the second day because likely the species of C_8 diol glycosides most sensitive to hydrolysis were cleaved on the first day. On the second day, glycosides of other aglycons were preferably hydrolyzed. When their concentration had decreased, now the less sensitive species of C_8 diol glycosides reacted on the third day. In the literature (Bohlmann and Le Van, 1977; Williams et al., 1982) there are examples for the presence of several glycosides of a defined aglycon. The parallel curves of related aglycons shown in Figures 3–5 also indicate the occurrence of different glycosides of defined aglycons in apples.

CONCLUSIONS

The results represented in this paper confirm earlier findings about the specificity of glycosidases toward glycosides with different aglycons. In our previous work carried out on β-glucosidase it was shown that the enzyme preferably catalyzed the liberation of aromatic aglycons, such as, e.g., benzyl alcohol and 2-phenylethanol; more interesting, aglycons like terpenoids exhibited much higher K_m values (Hartmann-Schreier and Schreier, 1987). Thus, repeating the enzymatic hydrolysis several times, as done in the present study, was the logical next step of our previous investigations. The development of a continuous process now opens the way for potential technological applications in which industrially attractive plant volatiles not commonly available will be accessible from conjugates. The system is generally suitable for enzymatic reactions in which nonpolar products were formed from polar substrates (Bednarski et al., 1987).

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Received for review February 9, 1988. Accepted May 31, 1988.

Structure–Odor Relationships for Disubstituted Pyrazines

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The odor characteristics, odor thresholds (T), and retention indices on Carbowax 20M ($I^{CW\ 20M}$) and OV-101 columns ($I^{OV\text{-}101}$) were determined for 60 disubstituted pyrazines. Irrespective of the type of substituents and positions of additional ring substituents for the alkylpyrazines, substituted methyl- or ethylpyrazines, in general, have nutty and/or brown notes, while the longer alkyl-substituted pyrazines tend to have green and/or burdock like odors. The ΔI ($=I^{CW\ 20M} - I^{OV\text{-}101}$) values have been applied to quantitative structure–activity relationships in olfaction. The relationship between odor threshold and ($\sum\delta I_R - \Delta\Delta I$), where δI_R is the parameter for a substituent group and $\Delta\Delta I$ ($=\Delta I - \Delta I_P$) is the difference between the ΔI of the disubstituted pyrazine and that of pyrazine (ΔI_P), for the 60 disubstituted pyrazines is as follows: $\log (1/T) = 0.04(\sum\delta I_R - \Delta\Delta I) + 6.2$. The difference between the observed and calculated threshold value is within 1 order of magnitude.

The pyrazines possess roasted or green odors and low threshold values (Masuda and Mihara, 1986). They have been widely used as flavor constituents or perfume components. Takken et al. (1975) and Seifert et al. (1972) mentioned that the most potent odors for alkylmethoxypyrazines were found when an alkyl substituent occupies the position ortho to a methoxy function. One should be careful to drawing conclusions from a comparison of threshold values determined by different groups, even when they have used the same procedure (Maga, 1982; Takken et al., 1975). In order to elucidate the structure–odor relationships for disubstituted pyrazines, we investigated the olfactive properties for some disubstituted pyrazines.

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EXPERIMENTAL SECTION

Instrumentation. The IR, ^1H NMR, ^{13}C NMR, and GC/MS were recorded on a Hitachi 260-10, a JNM-PMX 60, a Bruker AM-400, and a Hitachi M-80B spectrometer, respectively.

GC analyses were carried out on a Hewlett-Packard Model 5710A gas chromatograph equipped with a flame

0021-8561/88/1436-1242$01.50/0

ionization detector and a fused-silica capillary column. Two types of wall-coated open tubular fused-silica capillary columns were used: 50 m × 0.22 mm (i.d.) coated with Carbowax 20 M (CW 20M; polar phase) and 50 m × 0.22 mm (i.d.) coated with OV-101 (nonpolar phase). These columns were prepared in our laboratory (Shibamoto, 1982). The column temperature was programmed from 80 to 200 °C at 2 °C/min. The preparative TLC was carried out on 20 × 20 cm glass plates coated with Macherey-Nagel silica gel SIL G-200 UV_{254} precoated sheets (2.0 mm) containing a fluorescent indicator. The preparative HPLC was preformed on an Japan Analytical Industry LC-80 instrument equipped with a refractive index detector and a GPC column using chloroform as the eluant.

Materials. All starting chemicals were obtained from reliable commercial sources and used without further purification. 2,3-Dimethyl-, 2-methoxy-3-methyl-, 2-ethoxy-3-methyl-, 2-acetyl-3-methyl-, 2-ethyl-3-methyl-, 2-methyl-3-propyl-, and 2-isopropyl-3-methylpyrazine (**1**, **4**, **7**, **13**, **16**, **19**, **22**) were obtained from reliable commercial sources and used without further purification.

