Prenyl Alcohol—Source for Odorants in Roasted Coffee

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The identification and characterization of the sulfur-containing flavor components 3-methyl-2-butene-1-thiol, 3-mercapto-3-methylbutanol, and 3-mercapto-3-methylbutyl formate are described. The volatiles were isolated by simultaneous distillation/extraction. After preseparation by means of column chromatography, preparative HPLC, and GC, the aroma extracts were investigated by capillary GC, GC–MS, and simultaneous GC/sniffing. Identification was carried out by retention and spectroscopic data. The proposed structures were confirmed by synthesis. The results of sensory trials indicate that these components may contribute to the flavor of roasted coffee. As the identified components are very likely to be related to each other by their common precursor prenyl alcohol (3-methyl-2-buten-1-ol), the formation pathway was investigated by means of model reactions.

The literature about volatiles in roasted coffee is undoubtedly vast. Nevertheless, little is known about the individual contribution of the hundreds of components to the sensory impression of roasted coffee flavor. In a recent paper, we described the investigation of the character-impact compounds in roasted Colombian coffee (Holscher et al., 1990). Results indicated that only a relatively small number of volatiles (about 60) are of importance for roasted coffee flavor. By means of aroma dilution analysis (Ullrich and Grosch, 1987) the individual contributions of these components to the overall perceptible coffee aroma were estimated. Among these were some sulfur-containing flavor components related to each other by the common precursor prenyl alcohol (I, see Figure 4). In this paper, the details of their identification and sensory characterization are described.

EXPERIMENTAL PROCEDURES

Isolation of Volatiles. The isolation of volatiles from Arabica coffee (medium roast) by simultaneous distillation/extraction (SDE) and the preseparation and enrichment steps for the isolation of 3-methyl-2-butene-1-thiol (II), 3-mercapto-3-methylbutanol (III), and 3-mercapto-3-methylbutyl formate (IV) were carried out as earlier described (Holscher et al., 1990). The identification of compounds II and III required the cleanup of a total amount of 5 kg of roasted coffee and 20 kg in the case of compound IV.

Enrichment of 3-Mercapto-3-methylbutyl Formate. For column chromatography 0.5 mL of aroma concentrate obtained from each 250-g sample of roasted coffee (see above) was transferred onto a water-cooled column (250 mm × 10 mm; 10 °C) filled with 5.3 g of silica gel 60 (60–200 μm, deactivated with 4.7%, g/g, distilled water) and eluted with 50 mL of *n*-pentane/dichloromethane (60/40 v/v). The eluates of a total amount of 20 kg of roasted coffee were combined and concentrated to a volume of 20 mL. Preparative HPLC was performed with a Merck-Hitachi system on a stainless steel column (250 mm × 20 mm) filled with silica gel 100 (5 μm) protected by a self-packed precolumn (25 × 4 mm; silica gel 60, 25–40 μm), and both columns were cooled with a HPLC oven to 10 °C. The mobile phase was *n*-pentane/diethyl ether (95/5 v/v) at a flow rate of 10 mL/min. Twenty-three runs were performed, each using a sample volume of 900 μL; separation was monitored at 254 nm. In each run the eluate was collected during a certain time window (20.2–21.2 min). The subfractions obtained by preparative HPLC were combined and concentrated to about 100 μL according to the method described by Dünges (1979). Three microliters of each subfraction was injected onto a nonpolar thick-film capillary column (see next paragraph). The peak of interest was cryotrapped 25 times by means of a small glass tube cooled with dry ice; about 10–15 μg was obtained. The isolate was eluted with about 200 μL of tetrachloromethane and submitted to ^{1}H NMR spectroscopy. Rechromatography of the isolate on a DB-Wax capillary column showed a purity of the isolated formate of about 94%.

High-Resolution Gas Chromatography (HRGC). HRGC was performed on Carlo Erba gas chromatographs (type 5300 Mega series and 4200) using DB-Wax (60 m × 0.32 mm; film thickness 0.25 μm) and alternatively CP-Sil8-CB columns (50 m × 0.32 mm, film thickness 0.25 μm) in connection with a retention gap (5 m × 0.32 mm). Helium was used as carrier gas at a flow rate of 2–3 mL/min. Injection volume was 0.5–1 μL applied by the "on column" technique. The temperature program was as follows: 35 °C hold for 2 min, 40 °C/min to 60 °C, 2 min isotherm, and finally 2 °C/min to 220 °C. Flame ionization detectors were held at 220 °C. For HRGC/sniffing the same capillary columns mentioned above were used with splitting of the effluent 1:4 for flame ionization detection as well as for simultaneous sensory evaluation by means of a sniffing port and applying the glass-cap-cross technique according to the method of Bretschneider and Werkhoff (1988). Preparative GC for the isolation of compound IV was carried out on a DB-5 column (30 m × 0.32 mm, film thickness 1 μm) programmed from 60 to 240 °C at 3 °C/min in the "split" mode (1:3) and an injector temperature of 220 °C. Retention data were calculated according to the method of Dool and Kratz (1963).

