

Analysis of Carrot Constituents: Myristicin, Falcarinol, and Falcarindiol

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Falcarinol, falcarindiol, and myristicin contents of carrots, Daucus carota L., were determined by a sequence of dichloromethane extraction, column chromatographic purification, and gas-liquid chromatographic analysis. High Color 9, Long Emperor 58, Danvers 126, and Spartan Bonus varieties were grown in Wisconsin (1979-1982), Florida (1980-1982), California (1980-1982), Arizona (1981), and Illinois (1981-1982). Gold Pak, Nantes Half Long, Red Cored Chantenay, and Royal Chantenay varieties were grown in Illinois (1980). The overall mean of falcarinol for 510 observations of these eight commercial varieties was 24.1 mg/kg; that of falcarindiol for 389 observations was 65.1 mg/kg. The standard error of a mean based on 2 samples of 4 carrots, with 2 aliquots per sample, was 2.8 for falcarinol and 4.8 for falcarindiol. Varietal means ranged from 11.3 to 28.2 for falcarinol and 53.3 to 106.9 for falcarindiol. Myristicin was detected in only one variety of carrots (Spartan Bonus) harvested in Wisconsin in 1981; the mean of 12 observations, 2 samples, was 1.4 mg/kg with a range of 1.3 to 1.5 mg/kg.

Crosby and Aharonson (1), in the course of their investigation of naturally occurring toxicants in foods, discovered that an acetone extract of carrots was toxic to the organism Daphnia magna Straus. The purified toxin had an LD₅₀ in mice of 100 mg/kg. They gave this substance the trivial name "carotatoxin" and published a tentative structure. Bentley and Thaller (2) published a corrected structure and gave proof that the compound Crosby and Aharonson isolated was falcarinol, a polyacetylenic alcohol (I) (Figure 1) first isolated by Bohlmann et al. (3), from Falcaria vulgaris Bernh. Other such compounds have since been isolated from carrots (falcarindiol II, acetylfalcarindiol III, and falcarinolone IV) (4). Recently, the function of falcarindiol as an antifungal agent in the disease response mechanism of carrots was recognized (5-7). The LD₅₀, ip, in mice of falcarindiol was found to be 133 mg/kg (8).

Myristicin V, a phenylpropenoid, is frequently found in carrots (9). It is known to stimulate central nervous system activity (10) and to enhance the activity of certain insecticides (11), and it is suspected of being involved in the disease response mechanism of carrots (12).

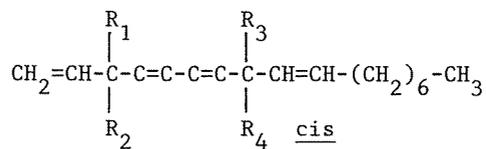
Carrots were analyzed for falcarinol, falcarindiol, and myristicin to determine if concentrations varied with respect to genetic (variety) and/or environmental changes (location and year). The data generated define the level of those toxicants normally present in carrots grown for processing (Danvers 126, Spartan Bonus, Royal Chantenay, Red-Cored Chantenay, and Nantes Half Long), for fresh market (Long Imperator 58, High Color 9, and Gold Pak), and for genetic studies (B10138, B9304, B0493, B3615, and B10720).

Experimental Section

Plant Materials. Carrot roots, Daucus carota L., were grown at NRRC or at the following locations as directed by the Department of Horticulture, University of Wisconsin, Madison: Wisconsin, Florida, California, and Arizona.

Carrots were harvested by hand and shipped unwashed to NRRC in plastic bags. Analysis of the four varieties was completed 1 to 2 weeks after each harvest sample was received. Carrots grown in Illinois (NRRC) were harvested by hand and analyzed immediately.

Chromatography Equipment and Conditions. Gas-liquid chromatography was performed with a Bendix 2600 instrument (flame ionization detectors); injector temperature was 250°C and detector temperature was 270°C, with helium carrier gas at 10-20 ml/min, air at 500-600 ml/min, and hydrogen at 50 ml/min. Columns were programmed from 80 to 250°C at 4°C/min with a



	R_1, R_2	R_3, R_4
I Falcarinol	(OH, H)	(H, H)
II Falcarindiol	(OH, H)	(OH, H)
III Acetylfalcarindiol	(OAc, H)	(OH, H)
IV Falcarinolone	(=O)	(OH, H)

V Myristicin

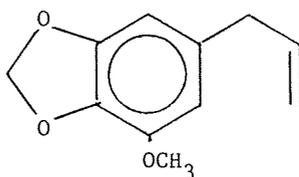


Figure 1. Structures of carrot constituents.

