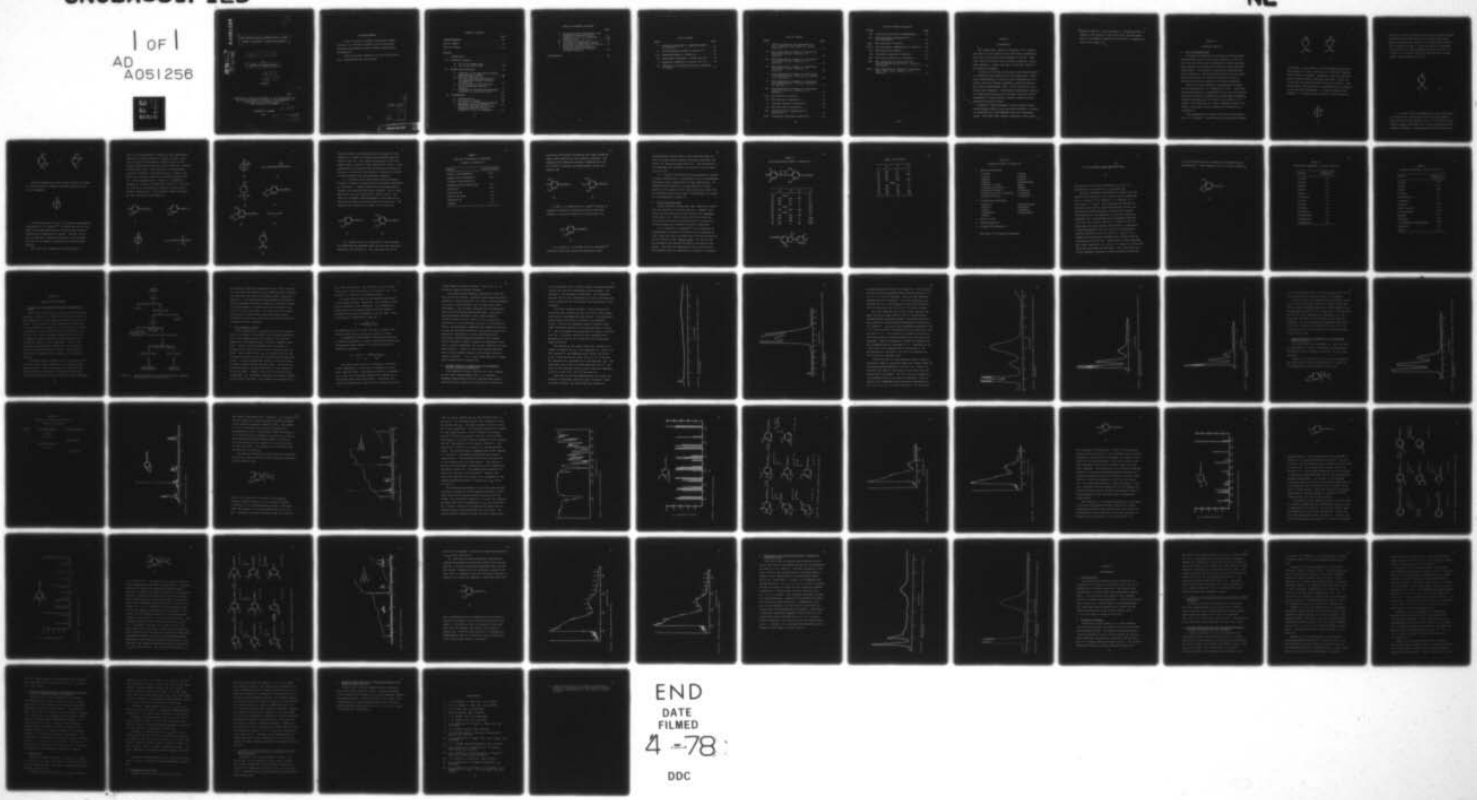
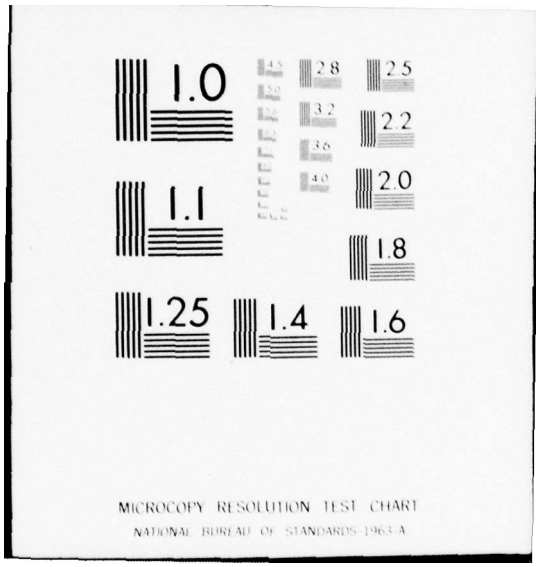
AD-A051 256 ALABAMA UNIV UNIVERSITY DEPT OF CHEMISTRY F/G 7/3
HIGH PRESSURE LIQUID CHROMATOGRAPHY OF SOME AROMATIC COMPOUNDS --ETC(U)
1977 T V ABERCROMBIE

UNCLASSIFIED NL

1 OF 1
AD
A051256



END
DATE
FILMED
4-78
DDC


1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

(1)

HIGH PRESSURE LIQUID CHROMATOGRAPHY OF SOME AROMATIC COMPOUNDS OF NUTMEG AND CARROTS.

(9) Master's thesis,

by

(10) THOMAS VICTOR ABERCROMBIE

(11) 1977

(12) 75 P.

A THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Chemistry in the Graduate School of The University of Alabama

UNIVERSITY, ALABAMA

1977

JD B

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude and appreciation to Dr. William W. Paudler for his invaluable assistance and guidance and his unending patience and encouragement.

I shall be forever grateful to my wife for her comfort, understanding and wise council.

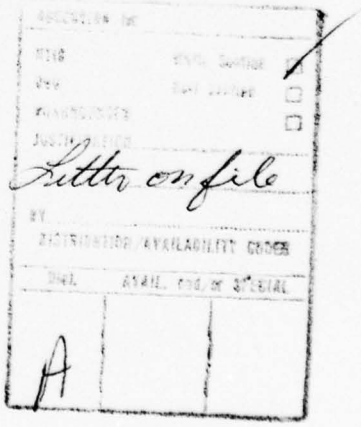


TABLE OF CONTENTS

TABLE OF CONTENTS--Continued

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES--Continued

CHAPTER I

INTRODUCTION

The nutmeg tree, "Myristica fragrans," is a tropical tree native to the islands of the East Indian archipelago. The fruit of the nutmeg tree resembles an apricot. When ripe, this fruit splits into two halves revealing a shiny brown seedcoat. Inside this shell is the seed, which is the nutmeg of commerce.

As early as the turn of the century scientists started to investigate the composition of the nutmeg seed. These early investigations, as well as those conducted more recently, have used thermal techniques, steam distillation, gas liquid chromatography (GLC), in the isolation of the nutmeg seed components. The elevated temperatures required for steam distillation and GLC analyses result in the decomposition of some thermally unstable compounds giving erroneous quantitative data.

Because of the development of high pressure liquid chromatography (HPLC), it has now become possible to analyze the components of the nutmeg seed at room temperature and with reasonable speed. Using HPLC some aromatic compounds of the nutmeg

seed are examined. Since myristicin, a compound present in nutmeg is also present in the carrot root, the HPLC spectrum of the aromatic fraction of carrot root is compared to that of the nutmeg oil.

CHAPTER II

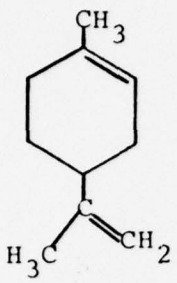
HISTORICAL SECTION

A. Oil of the Nutmeg Seed

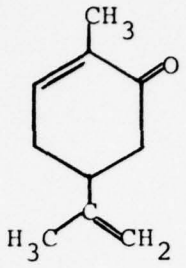
Although the essential oil distilled from nutmeg seed has been known for more than three centuries, it was not until the late 1800's that comprehensive research programs were undertaken to ascertain the nature of the nutmeg seed constituents. The results of these early investigations of the nutmeg oil, obtained by distillation from the nutmeg seed, are difficult to interpret because the source or genuineness of the oils used is not clearly documented.

One of the first important investigations of nutmeg oil was conducted by J. H. Galdstone[1] in 1864. Using fractional distillation, he separated the oil into two fractions. The lower boiling one consisted of a hydrocarbon resembling carvene (limonene) 1 and the higher boiling fraction consisted of an "oxidized" oil closely resembling carvol (carvone) 2. This higher boiling fraction was referred to as "myristicol."

The constituents of nutmeg oil were next investigated by C. R. A. Wright.[2] Using fractional distillation methods

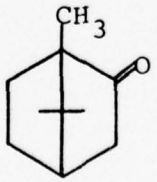


1



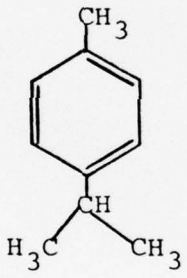
2

he obtained a large quantity of hydrocarbons boiling below 180 degrees and a small quantity of an "oxidized" compound boiling above 210 degrees. Apparently, this was the myristicol fraction earlier isolated by Galdstone. Wright observed that the purest myristicol boils at 212-218°. From an analysis of the fraction, he concluded that it contained, as the principal constituent, a compound isomeric with camphor 3. By repeated distillations of this fraction he



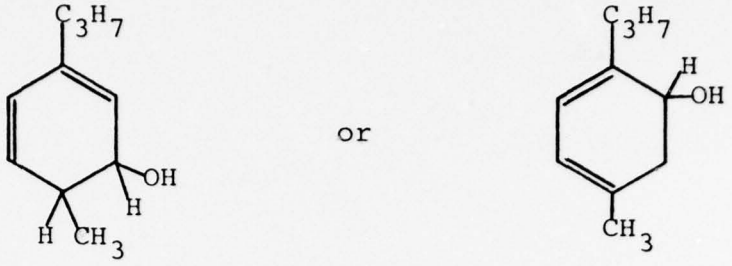
3

was able to obtain a portion which boiled at 250-265 degrees. He incorrectly assumed this to be a polymerized product. Wright determined with respect to the hydrocarbon fraction that Galdstone had been in error and that the hydrocarbon fraction was not a simple compound boiling below 167° but a mixture of a terpene, boiling at 163-164°C and a hydrocarbon, cymene 4 boiling at 177°C.

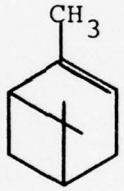


4

J. W. Bruhl,[3] after consideration of the previous publications concerning myristicol and from purely physical data, was led to the conclusion that, as an alcohol of the formula $C_{10}H_{16}O$, myristocol was a cyclic compound with two ethylenic linkages. He proposed the following structures:



Wallach[4] examined the lower boiling fraction of nutmeg oil and by analytical methods identified pinene 5 as one of the components:

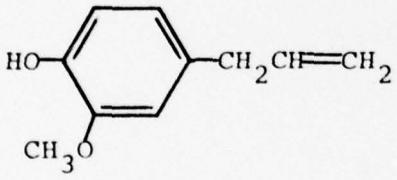


5

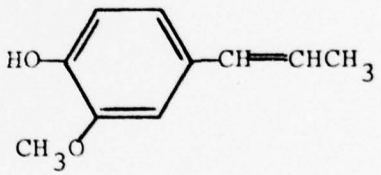
The investigation of the oil of nutmeg was subsequently undertaken by F. W. Semmler.[5-6] He noted that the oil supplied to him consisted entirely of terpenes but failed to investigate the composition in detail. However, he did note the absence of cymene and myristicol and concluded that the oil in question represented the steam-volatile portions.

The first truly comprehensive investigation to

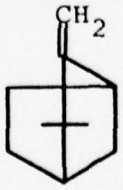
identify the constituents in nutmeg oil was conducted by Fredrich B. Power and Arthur H. Salway[7] in 1907. They used fractional distillation to separate the oil into sixteen fractions boiling from a low of 156°C to a fraction boiling above 275°C. Their analysis of the individual fractions resulted in the identification of several compounds not previously identified as being present in the oil. These compounds were: eugenol 6, isoeugenol 7, camphene 8, linalool 9, borneol 10, geroniol 11, terpineol 12, safrole 13, myristicin 14, myristic acid 15 and dipentene (limonene) 16. The previously identified pinene was also detected in the nutmeg oil.



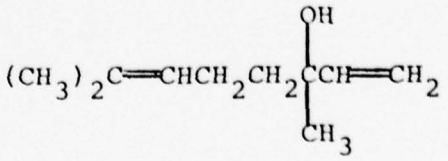
6



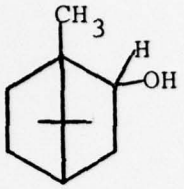
7



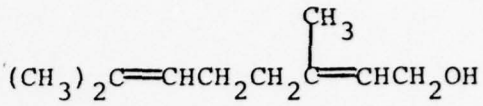
8



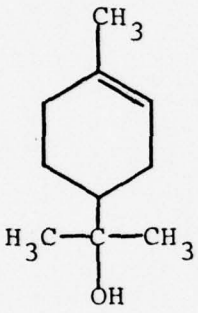
9



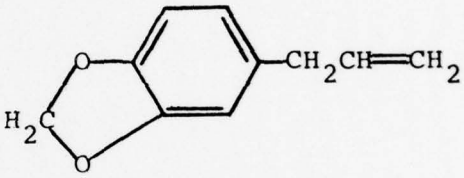
10



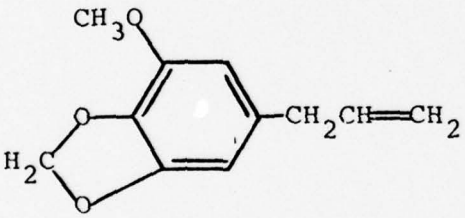
11



12



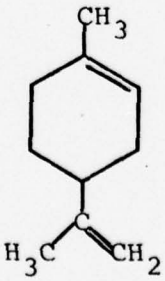
13



14

$CH_3(CH_2)_{12}COOH$

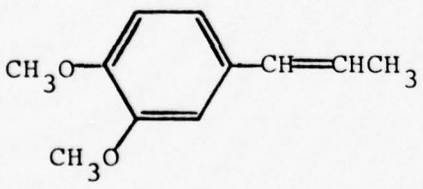
15



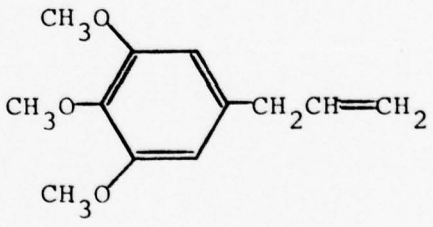
16

Power and Salway also determined that the amount of each compound in a sample of nutmeg varied depending upon the source of the oil. The relative proportions of the compounds found in nutmeg oil were reported (cf. Table I). Their investigation also proved that the fraction previously referred to as myristicol was actually a mixture of alcohols of which terpineol was the predominant compound.

A. T. Shulgin,[8] using vacuum distillation, isolated a fraction of nutmeg oil which had been previously identified as "myristicin." Shulgin employed gas chromatography to separate this fraction into three compounds. One of these myristicin, had been identified in earlier works. The other two compounds, methylisoeugenol 17 and elemicin 18 had not been reported as being present in nutmeg oil. The compounds were identified by their infrared spectra.



