Investigation of the Reaction between 4-Hydroxy-5-methyl-3(2*H*)-furanone and Cysteine or Hydrogen Sulfide at pH 4.5

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Reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone (HMF) with cysteine or hydrogen sulfide at pH 4.5 for 60 min at 140 °C produced complex mixtures of volatile compounds, the majority of which contained sulfur. Sixty-nine compounds were identified, some tentatively, by GC/MS. These included disulfides (26), thiols (7), dithiolanones (6), thiophenones (4), dithianones (3), and thienothiophenes (6). The main non-sulfur compounds were 2,3-pentanedione, 2,4-pentanedione, and 3,4-hexanedione. Both systems produced approximately the same total quantity of volatile compounds, but the reaction containing cysteine gave the larger number of individual compounds, with thiols quantitatively the dominant components. By comparison, the major products formed in the reaction with hydrogen sulfide were the dithiolanones. Reaction pathways are presented for the major products and, where applicable, possible reasons for the differences in composition of the two systems are discussed. The contribution of these reactions, and their products, to the flavor of roasted foods is considered.

Keywords: *Maillard reaction; aroma; volatiles; amino acids; pentoses; mercaptoketones*

INTRODUCTION

4-Hydroxy-5-methyl-3(2*H*)-furanone (HMF) is formed in Maillard reactions involving pentose sugars and results from the dehydration of 1-deoxypentosones (Feather, 1981). This compound, and its 2,5-dimethyl homologue, have been isolated from natural beef broth (Tonsbeek et al., 1968) and, as a consequence, they are considered to be involved in the formation of meaty flavors through their reaction with either hydrogen sulfide or sulfur-containing amino acids (van den Ouweland and Peer, 1975; Shu and Ho, 1988). Furthermore, as early as 1960 patents described the formation of meatlike flavors by heating cysteine with ribose, a precursor of HMF (Morton et al., 1960). In 1975, van den Ouweland and Peer (1975) identified 2-methyl-3-furanthiol, 2-methyl-3-thiophenethiol, and the corresponding dihydro and tetrahydro derivatives as the main volatile products from the reaction of hydrogen sulfide and HMF. In other studies, involving the reaction between 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and cysteine, neither of the above furan or thiophene thiols was reported (Shu and Ho, 1988). At pH 5.1, the volatiles were dominated by the dimethyl derivatives of 4-hydroxy-3(2*H*)-thiophenone and 1,2,4-trithiolane, although when the pH was increased to 7.1, pyrazines became major products. However, 2-thiophenethiol was identified in mixtures buffered at pH 2.2 (Shu and Ho, 1988).

In some of our recent studies of the reaction between cysteine and ribose at pH 5.6, we have demonstrated the formation of a wide range of compounds containing one or more atoms of sulfur (Mottram and Whitfield, 1995a,b). As a result of such studies, it was suggested that HMF was a possible intermediate in the formation of some of these compounds (Whitfield and Mottram, 1996). To further our understanding of the role of HMF in such reactions, we have now reacted this compound with cysteine or hydrogen sulfide and have identified the major products formed in the two systems. A pH of 4.5 was chosen for the reactions as this pH has been shown to favor the production of sulfur compounds with meatlike aromas (Madruga and Mottram, 1995). In addition, this pH is slightly on the acidic side of the isoelectric point of cysteine (pH 5.1), a condition that is known to favor the formation of sulfur-substituted furans (Zhang and Ho, 1991).

EXPERIMENTAL PROCEDURES

Materials. HMF was obtained as a gift from a flavor company. L-Cysteine was purchased from Sigma Chemical Co., St. Louis, MO, and a lecture bottle of hydrogen sulfide was obtained from Aldrich Chemical Co., Milwaukee, WI. Phosphate buffer (0.5 M, pH 4.5) was prepared from disodium hydrogen phosphate and sodium dihydrogen phosphate (BDH Chemicals, Poole, U.K.) in distilled water purified by filtration through a Milli-Q purification system (Millipore Corp., Bedford, MA). Authentic samples of reference compounds were either purchased from a range of laboratory chemical suppliers or obtained as gifts from flavor laboratories.

Reaction Mixtures. Separate solutions containing HMF (0.1 M) and cysteine (0.1 M) were prepared in the phosphate buffer. A saturated solution of hydrogen sulfide (~0.2 M) was prepared by passing hydrogen sulfide through phosphate buffer (pH 4.5), which was cooled in ice at 0 °C. Reaction mixtures were prepared by mixing equal quantities (1 mL each) of the solutions of HMF (containing 11.4 mg) with those of either cysteine (12.1 mg) or the hydrogen sulfide (~6.6 mg) in 5 mL glass ampules. The ampules were flame sealed and were then heated in an oven, controlled at 140 °C, for 60 min.

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The reaction of HMF with cysteine was also carried out at a higher concentration (2-fold) and in greater quantities (10 mL each) to obtain larger amounts of some trace volatile components.

Isolation of Volatile Reaction Products. After cooling, each reaction mixture was transferred to a 250 mL conical flask containing 20 mL of 0.5 M phosphate buffer (pH 4.5) and a magnetic stirrer bar. The reaction tube was rinsed twice with buffer solution (2 mL), and these washings were added to the conical flask. Methyl decanoate (100 μg in 0.1 mL of ethanol) was then added to the reaction mixture as an internal standard. The flask was fitted with a Drechsel head, and a glass-lined stainless steel tube (115 mm long × 0.75 mm i.d.) packed with Tenax GC (Scientific Glass Engineering Pty. Ltd., Australia) was attached by a stainless steel reducing union to the head outlet. During the collection of the volatile components, the dilute reaction solution was stirred slowly and maintained at 60 °C in a water bath, while the Tenax trap was maintained at room temperature. The volatiles were swept from the flask to the adsorbent in the trap using a flow of oxygen-free nitrogen (60 mL/min) for 1 h. At the end of this time, the flask was removed and the trap connected directly to the nitrogen supply for 5 min to remove moisture.

Gas Chromatography/Mass Spectrometry (GC/MS). A Varian 1440 gas chromatograph, fitted with a "Unijector" (Scientific Glass Engineering Pty. Ltd., Australia) set in the concentrator-headspace mode and fitted with a fused-silica capillary column (50 m × 0.32 mm i.d.) coated with BP5 (Scientific Glass Engineering Pty. Ltd., Australia), was used for all analyses. The GC was coupled directly to a Varian-MAT 311A double-focusing mass spectrometer controlled by a Finnigan-MAT Incos 2200 data system. The helium carrier gas was set at a flow of 1 mL/min. The trapped volatile components were thermally desorbed onto the GC column by heating the trap at 260 °C for 5 min, while the oven was cooled at 0 °C using a fine stream of liquid nitrogen as the coolant. By directing the cooling gas at the front of the column, efficient cryofocusing of the desorbed volatiles was achieved. After removal of the coolant, the oven temperature was increased rapidly to 60 °C, held at that temperature for 5 min, and then increased to 250 °C at a rate of 4 °C/min. C_6−C_{20} *n*-alkanes (100 ng of each in 1 μL of diethyl ether added to the trap), analyzed under the above conditions, were used as external standards for the calculation of linear retention indices of the volatile reaction products.

