

Investigation of the Reaction between Ammonium Sulfide, Aldehydes, and α -Hydroxyketones or α -Dicarbonyls To Form Some Lipid–Maillard Interaction Products Found in Cooked Beef

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Aqueous reaction mixtures containing α -dicarbonyls (2,3-butanedione or 2,3-pentanedione) or α -hydroxyketones (1-hydroxypropanone, 1-hydroxy-2-butanone, or 3-hydroxy-2-butanone), alkanals (C₄–C₁₀) and ammonium sulfide were heated at 140 °C for 30 min. Among the products formed were 3-thiazolines and thiazoles with C₃–C₉ alkyl substituents in the 2-position and methyl or ethyl in positions 4 and/or 5. Similar compounds have recently been reported in cooked beef, and their presence in the reaction mixtures confirmed the proposed route of formation, in cooked beef, from the reaction of lipid-derived aldehydes with dicarbonyls, ammonia, and hydrogen sulfide produced via the Maillard reaction. Trialkylpyridines, which had been observed in cooked beef, were also formed by the reaction of three molecules of aldehyde with one of ammonia. The mass spectra of all of these compounds are reported.

Keywords: Flavor; Maillard reaction; lipids; 3-thiazolines; thiazoles; alkylpyridines

INTRODUCTION

During the cooking of foods, flavor is generated from the interaction of a number of intermediates produced by the thermal degradation of natural flavor precursors. The Maillard reaction, between amino acids and reducing sugars, and the thermal degradation of lipid are the major reactions involved. Model systems have been used to elucidate the intermediates and the pathways involved in the formation of some of the flavor volatiles found in cooked foods (Vernin and Párkányi, 1982).

Alkylthiazoles and alkylthiazolines can have low odor threshold values and may contribute to nutty, roast, and meaty aroma characteristics in foods (Fors, 1983). They are found in heated foods, such as coffee, meat, and bread. Thiazoles have been found in model systems involving cysteine and reducing sugars (Mulders, 1973; Vernin and Párkányi, 1982). One of the most likely routes to the formation of alkylthiazoles and alkylthiazolines in foods and model systems is from the reaction of an α -dicarbonyl or an α -hydroxyketone (from sugar degradation), hydrogen sulfide, ammonia, and an aldehyde. The Strecker degradation of cysteine produces acetaldehyde, ammonia, and hydrogen sulfide, providing three key intermediates for thiazole and thiazoline formation. Takken et al. (1976) found 4-ethyl-2,5-dimethyl-3-thiazoline, 5-ethyl-2,4-dimethyl-3-thiazoline, and the corresponding thiazoles in a reaction mixture containing 2,3-pentanedione, acetaldehyde, hydrogen sulfide, and ammonia. Vernin et al. (1987) identified 2-isopropyl-4,5-dimethyl-3-thiazoline and 2-isopropyl-4,5-dimethylthiazole in a reaction mixture containing 3-hydroxy-2-butanone, 2-methylpropanal, and ammonium sulfide.

Recently, a number of alkylthiazoles with long *n*-alkyl chains in the 2-position were reported in the volatiles of cooked beef and chicken (Farmer and Mottram, 1994).

A number of these had chain lengths of between 13 and 16 carbons, and it was suggested that aldehydes, derived from meat plasmalogens, were involved in the formation of these compounds. Other 2-*n*-alkylthiazoles have been reported in fried chicken and fried potatoes, although these had chain lengths of C₃–C₈ (Ho and Carlin, 1989; Tang et al., 1983). We have also recently reported a large number of alkylthiazoles and alkyl-3-thiazolines, with C₃–C₉ alkyl substituents in the 2-position, in cooked beef (Elmore et al., 1997). However, the identifications made were tentative because no reference compounds were available. Mass spectral interpretation and comparison of the spectra of the unknowns with a limited number of literature spectra (Tang et al., 1983; Werkhoff et al., 1991; Jennings and Shibamoto, 1980) were the criteria used for the identification. It was suggested that these thiazoles and 3-thiazolines were derived from the interaction of aliphatic aldehydes, from lipid oxidation, with hydrogen sulfide, ammonia, and dicarbonyls or hydroxyketones, from the Maillard reaction.

In this paper we confirm this proposed route of formation of 3-thiazolines and thiazoles in cooked beef and report on the alkylthiazoles, alkyl-3-thiazolines, and alkylpyridines formed in aqueous reaction mixtures containing α -dicarbonyls (2,3-butanedione or 2,3-pentanedione) or α -hydroxyketones (1-hydroxypropanone, 1-hydroxy-2-butanone, or 3-hydroxy-2-butanone), alkanals, and ammonium sulfide.

EXPERIMENTAL PROCEDURES

Materials. 3-Methylbutanal, pentanal, octanal, (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal, decanal, 1-hydroxy-2-propanone (acetol), 1-hydroxy-2-butanone, 3-hydroxy-2-butanone (acetoin), 2,3-butanedione (diacetyl), 2,3-pentanedione, and ammonium sulfide (20% in water) were obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K.; 2-methylpropanal, hexanal, heptanal, and nonanal were from Lancaster Synthesis, Morecambe, U.K.

Preparation of Reaction Mixtures. A 0.1 M solution of ammonium sulfide was made up in deionized water. An aliquot (50 mL) was poured into a 100 mL autoclavable screw-

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Table 1. 3-Thiazolines, Thiazoles, and Pyridines Isolated from the Reactions of α -Hydroxyketones with Ammonium Sulfide and Aliphatic Aldehydes

aldehyde	products and relative amounts (as percentage of total chromatogram peak area)					
	3-thiazolines	%	thiazoles	%	pyridines	%
Reactions Using 3-Hydroxy-2-butanone						
2-methylpropanal	4,5-dimethyl-2-isopropyl ^a	26.0	4,5-dimethyl-2-isopropyl	0.6		
3-methylbutanal	4,5-dimethyl-2-isobutyl ^a	15.2	4,5-dimethyl-2-isobutyl	0.2	3,5-diisopropyl-2-isobutyl	0.8
pentanal	2-butyl-4,5-dimethyl ^a	18.2	2-butyl-4,5-dimethyl	0.2	2-butyl-3,5-dipropyl	2.4
hexanal	4,5-dimethyl-2-pentyl ^a	36.7	4,5-dimethyl-2-pentyl	1.7	3,5-dibutyl-2-pentyl	2.2
heptanal	4,5-dimethyl-2-hexyl ^a	41.8	4,5-dimethyl-2-hexyl	0.3	3,5-dipentyl-2-hexyl	9.7
octanal	4,5-dimethyl-2-heptyl ^a	33.5	4,5-dimethyl-2-heptyl	0.5	3,5-dihexyl-2-heptyl	22.6
nonanal	4,5-dimethyl-2-octyl ^a	31.4	4,5-dimethyl-2-octyl	0.4		
decanal	4,5-dimethyl-2-nonyl ^a	26.2	4,5-dimethyl-2-nonyl	0.7		
(<i>E</i>)-2-nonenal	4,5-dimethyl-2-hexyl ^a	0.9				
	4,5-dimethyl-2-(1-octenyl) ^a	0.2				
	4,5-dimethyl-2-octyl ^a	1.4				
(<i>E,E</i>)-2,4-nonadienal	2-butyl-4,5-dimethyl ^a	1.7				
	4,5-dimethyl-2-(1,3-octadienyl) ^a	0.7				
Reactions Using 1-Hydroxy-2-propanone						
3-methylbutanal	2-isobutyl-5-methyl ^a	1.8			3,5-diisopropyl-2-isobutyl	2.8
	2-isobutyl-4-methyl	6.6				
pentanal	2-butyl-5-methyl ^a	2.3			2-butyl-3,5-dipropyl	2.6
	2-butyl-4-methyl	5.7	2-butyl-4-methyl	<0.1		
hexanal	5-methyl-2-pentyl ^a	4.4			3,5-dibutyl-2-pentyl	4.7
	4-methyl-2-pentyl	18.3	4-methyl-2-pentyl	0.7		
heptanal	2-hexyl-5-methyl ^a	3.8	2-hexyl-5-methyl	<0.1	3,5-dipentyl-2-hexyl	39.6
	2-hexyl-4-methyl	14.3	2-hexyl-4-methyl	0.6		
octanal	2-heptyl-5-methyl ^a	1.5			3,5-dihexyl-2-heptyl	43.5
	2-heptyl-4-methyl	5.5	2-heptyl-4-methyl	0.5		
nonanal	5-methyl-2-octyl ^a	1.6				
	4-methyl-2-octyl	6.9	4-methyl-2-octyl	<0.1		
Reactions Using 1-Hydroxy-2-butanone						
hexanal	5-ethyl-2-pentyl ^a	15.2			3,5-dibutyl-2-pentyl	11.3
	4-ethyl-2-pentyl	16.0	4-ethyl-2-pentyl	1.2		
heptanal	5-ethyl-2-hexyl ^a	8.0	5-ethyl-2-hexyl	<0.1	3,5-dipentyl-2-hexyl	19.5
	4-ethyl-2-hexyl	8.3	4-ethyl-2-hexyl	1.0		
octanal	5-ethyl-2-heptyl ^a	4.8			3,5-dihexyl-2-heptyl	48.1
	4-ethyl-2-heptyl	7.6	4-ethyl-2-heptyl	0.6		
nonanal	5-ethyl-2-octyl ^a	10.5				
	4-ethyl-2-octyl	21.2	4-ethyl-2-octyl	0.8		

