3-Mercapto-2-methylpentan-1-ol, a New Powerful Aroma Compound†

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3-Mercapto-2-methylpentan-1-ol was first detected in a complex thermally processed flavor and finally isolated from raw onions. The chemical structure of this new compound was identified by MS and ^{1}H NMR measurement and synthesis of the proposed structure. Sensory evaluation at different concentrations indicated that the flavor quality is strongly dependent on concentration. At low concentration (0.5 ppb) a pleasant meat broth, sweaty, onion, and leek-like odor can be perceived. On the basis of some isolation experiments and volatiles occurring in raw onions, a formation pathway is proposed. As one intermediate 3-mercapto-2-methylpentanal, another new strong flavor compound, was suggested. The presence of this compound in raw onions was confirmed by synthesis and comparison of MS and chromatographic data.

Keywords: *3-Mercapto-2-methylpentan-1-ol; 3-mercapto-2-methylpentanal; onion; flavor; formation in onions*

INTRODUCTION

Volatile compounds that have sensory properties, that is, elicit flavor properties, belong to different chemical classes. Among them sulfur compounds play an important role because many of them have very low odor thresholds and thus contribute considerably to the overall odor impression even at low concentrations (Boelens and van Gemert, 1993). Sulfur-containing flavor impact compounds have been identified in different fruits such as passionfruit (Winter et al., 1976; Engel and Tressel, 1991), durian (Fischer, 1995), and grapefruit (Demole et al., 1982), in *Allium* species (Boelens et al., 1971), and in processed foods, that is, meat (Gasser and Grosch, 1988) and coffee (Blank et al., 1992; Semmelroch and Grosch, 1995), as well as in Maillard model reaction systems (Ho, 1996; Mottram and Whitfield, 1994).

The numerous Maillard reaction systems investigated are generally very simple, consisting only of one or a few carbohydrates and a limited number of amino acids. In contrast to this, investigations of complex flavors produced by heating of a variety of carbohydrates and amino acids in combination with several natural extracts, that is, meat extracts and vegetable extracts, are not reported to the best of our knowledge. Reaction of such complex mixtures may bear further new strong impact sulfur compounds.

During sensory screening of different thermally processed flavors, we selected one flavor having an interesting flavor profile. GC-sniffing analysis of the aroma extract obtained from this flavor indicated the presence of a very powerful flavor impression described as meaty, sweaty, and leek-like. This paper describes the detection, isolation, identification, and synthesis of this new strong and high-impact flavor compound.

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† Dedicated to Dr. Günther Ohloff on the occasion of his 75th birthday.

EXPERIMENTAL PROCEDURES

Syntheses. *3-Mercapto-2-methylpentanol.* Piperidine (0.14 g) was added to 2-methyl-2-pentenal (13.47 g) under an inert atmosphere at 10 °C. Then 15.73 g of thioacetic acid was added dropwise at 10 °C. After the addition, the reaction mixture was stirred for another 18 h at room temperature. The mixture was diluted with 100 mL of diethyl ether and washed with 20 mL of 1 N hydrochloric acid and twice with 20 mL of saturated sodium bicarbonate solution. After drying over sodium sulfate, the solvent was evaporated. The crude product (82% purity by GC) was used in the subsequent reduction reaction without further purification.

The crude product from the first step was slowly added to a suspension of 3.6 g of lithium aluminum hydride in 100 mL of dry diethyl ether at 0 °C under nitrogen. The internal temperature should not rise above 5 °C. After the addition, stirring was continued for another 0.5 h at room temperature. First, ammonium chloride solution and then 2 N hydrochloric acid at 0 °C were added for hydrolysis. The organic phase was separated, and the aqueous phase was extracted twice with 100 mL portions of diethyl ether. The combined organic phases were washed twice with 20 mL of saturated sodium bicarbonate solution, dried over sodium sulfate, and filtered, and the solvent was evaporated. Vacuum distillation (bp 44−46 °C/0.5 mbar) afforded 5.6 g (30% over two steps) of a 1:1 diastereomeric mixture of the desired product as a clear liquid of intense smell.

