TERPENE CONSTITUENTS OF CARROT SEED OIL

By

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1961

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1963

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ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. Leon H. Zalkow, thesis adviser, for his continued guidance and assistance throughout the course of this investigation.

The author is indebted to the Department of Chemistry for the use of their laboratory facilities and to the National Institutes of Health, Division of General Medical Sciences, for financial support under Predoctoral Fellowship No. GF 17,658. Sincere appreciation is extended to Sister M. Roger Brennan for running and interpreting the NMR spectra.

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CHAPTER I

THE VPC-VOLATILE CONSTITUENTS OF CARROT SEED OIL

A survey of the literature concerning the composition of the essential oil of Daucus Carota L. quickly reveals that, although quite a large amount of work has been done regarding the composition of the oil, there is little agreement between various research groups and even between reports by the same workers.

Early work on separation of the components was limited to fractional distillation, and because most of the components are liquids, the compounds were often isolated in various degrees of purity which greatly increased the problem of identification. In the last ten years, separation has been done by both distillation and adsorption chromatography on alumina, the latter of which is now standard procedure and probably the method most widely used. Vapor phase chromatography [VPC], both as a tool for identification and as a technique for separation, has recently been recognized as a convenient method for analyzing the terpene constituents of essential oils.[1] It is by this method that this study has been done.

Historical Background

The chemical composition of carrot seed oil [from cylindrical-root carrots] was first investigated by Richter[2] and later by Asahina and Tsukamoto[3] and the presence of the following constituents suggested: l-α-pinene, l-limonene, daucol, carotol, asaron, bisabolene, formic acid [?], acetic acid as the ester, free butyric acid [probably isobutyric acid], and free palmitic acid. Šorm and coworkers, in a paper published in 1951,[4] identified, after fractional distillation and repeated chromatographic separation on alumina, the following components by infrared examination and the preparation of various derivatives: α- and probably β-pinene, dipentene [racemic limonene], p-cymene, carvone, geranyl acetate, caryophyllene, bergamotene, bisabolene, carotol [principal component], a sesquiterpene aldehyde $C_{15}H_{24}O$, and a high-boiling fraction containing a mixture of diterpenic hydrocarbons and daucol. The essential oil of the fruit[+] of Daucus Carota L., which is widely distributed in the European part of the Soviet Union, in middle Asia, and in Caucasus, contains according to the work of Pigulevskii and Kovaleva[5] a relatively large portion of geranyl acetate, the percentage varying from 24 to 50%, depending upon the region from which

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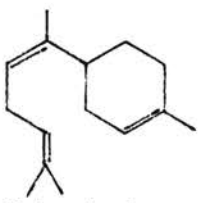
[+]The oil obtained from the seeds of the plant is referred to in the literature as both seed oil and fruit oil, as opposed to the oil which is obtained from either the root or from the stalks and stems. In this paper it will be referred to as carrot seed oil.

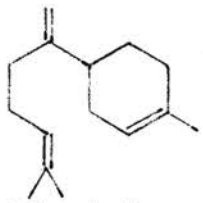
the oil was obtained. Also present in the oil are l-sabinene, nopinene [probably], and an additional $C_{10}H_{16}$ terpene, a small amount of a $C_{30}H_{62}$ paraffin hydrocarbon, two very similar sesquiterpenes, one of which is bicyclic and gives upon treatment with selenium a blue and violet azulene mixture. In later publications these workers reported the presence of bergamotene and β-bisabolene,[6] obtained by column chromatography on alumina and identified by their infrared [IR] and Raman spectra, and a sesquiterpene hydrocarbon, daucene,[7] which was identified as dehydrocarotol. Makarova and Borisyuk[8] reported l-α-pinene with a slight admixture of β-pinene, as well as citronellol [1.5-2%], geraniol [25%], citral aldehyde [2%], and caryophyllene [3%]. The most recent study of the composition of the essential oil of Daucus Carota L. from Central Asia was again reported by Pigulevskii.[9] The oil was fractionally distilled into five fractions. From fraction 1 [b.p. 40-49°/10 mm., n_D^{20} 1.4612, 2.5% of oil] were isolated after an additional fractional distillation l-α-pinene [2%] and dipentene [0.5%]. From fraction 2 [b.p. 70-94°/3 mm., n_D^{20} 1.4851, 0.8% of oil] after chromatography on neutral alumina [activity II] geraniol was isolated [0.5%] and identified by infrared spectroscopy [IR]. Repeated chromatography of combined fractions 3 and 4 [b.p. 94-102°/3 mm., n_D^{20} 1.4902, 6.1% of oil, and b.p. 103-117°/3 mm., n_D^{20} 1.4930, 31.6% of oil] on alumina [activity I] gave d-daucene [2%] and β-bisabolene [35%]. β-bisabolene was identified as the trihydrochloride and by IR. From fraction 5 [b.p. 117-123°/3 mm.,

n_D^{20} 1.4970, 59% of oil] after chromatography on alumina [activity III] were obtained carotol [55%], asaron [4%], and daucol [0.5%]. Carotol and daucol were identified by their IR spectra; asaron was identified by its U. V. spectrum. It should be noted that although geranyl acetate, l-sabinene, and bergamotene had previously been reported[5, 6] by these workers as constituents of the same oil, these compounds were not reported in the latest work; no explanation for this discrepancy was advanced by the authors.

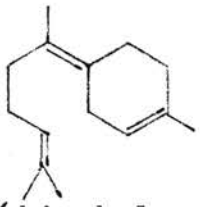
Up to the 1959 report of Pigulevskii, the isomeric form of the bisabolene present in the oil had not been established. Identification had been established by comparison of physical constants and preparation of the trihydrochloride derivative. All three isomers, however, can be transformed into the same trihydrochloride, according to Ruzicka and van Veen.[10]



α-bisabolene



β-bisabolene



γ-bisabolene

Neither is the rotatory power displayed by the bisabolenes a good indication of the isomer present, as several bisabolene fractions of varying optical activity have been isolated, and there is no agreement in the literature as to the rotation of the pure α- and β-isomers. The γ-product, believed to predominate in nature,[10] is optically inactive. Pigulevskii and Kovaleva[6] based their report of the presence of the beta isomer on infrared spectral comparison with that of Sorm for

β-bisabolene, and the Raman spectrum which indicated the presence of one primary-tertiary and two secondary-tertiary double bonds.

Discussion of Results

Vapor phase chromatographic analysis of natural carrot seed oil indicated the presence of the following compounds: limonene, α-pinene, β-pinene, caryophyllene, β-bisabolene, bergamotene [?], geranyl acetate, geraniol, carotol and daucol. Of the other compounds which have been reported to be present in the oil, citronellol, citronellal, and carvone were not found in this oil.

Caryophyllene, which was reported by Šorm but was not indicated as a constituent by Pigulevskii, was found to be 10% of the total amount of material observed by VPC. Identity of this hydrocarbon was established by comparison of retention times in VPC, and by NMR spectral comparison with an authentic sample.

The sesquiterpene hydrocarbon constituents of carrot seed oil were obtained by chromatography of a distillate fraction [b.p. 80-94°/0.1 mm.] on Merck base-washed alumina in a manner similar to the procedures of Šorm and Pigulevskii. Both of these workers reported that they were able to isolate, after repeated chromatography by this method, pure samples of bergamotene and β-bisabolene. The physical measurements [b.p. and refractive index] of the hydrocarbon constituents which were obtained in the same manner for this study were found to agree

within reasonable experimental limits with those of the previous workers. The VPC chromatogram, however, indicated the samples to be mixtures of three major components, the relative amount of each varying slightly according to the relative retention time of the sample on the basic alumina column.