17



18

A. T. Shulgin and H. O. Kerlinger,[9] in 1964, isolated and identified two compounds which had not been previously detected in the nutmeg oil. They separated the oil by

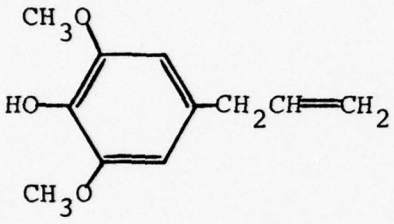
TABLE I

Relative Percentages of Compounds

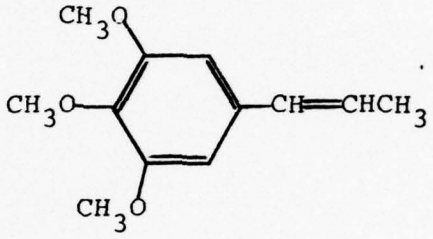
Present in Nutmeg Oil

Compound	Percent Present
Pinene 5 and camphene 8	80.0
eugenol 6 and isoeugenol 7	0.2
linalool 9 and borneol 10	
terpineol 11 and geraniol 12	6.0
safrole 13	0.6
myristicin 14	4.0
myristic acid 15	0.3
dipentene 16	8.0
unknown	0.1

fractional distillation followed by Gas Liquid Chromatographic (GLC) separation of the different fractions. The compounds were identified through a combination of nmr and infrared analyses, as methoxyeugenol 19 and isoelemicin 20.

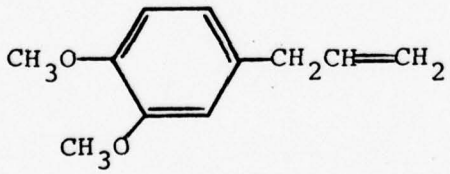


19



20

In 1968, G. M. Sammy and W. W. Nawar[10] used GLC to isolate, in addition to several previously undetected terpines, a compound identified as methyleugenol 21.



21

T. P. Forrest, J. E. Forrest and R. A. Heacock,[11-13] using both silica gel column and preparative layer

chromatography isolated from a light petroleum ether extract of ground nutmeg numerous previously undetected compounds (cf. 22-29 and 33-36, Table II). The structures of the compounds were determined by analysis of nmr and mass spectral data.

D. J. Harvey[14] examined the diarylpropanoids of nutmeg as their trimethylsilyl, triethylsilyl and tri-n-propylsilyl derivatives using combined GLC and mass spectrometry. He identified eight additional diarylpropanoids as being present in the nutmeg oil (cf. 30-32 and 37-41, Table III). The compounds identified to date as being present in nutmeg oil are summarized in Table III.

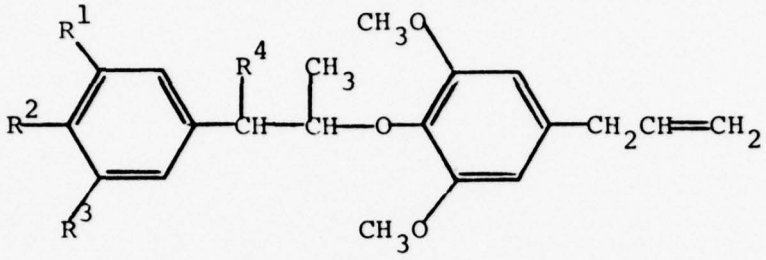
B. Oil of the Carrot Root

Fairly extensive studies have been conducted to determine the composition of carrot seed oil. However, very little work has been done on the volatile oil components of the carrot root. The oil is quite different in odor and taste from carrot seed oil and as such might well be expected to contain compounds of somewhat different composition

D. G. Crosby and N. Aharonson[15] in the course of an investigation of naturally occurring toxic substances in food, found that one of the most toxic ones was the extract of the ordinary carrot, Daucus carota. The carrot root was extracted with acetone followed by reextraction into hexane. The toxin was separated by TLC on silicic acid. The isolated toxin was identified by analysis of infrared

TABLE II

Diarylpropenoids Present in Nutmeg Oil



	R^1	R^2	R^3	R^4
22	OCH_2O		H	OH
23	OMe	OMe	OMe	OH
24	OMe	OH	H	OH
25	OMe	OAc	H	OAc
26	OCH_2O		H	OAc
27	OMe	OMe	OAc	OAc
28	OCH_2O		H	OBz
29	OMe	OMe	OMe	H
30	OMe	OMe	H	OTMS
31	OH	OMe	H	OTMS
32	OMe	H	OMe	OTMS

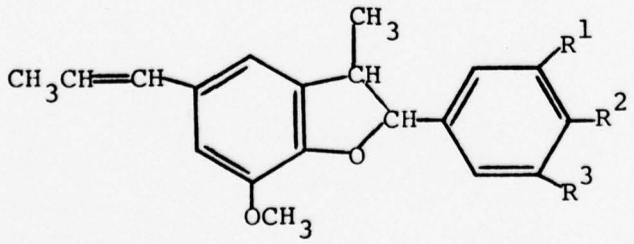


TABLE II--Continued

	R^1	R^2	R^3
33	OMe	OH	H
34	OMe	OH	OMe
35	OMe	OAc	H
36	OMe	OAc	OMe
37	OCH_2O		H
38	OMe	OMe	H
39	OMe	OH	H
40	OMe	OMe	OMe
41	OMe	OH	OMe

TABLE III

Compounds Present in Nutmeg Oil

1. Terpene hydrocarbons

α-pinene	toluene
β-pinene	p-cymene
camphene	linolool
sabinene	geranylacetate
p-mentha-1,4-diene	cineol
p-mentha-1,4(8)-diene	camphor
p-mentha-1,8-diene	citronellol
p-menth-1-en-4-ol (4-terpenol)	citronellal
p-menth-1-en-8-ol	(+)-borneol

2. Allylbenzene derivatives

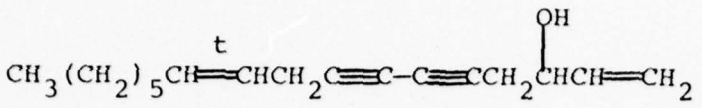
myristicin	methylisoeugenol
elemicin	isoeugenol
safrole	isoelemicin
methyeugenol	methoxyeugenol
eugenol	

3. Myristic acid

4. Diarylpropanoids*

5. Unidentified substances

*See Table II for detailed breakdown.

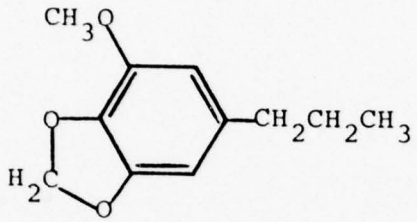


42

ultraviolet mass and nmr spectrometry as trans-1,10-heptadecadrene 5,7-diyn-3-ol or carotatoxin 42.

The most extensive investigation to identify the components of the volatile oil of carrot roots was conducted by R. G. Buttery, R. M. Serfert, D. G. Guadagne, D. R. Black and L. C. Ling.[16] They extracted the volatile oil from the carrot root by steam distillation with the condensed water being continually extracted with pentane. The extracted oil was placed on a silica gel column and separated into a hydrocarbon and an oxygenated fraction. Using GLC, they isolated twenty-nine compounds from the hydrocarbon fraction and were able, through a combination of mass spectrometry, GLC and infrared spectrometry, to identify thirteen of these (cf. Table IV). A total of one hundred and thirty-eight compounds were isolated from the oxygenated fraction by GLC. Twenty-four of these compounds were either positively (cf. Table V) or tentatively identified using the techniques outlined above. The tentatively identified compounds consisted of seven oxygenated terpenoids,

two sesquiterpenoids and 3-methoxy-4,5-methylenedroxypropylbenzene 43. These compounds were not further identified.



43

TABLE IV

Hydrocarbon Components in Carrot Root Oil

Compound	Relative % in whole oil
α-Pinene	0.6
β-Pinene	0.1
camphene	0.2
sabinene	4.0
myrcene	0.8
α-Terpinene	0.7
p-cymene	0.3
Lemonene	3.8
Terpinene	5.4
Terpinolene	38.0
Caryophyllene	5.1
β-Bisabolene	2.9
γ-Bisabolene	6.6

TABLE V

Oxygenated Components in Carrot Root Oil

Compound	Relative % in whole oil
Heptanal	0.05
Octanal	0.2
Nonanal	0.02
2-Nonenal	0.3
Terpinene-4-ol	0.7
γ-Terpineol	0.7
2-Decenal	0.04
Bornyl acetate	0.6
2,4-Decadienal	0.01
Biphenyl	0.1
Dodecanal	0.02
3,4-Dimethosy-1-allylbenzene	0.02
Myristicin	0.4
Carotol	0.2

CHAPTER III

RESULTS AND DISCUSSION

A. Isolation of the Oxygenated Aromatic Compounds From Nutmeg

Most of the early efforts to isolate and identify the compounds in nutmeg oil involved the exposure of the oil to high temperatures at some stage during the isolation procedure. Since exposure to high temperatures causes many of the compounds to decompose, it was necessary to develop a procedure to extract the nutmeg oil avoiding these conditions. The acidic compounds in nutmeg oil were not to be examined, therefore the extraction procedure had to include the removal of these compounds. Finally, the nonoxygenated hydrocarbon fraction had to be separated from the oxygenated aromatic fraction. The procedure developed to accomplish these objectives is outlined in Figure I.

The ground nutmeg, obtained from a local grocery, was extracted with pentane, resulting in a residue, and the pentane extract. After the pentane was evaporated, the yellow residue was dissolved in chloroform and treated with dilute sodium hydroxide to remove any acidic compounds.

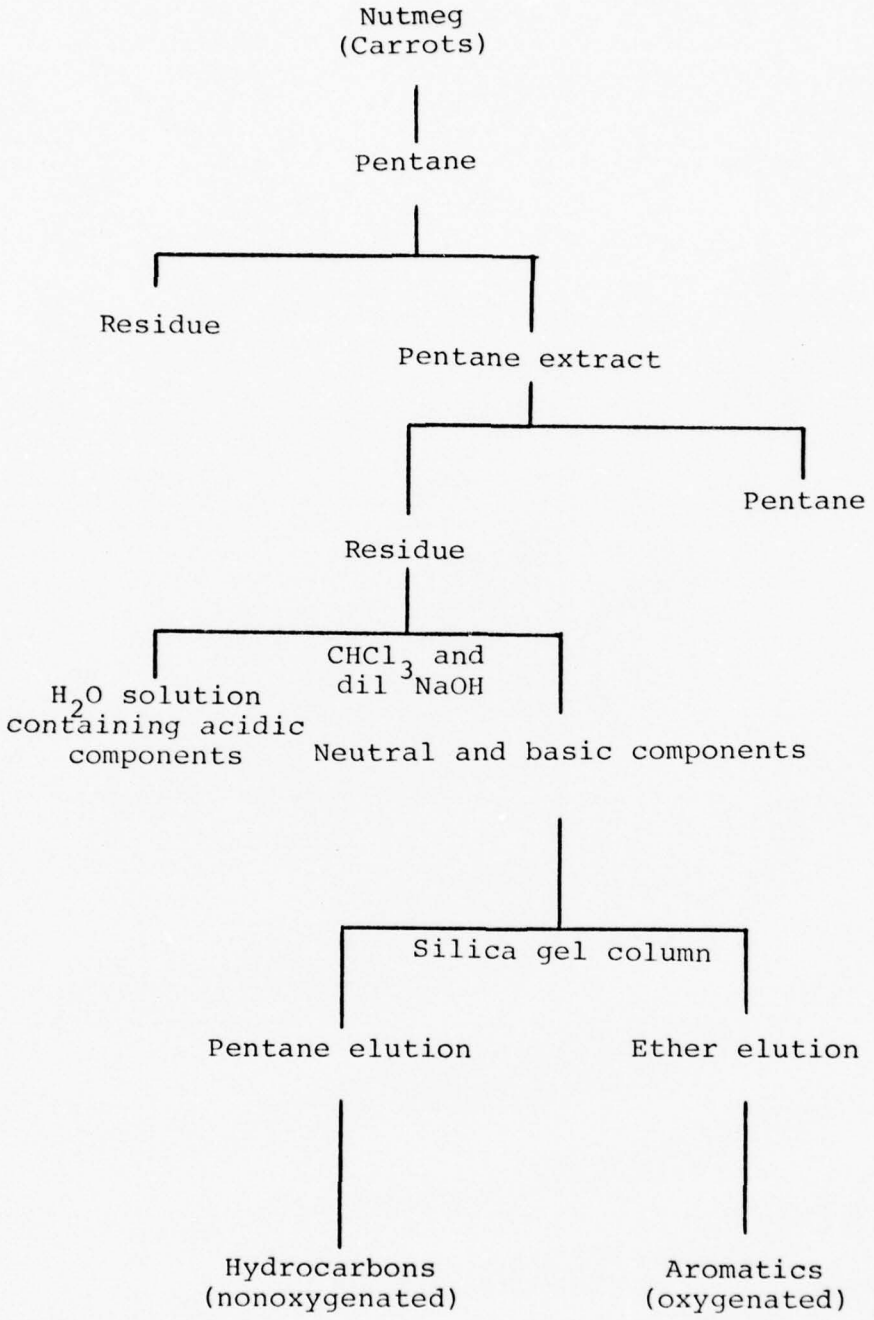


Figure I. Isolation scheme for oxygenated aromatic compounds from nutmeg oil (carrot root oil).

The resulting layers were separated giving a water solution (A) containing the undesired acidic compounds and a solution (B) containing the neutral and possibly basic compounds of nutmeg oil. The nonoxygenated hydrocarbons were separated from the oxygenated aromatic compounds by selective elution (a standard method) of residue (B) from the neutral solution on a silica gel column. The column was first eluted with pentane to remove the nonoxygenated hydrocarbons followed by elution with ethyl ether to obtain the desired oxygenated aromatic compounds.

B. Basic Concepts of HPLC

A HPLC chromatogram is characterized by four features which are important in describing the resulting separation. First, each compound leaves the column in the form of a symmetrical, bell-shaped band or Gaussian (standard error) curve. Second, each band emerges from the column at a characteristic time that can be used to identify that compound. This retention time, t_R, is measured from the time of sample injection to the time the band maximum leaves the column. A third characteristic feature is the difference in retention times between adjacent bands. The larger the difference between the bands the easier is the seperation of the two bands. Finally, each band is characterized by a bandwidth, t_w. Bandwidth is measured by drawing tangents to each side of the band. The tangents are extended until

they touch the baseline. The distance from one tangent to the other along the baseline is a measure of t_w. The separation is enhanced as t_w becomes smaller.

The usual goal of HPLC is the adequate separation of a given sample mixture. Resolution, R_s, provides a quantitative measure of the separation. R_s is defined as being equal to the distance between two band centers, divided by the average bandwidth of the two bands. This relationship is summarized in equation 1.

$$R_s = \frac{t_2 - t_1}{(1/2)(t_{w1} + t_{w2})} \qquad 1$$

The quantities t_1 and t_2 refer to the t_R values of two adjacent bands and t_{w1} and t_{w2} are their t_w values.

A fundamental relationship in HPLC which allows one to control resolution by varying α(separation selectivity), N(theoretical plate number) or k'(capacity factor) is outlined by equation 2.

$$R_s = \underset{\text{(i)}}{(1/4)}\underset{\text{(ii)}}{(\alpha - 1)}\underset{\text{(iii)}}{\sqrt{N}\left[\frac{k'}{(1 + k')}\right]} \qquad 2$$

The three terms (i)-(iii) of equation 2 are essentially independent, so that one can optimize first one term, then the other. Separation selectivity as measured by α, term (i), is varied by changing the composition of the mobile and/or stationary phases. Separation efficiency as measured by N, term (ii), is optimized by changing

column length or solvent velocity. Term (iii), k', is varied by changing solvent strength.

The column packing materials available for HPLC are many and varied, however, generally these materials can be grouped into three categories; (1) porous, high-performance, (2) porous, low-performance, and (3) pellicular, high-performance. Each of these categories can be subdivided into silica and alumina packing materials. The porous, high-performance adsorbents offer greater capacity thereby permitting larger sample sizes on preparative separations, and corresponding increases in detectability. Porous, low-performance adsorbents are inexpensive and can be used for purifying solvents or carrying out large scale preparative separations of easily resolved mixtures. Pellicular, high-performance adsorbents offer greater column efficiency (larger N values) and convenience, compared to porous adsorbents, but are more expensive and have lower capacity. Porasil A, the column packing material used in this research, belongs in the porous, high performance category. It is a silica bead spherical in shape with a surface area of 350-500 m^2/g.

C. The HPLC Analytical Separation of the Oxygenated Aromatic Fraction of Nutmeg Oil

The oxygenated aromatic fraction was first examined by thin layer chromatography (TLC) in an attempt to determine approximately how many compounds were present and which solvent system would give the best separation.

