sential for an efficient separation. Six bands designated 1-6 in the decreasing order of their R_f values were developed. Band 1 contained several components of which α-muurolene, δ-cadinene, and calamenene were identified (ir, T_r), while α-cubenene, copaene, and α-gurjunene were tentatively identified (T_r). α-*trans*-Bergamotene was identified in band 2, aromadendrene, γ-muurolene, and γ-cadiene in band 3, isocaryophyllene in band 4, caryophyllene in band 5, and α-humelene in band 6.

The mid-fraction (B) contained several esters (ir) of which *n*-pentyl *n*-butyrate, myrtenyl acetate, methyl perillate, perillyl acetate, and perillyl butyrate were identified. In addition, the last peak to exit from the GC in this fraction was the diterpene hydrocarbon (peak 60) characterized above. This compound was polar enough so that it was clearly separated from the hydrocarbon fraction A.

The rest of the oxygenated compounds (Table II) were identified in the polar fraction C. The residue remaining in the pot after fractional distillation was found to be rich in oxygenated sesquiterpenoids. However, only nerolidol and α-cadinol could be positively identified in this mixture.

While the pleasant fruity odor of the oil in the authors' judgment is undoubtedly due to methyl perillate and perillyl acetate, its camphoraceous by-note is mainly due to myrtenyl acetate and to a lesser extent to cineole. On the other hand, camphor, cineole, thujone, and, to a lesser extent, borneol and its acetate ester are responsible for the important camphoraceous odor in Dalmation sage oil, its substituents, or adultrants (Guenther, 1949; Lawrence et al., 1970, 1971). As no standard sample of myrtenol or its ir spectrum was found in our acquisition, myrtenyl acetate (peak 33) was reduced with $LiAlH_4$ and the ir and T_r of the produced myrtenol were identical with those of peak 37.

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Cycloalkapyrazines in Coffee Aroma

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The volatile components of roasted coffee were separated into basic and neutral components and analyzed on a 185 m × 0.31 mm i.d. glass capillary column coupled to a mass spectrometer. Seventeen alkylated five- and six-membered alicyclic pyrazines are reported for the first time in roasted coffee. The identities of bicyclic pyrazines were confirmed by direct comparison of their mass spectral and gas chromatographic retention data with those of authentic samples and with spectra given in the literature. Possible precursors of the bicyclic pyrazine compounds are discussed. The principal mass spectrometric fragmentation pathways of cyclopentapyrazines and tetrahydroquinoxalines are demonstrated.

Although extensive work has been done on the volatile constituents of coffee aroma, not a single carrier compound which could be responsible for coffee flavor has yet been elucidated. In order to simulate the natural product effectively one must first have an accurate knowledge of its chemical composition. However, in recent years there has been a tremendous increase in the volume of research effort devoted to the separation and identification of organoleptically important constituents of roasted coffee.

Walter and Weidemann (1969), in their comprehensive review of coffee constituents, list a large number of compounds which have been isolated from coffee aroma. The majority of the heterocyclic substances identified were furans, pyrroles, thiophenes, pyrazines, and pyridines.

During the last few years, evidence has been accumulated that heterocyclic nitrogen-containing compounds contribute directly to coffee aroma. Therefore, we studied some relationships between the basic fraction of roasted coffee and coffee flavor and identified 86 heterocycles in the basic coffee fraction of steam volatiles: oxazoles, thiazoles, pyrroles, acetylpyridines, acetyl- and furylpyrazines, quinoxalines, indoles, and quinolines (Vitzthum and Werkhoff, 1974a,b).

Pyrazines are important flavor constituents of a variety of roasted foods (Maga and Sizer, 1973). A considerable number of pyrazines have also been found in roasted coffee (Bondarovich et al., 1967; Goldman et al., 1967; Stoffelsma et al., 1968; Friedel et al., 1971). In addition to the compounds described by these investigators we wish to report here on 17 new volatile bicyclic pyrazine components occurring in coffee aroma. At present, cycloalkapyrazines have been identified in roasted food products such as peanuts (Walradt et al., 1971), filberts (Kinlin et al., 1972), cooked beef (Mussinan et al., 1973), roasted green tea (Yamanishi et al., 1973), and roasted sesame seed (Manley et al., 1974).

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EXPERIMENTAL SECTION

Preparation of Aroma Sample. The method of isola-

tion technique used in our work was described in detail in a previous paper (Vitzthum and Werkhoff, 1974b).

Gas Chromatography. Investigations were carried out using a Carlo Erba Model GI 450 gas chromatograph equipped with an 85 m × 0.31 mm i.d. glass capillary column coated with UCON HB 5100. Glass capillary columns were purchased from H. J. Jaeggi, 9043 Trogen, Switzerland. Injection was carried out without stream splitting (Grob and Grob, 1969a,b). The GC instrument is specially designed for direct injection onto capillary columns. A typical gas chromatographic separation of the total coffee aroma sample is shown in Figure 1.

Gas Chromatography–Mass Spectrometry. Mass spectral data of coffee aroma components were obtained on a single-focusing Varian MAT 111 instrument interfaced with a specially designed gas–liquid chromatograph (splitless-injection method) through a molecular slit separator. The instrument is equipped with a mass marker with upper limit m/e 999.

