

Sulfur-Containing Furans in Commercial Meat Flavorings

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The volatiles of eight commercially available meat flavorings were enriched by dynamic headspace extraction. The resulting aroma extracts were analyzed by high-resolution gas chromatography and gas chromatography/mass spectrometry (GC/MS). Among the compounds identified were 16 sulfur-containing furans. 2-Methyl-3-(ethylthio)furan, 1-[(2-methyl-3-furyl)dithio]-2-propanone, and the spectroscopic data of 3-[(2-methyl-3-furyl)dithio]-2-butanone, 2-[(2-methyl-3-furyl)dithio]-3-pentanone and 3-[(2-methyl-3-furyl)dithio]-2-pentanone are reported here for the first time. Possible formation pathways are discussed.

Keywords: Processed meat flavorings; dynamic headspace analysis; aroma chemicals; sulfur-containing furans

INTRODUCTION

Most of mankind enjoys the flavor of thermally treated meat. In the past 30 years there has been great effort to investigate the components responsible for flavor development during the thermal processing of meat and to analyze the chemical structures of the odorants that contribute to the meaty flavor impact. Since early work on meat flavor showed that the flavor compounds are generated from thermal reactions of small water-soluble precursor molecules, many workers have investigated model reactions consisting of only a few reactants to identify organoleptically interesting meat flavor compounds and to obtain information about their formation pathways. These studies showed that most of the odorants described with meaty aroma notes contain sulfur and that the reaction between sulfur-containing amino acids and reducing sugars (Maillard reaction) on the one hand and the thermal degradation of thiamin on the other hand are the most important reactions in generating meaty aroma compounds. The results from the analysis of natural meat flavors and model systems have led to the development of commercial meat flavorings, in which authentic constituents from meat are replaced by cheaper precursor sources. These so-called "processed meat flavors" are normally produced by thermal treatment of a reaction mixture consisting of an amino acid source, e.g., hydrolyzed vegetable protein (HVP) or autolyzed yeast extracts (AYE), a reducing sugar source, and an additional sulfur source (e.g., cysteine, methionine, or thiamin) which facilitates the formation of sulfur-containing aroma compounds. Varying the ingredients or the reaction conditions (e.g., pH, temperature, pressure, or processing time) and adding special aroma essences (e.g., essential oils, smoke essences, or synthetic aroma chemicals) yield different species-specific flavorings (e.g., pork, beef, or lamb) or products with aroma notes reminiscent of prepared meat and meat products. An excellent review dealing with processed meat flavorings is given by May (1991).

Although it is well established that meat flavor is due to a great number of volatiles from different chemical classes, many authors point out the importance of sulfur-containing furans for the aroma of thermally treated meat. 2-Methyl-3-furanthiol and some of its derivatives have been known as meaty aroma volatiles

from model systems for more than 20 years (Ouwenland and Peer, 1969; Evers *et al.*, 1975). MacLeod and Ames (1986) and in recent studies Gasser and Grosch (1988, 1990), Farmer and Patterson (1991), and Werkhoff *et al.* (1993) identified furans, substituted directly at the 3-position with sulfur in authentic meat aroma extracts and pointed out the meaty aroma notes of these compounds. In the last three studies cited, additionally some furans substituted indirectly with sulfur at the 2-position via a methylene group (furfurylthiol and its derivatives) were identified in the natural aroma extracts. Like the furans substituted at the 3-position with sulfur, these compounds possess very low odor threshold values (Gasser and Grosch, 1990b) and are found in several thermal aromas, e.g., coffee (Tressl and Silwar, 1981) or white bread (Baltes and Song, 1994).

Several papers have been published dealing with authentic meat aromas and meat aroma related model systems. On the other hand, there are only few studies about commercially available meat flavorings, whose ingredients and manufacturing processes are unknown. Gasser and Grosch (1990b) compared eight commercial meat flavorings by the so-called "aroma extract dilution analysis (AEDA)" and identified between 8 and 33 odorants with significant flavor dilution factors (FD factors). Four of these compounds were sulfur-containing furans.