It was determined that a solvent system of hexane/chloroform (70/30) gave the most advantageous solvent system. The presence of six compounds was detected. The information obtained from the TLC experiments was used to establish the initial conditions for an attempted HPLC separation of the compounds.

Since HPLC normally requires a solvent system less polar than that required for TLC, the initial mobile phase selected was isooctane/chloroform (90/10). The stationary phase selected was Porasil A packed in an analytical column. Eluting the oxygenated aromatic fraction under these conditions resulted in the spectrum shown in Figure II. Only one compound was satisfactorily eluted, retention time (t_R) 1.2 minutes, while the other compounds have excessive t_R's and are not eluted from the column under these conditions.

The polarity of the mobile phase was increased in an attempt to improve the t_R of the compounds (cf. Figure III). This resulted in two compounds being eluted, one with a t_R of 1.2 minutes and the other with a t_R of 7.8 minutes. The remaining four compounds still had excessive t_R's. The resolution (R_s) of the two eluted compounds was 4.7. Because of this extremely high R_s value, these two compounds, relative to each other could be obtained pure.

Since four of the six compounds were not eluted, the polarity of the mobile phase was again increased. Using a Porasil A column, the mobile phase was changed to

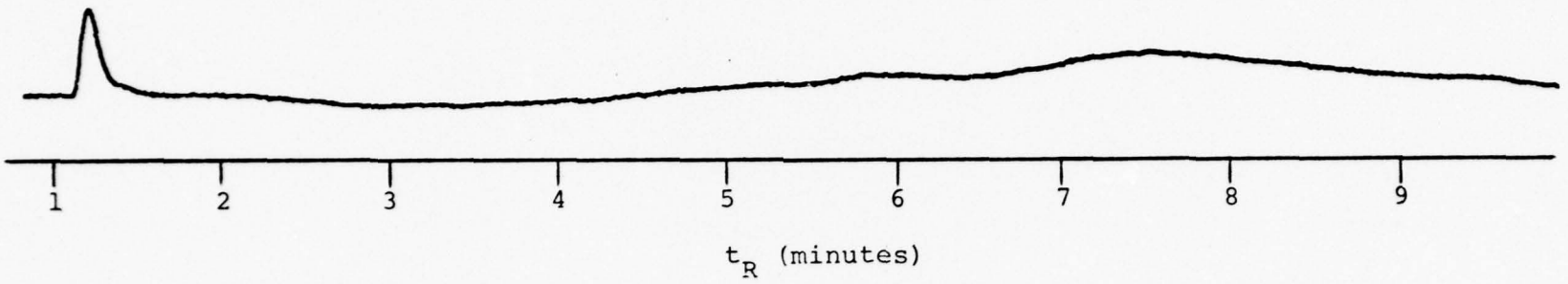


Figure II. HPLC separation of nutmeg oil (isooctane/chloroform (90/10); Porasil A; UV detector).

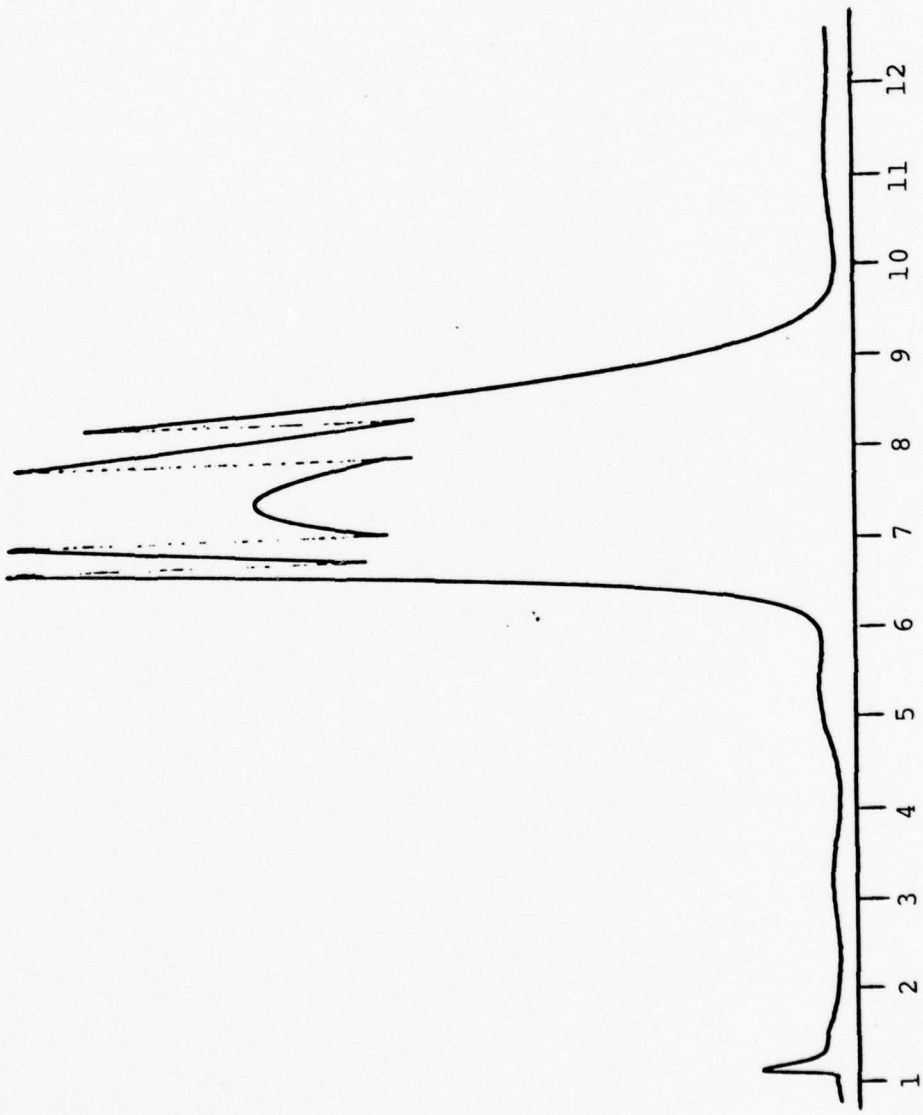


Figure III. HPLC separation of nutmeg oil (isooctane/chloroform (80/20); Porasil A; UV dector).

isooctane/chloroform (70/30) (cf. Figure IV). This resulted in five of the six compounds being eluted with t_R's of 1.1, 3.0, 6.1, 9.4 and 20.4 minutes. The t_R of the remaining compound was still excessive. Since the minimum R_s of the chromatogram is 1.2, these compounds, using these conditions, could be separated into fractions of 99 percent purity.

The t_R of compound five is still quite long and compound six has not been eluted by any of the previously attempted mobile/stationary phases. The mobile phase was next altered to isooctane/chloroform/methanol (80/19.9/0.1) (cf. Figure V). The t_R's of the compounds decreased to 0.9, 1.3, 2.0 and 3.8 however the minimum R_s of the chromatogram also decreased to 0.7. This reduction in minimum R_s is primarily due to the decrease in separation between the first and second compounds. When the separation of these two compounds is not considered then R_s increases to 1.2. Although the t_R's of the first four compounds and the minimum R_s of the chromatogram are acceptable, the last two compounds are not eluted with reasonable t_R's.

The best R_s and t_R's were obtained using a Porasil A analytical column as stationary phase and a mobile phase of isooctane/chloroform/methanol (70/29/1) (cf. Figure VI). The flow rate used for the elution of the first four compounds was 2.5 ml/minute. The flow rate was increased to 4.5 ml/minute to elute the last two compounds. This resulted in all compounds being eluted with acceptable t_R's of 2, 2.5, 3.3, 4.1, 6.3 and 8.0 minutes. The minimum R_s

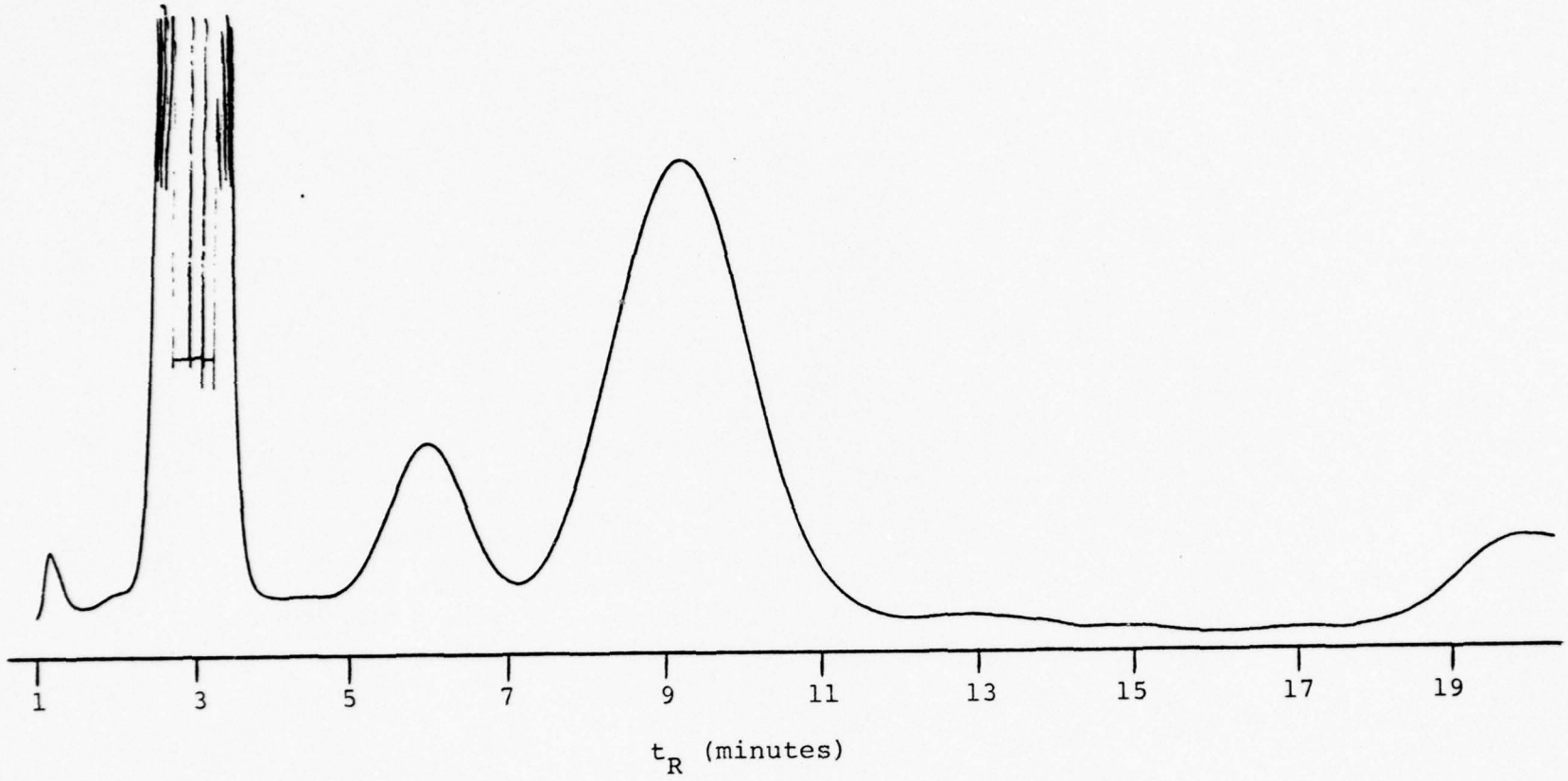


Figure IV. HPLC separation of nutmeg oil (isooctane/chloroform (70/30); Porasil A; UV detector).

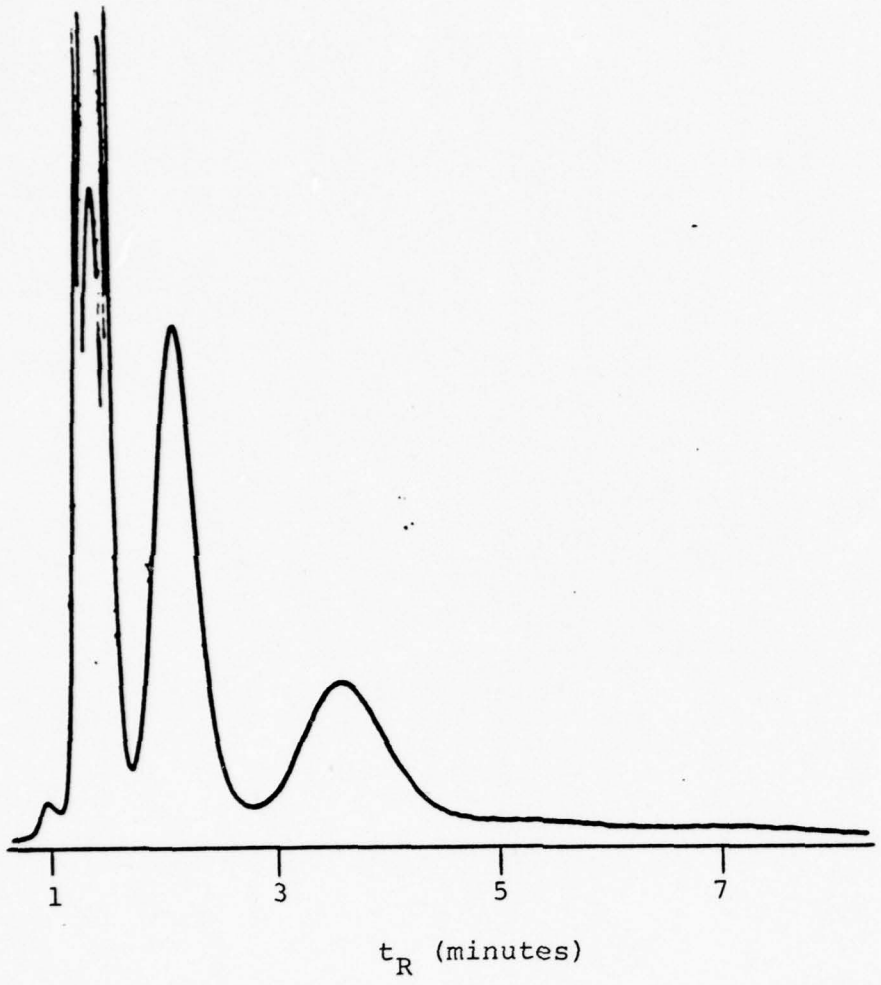


Figure V. HPLC separation of nutmeg oil (isooctane/chloroform/methanol (80/19.9/0.1); Porasil A; UV detector).

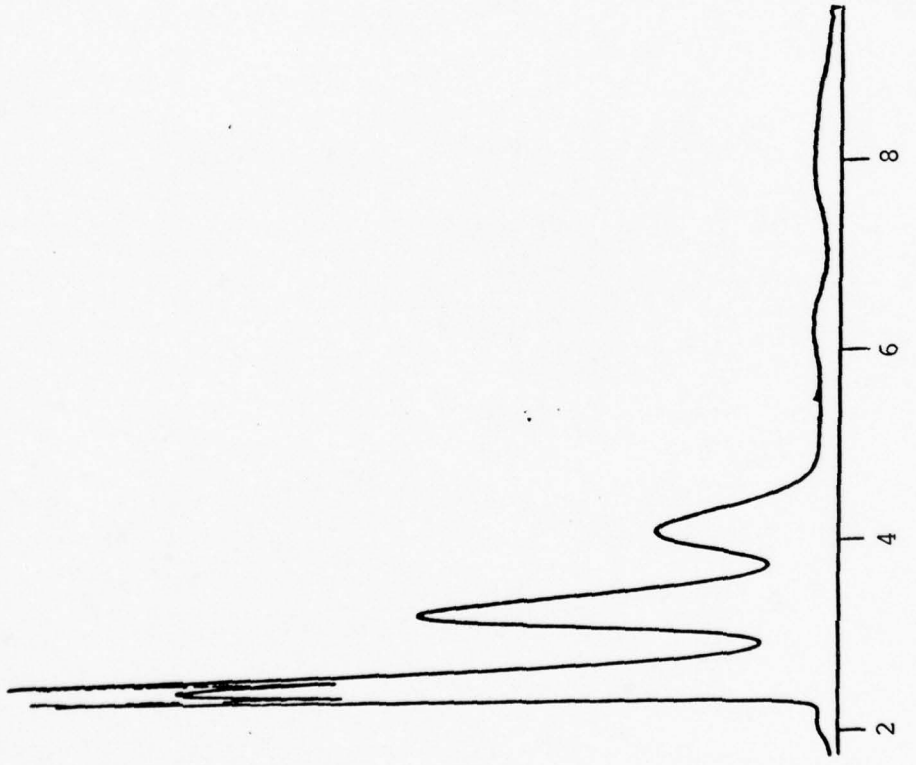


Figure VI. HPLC separation of nutmeg oil (isooctane/chloroform/methanol (70/19/1); Porasil A; UV detector).

