Identification of Potent Aroma Compounds in Thermally Treated Mixtures of Glucose/Cysteine and Rhamnose/Cysteine Using Aroma Extract Dilution Techniques

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Application of an aroma extract dilution analysis on extracts prepared from either thermally treated solutions (20 min, 145 °C) of glucose/cysteine (I) or rhamnose/cysteine (II) led to the identification of 2-furfurylthiol (roasty, coffee-like), 5-acetyl-2,3-dihydro-1,4-thiazine (roasty), 3-mercapto-2-butanone (sulfury, rotten), 3-mercapto-2-pentanone (catty), and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like) with the highest odor activities among the 34 odor-active volatiles detected in I. In II, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-hydroxy-6-methyl-2(2H)-pyranone (seasoning-like), 5-methyl-2-furfurylthiol (roasty, coffee-like), 2-furfurylthiol, and 5-acetyl-2,3-dihydro-1,4-thiazine appeared with the highest flavor dilution (FD) factors among the 18 compounds detected by HRGC/O. Among the flavor compounds identified, 2-propionyl-2-thiazoline is reported for the first time among the flavors of Maillard model reactions or foods, respectively. The odorant elicited an intense roasty, popcorn-like odor at the low odor threshold of 0.07 ng/L in air.

INTRODUCTION

The Maillard reaction between reducing sugars and cysteine is known to generate meat-like odors, and the volatiles produced by heating, especially, ribose/cysteine mixtures, have been extensively studied. By application of aroma extract dilution analysis, a method combining sensory evaluation with analytical chemistry (cf. reviews by Acree, 1993; Grosch, 1993; Schieberle, 1995), the key odorants of a thermally treated solution of ribose and cysteine eliciting an overall roasty, meat-like odor have been recently identified (Hofmann and Schieberle, 1995).

Compared to ribose, fewer studies on the volatiles generated from cysteine in the presence of glucose or rhamnose have been performed. The first report on glucose/cysteine mixtures was reported by Arroyo and Lillard (1970) and has led to the identification of methyl-, propyl-, and pentylmercaptan. Scanlan et al. (1973) reported thiophenes, such as 2-formylthiophene and 5-methyl-2-formylthiophene, as main products among the 31 volatiles identified. Recently, Martin (1988) reported the previously unknown 4-hydroxy-2,5-dimethyl-3(2H)-thiophenone (thiofuraneol) as an important reaction product in a thermally treated (90 min; 180 °C) glucose/cysteine solution. Microwave heating of similar mixtures (Yeo and Shibamoto, 1991) resulted in sulfury-burnt overall odors which were suggested to be evoked, especially, by 3-thiophenethiol and 2-furfurylthiol.

Only few data are available on the volatiles generated in rhamnose/cysteine mixtures (de Rijke et al., 1981; Martin, 1988; Silwar, 1992). However, at least 180 volatiles have been identified (Silwar, 1992) among which 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 3-hy-droxy-6-methyl-2(2H)-pyranone were reported to be formed as the main reaction products (Martin, 1988).

Systematic studies on the odor-active volatiles generated by thermal treatment of either cysteine/glucose or cysteine/rhamnose reaction flavors have not yet been performed. The aim of the following study was, therefore, (i) to elucidate the key odorants in both precursors mixture by aroma extract dilution techniques and (ii) to gain first insights into the different reaction pathways generating odors from cysteine in the presence of pentoses, hexoses and methyl pentoses.

EXPERIMENTAL PROCEDURES

Chemicals. 5-Methylfurfural, 3-bromothiophene, 2-acetylthiophene, 2-acetylfuran, and hex-3-yne-2,5-diol were from Aldrich (Steinheim, Germany). L-Cysteine, D-glucose, and L-rhamnose were from Sigma (Munich, Germany). Compounds **6**, **7**, **10**, **12**, **17**, **18**, **22**, **25**, **27**, **29**, and **33** (Table 1) were purchased from Aldrich (Steinheim, Germany). No. **9** was supplied by Lancaster (Mühlheim, Germany).

Syntheses. *5-Methyl-2-furfurylthiol (MFFT).* 5-Methylfurfural (10 mmol), dissolved in dry diethyl ether (40 mL), was dropwise added to a stirred suspension of lithium aluminum hydride (4 mmol) in dry diethyl ether (20 mL). After a 3 h reflux in an argon atmosphere, ice water was added (50 mL) and the mixture was acidified to pH 2.0 by dropwise addition of sulfuric acid (2 M). The 5-methyl-2-furfuryl alcohol formed was isolated by extraction with diethyl ether (total volume: 200 mL). 5-Methyl-2-furfurylthiol was then prepared by substitution of the hydroxy group by a thiol group using thiourea and by using the reaction conditions recently described for the preparation of 2-thenylthiol (Hofmann and Schieberle, 1995). MFFT which was obtained in a yield of 46%, was characterized by its mass spectral data and by ^{1}H-NMR measurements.

The MS/EI (Figure 1) agreed with data published previously by Tressl and Silwar (1981). MS/CI (isobutane), m/z (%): 95 (100; M$^+$ + 1 − H$_2$S); 129 (10; M$^+$ + 1). ^{1}H-NMR (CD$_2$Cl$_2$; 360 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity, H at relevant carbon atom in Figure 1]: 1.94 (t, $^3J_{SH,7}$ = 7.63, 1 H, SH); 2.28 (s, 3 H, C-6); 3.71 (d, $^3J_{7,SH}$ = 7.63, $^4J_{7,3}$ = 1.09, 2 H, C-7); 5.90 (dd, $^3J_{3,4}$ = 3.27, $^4J_{3,7}$ = 1.09, 1 H, C-3); 6.05 (dd, $^3J_{4,3}$ = 3.27, 1 H, C-4).

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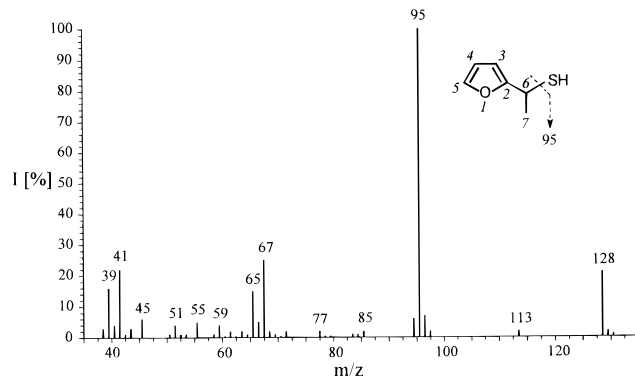
Aroma Compounds in Glucose/- and Rhamnose/Cysteine

