Study of the Fragmentation of 3(2*H*)-Furanones by Mass Spectrometry

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Fragmentation of 4-hydroxy-5-methyl-3(2*H*)-furanone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2*H*)-furanone was studied by gas chromatography/mass spectrometry and by gas chromatography/tandem mass spectrometry. Experiments were carried out using unlabeled reference compounds and isotopically labeled molecules. The latter were obtained either by synthesis or through the Maillard reaction based on xylose and alanine or glycine. A general fragmentation pathway of alkylated 4-hydroxy-3(2*H*)-furanones is proposed on the basis of cross-examination of the mass spectrometric data obtained from these different compounds.

Keywords: *4-Hydroxy-2,5-dimethyl-3(2H)-furanone; 4-hydroxy-5-methyl-3(2H)-furanone; 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2H)-furanone; mass spectrometry; GC/MS/MS; fragmentation; Maillard reaction; pentose*

INTRODUCTION

Mass spectrometry, particularly in combination with gas chromatography (GC/MS), is one of the most widely used tools in food analysis. The success of this technique for compound identification is largely due to its sensitivity and also to the availability of mass spectral libraries that help to identify unknown compounds. However, an unknown compound can be identified even if its spectrum has not yet been registered in a library. In such a case, its identification can be achieved if the fragmentation pathways of similar molecules are known. Moreover, the fragmentation pathways of compounds allow study of their formation mechanisms when using isotopically labeled precursors (Tressl et al., 1993).

3(2*H*)-Furanones are important compounds contributing to the flavor of many natural products and thermally processed foods. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol, **1**), 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(*2H*)-furanone (homofuraneol, **2**), and 4-hydroxy-5-methyl-3(2*H*)-furanone (norfuraneol, **3**) have been identified in foods and food-related model systems (Hodge et al., 1963; Nunomura et al., 1976; Severin and Seilmeier, 1967). Alkylated 4-hydroxy-3(2*H*)-furanones exist in the tautomeric forms **I** and **II** (Scheme 1). Tautomeric ratios from 1:2 (Re et al., 1973) to 1:3 (Huber, 1992) have been reported for the homofuraneol tautomers **2A** and **2B** (R = C_2H_5). The tautomers of **2** can be separated by GC on polar stationary phases (Blank and Fay, 1996; Blank et al., 1997). In contrast, Furaneol tautomers cannot be distinguished due to symmetry of the molecule.

The mass spectra of **1**−**3** were first published by Rodin et al. (1965), Re et al. (1973), and Tonsbeek et al. (1968), respectively. However, a complete description of their fragmentation is not yet available. To the best of our knowledge, only a few attempts have been made to interpret the fragmentation of 3(2*H*)-furanones. Some authors discussed a few fragments generated with conventional GC/MS with electron impact ionization (Nunomura et al., 1976; Preininger and Grosch, 1994).

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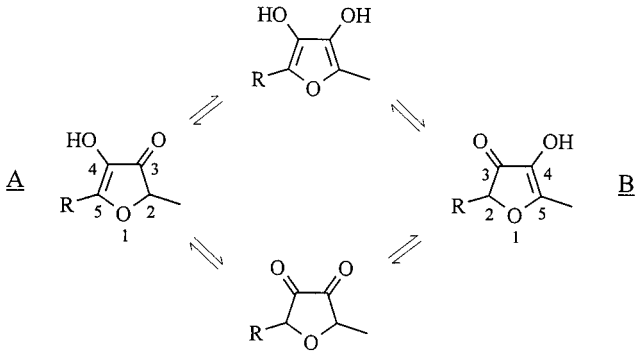
Scheme 1. Tautomerization of Alkylated 4-Hydroxy-3(2*H*)-furanones Resulting in the Tautomeric Forms A and B (R = CH_3 for Furaneol and R = C_2H_5 for Homofuraneol)



However, no systematic studies have yet been published. In contrast, fragmentation of isotetronic acids [3-hydroxy-2(5*H*)-furanones] and the corresponding methyl esters has been extensively studied by Bonini et al. (1980, 1981).

Recently, we discussed formation of Furaneol and homofuraneol through the Maillard reaction based on pentose sugars (Blank and Fay, 1996). Using isotopically labeled precursors, a mechanism for the formation of 3(2*H*)-furanones via Strecker-assisted chain elongation of the pentose moiety was proposed on the basis of data obtained by GC coupled with tandem mass spectrometry (GC/MS/MS). However, 3(2*H*)-furanones, particularly Furaneol, can also be generated by condensation of sugar fragmentation products (Blank et al., 1996).

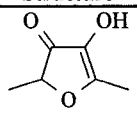
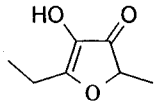
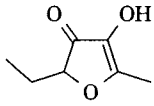
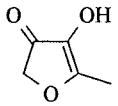
We report here on the mass spectrometric fragmentation of 3(2*H*)-furanones, which has been established using synthesized isotopically labeled analogues. In addition to GC/MS, GC/MS/MS was used because of its suitability for mechanistic studies (Fay et al., 1996). The proposed fragmentation schemes were applied to isotopomers of 3(2*H*)-furanones formed in Maillard model reactions.

