Formation of 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone and 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone through Maillard Reaction Based on Pentose Sugars

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The caramel-like smelling compounds 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF) were identified by GC−MS and GC−MS/MS in Maillard reaction systems based on pentoses. The reaction was performed in a phosphate buffer by heating xylose, ribose, or arabinose with glycine or L-alanine at 90 °C for 1 h. HEMF was detected in the system pentose/alanine. HDMF was formed in both pentose/glycine and pentose/alanine systems as well as directly from pentoses. Experiments using ^{13}C-labeled glycine and alanine suggest the incorporation of the Strecker degradation products formaldehyde and acetaldehyde into the pentose moiety, forming the furanones HDMF and HEMF, respectively. The presence of ^{12}C-HDMF, which was approximately 30% of the total HDMF amount found in xylose/glycine, indicates that HDMF is partly formed by sugar fragmentation. The proposed mechanism for the formation of the furanones is based on decomposition of the Amadori compound via 2,3-enolization, chain elongation by the Strecker aldehydes, and reduction of the resulting acetylformoin-type intermediates to the target molecules.

INTRODUCTION

4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF, Furaneol, a registered trademark of Firmenich S.A., Genève, Switzerland, **1**) and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF, homofuraneol, **2**) are
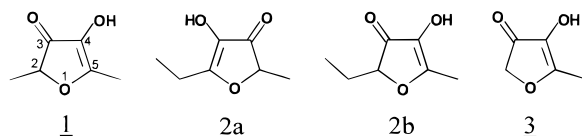
important flavor compounds contributing to the sensory properties of many natural products and thermally processed foods. HDMF has been found in various fruits such as pineapples (Rodin et al., 1965), strawberries (Ohloff, 1969), and grapes (Rapp et al., 1980) as well as in beef broth (Tonsbeek et al., 1968), roasted coffee (Tressl et al., 1978), bread crust (Schieberle, 1990), roasted beef (Cerny and Grosch, 1992), roasted sesame seeds (Schieberle, 1993), and stewed beef (Guth and Grosch, 1993). HEMF, which exists in the tautomeric forms **2a** and **2b** in the ratio of 1:2 (Re et al., 1973), has so far been reported in soy sauce (Nunomura et al., 1976), roasted coffee (Tressl et al., 1978), melon (Huber, 1992), lovage (Blank and Schieberle, 1993), and Emmentaler cheese (Preininger et al., 1994).

Both HDMF and HEMF smell caramel-like, sweet, and fruity and have low retronasal odor thresholds, i.e. 160 and 20 µg/kg water, respectively (Huber, 1992). According to Hodge (1967), the caramel-like flavor is associated with a planar enol−oxo group of cyclic dicarbonyl compounds.

As shown in model experiments, HDMF can be formed by thermal degradation of 6-deoxysugars, e.g. rhamnose (Hodge et al., 1963; Shaw and Berry, 1976), and of fructose (Mills and Hodge, 1976) in the presence of amines or amino acids. HDMF can also be generated directly from hexoses (Shaw et al., 1968; Fagerson, 1969) and hexose−phosphates (Schieberle, 1992). The latter author showed that acetylformoin [2,4-dihydroxy-2,5-dimethyl-3(2*H*)-furanone] is a key intermediate in the formation of HDMF from hexoses. The formation of furanones is generally explained to occur via the 2,3-enolization pathway leading to 1-deoxyosones as intermediates (Hodge et al., 1972).

HEMF is produced by yeast during shoyu fermentation (Sasaki et al., 1991). Intermediates of the pentose−phosphate cycle are important precursors in the biosynthesis of HEMF. Formation of HEMF during heat processing is not well understood. In analogy to the formation of HDMF from an Amadori compound of rhamnose, Huber (1992) speculated on the existence of 6,7-dideoxyheptose as an intermediate.

Similar to the decomposition of hexoses, the degradation pathway of pentoses proceeds via 2,3-enolization, forming 4-hydroxy-5-methyl-3(2*H*)-furanone (HMF, norfuraneol, **3**) (Feather, 1981). For this type of reaction the carbon skeleton of the sugar is determinant for the furanone formed. This means that HDMF is produced from hexoses and HMF from pentoses. To the best of our knowledge, no information is available about the formation of the furanones **1** and **2** from pentoses.

