

Tomato Leaf Volatile Aroma Components

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The volatiles of tomato leaves were isolated both by Tenax adsorbant trapping and by direct solvent extraction. These were analyzed by capillary GLC-MS and by packed-column GLC-batch infrared spectroscopy. An unusual major component identified was 2-carene (7 ppm of leaves). Additional evidence was also obtained for the presence of eight previously identified major terpene and sesquiterpene hydrocarbons including β -phellandrene (25 ppm), limonene (4 ppm), caryophyllene (3 ppm), and humulene (0.8 ppm). Evidence was also obtained for the identities of 2 other monoterpenes and 14 oxygenated components not previously reported. Blending of the leaves was found to give as much as 270 ppm of (*E*)-2-hexenal and 23 ppm of (*Z*)-3-hexenal. Determination of odor unit values for the volatile components indicated that the compounds contributing most to the leaf odor include (*Z*)-3-hexenal, limonene, hexanal, (*E*)-2-hexenal, eugenol, 1,8-cineole, caryophyllene, β -phellandrene, humulene, and linalool.

We have been carrying out a study to obtain a better definition of the factors responsible for the aroma and flavor of fresh ripe tomatoes (Buttery et al., 1987). Tomato leaves are well-known to give off an intense tomatolike aroma when they are handled. We felt that an accurate knowledge of the compounds causing this intense tomato leaf odor might help to give a better understanding of the aroma of the tomato fruit.

Knowledge of tomato leaf volatiles could also be useful in the understanding of the behavior (e.g., attraction to the plant) of pest insects of tomato. Some preliminary results of insect studies (including some identification of leaf volatiles) were already reported by one of us (Light, 1986). The volatiles of tomato leaves had been previously investigated by Andersson et al. (1980) who identified α -pinene, β -pinene, α -terpinolene, α -thujene, α -terpinene, limonene, β -phellandrene, *p*-cymene, γ -terpinene, δ -elemene, β -caryophyllene, and humulene. Urbasch (1981) identified the following additional components: hexanal, (*E*)-2-hexenal, (*Z*)- and (*E*)- β -ocimene, terpinolene, linalool, neral, geranial, methyl salicylate, nerol, geraniol, and 2-tridecanone. Methyl salicylate had also earlier been identified by Davis and Stanley (1960). The compound(s) responsible for the characteristic tomato leaf aroma was not identified in these previous studies, and we felt that further studies of tomato leaf volatiles were desirable.

EXPERIMENTAL SECTION

Materials. Three tomato (*Lycopersicon esculentum*) varieties used for this study were grown on an experimental plot in Albany, CA. These were Ace, Burpee Red Cherry, and Grivorski varieties. Leaves were generally obtained by cutting small branches from the main stem with a sharp knife. The leaves were kept at room temperature, and the isolation of volatiles was generally begun within 1-2 h after harvesting.

Isolation of Volatiles by Direct Hexane Extraction. Intact freshly picked tomato leaves (500 g) were placed in a 12-L flask and extracted by shaking gently but thoroughly with purified hexane (2 \times 300 mL). The hexane contained a trace (ca. 0.01%) of Ethyl antioxidant 330. The combined extract was dried over a small quantity of sodium sulfate and filtered and most of the hexane distilled off using a Vigreux distillation column to give a concentrate of ca. 3 mL. After being stored at -20 °C overnight, the concentrate was centrifuged and the upper liquid layer (ca.

2.6 mL) separated from the lower solid waxy material (ca. 0.4 g or ca. 0.1% of the leaf; IR spectra indicated that it consisted mostly of long-chain hydrocarbons). The upper liquid layer was used for the volatile analysis.

Isolation by Tenax Trapping. Intact freshly picked tomato leaves (400 g) were placed in a 12-L flask, and nitrogen (purified by passage through activated charcoal) was passed over the leaves at a flow rate of 1 L/min. The nitrogen left the flask through a Tenax trap (10 g; 14 \times 2.2 cm). The isolation was continued for 24 h at room temperature (ca. 25 °C). The Tenax trap was then eluted with freshly distilled diethyl ether (containing ca. 0.01% Ethyl antioxidant 330). The ether was concentrated (using a Vigreux column and a warm water bath) to ca. 50 μ L. In some isolations air was used as the sweep gas instead of nitrogen. No noticeable difference was found in the composition of the concentrates obtained.

