Heterocyclic Volatiles Formed by Heating Cysteine or Hydrogen Sulfide with 4-Hydroxy-5-methyl-3(2*H*)-furanone at pH 6.5

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The reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone (HMF) with cysteine or hydrogen sulfide at pH 6.5 for 60 min at 140 °C produced complex mixtures of volatile compounds, the majority of these containing either sulfur or nitrogen. Of the 68 compounds detected, 63 were identified, some tentatively, by GC-MS. Among the identified compounds were thiophenes (10), thiophenones (6), thienothiophenes (5), thiazoles (5), trithiolanes (4), pyrazines (6), and oxazoles (4). More compounds were produced in the reaction of HMF with cysteine (63) than were formed in the reaction with hydrogen sulfide (33). In both systems, thiophenones were major reaction products, accounting for 25−36% of the total volatiles formed. Possible reasons for the differences in the composition of the two systems are discussed. The contributions of these reactions, and their products, to the flavor of heated foods are considered.

Keywords: *Maillard reaction; aroma; volatiles; GC-MS analyses; amino acids; pentoses*

INTRODUCTION

4-Hydroxy-5-methyl-3(2*H*)-furanone (HMF) is formed in Maillard reactions involving pentose sugars such as ribose (*1*). Both HMF and its 2,5-dimethyl homologue have been isolated from beef broth (*2*) and are considered to be likely precursors of meaty flavors through their reaction with either hydrogen sulfide or sulfur-containing amino acids (*3*, *4*). In studies of the reaction of cysteine and ribose at pH 5.6 it was shown that a wide range of compounds were formed containing one or more sulfur atoms (*5*, *6*). As a consequence, it was suggested that HMF was a possible intermediate in the formation of some of these compounds (*7*). To further the understanding of the role of HMF in such reactions, this compound was reacted with either cysteine or hydrogen sulfide at pH 4.5 (*8*) and was shown to yield many of the sulfur-containing compounds previously identified in reaction mixtures involving ribose. These compounds included disulfides (26), thiols (7), dithiolanones (6), thiophenones (4), dithianones (3), and thienothiophenes (6). Of these compounds the disulfides and their thiol precursors were particularly interesting because of the association of this type of compound with meatlike aromas (*9*). However, it is known that pH can influence the products formed in such reactions (*4*). As a consequence, we have repeated the reactions of HMF with cysteine and hydrogen sulfide at pH 6.5. Although a similar number of compounds were identified in these reaction mixtures as were found at pH 4.5, the number of sulfur-containing compounds found at pH 6.5 (40) was far fewer than found at pH 4.5 (64).

MATERIALS AND METHODS

Materials. Details of reagents used are given in a previous paper (*8*). HMF was obtained as a gift from a flavor company. Phosphate buffer (0.5 M, pH 6.5) was prepared in distilled water. A saturated solution of hydrogen sulfide (~0.2 M) was prepared by passing hydrogen sulfide through phosphate buffer (pH 6.5) at 0 °C as described previously (*8*). Authentic samples of reference compounds were either purchased from a range of laboratory chemical suppliers or obtained as gifts from flavor laboratories.

Reaction Mixtures. Mixtures containing 11.4 mg of HMF and 12.1 mg of cysteine in phosphate buffer (2 mL) were prepared in 5 mL glass ampules. Other mixtures were prepared by mixing 1 mL of a solution containing 12.1 mg of cysteine, in buffer, and 1 mL of the saturated solution of hydrogen sulfide (~6.6 mg). The ampules were flame sealed and then heated in an oven, controlled at 140 °C, for 60 min.

Isolation of Volatile Reaction Products. After cooling, the reaction mixtures were diluted with 20 mL of 0.5 M phosphate buffer (pH 6.5), and volatiles were collected on glass-lined stainless steel traps containing Tenax GC (Scientific Glass Engineering Pty. Ltd., Melbourne, Australia) as descibed previously (*8*). Prior to volatile collection, methyl decanoate (100 μg in 0.1 mL of ethanol) was added to the reaction mixture as an internal standard. During the collection of the volatile components, the mixture was stirred slowly and maintained at 60 °C in a water bath.