Separation of these three major components by preparative gas chromatography gave samples which were then analyzed further on several VPC columns. Of these three components, the one having the longest retention time could be further resolved by VPC chromatography into the three isomeric bisabolenes. Reinjection of a mixture of the three isomers so obtained and the original carrot seed oil indicated that only one of these was present in the original oil. IR and NMR analysis of a sample of this isomer, collected by preparative VPC, showed this component to be the beta isomer. That this isomerization had not occurred on the VPC column was clearly demonstrated by reinjecting the pure β-bisabolene on the same column under identical conditions; a single, symmetrical peak was obtained. Comparison of the chromatograms of the original carrot seed oil and the distillate fraction used for the alumina chromatography with the chromatogram of the isomeric mixture obtained from column chromatography suggested that the isomerization must be occurring on the basic alumina column as only the beta isomer was present in the chromatograms of both oils.

Geranyl acetate was found to be a major component of the oil investigated in this study--second only to carotol in percentage of VPC-observable material. This compound was

reported by Šorm[4] and also by Pigulevskii in the work published in 1955,[5] the latter showing it to comprise as high as 50% of the oil. Yet later chromatographic analysis by the same workers,[9] accounting for 99.5% of the oil chromatographed, did not show this compound to be present in oil obtained from the same geographical location.

One factor which should not be overlooked in the comparison of the results of this study with those of other workers is the difference in geographical location from which the oil was obtained. While this might feasibly account for some of the differences observed, a VPC chromatogram of a sample of carrot seed oil from an entirely different source was very nearly identical to that of the oil used for this analysis, the only observable difference being a small variation in the relative amounts of the constituents present.

Experimental

VPC Instrumental Data

Natural carrot seed oil, obtained from Magnus, Mabee and Reynard, Inc. [New York, N. Y.], was analyzed by vapor phase chromatography. The experiments were carried out with an Aerograph A-350-B chromatograph using helium as the carrier gas, a thermal conductivity cell as detector, and Minneapolis-Honeywell strip-chart recorder with a 1 mv. scale and a chart speed of 0.5 inch per minute. Data for the four columns used are given in Table I, and the columns will be referred to by these numbers throughout the remainder of the discussion.

TABLE I

VPC CHROMATOGRAPHY COLUMNS

1	5% S. E. 30 on acid-washed chromosorb "W", 5' x 1/2" O. D., stainless steel.
2	20% Ucon Polar on chromosorb "W", 10' x 1/4" O. D., aluminum.
3	20% poly-m-phenyl ether [6 phenyl] on 45/60 chromosorb-6, 10' x 1/4" O. D., aluminum.
4	10% Craig polyester succinate on 45/60 chromosorb "W", 10' x 1/4" O. D., aluminum.

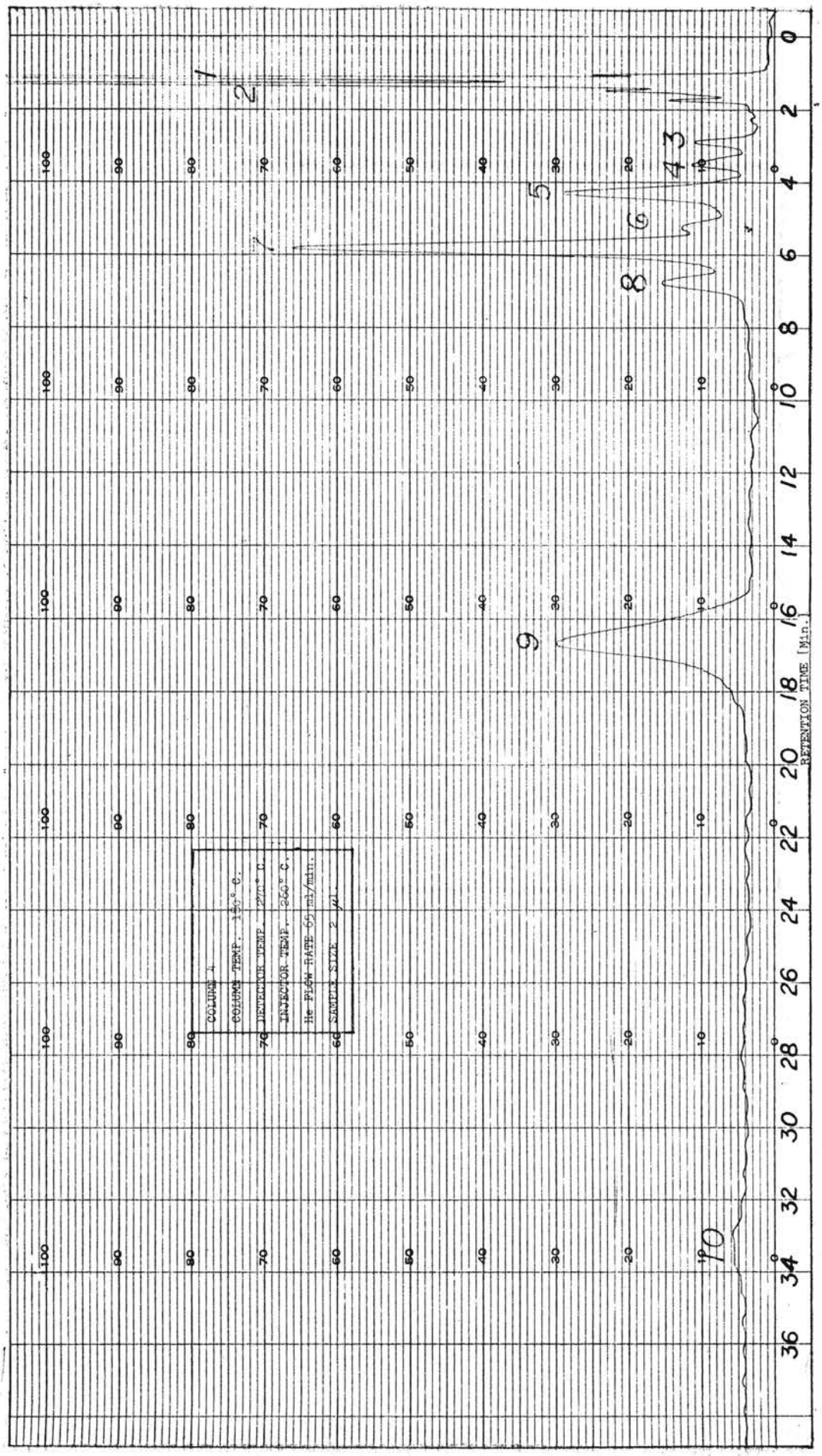
Chromatogram of Carrot Seed Oil

The chromatogram for the natural oil obtained on VPC Column 4 is shown in Plate I. The numbers of the peaks correspond to the same numbers, hence the compounds, in Table II. The various components were identified by comparison of retention times under identical conditions, and by injecting mixed samples of the carrot seed oil and an authentic sample if available, then observing the change in the area under the peak as related to the original chromatogram of the oil. Authentic samples of β-bisabolene and bergamotene were not available for direct comparison, and identification of these two is based on the IR and NMR spectra of the pure compound isolated and purified by preparative VPC. The percentages recorded in Table II are based on material observed in VPC.

An examination of the oil using Column 2 at 190° gave a better separation of the monoterpenes. The two pinenes

Plate I

VPC Chromatogram of Carrot Seed Oil



could be distinguished from each other, the α isomer having the shorter retention time. The sesquiterpene constituents could best be analyzed on VPC Column 4 at column temperatures of 180 and 200° C. The higher temperature was necessary in order to show carotol and daucol as sharply defined peaks on the chromatogram. However there was no change in the remainder of the chromatogram other than decreasing the retention times.