2,5-Dimethyl-, 2,6-dimethyl-, 2-methoxy-5-methyl-, 2-ethoxy-5-methyl-, 2-methyl-3-(methylthio)-, 2-methyl-5-(methylthio)-, 2-acetyl-5-methyl-, 2-acetyl-6-methyl-, 2-ethyl-5-methyl-, and 2-ethyl-6-methylpyrazine (**2**, **3**, **5**, **8**, **10**, **11**, **14**, **15**, **17**, **18**) and 2-chloro-6-methylpyrazine were obtained from reliable commercial sources and were purified by high-performance liquid chromatographic separation before use.

Synthesis of 2-Methoxy-6-methyl-, 2-Ethoxy-6-methyl-, and 2-Methyl-6-(methylthio)pyrazine (6, 9, 12). 2-Chloro-6-methylpyrazine was reacted with sodium methoxide, sodium ethoxide, or sodium thiomethoxide to obtain **6**, **9**, and **12**, respectively (Masuda et al., 1981).

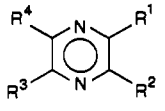
Synthesis of 2-Alkyl-3-alkoxy- and 2-Alkyl-3-(alkylthio)pyrazines 20, 21, and 23–33. The procedure used for the synthesis of these compounds was the same as that described previously (Bramwell et al., 1971, 1972; Masuda et al., 1981).

Synthesis of Alkenylpyrazines 34–36. The procedure used for the synthesis of these compounds was the same as that described previously (Chakrabartty and Levine, 1966; Rizzi, 1968).

Sensory Evaluation. Odor thresholds (T) for the pyrazines were determined by the "2/5 test" employed by Amoore (1970). The odor characteristics of the pyrazines were evaluated at about 100 times higher concentrations than the thresholds (Masuda and Mihara, 1986).

RESULTS AND DISCUSSION

The odor threshold (T) of the pyrazines means the olfactory detection threshold, that is, the ability of the test subject to distinguish between odorous water and water containing no odor. This should be contrasted with the recognition threshold, i.e. the ability of the subject to recognize the character or identity of the odor presented. The odor thresholds (T) and retention indices on OV-101 ($I^{OV\text{-}101}$) and Carbowax 20M ($I^{CW\ 20M}$) columns for 60 disubstituted pyrazines I are listed in Table I.



I

The yields and odor descriptions of the new pyrazines I are shown in Table II. The odor descriptions for disubstituted pyrazines not reported previously are as follows: **6**, nutty, sweet, chemical; **8**, sweet, fruity, nutty, roasted; **9**, fruity, sweet, nutty; **12**, nutty, roasted, sweet; **18**, medicine-like, sweet, nutty, roasted; **19**, roasted, burdock-like, ginseng-like, green; **22**, earthy, roasted, vegetable, burdock-like; **24**, green, earthy, woody, burdock-like, ginseng-like. Irrespective of the type of substituents and positions of additional ring substituents for the alkylpyrazines, substituted methyl- or ethylpyrazines, in general, have nutty and/or brown notes, while the longer alkyl-substituted pyrazines tend to have green and/or burdock-like odors (Masuda and Mihara, 1986, 1988).

The spectral data for the new pyrazines are listed in Table III.

Fors and Olofsson (1985) already mentioned that lipophilicity and water solubility are factors that significantly influence the odor thresholds of the pyrazines. A method based on gas–liquid chromatography for establishing solubility factors (or parameters) of solutes has been applied to quantitative structure–activity relationships in olfaction (Laffort and Patte, 1987). The hypothesis of Laffort concerning the recognition of odorous substances by olfactory receptor cells is that the intermolecular forces involved in this phenomenon are similar to those involved in solutions. In order words, these forces are only of the van der Waals and hydrogen-bonding types and not the highly specific lock and key type, as generally encountered in pharmacology. Mozell and Jagodowicz (1973, 1974) found a strong correlation between the retention times measured across the frog olfactry mucosa and that measured across a Carbowax 20M column.

Recently, Mihara and Masuda (1987) showed that the retention indices of 113 pyrazines on OV-101 (nonpolar) and Carbowax 20M (CW 20M; polar) columns can be represented as the sum of the retention index of pyrazine, the increments for the substituents, and the increments for the relative positions of the ring substituents

$$I^{st\ ph} = I_P{}^{st\ ph} + \sum \delta I_X{}^{st\ ph} + \sum \delta I_{XpY}{}^{st\ ph} \quad (1)$$

where $I^{st\ ph}$ is the retention index of the pyrazine derivative on a stationary phase, $I_P{}^{st\ ph}$ is the retention index of pyrazine, $\delta I_X{}^{st\ ph}$ is the increment of $I^{st\ ph}$ for a substituent group (X), and $\delta\ I_{XpY}{}^{st\ ph}$ is the increment of $I^{st\ ph}$ arising from the relative positions p (α, β, and γ positions) of two of the ring substituents (X and Y).