MATERIALS AND METHODS

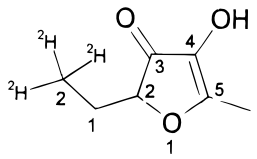
The following nomenclature was used (*A Guide to IUPAC. Nomenclature of Organic Compounds*, recommendation 1993; Blackwell Scientific Publications: Oxford, U.K., 1993): 2-([2,2,2-

S0021-8561(97)00230-6 CCC: $14.00

Table 1. Fragmentation of the Molecular Ions of the Reference 3(2*H*)-Furanones under CID Conditions

Compound Structure	Compound Name	Compound Number	Main daughter ions of the molecular ion *m/z* (relative intensity)
	Furaneol	1	128(60), 110(25), 100(5), 85(100), 72(60), 57(10), 43(15)
	Homofuraneol A	2A	142(10), 127(2), 114(1), 99(1), 86(1), 85(<1), 72 (<1), 71(1), 57(100), 43(1)
	Homofuraneol B	2B	142(70), 127(100), 114(35), 99(55), 86(10), 85(<1), 72(10), 71(15), 57(50), 43(10)
	Norfuraneol	3	114(20), 96(<1), 85(<1), 71(1), 58(35), 57 (<1), 43(100)

$^2H_3$]eth-1-yl)-4-hydroxy-5-methyl-3(2*H*)-furanone corresponding to the following formula:



Materials. The unlabeled reference compounds Furaneol (registered trademark of Firmenich S.A., Geneva, Switzerland, **1**) and homofuraneol (**2**) were commercially available from Aldrich (Steinheim, Germany) and Givaudan-Roure (Dübendorf, Switzerland), respectively. Unlabeled norfuraneol (**3**) was prepared by reacting D-xylose with glycine, both from Fluka (Buchs, Switzerland), in Maillard model reactions (Blank and Fay, 1996). 4-Hydroxy-2(or 5)-[^{13}C]methyl-5(or 2)-methyl-3(2*H*)-[2(or 5)-^{13}C]furanone (**1a**) and 2(or 5)-([2,2,2-$^2H_3$]eth-1-yl)-4-hydroxy-5(or 2)-methyl-3(2*H*)-furanone (see following paragraph) (**2a**) were synthesized as recently described (Blank et al., 1997). 4-Hydroxy-2,5-di[^{13}C]methyl-3(2*H*)-furanone (**1b**) was synthesised according to the method of Sen et al. (1991).

The following labeled 3(2*H*)-furanones were prepared in Maillard model reactions (Blank and Fay, 1996; Blank et al., 1996): 4-hydroxy-2(or 5)-[^{13}C]methyl-5(or 2)-methyl-3(2*H*)-furanone (**1c**) from D-xylose/[2-^{13}C]glycine and from [1-^{13}C]-D-xylose/glycine; 4-hydroxy-2,5-di[^{13}C]methyl-3(2*H*)-furanone (**1b**) from [1-^{13}C]-D-xylose/[2-^{13}C]glycine; 2(or 5)-([2-^{13}C]eth-1-yl)-4-hydroxy-5(or 2)-methyl-3(2*H*)-furanone (**2b**) from D-xylose/[3-^{13}C]-L-alanine; 2(or 5)-ethyl-4-hydroxy-5(or 2)-[^{13}C]methyl-3(2*H*)-furanone (**2c**) from [1-^{13}C]-D-xylose/L-alanine; and 2(or 5)-([2-^{13}C]eth-1-yl)-4-hydroxy-5(or 2)-[^{13}C]methyl-3(2*H*)-furanone (**2d**) from [1-^{13}C]-D-xylose/[3-^{13}C]-L-alanine.

GC/MS. GC/MS analyses were performed on an HP-5971 mass spectrometer connected to an HP-5890 gas chromatograph equipped with an HP-7673 autosampler (Hewlett-Packard, Geneva, Switzerland). A capillary column (J&W Scientific, MSP Friedli, Koeniz, Switzerland) with Carbowax stationary phase was employed (DB-Wax, 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), and 70 °C/min to 220 °C (2.5 min). 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 (EI) mode at 70 eV was held at about 180 °C.

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 performed on the same DB-Wax 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 °C, and 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 EI mode at 70 eV was held at 150 °C. 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. Pressure of the collision gas argon was set to 1.1 mTorr.

RESULTS AND DISCUSSION

Fragmentation of Unlabeled 3(*2H*)-Furanones under CID Conditions. Most of the experiments were carried out using GC/MS/MS because of its greater selectivity and sensitivity compared to GC/MS. The GC/MS and GC/MS/MS fragmentation patterns of the compounds studied were similar. Only the relative intensities of the fragments varied.

On the basis of the main daughter ions obtained from the molecular ions of each of the reference compounds (Table 1), possible fragmentation mechanisms of the 3(*2H*)-furanones were elaborated. Breakdown of the bonds O−C_2 (or O−C_5) and C_4−C_5 (or C_2−C_3) of the furanone cycle and the formation pathway of the low mass daughter ions *m/z* 43, 57, 71, 85, and 99 obtained from molecular ions of the three reference compounds are illustrated in Figure 1. The hydroxycyclopropanone ions *m/z* 71, 85, and 99 were formed from Furaneol (**1**), homofuraneol (**2**), and norfuraneol (**3**), respectively. Their further decomposition led to the ions at *m/z* 43, 57, and 71 found in the mass spectra of all 3(*2H*)-furanones studied.