TABLE II

COMPONENTS OF CARROT SEED OIL
AS IDENTIFIED BY VPC

No.	Compound	Retention Time	Percent
1	α-pinene β-pinene	1.1 min.	12%
2	limonene	1.2 min.	15%
3		2.9 min.	2%
4		3.5 min.	2%
5	caryophyllene	4.3 min.	10%
6	β-bisabolene	5.2 min.	3%
7	geranyl acetate	5.8 min.	22%
8	geraniol	6.7 min.	5%
9	carotol	16.6 min.	27%
10	daucol	33.2 min.	2%

Injection of samples of citronellol [5.6 min.], citronellal [2.7 min.], and carvone [7.7 min.] on Column 4 at a column temperature of 180° C. gave peaks which did not correspond to any of those in the chromatogram of the carrot seed oil run under identical conditions and can be assumed absent.

Isolation and Identification of Sesquiterpene Hydrocarbons

The sesquiterpene hydrocarbon fraction of carrot seed

oil was obtained by chromatography of the distillate fraction of carrot seed oil, b.p. 80-94°/0.1 mm., on Merck base-washed alumina [activity I]. The first fraction, eluted with carbon tetrachloride, was composed of a mixture of olefinic hydrocarbons [b.p. 85-90°/1 mm., n_D^{29} 1.4938] from which the major components were separated by preparative gas chromatography on VPC Column 1.

Caryophyllene and Bergamotene

The first major fraction separated by VPC from the alumina chromatography hydrocarbon mixture [$n_D^{27.5}$ 1.4915] appeared to be homogeneous when reinjected on the same column [1]; however, two components were indicated on VPC Column 2. The same sample on VPC Columns 3 and 4 showed, in addition to the two major components, several small peaks comprising less than 3% of the total area. Of the two major components, the compound having the shorter retention time corresponds to compound 4 in Plate I, while the second corresponds to compound 5. The latter was identified as caryophyllene by comparison of the retention time with that of an authentic sample. The NMR spectrum[+] of the mixture [approximately 1:1] is shown in Plate II. The spectrum of a pure sample of caryophyllene is shown in Plate III. By disregarding those peaks in Plate II which can be assigned

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[+]All NMR spectra were run with a Varian A-60 High Resolution Spectrometer using CCl_4 as solvent and tetramethyl silane as internal standard [$\delta = 0$].

Plate II

NMR Spectrum of Caryophyllene-Bergamotene

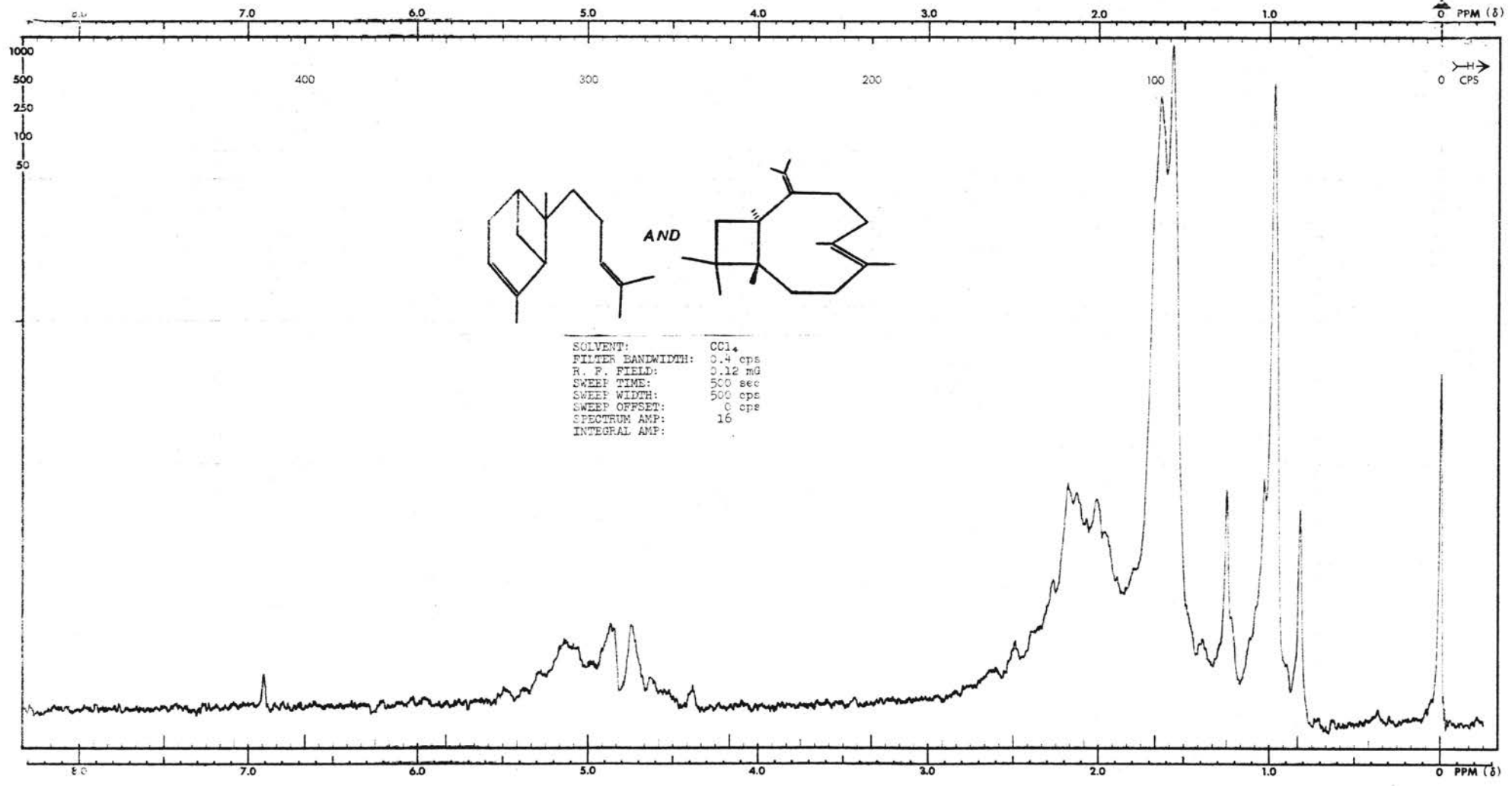
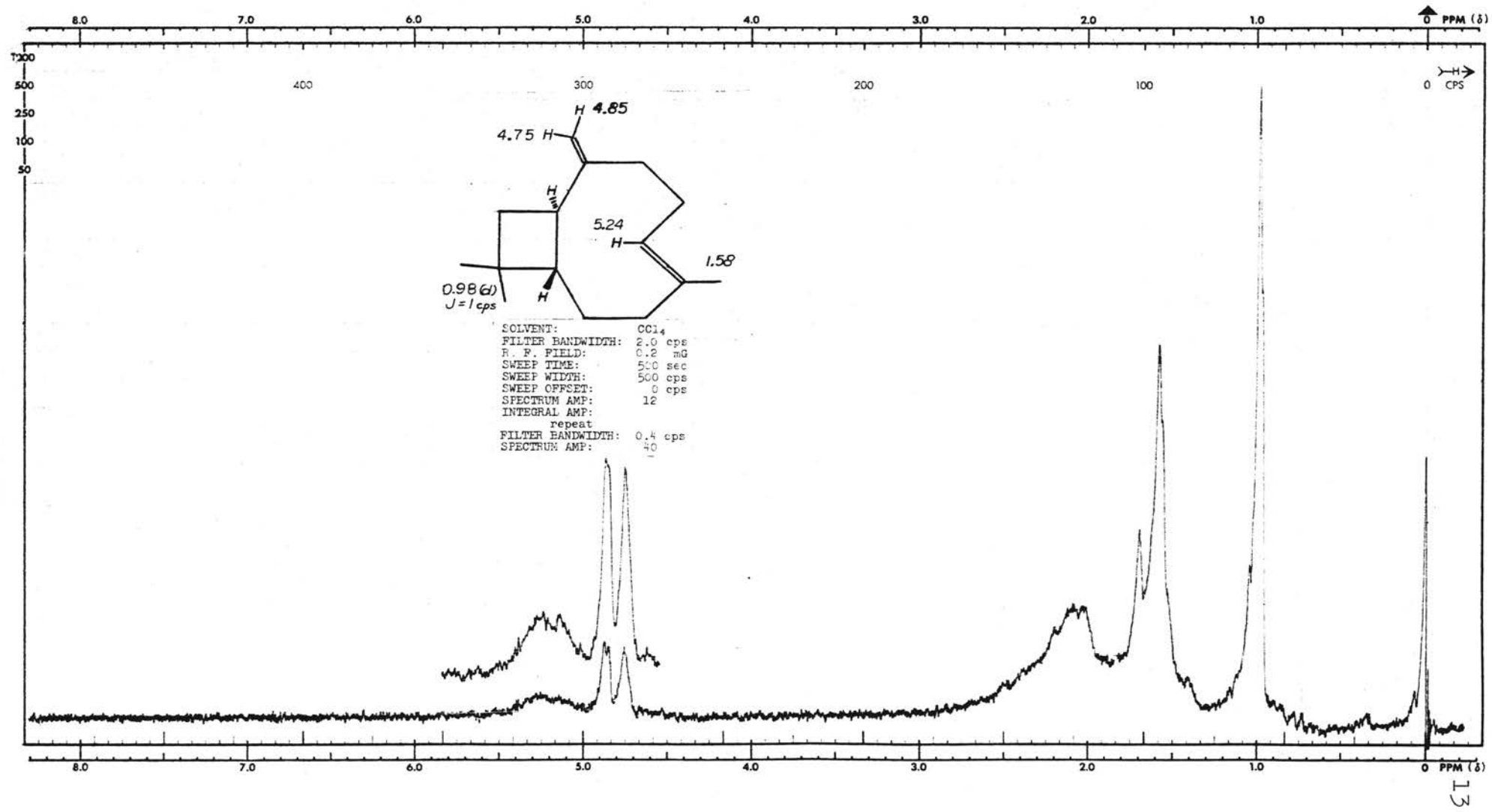