Gas Chromatography—Mass Spectrometry (GC-MS). A Varian 1440 gas chromatograph fitted with a "Unijector" (Scientific Glass Engineering Pty. Ltd.), and a fused-silica capillary column (50 m × 0.32 mm i.d.) coated with 5% phenyl methylsilicone, BP5 (Scientific Glass Engineering Pty Ltd.), 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 trapped volatile components were thermally desorbed onto the GC column by heating the trap at 260 °C for 5 min and were cyrofocused at the front of the GC column. Full details of the GC-MS conditions have been published previously (*8*). C_6−C_{20} *n*-alkanes (100 ng of each in 1 μL of diethyl ether added to a Tenax trap) were used as external standards for the calculation of linear retention indices of the volatile reaction products. 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 (*10*), and in previously published litera-

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10.1021/jf0008644 CCC: $20.00
Published on Web 01/30/2001

ture. When possible, confirmations of identifications were carried out by comparing linear retention indices (LRI) with those of authentic compounds. Masslib Software (MSP Friedli and Co., Köniz, Germany) was used to establish possible structures for some compounds with no reference mass spectra in the literature.

To obtain estimates of the relative quantities of the identified 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 68 compounds, including 5 not identified, were found in the headspace volatiles of reaction mixtures containing HMF and cysteine or hydrogen sulfide (Table 1). These included thiophenes (10), thiophenones (6), thienothiophenes (5), thiazoles (5), trithiolanes (4), pyrazines (6), and oxazoles (4). Whenever possible, identities were confirmed by comparison of the mass spectra and LRI with those of authentic compounds. For components for which no reference compounds were available, tentative identities were determined either by comparison with published data or by interpretation of their mass spectrum and comparison with related compounds. Except for the oxazoles, most of the other classes of these compounds had been previously identified in cysteine−ribose reaction mixtures (*5*, *11*−*15*). This was to be expected as HMF is a major product of the degradation of pentoses in Maillard and caramelization reactions (*1*).

The aromas of the reaction mixtures were evaluated by three assessors who worked in the laboratory. The aroma of the reaction between HMF and cysteine was described as caramel, roasted, and fried bacon rind, whereas that between HMF and hydrogen sulfide had an aroma described as caramel, metallic, and medicinal. Neither system had the meatlike aromas previously observed when these mixtures were reacted at pH 4.5 (*8*).

Of the 63 identified and tentatively identified compounds reported in Table 1, 58 were found in the volatiles formed in the reaction between HMF and cysteine, and 32 were found in the volatiles formed in the reaction between HMF and hydrogen sulfide. Both reaction mixtures gave similar quantities of total volatiles; those formed in the reaction with cysteine were 369 and 354 μg/10 mg of HMF for the duplicated reactions, whereas those formed in the reaction with hydrogen sulfide were 367 and 417 μg/10 mg of HMF.

There were major differences between the products of these reaction systems and those found previously in similar reactions carried out at pH 4.5 (*8*). The dominant compounds produced at pH 4.5, both qualitatively and quantitatively, were sulfur compounds, whereas at pH 6.5 far fewer sulfur compounds were produced in both systems. In the reaction of HMF with cysteine, nitrogen compounds accounted for 41% of the total volatiles produced (Table 2). The major classes of sulfur compounds not formed at pH 6.5 were mercaptoketones, furanthiols, thiophenethiols, and their related disulfides, which suggests that the reactions involved in their formation were promoted by acidic conditions. In addition, dithiolanones and dithianones, major compounds formed in both systems at pH 4.5, either were not formed or were formed in greatly reduced quantities at the higher pH (Table 2). At pH 6.5, the main sulfur compounds in both systems were thiophenones, whereas thiophenes were also major products of the reaction of HMF with hydrogen sulfide. Alkyl-substituted trithiolanes and trithianes were also found at pH 6.5 but were absent from the reaction performed at pH 4.5 (Table 2).

