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Formation of Aroma Compounds from Ribose and Cysteine during the Maillard Reaction

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The headspace volatiles produced from a phosphate-buffered solution (pH 5) of cysteine and a 1 + 1 mixture of ribose and [$^{13}C_5$]ribose, heated at 95 °C for 4 h, were examined by headspace SPME in combination with GC-MS. MS data indicated that fragmentation of ribose did not play a significant role in the formation of the sulfur aroma compounds 2-methyl-3-furanthiol, 2-furfurylthiol, and 3-mercapto-2-pentanone in which the carbon skeleton of ribose remained intact. The methylfuran moiety of 2-methyl-3-(methylthio)furan originated from ribose, whereas the methylthio carbon atoms came partly from ribose and partly from cysteine. In 3-mercapto-2-butanone one carbon unit was split from the ribose chain. On the other hand, all carbon atoms in 3-thiophenethiol stemmed from cysteine. In another trial cysteine, 4-hydroxy-5-methyl-3(2*H*)-furanone and [$^{13}C_5$]ribose were reacted under the same conditions. The resulting 2-methyl-3-furanthiol was mainly $^{13}C_5$-labeled, suggesting that it stems from ribose and that 4-hydroxy-5-methyl-3(2*H*)-furanone is unimportant as an intermediate. Whereas 2-mercapto-3-pentanone was found unlabeled and hence originated from 4-hydroxy-5-methyl-3(2*H*)-furanone, its isomer 3-mercapto-2-pentanone was formed from both 4-hydroxy-5-methyl-3(2*H*)-furanone and ribose. A new reaction pathway from ribose via its 1,4-dideoxyosone is proposed, which explains both the formation of 2-methyl-3-furanthiol without 4-hydroxy-5-methyl-3(2*H*)-furanone as an intermediate and a new way to form 3-mercapto-2-pentanone.

KEYWORDS: Maillard reaction; 2-methyl-3-furanthiol; 2-furfurylthiol; 2-mercapto-3-pentanone; 3-mercapto-2-pentanone; ribose; [$^{13}C_5$]ribose; cysteine; 4-hydroxy-5-methyl-3(2*H*)-furanone; stable isotope labeling; solid-phase microextraction; 1,4-dideoxyosone; meatlike flavor

INTRODUCTION

The Maillard reaction plays a substantial role in flavor generation during the cooking of meat. Besides thiamine, the precursors ribose and cysteine are important for meat aroma and are consequently employed in the production of process flavors (*1*). Ribose is frequently replaced by its less expensive isomer xylose. Among other compounds, the thiols 2-methyl-3-furanthiol, 2-furfurylthiol, and 3-mercapto-2-pentanone belong to the most important aroma impact compounds formed during the thermal reaction of ribose and cysteine. These molecules are also found in cooked meat (*2*−*4*), as well as in commercial meat flavorings (*5*), and contribute significantly to their aroma.

Numerous model reactions with ribose and cysteine have been carried out to study meat flavor generation (*6*−*14*). Further studies used hypothetical intermediates that could be formed during these reactions. The diketones 2,3-butanedione and 2,3-pentanedione seem to be intermediates in the formation of the α-mercaptoketones 3-mercapto-2-butanone, 3-mercapto-2-pentanone, and 2-mercapto-3-pentanone (*15*, *16*). Model experiments with hydrogen sulfide and 2-furaldehyde, formed in the Maillard reaction from pentoses via the 3-deoxyosone, showed that this intermediate is important for the formation of 2-furfurylthiol (*7*, *17*). Another Maillard reaction pathway from pentoses via the 1-deoxyosone leads to 4-hydroxy-5-methyl-3(2*H*)-furanone (*18*). 4-Hydroxy-5-methyl-3(2*H*)-furanone is considered to be an important meat aroma precursor (*19*), and model reactions of 4-hydroxy-5-methyl-3(2*H*)-furanone with cysteine and hydrogen sulfide confirmed that 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone, and 2-methyl-3-thiophenethiol are generated from it (*15*, *19*−*21*).

Isotopically labeled compounds are very useful in aroma research. Aroma compounds can be accurately quantified with the help of their corresponding labeled compounds in isotope dilution analysis (*22*). Labeled precursors in model reactions are employed to elucidate formation pathways, for example, in pyrazine formation (*23*). Recently the use of defined mixtures of labeled and unlabeled precursors in model studies was proposed to gain information on the fragmentation of this precursor during aroma formation (*24*−*26*). This method allows, for example, the determination of the extent to which a carbon

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Table 1. Model Reactions[a]

	amount (μmol)				
	A	B	C	D	E
cysteine	48.3	48.3	48.3	48.3	48.3
ribose	144.9		72.5		
[$^{13}C_5$]ribose			72.5	72.5	72.5
4-hydroxy-5-methyl-3(2*H*)-furanone		72.5		72.5	
2-furaldehyde					72.5

[a] Reaction in phosphate buffer (0.5 mol/L; pH 5.00) at 95 °C (4 h).

chain of a sugar precursor remains intact during the Maillard reaction and hence gives insight into the significance of different formation pathways. This approach was called "carbohydrate module labeling" (*25*) or "carbon module labeling" (*26*), respectively, and CAMOLA was used as acronym in both cases.