Only experimental conditions will be described below, since the principle of the method has been described elsewhere (Vitzthum and Werkhoff, 1974a,b). A 185 m × 0.31 mm i.d. high-resolution glass capillary column coated with UCON HB 5100 was used. The column temperature was programmed from 20 to 180° at 1°/min, with helium carrier gas at a flow rate of 4 ml/min. Conditions for the mass spectral scans were: ionization voltage, 80 eV; emission current, 270 μA; ion source temperature, 300°; ion source pressure, 3×10^{-6} Torr; separator temperature, 220°; inlet line, 230°.

Nuclear Magnetic Resonance. Proton magnetic resonance spectra of reference samples in CCl_4 solution were recorded using a Perkin-Elmer-Hitachi R-24 60-MHz spectrometer. Tetramethylsilane was the internal reference compound. Chemical shifts are given in parts per million with Me_4Si as 0.00 ppm.

Synthetic Routes. Reference standards were synthesized by procedures described in the literature.. Alkyl-6,7-dihydro-5*H*-cyclopenta[*b*]pyrazines were prepared by condensation of cyclopentenolones with alkylenediamines or of aliphatic α-diketones with 1,2-diaminocyclopentane according to the procedure of Flament et al. (1973). Products so obtained were dehydrogenated using a copper chromite Girdler G13 catalyst.

Alkyl-5,6,7,8-tetrahydroquinoxalines were obtained by a two-step procedure starting with 1,2-diaminocyclohexane and mono- or disubstituted dioxoalkanes or with an aliphatic ethylenediamine or a monoalkyl or dialkyl derivative thereof and 1,2-cyclohexadione. These substances were allowed to react as described in British Patent 1,310,771 (International Flavors and Fragrances, 1973). The bicyclic intermediates were then oxidized with KOH in ethylene glycol at 140–190° for 20–60 min to yield the fused pyrazine ring compounds according to Nakatani and Yanatori (1973).

In the course of this work, a new bicyclic pyrazine not previously described in the literature was synthesized. 2-Ethyl-5,6,7,8-tetrahydroquinoxaline was prepared by condensing ethyl glyoxal in ethanol with 1,2-diaminocyclohexane at −20°. The alcoholic solution was made alkaline and the intermediate was then aromatized with oxygen to the corresponding bicyclic pyrazine. Recently, Pittet et al. (1974) also reported on the synthesis and properties of alkylated five- and six-membered alicyclic pyrazines.

RESULTS AND DISCUSSION

In the present study, basic organic compounds were separated from volatiles of roasted coffee and then examined by GC followed by identification using a combination instrument of GC–mass spectrometer. Seventeen five- and six-membered alicyclic pyrazines were identified, not previously reported in the aroma of roasted coffee. Only those

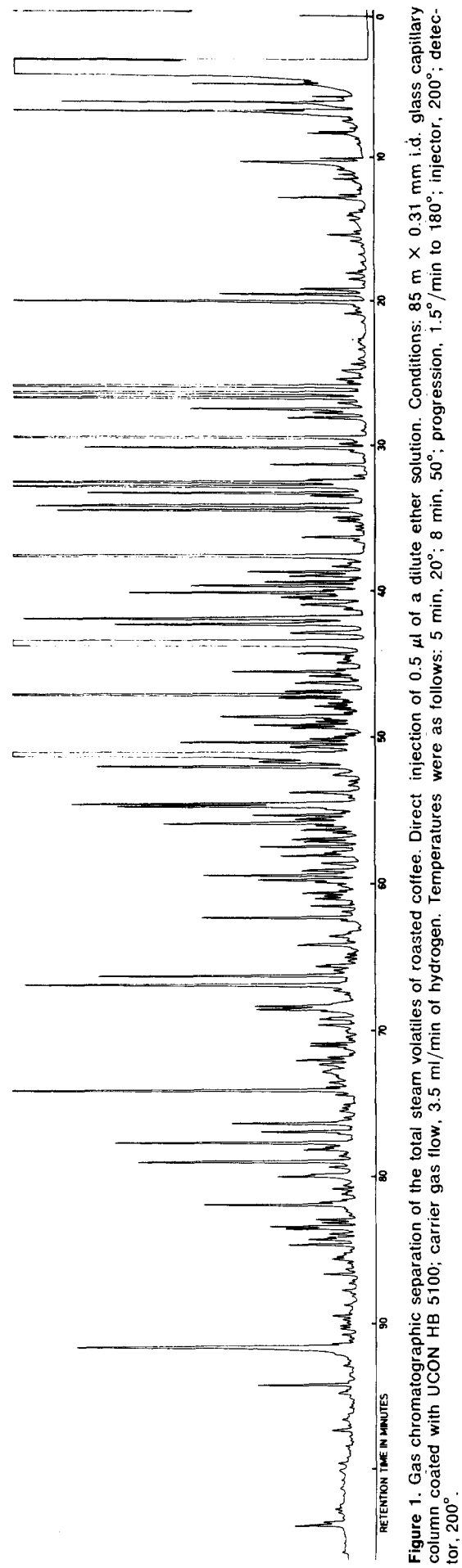


Figure 1. Gas chromatographic separation of the total steam volatiles of roasted coffee. Direct injection of 0.5 μl of a dilute ether solution. Conditions: 85 m × 0.31 mm i.d. glass capillary column coated with UCON HB 5100; carrier gas flow, 3.5 ml/min of hydrogen. Temperatures were as follows: 5 min, 20°; 8 min, 50°; progression, 1.5°/min to 180°; injector, 200°; detector, 200°.