J. Agric. Food Chem., Vol. 45, No. 3, 1997 **899**

Table 1. Intense Odorants (FD = 4) Formed by Reacting Glucose and Cysteine in Aqueous Solution[a]

no.	odorant[b]	RI on FFAP	RI on SE-54	FD factor[c] I	FD factor[c] II	reported as volatile compound[d]
1	3-mercapto-2-butanone	1282	820	512	<1	−
2	ethyl 2-mercaptopropionate	1300	869	8	<1	−
3	3-mercapto-2-pentanone[e]	1347	907	512	<1	−
4	mercapto-2-propanone	1357	<800	8	<1	1
5	2-(1-mercaptoethyl)furan	1396	951	256	<1	−
6	2-furfurylthiol	1431	909	1024	<1	2,3,4
7	2,3-diethyl-5-methylpyrazine[f]	1479	1163	<1	32	−
9	2-methyltetrahydrothiophen-3-one	1512	1017	8	<1	5,6,7
10	2-acetylthiazol	1615	1020	<1	32	1,7,8
11	2-(1-mercaptoethyl)thiophene	1655	1136	16	<1	−
12	2-formylthiophene	1669	1000	<1	128	1,2,6,7,9
13	2-thenylmercaptan	1682	1092	32	<1	2,3
14	2-propionylthiazol	1700	1107	<1	64	−
16	2-acetyl-2-thiazoline	1720	1111	<1	128	8
17	2-acetylthiophene	1740	1090	<1	128	1,3,6,7,9
18	5-methyl-2-formylthiophene	1744	1125	<1	256	1,2,6,7,9
20	unknown (earthy, roasty)	1784	−	<1	128	−
22	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2016	1100	512	<1	1,4
24	4-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone	2040	1181	} 128	<1	1
25	2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone[f]	2050	1178			−
26	2-mercaptopropionic acid	2130	1057	16	<1	1
27	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (Sotolon)[f]	2153	1112	32	<1	
28	5-acetyl-2,3-dihydro-1,4-thiazine	2177	1374	<1	1024	−
29	3-mercaptopropionic acid	2193	1057	4	<1	1
30	5-propionyl-2,3-dihydro-1,4-thiazine	2235	1456	<1	8	−
31	bis(1-(2′-furyl)-1-ethyl) disulfide	2422	1732	8	8	−
32	(2-furfuryl)-(1-(2′-furyl)-1-ethyl) disulfide	2449	1696	8	8	−
33	bis(2-furfuryl) disulfide	2465	1673	8	8	−

[a] A mixture of glucose (10 mmol) and cysteine (3 mmol) was reacted for 20 min in phosphate buffer (100 mL; 0.5 M; pH 5.0). [b] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention indices (RI) on two HRGC stationary phases given in the table, mass spectra obtained by MS(EI) and MS(CI), and odor quality and odor intensity perceived at the sniffing port. [c] Flavor dilution (FD) factor determined in the extracts containing the acidic (I) or the neutral/basic volatiles (II). Analyses were performed by two assessors in duplicates. The data differed by fewer than two FD factors. [d] Reported in the literature as volatile product reacting with cysteine, cystine, cysteamine, or hydrogen sulfide in the presence of glucose. (1) Martin, 1988; (2) Shibamoto and Russel, 1976; (3) Shibamoto and Russel, 1977; (4) Yeo and Shibamoto, 1991; (5) Kato et al., 1973; (6) Sakaguchi and Shibamoto, 1978a; (7) Zhang and Ho, 1991; (8) Sakaguchi and Shibamoto, 1978b; (9) Scanlan et al., 1973. [e] 3-Mercapto-2-pentanone contained smaller amounts (25%) of its isomer 2-mercapto-3-pentanone. [f] 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.
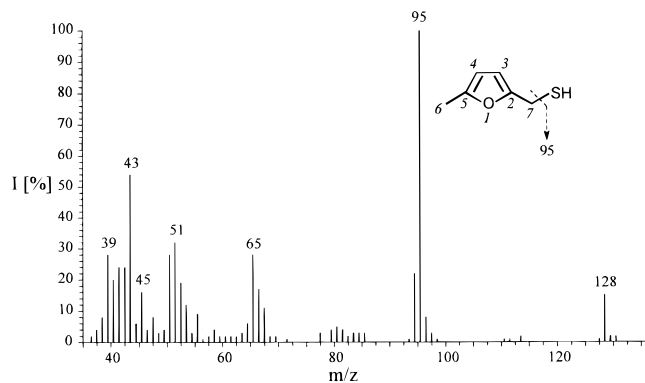
Figure 1. Mass spectrum (MS/EI) of 5-methyl-2-furfurylthiol.

Figure 2. Mass spectrum (MS/EI) of 2-(1-mercaptoethyl)-furan.

2-(1-Mercaptoethyl)furan (MEF). The compound was prepared from 2-acetylfuran following closely the route described above for the MFFT and was obtained in a 52% yield of the theory. Analysis of the compound on a chiral stationary phase (Lipodex E; 25 m × 0.25 mm fused silica capillary; Machery & Nagel, Düren, Germany) revealed the presence of two enantiomers in nearly equal amounts. The target compound was characterized by its mass spectra and by ^{1}H-NMR measurements. The MS/EI data (Figure 2) were in good agreement with a spectrum published previously by Güntert et al. (1990). The MS/EI is very similar to that of the isomeric 5-methyl-2-furfurylthiol (Figures 1 and 2). MS/CI (isobutane), m/z (%): 95 (100, M$^+$ + 1 − H$_2$S); 128 (6); 129 (5, M$^+$ + 1). ^{1}H-NMR (CD$_2$Cl$_2$, 360 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity, H at the relevant carbon atom in Figure 2]: 1.68 (d, $^3J_{7,6}$ = 7.09, 3 H, C-7); 2.19 (d, $^3J_{SH,6}$ = 6.00, 1 H, SH group); 4.23 (quin, $^3J_{6,SH}$ = 6.00, $^4J_{6,7}$ = 7.09, 1 H,

C-6); 6.18 (dd, $^3J_{3,4}$ = 3.27, 1 H, C-3); 6.34 (dd, $^3J_{4,3}$ = 3.27, $^3J_{4,5}$ = 1.09, 1 H, C-4); 7.38 (dd, $^3J_{5,4}$ = 1.09, 1 H, C-5).

5-Methyl-2-thenylthiol (5-MTT). 5-Methyl-2-thenylthiol was prepared in a 49% yield from 2-formyl-5-methylthiophene following closely the route described above for 5-methyl-2-furfurylthiol. The structure was verified by mass spectral and by ^{1}H- and ^{13}C-NMR measurements.

The MS/EI (Figure 3) and the MS/CI data (isobutane), m/z (%): 111 (100; M$^+$ + 1 − H$_2$S); 149 (9; M$^+$ + 1) were in good agreement with the structure displayed in Figure 3. ^{1}H-NMR (CD$_2$Cl$_2$, 360 MHz), δ in ppm (multiplicity, coupling constant (Hz), intensity, relevant hydrogen at carbon atom in Figure 3]: 1.90 (t, $^3J_{SH,7}$ = 7.63, 1 H, SH); 2.33 (s, 3 H, C-6); 3.77 (d, $^3J_{7,SH}$ = 7.63, $^4J_{7,3}$ = 1.09, 2 H, C-7); 6.44 (dd, $^3J_{3,4}$ = 3.27, $^4J_{3,7}$ = 1.09, 1 H, C-3); 6.51 (dd, $^3J_{4,3}$ = 3.27, 1 H, C-4). ^{13}C-NMR, δ in ppm (carbon number refers to Figure 3): 16.1 (CH$_3$, C-6);
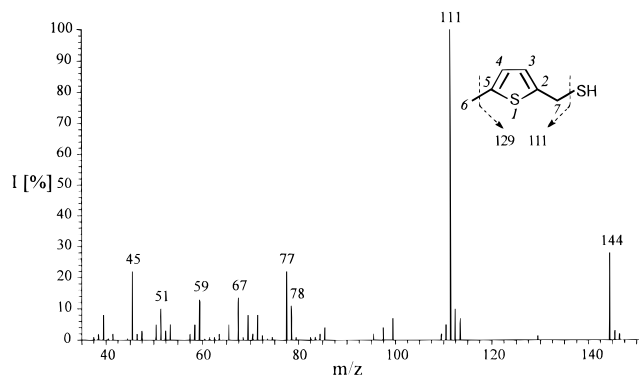
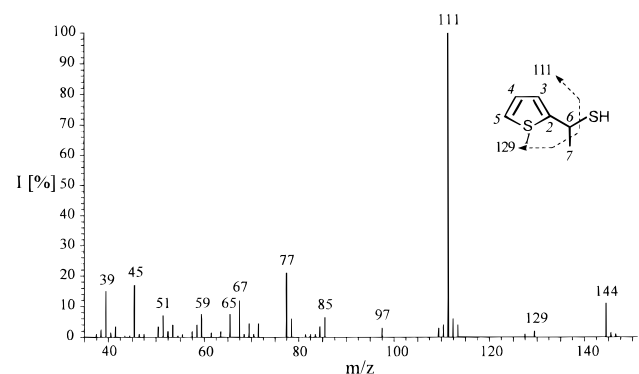**Figure 3.** Mass spectrum (MS/EI) of 5-methyl-2-thenylthiol.

