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# Identification of Additional Volatile Constituents of Carrot Roots

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Nine additional components of the oxygenated fraction of the steam volatile oil of carrot roots were identified using direct-capillary gas–liquid chromatography–mass spectrometry and packed-column gas–liquid chromatography–batch infrared spectrometry. These components were geranyl 2-methylbutyrate, geranyl isobutyrate, β-ionone, geranylacetone, *p*-cymen-8-ol, elemicin, eugenol, *p*-vinylguaiacol, and 4-methylisopropenylbenzene. A compound with a raw carrotlike aroma was isolated but could not be positively identified.

The systematic control of the flavor of carrots in both their production and processing requires a knowledge of the volatile constituents of carrots important to their aroma and flavor. A knowledge of the nature of the volatile constituents of carrots is also of interest from the point of view of food safety. The Food and Drug Administration has already been concerned with the quantitative variation of carotatoxin (falcarinol) and myristicin in carrots (Branen and Nagel, 1977).

Some of the authors had previously studied the volatile constituents of carrots (Buttery et al., 1968). However, using odor thresholds they had found that the compounds identified at that time only accounted for a portion of the total carrot odor and that other, then unidentified, compounds must also contribute considerably. More recent studies were carried out by four groups of workers. One of these groups (Heatherbell et al., 1971) generally confirmed the earlier work. Two other groups (Murray and Whitfield, 1975; Cronin and Stanton, 1976) identified 2-methoxy-3-*sec*-butylpyrazine as an additional important contributor to carrot aroma. More recently some of the authors (Seifert and Buttery, 1978) identified some additional sesquiterpene hydrocarbons. However, the present authors still felt that there were other compounds involved in the main carrot aroma that had not then been characterized, and this work reports the results of further studies.

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## EXPERIMENTAL SECTION

**Materials.** Carrots (*Daucus carota* L. var. *Sativa*, type Imperator) were obtained from a local wholesale market.

Authentic samples of organic compounds were generally obtained from reliable commercial sources or synthesized by established methods. Authentic carotol was obtained from commercial carrot seed oil. Samples were purified by gas–liquid chromatography (GLC) before use.

**Isolation of Steam Volatile Oxygenated Fraction.** Carrots (45 kg) were diced into cubes of ca. 1-cm sides and placed in a 90-L glass-lined, steam-jacketed, steel vessel. Attached to the top of the vessel was a large-scale Likens-Nickerson steam distillation continuous extraction head. Freshly distilled diethyl ether (150 mL) containing a trace of Ionox 330 antioxidant was placed in a 250-mL flask attached to the solvent arm of the head. The isolation was carried out at atmospheric pressure for 4 h. The extract obtained was dried over sodium sulfate and the ether removed by distillation using low hold-up Vigreux distillation columns. The concentrate (1.8 g) was taken up in hexane and put onto a column (3.3 × 12 cm) of silica gel (Mallinckrodt SilicAR CC-7). The hydrocarbons were first eluted with hexane (500 mL). The oxygenated fraction was then eluted with ether. The ether was then removed from the oxygenated fraction by distillation using low hold-up distillation columns to give the oxygenated fraction (0.5 g).

**Capillary GC–MS Analysis of the Oxygenated Fraction.** Several different combined gas chromatography–mass spectrometry (GC–MS) studies were made using different GLC conditions and columns, but the main one was that described below. The GLC instrument was

Table I. Additional Components Identified in the Oxygenated Fraction of the Volatile Oil of Carrot Roots

| peak[a] no. | compound[b] | important mass spectral ions | % whole oil |
|---|---|---|---|
| 31 | 4-methylisopropenylbenzene, MS, RT | 117, 132, 91, 115, 39 | |
| 55 | *p*-cymen-8-ol (1), MS, IR, RT | 135, 91, 117, 132, 150 | 0.9 |
| 78 | *p*-vinylguaiacol, MS, IR, RT | 150, 135, 39, 77, 51 | 0.4 |
| 88 | eugenol, MS, IR, RT | 164, 149, 77, 55, 131 | 0.7 |
| 94 | geranylacetone, MS, RT | 43, 69, 93, 136, 151, 194 | 0.03 |
| 98 | β-ionone, MS, IR, RT | 177, 43, 122, 91, 135, 192 | 0.03 |
| 101 | geranyl isobutyrate, MS, IR, RT | 69, 43, 68, 71, 93 | 0.05 |
| 106 | elemicin (II), MS, IR, RT (3,4,5-trimethoxyallylbenzene) | 208, 193, 77, 91, 79 | 0.2 |
| 116 | geranyl 2-methylbutyrate (III), MS, IR, RT | 69, 57, 68, 41, 93 | 0.3 |

