Some Unusual Minor Volatile Components of Tomato

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The identities and possible origins of three minor unusual volatile components of tomato are discussed. These are pentyl nitrate, the first identification of a volatile alkyl nitrate in a fresh vegetable or fruit; 5-ethyl-2(5*H*)-furanone, which, besides its presence in tomato, was found to be a major autoxidation product of pure (*Z*)-3-hexenal; and 5-ethylcyclopentene-1-carbaldehyde, a likely unusual linolenic acid oxidation product.

KEYWORDS: Tomato; volatiles; pentyl nitrate; 5-ethyl-2(5*H*)-furanone; 5-ethylcyclopentene-1-carbaldehyde

INTRODUCTION

In studies carried out at the authors' laboratory (see, e.g., refs *1−3*), over several years, a number of compounds were detected in fresh tomatoes in small concentrations, which at the time were not considered important enough for further study. Many of these seemed to have no direct contribution to the aroma of the tomato. They could be important, however, to the understanding of how other more significant aroma compounds are formed or degraded. This study discusses the identities of three unusual minor components and their possible origins.

MATERIALS AND METHODS

Materials. Samples of fresh ripe tomatoes were obtained from local retail markets including (a) the ordinary field-grown fresh market tomatoes, which are picked at the mature green stage and ripened with ethylene, and (b) "hot house" tomatoes, which are ripened on the plant. Fresh tomatoes were also obtained locally from plants grown in El Cerrito, CA. Samples of "mature green" tomatoes were obtained from the locally grown plants, which were also used as a source of tomato leaves. Solvents and other chemical compounds were obtained and purified as described previously (*2*).

Pentyl nitrate was synthesized by dropwise addition of pentanol to an ice-cooled stirred mixture of fuming sulfuric acid and concentrated nitric acid. 3-Methylbutyl nitrate was synthesized in a similar way from 3-methylbutanol. These compounds were purified by preparative gas chromatography (GC).

Isolation of Volatiles. Two principal isolation methods were used, both involving high-flow dynamic headspace trapping. These have been described previously (*2*). In the first method a 100 g sample of the fresh ripe tomato was blended (using a Waring blender). The mixture was held for 3 min, and then a saturated CaCl₂ solution (100 mL) was added and mixed thoroughly. The mixture was transferred to a 1 L flask containing an efficient magnetic stirring bar. A suitable head was attached to the flask and connected to a closed-loop dynamic headspace system containing a large (10 g) Tenax trap. The air in the system was displaced with purified nitrogen, and the volatiles were swept (via a

Teflon diaphragm pump: all Teflon or Pyrex surfaces) to the trap with a 6 L/min recirculating nitrogen flow for 3 h. The volatiles were isolated from the Tenax trap by extraction with diethyl ether, which was then concentrated to ∼50 µL.

The other principal method used a similar closed-loop system except with a 30 g sample of the fresh tomato, which after blending and holding for 3 min was mixed thoroughly with 240 g of anhydrous sodium sulfate. The mixture was packed into a Pyrex column, which was connected into the closed-loop system containing the Tenax trap and isolated as above.

In addition with 5-ethylcyclopenten-1-carbaldehyde, to get enough material for infrared spectra (IR), isolation was carried out with ∼5 kg amounts of blended tomato using steam distillation−continuous extraction (SDE) under reduced pressure (80 mm) with hexane as solvent.

Quantitative Determination. Measured amounts (usually 1.0 mL) of a dilute water solution, containing 20, 20, and 5.0 ppm, respectively, of the internal standards, 3-pentanone, 2-octanone, and anethole, were added to the blended tomato before the isolation of volatiles described above. Concentrations were determined relative to the internal standards from the GC peak areas using a flame ionization detector. In the case of these minor components the assumption was made that their recoveries and GC detector responses were the same as those of the standards.

GC-MS Analysis. GC-MS analysis was carried out using fused silica capillary columns (60 m *l* × 0.25 mm i.d.) coated with either DB-Wax or DB-1 (J&W Scientific, Folsom, CA) in an HP5890 GC instrument coupled to an HP 5971 quadrupole mass spectrometer.

Infrared Absorption Spectra. 5-Ethylcyclopenten-1-carbaldehyde was isolated using consecutive separations on silicone SF96 and Carbowax 20-M packed GC columns and IR measurement made using carbon tetrachloride solution with an ultramicrocavity cell and reflecting beam condensor on a Perkin-Elmer 237 instrument. Measurement of the IR spectrum of 5-ethyl-2(5*H*)-furanone was carried out in the vapor phase with an HP-5965B FT-IR instrument, which received the sample from a fused silica capillary (30 m *l* × 0.50 mm i.d.) coated with DB-1 in an HP5890 gas chromatograph.