The objective of this study was to elucidate the formation pathways for odorants formed from ribose and cysteine during the Maillard reaction. The CAMOLA approach should explain to what extent the ribose skeleton remains intact during the formation of odorants and to what extent breakdown products play a role. Additional experiments reacting a mixture of a carbohydrate (ribose) and an intermediate [4-hydroxy-5-methyl-3(2*H*)-furanone and 2-furaldehyde, respectively] with cysteine should elucidate the role of the intermediate in the formation of the aroma compounds. In this approach the intermediate and the sugar contain different carbon isomer labels (^{13}C and ^{12}C), which allows the actual contribution of the intermediate to the formation of specific aroma compounds to be estimated.

MATERIALS AND METHODS

Chemicals. Ribose and cysteine were from Fluka (Buchs, Switzerland), 2-furaldehyde was from Aldrich (Buchs, Switzerland), dipotassium hydrogenphosphate and potassium dihydrogenphosphate were from Merck (Darmstadt, Germany), 4-hydroxy-5-methyl-3(2*H*)-furanone was from Advanced Biotech (Paterson, NJ), and [$^{13}C_5$]ribose (98% enrichment) was from Cambridge Isotope Laboratories (Andover, MA). All chemicals were of analytical grade.

Reactions. The reactants listed in **Table 1** were dissolved in potassium phosphate buffer (0.5 mol/L; pH 5.00) to a total amount of 500 mg. The solutions were filled into silanized 2 mL glass vials and the septum-closed vials thermally treated for 4 h in a heated metal block (Reacti-Therm, stirring/heating module, Pierce Chemical Co., Rockford, IL) at 95 °C. The molar ratio between cysteine and the other precursors was 1:3, except for the reaction between cysteine and 4-hydroxy-5-methyl-3(2*H*)-furanone (2:3).

Analysis. All samples were analyzed in duplicate by headspace solid-phase microextraction in combination with gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS). After at least 1 h of equilibration at 20 °C, the fiber [polydimethylsiloxane−divinylbenzene (PDMS-DVB), film thickness = 65 μm, Supelco] was exposed for 60 min at 20 °C to the headspace above the samples in the glass vials without agitation. After sampling, the SPME device was placed for 5 min in the GC injector, equipped with a 0.75 mm i.d. liner (Supelco), and heated at 250 °C. GC-MS analyses were performed on a GC 6890A coupled to an MSD 5973 (both Agilent, Palo Alto, CA) using an HP-PONA column (50 m × 0.20 mm × 0.50 μm, Agilent). After insertion of the SPME device into the injector, the oven temperature program was started and the temperature raised at 6 °C/min from 35 to 240 °C and held for 10 min isothermally. Mass spectra in the electron impact mode (EI) were generated at 70 eV and at a scan range from *m/z* 28 to 350.

RESULTS AND DISCUSSION

Reaction of Ribose, [$^{13}C_5$]Ribose, and Cysteine. Cysteine and ribose (molar ratio 1:3) were reacted at pH 5 in phosphate buffer at 95 °C for 4 h. These conditions are within the frame of process flavor manufacturing conditions as defined by the Council of Europe, whose guidelines limit the reaction temperature to 180 °C, the reaction time to 24 h (for temperatures ≤ 110 °C), and the pH value to a maximum of 8.0 (*27*). A similar reaction was carried out by replacing ribose with a mixture (1:1) of unlabeled and $^{13}C_5$-labeled ribose. Both reaction mixtures were analyzed by SPME using an absorption time of 60 min and a PDMS-DVB fiber, followed by desorption and GC-MS. 2-Methyl-3-furanthiol (**7**), 3-mercapto-2-pentanone (**8**), 2-furfurylthiol (**9**), and many other aroma compounds were successfully detected with this method in both mixtures. The structures of the identified volatiles are shown in **Figure 1**.

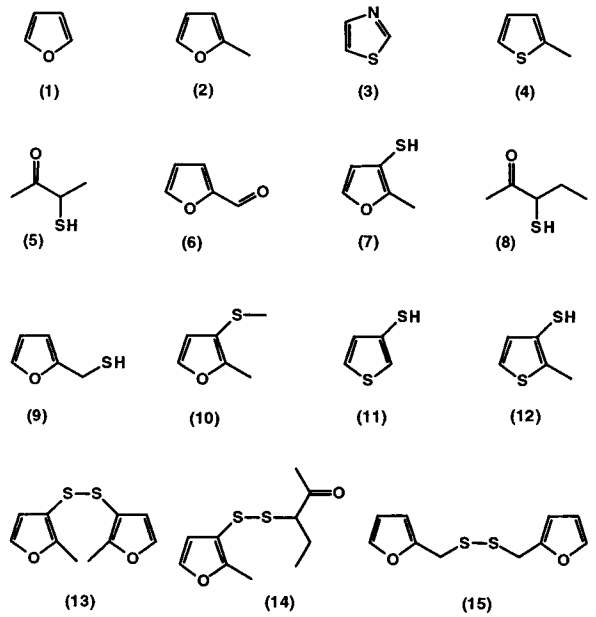

Figure 1. Compounds formed from ribose and cysteine (numbering refers to **Table 2**).

