of [6-^{13}C]glucose to a similar extent as demonstrated at the mole peak.

The fragmentation pathway of 2-acetylpyrido[3,4-*d*]-imidazole is demonstrated in Figure 5. From the result of isotope labeling experiments it can easily be derived that the fragment ion *m/z* 133 was formed by CO elimination and rearrangement of the terminal methyl group. Such CO elimination was already described by Tomer et al. (1973) at 2-acetylpyridine. On the other hand, the main fragmentation pathway from the molecule ion *m/z* 161 shows via an α-cleavage the separation of CH_2CO, which by addition of H^+ appears as *m/z* 43, while the remaining fragment forms the base peak *m/z* 119. The accompanied masses *m/z* 118 and 120 may have been formed by deprotonation or protonation. The

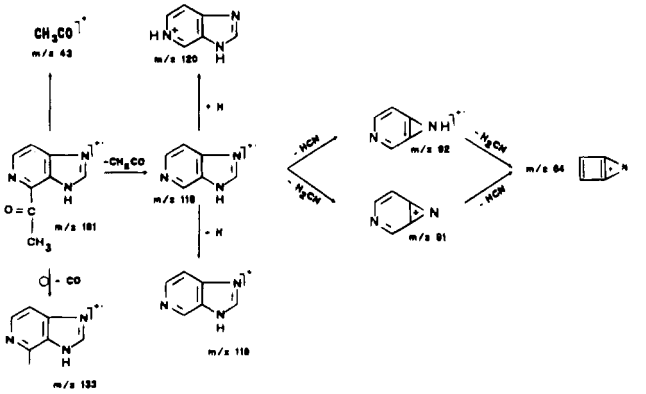

Figure 5. Postulated mass fragmentation pathway of 2-acetylpyrido[3,4-*d*]imidazole.

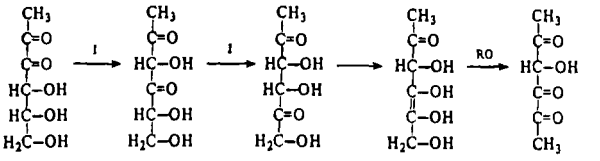

Figure 6. Possible transformation of α-dicarbonyl compounds derived from hexose, via isomerization (I) and redox process (RO).

further fragment ions *m/z* 91, 92, and 64 could be formed from the base peak *m/z* 119 by subsequent elimination of HCN or H_2CN, respectively.

According to the isotope labeling experiments the formation of pyruvic aldehyde from glucose during the Maillard reaction must be considered at least on two routes because the ^{13}C labeled 2-acetylpyrido[3,4-*d*]-imidazole possessed the ^{13}C labeling at the acetyl group in similar amounts from the reaction of 1-^{13}C or 6-^{13}C isotope labeled glucose.

Pyruvic aldehyde, which is one of the most important sugar degradation products, is easily derived from the well-known diacetylformoin (**2**) by a retro aldol scission. Diacetylformoin, in turn, is obtained from 1-deoxyglucosone (**1**) via two isomerizations and a dehydration reaction (Figure 6). The inclusion of the C-6 atom of glucose as a methyl group of pyruvic aldehyde enhances this probability. Hodge et al. (1963) considered diacetylformoin to be an important intermediate for the formation of piperidinohexosereductone. Simon (1962, 1965) carried out isotope labeling experiments and postulated the formation pathway of this reductone via diacetylformoin.

To investigate the formation of 2-oxobutyraldehyde (which forms 2-propionylpyrido[3,4-*d*]imidazole) in the course of sugar degradation, the mass spectra from the reaction of unlabeled and 1-^{13}C and 6-^{13}C isotope labeled glucose with histidine were compared (Figure 7). It was found that the following mass units were changed from unlabeled glucose: *m/z* 175 to 175/176, ratio about 1:1 (molecule peak); *m/z* 147 to 147/148, ratio about 2:1 (M − CO); *m/z* 57 to 58 (C_2H_5CO) (small peak).