Two distinct ion species are proposed for the fragments *m/z* 43 and 57, i.e. **IIb**/**IVa** and **IIc**/**IVb**, respectively, which correspond to different parts of the 3(2*H*)-furanone structure. Therefore, fragment *m/z* 57 found in both **1** and **2** does not necessarily represent the same ion species. However, even for one compound a fragment may correspond to different parts of the molecule as shown for Furaneol (Figure 1), e.g. *m/z* 43 (**IIb**/**IIb′**), *m/z* 57 (**IVb**/**IVb′**), and *m/z* 85 (**IIIb**/**IIIb′**), the latter being the base peak in the mass spectrum of **1** (Table 1). In the case of norfuraneol, the acyl ion at *m/z* 43

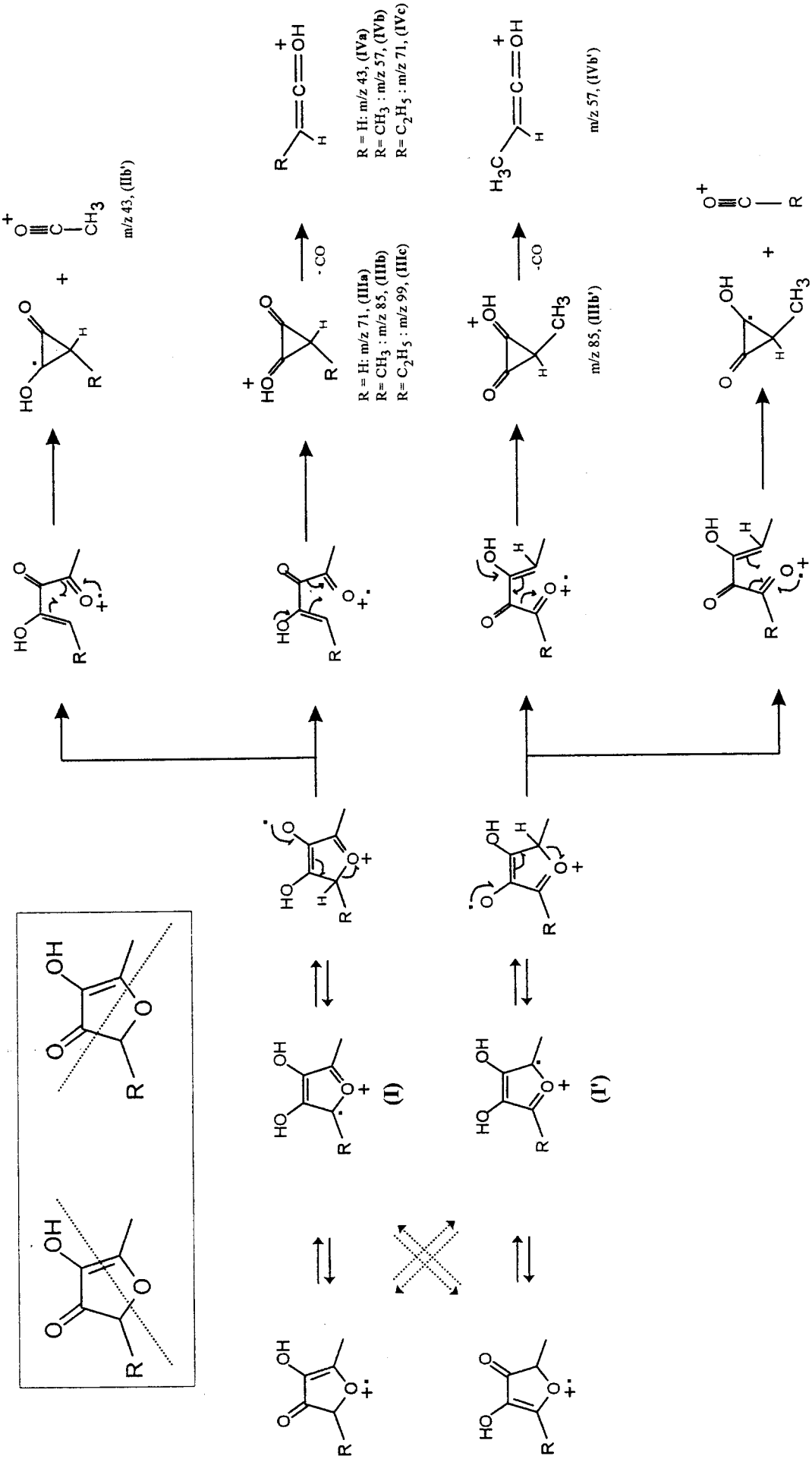


Figure 1. Fragmentation of the molecular ions of the reference compounds norfuraneol (R = H), Furaneol (R = CH_3), and homofuraneol (R = C_2H_5) by GC/MS/MS under CID conditions and proposed fragmentation pathways leading to the opening of the bonds O−C_2 (or O−C_5) and C_4−C_5 (or C_2−C_3) of the 3(2*H*)-furanone cycle. Full arrow and fishhook correspond to the transfer of an electron pair and a single electron, respectively (McLafferty and Turecek, 1993).