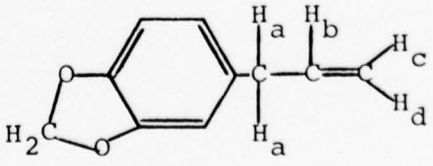
for the chromatogram was 0.9 and the expected purity of the worst fraction after separation would be 96 percent.

The best R_s and t_R's obtained using a Porasil A analytical column as stationary phase and a mobile phase that did not contain chloroform was with a mobile phase of isooctane/methanol (99.5/0.5) (cf. Figure VII). The flow rate for the first four compounds was maintained at 1.5 ml/minute and increased to 4.5 ml/minute for the last two compounds. The t_R's obtained were comparable with those of the above system but the minimum R_s value of 0.7 was considerably lower and the purity of the individual fractions would be less.

D. Identification of the Compounds in the Oxygenated Aromatic Fraction

The identities of the compounds (cf. Table VI) producing the peaks in the HPLC chromatogram were determined by analysis of nmr, infrared, ultraviolet and mass spectral data.

The nmr (cf. Figure VIII) of the compound producing the first peak in the HPLC chromatogram (cf. Figure VI) is consistent with the structure of safrole 13



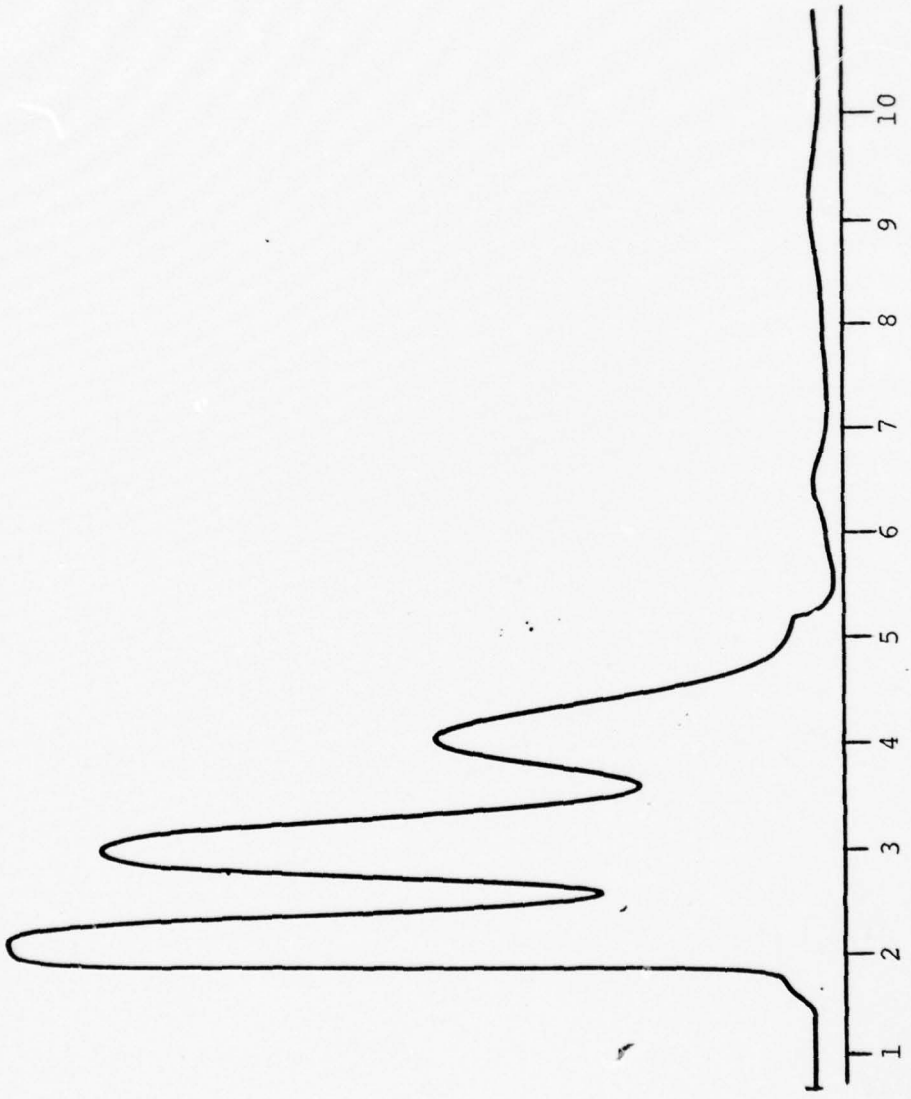


Figure VII. HPLC separation of nutmeg oil (isooctane/methanol (99.5/0.5); Porasil A; UV detector).

TABLE VI

Identities of Oxygenated Aromatic

Components of Nutmeg

Peak No.	Confirmed Identity	Tentative Identity
1	Safrole	
2	Myristicin	
3		Mythyleugenol
4	Methylisoeugenol	
5	Elemicin	
6		Isoelemicin

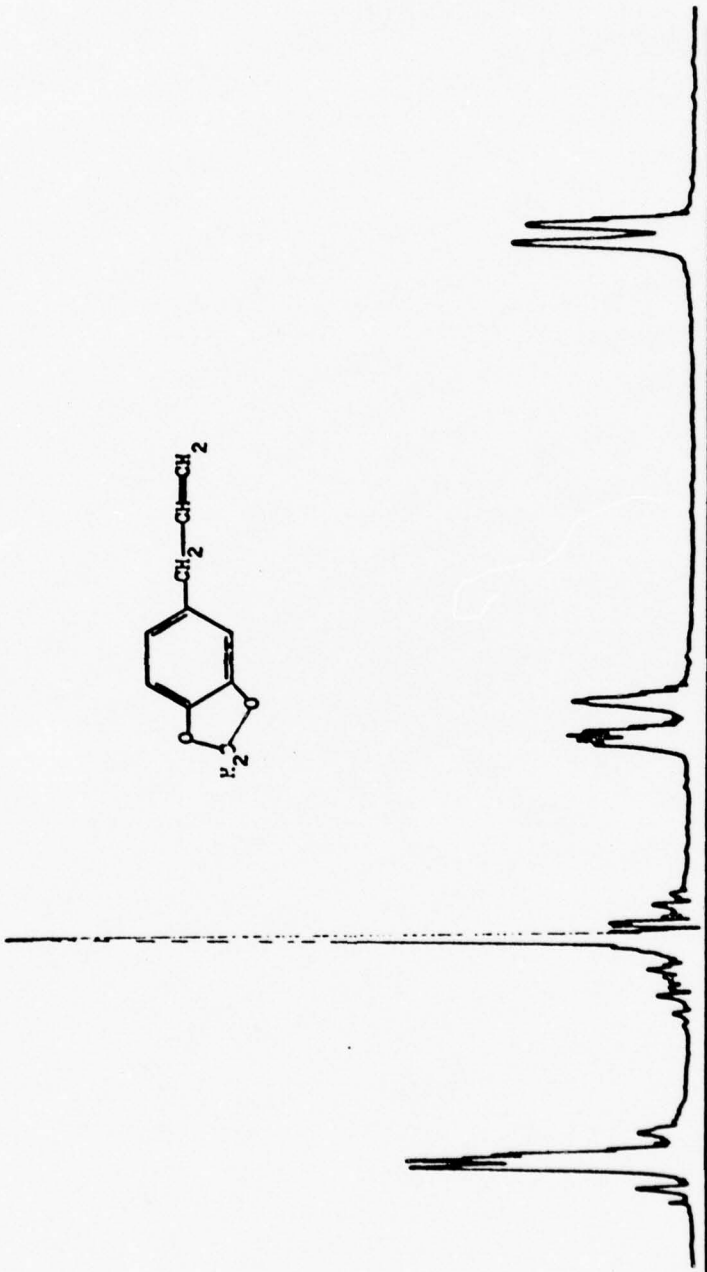
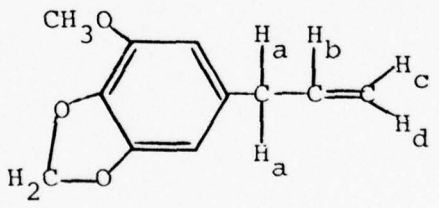


Figure VIII. NMR spectrum of safrole.

The doublet overlapping with a singlet at τ3.33 results from from the coupling of the two aromatic protons overlapped by the remaining uncoupled aromatic proton. The singlet at τ4.15 is characteristic of the two protons of a methylenedioxy group. The multiplet under the singlet at τ4.15 results from the coupling of H_b with H_a, H_c and H_d. The multiplet and poorly defined triplet at τ4.90 and τ5.02 are produced by the coupling of H_c and H_d with H_b and with each other. The doublet at τ6.74 results from the coupling of H_a with H_b.

The compound producing the second peak was identified as myristicin 14 by analysis of nmr, infrared, ultraviolet and mass spectral data.



The nmr (cf. Figure IX) is typical of the compound myristicin with a singlet at τ3.62 (aromatic protons), a singlet at τ4.15 (methylenedioxy protons), a multiplet under the singlet at τ4.15 (H_b coupled with H_a, H_c and H_d), a multiplet and poorly defined triplet at τ4.90 and

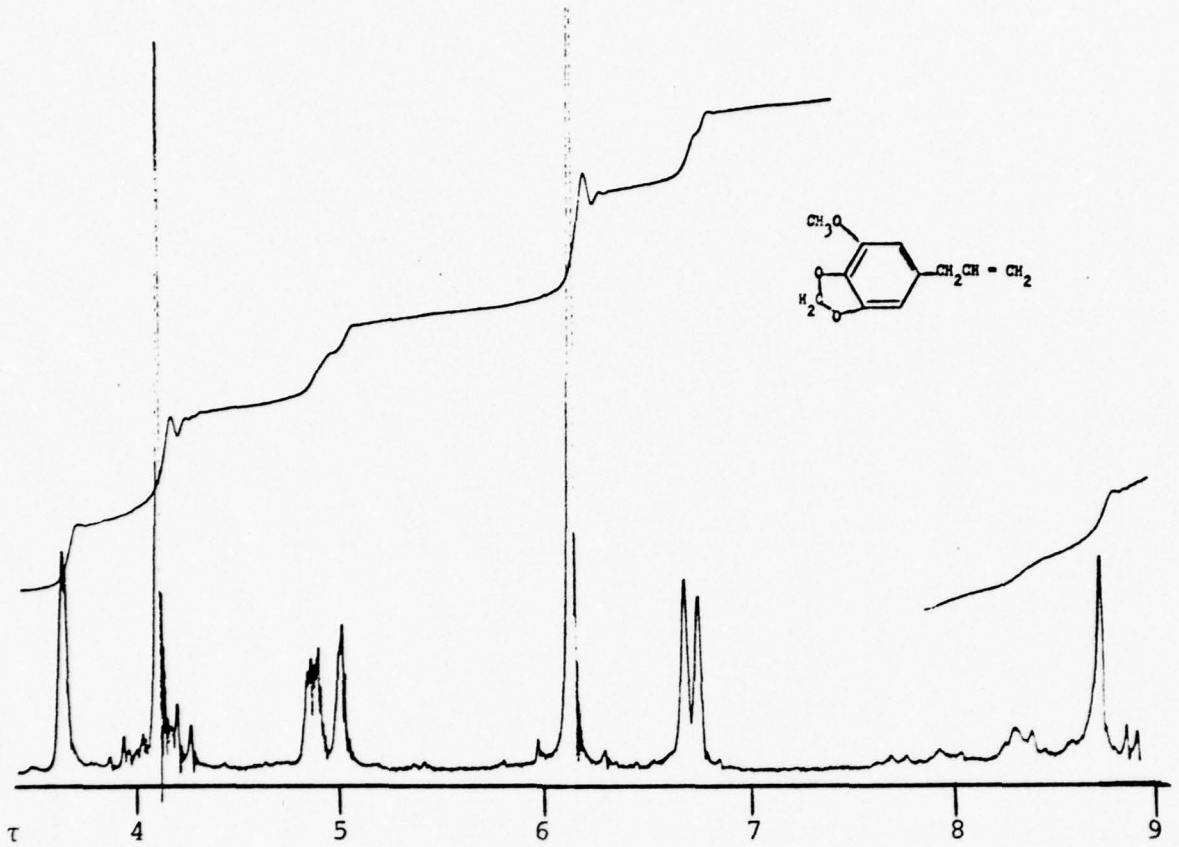


Figure IX. NMR spectrum of myristicin.

τ5.02 (H_e and H_d coupled with H_b and with each other), a singlet at τ6.12 (methoxy protons) and a doublet at τ6.74 (H_a coupled with H_b). The peaks between τ8.0 and τ9.0 are due to minor impurities. The infrared spectrum (cf. Figure X) of the compound shows it to be aromatic (C-H stretch, 3008 cm^{-1} and C=C ring stretch, 1610 cm^{-1}) and confirms the presence of an ether function (asymmetric C-O-C stretch, 1240 cm^{-1} and symmetric C-O-C stretch, 1040 cm^{-1}). The absorption at 1630 cm^{-1} is typical of a monosubstituted olefin. The electron-impact fragmentations of the compound (cf. Figure XI) considerably strengthens the structure identification. The molecular ion and the base peak are at the calculated m/e 192 (100 percent). The ions produced by electron-impact fragmentation of the compound are outlined in Figure XII. The fragmentations observed are typical for this type allyl benzene.[17] Finally, the ultraviolet spectrum (cf. Figure XIII) corresponds to the reported absorption pattern for myristicin (λ_{max} 278 nm and 285 nm).

The compound corresponding to the third peak has been tentatively identified as methyleugenol 21 based on ultraviolet data and the expected elution sequence from the column. The ultraviolet spectrum (cf. Figure XIV) compares favorably with that of methyleugenol (λ_{max} 282 nm and 290 nm). However, because of the presence of impurities its identity cannot be confirmed from this data alone. The expected elution sequence provides the best evidence that

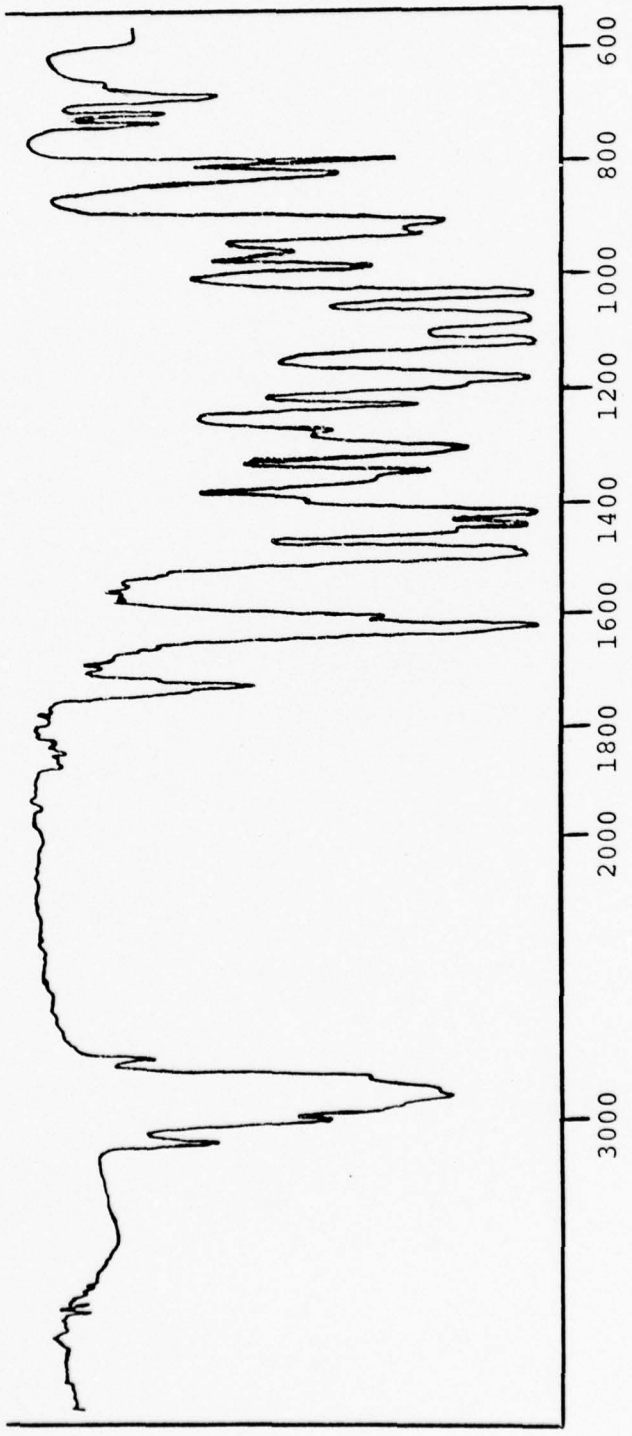


Figure X. Infrared spectrum of myristicin.