In the present study, we report on the identification of HDMF and HEMF in Maillard reaction systems based on pentoses and the amino acids glycine and alanine. The formation of these furanones was investigated using ^{13}C-labeled amino acids. GC−MS/MS was applied to identify the labeled and nonlabeled furanones. On the basis of the results, a formation pathway is proposed indicating the role of Strecker aldehydes in generating odor-active furanones.

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EXPERIMENTAL PROCEDURES

Materials and Reagents. D-Xylose, D-ribose, D-arabinose, glycine, and L-alanine of highest purity (>99%) were obtained from Fluka (Buchs, Switzerland). [2-^{13}C]Glycine and [1-^{13}C]-D-xylose were from Cambridge Isotope Laboratories (Andover, MA), and [3-^{13}C]-L-alanine was from Tracer Technologies (Somerville, MA). The isotopic content of the labeled compounds was 99%. Sodium sulfate, disodium hydrogen phosphate dihydrate, and diethyl ether were from Merck (Darmstadt, Germany). The reference compounds HDMF (Furaneol, **1**) and HEMF (homofuraneol, **2**) are commercially available from Aldrich (Steinheim, Germany) and Givaudan (Dübendorf, Switzerland), respectively. The organic solvents were purified by slow distillation on a Vigreux column (1 m × 1 cm).

Sample Preparation. In a 15 mL Pyrex tube, 5 mmol of pentose (xylose, ribose, or arabinose) and 5 mmol of amino acid (glycine or alanine) were dissolved in 5 mL of phosphate buffer (0.2 mol/L Na_2HPO_4, pH 6.0). The tube was sealed with a screw cap and heated at 90 °C for 1 h in an oil bath while stirring with a magnetic stirrer. The reaction was stopped by rapid cooling with tap water. During the reaction, the pH dropped to 5.0 (xylose/glycine) and 5.3 (xylose/alanine). Then, 40 mL of water was added to the dark brown reaction mixture, which was then saturated with 16 g of NaCl. The pH was adjusted to 4.0 (aqueous HCl, 2 mol/L), and the neutral compounds were continuously extracted with 50 mL of diethyl ether overnight using a rotation perforator (Normag, Weinheim, Germany). The organic phase was separated, dried over sodium sulfate at 4 °C, and concentrated to 0.5 mL using a Vigreux column (50 cm × 1 cm) and a microdistillation device according to the procedure of Bemelmans (1979). Experiments with the labeled compounds were performed in the same way as described above.

Gas Chromatography–Olfactometry (GC–O). GC–O was performed with a Carlo Erba instrument (Mega 2) equipped with an automatic cold "on-column" injector. At the end of the capillaries, the effluent was split 1:1 into the FID and a sniffing port (Blank and Schieberle, 1993). Fused silica capillaries of medium (OV-1701) and high polarity (FFAP) were used, both 30 m × 0.32 mm with a film thickness of 0.25 μm (J&W capillaries, Fisons Instruments, Brechbühler, Schlieren, Switzerland). The temperature program was as follows: 50 °C (2 min), 6 °C/min to 180 °C, 10 °C/min to 240 °C (10 min). Linear retention indices were calculated according to the method of van den Dool and Kratz (1963).

Gas Chromatography–Mass Spectrometry (GC–MS). GC–MS analyses were performed on an HP-5971 mass spectrometer (Geneva, Switzerland) connected to an HP-5890 gas chromatograph equipped with an HP-7673 autosampler. A capillary column with Carbowax stationary phase was employed (30 m × 0.32 mm, 0.25 μm film thickness). Helium was used as carrier gas at a pressure of 10 psi. The oven program was 20 °C (0.5 min), 30 °C/min to 100 °C, 4 °C/min to 145 °C (10 min), 70 °C/min to 220 °C (2.5 min). The samples were injected using a splitless injector heated at 250 °C, and the interface was kept at 220 °C. The ion source working in electron impact mode at 70 eV was held at about 180 °C.

