# Characterization of Some Previously Unidentified Sesquiterpenes in Carrot Roots

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Some previously unidentified sesquiterpenes from the steam volatile oil of carrot roots have been isolated by gas chromatography (GLC) and identified by combinations of mass spectrometry, infrared spectrometry (IR), proton magnetic resonance ($^1$H NMR), and GLC relative retention times. $\alpha$-Humulene and $\beta$-farnesene were characterized by IR, mass spectra, and GLC retention times for both authentic samples and published data. Published mass spectra and retention data were consistent with those found for $\alpha$-bergamotene. $\gamma$-Muurolene was tentatively identified from its mass spectra and retention data. A pair of compounds, previously identified as $\beta$- and $\gamma$-bisabolene, were isolated by GLC. The IR spectra of both compounds were consistent with that published for $\gamma$-bisabolene. Additional data from $^1$H NMR spectra and GLC retention time indicated that these two sesquiterpenes are probably both geometric isomers of $\gamma$-bisabolene, the major isomer being tentatively assigned the $E$ configuration and the minor isomer the $Z$ configuration (based on literature GLC retention values). No $\beta$-bisabolene was found.

The first comprehensive study of carrot root volatiles was made by Buttery et al. (1968). Since that study other investigations and especially that of Heatherbell et al. (1971) confirmed the volatile composition of carrot roots found by Buttery et al. and in addition characterized the terpene $\alpha$-phellandrene and some lower boiling components. Three sesquiterpenes had been identified by Buttery et al. (1968). Carophyllene was identified by its gas chromatography (GLC) retention time, mass spectra, and infrared spectra (IR). $\gamma$-Bisabolene was identified by proton magnetic resonance ($^1$H NMR) and by IR. Only mass spectral evidence was available in earlier studies for the identification of $\beta$-bisabolene. The authors have reexamined the sesquiterpene hydrocarbon fraction of the volatile oil from large amounts of carrot root. The larger sample facilitated the characterization of sesquiterpenes, especially those at low concentration.

## EXPERIMENTAL SECTION

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

**Isolation of the Volatile Oil and Fractionation.** Forty-kilogram lots of carrots were treated in a large-scale steam distillation extractor with a Likens head similar in design to that used in the characterization of carrot volatiles (Buttery et al., 1968). Hexane was used as a solvent in the atmospheric extraction that yielded ca. 40 ppm of volatile oil. The hydrocarbon fraction was obtained in ca. 58% yield by selective adsorption of the volatile oil on a silica gel column (17 cm × 3.5 cm i.d.) and elution with pentane (600 mL). The hydrocarbon sample was concentrated on a steam bath and examined by GLC.

**Capillary GLC–Mass Spectrometry.** A 0.75 mm i.d. × 300 m long stainless steel capillary column coated with Tween 20 containing 5% Igepal CO-880 was used. The carrier gas, helium, had an inlet pressure of 8 psi. Hydrogen flame detection was used. The injector temperature was at 175 °C. The column was programmed from 100 to 140 °C at 0.5 °C/min and held at 140 °C. A splitter allowed a portion of the sample from the column to go to a modified Consolidated 21-620 cycloidal type mass spectrometer using a silicone membrane separator. Mass spectra of the sesquiterpenes were obtained with this system.

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**Packed Column GLC.** A 0.64 cm i.d. × 3.1 m stainless steel column packed with 80–100 mesh Chromosorb G, treated with DMCS, and coated with 2% Carbowax 20M was used to separate the sesquiterpenes for IR analysis. The injector temperature was at 200 °C, detector 200 °C, and column manually programmed at a nonlinear rate from 70 to 150 °C and held at 150 °C for most separations. Separations of the bisabolene isomers required a slower program from 70 to 130 °C. The detector was a thermal-conductivity type.

**Infrared Spectra.** The IR was obtained from a Perkin Elmer 237 infrared spectrophotometer with samples run neat on micro salt plates.

**Nuclear Magnetic Resonance Spectra.** Samples were measured in $CDCl_3$ at 100 MHz using a Varian HA-100 instrument.

## RESULTS AND DISCUSSION

The GLC patterns obtained from both the Carbowax 20M packed column and the Tween 20 capillary column were similar to that obtained by Buttery et al. (1968) using a silicone SF 96 capillary column. A typical chromatogram of the sesquiterpenes on the Tween 20 capillary is shown in Figure 1.

