Detection of Odor-Active Ethenylalkylpyrazines in Roasted Coffee

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In roasted Robusta coffee, two potent earthy smelling compounds were identified as 2-ethenyl-3,5-dimethylpyrazine and 2-ethenyl-3-ethyl-5-methylpyrazine by comparison of gas chromatography and mass spectrometry data with those of the corresponding reference substances. The odor threshold values (0.014 ng/L, air) of the new pyrazines were as low as those of 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine. 3-Ethenyl-2-ethyl-5-methylpyrazine was also detected in coffee, and its odor threshold value was 8000 times higher than that of the 2-ethenyl-3-ethyl-5-methylpyrazine. After addition of HBr, the two ethenylethylmethylpyrazine isomers were separated by capillary gas chromatography. The results indicate the presence of the two isomers in a 1:1 ratio in coffee.

Keywords: *Roasted coffee; aroma; 2-ethenyl-3,5-dimethylpyrazine; 2-ethenyl-3-ethyl-5-methylpyrazine; 3-ethenyl-2-ethyl-5-methylpyrazine; detection; synthesis; odor threshold*

INTRODUCTION

Recently, aroma models were studied to verify the character-impact odorants of brews prepared from roasted Arabica (*Coffea arabica*) and Robusta coffees (*Coffea canephora* var. Robusta; Semmelroch and Grosch, 1996). The models consisted of aqueous solutions of 23 compounds that had been evaluated as potent odorants by aroma extract dilution analysis (AEDA), gas chromatography-olfactometry of headspace samples (GCO-H), and quantitative measurements (Blank et al., 1992; Semmelroch and Grosch, 1995, 1996). The overall odors of the models were clearly coffee-like. However, there were some differences between the odor profile of the model and that of the corresponding brew. In particular, the intensity of the earthy/musty odor note was lower in the model (Semmelroch and Grosch, 1996).

AEDA and GCO-H analyses of coffee brews indicated that 2-ethyl-3,5-dimethylpyrazine (**1**) and 2,3-diethyl-5-methylpyrazine (**2**) were the most potent odorants with an earthy smell (Blank et al., 1992; Semmelroch and Grosch, 1995), and hence they were considered to be constituents of the aroma models (Semmelroch and Grosch, 1996). In addition to these pyrazines, AEDA and GCO-H analyses revealed two earthy smelling compounds that appeared on an apolar GC capillary; that is, SE-54 at retention indexes (RI) of 1103 and 1182, respectively (Blank et al., 1992; Semmelroch and Grosch, 1995). The odor quality of the unknown odorants agreed with those of **1** and **2**, so we suggested that the unknown compounds were also alkylated pyrazines.

3-Ethenyl-2,5-dimethylpyrazine, an ethenyldimethylpyrazine isomer and an ethenylmethylpyrazine isomer, have been tentatively identified on the basis of mass spectral data in potato chips (Buttery et al., 1971), roasted coffee (Baltes and Bochmann, 1987), and shallow-fried beef (Specht and Baltes, 1994). Furthermore, Shibamoto and Bernhard (1977) have detected alkylated pyrazines bearing an ethenyl group as Maillard reaction products of glucose-ammonia model systems. These results, in combination with the detection of **1** and **2** as potent odorants, led to the postulation that similarly structured ethenyl pyrazines might occur in roasted coffee [e.g., 2-ethenyl-3,5-dimethylpyrazine (**3**), 2-ethenyl-3-ethyl-5-methylpyrazine (**4**), and 3-ethenyl-2-ethyl-5-methylpyrazine (**5**)]. To prove this hypothesis, the pyrazines **3** to **5** were synthesized, and their chromatographic, spectroscopic, and sensory properties were determined and then compared with those of the unknown earthy smelling odorants of Robusta coffee.

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EXPERIMENTAL PROCEDURES

Coffee. The Robusta coffee was from Indonesia. The beans were medium roasted (3 min) with a Jetzone roaster. The coffee beans were packed in 300-g portions that were sealed and stored at −35 °C. Before extraction, the beans were frozen in liquid nitrogen, ground with an ultracentrifugation mill (type ZM1; Retsch, Haan), and sieved (diameter of the pores: 2 mm).