Expressed as a percentage of total volatiles found, the major compounds obtained from the reaction involving cysteine at pH 6.5 were 4,5-dihydro-2-methylthiophen-3(2*H*)-one (**28**), 11%; (*E* and *Z*) 4,5-dihydro-2,5-dimethylthiophen-3(2*H*)-one (**33** and **34**), 10%; unknown N compound **A** (**40**), 9%; unknown N compound **C** (**58**), 7−8%; 2,5-dimethylthiophene (**10**), 2−6%; 2,4,5-trimethyloxazole (**9**), 4−5%; 2,5(or 2,6)-dimethylpyrazine (**18**), 3−4%; and 4,5-dimethylthiazole (**23**), 3−4%. By comparison, the major compounds formed in the reaction between HMF and hydrogen sulfide at pH 6.5 were 2-acetylthiophene (**41**), 15−18%; 4,5-dihydro-2-methylthiophen-3(2*H*)-one (**28**), 13−18%; (*E* and *Z*) 4,5-dihydro-2,5-dimethylthiophen-3(2*H*)-one (**33** and **34**), 6−8%; 3-ethyl-2-formylthiophene (**57**), 5−6%; 2,5-dimethylthiophene (**10**), 1−7%; 4,5-dihydro-2-ethylthiophen-3(2*H*)-one (**39**), 5%; and 4,5-dihydro-2-methyl-3(2*H*)-furanone (**6**), 2−4%. Of these 13 compounds only the dihydrothiophenones **28**, **33**, and **34** and the thiophene **10** were major components in both reaction systems. Although individually present in minor concentrations, the thienothiophenes (**63**−**67**) and trithiolanes (**48**, **49**, **59**, and **60**) represented 4−6% and 4−7% of the total volatiles found in the reactions involving cysteine and 1−4% and 2−3% of those found in the reactions with hydrogen sulfide, respectively. Accordingly, as classes the thienothiophenes and trithiolanes make significant contributions to the volatile content of these reaction systems. However, major differences exist between the quantitative and qualitative compositions of the volatile components generated in the two systems. As a consequence, the remainder of this paper will concentrate on providing reasons for these differences.

Thiophenones. Reaction between HMF and cysteine gave six thiophenones (**27**, **28**, **33**, **34**, **39**, and **43**) that accounted for 25−27% of the total volatiles formed, whereas in the reaction between HMF and hydrogen sulfide they accounted for 29−36% of the volatiles recovered. All six compounds were formed in both systems. As such, they are major products of these reactions. However, with the exception of the stereoisomers **33** and **34** the other thiophenones were all formed in greater quantities in the reaction between HMF and hydrogen sulfide. Previously it had been proposed (*8*) that in comparable reactions performed at pH 4.5 compounds **27**, **28**, **33**, and **34** were formed from acetaldehyde (derived from cysteine) and pyruvaldehyde or 2,3-butanedione (both from HMF). To account for the current results, we now suggest alternative possible reaction sequences involving hydroxyacetaldehyde (in place of acetaldehyde), the above dicarbonyl compounds, and 2,3-pentanedione (all four from HMF, see ref *8*) for the formation of **27**, **28**, **33**, **34**, **39**, and **43**. A similar reaction sequence could account for the formation of 4,5-dihydro-2-ethylthiophen-3(2*H*)-one (**39**) with formalde-