The mass spectrometer was operated in the electron-impact mode with an electron energy of 70 eV and an ion source temperature of 250 °C. A continuous scan mode was employed over a mass range of 34−340 amu with a scan rate of 2 s/decade. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley mass spectral databases, in collections of mass spectra of flavor compounds (ten Noever de Brauw et al., 1979), and in previously published literature. When possible, confirmations of identifications were carried out by comparing linear retention indices (LRI) with those of authentic compounds.

To obtain estimates of the relative quantities of the components, the approximate concentrations of selected compounds were determined by comparing their GC/MS chromatogram peak areas with the area of the internal standard, methyl decanoate, which was taken as 100 μg, and assuming all response factors were 1. The concentrations of these compounds are reported as micrograms per 10 mg of HMF used in the reaction. The detection limit for individual compounds present in the reaction mixture was estimated to be 0.1 μg/10 mg of HMF, based on 3 times background noise. Compounds described as "trace" components were present in concentrations between 0.1 and 1 μg/10 mg of HMF.

RESULTS AND DISCUSSION

A total of 69 compounds was found in the headspace volatiles of the reaction mixtures containing HMF and cysteine or hydrogen sulfide (Table 1). These included disulfides (26), thiols (7), dithiolanones (6), dihydrothiophenones (4), dithianones (3), alkanediones (3), and thienothiophenes (6). Whenever possible, identities were confirmed by comparison of the mass spectra and LRI with those authentic compounds. For components for which no reference compounds were available, tentative identities were determined by comparison with published spectra of the compound or related compounds. With the exception of the disulfides, most of the compounds had been previously identified in cysteine−ribose reaction mixtures (Mulders, 1973; Farmer et al., 1989; Farmer and Mottram, 1990; Güntert et al., 1990; Hofmann and Schieberle, 1995; Mottram and Whitfield, 1995a). This was not surprising because HMF is a major product of the degradation of pentoses in the Maillard and caramelization reactions. However, the majority of the disulfides had not been reported in such systems, and 20 of these compounds had not been reported previously in any food or model system. These disulfides, together with their thiol precursors, were particularly interesting, because of the association of this type of compound with meatlike aromas (Mottram, 1991).

The aromas of the reaction mixtures were evaluated by three assessors who worked in the laboratory. The system containing cysteine and HMF was described as sulfurous, rubbery, and boiled meat, and it was noted that, when diluted, the mixture had increased meatlike characteristics. The system containing hydrogen sulfide was dominated by the smell of hydrogen sulfide when the reaction vial was first opened, but as this odor disappeared, a caramel, meatlike aroma developed.

Of the 69 compounds reported in Table 1, 65 were found in the reaction mixtures containing cysteine, whereas only 43 compounds were found in the reaction of HMF with hydrogen sulfide. Accordingly, the majority of this paper will discuss the reasons for the differences in volatile compositions of the two reaction systems and the possible pathways involved in the formation of key components.

Alkanediones and Mercaptoalkanones. Three alkanediones, 2,3-pentanedione (**1** in Table 1), 2,4-pentanedione (**2**), and 3,4-hexanedione (**3**), were identified in the reaction mixtures containing cysteine, whereas only **2** was detected in those containing hydrogen sulfide alone (Table 1). In the latter system, **2** was one of the major products. Five mercaptoketones were found in the reaction mixtures, three of which [3-mercaptobutan-2-one (**5**), 3-mercaptopentan-2-one (**8**), and 3-mercaptopentan-2-one (**9**)] had been reported previously in cysteine−ribose model systems. Two other mercaptoketones, which had not been previously reported in meat or model systems, were tentatively identified from their mass spectra. The spectra were similar to those of compounds **5**, **8**, and **9**, with small molecular ions and major fragments corresponding to fission of the alkyl chain adjacent to the carbonyl group. Thus, 1-mercaptobutan-2-one (**7**) had a base peak at *m*/*z* 57 ($C_2H_5CO^+$) and a major fragment at *m*/*z* 47 (CH_2SH^+), whereas 5-mercaptohexan-2-one (**14**) showed a base peak at *m*/*z* 43 (CH_3CO^+) and fragments at *m*/*z* 90 ($C_4H_9SH^+$), *m*/*z* 71 ($C_3H_7CO^+$), and *m*/*z* 61 (CH_3CHSH^+).

Mercaptoalkanones, as well as their corresponding disulfides, are known to form readily by the reaction of α-dicarbonyl compounds with hydrogen sulfide (Mottram et al., 1995). Thus, the formation of α-dicarbonyls from HMF is a key step in the formation of mercaptoal-

Table 1. Volatile Compounds Obtained from Reactions between 4-Hydroxy-5-methyl-3(2*H*)-furanone and Cysteine or Hydrogen Sulfide