Separation into Hydrocarbon and Oxygenated Fractions. This was carried out by taking a portion of the Tenax-trapped volatile concentrate and placing it on a column of neutral chromatographic silica gel (Mallinckrodt SilicAR CC-7, 100-200 mesh). Elution with excess hexane and subsequent removal of solvent gave the "hydrocarbon fraction". This was followed by elution with diethyl ether (freshly distilled), which on removal of solvent gave the "oxygenated fraction".

Isolation of Volatiles from Blended Leaves. The method was similar to that developed by some of us previously for tomato fruit (Buttery et al., 1987). Tomato leaves (40 g) were cut into pieces ca. 4 cm \times 2 cm and placed in a Waring blender with 100 mL of water. The mixture was blended for 30 s and then held for different time periods (0, 1.5, 3 min). Saturated CaCl₂ solution (100 mL) was then added and the mixture blended for 10 s (for zero-time holding, the saturated CaCl₂ solution was added before blending). An internal standard 2-octanone (10.0 mL of 50 ppm solution in water) was then added and the mixture blended again for 10 s. The mixture was then placed in a 1-L flask with an efficient magnetic stirrer. Purified air (3 L/min) was led into the flask via a Teflon tube and passed over the vigorously stirred mixture and out of the flask through a Tenax trap of the same dimensions as described above. The volatiles were extracted from the trap and concentrated also as described above.

GLC-MS Analysis. Two main capillary gas-liquid chromatography (GLC) columns were used at different times. They were both of Pyrex glass, 150-m length by 0.66-mm i.d., wall coated. One column was coated with Silicone OV-3. This column was used with a temperature program rate of 1 °C/min from 20 to 170 °C and held at

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170 °C. The other column was coated with Carbowax 20-M and was used with oven temperature conditions that involved holding the column at 60 °C for the first 40 min and then temperature programming at 1 °C/min from 60 to 170 °C and holding at 170 °C. The columns were coupled to the mass spectrometer (a modified Consolidated 21-620 cycloidal instrument) using a single-stage silicone rubber membrane molecular separator. A splitless injector was used for the Tenax-isolated concentrates. The concentrates isolated by direct extraction were injected directly onto a precolumn to prevent problems from extracted nonvolatile material.

Authentic chemical samples were obtained from commercial sources, synthesized by established methods, or isolated from known essential oils. (+)-2-Carene was obtained from Fluka Chemical Corp. β -Phellandrene was isolated from Angelica seed oil, and caryophyllene and humulene were from hop oil. Epoxides were obtained by oxidizing the corresponding hydrocarbon with chloroperbenzoic acid. The identities of authentic samples were verified by comparison of spectral (MS, IR) and GLC data with published data.

Packed-Column GLC-Batch IR. Components were separated from the tomato leaf volatile oil on a 3 m \times 0.64 cm (o.d.) Pyrex glass column packed with 80–100-mesh Chromosorb G-DMCS coated with 1% Carbowax 20-M. The column was programmed from 50 to 170 °C at 2 °C/min. Samples were collected in 3-mm o.d. \times 14-cm length Pyrex tubes. The infrared (IR) spectra were measured as thin films between ultramicro salt plates or as solutions in an ultramicrocavity cell on a Perkin-Elmer 197 instrument.

Odor Threshold Determinations. This was carried out following the procedure described previously by some of us (Guadagni et al., 1966) using odor-free Teflon bottles equipped with Teflon tubes.

