Identification and Formation of Some Selected Sulfur-Containing Flavor Compounds in Various Meat Model Systems†

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The volatiles of various meat model systems containing thiamin, cysteine, cystine, and various carbohydrates were isolated by simultaneous steam distillation–extraction. The extracts were preseparated by medium-pressure liquid chromatography on silica gel using a pentane/diethyl ether gradient. The resulting fractions were analyzed by high-resolution gas chromatography and coupled gas chromatography–mass spectrometry (GC/MS). Identifications were focused on sulfur-containing constituents. Individual components were isolated by preparative capillary GC and characterized by MS, IR, and ^{1}H NMR spectroscopies. Various sulfur-containing compounds were identified for the first time in meat model systems or even in the context of flavor chemistry. In most cases, their structures were confirmed by synthesis. Formation pathways, sensory properties, and spectroscopic data of various compounds are described.

INTRODUCTION

Meat flavor is formed from chemical reactions occurring when numerous flavor precursors are heated. Meat contains amino acids, sugars, lipids, and vitamins that can act as flavor precursors. Meat flavor has been extensively studied, and the chemistry of its formation has been reviewed by van den Ouweland et al. (1978), MacLeod and Seyyedain-Ardebili (1981), and MacLeod (1986). A comprehensive list of compounds identified in natural meat aroma has been published by Shahidi et al. (1986). Recently, the thermal generation of aromas has been one of the subjects of the 196th ACS meeting. Many interesting papers have been published in the symposium book edited by Parliment et al. (1989).

The sulfur-containing amino acids cysteine and cystine as well as thiamin (vitamin B_1) are important precursors for the resulting roasted or cooked meat aroma. The decisive reactions leading to the formation of meat flavor include (1) thermal degradation of thiamin, (2) Strecker degradation of the amino acids, (3) nonenzymatic browning reaction between carbohydrates and amino acids, and (4) reactions of the degradation products formed by the preceding reactions.

Though many constituents have been identified in cooked and roasted meats, the further identification of trace levels of nitrogen- and sulfur-containing compounds remains a challenge and is important for the composition of "nature-identical" aromas. In particular, sulfur compounds undoubtedly play a significant role in meat flavor due to their interesting olfactory properties and generally low odor and taste thresholds. Since many of these are new compounds, their identification necessitates isolation and subsequent characterization by spectroscopic methods. Due to the difficulties of identifying trace constituents in cooked and roasted meats, many researchers have investigated model systems. Studies of model systems containing defined precursors at higher concentrations than occur in meat have led to the identification of important sulfur compounds whose presence could then be investigated in natural flavors. Additionally, the mechanisms of their formation can be more easily understood from defined precursors. This knowledge may lead to further structure proposals.

EXPERIMENTAL PROCEDURES

Model Systems. *I.* Thiamin hydrochloride (200 g) in 500 mL of distilled water (pH 2.5) was heated in an autoclave to 120 °C for 5 h. Thiamin hydrochloride (200 g) in 500 mL of distilled water (pH adjusted with NaOH to 8.0) was heated in an autoclave to 120 °C for 5 h.

II. A mixture of 100 g of cysteine and 100 g of ribose in 1 L of propylene glycol (pH 5.6) was refluxed at 120 °C for 2 h.

III. A mixture of 100 g of cystine, 500 g of lactose, 100 g of ascorbic acid, and 500 g of monosodium glutamate in 2 L of distilled water was heated in an autoclave to 120 °C for 0.5 h.

IV. A mixture of 100 g of cystine, 500 g of xylose, 100 g of lactose, 100 g of ascorbic acid, 100 g of thiamin-HCl, and 500 g of monosodium glutamate in 2 L of distilled water was heated in an autoclave to 120 °C for 0.5 h.

V. A mixture of 100 g of cystine, 100 g of thiamin-HCL, 100 g of ascorbic acid, and 500 g of monosodium glutamate in 2 L of distilled water was heated in an autoclave to 120 °C for 0.5 h (Werkhoff et al., 1989a, 1990).

Isolation of Volatiles by Simultaneous Distillation–Extraction. The respective reaction mixture was exposed for 7 h to a simultaneous distillation–extraction procedure with $CFCl_3$ (III, IV) or a 1:1 mixture of pentane/diethyl ether (I, II, V) at atmospheric pressure according to the method of Likens and Nickerson (1964). The resulting extract was dried over Na_2SO_4, and the organic solvent was carefully removed on a 25 cm × 1 cm Vigreux distillation column. The concentrated extract was stored under N_2.

Preseparation by Adsorption Chromatography. The respective concentrated flavor extracts were separated into 20 fractions by medium-pressure liquid chromatography on silica gel using a pentane/diethyl ether gradient (II, III, IV, V). The aroma concentrates were placed on a cooled column [480 mm × 37 mm (i.d.)] filled with 240 g of silica gel (25–40 μm). The pressure was 10–20 bar. The flow rate was 10 mL/min. All eluates were dried over Na_2SO_4. Each fraction was concentrated to a final volume of 1 mL. Further concentration to about 100 μL was performed by using a procedure described by Dünges (1979). The resulting extracts of I were not preseparated but

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used directly for the following chromatographic and spectroscopic applications.

Capillary Gas Chromatography. Analytical separations were performed on the following three gas chromatographs: Varian 3700, Carlo Erba 5360 Mega Series, and Carlo Erba 4200. The Varian 3700 gas chromatograph was equipped with a modified hot split/splitless injector (Gerstel/Mülheim, FRG) and a commercially available inlet splitter which allowed simultaneous injection into two capillaries of different polarities. The Carlo Erba 5360 gas chromatograph was equipped with the so-called "glass cap cross" (Seekamp/Achim, FRG) inlet splitting system. This system for dual column analysis was combined with a cold on-column technique (Bretschneider and Werkhoff, 1988a,b). The Carlo Erba 4200 gas chromatograph was equipped with a flame ionization detector (FID), a flame photometric detector (FPD) operated in the sulfur mode (394 nm), and a nitrogen–phosphorus detector (NPD). The column effluent was simultaneously split to the three different detectors by using a modified (five "arms" instead of four) glass cap cross.

The columns employed were a 60 m × 0.32 mm (i.d.) DB-Wax fused silica capillary (d_f = 0.25 μm) and a 60 m × 0.32 mm (i.d.) DB-1 fused silica capillary (d_f = 0.25 μm). The columns were obtained from J&W Scientific, Folsom, CA.

The columns were programmed from 60 to 220 °C (3 °C/min). Helium carrier gas was used at a flow rate of 2–3 mL/min.

The preparative separations were performed on wide bore DB-1 and DB-Wax fused silica columns (30 m × 0.53 mm i.d.; film thickness 1.0–3.0 μm) in an all-glass system. The condensed compounds (low microgram range) were analyzed by capillary GC prior to spectroscopic investigations for verifying the purity.

Gas Chromatography–Mass Spectrometry (GC/MS). A Finnigan MAT Series 8230 (sector field) interfaced by an open split coupling via a flexible heated (250 °C) transfer line to a Carlo Erba 5360 Mega Series GC was employed.

The operating conditions were as follows: ion source 220 °C, EI 70 eV, cathodic current 1 mA, accelerating voltage 3 kV, resolution 900, scan speed about 1 s/dec.

A Finnigan MAT Series 112S (sector field) directly coupled to a Varian 3700 Series GC was also used.

The operating conditions were as follows: ion source 230 °C, EI 70 eV, cathodic current 0.7 mA, accelerating voltage 800 V, resolution 800, scan speed about 1.3 s/dec.

Infrared (IR) and Nuclear Magnetic Resonance (NMR) Analyses. Infrared spectra of isolated samples were obtained in CCl_4 by using a Perkin-Elmer 983G type instrument.

NMR spectra of collected and of synthesized samples were measured at 200 MHz in $CDCl_3$ or C_6D_{12} on a Varian XL-200 instrument with $Si(CH_3)_4$ as internal standard.

Component Identification. Sample components were identified by comparison of the compound's mass spectrum and Kovats index with those of a reference standard. Reference compounds were obtained commercially or synthesized in our laboratory. The respective structures were confirmed by NMR, MS, and IR spectroscopies.

Identifications based solely on mass spectral data were considered tentative.

Sensory Evaluation. The olfactory evaluation of selected compounds (Table II) was performed by an expert panel of flavorists. The synthesized compounds were evaluated in water at certain concentrations. The isolated samples were dissolved in ethanol and tested on a smelling blotter.

Synthesis of Reference Compounds. 3-Acetyl-1,2-dithiolane (**16**) was synthesized in four steps starting with the preparation of 5-bromo-2-pentanone by reacting 5-hydroxy-2-pentanone with HBr and, subsequently, the bromination with Br_2 to 3,5-dibromo-2-pentanone. The following reaction with potassium thioacetate in methanol led to 3,5-bis(thioacetyl)-2-pentanone, which could be cyclized with sodium methylate under influence of oxygen to **16**. The spectroscopic data (^1H NMR, MS) are shown in Figure 6.

2-(2-Thienyl)furan (**46**) was synthesized according to the method of Ullenius (1972) by coupling 2-furylcopper with 2-iodothiophene. The spectroscopic data are shown in Table III.

2-Methoxy-2-methyl-4,5-dihydro-3(2*H*)-thiophenone (**57**) was synthesized by reacting 2-methyl-4,5-dihydro-3(2*H*)-thiophenone (**40**) with *N*-chlorosuccinimide in toluene under N_2 atmosphere at −20 °C. Then sodium methylate was added to the resulting 2-chloro-2-methyl-4,5-dihydro-3(2*H*)-thiophenone. The spectroscopic data are shown in Figure 8.

2-Tetrahydrothiophenethiol (**44**) was synthesized according to the method of Gais (1977). The spectroscopic data are shown in Table III.

2-Methyl-2-tetrahydrothiophenethiol (**37**) was synthesized according to a procedure described by van den Bosch (1978). Its spectroscopic data are shown in Figure 7.

RESULTS AND DISCUSSION

The investigation of the five model systems focused on the identification of volatile sulfur-containing compounds generated by reactions of the important meat flavor precursors, thiamin, cysteine, and cystine. Both the thermal degradation of the pure amino acids and of thiamin and the reactions between amino acids, certain sugars (Maillard type), and thiamin were studied. Table I shows S-containing flavor compounds identified in these model systems. The constituents are listed with their Kovats retention indices, their identification status, and the model system(s) in which they were identified. Spectroscopic data, sensory properties, and formation pathways of newly identified sulfur constituents will be discussed shortly.

Figure 1 shows three chromatograms of a medium polar fraction of model system IV. The effluent stream was split in three parts by using the glass cap cross as an effluent splitter. The chromatography was performed on an apolar fused silica capillary, and the separated compounds were detected simultaneously by an FID (top), an NPD (middle), and an FPD (bottom). In this way, it is possible to trace N and S compounds of interest in very complex reaction mixtures. Some structures of S-containing flavor volatiles of model system IV (see Table I) are shown on the chromatogram. A few other well-known N-containing flavor substances identified in this meat model but not discussed any further are also shown.

