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Evaluation of the Key Odorants in a Thermally Treated Solution of Ribose and Cysteine by Aroma Extract Dilution Techniques

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Application of the aroma extract dilution analysis on a solvent extract isolated from a thermally treated solution (145 °C; 20 min) of cysteine/ribose led to the identification of 2-furfurylthiol, 3-mercapto-2-pentanone, 2-methyl-3-furanthiol, 5-acetyl-2,3-dihydro-1,4-thiazine, 3-mercapto-2-butanone, and bis(2-methyl-3-furyl) disulfide showing the highest flavor dilution factors among the 29 odor-active volatiles. HRGC/olfactometry of decreasing headspace volumes established especially 2-furfurylthiol and 2-methyl-3-furanthiol as important odorants and revealed 2-thenyl mercaptan and ethyl mercaptan as further key contributors to the overall roasty, meatlike, sulfury odor of the model mixture. 5-Acetyl-2,3-dihydro-1,4-thiazine, identified for the first time among the volatiles of Maillard model reactions or foods, exhibited an intense roasty, popcorn-like odor at the low odor threshold of 0.06 ng/L of air, which was of the same order of magnitude as those reported in the literature for the roasty-smelling odorants 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline.

Keywords: *Aroma extract dilution analysis; nonenzymatic browning; Maillard reaction; ribose; cysteine; flavor; 5-acetyl-2,3-dihydro-1,4-thiazine*

INTRODUCTION

The Maillard reaction between reducing sugars and amino acids is known to generate flavors similar to those of certain processed foods [cf. Lane and Nursten (1983)] and, in particular, thermally treated model mixtures containing cysteine elicit odors resembling meat flavor (Morton et al., 1960). Therefore, precursor mixtures used in the industrial production of meat flavorings (reaction flavors, processed flavors) usually contain cysteine (van den Ouweland et al., 1978; MacLeod and Seyyedain-Ardebili, 1981; MacLeod, 1986).

In recent studies on the volatile fractions of model systems containing cysteine, an increasing number of reaction products have been identified (Martin, 1988; Whitfield et al., 1988; Tressl et al., 1989; Farmer et al., 1989; Güntert et al., 1990; Werkhoff et al., 1991; Silwar, 1992), giving insights into the complex reaction pathways governing the degradation of cysteine in the presence of carbohydrates, phospholipids, or thiamin. Sulfur-containing compounds especially predominate and were suggested as important odorants, e.g., in a reaction system containing thiamin and cysteine (Güntert et al., 1993).

Although very odor-active compounds have been identified, until now, a correlation between the overall odor of a reaction mixture containing exclusively cysteine and ribose and the compounds responsible for the flavor notes has not been established. However, a systematic evaluation of the odorants rendering the typical odor to such Maillard reaction mixtures would be helpful, e.g., in optimizing the processing conditions in the manufacturing of reaction flavors.

Application of aroma extract dilution techniques allows the detection and evaluation of key odorants in food flavor extracts [cf. reviews by Acree (1993) and Grosch (1993)], and recently such techniques have also been successfully applied to extracts obtained from thermally treated solutions of proline/2-oxopropanal (Schieberle, 1990), proline/glucose (Roberts and Acree, 1994), and thiamin/cysteine (Güntert et al., 1993).

The purpose of this investigation was, therefore, to elucidate the most odor-active compounds in a thermally treated cysteine/ribose mixture by application of aroma extract dilution techniques.

* Author to whom correspondence should be addressed [telephone (089) 3209-4170; fax (089) 3209-4183].

EXPERIMENTAL PROCEDURES

Chemicals. 1-Chloro-2-propanone, 2-(hydroxymethyl)thiophene (2-thienylmethanol), methyl mercaptan, and ethyl mercaptan were from Aldrich (Steinheim, Germany). 2,3,5,6-Tetramethyl-1,4-dioxane-2,5-diol was from Lancaster (Mühlheim, Germany). L-Cysteine and D-(−)-ribose were from Sigma (Munich, Germany). Compounds **2**, **5**, **8**, **9**, **12**, **15**, **17**, **21**, **23**, **25**, and **30** (Table 1) were also from Aldrich, and **6** was from Lancaster.

Syntheses. *2-Methyl-3-thiophenethiol.* To a stirred solution of diisopropylamide (10 mmol) in dry tetrahydrofuran (10 mL) maintained in an atmosphere of pure argon was added dropwise *n*-butyl lithium (10 mmol) in *n*-hexane (2.5 mL) at −78 °C within 10 min. After the addition of 3-bromothiophene (10 mmol); the mixture was stirred for a further 180 min at −78 °C and then methyl iodide (10.5 mmol), dissolved in dry tetrahydrofuran, was dropwise added. After stirring for a further 30 min, the mixture was allowed to react at room temperature for 16 h. After the addition of water (20 mL), the 3-bromo-2-methylthiophene formed (yield 69%) was extracted with diethyl ether (total volume 90 mL) and the solution dried over Na_2SO_4. After evaporation of the solvent, the crude 3-bromo-2-methylthiophene (6.9 mmol) was dissolved in dry diethyl ether (10 mL), which was stirred at −78 °C in an argon atmosphere. After the dropwise addition of *n*-butyl lithium (7.3 mmol in *n*-hexane), the mixture was allowed to react for a further 20 min and, after the addition of powdered sulfur (6.9 mmol), reacted for 60 min with rising temperature to −10 °C. The solution was then poured onto ice (30 g) and, after the pH was adjusted to 3.0, the target compound was extracted with diethyl ether (total volume 100 mL). The 2-methyl-3-thiophenethiol was purified by microdistillation (yield 58%) and characterized by its mass spectrum.