Chemicals. The following compounds were obtained from Aldrich (Steinheim, Germany): 2,6-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine (**2**), vinylmagnesium bromide (1 mol/L) in tetrahydrofurane (THF), *N*-bromosuccinimide (NBS), dicyclohexylamine, and α,α′-azodiisobutyronitrile. Thin-layer chromatography (TLC) plates coated with silica gel 60 F254 (0.25 mm thickness) and with a zone for the concentration of the applied sample were from Merck (Darmstadt, Germany).

Synthesis. *2-Ethenyl-3,5-dimethylpyrazine (**3**).* The synthetic route followed the indications of Tas and Kleipool (1974) for trialkylated pyrazines. In a three-necked flask (100 mL), fitted with a dropping funnel and an argon inlet, was placed a solution of vinylmagnesium bromide (1.31 g, 10 mmol) in THF (10 mL). While stirring, the solution was warmed to 40 °C, and then a solution of 2,6-dimethylpyrazine (1.08 g, 10 mmol) in THF (20 mL) was added to the stirred Grignard reagent over a 30 min period via the dropping funnel. The mixture was refluxed with stirring for 2 h. The reaction mixture was then cooled to room temperature, and water (20 mL) was added in a dropwise manner. The orange emulsion formed was extracted with diethyl ether (3 × 50 mL). After drying over Na_2SO_4, the ethereal solution was concentrated to 5 mL by distilling off the solvent. Pyrazine **3** was purified by TLC on silica gel 60. After elution with pentane:diethyl ether (7:3, v/v), pyrazine **3** was visualized by UV (R_f value, 0.39) and then extracted with diethyl ether; yield 5%; electron impact−mass spectra (EI-MS) of **3**, 133 (100%), 134 (M^+, 60%), 54 (50%), 39 (30%), 42 (28%); ^{1}H NMR ($CDCl_3$, 360 MHz; assignment of the hydrogen atoms refers to formula of **3**) δ: 2.52 (s, 3H, H-9/10), 2.58 (s, 3H, H-9/10), 5.54 (dd, J_{8a-8b} = 1.8 Hz, J_{8a-7} = 10.8 Hz, $1H_a$, H-8), 6.35 (dd, J_{8b-8a} = 1.8 Hz,

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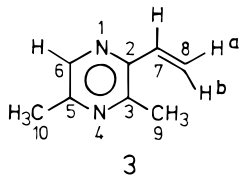

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J_{8b-7} = 17.0 Hz, $1H_b$, H-8), 6.95 (dd, J_{7-8a} = 10.8 Hz, J_{7-8b} = 17.0 Hz, 1H, H-7), 8.26 (s, 1H, H-6).