^a For each 5-substituted 3-thiazoline two isomers (*E* and *Z*) were found in approximately equal quantities. Combined percentage peak areas are given.

top jar fitted with a PTFE-lined cap. To this solution were added 0.005 mol of aldehyde and 0.005 mol of hydroxyketone (or dione). The components of these reaction mixtures are shown in Tables 1 and 2. The mixtures were heated in an autoclave for 30 min at 140 °C.

The reaction mixtures were each extracted with 30 mL of high-purity grade dichloromethane (Fisons, Loughborough, U.K.), and the dichloromethane layer was separated off and stored in a brown glass bottle at 4 °C before analysis by gas chromatography/mass spectrometry.

GC/MS. All analyses were performed on a Hewlett-Packard 5972 mass spectrometer, fitted with a HP 5890 Series II gas chromatograph and controlled by a G1034C Chemstation. Samples (1 μ L) were injected in split mode at 250 °C (split ratio 40:1) onto a BPX5 fused silica capillary column (50 m \times 0.32 mm i.d., 0.5 μ m film thickness; Scientific Glass Engineering Ltd., Milton Keynes, U.K.). The oven was held at 60 °C for 2 min and then heated at 10 °C/min to 280 °C, at which it was held for 10 min. Helium at 8 psi was used as the carrier gas, resulting in a flow of 1.75 mL/min at 40 °C. A series of *n*-alkanes (C₆–C₂₂) was analyzed under the same conditions to obtain linear retention index (LRI) values for the reaction mixture components.

The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μ A. The mass spectrometer scanned from *m/z* 28 to 450 at 1.7 scans/s.

RESULTS AND DISCUSSION

The chromatograms from the reaction mixtures containing 3-hydroxy-2-butanone and the alkane- α -diones

contained between 5 and 10 major peaks, whereas the 1-hydroxy-2-ketones were more complex. Each reacting aldehyde and hydroxyketone/dione was present at the end of the reaction at concentrations below 10% of the total peak area, except for the reaction between nonanal and 1-hydroxy-2-propanone (35% of total peak area) and the reaction between nonanal and 2,3-butanedione (33%). Total peak areas for all the reaction mixtures were similar.

Thiazoles and 3-Thiazolines. All of the reaction mixtures produced 3-thiazolines and thiazoles in which the 2-position was occupied by an alkyl group derived from the aliphatic aldehyde, while methyl or ethyl substituents in the 4- or 5-position were from the dicarbonyl or hydroxyketone (Figure 1). The relative proportions of these products varied substantially, depending on whether a hydroxyketone or dione was used and which aldehyde was present (Tables 1 and 2). The highest yields of 3-thiazolines were obtained with 3-hydroxy-2-butanone, which gave 4,5-dimethyl-2-alkyl-3-thiazolines. The 1-hydroxy-2-alkanones gave a mixture of the 4-methyl- and 5-methyl-2-alkyl-3-thiazolines, but the 4-isomers were present in greater quantities than the 5-isomers, especially for the reactions involving the longer chain aldehydes. For all of the 3-thiazolines containing a substituent in the 5-position, a pair of geometric isomers can be predicted, and for the dimethyl-3-thiazolines (Table 1) and the methylethyl-3-thiazolines (Table 2) these eluted as characteristic

Table 2. Thiazolines, Thiazoles, and Pyridines Isolated from the Reactions of Alkane- α -diones with Ammonium Sulfide and Aliphatic Aldehydes

aldehyde	products and relative amounts (as percentage of total chromatogram peak area)					
	3-thiazolines ^a	%	thiazoles	%	pyridines	%
Reactions Using 2,3-Butanedione						
pentanal	2-butyl-4,5-dimethyl	13.4	2-butyl-4,5-dimethyl	12.8	2-butyl-3,5-dipropyl	3.5
hexanal	4,5-dimethyl-2-pentyl	15.7	4,5-dimethyl-2-pentyl	16.8	3,5-dibutyl-2-pentyl	8.1
heptanal	4,5-dimethyl-2-hexyl	8.0	4,5-dimethyl-2-hexyl	6.9	3,5-dipentyl-2-hexyl	29.4
octanal	4,5-dimethyl-2-heptyl	4.9	4,5-dimethyl-2-heptyl	3.3	3,5-dihexyl-2-heptyl	27.6
nonanal	4,5-dimethyl-2-octyl	9.4	4,5-dimethyl-2-octyl	2.6		
Reactions Using 2,3-Pentanedione						
3-methylbutanal	4-ethyl-2-isobutyl-5-methyl	6.0	4-ethyl-2-isobutyl-5-methyl	3.1	3,5-diisopropyl-2-isobutyl	0.7
	5-ethyl-2-isobutyl-4-methyl	7.8	5-ethyl-2-isobutyl-4-methyl	3.0		
pentanal	2-butyl-4-ethyl-5-methyl	10.4	2-butyl-4-ethyl-5-methyl	6.0	2-butyl-3,5-dipropyl	2.9
	2-butyl-5-ethyl-4-methyl	10.1	2-butyl-5-ethyl-4-methyl	4.4		
hexanal	4-ethyl-5-methyl-2-pentyl	14.8	4-ethyl-5-methyl-2-pentyl	7.1	3,5-dibutyl-2-pentyl	6.4
	5-ethyl-4-methyl-2-pentyl	10.4	5-ethyl-4-methyl-2-pentyl	5.2		
heptanal	4-ethyl-2-hexyl-5-methyl	3.8	4-ethyl-2-hexyl-5-methyl	4.0	3,5-dipentyl-2-hexyl	12.2
	5-ethyl-2-hexyl-4-methyl	5.8	5-ethyl-2-hexyl-4-methyl	4.2		
octanal	4-ethyl-2-heptyl-5-methyl	4.6	4-ethyl-2-heptyl-5-methyl	1.3	3,5-dihexyl-2-heptyl	29.1
	5-ethyl-2-heptyl-4-methyl	4.9	5-ethyl-2-heptyl-4-methyl	1.0		
nonanal	4-ethyl-5-methyl-2-octyl	10.4	4-ethyl-5-methyl-2-octyl	4.2		
	5-ethyl-4-methyl-2-octyl	12.5	5-ethyl-4-methyl-2-octyl	4.0		
decanal	4-ethyl-5-methyl-2-nonyl	18.7	4-ethyl-5-methyl-2-nonyl	7.3		
	5-ethyl-4-methyl-2-nonyl	18.9	5-ethyl-4-methyl-2-nonyl	5.7		

^a For each 3-thiazoline two isomers (*E* and *Z*) were found in approximately equal quantities. Combined percentage peak areas are given.