The difference between the retention indices on a polar (P) and nonpolar (NP) stationary phase ($I^P - I^{NP} = \Delta I$) for a given substance as suggested by Kovats was a measure of the contributions of the adhering zones of the soluble molecule, which are the zones that interact more strongly with the polar phase than with the nonpolar one (Wehrli and Kovats, 1959; Kovats, 1961). By subtracting the ΔI of the pyrazine ($\Delta I_P = 469$) from that of the disubstituted pyrazine (ΔI), eq 3 is obtained (Mihara and Masuda, 1987). $\Delta\Delta I$ represents the strength of a polar interaction between substituents and Carbowax 20M column.

$$\Delta I_P = I_P{}^{CW\ 20M} - I_P{}^{OV\text{-}101} = 1179 - 710 = 469 \quad (2)$$

$$\Delta\Delta I = \Delta I - \Delta I_P \quad (3)$$

For example, the $\Delta\Delta I$ value of 2,3-dimethylpyrazine (**1**) is calculated as follows (see Table I):

$$\Delta\Delta I_1 = (I_1{}^{CW\ 20M} - I_1{}^{OV\text{-}101}) - \Delta I_P = 1309 - 897 - 469 = -57$$

The $\Delta\Delta I$ value of **1**, which has two nonpolar methyl group, is smaller than that of **13** ($\Delta\Delta I_{13} = 37$), which has a polar acetyl group and a nonpolar methyl group.

A logarithmic or geometric scale is needed for a threshold value (T) because biological sensory responses

Table I. Odor Thresholds and Retention Indices on OV-101 (I^{OV-101}) and Carbowax 20M ($I^{CW\ 20M}$) Columns for Disubstituted Pyrazines I