RESULTS AND DISCUSSION

The main study was concentrated on volatiles isolated from freshly picked intact leaves. The volatile oil was isolated by two main methods: Tenax trapping and direct solvent extraction. The concentration of volatile oil by Tenax trapping varied from 10 to 50 ppm. Direct hexane extraction of the leaves gave 50–80 ppm volatile oil. In both cases GLC peak area measurements were used in the calculation of concentrations. A nonvolatile wax was also obtained by the solvent extraction, amounting to ca. 0.1% of the leaves and consisting primarily of long-chain hydrocarbons (from IR spectra) commonly found in leaf waxes (Hadley, 1981). Capillary GLC-MS studies were initially carried out on the whole volatile oils. Later the oils were separated into hydrocarbon and oxygenated fractions and additional GLC-MS studies carried out on these fractions. For the intact leaves the hydrocarbon components made up ca. 96% of the oil and the oxygenated components 4%. Damage to the leaves and the production of (*E*)-2-hexenal and related compounds change this ratio considerably. The volatiles in damaged leaves are discussed later.

The components identified are listed in Table I together with figures giving some idea of their concentration in the intact leaves (based on GLC peak area measurements). The analysis shown is for the Red Cherry variety. The composition of the other varieties was generally similar qualitatively and quantitatively for the major components with some quantitative differences for the minor components.

One major component (7 ppm of the leaves) not previously identified was 2-carene (see Figure 1). A GLC

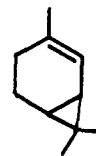


Figure 1. Structure of 2-carene.

peak corresponding to the retention time of this compound had been detected by the earlier workers Andersson et al. (1980) and Urbasch (1981) but had not been identified correctly. Andersson et al. had labeled the compound " α -terpinolene" but gave no idea of its structure. Terpinolene itself has quite different GLC retention properties and the prefix " α " is not usually used.

The samples of 2-carene isolated in the present work showed mass, infrared, and ^1H NMR spectra and capillary GLC retention data identical with that of a commercially obtained authentic sample of (+)-2-carene. The 2-carene showed the following mass spectrum (two major ions each 14 mass units above m/z 34; intensities in parentheses): 39 (49), 41 (60); 53 (21), 55 (22); 65 (12), 67 (13); 77 (48), 79 (48); 91 (45), 93 (100); 105 (17), 107 (13); 119 (0.5), 121 (83); 136 (41), 137 (4). The following IR absorption spectrum was obtained: strong bands at 3000, 2900, 1450, 1370, 855, 835 cm^{-1} ; moderate bands at 2730, 1660, 1300, 1215, 1125, 1020, 1006, 982, 965, 950 cm^{-1} ; weaker bands at 1345, 1060, 900, 790, 770, 760 cm^{-1} . The sample of 2-carene isolated was too small to determine its optical rotation.

Two unidentified major components of the oxygenated fraction, called unknown A (MW 152) and unknown B (MW 152), had mass spectra that indicated that they are probably monoterpenoid epoxides or epoxide rearrangement products. It was first suspected that these compounds were epoxide derivatives of the major monoterpene β -phellandrene. Peracid oxidation of β -phellandrene did not, however, give any compounds with mass spectra similar to that of unknowns A or B. It has been known for many years that the main product of peracid oxidation of β -phellandrene is 4-isopropyl-2-cyclohexenone (Macbeth et al., 1938), but this compound was not found in the tomato leaves. Known oxidation products of other terpenoid hydrocarbons such as limonene epoxide, caryophyllene epoxide, humulene epoxide, and 2-carene epoxide were identified. An authentic sample of 2-carene epoxide was obtained by chloroperbenzoic acid oxidation of (+)-2-carene and had the following mass spectrum: 41 (47), 43 (42); 53 (13), 55 (15); 65 (14), 67 (16); 77 (19), 79 (17); 91 (45), 93 (11); 105 (9), 109 (16); 117 (15), 119 (100); 134 (39), 137 (6); 152 (2). The phenols guaiacol and eugenol are unexpected components of the intact leaves because they are more commonly associated with cooked foods. Methyl salicylate, 2-phenylethanol, and benzyl alcohol are commonly found in plant materials. 2-Tridecanone previously identified in the leaves of some wild tomato varieties (Williams et al., 1980) was not detected in the leaves of the domestic varieties used in the present studies.