In a recent study (Ruther and Baltes, 1994) we characterized commercial meat flavorings by analyzing the volatiles using the dynamic headspace technique. Among the approximately 300 compounds identified by gas chromatography/mass spectrometry (Ruther, 1993) there were 16 sulfur-containing furans. Because of the importance of this chemical class for the aroma of thermally treated meat, we want to report on these compounds separately in this paper.

EXPERIMENTAL PROCEDURES

Samples. Eight commercially available meat flavorings (six beef flavorings, one pork flavoring, one bouillon flavoring) were analyzed. All samples were powders ranging from light to dark brown and possessed more or less intensive meat aroma; some samples additionally provided notes reminiscent of spices.

Isolation of the Volatiles by Dynamic Headspace Extraction. *Preparation of the Adsorption Tube.* A Pasteur pipet was sealed with quartz wool and filled with 250 mg of

Table 1. Furans Substituted with Sulfur Identified in Commercial Meat Flavorings

no.	compound	Kovats index		status of identification ^a	MS ref
		DB-Wax	DB-1		
1	<i>cis/trans</i> -2-methyl-3-tetrahydrofurylthiol	1299/1381		L	Ouwenland and Peer (1969)
2	2-methyl-3-furanthiol	1305	848	A	Evers <i>et al.</i> (1976)
3	2-methyl-3-(methylthio)furan	1343	933	L	MacLeod and Ames (1986)
4	2-methyl-3-(ethylthio)furan	1388	1007	L	Werkhoff <i>et al.</i> (1993)
5	furfurylthiol	1430	883	A	Tressl and Silwar (1981)
6	2-methyl-3-(methyldithio)furan	1667	1155	L	Gasser and Grosch (1990)
7	2-methyl-3-furanthiol acetate	1682	1096	L	Werkhoff <i>et al.</i> (1991a)
8	2-methyl-3-(ethylthio)furan	1725	1238	Sy	Figure 1
9	furfuryl methyl disulfide	1806		A	Tressl and Silwar (1981)
10	3-[(2-methyl-3-furyl)dithio]-2-butanone	2173	1482	Sy	Figure 2
11	bis(2-methyl-3-furyl) disulfide	2156	1527	A	Werkhoff <i>et al.</i> (1990)
12	3-[(2-methyl-3-furyl)dithio]-2-pentanone	2205	1557	Sy	Figure 3
13	2-[(2-methyl-3-furyl)dithio]-3-pentanone	2222	1571	Sy	Figure 4
14	difurfuryl sulfide	2223		A	Tressl and Silwar (1981)
15	1-[(2-methyl-3-furyl)dithio]-2-propanone	2235	1445	Sy	Figure 5
16	furfuryl 2-methyl-3-furyl disulfide	2398	1620	Sy	Zhang and Ho (1990)

^a Identification by comparison of (A) mass spectrum and Kovats indices with those of a commercially available reference compound, (L) mass spectrum with literature spectrum, and (Sy) mass spectrum and Kovats indices with those of synthesized reference compounds.

Tenax GC (Alltech, Unterhaching, Germany); after the top was sealed with another piece of quartz wool, the adsorption tube was rinsed with 5 mL of diethyl ether (twice distilled) and dried in a gentle stream of nitrogen.

Each sample (5 g) plus 50 mL of distilled water was placed in a 250 mL round-bottom two-neck flask and held at 50 °C. One neck was connected to a Liebig condenser (removal of the water from the gas flow), and at the end of the condenser the adsorption tube was positioned via a quick-fit connector (Schott, Mainz, Germany). A nitrogen stream of 60 mL/min (purified by a charcoal trap) was led via a gas inlet tube through the second neck of the flask for 19 h. The Tenax trap was eluted with diethyl ether, and the eluate was collected in a microtube (fused Pasteur pipet, calibrated to 1 mL and 50 μ L) up to the 1 mL mark. After the eluate was concentrated in a water bath at 40 °C to the 50 μ L mark (the diethyl ether level was adjusted to the water level continuously), 1 μ L was injected into the gas chromatograph.