MS/EI: m/z (%) 130 (100), 97 (72), 45 (68), 129 (55), 69 (34), 59 (32), 85 (20). The data agreed with those previously reported by van den Ouweland and Peer (1975).

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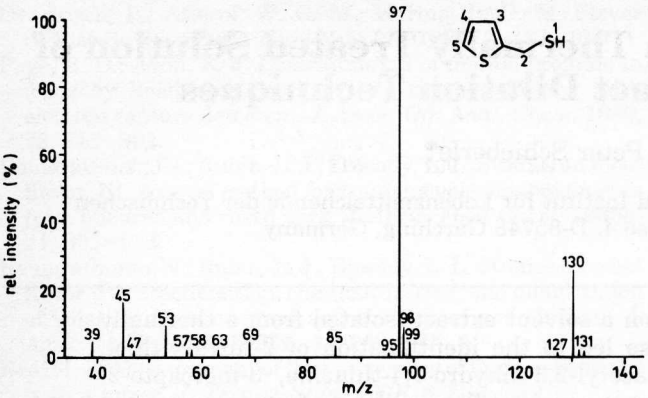

Figure 1. Mass spectrum of 2-thenyl mercaptan.

2-Thenyl Mercaptan. 2-(Hydroxymethyl)thiophene (3 mmol) was added to a solution of thiourea (4 mmol) in hydrochloric acid (4 mL; 0.3% by weight), which was maintained under argon atmosphere. After stirring for 4 h at room temperature, aqueous sodium hydroxide (3 mL; 5% by weight) followed by diethyl ether (10 mL) was added and the mixture stirred in argon atmosphere for a further 30 min. The organic layer was discarded and the aqueous layer extracted twice with diethyl ether (total volume 50 mL) to remove the unreacted (hydroxymethyl)thiophene and byproducts. After cooling with ice water, the aqueous phase was acidified to pH 3.0 (hydrochloric acid, 10% by weight) and the target compound was extracted with diethyl ether (total volume 80 mL). After drying over Na_2SO_4, the extract was concentrated to 40 mL by using a Vigreux column and then purified by sublimation in vacuo (yield 39%) using the apparatus reported earlier (Sen et al., 1991). The compound was characterized by its ^{1}H-NMR data in CD_2Cl_2 (assignment of the atoms bearing the H atoms refers to Figure 1): δ 2.08 (t, 1H, J_{1-2} = 7.63 Hz, H at 1), 3.98 (d, 2H, J_{2-1} = 7.63 Hz, H at C2), 6.93 (dd, 1H, J_{3-4} = 3.27 Hz, H at 3), 6.96 (dd, 1H, J_{4-3} = 3.27 Hz, J_{4-5} = 1.09 Hz, H at 4), 7.22 (dd, 1H, J_{5-4} = 1.09 Hz, H at C5) and its mass spectrum (Figure 1).

3-Mercapto-2-butanone. Acetoin (1 mmol) was liberated from its dimer 2,3,5,6-tetramethyl-1,4-dioxane-2,5-diol by weak acid treatment (SiO_2 in dichloromethane/water; 10 + 1 by vol) and subsequently dissolved in ethanol/water (6 mL; 5 + 1 by vol). The solution was saturated with H_2S at 0 °C and then stirred for 12 h at 80 °C in a closed vessel. After the addition of an aqueous sodium hydroxide solution (30 mL; 2 mol/L), the unreacted acetoin was extracted with diethyl ether (total volume 45 mL). The aqueous layer was then acidified to pH 3 with hydrochloric acid (1 mol/L), and the 3-mercapto-2-butanone was isolated by extraction with diethyl ether (three times; total volume 60 mL; yield 10%).

MS(EI): m/z (%) 43 (100), 61 (58), 104 (28), 60 (20), 72 (6), 71 (4), 106 (2). The spectrum was in good agreement with data published by Werkhoff et al. (1989).

1-Mercapto-2-propanone (Asinger et al., 1964). Under a constant stream of H_2S, 1-chloro-2-propanone (10 mmol) was added dropwise within 10 min to an ice-cooled stirred solution of sodium hydroxide (1.3 g) in water (6 mL), which had been presaturated with H_2S. After stirring for a further 60 min, an aqueous solution of sodium hydroxide was added (30 mL; 2 mol/L) and the excess of 1-chloro-2-propanone extracted with diethyl ether (45 mL). The aqueous phase was acidified to pH 3 with hydrochloric acid (1 mol/L), and the title compound (71% yield) was extracted with diethyl ether (total volume 60 mL) and characterized by mass spectrometry.

MS(EI): m/z (%) 43 (100), 90 (36), 47 (23), 45 (12), 73 (6), 58 (4), 71 (4), 92 (3).

*Mixed Disulfides (**20, 22,** and **27–29** in Table 1).* The mixed disulfides were synthesized according to the following general procedure: The corresponding thiols (0.1 mmol each) were dissolved in diethyl ether (15 mL) and, after the addition of an aqueous solution of copper(II) sulfate (3 mol/L; 15 mL), shaken for 35 min at room temperature. After complexation of the excess copper ions by the addition of ammonium acetate (2 g), the ethereal layer was separated, washed three times with brine (total volume 60 mL), and then dried over Na_2SO_4. The mixed disulfide was separated from the two bis-substituted disulfides formed in the reaction mixtures by HPLC on a RP-18 stationary phase as described for the isolation of bis(2-mercaptoethyl)furan (Hofmann and Schieberle, unpublished results). The mass spectral data of the synthesized compounds are shown in Figure 2. The spectra reported for 2-methyl-3-furyl 2-oxo-3-butyl disulfide and 2-methyl-3-furyl 2-oxo-3-pentyl disulfide were in good agreement with those published recently by Ruther and Baltes (1994).