Table I. Summary of Bicyclic Pyrazines Identified in the Basic Fraction of Roasted Coffee

Compound	First report in foodstuffs	Authentic ref compd	GLC	MS, *m/e*, %
6,7-Dihydro-5*H*-cyclopenta-pyrazine		+	+	120 (89), 119 (100), 93 (18), 92 (11), 78 (39), 67 (9), 66 (23), 65 (23), 54 (7), 53 (9), 52 (13), 41 (20), 39 (20)
2-Methyl-		+	+	134 (100), 133 (96), 118 (5), 107 (18), 106 (6), 95 (7), 93 (10), 78 (11), 66 (37), 65 (11), 54 (9), 53 (6), 42 (11), 41 (9), 39 (26)
5-Methyl-		+	+	134 (54), 133 (54), 119 (100), 92 (23), 80 (6), 79 (12), 78 (30), 66 (10), 65 (13), 54 (6), 53 (10), 52 (13), 51 (8), 41 (10), 39 (15)
2(or 3),5-Dimethyl-		+	+	148 (44), 147 (26), 133 (100), 118 (6), 80 (6), 79 (10), 78 (15), 66 (6), 65 (7), 39 (19)
3(or 2),5-Dimethyl-		+	+	148 (35), 147 (12), 133 (100), 118 (6), 80 (6), 79 (10), 78 (18), 66 (6), 65 (9), 39 (26)
2,3-Dimethyl-		+	+	148 (100), 147 (47), 133 (7), 107 (31), 106 (17), 80 (9), 79 (10), 66 (48), 65 (10), 54 (5), 53 (10), 52 (7), 42 (10), 41 (5), 39 (14)
5,7-Dimethyl-	+	+	+	148 (48), 147 (17), 133 (100), 132 (10), 131 (9), 119 (17), 118 (10), 92 (5), 79 (7), 78 (5), 77 (5), 66 (5), 54 (5), 52 (7), 41 (7), 39 (7)
2-Ethyl-				148 (96), 147 (100), 133 (9), 121 (6),120 (17), 119 (6), 93 (5), 66 (11), 65 (13), 54 (5), 53 (9), 52 (5), 41 (9), 39 (24)
5-Ethyl-	+			148 (25), 147 (13), 133 (6), 120 (100), 119 (71), 93 (8), 92 (12), 78 (14), 66 (5), 65 (6), 41 (12), 39 (17)
2,5,7-Trimethyl-	+	+	+	162 (44), 161 (14), 147 (100), 132 (7), 121 (5), 106 (5), 80 (13), 79 (16), 78 (5), 77 (8), 65 (9), 54 (7), 53 (13), 52 (11), 51 (7), 42 (5), 41(5), 39 (10)
2,3,5-Trimethyl-	+	+	+	162 (37), 161 (13), 147 (100), 132 (13), 121 (5), 106 (5), 80 (8), 79 (15), 78 (9), 77 (9), 65 (7), 54 (8), 53 (10), 42 (8), 41 (12), 39 (16)
2-Methyl-3-ethyl-	+	+	+	162 (82), 161 (100), 147 (18), 134 (21), 133 (13), 80 (5), 79 (6), 78 (5), 77 (5), 67 (9), 66 (21), 65 (18), 53 (12), 52 (7), 42 (12), 41 (14), 39 (18)
5,6,7,8-Tetrahydro-quinoxaline		+	+	134 (100), 133 (78), 130 (18), 119 (27), 107 (9), 106 (31), 105 (9), 103 (9), 92 (13), 80 (10), 79 (22), 78 (13), 77 (7), 76 (10), 69 (9), 67 (16), 66 (16), 65 (9), 54 (11), 53 (9), 52 (22), 51 (9), 50 (8), 41 (17), 39 (13)
2-Methyl-		+	+	148 (100), 147 (68), 133 (17), 132 (7), 121 (10), 120 (22), 119 (14), 106 (9), 80 (10), 79 (27), 53 (7), 52 (11), 42 (8), 41 (12), 39 (15)
5-Methyl-	+			148 (100), 147 (64), 133 (85), 121 (5), 120 (9), 106 (12), 94 (5), 93 (8), 79 (6), 66 (10), 65 (4), 41 (14), 39 (18)
2,3-Dimethyl-	+	+	+	162 (100), 161 (49), 147 (16), 146 (5), 134 (17), 133 (7), 121 (29), 120 (14), 106 (9), 93 (12), 80 (23), 79 (42), 77 (10), 53 (10), 52 (34), 42 (7), 41 (5), 39 (10)
2-Ethyl-	+	+	+	162 (65), 161 (100), 147 (5), 146 (5), 135 (6), 134 (18), 133 (10), 120 (5), 119 (6), 107 (5), 106 (6), 79 (7), 53 (5), 52 (8), 39 (6)