5-min final hold. Glass columns 6 ft by 1/8 in. were used, one packed with 3% SE-52 (Applied Science Laboratories, Inc.) on 80-100 mesh Gas-Chrom Q (nonpolar), and one packed with 3% OV-17 (Supelco Inc.) on 100-120 mesh Chromosorb W HP (intermediate polarity).

Dichloromethane Extraction, Column Chromatographic Purification and Gas-Liquid Chromatographic (DE-CCP-GLC)

Analysis of Carrots. A longitudinal quarter was removed from each carrot included in the sample. Each quarter was cut into 2-mm cross sections, and these were immediately placed in a 2 l stainless steel blending container. Antioxidant (Antioxidant 330, Ethyl Corp. or Ionox 330, Shell Chemicals) (2 mg/5 g carrot fresh weight) and dichloromethane (10 ml/5 g carrot) were added quickly, and the constituents were blended in a commercial Waring Blendor at moderate speeds for a total of 12 min. Alternate cycles of blending and cooling (4 min each) reduce evaporation of dichloromethane. Any solvent losses were corrected for by weighing the capped blender and contents before and after blending and then replacing the lost solvent. Maximum blending efficiency was obtained with 300- to 500-g of carrots. Teflon seals were inserted in the blender cup blade assembly to replace the standard seals. After dichloromethane lost by evaporation was replaced, the mixture was blended for an additional 30 s and aliquots transferred to 250-ml centrifuge bottles capped with a screw cap or Saran Wrap. The mixture was centrifuged for 15 min at 1500 g, and then 50 ml of the clear extract was removed with a large syringe fitted with a long 17-gauge needle passed through the pulp. One mg of methyl palmitate as internal standard was added to each of two 50-ml aliquots of centrifuged extract. These aliquots were concentrated under vacuum on a rotating evaporator at 25°C to about 3 ml; 7 ml of hexane was added and the solution was reconcentrated to 3 ml; 4 ml of hexane was added and the solution was reconcentrated to 1 ml. The resulting hexane solution was chromatographed on 2 g of Silica gel (70-325 mesh; EM Reagents). Glass columns 6 X 140 mm, fitted with 50-ml reservoirs, were constructed from 8 mm o.d. glass tubing and 25 X 140 mm test tubes. Yellow pigments were eluted with hexane until the hexane eluate was colorless (40 to 60 ml). The fraction of interest was eluted with 20 ml of ether-hexane (45:55). The eluate was concentrated to about 0.75 ml and transferred to a ½ dram vial with teflon-lined cap. Analysis by GLC (3-6 µl injection) gave symmetrical peaks, which were integrated electronically.

Quantities of individual toxicants were calculated by using the formula

$$\frac{\text{mg of toxicant}}{\text{kg of sample}} = F_t \frac{A_t W_p V_a}{A_p W_c V_r} \times 10^3$$

where F_t is the response factor of the toxicant in relation to methyl palmitate, A_t and A_p are the areas of the toxicant and methyl palmitate gas chromatographic peaks, respectively, W_p is the quantity of methyl palmitate added in milligrams, W_c is the initial weight of carrots used, V_a is the initial volume of solvent used for extraction, and V_r is the volume of solvent taken after centrifuging as the analysis aliquot. Response factors were determined by chromatographing a mixture of known weights of the toxicants myristicin, falcarinol, and falcariindiol, with a known weight of methyl palmitate (13).

Results and Discussion

Carrots (*Daucus carota* L.) make a significant contribution to the American diet by providing fiber, minerals, and vitamins (14). These essential nutrients must be maintained at present levels or improved as the plant breeder attempts to develop carrots of better culinary quality. Other components of carrots, those that contribute to the plant's defense against damage, disease, and insects, must be monitored to ensure that they do not increase or decrease to a troublesome level. Therefore, today more than ever, the plant breeder and chemist need to cooperate in developing new varieties.

Most of the carrots grown in the USA (80%) are for fresh market, the remainder are grown for processing (15). An unestimated, but significant, carrot crop also is produced in the home garden. The main thrust of this study involved four commercial varieties (for fresh markets, Long Imperator 58 and High Color 9; for processing, Danvers 126 and Spartan Bonus) grown in Wisconsin, Florida, California, Arizona, and Illinois during 1979-1982. Other varieties commonly grown in home gardens also were examined (Royal Chantenay, Red Cored Chantenay, Gold Pak, Nantes Half Long) as well as experimental genetic material from the USDA Carrot Improvement Program (low volatile inbreds B10138 and B9304, high volatile inbreds B0493 and B3615, and white carrot B10720).