Gas Chromatography–Tandem Mass Spectrometry (GC–MS/MS). The experiments were carried out using a Finnigan TSQ-700 mass spectrometer (Bremen, Germany) connected to an HP-5890 gas chromatograph equipped with an HP-7673 autosampler. The chromatographic separation was done using the FFAP capillary column as described above. The samples were injected in splitless mode (280 °C), and the oven program was 60 °C (1 min), 10 °C/min to 200, 30 °C/min to 240 °C (2 min). Helium was used as carrier gas at a pressure of 10 psi. The ion source working in electron impact mode at 70 eV was held at 150 °C. The detection was achieved by tandem mass spectrometry after collision-induced dissociation (CID) of the molecular ion of the compounds. The daughter spectra were recorded from 20 to 200 Da. A collision energy of 10 eV in the laboratory frame was used. The collision gas argon was set to 1.1 mTorr.

Table 1. Sensory Contribution of HDMF and HEMF Estimated by GC–Olfactometry[a] in Maillard Systems Based on Pentoses and either Glycine (Gly) or L-Alanine (Ala) and in Model Reactions Containing Only Pentose[b]

compd	linear retention index		pentose/ Gly[c]	pentose/ Ala[c]	pentose[c]
	OV-1701	FFAP			
HDMF **1**	1235	2040	++	+	+
HEMF **2a**	1310	2090	−	+++	−
HEMF **2b**	1325	2190	−	+[d]	−

[a] GC–O data are presented in terms of odor intensities perceived at the sniffing port (+: weak; +++: intense). [b] The model mixtures were heated in a phosphate buffer (0.2 mol/L, pH 6) at 90 °C for 1 h. [c] The pentoses used were ribose, xylose, and arabinose. [d] The tautomer **2b** was sensorily detectable only in highly concentrated samples.

RESULTS AND DISCUSSION

Identification of HDMF and HEMF. The overall aroma of Maillard reaction systems mainly depends on the composition of sugars and amino acids as well as on the reaction conditions. Simple Maillard systems consisting of pentoses and glycine or alanine develop sweet, caramel-like notes under relatively mild reaction conditions. We produced such aroma qualities by reacting the pentoses xylose, ribose, and arabinose with glycine or alanine in a phosphate buffer at 90 °C for 1 h. Similar notes were also obtained when using pentoses alone in the model reaction.

Gas Chromatography–Olfactometry. A sensory-directed chemical analysis was performed to identify the odorants responsible for the caramel-like character. The odor-active compounds were detected by GC–O. The potential of the GC–O technique to select odorants from odorless volatiles has recently been reviewed by Acree (1993) and Grosch (1993). Caramel-like smelling regions were found in the chromatograms from the model reactions containing a pentose and glycine or alanine. They were tentatively identified as 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF, **1**) and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF, **2**) on the basis of their characteristic aroma qualities and the retention indices on two capillaries of different polarity (Table 1). The sensory and chromatographic properties were identical to those of the reference compounds.

HDMF was mainly responsible for the caramel-like note of the reaction mixture containing pentose/glycine or only a pentose (Table 1). In the system pentose/alanine, both HDMF (**1**) and HEMF (**2**) contributed to the caramel-like, sweet character. Both furanones were represented by minor peaks in the gas chromatograms. Rough estimates of the amounts of HDMF and HEMF formed from pentoses were in the low milligrams per kilogram (parts per million) range. The tautomers of HEMF were base-line separated on both capillaries used. GC–O indicated that the odor activity of **2a** was significantly higher than that of **2b** (Blank, unpublished results, 1995). As in this paper we are mainly concerned with the mechanistic aspect, results concerning the identification of further odorants and volatiles will be published elsewhere. It should only be mentioned that HMF (**3**), which was the major volatile in the pentose/amino acid systems, did not contribute much to the caramel-like aroma. This can be explained by its relatively high retronasal odor threshold of 8300 μg/kg of water (Huber, 1992).