C_6 Aldehydes in Damaged Tomato Leaves. The concentrations of (*E*)-2-hexenal, (*Z*)-3-hexenal, and hexenal are relatively low (less than 1 ppm each) in the intact leaves. However, if the leaves are crushed or otherwise damaged, the concentrations of these aldehydes increase dramatically. This type of C_6 aldehyde formation has been extensively studied especially in regard to tea leaves (Hatanaka et al., 1973). It has been well established that the formation results from enzymatic catalyzed oxidation of the unsaturated fatty acids of the plant. In some recent studies with tomato fruit (Buttery et al., 1987) we found saturated CaCl_2 to be effective in deactivating tomato

Table I. Volatiles Identified in Intact Tomato Leaves Using GLC-MS and in Some Cases GLC-IR (Some Idea of the Concentration of the Volatile in the Leaves Also Listed)

| compound ^a | major MS ions ^b | Kovat's index ^c | concn, ^d ppm |
|--|-----------------------------------|----------------------------|-------------------------|
| Terpene Hydrocarbons | | | |
| α -pinene | 93, 77, 41, 121, <i>136</i> , 105 | 980 | 0.002 |
| 2-carene (IR) ^e | 93, 121, 41, <i>136</i> , 79, 55 | 1110 | 7.0 |
| myrcene | 93, 41, 69, 79, 53, 121 | 1145 | 0.03 |
| α -phellandrene (IR) ^e | 93, 77, <i>136</i> , 41, 121, 65 | 1150 | 1.3 |
| α -terpinene | 121, 93, <i>136</i> , 77, 105, 41 | 1165 | 0.4 |
| limonene | 68, 93, 41, <i>136</i> , 53, 79 | 1180 | 4 |
| β -phellandrene (IR) ^e | 93, 77, <i>136</i> , 41, 121, 69 | 1200 | 25 |
| terpinolene | 121, 93, <i>136</i> , 79, 41, 105 | 1290 | 0.4 |
| Sesquiterpene Hydrocarbons | | | |
| δ -elemene | 121, 93, <i>136</i> , 41, 161, 77 | 1440 | 0.05 |
| caryophyllene (IR) ^e | 41, 69, 93, 79, 133, 55 | 1570 | 3.1 |
| humulene (IR) ^e | 93, 121, 80, 41, 147, 107 | 1640 | 0.8 |
| Oxygenated Monoterpenoids | | | |
| 1,8-cineole | 43, 81, 71, 93, 55, 108 | 1210 | 0.03 |
| 2-carene epoxide | 119, 41, 91, 134, 109, 77 | 1430 | 0.005 |
| limonene epoxide | 41, 67, 55, 81, 94, 109 | 1450 | 0.02 |
| unknown A (MW 152) | 137, 109, 55, 41, 69, 81 | 1520 | 0.1 |
| linalool | 93, 71, 41, 55, 80, 121 | 1545 | 0.03 |
| unknown B (MW 152) | 109, 41, 91, 119, 79, 53 | 1690 | 0.1 |
| geraniol | 69, 41, 93, 53, 84, 121 | 1780 | 0.02 |
| Oxygenated Sesquiterpenoids | | | |
| caryophyllene epoxide | 41, 79, 93, 69, 55, 107 | 1950 | 0.02 |
| humulene epoxide | 43, 109, 138, 96, 67, 55 | 1970 | 0.005 |
| Aliphatic Compounds | | | |
| hexanal | 44, 56, 72, 82 | 810 | 0.01 |
| (<i>Z</i>)-3-hexenal | 41, 55, 69, 83, <i>98</i> | 1100 | 0.02 |
| (<i>E</i>)-2-hexenal | 41, 55, 69, 83, <i>98</i> | 1190 | 0.02 |
| hexanol | 56, 43, 31, 69, 84 | 1330 | 0.03 |
| (<i>Z</i>)-3-hexenol | 41, 67, 55, 31, 82, <i>100</i> | 1370 | 0.15 |
| Aromatic Compounds | | | |
| 2-acetylfuran | 95, <i>110</i> , 39, 68, 53, 81 | 1490 | 0.004 |
| benzaldehyde | 77, 105, 51, 39, 63 | 1520 | 0.005 |
| methyl salicylate | 120, <i>152</i> , 92, 65, 39, 53 | 1730 | 0.006 |
| guaiacol | 109; <i>124</i> , 81, 53, 39 | 1830 | 0.01 |
| benzyl alcohol | 108, 79, 51, 39, 91, 65 | 1840 | 0.03 |
| 2-phenylethanol | 91, <i>122</i> , 65, 39, 51, 77 | 1890 | 0.04 |
| eugenol | <i>164</i> , 149, 77, 55, 39, 131 | 2080 | 1.0 |