no.	R^1	R^2	R^3	R^4	odor threshold, ppm	I^{OV-101}	$I^{CW\ 20M}$	ref
1	CH_3	CH_3	H	H	8.0×10^{-1}	897[a]	1309[a]	*b*
2	CH_3	H	CH_3	H	8.0×10^{-2}	889[a]	1290[a]	*c*
3	CH_3	H	H	CH_3	4.0×10^{-1}	889[a]	1300[a]	*c*
4	CH_3	OCH_3	H	H	7.0×10^{-3}	954[a]	1339[a]	*e*
5	CH_3	H	OCH_3	H	2.0×10^{-2}	971	1371	*c, e*
6	CH_3	H	H	OCH_3	1.7×10^{-2}	972	1371	*f*
7	CH_3	OC_2H_5	H	H	8.0×10^{-4}[d]	1029[a]	1385[a]	
8	CH_3	H	OC_2H_5	H	1.2×10^{-2}	1047[a]	1418[a]	
9	CH_3	H	H	OC_2H_5	5.0×10^{-3}	1048	1419	*g*
10	CH_3	SCH_3	H	H	4.0×10^{-3}[d]	1151[a]	1616[a]	*e*
11	CH_3	H	SCH_3	H	6.0×10^{-2}	1166	1678	*e*
12	CH_3	H	H	SCH_3	2.0×10^{-2}	1166	1676	*h*
13	CH_3	$COCH_3$	H	H	2.0×10^{-2}	1061[a]	1567[a]	*i*
14	CH_3	H	$COCH_3$	H	4.0×10^{-1}	1093[a]	1625[a]	*j*
15	CH_3	H	H	$COCH_3$	3.0×10^{-1}	1088[a]	1618[a]	*j*
16	C_2H_5	CH_3	H	H	5.0×10^{-1}	984	1367	*b, i*
17	C_2H_5	H	CH_3	H	1.6×10^{-2}	980[a]	1357[a]	*i, k*
18	C_2H_5	H	H	CH_3	4.0×10^{-2}	977[a]	1353[a]	*k*
19	C_3H_7	CH_3	H	H	6.0×10^{-2}	1072[a]	1438[a]	
20	C_3H_7	OCH_3	H	H	1.2×10^{-4}[l]	1121	1470	*m*
21	C_3H_7	SCH_3	H	H	1.0×10^{-3}	1320	1764	
22	$CH(CH_3)_2$	CH_3	H	H	1.6×10^{-2}	1025	1362	*n*
23	$CH(CH_3)_2$	OCH_3	H	H	2.4×10^{-5}	1078[a]	1400[a]	*e, m*
24	$CH(CH_3)_2$	SCH_3	H	H	4.7×10^{-5}	1273[a]	1692[a]	
25	C_4H_9	OCH_3	H	H	5.0×10^{-5}[l]	1219	1575	*m*
26	$CH_2CH(CH_3)_2$	OCH_3	H	H	4.5×10^{-5}	1166	1494	*e, o*
27	$CH(CH_3)C_2H_5$	OCH_3	H	H	4.0×10^{-5}	1159	1475	*m*
28	C_5H_{11}	OCH_3	H	H	2.0×10^{-5}[l]	1312	1667	
29	$(CH_2)_2CH(CH_3)_2$	OCH_3	H	H	6.3×10^{-6}	1275	1616	*m*
30	$CH_2CH(CH_3)C_2H_5$	OCH_3	H	H	1.2×10^{-5}	1267	1609	
31	C_6H_{13}	OCH_3	H	H	7.0×10^{-5}[l]	1412	1767	*m*
32	$(CH_2)_3CH(CH_3)_2$	OCH_3	H	H	6.0×10^{-6}	1388	1711	
33	$CH_2CH(CH_3)C_3H_7$	OCH_3	H	H	8.0×10^{-6}	1351	1677	
34	$(CH_2)_3CH{=}CH_2$	OCH_3	H	H	3.0×10^{-5}	1302	1700	
35	$(CH_2)_2CH{=}CHCH_3$ (*E*)	OCH_3	H	H	1.3×10^{-4}	1311	1717	
36	$(CH_2)_2CH{=}CHCH_3$ (*Z*)	OCH_3	H	H	5.0×10^{-4}	1319	1729	
37	CH_3	SC_2H_5	H	H	7.0×10^{-2}[l]	1215[a]	1655[a]	
38	CH_3	OC_6H_5	H	H	2.0×10^{-1}[l]	1465[a]	2103[a]	
39	CH_3	SC_6H_5	H	H	3.0×10^{-1}[l]	1658[a]	2399[a]	
40	C_2H_5	OCH_3	H	H	1.0×10^{-2}[l]	1037[a]	1400[a]	*m*
41	C_2H_5	OC_2H_5	H	H	2.0×10^{-2}[l]	1101[a]	1439[a]	*p*
42	C_2H_5	SCH_3	H	H	4.0×10^{-2}[l]	1237[a]	1695[a]	
43	C_2H_5	SC_2H_5	H	H	6.0×10^{-2}[l]	1297	1723	
44	C_3H_7	SC_6H_5	H	H	9.0×10^{-2}[l]	1817	2532	
45	C_4H_9	SC_2H_5	H	H	4.0×10^{-3}[l]	1477	1894	
46	C_5H_{11}	OC_2H_5	H	H	8.0×10^{-5}[l]	1376	1691	
47	C_5H_{11}	OC_6H_5	H	H	5.0×10^{-2}[l]	1816	2430	
48	C_5H_{11}	SCH_3	H	H	1.2×10^{-4}[l]	1518	1970	
49	C_5H_{11}	SC_2H_5	H	H	1.0×10^{-3}[l]	1576	1995	
50	C_5H_{11}	SC_6H_5	H	H	1.0×10^{-2}[l]	2012	2717	
51	C_7H_{15}	OCH_3	H	H	2.6×10^{-5}[l]	1519	1867	
52	C_8H_{17}	OCH_3	H	H	6.0×10^{-5}[l]	1613	1966	
53	C_8H_{17}	OC_2H_5	H	H	2.0×10^{-3}[l]	1670	1995	
54	C_8H_{17}	SCH_3	H	H	7.0×10^{-4}[l]	1825	2287	
55	C_8H_{17}	SC_2H_5	H	H	2.0×10^{-3}[l]	1879	2308	
56	C_8H_{17}	SC_6H_5	H	H	8.0×10^{-2}[l]	2310	3016	
57	$C_{10}H_{21}$	OCH_3	H	H	4.0×10^{-2}[l]	1817	2171	
58	$C_{10}H_{21}$	OC_2H_5	H	H	6.0×10^{-2}[l]	1867	2200	
59	$C_{10}H_{21}$	SCH_3	H	H	2.0×10^{-2}[l]	2032	2499	
60	$C_{10}H_{21}$	SC_2H_5	H	H	1.2×10^{-1}[l]	2082	2520	

[a] Mihara and Masuda (1987). [b] Pittet and Hruza (1974). [c] Vernin (1979). [d] Masuda and Mihara (1986). [e] Calabretta (1978). [f] Nakel and Haynes (1972). [g] Kung and Epstein (1974). [h] Kolor and Rizzo (1971). [i] Coleman and Ho (1980). [j] Roberts (1968). [k] Goldman et al. (1967). [l] Masuda and Mihara (1988). [m] Parliment and Epstein (1973). [n] Flament and Stoll (1967). [o] Takken et al. (1975). [p] Seifert et al. (1972).