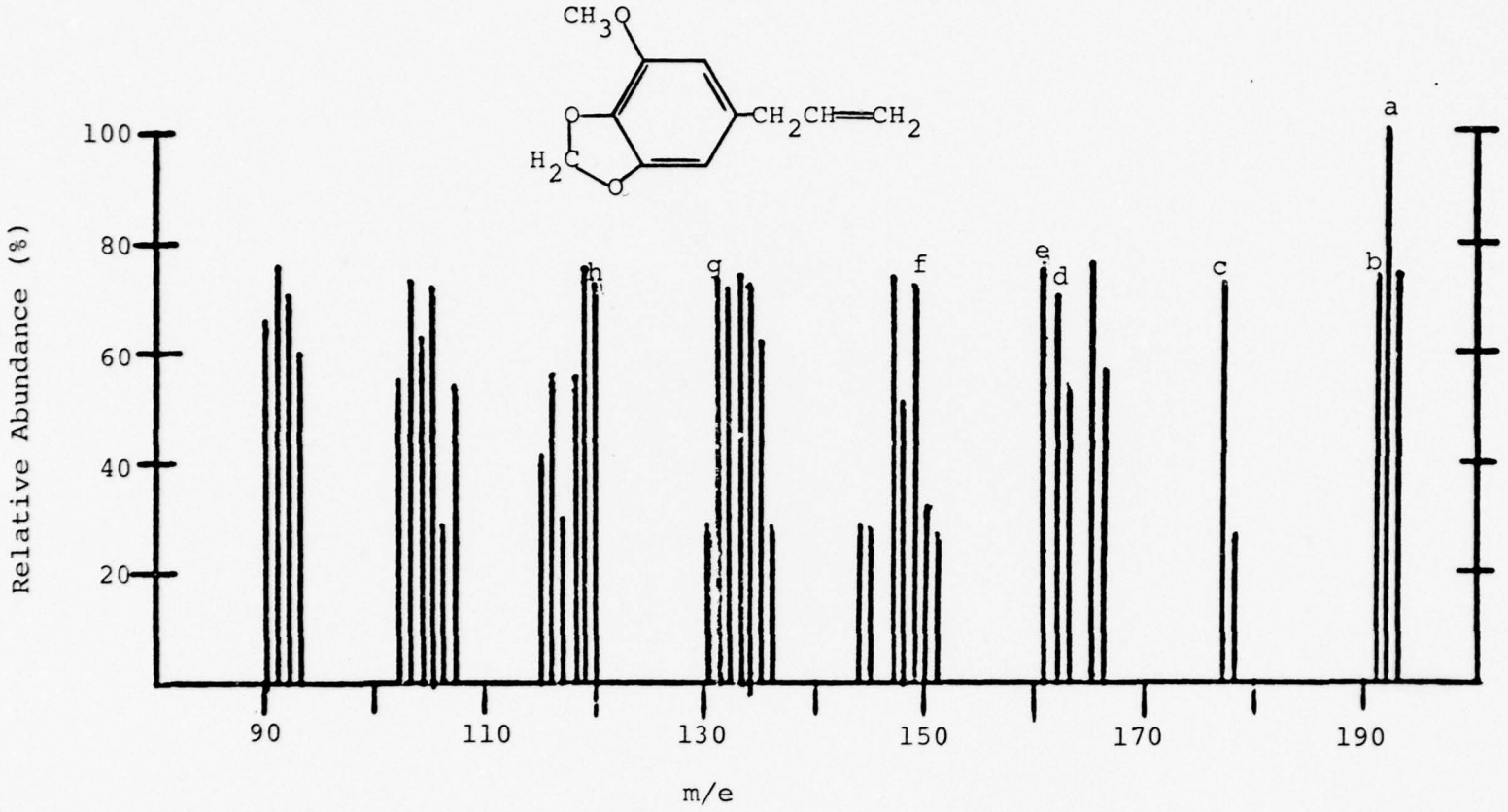


Figure XI. Mass spectrum of myristicin.

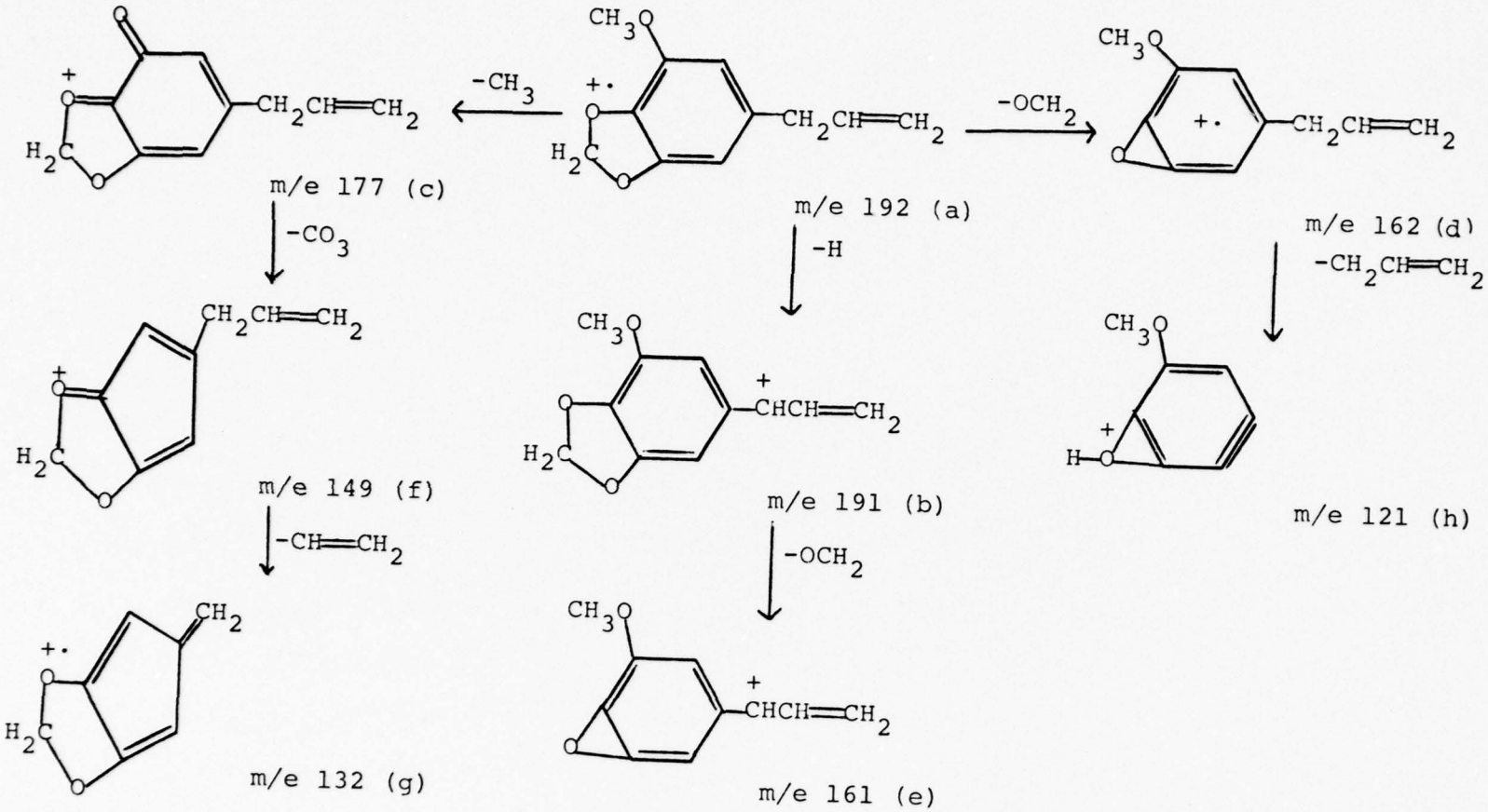


Figure XII. Electron-impact fragmentation of myristicin.

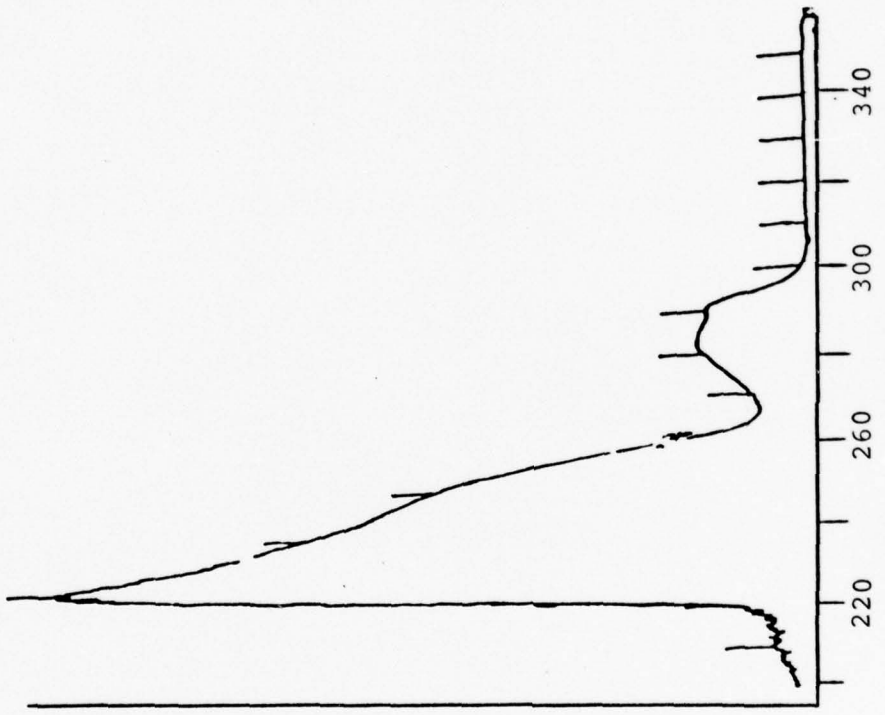


Figure XIII. Ultraviolet spectrum of myristicin.

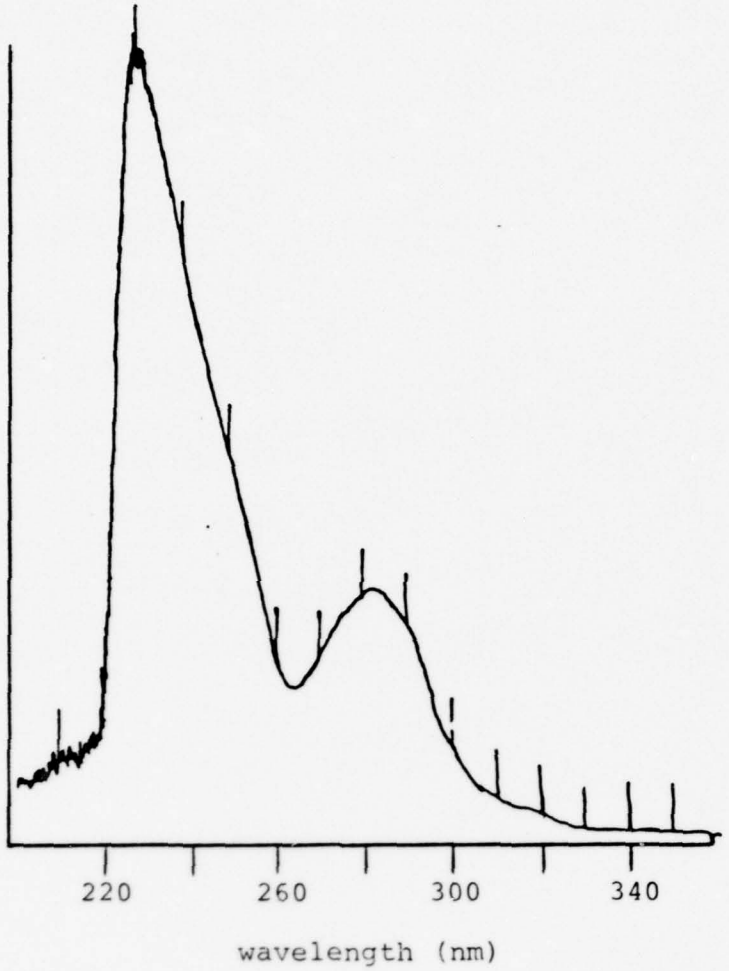
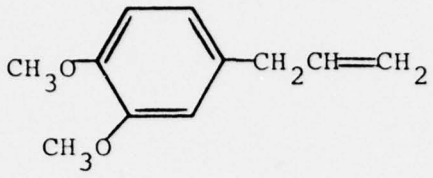


Figure XIV. Ultraviolet spectrum of methyleugenol.



21

this compound is methyleugenol. Since the column packing material used was Porasil A, the stationary phase was polar. Consequently the more polar a compound, the longer it will adhere to the stationary phase. This will result in more polar compounds having longer retention times than less polar compounds. The polarities of the remaining compounds increase in the order methyleugenol/methylisoeugenol and elemicin/isoelemicin due to the increase in methoxy groups. Therefore, the third compound to elute from the column should be either methyleugenol or methylisoeugenol. Since methylisoeugenol has been established as the compound producing the fourth peak in the HPLC chromatogram, there is a high probability that the third peak is produced by methyleugenol.