Table II. Nuclear Magnetic Resonance Data of Reference Compounds

Compound	NMR data, ppm
6,7-Dihydro-5*H*-cyclopentapyrazine	2.12 (quint., 2 H, $CH_2CH_2CH_2$), 2.97 (t, 4 H, N=CCH_2), 8.10 (s, 2 H, N=C*H*, arom.)
2-Methyl-	2.10 (quint., 2 H, $CH_2CH_2CH_2$), 2.40 (s, 3 H, CH_3), 2.90 (t, 4 H, N=CCH_2), 7.94 (s, 1 H, N=C*H*, arom.)
5-Methyl-	1.35 (d, 3 H, CH_3), 1.55–2.0 (m, 1 H, $CH_3CHCH_2CH_2$), 2.12–2.70 (m, 1 H, $CH_3CHCH_2CH_2$), 2.80–3.40 (m, 3 H, $CH_3CHCH_2CH_2$)
Mixture of 2(or 3),5-dimethyl- and 3(or 2),5-dimethyl-	1.28 (d, 3 H, CH_3), 1.5–2.0 (m, 1 H, $CH_3CHCH_2CH_2$), 2.0–2.6 (m, 1 H, $CH_3CHCH_2CH_2$), 2.45 (s, 3 H, CH_3), 2.75–3.35 (m, 3 H, $CH_3CHCH_2CH_2$), 8.0 (s, 1 H, N=C*H*, arom.)
2,3,5-Trimethyl-	1.30 (d, 3 H, CH_3), 1.50–2.0 (m, 1 H, $CH_3CHCH_2CH_2$), 2.0–2.5 (m, 1 H, $CH_3CHCH_2CH_2$), 2.40 (s, 6 H, CH_3), 2.65–3.30 (m, 3 H, $CH_3CHCH_2CH_2$)
5,7-Dimethyl-	1.29 and 1.35 (2 d, 6 H, CH_3, two isomeres), 1.65–3.5 (m, 4 H, $CH_3CHCH_2CHCH_3$), 8.16 (s, 2 H, N=C-*H*, arom.)
2,3-Dimethyl-	2.05 (quint., 2 H, $CH_2CH_2CH_2$), 2.38 (s, 6 H, CH_3), 2.90 (t, 4 H, N=C-CH_2)
2,5,7-Trimethyl-	1.25 and 1.30 (2 d, 6 H, CH_3, two isomeres), 1.85–3.65 (m, 4 H, $CH_3CHCH_2CHCH_3$), 2.30 (s, 3 H, CH_3), 8.08 (s, 1 H, N=C-*H*, arom.)
2-Methyl-3-ethyl-	1.24 (t, 3 H, CH_3CH_2), 2.10 (m, 2 H, $CH_2CH_2CH_2$), 2.42 (s, 3 H, CH_3), 2.48–3.1 (m, 6 H, CH_3CH_2 and $CH_2CH_2CH_2$)
5,6,7,8-Tetrahydroquinoxaline	1.80–2.03 (m, 4 H, $CH_2CH_2CH_2CH_2$), 2.76–3.0 (m, 4 H, $CH_2CH_2CH_2CH_2$), 8.16 (s, 2 H, N=C*H*, arom.)
2-Methyl-	1.75–2.0 (m, 4 H, $CH_2CH_2CH_2CH_2$), 2.41 (s, 3 H, CH_3), 2.70–2.95 (m, 4 H, $CH_2CH_2CH_2CH_2$), 8.02 (s, 1 H, N=C*H*, arom.)
2,3-Dimethyl-	1.78–2.05 (m, 4 H, $CH_2CH_2CH_2CH_2$), 2.42 (s, 6 H, CH_3), 2.70–2.92 (m, 4 H, $CH_2CH_2CH_2CH_2$)
2-Ethyl-	1.30 (t, 3 H, CH_2CH_3), 1.75–2.0 (m, 4 H, $CH_2CH_2CH_2CH_2$), 2.72 (q, 2 H, CH_2CH_3), 2.75–2.98 (m, 4 H, $CH_2CH_2CH_2CH_2$), 8.05 (s, 1 H, N=C*H*, arom.)

compounds considered to be positively identified are listed in Table I. Furthermore, mass spectral evidence was obtained for 5,7-dimethyl-5,6,7,8-tetrahydroquinoxaline and 5,8-dimethyl-5,6,7,8-tetrahydroquinoxaline, but these compounds can only be considered to be tentatively identified, because gas chromatographic separations were incomplete and mixed spectra were obtained.

Due to low bleeding of stationary phase the background in the mass spectra is negligible, thus making the registration of small peaks possible. However, it became evident that some peaks were still composed of two or more substances. Therefore, a good number of coffee aroma compounds still remain unidentified. Although these components are present in lower concentrations than others, it may be assumed that they also contribute to coffee flavor. We estimate that the 485 coffee aroma compounds which have been identified up to now represent less than half of the actual number of components yet to be found.

Naturally occurring compounds were identified by comparing their mass spectra with published spectra (Flament et al., 1973; Pittet et al., 1974). Furthermore, mass spectra obtained from basic coffee aroma components were in satisfactory agreement with those of authentic samples, except in a few cases where compounds were not available for comparison purposes. MS data are given in detail. The *m/e* values of significant mass peaks are shown in Table I (intensities in parentheses with base peak taken as 100).