[a] Peak numbers correspond to Figure 1 in Buttery et al. (1968). [b] MS, IR, RT = mass spectral, infrared absorption spectral, and GLC retention evidence, respectively. Data consistent with that of an authentic sample measured on the same instrument.

a Hewlett-Packard 5721A and the column was a 115 m × 0.75 mm i.d. stainless steel capillary coated with Silicone SF96(50) containing 5% Igepal CO-880. The injector was held at 170 °C and the column oven was programmed from 80–160 °C at 2 °C/min. The end of the column was directly interfaced with the mass spectrometer through a glass capillary restrictor (0.1 mm i.d.) held at 200 °C. The mass spectrometer was a VG-Micromass 70/70F double-focusing instrument equipped with a dual electron ionization (EI) and chemical ionization (CI) ion source. The source temperature was held at 200 °C. Separate GLC runs, under essentially identical GLC conditions, were made for EI (70 eV) and CI (isobutane reagent gas).

**Packed Column GLC–Infrared Spectra Analysis.** Samples were separated from the oxygenated fraction using a 3 m long by 0.64 cm o.d. stainless steel GLC column packed with 80–100 mesh Chromosorb G-DMCS coated with 2% Silicone SF96(50). The column was temperature programmed from 80–160 °C at 2 °C/min. Samples were collected in 3 mm o.d. × 14 cm long Pyrex tubes. Some samples were rechromatographed on a Carbowax 20-M column of otherwise similar description to the silicone column. The infrared absorption (IR) spectra were measured as thin films between ultramicro salt plates or as solutions in $CS_2$ with an ultramicro cavity cell using a reflecting beam condenser on a Perkin-Elmer Model 237 instrument.

## RESULTS AND DISCUSSION

The volatile oil was obtained by atmospheric steam distillation continuous extraction of the carrots. The oxygenated fraction was then separated by selective adsorption on silica gel. Components were characterized using the information from both EI and CI GC–MS analyses. EI spectra were compared with those of the authentic samples. Packed column GLC separation with batch infrared spectrometry was also used to confirm and aid the mass spectral identification. Table I lists the components identified, together with the means of identification and some idea of the relative percent of the component in the whole oil. Although several lots of carrots were examined and these quantitative figures seem fairly representative of these samples, they are only meant to give a rough idea of the concentration in a typical oil. No systematic quantitative study was made.

The GLC chromatograms obtained on the silicone capillary column were quite comparable to that obtained in the earlier work done in this laboratory, and the peak numbers in Table I (and mentioned later in the text) correspond to that used in Figure 1 in the earlier publication (Buttery et al., 1968).

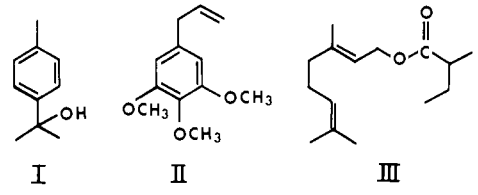


**Figure 1.** Structures of some of the components. I is *p*-cymen-8-ol, II is elemicin, and III is geranyl 2-methylbutyrate.

With the amount of β-carotene in carrots it is not surprising to find β-ionone. β-Ionone (as well as α-ionone) had been found previously in oxidized dehydrated carrots (Ayers et al., 1964). With its potent aroma [odor threshold 0.007 parts per billion (ppb) in water] β-ionone must certainly contribute to the total aroma.

Although geraniol esters such as acetate and isobutyrate are fairly common in essential oils, geranyl 2-methylbutyrate (III) is unusual. It has a pleasant "sweet" aroma and occurs in reasonable amount (0.3%) in the whole carrot oil. The more common geranyl isobutyrate (odor threshold 13 ppb in water) was also characterized.

Both eugenol (odor threshold 6 ppb in water) and *p*-vinylguaiacol formed rather broad tailing peaks on the stainless steel silicone capillary GLC column. They were better resolved on a glass Tween 20 capillary column. There were several other components detected which seemed to be related to the previously characterized myristicin but the only one that could be identified was elemicin (II).

The compound 4-methylisopropenylbenzene, being a hydrocarbon, does not really belong in the oxygenated fraction. It seems to be formed by dehydration of *p*-cymen-8-ol (I) in the injector of the gas chromatograph although the injector was only kept at a moderate temperature (170 °C).

The previous identification of carotol by mass spectra and GLC retention time (Buttery et al., 1968) was confirmed by isolation using packed column GLC and comparison of its infrared spectrum with that of an authentic sample of carotol isolated from carrot seed oil.