*2-(1-Bromoethyl)-3-ethyl-5-methylpyrazine (**6**) and 3-(1-Bromoethyl)-2-ethyl-5-methylpyrazine (**7**) (cf. Figure 1).* A stirred solution of pyrazine **2** (3.0 g, 20 mmol), NBS (3.56 g, 20 mmol), and α,α′-azodiisobutyronitrile (40 mg) in carbon tetrachloride (40 mL) was refluxed. After formation of a yellow color, thus indicating the beginning of the reaction, the mixture was refluxed for 1 h. Then the mixture was cooled, and the succinimide produced from NBS was filtered off. After concentration to 10 mL by distilling off the solvent, the reaction products were separated by TLC on silica gel 60 with pentane:diethyl ether (7:3, v/v) as eluent. Seven zones (R_f 0.15, 0.32, 0.52, 0.65, 0.84, 0.93, 1.0) were located on the plate by UV light. Each zone was extracted from the plate with diethyl ether and analyzed by high-resolution gas chromatography−mass spectrometry (HRGC-MS). The mixture of **6** and **7** was detected in the fourth zone (R_f 0.65) and the yield of both isomers was 1.2 g (5.2 mmol). This fraction was rechromatographed by TLC on silica gel 60, with toluene:ethyl acetate (97:3, v/v) as eluent. Each of the three zones (R_f 0.17, 0.25, 0.62), located by UV light, was analyzed by HRGC-MS. The mixture of **6** and **7** was detected in the zone of R_f 0.25 and then extracted from the plate with diethyl ether. The yield of both isomers was 0.8 g (3.6 mmol). Pyrazines **6** and **7** were separated by reversed-phase high-performance liquid chromatography (RP-HPLC). The extract, in portions of 50 μL, was applied onto a stainless steel column (25 × 0.92 cm) packed with Shandon RP 18 (100 Å, 5 μm; Shandon Products, Astmoor, U.K.). Elution (rate, 6 mL/min) was performed with acetonitrile:water (3:7, v/v), and the effluent of the column was monitored at 220 nm. The two peaks appearing in the chromatogram were collected separately. The material obtained in several runs was pooled until the volume of the effluent per peak amounted to 100 mL. Each effluent was diluted with water (900 mL) and then extracted with diethyl ether (5 × 100 mL). The combined ethereal solutions were dried over Na_2SO_4, and the solvent was distilled off. The residue (∼50 mg, 0.2 mmol of each isomer) was dissolved in $CDCl_3$ (0.5 mL), and then the solvent was evaporated. This treatment was repeated, and finally the residue was dissolved in $CDCl_3$ (0.5 mL) for nuclear magnetic resonance (NMR) measurements: ^{1}H-NMR ($CDCl_3$, 500 MHz) spectra of **6** and **7** (assignment of the hydrogen atoms refers to Figure 1): pyrazine **6**: δ 1.27 (t-like, J_{10-9} = 7.6 Hz, 3H, H-10), 2.03 (d, J_{8-7} = 6.8 Hz, 3H, H-8), 2.48 (s, 3H, H-11), 2.85 (q-like, J_{9-10} = 7.6 Hz, 2H, H-9), 5.35 (q, J_{7-8} = 6.7 Hz, 1H, H-7), 8.25 (s, 1H, H-6); pyrazine **7**: δ 1.28 (t-like, J_{8-7} = 7.6 Hz, 3H, H-8), 2.03 (d, J_{10-9} = 6.7 Hz, 3H, H-10), 2.48 (s, 3H, H-11), 2.88 (q-like, J_{7-8} = 7.5 Hz, 2H, H-7), 5.33 (q, J_{9-10} = 6.7 Hz, 1H, H-9), 8.25 (s, 1H, H-6). The EI-MS of pyrazines **6** and **7** were identical: 149 (100%), 150 (49%), 133 (38%), 39 (36%), 134 (27%), 54 (20%), 147 (18%), 121 (16%), 228 ($M^+ - 2$, 14%), 230 (M^+, 14%). The chemical ionization-mass spectra (CI-MS) of **6** and **7** were 149 (100%), 231 (M^+, 48%), 229 ($M^+ - 2$, 45%).

*2-Ethenyl-3-ethyl-5-methylpyrazine (**4**).* 2-(1-Bromoethyl)-3-ethyl-5-methylpyrazine (**6**, 50 mg) was dissolved in diethyl ether (2 mL) and, after addition of dicyclohexylamine (100 mg), the ether was evaporated. The residue was heated with stirring and refluxed for 5 min at 140 °C. After cooling, the residue was treated with diethyl ether (10 mL), and the solution obtained was filtered. Pyrazine **4** was purified by TLC on silica gel 60, with pentane:diethyl ether (7:3, v/v) as the developing solvent. After location at R_f 0.55, pyrazine **4** was extracted from the plate with diethyl ether (20 mL). 3-Ethenyl-2-ethyl-5-methylpyrazine (**5**) was prepared from 3-(1-bromoethyl)-2-ethyl-5-methylpyraine (**7**) by the procedure reported for **4**. The yield of each isomer amounted to 0.5 mg (3

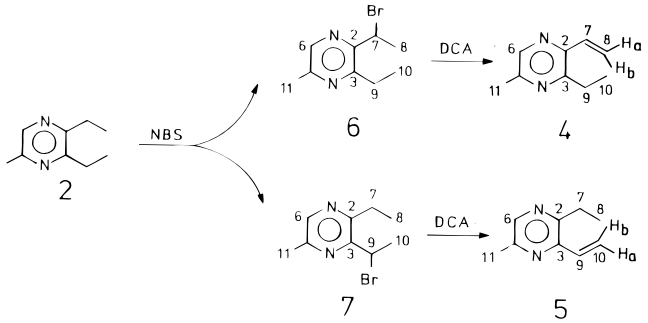

Figure 1. Synthetic route used for the preparation of 2-ethenyl-3-ethyl-5-methylpyrazine (**4**) and 3-ethenyl-2-ethyl-5-methylpyrazine (**5**) (NBS, *N*-bromosuccinimide; DCA, dicyclohexylamine).