Mass Spectrometric Identification of HDMF and the HEMF Tautomers. HDMF (**1**) and the two HEMF tautomers (**2a** and **2b**) were identified on the basis of GC–MS data by comparison of their retention indices
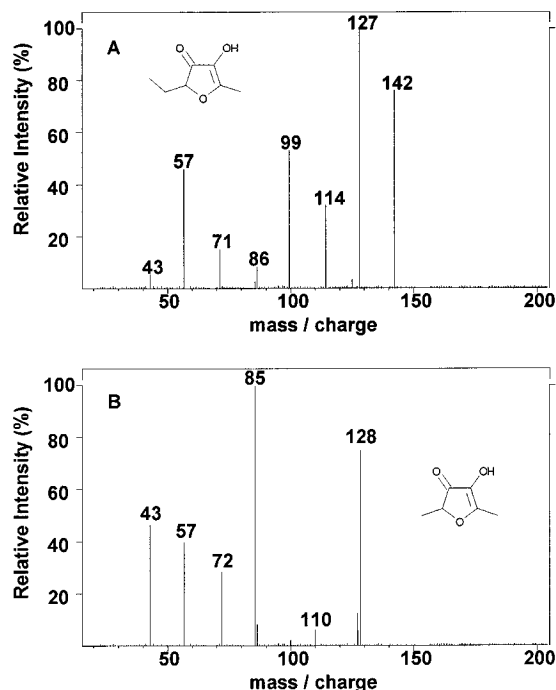
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Figure 1. GC−MS/MS spectra of the HEMF tautomer **2b** (A) and of HDMF (B) obtained in the reaction systems D-xylose/L-alanine and D-xylose/glycine.

Figure 2. GC−MS/MS spectra of the tautomer 2-[2-^{13}C]ethyl-4-hydroxy-5-methyl-3(2H)-furanone **2b** (A) and of 4-hydroxy-2-[^{13}C]methyl-5-methyl-3(2H)-furanone (B) obtained in the reaction systems D-xylose/[3-^{13}C]-L-alanine and D-xylose/[2-^{13}C]glycine (the labeled positions are marked with ■).

and mass spectra with the data available in our libraries. However, in such complex matrices, some interferences can contaminate the mass spectra, particularly at the low mass ranges (20−100 Da) where characteristic fragments of HDMF and HEMF are observed. To achieve unambiguous identification of these compounds, we used tandem mass spectrometry coupled to gas chromatography to obtain interference-free spectra. The molecular ion of each molecule was fragmented by collision-induced dissociation (CID), and the daughter spectra were recorded. Comparison of the retention indices (Table 1) and the daughter spectra obtained from pure compounds and from the samples allowed identification of the molecules. The mass spectra of the furanones **2b** and **1**, shown in parts A and B of Figure 1, respectively, were identical to those of the reference compounds and unequivocally demonstrate the presence of HDMF (**1**) and HEMF (**2**). To the best of our knowledge, this is the first time that the furanones **1** and **2** have been reported in Maillard reaction systems based on pentoses.

Labeling Experiments. Model experiments using labeled precursors have been proven promising in providing data from which hypotheses can be drawn on formation mechanisms. Recently, Tressl et al. (1993) showed the potential of using 1-^{13}C-labeled sugars to explain the formation of amino acid specific Maillard reaction products. As shown in Table 1, we found that glycine and alanine play a key role in generating HDMF (**1**) and HEMF (**2**) from pentoses. On the basis of these findings, we used [2-^{13}C]glycine and [3-^{13}C]-L-alanine to obtain insight into the formation mechanisms of HDMF and HEMF from pentoses using xylose as example.