The mass spectra of the many volatiles isolated from the ribose/[$^{13}C_5$]ribose/cysteine trials showed signals with mass differences up to $M^+ + 10$ as compared to the spectra of the corresponding volatiles from the ribose/cysteine trial. These signals correspond to compounds bearing ^{13}C atoms originating from [$^{13}C_5$]ribose. **Table 2** lists the identified volatiles and the proportion of their ^{13}C-labeled isotope molecules. Values for the $M^+ + 1$ and $M^+ + 2$ signals were corrected by subtracting the naturally occurring percentages of ^{13}C (1.10%), ^{33}S (0.76%), and ^{34}S (4.20%). The loss of hydrogen frequently observed with the molecular ion in EI-MS was also corrected in the labeled molecular ions by the ratio $(M^+ - 1)/M^+$.

In the case of 2-methyl-3-furanthiol (**7**) an isotope peak *m/z* 119 appeared in the mass spectrum in addition to *m/z* 114 (M^+). The unlabeled and the 5-times-labeled 2-methyl-3-furanthiol occurred at an approximate ratio of 1:1. Other labeling for 2-methyl-3-furanthiol (*m/z* 115−118) was practically not observed, indicating that during 2-methyl-3-furanthiol formation the ribose carbon skeleton remained intact. During studies on several intermediates Hofmann and Schieberle found the highest yields for 2-methyl-3-furanthiol in the reaction between hydroxyacetaldehyde and mercapto-2-propanone (*28*). In the present study, however, pathways via ribose fragmentation were not relevant.

2-Furfurylthiol (**9**) and 2-furaldehyde (**6**) showed similar formation patterns with either only unlabeled or completely labeled compound in a ratio of 1:1. These data support the

Table 2. Proportion of Isotopomers from the Reaction between Ribose, [$^{13}C_5$]Ribose, and Cysteine

			proportion of labeled carbon atoms in the molecule[a] (%)							
no.	compound[b]	*m/z* (M^+)	0[c]	1	2	3	4	5	6	10
1	furan	68	49	0	0	1	50			
2	2-methylfuran	82	51	0	0	1	9	39		
3	thiazole	85	56	44	0	0				
4	2-methylthiophene	98	45	7	0	0	3	44		
5	3-mercapto-2-butanone	104	49	0	0	1	50			
6	2-furaldehyde	96	47	0	0	2	5	45		
7	2-methyl-3-furanthiol	114	49	1	0	1	3	46		
8	3-mercapto-2-pentanone	118	49	1	0	1	2	47		
9	2-furfurylthiol	114	48	0	1	1	2	47		
10	2-methyl-3-(methylthio)furan	128	36	11	1	0	2	36	13	
11	3-thiophenethiol	116	95	1	2	1	1			
12	2-methyl-3-thiophenethiol	130	44	2	6	14	10	24		
13	bis(2-methyl-3-furyl) disulfide	226	26	0	0	0	3	48	0	22
14	(2-methyl-3-furyl) (2-oxo-3-pentyl) disulfide[d]	230	27	0	1	0	3	49	0	20
15	bis(2-furfuryl) disulfide	226	28	0	0	0	0	54	0	18

[a] Values are corrected by subtracting the naturally occurring percentages of ^{13}C (1.10%), ^{33}S (0.76%), and ^{34}S (4.20%) in $M^+ + 1$ and $M^+ + 2$. The loss of hydrogen observed with the molecular ion in EI-MS was also corrected in the labeled molecular ions by the ratio $(M^+ - 1)/M^+$. [b] Compounds were identified in the corresponding reaction between ribose and cysteine by comparing the mass spectra and retention indices with those of authentic compounds analyzed in our laboratory or in literature data. [c] Number of ^{13}C atoms in the molecule. [d] Mass spectrum of the unlabeled compound *m/z* (%) 230 (M^+, 43), 187 (13), 145 (27), 114 (34), 113 (92), 85 (15), 81 (14), 43 (100).

formation of 2-furfurylthiol from ribose via 2-furaldehyde as postulated by Silwar and Tressl (*29*).

The α-mercaptoketones 3-mercapto-2-butanone (**5**) and 3-mercapto-2-pentanone (**8**) were not generated from ribose fragments; only completely unlabeled or completely $^{13}C_5$-labeled compounds in equal amounts were found. Obviously, **5** was formed from ribose through the loss of one carbon atom and **8** from the intact carbon chain. Surprisingly, 2-mercapto-3-pentanone was not detected. Reactions of ribose and cysteine in an autoclave during 30 min at 140 °C (*30*) and 20 min up to 145 °C (*8*) both revealed 3-mercapto-2-pentanone and 2-mercapto-3-pentanone, with 3-mercapto-2-pentanone dominating. Higher temperatures and shorter reaction times seem to favor a different reaction mechanism leading to both isomers. In the present study the formation of 3-mercapto-2-pentanone via 2,3-pentanedione and hydrogen sulfide as intermediates (*15*, *16*) apparently does not play an important role; otherwise, 2-mercapto-3-pentanone would have been formed at the same time.