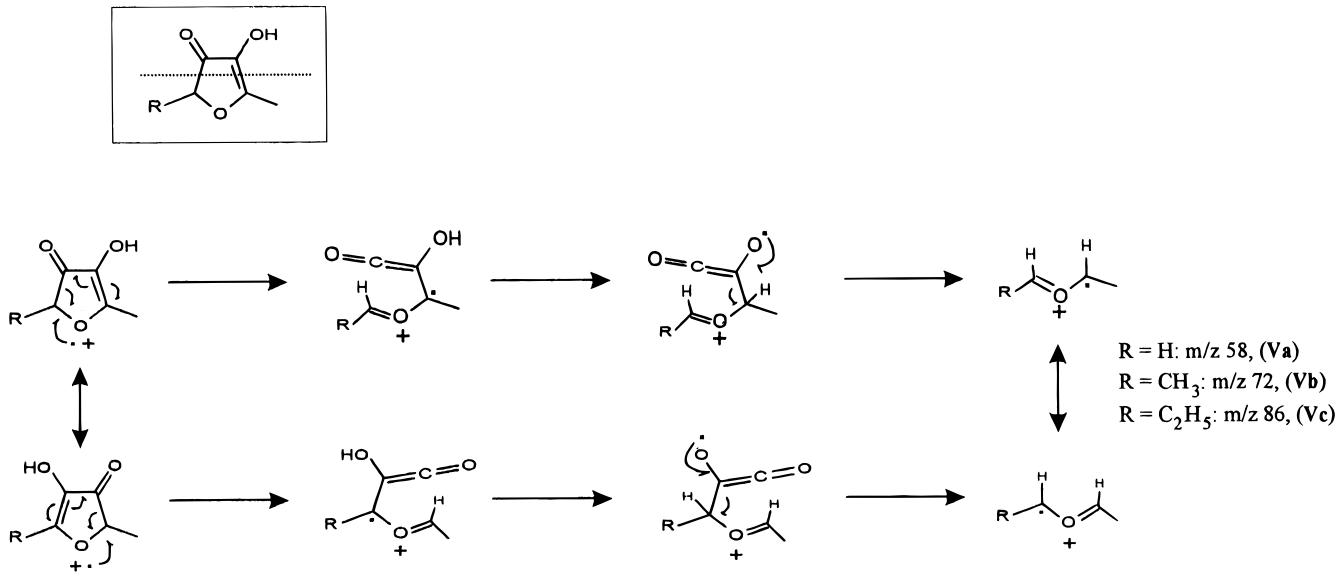


Figure 2. Fragmentation of the molecular ions of the reference compounds norfuraneol (R = H), Furaneol (R = CH_3), and homofuraneol (R = C_2H_5) by GC/MS/MS under CID conditions and proposed fragmentation pathways leading to the breakdown of the bonds C_2-C_3 and C_4-C_5 of the 3(2*H*)-furanone cycle. Fishhook corresponds to the transfer of a single electron (McLafferty and Turecek, 1993).

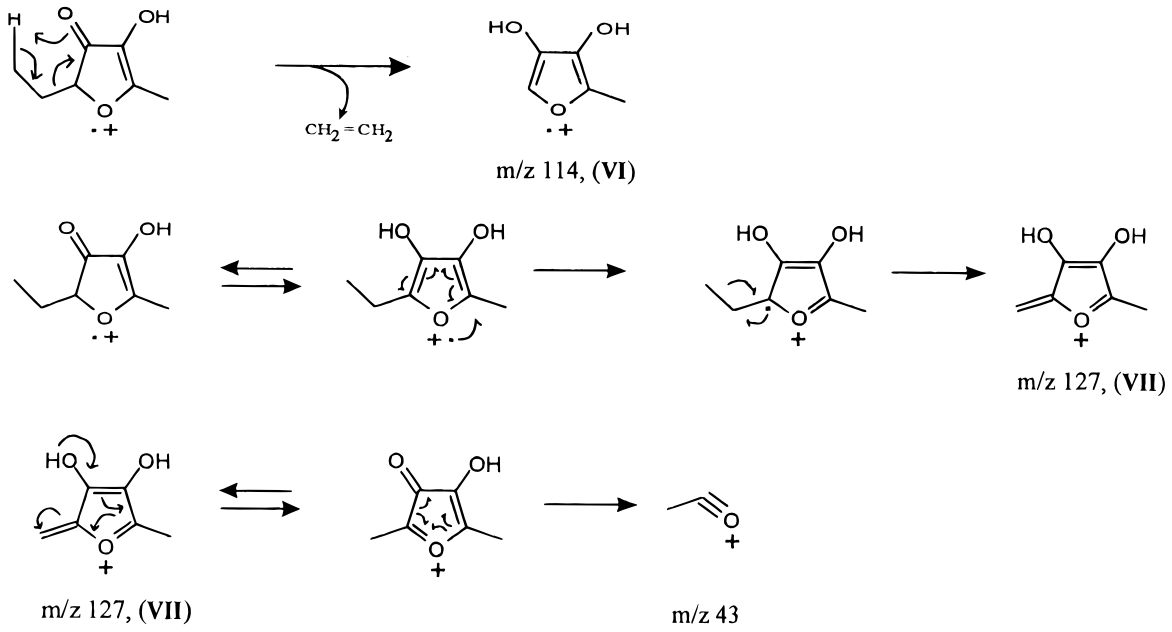


Figure 3. Fragmentation of the molecular ion of the reference compound homofuraneol by GC/MS/MS under CID conditions and proposed fragmentation pathways leading to a loss of the methyl and the ethyl group of the molecule. The ion at *m*/*z* 127 may further fragment to generate an ion at *m*/*z* 43. Full arrow and fishhook correspond to the transfer of an electron pair and a single electron, respectively (McLafferty and Turecek, 1993).

was the most stable fragment formed and, therefore, the base peak in the mass spectrum of **3** (Table 1).