Plate III

NMR Spectrum of Caryophyllene



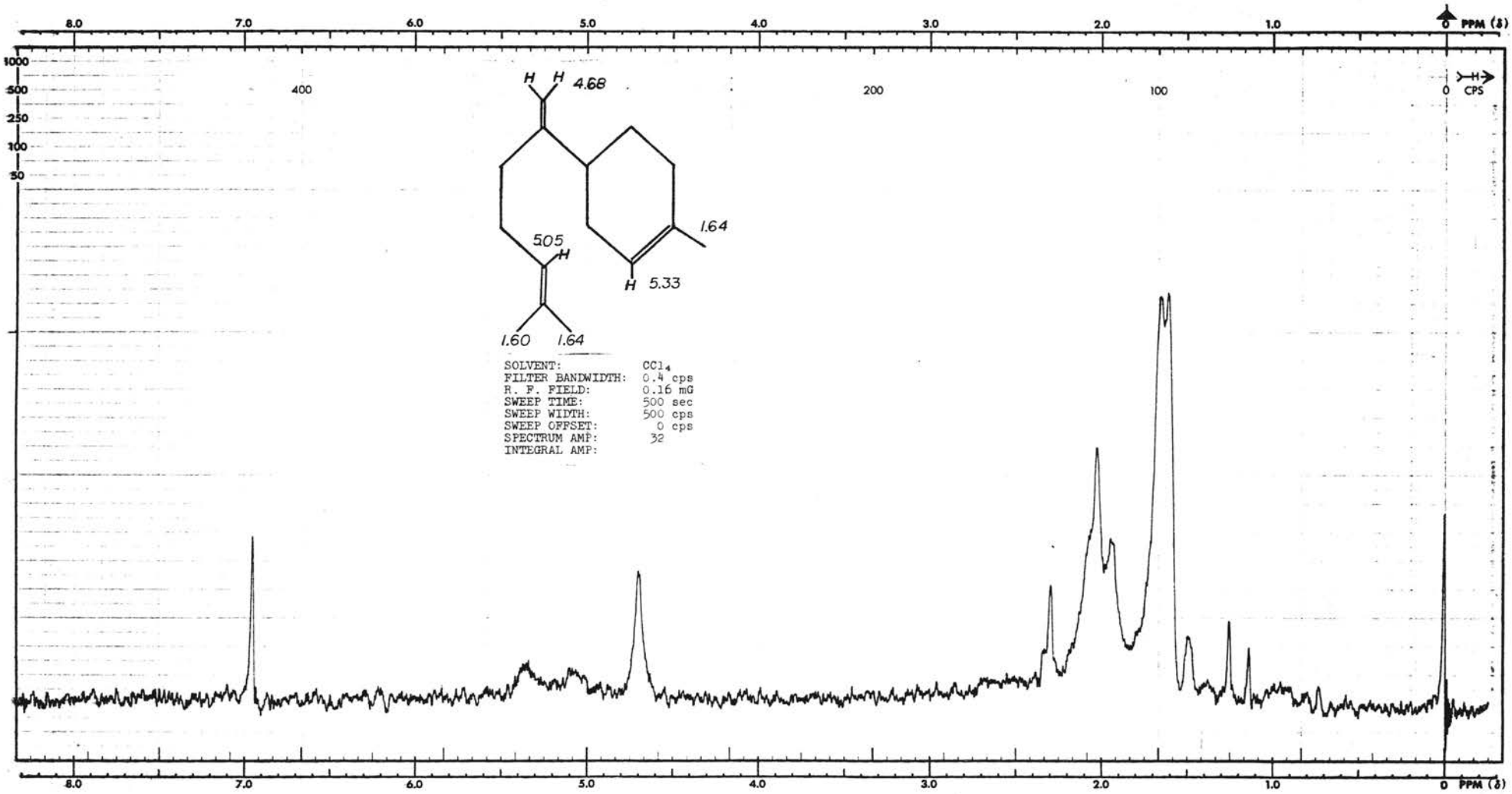
to caryophyllene by comparing this spectrum to Plate III, the remaining protons can be studied with regard to the second component. Of the compounds which have been previously reported as constituents of carrot seed oil, the NMR analysis most nearly fits the structure for bergamotene as given by Šorm.[11]

β-bisabolene

The second fraction collected by VPC [$n_D^{27.5}$ 1.4939] appeared to be homogeneous upon reinjecting on VPC Columns 1 and 2. On VPC Columns 3 and 4 this sample could be further resolved into three components, 58%, 34%, and 8% respectively. Collection of the major component and reinjection on the same column gave a single symmetrical peak, indicating that the three peaks were, in fact, three separate compounds, or, more probably, three isomers of the same compound. The NMR spectrum of this pure component, shown in Plate IV, is consistent with the structure of β-bisabolene. IR [0.1 mm cell] of the mixture was very similar to that reported by Šorm[12] for β-bisabolene. The NMR spectrum of the mixture was very similar to that of the pure component and supported the isomeric relationship of these three components. The number of protons in each of the peaks in the NMR spectrum varied slightly, but not by whole numbers, and a small doublet present in the mixture at 0.75 ppm [J = 4 cps] was not present in the NMR of pure β-bisabolene. Insufficient amounts of the two remaining components prevented NMR confirmation of the isomeric relationship. The VPC chromatogram of a mixed

Plate IV

NMR Spectrum of β-bisabolene



sample of the original carrot seed oil and the sample showing three components on VPC Column 4, however, indicated that β-bisabolene was the only one of the three present in the original oil, and that the other two isomers must have been formed in the basic-alumina chromatography as they were not present in the distillate fraction used for the chromatography.