increase merely logarithmically with stimulus intensity, according to the Fechner–Weber (or Weber–Fechner) law (Wright, 1982). The relationship between log $(1/T)$ and $\Delta\Delta I$ for six kinds of 3-position isomers of the disubstituted pyrazines and seven kinds of alkyl and alkenyl substituent isomers (**1–36**) is presented in Figure 1. The odor threshold is more significantly influenced by the kind of substituent rather than the position of the ring substituent. This figure also shows that there are different linear relationships between log $(1/T)$ and $\Delta\Delta I$ for each position isomer. The decreasing order of odor thresholds of the dialkylpyrazines is as follows: 2,5-position < 2,6-position < 2,3-position. Our measurements on dimethylpyrazines are in agreement with the odor thresholds published by Fors and Olofsson (1985) and Calabretta (1978). While, in the case of methylpyrazines having a polar group, such as an ethoxy, methoxy, methylthio, or acetyl, their order of decreasing odor thresholds is reversed: 2,3-position < 2,6-position < 2,5-position. Taking into account both the $\Delta\Delta I$ values and relative dipole moments, which are pre-

Table II. Yields and Odor Descriptions of New Pyrazines I

no.[a]	yield, %	odor description
21	29[b]	ginseng-like, green, burdock-like
30	36[b]	ginseng-like, earthy, nutty
32	42[b]	ginseng-like, dusty, nutty
33	40[b]	green, nutty, ginseng-like, earthy
34	30[c]	earthy, ginseng-like
35	27[c]	metallic, sharp, green, earthy
36	4[c]	sharp, earthy, ginseng-like, green

[a] See Table I for substituents. [b] Relative to the quantity of the corresponding alkylpyrazine. [c] Relative to the quantity of 2-methoxy-3-methylpyrazine.

dictable from the structures of these pyrazines, it appears that the odor thresholds decrease with increasing lipophilicity of the pyrazines within each isomer. However, as is seen from Figure 1, the correlations of log $(1/T)$ vs $\Delta\Delta I$ can never explain the entire phenomenon. One approach for solving this difficulty is to consider the specific receptor interactions of disubstituted pyrazines, shown in previous paper (Masuda and Mihara, 1988). In addition to the $\Delta\Delta I$ value, the parameter for a substituent group (δI_R) should be applied in order to evaluate this specific interactions. In this case, a plot of log $(1/T)$ vs $(\sum\delta I_R - \Delta\Delta I)$ is a straight line, where a and b are constant.

$$\log (1/T) = a(\sum\delta I_R - \Delta\Delta I) + b \quad (4)$$

The constants a and b and empirical values of 17 δI_R's (see Table IV) were determined from eq 4 and the log $(1/T)$ and $\Delta\Delta I$ values of pyrazine and 60 disubstituted pyrazines, which were calculated from the odor threshold (T) and $I^{st\,ph}$ in Table I, respectively. The δI_R values for the alkyl groups were negative. On the other hand, the δI_R values were positive for the polar groups, that is, the olefin groups, the groups containing oxygen or sulfur atoms except for the methoxy and ethoxy groups. Figure 2 shows the relationship between $(\delta\sum I_R - \Delta\Delta I)$ and the odor thresholds for the 60 disubstituted pyrazines. The odor thresholds of the disubstituted pyrazines vary within a wide range from 0.8 ppm to 0.006 ppb. The following expression was obtained from Figure 2:

$$\log (1/T) = 0.04(\sum\delta I_R - \Delta\Delta I) + 6.2 \quad (4')$$

The difference between the observed and calculated threshold value is within 1 order of magnitude. Taking