The identity of the compound producing the fourth peak in the HPLC chromatogram has been identified as methylisoeugenol 17 based on mass spectral data (cf. Figure XV). The molecular ion and base peak of the mass spectrum correspond to the calculated m/e 178 (100 percent) for

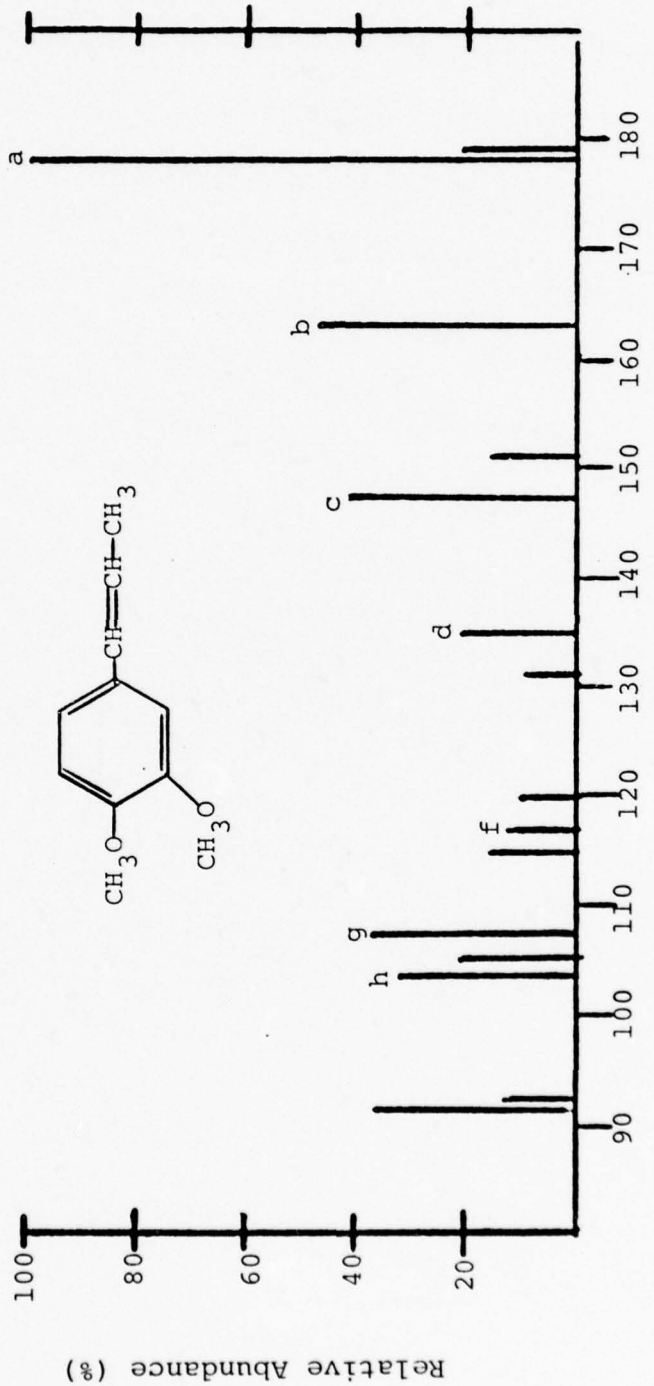
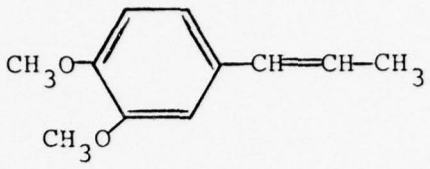


Figure XV. Mass spectrum of methylisoeugenol.



17

methylisoeugenol. The fragmentation of the compound is typical of an o-dimethoxybenzene derivative.[17] The ions resulting from the electron-impact fragmentation are listed in Figure XVI. This fragmentation pattern, due to the absence of a P-1 peak, shows conclusively that the compound in question is methylisoeugenol and not the corresponding compound methyleugenol. As was seen in the compound myristicin, when the olefin of the propenyl substituent is not conjugated with the aromatic ring an intense (75 percent) P-1 peak results. Since the olefin of the propenyl substituent of methylisoeugenol is conjugated with the aromatic ring a P-1 peak is not produced.

The identity of the compound producing the fifth peak in the HPLC chromatogram was established as elemicin 18 by a combination of ultraviolet, nmr and mass spectral data. The molecular ion in the mass spectrum (cf. Figure XVII) is at the calculated m/e 208 (40 percent). The base peak m/e 111 (100 percent) corresponds to a stable ion (species

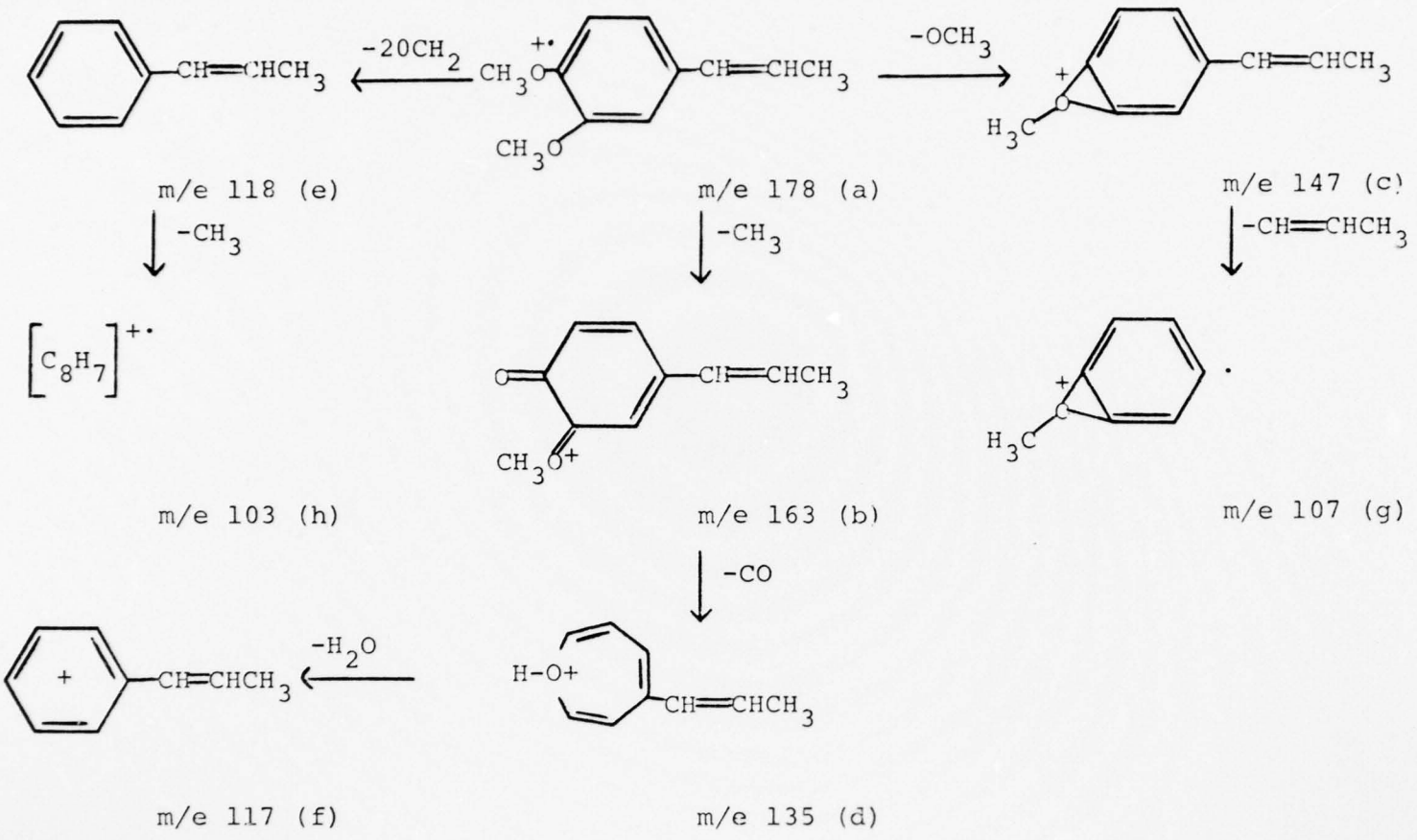


Figure XVI. Electron impact fragmentation of methylisoeugenal.

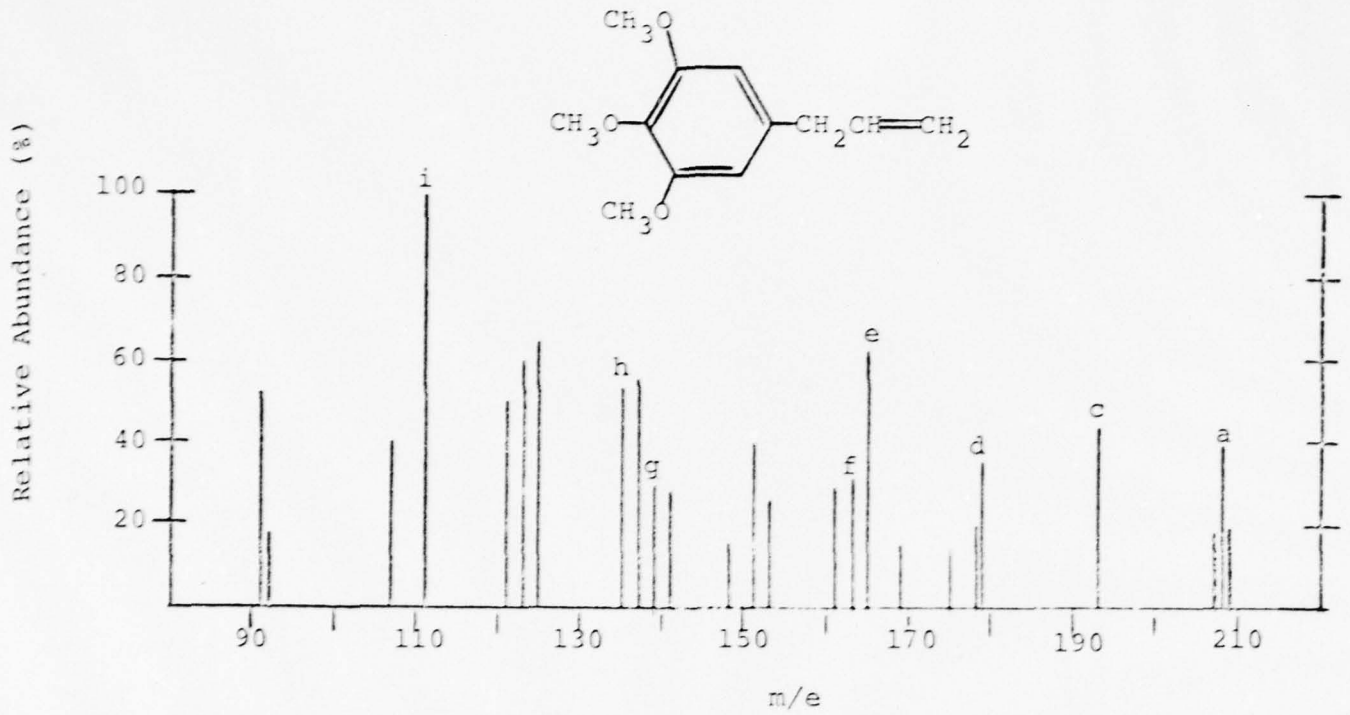
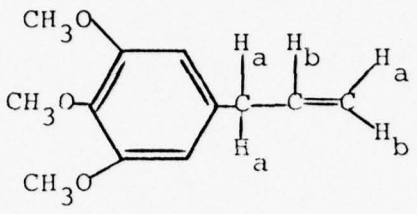


Figure XVII. Mass spectrum of elemicin.



18

(i) in Figure XVIII). The ions resulting from the electron-impact fragmentation are shown in Figure XVIII. This fragmentation is typical for pyrogallol trimethyl ether.[17] The nmr (cf. Figure XIX) shows a singlet at τ3.68 (aromatic protons), a multiplet and triplet at τ4.84 and τ4.98 (H_c and H_d coupled with H_b and with each other), two singlets with slightly different chemical shifts at τ6.14 and τ6.16 (singlet at τ6.14 is produced by the two methoxy groups meta to the propenyl substiuent and the singlet at τ6.16 results from the methoxy group para to the propenyl substituent) and a doublet at τ6.74 (H_a coupled with H_b). The multiplet produced by the coupling of H_b with H_a, H_c and H_d is concealed in the base line noise at τ4.15. The peaks from τ8.00 to τ9.00 are a result of minor impurities. Since elemicin is of the same basic structure as myristicin, six-membered aromatic ring with an unconjugated propenyl substituent, the ultraviolet spectrum was expected to correspond to the reported ultraviolet spectrum of myristicin (λ_{max} 278 nm and 285 nm). The ultraviolet absorption (cf.

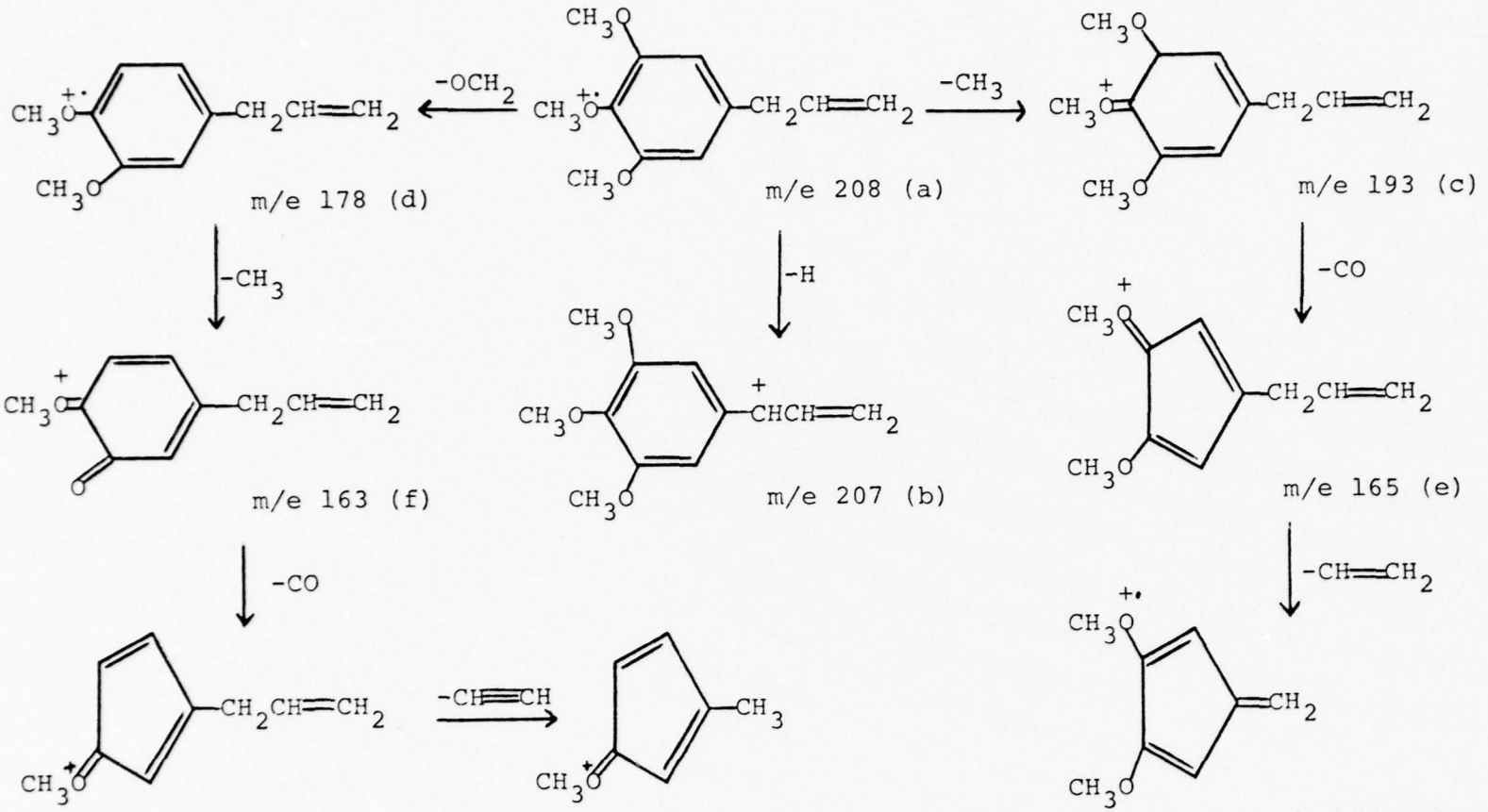


Figure XVIII. Electron-impact fragmentation of elemicin.