High-Resolution Gas Chromatography. Analytical separations were performed on a Carlo Erba Type 5169 Mega Series gas chromatograph, technical details as follows: (column a) DB-1, 60 m \times 0.32 mm, film thickness 1 μ m (J&W/Fisons, Mainz, Germany); injector, 280 °C; detector, FID, 280 °C; carrier gas, helium, 2 mL/min; temperature program; 35 °C, 5 min isotherm, 2 °C/min to 150 °C, 3 °C to 260 °C, 260 °C, 30 min isotherm; (column b) DB-Wax, 60 m \times 0.25 mm, film thickness 0.25 μ m (J&W/Fisons); injector, 250 °C; detector, FID, 250 °C; temperature program, 35 °C, 5 min isotherm, 2 °C/min to 210 °C, 210 °C, 30 min isotherm; injection volume, 1 μ L splitless; carrier gas, helium, 2 mL/min.

Gas Chromatography/Mass Spectrometry. Analytical separations were performed on a Finnigan Type 9610 gas chromatograph; the columns and chromatographic conditions were the same as described above. The mass spectra were obtained on a Finnigan MAT Type 4500 quadrupole instrument with INCOS 2100 data system, technical details as follows: direct coupling; ion source, 120 °C; ionization mode, EI, 70 eV; cyclic scan, 0.75 s; mass range, 35–350 amu.

Preparative Gas Chromatography. The synthesized disulfides of the 2-methyl-3-furanthiol were isolated from the diethyl ether extracts using a Gerstel Multi Column Switching (MCS) gas chromatographic system (Gerstel, Mühlheim/Ruhr, Germany), technical details as follows: precolumn, DB-1, 5 m \times 0.53 mm, film thickness 5 μ m (J&W/Fisons); analytical column, DB-1, 30 m \times 0.53 mm, film thickness 1.5 μ m (J&W/Fisons); temperature program, 100 °C, 5 min isotherm, 10 °C/min to 130 °C, 3 °C/min to 160 °C, 10 min isotherm, thermal column cleaning for 20 min at 280 °C, injection system, programmable cold injection system (CIS), starting temperature 100 °C, 1 s isotherm, 12 °C/min to 260 °C, 180 s isotherm, 12 °C/min to 350 °C, held for 60 s; detector, FID; carrier gas, helium, 5.0 mL/min; injection volume, 3 μ L; transfer line, 260 °C; cold trap temperature, –30 °C.

¹H-NMR Analysis. The ¹H-NMR spectra were obtained on a Bruker Type AC 400 instrument at 400 MHz in CDCl₃ with tetramethylsilane as an internal standard.

Organic Syntheses. Materials. 2-Methyl-3-furanthiol, furfurylthiol, ethanethiol, furfuryl methyl disulfide, bis(2-methyl-3-furyl) disulfide, and difurfuryl sulfide for organic synthesis and as reference compounds, respectively, were purchased from Aldrich (Steinheim, Germany). 2-Bromo-3-butanone and chloroacetone were bought from Lancaster (Mühlheim/Main, Germany). 3-Bromo-2-pentanone and 2-bromo-3-pentanone were synthesized according to the method of Gasser (1990) by α -halogenation of the corresponding ketones. Mercaptopropanone, 2-mercapto-3-butanone, 2-mercapto-3-pentanone, and 3-mercapto-2-pentanone were synthesized by substitution of the halogen atoms with hydrogen sulfide in alkaline media according to the procedure of Gasser (1990).

Synthesis of the 2-Methyl-3-furyl Disulfides. The synthesis of the 2-methyl-3-furyl disulfides was performed in a semi-microbatch by oxidative coupling of 2-methyl-3-furanthiol with the corresponding α -mercaptoketones, furfurylthiol, and ethanethiol, respectively, in a reaction medium consisting of two phases according to the method of Farmer and Patterson (1991). To give an example for the synthesis of the disulfides, which was the same for each compound, the synthesis of 3-[(2-methyl-3-furyl)dithio]-2-pentanone is described in more detail: About 50 mg each of 3-mercapto-2-pentanone and 2-methyl-3-furanthiol, respectively, were dissolved in 40 mL of diethyl ether. After 20 mL of a 1 M solution of CuSO₄ was added, the mixture was stirred for 30 min. The organic phase, which contained not only the desired 3-[(2-methyl-3-furyl)dithio]-2-pentanone but also bis(2-methyl-3-furyl) disulfide and two diastereomers of bis(2-oxo-3-pentyl) disulfide, was dried over anhydrous Na₂SO₄. Following concentration, this solution was taken for GC/MS analysis and for preparative gas chromatography.