Identification of Volatiles. The volatiles were identified on the basis of their retention data, mass spectral data, and sensory properties compared to those of authentic reference compounds.

Spectroscopic Measurements. For GC–MS, the GC system was connected either to an ion-trap detector (Finnigan MAT ITD 800) or a Finnigan MAT 8222 mass spectrometer. The EI spectra were generated at 70 eV. For chemical ionization (ITD 800), methanol was used as reagent gas. For GC-FTIR, the GC system was connected to a Bruker IFS 48. ^{1}H NMR spectroscopy was performed on a Bruker AC 250-MHz NMR spectrometer in $CDCl_3$ and with tetramethylsilane as internal standard. ^{1}H NMR of the isolated compound IV, obtained by preparative HPLC and GC (see above), was performed on a Varian XL 200-MHz spectrometer (15 700 scans, ca. 13 h).

Determination of Odor and Taste Thresholds. Stock solutions were prepared in acetone and diluted stepwise with twice-distilled water. Sensory evaluation was carried out by five trained testers as a triangular cup-testing according to the method

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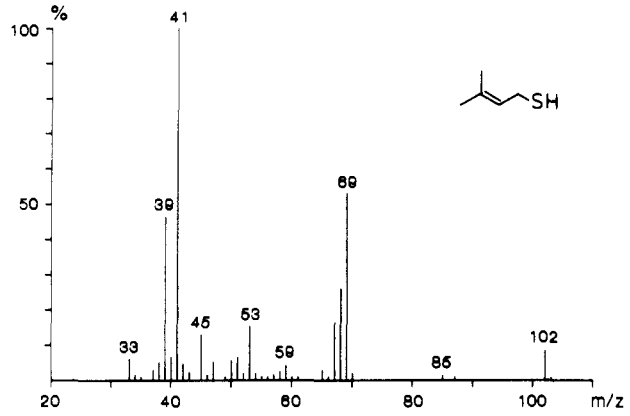


Figure 1. Mass spectrum (EI) of 3-methyl-2-butene-1-thiol.

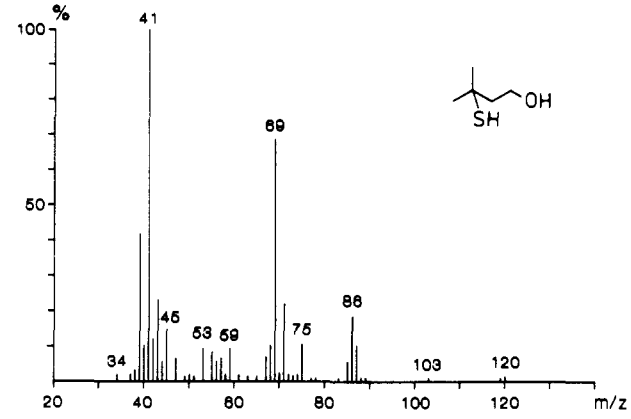


Figure 2. Mass spectrum (EI) of 3-mercapto-3-methylbutanol.

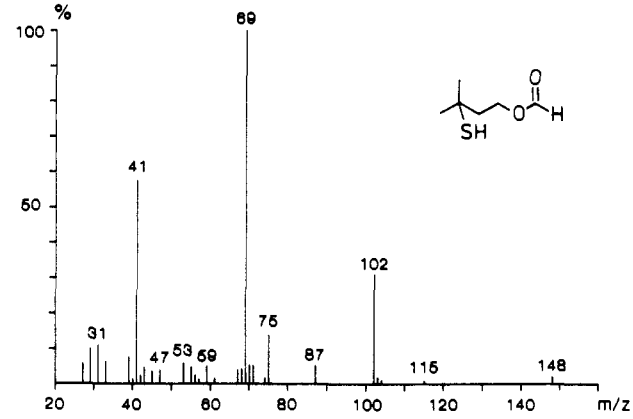


Figure 3. Mass spectrum (EI) of 3-mercapto-3-methylbutyl formate.