The even mass fragments *m*/*z* 58 (**Va**), *m*/*z* 72 (**Vb**), and *m*/*z* 86 (**Vc**) detected in the mass spectra of the furanones norfuraneol, Furaneol, and homofuraneol, respectively, were formed by cleavage of the C_2-C_3 and C_4-C_5 bonds of the 3(2*H*)-furanone cycle (Figure 2). The formation pathway of the odd-electron ions can be explained via a neutral loss of 56 (likely C_2O_2 or 2CO), i.e. opening of the bond C_2-C_3 (or C_4-C_5), hydrogen shift from the hydroxy group to the carbon, and then cleavage of the bond C_4-C_5 (or C_2-C_3). Fragments **Va**−**c** are 1,3-dipolar species similar to 1,3-dipoles found in carbanion chemistry and thus are stabilized by charge delocation over the three atom moieties. Parent scanning experiments were in agreement with the fragmentation scheme, showing that these fragments were directly formed from the molecular ions without any stable intermediates.

The ions *m*/*z* 114 (**VI**) and *m*/*z* 127 (**VII**) were characteristic fragments in the mass spectrum of homofuraneol, the latter being the base peak in the spectrum of tautomer **2B** (Table 1). As shown in Figure 3, fragment *m*/*z* 127 was formed by an α-cleavage of the methyl group, whereas ion *m*/*z* 114 was the result of a McLafferty rearrangement (McLafferty, 1959) releasing ethylene.

Homofuraneol was the only compound studied that could be separated by GC into the two tautomers 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (tautomer **2A**) and 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (tautomer **2B**). The tautomers showed comparable fragmentation patterns but with significant differences in relative intensity of the fragments (Table 1). This can

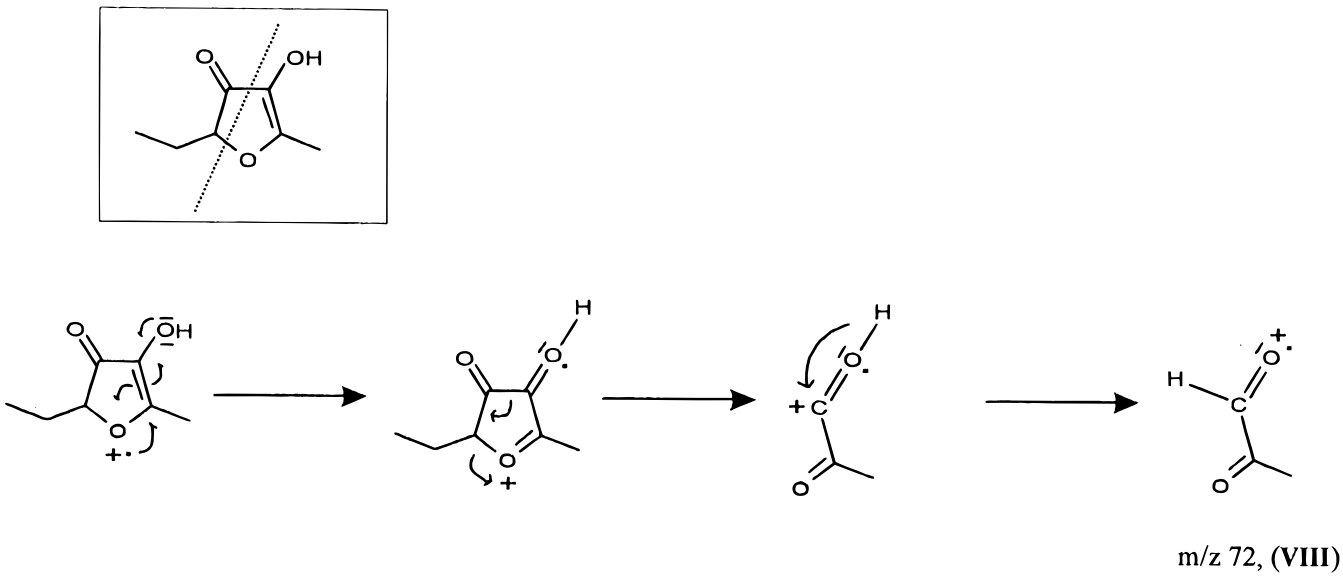


Figure 4. Fragmentation of the molecular ions of the reference compound homofuraneol by GC/MS/MS under CID conditions and proposed fragmentation pathways leading to the breakdown of the bonds O−C_2 and C_3−C_4 of the 3(2*H*)-furanone cycle. Full arrow and fishhook correspond to the transfer of an electron pair and a single electron, respectively (McLafferty and Turecek, 1993).