The following odorants were synthesized as reported in the literature: bis(2-methyl-3-furyl) disulfide (Evers et al., 1976); 3-mercapto-2-pentanone (Asinger et al., 1964); dimethyl trisulfide (Milligan et al., 1963); 5-acetyl-2,3-dihydro-1,4-thiazine and 5-propionyl-2,3-dihydro-1,4-thiazine (Hofmann and Schieberle, 1995); 2-acetyl-2-thiazoline (Cerny and Grosch, 1992, 1993); 3-hydroxy-2-pyranone [Wiley and Jarboe, 1956; mass spectra and ^{1}H-NMR data agreed with those published by Normington et al. (1986)]; and 4-hydroxy-5-methyl-3(2*H*)-furanone (Peer et al., 1968).

Model Reaction. L-Cysteine (3.3 mmol) and D-ribose (10 mmol) in phosphate buffer (100 mL, 0.5 mol/L, pH 5.0) were allowed to react in a laboratory autoclave (Model II; Roth, Karlsruhe, Germany) by raising the temperature within 20 min from 20 to 145 °C.

Isolation of the Volatile Reaction Products. After cooling, the dark yellow reaction mixture was extracted five times with diethyl ether (total volume 300 mL). The combined organic phases were treated three times with an aqueous sodium bicarbonate solution (0.5 mol/L; total volume 100 mL), and the ethereal solution containing the neutral/basic compounds (fraction NB) was washed twice with brine (total volume 100 mL) and then dried over Na_2SO_4. The bicarbonate solution containing the acidic compounds was adjusted to pH 3.0 with hydrochloric acid (1 mol/L), and the acidic volatiles were then extracted with diethyl ether (total volume 150 mL; fraction AF). After washing with brine, the etheral solution was dried over Na_2SO_4. Each fraction was distilled from the nonvolatile material in the apparatus described recently (Sen et al., 1991).

Column Chromatography. Fraction NB was concentrated to 1 mL by distilling off the solvent on a Vigreux column (60 cm × 1 cm). The solution was placed on the top of a water-cooled (10–12 °C) column, designed for flash chromatography (20 cm × 2 cm i.d.; J. T. Baker BV, Deventen, The Netherlands, no. 7022-01) and equipped with a reservoir (250 mL) and a pressure regulator. The column was then filled with a slurry of silica gel (30–60 μm, Baker analyzed reagent) in *n*-pentane. Chromatography was performed under nitrogen pressure maintaining a constant flow rate of 5 mL/min and by using the following solvents: *n*-pentane (150 mL; NB I), *n*-pentane/diethyl ether (150 mL; 95 + 5 by vol; NB II), *n*-pentane/diethyl ether (150 mL; 8 + 2 by vol; NB III), *n*-pentane/diethyl ether (150 mL; 1 + 1 by vol; NB IV), diethyl ether/methanol (150 mL; 99.5 + 0.5 by vol; NB V), and finally diethyl ether/methanol (150 mL; 9 + 1 by vol; NB VI).

Enrichment of Thiols by Affinity Chromatography (Full and Schreier, 1994). Fraction AF also containing the thiols was concentrated to 1 mL and, after the addition of *n*-pentane (1 mL), placed on the top of a water-cooled (10–12 °C) glass column (5 cm × 1 cm i.d.) filled with a slurry of Affi-Gel 501 (Bio-Rad, Munich, Germany) in 2-propanol (30 mL). Nonbound compounds were first eluted with *n*-pentane/dichloromethane (60 mL; 2 + 1 by vol; AF I), and then the complexed compounds were isolated by elution with with *n*-pentane/dichloromethane (30 mL; 2 + 1 by vol) containing 1,4-dithiothreitol (10 mmol; AF II). To separate the excess of the dithiol, the volatile material and the solvent were distilled off by using the apparatus described earlier (Sen et al., 1991).

High-Resolution Gas Chromatography (HRGC)/Mass Spectrometry (MS). HRGC was performed with a Type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) by using the following capillaries: FFAP (30 m × 0.32 mm fused silica capillary, free fatty acid phase, 0.25 μm; J&W Scientific, Fisons Instruments, Mainz, Germany) and SE-54 (30 m × 0.32

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Table 1. Intense Odorants (FD ≥ 4) Formed by Heating of an Aqueous Ribose/Cysteine Solution[a]