**Mass Spectra.** Capillary GLC–mass spectral data obtained for the sesquiterpenes, in order of their retention times, are as follows (two most intense ions every 14 mass units above $m/e$ 34, intensities in parentheses, molecular ion in boldface). $\alpha$-Bergamotene: 41 (55), 43 (21); 53 (12), 55 (50); 67 (12), 69 (44); 77 (29), 79 (18); 91 (35), 93 (100); 105 (23), 107 (28); 119 (68), 120 (18); 133 (6), 135 (4); 161 (14); **204** (3).

Unknown: 41 (82), 42 (10); 53 (24), 55 (34); 67 (22), 69 (100); 79 (32), 81 (30); 91 (30), 93 (74); 105 (16), 107 (20); 119 (20), 120 (16); 133 (26), 135 (8); 147 (8); 161 (14); **204** (6).

$\beta$–Farnesene: 41 (100), 43 (17); 53 (31), 55 (44); 67 (33), 69 (100); 79 (35), 81 (29); 91 (24), 93 (70); 105 (11), 107 (13); 119 (13), 120 (18); 133 (24), 134 (9); 147 (4); 161 (15); 189 (2); **204** (5).

$\alpha$-Humulene: 41 (73), 43 (23); 53 (32), 55 (30); 67 (24), 69 (21); 79 (32), 80 (56); 92 (30), 93 (100); 105 (22), 107 (26); 119 (16), 121 (47); 133 (6), 136 (8); 147 (18); 161 (12); 189 (2); **204** (8).

$\gamma$-Muurolene: 41 (29), 43 (21); 53 (8), 55 (17); 67 (8), 69 (14); 79 (18), 81 (24); 91 (26), 93 (23); 105 (33), 107 (15); 119 (26), 120 (24); 133 (13), 134 (8); 147 (7), 148 (2); 161 (100); **204** (13).

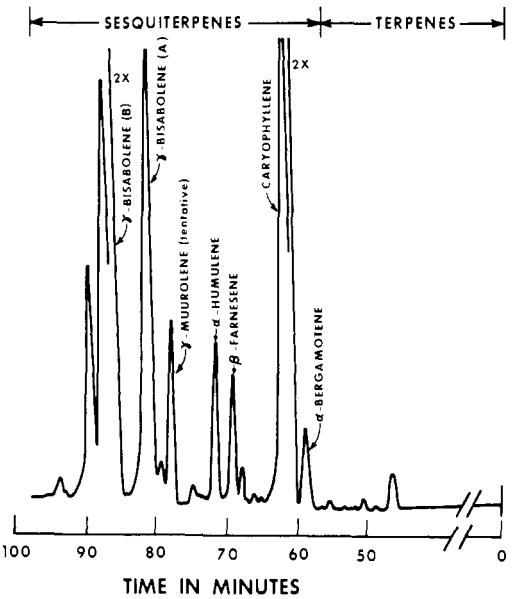


**Figure 1.** Capillary GLC chromatogram of the sesquiterpenes from the steam volatile oil of carrot roots using a 0.75 mm i.d. × 300 m long stainless steel capillary column coated with Tween 20 containing 5% Igepal CO-880 programmed from 100 to 140 °C at 0.5 °C/min and held at 140 °C.

The mass spectra for α-bergamotene and β-farnesene was consistent with that published respectively by Moshonas and Lund (1970) and by Murray (1969). The mass spectra for both β-farnesene and α-humulene were consistent with spectra of authentic samples obtained from hops by Buttery et al. (1966). The mass spectra for the tentatively identified γ-muurolene compared well with published spectra by Stenhagen et al. (1974). A sesquiterpene (retention time just before β-farnesene) has a mass spectra similar to the spectra of (*Z*)-β-farnesene published by Anet (1970) except intensities were much higher for many of the fragments indicating the possibility of a mixture. The retention times of the six possible α–β isomers of farnesene on the Carbowax 1540 column as found by Anet (1970) puts *Z*-β first followed closely by (*E*)-β-farnesene. The unknown sesquiterpene may be the (*Z*)-β-farnesene.