As was shown in the mass spectrum of 2-acetylpyrido-[3,4-*d*]imidazole, its propionyl homologue has its base peak at *m/z* 119, which was not changed by isotope labeling. Because of the similarity of labeling and fragmentation pathways of 2-acetyl- and 2-propionyl derivatives, it was concluded that the terminal methyl group of the propionyl moiety was derived from the C-1 as well as the C-6 atom of glucose. The amounts of ^{13}C isotope

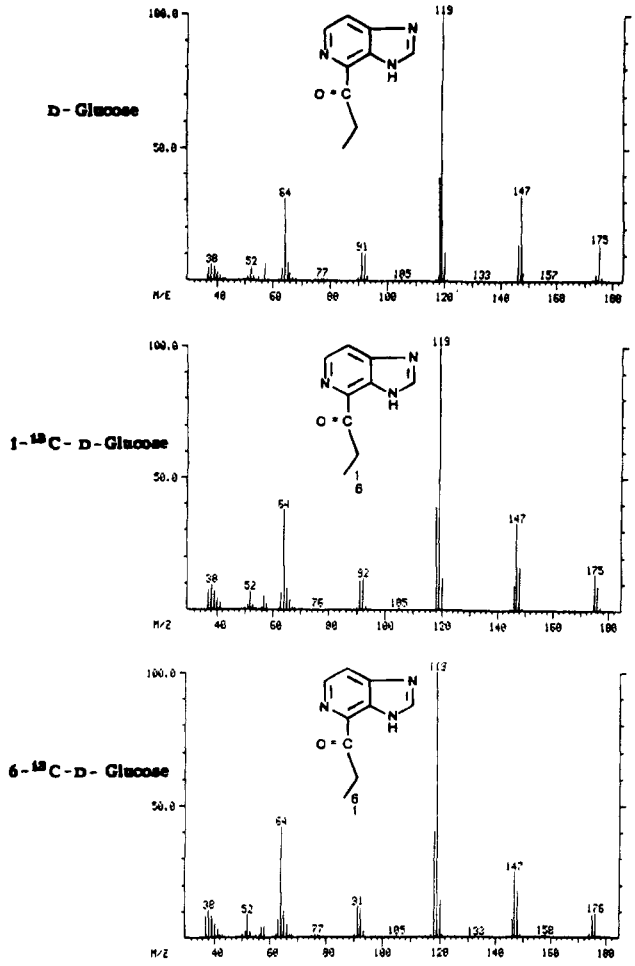

Figure 7. Mass spectra of 2-propionylpyrido[3,4-*d*]imidazole from the reaction of histidine with unlabeled and 1-^{13}C and 6-^{13}C isotope labeled glucose.

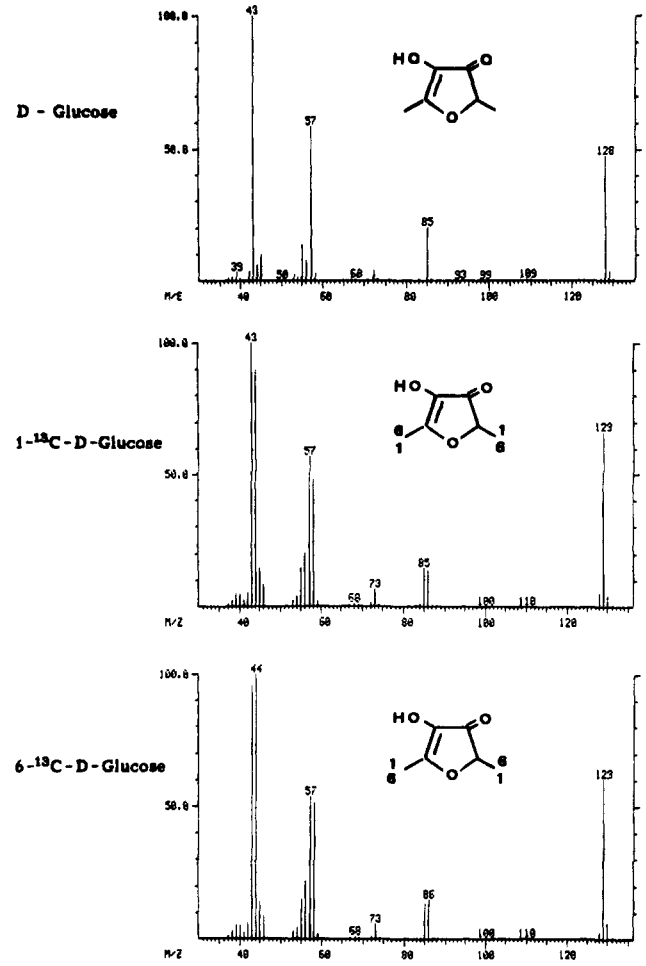

Figure 8. Mass spectra of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone from the reaction of histidine with unlabeled and 1-^{13}C and 6-^{13}C isotope labeled glucose.

labeling are in the ratio of about 40% [1-^{13}C]glucose to about 60% [6-^{13}C]glucose.