Figure 4. Mass spectrum (MS/EI) of 2-(1-mercaptoethyl)-thiophene.
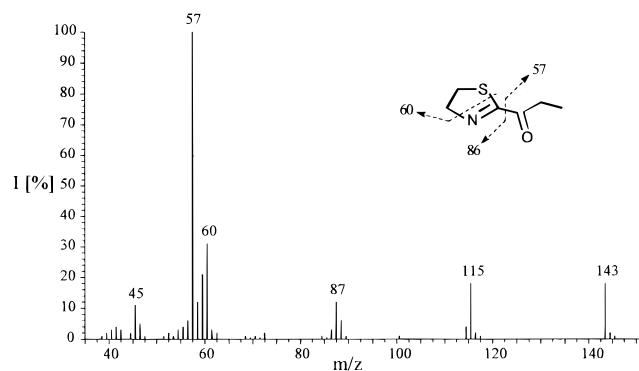

Figure 5. Mass spectrum (MS/EI) of 2-propionyl-2-thiazoline.

24.6 (CH$_2$, C-7); 125.7 (CH, C-3); 126.1 (CH, C-4); 140.4 (C, C-2); 143.3 (C, C-5).

2-(1-Mercaptoethyl)thiophene (MET). The target compound was prepared in a 79% yield of the theory from 2-acetyl-thiophene and following closely the route described above for 5-methyl-2-furfurylthiol.

The mass spectrum (MS/EI; Figure 4), the MS/CI [isobutane; m/z (%): 111 (100; M$^+$ + 1 − H$_2$S); 144 (6); 145 (5; M$^+$ + 1)], and the NMR data confirmed the structure of the 2-(1-mercaptoethyl)thiophene displayed in Figure 5. ^{1}H-NMR (CD$_2$-Cl$_2$, 360 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity, relevant carbon in Figure 4]: 1.76 (d, $^3J_{7,6}$ = 7.08, 3 H, C-7); 2.30 (d, $^3J_{SH,6}$ = 6.00, 1 H, SH); 4.23 (quin, $^3J_{6,7}$ = 7.08, $^3J_{6,7}$ = 7.08; $^3J_{6,SH}$ = 6.00, 1 H, C-6); 6.93 (dd, $^3J_{3,4}$ = 3.27, 1 H, C-3); 6.98 (dd, $^3J_{4,3}$ = 3.27, $^3J_{4,5}$ = 1.09, 1 H, C-4); 7.22 (dd, $^3J_{5,4}$ = 1.09, 1 H, C-5). ^{13}C-NMR, δ (ppm of relevant carbon in Figure 4): 28.28 (CH$_3$, C-7); 35.05 (CH, C-6); 124.55 (CH, C-4); 125.08 (CH, C-3); 127.57 (CH, C-5); 151.67 (C-2).

2-Propionyl-2-thiazoline. Cysteamine−hydrochloride (1 mmol) and 2-oxobutanal (1 mmol) were reacted in phosphate buffer (10 mL, 0.5 M; pH 7.0) for 1 h at 80 °C. After extraction with diethyl ether (total volume: 50 mL) the target compound (yield, 5%) was purified by preparative gas chromatography using the conditions previously reported (Schieberle, 1991).
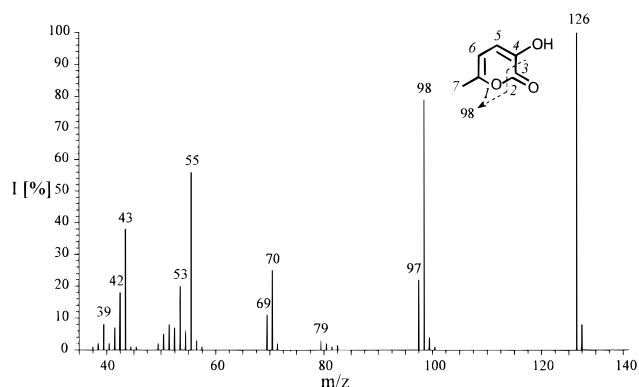

Figure 6. Mass spectrum (MS/EI) of 3-hydroxy-6-methyl-2(*2H*)-pyranone.
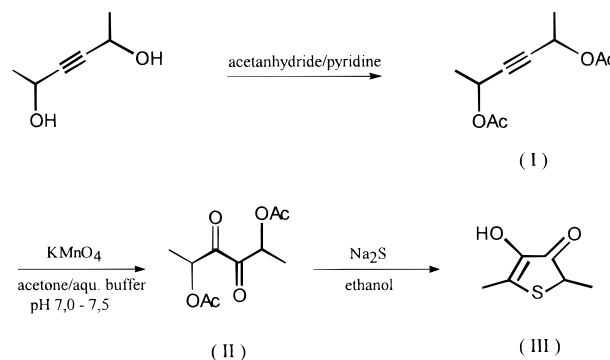

Figure 7. Synthetic route for the synthesis of 4-hydroxy-2,5-dimethyl-3(*2H*)-thiophenone.

The thiazoline was characterized by its mass spectra (MS/EI; cf. Figure 5; MS/CI, m/z (%): 144 (100; M$^+$ + 1)] and by ^{1}H-NMR measurements. ^{1}H-NMR (CDCl$_2$; 360 MHz), δ in ppm [multiplicity, coupling constant (Hz), intensity; hydrogen atom at relevant carbon number in Figure 5]: 0.78 (t, $^3J_{8,7}$ = 7.30, 3 H, C-8); 2.17 (quart, $^3J_{7,8}$ = 7.30, 2 H, C-7); 3.37 (t, $^3J_{5,4}$ = 8.41, 2 H, C-5); 4.55 (t, $^3J_{4,5}$ = 8.41, 2 H, C-4).

3-Hydroxy-6-methyl-2(2H)-pyranone (HMP). The HMP was prepared by oxidation of rhamnose to the corresponding 2,3,4,5-tetrahydroxypentanoic acid, cyclization to the δ-lactone followed by dehydration in phosphoric acid.