no.	compound	approx concn[a] cysteine		H_2S		method of ID[b]	LRI[c]	MS data, *m*/*z* (rel intensity), or reference[d]
1	2,3-pentanedione	4	15	−	−	MS + LRI	680	
2	2,4-pentanedione	12	16	47	31	MS + LRI	787	
3	3,4-hexanedione	4	4	−	−	MS + LRI	800	
4	4,5-dihydro-5-methyl-3(2*H*)-furanone	2	8	6	6	MS	808	
5	3-mercaptobutan-2-one[e]	84	100	6	5	MS + LRI	817	Mottram et al., 1995
6	2-methyl-3-furanthiol	15	15	16	8	MS + LRI	867	Evers et al., 1976
7	1-mercaptobutan-2-one	tr	−	8	6	ms	886	57 (100), 47 (28), **104** (19), 45 (10), 42 (9)
8	3-mercaptopentan-2-one	81	68	9	6	MS + LRI	902	Mottram et al., 1995
9	2-mercaptopentan-3-one	78	77	8	6	MS + LRI	908	Mottram et al., 1995
10	3-thiophenethiol	26	14	−	−	MS	972	Werkhoff et al., 1993
11	2-acetyl-5-methylfuran	tr	−	6	6	MS + LRI	977	
12	dihydro-5-methylthiophen-3(2*H*)-one	3	2	5	3	MS	982	Farmer et al., 1989
13	dihydro-2-methylthiophen-3(2*H*)-one	66	64	26	33	MS + LRI	990	
14	5-mercaptohexan-2-one[e]	10	5	−	−	ms	993	43 (100), 71 (50), 55 (22), 61 (20), 41 (15), 47 (13),90 (7), **132** (5)
15	4,5-dihydro-2,4-dimethylthiophen-3(2*H*)-one (*E* or *Z*)	22	17	2	3	MS	1016	Farmer et al., 1989
16	4,5-dihydro-2,4-dimethylthiophen-3(2*H*)-one (*E* or *Z*)	5	5	−	−	MS	1027	Farmer et al., 1989
17	2-methyl-3-thiophenethiol	10	5	9	−	MS	1060	van den Ouweland and Peer, 1975
18	3-methyl-1,2-dithiolan-4-one	2	1	204	196	MS	1071	Shu et al., 1985
19	2-ethyl-4,5-dihydrothiophen-3(2*H*)-one	3	tr	6	−	MS	1082	ten Noever de Brauw et al., 1979
20	2-acetylthiophene	tr	tr	−	tr	MS + LRI	1092	
21	3,5-dimethyl-1,2-dithiolan-4-one (*E* or *Z*)	16	10	112	116	MS	1098	Farmer et al., 1989
22	3,5-dimethyl-1,2-dithiolan-4-one (*E* or *Z*)	12	8	77	86	MS	1105	Farmer et al., 1989
23	2-formyl-5-methylthiophene	7	tr	−	tr	MS + LRI	1124	
24	(3-thienyl)−2-propanone	4	tr	−	−	MS	1134	Farmer et al., 1989
25	2-methyl-(3-methylthio)thiophene	tr	tr	−	−	MS	1141	Güntert et al., 1993
26	2-acetyl-5-methylthiophene	7	10	4	3	MS + LRI	1157	
27	3-ethyl-1,2-dithiolan-4-one	15	6	48	30	MS	1167	Farmer et al., 1989
28	1-(3-thienyl)-1-propanone	6	6	12	1	MS + LRI	1183	
29	3-ethyl-5-methyl-1,2-dithiolan-4-one (*E* or *Z*)	tr	tr	7	tr	MS	1193	Farmer et al., 1989
30	3-ethyl-5-methyl-1,2-dithiolan-4-one (*E* or *Z*)	tr	tr	3	2	MS	1197	Farmer et al., 1989
31	2,3-dihydro-6-methylthieno[2,3-*c*]furan	tr	−	tr	4	MS + LRI	1199	ten Noever de Brauw et al., 1979
32	3-ethyl-2-formylthiophene	57	17	−	−	MS	1206	Farmer et al., 1989
33	thieno[3,2-*b*]thiophene	tr	−	−	−	MS	1213	Farmer et al., 1989
34	3-methyl-1,2-dithian-4-one	3	3	−	5	MS	1220	Hartman et al., 1984
35	diformylthiophene[e]	8	3	−	−	MS	1245	
36	3,5-dimethyl-1,2-dithian-4-one (*E* or *Z*)	3	1	−	−	MS	1251	Farmer et al., 1989
37	3,5-dimethyl-1,2-dithian-4-one (*E* or *Z*)	1	0	−	−	MS	1261	Farmer et al., 1989
38	a dihydrothienothiophene	tr	−	−	−	MS	1314	Farmer et al., 1989
39	a methylthienothiophene	tr	0	5	1	MS	1317	Farmer et al., 1989
40	a methylthienothiophene	16	9	3	1	MS	1355	Farmer et al., 1989
41	a dihydromethylthienothiophene	tr	1	5	3	MS	1378	Farmer et al., 1989
42	a dihydromethylthienothiophene	18	14	tr	−	MS	1409	Farmer et al., 1989
43	a dihydromethylthienothiophene	3	2	tr	−	MS	1418	Farmer et al., 1989
44	bis(1-methyl-2-oxopropyl) disulfide[e,f]	2	1	−	−	MS + LRI	1469	Mottram et al., 1995
45	bis(1-methyl-2-oxopropyl) disulfide[e,f]	2	1	−	−	MS + LRI	1474	Mottram et al., 1995
46	1-[2-methyl-(3-furyldithio)]propan-2-one[e]	−	−	3	tr	ms	1476	43 (100), 113 (65), 114 (32), 45 (26), **202** (25), 85 (12), 51 (11), 81 (10)
47	3-[2-methyl-(3-furyldithio)]butan-2-one	6	4	5	1	MS + LRI	1501	Mottram et al., 1995
48	bis(2-methyl-3-furyl) disulfide	2	3	7	2	MS + LRI	1537	Mottram et al., 1995
49	3-(1-methyl-2-oxopropyldithio)pentan-2-one[e]	tr	tr	−	−	MS + LRI	1539	Mottram et al., 1995
50	3-(2-oxobutyldithio)butan-2-one[e]	−	−	1	−	ms	1547	43 (100), 57 (63), 59 (18), 104 (14), 103 (12), 61 (10), **206** (7)
51	2-(1-methyl-2-oxopropyldithio)pentan-3-one[e,f]	2	2	−	−	MS + LRI	1555	Mottram et al., 1995
52	2-(1-methyl-2-oxopropyldithio)pentan-3-one[e,f]	2	1	tr	−	MS + LRI	1561	Mottram et al., 1995
53	1-[2-methyl-(3-furyldithio)]butan-2-one[e]	−	−	4	1	ms	1572	57 (100), 113 (67), 114 (45), **216** (38), 43 (26), 45 (20), 81 (14), 85 (13), 51 (12)
54	3-[2-methyl-(3-furyldithio)]pentan-2-one	1	1	3	tr	MS + LRI	1574	Mottram et al., 1995
55	2-[2-methyl-(3-furyldithio)]pentan-3-one	5	3	4	1	MS + LRI	1589	Mottram et al., 1995
56	bis(2-oxobutyl) disulfide[e]	−	−	tr	−	ms	1616	57 (100), 43 (16), 104 (10), **45** (9), 71 (4), **206** (3)
57	3-(1-methyl-2-oxobutyldithio)pentan-2-one[e,f]	1	−	−	−	MS + LRI	1624	Mottram et al., 1995
58	3-(1-methyl-2-oxobutyldithio)pentan-2-one[e,f]	1	−	−	−	MS + LRI	1630	Mottram et al., 1995
59	bis(1-methyl-2-oxobutyl) disulfide[e,f]	tr*		−	−	MS + LRI	1647	Mottram et al., 1995
60	bis(1-methyl-2-oxobutyl) disulfide[e,f]	tr*		−	−	MS + LRI	1651	Mottram et al., 1995
61	3-(3-thienyldithio)butan-2-one[e]	3	1	−	−	ms	1657	43 (100), **218** (46) 115 (39), 71 (36), 116 (32), 45 (28), 59 (18), 141 (17), 111 (17), 57 (13)
62	2-methyl-3-(3-thienyldithio)furan[e]	1	−	−	−	ms	1697	113 (100), **228** (54), 43 (43), 45 (43), 71 (40), 164 (24), 114 (21), 115 (20), 116 (15), 51 (14)