MS (EI, 70 eV), *m*/*z* (%) (diastereomer A) 134 (M^+, 21), 100 (42), 83 (18), 75 (51), 74 (100), 71 (60), 55 (48), 47 (30), 45 (27), 41 (96), 31 (33); (diastereomer B) 134 (M^+, 21), 100 (42), 83 (22), 75 (52), 74 (100), 71 (60), 55 (50), 47 (31), 45 (31), 41 (97), 31 (34).

^{1}H NMR (C_6D_6) δ 0.73 (d, 7 Hz, 3 H, 2-Me, B), 0.88 (d, 7 Hz, 3 H, 2-Me, A), 0.92 (t, 7 Hz, 3 H, 5-H, A or B), 0.925 (d, 8.5 Hz, 1 H, SH, B), 0.93 (t, 7 Hz, 3 H, 5-H, B or A), 1.15 (d, 8 Hz, 1 H, SH, A), 1.26 (br m, 1 H, 4-Ha, A), 1.38 (m, 2 H, 4-H, B), 1.55 (dqd, 4 Hz, 7 Hz, 15 Hz, 4-Hb A), 1.68 (br sept, ∼7 Hz, 1 H, 2-H, A), 1.74 (br m, 1 H, 2-H, B), 2.26 (br s, 2 H, OH), 2.62 (dddd, 4 Hz, 5.5 Hz, 8 Hz, 10 Hz, 1 H, 3-H, A), 2.89 (ddt, 4 Hz, 6 Hz, 9 Hz, 1 H, 3-H, B), 3.35 (dd, 6 Hz, 10 Hz, 1 H, 1-Ha, B), 3.43 (dd, 6 Hz, 10.5 Hz, 1 H, 1-Ha, A), 3.47 (dd, 7 Hz, 10.5 Hz, 1 H, 1-Hb, A), 3.52 (dd, 8 Hz, 10.5 Hz, 1 H, 1-Hb, B).

^{13}C NMR (C_6D_6) δ 10.5 (1°, 2-Me, B), 12.2; 12.6 (1°, C-5, A and B), 14.4 (1°, 2-Me, A), 28.3 (2°, C-4, A), 30.6 (2°, C-4, B), 40.3 (3°, C-2, B), 42.2 (3°, C-2, A), 44.4 (3°, C-3, B), 45.7 (3°,

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Table 1. Ingredients of Different Reaction Mixtures

expt	composition
1	amino acids (330 g) + water (170 g)
2	amino acids (282 g) + fructose (48 g) + water (170 g)
3	meat extract (33 g) + chicken broth extract (66 g) + yeast extract (198 g) + onion extract (66 g) + water (170 g)
4	chicken broth extract (73 g) + yeast extract (220 g) + onion extract (37 g) + water (170 g)
5	yeast extract (283 g) + onion extract (47 g) + water (170 g)
6	yeast extract (330 g) + water (170 g)
7	onion extract (330 g) + water (170 g)

C-3, A), 65.3 (2°, C-1, A), 65.9 (2°, C-1, B) (1° = primary, 2° = secondary, 3° = tertiary C atom).

3-Mercapto-2-methylpentanal. Hydrogen sulfide was bubbled through a stirred solution of 321 g of 2-methylpent-2-enal, 0.3 g of hydroquinone, and 32 mL of triethylamine in 600 mL of dry tetrahydrofuran at 40 °C. The excess hydrogen sulfide was absorbed in aqueous sodium hydroxide solution. After 6 h, the addition was complete and nitrogen was bubbled through the solution for 5 min to remove excess hydrogen sulfide. The solution was diluted with 600 mL of diethyl ether and then successively washed with water, 1 N aqueous hydrochloric acid, saturated aqueous sodium bicarbonate solution, and brine. The organic phase was dried over sodium sulfate and filtered, and the solvent was removed in vacuo to yield a viscous oil. A small sample was chromatographed over silica gel. As the compound forms dimers (Kleipool et al., 1976), the NMR spectra were very complex.

MS (EI, 70 eV), *m*/*z* (%) (diastereomer A) 132 (M^+, 12), 114 (13), 99 (30), 75 (33), 70 (100), 61 (24), 55 (82), 43 (39), 41 (97); (diastereomer B) 132 (M+, 13), 114 (18), 99 (41), 75 (39), 70 (75), 61 (24), 55 (67), 43 (43), 41 (100).