no.	odorant[b]	fraction[c]	RI on FFAP	RI on SE-54	FD factor[d] I	FD factor[d] II	reported as volatile compound[e]
1	3-mercapto-2-butanone	AF II	1282	820	128	<1	5, 7
2	2-methyl-3-furanthiol	AF II	1300	869	256	<1	4, 6
3	3-mercapto-2-pentanone[f]	AF II	1347	907	512	<1	6, 7
4	1-mercapto-2-propanone	AF II	1357	<800	16	<1	5
5	2-furfurylthiol	AF II	1431	909	1024	<1	3–7
6	2-methyltetrahydrothiophen-3-one	AF I/NB III	1512	1017	8	16	3–7
7	2-methyl-3-thiophenethiol	AF II	1559	1059	16	<1	2, 6
8	2-acetylthiazole	NB III	1615	1142	<1	8	1, 3–6, 11
9	2-formylthiophene	NB III	1669	1000	<1	64	4–6
10	2-thenyl mercaptan	AF II	1682	1092	8	<1	4, 5, 12
11	2-acetyl-2-thiazoline	NB IV	1720	1111	<1	64	8
12	acetylthiophene	NB IV	1740	1090	<1	16	4–6, 11
13	unknown		1784		<1	16	
14	unknown		1800		<1	8	
15	2-(hydroxymethyl)thiophene	AF I/NB IV	1917	1030	2	<1	5
16	3-hydroxy-2*H*-pyran-2-one	AF II	1965	989	16	<1	5
17	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	AF II	2016	1100	32	<1	5, 11
18	bis(2-methyl-3-furyl) disulfide	AF I/NB I	2100	1526	128	16	7, 8, 10
19	4-hydroxy-5-methyl-3(2*H*)-furanone (norfuraneol)	AF II	2105	1044	64	<1	5, 6
20	2-methyl-3-furyl 2-oxo-3-butyl disulfide	AF I	2127	1491	4	<1	9
21	2-mercaptopropanoic acid	AF II	2130	1057	4	<1	5
22	2-methyl-3-furyl 2-oxo-3-pentyl disulfide	AF I	2145	1561	8	<1	9
23	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)[g]	AF II	2153	1112	16	<1	
24	5-acetyl-2,3-dihydro-1,4-thiazine	NB VI	2177	1374	<1	256	
25	3-mercaptopropanoic acid	AF II	2193	1057	16	<1	5
26	5-propionyl-2,3-dihydro-1,4-thiazine	NB VI	2235	1456	<1	8	
27	2-furfuryl 2-methyl-3-furyl disulfide	AF I/NB I	2323	1624	16	4	10
28	2-furfuryl 2-oxo-3-butyl disulfide	AF I	2352	1577	4	<1	
29	2-furfuryl 2-oxo-3-pentyl disulfide	AF I	2385	1649	4	<1	
30	bis(2-furfuryl) disulfide	AF I/NB I	2465	1673	16	4	10

[a] A mixture of ribose (10 mmol) and cysteine (3.3 mmol) was reacted for 20 min in phosphate buffer (100 mL; 0.5 mol/L; pH 5.0). [b] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on two capillary columns given in the table, mass spectra obtained by MS (EI) and MS (CI), and odor quality perceived at the sniffing port. [c] Fraction in which most of the compound appeared after chromatography on silica gel or Affi-Gel, respectively. [d] Flavor dilution (FD) factor determined in extracts containing the acidic (I) or the neutral/basic volatiles (II). Analyses were performed by two assessors in duplicates. The data differed to not more than two FD factors. [e] Reported in the literature as volatile reaction product of cysteine, cystine, or cysteamine reacted in the presence of carbohydrates or carbohydrate degradation products. (1) Mulders (1973); (2) van den Ouweland and Peer (1975); (3) Mussinan and Katz (1973); (4) Whitfield et al. (1988); (5) Martin (1988); (6) Farmer et al. (1989); (7) Güntert et al. (1990); (8) Sakaguchi and Shibamoto (1978); (9) Whitfield et al. (1993); (10) Farmer and Patterson (1991); (11) Silwar (1992); (12) Güntert et al. (1993). [f] 3-Mercapto-2-pentanone contained small amounts of its isomer 2-mercapto-3-pentanone. [g] The MS signals were too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote *b*.

mm fused silica capillary DB-5; 0.25 μm; J&W Scientific, Fisons Instruments). The samples were applied by the on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (SE-54) or 60 °C (FFAP), respectively, held for 5 min isothermally, then raised at 6 °C/min to 230 °C, and held for 15 min. The flow of the carrier gas helium was 2.5 mL/min. At the end of the capillary, the effluent was split 1:1 (by vol) into an FID and a sniffing port using deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm). The FID and the sniffing port were held at 180 °C. Linear retention indices (RI) of the compounds were calculated from the retention times of *n*-alkanes by using a computer program (Halang et al., 1978). MS analysis was performed with an MS-8230 (Finnigan, Bremen, Germany) in tandem with the capillaries described above. Mass spectra in the electron impact mode (MS/EI) were generated at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with isobutane as reactant gas.

Aroma Extract Dilution Analysis (AEDA); Approximation of Odor Threshold Values. The flavor dilution (FD) factors of the odor-active compounds were determined by AEDA (Ullrich and Grosch, 1987; Schieberle and Grosch, 1987) of the following dilution series: The original extract (100 μL) of either fraction NB or fraction AF obtained from 100 mL of the model reaction was stepwise diluted with diethyl ether (1 + 1). HRGC/olfactometry was performed with aliquots (1.0 μL) using capillary FFAP (fraction AF) and capillary DB-5 (fraction NB), respectively.

Static Headspace Analysis/Olfactometry (SHO). The reaction mixture (100 mL) was pipetted into a septum-sealed, thermostated vessel (240 mL total volume) and equilibrated for 1 h at 40 °C. Decreasing headspace volumes (20 to 0.02 mL) were taken off by means of a gastight syringe and then analyzed by SHO on the FFAP column as detailed by Guth and Grosch (1993). The perceived odors were attributed to the odorants identified in the preceding experiments using solvent extracts by means of odor quality, odor intensity, and retention index. The FD factors given in Table 2 were calculated by dividing the largest volume analyzed (20 mL; FD = 1 by definition) by the lowest volume in which the respective odorant was still detectable.