μmol). The EI-MS of **4** and **5** are displayed in Figure 2. The ^{1}H NMR ($CDCl_3$, 360 MHz) spectra of **4** and **5** (assignment of the hydrogen atoms refer to Figure 1) were identical: δ 1.30 (t-like, J_{10-9} = 7.6 Hz, 3H, H-10), 2.50 (s, 3H, H-11), 2.77 (q-like, J_{9-10} = 7.6 Hz, 2H, H-9), 5.45 (dd, J_{8d-8b} = 1.1 Hz, J_{8a-7} = 10.7 Hz, $1H_a$, H-8), 6.20 (dd, J_{8b-8a} = 1.1 Hz, J_{8b-7} = 17.7 Hz, $1H_b$, H-8), 6.71 (dd, J_{7-8a} = 10.7 Hz, J_{7-8b} = 17.7 Hz, 1H, H-7), 8.27 (s, 1H, H-6).

Isolation of 3, 4, and 5 from Coffee. Ground coffee (25 g) was stirred for 3 h with diethyl ether (500 mL) saturated with water. The suspension was filtered, and the residue was extracted for 16 h with the same solvent. After filtration, the ethereal extracts were combined and then concentrated to 100 mL by distilling off the solvent on a Vigreux column (100 × 2 cm) at 40 °C. The volatiles and the solvent were distilled under reduced pressure (5 mPa) at 70 °C (Semmelroch and Grosch, 1996). The condensate was freed from the volatile acids by extraction with aqueous $NaHCO_3$ (0.5 mol/L, 2 × 100 mL), dried over Na_2SO_4, and then concentrated to 2 mL by distilling off the solvent on a Vigreux column (40 × 1 cm). This concentrate was separated by TLC on silica gel 60, with pentane:diethyl ether (7:3, v/v) as the eluent. The zones R_f 0.30−0.40 (pyrazine **3**) and R_f 0.50−0.60 (pyrazines **4** and **5**) were extracted with diethyl ether. Each extract was concentrated to 0.1 mL and analyzed by HRGC-MS. To differentiate between pyrazine **4** and **5**, the extract (1 mL), containing the substances of the TLC zone appearing at R_f 0.50−0.60, was diluted with aqueous HBr (1 mol/L, 5 mL). The reaction mixture was stirred for 30 min at room temperature, then its pH was shifted to >10 by the addition of aqueous Na_2CO_3 (0.5 mol/L), and finally the pyrazines **6** and **7** formed were extracted with diethyl ether (10 mL). After filtration, the ethereal solution of the pyrazines **6** and **7** was dried over Na_2SO_4 and then concentrated to 0.1 mL.

HRGC-MS Analysis. HRGC analysis of the pyrazines was performed with a Carlo Erba gas chromatograph (type 4200; Carlo Erba; Hofheim, Germany). A fused silica capillary SE-54 (30 m × 0.32 mm, 0.25 μm film thickness), supplied from Chrompack (Frankfurt a.M., Germany), was used. After application of the sample (0.5 μL) by the on-column injection technique at 40 °C, the temperature of the capillary was held for 2 min at 40 °C, then raised at 10 °C/min to 50 °C, held isothermal for 2 min, then raised at 6 °C/min to 180 °C, and finally raised at 20 °C/min to 250 °C. The latter temperature was held for 15 min before the oven was cooled. The flow of the carrier gas helium was 2.0 mL/min. Retention data of the compounds are presented as retention indices (RI) that were calculated from the retention times of alkanes with a program for cubic spline interpolation (Halang et al., 1978).

MS analyses were performed on an MS 8230 (Finnigan MAT, Bremen, Germany) in tandem with the SE-54 capillary just described. The conditions used for HRGC were the same as already described. EI-MS were generated at 70 eV and CI-MS were generated at 175 eV, with isobutane as reagent gas. Mass chromatograms were recorded in the CI mode.

