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GRADUATE COLLEGE

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III. GERMACRENE-A: AN ELUSIVE SESQUITERPENE HYDROCARBON

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

by

WILLIAM WAYNE YOUNGBLOOD

Norman, Oklahoma

1969

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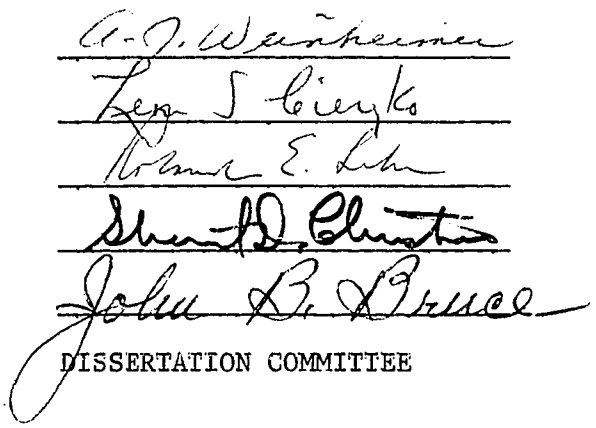
APPROVED BY



DISSERTATION COMMITTEE

ACKNOWLEDGMENT

The author is deeply indebted to Professor A. J. Weinheimer, who suggested these problems, for his counsel during the course of this work, and to the National Institute of Health for generous financial support (Grant HE-05675) in the form of a predoctoral fellowship.

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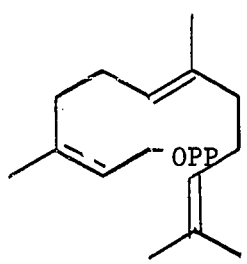
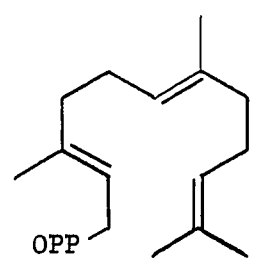
I. SESQUITERPENOIDS ISOLATED FROM OCTOCORALLIA

INTRODUCTION

Sesquiterpene hydrocarbons (STHC) are a class of compounds, $C_{15}H_{24}$, which are generally observed in photosynthesizing organisms. Appearing in a vareity of mono-and fused ring structures, the skeletons of the sesquiterpene hydrocarbons can be derived by a suitable cyclization of either cis-farnesyl pyrophosphate (1) or trans-farnesyl pyrophosphate (2).[1]



(1)



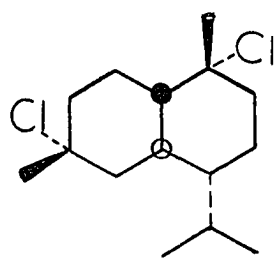
(2)

OPP = pyrophosphate

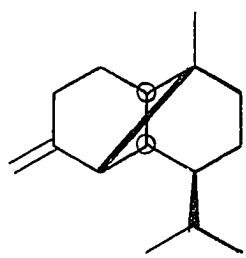
Although exact biosynthetic pathways have not been extended beyond formation of farnesyl pyrophosphate, reasonable cyclization mechanisms are argued by analogy and by comparison of structural similarity observed in sesquiterpene mixtures.[2]

The investigation of sesquiterpene hydrocarbons has been and is an active area of study. However, the studies of sesquiterpene hydrocarbon mixtures of marine origin have been to date restricted to one group of Japanese workers[3-7] studying species of brown and red marine algae, and to the group at the University of Oklahoma studying gorgonians.[8-13]

Sifford[11] reported the isolation of (+)-cadinene-dihydrochloride (3) when dry HCl was passed through an ethereal solution of the sesquiterpene hydrocarbon mixture from Pseudoplexaura porosa. Washecheck[12] in examining the same hydrocarbon mixture isolated one component which he identified as (+)-β-ylangene (4).[12]



(3)



(4)

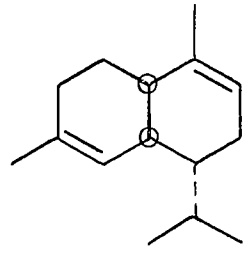
In the present work, the hydrocarbon fraction from Pseudoplexaura porosa has been examined in depth and nine compounds identified. Examination of hydrocarbon mixtures from Eunicea palmeri and Plexaurella dichotoma has permitted identification of two and five components, respectively.