Isolation of Geranyl Acetate

The second fraction eluted with CCl_4 from the basic alumina column showed four components on VPC Column 1, two of which were minor and corresponded to those in the preceding chromatography fraction [bisabolenes and caryophyllene-bergamotene]. The two major fractions were collected by preparative VPC. The first fraction showed a single, symmetrical peak when injected on each of the four columns and corresponded to compound 7 in Plate I; its IR spectrum showed bands at ν^{film} 1745, 1675, and 1235 cm^{-1}, indicating an unsaturated ester. The sample collected by VPC had a retention time identical with that of an authentic sample of geranyl acetate. The NMR spectrum, Plate V, is consistent with the structure of geranyl acetate.

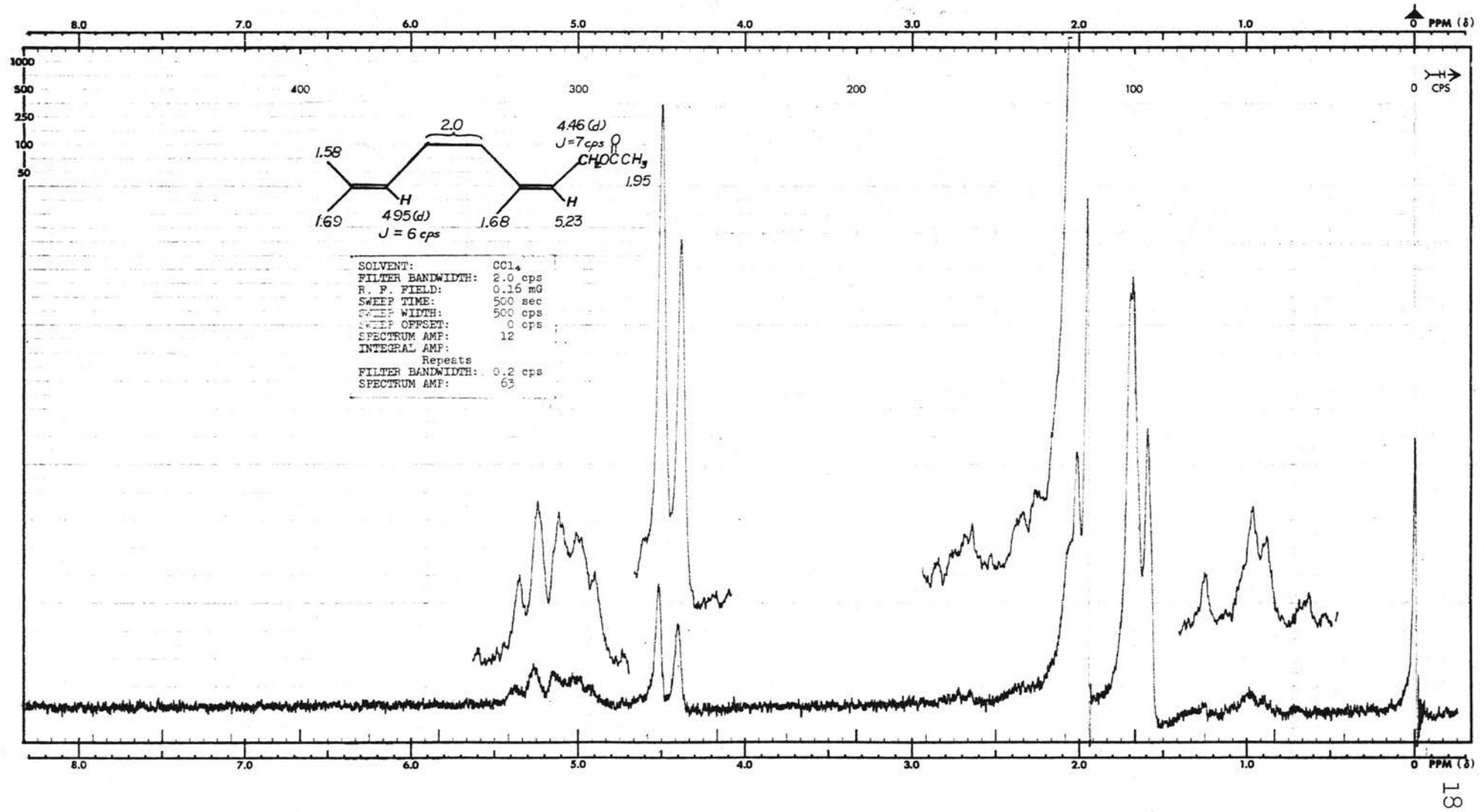
Isolation of Carotol

The second major component of the basic-alumina chromatography fraction two corresponded to compound 9 in Plate I, and had the same retention time as an authentic sample of

carotol. The NMR spectrum was identical with the spectrum previously reported.[13]

Plate V

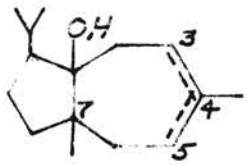
NMR Spectrum of Geranyl Acetate



CHAPTER II

CONTRIBUTIONS TO THE DETERMINATION OF THE ABSOLUTE CONFIGURATION OF CAROTOL AT C_7

Carotol, a sesquiterpenoid constituent of carrot seed oil, has recently been shown to have structure I.[13] [14] [15]



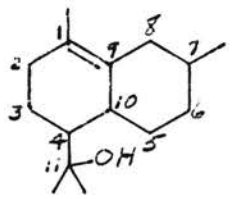
I

The position of the double bond [C_3-C_4 or C_4-C_5] has not been rigidly proved, and the relative and absolute configurations of the molecule are not known.

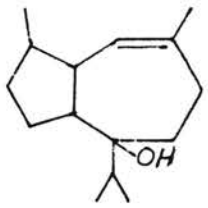
Historical Background

Carotol, $C_{15}H_{26}O$, was first isolated by Asahina and Tsukamoto[16] and later by Palfray and Lepesqueur[17] from the essential oil of Daucus carota L. Three different carbon skeletons have been proposed for the alcohol. Šorm and Urbanek[18] originally proposed formula II in which carotol was represented as having a hydroxy group at C_{11} and a double bond at C_1-C_9. These workers[19] later suggested that the hydroxy group was at C_4 and the double bond at C_6-C_7 or C_7-C_8. Very recently Šorm and coworkers[15] proposed formula I,

with the double bond at C_4-C_5, for carotol on the basis of new degradative experimental work. At the time of the latter report there appeared another publication in which Chiurdoğlu and Descamps[20, 21] offered evidence in support of formula III.