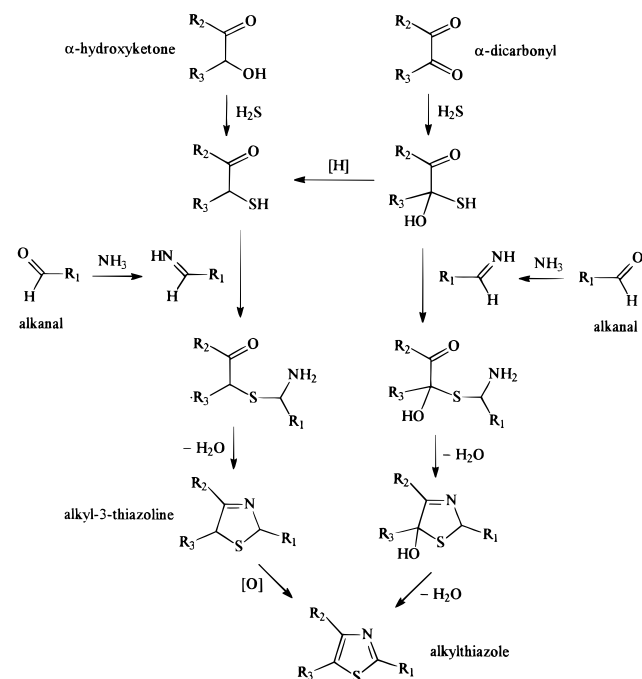


Figure 1. Formation of alkyl-3-thiazolines and alkythiazoles from the reaction of α -hydroxyketones or α -dicarbonyls with ammonia and hydrogen sulfide in the presence of aliphatic aldehydes

doublets in the chromatograms. In the case of the 5-methyl- and 5-ethyl-3-thiazolines (Table 1) the two peaks were poorly resolved.

Oxidation of thiazolines leads to thiazoles, and the reaction mixtures involving saturated aliphatic aldehydes all yielded thiazoles corresponding to the 3-thiazolines. In reaction mixtures using hydroxyketones, the thiazoles were present at much lower concentrations than the 3-thiazolines. When alkane- α -diones were used, the yields of thiazoles were much higher than for the reactions containing hydroxyketones, and the 3-thiazolines and thiazoles were present in approximately equal amounts.

All of the 3-thiazolines and thiazoles that we recently reported in the volatiles of pressure cooked beef were found in these reaction mixtures, and there was excellent agreement in LRI values and mass spectra. This confirms the identities of these cooked beef components and indicates their probable precursors. In foods, aliphatic aldehydes are products of lipid oxidation, and we have shown the importance of the interactions between lipid-derived components and intermediates of the Maillard reaction. Such interactions will modify the profile of volatiles from the Maillard reaction and hence may be important in determining the aroma characteristics of cooked foods (Farmer and Mottram, 1994).

The mass spectra of the 3-thiazolines and thiazoles are shown in Tables 3 and 4, respectively. The spectra for each type of thiazole and 3-thiazoline were very distinctive, and their base peaks are shown in Table 5. The fragmentation scheme for the thiazoles is straightforward. In each case the base peak is due to loss of an alkene from the 2-position via cleavage alpha to the thiazole ring. Most of the other fragments are derived from the base peak, due to losses of alkyl groups from the side chain in the 2-position. A mass spectral fragmentation scheme for the 3-thiazolines identified in the reaction mixtures is given in Figure 2. The double bond in the thiazoline is clearly in the 3-position because cleavage of the 2-alkyl chain occurred between the ring and the first carbon atom of the chain, i.e., alpha to the double bond. A 2-thiazoline would cleave alpha to the ring, i.e., between the first and second carbons of the alkyl chain.

The proposed scheme for the formation of these 3-thiazolines and thiazoles is shown in Figure 1. Formation of 3-thiazolines from an α -hydroxyketone involves the substitution of the $-\text{OH}$ group with an $-\text{SH}$. This is followed by nucleophilic attack by the sulfur at the carbon atom of an imine intermediate formed by the reaction between ammonia and an aldehyde. Subsequent ring closure, with the elimination of water, gives the 3-thiazoline. Thiazoles could result from the oxidation of the thiazoline. However, ammonium sulfide readily hydrolyzes to hydrogen sulfide, which would give

Table 3. Mass Spectral Data for Alkyl-3-thiazolines Produced in the Reaction of Ammonium Sulfide, Aldehydes, and α -Hydroxyketones or α -Dicarbonyls