Table 1. (Continued)

no.	compound	approx concn[a] cysteine		approx concn[a] H_2S		method of ID[b]	LRI[c]	MS data, *m/z* (rel intensity), or reference[d]
63	3-[2-methyl-(3-thienyldithio)]butan-2-one[e]	1	tr	1	−	ms	1711	129 (100), 43 (72), 97 (54), 34 (53), 130 (46), **232** (42), 59 (37), 57 (23), 85 (19), 125 (15)
64	2-methyl-3-[2-methyl-(3-thienyldithio)]furan	tr	tr	2	tr	MS	1744	Werkhoff et al., 1990
65	2-(3-thienyldithio)pentan-3-one[e]	2	tr	−	−	ms	1747	57 (100), 71 (25), **232** (24), 115 (22), 116 (20), 45 (17), 59 (13), 141 (12)
66	3-[2-methyl-(3-thienyldithio)]pentan-2-one[e]	tr	−	tr	−	ms	1780	43 (100), 129 (74), 97 (71), 45 (60), 130 (39), **246 (**36), 85 (21), 161 (18), 73 (17), 59 (14), 41 (14)
67	1-[2-methyl-(3-thienyldithio)]butan-2-one[e]	tr*		1	−	ms	1787	57 (100), 45 (84), 129 (67), 130 (60), 97 (49), 43 (42), 59 (32), 85 (19), 53 (10), 71 (9), 111 (8), **232** (8)
68	2-[2-methyl-(3-thienyldithio)]pentan-3-one[e]	tr	−	−	−	ms	1795	57 (100), 123 (94), 130 (50), 45 (50), **246** (45), 97 (45), 59 (37), 125 (19), 85 (19)
69	bis(2-methyl-3-thienyl) disulfide	tr*		−	−	ms	1955	129 (100), 45 (53), **258** (39), 130 (30), 85 (22), 97 (18), 59 (14), 131 (9)

[a] Concentrations (μg/10 mg of HMF) obtained by comparing GC/MS peak area with that from 100 μg of methyl decanoate internal standard added to the HMF solution before volatile collection; duplicate analyses are shown; −, not detected (limit of detection ~0.1 μg/10 mg of HMF); tr, <1 μg/10 mg of HMF; tr*, found only in reaction mixtures with 10 times higher quantities of cysteine and HMF. [b] MS + LRI, identified by comparison of mass spectrum and LRI with those of authentic compound; MS, tentative identification by comparison with mass spectrum reported in the literature; ms, tentative identification by interpretation of mass spectrum. [c] Linear retention index. [d] Where neither mass spectrum nor a reference is given, the reference spectrum can be found in the NIST/EPA/NIH mass spectral database. [e] Not reported previously in meat or meatlike model systems. [f] Pairs of diastereoisomers.

kanones, such as 3-mercaptopentan-2-one (**8**) and 2-mercaptopentan-3-one (**9**), and the disulfides derived from them.

2,3-Pentanedione may be formed by two pathways. In the first, the key steps are the acid hydrolysis of HMF to yield 1-deoxypentosone followed by the reduction and acid-catalyzed dehydration of the latter (Figure 1a). The second pathway requires the presence of acetaldehyde, which is known to be formed from the hydrolysis or Strecker degradation of cysteine (Obata and Tanaka, 1965; Mottram, 1991). Retroaldolization (RA) of the 1-deoxypentosone, produced from the hydrolysis of HMF, can yield 2-oxopropanal (pyruvaldehyde) and, subsequently, hydroxyacetone by reduction. Aldol condensation (AC) of the latter with acetaldehyde, followed by dehydration, would yield 2,3-pentanedione (Figure 1b). It could be expected that the first pathway would operate in both reaction systems. However, **1** was not isolated from the mixture containing hydrogen sulfide alone and, although the mercaptopentanones **8** and **9** were formed in this reaction mixture, the quantities were only ~10% of those found in the cysteine-containing reaction. The second pathway appears to dominate in the system containing cysteine, yielding moderate to high concentrations of **1** and the mercaptopentanones **8** and **9**. Both reaction systems will have reducing properties due to the presence of hydrogen sulfide as a reactant or as a degradation product of cysteine. Such conditions will also permit the reduction of other keto groups, which are also required in the proposed pathways.

There is strong evidence that two other α-dicarbonyl compounds were formed in the reactions, 2,3-butanedione (diacetyl), in both reaction systems, and 2-oxobutanal in the system containing hydrogen sulfide alone. Evidence for the formation of these compounds centers on the identification of the corresponding mercaptoalkanones, 3-mercaptobutan-2-one (**5**) and 1-mercaptobutan-2-one (**7**), in these systems (Table 1). Another compound that is also predicted for the cysteine-containing reaction system is 3-hydroxy-2-butanone. All of these compounds are relatively volatile and were not

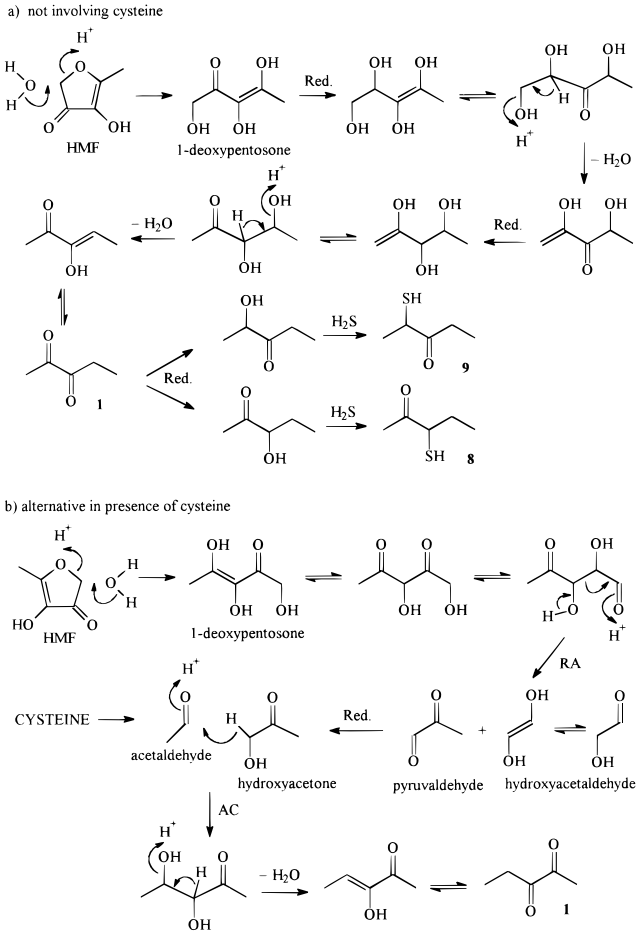

Figure 1. Two possible pathways for the formation of 2,3-pentanedione and two mercaptopentanones in the reaction of HMF with hydrogen sulfide or cysteine. (Red. = reduction.)