RESULTS AND DISCUSSION

Pentyl Nitrate. A minor peak, separated from fresh tomato volatiles on a DB-Wax column with a retention index of KI =

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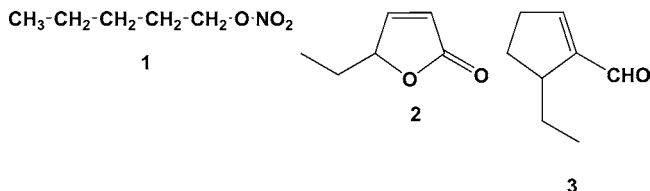
Figure 1. Structures of minor tomato volatiles discussed.

Figure 2. Possible reaction pathway from (*Z*)-3-hexenal to 5-ethyl-2(5*H*)-furanone initially involving autoxidation of (*Z*)-3-hexenal to the peracid.

1220, had a mass spectrum with unusual prominent ions at *m/z* 46 and 76. The GC retention position, on DB-Wax, closely follows that of (*E*)-2-hexenal (KI = 1214, DB-Wax), which, because of its high concentration in tomatoes, can easily obscure the KI = 1220 peak. The mass spectrum was similar to that of some alkyl nitrates and eventually was found to be identical to that of a sample of synthetic pentyl nitrate (**Figure 1**, structure **1**). In addition, the retention index on DB-Wax and DB-1 (KI = 905) was also the same as that of the synthetic pentyl nitrate.

Pentyl nitrate was found in both fresh ripe and mature green tomatoes at about the same concentrations (5−10 µg/kg).

Volatile nitrates are certainly unusual in food volatiles. This seems to be the first report of an alkyl nitrate in a fresh vegetable or fruit. Pentyl nitrate and other volatile alkyl nitrates are well-known (*4*) in cooked cured pork, for which relatively large amounts of sodium nitrite are used in the curing process. Our laboratory had previously identified 1-nitro-3-methylbutane and other nitro compounds in fresh tomatoes (*3*) and discussed the probability that these arise from amino acids. However, with pentyl nitrate we have an unbranched alkyl group that is attached through an oxygen to the nitro group. It might be noted that pentyl nitrate has a retention index clearly different from that of 3-methylbutyl nitrate (KI = 1170, DB-Wax). The mass spectra are similar but can be easily distinguished. Pentanol, a probable lipid-derived volatile, occurs in moderate concentration (30 ppb) in fresh tomatoes and in considerably higher amounts (430 ppb) in tomato plant leaves (*3*). Inorganic nitrates occur in concentrations as high as ∼0.1% in the tissue of many plants. It is possible that the pentyl nitrate might be formed by some kind of enzyme process involving pentanol and plant inorganic nitrate. However, the conditions in the plant are very different from the anhydrous conditions needed in the laboratory. A free radical mechanism had been suggested (*4*) for the formation of alkyl nitrates in cooked cured pork, and it is possible that pentyl nitrate in tomatoes is formed in a related way but by an enzymatic process.

It is interesting that pentyl nitrate, also called amyl nitrate, like glyceryl trinitrate (nitroglycerin) is a vasodilator. The nitrite form, as amyl (or isoamyl) nitrite, is a well-known vasodilator and used for this purpose in medicine. In the minute concentrations found in tomatoes it is doubtful whether pentyl nitrate can have this effect.

5-Ethyl-2(5*H*)-furanone. A compound, found by us previously in fresh tomatoes (*3*), with a retention index of KI = 1750 on DB-Wax and KI = 916 on DB-1, had a mass spectrum consistent with that reported by Winter and Enngist (*5*) for 5-ethyl-2(5*H*)-furanone, then named 2-hexen-4-olide (**Figure 1**, structure **2**), a component of raspberry. It had also been reported in asparagus (*6*) and bread (*7*). There were some differences in the GC retention index found in our laboratory and those reported. This compound was found in tomato fruit at a concentration of 74 µg/kg and also in tomato plant leaves at a concentration of 870 µg/kg. An infrared absorption spectrum of the compound was obtained using vapor phase GC-IR. This showed a strong absorption at 5.52 µm, which is consistent with the lactone structure.
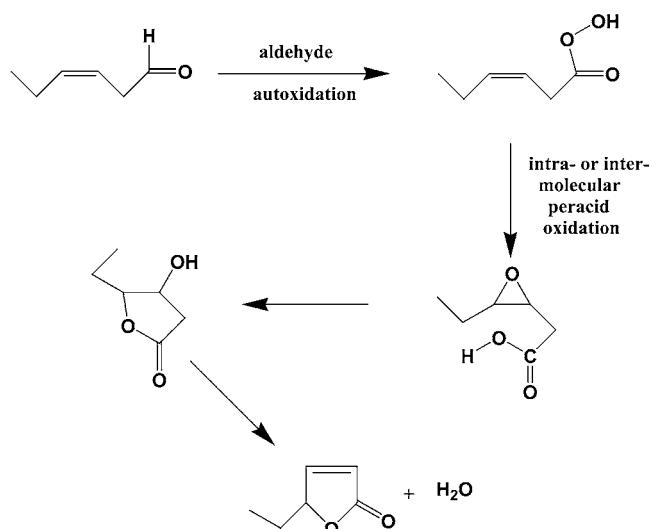
5-Ethyl-2(5*H*)-furanone is a homologue of angelica lactone, the β-form of angelica lactone being 5-methyl-2(5*H*)-furanone. The interconversion of the α-, β-, and γ-forms of angelica lactone under varying GC conditions has been described by Zviely et al. (*8*). They showed that the relative proportions of each isomer, each of which has a different GC retention time, can change depending on GC conditions. A similar isomerization might be expected for the 5-ethyl-2(5*H*)-furanone, which could explain the differences in the reported GC retention indices in the earlier literature.