3-thiazoline	LRI ^a	characteristic MS data, <i>m/z</i> (rel intensity)
4,5-dimethyl-2-isopropyl ^{b,c}	1155	114, 87 (27), 157 (22), 82 (18), 113 (12), 39 (11), 42 (11), 41 (10), 53 (10), 55 (10), 45 (9), 59 (8), 115 (8), 116 (8), 60 (7), 100 (5)
4,5-dimethyl-2-isopropyl ^{b,c}	1175	114, 87 (27), 157 (23), 82 (18), 39 (11), 42 (10), 53 (10), 55 (10), 116 (10), 41 (9), 45 (9), 59 (8), 60 (7), 115 (7), 100 (6)
2-isobutyl-4-methyl ^c	1228	100, 124 (35), 68 (28), 101 (24), 73 (20), 110 (17), 157 (16), 41 (15), 42 (14), 39 (11), 45 (11), 69 (9), 58 (8), 57 (7), 84 (6), 102 (6)
2-butyl-5-methyl ^b	1237	100, 96 (62), 124 (61), 73 (32), 68 (31), 41 (26), 39 (19), 45 (18), 101 (18), 54 (17), 86 (17), 82 (16), 97 (15), 55 (12), 69 (12), 157 (11)
2-butyl-5-methyl ^b	1239	100, 124 (66), 96 (49), 68 (31), 73 (30), 41 (27), 54 (24), 45 (22), 39 (20), 101 (19), 86 (17), 82 (16), 57 (15), 97 (15), 55 (14), 157 (14)
4,5-dimethyl-2-isobutyl ^{b,c}	1253	114, 68 (43), 87 (34), 138 (28), 171 (26), 42 (24), 41 (22), 39 (19), 71 (19), 100 (18), 53 (15), 55 (14), 45 (13), 59 (12), 101 (12), 115 (12)
4,5-dimethyl-2-isobutyl ^{b,c}	1260	114, 68 (53), 87 (37), 171 (32), 138 (28), 42 (25), 41 (22), 71 (21), 100 (20), 39 (16), 53 (16), 115 (16), 59 (15), 101 (15), 82 (14), 55 (13)
2-butyl-4-methyl ^c	1272	100, 124 (41), 68 (28), 73 (23), 41 (19), 42 (19), 110 (19), 39 (16), 45 (16), 101 (16), 82 (10), 57 (6), 102 (6), 111 (6), 157 (6)
2-butyl-4,5-dimethyl ^{b,c}	1298	114, 68 (31), 138 (31), 87 (27), 71 (25), 42 (20), 101 (18), 171 (15), 41 (14), 55 (12), 53 (11), 110 (11), 39 (10), 59 (10), 100 (10), 115 (10)
2-butyl-4,5-dimethyl ^{b,c}	1308	114, 138 (36), 68 (32), 71 (28), 87 (28), 101 (20), 171 (20), 42 (18), 41 (15), 53 (12), 55 (12), 59 (11), 39 (10), 45 (10), 100 (10), 115 (10)
4-ethyl-2-isobutyl-5-methyl ^{b,c}	1322	128, 82 (56), 41 (33), 85 (30), 101 (28), 185 (23), 59 (22), 87 (22), 69 (21), 56 (19), 114 (18), 152 (16), 124 (15), 96 (13), 39 (12), 129 (12)
4-ethyl-2-isobutyl-5-methyl ^{b,c}	1332	128, 82 (58), 41 (38), 101 (32), 85 (30), 87 (26), 59 (25), 69 (23), 185 (23), 56 (21), 114 (18), 152 (15), 39 (14), 129 (14), 96 (12), 130 (12)
5-ethyl-2-isobutyl-4-methyl ^{b,c}	1337	128, 68 (49), 100 (49), 101 (36), 41 (34), 185 (27), 152 (24), 42 (23), 85 (23), 59 (19), 69 (19), 110 (15), 156 (14), 39 (13), 96 (13), 129 (13)
5-methyl-2-pentyl ^b	1343	100, 110 (74), 138 (69), 73 (28), 68 (27), 83 (27), 41 (24), 45 (20), 55 (19), 54 (18), 39 (17), 82 (17), 86 (15), 115 (15), 96 (13), 171 (7)
5-methyl-2-pentyl ^b	1345	100, 138 (69), 110 (54), 41 (30), 83 (30), 68 (29), 73 (29), 115 (22), 82 (21), 39 (20), 86 (20), 45 (18), 54 (17), 55 (16), 111 (13), 171 (13)
5-ethyl-2-isobutyl-4-methyl ^{b,c}	1349	128, 68 (56), 100 (54), 41 (40), 101 (39), 42 (28), 185 (27), 85 (26), 152 (23), 59 (22), 69 (22), 39 (17), 45 (15), 129 (15), 96 (14), 110 (14)
2-butyl-4-ethyl-5-methyl ^{b,c}	1366	128, 82 (33), 101 (27), 152 (27), 41 (25), 85 (25), 56 (19), 59 (18), 185 (17), 69 (12), 86 (12), 110 (12), 124 (11), 39 (10), 130 (10)
4-methyl-2-pentyl ^c	1377	100, 138 (42), 68 (22), 73 (21), 83 (19), 41 (18), 42 (16), 45 (14), 115 (14), 39 (13), 82 (13), 124 (13), 55 (11), 58 (9), 114 (9), 171 (6)
2-butyl-4-ethyl-5-methyl ^{b,c}	1379	128, 82 (34), 152 (31), 101 (30), 41 (27), 85 (26), 185 (22), 56 (20), 59 (18), 110 (12), 130 (12), 69 (11), 124 (10), 39 (10)
2-butyl-5-ethyl-4-methyl ^{b,c}	1383	128, 152 (33), 100 (32), 68 (27), 41 (24), 101 (21), 156 (20), 185 (19), 42 (18), 85 (18), 59 (15), 69 (12), 73 (11), 110 (11), 115 (11)
2-butyl-5-ethyl-4-methyl ^{b,c}	1395	128, 152 (34), 100 (34), 68 (28), 101 (25), 41 (24), 85 (22), 185 (22), 156 (20), 42 (19), 59 (15), 69 (13), 115 (13), 45 (11), 84 (11)
4,5-dimethyl-2-pentyl ^{b,c}	1401	114, 152 (29), 115 (28), 68 (27), 87 (26), 42 (25), 71 (24), 55 (21), 41 (19), 29 (15), 53 (15), 185 (15), 39 (13), 59 (13), 83 (13)
4,5-dimethyl-2-pentyl ^{b,c}	1412	114, 152 (36), 115 (32), 68 (29), 87 (29), 42 (27), 71 (25), 55 (23), 41 (22), 185 (20), 39 (18), 29 (17), 53 (17), 59 (16), 45 (15), 83 (13)
5-ethyl-2-pentyl ^d	1443	114, 152 (76), 110 (56), 86 (52), 156 (28), 41 (25), 82 (25), 115 (25), 55 (23), 83 (22), 96 (19), 29 (18), 54 (18), 45 (16), 71 (15), 185 (11)
2-hexyl-5-methyl ^d	1451	100, 124 (82), 152 (76), 68 (31), 41 (28), 55 (24), 73 (24), 97 (22), 82 (20), 86 (19), 39 (17), 96 (17), 129 (15), 43 (14), 45 (14), 54 (14), 185 (8)
4-ethyl-2-pentyl ^c	1457	114, 152 (37), 82 (23), 83 (19), 115 (19), 56 (15), 55 (14), 41 (12), 87 (12), 72 (11), 138 (10), 29 (9), 45 (9), 96 (9), 39 (7), 71 (7), 86 (7), 185 (6)
4-ethyl-5-methyl-2-pentyl ^{b,c}	1469	128, 82 (33), 85 (31), 115 (27), 41 (26), 56 (24), 166 (24), 59 (19), 83 (18), 199 (14), 55 (13), 29 (12), 129 (11), 67 (10), 69 (10), 114 (10)
4-ethyl-5-methyl-2-pentyl ^{b,c}	1481	128, 82 (33), 115 (32), 85 (30), 166 (27), 56 (22), 41 (20), 199 (19), 59 (17), 83 (15), 184 (13), 55 (11), 114 (11), 129 (11), 110 (10)
2-hexyl-4-methyl ^c	1483	100, 152 (50), 97 (23), 68 (20), 138 (20), 41 (19), 73 (18), 42 (13), 129 (13), 45 (12), 55 (12), 82 (11), 39 (10), 128 (10), 101 (8), 185 (5)
5-ethyl-4-methyl-2-pentyl ^{b,c}	1485	128, 166 (30), 100 (28), 170 (23), 85 (21), 41 (20), 68 (20), 115 (20), 42 (18), 199 (17), 59 (13), 83 (13), 129 (12), 55 (11), 29 (10), 101 (10)
5-ethyl-4-methyl-2-pentyl ^{b,c}	1498	128, 166 (33), 100 (28), 170 (27), 115 (25), 85 (24), 199 (21), 41 (19), 68 (19), 42 (15), 129 (15), 83 (14), 59 (13), 101 (10), 29 (9), 55 (9), 69 (9)
4,5-dimethyl-2-hexyl ^{b,c}	1507	114, 166 (36), 41 (32), 42 (31), 68 (30), 87 (28), 29 (25), 55 (25), 129 (22), 53 (21), 39 (19), 71 (19), 45 (16), 59 (16), 97 (15), 184 (14), 199 (12)
4,5-dimethyl-2-hexyl ^{b,c}	1518	114, 166 (44), 87 (31), 129 (27), 68 (26), 41 (25), 42 (25), 71 (25), 55 (22), 184 (20), 97 (19), 199 (19), 29 (18), 53 (16), 39 (15), 45 (13), 59 (13)
5-ethyl-2-hexyl ^d	1551	114, 166 (93), 124 (74), 86 (52), 170 (35), 41 (31), 55 (28), 97 (25), 82 (24), 96 (23), 29 (20), 43 (20), 129 (20), 54 (17), 87 (17), 45 (16), 68 (16), 199 (11)
2-heptyl-5-methyl ^d	1558	100, 138 (92), 166 (89), 41 (31), 68 (27), 73 (25), 69 (24), 86 (21), 55 (20), 57 (20), 82 (20), 54 (18), 111 (18), 143 (18), 43 (17), 45 (17), 199 (6)
4-ethyl-2-hexyl ^c	1563	114, 166 (49), 97 (27), 82 (24), 55 (18), 41 (17), 56 (16), 129 (14), 152 (14), 87 (13), 29 (12), 96 (12), 45 (11), 72 (11), 115 (9), 199 (5)
2-heptyl-4-methyl ^c	1589	100, 166 (60), 41 (35), 152 (25), 68 (24), 42 (22), 73 (21), 39 (18), 29 (16), 69 (15), 111 (15), 82 (14), 143 (13), 101 (10), 43 (10), 199 (5)
4-ethyl-2-hexyl-5-methyl ^{b,c}	1573	128, 129 (35), 180 (31), 85 (26), 82 (21), 41 (19), 97 (18), 213 (18), 56 (17), 198 (15), 59 (14), 152 (13), 55 (11), 96 (10)