To explain the formation of 3-mercapto-2-pentanone without the simultaneous generation of 2-mercapto-3-pentanone, 5-hydroxy-3-mercapto-3-pentene-2-one (**19**) is proposed as an intermediate. This intermediate could be formed from the open chain or cyclic form of the 1,4-dideoxyosone (**16**) of ribose by the addition of hydrogen sulfide, originating from cysteine, and loss of water, as illustrated in **Figure 2**. 5-Hydroxy-3-mercapto-3-penten-2-one (**19**) would be transformed to 3-mercapto-2-pentanone (**8**) via a series of reaction steps including a Strecker-type reaction with cysteine, isomerization, hydrolysis, and reduction. Its 2-mercapto-3-pentanone isomer is not generated by this mechanism. On the other hand, dehydration of 2,5-dihydro-2-hydroxy-2-methyl-3-furanthiol (**20**), the cyclic form of 5-hydroxy-3-mercapto-3-penten-2-one, should yield 2-methyl-3-furanthiol (**7**). Alternatively, **7** could be formed by dehydration of 2,5-dihydro-2,3-dihydroxy-2-methylfuran (**17**, cyclic form of the 1,4-dideoxyosone of ribose), which yields 3-hydroxy-2-methylfuran (**18**). The addition of hydrogen sulfide to its keto form and a loss of water would lead to 2-methyl-3-furanthiol. Thus, if proven, the formation of 2-methyl-3-furanthiol via the 1,4-dideoxyosone would represent a new alternative pathway for the formation via 1-deoxyosone and 4-hydroxy-5-methyl-3(2*H*)-furanone (*31*).

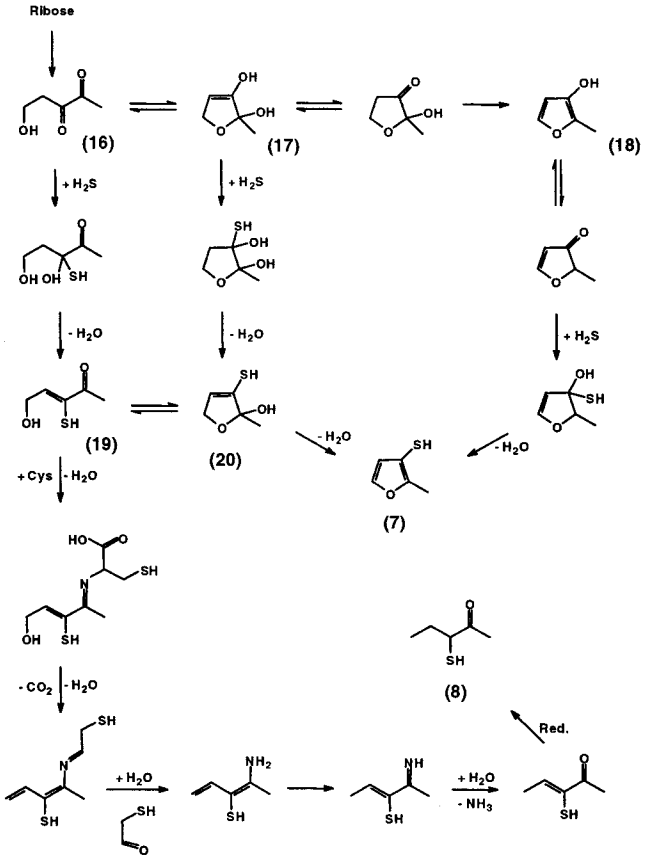

Figure 2. Proposed formation pathway for 3-mercapto-2-pentanone and 2-methyl-3-furanthiol from ribose and cysteine via the 1,4-dideoxyosone route.

The 1,4-dideoxyosone of ribose, 5-hydroxy-2,3-pentanedione, has been previously identified as an intermediate in the Maillard reaction between xylose and amino acids from hydrolyzed wheat protein (*32*). Various syntheses have been described by de Kimpe et al. (*33*, *34*). It also occurs in the alga *Laurencia spectabilis* (*35*) and is, therefore, named laurencione. The formation of 1,4-dideoxyosones has been explained by the dehydration of 1-deoxyketose obtained either by the reduction

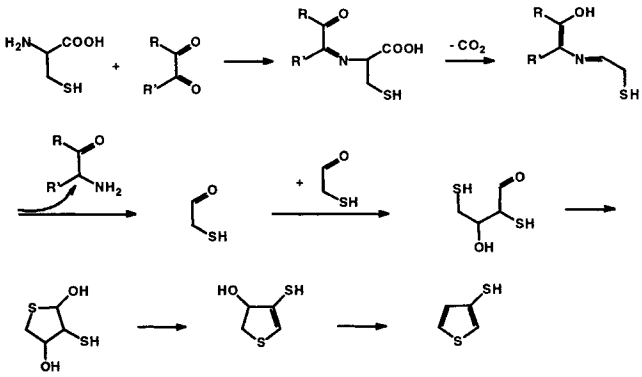

Figure 3. Proposed formation pathway for 3-thiophenethiol, adapted from ref *21*.

of 1-deoxyosone, for example, via Strecker degradation, or by its disproportionation (*32*, *36*). It can be also formed from oligosaccharides by a so-called "peeling off" mechanism (*37*).