Thermally Processed Flavor. Water (340 g), yeast extract (300 g), chicken broth extract (100 g), onion extract (50 g), meat extract (50 g), fructose (20 g), and a mixture of six amino acids (140 g) were heated under reflux for 1 h.

Thermally Processed Mixtures. The amounts of ingredients heated in trials 1−7 are listed in Table 1. Every mixture was heated under reflux for 1 h.

Isolation of Volatiles from the Processed Flavor and from Processed Mixtures. After cooling, the reaction mixture was continuously extracted with 400 mL of diethyl ether for 4 h by using a rotational perforator. The extract obtained was concentrated to 100 mL on a Vigreux column (50 × 1 cm) by distilling off the solvent at 40 °C. The volatile compounds were isolated on a high-vacuum distillation apparatus previously described by Sen et al. (1991). The distillate was treated two times with an aqueous sodium hydrogen carbonate solution (1 mol/L; total volume = 150 mL), and the ether solution was washed twice with brine (total volume = 100 mL) and then dried over Na_2SO_4. For HRGC-O-MS the extract was concentrated at 40 °C to 1 mL by distillation on a Vigreux column (50 × 1 cm).

Column Chromatography. The concentrated extract obtained from 10 kg of trial 5 was fractionated on a glass column (61 × 3.7 cm) packed with a slurry of silica gel (40 μm, J. T. Baker BV, Deventer, The Netherlands) in pentane. Chromatography was performed under nitrogen pressure, maintaining a constant flow rate of 5 mL/min and by using the following solvents: *n*-pentane (500 mL, fraction A), *n*-pentane/diethyl ether (500 mL; 95 + 5; fraction B), *n*-pentane/diethyl ether (500 mL; 90 + 10; fraction C), *n*-pentane/diethyl ether (500 mL; 80 + 20; fraction D), diethyl ether (500 mL; fraction E), and finally methanol (200 mL; fraction F). Each of the fractions was concentrated on a Vigreux column and then by microdistillation (Bemelmanns, 1979).

Isolation of Volatiles from Raw Onion. *Dynamic Headspace Enrichment.* Raw onions (200 g) were sliced into small cubes and transferred into a 2 L flask. The volatiles were enriched at room temperature on a glass tube packed with activated charcoal (100 mg of Carbotrap 15−40 mesh, Supelco) at a gas flow rate of 200 mL/min. Enrichment was performed using air or nitrogen. After 24 h, the volatiles were desorbed with 2 mL of diethyl ether. The extract was concentrated to 100 μL by microdistillation (Bemelmanns, 1979) and analyzed by HRGC-O-MS.

Simultaneous Distillation/Extraction (SDE). Two hundred grams of sliced raw onions and 800 mL of tap water were mixed in a 2 L round-bottom flask and continuously extracted for 2 h with 100 mL of diethyl ether in the apparatus designed by Nickerson and Likens (1966). The extract obtained was dried over anhydrous Na_2SO_4, concentrated to 100 μL by distillation and microdistillation, and subsequently analyzed by HRGC-O-MS.

In addition, SDE was performed under acidic conditions using the procedure described above. Therefore, the water/onion mixture was adjusted to pH 2.0 with hydrochloric acid (1 mol/L).

Vacuum Headspace Distillation. One kilogram of raw onions was sliced into small cubes and either directly transferred in a 2 L round-bottom flask of the high-vacuum distillation apparatus described by Sen et al. (1991) or first spread out on a tray, stored at room temperature for 2 h, and then transferred into the apparatus. The volatiles were condensed during 8 h at a vacuum of 0.1−1 mbar in three traps cooled with liquid nitrogen. At the end of the sampling time, the aqueous condensates were combined and were either extracted with diethyl ether in a rotational perforator or further fractionated by means of SPE.

Solid-Phase Extraction (SPE). The aroma-bearing aqueous distillate obtained by high-vacuum distillation was passed through a glass column (20 × 1 cm i.d.) filled with C-18 reversed phase material (40 μm, J. T. Baker BV). Subsequent desorption was performed with pentane (30 mL, fraction I), pentane/diethyl ether (30 mL, 9 + 1, fraction II), and diethyl ether (30 mL, fraction III). After drying over Na_2SO_4 and concentration to 100 μL, the fractions were analyzed by HRGC-O-MS.