RESULTS

In preliminary experiments, first, the influence of the pH (5.0 and 7.0) and of the ribose/cysteine ratio (1:1 to 10:1) on the overall odor of the thermally treated reaction mixture was studied. The results of the sensory evaluation (data not shown) revealed that sulfury, meatlike odors were generally more prominent in reaction mixtures treated in phosphate buffer at pH 5.0, whereas at pH 7.0 caramel, burnt notes predominated. Furthermore, heating of several ribose (100 mmol/L)/cysteine mixtures at pH 5.0 with lowered concentrations of cysteine (100 to 10 mmol/L) showed significant differences in the overall odors, which were described as rubbery, sulfury, and H_2S-like in a 1:1 mixture and burnt and caramel-like in the 10:1 mixture. The most prominent roasty, meatlike odor was detectable in a mixture containing 100 mmol/L ribose and 33 mmol/L cysteine. Therefore, this reaction mixture was chosen for the evaluation by aroma extract dilution analyses.

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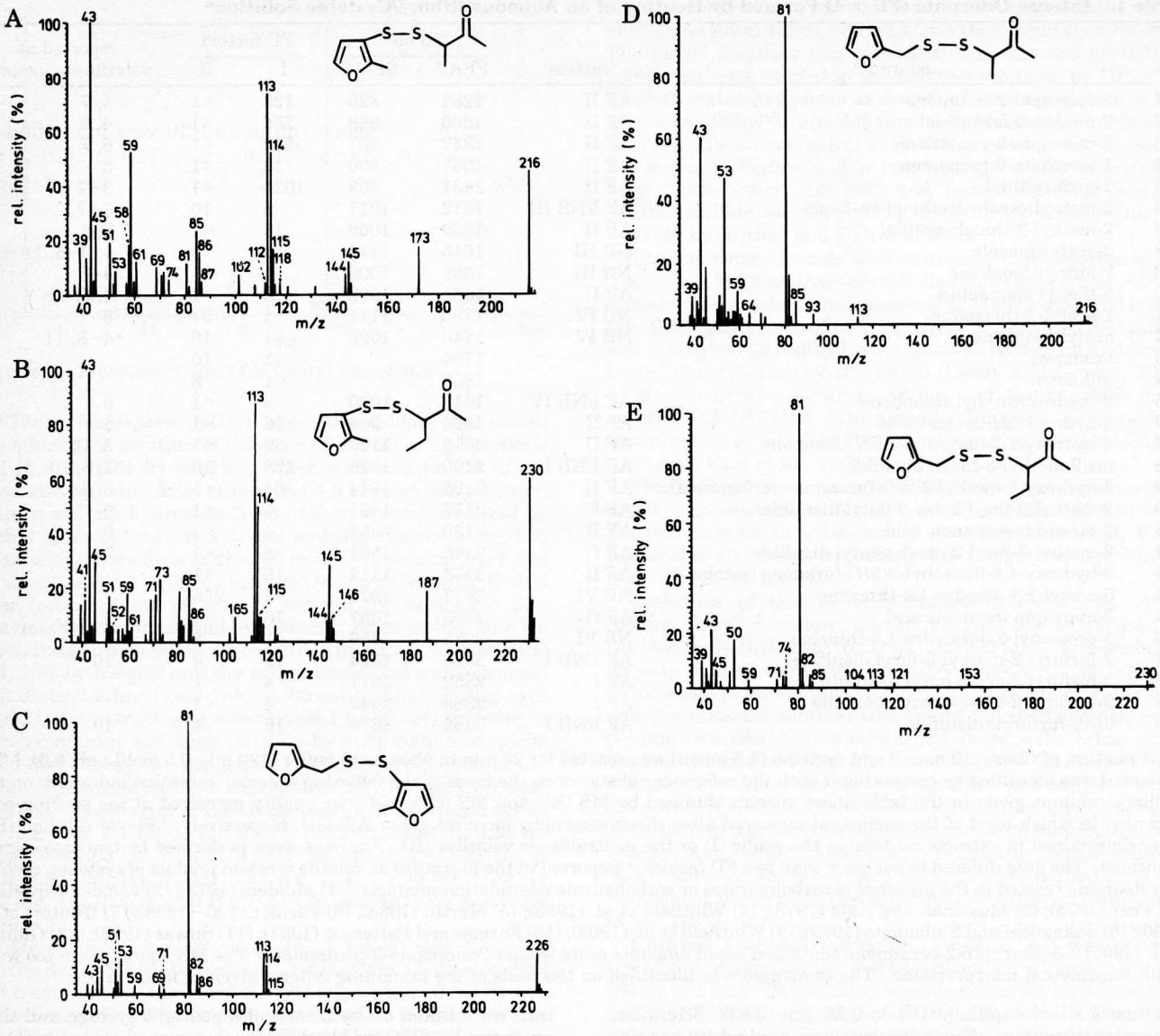

Figure 2. Mass spectra of mixed disulfides: (A) 2-methyl-3-furyl 2-oxo-3-butyl disulfide; (B) 2-methyl-3-furyl 2-oxo-3-pentyl disulfide; (C) 2-furfuryl 2-methyl-3-furyl disulfide; (D) 2-furfuryl 2-oxo-3-butyl disulfide; (E) 2-furfuryl 2-oxo-3-pentyl disulfide.