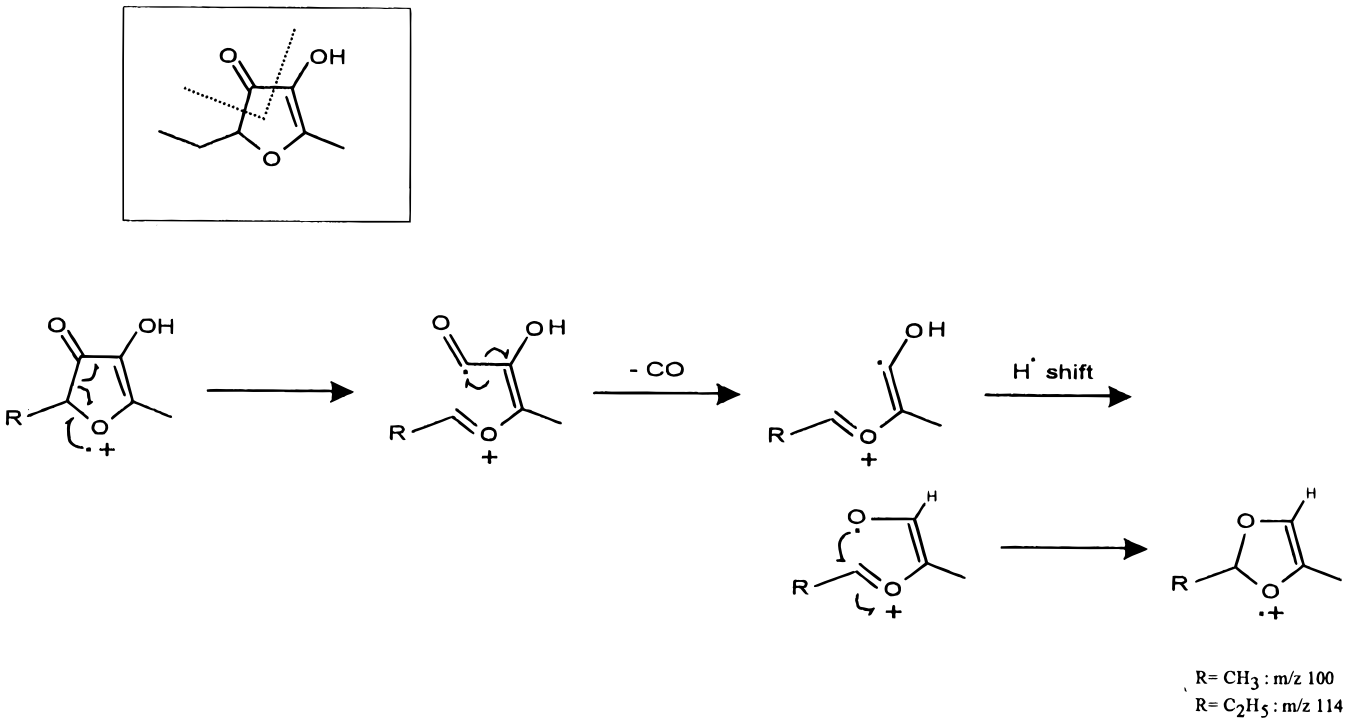


Figure 5. Fragmentation of the molecular ions of the reference compounds Furaneol and homofuraneol by GC/MS/MS under CID conditions and proposed fragmentation pathways leading to the loss of CO from the 3(2*H*)-furanone cycle. Fishhook corresponds to the transfer of a single electron (McLafferty and Turecek, 1993).

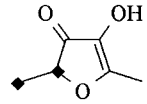
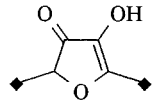
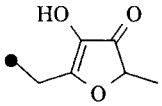
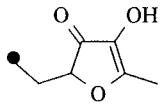
be explained by greater stability of the ion **(I)** that carries the radical on the carbon attached to the ethyl group compared to the ion **(I′)** in which the radical is located on the carbon attached to the methyl group (Figure 1). Tautomer **2B** may also be more susceptible to McLafferty rearrangement (McLafferty, 1959) due to the proximity of the ethyl group on C_2 and the ketone on the adjacent C_3 atom.

Formation of the low intensity ion at *m*/*z* 72 (**VIII**) detected in the mass spectrum of homofuraneol can be explained by subsequent breakdown of the bonds O−C_2 and C_3−C_4, thus liberating ethyl ketene (CH_3−CH_2−CH=C=O) as neutral molecule (Figure 4). This mechanism might also contribute to formation of the ion at *m*/*z* 72 in the mass spectrum of Furaneol by splitting off methyl ketene (CH_3−CH=C=O), in addition to the fragmentation pathway described in Figure 2. Therefore, similar to *m*/*z* 43, 57, and 85, two distinct ion species with *m*/*z* 72 can occur in the mass spectra of Furaneol, i.e. **Vb** and **VIII**.

Formation of the fragment $[M - CO]^+$ found in the spectra of Furaneol and homofuraneol at *m*/*z* 110 and 114, respectively, is described in Figure 5. Loss of H_2O occurs for norfuraneol and Furaneol, resulting in ions at *m*/*z* 96 and 110, respectively. This type of fragmentation was less favored in the case of homofuraneol.

Fragmentation of Labeled 3(*2H*)-Furanones Obtained by Synthesis. Synthesized labeled 3(*2H*)-

Table 2. Characteristic Ions in the Mass Spectra of the Synthesized Compounds*

Compound Structure	Compound Name	Compound Number	Main daughter ions of the molecular ion *m/z* (relative intensity)
	4-Hydroxy-2(or 5)-[^{13}C]methyl-5(or 2)-methyl-3(2*H*)-[2(or 5)-^{13}C]furanone	1a	**130(100)**, **112(35)**, **102(10)**, **87(80)**, 85(90), **74(90)**, **59(5)**, 57(5), **45(15)**, 43(10)
	4-Hydroxy-2,5-di-[^{13}C]methyl-3(2*H*)-furanone[a]	1b	**130(90)**, **86(30)**, **74(5)**, **58(75)**, **56(15)**, **44(100)**
	5-([2,2,2-^{2}H$_3$]Eth-1-yl)-4-hydroxy-2-methyl-3(2*H*)-furanone (Homofuraneol A)	2aA	**145(100)**, 127(<1), **115(<1)**, **102(5)**, **89(5)**, **87(15)**, **74(5)**, 72(10), **60(90)**, 57(50), 43(5)
	2-([2,2,2-^{2}H$_3$]Eth-1-yl)-4-hydroxy-5-methyl-3(2*H*)-furanone (Homofuraneol B)	2aB	**145(100)**, 127(45), **115(10)**, **102(40)**, **89(5)**, **87(5)**, **74(80)**, 72(15), 57(10), 55(35), 43(100)