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

no.	compound	approximate concn[a] cysteine		H_2S		method of ID[b]	LRI[c]	mass spectral data, *m/z* (rel intensity or reference)[d]
1	2,3-pentanedione	7	11	16	nd	MS + LRI	680	
2	3-penten-2-one	tr	nd	8	nd	MS + LRI	755	
3	4,5-dimethyloxazole	1	2	nd	nd	MS + LRI	771	
4	2-hexanone	1	1	4	29	MS + LRI	792	
5	3,4-hexanedione	3	3	6	nd	MS + LRI	800	
6	4,5-dihydro-2-methyl-3(2*H*)-furanone	6	8	15	8	MS	806	(*10*)
7	3-mercaptobutan-2-one	2	5	1	nd	MS + LRI	816	(*20*)
8	methylpyrazine	3	2	nd	nd	MS + LRI	825	
9	2,4,5-trimethyloxazole	20	14	nd	nd	MS + LRI	846	
10	2,5-dimethylthiophene	6	22	24	4	MS + LRI	867	
11	2,4-dimethylthiophene	nd	nd	9	11	MS + LRI	875	
12	2-methyl-2-thiazoline	6	4	nd	nd	MS + LRI	876	
13	2,4-hexanedione	tr	nd	9	7	MS + LRI	880	
14	2,4-dimethylthiazole	tr	nd	nd	nd	MS + LRI	883	
15	3,4-dimethylthiophene	1	4	1	nd	MS + LRI	887	
16	3-mercaptopentan-2-one	2	4	nd	nd	MS + LRI	902	(*20*)
17	2-methyl-2-cyclopenten-1-one	4	3	6	nd	MS + LRI	905	
18	2,5(or 2,6)-dimethylpyrazine	16	11	nd	nd	MS + LRI	910	
19	2,5-dimethylthiazole	1	nd	nd	nd	MS + LRI	915	
20	2,3-dimethylpyrazine	2	nd	nd	nd	MS + LRI	919	
21	4-ethyl-2,5-dimethyloxazole	1	nd	nd	nd	MS	919	
22	2-ethyl-4,5-dimethyloxazole	2	1	nd	nd	MS	925	
23	4,5-dimethylthiazole	13	11	nd	nd	MS + LRI	933	
24	2-ethyl-1*H*-pyrrole	1	2	nd	nd	MS + LRI	944	
25	1-(2-furyl)-2-propanone	3	4	nd	nd	MS	952	(*10*)
26	2-ethyl-5-methylthiophene	1	4	9	17	MS + LRI	959	
27	4,5-dihydro-5-methylthiophen-3(2*H*)-one	6	6	15	20	MS	982	(*12*)
28	4,5-dihydro-2-methylthiophen-3(2*H*)-one	39	40	48	75	MS + LRI	990	
29	2,4,5-trimethylthiazole	7	7	nd	nd	MS + LRI	997	
30	2-ethyl-(5 or 6)-methylpyrazine	8	7	nd	nd	MS + LRI	997	
31	2,3,5-trimethylpyrazine	10	7	nd	nd	MS + LRI	1002	
32	1-(2-furyl)-1-propanone	3	3	4	nd	MS + LRI	1008	
33	4,5-dihydro-2,5-dimethylthiophen-3(2*H*)-one (*E* or *Z*)[e]	31	28	17	29	MS	1016	(*12*)
34	4,5-dihydro-2,5-dimethylthiophen-3(2*H*)-one (*E* or *Z*)[e]	5	8	4	6	MS	1027	(*12*)
35	2,3-dimethyl-2-cyclopenten-1-one	6	4	7	10	MS + LRI	1040	
36	1-(5-methyl-2-furyl)-2-propanone	2	2	6	7	MS	1047	(*21*)
37	2-ethyl-3,6-dimethylpyrazine	3	2	nd	nd	MS + LRI	1078	
38	3-methyl-1,2-dithiolan-4-one	nd	nd	6	nd	MS	1071	(*22*)
39	4,5-dihydro-2-ethylthiophen-3(2*H*)-one	9	8	17	19	MS	1082	(*10*)
40	nitrogen compound **A**	34	31	nd	nd	ms	1089	**137** (100), 43 (82), 94 (62), 95 (58), 39 (57), 67 (20), 109 (18)
41	2-acetylthiophene	nd	nd	55	76	MS + LRI	1092	
42	3,5-dimethyl-1,2-dithiolan-4-one (*E* or *Z*)	1	nd	7	25	MS	1098	(*12*)
43	2-ethyl-5-methyl-4,5-dihydrothiophen-3(2*H*)-one	4	4	7	nd	ms	1104	74 (100), 41 (38), **144** (37), 45 (11), 39 (9), 42 (8), 59 (6)
44	2-thiazolyl-1-propanone	2	2	nd	nd	MS	1119	
45	2-formyl-5-methylthiophene	3	4	nd	nd	MS + LRI	1124	
46	3,4,5-trimethyl-2-furfural	2	2	nd	nd	MS	1130	
47	1-(3-thienyl)-2-propanone	1	nd	nd	nd	MS	1134	(*12*)
48	3,5-dimethyl-1,2,4-trithiolane (*E* or *Z*)	7	8	nd	nd	MS + LRI	1138	
49	3,5-dimethyl-1,2,4-trithiolane (*E* or *Z*)	7	14	nd	nd	MS + LRI	1144	
50	1-(dimethyl-2-furyl)-2-propanone	5	5	6	15	MS	1151	(*12*)
51	2-acetyl-5-methylthiophene	1	nd	6	nd	MS + LRI	1157	
52	nitrogen compound **B**	3	2	nd	nd	ms	1167	**151** (100), 108 (65), 43 (60), 109 (48), 53 (30), 80 (20), 123 (13)
53	3-ethyl-1,2-dithiolan-4-one	nd	nd	1	nd	MS	1169	(*12*)
54	sulfur compound MW 142	1	nd	9	19	ms	1171	**142** (100), 60 (64), 99 (34), 59 (27), 127 (21), 54 (20), 113 (13), 83 (13)
55	1-(3-thienyl)−1-propanone	2	nd	nd	nd	MS + LRI	1183	
56	2,3-dihydro-6-methylthieno[2,3*c*]furan	1	nd	nd	nd	MS + LRI	1199	(*10*)
57	3-ethyl-2-formylthiophene	nd	nd	22	21	MS	1206	(*12*)
58	nitrogen compound **C**	30	23	nd	nd	ms	1219	**151** (100), 108 (70), 43 (52), 109 (43), 53 (35), 80 (20), 136 (17)
59	3-ethyl-5-methyl-1,2,4-trithiolane (*E* or *Z*)	1	nd	4	7	MS	1242	(*12*)
60	3-ethyl-5-methyl-1,2,4-trithiolane (*E* or *Z*)	1	2	5	7	MS	1250	(*12*)
61	3-methyl-1,2,4-trithiane	3	3	nd	nd	MS + LRI	1254	
62	nitrogen compound **D**	4	2	nd	nd	ms	1314	**165** (100), 122 (63), 123 (33), 43 (32), 137 (17), 67 (16), 150 (12)
63	a dihydrothienothiophene	8	5	nd	nd	MS	1319	(*12*)