RESULTS AND DISCUSSION

A. Sesquiterpene Hydrocarbons from Plexaurella dichotoma (Esper).

The sesquiterpene hydrocarbon fraction of Plexaurella dichtoma (0.29% of dry weight) was isolated by chromatography (Florisil) of the volatile components of the hexane extract. Gas chromatography (20% Carbowax) indicated fourteen distinct peaks (Fig. 1). Distillation of the mixture through a Nester-Faust annular still permitted concentration of minor components A through I (Fig. 1) and afforded a sample of hydrocarbon containing the two major components J and K. Separation of the two components was accomplished by chromatography on silicic acid impregnated with silver nitrate.

Elemental analysis and mass spectrographic molecular weight determination (204 parent ion) of the first hydrocarbon eluted from the column indicated a composition of $C_{15}H_{24}$. The excellent correspondence of its physical properties, and infrared (IR) spectrum (Fig. 2), with published data[14] established the identity of the hydrocarbon as (+)-α-muurolene (5) shown below:



(5)

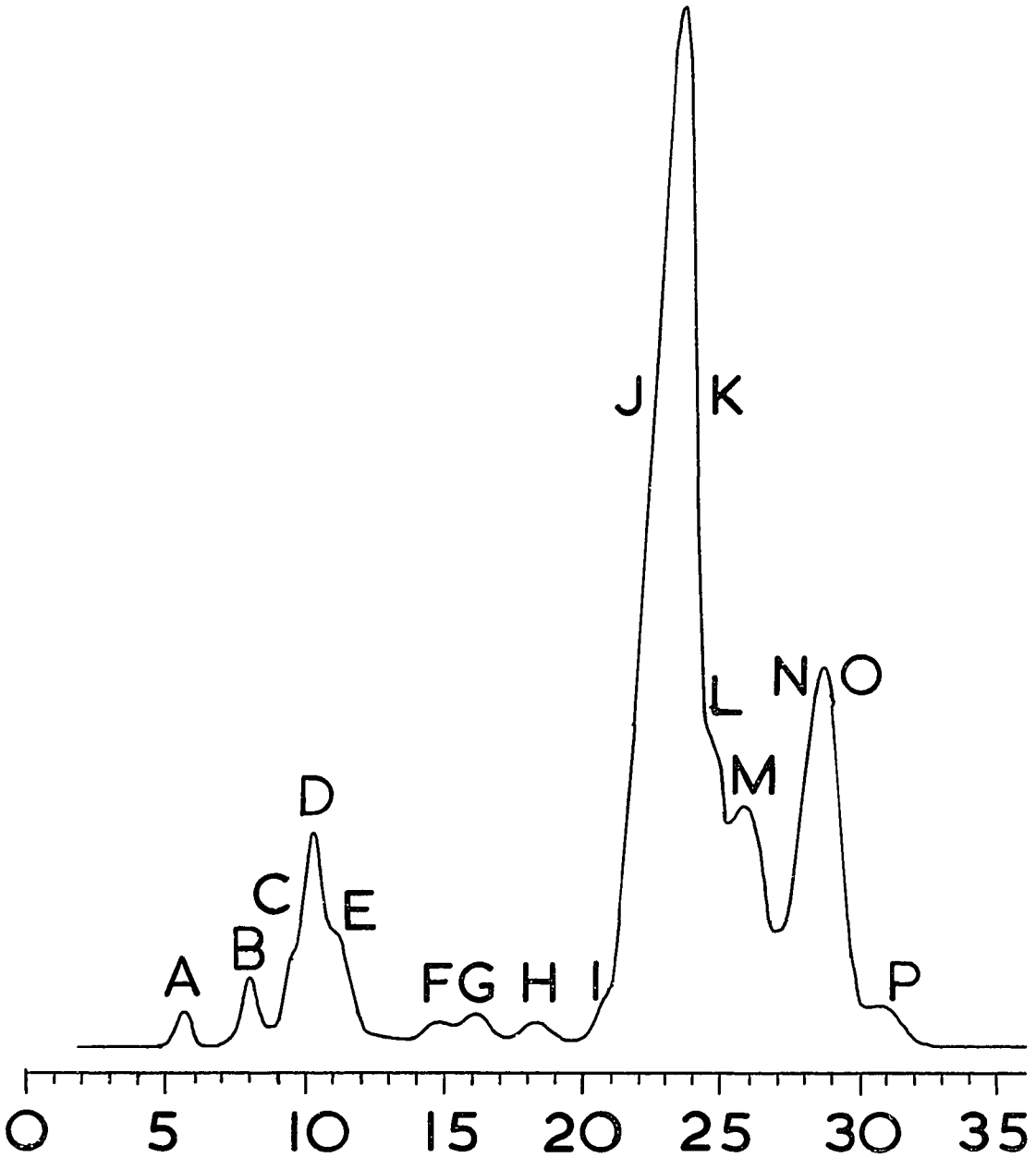


Figure 1

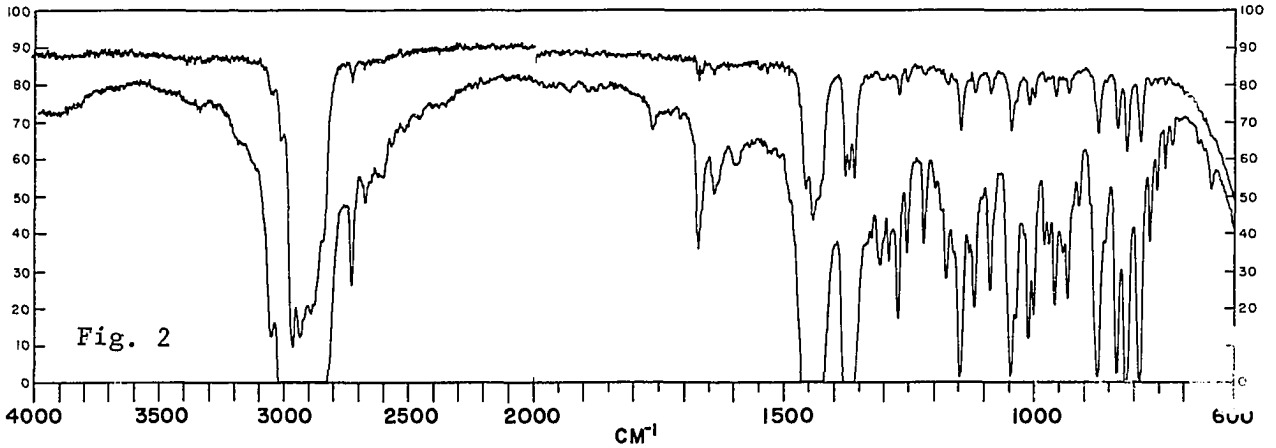
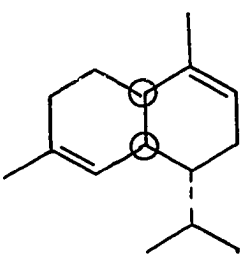


Fig. 2



(+)α-Muurolene

Fig. 3

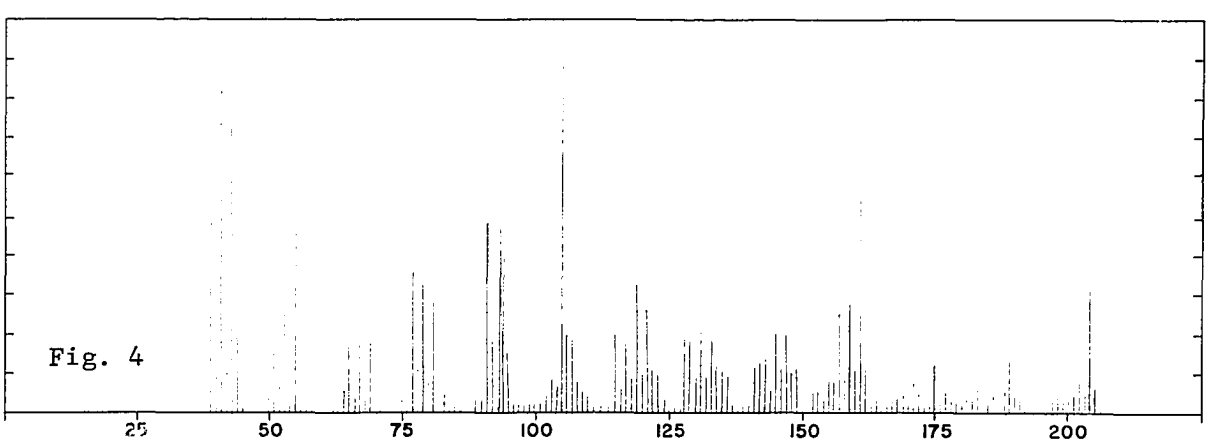


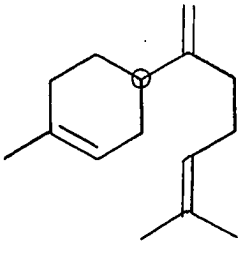
Fig. 4

The nmr (Fig. 3) and mass (Figure. 4) spectra were consistent with the structure proposed by Zabja, Romanuk, and Herout[15] for (-)-α-muurolene. The two vinyl protons appeared as a broad multiplet centered around δ5.36 with the vinyl methyls appearing as a broad singlet at δ1.67. The isopropyl group appeared as two doublets centered at δ0.88 and δ0.82 (J = 7 Hz). The allylic bridgehead and four other protons were assigned the position of δ1.88 where they appeared as a broad peak.