Novel compounds were isolated by preparative capillary gas chromatography and characterized by ^1H NMR, MS, and IR spectroscopies. An example of the isolation procedure is illustrated in Figure 2. Dihydro-3(2*H*)-thiophenone (**45**) was isolated from a medium polar silica gel fraction of model system IV using a poly(methylsiloxane) thick film wide bore capillary (middle). Its purity (92.7%) was subsequently checked on a bonded poly(ethylene glycol) column (bottom).

Model I contained only thiamin as thiamin hydrochloride and was reacted at different pH values. Early investigations of vitamin B_1 dealt primarily with the loss of its biological activity. In the 1960s researchers began to focus their interest on the resulting volatile compounds of thermal degradation. Therefore, it is very well-known from literature (Matsukawa and Iwatsu, 1950; Arnold et al., 1969; Dwivedi et al., 1972; Dwivedi and Arnold, 1972, 1973a,b; Wilson, 1975; van der Linde et al., 1979, Hopmann and Brugnoni, 1981; Tressl, 1983; Hartman et al., 1984a; Reineccius and Liardon, 1985) that the thermal degradation of thiamin leads to several main compounds depending on the pH value. These precursors react further to produce numerous volatile flavor compounds. The proposed pathways are shown in Figure 3. The brackets characterize unidentified compounds, which in most cases can be viewed as very reactive unstable intermediates. Others may not be amenable to gas chromatography. Many of the listed substances in Table I which were identified in model system I, IV, or V (containing thiamin) are explainable by these reaction schemes (Figure 3).

Table I. Selected S-Containing Volatile Flavor Compounds in Different Meat Model Systems

constituent	no.	MW	identification status	retention indices		meat model systems				
				DB-1	DB-Wax	I	II	III	IV	V
thiazole	1	85	*a*	707	1240				X	X
mercaptopropanone	11	90	*a*	739	1351	X				
3-mercapto-2-butanone	34	104	*a*	787	1283	X		X	X	X
4-methylthiazole	4	99	*a*	798	1279	X				X
2-methyl-4,5-dihydrothiophene	39	100	*a*	824	1156	X				X
2-methyl-3-furanthiol	30	114	*a*	844	1303	X				X
3-mercapto-2-pentanone	35	118	*a*	875	1343	X		X	X	X
furfurylthiol	49	114	*a*	885	1423		X	X	X	X
4,5-dimethylthiazole	2	113	*a*	909	1372	X			X	X
dihydro-3(2*H*)-thiophenone	45	102	*a*	916	1553	X		X	X	X
2-(1-mercaptoethyl)furan[f]	56	128	*a*	926	1413			X		X
2-methyl-4,5-dihydro-3-furanthiol	28	116	*d*	927		X				
3-thiophenethiol	50	116	*a*	940	1547		X			X
2-methyl-4,5-dihydro-3(2*H*)-thiophenone	40	116	*a*	947	1509	X	X	X	X	X
dihydro-2(3*H*)-thiophenone	52	102	*a*	952	1615			X	X	
cis-2-(mercaptomethyl)-4-methyl-1,3-dioxolane[e]	59	134	*b*	959	1454		X			
trans-2-(mercaptomethyl)-4-methyl-1,3-dioxolane[e]	60	134	*b*	959	1476		X			
2-acetylthietane[f]	18	116	*b*	972	1549	X				
3-mercaptopropyl acetate[f]	10	134	*b*	991	1549	X				
4-methyl-5-vinylthiazole	3	125	*a*	999	1512	X				
2-tetrahydrothiophenethiol[f]	44	120	*a*	1017	1561					X
2-methyl-2-tetrahydrothiophenethiol	37	134	*a*	1025	1488					X
2-methyl-3-thiophenethiol	22	130	*a*	1030	1584				X	X
2-methoxy-2-methyl-4,5-dihydro-3(2*H*)-thiophenone[e]	57	146	*a*	1074	1645				X	
1-methylbicyclo[3.3.0]-2,8-dioxa-4-thiaoctane	26	146	*b, c*	1075	1642	X				
2-formyl-5-methyl-4,5-dihydrothiophene	51	128	*b*	1078	1734		X			
1,2,3-trithia-5-cycloheptene	48	150	*c*		2224		X			
2-formyl-5-methylthiophene	58	126	*a*	1077	1759			X	X	X
1,3-dimethylbicyclo[3.3.0]-2,8-dioxa-4-thiaoctane	27	160	*b, c*	1083	1562	X				X
3-methyl-1,2-dithian-4-one	41	148	*b*	1168	1885	X			X	X
thieno[3,2-*b*]thiophene	53	140	*b, c*	1168	1843		X			
2-(2-thienyl)furan[f]	46	150	*a*	1189	1831	X		X	X	
3-acetyl-1,2-dithiolane	16	148	*a*	1199	1994	X			X	X
2-(2-furyl)thiazole	47	151	*a*	1207	1985				X	X
1*H*-pyrrolo[2,1-*c*]-1,4-thiazine[e]	54	137	*b*	1226	1977		X			
5-(2-hydroxyethyl)-4-methylthiazole	5	143	*a*	1241	2309	X			X	
1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane	31	162	*c*	1245		X				
2-methyl-5,7-dihydrothieno[3,4-*d*]pyrimidine	8	152	*a*	1339	2137				X	
bis(2-methyl-3-furyl) disulfide	33	226	*a*	1496	2141	X		X	X	X
bis(2-methyl-4,5-dihydro-3-furyl) disulfide	32	230	*a*	1676	>2500	X				X
bis(3-thienyl) disulfide	55	230	*d*		>2500		X			
bis(2-methyl-3-thienyl) disulfide	24	258	*a*	1868	>2500					X

[a] Identified by comparison of the compound's mass spectrum and retention index with those of an authentic reference standard. [b] Not synthesized but identified by means of the spectra (NMR, IR, MS) of the isolated sample. [c] Tentatively identified by comparison with a mass spectrum reported in the literature. [d] Tentatively identified by interpretation of the mass spectrum. [e] Not previously mentioned in the literature. [f] Cited in the literature but not in relation to flavor chemistry.

The acid degradation of thiamin (Figure 3a) is strongly influenced by the cleavage of the methylene bond between the pyrimidine and the thiazole ring systems and therefore leads mainly to 4-amino-5-(hydroxymethyl)-2-methylpyrimidine (**7**) and 5-(2-hydroxyethyl)-4-methylthiazole (**5**). The pyrimidine moiety **7** probably does not play an important role in flavor production, while the resulting thiazole part **5** (sulfurol) is supposed to be a key substance for the formation of processed flavors. Interestingly, Stoll et al. (1967) identified this thiazole in cocoa and judged the synthesized reference to be "nearly odorless in the very pure state", while Wilson (1975) reported it to be "smooth, meaty, sweet, woody, smokey, sharp, pungent, biting, harsh, or disagreeable".

The formation of flavor compounds with meaty sensory properties is favored by the thermal degradation of thiamin in a slightly alkaline pH value. The mechanism of degradation was described by Hopmann and Brugnoni (1981). The additional nucleophilic attack on the C-2 atom of the thiazole ring system leads after a rearrangement to the flavor precursors 4-amino-5-(aminomethyl)-2-methylpyrimidine (**6**), formic acid, and the key intermediates hydrogen sulfide and 3-mercapto-5-hydroxy-2-pentanone (**13**). The release of hydrogen sulfide is very important for the further degradation of thiamin. Apparently, **13** can form seven additional intermediates, 3,5-dimercapto-2-pentanone (**12**), 3,5-dihydroxy-2-pentanone (**13a**), 3-hydroxy-5-mercapto-2-pentanone (**13b**), and, by a keto–enol tautomerization of **13a**, 1,4-dihydroxy-3-pentanone (**13c**), 1-mercapto-4-hydroxy-3-pentanone (**13d**), 1-hydroxy-4-mercapto-3-pentanone (**14**), and 1,4-dimercapto-3-pentanone (**15**).

The described precursors **5**, **12**, **13**, **13b**, **13d**, **14**, and **15** lead predominantly to S-containing heterocycles. The identified thiazoles 4,5-dimethylthiazole (**2**) (Dwivedi and Arnold, 1973b; van der Linde et al., 1979; Tressl, 1983; Hartman et al., 1984a; Reineccius and Liardon, 1985) and 4-methyl-5-vinylthiazole (**3**) (Dwivedi and Arnold, 1973b; van der Linde et al., 1979; Tressl, 1983) are known flavor compounds of the thermal degradation of thiamin. 4-Methylthiazole (**4**) has been previously reported by Hartman et al. (1984a). On the other hand, thiazole (**1**) is a known compound but has not been reported as a degradation product of thiamin. All of these thiazoles are closely related structurally to **5** and probably originate from this precursor. In general, thiazoles are mainly formed by nonenzymatic browning reactions between reducing sugars and