Table 1 (Continued)

no.	compound	approximate concn[a] cysteine		H_2S		method of ID[b]	LRI[c]	mass spectral data, *m/z* (rel intensity or reference)[d]
64	a methylthienothiophene	4	3	9	5	MS	1357	(*12*)
65	a dihydromethylthienothiophene	1	nd	4	nd	MS	1378	(*8*)
66	a dihydromethylthienothiophene	4	2	nd	nd	MS	1409	(*8*)
67	a dihydromethylthienothiophene	7	3	nd	nd	MS	1418	(*8*)
68	3-(2-methyl-3-furyldithio)-2-butanone	1	1	nd	nd	MS + LRI	1501	(*20*)
	total	369	354	367	417			

[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; nd, not detected (limit of detection ~0.1 μg/10 mg of HMF); tr, between 0.1 and 1 μg/10 mg of 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] Previously incorrectly reported as the 2,4-isomer (*8*).

Table 2. Comparison of Classes of Compounds Found in Reactions between HMF and Cysteine or Hydrogen Sulfide at pH 4.5 and 6.5

compound class	approximate concn[a] cysteine pH 4.5[b]	cysteine pH 6.5	H_2S pH 4.5[b]	H_2S pH 6.5
dithiolanones and dithianones	41	1	443	20
trithiolanes and trithianes	nd	23	nd	12
thiophenes	94	25	21	128
thiophenones	94	94	39	129
aliphatic and alicyclic ketones	28	22	39	51
thiols and mercaptoketones	294	7	44	1
disulfides	24	1	18	nd
furan derivatives	5	23	12	31
thiazoles and oxazoles	nd	42	nd	nd
pyrazines	nd	36	nd	nd
other nitrogen compounds	nd	71	nd	nd
total	580	345	616	372

[a] Mean concentrations (μg/10 mg of HMF) from duplicate analyses; nd, not detected. [b] From ref *8*.

hyde replacing hydroxyacetaldehyde in its reaction with 2,3-pentanedione. The presence of the six dihydrothiophenones in comparable concentrations in both reaction systems (Table 1) strongly supports the proposal that these compounds are principally derived from breakdown products of HMF. The compound tentatively identified as 2-ethyl-5-methyl-4,5-dihydrothiophen-3(2*H*)-one (**43**) has a simple mass spectrum similar to that of the 2-ethyl derivative (**39**). Compound **43** has a base peak *m/z* 74 ($C_2H_2OS^+$) corresponding to the loss of the netural fragments C_2H_4 and C_3H_6 (Figure 1). It also has weak ions at *m/z* 116 ($C_5H_8OS^+$) corresponding to the loss of C_2H_4 and *m/z* 102 ($C_4H_6OS^+$) corresponding to the loss of C_3H_6.

Thiophenes. A total of 10 thiophenes (**10**, **11**, **15**, **26**, **41**, **45**, **47**, **51**, **55**, and **57**) were formed in the two reaction systems. These thiophenes accounted for 4−10% of the total volatiles produced in the system with cysteine but 31−34% of those formed in the system with hydrogen sulfide. The major compounds formed in the reaction between HMF and hydrogen sulfide were 2-acetylthiophene (**41**), 15−18%; 3-ethyl-2-formylthiophene (**57**), 5−6%; 2,5-dimethylthiophene (**10**), 1−7%; and 2-ethyl-5-methylthiophene (**26**), 2−4%. The thiophenes **41** and **57** were not detected in the system with cysteine, whereas the other two compounds were present in either the same concentrations or slightly less (Table 1). Compounds **10** and **41** can be formed from the same pair of HMF retro-aldol condensation products (*8*), hydroxyacetaldehyde and 2,3-butandione, and **26** from hydroxyacetaldehyde and 2,3-pentanedione.