Significant in the IR is the region between 1350 and 900 cm^{-1}. Independent workers[15,16] have shown that the fingerprint in this region differs for the trans-and cis-fused decalin systems and may be used to differentiate between the cadinene (trans-fused) and muuroline (cis-fused) isomers.

Elemental analysis and mass spectrographic molecular weight determination (204 parent ion) of the second hydrocarbon eluted from the column indicated a molecular composition of $C_{15}H_{24}$. Hydrogen uptake of three equivalents indicated the compound to be a monocyclic and comparison of the IR of the hydrogenated compound with that shown by Sôrm[17] for bisabolane established the skeleton of the compound.

Excellent correspondence of the physical properties and IR spectrum (Figure 5) with published data[18,19] established the identity of this hydrocarbon as (+)-β-bisabolene (6).



(6)

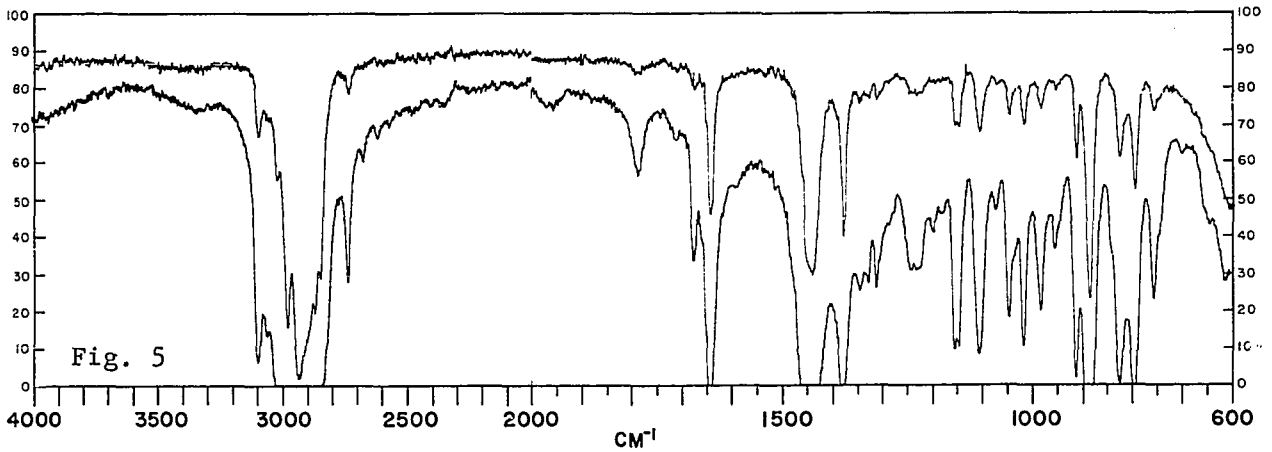
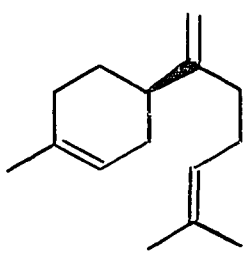