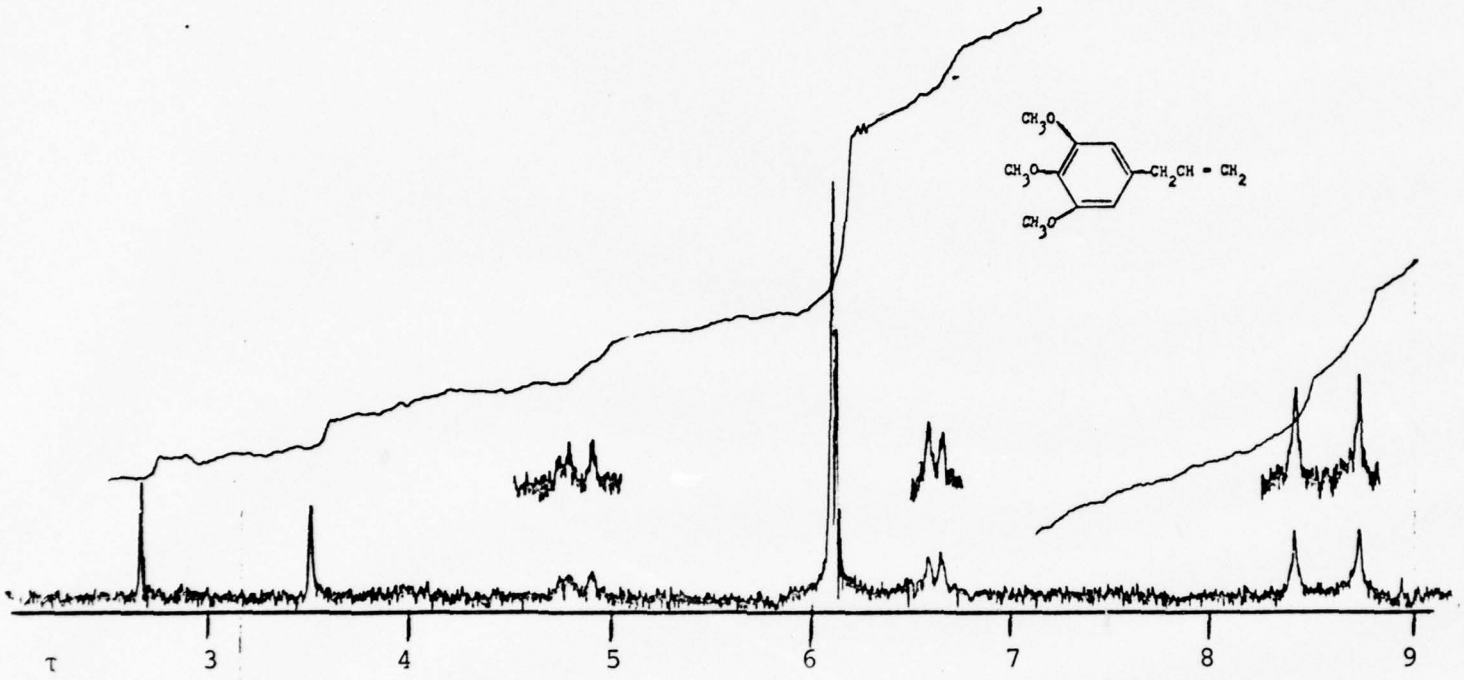
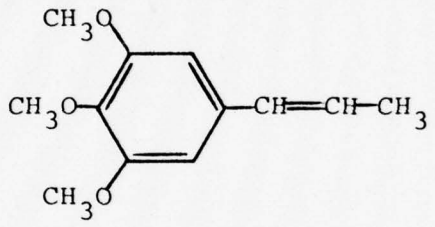


Figure XIX. MMR spectrum of elemicin.

Figure XX) of compound 5 contains the expected absorptions of λ_{max} 278 nm and 285 nm.

The sixth peak has been tentatively identified as isoelemicin 20 based on ultraviolet spectral data and the sequence in which the identified compounds elute from the HPLC column. Comparison of the ultraviolet spectrum (cf. Figure XXI) of compound 6 with the ultraviolet spectrum of elemicin (cf. Figure XX) compound 5, shows that there has



20

been a bathochromic shift accompanied by an increase in absorption intensity in the ultraviolet spectrum of compound 6. This shift is due to conjugation of the side chain with the aromatic ring which is not present in elemicin 18. Using the same rationale as discussed for methyleugenol, page 44, isoelemicin would be expected to elute from the HPLC column as compound 6.

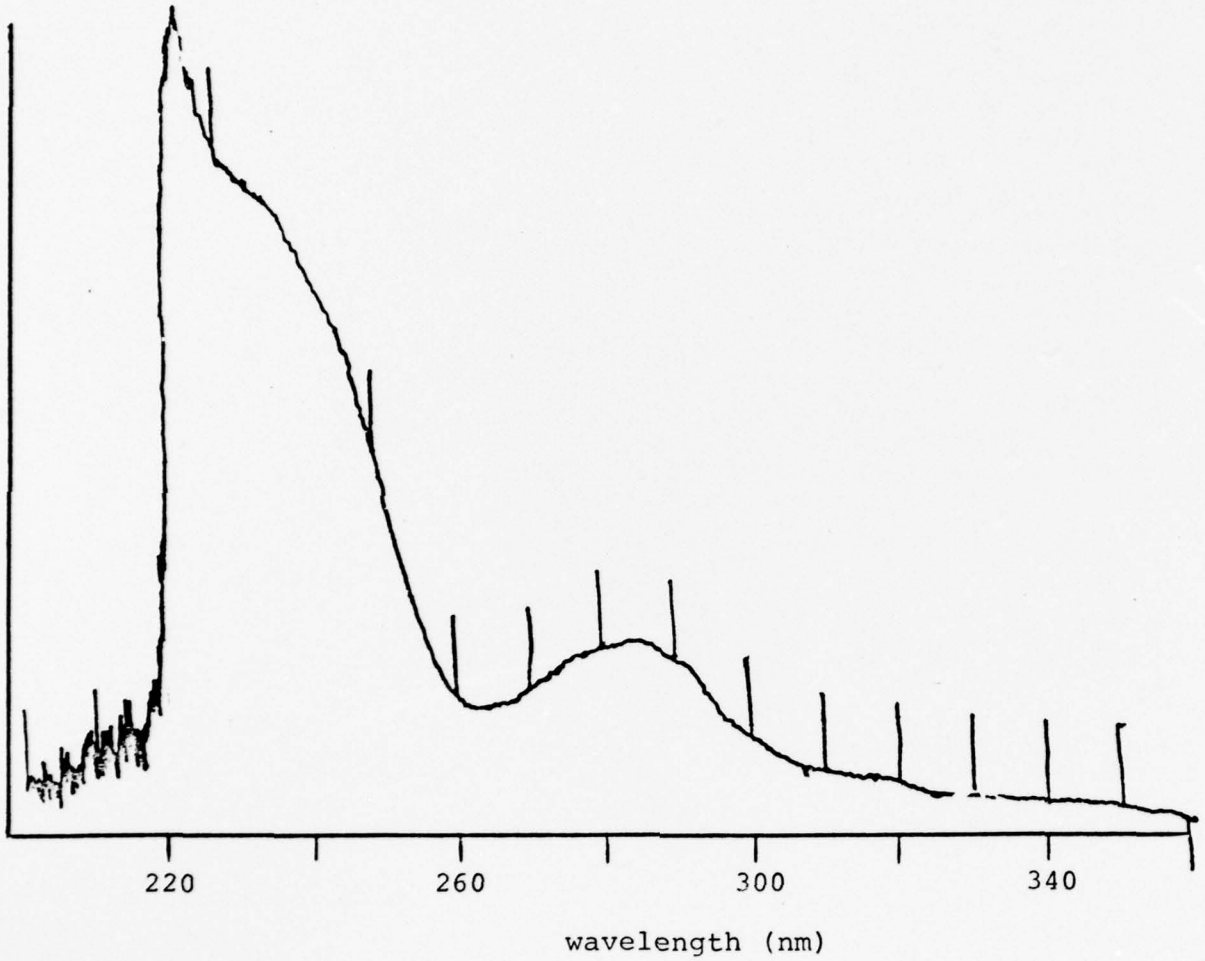


Figure XX. Ultraviolet spectrum of elemicin.

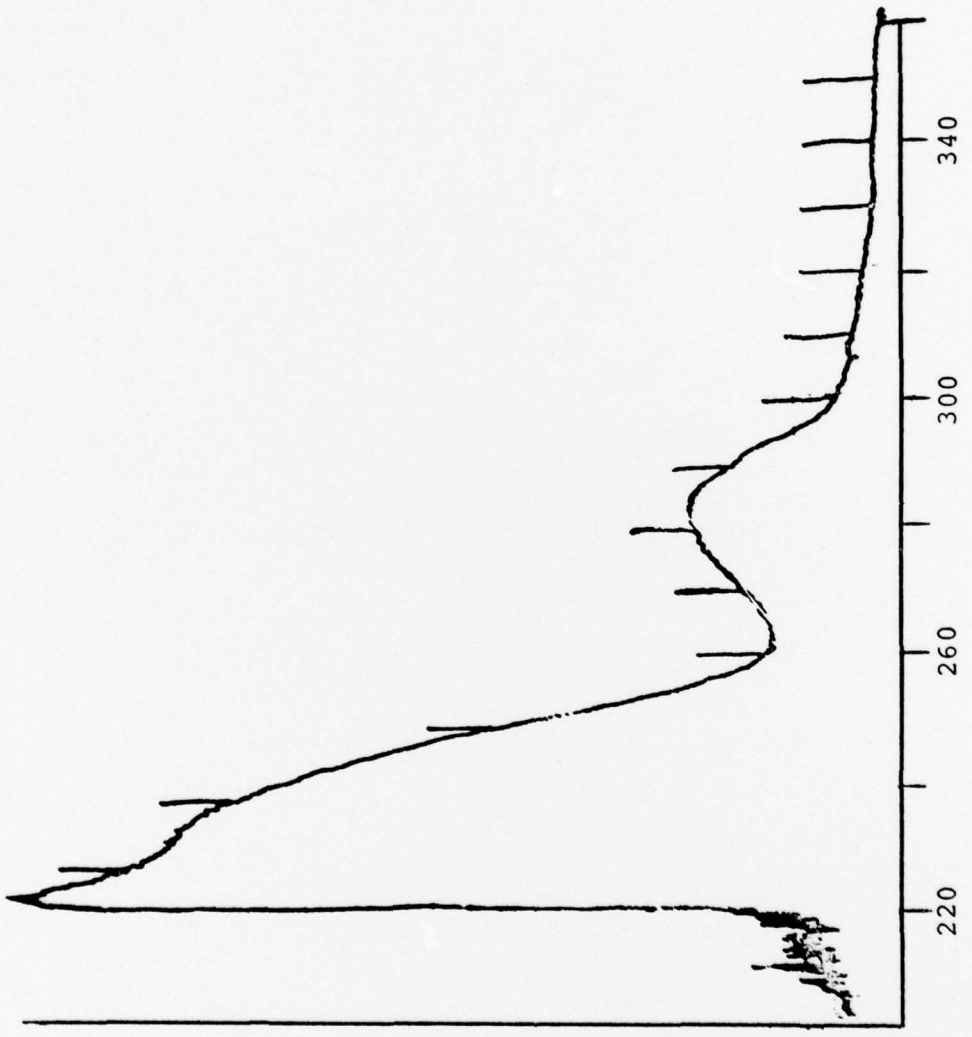


Figure XXI. Ultraviolet spectrum of isoelemicin.

E. Comparison of the HPLC Chromatograms of Nutmeg and Carrot Root Oils

The carrot root oil examined was extracted from the carrot root using the procedure outlined for the extraction of nutmeg oil (cf. Figure I). The best separation of the carrot root oil was obtained using a stationary phase of Porasil A and a mobile phase of isooctane/ethyl ether (95/5) (cf. Figure XXII). A total of six compounds were eluted with t_R's of 2.5, 3.0, 3.5, 4.1, 4.9 and 6.7 minutes. The HPLC of nutmeg oil under the same conditions produced a spectrum (cf. Figure XXIII) with the following t_R's: 2.7, 5.2, 12.7 minutes. The remaining compounds in nutmeg oil, methylisoeugenol, elemicin and isoelemicin had excessive t_R's using these conditions. A comparison of the t_R's of the compounds of carrot root oil and nutmeg oil reveals that the compounds are all different. Myristicin, a previously identified compound in carrot root oil, was eluted under these conditions with a t_R of 5.2 minutes. Since no compound in the carrot root oil was eluted with a t_R of 5.2 minutes, it is concluded that myristicin is not present in this sample of carrot root oil.

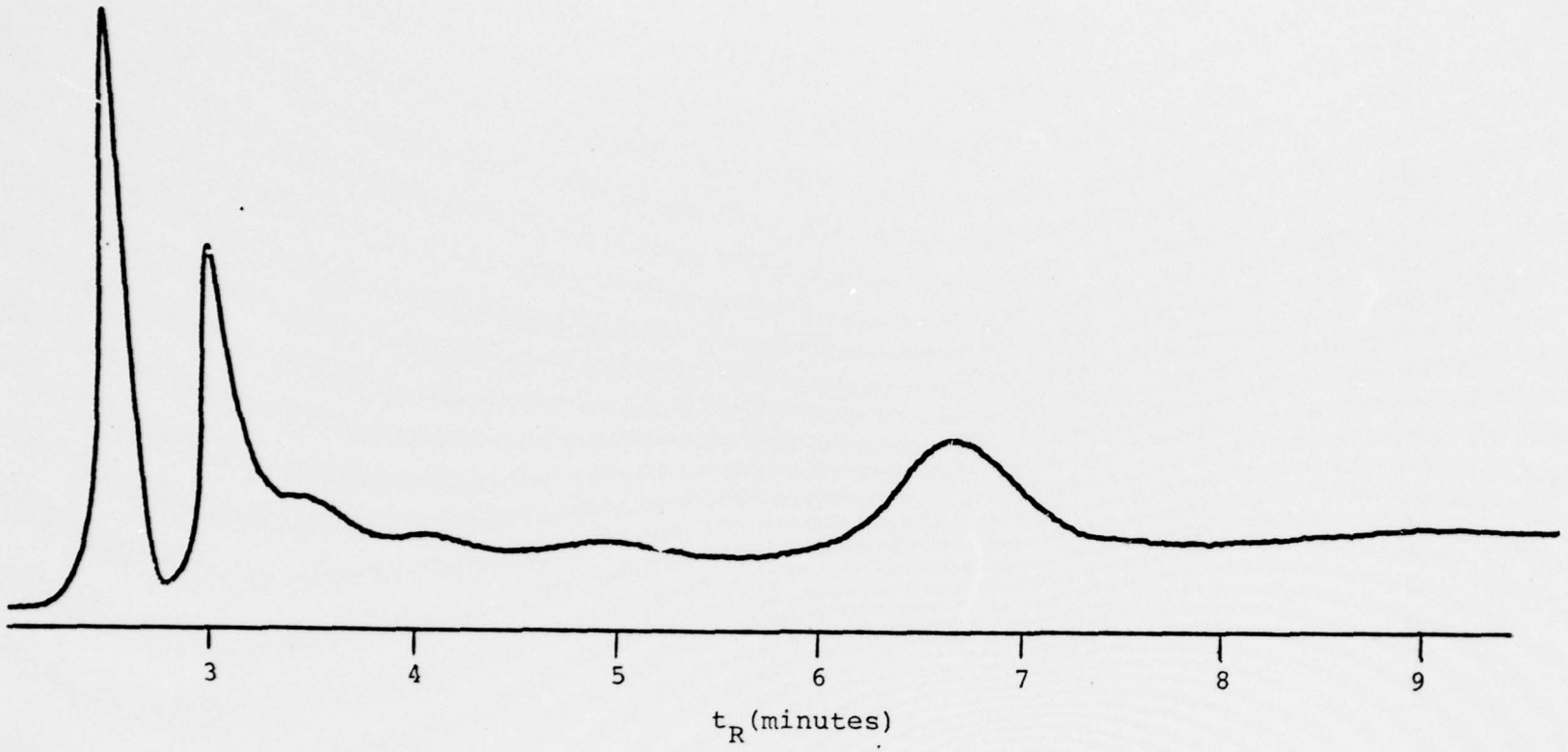


Figure XXII. HPLC spectrum of carrot root oil (isooctane/ethyl ether (95/5); Porasil A; UV detector).