Table III. Spectral Data of New Pyrazines I

no.	IR (neat), cm^{-1}	^1H NMR ($CDCl_3$), δ	^{13}C NMR ($CDCl_3$), δ	MS, m/z (%)
21	3040, 2950, 2920, 2860, 1515, 1460, 1450, 1380, 1370, 1330, 1320, 1260, 1200, 1155, 1100, 1070, 1065, 1040, 960, 915, 835, 730	1.01 (3 H, t, J = 7 Hz, CH_2CH_3), 1.5–2.2 (2 H, m, CH_2CH_3), 2.55 (3 H, s, SCH_3), 2.6–3.0 (2 H, m, $ArCH_2$), 8.12 (1 H, d, J = 3 Hz, ring H), 8.23 (1 H, d, J = 3 Hz, ring H)	12.6, 13.9, 20.5, 36.5, 138.0, 140.9, 155.1, 156.0	170 (2), 169 (5), 168 (M^+, 41), 154 (8), 153 (89), 141 (8), 140 (100), 138 (8), 135 (17), 120 (5), 119 (10), 107 (14), 94 (14), 93 (6)
30[a]	3030, 2980, 2930, 2900, 1540, 1460, 1450, 1400, 1380, 1360, 1300, 1270, 1250, 1220, 1170, 1100, 1050, 940, 850	0.88 (3 H, d, J = 6 Hz, $CHCH_3$), 1.00 (3 H, t, J = 7 Hz, CH_2CH_3), 1.2–1.5 (2 H, m, CH_2CH_3), 2.6–2.9 (2 H, m, $ArCH_2$), 3.96 (3 H, s, OCH_3), 7.87 (1 H, d, J = 3 Hz, ring H), 8.00 (1 H, d, J = 3 Hz, ring H)	11.3, 19.2, 29.7, 33.9, 39.4, 53.3, 135.6, 137.9, 148.0, 159.1	180 (M^+, 0.5), 165 (5), 151 (8), 125 (7), 124 (100), 95 (6), 94 (11), 93 (3), 81 (5)
32	3060, 2950, 2930, 2900, 2870, 1580, 1545, 1460, 1450, 1395, 1370, 1350, 1320, 1210, 1190, 1170, 1020, 890, 840	0.87 (6 H, d, J = 6 Hz, $CH(CH_3)_2$), 1.1–2.0 (5 H, m, $(CH_2)_2CH(CH_3)_2$), 2.6–3.0 (2 H, m, $ArCH_2$), 3.98 (3 H, s, OCH_3), 7.92 (1 H, d, J = 3 Hz, ring H), 8.03 (1 H, d, J = 3 Hz, ring H)	22.6, 25.3, 27.9, 32.7, 38.9, 53.3, 135.6, 137.9, 148.5, 158.7	194 (M^+, 2), 179 (2), 151 (8), 137 (10), 125 (7), 124 (100), 94 (7)
33	3040, 2940, 2920, 2850, 1580, 1540, 1475, 1455, 1445, 1390, 1350, 1340, 1310, 1270, 1210, 1180, 1160, 1110, 1070, 1050, 1030, 1010, 980, 930, 900, 840, 790, 760, 730	0.89 (3 H, d, J = 6 Hz, $CHCH_3$), 0.89 (3 H, t, J = 6 Hz, CH_2CH_3), 1.1–1.5 (4 H, m, $(CH_2)_2CH_3$), 1.8–2.2 (1 H, m, $CHCH_3$), 2.6–2.9 (2 H, m, $ArCH_2$), 3.97 (3 H, s, OCH_3), 7.94 (1 H, d, J = 3 Hz, ring H), 8.06 (1 H, d, J = 3 Hz, ring H)	14.2, 19.7, 20.1, 32.0, 39.5, 39.8, 53.3, 135.6, 137.9, 148.0, 159.1	194 (M^+, 2), 179 (4), 165 (16), 151 (27), 137 (11), 125 (21), 124 (100), 123 (13), 95 (23), 94 (43), 93 (16), 81 (24), 54 (11), 53 (16), 43 (32), 41 (31)
34	3050, 2930, 2850, 1640, 1540, 1460, 1450, 1390, 1350, 1310, 1180, 1170, 1010, 990, 910, 840	1.5–2.4 (4 H, m, $(CH_2)_2CH{=}CH_3$), 2.7–3.1 (2 H, m, $ArCH_2$), 3.97 (3 H, s, OCH_3), 4.8–5.3 (2 H, m, $CH{=}CH_2$), 5.5–6.3 (1 H, m, $CH{=}CH_2$), 7.90 (1 H, d, J = 3 Hz, ring H), 8.02 (1 H, d, J = 3 Hz, ring H)	26.6, 31.9, 33.5, 53.3, 114.7, 135.6, 138.0, 138.4, 148.1, 158.8	179 (7), 178 (M^+, 64), 177 (12), 163 (21), 149 (6), 137 (8), 124 (100), 95 (11), 94 (25), 81 (10)
35	3050, 2930, 2850, 1540, 1460, 1450, 1390, 1350, 1310, 1210, 1180, 1170, 1010, 965, 840	1.5–1.8 (3 H, m, $CH{=}CHCH_3$), 2.1–2.6 (2 H, m, $ArCH_2CH_2$), 2.6–3.1 (2 H, m, $ArCH_2$), 3.97 (3 H, s, OCH_3), 5.3–5.6 (2 H, m, CH=CH), 7.92 (1 H, d, J = 3 Hz, ring H), 8.03 (1 H, d, J = 3 Hz, ring H)	17.7, 30.2, 32.5, 53.3, 125.4, 130.4, 135.6, 138.0, 147.8, 158.7	179 (10), 178 (M^+, 90), 177 (16), 163 (100), 149 (26), 147 (10), 124 (38), 123 (13), 94 (10), 93 (29), 55 (9)
36	3050, 3020, 2950, 2860, 1650, 1580, 1540, 1460, 1450, 1395, 1370, 1350, 1310, 1180, 1170, 1140, 1010, 970, 840, 700	1.5–1.8 (3 H, m, $CH{=}CHCH_3$), 2.3–2.7 (2 H, m, $ArCH_2CH_2$), 2.7–3.1 (2 H, m, $ArCH_2$), 3.99 (3 H, s, OCH_3), 5.3–5.6 (2 H, m, CH=CH), 7.94 (1 H, d, J = 3 Hz, ring H), 8.05 (1 H, d, J = 3 Hz, ring H)	12.6, 24.7, 32.3, 53.4, 124.7, 129.3, 135.4, 138.1, 147.7, 158.8	179 (11), 178 (M^+, 86), 177 (16), 164 (10), 163 (100), 149 (26), 147 (12), 124 (41), 123 (15), 95 (11), 94 (12), 93 (32), 55 (11)

[a] IR spectrum was obtained for solution in $CHCl_3$.