High-Resolution Gas Chromatography/Olfactometry/Mass Spectrometry (HRGC-O-MS). HRGC-O and HRGC-MS were performed in combination by means of a GCD type mass spectrometer (Hewlett-Packard) equipped with a cold injection system (CIS, Gerstel GmbH, Mülheim, Germany) and a sniffing port (Gerstel GmbH, Mülheim an der Ruhr, Germany). The following columns were used: DB-1, (30 m × 0.25 mm fused silica, 0.25 μm; J&W Scientific, Fisons, Mainz, Germany) and DB-Wax (30 m × 0.25 mm fused silica, 0.25 μm; J&W Scientific). The samples were injected splitless into the CIS at 60 °C. After injection, the CIS was heated at 12 °C/s to 240 °C and the temperature of the oven was raised from 40 °C at 4 °C/min to 230 °C and then held for 15 min. At the end of the column, the effluent was split 1:4 (by volume) into the sniffing port and the mass detector using deactivated uncoated fused silica capillaries. The temperature of the sniffing port was held at 200 °C. Mass spectra in the electron ion impact mode were generated at 70 eV.

NMR Spectroscopy. ^{1}H NMR spectra of synthesized samples were recorded in C_6D_6 at 300 MHz on a Varian instrument. The ^{13}C NMR spectra of synthesized samples were recorded in C_6D_6 at 75 MHz each with $Si(CH_3)_4$ as internal standard. ^{1}H NMR spectra of collected samples were obtained in C_6D_6 at 500 MHz on a Varian INOVA 500 instrument with $Si(CH_3)_4$ as internal standard.

Preparative Capillary Gas Chromatography. For the preparative enrichment and isolation of 3-mercapto-2-methylpentanol, a mass flow controlled automated multidimensional switching system (MCS Gerstel GmbH, Mülheim/Ruhr, Germany) was employed in combination with an HP 6890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a cold injection system (CIS, Gerstel GmbH, Mülheim/Ruhr, Germany). A combination of a DB-1 column (15 m × 0.53 mm, 3 μm; J&W Scientific) and a DB-Wax column (30 m × 0.53 mm, 2 μm; J&W Scientific) was used. The samples were injected splitless into the CIS at 80 °C. After injection, the CIS was heated at 10 °C/s to 240 °C and the temperature of the oven was raised from 60 °C at 4 °C/min to 140 °C and then at 8 °C/min to 250 °C. Fractionation was performed with an automated fraction collecting system (Gerstel GmbH) cooled with liquid nitrogen.

Enantioselective MDGC Analysis. For the separation of the enantiomers, multidimensional gas chromatography was performed with a Siemens Sichromat 2-8 equipped with two independent column oven programs and a live-T-switching device for selective cutting from the first to the second column. Precolumn conditions were as follows: DB-Wax column (30 m × 0.25 mm, 0.25 μm; J&W Scientific), carrier gas, helium; gas flow rate, 1.07 mL/min; injector temperature, 200 °C, splitless injection; oven temperature, 60 °C raised at 4 °C/min to 200 °C. Main column conditions were as follows: MN Lipodex E column (25 m × 0.25 mm, 0.25 μm; Machery and Nagel, Düren, Germany), carrier gas, helium; gas flow rate, 1.26 mL/min; oven temperature, 60 °C raised at 2 °C/min to 160 °C.

Odor Thresholds in Water. A defined amount of each compound, dissolved in 0.1 mL of ethanol, was added to water (1 L). After stirring for 10 min, this stock solution was diluted stepwise with water (1 + 1, v/v) and stirred for 5 min after each dilution step. Odor threshold values were determined by triangle tests. Every dilution step was presented with three glass beakers each containing 20 mL of liquid. One of these beakers contained the solution with the compound, the remaining two contained water. The samples were presented in order of decreasing concentrations, and the odor threshold values evaluated by at least 15 judges were averaged.

RESULTS AND DISCUSSION

Investigation of the Processed Flavor. Sensory screening of different processed flavors, all based on Maillard type reactions using different ingredients, was performed with regard to an interesting flavor profile. One flavor showing a very interesting profile described as sour, meaty, savory, broth-like, and leek-like was selected for analysis.