**Infrared Spectra.** IR spectra were obtained on samples separated by packed column GLC and collected in borosilicate tubes. The IR spectra obtained for α-humulene in the 2000 to 700 $cm^{-1}$ region where vs means very strong, s strong, m medium, w weak, and vw very weak is as follows: vs 1445, 1388, 970; s 1365, 825; m 1665, 1213, 1180, 890; w 1470, 1295, 1270, 1147, 1100, 1030, 995, 845; vw 1455, 1325, 1135, 1070, 1005, 940, 925, 906, 875, 785, 775, 765, and 745 $cm^{-1}$. The spectra obtained for β-farnesene was: vs 890; s 1600, 1450, 1380, 990; m 1640, 1110, 830; w 1790, 1670, 1385, 1152, and vw 1300, 1090, 1055, 1025, 750 $cm^{-1}$. These spectra were in excellent agreement with spectra published by Wenninger et al. (1967) and with spectra of authentic samples obtained from hops as reported by Buttery et al. (1966).

The geometric configuration of β-farnesene is probably the cis or *E*-β isomer because there was IR absorption at 1152 $cm^{-1}$ (w) for our compound corresponding to absorption for $-C(CH_3)=CH$ at 1150 $cm^{-1}$ (w) as reported by Anet (1970) for the *E*-β isomer, while he found no band at 1150 for the *Z*-β isomer.

**Bisabolenes.** Different investigators (Anderson and Falcone, 1969; Nigam and Levi, 1966; Wenninger et al., 1967) obtained inconsistent GLC orders of elution for β- and γ-bisabolenes on similar or even the same stationary phases.

Samples of carrot root hydrocarbons were separated on a packed column (2% Carbowax 20M on chromosorb G) which was manually programmed from 70 to 130 °C. The three peaks which were resolved in the 80–90-min region of the capillary GLC run (see Figure 1) were well resolved on the packed column except for the last peak. The IR and $^1H$ NMR spectra obtained from the collected samples of these peaks were very similar to each other. The first peak should have been β-bisabolene if the previous mass spectral identification was correct. A comparison of the IR spectra of β-bisabolene published by Nigam and Neville (1968) showed none of the three peaks was β-bisabolene. The strong adsorptions for C=C stretch at 1636 $cm^{-1}$ and the out of plant bending of CH at 885 $cm^{-1}$ for $CH_2=C<$ characteristic of β were weak or absent in the bisabolenes from carrot root hydrocarbons. The IR spectra of the three compounds matched γ-bisabolene.

The $^1H$ NMR spectra of the bisabolenes was obtained in $CDCl_3$ on a Varian 100 instrument. Chemical shifts from 2.01 to 2.07 ppm and from 2.25 to 2.32 ppm of the methylene protons (probably the allylic ring methylenes) and the similarity of the rest of the spectra of the first two bisabolenes are consistent with an assignment of two geometric isomers (*Z* and *E*) for these compounds. The $^1H$ NMR spectrum published by Minyard et al. (1966) for (*Z*)-γ-bisabolene run in $CCl_4$ on a Varian A-60 instrument could not be compared directly to our data obtained under different conditions. The variations in chemical shift data especially the downfield signal areas at 5.02 (1 proton) and 5.26 (1 proton) found by Minyard (1966) compared to 5.12 and 5.37 ppm in our spectra would be reasonable differences due to different instrumentation and solvent effects. It is reasonable that the first two peaks are γ-bisabolenes.

Wolensky et al. (1976) reported that (*Z*)-γ-bisabolene had a GLC retention time before that of the (*E*)-γ-bisabolene on a 0.64 cm × 2 m column packed with chromosorb W containing 2% Carbowax 20M. Since we also used a 2% Carbowax 20M packed column for this identification work, the *Z* isomer is tentatively assigned as the first γ-bisabolene (labeled "A" in Figure 1) and the (*E*)-γ-bisabolene as the second peak (labeled "B" in Figure 1). The last peak of the trio was not completely separated. Its IR spectra was identical with γ-bisabolene with no absorptions incompatible with the γ isomer. The absence of significant amounts of β-bisabolene in the unknown peak is indicated by the lack of strong C=C stretch absorption at 1636 $cm^{-1}$ and strong CH out of plant bending at 885 $cm^{-1}$.