^aMass spectrum and GLC Kovat's retention index values are consistent with those of authentic sample. ^bThe most intense ion, each 14 mass units above m/z 34. Ions in descending order of intensity. Molecular ion in italic type if listed. ^cOn Carbowax 20-M Pyrex capillary GLC column. ^dConcentration of compound in parts per million parts (ppm) of the whole leaf. ^eInfrared absorption spectrum consistent with that of authentic sample in addition to a.

Table II. Concentration of C₆ Aldehydes Found in Blended Tomato Leaves Held at Different Times before Enzyme Deactivation

| compound | concn, ppm | | |
|------------------------|------------|---------|---------|
| | 0 min | 1.5 min | 3.0 min |
| hexanal | 0.5 | 2.5 | 18 |
| (<i>Z</i>)-3-hexenal | 8.9 | 11 | 23 |
| (<i>E</i>)-2-hexenal | 5.7 | 91 | 270 |

oxidative enzyme systems. This seems also to be the case with tomato leaves. Table II shows concentrations of the three C₆ aldehydes in blended tomato leaves when saturated CaCl₂ solution was added at various times after blending of the leaves. The concentration of (*E*)-2-hexenal at 270 ppm is very high in comparison to the 10 ppm concentration of this compound found previously by one of us in blended alfalfa leaves (Buttery and Kamm, 1980). It is interesting that the concentration of (*Z*)-3-hexenal does not change very much for the different holding times but that the concentration of (*E*)-2-hexenal increases almost 50 times for the 3-min holding period. Much of the (*Z*)-3-hexenal may be isomerized into (*E*)-2-hexenal as it is formed. The concentrations found for even zero-time blending was considerably higher than that found in the

intact leaves. Leaves whose surface was rubbed with clean aluminum foil produced the typical intense "tomato leaf" odor but showed concentrations of less than 1 ppm for each of the C₆ aldehydes.

Nature of Compound(s) Responsible for "Tomato Leaf" Odor. We have carried out some panel studies on the odor thresholds and odor quality of different GLC fractions from tomato leaf volatiles. But these studies have not indicated any one compound as being responsible for the typical "tomato leaf" aroma. The high concentrations of (*Z*)-3-hexenal and (*E*)-2-hexenal in blended leaves indicated that these could contribute significantly to the green aroma. The tomato component 2-isobutylthiazole was described (Viani et al., 1969) as being "gifted of a green aroma strongly recalling tomato leaves". This observation has been repeated in a number of reviews since this early publication. We are thoroughly familiar with the analysis of 2-isobutylthiazole in tomato fruit (Buttery et al., 1987) but in a number of studies have been unable to find any detectable amount of 2-isobutylthiazole in tomato leaves. If it is present in tomato leaves, it would be below the odor threshold concentration (3.5 ppb) and therefore it is doubtful whether it could contribute to the odor. We also submitted 2-isobutylthiazole (as a 1 ppm solution in water)

Table III. Odor Thresholds of Volatile Tomato Components in Water Solution and a Calculation of the log Odor Units of Each Compound for Tomato Intact Leaves (and Blended Leaves for C₆ Aldehydes)