RESULTS AND DISCUSSION

The volatiles of eight commercial meat flavorings were analyzed by dynamic headspace analysis. In six samples investigated in this study we found altogether 16 volatile sulfur-containing furans. These compounds are listed in Table 1. Twelve furans are substituted with sulfur directly at the 3-position, three compounds are substituted indirectly with sulfur at the 2-position via a methylene group, and one compound [furfuryl (2-methyl-3-furyl) disulfide, 16] contains both structures. 2-Methyl-3-furanthiol (2) can be formed during the thermal degradation of thiamin (Linde *et al.*, 1979) or by a Maillard-type reaction between cysteine on the one hand and ribose (Farmer *et al.*, 1989) or inosine 5'-

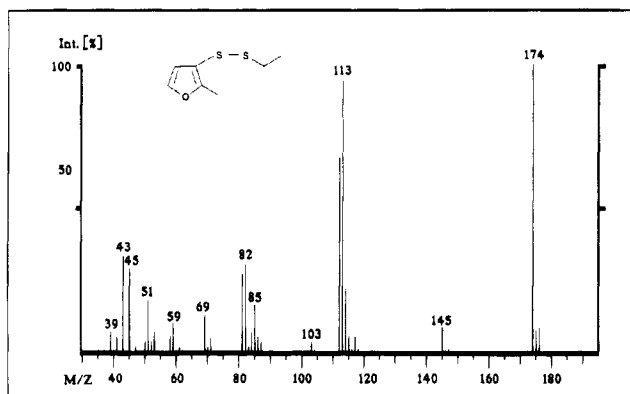


Figure 1. Mass spectrum of 2-methyl-3-(ethylthio)furan.

monophosphate (IMP) (Zhang and Ho, 1991) on the other. In a recent study Tressl *et al.* (1993) investigated model reactions of ^{13}C -labeled sugars with cysteine indicating that the methyl group of **2** derives from the C_1 atom of the sugar moiety. Model investigations showed that the formation of **2** leads via the well-known pentose degradation product 4-hydroxy-5-methyl-3(2H)-furanone (Ouwenland *et al.*, 1969; Mottram *et al.*, 1994). **2** has been described in several meat-related model systems (Hartman, 1984; Reineccius and Liardon, 1985; Werkhoff *et al.*, 1990, 1991b; Güntert *et al.*, 1990, 1992), in a yeast extract composition (Ames and MacLeod, 1985), and in natural meat aroma extracts from beef, pork, and chicken (Gasser and Grosch, 1988, 1990; Werkhoff *et al.*, 1993). The thiol function of **2** seems to be relatively reactive, and thus altogether nine derivatives of **2** were identified in the headspace extracts of the meat flavorings. Under oxidative conditions, for example in the presence of oxygen, **2** tends to form disulfides with other thiols. The compounds **6**, **8**, **10**–**13**, **15**, and **16** obviously originate from such mechanisms (compare Figure 6). The reaction of **2** with methanethiol and ethanethiol may form **6** and **8**; the corresponding monosulfides **3** and **4** alternatively may arise from the same reactants under the loss of hydrogen sulfide. Methanethiol is known to be one of the Strecker degradation products of methionine (Whitfield, 1992); ethanethiol has been found among the reaction products of the Maillard-type reaction between cysteine/cystine and ribose (Mulders, 1973). **3** has been found in authentic meat aroma extracts (MacLeod and Ames, 1986; Gasser and Grosch, 1988, 1990; Werkhoff *et al.*, 1993), in a yeast extract composition (Ames and MacLeod, 1985), and in coffee (Tressl and Silwar, 1981). In a recent study, Werkhoff *et al.* (1993) described **4** for the first time as a constituent of chicken flavor. The disulfide **6** was identified by Gasser and Grosch (1990b) in commercial meat flavorings but has also been found in a meat aroma model system (Misharina and Golovnja, 1988), in a yeast extract composition (Ames and MacLeod, 1985), in roasted coffee (Tressl and Silwar, 1981), and recently in aroma extracts from beef and pork (Werkhoff *et al.*, 1993). The corresponding ethyl derivative **8**, on the other hand, has not previously been reported in the literature; the mass spectrum is shown in Figure 1. Another compound that is described for the first time in this study is the disulfide **15**, whereas compounds **10**, **12**, and **13** most recently have been identified by Mottram *et al.* (1994) among the reaction products of 4-hydroxy-5-methyl-3(2H)-furanone and cysteine. However, no spectroscopic data have been given. Compounds **10**, **12**, **13**, and **15** may be formed by