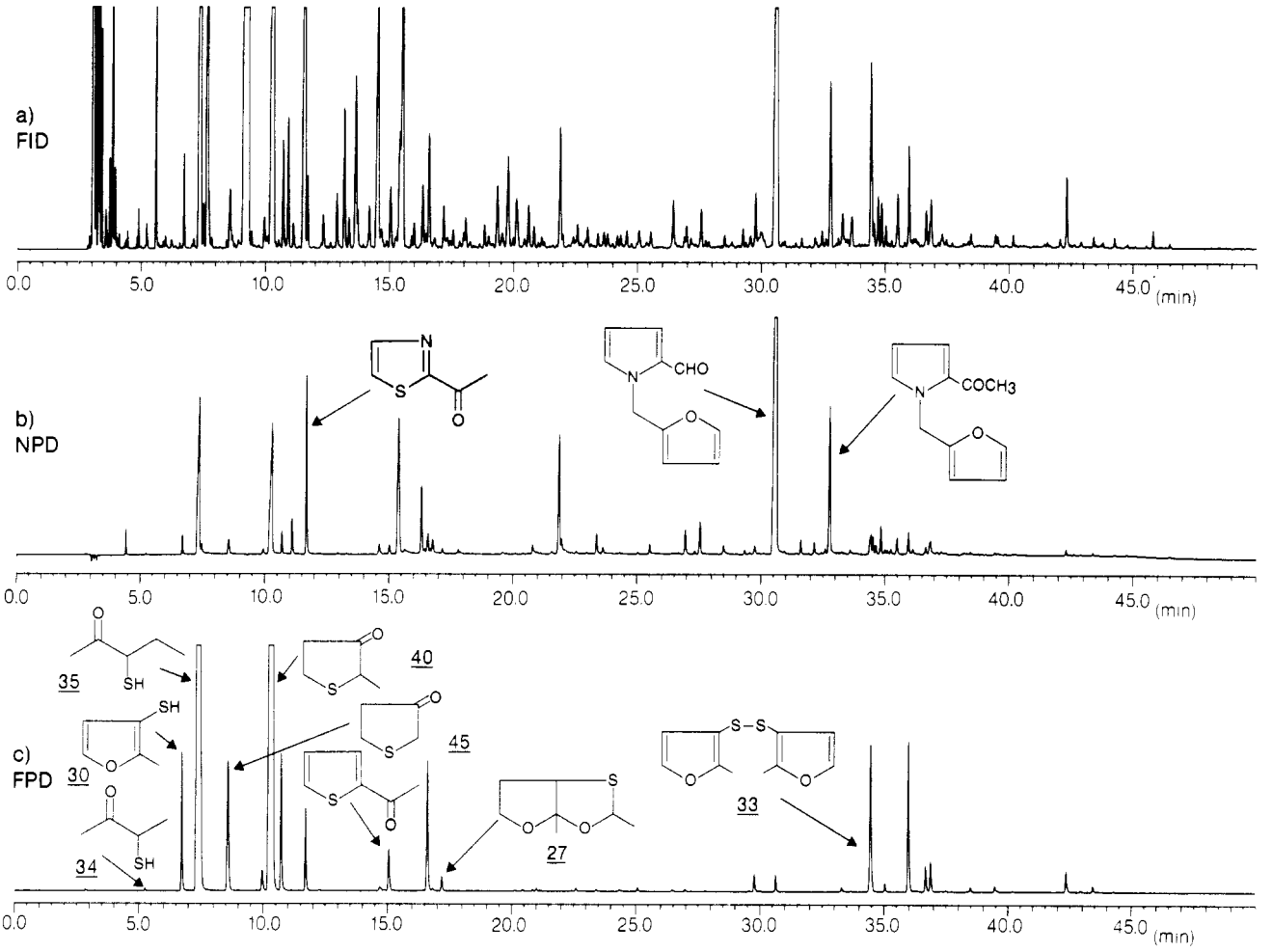


Figure 1. Capillary gas chromatogram of a medium polar silica gel fraction of model system IV showing simultaneous detector response to nitrogen-containing (b) and sulfur-containing (c) volatiles. Temperature was programmed from 60 to 250 °C at 3 °C/min on a 60 m × 0.32 mm (i.d.) DB-1 column (d_f = 0.25 μm). The column effluent was split to the three detectors with a "glass-cap-cross".

amino acids in the presence of H_2S. Therefore, thiazoles can be identified in nearly all cooked or roasted food aromas.

A new compound in context of flavors is 3-mercaptopropyl acetate (**10**). It was mentioned by Sverdlov et al. (1969) but not in relation to food. Its presence may be explained by 3-mercaptopropanol (**9**), which could derive from thiamin by an additional cleavage of the double bond between C-4 and C-5 in the thiazole moiety. Unfortunately, **9** could not be identified in our studies thus far, though it was reported as a degradation product of thiamin by Matsukawa and Iwatsu (1950). Nevertheless, **9** is a very uncommon compound not reported over the past years in regard to flavor chemistry. A second explanation for 3-mercaptopropyl acetate (**10**) could be its formation from the precursor 3-mercapto-5-hydroxy-2-pentanone (**13**) via the intermediate **25** and the S-ylide **61** (Figure 3c). The spectral data of **10** are listed in Table III.

As mentioned above, the intermediates **12**–**15** are important precursors for the degradation of thiamin (Figure 3). The dimercaptopentanones **12** and **15** can react to interesting cyclic S compounds under oxidative conditions by dehydration as well as by release of hydrogen sulfide. Apparently, in this way **12** leads to the formation of 3-acetyl-1,2-dithiolane (**16**) and 2-acetylthietane (**18**), while 3-methyl-1,2-dithian-4-one (**41**) and 2-methyl-4,5-dihydro-3(2*H*)-thiophenone (**40**) derive from **15**. 3-Acetyl-1,2-dithiolane (**16**) was mentioned by Schmidt et al. (1963) and later found by Tressl (1983) as a degradation product of thiamin. In our studies it was identified in the three model mixtures I, IV, and V which contained thiamin. Its organoleptic impression is described in Table II. Its spectroscopic data are shown in Figure 6. 2-Acetylthietane (**18**) is a new compound in relation to flavor chemistry. However, it was cited twice in connection with vitamin B_1 by Yonemoto (1957) and Kawasaki et al. (1957). Interestingly, it has not been identified in recent years despite the application of modern analytical methods. Its identification is based on spectral data of the collected sample, which are shown in Figure 5. The sensory impression is described in Table II.

2-Methyl-4,5-dihydro-3(2*H*)-thiophenone (**40**) is a well-known flavor volatile. Its identification as a thermal degradation product of thiamin has been described several times previously (Dwivedi and Arnold, 1973b; van der Linde et al., 1979; Tressl, 1983; Hartman et al., 1984a). Interestingly, it was found in all of our model systems I–V. Therefore, besides the obvious formation mechanism starting from the intermediates **13d**, **14**, and **15** (Figure 3c) in the thermal degradation of thiamin there must be an additional pathway for the formation of this flavor compound proceeding from the nonenzymatic browning reaction between cysteine/cystine and different reducing sugars. This point is strongly supported by the work of Martin (1988), who reported this thiolanone to be present in different model systems (e.g., cysteine/xylose, cysteine/ascorbic acid, cysteine/glucose). This compound has also been identified in yeast extract (Werkhoff et al., 1988). Finally, 3-methyl-1,2-dithian-4-one (**41**) has been identified as a degradation product of thiamin by Tressl (1983) and

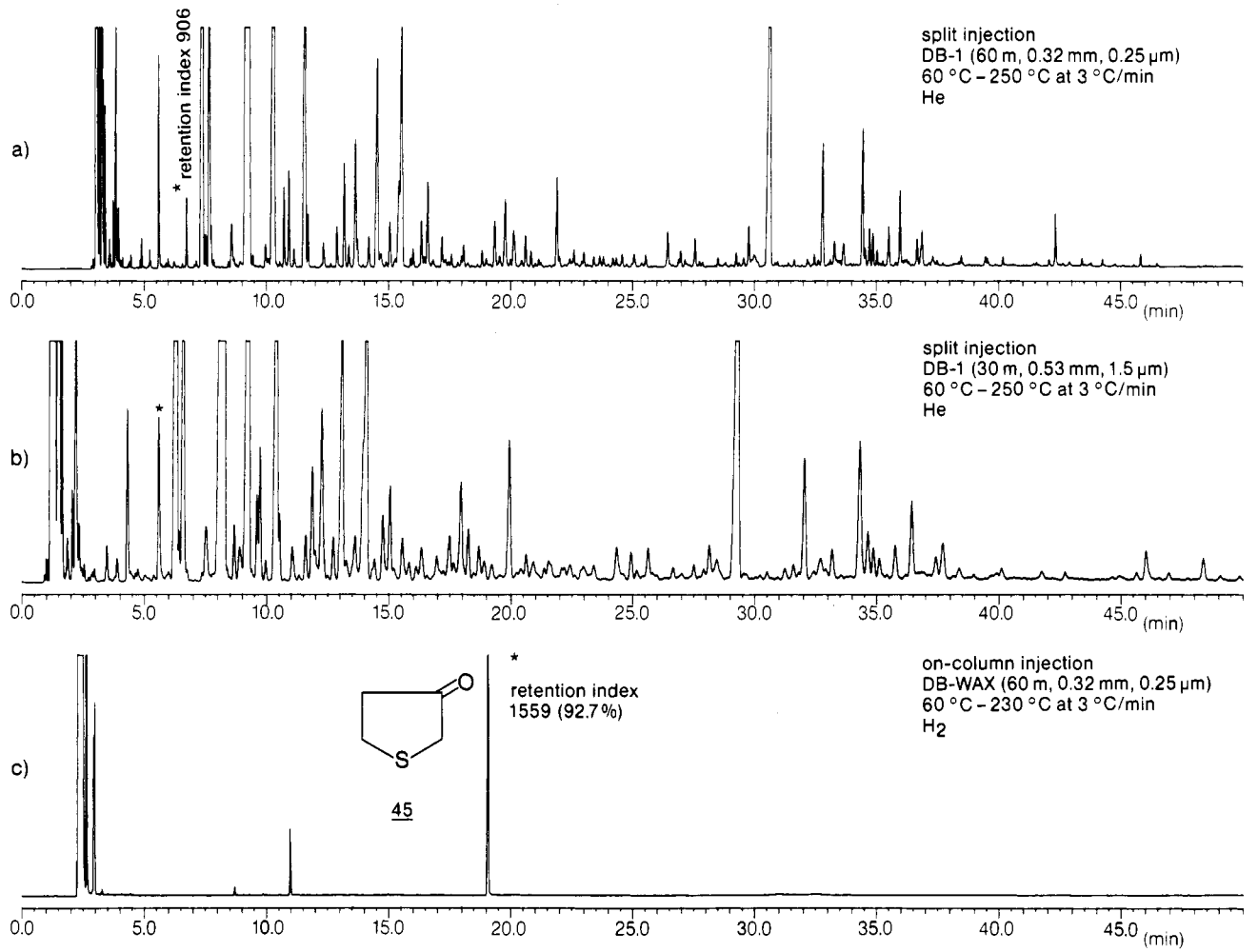


Figure 2. Isolation of the marked (*) dihydro-3(2*H*)-thiophenone (**45**) by capillary gas chromatography from a medium polar silica gel fraction of model system IV for subsequent structure elucidation by NMR, MS, and IR spectroscopies.