| compound | odor threshold, ppb | log odor units |
|-----------------------|---------------------|------------------------|
| hexanal | 4.5 | 0.3 (3.6) ^b |
| (Z)-3-hexenal | 0.25 | 1.9 (5.0) ^b |
| (E)-2-hexenal | 17 | 0.1 (4.2) ^b |
| (Z)-3-hexenol | 70 | 0.3 |
| α-pinene | 6 | -0.5 |
| (+)-2-carene | 4000 | 0.3 |
| myrcene | 13 | 0.3 |
| limonene | 10 | 2.6 |
| β-phellandrene | 500 | 1.7 |
| terpinolene | 200 | 0.3 |
| caryophyllene | 64 | 1.7 |
| humulene | 120 | 0.8 |
| 1,8-cineole | 1.3 | 1.7 |
| 2-carene epoxide | 500 | -2 |
| limonene epoxide | 100 | -0.7 |
| linalool | 6 | 0.7 |
| geraniol | 40 | -0.3 |
| caryophyllene epoxide | 200 | -1 |
| benzaldehyde | 350 | -2 |
| methyl salicylate | 40 | -0.7 |
| guaiacol | 3 | 0.5 |
| benzyl alcohol | 20000 | -3 |
| 2-phenylethanol | 1100 | -1.5 |
| eugenol | 6 | 2.2 |

^a ppb = parts (mL) of compound per billion (10⁹) parts (mL) of water. ^b Value in parentheses for blended tomato leaves.

to an odor panel of 16 judges for odor quality evaluation. In 32 judgments the most often used descriptions were green (28%), capsicum like (16%), onion like (16%), black pepper like (16%), and tomato like (2%). None of the judges described the odor as being like tomato leaves.

Another compound described in the literature as having an odor "like tomato leaves" is 2,5-epoxymegastigma-6,8-diene (Kaiser and Lamparsky, 1978). We have been unable to detect any compound with a mass spectrum like that of this compound in tomato leaves.

The odor thresholds in water solution of most of the compounds identified in tomato leaves have been measured by us in either previous or the present studies. These are listed in Table III. The number of odor units for any compound in a largely aqueous food has been defined as $U_o = \text{concentration of compound} / \text{odor threshold concentration}$ (Guadagni et al., 1966). These values have been calculated for the compounds in Table III (and listed as log odor units) with the concentrations determined for the tomato leaf listed in Table I. If a compound has a negative log odor unit value, then its concentration in the tomato leaves is lower than its odor threshold and it is doubtful that it can contribute anything significant to the total leaf odor. If we look at the threshold concentration of a compound as a separate quantity or unit, then the odor unit value for that compound gives the number of threshold concentrations of that compound available in the leaves. The probability of a compound's odor being detected should be greater the greater the number of odor units present. This value should then give some indication of that compound's importance to the total odor. It can be

seen that the compound with the most log odor units include (Z)-3-hexenal, limonene, hexanal, (E)-2-hexenal, eugenol, 1,8-cineole, caryophyllene, β-phellandrene, humulene, and linalool. The evidence indicates that these are the components most important to tomato leaf aroma, the C₆ aldehydes contributing much more to the blended leaves.

Insect Studies. Electroantennogram studies using the tomato horn worm, *Manduca quinquemaculata*, and the tobacco hornworm, *Manduca sexta*, have been carried out with many of the compounds in Table I. Preliminary results of these studies have been reported (Light, 1986) and will be published in full elsewhere.

Registry No. BuCH₂CHO, 66-25-1; (Z)-EtCH=CHCH₂CHO, 6789-80-6; (E)-PrCH=CHCHO, 6728-26-3; (Z)-EtCH=CH-(CH₂)₂OH, 928-96-1; PhCHO, 100-52-7; *o*-HOC₆H₄CO₂Me, 119-36-8; PhCH₂OH, 100-51-6; Ph(CH₂)₂OH, 60-12-8; α-pinene, 80-56-8; 2-carene, 554-61-0; myrcene, 123-35-3; α-phellandrene, 99-83-2; α-terpinene, 99-86-5; limonene, 138-86-3; β-phellandrene, 555-10-2; terpinolene, 586-62-9; δ-elemene, 20307-84-0; caryophyllene, 87-44-5; humulene, 6753-98-6; 1,8-cineole, 470-82-6; 2-carene epoxide, 62413-92-7; limonene epoxide, 1195-92-2; linalool, 78-70-6; geraniol, 106-24-1; caryophyllene epoxide, 1139-30-6; humulene epoxide, 19888-33-6; 2-acetylfuran, 1192-62-7; guaiacol, 90-05-1; eugenol, 97-53-0; hexanol, 25917-35-5.

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