NMR Spectroscopy. NMR spectra were recorded with an AMX 500 (Bruker, Karlsruhe, Germany). Carbon-13 spectra (WALTZ 16 decoupled) at 125.77 MHz and proton spectra at

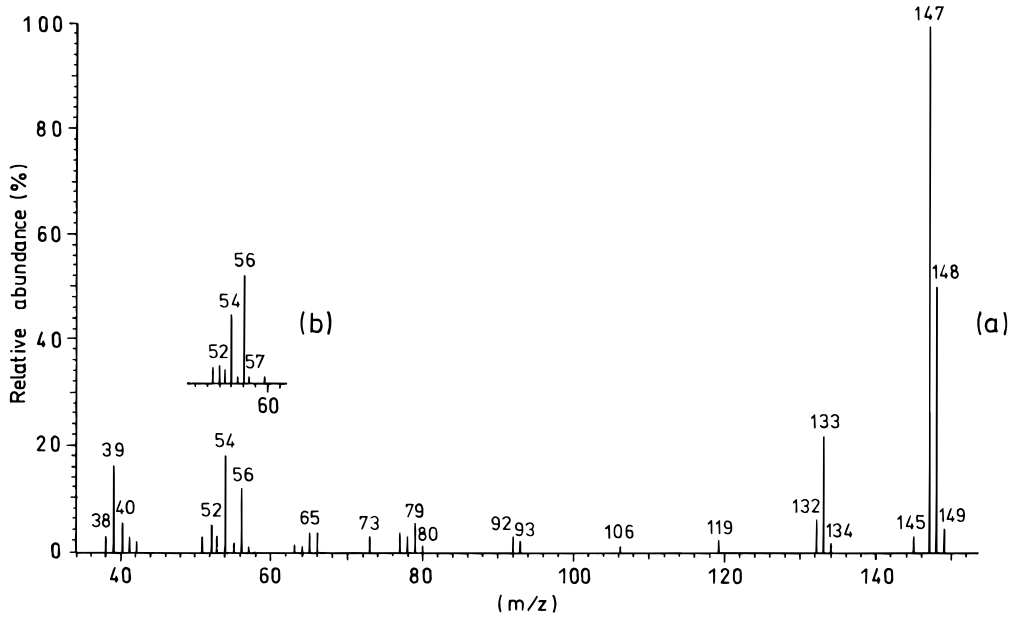

Figure 2. EI-MS of (a) 2-ethenyl-3-ethyl-5-methylpyrazine (**4**) and (b) 3-ethenyl-2-ethyl-5-methylpyrazine (**5**).

Table 1. Odor Threshold Values of Earthy/Musty Smelling Pyrazines in Air

compound	threshold[a] (ng/L)
2-ethyl-3,5-dimethylpyrazine (**1**)	0.007−0.014 (0.011)
2,3-diethyl-5-methylpyrazine (**2**)	0.009−0.018 (0.014)
2-ethenyl-3,5-dimethylpyrazine (**3**)	0.008−0.016 (0.012)
2-ethenyl-3-ethyl-5-methylpyrazine (**4**)	0.009−0.018 (0.014)
3-ethenyl-2-ethyl-5-methylpyrazine (**5**)	73−146 (109.5)

[a] The range was established by the lowest and the highest value found by two judges. The reference substance for the calculation of the values was (*E*)-2-decenal, which has an odor threshold in air of 2.7 ng/L (Boelens and Van Gemert, 1986). The mean odor threshold value is given in parentheses.

500.13 MHz were recorded under standard conditions. The ^{1}H-detected heteronuclear multiple bond correlation with low pass J-filter was acquired with the pulse sequence described by Bax and Summers (1986) and applying the following parameters: spectral width in ^{1}H NMR of 5150 Hz; spectral width in ^{13}C NMR of 86 400 Hz; 4K real increments in F2 and 512 real increments in F1; relaxation delay, D1 = 1.00 s; evolution delay, 50 ms; eight scans per increment; processing with a squared cosine bell as window function in both dimensions; time proportional phase increment in F1; magnitude calculation in F2. The sample was not rotated to suppress t1-noise.

The ^{1}H NMR spectra of pyrazines **3**, **4**, and **5** as well as the ^{13}C DEPT (distorsionless enhancement by polarization transfer) spectra of pyrazines **6** and **7** were measured on an AM 360 MHz spectrometer (Bruker, Karlsruhe, Germany). A 135° selection pulse was used for the ^{13}C DEPT spectra.

Odor Threshold Values. The values in air were determined by GCO with (*E*)-2-decenal as internal standard (Ullrich and Grosch, 1987; Schieberle and Grosch, 1988). HRGC was performed on the capillary SE-54 as already described.

RESULTS

Pyrazine **3** was synthesized, and its structure was corroborated by ^{1}H NMR measurements. The compound smelled earthy. Its odor threshold in air was as low as those of pyrazines **1** and **2** (Table 1). The values found for the latter two pyrazines agreed with the corresponding data reported earlier (Cerny, 1993; Cerny and Grosch, 1994).