A solution of rhamnose (10 mmol), bromine (12 mmol), and barium benzoate (15 mmol, freshly prepared from sodium benzoate and barium chloride) in water (75 mL) was stirred for 36 h in the dark at room temperature. The excess of bromine was then removed by treatment with sodium thio-sulfate (0.1 M), and barium ions were precipitated by adding sulfuric acid (0.1 M). After extraction with dichloromethane (total volume: 100 mL), the aqueous phase containing the intermediate 3,4,5-trihydroxy-6-methyltetrahydropyran-2-one was adjusted to pH 4.0 with phosphoric acid (1 M) and heated in a laboratory autoclave for 45 min at 150 °C.

After extraction with dichloromethane (total volume: 100 mL) the target compound, obtained in a 23% yield of the theory, was characterized by mass spectral and ^{1}H-NMR measurements.

The MS/EI (Figure 6), which was consistent with a spectrum published previously by Martin (1988), and the MS/CI [m/z (%): 127 (100; M$^+$ + 1)] corroborated the structure displayed in Figure 6. ^{1}H-NMR (CD$_2$Cl$_2$, 360 MHz), δ in ppm [multiplicity, coupling constants (Hz), intensity, hydrogen atom at carbon number in Figure 6]: 2.21 (s, 3 H, C-7); 5.94 (d, ^{3}H$_{3,4}$ = 7.08, 1 H, C-4); 6.03 (broad s, 1 H, OH group); 6.61 (d, $^3J_{5,4}$ = 7.08, 1 H, C-5).

4-Hydroxy-2,5-dimethyl-3(2H)-thiophenone (HDMT). The scheme of the synthetic route is displayed in Figure 7.

Hex-3-yne-2,5-diol (10 mmol) was reacted with acetanhy-dride (20 mmol) by refluxing in dry pyridine (22 mmol) for 4 h. After addition of ice water (200 mL), the solution was acidified to pH 3.0 with hydrochloric acid (1 M), and compound
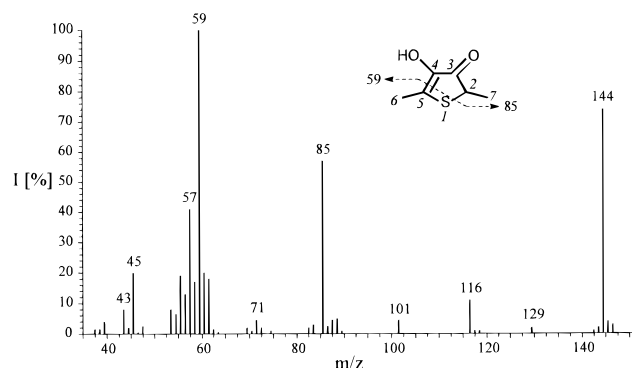
Aroma Compounds in Glucose/- and Rhamnose/Cysteine

J. Agric. Food Chem., Vol. 45, No. 3, 1997 **901**

Figure 8. Mass spectrum (MS/EI) of 4-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone.

I (Figure 7) was isolated by extraction with diethyl ether. I was taken up in a mixture of acetone (51.5 mL), sodium hydrogencarbonate (0.88 mmol), magnesium sulfate (2.84 mmol), and water (30.9 mL) and then oxidized by addition of potassium permanganate (10 mmol). After 2 h of stirring at 25 °C and maintaining the pH between 7.0 and 8.0 by dropwise addition of hydrochloric acid (1 M), the 2,5-bisacetoxyhexane-3,4-dione formed (II; Figure 7), which was isolated by extraction with diethyl ether. II (3.9 mmol) was then dissolved in ethanol (20 mL) containing sodium sulfide (8 mmol) and stirred for 12 h at room temperature. After addition of dilute hydrochloric acid (50 mL; pH 3.0), the mixture was extracted with diethyl ether (total volume: 100 mL). To purify the target compound from byproducts, the organic phase was then extracted with sodium bicarbonate (0.5 M; total volume: 60 mL), the combined organic phases were discarded, and the target compound was re-extracted into diethyl ether after adjusting the pH of the combined aqueous phases to 3.0.

The HDMT (III; Figure 7) was further purified by flash chromatography using the same equipment as described recently (Hofmann and Schieberle, 1995). After the column was flushed with pentane (150 mL), followed by pentane/diethyl ether (150 mL, 7 + 3 by vol) and pentane/diethyl ether (150 mL, 1 + 1 by vol), the target compound, which was obtained in a yield of 6%, was eluted with diethyl ether (150 mL).

The MS/EI (Figure 8) data were in agreement with a spectrum published by Martin (1988). In addition, the MS/CI (isobutane), *m/z* (%) [145 (100; M$^+$ + 1)], and the ^{1}H-NMR data confirmed the structure for HDMT displayed in Figure 8. ^{1}H-NMR, δ in ppm [multiplicity, coupling constant (Hz), intensity, relevant carbon in Figure 8]: 1.55 (d, $^3J_{7,2} = 7.08$, $^4J = 1.77$, 3 H, C-7); 2.51 (s, $^4J = 1.77$, 3 H, C-6); 3.70 (quart, $^3J_{2,7} = 7.08$, $^4J = 1.77$, 1 H, C-2); 5.25 (broad s, 1 H, OH group).

Bis(1-(2′-furyl)-1-ethyl) Disulfide (MEF−MEF) and 2-Furfuryl-(1-(2′-furyl)-1-ethyl) Disulfide (FFT-MEF). 2-(1-Mercaptoethyl)furan and 2-furfurylthiol (0.1 mmol each) were dissolved in diethyl ether (15 mL) and oxidized to the corresponding disulfides by copper(II) sulfate as previously described (Hofmann and Schieberle, 1995). The mixture of the three disulfides formed was separated by high-resolution column chromatography (HPLC) using a Beckman model 100 A pump and a Rheodyne valve (type 7125). The sample was applied onto a stainless steel column (25 cm × 0.46 cm i.d.) filled with ODS-Hypersil (Shandon, Eastmore, GB; 5 µm), and the target compounds were eluted with a mixture of acetonitrile/water (65 + 35 by vol). The effluent was monitored by an UVIDEC-100-III spectrophotometer (Jasco, Tokyo, Japan) at 254 nm. Eluates of 20 repeated injections were collected (MEF−MEF, 7.8 mL to 9.2 mL; MEF−FFT, 9.4 mL to 12.2 mL) and then diluted with water, and the mixture was extracted with diethyl ether. The mass spectra of MEF−MEF and FFT−MEF are displayed in Figure 9A and 9B. The molecular weights were established by MS/CI (isobutane).

The following odorants (cf. Tables 1 and 2) were synthesized as reported in the literature: nos. **1**, **4**, and **13** (Hofmann and Schieberle, 1995); nos. **28** and **30** (Hofmann et al., 1995); no. **3** (Asinger et al., 1964); no. **14** (Zehentbauer and Grosch, in

preparation); no. **16** (Cerny and Grosch, 1992). Ethyl 2-mercaptopropionate MS/EI data, *m/z* (%): 61 (100); 134 (40); 88 (26); 60 (22); 59 (18); 45 (15); 43 (14); 55 (12); 73 (8); 106 (4), were obtained by reacting 2-mercaptopropanoic acid and ethanol as recently reported for the synthesis of ethyl cyclohexanoate (Guth and Grosch, 1991). 2-Oxobutanal was prepared by oxidation of 1-hydroxy-2-butanone using bismutoxide in acetic acid.