In previous studies of the reaction of HMF with cysteine or hydrogen sulfide at pH 4.5 (*8*), 3-ethyl-2-formylthiophene (**57**) was obtained in relatively high yield (17−57 μg/10 mg of HMF) in reactions containing cysteine but was not detected in the corresponding reactions with hydrogen sulfide. As a consequence, it was proposed that acetaldehyde from cysteine was a key precursor in the formation of **57**. However, in the current reactions at pH 6.5 the reverse was observed with significant concentrations of **57** (21−22 μg/10 mg of HMF) found in the volatiles obtained from the reaction of HMF with hydrogen sulfide, but **57** was not detected in those reactions involving cysteine (Table 1). Accordingly, an alternative reaction sequence involving precursors derived only from HMF (1-deoxypentosone and hydroxyacetaldehyde) is now proposed for the formation of **57** at pH 6.5.

Of the remaining thiophenes, **11** was formed only in the reaction with hydrogen sulfide, whereas **45** was formed only in small quantities in the reaction with cysteine (Table 1). The remaining four thiophenes (**15**, **47**, **51**, and **55**) were formed in only minor quantities in either one or other of the reaction systems (Table 1).

Trithiolanes and Dithiolanones. The four trithiolanes (**48**, **49**, **59**, and **60**) accounted for 4−7% of the total volatiles formed in the reaction with cysteine and 2−3% of those formed in the system with hydrogen sulfide. The trithiolanes **48** and **49** were formed only in the reaction with cysteine, whereas **59** and **60** were preferentially formed in the reaction with hydrogen sulfide (Table 1). It has been suggested (*16*) that **48** and **49** can be formed by the reaction of two molecules of acetaldehyde with hydrogen sulfide. In this sequence, acetaldehyde is derived from the decomposition of cysteine (*17*). The absence of a ready source of acetaldehyde in the reaction of HMF with hydrogen sulfide would account for the absence of **48** and **49** from the subsequent reaction products (Table 1). The trithiolanes **59** and **60** could also be formed by the same reaction sequence (*16*) by the reaction of propanal and acetaldehyde with hydrogen sulfide. However, **59** and **60** are formed in greater quantities in the reaction between HMF and hydrogen sulfide (a system with no ready source of acetaldehyde) than in the reaction of HMF with cysteine (Table 1). It is feasible that acetaldehyde can be formed by the breakdown of HMF, although the failure to detect the trithiolanes **48** and **49** as products of the reaction of HMF with hydrogen sulfide (Table 1) would indicate that acetaldehyde is not formed in significant quantities by this process. Furthermore, none of these trithiolanes were found when the reaction was carried out at pH 4.5 (*8*). The formation of **59** and

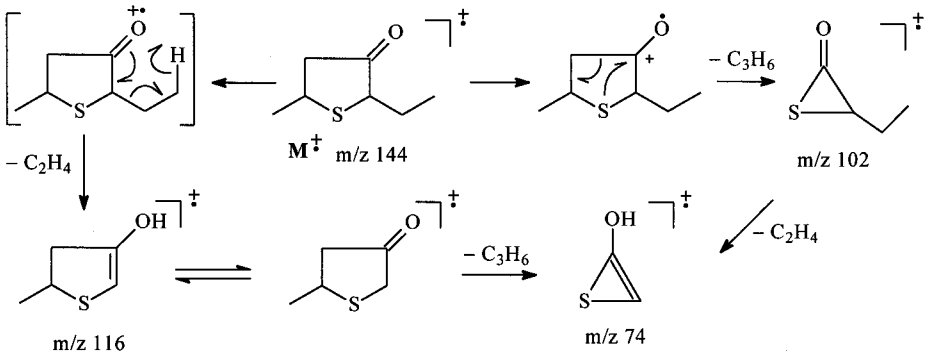

Figure 1. Proposed mass spectral fragmentation of 2-ethyl-5-methyl-4,5-dihydrothiophen-3(2*H*)-one.