**Capillary Column GLC Retention Times.** Relative retention times (caryophyllene = 1.00) found on the Tween 20 capillary column were bergamotene 0.90, β-farnesene 1.19, α-humulene 1.30, and γ-muurolene 1.53. These retention times were reasonably consistent with data published by Nigam and Levi (1966), Wenninger et al. (1967), and with Anderson and Falcone (1969) and also for authentic samples of β-farnesene and α-humulene from styrian hop oil run on the Tween 20 capillary.

The percentage of these newly identified sesquiterpenes is given in Table I. Peak areas (peak height times peak width at half-height) obtained from a chromatogram of the hydrocarbon fraction on Tween 20 capillary column and the percentage of hydrocarbons obtained by silica gel column chromatography of the whole carrot root oil were used to calculate the relative percentages of the sesquiterpenes reported. Most of the sesquiterpenes are found in relatively small amounts. The (*E*)-γ-bisabolene is the largest peak while the next largest is the previously

Table I. Sesquiterpenes Characterized in the Steam Volatile Oil of Carrot Roots

| | Rel % of hydrocarbon fraction | Rel % of whole oil |
|---|---|---|
| α-Bergamotene | 0.4 | 0.2 |
| Caryophyllene | 8.8 | 5.1 |
| β-Farnesene | 0.5 | 0.3 |
| α-Humulene | 0.5 | 0.3 |
| γ-Muurolene[a] | 0.5 | 0.3 |
| γ-Bisabolene (A)[b] | 0.4 | 0.2 |
| γ-Bisabolene (B)[c] | 11.3 | 6.7 |

[a] Tentative. [b] γ-Bisabolene (A) tentatively assigned as *Z* isomer. [c] γ-Bisabolene (B) tentatively assigned as *E* isomer.

identified caryophyllene with 5.1% of the whole oil.

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# Formation of Heterocyclic Compounds from the Reaction of L-Rhamnose with Ammonia

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The reaction of L-rhamnose with ammonium hydroxide in an aqueous system was investigated, and 95 volatile reaction products were isolated using continuous liquid–liquid extraction with dichloromethane for 16 h. Qualitative and quantitative analyses of the reaction products were made using GC–MS techniques; 65 compounds were positively identified and 20 others tentatively identified. The principal constituents of the extracts were pyrazines, pyrroles, and imidazoles. Eight compounds hitherto unreported to be present in foods or sugar–amine model systems were identified. These are: 2-methyloxazole, 2-ethylpyrrole, 2-ethyl-5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 4,5-dimethyloxazole-2-carboxaldehyde, 5-methyl-5*H*-cyclopentapyrazine, 2-ethyl-5*H*-cyclopentapyrazine, 2-ethyl-3-methyl-5,8-dihydroquinoxaline, and 2-amino-5-methylpyridine. A reaction mechanism for the formation of cyclic pyrazines featuring addition of ammonia to cyclopentenone derivatives and subsequent condensation with α-amino carbonyl compounds is advanced, as is also a possible mechanism for the formation of imidazoles from α-amino carbonyl compounds and carboxylic acids.

It is a well-known fact that the reaction of sugars with aqueous ammonia produces many heterocyclic compounds which include pyrazines, imidazoles, pyridines, and piperazines (Rizzi, 1974; van Praag et al., 1968; Jezo and Luzak, 1966). The reaction products of sugars and amine compounds have been studied intensively over the last several years, since pyrazines were recognized as important flavor constituents of a variety of roasted or toasted foods (Guadagni et al., 1972). Formation pathways for those heterocyclic compounds have been proposed by many researchers (Rizzi, 1974; Shibamoto and Bernhard, 1977 a,b; Velisek et al., 1976). The present view among most investigators suggests that there are two possible formation pathways. One is that the decomposition of sugars produces unstable α-diketones and aldehydes and that those carbonyls subsequently react with amines to form heterocyclic compounds (Walradt et al., 1971). The other is that sugars react with amines followed by formation of α-amino carbonyl intermediates, and these intermediates produce heterocyclic compounds (Jezo and Luzak, 1966). Recently, Shibamoto and Bernhard (1977b) reported the proposed formation pathways of 12 alkylpyrazines which were isolated from various sugar (D-glucose, L-rhamnose, 2-deoxy-D-glucose)–ammonia model systems and the authors' investigations support the second hypothesis. Ten α-amino carbonyl intermediates were proposed in order to explain the formation pathways of pyrazines from the sugar–ammonia model systems. The present paper covers

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