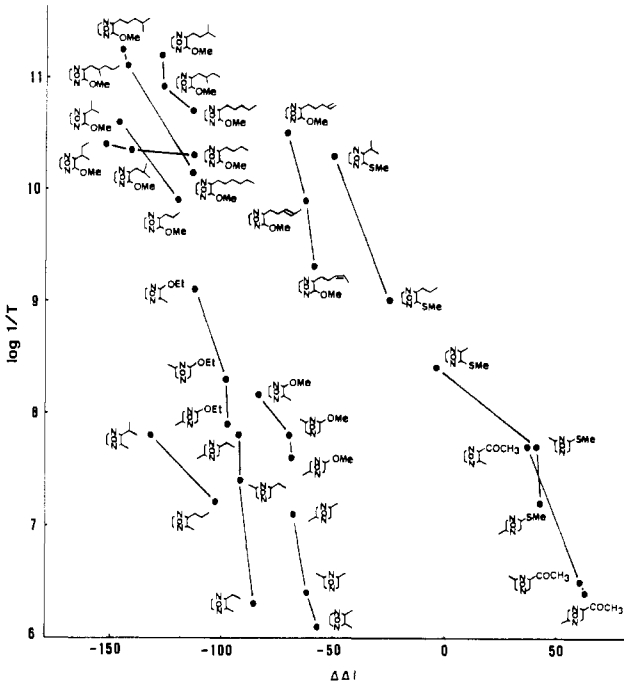


Figure 1. Correlation of log $(1/T)$, where T is the odor threshold, with $\Delta\Delta I$ for disubstituted pyrazines.

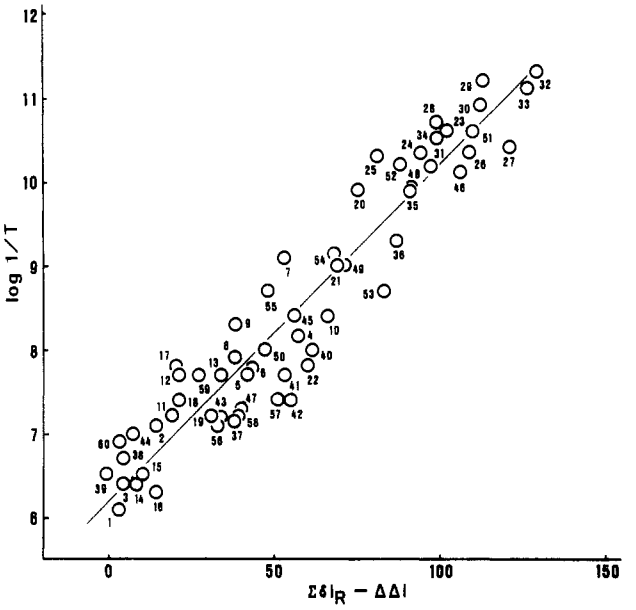


Figure 2. Correlation of log $(1/T)$, where T is the odor threshold, with $(\delta I_R - \Delta\Delta I)$ for disubstituted pyrazines. See Table I for chemical names.

Table IV. Parameters for Substituents (δI_R) of Pyrazines I

R	δI_R	R	δI_R
CH_3	−27	pentenyl	28
C_2H_5	−45	(C_5H_9)	
C_3H_7	−45	OCH_3	0
C_4H_9	−32	OC_2H_5	−33
C_5H_{11}	−15	OC_6H_5	200
C_6H_{13}	−17	SCH_3	89
C_7H_{15}	−11	SC_2H_5	36
C_8H_{17}	−28	SC_6H_5	298
$C_{10}H_{21}$	−64	$COCH_3$	98

into account our experimental error of replication in determining odor threshold, the calculated values appear to be in fairly good agreement with the observation data.