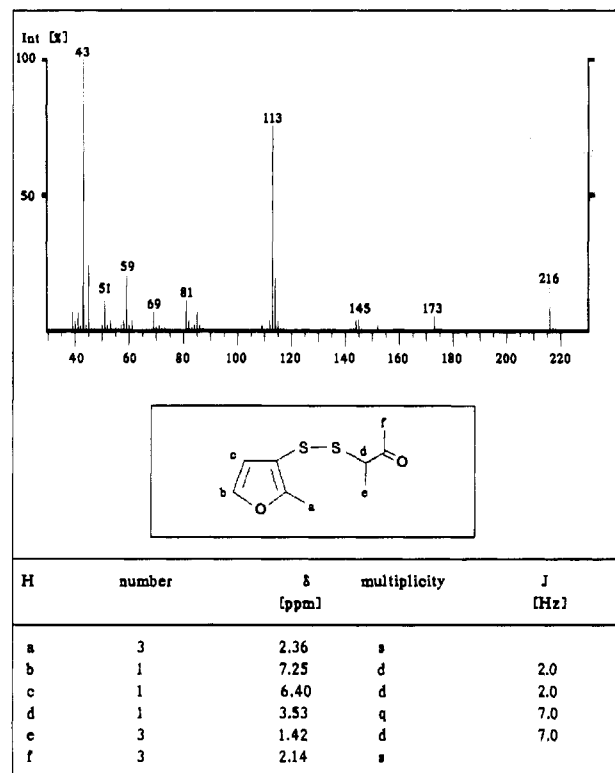


Figure 2. Mass spectrum and ^1H -NMR data of 3-[(2-methyl-3-furyl)dithio]-2-butanone.

oxidative coupling of **2** with 2-mercapto-3-butanone, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone, and mercaptoacetone, respectively, which are all known from the literature as thiamin degradation products (Güntert *et al.*, 1991). Mottram *et al.* identified the α -mercapto ketones, with the exception of mercaptoacetone in the cited study, and showed that the formation of these compounds and the corresponding disulfides **10**, **12**, and **13** is favored at low pH values. Identification of **10**, **12**, **13**, and **15** in our study was performed by comparison of the mass spectra and the Kovats indices with those of synthesized reference compounds. The synthesis products were additionally characterized by ^1H -NMR spectroscopy after a cleanup step via preparative gas chromatography. The spectroscopic data are shown in Figures 2–5. **10**, **12**, **13**, and **15** possess similar organoleptic properties reminiscent of cat urine and onions, providing also meatlike aroma notes when more diluted.

2-Methyl-3-furanthiol acetate (**7**), which may derive from the reaction of **2** with acetic acid or its anhydride, has been identified by Werkhoff *et al.* (1991a) in yeast extract. The dimerization of **2** leads to bis(2-methyl-3-furyl) disulfide (**11**), which like **2** is well-known to be a potent meat aroma compound and has been identified in several meat aroma model systems (Evers *et al.*, 1976; Hartman, 1984; Reineccius and Liardon, 1985; Werkhoff *et al.*, 1990, 1992; Güntert *et al.*, 1990, 1992; Misharina *et al.*, 1988; Grosch and Zeiler-Hilgart, 1992) and also in natural meat aroma extracts (Gasser and Grosch, 1988; Farmer and Patterson, 1991; Werkhoff *et al.*, 1993) and yeast extract (Ames and MacLeod, 1985). Gasser and Grosch (1990) described **11** as a constituent of commercial meat flavorings. Furfuryl (2-methyl-3-furyl) disulfide (**16**) may be formed by oxidative coupling of **2** and furfuranthiol (**5**). Farmer and Patterson (1991) identified **16** in aroma extracts from different bovine muscles; Zhang and Ho (1991) described **16** among the volatiles formed in a cysteine/IMP model system. As