Peak 104 was identified as the commercial antioxidant BHT. It probably results from plastic packaging used in transporting the carrots. There are still a considerable number of peaks that could not be identified even though both mass and infrared spectra were obtained. These generally seem to be either oxygenated sesquiterpenes (e.g., peaks 112 and 120–138) or oxygenated terpenes (e.g., peaks 40 and 65 and minor peaks from ca. 40–94). Comparison with oil isolated under vacuum showed that a number of the $C_{10}$ oxygenated terpenoids seemed to increase in

concentration considerably as a result of atmospheric isolation. These may possibly result from some nonvolatile peroxide type precursor. It was noticed that terpinolene, the major carrot hydrocarbon, very readily underwent autoxidation when isolated in the pure state. This process probably also happens to some extent in the carrot. The peroxides formed by such oxidation might be expected to break down on heating, e.g., when carrots are cooked or during steam distillation at atmospheric pressure. Terpinolene has a methylene group between two double bonds (doubly allylic). Such methylene groups are well known to be very susceptible to autoxidation. It is fairly easy to see how $\alpha$-terpineol and terpenin-4-ol could arise from terpinoline through oxidative processes in which the tetra-substituted double bond is attacked. When carrots are blended (which could increase enzymatic oxidation) these two alcohols increase in concentration relative to other components.

Peak 65 (ca. 0.2% of the whole oil), in our opinion, has a moderately intense raw carrotlike aroma. It could not be characterized with certainty even though mass, IR, NMR, and UV spectra were obtained. The mass spectrum measured on Time of Flight mass spectrometer was as follows (two most intense ions each 14 mass units above $m/e$ 34, intensities in parentheses, molecular ion in boldface type): 39 (24), 41 (19); 53 (14), 55 (14); 65 (16), 67 (22); 79 (34), 82 (41); 91 (36), 93 (29); 105 (24), 107 (17); 117 (29), 121 (19); 135 (100), 136 (19); 149 (22), **150** (43). This mass spectrum was somewhat similar to that of carvacrol. CI mass spectrometry confirmed the molecular weight of 150. IR and NMR spectra, however, showed that there was no aromatic ring. IR absorption spectra indicated a probable alcohol with a band at 2.78 $\mu$m and a strong band at 9.6 $\mu$m. A $^1$H NMR spectrum (90 MHz, $CDCl_3$) showed $\delta$ 1.5 (s, 1 H), 1.62 (s, 2 H), 2.08 (s, 2 H), 2.5 (m, 1 H), 4.5 (s, 2 H), 5.06 (s, 1 H), 5.55 (s, 1 H). A UV absorption spectrum in methanol showed an absorption maximum at 2600 Å with $\epsilon$ 2000 (ca.). Catalytic hydrogenation (Pd on charcoal, 10 psi H, 25 °C, 15 h) gave three isomeric alcohols with molecular weight 154 and some 4-isopropylmethylcyclohexane (two isomers). Unfortunately the authors could not come up with a structure that fitted all of these data. It is possible that the compound is changing during the handling.

The mass and infrared spectra of the components in Table I were compared directly with those of authentic samples. The mass spectra of most of the components are also available in the literature (e.g., Stenhagen et al., 1974) except for that of geranyl 2-methylbutyrate, which is listed below: 39 (5), 41 (49); 53 (5), 57 (69); 68 (53), 69 (100); 80 (17), 85 (21); 92 (6), 93 (32), 107 (3), 108 (1); 121 (13), 123 (2); 136 (11), 137 (2); 154 (1); 169 (1) (no molecular ion).

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# On the Electrophoretical Differentiation and Classification of Proteins. 10. Comparative Investigation of Yeast Proteins of Various Genera by Means of Isoelectric Focusing in Polyacrylamide Gels

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The water-soluble proteins of eight different yeast genera (*Brettanomyces claussenii, Candida utilis, Cryptococcus laurentii, Debaryomyces hansenii, Kloeckera apiculata, Kluyveromyces lactis, Saccharomyces cerevisiae, Schizosaccharomyces pombe*) obtained by cell disruption with freeze-pressing were resolved by means of isoelectric focusing in cylindrical polyacrylamide gels. In the pH range of 3.5–10.0 carrier ampholytes, about 30 protein bands were found with isoelectric points of 4.5 to 9.7. By using a special one-rod electrode, the pH of the gel slices in minute quantities of distilled water were measured, and the trend of the pH gradient in the whole gel was determined. By means of densitometer tracing of the protein pattern in connection with the pH gradient, the isoelectric points of particular proteins were established. The protein patterns were dissimilar and reproducible under constant working conditions.

In the identification of microorganisms, increasing attention has been paid to physicochemical methods which are more rapid compared with morphological, physiological, and serological methods and which can ideally supplement them (Hedén and Illéni, 1975; Mitruka, 1975). Among these, electrophoresis of proteins has been proved to be a convenient method for diagnostic and taxonomic purposes.

In spite of the rapid increase in the use of electrophoretic methods in bacteriology, it has been used to a very limited extent with yeasts.

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