Acidic Volatile Fraction. To avoid interferences with the gas chromatographic separations, the acidic volatiles from the reaction/mixture (fraction AF) were separated by treating the flavor extract with sodium bicarbonate. Application of the AEDA on fraction AF, eliciting a strong sulfury note, revealed 21 odor-active compounds in the FD factor range 4−1024 (column I in Table 1). On the basis of their high FD factors five compounds (**1**−**3**, **5**, and **18**), exhibiting sulfury, roasty, meatlike odors, were the main contributors to the overall odor of this fraction.

To get enough material for identification, 25 samples of the model reaction were combined (total volume 2.5 L); the acidic volatiles were isolated by treatment of the extract with sodium bicarbonate and then separated by affinity chromatography with phenylmercury chloride. This method has been recently developed (Full and Schreier, 1994) to enrich thiols from flavor extracts. By application of HRGC/olfactometry and HRGC/MS (Table 1; footnote *b*) to fraction AF II containing the compounds, which had been bound as mercury adducts, four of the five most important odorants in fraction AF could be identified (Table 1): **5**, 2-furfurylthiol (roasty, coffee-like); **3**, 3-mercapto-2-pentanone (sulfury, catty); **2**, 2-methyl-3-furanthiol (meatlike, sulfury); and **1**, 3-mercapto-2-butanone (sulfury, rotten). In addition, **19** [4-hydroxy-5-methyl-3(2*H*)-furanone (norfuraneol; caramel-like)], **17** [4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDF, caramel-like)], **23** [3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon, seasoning-like)], **16** [3-hydroxy-2*H*-pyran-2-one (seasoning-like)], **7** [2-methyl-3-thiophenethiol (meatlike, sulfury)], **4** [1-mercapto-2-propanone (sulfury)], **10** [2-thenyl mercaptan (roasty)], and **21** and **25** [2- and 3-mercaptopropanoic acid (sulfury, seasoning-like)], contributing with lower FD factors to the overall odor, were identified in fraction AF II (Table 1). It is interesting to note that the affinity chromatography with phenylmercury chloride seems to be useful also in the enrichment of enoloxo compounds, such as norfuraneol, HDF, 3-hydroxy-2*H*-pyran-2-one, or sotolon (**19**, **17**, **16**, and **23**; Table 1).

The fifth key odorant, **18**, was identified in fraction AF I as bis(2-methyl-3-furyl) disulfide.

Neutral Basic Fraction. Application of the AEDA on the neutral/basic volatile fraction (fraction NB) exhibiting an intense roasty, popcorn-like odor revealed 12 odorants (column II in Table 1) of which four compounds showing moderate FD factors [**6**, 2-methyltetrahydrothiophen-3-one; **18**, bis(2-methyl-3-furyl) disulfide; **27**, 2-furfuryl 2-methyl-3-furyl disulfide; and **30**,

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Table 2. Results of Static Headspace Analysis/ Olfactometry (SHO) of the Cysteine/Ribose Model Solution

odorant[a]	RI on FFAP	vol[b] (mL)	FD factor[c]
hydrogen sulfide[d]	<900	5	4
methyl mercaptan	<900	2.5	8
ethyl mercaptan	<900	0.16	125
2,3-butanedione (diacetyl)	968	20	1
3-mercapto-2-butanone	1282	2.5	8
2-methyl-3-furanthiol	1300	0.08	250
3-mercapto-2-pentanone[d]	1347	0.64	32
dimethyl trisulfide	1356	20	1
2-furfurylthiol	1431	0.02	1000
unknown (sulfury)	1438	10	2
unknown (sulfury)	1511	5	4
2-methyl-3-thiophenthiol	1559	0.64	32
2-thenyl mercaptan	1682	0.16	125
2-acetyl-2-thiazoline[e]	1720	20	1
5-acetyl-2,3-dihydro-1,4-thiazine[e]	2177	20	1

[a] The compound was identified by comparing its mass spectra, retention index, odor quality, and odor activity (displayed by the odor threshold in air) with the reference compound (cf. Table 1). [b] Lowest headspace volume required to perceive the odorant at the sniffing port. [c] Calculated by dividing the largest volume analyzed (20 mL) by the lowest headspace volume required to perceive the odorant at the sniffing port. [d] 3-Mercapto-2-pentanone contained a smaller proportion of its isomer 2-mercapto-3-pentanone. [e] MS signals were too weak for an unequivocal interpretation.

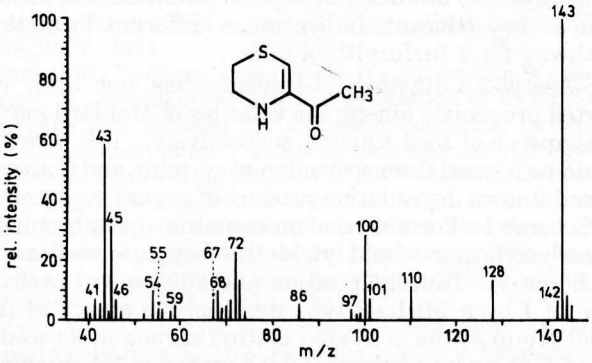


Figure 3. Mass spectrum of peak 24: 5-acetyl-2,3-dihydro-1,4-thiazine.

bis(2-furfuryl) disulfide] had already been identified in the acidic fraction (column I, Table 1). Among the remaining eight compounds (**8**, **9**, **11**–**14**, **24**, and **26**) odorant **24**, exhibiting an intense roasty, popcorn-like odor, showed the highest odor activity in the neutral basic fraction.

