Effect of pH on the Maillard Reaction of [^{13}C$_5$]Xylose, Cysteine, and Thiamin

Christoph Cerny* and Matthieu Briffod

Corporate R&D Division, Firmenich SA, P.O. Box 148, CH-1217 Meyrin, Geneva 2, Switzerland

The influence of different pH values, ranging from 4.0 to 7.0, on the formation of sulfur volatiles in the Maillard reaction was studied using a model system with [^{13}C$_5$]xylose, cysteine, and thiamin. The use of ^{13}C-labeled xylose allowed, by analysis of the mass spectra, volatiles that incorporated xylose carbons in the molecule from other carbon sources to be discerned. For 2-furaldehyde and 2-furfurylthiol, which were favored at low pH, the labeling experiments clearly indicated that xylose was the exclusive carbon source. On the other hand, xylose was virtually not involved in the formation of 3-mercapto-2-butanone, 4,5-dihydro-2-methyl-3-furanthiol, and 5-(2-hydroxyethyl)-4-methylthiazole, which apparently stemmed from thiamin degradation. Both xylose and thiamin seemed to significantly contribute to the formation of 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, and 2-mercapto-3-pentanone, and therefore different formation pathways must exist for each of these molecules. In general, the pH determined strongly which volatiles were formed, and to what extent. However, the relative contribution of xylose to the C-skeleton of a particular compound changed only slightly within the investigated pH range, when both xylose and thiamin were involved in the formation.

INTRODUCTION

The Maillard reaction is the main pathway for aroma generation when meat is cooked. Capitalizing on this fact, the flavor industry also utilizes the Maillard reaction for the production of thermally generated flavorings, the so-called process or reaction flavors (*1*). Cysteine and thiamin are two important ingredients for meat-like reaction flavors as they act as important aroma precursors for meat aroma, in particular for sulfur-containing odorants (*2*). Gas chromatography−olfactometry and aroma extract dilution analysis (AEDA) have detected 3-mercapto-2-pentanone, 2-methyl-3-furanthiol, 4,5-dihydro-2-methyl-3-furanthiol, bis(2-methyl-3-furyl) disulfide, 5-(2-acetoxyethyl)-4-methylthiazole, 5-(2-hydroxyethyl)-4-methylthiazole, and others as key odorants in heated thiamin solutions (*3*, *4*). 2-Methyl-3-furanthiol, its disulfide bis(2-methyl-3-furyl) disulfide, and 3-mercapto-2-pentanone were also found to be important aroma compounds in thermally treated solutions of cysteine and ribose (*3*, *5*), together with 2-furfurylthiol, 2-methyltetrahydrothiophen-3-one, 3-mercapto-2-butanone, 5-acetyl-2,3-dihydro-1,4-thiazine, 2-acetyl-2-thiazoline, 2-thenylthiol, and ethyl mercaptan.

When both thiamin and cysteine/pentose are present as precursors, for example, when meat is boiled or a process flavor is prepared (*6*), the question arises concerning the relative contribution of each of the precursor systems to the formation

of aroma compounds such as 2-methyl-3-furanthiol. Grosch and Zeiler-Hilgart (*7*) found that the formation of 2-methyl-3-furanthiol from cysteine/ribose drastically increased in model experiments when thiamin was present. The authors used very low concentrations of the reactants in order to approach the conditions in meat. They concluded that thiamin was more efficient in generating 2-methyl-3-furanthiol than cysteine/ribose under these conditions. Bolton and co-workers used [^{34}S]cysteine in a model system comprising, in addition, thiamin, xylose, and other ingredients, to determine how much sulfur in the 2-methyl-3-furanthiol stemmed from cysteine (*8*). The reactant concentrations were between 0.2 and 1.1% and simulated process flavor conditions. Only a low amount of [^{34}S]-2-methyl-3-furanthiol (8%) was found, indicating that cysteine contributed to only a small extent to its formation.