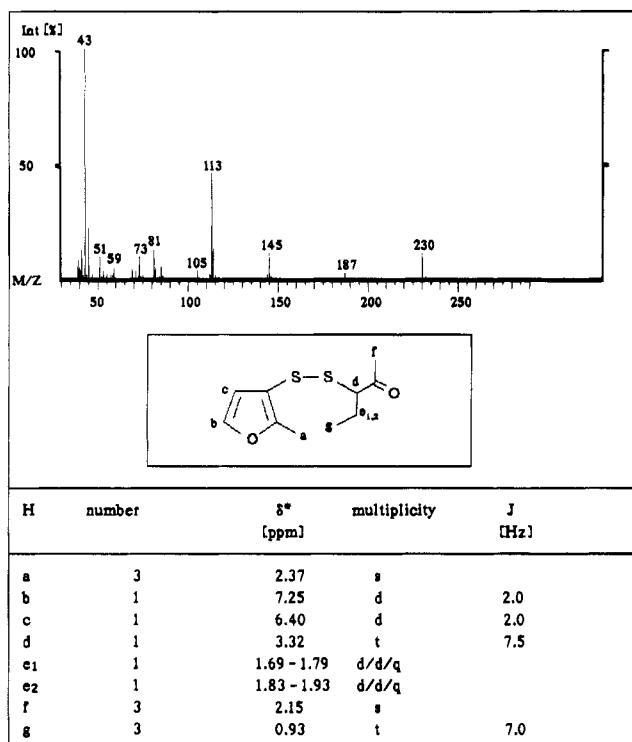


Figure 3. Mass spectrum and $^1\text{H-NMR}$ data of 3-[(2-methyl-3-furyl)dithio]-2-pentanone.

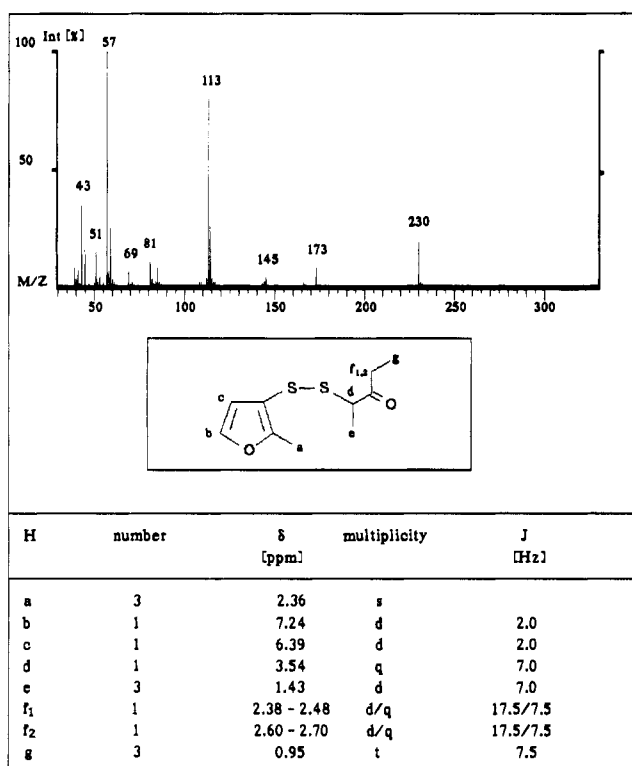


Figure 4. Mass spectrum and $^1\text{H-NMR}$ data of 2-[(2-methyl-3-furyl)dithio]-3-pentanone.