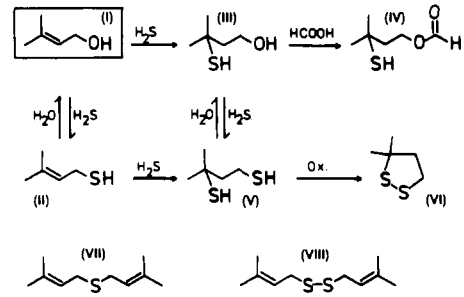


Figure 4. Genesis of sulfur-containing odorants derived from prenyl alcohol [scheme proposed by Tressl et al. (1983) with modifications suggested by the present work].

DIN 10951 (1986), taking twice-distilled water as the reference. The threshold value is defined as a concentration at which the stimulus cannot be identified but a sensory deviation compared to the reference is just perceivable (Teranishi et al., 1987).

Synthesis of 3-Methyl-2-butene-1-thiol. Synthesis was carried out in a modified manner according to the method of Kofod (1955): 0.05 mol of thiourea was dissolved in 9 mL of 6 N hydrochloric acid and cooled to about 10 °C; 0.05 mol of 3-methyl-2-buten-1-ol (prenyl alcohol) was added, and the mixture was briefly heated to boiling and then left at 60 °C overnight. The solution was made alkaline (pH 9.0–10.0) with sodium hydroxide (50% in water, g/g) and held at room temperature for 2 h. Fifty milliliters of water was added, and a steam distillation was carried out until the distillate contained no oily droplets. The thiol was separated from the aqueous phase with 2 × 20 mL of *n*-pentane. The organic layers were combined, dried over anhydrous sodium sulfate, and evaporated. Finally, the thiol was purified by vacuum distillation (0.1 bar, 70 °C) under flushing with nitrogen (yield 63%). For sensory evaluations the thiol was further cleaned by means of preparative GC on a DB-5 column (30 m × 0.53 mm, film thickness 1 μm). The temperature program was a 45 °C hold for 5 min and then the temperature was raised at 6 °C/min to 220 °C.

Spectroscopic Data of 3-Methyl-2-butene-1-thiol. GC-FTIR 833 (=C-H deform.), 1674 (C=C valence), 2975, 2930 cm^{-1} (CH_3 valence, CH valence); MS (ITD 800) see Figure 1; ^1H NMR (250 MHz, $CDCl_3$) δ 1.42 (t, 1 H, SH, $^3J(CH_2)$ = 7.1 Hz), 1.66 (s, 3 H, CH_3), 1.72 (s, 3 H, CH_3), 3.15 (dd, 2 H, CH_2, $^3J(SH)$ = 7.1 Hz, $^3J(=CH)$ = 7.7 Hz), 5.33 (compl m, 1 H, =CH).

Synthesis of 3-Mercapto-3-methylbutanol and 3-Mercapto-3-methylbutyl Formate. Synthesis was carried out according to the method of Stoffelsma and Pijpker (1973). The crude products containing several aroma potent impurities were purified by means of preparative HPLC (see above) and refractometric detection using diethyl ether as mobile phase in the case of the alcohol and *n*-pentane/diethyl ether (95/5 v/v) for the formate. After evaporation of the solvent in a stream of nitrogen, the purity was >99% (GC). The absence of further sensorially important impurities could be proven by GC/sniffing analysis.

Spectroscopic Data of 3-Mercapto-3-methylbutanol. GC-FTIR 1028 (CO valence), 3650 (OH valence), 2972, 2931 cm^{-1} (CH_3 valence, CH valence); MS (ITD 800) see Figure 2; ^1H NMR (250 MHz, $CDCl_3$) δ 1.43 (s, 6 H, CH_3), 1.81 (s, 1 H, SH), 1.89 (t, 2 H, C-2, ^3J(C-1) = 6.6 Hz), 1.93 (s, 1 H, OH), 3.86 (t, 2 H, C-1, ^3J(C-2) = 6.6 Hz).

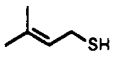
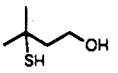
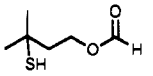
Spectroscopic Data of 3-Mercapto-3-methylbutyl Formate. GC-FTIR 1167 (COC valence), 1743 (C=O valence), 2972, 2931 cm^{-1} (CH_3 valence, CH valence); MS (MAT 8222) see Figure 3; ^1H NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 6 H, CH_3), 1.75 (s, 1 H, SH), 1.97 (t, 2 H, C-2, ^3J(C-1) = 7.2 Hz), 4.39 (dt, 2 H, C-1, ^3J(C-2) = 7.2 Hz, ^4J(formyl H) = 0.8 Hz), 8.06 (s, 1 H, formyl H).