Further compounds formed from ribose without chain cleavage are 2-methylfuran (**2**) and 2-methylthiophene (**4**). An unambiguous determination of the isotopomer ratio for 2-methyl-3-thiophenethiol (**12**), the thiophene analogue of 2-methyl-3-furanthiol, was not possible because it coeluted with another compound.

For thiazole (**3**), a molecular ion that was 56% unlabeled (*m/z* 85) and 44% singly labeled (*m/z* 86) was found. The signal at *m/z* 58 reveals the loss of *m/z* 27 and 28, corresponding to a loss of hydrogen [^{12}C]cyanide and hydrogen [^{13}C]cyanide, respectively. The labeling position is assigned to the C-2 atom of thiazole, suggesting that it has been formed from cysteamine derived from cysteine and formaldehyde stemming from ribose to yield thiazolidine and thiazole by subsequent oxidation of the former, as proposed by Sakaguchi and Shibamoto (*38*).

Almost no labeling was observed in 3-thiophenethiol (**11**), suggesting its formation from cysteine rather than from ribose. This is in accordance with the finding that 3-thiophenethiol is a thermal degradation product of cysteine (*39*) and that in model reactions with 4-hydroxy-5-methyl-3(2*H*)-furanone it occurred only in cysteine-containing systems (*21*). Shu et al. (*39*) postulated the formation of this compound from two molecules of mercaptoacetaldehyde, the Strecker aldehyde of cysteine. **Figure 3** gives an overview of the pathway.

An interesting labeling pattern was found for 2-methyl-3-(methylthio)furan (**10**). The ratio between the unlabeled, singly labeled, and the 5- and 6-times-^{13}C-labeled molecules was found to be 3:1:3:1, suggesting, according to the mass spectra (not shown), the labeling positions shown in **Figure 4**. Obviously, the 2-methylfuran moiety stems exclusively from ribose with the C-skeleton remaining intact as indicated by the 1:1 ratio between the unlabeled and the 5-times-labeled molecules. On the other hand, only part of the carbon atoms of the methylthio group originate from ribose; otherwise, the labeling pattern would have been 1:1:1:1. It can be concluded that half of the thiomethyl carbon atoms stem from cysteine, the reaction mechanism being still unclear.

In addition to the mercapto compounds 2-methyl-3-furanthiol and 2-furfurylthiol, the corresponding disulfides bis(2-methyl-3-furyl) disulfide (**13**), bis(2-furfuryl) disulfide (**15**), and the mixed disulfide between 2-methyl-3-furanthiol and 3-mercapto-2-pentanone [(2-methyl-3-furyl-2-oxo-3-pentyl) disulfide (**14**)] were detected. The isotopomer ratio of approximately 1:2:1 (unlabeled/[$^{13}C_5$]/[$^{13}C_{10}$]) indicates that they are formed by oxidation of the corresponding thiols **7**−**9** as these thiols are either unlabeled or completely $^{13}C_5$-labeled. According to

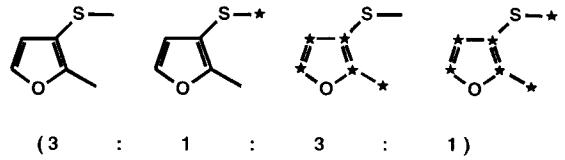

Figure 4. ^{13}C-Labeling positions in 2-methyl-3-(methylthio)furan (* = ^{13}C).

Hofmann et al. (*40*) these disulfides can be formed as artifacts from the corresponding monothiols during extraction with organic solvents and storage of the extracts. However, solvent extraction was avoided in the present study, and the samples were analyzed directly by HS-SPME-GC-MS. According to van Seeventer (*41*) disulfide formation is not the cause of 2-methyl-3-furanthiol instability in aqueous solutions of reacted cysteine/ribose kept at 50 °C. To verify whether the disulfides represent true reaction products or are formed as artifacts during SPME, additional experiments would be necessary.

Reaction between 4-Hydroxy-5-methyl-3(2*H*)-furanone and Cysteine. 4-Hydroxy-5-methyl-3(2*H*)-furanone and cysteine were reacted in a molar ratio of 3:2 using the same reaction conditions as for the ribose/cysteine experiment. Among other sulfur compounds 3-mercapto-2-butanone, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone, and bis-(2-methyl-3-furyl) disulfide were identified. Because both 2-mercapto-3-pentanone and 3-mercapto-2-pentanone are formed during the reaction, the reaction pathway via the α-diketones, as depicted in **Figure 5**, is postulated. The addition of the amino group of cysteine causes ring opening of 4-hydroxy-5-methyl-3(2*H*)-furanone (**21**). The resulting Schiff base of the 1-deoxypentosone with cysteine undergoes Strecker degradation and yields 2-amino-4,5-dihydroxy-3-pentanone (**22**). The retroaldol cleavage of the compound forms 2-amino-4-hydroxy-3-butanone (**23**), which by a series of reaction steps, including enolization, dehydration, and hydrolysis, is converted to 2,3-butandione (**24**). This compound gives 3-mercapto-2-butanone (**5**) upon reaction with hydrogen sulfide and reduction. If, instead of a retroaldol cleavage, **22** loses ammonia, 1-hydroxy-2,3-pentadione (**25**) is formed. Strecker degradation with cysteine and loss of water yield 2,3-pentadione (**26**). Whitfield and Mottram (*21*) postulate a similar mechanism that, however, does not involve cysteine. If cysteine is present, the authors propose a mechanism including an aldol reaction between 2-oxopropanal, which is generated by the fragmentation of 4-hydroxy-5-methyl-3(2*H*)-furanone, and acetaldehyde stemming from cysteine degradation. CAMOLA experiments with 4-hydroxy-5-methyl-3(2*H*)-furanone/cysteine would elucidate whether α-mercaptoketones are formed from an intact 4-hydroxy-5-methyl-3(2*H*)-furanone chain or from fragments from 4-hydroxy-5-methyl-3(2*H*)-furanone and cysteine. Further experiments are currently under way to answer this question.