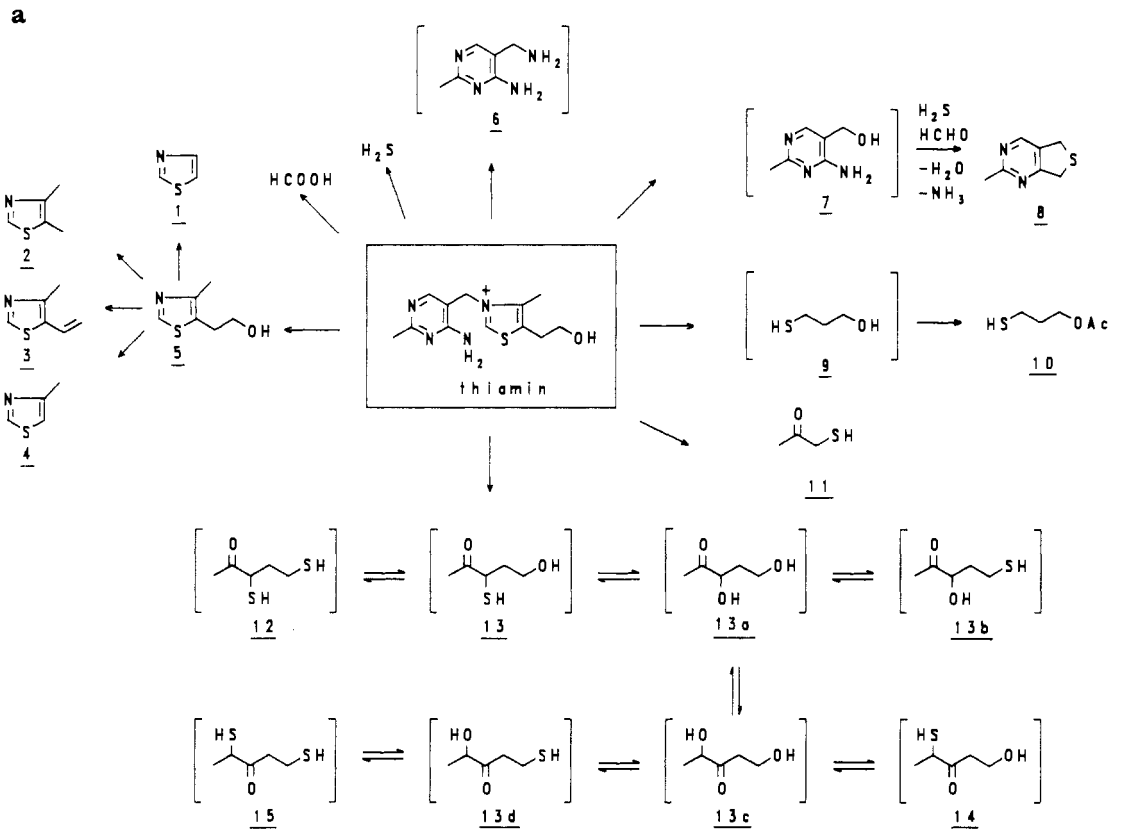
Hartman et al. (1984a) and in a model system containing monosodium glutamate, ascorbic acid, thiamin hydrochloride, and cystine by Hartman et al. (1984b,c). It also has been recently identified in yeast extract by Werkhoff et al. (1989b).

Another key intermediate in the reaction schemes (Figure 3) is 2-methyl-4,5-dihydro-3-furanthiol (**28**). This compound reportedly has a roasted meat aroma and was patented as a flavoring compound by van den Ouweland and Peer (1970). The same authors (van den Ouweland and Peer, 1975) reported its formation from the reaction of the sugar degradation component 4-hydroxy-5-methyl-3(2*H*)-furanone (norfuraneol) with H_2S. Though **28** seems to be a well-known compound in the thermal degradation of thiamin (van der Linde et al., 1979; Hartman et al., 1984a; van Dort et al., 1984), we could only tentatively identify this compound on the basis of its mass spectrum since it was too unstable to isolate by preparative GC. Additional supporting evidence was the finding of the corresponding heterocycle 2-methyl-4,5-dihydro-3-thiophenethiol (**19**). These two compounds, **19** and **28**, are probably formed from the unstable intermediates 2-methyl-2-hydroxy-3-tetrahydrothiophenethiol (**17**) and 2-methyl-2-hydroxy-3-tetrahydrofuranthiol (**25**) (Figure 3b).

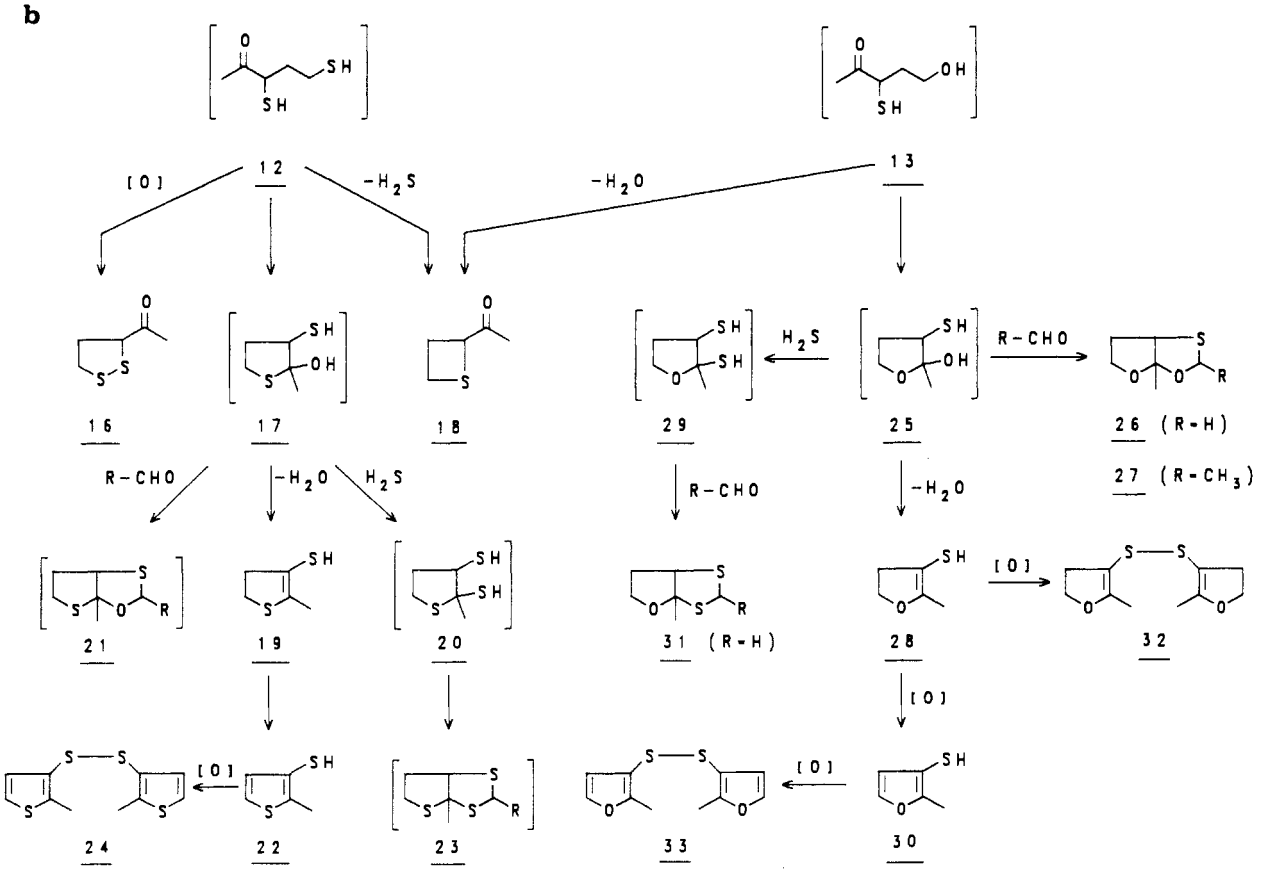
Two other unstable intermediates are 2-methyl-2-mercapto-3-tetrahydrothiophenethiol (**20**) and 2-methyl-2-mercapto-3-tetrahydrofuranthiol (**29**), which are probably very difficult to analyze by gas chromatography. Cyclization with a respective aldehyde leads to the general structure type of the 1-methylbicyclo[3.3.0]octane with three heteroatoms (O/S) in the 2-, 4-, and 8-positions. These are typical degradation products of thiamin. Their formation seems quite obvious (Figure 3b). To our knowledge, only 1-methylbicyclo[3.3.0]-2,8-dioxa-4-thiaoctane (**26**), 1,3-dimethylbicyclo[3.3.0]-2,8-dioxa-4-thiaoctane (**27**), and 1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane (**31**) are known in the literature (Tressl, 1983; Hartman et al., 1984a,b; Reineccius and Liardon, 1985; van Dort et al., 1984). The first flavor volatile of this type, 1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane (**31**) was identified by Seifert et al. (1978) in the photolysis (at 253.7 nm) of thiamin hydrochloride. In our studies we were able to identify **26**, **27**, and **31**. In our opinion, the formation of 1-methylbicyclo[3.3.0]-4,8-dithia-2-oxaoctane (**21**) as well as 1-methylbicyclo[3.3.0]-2,4,8-trithiaoctanes (**23**) with different substituents in the 3-position should also be possible. This assumption was supported by the finding of 1-methylbicyclo[3.3.0]-2,4,8-trithiaoctane (**23**) by Tressl (1983) among the degradation products of thiamin.

Further flavor compounds identified in our studies (Table I) were the well-known thiols 2-methyl-3-furanthiol (**30**) (model systems I, V) and 2-methyl-3-thiophenethiol (**22**) (model systems IV, V). The respective disulfides, bis(2-methyl-3-furyl) disulfide (**33**) (model systems I, III, IV, V) and bis(2-methyl-3-thienyl) disulfide (**24**) (model system V), were also identified. However, only **30** and its oxidation product **33** were found among the volatiles of the degradation of pure thiamin. Nevertheless, we believe that all these compounds can be formed by thermally degrading thiamin. They probably exist in traces and need to be enriched prior to their identification. The mechanism of formation of the thiols and subsequently

a


HCOOH
H2S
H2S
HCHO
-H2O
-NH3
thiamin

b


[O]
-H2S
-H2O
H2S
R-CHO
-H2O
H2S
R-CHO
-H2O
[O]
[O]
[O]
R-CHO
(R-H)
(R-CH3)

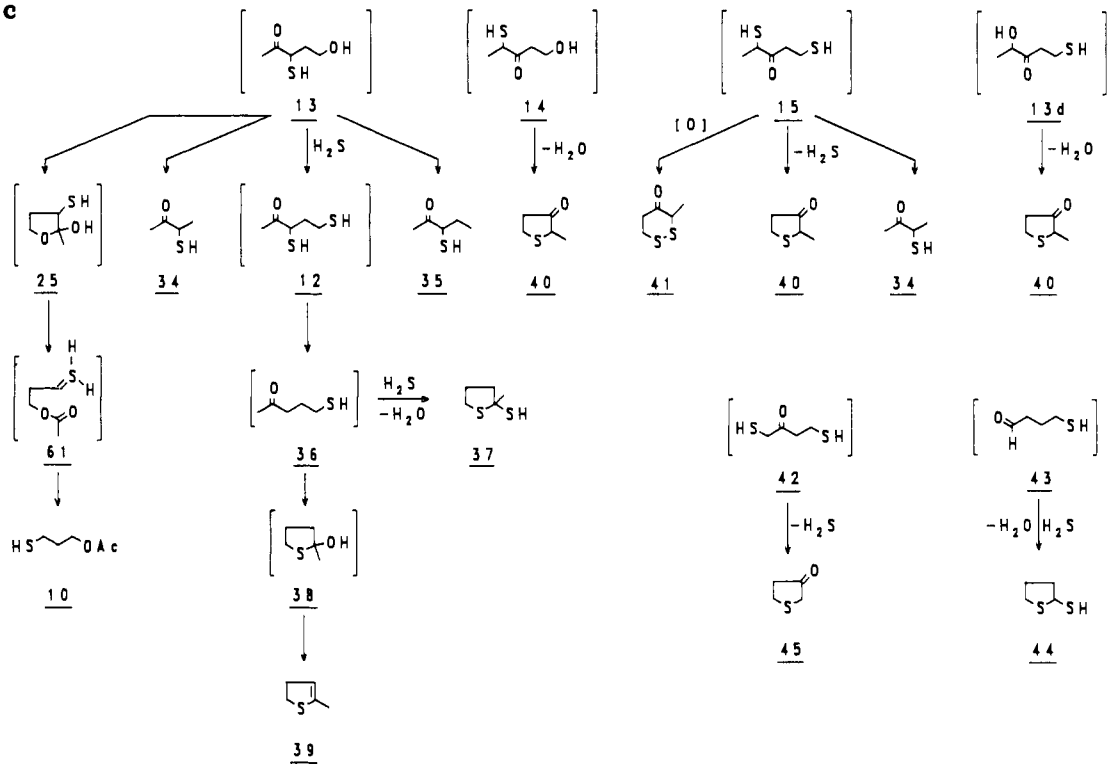


Figure 3. Proposed pathways of the thermal degradation of thiamin.