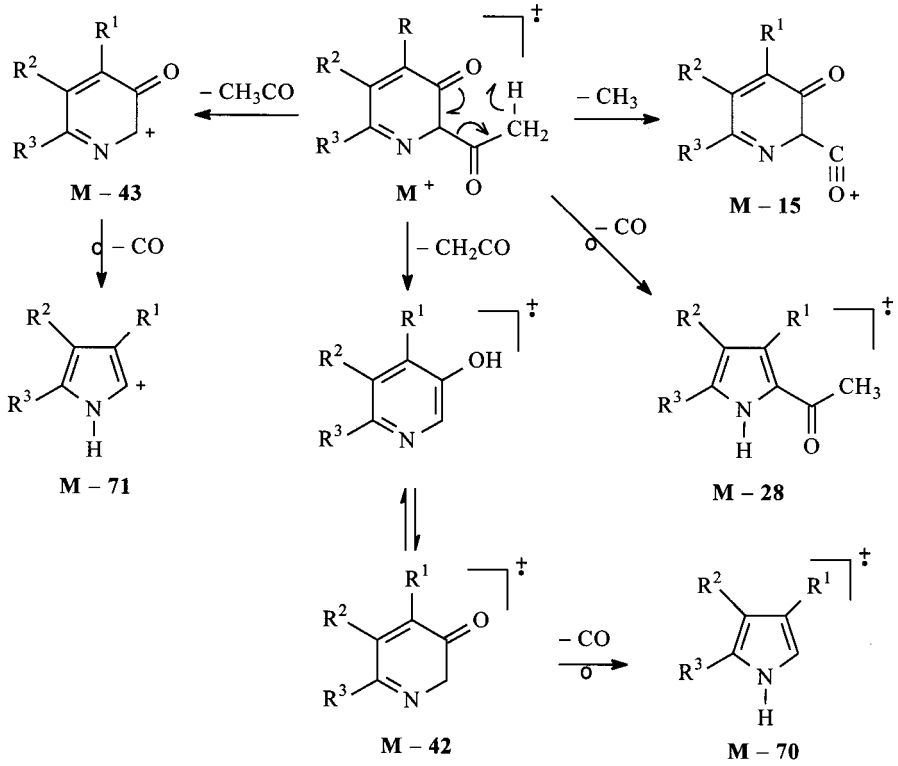

Figure 2. Proposed mass spectral fragmentation of acetylalkylpyridinones tentatively identified in the reaction of HMF with cysteine.

60 in the reaction between HMF and hydrogen sulfide warrants further study.

The three dithiolanones (**38**, **42**, and **53**) were found only in trace quantities in the reaction of HMF with cysteine but accounted for 4−6% of the total volatiles formed in the reaction of HMF with hydrogen sulfide. Significantly, these compounds were major products (>70%) of the reaction of HMF with hydrogen sulfide at pH 4.5 (*8*). The difference in yields of the dithiolanones **38**, **42**, and **53** from the reactions carried out at pH 4.5 and 6.5 indicates that pH has a profound effect on the course of the reactions leading to these compounds. Possible pathways for the formation of **38**, **42**, and **53** from the breakdown products of HMF have been described (*8*).

Thienothiophenes. Five thienothiophenes (**63**−**67**), accounting for 4−7% of the total volatiles, were formed in the reaction of HMF with cysteine. However, only two of these, **64** and **65**, accounting for 1−4% of the volatiles, were formed in the reaction of HMF with hydrogen sulfide (Table 1). Possible pathways to the three dihydromethylthienothiophenes **65**−**67** involving the reaction of the dihydromethylthiophen-3(2*H*)-ones **27** and **28** with mercaptoacetaldehyde have been described (*8*). The same reaction sequences can be used to account for the formation of the dihydrothienothiophene **63** from the reaction of tetrahydrothiophen-3(2*H*)-one and mercaptoacetaldehyde and of the methylthienothiophene **64** from the reaction of (2 or 5)-methylthiophen-3(2*H*)-one with mercaptoacetaldehyde. However, further work would be necessary to assign structures to these compounds. Of interest, although most of these thienothiophenes were formed in higher yields in the reaction of HMF with cysteine at pH 4.5 (*8*), the dihydrothienothiophene **63** was found in significant quantities only when this reaction was performed at pH 6.5.

Pyrazines, Thiazoles, Oxazoles, and Other Nitrogen Heterocyclic Compounds. Six pyrazines (**8**, **18**, **20**, **30**, **31**, and **37**), five thiazoles (**14**, **19**, **23**, **29**, and **44**), four oxazoles (**3**, **9**, **21**, and **22**), and four unidentified nitrogen compounds (**40**, **52**, **58**, and **62**) were formed in the reaction of HMF with cysteine (Table 1). The pyrazines account for 8−11% of the total volatiles, thiazoles, 6%; oxazoles, 5−7%; and the unidentified compounds, 16−19%.