In a recent study (*9*), we heated a solution of [^{13}C$_5$]xylose, cysteine, and thiamin (molar ratio 3:1:1) in phosphate buffer (pH 5.0) at 145 °C for 20 min. Analysis of the mass spectra revealed that 2-furaldehyde and 2-furfurylthiol were exclusively ^{13}C$_5$-labeled, indicating that the carbon atoms stem from [^{13}C$_5$]-xylose. On the other hand, other volatiles such as 3-mercapto-2-butanone, 4,5-dihydro-2-methyl-3(2*H*)-furanone, and 4,5-dihydro-2-methyl-3-furanthiol were found to be virtually unlabeled, suggesting their origin was thiamin. Finally a balanced mixture of unlabeled and ^{13}C$_5$-labeled 2-methyl-3-furanthiol and 3-mercapto-2-pentanone, respectively, was formed, indicating that thiamin and xylose were equally important as carbon source. If cysteine was omitted from the reaction, all

* Author to whom correspondence should be addressed (telephone +41 22 780 22 16; fax +41 22 780 27 35; e-mail christoph.cerny@firmenich.com).

Maillard Reaction of [^{13}C$_5$]Xylose, Cysteine, and Thiamin and pH Effect

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Table 1. Model Reactions[a]

	A	B	C	D	E	F	G	H	I
pH	4.00	5.00	6.00	7.00	4.00	5.00	5.50	6.00	7.00
cysteine[b]	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
[^{13}C$_5$]xylose[b]	22.50	22.50	22.50	22.50					
xylose[b]					22.50	22.50	22.50	22.50	22.50
thiamin hydrochloride[b]	16.85	16.85	16.85	16.85	16.85	16.85	16.85	16.85	16.85

[a] Reaction in phosphate buffer (0.5 mol/L; 1.00 mL) at 145 °C (20 min). [b] Amount (mg).

Table 2. Influence of the pH on the Formation of Sulfur Volatiles from Xylose, Cysteine, and Thiamin (GC-TIC Peak Areas $\times$ 10^6)

no.	compound[a]	RI[b]	pH 4.0	pH 5.0	pH 5.5	pH 6.0	pH 7.0
1	4,5-dihydro-2-methyl-3(2H)-furanone	800	17	18	18	94	104
2	3-mercapto-2-butanone	807	69	81	83	59	73
3	2-furaldehyde	831	208	158	165	0	0
4	2-methyl-3-furanthiol	871	415	336	371	539	391
5	3-mercapto-2-pentanone	901	145	141	147	62	12
6	2-mercapto-3-pentanone[c]	911	0	0	0	19	5
7	2-furfurylthiol	915	431	368	364	185	0
8	4,5-dihydro-2-methyl-3-furanthiol[c]	939	226	43	41	18	7
9	2-methyl-3-(methylthio)furan	950	14	12	11	11	0
10	4,5-dihydro-2-methyl-3(2H)-thiophenone	988	0	0	0	372	470
11	3-methyl-1,2-dithian-4-one[c]	1162	0	0	0	75	82
12	3-acetyl-1,2-dithiolane[c]	1201	0	0	0	117	240
13	5-(2-hydroxyethyl)-4-methylthiazole	1261	525	54	43	161	80

[a] If not indicated otherwise, compounds were identified in the corresponding reaction between xylose, cysteine, and thiamin by comparing the mass spectra and retention indices with those of authentic reference compounds. [b] Retention index on HP-5MS. [c] The compound was tentatively identified by comparing the mass spectra and retention index with literature data (*11–14*).

2-methyl-3-furanthiol and 3-mercapto-2-pentanone were found to be unlabeled, showing that xylose was an effective precursor for these compounds only when cysteine was present.

The present study is designed to elucidate the influence of the pH on the reaction between thiamin, cysteine, and [^{13}C$_5$]-xylose. The pH value was varied between 4.0 and 7.0, peaks of selected volatiles were integrated in the total ion chromatogram (TIC) obtained from GC-MS, and the corresponding mass spectra were analyzed to assess the relative contribution of thiamin to their formation.