Table 3 (Continued)

3-thiazoline	LRI ^a	characteristic MS data, <i>m/z</i> (rel intensity)
4-ethyl-2-hexyl-5-methyl ^{b,c}	1585	128, 129 (35), 180 (31), 85 (27), 97 (20), 213 (19), 82 (18), 41 (16), 56 (15), 198 (15), 59 (14), 55 (12), 96 (12), 69 (10), 130 (10)
5-ethyl-2-hexyl-4-methyl ^{b,c}	1591	128, 180 (31), 129 (30), 100 (29), 41 (27), 85 (25), 184 (24), 68 (21), 97 (20), 42 (17), 59 (15), 213 (13), 55 (12), 101 (12), 29 (11), 69 (10)
5-ethyl-2-hexyl-4-methyl ^{b,c}	1603	128, 180 (34), 129 (33), 100 (31), 184 (31), 85 (27), 41 (24), 68 (19), 97 (19), 213 (17), 42 (16), 59 (14), 55 (13), 101 (13), 43 (11), 69 (11)
4,5-dimethyl-2-heptyl ^{b,c}	1612	114, 180 (34), 41 (30), 71 (29), 87 (29), 42 (28), 68 (26), 29 (24), 55 (24), 143 (21), 53 (16), 69 (16), 39 (15), 111 (14), 43 (12), 198 (12), 213 (10)
4,5-dimethyl-2-heptyl ^{b,c}	1623	114, 180 (39), 71 (35), 41 (33), 87 (31), 42 (29), 68 (28), 29 (25), 55 (25), 143 (27), 53 (18), 69 (17), 198 (17), 111 (16), 39 (15), 43 (13), 213 (12)
5-ethyl-2-heptyl ^d	1657	114, 138 (92), 180 (85), 41 (38), 184 (33), 82 (27), 55 (25), 69 (24), 29 (23), 96 (22), 43 (19), 139 (19), 87 (17), 45 (15), 68 (15), 213 (8)
5-methyl-2-octyl ^d	1669	152, 100 (94), 180 (72), 41 (42), 68 (29), 43 (28), 55 (28), 69 (26), 73 (26), 82 (21), 29 (17), 39 (17), 54 (17), 85 (17), 83 (16), 57 (15), 213 (4)
4-ethyl-2-heptyl ^c	1669	114, 180 (55), 41 (29), 29 (24), 82 (24), 56 (21), 55 (19), 45 (15), 87 (15), 166 (15), 69 (14), 143 (13), 39 (12), 96 (11), 53 (10), 72 (10), 213 (5)
4-ethyl-2-heptyl-5-methyl ^{b,c}	1678	128, 194 (35), 85 (25), 143 (23), 41 (22), 29 (19), 82 (19), 111 (16), 227 (16), 56 (15), 212 (15), 59 (14), 69 (14), 101 (13), 114 (13), 166 (11)
4-ethyl-2-heptyl-5-methyl ^{b,c}	1691	128, 41 (39), 194 (36), 143 (34), 85 (33), 82 (23), 56 (21), 29 (20), 69 (19), 59 (18), 111 (18), 101 (16), 212 (15), 227 (15), 55 (13), 67 (12)
4,5-dimethyl-2-(1-octenyl) ^{b,c}	1696	114, 87 (14), 115 (9), 116 (7), 41 (6), 55 (4), 53 (3), 165 (3), 210 (3), 225 (2)
5-ethyl-2-heptyl-4-methyl ^{b,c}	1697	128, 41 (36), 194 (36), 198 (27), 85 (25), 100 (25), 143 (23), 42 (22), 68 (21), 29 (18), 69 (17), 59 (14), 111 (14), 55 (13), 101 (13), 227 (12)
4-methyl-2-octyl ^c	1699	100, 180 (54), 41 (28), 68 (23), 166 (23), 73 (19), 42 (15), 55 (15), 69 (14), 82 (13), 157 (13), 45 (12), 29 (11), 39 (11), 43 (11), 213 (4)
4,5-dimethyl-2-(1-octenyl) ^{b,c}	1708	114, 87 (17), 115 (9), 116 (8), 41 (8), 210 (6), 165 (5), 225 (0)
5-ethyl-2-heptyl-4-methyl ^{b,c}	1709	128, 194 (34), 100 (30), 85 (29), 143 (26), 41 (25), 198 (23), 42 (14), 68 (14), 101 (14), 111 (14), 29 (13), 59 (13), 69 (12), 67 (10), 45 (9), 227 (6)
4,5-dimethyl-2-octyl ^{b,c}	1718	114, 194 (39), 71 (30), 87 (29), 41 (23), 68 (23), 55 (22), 157 (22), 42 (19), 212 (15), 29 (14), 53 (14), 69 (14), 43 (12), 227 (12), 82 (11)
4,5-dimethyl-2-octyl ^{b,c}	1729	114, 194 (50), 71 (34), 87 (32), 157 (32), 41 (24), 68 (24), 55 (23), 212 (23), 42 (19), 227 (16), 29 (14), 53 (14), 69 (14), 43 (12), 82 (12)
5-ethyl-2-octyl	1765	152, 194 (90), 114 (88), 86 (42), 41 (32), 198 (30), 55 (24), 82 (19), 157 (19), 87 (18), 153 (18), 29 (17), 43 (17), 96 (16), 69 (15), 227 (6)
4-ethyl-2-octyl ^c	1775	114, 194 (69), 82 (24), 41 (22), 55 (19), 180 (18), 56 (16), 87 (15), 157 (14), 29 (13), 69 (13), 96 (12), 125 (12), 45 (11), 195 (10), 212 (10), 227 (6)
2-(1,3-octadienyl)-4,5-dimethyl ^{b,c}	1777	114, 87 (13), 53 (6), 67 (6), 115 (6), 41 (5), 116 (4), 223 (4)
4-ethyl-5-methyl-2-octyl ^{b,c}	1782	128, 208 (39), 85 (29), 157 (29), 82 (21), 41 (20), 226 (16), 241 (16), 69 (14), 56 (13), 101 (13), 59 (12), 125 (12), 180 (12), 67 (10)
2-(1,3-octadienyl)-4,5-dimethyl ^{b,c}	1792	114, 87 (18), 53 (10), 115 (8), 41 (6), 67 (6), 116 (6), 223 (3)
4-ethyl-5-methyl-2-octyl ^{b,c}	1795	128, 208 (59), 157 (40), 85 (38), 226 (31), 241 (25), 82 (21), 41 (17), 101 (16), 56 (13), 59 (13), 125 (13), 69 (12), 55 (11), 129 (11), 180 (11)
5-ethyl-4-methyl-2-octyl ^{b,c}	1802	128, 208 (45), 212 (35), 157 (26), 85 (25), 100 (21), 41 (16), 241 (15), 69 (14), 101 (13), 55 (12), 59 (12), 68 (12), 42 (10), 166 (9)
5-ethyl-4-methyl-2-octyl ^{b,c}	1814	128, 208 (46), 212 (41), 85 (32), 157 (31), 100 (25), 41 (23), 68 (17), 241 (17), 69 (15), 101 (15), 55 (12), 42 (11), 59 (11), 67 (10)
4,5-dimethyl-2-nonyl ^{b,c}	1824	114, 208 (49), 71 (29), 87 (29), 41 (28), 171 (26), 55 (24), 68 (23), 226 (21), 42 (19), 29 (16), 43 (16), 241 (14), 53 (13), 180 (12)
4,5-dimethyl-2-nonyl ^{b,c}	1836	114, 208 (51), 41 (42), 71 (37), 87 (37), 171 (32), 55 (31), 42 (28), 68 (27), 226 (25), 29 (24), 43 (24), 53 (20), 39 (15), 82 (14), 241 (14)
4-ethyl-5-methyl-2-nonyl ^{b,c}	1887	128, 222 (38), 85 (34), 171 (31), 41 (24), 82 (21), 240 (18), 56 (15), 59 (14), 255 (14), 55 (13), 83 (13), 101 (13), 69 (12), 67 (11), 87 (11)
4-ethyl-5-methyl-2-nonyl ^{b,c}	1900	128, 85 (43), 222 (38), 171 (36), 41 (35), 82 (26), 56 (20), 240 (20), 59 (19), 55 (16), 101 (16), 69 (15), 83 (15), 43 (14), 67 (14), 255 (14)
5-ethyl-4-methyl-2-nonyl ^b	1907	128, 222 (42), 226 (31), 85 (29), 41 (25), 171 (25), 100 (24), 68 (17), 59 (13), 101 (13), 255 (13), 42 (12), 43 (12), 55 (12), 69 (11), 67 (10)
5-ethyl-4-methyl-2-nonyl ^b	1920	128, 222 (50), 226 (43), 85 (37), 171 (33), 100 (26), 41 (25), 68 (17), 255 (15), 59 (14), 101 (14), 42 (13), 43 (13), 55 (13), 67 (12), 69 (12)