Reaction of Xylose with [3-^{13}C]-L-Alanine. The reaction of xylose and [3-^{13}C]-L-alanine gave rise to unlabeled HDMF and monolabeled HEMF tautomers. The daughter mass spectrum of the tautomer **2b** shown in Figure 2A was obtained by GC−MS/MS of the parent molecular ion at m/z 143 using the CID fragmentation technique. The daughter ions at m/z 127 [M − ^{13}CH$_3$]$^+$
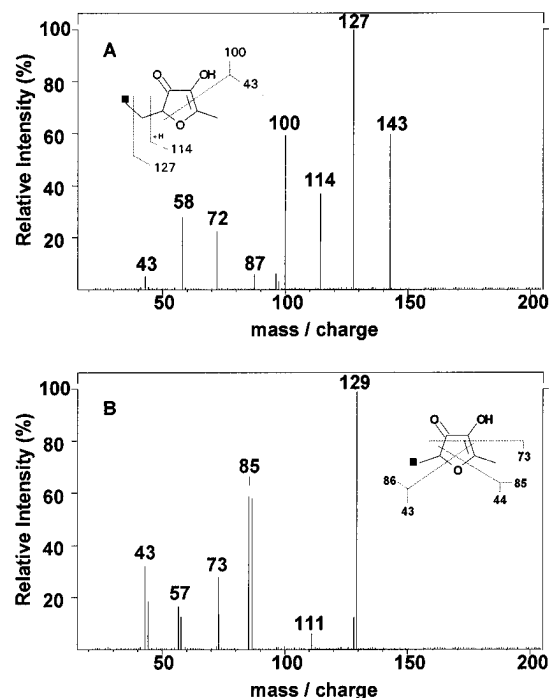
and at m/z 114 [M − CH$_2$ − ^{13}CH$_3$]$^+$ revealed the presence of the ^{13}C atom in the ethyl group. The incorporation of the ^{13}C was also indicated by the fragments at m/z 58, 72, 87, and 100, which correspond to the ions 57, 71, 86, and 99, respectively, in the mass spectrum of the unlabeled HEMF (Figure 1A).

As indicated by the fragment [M − CO − CH$_3$]$^+$ at m/z 100 for the labeled HEMF and at m/z 99 for the unlabeled HEMF, as well as by the fragment [CO − CH$_3$]$^+$ at m/z 43 for both compounds, the methyl group of the labeled HEMF does not bear the ^{13}C atom. Therefore, the data strongly support the presence of a ^{13}CH$_3$CH$_2$ group attached to C-2 of HEMF, i.e. 2-([2-^{13}C]ethyl)-4-hydroxy-5-methyl-3(2H)-furanone.

Reaction of Xylose with [2-^{13}C]Glycine. In the model reaction based on xylose and [^{13}C]glycine, a mixture of monolabeled and unlabeled HDMF was detected by GC−MS. The intensity of the molecular ions at m/z 128 and 129 revealed that about 70% of HDMF was labeled, thus indicating that HDMF was preferentially, but not exclusively, produced by incorporation of the labeled carbon of glycine. The remaining 30% might be formed by sugar fragmentation.

The daughter mass spectrum of the labeled HDMF obtained by GC−MS/MS of the parent molecular ion at m/z 129 using the CID technique is shown in Figure 2B. The molecular ion and the daughter fragments at m/z 86, 73, 58, and 44 revealed the incorporation of one ^{13}C atom into the molecule. The ion pairs 43/44, 57/58, and 85/86 of nearly equal intensity correspond to the symmetric structure of HDMF which may occur as a 1:1 mixture of two isotopomers. Recently, a similar mass spectrum was reported for singly ^{13}CH$_3$-labeled HDMF detected in Maillard reaction systems based on [1-^{13}C]-hexoses (Tressl et al., 1993), which is in good agreement with the MS data of the singly labeled HDMF found in this work (Table 2).

The mass spectrum (Figure 2B) indicates further that the labeled carbon should not be located at positions C-3

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Table 2. Mass Spectral Data of Nonlabeled and ^{13}C-Labeled HDMF

compd	fragmentation pattern (m/z)					technique	ref
HDMF	128	85	72	57	43	GC–MS	Sen et al. (1991)
HDMF[a]	128	---	72	---	---	GC–MS/MS	this work
^{13}CH$_3$-HDMF[b]	129	85/86	73	57/58	43/44	GC–MS	Tressl et al. (1993)
[2,5-^{13}CH$_3$]-HDMF	130	86	74	58	44	GC–MS	Sen et al. (1991)