For identification of the flavor compounds responsible for this profile, the volatiles of the processed flavor consisting of water, yeast extract, chicken broth extract, onion extract, and meat extract, as well as fructose and a mixture of six amino acids, were isolated by continuous solvent extraction using diethyl ether. GC-O-MS analysis of the aroma extract obtained indicated *one* position in the gas chromatogram at RI 1796 on DB-Wax having a strong meaty, sweaty, and leek-like flavor quality that seemed to contribute strongly to the overall flavor profile of the processed flavor; however, no MS signal for unequivocal interpretation could be obtained.

On the basis of the different ingredients of the processed flavor, a great number of volatile compounds are formed during the heating process, leading to a very complex gas chromatogram. To achieve more information on possible precursors for the unknown flavor compound and to reduce the number of volatiles formed during heating, the composition of the reaction mixture was systematically varied. The ingredients necessary for the formation of the unknown compound were determined by systematic omission of constituents. Every mixture was heated under reflux for 1 h. After cooling, the mixture was continuously extracted with diethyl ether, and the volatiles were subsequently separated from the nonvolatile material by high-vacuum distillation. The results obtained by HRGC-O-MS analysis of the flavor distillate are shown in Table 2.

In the first experiment all amino acids were heated. GC-sniffing analysis indicated that the unknown compound was not formed in detectable amounts. The same result was obtained after addition of fructose in experiment 2. In contrast to this, the target compound could be strongly perceived after heating of all extracts (yeast extract, chicken broth extract, meat extract, and onion extract) as shown in experiment 3. Subsequent omission of meat extract in experiment 4 and further omission of chicken broth extract in experiment 5 revealed that the unknown compound can be produced by heating yeast and onion extract, whereas meat extract and chicken broth extract have no effect on its formation. In experiments 6 and 7, yeast extract and onion extract were heated separately. The GC-sniffing analysis indicated the presence of the interesting compound only after heating of onion extract, but its perceived signal was very weak. These results suggest that some ingredients of the yeast extract enhance the formation of the unknown molecule during heating of onion extract. Furthermore, experiment 8 showed that the target compound already exists in the untreated onion extract.

Table 2. Precursors of the Unknown Flavor Molecule

expt[a]	composition[b]	detection[c]
1	amino acids	−
2	amino acids + fructose	−
3	all extracts	+++
4	trial 3 without meat extract	+++
5	trial 4 without chicken broth extract (= yeast and onion extract)	+++
6	yeast extract	−
7	onion extract	+
8	unheated onion extract	+

[a] The mixtures are heated under reflux for 1 h and are extracted with diethyl ether. [b] Quantitative amounts are listed in Table 1 (Experimental Procedures). [c] Extracts were analyzed by GC-sniffing: −, 3-mercapto-2-methylpentanol could not be perceived; +, a weak sensory signal of 3-mercapto-2-methylpentanol was perceived; +++, a strong sensory signal of 3-mercapto-2-methylpentanol was perceived.

Unfortunately in all experiments, the concentration of the interesting flavor compound was too low for mass spectral interpretation.

To produce more material necessary for the identification experiments, the volatiles obtained from 10 kg of experiment 5 were combined, concentrated to 6 mL, and fractionated on silica gel. The unknown compound could be strongly detected by GC-sniffing in fraction E, but its concentration was very low. Although the amount collected was enough to give an MS signal, an unequivocal interpretation was not possible because of peak overlapping.

Investigation of Raw Onions. The results of experiment 8 (Table 2) revealed the presence of the unknown compound even in untreated onion. Therefore, in the next steps the investigations were focused on the analysis of raw onions.

Different analytical enrichment methods were used first to prove the presence of the interesting component in raw onions and second to find the best and most effective isolation and enrichment procedure. Furthermore, we hoped to obtain some information on the biogeneration of this molecule.