After separation of the neutral/basic volatiles from 2.5 L of the reaction mixture by flash chromatography on silica gel, the mass spectrum shown in Figure 3 was obtained for compound **24** in fraction NB VI. As indicated in the figure, purification by preparative GLC and application of high-resolution mass spectrometry as well as various NMR techniques led to its structure being identified as 5-acetyl-2,3-dihydro-1,4-thiazine. The details of the NMR, MS, and synthetic experiments demonstrating the chemical identity of compound **24** will be published shortly (Hofmann et al., 1995a).

On the basis of their FD factors, compounds **9** and **11**, also exhibiting roasty odors, followed next in rank to 5-acetyl-2,5-dihydro-1,4-thiazine. The identification experiments (Table 1, footnote *b*) revealed that 2-formylthiophene (**9**) and 2-acetyl-2-thiazoline (**11**) evoked the roasty odors detected by AEDA in the corresponding odor-active regions.

Compound **26**, contributing with a lower FD factor to the odor of the neutral/basic fraction, also exhibited a popcorn-like, roasty odor. On the basis of mass spectral data [MS/EI m/z (%) 157 (100), 100 (64), 72 (42), 45 (40), 57 (34), 101 (22)] and NMR experiments followed by synthesis, compound **26** was identified as 5-propionyl-2,3-dihydro-1,4-thiazine. The analytical details will be reported shortly (Hofmann et al., 1995a).

Eight disulfides (**18**, **20**, **22**, and **27**–**30**), known as oxidation products of the corresponding thiols (Farmer and Patterson, 1991; Whitfield et al., 1993), were additionally identified which, except for bis(2-methyl-3-furyl) disulfide (**18**; Table 1), contributed with low FD factors to the overall odor of the reaction mixture. Unexpectedly, the FD factors and, therefore, the concentrations of these neutral compounds were higher in the acidic than in the neutral/basic fraction (cf. columns I and II, Table 1), suggesting that the disulfides were formed as artifacts during the workup procedure. This assumption was very recently corroborated by our findings (Hofmann et al., 1995b) that after 10 days storage of, e.g., 2-methyl-3-furanthiol in diethyl ether at 6 °C, more than 50% of the thiol had been converted into the corresponding disulfide.

Static Headspace Analysis/Olfactometry (SHO). The results of the static headspace analysis are summarized in Table 2. In a 20 mL sample of air above the model solution, 15 odor-active compounds were detectable by GC/Olfactometry, of which 12 compounds could be identified (Table 2). Eight of these odorants had already been identified in the AEDA of the solvent extract (**1**–**3**, **5**, **7**, **10**, **11**, and **24**, Table 1), whereas hydrogen sulfide, methyl mercaptan, ethyl mercaptan, diacetyl, and dimethyl trisulfide had not. This is undoubtedly due to the high volatility of these four odorants causing losses during concentration of the solvent extracts. On the other hand, possibly due to their lower vapor pressure, compounds sensorially detected in the solvent extract (**4**, **6**, **8**, **9**, **12**, **15**–**23**, and **25**–**30**, Table 1) were not sensorily detectable in the headspace analysis.

A stepwise reduction of the headspace volume elicited the most odor-active compounds in the headspace of the reaction mixture. 2-Furfurylthiol followed by 2-methyl-3-furanthiol, 2-thenyl mercaptan and ethyl mercaptan were detected in the lowest headspace volumes analyzed (0.02–0.16 mL; Table 2). The two first mentioned thiols were also detected with the highest FD factors in the solvent extract, whereas ethyl mercaptan was sensorially not detectable and the 2-thenyl mercaptan occurred in the extract with a much lower FD factor (cf. **10**, Table 1). Presumably, the last thiol is relatively labile, which would lead to significant losses of this odorant during concentration of the extract.

Interestingly, no disulfide was detected in the headspace analysis, corroborating the assumption that a significant proportion of these flavor compounds detected by AEDA result as artifacts during the workup procedure.

5-Acetyl-2,3-dihydro-1,4-thiazine (ADT), which was detected among the key odorants of the solvent extract, only showed a low FD factor in the headspace analysis. Since low odor thresholds in air and water were determined for ADT (Table 3), a significant retronasal flavor contribution of this odorant is to be assumed. However, this needs to be proved by further quantitative experiments.

Table 3. Odor Thresholds of Selected Sulfur-Containing Odorants Identified in the Cysteine/Ribose System

odorant	odor threshold[a] (ng/L of air)
2-methyl-3-thiophenethiol	0.0032–0.0128
2-thenyl mercaptan	0.003–0.012
5-acetyl-2,3-dihydro-1,4-thiazine[c]	0.02–0.08
2-acetyl-2-thiazoline	0.02–0.08
3-mercapto-2-pentanone	0.05–0.2
3-mercapto-2-butanone	0.2–0.8
1-mercapto-2-propanone	1.7–6.8
2-furfurylthiol	0.0025–0.01[b]
2-methyl-3-furanthiol	0.0025–0.01[b]
bis(2-methyl-3-furyl) disulfide	0.0006–0.0024[b]
bis(2-furfuryl) disulfide	0.00015–0.0006[b]
2-furfuryl 2-methyl-3-furyl disulfide	0.0004–0.0016
2-methyl-3-furyl 3-oxo-2-butyl disulfide	0.01–0.04
2-furfuryl 3-oxo-2-butyl disulfide	0.004–0.016

[a] Determined by HRGCO/olfactometry as recently described (Ullrich and Grosch, 1987). (*E*)-2-Decenal (odor threshold = 2.7 ng/L of air) instead of hexanal was used as the reference odorant. [b] Adapted from the literature (Gasser and Grosch, 1990b). [c] Threshold in water = 0.6 μg/L (retronasally) and 1.7 μg/L (nasally) (unpublished results).