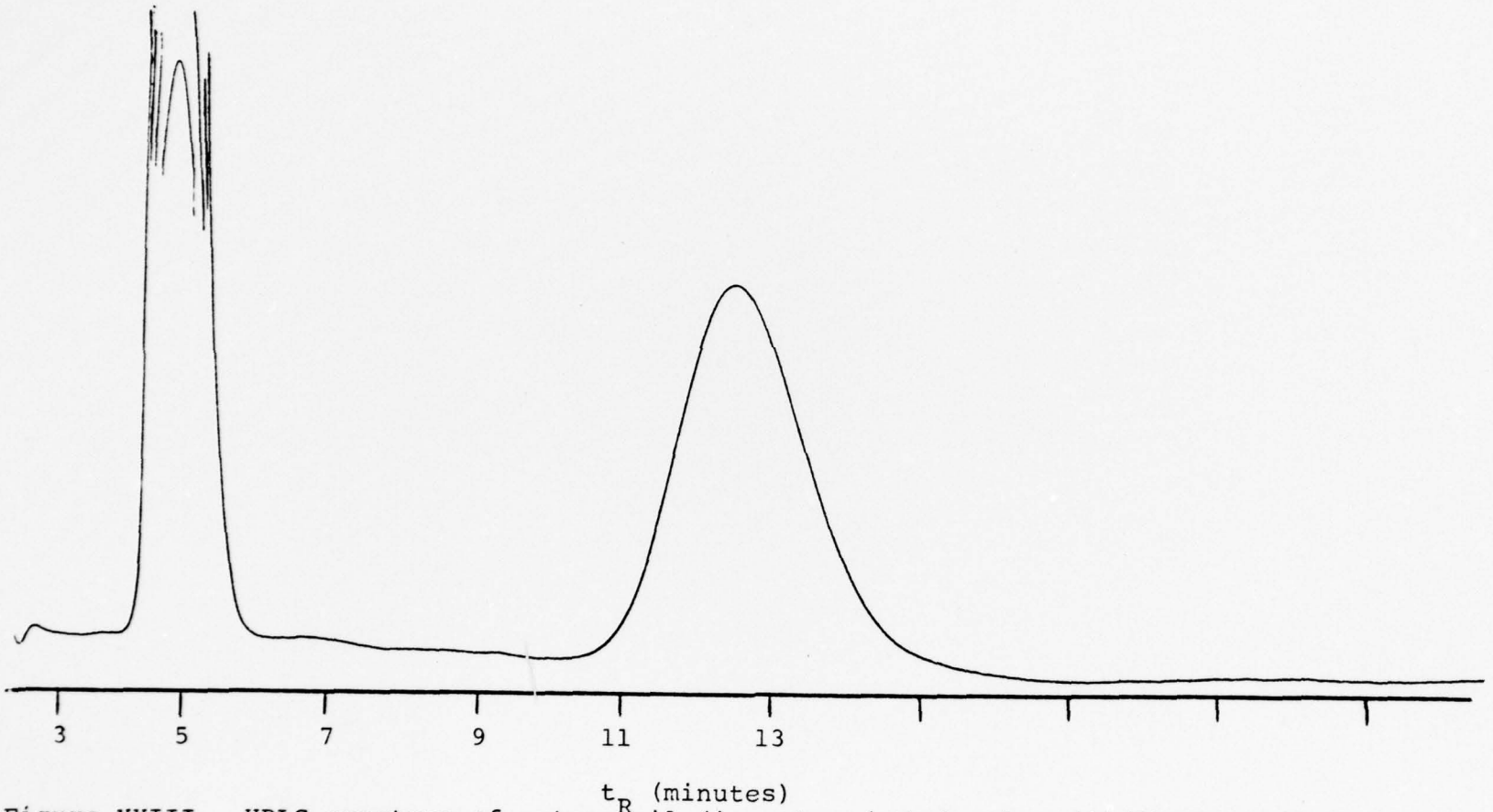


Figure XXIII. HPLC spectrum of nutmeg oil (isooctane/ethyl ether (95/5); Porasil A; UV detector).

CHAPTER IV

EXPERIMENTAL

A. Instrumentation

Nmr spectra were determined with a Varian HA 100 instrument for solutions in deuterochloroform with tetramethylsilane as internal reference. Mass spectra were recorded with a Hitachi-Perkin Elmer RMU-6M mass spectrometer. Infrared spectra were measured with a Beckman Acculab I recording spectrophotometer. Ultraviolet spectral were recorded with a Beckman Model 25 spectrophotometer. HPLC separations were performed using a Waters ALC 202 liquid chromatograph; an ultraviolet detector and a 2' X 2.5 mm column of Porasil A were used.

B. Extraction of Nutmeg

Commercially ground nutmeg (R. T. French Company) (453.6 g) was extracted with pentane (1.5 l) for 18 hours with constant stirring. The extract was concentrated on a rotary evaporator to give a yellow residue (A) (90.9 g). This residue was redissolved in chloroform and extracted with 0.5 N aqueous sodium hydroxide solution. The residual chloroform fraction was dried (anhydrous Na_2CO_3) and

concentrated on a rotary evaporator to give a yellow residue (B) (58.7 g) containing the neutral and basic compounds. Residue B was dissolved in chloroform (100 ml) and added to silica gel (100 g). The solution was evaporated on a rotary evaporator and dried on a vacuum pump for four hours. The resulting residue (C) was placed on a silica gel (grade II) column (89 cm X 5 cm), and eluted with pentane (3.3 l) followed by elution with ethyl ether (3 l). The ethyl ether fraction was concentrated on a rotary evaporator to give a yellow residue (D) (55.8 g) containing the polar (oxygenated aromatic) compounds of nutmeg.

C. R_f Values of the Oxygenated Aromatic Compounds of Nutmeg (Residue D)

A portion of Residue D was dissolved in chloroform. Thin layer chromatography, (TLC) (silica gel) of the resulting solution using a hexane/chloroform (70/30) mobile phase showed the presence of at least six components (R_f: 0.10, 0.15, 0.40, 0.45, 0.60 and 0.80). Visualization of the developed TLC was obtained by using iodine vapors.

D. Analytical HPLC Separation of the Oxygenated Aromatic Fraction (Residue D) Into Its Components

A sample of residue D (1.0 g) was dissolved in an isooctane/chloroform (90/10) solution (25 ml). Fifteen microliters of the solution was injected by syringe through the septum injector into the HPLC instrument. Using a flow rate of 2.5 ml/min. and a mobile phase of isooctone/chloroform (90/10), one compound was eluted with a t_R of

1.2 minutes (cf. Figure II). TLC (silica gel) in hexane/chloroform (70/30) of the eluted compound gave an R_f of 0.70.

Residue D (1.0 g) was dissolved in an isooctane/chloroform (80/20) solution (25 ml). Fifteen microliters of this solution was injected into the HPLC instrument through the septum injector using a syringe. Using a flow rate of 2.5 ml/min. and a mobile phase of isooctane/chloroform (80/20), two compounds were eluted with t_R's of 1.2 and 7.8 minutes, respectively (cf. Figure III). Resolution of the two eluted compounds was 4.7. TLC (silica gel) of the material eluted from the column using a mobile phase of isooctane/chloroform (80/20) showed two compounds with R_f's of 0.9 and 0.75, respectively.

Residue D, (1.0 g) was redissolved in an isooctane/chloroform (70/30) solution (25 ml) and injected (15 ml) through the septum injector of the HPLC instrument. The conditions were: flow rate 2.5 ml/min. and mobile phase isooctane/chloroform (70/30). The five eluted compounds had t_R's of 1.1, 3.0, 6.1, 9.4 and 20.4 minutes, respectively. The resolution of the chromatogram was 1.2 (cf. Figure IV).

Residue D (1.0 g) was dissolved in an isooctane/chloroform/methanol (80/19.9/0.1) solution (25 ml). Fifteen microliters of this solution was injected into the HPLC instrument through the septum injector. Using a flow rate of 2.5 ml/min. and a mobile phase of isooctane/

chloroform/methanol (80/19.9/0.1), four compounds were eluted with t_R's of 0.9, 1.3, 2.1 and 3.8 minutes, respectively. Resolution was 0.7 (cf. Figure V).

Residue D (1.0 g) was dissolved in an isooctane/chloroform/methanol (80/19/1) solution (25 ml). Ten microliters of the solution were injected through the septum injector. The mobile phase was isooctane/chloroform/methanol (80/19/1). The iniital flow rate was 2.5 ml/min. for five minutes. After five minutes, the flow rate was increased to 4.5 ml/min. Six compounds were thus detected with t_R's of 2, 2.5, 3.3, 4.1, 6.3 and 8.0 minutes (cf. Figure VI). Chromatogram minimum resolution was 0.9. TLC (silica gel) of this eluted material showed six compounds of R_f's 0.90, 0.70, 0.50, 0.40, 0.15 and 0.10 using a mobile phase of hexane/chloroform (70/30).

Sample D (1.0 g) was dissolved in an isooctane/methanol (99.5/0.5) solution (25 ml) and injected (10 into the HPLC instrument. Using mobile phase of isooctane/methanol (99.5/0.5) and a flow rate of 2.5 ml/min. for 5.1 minutes increased to 4.5 ml/min., the sample was separated into six compounds with t_R's of 1.7, 2.2, 3.0, 4.2, 6.6 and 9.3 minutes. Minimum resolution was 0.7 (cf. Figure VII).

Sample D (1.0 g) was dissolved in an isooctane/ethyl ether (80/20) solution (25 ml). Fifteen microliters of this solution were injected into the HPLC instrument through the septum injector. Using a flow rate of 2.5 ml/

min. and a mobile phase of isooctane/ethyl ether (80/20), three compounds were eluted with t_R's of 2.7, 5.1 and 12.7 (cf. Figure XXIII).

E. Preparative HPLC Separation of the Oxygenated Aromatic Fraction (Residue D) into its Components

Residue D (1.0 g) was dissolved in an isooctane/chloroform/methanol (80/19/1) solution (20 ml). Twenty microliters of this solution were injected into the HPLC instrument through the septum injector. The conditions were: flow rate of 2.5 ml/min. for five minutes, increased to 4.5 ml/min until all compounds are eluted and a mobile phase of isooctane/chloroform/methanol (80/90/1). Six fractions were collected. The t_R's of the collected fractions were: fraction A 2 minutes, fraction B 2.5 minutes, fraction C 3.3 minutes, fraction D 4.1 minutes, fraction E 6.3 minutes and fraction F 8.0 minutes. TLC (silica gel) of fractions gave the following R_f's for major components: fraction A 0.9; fraction B 0.7; fraction C 0.6; fraction D 0.4; fraction E 0.2; fraction F 0.1. A total of 40 separations were conducted to amass each fraction.

F. Compound Data

Fraction A, safrole was an oil, $\tau(CDCl_3)$ 3.33 (2H,d), 3.33 (1H,s), 4.15 (2H,s), 4.15 (1H,m), 4.90 and 5.02 (2H,m, t), 6.74 (2H,d), t_R (min.) [Porasil A, isooctane/chloroform/methanol (80/19/1)] 2.0.

Fraction B, myristicin was an oil, λ_{max} 278, 285 nm,

$\tau(CDCl_3)$ 3.62 (2H,s), 4.15 (2H,s), 4.15 (1H,m), 4.90 and 5.02 (2H,m,t), 6.12 (3H,s), 6.74 (2H,d), m/e 152 (M^+, 100%), 191(74), 177(72), 165(76), 162(71), 161(75), 149(72), 135(62), 134(72), 132(72) and 121(65). cm^{-1} 1040, 1080, 1130, 1190, 1240, 1430, 1450, 1505, 1610, 1630, 2920, 3008. t_R (min.) [Porasil A, isooctane/chloroform/methanol (80/19/1)] 2.5 (cf. page 38 for comparison to known data.

Fraction C, methyleugenol was an oil, λ_{max} 282 and 290 nm, t_R (min.) [Porasil A, isooctane/chloroform/methanol (80/19/1)] 3.3 (cf. page 38 for comparison to known data.

Fraction D, methylisoeugenol was an oil, m/e 178 (M^+, 100%); 163(48), 147(42), 135(22), 117(28), 107(38) and 103(33), t_R (min.) [isooctane/chloroform/methanol (80/19/1)] 4.1 (cf. page 44 for comparison to known data.

Fraction E, elemicin was an oil, λ_{max} 278, 284 nm, $\tau(CDCl_2)$ 3.58 (2H,s), 4.15 (1H,m), 4.84 and 4.98 (2H,m,d), 6.14 (3H,s), 6.16 (3H,s) and 6.74 (2H,d), m/e 208 (M^+, 40%), 207(19), 193(44), 178(20), 165(62), 163(32), 151(40), 148 (16), 137(56), 135(54), 121(50), 111(100) and 91(56). t_R (min.) [Porasil A, isooctane/chloroform/methanol (80/19/1)] 6.3.

Fraction F, isoelemicin was an oil, λ_{max} 277, 282 and 289 nm, t_R (min.) [isooctane/chloroform/methanol (80/19/1)] 8.0.

G. Extraction of Carrot Root

Blender ground carrot roots (2.2 kg) with leaves

removed were extracted with pentane (1.5 l) for 12 hours with constant stirring. The extract was concentrated on a rotary evaporator to give a reddish yellow oil (A) (3.2 g) which was redissolved in chloroform and extracted with 0.5 N aqueous sodium hydroxide solution. The residual chloroform fraction was dried (anhydrous Na_2CO_3) and concentrated on a rotary evaporator to give a reddish-yellow oil (B) 3.0 g) containing the neutral and basic compounds. Chloroform (100 ml) and silica gel (grade II) (5 g) were added to oil (B) and evaporated to dryness on a rotary evaporator. The resulting residue (C) was placed on a vacuum pump for four hours to remove remaining chloroform. Residue (C) was placed on a silica gel (grade II) column (89 cm X 5 cm) and eluted with pentane (5.5 l) followed by elution with ethyl ether (6.0 l). Fraction (D) was concentrated on a rotary evaporator giving a deep red oil (E) (2.2 g) containing the polar (oxygenated aromatic) compounds of carrot root oil.

H. R_f Values of Oxygenated Aromatic Compounds of Carrot Root Oil (Oil F)

A portion of oil F was dissolved in hexane. TLC (silica gel) of the resulting solution using a hexane/ethyl ether (80/20) mobile phase showed the presence of at least five components (R_f: 0.05, 0.15, 0.25, 0.50 and 0.75). Visualization of the developed TLC was obtained by using iodine vapor.

I. Analytical HPLC Separation of Oxygenated Aromatic Compounds of Carrot Root Oil

The oil (F) (1.0 g) was combined with an isooctane/ethyl ether (95/5) solution (25 ml). Fifteen microliters of the solution was injected into the HPLC instrument through the septum injector. Using a flow rate of 2.5 ml/min. and a mobile phase of isooctane/ethyl ether (95/5), six compounds were eluted with t_R's of 2.5, 3.0, 3.5, 4.5, 5.0 and 6.7 minutes (cf. Figure XXII).

BIBLIOGRAPHY

1. J. H. Galdstone, J. Chem. Soc., 17, 11(1864).
2. C. R. A. Wright, J. Chem. Soc., 26, 549(1873).
3. J. W. Bruhl, Ber., 21, 472(1888).
4. Wallach, Annalen, 252, 105(1889).
5. J. W. Semmler, Ber., 23, 1803(1890).
6. J. W. Semmler, Ber., 24, 3818(1891).
7. F. B. Power and A. H. Salway, J. Chem. Soc., 91, 2037(1907).
8. A. T. Shulgin, Nature, 197, 379(1963).
9. A. T. Shulgin and H. O. Kerlenger, Naturewissenshaften, 51, 360(1964).
10. G. M. Sammy and W. W. Nawar, Chem. Ind. (London), 38, 1278(1968).
11. T. P. Forrest, Nautrewissenshaften, 60, 257(1973).
12. J. E. Forrest, R. A. Heacock and T. P. Forrest, Experientea, 29, 139(1973).
13. J. P. Forrest, R. A. Heacock and T. P. Forrest, J. Chem. Soc. Perkin Trans., 205(1974).
14. D. J. Harvey, J. Chromatogr., 110, 91(1975).
15. D. G. Crosby and N. Aharonson, Tetrahedron, 23, 465(1967).
16. R. G. Buttery, R. M. Seifert, D. G. Guadagni, D. R. Black and L. C. Ling, J. Agr. Food Chem., 16, 1009 (1968).

17. Herbert Budzikiewicz, Carl Djerassi and Dudley H. Williams, "Interpretation of Mass Spectra of Organic Compounds," (Holden-Day Inc., San Francisco, 1964), p. 181.