adequately entrained or chromatographed by the analytical procedures employed. The formation of diacetyl from HMF could follow pathways similar to those involved in the formation of 2,3-pentanedione, one not requiring acetaldehyde and the other utilizing cysteine-

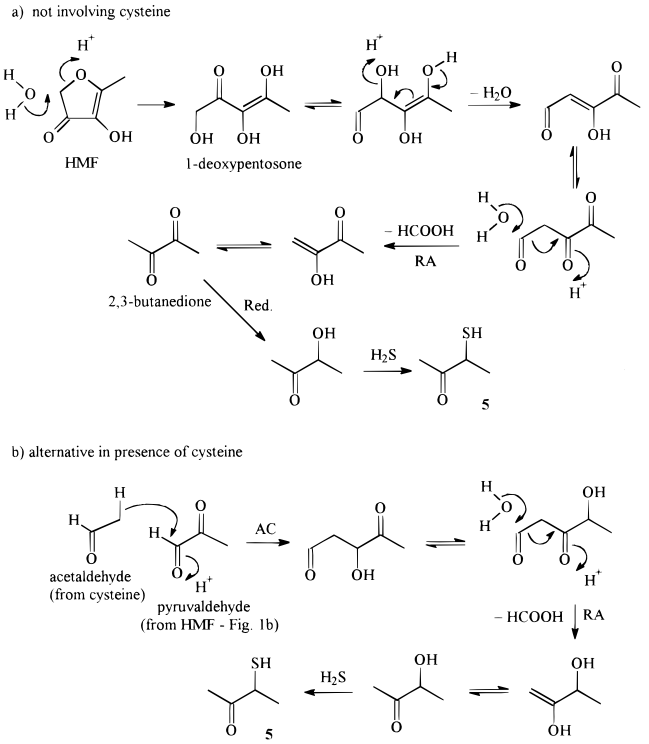

Figure 2. Possible pathways for the formation of 3-mercaptobutan-2-one from HMF by reaction with hydrogen sulfide showing an alternative pathway that could be involved in systems containing cysteine. (Red. = reduction.)

derived acetaldehyde. In the former, hydrolysis of HMF is the first step, followed by dehydration of the intermediate 1-deoxypentosone (Figure 2a). The loss of one carbon to give diacetyl occurs via RA with the elimination of formic acid. In the presence of cysteine, the suggested pathway for the formation of **5** requires AC between pyruvaldehyde and acetaldehyde followed by the elimination of formic acid by RA The resulting hydroxybutanone readily reacts with hydrogen sulfide to yield **5**.

In all of these schemes for the formation of mercaptoalkanones, with the exception of the latter pathway for **5**, reduction of the alkanedione to a hydroxyalkanone is required prior to substitution of −OH by −SH. The other essential steps in the pathways, shown in Figures 1 and 2, are keto−enol tautomerism, AC, RA, acid-catalyzed dehydration, and ring opening of HMF by acid-catalyzed hydrolysis. Important intermediates include acetaldehyde, pyruvaldehyde, hydroxyacetone, hydroxyacetaldehyde, and the different tautomers of 1-deoxypentosone. These intermediates are either too reactive or too water soluble to be isolated by the headspace analysis technique used in this work. Routes to the formation of most of the other major products of the reaction of HMF with hydrogen sulfide or cysteine can be explained using appropriate combinations of these reaction steps and these intermediates.

A scheme for the formation of 5-mercaptohexan-2-one (**14**) is presented in Figure 3. This compound was produced only in the reaction containing cysteine, which implicates the cysteine breakdown product, acetaldehyde, in its formation. The six-carbon chain is achieved by the AC of acetaldehyde and diacetyl. Reduction and dehydration of the resulting hydroxyhexanedione yields a hydroxyhexanone, which readily gives **14** by reaction with hydrogen sulfide.

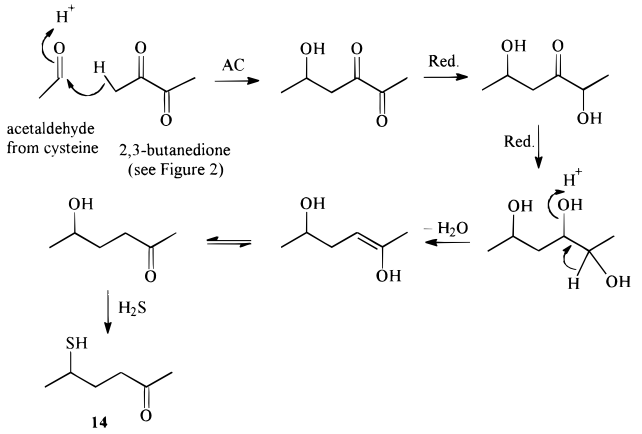

Figure 3. Possible pathway for the formation of 5-mercaptohexan-2-one from intermediates formed in the reaction of HMF with cysteine. (Red. = reduction.)

Furanthiols and Thiophenethiols. Thiol substitution in the 3-position on furan or thiophene rings generally gives volatile compounds with savory and meaty aromas and very low odor threshold values (Evers et al., 1976; Buttery et al., 1984; Werkhoff et al., 1993). Three such compounds were found in the HMF reaction mixtures, 2-methy-3-furanthiol (**6**), 2-methyl-3-thiophenethiol (**17**), and 3-thiophenethiol (**10**). The identity of **6** was confirmed by comparison of the mass spectrum and LRI with those of the authentic compound, but compounds **10** and **17** were only tentatively identified by comparing the spectra and retention data with those reported in the literature (Hofmann and Schieberle, 1995; Güntert et al., 1993).

Compounds **6** and **17** have both been found in cysteine−ribose model systems (Farmer et al., 1989; Hofmann and Schieberle, 1995). They were also reported by van den Ouweland and Peer (1975) to be major products of the reaction between HMF and hydrogen sulfide. However, these authors also reported that the di- and tetrahydro derivatives of **6** and **17** were also formed. In the present work, none of these hydrogenated derivatives were detected. Reaction conditions were different, with the earlier work carried out at higher concentrations of both reactants and a 100-fold excess of hydrogen sulfide, whereas the present work involved dilute aqueous solutions with similar concentrations of each reactant.