^a Linear retention index. ^b Two isomers (*E* and *Z*) were found for these 3-thiazolines. ^c Previously reported in cooked beef (Elmore et al., 1997). ^d Spectrum of two unresolved *E* and *Z* isomers.

reducing conditions in the reaction mixture, discouraging thiazole formation.

A slightly different mechanism could occur with the alkane- α -diones. The initial step requires nucleophilic addition of -SH to one of the carbonyl groups. This intermediate could be reduced to a mercaptoketone, or it could react with an imine intermediate leading to a thiazole. This route offers an explanation for the larger quantities of thiazoles found in the dione systems.

Saturated aliphatic aldehydes all gave 3-thiazolines and thiazoles with a saturated side chain in the 2-position containing one fewer carbon atom than the aldehyde. Unsaturated aldehydes are important products

from the oxidative degradation of lipids, and therefore two reactions were carried out using an alkenal and an alkadienal. When (*E*)-2-nonenal and (*E,E*)-2,4-nonadienal were reacted with 3-hydroxy-2-butanone and ammonium sulfide, 3-thiazolines and thiazoles with C₈ unsaturated 2-alkyl substituents were found only in very small amounts. However, the (*E*)-2-nonenal reaction also gave small amounts of 2-hexyl-4,5-dimethyl-3-thiazoline, and (*E,E*)-2,4-nonadienal produced small amounts of 2-butyl-4,5-dimethyl-3-thiazoline. This suggested that, in both cases, oxidative fragmentation of the double bonds occurred during the reaction. 2-Octyl-4,5-dimethyl-3-thiazoline formed in the (*E*)-2-nonenal

Table 4. Mass Spectral Data for Alkylthiazoles Produced in the Reaction of Ammonium Sulfide, Aldehydes, and α -Hydroxyketones or α -Dicarbonyls