During the early stages of the study (1979-1980), only falcariindiol was measured; later measurements (1980-1982) included falcariindiol and myristicin. Identification of peaks in the chromatographic record was based on elution times relative to methyl palmitate (internal standard) in nonpolar (3% SE-52) and intermediate polarity (3% OV-17) packed columns. Identification of myristicin, a small peak, was generally confirmed by GC/MS.

The data acquired were divided into three main groups, depending on sampling and analysis protocol, for further evaluation: (a) Type 1: 22 selections, with four samples each; sample weight was 50 g, with 4 carrots per sample. Aliquots of each sample were analyzed. (b) Type 2: 5 selections, with 5 to 18 carrots per sample. Carrots were quartered, and aliquots of two quarters each were analyzed in duplicate. (c) Type 3: 28 selections with two samples each; sample weight was 300 to 500 g, with 10 to 30 carrots per sample. Aliquots of each sample were analyzed in duplicate. Although sampling procedures differed, there were 8 observations of each toxicant for each carrot selection. Analysis of variance was computed for each group; the least significant difference (LSD, 0.05 level) is reported where appropriate (16).

The largest sources of variation in the analysis are due to sampling and lack of agreement between GC columns. Larger composite samples (Type 3) gave less variation for falcarindiol; falcarinol generally gave good agreement with all sampling types. Agreement between GC columns also was much better for falcarinol than for falcarindiol. One probable explanation for these results is that the elution time for falcarinol is very close to that of methyl palmitate, whereas elution time for falcarindiol was somewhat later. Also, in the OV-17 column, establishment of a base line at the falcarindiol peak was complicated because of minor peaks that were not completely resolved from falcarindiol. Minor constituents may have coeluted with falcarindiol.

Myristicin was detected in only one variety of carrots (Spartan Bonus) harvested in Wisconsin in 1981; the mean of 12 observations, 2 samples, was 1.4 mg/kg with a range of 1.3 to 1.5 mg/kg. Wulf et al. (9) also report myristicin in carrots. They show 15 mg/kg for the variety Imperator, with lesser amounts for other varieties. The polyacetylenes, on the other hand, were found in all varieties (Table I).

The overall mean of falcarinol for 510 observations of all commercial varieties was 24.1 mg/kg; that of falcarindiol for 389 observations was 65.1 mg/kg. Previous reports on toxicant levels, except for those relating to disease response, tended to overlook the falcarindiol content. The mean of the falcarindiol/falcarinol ratio was 4.4, reflecting the higher concentration of falcarindiol.

The standard error of a mean based on 2 samples (4 carrots per sample), 2 aliquots per sample, and 2 runs per sample, or 8 observations, was 2.8 for falcarinol and 4.8 for falcarindiol. For single observations, the respective standard deviations were 15.2 and 24.8. About 70% of the variation for falcarinol was associated with sample, but only about 25% for falcarindiol. For falcarindiol, variation associated with the assay differences contributed most of the variation. Precision for both toxicants

Table I. Summary of Means of Falcarinol and Falcarindiol for Thirteen Carrot Varieties¹

Variety	Falcarinol		Falcarindiol		Mean ratio
	N ²	mg per kg	N	mg per kg	falcarindiol/ falcarinol
High Color 9	104	11.3	72	54.5	9.0
Long Imperator 58	114	28.1	85	69.0	2.8
Danvers 126	124	28.2	92	59.3	2.7
Spartan Bonus	<u>118</u>	<u>28.1</u>	<u>90</u>	<u>74.4</u>	<u>4.2</u>
Mean	460	24.3	339	64.7	4.4
Gold Pak	10	13.4	10	106.9	8.0
Nantes Half Long	8	22.2	8	59.9	2.7
Red Cored Chantenay	8	22.6	8	67.8	3.1
Royal Chantenay	<u>24</u>	<u>25.6</u>	<u>24</u>	<u>53.3</u>	<u>2.6</u>
Mean	50	22.1	50	67.4	3.8
Overall mean ³	510	24.1	389	65.1	4.4
Low volatiles B10138	8	10.5	8	78.0	7.6
Low volatiles B9304	8	12.3	8	38.0	3.1
High volatiles B0493	8	6.1	8	58.9	9.8
High volatiles B3615	12	9.2	12	284.6	52.4
White carrot B10720	<u>8</u>	<u>9.4</u>	<u>8</u>	<u>129.1</u>	<u>14.2</u>
Mean	44	9.5	44	132.9	20.6
Overall means ³	564	22.7	433	76.8	7.1
Minimum single value		0.4		16.7	0.8
Maximum single value		53.5		384.2	82.9

¹ High Color 9, Long Imperator 58, Danvers 126, and Spartan Bonus varieties were grown in Wisconsin (1979-1982), Florida (1980-1982), California (1980-1982), Arizona (1981), and Illinois (1981-1982). Gold Pak, Nantes Half Long, Red Cored Chantenay, and Royal Chantenay varieties were grown in Illinois (1980), and the experimental genetic materials were grown in Wisconsin (1980-1981).