II

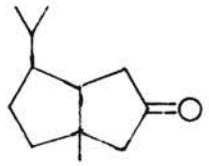


III

The oxygen atom in carotol was recognized to be present as a tertiary alcoholic grouping[22] because of its unreactive character towards acylating agents and its ease of elimination. Šorm and Urbanek[18] found that on catalytic hydrogenation the saturated dihydrocarotol, $C_{15}H_{28}O$, was formed. The presence of only one ethylenic linkage, and hence two rings, implied by this experiment was confirmed by the formation of a crystalline carotol oxide, $C_{15}H_{26}O_2$, upon treatment with perphthalic acid. Dehydrogenation of carotol with palladium on charcoal gave an aromatic hydrocarbon identical with synthetic 1,7-dimethyl-4-isopropylnaphthalene together with a small amount of unidentified azulenes. The position of the double bond was determined from the fact that oxidation of the triol derived from carotol with $KMnO_4$ apparently gave a diketone which was also a methyl ketone. Based upon this evidence, Šorm and Urbanek proposed structure II for carotol. This skeleton can be constructed from three isoprenoid residues, but not if these are linked as in the conventional farnesol chain; therefore, it was suggested that carotol may be a

member of the cadalene group in which methyl migration has occurred in the last stage of the biosynthesis of the carbon skeleton.

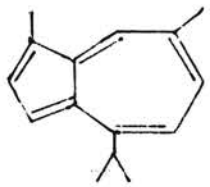
In August of 1959 Šorm and coworkers suggested[19] that their original structure was incorrect, and this report was followed by a paper[15] in which they proposed structure I. This structure was based upon extensive degradative experimental work in which several oxidations followed by pyrolysis led to a five-membered ketone of possible formula IV. The assignment of the double bond at C_4-C_5 was based upon the isolation of a small amount of succinic acid from the nitric acid oxidation of the triol.[23]



IV

At almost the same time that this last report came from Šorm's laboratory, another came from the laboratory of Chiurdoglu and Descamps in Belgium. These workers[20, 21] proposed structure III for carotol; the carbon skeleton was based on dehydrogenation studies. On treatment with selenium at 280° C. for two hours, carotol gave a previously unreported azulene in 7% yield which was assigned structure V. Under more drastic conditions, V was found to rearrange to 1,7-dimethyl-4-isopropylnaphthalene, previously isolated and synthesized by Šorm.[24] The ultraviolet spectrum calculated for structure V was also consistent with that observed for the unknown

azulene. This data, plus additional chemical evidence led to the proposed structure III.

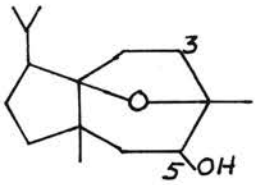


V

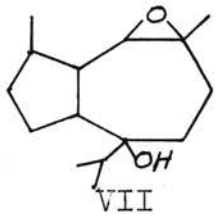
Work presented by Zalkow and associates[13] supports that of Šorm in showing that formula I, or its isomer with the double bond at C_3-C_4, represents the correct structure of carotol. NMR data presented by these workers for carotol and several of its derivatives are consistent with structure I [double bond at C_3 or C_4] for carotol, and does not support structure II proposed earlier by Šorm or the recently proposed structure III of Chiurdoglu and Descamps.

Closely related to carotol is another sesquiterpenic constituent of carrot seed oil, daucol, $C_{15}H_{26}O_2$. This compound was first reported by Richter,[2] and the properties obtained by Šorm, Chiurdoglu, and Zalkow for the oxide which arises from carotol upon treatment with peracids are in fairly close agreement with those recorded by Richter for daucol. Originally, daucol was believed to be the epoxide of carotol, but it was recently shown by Šorm"s group that oxidation of daucol gives a keto ether, $C_{15}H_{24}O_2$; and formula VI, which is a hydroxy ether of I, was proposed as the structure of daucol. Chiurdoglu and Descamps[20, 21] proposed structure VII, which is simply the epoxide of their structure III for carotol. Chemical and NMR data obtained by Zalkow[13] is

consistent only with structure VI [OH at C_3 or C_5] for daucol.



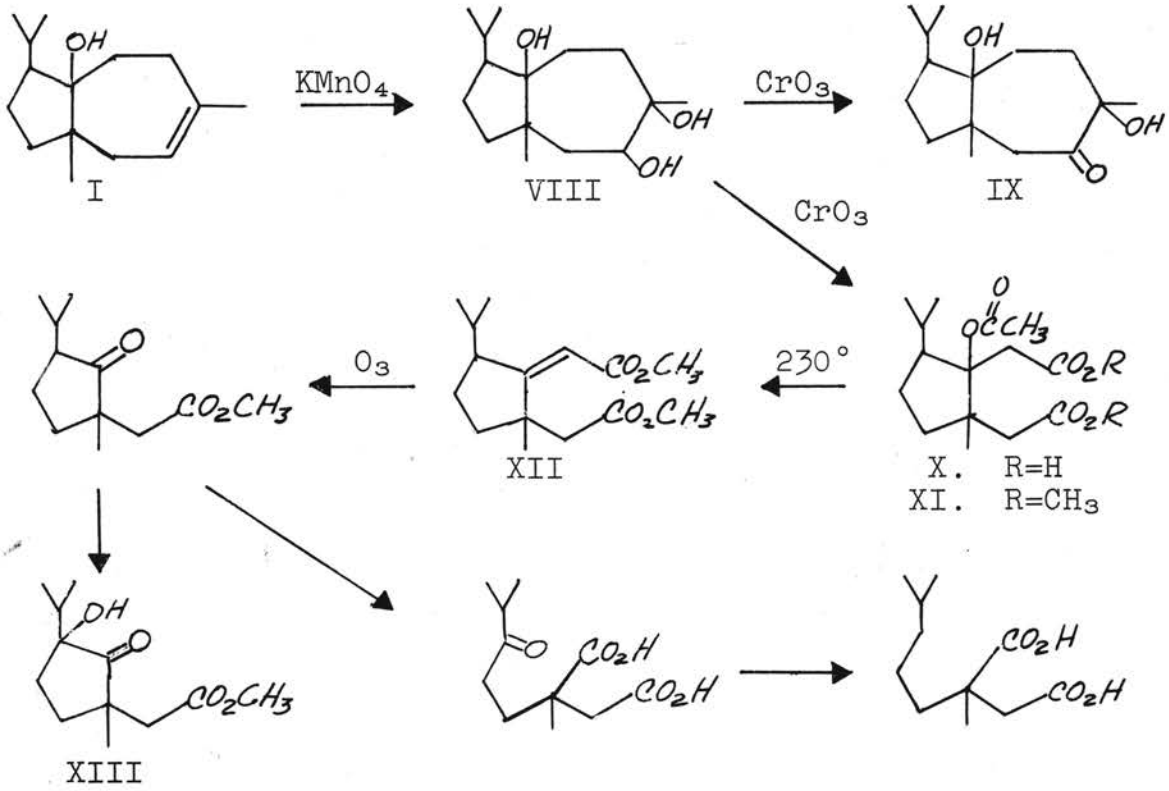
VI



VII

Theoretical Discussion

In order to determine the absolute configuration of carotol [and daucol] at C_7, it was proposed that carotol be degraded according to the following scheme to 2-methyl-2-[4-methylpentyl]succinic acid which could then be compared by the quasi-racemate method[25] with [+] and [-] α-methyl-α-isopropyl succinic acid.