MATERIALS AND METHODS

Chemicals. Chemicals were of analytical grade. L-Cysteine, xylose, dipotassium hydrogenphosphate and potassium dihydrogenphosphate were from Fluka (Buchs, Switzerland), [^{13}C$_5$]xylose (99% enrichment) was from Cambridge Isotope Laboratories (Andover, MA), and thiamin hydrochloride (aroma grade) was from Firmenich.

Reactions. The reagents in **Table 1** were dissolved in 1.00 mL of potassium phosphate buffer (0.5 mol/L). Different buffers with pH values from 4.0 to 7.0 were used. The mixtures were heated while stirring in Teflon vials (Infochroma, Zug, Switzerland) in a heated metal block (Reactitherm, stirring/heating module, Pierce Chemical Co., Rockford, IL) for 20 min at 145 °C. Trials with unlabeled xylose (E–I) were carried out in triplicate.

Analysis. All samples were analyzed by headspace solid-phase microextraction in tandem with gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS) as previously described (*10*). Absorption was carried out at 40 °C for 35 min. The oven temperature was 40 °C during 5 min, then raised by 5 °C/min to 260 °C and finally by 15 °C/min to 280 °C. The isotopomer ratios in the trials with [^{13}C$_5$]-xylose were calculated using the relative signal intensities of the analyzed ions (*m/z*) in the mass spectrum of the respective compound. Peak areas were integrated using the TIC signals from the experiments with unlabeled xylose and expressed as mean values from triplicates. The standard deviation did not exceed 23%.
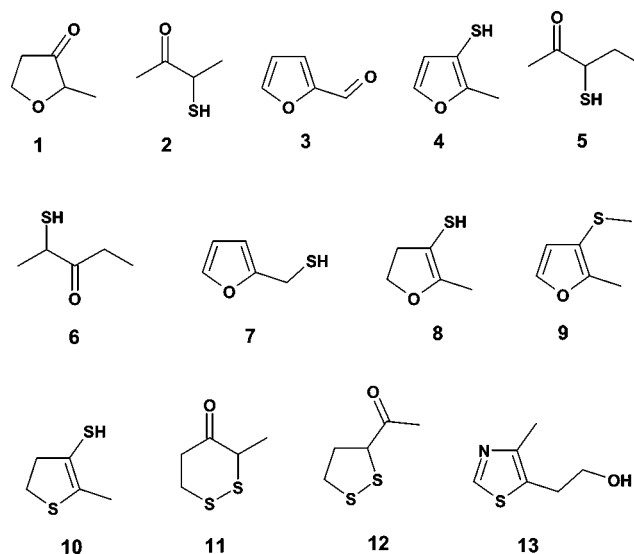

Figure 1. Identified volatiles from the reactions of xylose, cysteine, and thiamin.

RESULTS AND DISCUSSION

Small-scale reactions between cysteine (50 μmol), thiamin (50 μmol), and xylose (unlabeled and ^{13}C$_5$-labeled, respectively; 150 μmol) were effected in phosphate buffer at 145 °C for 20 min. The pH value for the different experiments varied between 4.0 and 7.0 (**Table 1**). The analysis was carried out after transfer of the reacted solutions to 20-mL headspace vials, by SPME-GC-MS at 40 °C. The identified volatiles in **Table 2** include the major sulfur-containing compounds in the gas chromatogram. Their chemical structures are shown in **Figure 1**. The identification is based on the comparison of the mass spectrum and the retention index with reference compounds. No commercial reference compounds exist for 2-mercapto-3-pentanone

Table 3. Isotope Ratios (Percent) of Sulfur Volatiles Formed from [^{13}C$_5$]Xylose, Cysteine, and Thiamin, Reacted at Different pH Values