Roast Model Reactions. Two millimoles of cysteine (methionine) was carefully mixed with 50 g of purified sea sand and treated with 2 mmol of prenyl alcohol. The mixture was transferred into a bomb tube, which was tightly closed and held 2 h at 190 °C. After the mixture cooled to room temperature, 500 mL of distilled water was added. The volatiles were extracted by means of SDE (Holscher et al., 1990) and investigated by combined GC–MS/sniffing evaluation subsequently. Blind samples were carried out in the same manner but without the prenyl alcohol.

RESULTS AND DISCUSSION

Tressl et al. (1983) proposed a possible pathway for the formation of roasted coffee volatiles under roasting conditions (Figure 4), but no experimental data were given. One key compound of this pathway is prenyl alcohol (I). Prenyl alcohol is a well-known constituent of roasted coffee volatiles (Stoll et al., 1967) and was also identified in green coffee in amounts of about 0.5 mg/kg (Holscher, 1991). The diphosphate of prenyl alcohol, an isomer of the "active isopren", is a key compound for the biosynthesis of isoprenoids. Under roasting conditions, many kinds of chemical reactions are possible in the whole coffee bean at elevated pressures and temperatures. Sulfur-containing amino acids will liberate hydrogen sulfide, which may react with the double bond of the prenyl alcohol according to

Table I. Retention Data and Sensory Characteristics of Newly Identified Roasted Coffee Odorants[a]

compound			
retention index (DB-Wax)	1098	1502	1650
retention index (CP-Sil8)	821	1023	970
odor description			
concentrated (pure substance)	pungent, leek-like	sweet, soup-like	sweaty, fruity
diluted (ca. 0.1 mg/L)	foxy, animal-like, skunky	soup-like, cooked meal, spicy	sweaty, black currant-like, catty
odor threshold, μg/L	0.0002–0.0004	2–6	0.002–0.005
taste threshold, μg/L	0.0002–0.0003	8–10	0.005–0.008

[a] Highest and lowest values found by the testers are given for each compound as micrograms per liter of twice-distilled water.

the rule of Markovnikov to form 3-mercapto-3-methylbutanol (III). On the other hand, the hydroxy group of the prenyl alcohol may be substituted to form 3-methyl-2-butene-1-thiol (II). 2-Methylbutane-2,4-dithiol (V), 3,3-dimethyl-1,1-dithiolane (VI), bis(3-methyl-2-butene) sulfide (VII), and bis(3-methyl-2-butene) disulfide (VIII) are therefore hypothetical compounds arising from the precursor prenyl alcohol. Compound VI has been identified in roasted coffee earlier (Silwar, 1982). According to Holscher et al. (1990), the contribution of the latter four compounds to the flavor of roasted coffee seems to be very low. Compounds II and III were newly identified and are of greater importance for the sensorially perceptible aroma of roasted coffee. In addition to compounds predicted by Tressl et al. (1983), 3-mercapto-3-methylbutyl formate (IV) could be identified as an aroma potent constituent of roasted coffee flavor. The spectroscopic data of the authentic compound isolated from roasted coffee were identical compared to those of the synthetic reference substance especially with regard to the NMR data. Compounds III and IV have not been identified in other kinds of aromas arising from natural sources yet. Table I makes plain their remarkable sensory potencies. Odor thresholds down to the picograms per liter level have been recognized in the case of 3-methyl-2-butene-1-thiol. The mass spectrum of the peak with the odor description "foxy" was identical to that of the synthetic reference substance and literature data (Kattein et al., 1988). The pure substance smells pungent or leek-like, whereas a penetrating animal-like, "foxy" or even "skunky" (Soeltoft, 1988), odor dominates at low concentrations. Kuroiwa and Hashimoto (1961) found that 3-methyl-2-butene-1-thiol causes off-flavors in certain types of beers. 3-Mercapto-3-methylbutanol is not as aroma potent as the thiol with respect to its threshold values. Esterification with formic acid gives 3-mercapto-3-methylbutyl formate, a compound having a much lower odor threshold than the alcohol. The latter two mercapto compounds were used for artificial aromatizing of various kinds of foods (Stoffelsma and Pijpker, 1973). At low concentrations, the formate possesses a characteristic penetrating sweaty odor, generally described as "catty" due to its similarity to the excrements of cats (Pearce, 1967). Catty-smelling compounds belong to the most potent odorants known so far. According to Polak et al. (1988), their essential structure element is the tertiary amylmercaptan group; the functional group influences the aroma quality. The polar hydroxy group in 3-mercapto-3-methylbutanol causes a total loss of the catty note and a conversion of its odor properties into a sweet, cooked meal-like or spicy aroma impression.