Table II. Organoleptic Judgments of Some S-Containing Flavor Volatiles

constituent	no.	isolated sample,[a] μg/100 mg	synthesized sample,[b] ppm	impression
mercaptopropanone	11		3.7×10^{-2}	sulfurous, meaty, pickled
2-(1-mercaptoethyl)furan	56		1.5×10^{-2}	mocha, roasty, elderberry
2-acetylthietane	18	5		onion, pungent, radish, sulfurous
2-tetrahydrothiophenethiol	44		4.5×10^{-1}	onion, roasty, tropical fruit, meaty, sulfurous
2-methyl-2-tetrahydrothiophenethiol	37		1.1×10^{-3}	tropical fruit, sulfurous, buccu, meaty, black currant
2-methoxy-2-methyl-4,5-dihydro-3(2*H*)-thiophenone	57		5×10^{1}	rubber, anaesthetic, sulfurous
2-(2-thienyl)furan	46		7.5×10^{-1}	sulfurous, naphtha
3-acetyl-1,2-dithiolane	16		1.5×10^{-1}	meaty, onion, shiitake, liver
2-(2-furyl)thiazole	47		2.2	coffee
1*H*-pyrrolo[2,1-*c*]-1,4-thiazine	54	5		meaty, roasty

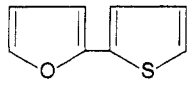
[a] Dissolved in ethanol and tested on a smelling blotter. [b] Tested in water.

of the disulfides from thiamin can be explained as an oxidation reaction starting from **28** and **19**, respectively. The spectral data of **22**, **24**, **30**, and **33** were previously reported by Werkhoff et al. (1989a, 1990). **30** has been found in thermally degraded thiamin by Tressl (1983), Hartman et al. (1984a), and Reineccius and Liardon (1985) as well as in a flavor model system by Hartman et al. (1984b,c). Recently, **30** was identified as a natural compound in the steam distillate from canned tuna fish by Withycombe and Mussinan (1988) and in cooked beef by Gasser and Grosch (1988). The disulfide **33** is a compound with a remarkably low odor threshold (Buttery et al., 1984). It was first identified by Evers et al. (1976) in a model system containing cysteine, thiamin, and a vegetable protein hydrolysate. Later, this disulfide was also identified among the degradation products of thiamin (Tressl, 1983; Hartman et al., 1984a; Reineccius and Liardon, 1985) and the above-mentioned flavor model system (Hartman et al., 1984b,c) and recently by Golovnya et al. (1983) in a simulated meat flavor. Moreover, its presence in cooked beef was reported by Gasser and Grosch (1988). **22** was found by van den Ouweland and Peer (1975) in a heated model system of 4-hydroxy-5-methyl-3(2*H*)-furanone (norfuraneol) with H_2S. Tressl (1983) traced it as a degradation product of thiamin. Besides in our own work (Werkhoff et al., 1989a, 1990) the disulfide **24** was only mentioned by Gol'dfarb et al. (1967). Recently, we were able to identify **22**, **30**, and **33** in yeast extract (Werkhoff et al., 1988, 1989b).

Another disulfide found as a degradation product of thiamin was bis(2-methyl-4,5-dihydro-3-furyl) disulfide (**32**). Its formation from 2-methyl-4,5-dihydro-3-furanthiol (**28**) follows easily by an oxidation step. It was identified in model systems I and V. Its presence in model system V was described previously (Werkhoff et al., 1989a, 1990). However, the identification was considered tentative due to lack of a reference standard. The identification has now been confirmed by synthesis with the spectroscopic data shown in Figure 9.

Other interesting thiamin degradation products include mercaptopropanone (**11**), 3-mercapto-2-butanone (**34**), and 3-mercapto-2-pentanone (**35**). The mutual structure moiety of 3-mercapto-2-alkanone bears strong evidence for the same origin from the thiazole part of thiamin. Mercaptopropanone (**11**) (model system I) and 3-mercapto-2-butanone (**34**) (model systems I, III, IV, V) have not been reported as thiamin degradation products. However, they are known as food volatiles. Uchman and Jennings (1977) found **11** in ground pork meat. The identification of **34** along with its spectroscopic data was recently reported as

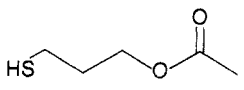
Table III. Spectroscopic Data of Some S-Containing Compounds



2-(thienyl)furan 46

MS-data (m/z, %)
150 (100) 121 (68) 122 (28) 78 (13) 77 (12)
96 (12) 151 (9) 39 (9) 45 (8) 75 (8)

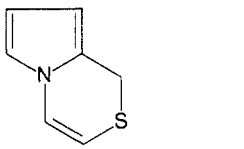
^{1}H-NMR-data (200 MHz, C_6D_{12}, TMS)
7.25 ppm (1H; dd; 1.7, 0.7 Hz) } furyl-
6.35 ppm (1H; dd; 3.4, 0.8 Hz) } furyl-
6.29 ppm (1H; dd; 3.4, 1.8 Hz) } furyl-
7.15 ppm (1H; dd; 3.6, 1.2 Hz) } thienyl-
7.05 ppm (1H; dd; 5.1, 1.2 Hz) } thienyl-
6.89 ppm (1H; dd; 5.0, 3.6 Hz) } thienyl-



3-mercaptopropylacetate 10

MS-data (m/z, %)
43 (100) 74 (81) 41 (37) 46 (14) 47 (10)
61 (10) 45 (9) 73 (6) 27 (6) 59 (4)

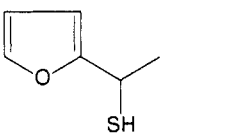
^{1}H-NMR-data (200 MHz, $CDCl_3$, TMS)
4.19 ppm (2H; t; 6.2 Hz)
2.61 ppm (2H; q; 7.7 Hz)
2.06 ppm (3H; s)
1.94 ppm (2H; quint; 6.8 Hz)
1.40 ppm (1H; t; 8.0 Hz)



1H-pyrrolo[2,1-c] [1,4]thiazine 54

MS-data (m/z, %)
136 (100) 137 (76) 138 (10) 39 (9) 109 (7)
78 (7) 58 (7) 65 (5) 104 (4) 51 (4)

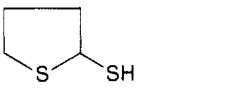
^{1}H-NMR-data (200 MHz, C_6D_{12}, TMS)
6.97 ppm (1H; d; 7.4 Hz)
6.59 ppm (1H; m)
6.02 ppm (1H; t; 3.2 Hz)
5.77 ppm (1H; m)
5.60 ppm (1H; d; 7.4 Hz)
3.84 ppm (2H; s)



2-(1-mercaptoethyl)furan 56

MS-data (m/z, %)
95 (100) 128 (22) 67 (19) 94 (17) 65 (17)
41 (13) 39 (12) 96 (7) 66 (6) 45 (4)

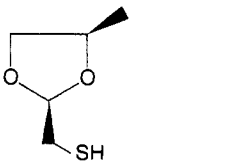
^{1}H-NMR-data (200 MHz, $CDCl_3$, TMS)
7.36 ppm (1H; dd; 1.8, 0.9 Hz)
6.29 ppm (1H; dd; 3.1, 1.9 Hz)
6.15 ppm (1H; dd; 3.1, 0.9 Hz)
4.21 ppm (1H; quint; 6.7 Hz)
2.12 ppm (1H; d; 6.5 Hz)
1.67 ppm (3H; d; 6.9 Hz)



2-tetrahydrothiophenethiol 44

MS-data (m/z, %)
87 (100) 45 (48) 120 (26) 58 (15) 85 (14)
53 (14) 57 (9) 47 (9) 41 (9) 27 (9)

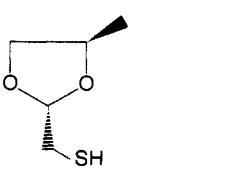
^{1}H-NMR-data (200 MHz, $CDCl_3$, TMS)
4.6 - 4.7 ppm (1H; m)
2.8 - 3.3 ppm (2H; m)
1.9 - 2.4 ppm (4H; m)
2.30 ppm (1H; d; 6.1 Hz)



cis-2-mercaptomethyl-4-methyl-1,3-dioxolane 59

MS-data (m/z, %)
87 (100) 59 (50) 31 (38) 41 (31) 47 (14)
45 (9) 29 (9) 43 (8) 61 (7) 27 (6)

^{1}H-NMR-data (200 MHz, $CDCl_3$, TMS)
5.06 ppm (1H; t; 4.3 Hz)
4.22 ppm (1H; ddq; 7.4, 6.1, 6.0 Hz)
4.01 ppm (1H; dd; 7.9, 7.1 Hz)
3.48 ppm (1H; t; 7.5 Hz)
2.72 ppm (2H; dd; 8.5, 4.3 Hz)
1.55 ppm (1H; t; 8.6 Hz)
1.33 ppm (3H; d; 6.0 Hz)



trans-2-mercaptomethyl-4-methyl-1,3-dioxolane 60

MS-data (m/z, %)
87 (100) 59 (41) 31 (39) 41 (30) 47 (16)
29 (9) 45 (8) 43 (7) 61 (7) 88 (7)

^{1}H-NMR-data (200 MHz, $CDCl_3$, TMS)
5.18 ppm (1H; t; 4.4 Hz)
4.30 ppm (1H; ddq; 7.1, 5.9, 6.0 Hz)
4.16 ppm (1H; dd; 7.9, 5.8 Hz)
3.45 ppm (1H; dd; 7.9, 7.1 Hz)
2.69 ppm (2H; dd; 8.6, 4.4 Hz)
1.53 ppm (1H; t; 8.6 Hz)
1.28 ppm (3H; d; 6.1 Hz)