The structures of all reference compounds were confirmed by their ^1H NMR spectra (Table II). The coincidence of GLC retention times of authentic compounds with those of unknown samples provided information confirming mass spectral interpretations. Other bicyclic pyrazines are also present by evidence of GLC–mass spectrometry; however, these minor constituents could not be identified, because reference compounds were not yet available.

Alkyl-substituted pyrazines are present in much greater quantity than five- and six-membered alicyclic pyrazines which only occur in traces in coffee aroma. The concentrations of cyclopentapyrazines and tetrahydroquinoxalines in roasted coffee are estimated to be approximately 1–10 ppb.

Removal of the basic components from the total volatiles of roasted coffee resulted in a considerable loss of the coffee aroma, indicating that the basic compounds played some important role in the flavor of roasted coffee. On the other hand, the basic fraction alone does not provide the full aroma recognized as roasted coffee.

Of particular interest in recent literature have been the reports on the mechanism of pyrazine formation in foods. Several theories exist for the formation of various types of pyrazines and are discussed in detail by Maga and Sizer (1973). The significance of the nonenzymic browning or Maillard reaction between amino acids and sugars for the formation of flavoring components in food products has been well established. Walradt et al. (1971) have postulated that some of the cyclopentapyrazines could form from the interaction of glyoxal or pyruvaldehyde with amino acids and the carbohydrate degradation product 2-hydroxy-3-methyl-2-cyclopenten-1-one.

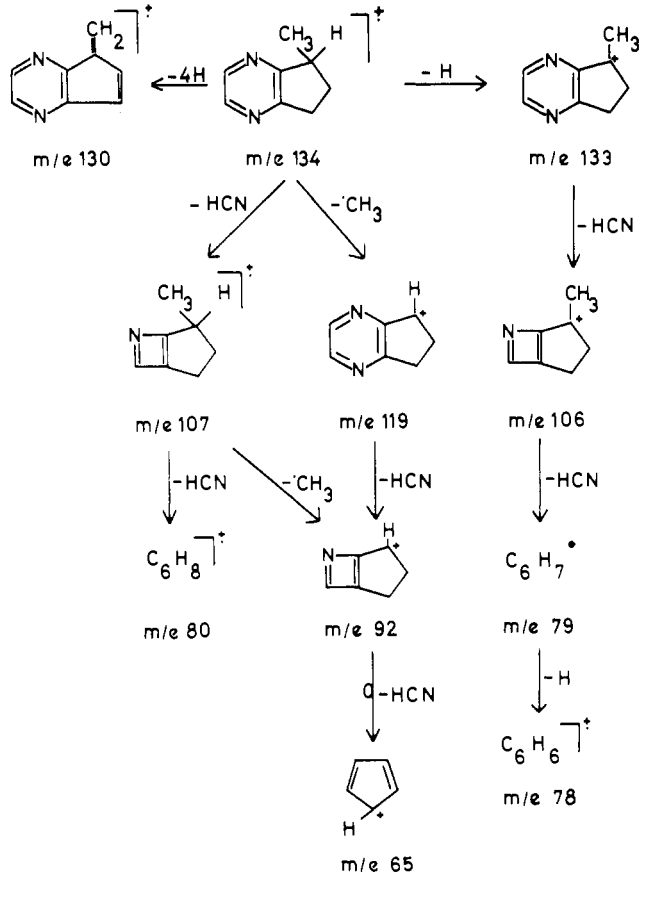
Mulders (1973) isolated cyclopentapyrazines from the volatiles obtained by heating the mixture of cysteine–cystine–ribose. The mass spectra of two peaks agree well with those of 6,7-dihydro-5*H*-cyclopentapyrazine and its 2-methyl homolog. The formation of the alkylated five- and six-membered alicyclic pyrazines, now identified in coffee aroma, may be explained as the result of the interaction between cyclic 1,2-diketones, glyoxals, and amino acids. We suggest that cyclopentane-1,2-dione, 3-methylcyclopentane-1,2-dione, 3,4-dimethylcyclopentane-1,2-dione, 3,5-dimethylcyclopentane-1,2-dione, 3-ethylcyclopentane-1,2-dione, and 3-methylcyclohexane-1,2-dione,

previously identified in roasted coffee by Gianturco et al. (1963), may be the precursors in the formation of cycloalkapyrazines and tetrahydroquinoxalines. E.g., the formation of 5-methyl-5,6,7,8-tetrahydroquinoxaline may come from condensation of 3-methylcyclohexane-1,2-dione and glyoxal (a frequently occurring product of carbohydrate degradation) with amino acids to produce α-amino ketone intermediates (Strecker degradation), which in subsequent steps of self-condensation and oxidation can form a bicyclic pyrazine. Other bicyclic pyrazines may be formed by a similar mechanism where other α,β-dicarbonyls may be participating.

The mass spectra of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 2-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 2,3-dimethyl-5,6,7,8-tetrahydroquinoxaline, and 2-ethyl-5,6,7,8-tetrahydroquinoxaline are shown in Figures 2-5, respectively. The most common feature of the mass spectra of cyclopentapyrazines with methyl substituents on the alicyclic ring is the occurrence of a characteristic M − 15 ion. Thus, 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine gives a base peak (*m/e* 119) due to the loss of a methyl radical. Subsequent loss of a hydrogen cyanide leads to an ion of mass 92, which likewise ejects HCN to give the $[C_5H_5]^+$ cation at *m/e* 65. Of some significance in the mass spectrum of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine is the M − 1 species (*m/e* 133), which is of approximately equal abundance in comparison with the molecular ion. Two consecutive losses of hydrogen cyanide from *m/e* 133 are observed.