[a] Parent scan of m/z 72 obtained by GC–MS/MS using commercially available HDMF. [b] MS data reported for a 1:1 mixture of two singly labeled isotopomers [2-^{13}CH$_3$]HDMF and [5-^{13}CH$_3$]HDMF.
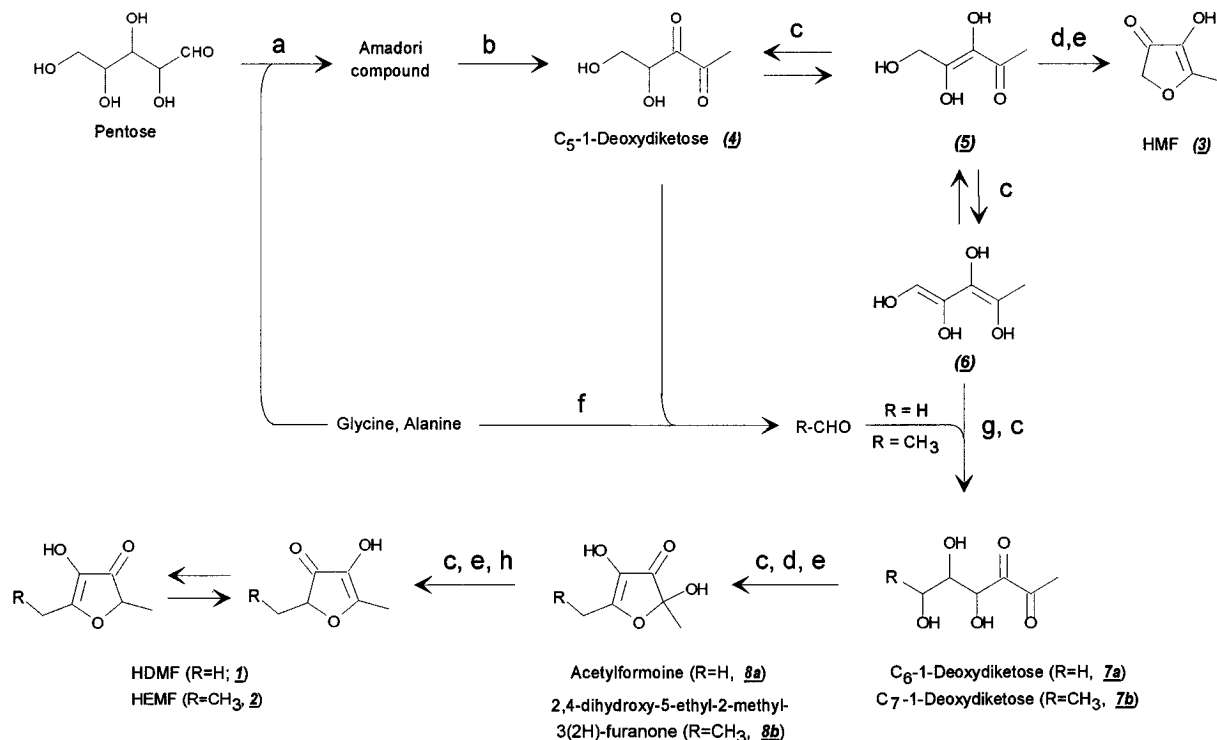

Figure 3. Hypothetical formation pathway of HDMF (**1**) and HEMF (**2**) from pentoses (explanation in the text): a, early stage of Maillard reaction including Amadori rearrangement; b, degradation via 2,3-enolization; c, keto/enol or vinylogous keto/enol tautomerization; d, cyclization; e, dehydration; f, Strecker reaction; g, aldol condensation; h, reduction

and C-4 due to the presence of the singly labeled fragment [M − 2 × CO]$^+$ at m/z 73. The corresponding fragment at m/z 72 of the nonlabeled HDMF was formed exclusively from the molecular ion as verified by a parent scan (Table 2). The origin of this fragment is also supported by the data reported by Sen et al. (1991) showing the presence of the ion at m/z 74 in the spectrum of [2,5-^{13}CH$_3$]HDMF (Table 2). On the basis of the fragmentation pattern and literature data, the ^{13}C atom might be located in the methyl group, by analogy to HEMF preferentially at C-2, i.e. 4-hydroxy-2-[^{13}C]methyl-5-methyl-3(2H)-furanone.

Formation of HDMF (**1**) *and HEMF* (**2**). Considering the results from labeling experiments, the furanones **1** and **2**, having six and seven carbon atoms, respectively, might be formed by the following reaction:

$$\text{C5} + \text{C1(2)} \rightarrow \text{C6(7)}$$
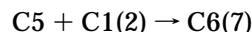
C5 represents the pentose, indicating that the pentose carbon chain remains intact. The C1 and C2 units may be formaldehyde and acetaldehyde, the Strecker degradation products of glycine and alanine, respectively.