The isolation and enrichment methods as well as the results obtained by HRGC-O-MS analysis are summarized in Table 3. In experiments 9 and 10 the volatiles were isolated by dynamic headspace enrichment. If air was used for the purging procedure, the unknown compound could be perceived during the subsequent GC-sniffing analysis, whereas, after usage of nitrogen, the interesting target compound was not detectable. Performance of dynamic headspace enrichment of raw onions treated with calcium chloride (experiment 11) inhibited the formation of the unknown molecule even if the volatiles were trapped with air. On

Table 3. Investigation of Raw Onions: Comparison of Different Isolation Techniques

expt	method[a]	HRGC-O[b]	HRGC-MS[c]
9	dynamic headspace enrichment; air	+	−
10	dynamic headspace enrichment; nitrogen	−	−
11	dynamic headspace enrichment; air; $CaCl_2$	−	−
12	SDE	−	−
13	SDE; acidic conditions	−	−
14	vacuum headspace distillation	+	−
15	vacuum headspace distillation; storage of onions	+++	+

[a] Performance see Experimental Procedures. [b] Extracts were analyzed by GC-sniffing: −, 3-mercapto-2-methylpentanol could not be perceived; +, a weak sensory signal of 3-mercapto-2-methylpentanol was perceived; +++, a strong sensory signal of 3-mercapto-2-methylpentanol was perceived. [c] Extracts were analyzed by GC/MS: −, no MS signal; +, weak MS signal.

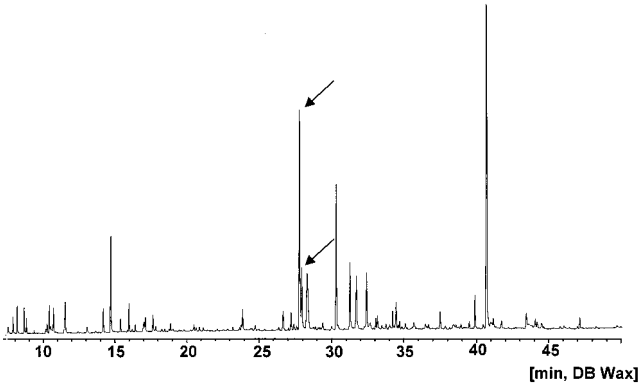

Figure 1. Gas chromatogram of the volatiles of stored raw onions (fraction II).

the basis of these results, it was concluded that enzymes are involved in the formation of the target compound as calcium chloride is known as an enzyme inhibitor (Buttery et al., 1987). In experiments 12 and 13 the volatiles were isolated using SDE extraction. The absence of detectable amounts of the unknown component in experiment 12 might be an effect of enzyme inhibition caused by heating. As addition of hydrochloric acid to the onion water mixture in experiment 13 did not influence this result, the presence of a glycosidic bound form of the molecule could be excluded (Humpf et al., 1991). During HRGC-O-MS analysis of the distillate obtained by vacuum headspace distillation at room temperature (experiment 14), a strong sensory signal and a weak MS signal were detectable. It was therefore concluded that vacuum distillation at room temperature was the most effective method. This technique has already been described as a suitable method for the isolation of fruit volatiles (Idstein et al., 1984; Fischer and Hammerschmidt, 1992; Werkhoff et al., 1998).

Storage of onions at room temperature after they had been cut into cubes increases the yield of the interesting component during the isolation procedure (experiment 15). After enrichment and purification of the distillate by SPE on C-18 reversed phase, high amounts of the target molecule were obtained in the fraction eluted with pentane/diethyl ether (9 + 1, fraction II). The gas chromatogram of this fraction recorded on a DB-Wax capillary is shown in Figure 1.

HRGC-O-MS analysis of fraction II revealed the presence of a second compound in the gas chromatogram having a flavor quality and a mass spectrum similar to

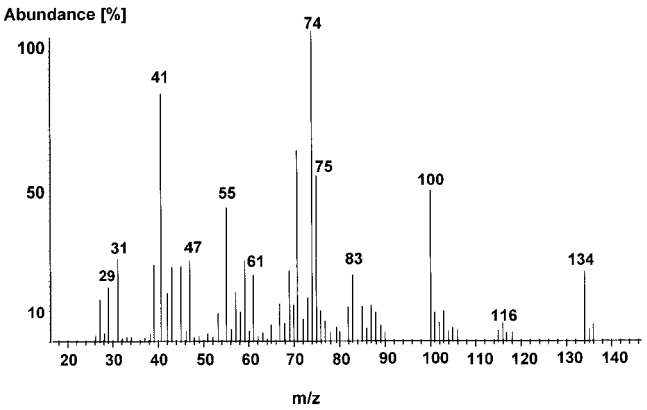

Figure 2. Mass spectrum of 3-mercapto-2-methylpentanol.