Additional features of interest in the spectrum of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine are the peaks of *m/e* 107 and 80 of low abundance due to the consecutive expulsion of hydrogen cyanide from the molecular ion. The reaction sequence is given schematically below (Scheme I).

Scheme I. Fragmentation Pathway for 5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine



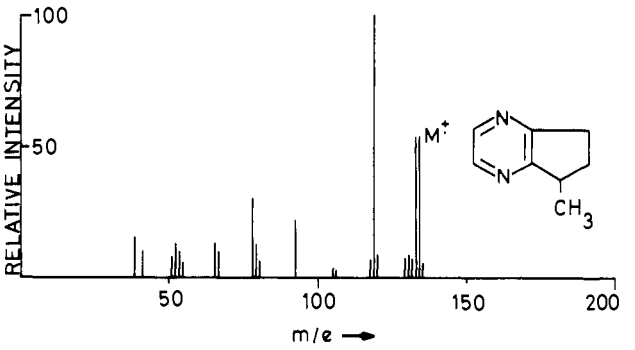
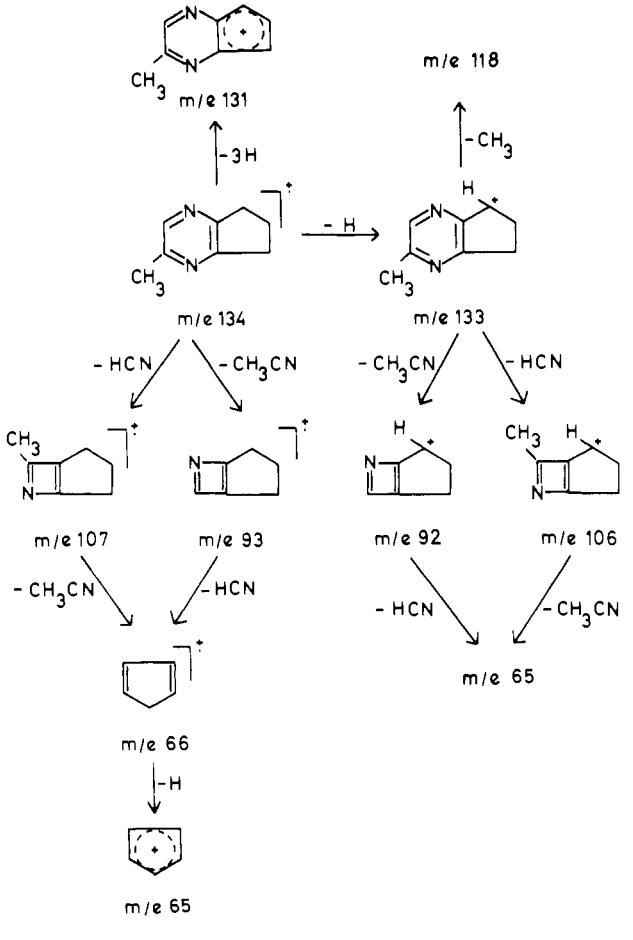


Figure 2. Mass spectrum of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine.

The base peak in the mass spectrum of 2-methyl-6,7-dihydro-5*H*-cyclopentapyrazine is the molecular ion. The first step in the fragmentation sequence is expulsion of a hydrogen atom from the molecular ion, giving a large M − 1 ion (*m/e* 133). Loss of 27 and 41 mass units corresponds to the elimination of hydrogen cyanide and methyl cyanide, forming *m/e* 107 and 93. Both, *m/e* 107 and 93 yield as an important fragment the ion *m/e* 66. Whereas 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine leads to an abundant M − 15 fragment, such an analogous ion is of only minor importance in 2-methyl-6,7-dihydro-5*H*-cyclopentapyrazine. Loss of hydrogen cyanide from *m/e* 133 and subsequent elimination of methyl cyanide from *m/e* 106 are also of minor importance (Scheme II).

Scheme II. Fragmentation Pathway for 2-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine



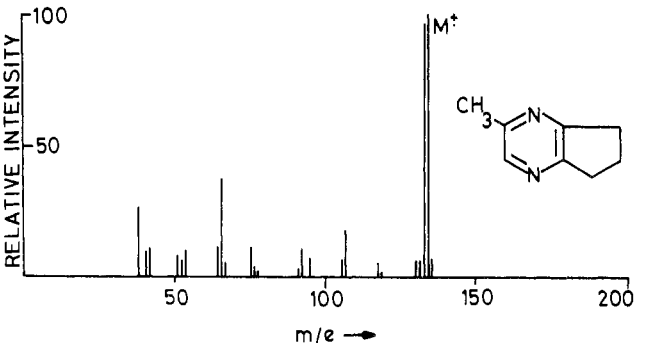


Figure 3. Mass spectrum of 2-methyl-6,7-dihydro-5*H*-cyclopentapyrazine.