Model Reaction. L-Cysteine (3.3 mmol) and either D-glucose (10 mmol) or L-rhamnose (10 mmol) were dissolved in phosphate buffer (100 mL; 0.5 M; pH 5.0) and allowed to react in a laboratory autoclave (model II, 200 mL total volume; Roth, Karlsruhe, Germany) by raising the temperature within 20 min from 20 to 145 °C.

Isolation of the Volatiles and Aroma Extract Dilution Analysis. The volatile reaction products formed were isolated by extraction with diethyl ether and sublimation in vacuo as recently described for the ribose/cysteine mixture (Hofmann and Schieberle, 1995). The distillates of both model solutions were each separated into an acidic volatile fraction (I in Tables 1 and 2) and a neutral/basic volatile fraction (II in Tables 1 and 2) by treatment with sodium bicarbonate, and then the odor-active compounds were evaluated by aroma extract dilution analysis (AEDA; cf. review by Schieberle, 1995) as recently described (Hofmann and Schieberle, 1995).

High-Resolution Gas Chromatography (HRGC)/Olfactometry and HRGC/Mass Spectrometry (MS; Approximation of Odor Thresholds). HRGC was performed with a type 5160 gas chromatograph (Fisons, Mainz, Germany) by using capillary FFAP (30 m × 0.32 mm fused silica capillary, free fatty acid phase, 0.25 µm; J&W Scientific, Fisons, Mainz, Germany) and capillary SE-54 (30 m × 0.32 mm fused silica capillary DB-5, 0.25 µm; J&W Scientific, Fisons, Mainz, Germany). The samples were applied by the "cold on-column" 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, and then raised at 6 °C/min to 230 °C and held for 15 min. The flow of the carrier gas helium was between 2.3 and 2.5 mL/min. At the end of capillary, the effluent was split 1:1 (by vol) into an FID and an odor port using deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm). The FID and the odor port were maintained at 180 °C. Calculation of the linear retention indices (RI) and mass spectral measurements were performed as recently described (Hofmann and Schieberle, 1995). Odor thresholds were approximated by HRGC/O as previously reported (Hofmann and Schieberle, 1995).

RESULTS

Glucose/Cysteine. A solution of glucose and cysteine was thermally treated for 20 min at 145 °C, and, after cooling, the overall odor was characterized by a six-membered sensory panel. The intensity of ten odor qualities had to be evaluated on a seven-point scale ranging from 0 (no odor) to 3.0 (most intense odor) as previsouly described (Hofmann and Schieberle, 1996). In Figure 10A, mean values of intensities of each odor note are displayed as a spider web diagram. Sulfury, roasty, and caramel-like notes were the most intense, and the overall odor was described as somewhat resembling roast chicken.

To identify the odorants responsible for these odors, the volatiles were isolated by extraction with diethyl ether followed by high-vacuum distillation. The distillate was separated into the acidic volatiles (fraction I; Table 1) and the neutral/basic volatiles (fraction II; Table 1), and then the most odor-active compounds in both fractions were selected by aroma extract dilution analysis (AEDA).

In fraction I, 17 odor-active compounds were detected in the flavor dilution (FD) factor range of 4−1024 (first column in Table 1). The highest FD factors were found
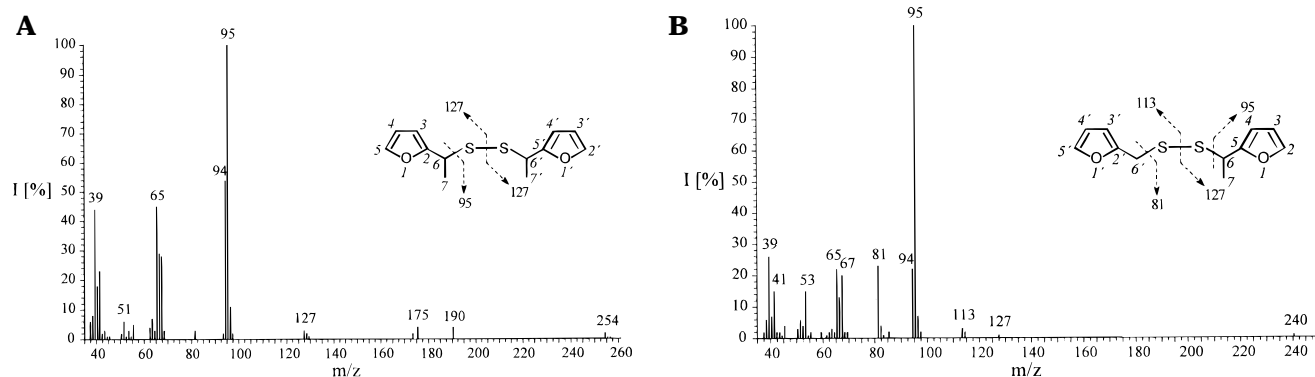
Figure 9. Mass spectra (MS/EI) of bis(1-(2′-furyl)-1-ethyl) disulfide (A) and 2-furfuryl-1-(2′-furyl)-1-ethyl disulfide (B).

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

odorant[a]	RI on FFAP	vol[b] (mL)	FD factor[c]
hydrogen sulfide	<900	5.0	1
methylmercaptan	<900	1.25	4
ethylmercaptan	<900	0.31	16
3-mercapto-2-butanone	1282	2.5	2
2-methyl-3-furanthiol[e]	1300	0.63	8
3-mercapto-2-pentanone[d]	1347	1.25	4
2-(1-mercaptoethyl)furan[e]	1396	0.31	16
2-furfurylthiol[e]	1431	0.08	64
2-(1-mercaptoethyl)thiophene[e]	1655	2.5	2
2-thenylthiol[e]	1682	1.25	4

[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.

for seven compounds (nos. **1**, **3**, **5**, **6**, **22**, and **24/25**) exhibiting either sulfury, roasty, or caramel-like odors and were, therefore, suggested as the main contributors to the overall odor of this fraction.

In a flavor extract prepared from 25 samples of the model reaction (total volume: 2.5 L) six of the seven odorants could be identified by comparison of the analytical and sensory data with those of reference compounds (cf. footnote *a* in Table 1): **1**, 3-mercapto-2-butanone (sulfury, rotten); **3**, 3-mercapto-2-pentanone (sulfury, catty); **6**, 2-furfurylthiol (roasty, coffee-like); **22**, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel-like); **24**, 4-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone (caramel-like), and **25**, 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (caramel-like).

The mass spectrum of compound **5** (Table 1) exhibiting a burnt, sulfury flavor note was very similar to a spectrum reported in the literature for 5-methyl-2-furfurylthiol (MFFT) (Tressl and Silwar, 1981; cf. Figure 1). However, the retention indices of the synthesized MFFT did not agree with those of compound **5** and the odors in the sniffing port were also quite different. Due to the difference of 104 units in the retention indices of MFFT (cf. Table 3) and compound **5** on the polar FFAP column, a secondary thiol structure was assumed for **5**. By synthesis, **5** was identified as 2-(1-mercaptoethyl)-furane.