The isotope labeling from [1-^{13}C]- as well as [6-^{13}C]-glucose to the terminal methyl group of the propionyl moiety requires a 1,2- or 5,6-dideoxysugar as a precursor of 2-oxobutyraldehyde. Such compounds were not described, up to now. On the other hand, the absence of a double isotope labeling of this compound demonstrates that 2-oxobutyraldehyde cannot be formed via aldol condensation reactions of aldehydes, e.g. acetaldehyde. Because of almost similar isotope distributions of *m/z* 175/176, 147/148, and 57/58 from 1-^{13}C and 6-^{13}C isotope labeled glucose, it was concluded that 2-oxobutyraldehyde cannot be a primary sugar degradation product; rather, it must have been formed from a secondary product. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (commercially known as Furaneol) is likely such a secondary sugar degradation product. Rodin et al. (1965) isolated this compound as colorless crystals from pineapples for the first time. They postulated its structure and possible tautomer forms via IR and ^{1}H NMR spectra. Mills et al. (1969) proposed the formation pathway of this compound from 1-deoxyhexosone via intermediate diacetylformoin. Indeed, the mass spectra of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, which was obtained as one of the main products after reaction of histidine with 1-^{13}C or 6-^{13}C isotope labeled glucose, were quite identical (Figure 8). Both spectra showed the same ratio of *m/z* 85 and 86 which were formed from the molecular peak after cleavage of *m/z* 43 or 44 (→ $^{12}CH_3CO^{\bullet}$ or $^{13}CH_3CO^{\bullet}$). This means that the isotope distributions

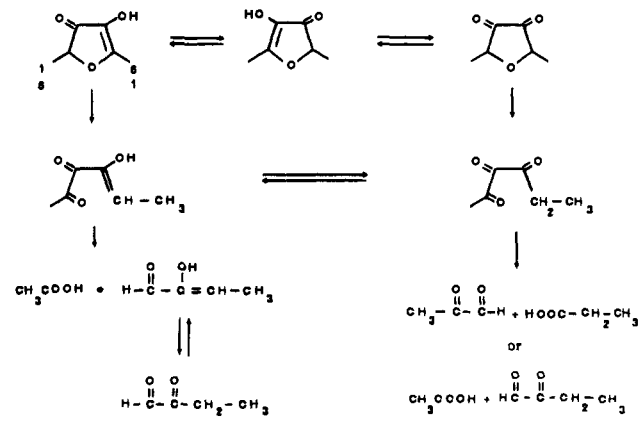

Figure 9. Postulated degradation pathway of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone relying on Shu (1985).

in both methyl groups are identical and it does not matter if the product was formed via a reaction of 1-^{13}C or 6-^{13}C isotope labeled glucose. Consequently, both methyl groups can be derived from the C-1 as well as the C-6 atom of glucose.

It has been discussed several times (Shu et al., 1985; Reese, 1989; Kunert-Kirchhoff, 1990) that relatively large amounts of 1-hydroxy-2-butanone were formed as a thermal degradation product by heating of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone in an aqueous medium. The possible tautomerism of this compound and its decomposition pathway are shown in Figure 9. After ring opening of this compound, 2-oxobutyraldehyde as

well as pyruvic aldehyde from three different tautomer forms could be formed, where the ^{13}C isotope labeling at C-1 as well as C-6 of glucose is found at both terminal methyl groups in each case.

To prove the possible formation of 2-oxobutyraldehyde from 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, we reacted Furaneol with histidine in 1 M phosphate buffer (pH 5.8) at 150 °C in an autoclave. From this reaction relatively high concentrations of 2-propionylpyrido[3,4-*d*]imidazole and negligible amounts of 2-acetylpyrido-[3,4-*d*]imidazole were found. This result proves our mechanism of the formation of 2-oxobutyraldehyde and 2-propionylpyrido[3,4-*d*]imidazole via 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone in the course of the Maillard reaction.

As we have already reported (Gi and Baltes, 1993), 2-acetylpyrido[3,4-*d*]imidazole is formed by heating of D-glucose with fresh tuna meat. The corresponding propionyl derivative has not been identified in food up to now probably because of its lower amounts. We have just begun synthesis for sensorial and toxicological investigations.

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