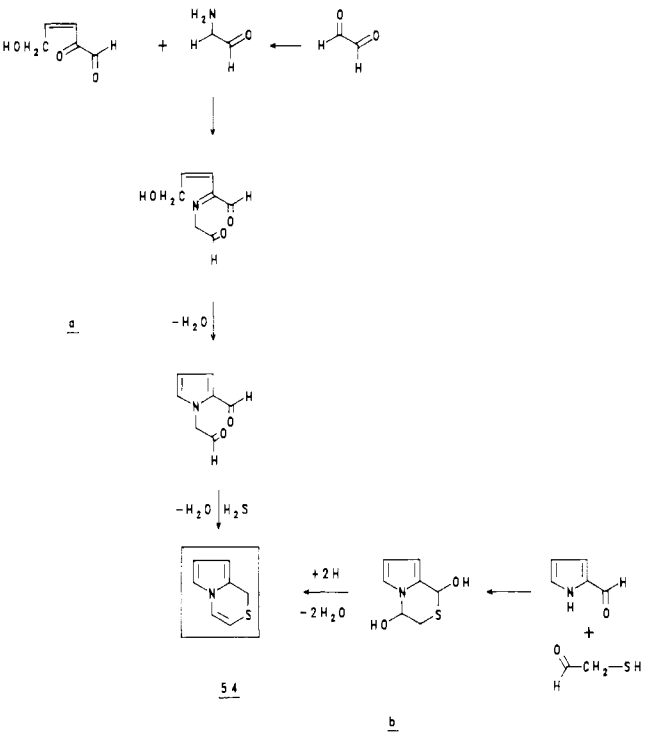


Figure 4. Possible mechanisms a and b for the formation of 1*H*-pyrrolo[2,1-c]-1,4-thiazine by a nonenzymatic browning reaction between ribose and cysteine.

a constituent of yeast extract by Werkhoff et al. (1989b). 3-Mercapto-2-pentanone (**35**) (model systems I, III, IV, V) is a more common flavor compound. Its identification as a degradation product of thiamin was reported by van der Linde et al. (1979) and by Hartman et al. (1984a). It was also found by Shu et al. (1985c) in a reaction mixture between cystine and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (furaneol). In general, the substance class of α-ketothiols has been patented for use in meat flavors [e.g., Katz et al. (1973a)]. Their formation has been discussed by Cramer (1983).

An interesting flavor compound is 2-methyl-5,7-dihydrothieno[3,4-*d*]pyrimidine (**8**). It was claimed in two patents by Katz et al. (1972b, 1973b). The odor is described as "sharp, fresh roasted, sweet nut with a popcorn character, sweet roasted peanut". Surprisingly, we could identify this compound in only one model system (IV) containing thiamin. Its presence among the volatiles of the thermal degradation of pure thiamin has not been established thus far. Nevertheless, its formation from the pyrimidine moiety of thiamin with formic aldehyde and H_2S seems obvious (Figure 3a). Perhaps, the precursor **7** is formed in less yield by the different reaction conditions in model system I (pure thiamin hydrochloride) compared to model system IV.

Another well-known food volatile is dihydro-3(2*H*)-thiophenone (**45**). However, it was not previously mentioned in the literature as a compound resulting from the thermal degradation of thiamin. The mechanism of its formation remains unclear. The probable precursor of **45** is 1,4-dimercapto-2-butanone (**42**) (Figure 3c). The flavor compound **45** could also be identified in the model systems I, III, IV, and V. Its formation from the amino acids cystine and cysteine by condensation of two molecules of mercaptoacetaldehyde (main Strecker degradation product) is much easier to explain. **45** has been identified in a cysteine/xylose model mixture (Martin, 1988), thermally degraded cysteine (Shu et al., 1985b), pressure-

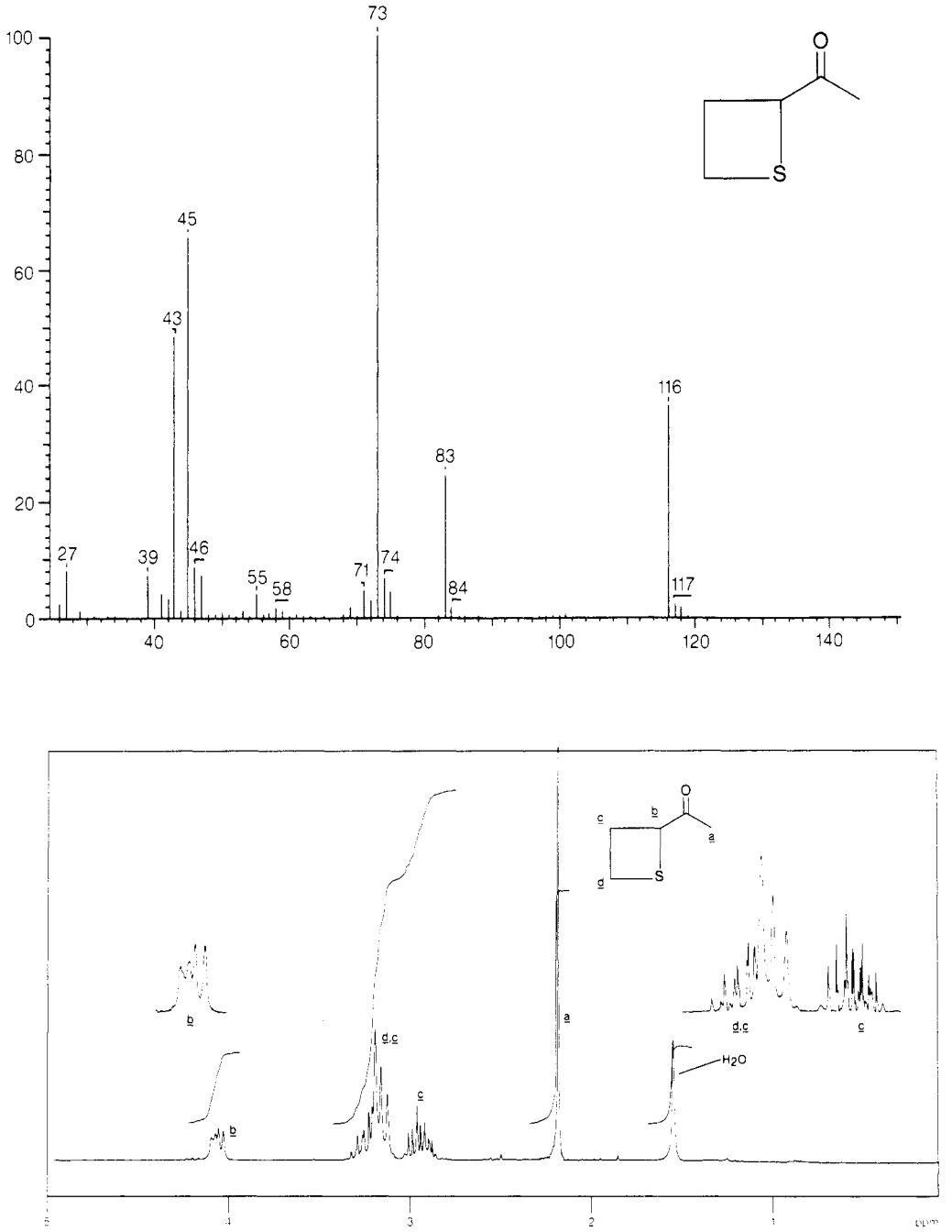


Figure 5. Spectral data (MS, 200-MHz ^{1}H NMR) of an isolated sample of 2-acetylthietane.

cooked beef (Wilson et al., 1973), and yeast extract (Werkhoff et al., 1989b).

Another thiolanone identified in our model systems III and IV was dihydro-2(3*H*)-thiophenone (γ-thiobutyrolactone) (**52**). It was also identified in pressure-cooked beef (Wilson et al., 1973), as a degradation product of a cysteine/xylose mixture (Martin, 1988), and in thermally degraded cystine (Shu et al., 1985a). It seems to be a pure degradation product of cystine/cysteine as reported by Shu et al. (1985a) and Ledl and Severin (1973).

Further compounds identified in our studies that bear a close relation to the thermal degradation of thiamin are 2-methyl-4,5-dihydrothiophene (**39**), 2-tetrahydrothiophenethiol (**44**), and 2-methyl-2-tetrahydrothiophenethiol (**37**). However, only **39** could be found in model system I (pure thiamin). All three mentioned S compounds were identified in model system V. The formation of **44** as a C-4 compound is difficult to explain. Its probable precursor is 4-mercaptobutanal (**43**) (Figure 3c). Its homologue **37** requires 5-mercapto-2-pentanone (**36**) as a direct precursor, which could derive from the intermediate 3,5-dimercapto-2-pentanone (**12**) by the loss of sulfur in the 3-position (Figure 3c). Van der Linde et al. (1979) described the probable mechanism for the formation of 5-hydroxy-2-pentanone from 3-mercapto-5-hydroxy-2-pentanone by the loss of an HS^- ion. They cite work by Mayer (1970) as reference for this reaction which can occur in mercapto ketones. Accordingly, **36** and subsequently **37** can be formed analogously in our studies (Figure 3c). The subsequent formation of **39** via the intermediate 2-hydroxy-2-methyltetrahydrothiophene (**38**) seems also possible by this pathway. The presence of ascorbic acid in model system V and the preparation under autoclave conditions probably favor the reduction step from **12** to **36**. This could be one reason **37** and **44** were not identified among the volatiles of thermally degraded thiamin (model system I) but in model system V. The olfactory impression (Table II) and the spectroscopic data (Table III, Figure 7) of the tetrahydrofurans **37** and **44** are given. 2-Tetrahydrothiophenethiol (**44**) is mentioned by