m/z	[^{12}C$_5$] a	pH 4	pH 5	pH 6	pH 7	m/z	[^{12}C$_5$] a	pH 4	pH 5	pH 6	pH 7
		2-methyl-3-furanthiol (**4**)						3-mercapto-2-pentanone (**5**)			
112	0.6	0.5	0.6	0.5	<0.1	116	0.5	1.0	1.3	3.1	2.1
113	17.8	13.3	15.1	16.1	14.2	117	<0.1	<0.1	<0.1	<0.1	<0.1
114	**72.4**	**53.6**	**61.0**	**65.3**	**57.7**	**118**	**89.1**	**67.1**	**75.9**	**75.5**	**76.0**
115	5.4	3.9	4.5	4.7	4.2	119	5.6	4.6	4.9	6.5	4.6
116	3.6	2.7	3.1	3.2	2.9	120	4.3	3.4	3.9	4.0	4.3
117	0.2	0.7	0.5	0.4	0.5	121	0.3	0.6	0.4	1.0	0.5
118	<0.1	5.8	3.5	2.3	4.7	122	<0.1	1.4	0.8	0.7	1.3
119	**<0.1**	**18.2**	**10.8**	**7.0**	**14.7**	**123**	**<0.1**	**20.4**	**12.1**	**8.2**	**10.2**
120	<0.1	0.5	0.3	0.2	0.4	125	<0.1	0.3	0.2	0.4	0.2
121	<0.1	0.8	0.6	0.3	0.7	126	<0.1	1.2	0.5	0.6	0.8
		4,5-dihydro-2-methyl-3-furanthiol (**8**)						5-(2-hydroxyethyl)-4-methylthiazole (**13**)			
114	0.7	1.4	1.3	1.3	na b	141	0.2	0.4	0.3	0.2	<0.1
115	6.1	6.1	6.0	6.3	na	142	<0.1	<0.1	<0.1	<0.1	<0.1
116	**83.3**	**82.4**	**82.4**	**83.0**	**na**	**143**	**91.3**	**88.3**	**88.5**	**87.6**	**88.4**
117	5.6	5.8	5.9	5.3	na	144	5.0	6.6	6.7	6.9	6.7
118	4.1	3.8	3.9	3.7	na	145	3.3	4.2	4.3	4.6	4.4
119	0.3	0.5	0.5	0.4	na	146	0.2	0.3	0.2	0.5	0.3
120	<0.1	<0.1	<0.1	<0.1	na	147	<0.1	0.2	<0.1	<0.1	<0.1
121	<0.1	<0.1	<0.1	<0.1	na	148	<0.1	<0.1	<0.1	0.2	0.2
122	<0.1	<0.1	<0.1	<0.1	na	149	<0.1	<0.1	<0.1	<0.1	<0.1
123	<0.1	<0.1	<0.1	<0.1	na	150	<0.1	<0.1	<0.1	<0.1	<0.1
						151	<0.1	<0.1	<0.1	<0.1	<0.1

[a] Isotope ratio (percent) for the unlabeled compound from the reaction with unlabeled xylose at pH 4. [b] Not analyzed.

(**6**), 4,5-dihydro-2-methyl-3-furanthiol (**8**), 3-methyl-1,2-dithian-4-one (**11**), and 3-acetyl-1,2-dithiolane (**12**). Their mass spectra and retention indices were compared only with literature data (*11−14*), and thus the identification is only tentative.

The data in **Table 2** represent integrated peak areas and are not corrected by MS response factors or taking into account the different partition coefficients of volatiles between the sample matrix and the SPME fiber. Also, for certain compounds, the pH might have an effect on the partition coefficient. Nonetheless, **Table 2** illustrates to a certain extent the influence of the pH on the formation of the respective volatiles. Compounds **6**, **11**, **12**, and 4,5-dihydro-2-methyl-3(2*H*)-thiophene (**10**) were detected only when the reaction was carried out at pH 6.0 and 7.0, and not under more acidic conditions; the formation of 4,5-dihydro-2-methyl-3(2*H*)-furanone (**1**) was also favored at higher pH values. On the other hand, 2-furaldehyde (**3**), 2-furfurylthiol (**7**), and 2-methyl-3(methylthio)furan (**9**) were formed only at acidic pH, and no traceable amounts were detected at pH 7.0. Peak areas decreased with increasing pH value for **8** and 3-mercapto-2-pentanone (**5**). The pH had no obvious influence on the formation of 3-mercapto-2-butanone (**2**), 2-methyl-3-furanthiol (**4**), and 5-(2-hydroxyethyl)-4-methylthiazole (**13**).