We also found this compound associated with samples of purified synthetic (*Z*)-3-hexenal and that the compound was formed when solutions of purified (*Z*)-3-hexenal had contact with air for a few hours. Its presence in the volatile concentrates from tomato fruit and leaves probably is due to a similar autoxidation of the natural (*Z*)-3-hexenal. This may also be the reason for its occurrence (*5*−*7*) in other fruits, vegetables, and bread. Whether this occurs in the food or is formed only during the isolation may not be easy to determine.

A possible pathway of the formation of this compound is shown in **Figure 2**. The general mechanism of autoxidation of aldehydes has been thoroughly studied, and it has been found that the first step involves conversion to the peracid. In this case the peracid could then oxidize the (*Z*)-3-double bond to the epoxide. This might occur by intermolecular reaction between different molecules, or it may occur in an intramolecular way. Models indicate that the peroxide group would probably be in sufficiently close proximity to the double bond. This would lead to the epoxide acid, which could (again in an intramolecular process) open the epoxide ring to first form the hydroxy lactone, which could then dehydrate to the unsaturated lactone as shown.

5-Ethylcyclopentene-1-carbaldehyde. A compound isolated at the author's laboratory (*9*) some years ago from a number of vegetables, but particularly potatoes and tomatoes, had a mass spectrum with a molecular ion *m/z* 124 with the most intense ion being at *m/z* 67. Its concentration was ∼1−10 µg/kg. Its GC retention index was KI = 1010 on DB-1 and KI = 1410 on DB-Wax, which is between that of (*E*)-2-heptenal and (*E*)-2-octenal. An infrared spectrum (in CCl₄) showed a carbonyl absorption maxima at 5.9 µm and an aldehyde hydrogen absorption at 3.69 µm. These were both consistent with an aldehyde group conjugated with a single double bond. The

unknown seemed to be a lipid oxidation product because concentration changes in the unknown also coincided with concentration changes in other known lipid oxidation products. A number of compounds were synthesized on the basis of possibilities from interpretation of the mass spectrum, but none matched that of the unknown. In 1993 Werkhoff et al. (*10*) published the results of some studies on the identification of meat volatiles. One of the unusual compounds they identified was 5-ethylcyclopentene-1-carbaldehyde (**Figure 1**, structure **3**), the identity of which they had confirmed by synthesis. The mass spectrum of this compound was consistent with that of the tomato unknown. In addition, the GC retention data [personal communication, G. E. Krammer, Haarmann and Reimer GmbH (now Symrise GmbH), Germany] on both DB-1 (KI = 1008) and DB-Wax (KI = 1422) were also consistent.

This compound is very likely formed by linolenic acid oxidative degradation (possibly enzymatically induced) by a variation of the well-known mechanisms already established for the formation of the aliphatic noncyclic aldehydes (cf. ref *11*). With linolenic acid, formation of a free radical at carbon position 14 could lead to a pentadienyl radical between positions 12 and 16, which might ring close to give an ethyl cylopentenyl system. The other requirement would be for the modified linolenic acid molecule to break between positions 11 and 10, with position 11 being converted to an aldehyde group. Possibly this occurs through β-scission via a position 11 hydroperoxy group. The detailed mechanism of how this whole process occurs is, however, not clear at present.

Conclusions. Of the three minor components discussed, probably the most interesting is 5-ethyl-2(5*H*)-furanone because its formation represents an additional pathway for the degradation of the key tomato aroma component, (*Z*)-3-hexenal.

The identification of pentyl nitrate is interesting because no volatile alkyl nitrates had previously been reported in vegetables and fruits, and the fact that it is a vasodilator may be important in future studies on compounds in foods which could affect human physiology.

The identification of 5-ethylcyclopentene-1-carbaldehyde shows that this unusual lipid oxidation product can occur in vegetables besides its first discovery in meat products.

Although the compounds discussed occur in minute amounts in the tomato samples studied, there may be cases in breeding new varieties, or in using different fertilizing and other growing conditions, when these compounds could occur in much higher amounts.

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