In the fraction of the neutral/basic volatiles, 13 odorants were detected (sixth column in Table 1) among which compound **28** predominated with the highest FD factor and a roasty note the overall odor of the flavor concentrate. After enrichment by column chromatography (cf. Hofmann and Schieberle, 1995), the odorant was identified as 5-acetyl-2,3-dihydro-1,4-thiazine. In addition, 2-acetyl-2-thiazoline (no. **16**; roasty), 5-methyl-2-formylthiophene (no. **18**; earthy-roasty), 2-acetylthiophene (no. **17**; earthy-roasty), 2-formylthiophene (no. **12**; earthy-roasty), and an unknown odorant (no. **20**) with a roasty, earthy odor contributed with high FD factors to the odor of the thermally treated mixture.

Among the 28 odor-active compounds identified (Table 1), 15 odorants had previously not been reported as volatile reaction products in thermally treated glucose/cysteine solutions. Among them, due to their high FD factors, 3-mercapto-2-butanone, 3-mercapto-2-pentanone, 5-acetyl-2,3-dihydro-1,4-thiazine, and 2-(1-mercapto-ethyl)furan are of special interest. Furthermore, 2-propionylthiazol (no. **14**; Table 1) is an additional contributor to the overall roasty odor.

Our previous study on a ribose/cysteine solution (Hofmann and Schieberle, 1995) had shown that during the isolation procedure some labile flavor compounds, such as 2-thenylthiol and, also, volatile odorants, e.g., ethyl mercaptane, are lost to a certain degree. Applica-
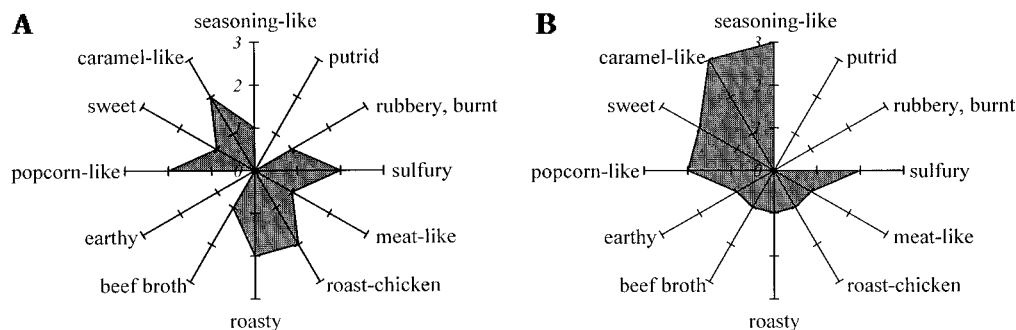

Figure 10. Odor profiles of (A) the glucose/cysteine model and (B) the rhamnose/cysteine model.

Aroma Compounds in Glucose/- and Rhamnose/Cysteine

J. Agric. Food Chem., Vol. 45, No. 3, 1997 **903**

Table 3. Intense Odorants (FD ≥ 4) Formed by Reacting Rhamnose with Cysteine in Aqueous Solution[a]

no.	odorant[b]	RI on FFAP	RI on SE-54	FD factor[c] I	FD factor[c] II	reported as volatile compound[d]
1	3-mercapto-2-butanone	1282	820	32	<1	−
3	3-mercapto-2-pentanone	1347	907	128	<1	−
6	2-furfurylthiol	1431	909	512	<1	−
7	2,3-diethyl-5-methylpyrazine	1479	1163	<1	64	−
8	5-methyl-2-furfurylthiol	1500	995	2048	<1	1,2
15	unknown (sulfury)	1707	−	32	<1	−
16	2-acetyl-2-thiazoline	1720	1111	<1	256	−
17	2-acetylthiophene	1740	1090	<1	32	1,2,3
18	5-methyl-2-formylthiophene	1744	1125	<1	256	−
19	5-methyl-2-thenylthiol	1755	1179	32	<1	−
20	unknown (earthy, roasty)	1784	−	<1	64	−
21	2-propionyl-2-thiazoline	1803	1196	<1	32	−
22	4-hydroxy-2,5-dimethyl-3(2H)-furanone	2016	1100	65536	<1	1,2
23	3-hydroxy-6-methyl-2(2H)-pyranone	2035	1047	16384	<1	1,2
25	2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone	2050	1178	64	<1	−
27	3-hydroxy-4,5-dimethyl-2(5H)-furanone	2153	1112	128	<1	−
28	5-acetyl-2,3-dihydro-1,4-thiazine	2177	1374	<1	512	−
30	5-propionyl-2,3-dihydro-1,4-thiazine	2235	1456	<1	32	−

[a] A mixture of rhamnose (10 mmol) and cysteine (3.3 mmol) was reacted for 20 min in phosphate buffer (100 mL; 0.5 M; pH 5.0). Footnotes *b* and *c*, cf. Table 1. [d] Reported in the literature as volatile reaction product reacting either cysteine, cystine, cysteamine, or hydrogen sulfide in the presence of rhamnose. (1) Martin, 1988; (2) Silwar, 1992; (3) Yamagushi et al., 1979.

Table 4. Results of the Static Headspace Analysis/Olfactometry (SHO) of the Rhamnose/Cysteine Model

odorant[a]	RI on FFAP	vol[b] (mL)	FD factor[c]
hydrogen sulfide	<900	2.5	2
methylmercaptan	<900	2.5	2
ethylmercaptan	<900	0.3	16
unknown (sulfury)	1000	5.0	1
2-methyl-3-furanthiol	1300	5.0	1
3-mercapto-2-pentanone[d]	1347	2.5	2
dimethyl trisulfide	1356	5.0	1
2-furfurylthiol	1431	0.3	16
5-methyl-2-furfurylthiol	1500	0.04	125
2-thenylmercaptan	1682	5.0	1
5-methyl-2-thenylthiol	1755	1.25	4

[a–d] For footnote explanations, see Table 2.

Table 5. Odor Thresholds of Selected Sulfur-Containing Odorants in the Cysteine/Carbohydrate Mixtures

odorant	odor threshold[a] (ng/L of air)
2-(1-mercaptoethyl)furan	0.009
5-methyl-2-furfurylthiol	0.006
2-(1-mercaptoethyl)thiophene	0.016
5-methyl-2-thenylthiol	0.019
3-hydroxy-6-methyl-2(2H)-pyranone	0.76
4-hydroxy-2,5-dimethyl-3(2H)-thiophenone	1.6
bis(2-(1-mercaptoethyl)furane) disulfide	0.005

[a] Determined by HRGC/olfactometry as described by Ullrich and Grosch (1987). (E)-2-Decenal (odor threshold = 2.7 ng/L air) instead of hexanal was used as the reference odorant.