The mass spectra of the compounds from [^{13}C$_5$]xylose, cysteine, and thiamin were analyzed on the basis of the mass-to-charge ratios (*m/z*) of the molecular ions of the isotopomers. **Table 3** compares the isotope ratios of **4**, **5**, **7**, **8**, and **13**. No other compounds coeluted with them under the chromatographic conditions of the study, thus allowing an unequivocal analysis of their mass spectra. They were either unlabeled, fully ^{13}C-labeled, or a mixture of unlabeled and fully labeled isotopomers. Other isotopomers, singly, doubly, triply, or quadruply ^{13}C-labeled, were virtually absent, indicating that the molecules did not consist of a mixture of labeled and unlabeled carbon fragments. The carbon skeletons of **8** and **13** were unlabeled (*m/z* 116 and 143, respectively) and hence derived not from [^{13}C$_5$]xylose, but rather from thiamin, because both volatiles are known thiamin degradation products (*15, 16*). On the other hand, **7** was exclusively 5 times labeled (*m/z* 119), clearly

demonstrating its origin from xylose. Compounds **4** and **5** consisted of a mixture of ^{13}C$_5$-labeled and unlabeled compounds with a lower share of the labeled isomer ([^{13}C$_5$]**4**, 10−25%; [^{13}C$_5$]**5**, 10−23%). According to their mass spectra, the other volatiles in **Table 2** revealed also either unlabeled, fully ^{13}C-labeled, or a mix of nonlabeled and fully labeled isotopomers for the reactions at pH 4.0−7.0 (**Table 4**). The thio ether **9** was an exception, because it was composed of four isotope molecules with molecular masses of 128, 129, 133, and 134, indicating that both the 2-methyl-3-furyl and the thiomethyl fragment can be either nonlabeled or fully ^{13}C-labeled, resulting in four possible combinations. The unlabeled 2-methyl-3-furyl part supposedly stems from thiamin, whereas the unlabeled thiomethyl fragment probably originates from cysteine degradation. Cysteine can be considered as a precursor for the thiomethyl group of **9**, because in the reaction product between [^{13}C$_5$]xylose and cysteine about 36% of **9** was found to be 5 times labeled, highlighting the origin of the unlabeled methyl carbon from cysteine (*9*). The pH during the reaction had no major influence on the isotopomer distribution of the volatiles (**Table 4**), in contrast to the strong effect on the pattern of volatiles and the pH-dependent formation of most compounds (**Table 2**).

Few studies have systematically investigated the influence of the pH value on the formation of volatiles from thiamin degradation and/or Maillard reaction between cysteine and pentoses. Güntert and co-workers (*17*) have heated thiamin solutions at different pH values at 130 °C for 6 h. The main compounds at pH 1.5 were 1-methyl-2,4-dithia-8-oxabicyclo-[3.3.0]octane, 2-methyl-3(2*H*)furanone, **1**, **8**, 1-methyl-2,8-dioxa-4-thiabicyclo[3.3.0]octane, and 4-acetyl-5-methyl-1,3-dithiolane. The highest yields at neutral pH 7.0 were obtained for **13**, its formate and acetate esters, **1**, 1-methyl-2,8-dioxa-4-thiabicyclo-[3.3.0]octane, and **4**. The treatment under basic conditions produced the most complex mixture, and also the highest yield of volatiles. The most prominent ones were **10**, 4,5-dimethylthiazole, 2-methyl-2-hydroxytetrahydrothiophene, (*E/Z*)-3-penten-1-ol, 4,5-dihydro-2-methylthiophene, 3-hydroxy-2-pentanone, and 2-methyl-3-thiophenethiol. The authors remarked that elemental sulfur and the generation of relatively high