The precursors of the pyrazines **4** and **5** were synthesized by bromination of pyrazine **2** (Figure 1). After purification by TLC, the two isomers **6** and **7** were separated by RP-HPLC (baseline resolution) and then analyzed by NMR measurements. ^{1}H,^{13}C correlation experiments, the results of which are summarized in Table 2, were suitable to differentiate pyrazine **6** from **7**. The structure of pyrazine **6**, which eluted before **7** in the RP-HPLC system used here, was established by the vicinal coupling of carbon 2 (δ 150.3) with the three protons (δ 2.03) at carbon 8 and by the coupling of carbon 3 (δ 154.2) with the three protons (δ 1.27) at carbon 10. The reverse was found for pyrazine **7**; carbon 2 (δ 151.6) coupled with the three protons (δ 1.28) at carbon 8 and carbon 3 (δ 152.5) with the three protons (δ 2.03) at carbon 10.

Table 2. Assignment of ^{13}C NMR Signals ($CDCl_3$) of Pyrazines 6 and 7[a]

C atom[b]	δ (ppm)	DEPT analysis	heteronuclear ^{1}H, ^{13}C multiple-quantum coherence via $^{2,3}J$ (C, H)
			Pyrazine **6**
2	150.3	C	^2J (H-C7); ^3J (H-C6); ^3J (3H-C8); ^3J (2H-C9)
3	154.2	C	^2J (2H-C9); ^3J (H-C7); ^3J (3H-C10)
5	152.3	C	^2J (H-C6); ^2J (3H-C11)
6	141.6	CH	^3J (3H-C11)
7	44.3	CH	^2J (3H-C8)
8	23.6	CH_3	^2J (H-C7)
9	27.2	CH_2	^2J (3H-C10)
10	13.2	CH_3	^2J (2H-C9)
11	21.1	CH_3	
			Pyrazine **7**
2	151.6	C	^2J (2H-C7); ^3J (H-C6); ^3J (3H-C8); ^3J (H-C9)
3	152.5	C	^2J (H-C9); ^3J (2H-C7); ^3J (3H-C10)
5	151.5	C	^2J (H-C6); ^2J (3H-C11)
6	141.9	CH	^3J (3H-C11)
7	26.4	CH_2	^2J (3H-C8)
8	12.9	CH_3	^2J (2H-C7)
9	44.3	CH	^2J (3H-C10)
10	23.2	CH_3	^2J (H-C9)
11	21.1	CH_3	

[a] Observed heteronuclear ^{1}H,^{13}C connectivities in 6 and **7** by 135° DEPT analysis and inverse ^{1}H,^{13}C multiple quantum coherence experiments. The signal assignment was confirmed by heteronuclear ^{1}H,^{13}C multiple-quantum coherence measurements as detailed by Hofmann et al. (1995). [b] Assignment of the carbon atoms refers to Figure 1.

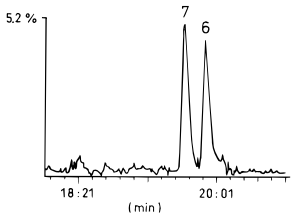

Figure 3. Mass chromatograms (cutting) of a fraction obtained from Robusta coffee after treatment with HBr, showing detection of pyrazines **6** and **7**.

Pyrazines **4** and **5** were prepared by dehydrobromination of the pyrazines **6** and **7** (Figure 1). Their EI-MS (Figure 2) were almost identical; only the ratio of the relative abundances of the fragment ions at *m/z* 54 and *m/z* 56 were somewhat different. In the EI-MS of **4**, the relative abundance of *m/z* 54 was slightly greater than that of *m/z* 56 (Figure 2a), whereas the reverse was found for pyrazine **5** (Figure 2b). The odor threshold of pyrazine **5** in air was 8000 times higher than that of **4** smelling earthy at a threshold which was as low as that of the pyrazines **1** to **3** (Table 1). This difference in the odor threshold values of alkylpyrazine isomers is not uncommon; for example, the threshold values of **1** and 2-ethyl-5,6-dimethylpyrazine differ by a factor of $>10^4$ (Cerny and Grosch, 1994).

An extract containing the volatiles of a Robusta coffee sample was separated by TLC to detect pyrazines **3** and **4**. Two fractions, which moved at the same rate as authentic samples of **3** and **4**, were separately extracted from the TLC plate and then analyzed by HRGC-MS. Pyrazine **3** was identified in the coffee extract by cochromatography with the reference substance (RI 1102 on capillary SE-54) and by the agreement of the EI-MS with that of the reference.