thiazole	LRI ^a	characteristic MS data, <i>m/z</i> (rel intensity)
4,5-dimethyl-2-isopropyl	1139	NIST ^b
2-butyl-4-methyl	1222	NIST ^b
4,5-dimethyl-2-isobutyl ^c	1225	NIST ^b
4-ethyl-2-isobutyl-5-methyl	1278	141, 140 (24), 85 (15), 168 (15), 59 (13), 142 (10), 41 (8), 126 (8), 183 (8), 39 (5), 182 (5)
2-butyl-4,5-dimethyl	1287	NIST ^b
5-ethyl-2-isobutyl-4-methyl	1297	141, 126 (32), 140 (18), 168 (17), 99 (14), 41 (12), 85 (12), 59 (11), 142 (10), 183 (10), 65 (9), 45 (8), 39 (7), 182 (5)
4-methyl-2-pentyl	1322	113, 126 (35), 71 (17), 112 (17), 140 (14), 72 (13), 114 (10), 41 (9), 73 (9), 127 (9), 45 (7), 169 (7) 39 (6)
2-butyl-4-ethyl-5-methyl	1334	NIST ^b
2-butyl-5-ethyl-4-methyl	1356	141, 126 (31), 154 (24), 85 (11), 142 (9), 59 (8), 99 (7), 155 (7), 168 (7), 41 (6), 45 (6), 183 (6), 143 (5)
2-hexyl-5-methyl	1381	113, 126 (29), 112 (20), 45 (19), 140 (18), 71 (11), 72 (11), 127 (11), 183 (9)
4,5-dimethyl-2-pentyl ^c	1384	127, 140 (34), 126 (18), 71 (15), 86 (13), 154 (11), 59 (9), 128 (9), 41 (8), 85 (8), 141 (8), 183 (8), 29 (6), 45 (6), 53 (6), 39 (5), 129 (5)
4-ethyl-2-pentyl	1412	127, 140 (41), 112 (20), 154 (11), 128 (10), 141 (9), 71 (8), 85 (7), 126 (7), 45 (6), 183 (6), 39 (5), 41 (5)
2-hexyl-4-methyl	1426	113, 126 (37), 112 (15), 140 (13), 114 (11), 71 (10), 72 (10), 127 (10), 115 (6), 154 (6), 183 (5)
4-ethyl-5-methyl-2-pentyl ^c	1432	141, 154 (32), 85 (15), 59 (14), 168 (12), 140 (11), 142 (11), 41 (8), 126 (8), 155 (8), 197 (6), 29 (5), 39 (5), 45 (5), 143 (5)
5-ethyl-4-methyl-2-pentyl	1454	141, 154 (40), 126 (28), 168 (14), 140 (11), 85 (10), 155 (10), 142 (9), 197 (9), 59 (8), 41 (7), 99 (7), 65 (6), 45 (5), 143 (5)
5-ethyl-2-hexyl	1457	127, 140 (38), 71 (16), 128 (11), 154 (11), 126 (9), 141 (8), 129 (6), 168 (6), 197 (5)
4,5-dimethyl-2-hexyl ^c	1485	127, 140 (33), 126 (15), 71 (13), 86 (11), 128 (9), 154 (9), 41 (7), 59 (7), 85 (7), 141 (7), 45 (6), 53 (6), 197 (6), 29 (5), 129 (5)
4-ethyl-2-hexyl	1516	127, 140 (37), 112 (15), 154 (12), 126 (9), 141 (9), 128 (8), 71 (7), 41 (6), 168 (6), 45 (5), 85 (5), 129 (5), 197 (5)
2-heptyl-4-methyl	1532	113, 126 (39), 112 (14), 127 (10), 168 (10), 41 (7), 71 (7), 114 (7), 140 (7), 72 (6), 154 (6), 39 (5), 73 (5), 115 (5), 197 (5)
4-ethyl-2-hexyl-5-methyl ^c	1533	141, 154 (31), 85 (11), 140 (10), 168 (10), 59 (9), 142 (9), 155 (7), 211 (7), 41 (6), 126 (6), 143 (5), 182 (5)
5-ethyl-2-hexyl-4-methyl	1556	141, 154 (39), 126 (22), 168 (11), 140 (10), 142 (9), 155 (9), 211 (9), 85 (8), 41 (7), 59 (6), 99 (6), 182 (6), 65 (5)
4,5-dimethyl-2-heptyl ^c	1588	127, 140 (30), 126 (14), 71 (11), 41 (10), 86 (10), 128 (9), 141 (8), 182 (8), 29 (7), 45 (6), 53 (6), 59 (6), 85 (6), 154 (6), 211 (6)
4-ethyl-2-heptyl	1621	127, 140 (41), 112 (14), 182 (10), 86 (10), 126 (8), 128 (8), 141 (8), 154 (8), 211 (6), 71 (5), 85 (5)
4-methyl-2-octyl	1624	113, 126 (31), 112 (13), 71 (12), 168 (10), 73 (7), 115 (7), 127 (7), 211 (7), 72 (6), 114 (6), 140 (6), 41 (5), 45 (5), 182 (5)
4-ethyl-2-heptyl-5-methyl ^c	1634	141, 154 (29), 142 (11), 85 (9), 140 (8), 59 (7), 155 (7), 196 (7), 41 (6), 126 (5), 143 (5), 168 (5), 225 (5)
5-ethyl-2-heptyl-4-methyl	1657	141, 154 (36), 126 (20), 142 (9), 196 (9), 155 (8), 225 (7), 85 (6), 168 (6), 41 (5), 59 (5), 143 (5), 182 (5)
4,5-dimethyl-2-octyl ^c	1691	127, 140 (33), 126 (11), 128 (8), 182 (8), 71 (7), 86 (7), 141 (7), 41 (6), 85 (5), 129 (5), 225 (5)
4-ethyl-2-octyl-	1726	127, 140 (41), 112 (13), 126 (10), 182 (10), 128 (8), 196 (8), 41 (7), 141 (7), 71 (6), 154 (6), 225 (5)
4-ethyl-5-methyl-2-octyl ^c	1736	141, 154 (29), 142 (9), 85 (8), 140 (8), 196 (8), 155 (7), 59 (6), 41 (5), 126 (5), 143 (5), 168 (5), 239 (5)
5-ethyl-4-methyl-2-octyl ^c	1760	141, 154 (37), 126 (15), 142 (9), 140 (8), 196 (8), 155 (7), 41 (6), 239 (6), 85 (5), 143 (5), 168 (5), 210 (5)
4,5-dimethyl-2-nonyl	1795	127, 140 (36), 126 (12), 128 (9), 141 (7), 182 (7), 86 (6), 154 (6), 196 (6), 239 (6), 71 (5)
4-ethyl-5-methyl-2-nonyl	1839	141, 154 (28), 142 (10), 85 (7), 155 (7), 59 (6), 196 (6), 41 (5), 143 (5), 210 (5), 253 (5)
5-ethyl-4-methyl-2-nonyl	1864	141, 154 (35), 126 (15), 142 (9), 155 (8), 196 (8), 210 (7), 253 (7), 41 (6), 168 (6), 85 (5), 143 (5)

^a Linear retention index. ^b Spectrum reported in NIST/NIH/EPA mass spectral database. ^c Previously reported in cooked beef (Elmore et al., 1997).

Table 5. Mass Spectral Base Peaks for 2-Alkylthiazoles and 2-Alkyl-3-thiazolines

substituent	3-thiazoline	thiazole
2-alkyl-4-methyl	<i>m/z</i> 100	<i>m/z</i> 113
2-alkyl-5-methyl	<i>m/z</i> 100 (below octyl) M ⁺ - 61 (octyl and above)	<i>m/z</i> 113
2-alkyl-4-ethyl	<i>m/z</i> 114	<i>m/z</i> 127
2-alkyl-5-ethyl	<i>m/z</i> 114 (below octyl) M ⁺ - 75 (octyl and above)	<i>m/z</i> 127
2-alkyl-4,5-dimethyl	<i>m/z</i> 114	<i>m/z</i> 127
2-alkyl-4-ethyl-5-methyl	<i>m/z</i> 128	<i>m/z</i> 141
2-alkyl-5-ethyl-4-methyl	<i>m/z</i> 128	<i>m/z</i> 141

reaction may have arisen from traces of nonanal in the nonenal. The main products from these reaction systems are described later in the paper.

The reactions involving 1-hydroxy-2-alkanones produced smaller amounts of the expected 3-thiazolines than the 3-hydroxy-2-butanone systems, but significant amounts of other 3-thiazolines were found, which have not been fully characterized. For example, the reaction mixture containing 1-hydroxy-2-propanone and pentanal gave four 3-thiazolines with a molecular weight of 227. These 3-thiazolines were present at 12% of the total peak area, higher than the total peak area of the

3-thiazolines that were expected to be present. It appeared that two molecules of pentanal were incorporated into these compounds. The mass spectra of these 3-thiazolines suggested identities of 2-butyl-4-methyl-5-pentyl-3-thiazoline and 2-butyl-5-methyl-4-pentyl-3-thiazoline, each present as pairs of isomers, although we have not yet confirmed this. The equivalent four 3-thiazolines was found in each of the reaction mixtures containing a 1-hydroxy-2-alkanone but, as the chain length of the aldehyde increased, the relative amounts of these 3-thiazolines decreased.