Compounds **6** and **17** were formed in similar quantities in the hydrogen sulfide and cysteine systems, which suggests that the pathways involved require only hydrogen sulfide and not other cysteine degradation products. A simple pathway for the formation of **6** is given in Figure 4. This involves the initial reduction of HMF to give a hydroxydihydrofuranone, which undergoes acid-catalyzed dehydration to 2-methyl-3-hydroxyfuran. Ketonization of this, followed by the addition of hydrogen sulfide and the elimination of a molecule of water, gives the thiol **6**. The substitution of the ring oxygen in **6** by a sulfur atom via ring opening has been suggested previously (van den Ouweland and Peer, 1975), and a pathway for the reaction is also shown in Figure 4, with the subsequent formation of the thiophenethiol **17** via a route similar to that proposed above for the furanthiol. If the intermediate 2-methyl-3(2*H*)-furanone or 2-methyl-3(2*H*)-thiophenone were reduced to the corresponding hydroxy derivatives prior to substitution of the −OH by −SH, 4,5-dihydro-2-methyl-3-

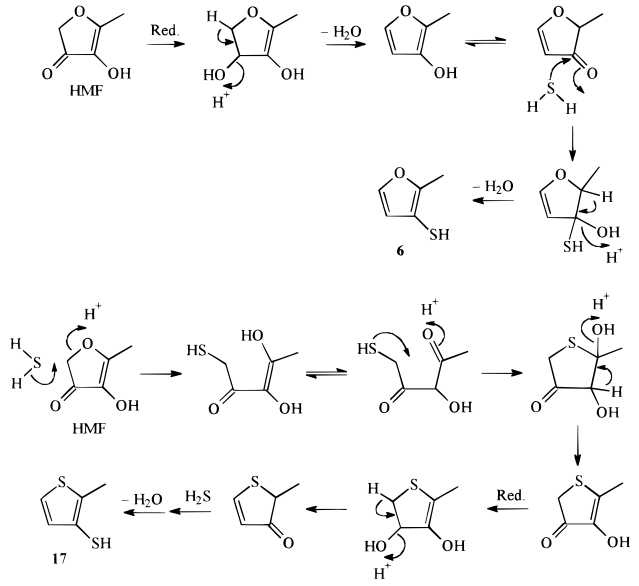

Figure 4. Possible pathways for the formation of 2-methyl-3-furanthiol and 2-methyl-3-thiophenethiol in the reaction of HMF with hydrogen sulfide. (Red. = reduction.)

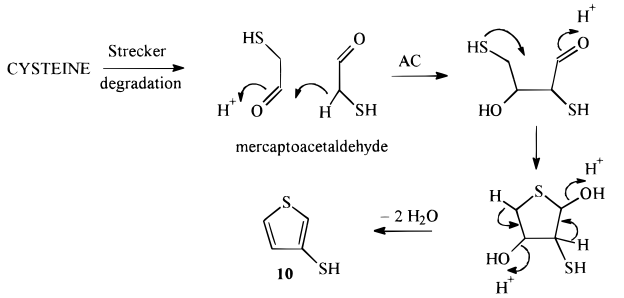

Figure 5. Suggested route for the formation of 3-thiophenethiol from mercaptoacetaldeyde derived from the Strecker degradation of cysteine. [Adapted from Shu et al. (1985).]

furanthiol and the corresponding thiophenethiol would result. Neither of these compounds were found in the reaction systems, suggesting that the latter pathway was not favored by the conditions used. Interestingly, dihydro-2-methyl-3-furanthiol and dihydro-2-methyl-3-thiophenethiol were found by van den Ouweland and Peer (1975) when they examined the reaction of HMF with a large excess of hydrogen sulfide.

Compound **10** was found only in the cysteine-containing system. It has been reported previously as a product of the thermal degradation of cysteine (Shu et al., 1985), and it has also been found in cooked meat (Werkhoff et al., 1993). Its aroma has been described as fatty, onion, and cooked meat, and it is reported to have an odor threshold value of 5−10 μg/kg. Shu et al. (1985) suggested that the compound could be derived from two molecules of mercaptoacetaldehyde, which is the Strecker aldehyde of cysteine. A pathway for this reaction is given in Figure 5.

Disulfides. Twenty-six disulfides were identified in the HMF reaction mixtures. They were derived from the mercaptoalkanones, furan and thiophene thiols, which were produced in the heated mixtures. They included symmetrical disulfides, formed from two molecules of the same thiol, and mixed disulfides containing different thiol moieties. Disulfides **44**, **45**, **51**, **52**, and **57**−**60**, derived from the mercaptoalkanones **5**, **8**, and **9**, possessed two asymmetric centers and, therefore, were present as pairs of diastereoisomers with almost identical mass spectra but slightly different retention times. Many of the disulfides had been isolated previously from the reactions of 2,3-butanedione or 2,3-pentanedione with hydrogen sulfide and 2-methyl-3-furanthiol (Mottram et al., 1995), but several (**46**, **50**, **53**, **56**, **61**−**63**, and **65**−**69**) are reported for the first time. All of the disulfides showed molecular ions and, except for compounds with small M^+ ions, they also contained M + 2 ions arising from the ^{34}S isotope. The main fragment ions showed patterns very similar to those of the parent thiols. Thus, disulfides derived from mercaptoalkanones contained a fragment of low intensity due to the oxoalkylthio ion from the fission of the disulfide bond, and strong *m*/*z* 43 or 57 ions depending on whether the keto group was in the 2- or 3- position. Compounds containing 2-methyl-3-furyldithio, 2-methyl-3-thienyldithio, or 3-thienyldithio moieties showed intense ions at *m*/*z* 113, 129, or 115, respectively, from the thio ion arising from the fission of the disulfide bond.

Disulfides containing 2-methyl-3-furyl and 2-methyl-3-thienyl moieties were found in both the cysteine and hydrogen sulfide systems, but those containing the 3-thienyl group were found only in the cysteine systems. Furthermore, where both of the moieties of the disulfide were derived from the mercaptoalkanones **5**, **8**, and **9**, the disulfides were, with one exception, found only in the cysteine system. Disulfides derived from 1-mercapto-2-butanone (**7**) were found only in the hydrogen sulfide system. These observations reflect the occurrences of the different thiols in the reaction mixtures. The furan and thiophene thiols **6** and **17** were present in similar quantities in both systems, whereas 3-thiophenethiol (**10**) was present only in the cysteine system. The mercaptoalkanones were present in higher concentrations in the cysteine reaction mixture, with the exception of **7**, which was found only in the hydrogen sulfide system.

Many disulfides are characterized by meaty or savory aromas and very low odor threshold values. Those disulfides containing the 2-methyl-3-furyl moiety possess meaty aromas (Evers et al., 1976; Gasser and Grosch, 1988; Mottram et al., 1995), and bis(2-methyl-3-furyl) disulfide has one of the lowest reported odor threshold values at 2×10^{-5} μg/kg (Buttery et al., 1984).

The formation of disulfides from thiols requires the elimination of two hydrogens. It has been shown that during the storage of solutions of 2-methyl-3-furanthiol in diethyl ether, the corresponding disulfide is formed by oxidation (Hofmann et al., 1996). However, the conditions in the reaction systems were not conducive to aerial oxidation, because of the relatively high concentrations of hydrogen sulfide. Furthermore, recent observations on the stability of thiols and disulfides during headspace collection and thermal desorption have shown that thiols are not converted to disulfides during the analysis procedures employed in this work (Mottram et al., 1998). Therefore, another redox system must be involved in the formation of the disulfides. In the formation of mercaptoalkanones, it has been proposed that α-dicarbonyls are reduced to hydroxyalkanones before substitution by hydrogen sulfide (Figures 1 and 2). This could provide the redox system required for the formation of the disulfides (Figure 6).