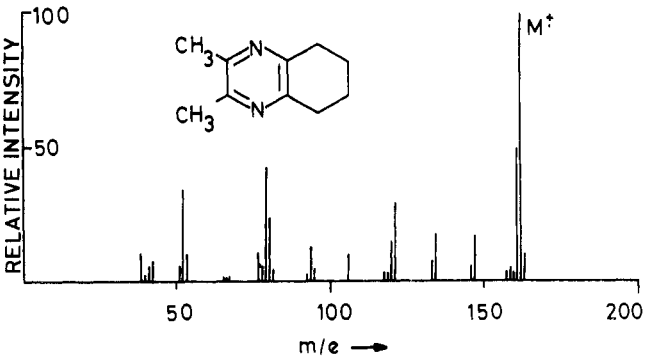


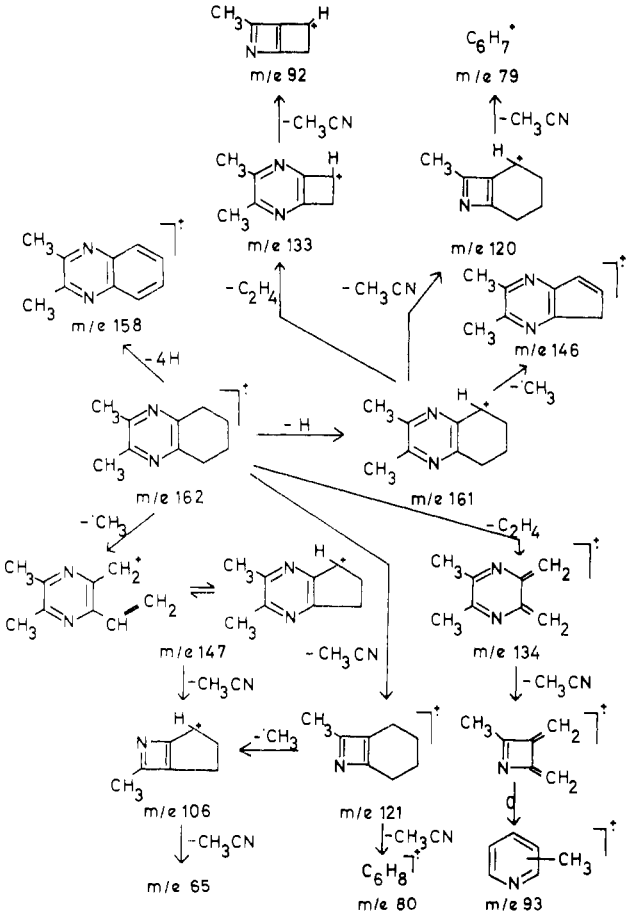
Figure 4. Mass spectrum of 2,3-dimethyl-5,6,7,8-tetrahydroquinoxaline.

The spectrum of 2,3-dimethyl-5,6,7,8-tetrahydroquinoxaline shown in Figure 4 has a large M − 1 ion resulting from loss of a hydrogen atom, the base peak corresponding to the molecular ion. The sequences *m/e* 162 → *m/e* 121 → *m/e* 80 and *m/e* 161 → *m/e* 120 → *m/e* 79 occur in the spectrum of 2,3-dimethyl-5,6,7,8-tetrahydroquinoxaline by the mechanism shown in Scheme III. The ion *m/e* 147 is produced by the loss of a methyl radical from the molecular ion. The retro-Diels–Alder decomposition of the ions *m/e* 162 and 161 yields two important peaks at *m/e* 134 and 133, which then lose methyl cyanide to give the ions *m/e* 93 and 92, respectively.

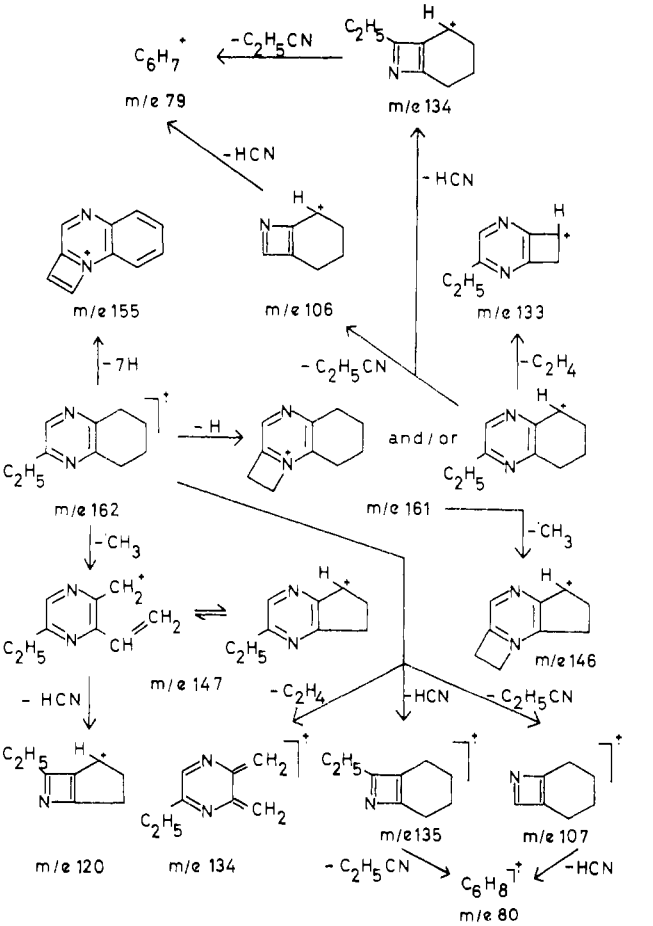
The mass spectrum of 2-ethyl-5,6,7,8-tetrahydroquinoxaline reflects the great stability of the molecular ion. The base peak in this heterocyclic system is the M − 1 ion, resulting from loss of a hydrogen atom from the alicyclic ring system as well as from the ethyl group; the next most abundant species is the molecular ion peak. Elimination of ethylene from *m/e* 162 and 161 is an important fragmentation process, whereas ejection of ethyl cyanide occurs to only a minor extent. The ubiquitous feature for nitrogen-containing aromatic heterocyclics, namely the loss of HCN, is evident (Scheme IV).