Table 4. Retention Times and Distribution of 3-Mercapto-2-methylpentanol Enantiomers in Stored Raw Onions

		distribution (%)	
enantiomer	retention time[a]	enantiomer	diastereomer
2*S*,3*R*	49.09	47	
2*R*,3*S*	49.69	53	4
2*S*,3*R*	49.08	54	
2*R*,3*S*	50.17	46	1

[a] For chromatographic conditions see Experimental Procedures.

those of the compound studied. Comparison of odor strength indicated a weaker sensory signal for the later eluting compound. These results strongly suggested the existence of two isomers.

Identification of 3-Mercapto-2-methylpentanol. The mass spectrum of the unknown compound obtained by HRGC-MS analysis of the volatiles isolated from raw onions is shown in Figure 2. The fragmentation pattern strongly indicated the presence of a thiol group—due to the loss of *m/e* 34—and a hydroxy group—due to the loss of *m/e* 18. To collect more information for an unequivocal structure elucidation, the volatiles from a total of 20 kg of raw onions were isolated by vacuum distillation and solid-phase extraction. Subsequently, one pure diastereoisomer was isolated from the combined SPE fractions II by preparative capillary gas chromatography in a total amount of 10 μg. The purity and the concentration of this isolate were sufficient for ^{1}H NMR measurement.

Interpretation of the NMR and the mass spectrum leads to the general molecular structure of the 3-mercapto-2-methylpentanol, which has not yet been described.

To confirm the proposed structure, 3-mercapto-2-methylpentan-1-ol was synthesized. Thioacetic acid was added by Michael addition to 2-methyl-2-pentenal, and the resulting acetylthio aldehyde was reduced with lithium aluminum hydride to the corresponding alcohol. The two diastereoisomeric 3-mercapto-2-methylpentan-1-ols were obtained after distillation as a light yellow liquid of intense smell.

Distribution of 3-Mercapto-2-methylpentanol Isomers in Raw Onions. Gas chromatography of the synthesized four enantiomers (Sabater Lüntzel et al., 2000) on DB-Wax indicated that 2*R*,3*S* and 2*S*,3*R* isomers having the thiol and methyl group in *anti* configuration coeluted before the 2*R*,3*R* and 2*S*,3*S* isomers in which the two groups have a *syn* configuration. The peak areas in the gas chromatogram of Figure 1 show that the *anti* and the *syn* diastereoisomers of

Table 5. Odor Description and Odor Thresholds of 3-Mercapto-2-methylpentanol and 3-Mercapto-2-methylpentanal

compound	odor description	odor threshold[a] (μg/L of water)
3-mercapto-2-methylpentanol	at 1 ppm in 5% saltwater: sulfuric, burnt gum, sweaty, onion at 0.5 ppb in saltwater: meat broth, sweaty, onion, leek	0.15
3-mercapto-2-methylpentanal	at 1 ppm in 5% saltwater: sulfuric, pungent, meaty, sweaty, onion, roasty at 5 ppb in saltwater: meat broth, cooked meat, roasty, sweaty	0.95

[a] For determination see Experimental Procedures.

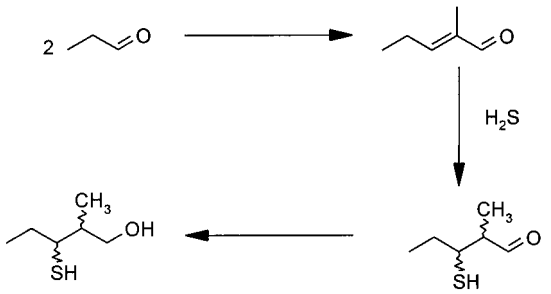

Figure 3. Hypothesis for the formation of 3-mercapto-2-methylpentanol.

3-mercapto-2-methylpentanol are formed in raw onions in a 4:1 ratio.

To determine the distribution of the *anti* and *syn* enantiomers, enantioselective multidimensional gas chromatography was performed. Synthetic enantiomers were used to verify the retention times. The retention time of each enantiomer and their ratios are given in Table 4. The results show that the 2*R*,3*S* and the 2*S*,3*R* enantiomer as well as the 2*R*,3*R* and the 2*S*,3*S* enantiomer are formed in raw onions in a ratio of about 1:1.