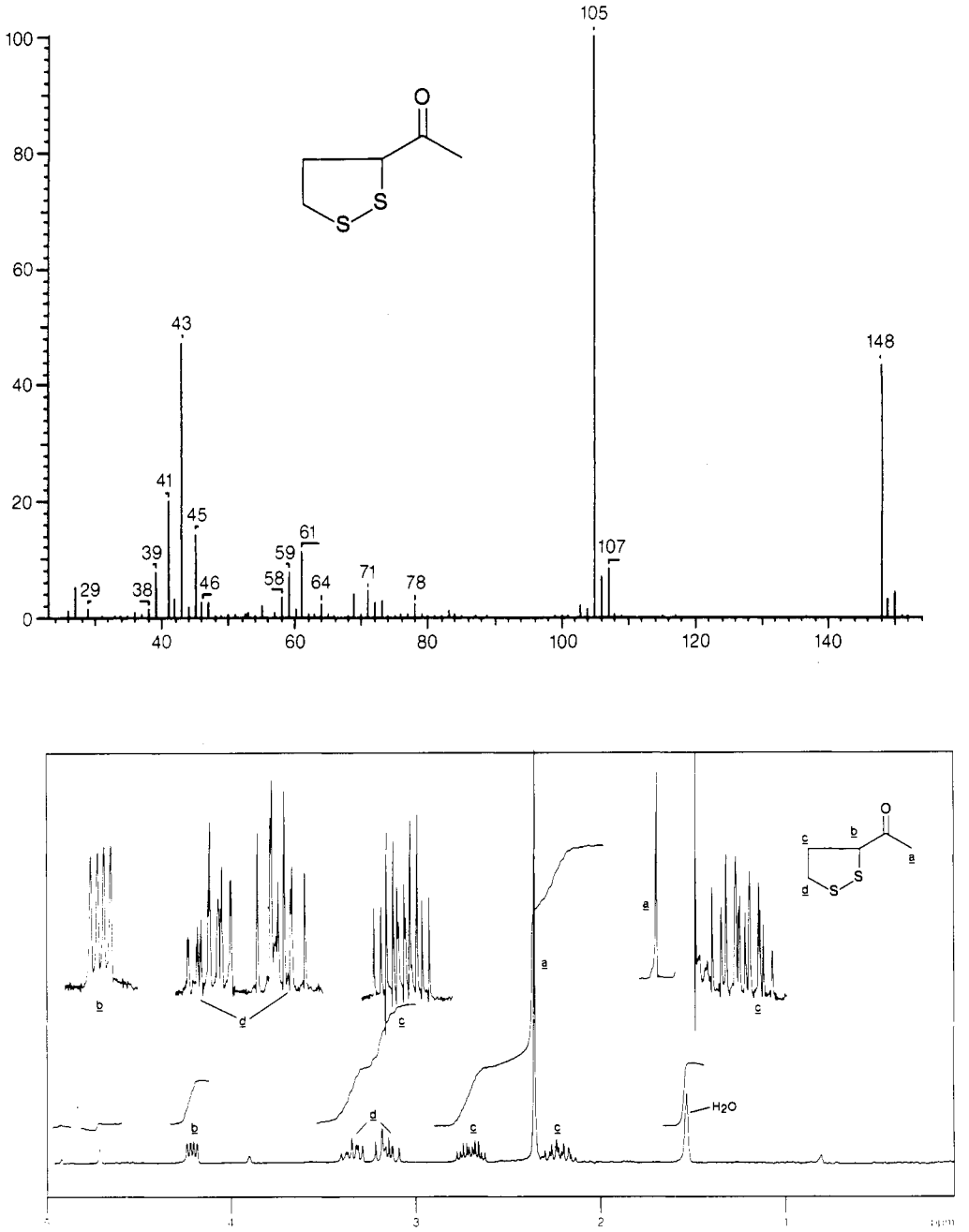


Figure 6. Spectral data (MS, 200-MHz ^{1}H NMR) of synthesized 3-acetyl-1,2-dithiolane (**16**).

Gais (1977), though not in relation to flavor chemistry. 2-Methyl-2-tetrahydrothiophenethiol (**37**) was mentioned in patents of the flavor and fragrance company PFW (van den Bosch, 1976, 1978).

A new compound in context with flavor chemistry is 2-(2-thienyl)furan (**46**). On the other hand, its close relative 2-(2-furyl)thiazole (**47**) is a well-known flavor constituent. It was reported by Tressl et al. (1981) in malt and roasted coffee as well as by Mulders (1973a) in white bread. Additionally, it was identified as product of the nonenzymatic browning reaction between cysteine and ribose (Kleipool and Tas, 1973) and in a cysteine/cystine–ribose system (Mulders, 1973b). It seems likely that these compounds are formed by radical reactions. The respective furan, thiophene, and thiazole moieties are imaginable in thermally degraded thiamin as well as in model reactions with cysteine or cystine and reducing sugars.

2-(2-Thienyl)furan (**46**) is reported for the first time as a degradation product of thiamin (model system I) and was also found in the model reactions III and IV. Its organoleptic impression and spectroscopic data are described in Tables II and III, respectively. The synthesis of **46** was performed according to the method of Ullenius (1972) (see Experimental Procedures). Both compounds **46** and **47** were also identified in yeast extract (Werkhoff et al., 1988).

Besides the thermal degradation of thiamin, the amino acids cystine and cysteine are well-known precursors of meat flavor. There are many patents and publications concerning this point. The most recent investigations into flavor compounds derived from these sulfur-containing amino acids were done by Golovnya and Rothe (1980), de Rijke et al. (1981), Shu et al. (1985a–c, 1986), Zhang et al. (1988), and Martin (1988). The decisive reaction mechanism for the formation of meat flavor from cystine/cysteine is the Strecker degradation with α-diketones leading to mercaptoacetaldehyde, acetaldehyde, and H_2S. These very volatile primary degradation products can react further by interaction with each other or with the degradation compounds of sugars.

1,2,3-Trithia-5-cycloheptene (**48**) is a S compound

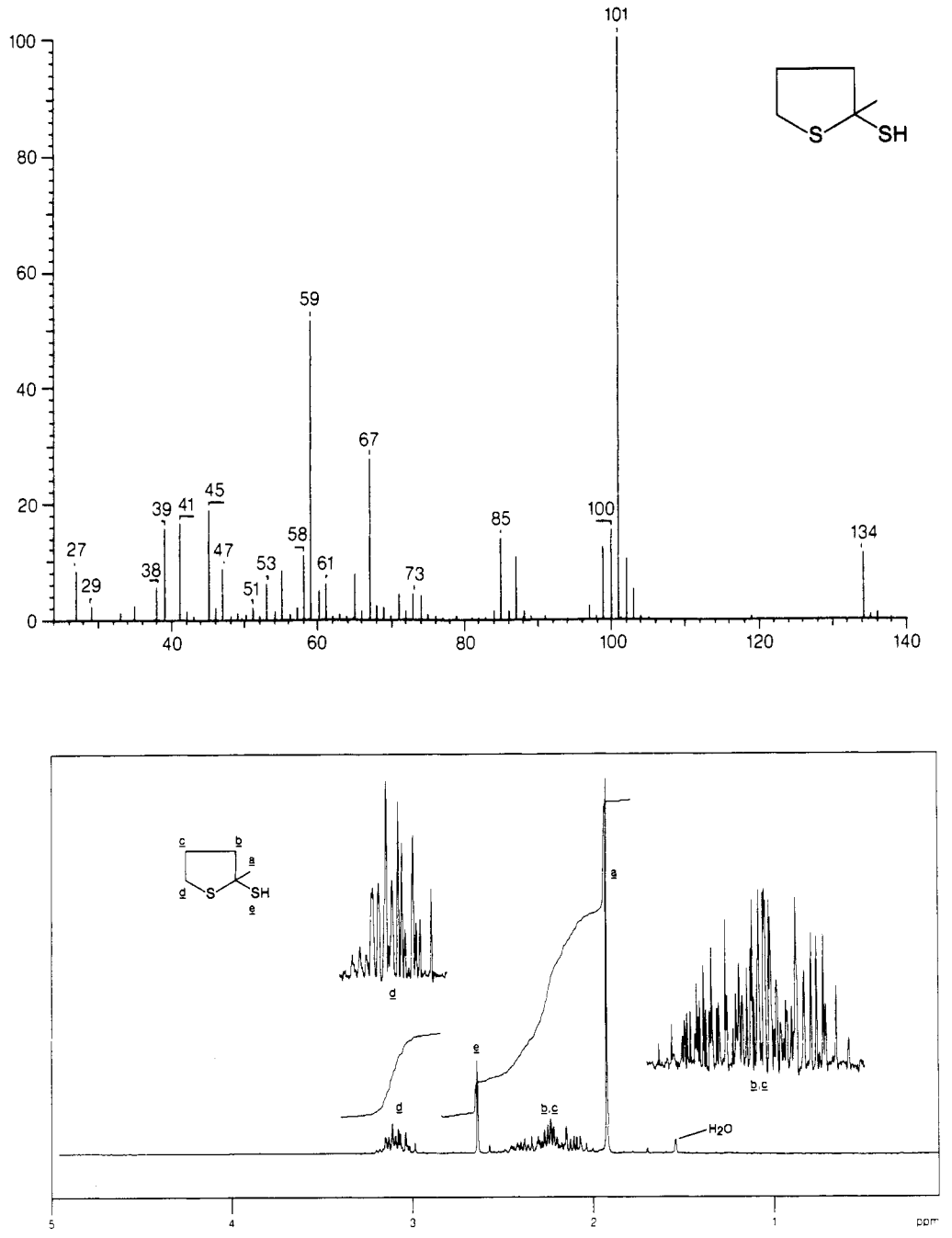


Figure 7. Spectral data (MS, 200-MHz ^{1}H NMR) of synthesized 2-methyl-2-tetrahydrothiophenethiol (**37**).

identified recently by Shu et al. (1985b) as a degradation product of cysteine. We tentatively identified this compound in model system II by comparing its mass spectrum to that reported by Shu et al. (1985b). Further volatiles identified in model system II (cysteine and ribose) were furfurylthiol (**49**), 3-thiophenethiol (**50**), 2-methyl-4,5-dihydro-3(2*H*)-thiophenone (**40**), 2-formyl-5-methyl-4,5-dihydrothiophene (**51**), thieno[3,2-*b*]thiophene (**53**), 1*H*-pyrrolo[2,1-*c*]-1,4-thiazine (**54**), and bis(3-thienyl) disulfide (**55**). This is only a selection of compounds since the use of propylene glycol as solvent implies the formation of many dioxolanes, which are not reported here with the exception of the cis and trans isomers of 2-(mercaptomethyl)-4-methyl-1,3-dioxolane (**59**, **60**) (Table I). Indirectly, these dioxolanes demonstrate the presence of mercaptoacetaldehyde as the main thermal degradation product of cysteine in this model system. They are reported for the first time, and their spectroscopic data are shown in Table III. Their stereochemistry was determined by the ^{1}H NMR spectra.

3-Thiophenethiol (**50**) is known as a degradation compound of cysteine (Shu et al., 1985b). Additionally, it was recently identified by Misharina et al. (1975) in model systems with meat odor and by Whitfield et al. (1988) in heated aqueous solutions of amino acids and ribose. Its oxidation product, bis(3-thienyl) disulfide (**55**), has been patented as a useful flavor compound (Katz et al., 1972a). The identification of **55** is considered tentative since it is based solely on mass spectral data and the presence of the respective thiol **50** in model system II. Interestingly, Martin (1988) does not report these two compounds in his studies.

2-Formyl-5-methyl-4,5-dihydrothiophene (**51**) was recently identified in yeast extract (Werkhoff et al., 1989b). Its formation cannot be easily explained since model system II contains only a C-5 sugar (ribose) and cysteine. It is possible that mercaptoacetaldehyde reacts with ribose by cyclization and subsequent decarbonylation. Its identification was based only on ^{1}H NMR and mass spectra of the collected sample.