Reaction between 4-Hydroxy-5-methyl-3(2*H*)-furanone, [$^{13}C_5$]Ribose, and Cysteine. In this experiment, [$^{13}C_5$]ribose and cysteine were reacted with 4-hydroxy-5-methyl-3(2*H*)-furanone. Because [$^{13}C_5$]ribose and 4-hydroxy-5-methyl-3(2*H*)-furanone carried different carbon isomer labels, it was possible to estimate the contribution of ribose and 4-hydroxy-5-methyl-3(2*H*)-furanone to the formation of aroma compounds. **Table 3** shows the identified compounds, the number of ^{13}C-labeled atoms in the molecules, and the ratios of the isotopomers. The two mercaptoketones 3-mercapto-2-butanone and 2-mercapto-3-pentanone were mainly unlabeled (94 and 96%, respectively) and, hence, almost exclusively formed from 4-hydroxy-5-methyl-3(2*H*)-furanone, whereas 3-mercapto-2-pentanone (42% unlabeled and 58% $^{13}C_5$-labeled) was formed from both 4-hy-

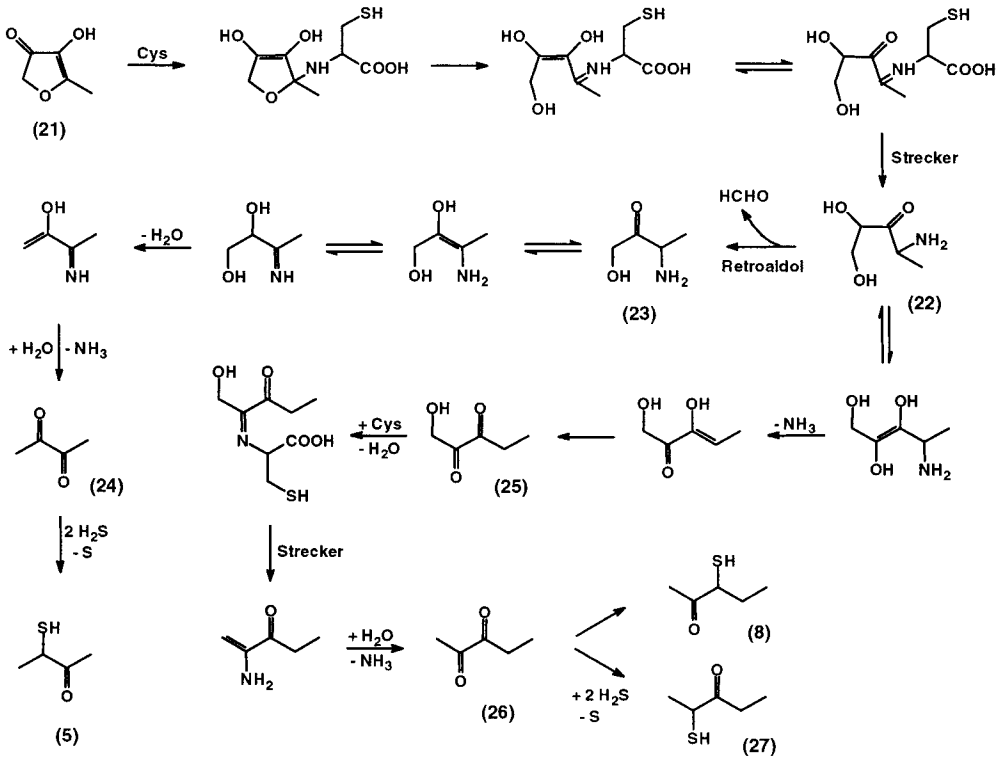

Figure 5. Proposed formation pathway for α-mercaptoketones from 4-hydroxy-5-methyl-3(2*H*)-furanone.