model reactions indicate, **5** may derive from the reaction of furfural with hydrogen sulfide (Shibamoto, 1977). Tressl *et al.* (1983) investigated the model system furfural/cysteine/methionine and identified not only **5** but additionally some further derivatives, among others furfuryl methyl disulfide (**9**) and difurfuryl sulfide (**14**), which had been found in the headspace of commercial meat flavorings in this study. The possible formation pathway of **9** and **14** in the flavorings is given by the

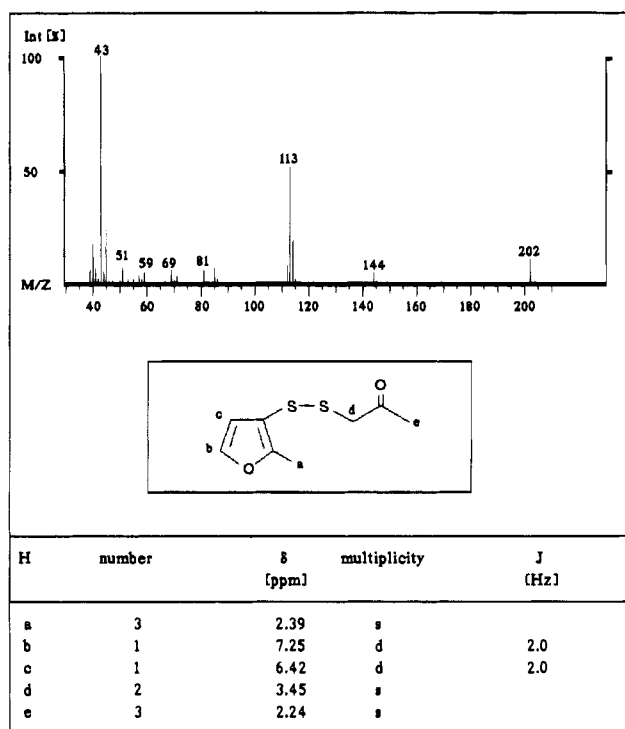


Figure 5. Mass spectrum and $^1\text{H-NMR}$ data of 1-[(2-methyl-3-furyl)dithio]-2-propanone.

cited model system, because furfural is a typical sugar degradation product and cysteine and methionine are common ingredients for the manufacture of commercial meat flavorings. **5** and its derivatives play an important role in coffee aroma (Tressl and Silwar, 1981) but also have been found in other thermal derived aromas, e.g., the aroma of white bread (Baltes and Song, 1994). Gasser and Grosch (1990b) described **5** as one of the primary odorants of chicken broth and several commercial meat flavorings.

Many of the sulfur-containing furans identified in this study (**1-3**, **6**, **9**, **11**, and **14**) are known from the literature as aroma chemicals, used to improve the sensory properties of meat flavorings, gravies, soups, and other convenience foods (Fenaroli, 1975; MacLeod and Seyyedain-Ardebili, 1981; Vernin, 1982; May, 1991). This has to be taken into consideration when the possible origins of the volatiles from commercial meat flavorings are discussed. In many cases it will be very difficult to identify these compounds as synthetic odorants, because most of them can also derive from thermal reactions of common ingredients. Only the occurrence of single compounds in striking quantities and the absence of typical accompanying substances may indicate the use of synthetic aroma chemicals. In this context, the two diastereomers of 2-methyl-3-tetrahydrofuranthiol (**1**, Figure 7) which has been found in the headspace extract of one sample as a main constituent seems to be very interesting. In contrast to the corresponding dihydrofuran- and furanthiol, the tetrahydrofuranthiol is only rarely described in the literature. Ouwenland and Peer (1969) identified **1** and several other sulfur-containing heterocycles among the reaction products of a model system consisting of 4-hydroxy-5-methyl-3(2*H*)-furanone and hydrogen sulfide. Due to the fact that the other heterocyclic compounds described in the cited model system were absent in the headspace extract of the sample concerned, it can be assumed that **1** had been used as a synthetic aroma chemical in this case as had been claimed in the cited