Odor Threshold Values. The odor threshold values of selected sulfur-containing odorants in the cysteine/ribose system were approximated by a HRGC/O method. With the exception of 1-mercapto-2-propanone, all compounds showed very low odor thresholds below 1 ng/L of air (Table 3). The lowest value was found for bis(2-furfuryl) disulfide, which was in very good agreement with data reported earlier by Gasser and Grosch (1990a). The odor threshold of the newly identified flavor compound 5-acetyl-2,3-dihydro-1,4-thiazine was of the same order of magnitude as the odor threshold of 2-acetyl-2-thiazoline, recently established as a potent odorant in roast beef (Cerny and Grosch, 1993).

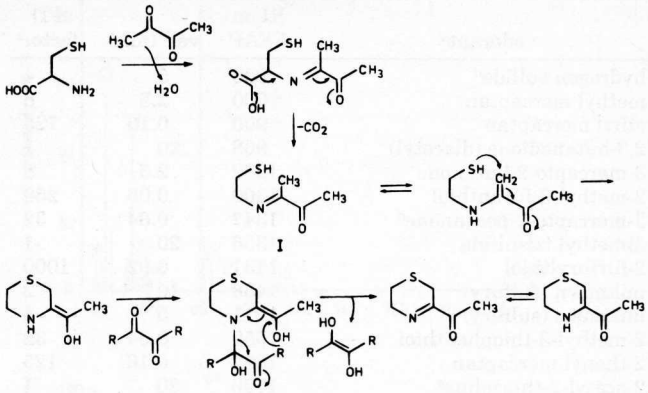
DISCUSSION

The results indicated 2-furfurylthiol, 2-methyl-3-furanthiol, 2-thenyl mercaptan, and ethyl mercaptan to be as the main contributors to the overall roasty, meatlike odor of a thermally treated aqueous cysteine/ribose solution and suggested 3-mercapto-2-pentanone, 5-acetyl-2,3-dihydro-1,4-thiazine, 3-mercapto-2-butanone, and bis(2-methyl-3-furyl) disulfide as further key flavor compounds in the reaction mixture.

3-Mercapto-2-pentanone, 2-methyl-3-furanthiol, and thenyl mercaptan have also been reported among the most odor-active compounds of a thermally treated thiamin/cysteine mixture containing no carbohydrate (Güntert et al., 1993), and quantitative investigation (Grosch and Zeiler-Hilgart, 1993) has established that thiamin/cysteine is a much more effective precursor mixture in the formation of 2-methyl-3-furanthiol than, e.g., cysteine/ribose. However, 2-thenyl mercaptan was not detected among the key odorants when cysteine was omitted from the reaction mixture (Güntert et al., 1993), suggesting that cysteine is the main precursor of this odorant.

On the basis of AEDA results 2-furfurylthiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, and bis(2-methyl-3-furyl) disulfide have also been detected as key odorants in a commercial meat flavoring (flavoring D; Gasser and Grosch, 1990a). Furthermore, 2-methyl-3-furanthiol and 2-furfurylthiol were established as key odorants in cooked beef and chicken broth (Gasser, 1990; Gasser and Grosch, 1990b). In addition, 3-mercapto-2-pentanone significantly contributed to the overall odor of chicken broth but did not much contribute to the flavor of cooked beef, whereas the reverse was found for bis-(2-methyl-3-furyl)-disulfide (Gasser, 1990).

Scheme 1. Hypothetical Pathway Leading from Cysteine and 2,3-Butandione to 5-Acetyl-2,3-dihydro-1,4-thiazine



2-Furfurylthiol and 2-methyl-3-furanthiol have also been established as key odorants in roasted coffee (Holscher et al., 1990; Blank et al., 1992), suggesting cysteine and/or thiamin as the precursors in the coffee beans. However, in freshly prepared popcorn (Schieberle, 1991) and roasted sesame seeds (Schieberle, 1993) 2-furfurylthiol but not 2-methyl-3-furanthiol was identified as key odorant, indicating a different formation pathway for 2-furfurylthiol here.

5-Acetyl-2,3-dihydro-1,4-thiazine has not been reported previously among the volatiles of Maillard model systems or of food flavors, respectively. The odorant could be formed from a reaction of cysteine and diacetyl, a well-known degradation product of sugars, as detailed in Scheme 1. Formation of an enamine and subsequent decarboxylation would yield the key intermediate **I** (Scheme 1). Tautomerization and subsequent cyclization of **I** by a Michael-type nucleophilic attack of the thiol group at the activated methyl carbon atom would yield 5-(2-hydroxyethenyl)-2,3,6-trihydro-1,4-thiazine. Oxidation of this enaminol, either by a second molecule of diacetyl or by a further dicarbonyl compound present in the reaction system, would then result in 5-acetyl-2,3,5-trihydro-1,4-thiazine, which due to the electronegativity of the sulfur atom tautomerizes into the more stable 5-acetyl-2,3-dihydro-1,4-thiazine.

A similar reaction of 2,3-pentanedione would result in 5-propionyl-2,3-dihydro-1,4-thiazine, which was also detected among the odorants of the reaction mixture.

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