Table 4. Proportion of Isotopomers Formed from [^{13}C$_5$]Xylose, Cysteine, and Thiamin at pH 4–7

no.	compound	m/z (analyzed ions)	pH 4.0 [^{12}C]^a (%)	[^{13}C]^b (%)	pH 5.0 [^{12}C] (%)	[^{13}C] (%)	pH 6.0 [^{12}C] (%)	[^{13}C] (%)	pH 7.0 [^{12}C] (%)	[^{13}C] (%)	labeled carbon atoms
1	4,5-dihydro-2-methyl-3(2*H*)-furanone	100; 105	>99	<1	>99	<1	>99	<1	>99	<1	5
2	3-mercapto-2-butanone	104; 108	>95	<5	>98	<2	>98	<2	>95	<5	4
3	2-furaldehyde	96; 101	<1	>99	<1	>99					5
4	2-methyl-3-furanthiol	114; 119	75	25	85	15	90	10	80	20	5
5	3-mercapto-2-pentanone	118; 123	77	23	86	14	90	10	88	12	5
6	2-mercapto-3-pentanone	118; 123					6	94	<5	>95	5
7	2-furfurylthiol	114; 119	<1	>99	<1	>99	<1	>99			5
8	4,5-dihydro-2-methyl-3-furanthiol	116; 121	>99	<1	>99	<1	>99	<1	>99	<1	5
9	2-methyl-3-(methylthio)furan	128; 129; 133; 134	35	3; 8; 54	39	3; 8; 50	40	2; 9; 49			1; 5; 6
10	4,5-dihydro-2-methyl-3(2*H*)-thiophenone	116; 121					>99	<1	>98	<2	5
11	3-methyl-1,2-dithian-4-one	148; 153					91	9	92	8	5
12	3-acetyl-1,2-dithiolane	148; 153					>99	<1	>99	<1	5
13	5-(2-hydroxyethyl)-4-methylthiazole	143; 149	>99	<1	>99	<1	>99	<1	>99	<1	6

^{a} Unlabeled carbon atoms (percent). ^{b} Labeled carbon atoms.

amounts of hydrogen sulfide played a more important role under alkaline conditions and that more thiophenes and no furans were formed. They also speculated that, in reaction mixtures containing xylose, cysteine, and thiamin, some of the compounds are probably formed via two different pathways. Our results can confirm this assumption, because the different isotopomers of **4**, **5**, **6**, **9**, and **11** indicate different formation pathways with and without involving xylose as precursor. In the present study, the thiophene **10**, as well as the two compounds **11** and **12**, both bearing two sulfur atoms in the ring, were formed only at the higher pH values of 6.0 and 7.0. This finding is in agreement with the stronger involvement of hydrogen sulfide at higher pH, and a formation pathway involving its reaction with 5-hydroxy-3-mercapto-2-pentanone (*17*), which is generally accepted as a key intermediate of thiamin degradation (*16*).

Dwivedi and Arnold (*18*) heated solutions of radiolabeled [^{35}S]thiamin in phosphate buffer ranging from pH 3.5 to 8.0 and analyzed the TLC radiochromatograms. At pH 6.0 or below, the principal sulfur compound from thiamin degradation was **13**, whereas at pH 7.0 and 8.0 other sulfur compounds dominated, and hydrogen sulfide appeared to be the main volatile (*18*, *19*). In our study **13** was the major peak in the TIC for the reaction at pH 4.0. At pH 5.0–7.0 it generated a major peak, but was not dominant.

Shu and co-workers (*20*) studied the degradation of cysteine in aqueous solution at pH 2.2, 5.1, and 7.1. Strong formation of volatiles occurred at pH 5.1, but only a low yield was obtained at pH 2.2 and even less at 7.1. The main volatile at pH 2.2 was 1,2,3-trithia-5-cycloheptene; at pH 5.1 acetone and 3,5-dimethyl-1,2,4-trithiolane were formed; and at pH 7.1, 3,5-dimethyl-1,2,4-trithiolane was the main volatile. None of the compounds that have been found by Shu and co-workers were identified in the present study, but Maillard reaction products and thiamin degradation products dominated (**Table 2**).