Table 6. Comparison of the Key Odorants (FD Factors >128 to 65536) Formed by Reacting Cysteine in the Presence of Carbohydrates

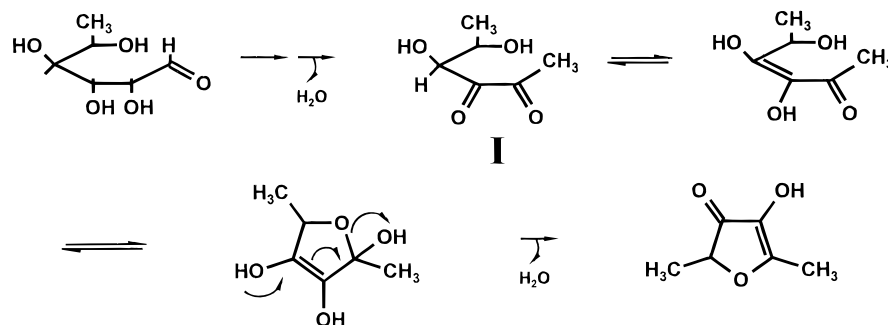| no.	odorant	FD factor glucose	FD factor rhamnose	FD factor ribose[a]
6	2-furfurylthiol	1024	512	4096
28	5-acetyl-2,3-dihydro-1,4-thiazine	1024	512	1024
8	5-methyl-2-furfurylthiol	<1	2048	<1
3	3-mercapto-2-pentanone	512	128	2048
1	3-mercapto-2-butanone	512	32	512
22	4-hydroxy-2,5-dimethyl-3(2H)-furanone	512	65536	128
5	2-(1-mercaptoethyl)furan	256	<1	<1
16	2-acetyl-2-thiazoline	128	256	256
23	3-hydroxy-6-methyl-2(2H)-pyranone	<1	16384	<1
27	3-hydroxy-4,5-dimethyl-2(5H)-furanone	16	128	64
A	2-methyl-3-furanthiol	<1	<1	1024
B	4-hydroxy-5-methyl-3(2H)-furanone	<1	<1	256
24	4-hydroxy-2,5-dimethyl-3(2H)-thiophenone	128[b]	<1	<1

[a] Adapted from the previous publication (Hofmann and Schieberle, 1995). [b] Coelution with 2-ethyl-4-hydroxy-5-methyl-3(2H)furanone (ethylfuraneol).

yl-3-furanthiol had not. The latter odorant has been identified as one of the key odorants generated by reacting pentoses in the presence of cysteine (Hofmann and Schieberle, 1995). However, it has not yet been reported to be formed from cysteine in the presence of hexoses.

Rhamnose/Cysteine. In a further model system, glucose was substituted by the 6-desoxy sugar rhamnose and, after thermal treatment, the overall odor was compared to that of the glucose model. In Figure 10 the odor profiles of both model systems are contrasted. In the rhamnose system (Figure 10B), caramel-like and spicy odor notes predominated, whereas the meat-like, roasty note was significantly lower than in the glucose system (Figure 10A).

To elucidate, which compounds are responsible for the flavor differences, the volatile fraction from the rhamnose/cysteine mixture was isolated and evaluated by AEDA. In Table 3, the results of the sensory evaluation and the identification experiments are summarized.

In the acidic fraction (first column; Table 3) the caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2H)-

tion of HRGC/olfactometry on static headspace samples (SHO) can overcome this problem (Schieberle, 1995).

The results of the application of SHO on the thermally treated glucose/cysteine solution are summarized in Table 2. In a 5 mL sample of air above the model, 10 odor-active regions were detectable after cryofocusing of the volatiles in a trap followed by HRGC separation and sniffing. The identification experiments, performed on the basis of retention indices and odor quality, in combination with a stepwise reduction of the headspace volume (Table 2) revealed 2-furfurylthiol as the key odorant, thereby corroborating the AEVA results (Table 1). The odorant was detectable in headspace volumes down to 0.08 mL. Seven of the 9 additional aroma compounds had also been identified in the AEDA of the solvent extract, whereas ethyl mercaptane and 2-meth-

Scheme 1. Reaction Pathway Leading from Rhamnose to 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone

Scheme 2. Reaction Pathway Leading from the 3-Desoxyosone of Rhamnose and H₂S to 5-Methyl-2-furfurylthiol via 5-Methylfurfural (I) as the Intermediate
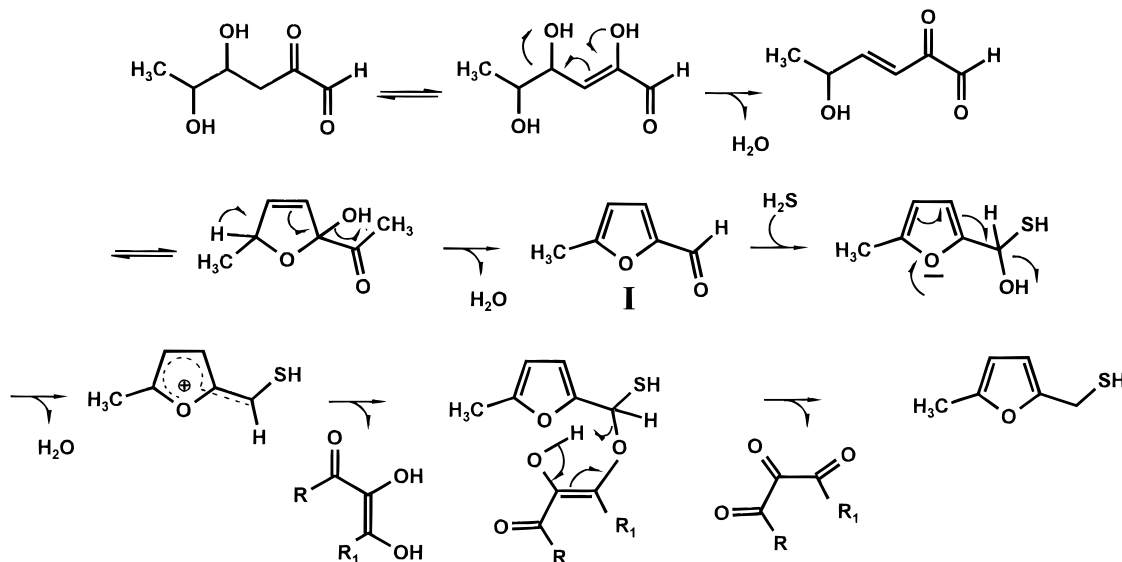
Table 7. Comparison of Odor Threshold of Roast Smelling Odorants Established as Contributors to Food Flavors or Reaction Flavors, Respectively

odorant	odor threshold (ng/L in air)	identified in [ref][a]
2-acetyl-1-pyrroline	0.02	bread crust [1]; popcorn [2]; proline/carbohydrates [3,4]; ornithine/carbohydrates [5]
2-propionyl-1-pyrroline	0.02	popcorn [2]
6-acetyl-tetrahydropyridine	0.06	bread crust [1]; popcorn [2]
5-acetyl-2,3-dihydro-1,4-thiazine	0.05	cysteine/ribose [6]
5-propionyl-2,3-dihydro-1,4-thiazine	0.10	cysteine/ribose [6]
2-acetylthiazol	4.10	cooked meat [7]
2-propionyl-2-thiazoline	0.07	cysteine/rhamnose
2-acetyl-2-thiazoline	0.05	roast meat [8]; cysteine/ribose [6]
2-acetylpyrazine	0.4	wheat bread crust [1]; popcorn [2]

[a] [1] Schieberle and Grosch, 1987; [2] Schieberle, 1991; [3] Schieberle, 1990a; [4] Roberts and Acree, 1994; [5] Schieberle, 1990b; [6] Hofmann and Schieberle, 1995; [7], Gasser and Grosch, 1988; [8] Cerny and Grosch, 1992.

furanone (no. **22**) and 3-hydroxy-6-methyl-2(2*H*)-pyranone (no. **23**), smelling like hydrolyzed protein showed by far the highest FD factors suggesting both compounds as the key odorants in this reaction flavor model. In addition, 5-methyl-2-furfurylthiol (no. **8**; coffee-like) and 2-furfurylthiol (no. **6**; roasty, coffee-like) were identified as important flavor contributors.