Formation of 3-Mercapto-2-methylpentanol in Onions. The investigations of raw onions strongly indicate that enzymes are involved in the formation of 3-mercapto-2-methylpentanol. On the basis of the importance of storage time for the amounts formed, it was assumed that additional nonenzymatic chemical reactions are involved.

One of the first important volatile compounds formed after cutting of onions is propanal (Boelens et al., 1971). Its formation was already suggested by Virtanen in 1967. The flavor precursor *S*-propenylcysteine-*S*-oxide is first transformed to the unstable lachrymatory factor thiopropanal-*S*-oxide (Brodnitz and Pascale, 1970) through the action of a C-S-lyase. Subsequently, this compound rearranges spontaneously to form propanal and sulfur.

On the basis of the fact that propanal is immediately formed after cutting of onions, the pathway for the formation of 3-mercapto-2-methylpentanol shown in Figure 3 is suggested. After propanal is formed in sufficient amounts, 2-methyl-2-pentanal can be formed by aldol condensation. Addition of hydrogen sulfide to 2-methyl-2-pentenal leads to 3-mercapto-2-methylpentanal, which is finally reduced to the 3-mercapto-2-methylpentanol.

The formation of 2-methyl-2-pentenal in raw onions by aldol condensation was already described by Boelens et al. (1971). In addition, these authors could show that the concentrations of 2-methyl-2-pentenal in the headspace of cut onions were considerably higher after 2 h of storage than after 15 min. These results are in good agreement with the fact that higher yields of 3-mercapto-2-methylpentanol could be isolated after storage of raw onions at room temperature than after direct investigation without storage.

Hydrogen sulfide could also be shown as a volatile compound occurring in raw onions (Carson et al., 1961; Boelens et al., 1971). On the basis of the fact that the *anti* diastereoisomer is preferrably formed in raw onions over the *syn* diastereoisomer (which could not be observed during chemical synthesis), it can be assumed that the addition of hydrogen sulfide may be catalyzed by enzymes. Contrary to this, the addition of H_2S seems not to be enantioselective as the ratio of the 2*R*,3*S* and 2*S*,3*R* enantiomers as well as the ratio of the 2*R*,3*R* and 2*S*,3*S* in raw onions is about 1:1.

Propanal and 2-methyl-2-pentenal are known flavor compounds of raw onions, whereas the existence of 3-mercapto-2-methylpentanal was not mentioned in raw onions or other food materials, nor has its chemical structure ever been described before. To prove the presence of 3-mercapto-2-methylpentanal in raw onions, the compound was synthesized by Michael addition of hydrogen sulfide onto 2-methyl-2-pentenal. On the basis of the retention time and the mass spectrum of this new compound, an extract obtained from 2 kg of raw onions was investigated by HRGC-O-MS during which 3-mercapto-2-methylpentanal could be identified. This compound has a very interesting and intense meaty odor quality even at low concentrations.

The reduction of aldehydes to alcohols as postulated in the last step of the formation pathway is a well-known enzyme-catalyzed reaction in nature.

Odor Characteristics of 3-Mercapto-2-methylpentanol and 3-Mercapto-2-methylpentanal. Table 5 summarizes the flavor descriptions of the racemic 3-mercapto-2-methyl-pentanol and of 3-mercapto-2-methylpentanal at different concentrations. The results show that the flavor quality is strongly dependent on the concentration of the compounds. At high concentrations (1 ppm, 10^{-3}g/L!) 3-mercapto-2-methylpentanol causes a very strong and unpleasant odor, which was described as sulfuric, burnt gum-like, sweaty, and onion-like. At lower concentrations (0.5 ppb, 5×10^{-7}g/L) a very pleasant broth-like, slightly sweaty, onion-like, and leek-like flavor quality could be perceived. The odor threshold of the racemic mixture in water is 0.15 ppb.

The sensory impression of 3-mercapto-2-methylpentanal also depends on its concentration. In low amounts (5 ppb) it has a very pleasant meaty and roasty flavor profile, whereas at high concentration (1 ppm) its flavor quality was mainly described as sulfuric, pungent, and meaty. The odor threshold of 3-mercapto-2-methylpentanal is 0.95 ppb.

The data show that these new flavor compounds have high impact flavor qualities as well as high odor strength.

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