Registry No. 1, 5910-89-4; 2, 123-32-0; 3, 108-50-9; 4, 2847-30-5; 5, 2882-22-6; 6, 2882-21-5; 7, 32737-14-7; 8, 67845-34-5; 9, 53163-97-6; 10, 2882-20-4; 11, 2884-14-2; 12, 2884-13-1; 13, 23787-80-6; 14, 22047-27-4; 15, 22047-26-3; 16, 15707-23-0; 17, 13360-64-0; 18, 13925-03-6; 19, 15986-80-8; 20, 25680-57-3; 21, 68560-83-8; 22, 15986-81-9; 23, 25773-40-4; 24, 67952-59-4; 25, 32737-11-4; 26, 24683-00-9; 27, 24168-70-5; 28, 32737-12-5; 29, 32737-13-6; 30, 115652-10-3; 31, 25680-56-2; 32, 68844-95-1; 33, 115652-11-4; 34, 115652-12-5; 35, 115652-13-6; 36, 115652-14-7; 37, 32740-98-0; 38, 91137-78-9; 39, 91091-02-0; 40, 25680-58-4; 41, 35243-43-7; 42, 72987-62-3; 43, 113685-90-8; 44, 113685-95-3; 45, 113685-91-9; 46, 113685-82-8; 47, 113685-85-1; 48, 113685-87-3; 49, 113685-92-0; 50, 113685-96-4; 51, 113685-79-3; 52, 113685-80-6; 53, 113685-83-9; 54, 113685-88-4; 55, 113685-93-1; 56, 113685-97-5; 57, 113685-81-7; 58, 113685-84-0; 59, 113685-89-5; 60, 113685-94-2; 2-chloro-6-methylpyrazine, 38557-71-0.

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Received for review January 12, 1988. Accepted April 20, 1988.

Quantitative Studies on Origins of Fresh Tomato Aroma Volatiles

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A method developed for the quantitative analysis of tomato volatiles has been extended to cover the C_5–C_{13} range of fresh tomato aroma volatiles. This method showed that the pulp and skin (+ some adhering pulp) had the highest concentrations of volatile aroma compounds of the tomato. The fluid showed smaller concentrations of (*Z*)-3-hexenal. No significant contribution was found from the seeds. A study of some unusual varieties containing high and low levels of carotenoids showed differences that were generally consistent with the view that 6-methyl-5-hepten-2-one, geranylacetone, β-ionone, and related compounds are derived from oxidative carotenoid breakdown.

Although there have been numerous qualitative studies of the aroma volatiles of foods, there have been very few quantitative studies. This is especially true with tomatoes. This is partly due to the complex mixtures of components involved, their usual very low concentration, and the variability with different isolation methods. A knowledge of the concentrations of aroma volatiles in a food is, however, very important to the understanding of each compound's role in the perceived aroma of that food. Some of the authors had previously developed a workable method for the quantitative analysis of C_5–C_9 tomato volatiles (Buttery et al., 1987). It seemed desirable to extend this method to a wider range of volatiles and to apply the method to determine the principal location of volatile aroma compound production in the tomato. This information could be useful in the basic understanding of tomato aroma.

Considerable evidence regarding the lipids as the source of the C_6 aldehydes in tomatoes has been published by other authors (Kazeniac and Hall, 1970; Galliard and Matthew, 1977) and also for other foods such as tea leaves (Hatanaka and Harada, 1973). Buttery et al. (1969) and Stevens (1970) have thought that C_8, C_{13}, and C_{18} terpenoid-like aroma volatiles of tomato might be derived by oxidative breakdown of carotenoids. The availability of the method for the quantitative analysis of tomato volatiles gave the authors the opportunity of supplying some additional information in this area.

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EXPERIMENTAL SECTION

Materials. Tomatoes were grown on experimental fields at Davis, CA, during 1987. Tomato breeding lines used included the following: E6203, LA1563, ACC29 (Ace Yellow), ACC36 (High Beta), FM785, V80007-2-2-4, ACC81, XPH5498, Lassen, and GS-12 (Goldsmith-12). Freshly picked vine-ripe tomato samples were stored in the laboratory at room temperature under normal laboratory (fluorescent) lighting and used within 3 days. Authentic reference chemicals were obtained from reliable commercial sources or synthesized by well-established methods. They were purified by GLC separation before use. Freshly distilled diethyl ether and saturated $CaCl_2$ solutions were prepared as described previously (Buttery et al., 1987) except that it was found necessary to distill the ether through a fairly efficient (ca. 10-plate) glass helices packed column to remove trace amounts of volatile impurities.

Isolation of Volatiles from Tomato Samples. *From Whole Tomatoes.* Isolation was carried out largely as previously described (Buttery et al., 1987). The whole tomato sample (100 g at 25 °C) of pieces cut from three different tomatoes was blended for 30 s. The mixture was allowed to stand at room temperature for 180 s longer, then saturated $CaCl_2$ solution (100 mL) added, and the mixture blended for 10 s. A standard solution (5.0 mL) containing 20.0 ppm 2-octanone, 20.0 ppm 3-pentanone, and 5.0 ppm anethole in water was then added and the mixture blended again for 10 s. The resultant mixture was then poured into a round-bottomed 1-L flask containing an efficient magnetic stirrer. Purified air (3 L/min) was then led into the flask and passed over the vigorously stirred mixture and out of the flask through a Tenax trap (14-cm length ×