Thiophenones and Dithiolanones. Two dihydromethylthiophen-3(2*H*)-ones (**12** and **13**) and their 2,4-dimethyl homologue **15** were found in both reaction systems, although only small amounts of **15** and none

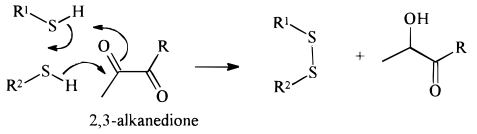

Figure 6. Proposed redox reaction between thiols and α-dicarbonyl compounds.

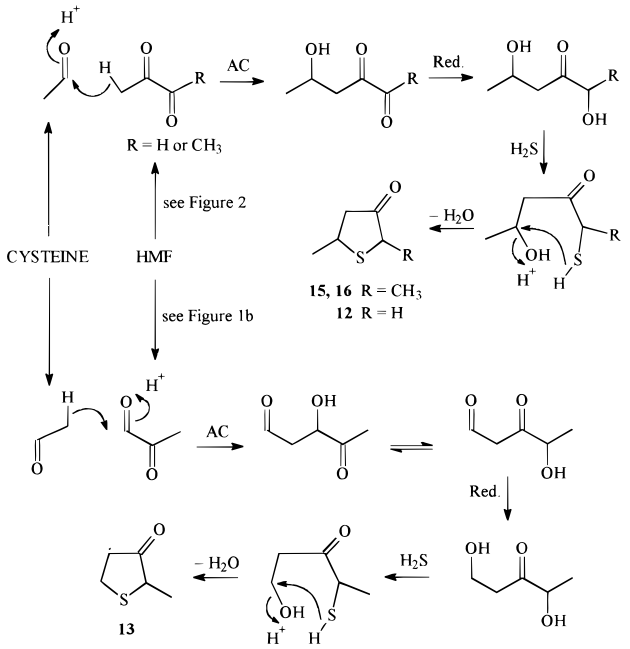

Figure 7. Possible pathways for the formation of dihydrothiophenones in reaction systems containing HMF and hydrogen sulfide or cysteine. (Red. = reduction.)

of its stereoisomer **16** were present in the hydrogen sulfide system. All of these compounds were shown previously to be major products in the reaction of cysteine and ribose (Farmer et al., 1989). Compound **12** has been found in a number of different processed foods, including meat and coffee, and it has been described as having a burnt coffee aroma (Flament, 1991).

A possible pathway for the formation of the isomers **15** and **16** is given in Figure 7. It involves AC between acetaldehyde, derived from cysteine, and diacetyl, the formation of which from HMF has already been discussed in Figure 2. The pathway is closely related to the formation of another C6 sulfur compound, **14** (Figure 3). The need for acetaldehyde in this pathway explains the very much larger quantities found in the cysteine system. A similar pathway, involving pyruvaldehyde instead of diacetyl, would explain the formation of **12** in the cysteine system, and an alternative AC between acetaldehyde and pyruvaldehyde produces the more abundant dihydromethylthiophenone (**13**). However, a different pathway is required to explain the smaller quantities of **12** and **13** that were found in the hydrogen sulfide reaction.

Several alkyl-1,2-dithiolan-4-ones (**18**, **21**, **22**, **27**, **29**, **30**) were tentatively identified in the HMF reaction mixtures. Although they were present in both reaction systems, their concentrations were very much higher in the hydrogen sulfide systems. All of these dithiolanones have been reported previously in the volatiles from heated cysteine−ribose model systems, where they were among the major products (Farmer et al., 1989).

Pathways to the formation of 1,2-dithiolanones necessitate the introduction of two −SH groups on carbon

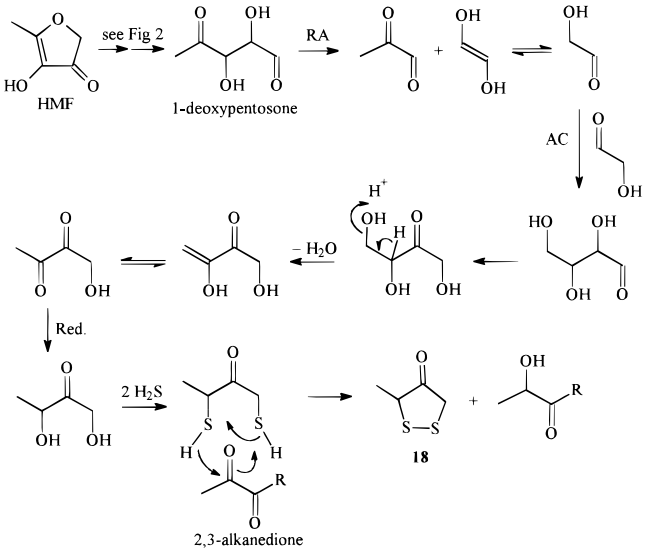

Figure 8. Possible pathways for the formation of 3-methyl-1,2-dithiolan-4-one in HMF reaction systems. (Red. = reduction.)

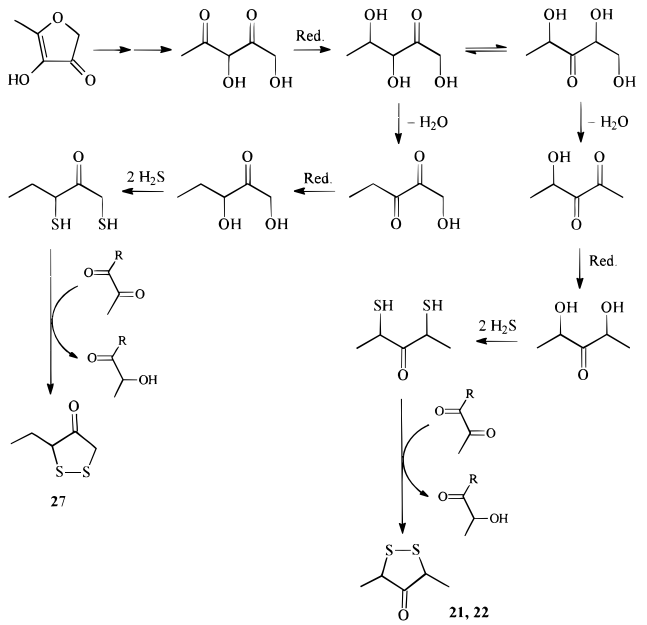

Figure 9. Possible pathways for the formation of 3,5-dimethyl-1,2-dithiolan-4-one and 3-ethyl-1,2-dithiolan-4-one in HMF reaction systems. (Red. = reduction.)

atoms separated by a carbonyl group. In the case of the monomethyl derivative **18**, the proposed pathway (Figure 8) involves the AC of two hydroxyacetaldehyde molecules formed, along with pyruvaldehyde, by the RA of 1-deoxypentosone, as discussed in Figure 1b. Reduction of the product followed by substitution with two molecules of hydrogen sulfide yields 1,3-dimercaptobutan-2-one. Conversion to the 1,2-dithiolan-4-one requires ring closure with the loss of two hydrogens. It is proposed that the redox system involved is an α-dicarbonyl in a pathway similar to that described above for the formation of disulfides from thiols.