The formation mechanism proposed in Figure 3 is based on the formation of the Amadori compound of the pentose and its subsequent decomposition via 2,3-enolization to form a C$_5$ 1-deoxydiketose (**4**). This compound and other reductones react with the amino acids forming the corresponding aldehydes by Strecker degradation, i.e. formaldehyde and acetaldehyde. Further enolization of the 1-deoxyosone (**4**) may lead via compound (**5**) to the intermediate (**6**) which reacts with the Strecker aldehydes via an aldol-type condensation. The chain elongation by the Strecker aldehydes results in the intermediates **7a** and **7b** containing six and seven carbon atoms, respectively. Enolization and dehydration give rise to the cyclic intermediates acetylformoin (**8a**) and the corresponding ethyl homologue (**8b**). The target molecules HDMF (**1**) and HEMF (**2**) are formed by reduction, enolization, and water elimination of the intermediates (**8a**) and (**8b**). The reduction may occur either by a dismutation or by a reaction with further enoloxo compounds, as recently reported by Schieberle (1992) for the formation of HDMF from (**8a**) in a model reaction based on hexoses.

To support the hypothesis shown in Figure 3, [1-^{13}C]-D-xylose was reacted with [3-^{13}C]-L-alanine to form the double-labeled tautomers of HEMF (**2**) which, on the basis of the hypothesis, should additionally be labeled in the methyl group. Indeed, the double-labeled HEMF tautomers were detected by GC–MS/MS. In agreement with the mass spectrum of the singly labeled HEMF (Figure 2A), the fragment at m/z 100 in Figure 4A indicated the incorporation of one ^{13}C into the C-2 ethyl group. On the other hand, the fragment at m/z 44, represented by the ion [CO−^{13}CH$_3$]$^+$, suggested the second labeling position in the C-5 methyl group. In concordance with the proposed formation pathway

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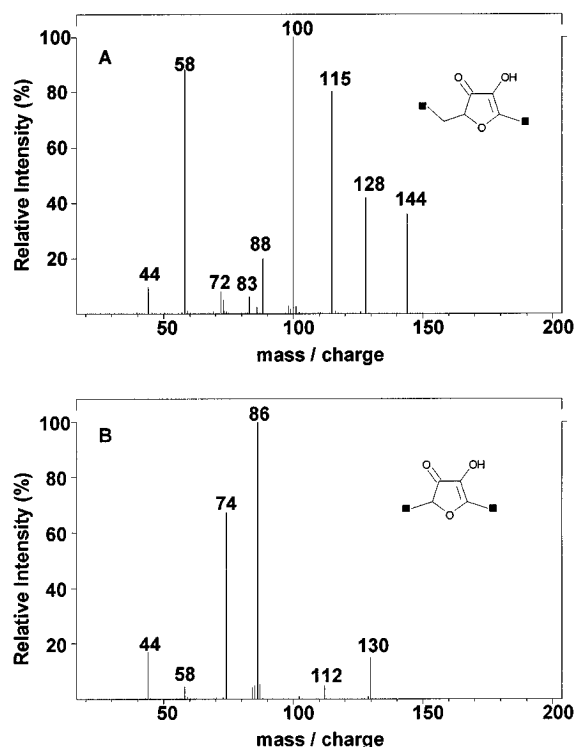*J. Agric. Food Chem.,* Vol. 44, No. 2, 1996 **535**

Figure 4. GC−MS/MS spectra of the tautomer 2-[2-^{13}C]ethyl-4-hydroxy-5-[^{13}C]methyl-3(2*H*)-furanone **2b** (A) and of 4-hydroxy-2,5-[^{13}C]dimethyl-3(2*H*)-furanone (B) obtained in the reaction systems [1-^{13}C]-D-xylose/[3-^{13}C]-L-alanine and [1-^{13}C]-D-xylose/[2-^{13}C]glycine (the labeled positions are marked with ■).

(Figure 3), the data strongly support the presence of 2-[2-^{13}C]ethyl-4-hydroxy-5-[^{13}C]methyl-3(2*H*)-furanone formed in the system [1-^{13}C]-D-xylose/[3-^{13}C]-L-alanine.