It is worth mentioning that molecular ions of cyclopentapyrazines show weak but clear multiple hydrogen abstraction ending up in the most stable unsaturated ion species. Typically, cyclopentapyrazine itself and 2- and 2,3-substituted cyclopentapyrazines lose three hydrogens to give the cyclopentadienylium ion. In contrast, 5-methyl-substituted cyclopentapyrazines split off four

Scheme III. Fragmentation Pathway for 2,3-Dimethyl-5,6,7,8-tetrahydroquinoxaline



Scheme IV. Fragmentation Pathway for 2-Ethyl-5,6,7,8-tetrahydroquinoxaline



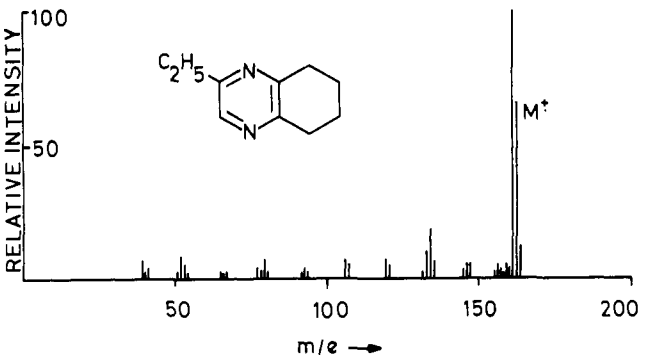


Figure 5. Mass spectrum of 2-ethyl-5,6,7,8-tetrahydroquinoxaline.

hydrogens to form the fulvene radical ion. The same is true with the tetrahydroquinoxalines, yielding radical ions of quinoxalines under consecutive elimination of four hydrogen atoms.

Because no high-resolution measurements of the key fragment ions have been made yet, structures and compositions given in the schemes are based on fragmentation patterns found to be identical in a series of mono-, di-, and trisubstituted bicyclic pyrazine compounds. Modifications of the fragmentation pathways can be clearly understood by the nature and the positions of the substituents. In some cases, however, high-resolution proof appears to be necessary. Thus, for example, the fragment ions *m/e* 120 and 119 in the mass spectrum of 2-ethyl-5,6,7,8-tetrahydroquinoxaline (Scheme IV) may result from loss of C_3H_6 from the molecular ion (*m/e* 162) or the M − 1 ion, respectively, although this appears to be unlikely from comparable examples.

The isomeres 2(or 3),5-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine and 3(or 2),5-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine are separable by capillary GC, but their mass spectra are so similar that structural assignment was not possible. Other workers (Pittet et al., 1974) encountered the same problem in structural assignments for the two isomeric cyclopentapyrazines.

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Characterization of Some Volatile Constituents of Dry Red Beans

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Dry red beans (*Phaseolus vulgaris*) were treated with a steam distillation continuous extraction apparatus, both under vacuum and at atmospheric pressure. The volatile oils obtained were analyzed by the direct combination of capillary gas chromatography and mass spectrometry. Major components characterized in the vacuum isolated oil included oct-1-en-3-ol, oct-*cis*,5-en-2-one, oct-*cis*,5-en-2-ol, hex-*cis*,3-enol, hexanol, and octa-3,5-dien-2-one. Additional major components characterized in the atmospheric pressure isolated oil included thialdine, *p*-vinylguaiacol, 3,5-dimethyl-1,2,4-trithiolane, 2,4,5-trimethylthiazole, 2,4-dimethyl-5-ethylthiazole, 2,5-dimethyl-4-ethylthiazole, 2-acetylthiazole, 2-isopropyl-4,5-dimethylthiazole, 2-isobutyl-4,5-dimethylthiazole, 2,4,5-trimethyl-2-thiazoline, and 2-methyl-5-ethylpyridine.

Dry beans (*Phaseolus vulgaris*) are becoming a more important food product. This is largely because of their high protein yield per acre and their good storage stability. Off-aromas and off-flavors occasionally develop during storage of dry beans, resulting in the rejection of large quantities of beans for human food purposes. This study was carried out to learn more about the compounds responsible for dried bean aroma and flavor, and to make possible better control of these flavor problems.

Studies have been made on the volatile components from soybeans (Arai et al., 1967; Mattick and Hand, 1969;

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