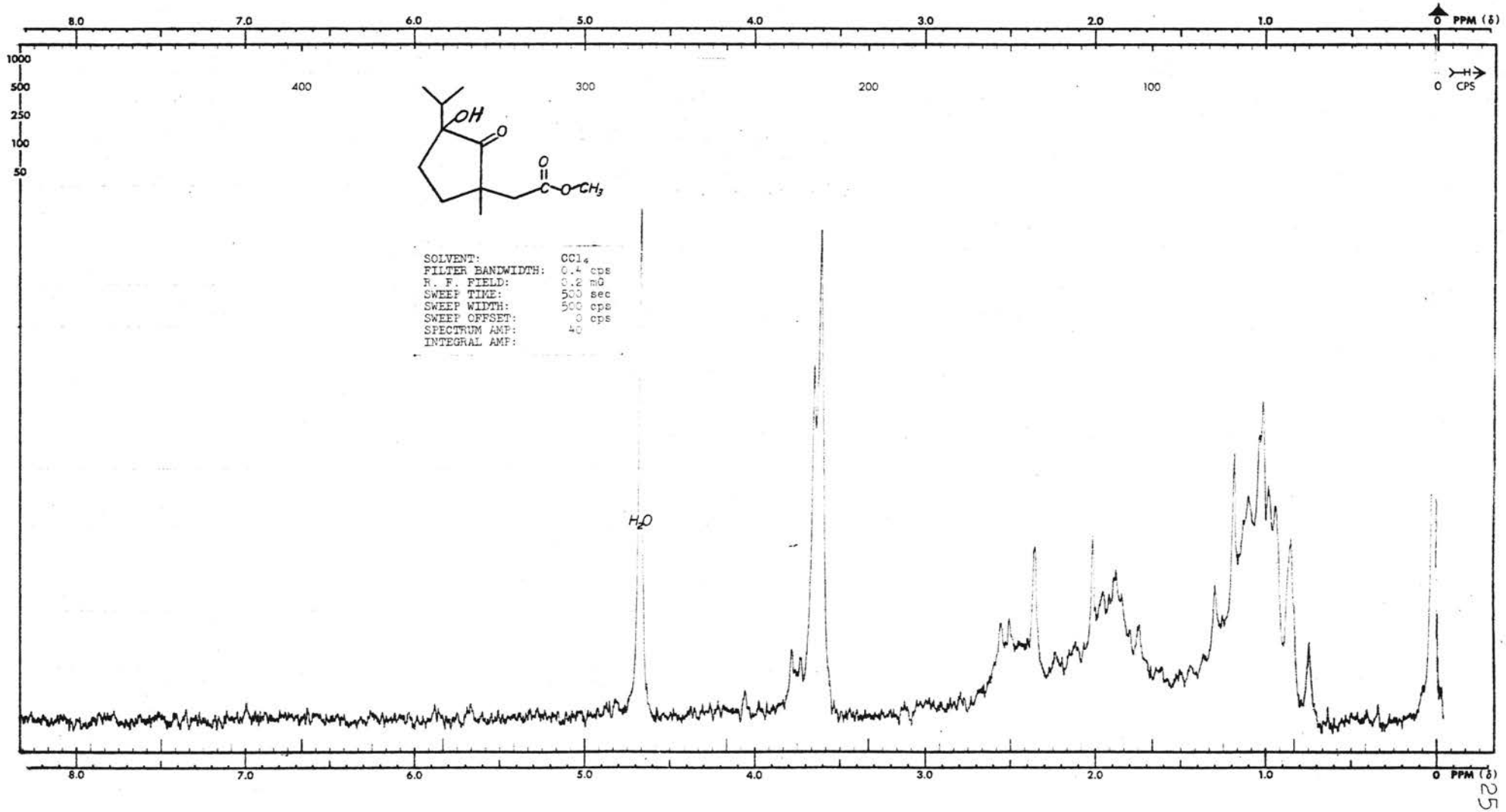


Carotol, which was obtained by fractional distillation of commercial carrot seed oil, was oxidized with potassium permanganate to the crystalline triol VIII by the method of Šorm and Urbanek.[18] On treatment with chromic acid in acetic acid, the triol afforded both acetoxy dicarboxylic acid X and the dihydroxy ketone IX. The latter, upon reoxidation with chromic acid, afforded the acid X as well. Pyrolysis of the dimethyl ester XI at 230° C. and atmospheric pressure gave an unsaturated diester. The ultraviolet spectrum, $\lambda_{max.}^{ab.\ EtOH}$ 228 mμ [log ϵ = 3.98], indicated the reaction product to be predominantly the α,β unsaturated ester XII. Integration of its NMR spectrum showed 0.7 olefinic protons, which would suggest 70% ± 10% of the conjugated isomer; the isomer with the double bond within the five-membered ring would show no olefinic proton and would have no U. V. maximum above 200 mμ.

Ozonolysis of the olefinic diester at room temperature in acetic acid for 24 hours, followed by decomposition of the ozonide with Zn, gave a compound for which the structure XIII is suggested. The IR spectrum supports this structure in that it shows two carbonyl bands at 1742 and 1790 cm^{-1} and an O-H band at 3470 cm^{-1}. The carbonyl band at 1790 is very weak and appears as a shoulder on the 1742 band. The NMR spectrum, shown in Plate VI, did not change upon addition of D_2O; however, this is not uncommon for a tertiary alcohol as the alcohol proton of carotol does not show an exchange either. Integration of the NMR spectrum, based on the peak

Plate VI

NMR Spectrum of Keto-ester XIII



at 3.58 ppm as the three protons of the methyl ester, indicates a total of 20 protons. Elemental analysis of the pure compound, which was obtained by preparative VPC, agrees with the molecular formula $C_{12}H_{20}O_4$. The optical rotatory dispersion [ORD] curve, shown in Plate VII, exhibits a positive Cotton effect; hence it confirms the presence of the keto-carbonyl. Addition of hydrochloric acid did not change the curve significantly, which would support the location of the hydroxyl group as shown.

A means by which the alcoholic group could be obtained would be allylic oxidation during the ozonolysis process. Investigations in this laboratory have shown that oxidation of this type occurs quite readily when ozonolysis of a compound containing a similar grouping is carried out in glacial acetic acid over a long period of time. Although two tertiary positions are available in compound XIII, one of these is adjacent to the carbonyl and should be the more stable of the two, which would give rise to the proposed compound.