Meynier and Mottram (*21*) investigated the model reaction between cysteine and ribose at pH 4.5 to 6.5. Seven of 20 identified compounds, namely, **1**, **3**, **4**, **5**, **7**, **10**, and **11**, have also been found in the present work. The authors observed that the concentrations were pH-dependent and that the quantities of **4**, **5**, and **7** were highest at pH 4.5 and decreased with increasing pH. For the dithiane derivative **11** the opposite effect was observed with slightly increasing quantities at higher pH values. Our results show a similar trend for **5** and **7**, whereas the peak areas for **4** remained relatively constant over the pH range from 4.0 to 7.0. However, in our study thiamin was an additional reagent with xylose and cysteine. We could not detect

11 at pH 4–5.5, but only at pH 6.0 and 7.0. We suggest that, in the presence of thiamin, the formation pathway from xylose and cysteine to **4**, **5**, and **11** played only a minor role under the experimental conditions, because the majority (75–92%) of these molecules were found to be unlabeled and likely arose from thiamin degradation.

The formation of the α-mercaptocarbonyl compounds **5** and **6** appears to follow different pathways. Whereas **5** was formed throughout the whole pH range, **6** was formed only at pH 6 and 7. The majority of **5** was found to be unlabeled (**Table 3**), and consequently thiamin appears to be the main precursor under the experimental conditions, whereas **6** was >94% ^{13}C-labeled and thus originated mainly from xylose. Previous studies on the reaction between ribose and cysteine revealed both **5** and **6** as volatile products at pH 5.0 (*22*) and pH 5.6 (*23*), respectively, with predominantly **5**. Both mercaptoketones have been previously found in reactions between 2,3-pentanedione and hydrogen sulfide (*14*, *24*). Another study detected only **5** in the reaction product between xylose and cysteine (*25*), and consequently a different formation pathway via 5-hydroxy-2,3-pentanedione as intermediate and hydrogen sulfide was proposed, as the formation of **5** via 2,3-pentanedione and hydrogen sulfide without the concurrent generation of **6** appeared to be unlikely.

Not only do the pH, reaction temperature, and time have an influence on the reaction pathways to **4** and **5**, but also the concentration of the precursor molecules may play a crucial role. In a previous study (*9*) the reaction between [^{13}C$_5$]xylose, cysteine, and thiamin at pH 5.0 was carried out at exactly the same reaction conditions, but with twice the precursor concentration as compared to the present study. Inspection of the results (**Table 5**) reveals that a higher precursor concentration (**A**) affords a higher proportion (54 and 60%, respectively) of ^{13}C-labeled **4** and **5**, indicating that xylose is the predominant precursor. At lower precursor concentration (**B**), the unlabeled molecules dominate and xylose becomes less important. Zeiler (*26*) showed that the degradation of a thiamin solution (120 mmol/L) at pH 5.7 produced 13 times more **4** (45 vs 3.4 μg/L) than the reaction of ribose and cysteine (both 120 mmol/L) under the same conditions. She also showed that thiamin generates 1500 times more **4** than the reaction between ribose and cysteine when the reagent concentrations are as low as in meat (*27*). Further studies are necessary to elucidate the role of the reactant concentration, as well as small pH changes between 5.5 and 6.0, on the formation of sulfur odorants.

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Table 5. Proportion of Isotopomers from the Reaction of [$^{13}C_5$]Xylose, Cysteine, and Thiamin (pH 5) at Different Precursor Concentrations

no.	compound	m/z (analyzed ions)	unlabeled:labeled (%) A[a]	B[a]
4	2-methyl-3-furanthiol	114; 119	54:46	85:15
5	3-mercapto-2-pentanone	118; 123	60:40	86:14

[a] Precursor concentrations: **A**, xylose (0.3 m), cysteine (0.1 m), thiamin (0.1 m); **B**, xylose (0.15 m), cysteine (0.05 m), thiamin (0.05 m).

ABBREVIATIONS USED

DVB/CAR/PDMS, divinylbenzene/carboxen/polymethylsiloxane; GC-MS, gas chromatography–mass spectrometry; SPME, solid-phase microextraction; TIC, total ion chromatogram; TLC, thin-layer chromatogram.

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