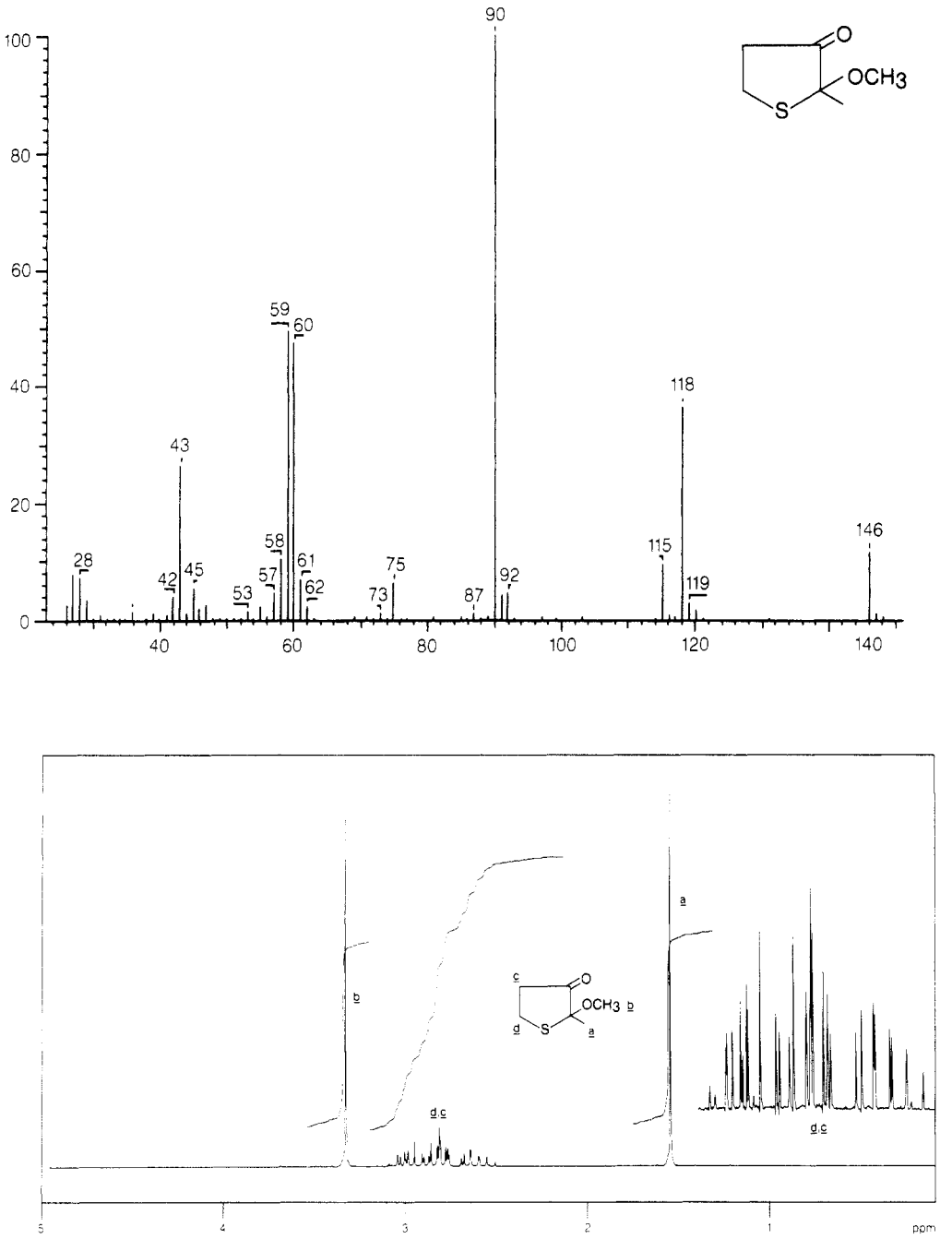


Figure 8. Spectral data (MS, 200-MHz ^{1}H NMR) of synthesized 2-methoxy-2-methyl-4,5-dihydro-3(2*H*)-thiophenone (**57**).

An interesting and new compound is 1*H*-pyrrolo[2,1-*c*]-1,4-thiazine (**54**). The elucidation of its structure was based on spectral data of the isolated compound (Table III). We first proposed the isomeric structure of 1*H*-pyrrolo[1,2-*c*]-1,3-thiazine. However, after synthesis of this heterocycle (Thielmann et al., 1989), it was clear that the spectral data of our compound represented structure **54**. Its organoleptic impression is described in Table II. Figure 4 shows the possible formation mechanisms (a, b) of **54**. Flament et al. (1977) previously reported the identification of pyrrolo[1,2-*a*]pyrazines in grilled meat (beef). The formation of **54** is possible in a similar way by a nonenzymatic browning reaction between ribose and cysteine (a). A second pathway (b) could start from the well-known pyrrole-2-carboxaldehyde and mercaptoacetaldehyde, though this reaction requires a reduction step. One possible reducing agent is the enaminol which is generated from the Strecker degradation of cysteine and causes the cleavage of mercaptoacetaldehyde to acetaldehyde and hydrogen sulfide. This reaction was first described by Kobayasi and Fujimaki (1965) [see also Martin (1988)].

A well-known flavor volatile in the context of reactions between cysteine/cystine and reducing sugars is thieno-[3,2-*b*]thiophene (**53**) (Mulders, 1973a,b; Zhang et al., 1988; Whitfield et al., 1988). Shu et al. (1985a,b) reported one "thienothiophene" in thermally degraded cysteine and two "thienothiophenes" in thermally degraded cystine. Though the structures were not differentiated (i.e., [3,2-*b*], [2,3-*b*], and [3,4-*b*]), the formation of this structure type from pure cysteine/cystine seems to be possible without any reducing sugar.

2-Methoxy-2-methyl-4,5-dihydro-3(2*H*)-thiophenone (**57**) is a new compound identified in model system IV. Its organoleptic impression is described in Table II, and its spectroscopic data are shown in Figure 8. Its formation is not easily understood, but the structure bears a striking similarity to 2-methyl-4,5-dihydro-3(2*H*)-thiophenone (**40**). Since the methoxy group is quite uncommon, one possible

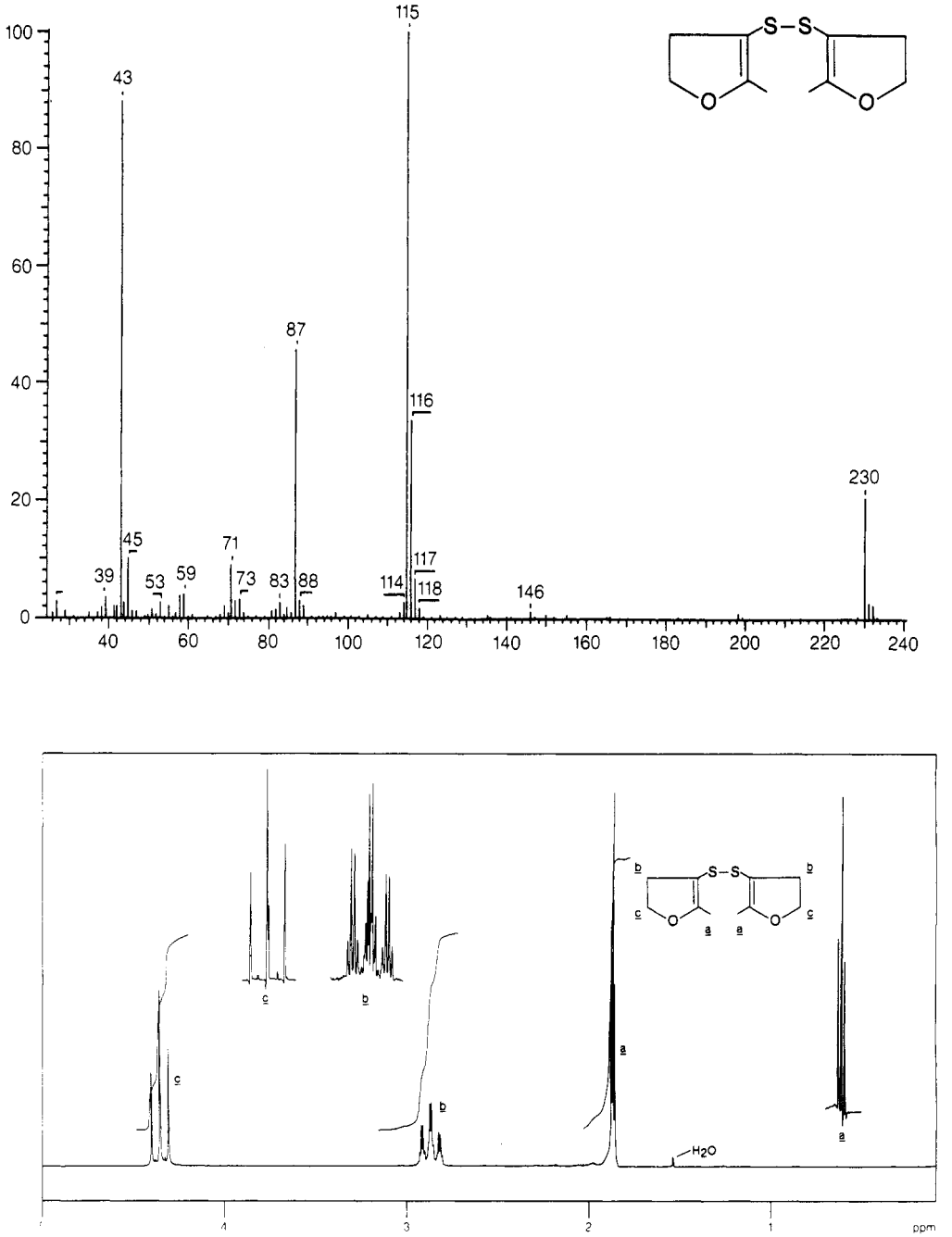


Figure 9. Spectral data (MS, 200-MHz ^1H NMR) of synthesized bis(2-methyl-4,5-dihydro-3-furyl) disulfide (**32**).

precursor could be a compound like 2-hydroxy-2-methyl-4,5-dihydro-3(2*H*)-thiophenone, leading to **40** by reaction with a methyl radical.

2-(1-Mercaptoethyl)furan (**56**) is mentioned twice in the literature, though not in relation to flavor chemistry. It was found in model systems III and V. In these two reaction mixtures C-6 carbohydrates (ascorbic acid, lactose) were present. Therefore, it is likely that **56** is formed directly by the degradation of the reducing carbohydrate followed by reaction with H_2S. The organoleptic impression is described in Table II, and its spectral data are shown in Table III.

2-Formyl-5-methylthiophene (**58**) is a known flavor volatile. It was present in model systems III, IV, and V. It was reported by Martin (1988) in mixtures of cysteine with different sugars. Additionally, **58** was identified in cooked beef by Wilson et al. (1973) and Gasser and Grosch (1988). Its formation may be explained similarly to the formation of 2-formyl-5-methyl-4,5-dihydrothiophene (**51**) from a C-5 sugar with mercaptoacetaldehyde or by the thermal degradation of a C-6 carbohydrate followed by reaction with H_2S.

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