In the case of the dimethyl and ethyldithiolanones **21**, **22,** and **27**, the later stages of the reaction proceed by the same pathway (Figure 9). However, the need for five carbons in the product means that the initial stages of the pathway could proceed via the formation of the intermediate dimercaptoketone by the reduction and dehydration of 1-deoxypentosone.

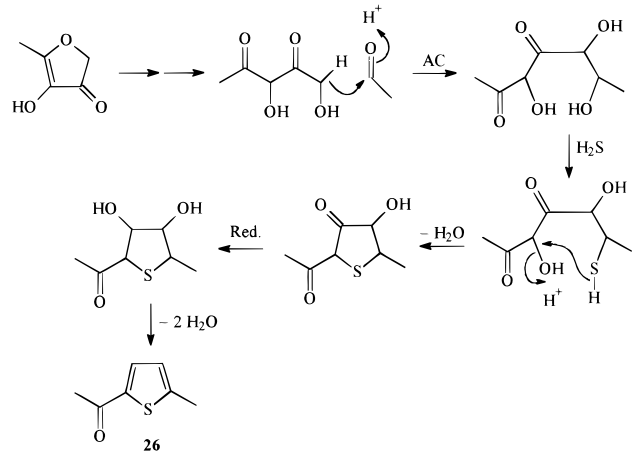

Figure 10. Possible pathways for the formation of 2-acetyl-5-methylthiophene in reaction systems containing HMF and hydrogen sulfide or cysteine. (Red. = reduction.)

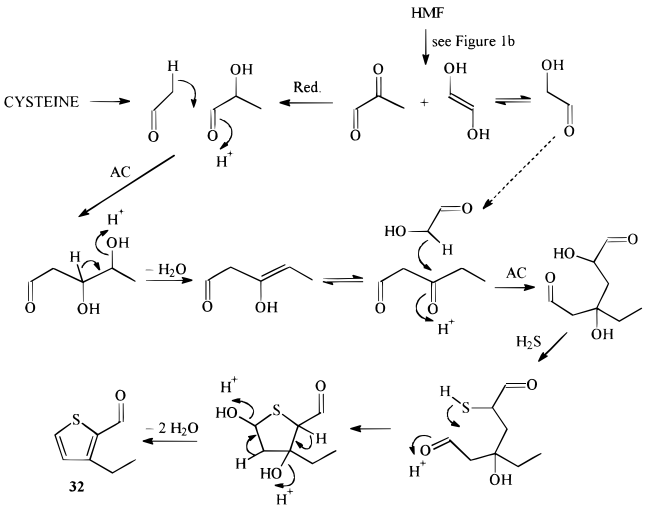

Figure 11. Possible pathway for the formation of 3-ethyl-2-formylthiophene in reaction systems containing HMF and cysteine. (Red. = reduction.)

Other Thiophenes. Among the products of the reaction mixtures were two acetylthiophenes, **20** and **26**, and three formylthiophenes, **23**, **32**, and **35**. The former were found in both reaction systems at similar concentrations, whereas the latter were associated with the cysteine−HMF reaction. All of these compounds were major volatile products of the reaction between cysteine and ribose (Farmer et al., 1989). Possible pathways to the formation of the two most abundant of these compounds, 2-acetyl-5-methylthiophene (**26**) and 3-ethyl-2-formylthiophene (**32**), are given in Figures 10 and 11. These pathways present further examples of the differences between the cysteine and hydrogen sulfide systems, showing the particular role of cysteine degradation products.

Several bicyclic thienothiophenes were also tentatively identified in the reaction mixtures. Similar compounds had been reported previously in cysteine−ribose model systems, and their mass spectra were discussed (Farmer et al., 1989). Other workers have found thieno-[3,2-*b*]thiophene in cysteine-ribose model systems (Mulders, 1973; Güntert et al., 1990). Dihydromethylthienothiophenes were the most abundant of the bicyclic compounds found in the HMF reactions, and possible pathways to three such compounds are shown in Figure 12. Further work would be necessary to assign these different structures to the GC/MS components.

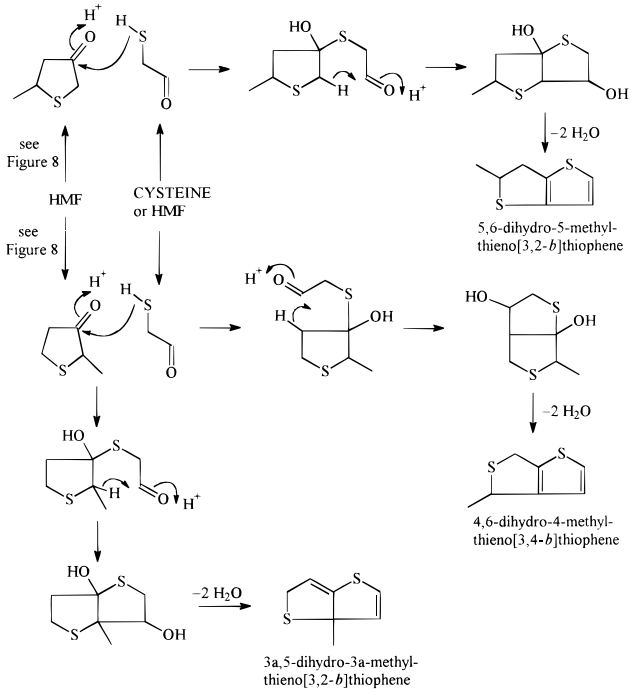

Figure 12. Possible pathway for the formation of dihydromethylthienothiophenes in reaction systems containing HMF and cysteine.

Conclusions. The reaction of HMF with hydrogen sulfide or cysteine in heated aqueous solution results in the formation of a complex mixture of aroma volatiles, most of which contain sulfur. Many of these volatiles are formed in model systems containing cysteine and ribose and make important contributions to meaty and savory aromas in cooked foods. The origin of many of the compounds has been explained, in this paper, by pathways involving the interaction of hydrogen sulfide with HMF, or its hydrolysis product, 1-deoxypentosone, or degradation products of these two compounds. Some compounds were found only in reactions containing cysteine and HMF, and pathways to these compounds necessitated the presence of acetaldehyde which, along with hydrogen sulfide, is a degradation product of cysteine. These reaction pathways may explain the routes to important aroma compounds found in heated foods.

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