*Compound **1a** was analyzed by GC/MS/MS (fragmentation under CID conditions of the molecular ion), whereas compounds **1b**, **2aA**, and **2aB** were analyzed by GC/MS after electron impact ionization at 70 eV. Isotopically labeled ions are marked with bold characters. Symbols ◆ represent the ^{13}C-labeled atoms and symbols ● the C atoms labeled with ^{2}H. [a] These GC/MS data were published by Sen et al. (1991).

furanones were analyzed by GC/MS and GC/MS/MS using the same conditions as reported for the corresponding unlabeled compounds. The results are presented in Table 2. Doubly labeled 4-hydroxy-2(or 5)-[^{13}C]methyl-5(or 2)-methyl-3(2*H*)-[2(or 5)-^{13}C]furanone (**1a**) showed a molecular ion at *m/z* 130. As labeling was not symmetrical, opening of the bonds O−C$_2$ (or O−C$_5$) and C$_4$−C$_5$ (or C$_2$−C$_3$) (Figure 1) gave rise to doubly labeled fragments at *m/z* 87 and 59 associated with unlabeled fragments at *m/z* 85 and 57 with similar relative intensities. In agreement with that, nonsymmetrical labeling allowed the formation of a doubly labeled acyl ion at *m/z* 45 associated with its unlabeled equivalent at *m/z* 43. As the labeling was not located on the C$_3$ or C$_4$ atom, the fragment corresponding to the breakdown of the C$_2$−C$_3$ and C$_4$−C$_5$ bonds was a doubly labeled ion found at *m/z* 74. The fragment at *m/z* 102 indicated loss of CO located at C$_3$ and/or C$_4$ (see Figure 5).

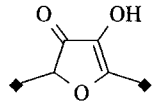
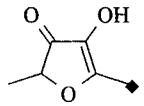
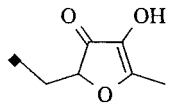
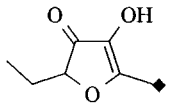
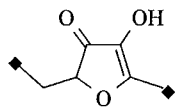
Doubly labeled 4-hydroxy-2,5-di[^{13}C$_2$]methyl-3(2*H*)-furanone (**1b**) showed a molecular ion at *m/z* 130. Because of the symmetry of the molecule, the fragments at *m/z* 86 and 58, corresponding to opening of the O−C$_2$ (or O−C$_5$) and C$_4$−C$_5$ (or C$_2$−C$_3$) bonds (for explanation see Figure 1), were singly labeled and not associated with the corresponding unlabeled ions. For the same symmetry reasons, the loss of an acyl ion led to a singly labeled fragment at *m/z* 44; hence, the fragment at *m/z* 43 did not appear in the mass spectrum. On the other hand, breakdown of C$_2$−C$_3$ and C$_4$−C$_5$ bonds allowed formation of the doubly charged ion at *m/z* 74 (Figure 2).

The GC/MS fragmentation pattern of the homofuraneol tautomers 2(or 5)-([2,2,2-^{2}H$_3$]eth-1-yl)-4-hydroxy-5(or 2)-methyl-3(2H)-furanone (**2aA** and **2aB**) was also in good agreement with the proposed schemes. The fragment at *m/z* 115, bearing one deuterium atom, helped to confirm the neutral loss of C$_2$H$_2$D$_2$ formed by a McLafferty rearrangement (McLafferty, 1959) of the molecular ion at *m/z* 145, particularly in **2aB** (Figure 3). The fragment at *m/z* 127 formed by α-cleavage indicated that the methyl group was exclusively released from the ethyl moiety (Figure 3). The labeled fragments at *m/z* 102, 89, and 74 correspond to the signals at *m/z* 99, 86, and 71 of **2A**/**2B** (Figures 1 and 2), respectively. The presence of ions at *m/z* 72 and 43 is in agreement with the labeling position in **2aA**/**2aB** (Figures 4 and 1, respectively). The presence of both *m/z* 57 and 60, particularly in the mass spectra of tautomer **2aA**, was compatible with the assumption about two ion species for *m/z* 57 shown in Figure 1, i.e. **IIc** and **IVb′**. In summary, MS data of labeled 3(2*H*)-furanones obtained by synthesis confirmed the fragmentation pathways previously presented.

Fragmentation of Labeled 3(*2H*)-Furanones Formed through the Maillard Reaction. As previously reported, furanones **1** and **2** can be generated from pentose sugars in the presence of glycine and L-alanine. The key step is the incorporation of the Strecker aldehyde into the pentose moiety (Blank and Fay, 1996). The proposed reaction mechanism was corroborated in model experiments by reacting labeled or unlabeled D-xylose (Xyl) with labeled or unlabeled glycine (Gly) or L-alanine (Ala) (Blank et al., 1996). In the following, fragmentation of the differently labeled compounds obtained in such model experiments will be discussed in more detail as related to structure elucidation (Table 3). In the case of homofuraneol (**2**), only the second tautomer will be considered because of the higher abundance of the ions. Differences between the mass spectra of both tautomers are mainly due to differences in relative intensities of the fragments.

The mass spectrum of 4-hydroxy-2,5-di[^{13}C]methyl-3(2*H*)-furanone (**1b**) obtained in the model system [1-^{13}C]Xyl/[2-^{13}C]Gly was very close to that of the synthesized compound (see Table 2).