Following the hypothesis, the corresponding reaction of [1-^{13}C]-D-xylose and [2-^{13}C]glycine should result in ^{13}C$_2$-HDMF labeled in both methyl groups. The mass spectrum shown in Figure 4B indicated the incorporation of two ^{13}C atoms, i.e. *m/z* 130 and 74. The fragment at *m/z* 44 ([CO−^{13}CH$_3$]$^+$) suggested that both methyl groups were labeled, because the fragment at *m/z* 43 was not at all present in the mass spectrum. The fragmentation pattern was in good agreement with that of synthesized [2,5-^{13}CH$_3$]HDMF (Table 2) reported by Sen et al. (1991), thus suggesting that both methyl groups were labeled in the ^{13}C$_2$-HDMF produced, i.e. 4-hydroxy-2,5-[^{13}C]dimethyl-3(2*H*)-furanone.

The total HDMF amount detected in the model reaction based on [1-^{13}C]-D-xylose and [2-^{13}C]glycine was composed of unlabeled (4%), singly labeled (12%), and doubly labeled (84%) HDMF. The corresponding figures for the system [1-^{13}C]-D-xylose/[3-^{13}C]-L-alanine were 15%, 47%, and 38%, respectively. The data indicate that HDMF was generated by different types of retro−aldol fragmentation of the pentose sugar. In the presence of glycine, HDMF was preferably formed involving the Strecker reaction.

Conclusions. The labeling experiments suggest that the furanones **1** and **2** are mainly formed via Maillard reaction of pentoses with the amino acids glycine and alanine, respectively (Figure 5). The C$_5$ moiety of the sugar is prolonged by a C$_1$ or C$_2$ unit, i.e. formaldehyde or acetaldehyde formed by Strecker degradation of the corresponding amino acids. Alternatively, HDMF can also be produced without direct interaction of glycine.
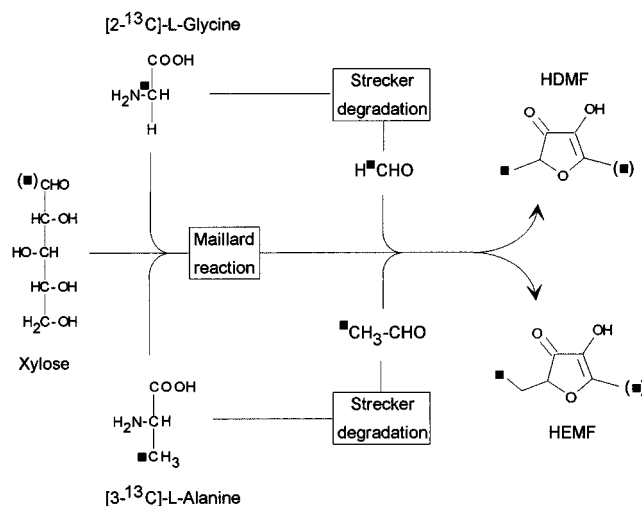

Figure 5. Schematic formation of labeled HDMF and HEMF as indicated by labeling experiments (the labeled carbons are marked with ■).

The C$_1$ unit formaldehyde may originate from both Strecker degradation and sugar fragmentation, i.e. by retro−aldol cleavage of 1-deoxyosones (Ledl and Schleicher, 1990). This explains the presence of unlabeled HDMF in the model reactions containing only pentoses, xylose/[2-^{13}C]glycine, and xylose/[3-^{13}C]-L-alanine. HDMF can be generated by recombination of pentose fragmentation products which may also include the fragments C$_2$, C$_3$, and C$_4$ formed by retro−aldol reaction.

The results presented in this paper show the manifold role of Strecker aldehydes. They may contribute to flavor on their own (methional, phenylacetaldehyde, etc.) or participate in the formation of other key odorants. As shown in our laboratory, alanine and glycine are actively involved in the formation of sensory relevant 3(2*H*)-furanones (this paper) and alkylpyrazines (Amrani-Hemaimi et al., 1995). Although the furanones are formed by a side reaction, these compounds may significantly contribute to the overall flavor due to their low threshold values. As a continuation of this study, the amounts of HDMF and HEMF are being quantified and parameters affecting this reaction are currently being studied.

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