Table 3. Proportion of Labeling in Compounds from the Reaction between 4-Hydroxy-5-methyl-3(2*H*)-furanone, [$^{13}C_5$]Ribose, and Cysteine

no.	compound[a]	*m/z* (M^+)	no. of ^{13}C atoms in the labeled molecule	unlabeled compound (%)	^{13}C-labeled compound (%)
5	3-mercapto-2-butanone	104	5	94	6
6	2-furaldehyde	96	5	2	98
7	2-methyl-3-furanthiol	114	5	7	93
8	3-mercapto-2-pentanone	118	5	42	58
16	2-mercapto-3-pentanone	118	5	96	4
9	2-furfurylthiol	114	5	0	100
10	2-methyl-3-(methylthio)furan	128	5, 6	6	84, 10
11	3-thiophenethiol	116	4	98	2
13	bis(2-methyl-3-furyl) disulfide	226	5, 10	1	15, 84

[a] Compounds were identified in the corresponding reaction between ribose and cysteine by comparing the mass spectra and retention indices with those of authentic compounds analyzed in our laboratory or in literature data.

droxy-5-methyl-3(2*H*)-furanone and ribose. Both reaction pathways shown in **Figures 2** and **5** seem to contribute to the formation of 3-mercapto-2-pentanone. These findings, however, do not allow conclusions on the role of 4-hydroxy-5-methyl-3(2*H*)-furanone as intermediate in the formation of the mercaptoketones 3-mercapto-2-butanone and 2-mercapto-3-pentanone from ribose and cysteine alone. 2-Furaldehyde and 2-furfurylthiol, which were ~100% labeled, stem from ribose.

Surprisingly, 2-methyl-3-furanthiol was mostly labeled (93%), indicating that it originates mainly from ribose and only to a small extent from 4-hydroxy-5-methyl-3(2*H*)-furanone. Also, most of the corresponding disulfide **13** was found fully labeled. These findings suggest that 4-hydroxy-5-methyl-3(2*H*)-furanone plays only a minor role as an intermediate in the formation of 2-methyl-3-furanthiol from ribose and cysteine under the conditions studied. 2-Methyl-3-furanthiol formation from ribose by recombination of fragments can be excluded, too, according to the results from the first experiment.

As postulated in **Figure 2**, 1,4-dideoxypentosone could be a suitable precursor of 2-methyl-3-furanthiol. However, if this mechanism is correct, the results imply that either the formation of 1-deoxypentosone from 4-hydroxy-5-methyl-3(2*H*)-furanone is not favored under the conditions studied or 1,4-dideoxypentosone arises from ribose by a mechanism other than the reduction or disproportionation of 1-deoxypentosone. Otherwise, 2-methyl-3-furanthiol should be formed also from 4-hydroxy-5-methyl-3(2*H*)-furanone.

The alternative pathways leading to 1,4-dideoxypentosone from ribose are proposed in **Figure 6**. The reaction of ribose with cysteine leads via Amadori rearrangement to 1-cysteino-1,4-dideoxypentosone (**28**). The latter compound is transformed via Strecker degradation and hydrolysis to 1,4-dideoxypentosone (**16**). This mechanism is analogous to that proposed by Nedvidek et al. (*32*) for the formation of 1,4-dideoxyosones from 1-deoxyosones. Recently, the high mobility of the carbonyl group in 1-amino-1,4-dideoxyosones has been demonstrated (*42*). Thus, 1,4-dideoxypentosone could also be formed from 1-cysteino-1,4-dideoxypentosone by enolizations yielding the Schiff base of 4-deoxypentosone with cysteine (**29**). Strecker degradation of the latter compound followed by elimination of ammonia would yield 1,4-dideoxypentosone. Further experiments are necessary to verify whether the new pathway proposed