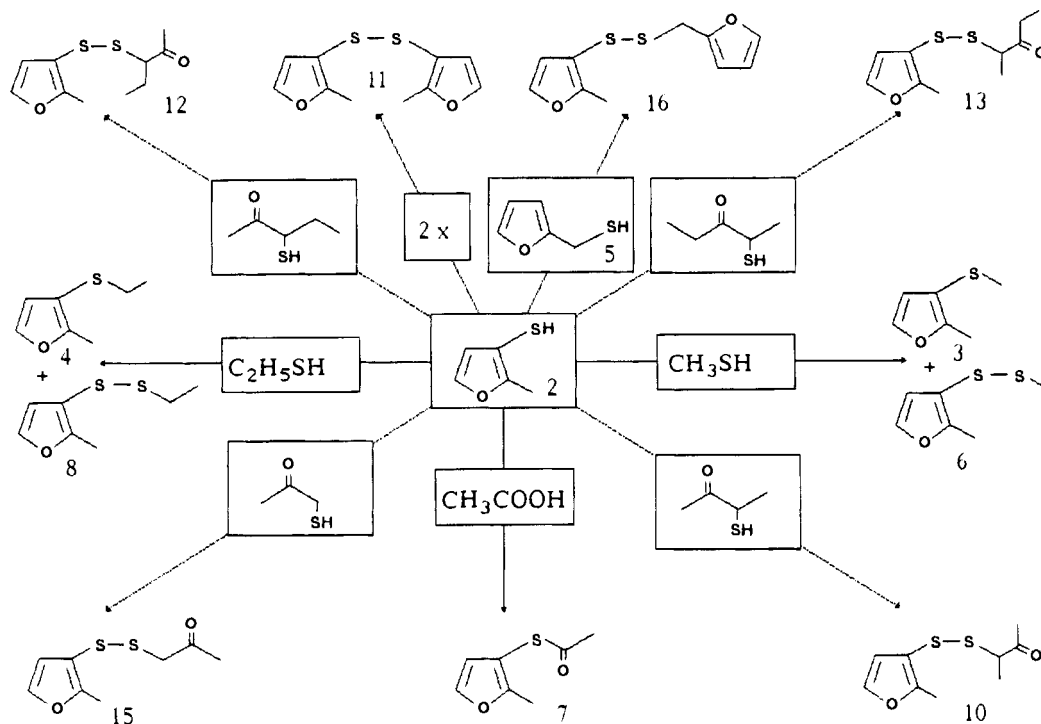


Figure 6. Possible formation pathways of 2-methyl-3-furyl (di)sulfides.

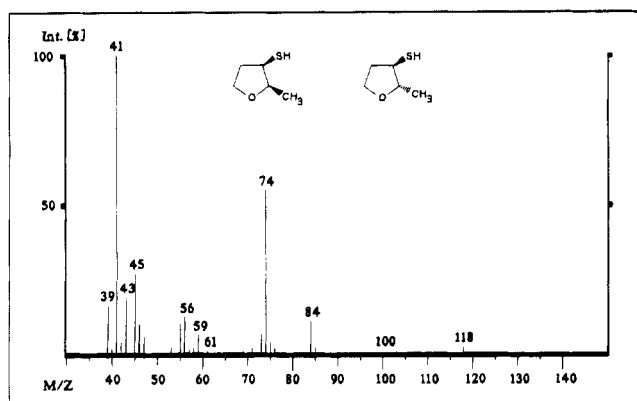


Figure 7. Mass spectrum of *cis/trans*-2-methyl-3-tetrahydrofuranthiol.

patent. In another patent, Heyden and Schutte (1971) claim the use of *O-tert*-alkyl thiocarbonates as flavoring agents for the application of thiols, releasing the thiol (compound 1 and others) continuously during heating. In our study we did not find any *O-tert*-alkyl thiocarbonates. However, these precursors are nonvolatile and therefore not detectable by headspace methods.

ACKNOWLEDGMENT

The work was supported by means of the industrial research community (Bundesministerium für Wirtschaft/AiF) via the Forschungskreis der Ernährungsindustrie (FEI). We thank Ms. Chr. Kloth for performing the preparative gas chromatography, Mr. W. Seidl for technical assistance, and Mr. R. Hatton for assistance with translation.

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Received for review March 22, 1994. Revised manuscript received July 5, 1994. Accepted July 11, 1994.®

® Abstract published in *Advance ACS Abstracts*, August 15, 1994.