In the neutral/basic fraction (second column; Table 3), three compounds appeared with the highest FD factors (nos. **16**, **18**, and **28**) which were identified as the roasty smelling 5-acetyl-2,3-dihydro-1,4-thiazine (no. **28**), 2-acetyl-2-thiazoline (no. **16**; roasty), and 5-methyl-2-formylthiophene (no. **18**; roasty, earthy).

Among the nine odorants appearing with the highest FD factors in the rhamnose/cysteine mixture (cf. Table 3), five compounds (nos. **3**, **6**, **18**, **27**, and **28**) are reported for the first time as volatile reaction products in

processed flavors containing rhamnose and cysteine. Furthermore, 2-propionyl-2-thiazoline (no. **21**), eliciting an attractive roasty, cracker-like odor note, is reported for the first time among the food or reaction flavors, respectively.

An evaluation of the thermally treated rhamnose/cysteine solution by SHO revealed 5-methyl-2-furfurylthiol as the most potent odorant (Table 4), followed by 2-furfurylthiol and ethyl mercaptan. The data confirmed the two first mentioned thiols as key odorants in the mixture. However, it is interesting to note that the flavor contribution of further important odorants, such as the 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone or the 5-acetyl-2,3-dihydro-1,4-thiazine (cf. Table 3) would have been overlooked by applying only the SHO method. So, quantitative measurements and sensory studies to mimic the overall odor by using the reference odorants

are necessary further steps to determine exactly the flavor contribution of the odorants identified.

Odor Threshold Values. The odor threshold values of selected key odorants identified in the two reaction flavor model were approximated by HRGC/olfactometry (Table 5). The values obtained for the four thiols, 2-(1-mercaptoethyl)furane, 5-methyl-2-furfurylthiol, 2-(1-mercaptoethyl)thiophene, and 5-methyl-2-thenylthiol, were very low and were in the same order of magnitude as previously reported for the very intense food odorants 2-furfurylthiol (coffee-like) and 2-methyl-3-furanthiol (cooked meat). The seasoning-like smelling 3-hydroxy-6-methyl-2(2*H*)-pyranone showed a higher odor threshold than the well-known seasoning-like smelling food odorants Sotolon [3-hydroxy-4,5-dimethyl-2(5*H*)-furanone; 0.015 ng/L] and Abhexone [3-hydroxy-5-ethyl-4-methyl-2(5*H*)-furanone; 0.003 ng/L], respectively. The odor threshold of the so-called "thiofuraneol", 4-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone, was almost identical with the value of 1 ng/L in air (Blank and Schieberle, 1993) reported for the corresponding 3(2*H*)-furanone "Furaneol".

DISCUSSION

The results indicated 2-furfurylthiol, 5-acetyl-2,3-dihydro-1,4-thiazine, 3-mercapto-2-pentanone, 3-mercapto-2-butanone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 2-(1-mercaptoethyl)furan as the main contributors to the overall roasty, sulfury odor of a thermally treated glucose/cysteine mixture (cf. Table 6).

Substitution of the hexose by the 6-desoxy sugar rhamnose significantly changed the overall odor note. This was reflected by a change in the key odorants with 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-hydroxy-6-methyl-2(2*H*)-pyranone and 5-methyl-2-furfurylthiol being the most important odorants in the rhamnose solution (Table 6).

A comparison of the data presented here with our recent results on a ribose/cysteine solution (Hofmann and Schieberle, 1995) revealed (cf. Table 6) that the FD factors of 2-furfurylthiol (no. **6**; Table 6), 5-acetyl-2,3-dihydro-1,4-thiazine (no. **28**), and 2-acetyl-2-thiazoline (no. **16**) were not significantly influenced by the carbohydrate moiety. In addition, 3-mercapto-2-pentanone (no. **3**), 3-mercapto-2-butanone (no. **1**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (no. **24**), and 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon; no. **27**) were formed from cysteine in the presence of the three carbohydrates studied. However, differences in their FD factors or concentrations, respectively, were observable, e.g., 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDF) was preferentially formed from rhamnose. This is in agreement with its easy formation from the 1-desoxyosone of rhamnose as detailed in Scheme 1. However, because the formation of the 1,6-dimethyl-2,3-dioxo intermediate I (Scheme 1) is not likely in the case of glucose and is impossible from ribose, an alternative reaction pathway undoubtedly occurs during formation of the HDF from these two carbohydrates. Recent results (Schieberle and Hofmann, 1996) proposed retro-aldol type reactions of the C-3 carbohydrate cleavage products 2-oxopropanal and 1-hydroxypropanone to generate HDF.

Contrary, a few odorants were only formed in the presence of one carbohydrate. For instance, 2-(1-mercaptoethyl)furan (no. **5**; Table 6) is exclusively formed in the presence of glucose, whereas the isomeric 5-methyl-2-furfurylthiol (MFFT) is exclusively generated from rhamnose. A reaction pathway for the formation of MFFT is proposed in Scheme 2. Starting from the 3-desoxyosone of rhamnose, dehydration reactions lead to 5-methylfurfural as the key intermediate. Formation of a thiohemiacetal, elimination of water, assisted by the ring oxygen, and subsequent reduction of the intermediate cation, e.g., by an enol−oxo compound, gives rise to 5-methylfurfurylthiol. In a model experiment, using the same reaction conditions as in the carbohydrate/cysteine model, a mixture of 5-methylfurfural and H_2S (1 mmol each) generated the MFFT in a yield of 0.4% (data not shown). The yield was significantly dependent on the H_2S concentration, with higher amounts of H_2S increasing the MFFT formation. These data are well in line with the mechanism shown in Scheme 2.

2-(1-Mercaptoethyl)furan was formed in model studies to the same extent from 2-acetylfuran as the intermediate (data not shown) and a similar reaction pathway can, therefore, be proposed. Because 5-methylfurfural is much easier formed from rhamnose than from glucose and, on the other hand, the generation of 2-acetylfuran is very unlikely from rhamnose, the proposed reaction pathway is in good agreement with the exclusive formation of thiols no. **5** (from glucose) and no. **8** (from rhamnose).

2-Propionyl-2-thiazoline and its oxidation product 2-propionylthiazol are reported here for the first time among the food flavors or processed flavors, respectively. Both are additional roast-smelling compounds having an α-oxo-imino structure with R_2 and R_3 being part of a ring structure (Schieberle, 1991):
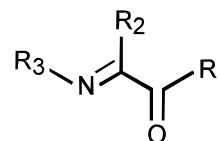
As summarized in Table 7, the odor threshold of 2-propionyl-2-thiazoline is in the same order of magnitude as the value reported in the literature (Schieberle, 1991) for the popcorn-like smelling intense odorant 2-acetyl-1-pyrroline.

In comparison to 2-acetyl-2-thiazoline, the 2-acetylthiazole, however, showed a significantly higher odor threshold. Obviously, not only the length of the alkyl side chain R (Schieberle, 1991; Hofmann et al., 1995) but also the aromatic ring system significantly increases the odor threshold of compounds having the structural element shown above. This is most significant for 2-acetylpyrrol, which does not smell roasty and has an odor value of >2000 ng/L in air.

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906 *J. Agric. Food Chem.,* Vol. 45, No. 3, 1997

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