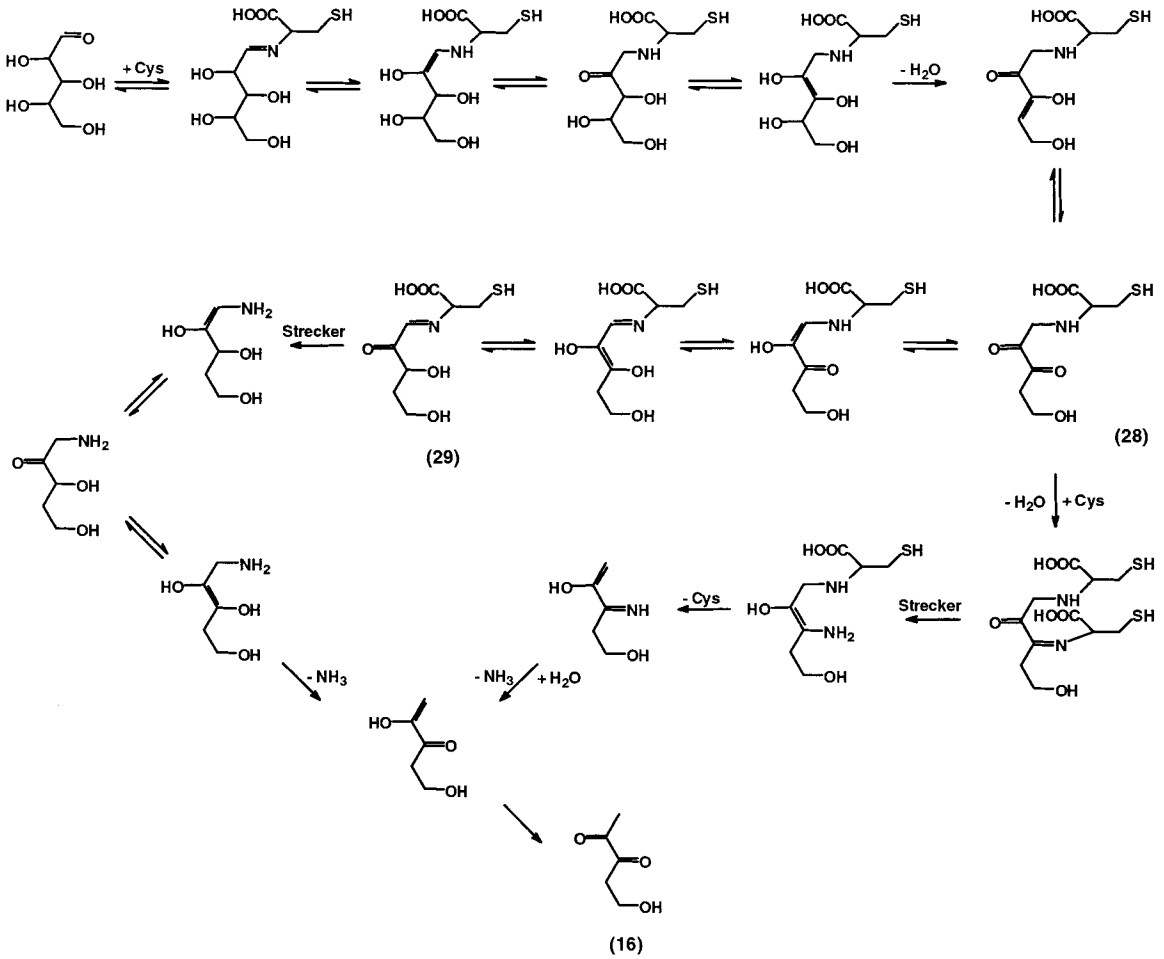

Figure 6. Proposed formation of 1,4-dideoxypentosone from ribose and cysteine.

in **Figure 2** can be considered to be the main reaction mechanism and the 1,4-dideoxyosone as a new key intermediate for 2-methyl-3-furanthiol formation in the reaction of ribose and cysteine under cooking conditions.

Reaction of 2-Furaldehyde, [$^{13}C_5$]Ribose, and Cysteine. 2-Furaldehyde was reacted with [$^{13}C_5$]ribose and cysteine to elucidate its role as a 2-furfurylthiol precursor. During HS-SPME-GC-MS analysis only a few compounds were detected due to saturation of the PDMS-DVB fiber with unreacted 2-furaldehyde. However, 2-furfurylthiol was identified, and the ratio between unlabeled and $^{13}C_5$-labeled isotopomer was found to be 92:8 according to the ratio between the ion signals *m/z* 114 and 119. This indicates that most 2-furfurylthiol was formed from 2-furaldehyde. Because ribose and 2-furaldehyde were employed in equimolar amounts in the reaction, it can be concluded that 2-furaldehyde is a much more efficient precursor than ribose. In contrast to 4-hydroxy-5-methyl-3(2*H*)-furanone, its role as an intermediate for 2-methyl-3-furanthiol formation under the conditions studied being doubtful, this experiment confirmed the role of 2-furaldehyde as an important intermediate for 2-furfurylthiol generation during the Maillard reaction between ribose and cysteine.

Conclusions. The reaction between ribose and cysteine in aqueous phosphate buffer (pH 5) generates numerous sulfur-containing aroma compounds. The ribose carbon chain remains intact in the reaction yielding 2-methyl-3-furanthiol, 2-furfurylthiol, and 3-mercapto-2-pentanone. 2-Furaldehyde could be confirmed as an important intermediate for 2-furfurylthiol formation. On the other hand, 4-hydroxy-5-methyl-3(2*H*)-furanone plays only a minor role in the formation of 2-methyl-3-furanthiol under cooking conditions. A new reaction mechanism with the 1,4-dideoxyosone as key intermediate is proposed. In the reaction of ribose, 4-hydroxy-5-methyl-3(2*H*)-furanone, and cysteine, 2-mercapto-3-pentanone was formed almost exclusively from 4-hydroxy-5-methyl-3(2*H*)-furanone, whereas 3-mercapto-2-pentanone was formed from both 4-hydroxy-5-methyl-3(2*H*)-furanone and ribose. These data suggest that the formation of 3-mercapto-2-pentanone from ribose by an alternative pathway is more important than its formation via 4-hydroxy-5-methyl-3(2*H*)-furanone under the conditions studied. The pathway involving the addition of hydrogen sulfide to the cyclic form of the 1,4-dideoxyosone explains why 3-mercapto-2-pentanone rather than 2-mercapto-3-pentanone is formed from ribose in the Maillard reaction with cysteine.

ABBREVIATIONS USED

CAMOLA, carbohydrate module labeling; HS-SPME-GC-MS, headspace solid-phase microextraction−gas chromatography−mass spectrometry; PDMS-DVB, poly(dimethylsiloxane)−divinylbenzene; TIC, total ion count.

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