Trialkylpyridines. A large GC peak was present in each of the reactions, which eluted after the 3-thiazolines and thiazoles when the column had reached the maximum temperature (280 °C). The mass spectra (Table 6) were consistent with trialkylpyridines and matched the spectra of compounds that we have recently found in samples of pressure cooked beef (J.S.E. and D.S.M., unpublished results). Trialkylpyridines have been reported by Shu et al. (1985) and Hwang et al. (1986) in reaction mixtures containing aldehydes and ammonium sulfide, and they proposed a mode of formation involving three aldehyde molecules condensing with ammonia. Many different trialkylpyridines were found

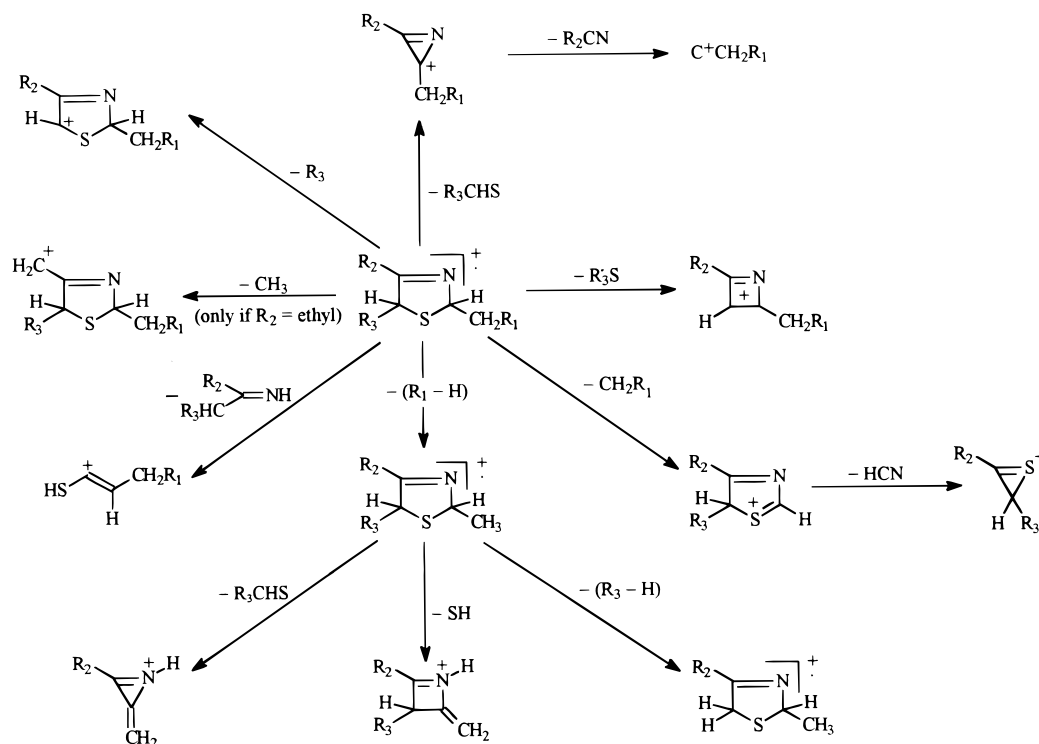


Figure 2. Proposed mass spectral fragmentation pattern for alkyl-3-thiazolines (R_1 = alkyl C_3 – C_9 ; R_2, R_3 = H, methyl, ethyl).

Table 6. Mass Spectra of Alkylpyridines Produced in the Reaction of Ammonium Sulfide, Aldehydes, and α -Hydroxyketones or α -Dicarbonyls

pyridine	LRI ^a	characteristic MS data, m/z (rel intensity)
2-isobutyl-3,5-isopropyl	1480	NIST ^b
2-butyl-3,5-dipropyl	1643	149, 162 (54), 177 (33), 204 (22), 176 (20), 190 (19), 150 (12), 135 (9), 160 (9), 163 (9), 120 (8), 91 (7), 29 (6), 41 (6), 132 (6), 219 (4)
3,5-dibutyl-2-pentyl	1918	163, 190 (39), 232 (28), 205 (13), 164 (11), 176 (11), 218 (11), 177 (10), 120 (8), 146 (6), 191 (6), 174 (5), 261 (3)
3,5-dipentyl-2-hexyl	2200	177, 218 (55), 204 (27), 260 (26), 190 (20), 246 (17), 191 (15), 178 (13), 120 (11), 233 (11), 219 (9), 274 (7), 303 (3)
3,5-dihexyl-2-heptyl	>2200	191, 246 (51), 204 (27), 218 (23), 288 (20), 274 (17), 192 (14), 205 (14), 261 (13), 120 (9), 247 (9), 260 (7), 216 (6), 219 (6), 302 (6), 316 (6), 345 (3)

^a Linear retention index. ^b Spectrum reported in NIST/NIH/EPA mass spectral database.

in the cooked beef. They could not be individually identified, because they were not fully resolved under the gas chromatographic conditions used but appeared to arise from various combinations of the many aldehydes present in the cooked meat. In the present work, the largest quantities of the trialkylpyridines were found in reaction mixtures using the longer chain aldehydes (Tables 3 and 4), although they appeared to be absent from the reaction mixtures containing nonanal and decanal, probably because the molecules were too large to elute from the column under the conditions used.

Other Compounds. A number of other compounds were formed in the reaction mixtures at levels ranging from trace to 30% of the total GC peak area. These included pyrazines, pyrroles, imidazoles, oxazoles, and alkanethiols. As expected, aldol condensation products of the aldehydes were found in all of the reaction mixtures and, as the alkanal chain length increased, the relative amounts of such compounds increased. For mixtures containing nonanal and decanal, 2-heptyl-2-undecenal and 2-octyl-2-dodecenal were present at approximately 20% of the total peak areas of the respective chromatograms.

The reaction mixtures containing 3-hydroxy-2-butanone contained between 12% and 30% tetramethylpyrazine, formed by the condensation of two molecules

of the intermediate, 3-amino-2-butanone, produced by the action of ammonia on the hydroxyketone. However, reaction mixtures containing 2,3-butanedione produced much lower quantities of tetramethylpyrazine, between 0.3% and 2.0%.

In the dione reaction mixtures, 2-alkyl-4,5-dimethylimidazoles were produced, which are similar in structure to thiazoles but with a second nitrogen replacing the sulfur. However, they were not found in the hydroxyketone systems. This may be explained by a modification of the proposed mechanism given in Figure 1, in which both keto groups react with ammonia. Substitution of the hydroxy group of a hydroxyketone with $-NH_2$ does not occur; hence, imidazoles are not formed. In systems containing 2,3-butanedione and pentanal, the level of 2-butyl-4,5-dimethylimidazole was 28%; the amount of imidazole decreased as the length of the alkanal chain increased, with only 1.4% when nonanal was present. When 2,3-pentanedione was the dione the level of imidazole produced was 27% for 3-methylbutanal and 10% for decanal.

The systems involving unsaturated aldehydes contained only very small amounts of 3-thiazolines with no thiazoles or trialkylpyridines. However, tetramethylpyrazine was formed in large amounts in both reaction mixtures [16% for (*E*)-2-nonenal and 30% for (*E,E*)-2,4-nonadienal]. 2-Pentylthiophene was a major product in

the reaction mixture containing (*E,E*)-2,4-nonadienal (21%). Similar alkylthiophenes have been reported in Maillard reactions between cysteine, ribose, and phospholipids, in which the latter oxidized to give unsaturated aldehydes (Farmer and Mottram, 1992). Two large peaks in the reaction mixture from (*E*)-2-nonenal were suggested by structure elucidation as being the two isomers of 3,4-diheptyl-2(5*H*)-thiophenone (18% and 11%), formed from two molecules of (*E*)-2-nonenal and one molecule of hydrogen sulfide.

Conclusion. The 3-thiazolines and thiazoles tentatively identified in pressure cooked beef were all formed in reaction mixtures containing an α -hydroxyketone or α -dione, an aliphatic aldehyde, and ammonium sulfide. Other 3-thiazolines and thiazoles were also produced. The reaction mixtures also contained other products including trialkylpyridines, pyrazines, pyrroles, imidazoles, oxazoles, and alkanethiols. The results confirmed routes to the formation of 3-thiazolines and thiazoles in cooked foods and demonstrated the importance of interactions between the Maillard reaction and lipid-derived compounds in the flavor chemistry of heated foods.

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