The TLC fraction containing pyrazines **4** and **5** was also analyzed by HRGC-MS. The mass chromatogram, which was recorded for the protonated molecular ion of the two pyrazines at *m/z* 149, showed only a single peak at RI 1180. The EI-MS of this peak was identical with that shown in Figure 3 for pyrazines **4** and **5**.

As it was not possible to separate **4** and **5** by HRGC, the fraction of coffee volatiles containing these pyrazines was treated with hydrogen bromide, and the **6** and **7** formed were analyzed by HRGC-MS. The mass chromatogram that was recorded for the base ion at *m/z* 149 indicated two peaks (Figure 3) that were identified on the basis of their EI-MS as pyrazines **7** and **6**. The elution sequence **6** after **7** in HRGC analysis was reverse in order with that in RP-HPLC. As the areas of the two peaks were nearly of equal size (Figure 3), it was concluded that equal amounts of pyrazines **4** and **5**, the precursors of **6** and **7**, were present in the coffee sample.

Analysis of roasted Arabica coffee indicated that pyrazines **3** and **4** also occur in this coffee variety. The concentrations found for **3** and **4** in Arabica coffee from Colombia amounted to 53 and 14 μg/kg, respectively (Grosch et al., 1996). Pyrazine **4** was found, in addition, in French fries (Wagner and Grosch, 1996).

3-Ethenyl-2,5-dimethylpyrazine, which has been tentatively identified in potato chips (Buttery et al., 1971), was not a potent odorant; its odor threshold in air was 7×10^4 times higher than that of its isomer **3** (unpublished results).

DISCUSSION

Pyrazines **3** and **4** were detected as additional earthy smelling volatiles of roasted coffee. Their formation during the roasting process can be explained as a side reaction on the pathway to pyrazines **1** and **2**. The amino acid alanine and 2-oxopropanal, a breakdown product of glucose and fructose (Hodge, 1967; Weenen and Tjan, 1992), are the precursors of **1** (Cerny and Grosch, 1994). A Strecker degradation reaction of the precursors yields aminoacetone, 2-aminopropanal, and acetaldehyde. According to a mechanism that was proposed by Shibamoto et al. (1979) and recently confirmed by labeling experiments (Amrani-Hemaimi et al., 1995), pyrazine **1** is formed by the condensation of aminoacetone with 2-aminopropanal. 2-(1-Hydroxyethyl)-3,5-dimethyldihydropyrazine (**8** in Figure 4) has been proposed as an intermediate on the reaction route to pyrazine **1** (Grosch and Cerny, 1994). We suggest that pyrazine **3** is also formed from **8** by dehydration and oxidation (Figure 4).

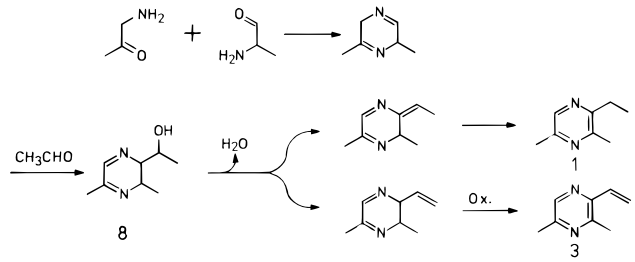

Figure 4. Proposed reaction route for the formation of pyrazines **1** and **3** in roasted coffee.

The reaction route to pyrazine **4** is less clear. To explain the formation of pyrazine **2** in the model system glucose/[^{13}C]alanine, Amrani-Hemaimi et al. (1995) postulated the formation of 3-ethyl-2(1-hydroxyethyl)-5-methyldihydropyrazine as an intermediate. Pyrazine **4** can be derived by dehydration and oxidation from this intermediate. However, the Maillard reaction of the components of the model system to 3-ethyl-2-(1-hydroxyethyl)-5-methyldihydropyrazine is an open question. The authors proposed a reaction of aminoacetone, acetaldehyde, and 1-amino-2-butanone, but they admit that the formation of the latter compound from glucose is not clear.

CONCLUSION

Three ethenylalkylpyrazines are formed during the roasting of coffee. On the basis of low odor threshold values, two of them contribute to the earthy note in the aroma profile of coffee.

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