² N Number of analyses ignoring sampling design.

³ Means of all varieties listed above.

Table II. Falcarinol and Falcarindiol Means by
Location, Year, and Variety

	Falcarinol	Falcarindiol
	mg/kg	mg/kg
<u>Location</u>		
Illinois (NRRC)	15.6 ¹	75.1
Wisconsin	15.1	52.4
Florida	33.5	62.8
California	25.2	56.2
LSD ²	5.0	8.8
<u>Year</u>		
1980	22.5	--
1981	25.9	65.8
1982	25.4	57.6
LSD	5.0	6.2
<u>Variety</u>		
High Color 9	12.1	46.9
Long Imperator 58	28.8	65.2
Danvers 126	27.9	57.4
Spartan Bonus	29.6	70.2
LSD	5.8	8.8

¹ Based on 1981 and 1982 only.

² Least significant difference (0.05 level)
between two means.

Table III. Falcarinol and Falcarindiol Means Associated with the Interaction of Location and Year¹

Location	Year		
	1980	1981	1982
	<u>Falcarinol mg/kg</u>		
Wisconsin	18.0	27.2	21.9
Florida	13.4	40.5	23.7
California	13.8	32.8	29.6
LSD ² = 8.6			
	<u>Falcarindiol mg/kg</u>		
Illinois	--	95.3	54.9
Wisconsin	--	56.8	48.1
Florida	--	56.9	68.8
California	--	54.0	58.4
LSD = 12.4			

¹ Each mean is for High Color 9, Long Emperor 58, Danvers 126, and Spartan Bonus varieties.

² Least significant difference (0.05 level) between two means.

Table IV. Falcarinol and Falcarindiol Means by Variety and Location

Variety	Illinois	Wisconsin	Florida	California
	<u>Falcarinol mg/kg¹</u>			
High Color 9	--	6.2	14.9	13.2
Long Imperator 58	--	22.3	37.1	29.8
Danvers 126	--	18.6	40.5	22.5
Spartan Bonus	--	13.3	41.5	34.8
LSD = 10.0				
	<u>Falcarindiol mg/kg²</u>			
High Color 9	49.6	69.6	56.8	48.8
Long Imperator 58	60.4	67.6	71.2	51.6
Danvers 126	81.6	90.0	74.8	54.0
Spartan Bonus	48.8	59.2	68.4	33.2
LSD = 16.8				

¹ 1980, 1981, 1982.² 1981, 1982.

was improved by a factor of 2 when sample size was increased from 4 to 18 carrots.

The means by location, year, and variety for four selected commercial varieties are shown in Table II. The combination of data for all years and varieties shows that the Florida location produced the highest concentration of falcarinol. However, location differences are dependent on year; the high value of falcarinol occurred at a different location each year (Table III). Table II also shows that falcarindiol concentration is highest in those carrots grown in Illinois; however, Table III again shows that the high toxicant level is in a different location each year. Therefore, the significant effects of location in Table II must be judged in the light of a significant year-location interaction.

Tables II and III indicate that the levels of both toxicants were highest in 1981. Perhaps these results account for variation in resistance to rot that is observed in carrots from year to year (6). The interaction of varieties with year was not significant, suggesting that varietal differences tended to remain the same from year to year.

An interaction of variety and location was observed for falcarinol (Table IV). Long Emperor 58, Danvers 126, and Spartan Bonus show considerable variation among locations, but High Color 9 has the lowest values at all three locations. High Color 9 has low levels of falcarindiol, whereas Danvers 126 consistently had high levels.

Sensory scores on a scale of 1 to 3 were tabulated by one of the authors with a limited number of carrots for four sensory evaluations: sweetness, harsh aftertaste, crispness, and overall preference. There was no indication of association between toxicant levels and sensory evaluations in a plot of toxicants versus sensory evaluation. Therefore, it seems highly probable that culinary quality can be improved without increasing toxicant levels.

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