Five of the six pyrazines have a feature common to

each of their structures. They have a methyl adjacent to an unsubstituted ring carbon atom on one side of the molecule. This would suggest that these pyrazines have a common precursor, possibly hydroxyacetone formed by the reduction of the HMF hydrolysis product, pyruvaldehyde. Other compounds that could be involved in the formation of these pyrazines would be glyoxal, pyruvaldehyde, 1,2-butanedione, 2,3-butanedione, and 2,3-pentanedione, all feasible hydrolysis products of HMF (*8*). The key steps in the formation of these pyrazines are the reaction of ammonia with 2-hydroxypropanal (from the reduction of pyruvaldehyde) to form an aminoacetone and the condensation of this compound with appropriate α-dicarbonyl compounds (*18*).

With the exception of the thiazoles **23** and **44** and the oxazoles **3** and **22**, all other thiazoles and oxazoles formed in the reaction of HMF with cysteine have a methyl substituent in the 2-position (Table 1). Accordingly, it is likely that one of the precursors to all of these compounds is acetaldehyde, derived from the decomposition of cysteine (*17*). Other compounds likely to be involved in the formation of such 2-methylthiazoles and oxazoles would be the HMF hydrolysis products pyruvaldehyde (for compounds **14** and **19**), 2,3-butanedione (for **9** and **29**), and 2,3-pentanedione (for **21**). The key steps in the reaction sequences to these compounds are the reaction of ammonia with acetaldehyde to form 1-aminoethanol and the condensation of this compound with appropriate α-dicarbonyl compounds. Subsequent cyclization of one of these condensation products followed by dehydration would lead to the oxazoles **9** and **21**. The thiazoles **14**, **19**, and **29** could be formed from the same condensation products by reaction with hydrogen sulfide followed by cyclization and dehydration (*19*).

The four unidentified nitrogen compounds (**40**, **52**, **58**, and **62**) are major volatile products (16−19%) of the reaction of HMF with cysteine. Only the thiophenones (25−27%) were produced in greater quantities in this system. All four compounds gave mass spectra with the molecular ions as base peaks, and all had fragment ions at M − 15 (M − CH_3), M − 28 (M − CO), M − 42 (M − CH_2=C=O), M − 43 (M − CH_3CO), M − 42 − 28 (M − CH_2=C=O−CO), and M − 43 − 28 (M − CH_3CO−CO). Interpretation of their mass spectra using Masslib Software indicated that **40** could be 2-acetyl-3(2*H*)-pyridinone and that **52**, **58**, and **62** could also be 2-acetyl-3(2*H*)-pyridinones with alkyl substituents in the 4-, 5-, or 6-position. The fragmentation patterns of these four components can be rationalized as shown in Figure 2. However, a search of the literature has shown that these compounds have not been synthesized. Accordingly, studies are currently in progress to establish the structures of the major reaction products **40** and **58**.

In summary, the reactions of HMF with cysteine or hydrogen sulfide in heated aqueous solutions at either pH 4.5 or 6.5 produced complex mixtures of volatiles. However, the compositions of the mixtures formed at different pH values were significantly different. At pH 4.5 the dominant compounds produced, both qualitatively and quantitatively, were sulfur compounds (*8*), whereas at pH 6.5 41% of the total volatiles formed in the reaction of HMF with cysteine were nitrogen compounds and far fewer sulfur compounds were produced in both the cysteine and hydrogen sulfide systems. These results further demonstrate the important role of pH in determining the course of the later stages of the Maillard reaction. In particular, at pH 4.5 ammonia from the decomposition of cysteine is essentially not available for reaction with the HMF hydrolysis products to form nitrogen compounds such as pyrazines, thiazoles, and oxazoles. However, lower pH values are essential for the reaction of hydrogen sulfide with such HMF products to form mercaptoketones, furan- and thiophenethiols, and dithiolanones. These compounds make important contributions to the meaty characteristics of cooked meats. Accordingly, the reactions described in this, and in our earlier work (*8*), may explain not only the routes to some important aroma compounds in heated foods but also the conditions necessary for their formation. Future studies should therefore be directed toward establishing whether such reaction pathways and reaction conditions are of similar importance in the formation of key cooked flavor compounds in natural systems.

ACKNOWLEDGMENT

We gratefully acknowledge the assistance of Kevin Shaw in carrying out the GC-MS analyses and Franz Hammerschmidt (DRAGOCO, Holzminden, Germany) for the Masslib Software interpretations of unidentified mass spectra.

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Received for review July 11, 2000. Revised manuscript received December 5, 2000. Accepted December 5, 2000.

JF0008644