On the basis of the hypothetical pathway formulated in Figure 4, roast model reactions of prenyl alcohol and sulfur-containing amino acids were carried out, thus proving that compounds like 3-methyl-2-butene-1-thiol and 3-mercapto-3-methylbutanol are principally formed under pyrolytic conditions (Figure 5). In the cysteine as well as in the methionine system, both compounds could be identified as the most aroma-intense ones, besides numerous volatiles of much lower odor intensity. On the other hand, addition of small amounts of formic acid to the model reactions did not lead to the formation of 3-mercapto-3-methylbutyl formate. It can be assumed that its formation only is possible under specific conditions occurring in the whole coffee bean and that the biogenic precursor prenyl diphosphate may be more reactive. Overall results indicate that the formation of volatiles derived from the precursor prenyl alcohol seems to be an important pathway for the formation of roasted coffee flavor in addition to the well-known Strecker degradation and the Maillard reaction.

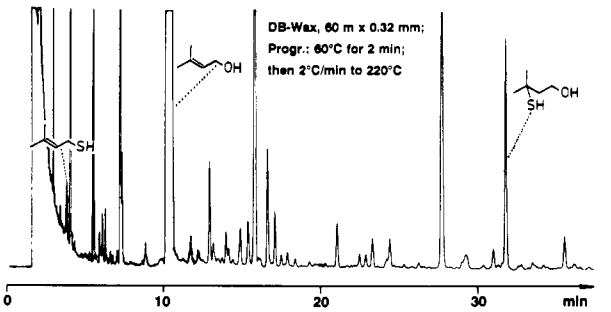


Figure 5. GC separation of volatiles formed during cysteine/prenyl alcohol roast model reactions.

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LITERATURE CITED

Bretschneider, W.; Werkhoff, P. Progress in All-Glass Stream Splitting Systems in Capillary Gas Chromatography. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1988**, *11*, 543–546.

DIN 10 951. Deutsche Industrie Norm, Dreiecksprüfung, 1986.

Dool, H.; Kratz, P. A Generalization of the Retention Index System Including Linear Temperature Programmed Gas-Liquid Partition Chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.

Dünges, W. *Prächromatographische Mikromethoden*; Hüthig Verlag: Heidelberg, 1979.

Holscher, W. Thesis, University of Hamburg, FRG, 1991.

Holscher, W.; Vitzthum, O. G.; Steinhart, H. Identification and Sensorial Evaluation of Aroma-Impact-Compounds in Roasted Colombian Coffee. *Cafe, Cacao, The* **1990**, *34*, 205–212.

Kattein, U.; Miedaner, H.; Narziss, L. *Monatsschr. Brauwiss.* **1988**, *41*, 205–208.

Kofod, H. 2-Furfurylmercaptan. *Org. Synth.* **1955**, *35*, 66–68.

Kuroiwa, Y.; Hashimoto, N. Composition of Sunstruck Flavor Substance and Mechanism of Its Evolution. *Proc. Am. Soc. Brew. Chem.* **1961**, 28–36.

Pearce, T. J. P.; Peacock, J. M.; Aylward, F.; Haisman, D. R. *Chem. Ind.* **1967**, 1562–1563.

Polak, E.; Fetison, G.; Fombon, A. M.; Skalli, A. J. Structure-Odor Relationships for "Catty"-Smelling Mercapto Compounds in Humans. *J. Agric. Food Chem.* **1988**, *36*, 355–359.
Silwar, R. Thesis, Technical University of Berlin, FRG, 1982.
Soeltoft, M. Flavor Active Sulfur Compounds in Beer. *Brygmesteren* **1988**, *45*, 18–20.
Stoffelsma, J.; Pijpker, J. DE Patent 23 16 465 C2, 1973.
Stoll, M.; Winter, M.; Gautschi, F.; Flament, I.; Willhalm, B. *Helv. Chim. Acta* **1967**, *50*, 628–694.
Teranishi, R.; Buttery, R. G.; Schamp, N. The Significance of Low Threshold Odor Compounds in Aroma Research. In *Flavour Science and Technology*; Martens, M., Dalen, G. A., Russwurm, H., Eds.; Wiley: New York, 1987; pp 515–527.
Tressl, R.; Holzer, M.; Kamperschroer, H. *Colloq. Sci. Int. Cafe* **1983**, *10*, 279–292.
Ullrich, F.; Grosch, W. Identification of the Most Intensive Volatile Flavor Compounds Formed During Autoxidation of Linoleic Acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.

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Registry No. I, 556-82-1; II, 5287-45-6; III, 34300-94-2; IV, 50746-10-6; thiourea, 62-56-6; cysteine, 52-90-4; methionine, 63-68-3.