Table 3. Fragmentation under CID Conditions of the Molecular Ions of the Labeled 3(2*H*)-Furanones Formed through the Maillard Reaction Based on D-Xylose (Xyl), Glycine (Gly), and L-Alanine (Ala)[a]

Compound Structure	Compound Name	Compound Number	Precursors	Main daughter ions of the molecular ion *m/z* (relative intensity)
	4-Hydroxy-2,5-di-[^{13}C]methyl-3(2*H*)-furanone	**1b**	[1-^{13}C]Xyl + [2-^{13}C]Gly	**130(25), 86(45), 74(80), 58(40), 44(100)**
	4-Hydroxy-2(or 5)-[^{13}C]methyl-5(or 2)-methyl-3(2*H*)-furanone	**1c**	Xyl+[2-^{13}C]Gly and [1-^{13}C]Xyl+Gly	**129(100), 111(10), 86(60)**, 85(60), **73(30), 58(15)**, 57(20), **44(20)**, 43(35)
	2-([2-^{13}C]Eth-1-yl)-4-hydroxy-5-methyl-3(2*H*)-furanone	**2bB**	Xyl + [3-^{13}C]Ala	**143(65)**, 127(100), 114(35), **100(65), 87(10), 72(25), 58(30)**, 43(10)
	2-Ethyl-4-hydroxy-5-[^{13}C]methyl-3(2*H*)-furanone	**2cB**	[1-^{13}C]Xyl + Ala	**143(45), 128(40), 115(25)**, 99(45), **87(10), 73(30)**, 71(50), 57(100), **44(45)**, 43(15)
	2-([2-^{13}C]Eth-1-yl)-4-hydroxy-5-[^{13}C]methyl-3(2*H*)-furanone	**2dB**	[1-^{13}C]Xyl + [3-^{13}C]Ala	**144(40), 128(35), 115(25), 100(40), 88(10), 73(30)**, ***72(45)***, **58(100), 44(55)**

[a] Isotopically labeled ions are marked with bold characters.

Fragments of 4-hydroxy-2(or 5)-[^{13}C]methyl-5(or 2)-methyl-3(2*H*)-furanone (**1c**) formed in Xyl/[2-^{13}C]Gly and [1-^{13}C]Xyl/Gly are presented in Table 3. The labeling position in one of the two methyl groups was clearly indicated by the fragment pairs at *m/z* 85/86, 57/58, and 43/44.

Localization of the labeling position in 2-([2-^{13}C]eth-1-yl)-4-hydroxy-2-methyl-3(2*H*)-furanone (**2B**) formed in Xyl/[3-^{13}C]Ala was established on the basis of Figure 3. The fragments at *m/z* 127 and 114 indicated the loss of labeled $^{13}CH_3$ and $^{13}CH_2{=}CH_2$ from the molecular ion at *m/z* 143, respectively, and so suggested labeling of the methyl group in the ethyl moiety. The remaining fragments were consistent with the proposed fragmentation pathways.

In the mass spectrum of 2-ethyl-4-hydroxy-5-[^{13}C]-methyl-3(2*H*)-furanone (**2cB**) detected in [1-^{13}C]Xyl/Ala, the ions at *m/z* 71 and 73 allowed the labeling position to be located in the methyl group. The labeled ion at *m/z* 73 corresponded to the low-intensity ion at *m/z* 72 in the mass spectra of unlabeled homofuraneol (Table 1), which arose from subsequent breakdown of the C_3-C_4 and $O-C_2$ bonds (Figure 4). It was also clearly visible in the mass spectrum of compound **2aB** (Table 2). In addition, the fragment at *m/z* 44 ($[^{13}CH_3C{\equiv}O]^+$) was a further indication of the presence of a labeled methyl group attached to the ring. The remaining fragments were in agreement with the proposed structure. The low-intensity ion found at *m/z* 43 indicates subsequent fragmentation of the ion $[M - CH_3]^+$ at *m/z* 127 (ion **VII**) by cleavage of the bonds C_2-C_3 and $O-C_5$ (see Figure 3).

The molecular ion at *m/z* 144 and the fragment at *m/z* 88 (see Figure 2) in the mass spectrum of 2-([2-^{13}C]eth-1-yl)-4-hydroxy-5-[^{13}C]methyl-3(2*H*)-furanone (**2dB**) obtained by reacting [1-^{13}C]Xyl with [3-^{13}C]Ala revealed doubly labeled homofuraneol. The singly labeled fragments at *m/z* 128 and 115 suggested one labeling position in the methyl group of the ethyl moiety (see Figure 3). This was in agreement with the fragments at *m/z* 100, 72, and 58 (see Figure 1). On the other hand, the fragments at *m/z* 73 (see Figure 4) and *m/z* 44 indicated that the second labeling position was in the methyl group attached to the ring.

Conclusions. The fragmentation pathway of the 3(2*H*)-furanones norfuraneol, Furaneol, and homofuraneol was elucidated on the basis of isotopically labeled synthesized reference compounds. This knowledge was successfully applied to localize labeling atoms in 3(2*H*)-furanones generated through the Maillard reaction based on labeled precursors. The origin of the fragments detected in the mass spectra of various isotopomers could be explained. The structures obtained were consistent with the formation mechanisms proposed earlier (Blank and Fay, 1996). This paper indicates the importance of a more precise knowledge about fragmentation patterns for mass spectrometric structure elucidation of reaction products and for formulation of formation mechanisms.

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