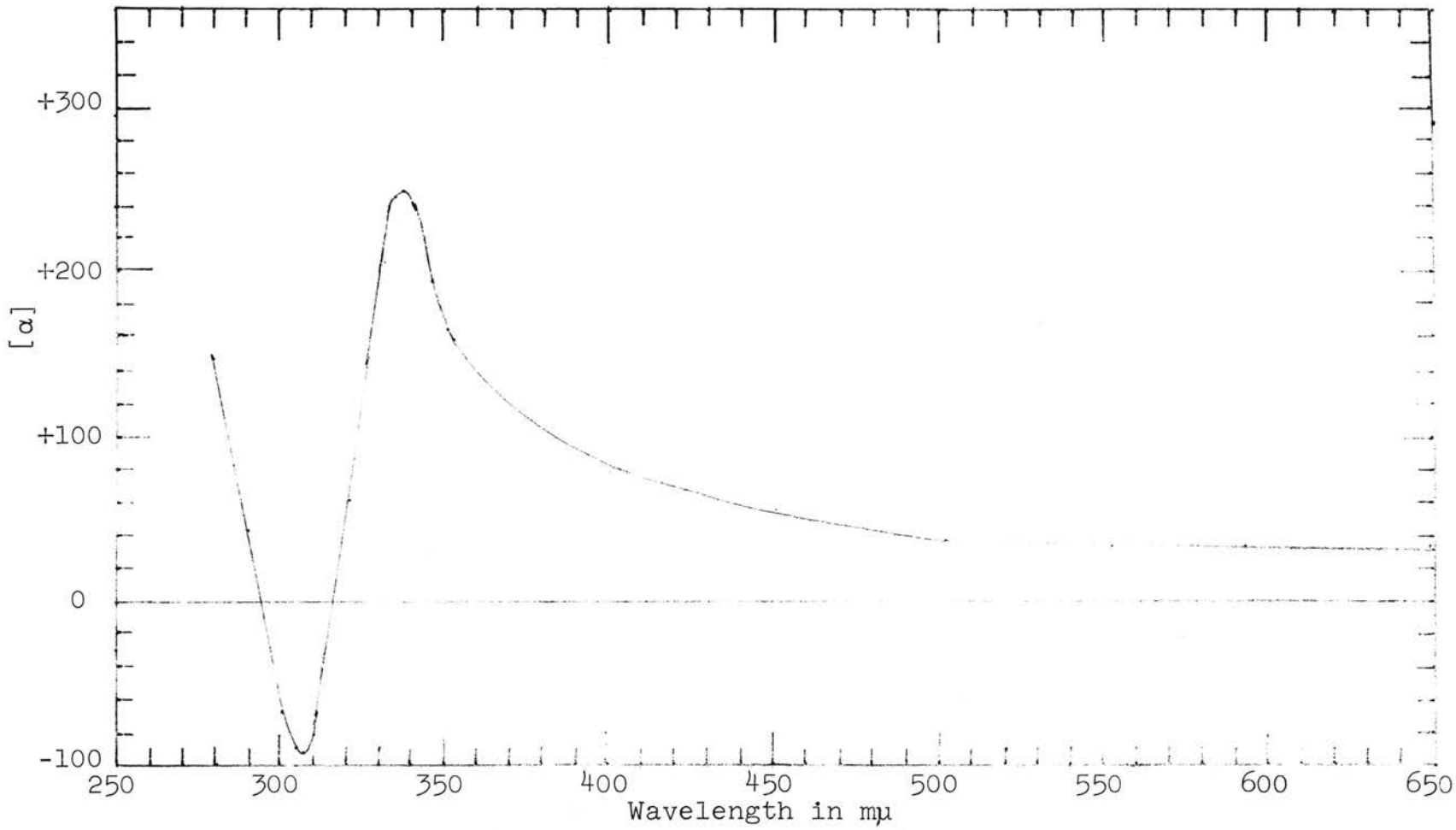
Experimental

Distillation of Carrot Seed Oil

Carotol was obtained by fractional distillation of commercial natural carrot seed oil [Magnus, Mabee and Reynard, Inc., New York, N. Y.] under a nitrogen atmosphere. The fraction of boiling point 91-95°/0.7-0.9 mm., n_D^{25} 1.4967, was collected. Gas chromatography indicated this fraction contained at least 95% carotol. IR: ν^{film} 3480, 1635 cm^{-1}.

Plate VII

Optical Rotatory Dispersion Curve of Keto-ester XIII



Preparation of Carotol Triol VIII

Carotol [10 g.] was oxidized to the triol using the method of Šorm and Urbanek[18] with several modifications. After oxidation at 0° C. under the conditions given in the above reference, the precipitated MnO_2 was filtered and washed thoroughly with acetone, and the filtrate concentrated under vacuum until most of the acetone had been removed. The white solid which precipitated at this point was filtered, and the filtrate then extracted continuously for 38 hours with $CHCl_3$. Upon removal of the chloroform and cooling to room temperature, white crystalline triol [5.72 g.] was obtained. Filtration, followed by recrystallization from benzene and hexane [1:1], afforded 4.61 g. triol, m.p. 141-142° C. Lit. m.p. 142° C.[18] IR: ν^{KBr} 3300, 3400 cm^{-1}.

The residual oil from the oxidation showed IR bands at ν^{film} 3400, 1710, and 1650 cm^{-1}. Chromatography on acid-washed alumina failed to give good separation; however, repeated recrystallization of the fractions eluted with ethyl ether gave a small amount of triol. Upon oxidation of the residual oil under the conditions for preparation of the acetoxydicarboxylic acid [see below], the diacid could be obtained in approximately 10% yield.

Preparation of Acetoxydicarboxylic Acid X

A solution of chromic acid [30 g.] in acetic acid was added dropwise to a solution of triol VIII [18 g.] in acetic acid and the solution stirred at room temperature for 36

hours. The acetic acid was distilled off in vacuo, the residue diluted with water and stirred until all the solid residue dissolved, and the solution then extracted continuously with ether. The acidic material was extracted with sodium carbonate solution, and upon acidification with HCl, a white solid precipitated. After extraction with ether, drying over anhydrous Na_2SO_4, and removal of the solvent, 4.82 g. of white solid were obtained which on recrystallization from ethyl acetate afforded the acid X, m.p. 172.5° C. Lit. m.p. 169-172° C.[23] IR: ν^{KBr} 3390, 2850, 1735, 1700, and 1255 cm^{-1}.

The ethereal solution containing the neutral material was dried over sodium sulfate and the solvent evaporated to give a colorless liquid. IR, ν^{film} 3440 and 1705 cm^{-1}, indicated the oil to be primarily the keto-diol IX. Reoxidation under the same conditions as used for preparing the acetoxydiacid gave the diacid in 36% yield, m.p. 176° C. The IR spectrum was identical to that of the acetoxydiacid.

Preparation of unsaturated diester XII

The dimethyl ester XI of acetoxydicarboxylic acid X was prepared with diazomethane in ether solution, b.p. 145°/0.15 mm., $n_D^{28.5}$ 1.4670. Lit. b.p. 155-160 [bath]/0.06 mm.[23] IR: ν^{film} 1255, 1740 cm^{-1}.

Pyrolysis of the resulting acetoxydiester XI at 230° C. gave a yellow liquid, which was taken up in ether, then washed with Na_2CO_3 solution until the yellow color had been removed.

After washing with water and drying over anhydrous $MgSO_4$, the solvent was removed, and the colorless liquid distilled. IR: ν^{film} 1720, 1740, and 1638 cm^{-1}. UV: $\lambda_{max.}^{abs.\ EtOH}$ 228 mμ [log ε = 3.98]. B.p. 70-75° C./0.2 mm.; Lit. b.p. 140° [bath]/0.3 mm.[23]

Analysis: Calc. for $C_{15}H_{24}O_4$: C, 67.13; H, 9.01; O, 23.85. Found: C, 67.51; H, 9.16; O, 23.56.

Preparation of keto-ester XIII

The unsaturated diester XII [1.41 g.] was dissolved in acetic acid and ozonized for 26 hours at room temperature. Zinc dust was added, and the solution was stirred for 5 hours. After filtration of the zinc, the filtrate was poured into water, extracted with ether, and the ether layer washed with sodium carbonate solution, then water. Upon removal of the solvent, a yellow oil [0.48 g.] was obtained. IR: ν^{film} 1742, 1790 [weak], 3470, 1640 [very weak], and 1242 cm^{-1}. A sample purified by preparative thin layer chromatography for analysis had R_f = 0.7 on Silica gel with $4CHCl_3$:1 abs. EtOH. The NMR spectrum is shown in Plate VI. The ORD curve is shown in Plate VIII [methanol].

Analysis: Calc. for $C_{12}H_{20}O_4$: C, 63.18; H, 8.83; O, 28.04. Found: C, 62.80, 62.57, 62.46, 64.12; H, 8.68, 8.39, 8.50, 8.62.

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