Fig. 5



(+) β-Bisabolene

Fig. 6

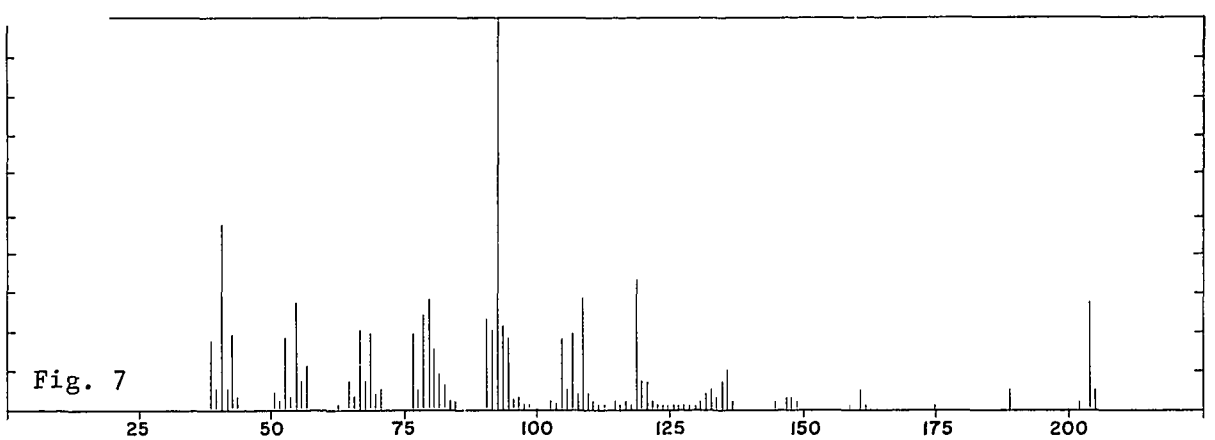


Fig. 7

The nmr (Fig. 6) and mass (Fig. 7) spectra were consistent with the structure proposed by Mills[20] for (-)-β-bisabolene. In addition to vinyl methyls at δ1.95, 1.65, and 1.61, the nmr spectrum showed a signal at δ5.43 (1H) appearing as a multiplet, a signal at δ5.22 (1H) appearing as a multiplet, and a signal at δ4.84 (2H) appearing as a singlet corresponding to two trisubstituted double bond protons and one exo-methylene, respectively. The nine allylic protons appeared in bands between δ2.11 and 1.95.

During the course of the distillation described above, the pot material changed from colorless to deep orange-yellow. Following the removal of a fraction of the (+)-α-muurolene and (+)-β-bisabolene, the distillation was interrupted and the contents of the pot were chromatographed (Florisil) with hexane. Gas chromatography of the mixture revealed that component P (Fig. 1) was no longer present and that component M (Fig. 1) had been greatly reduced. In order to avoid the loss of component M, the distillation was not resumed although components J and K were still the major constituents of the mixture.

Chromatography of the pot hydrocarbons on silver nitrate-impregnated silicic acid led to the isolation of components M, N, and O (Fig. 1). The first component eluted from the column was the previously identified (+)-α-muurolene. Continued elution (8% benzene in hexane) provided a sample of hydrocarbon identified by retention time (G.C.) to be component N (Fig. 1). Elemental analysis and molecular weight determination (202 m.s.) indicated a composition of $C_{15}H_{22}$. Excellent correspondence of its physical properties, IR[21] (Fig. 8), mass spectra[22] (Fig. 10), and UV data[22] with that cited in the literature established the identity of

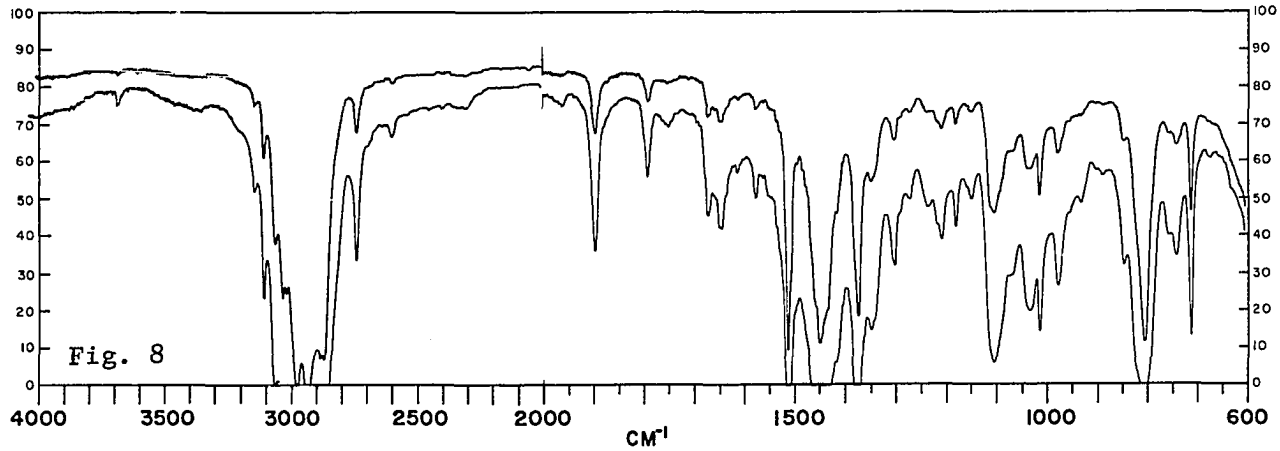
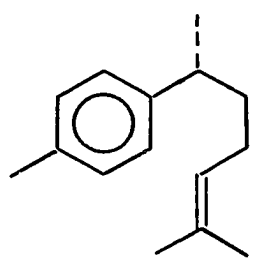


Fig. 8



(+) α-Curcumene

Fig. 9

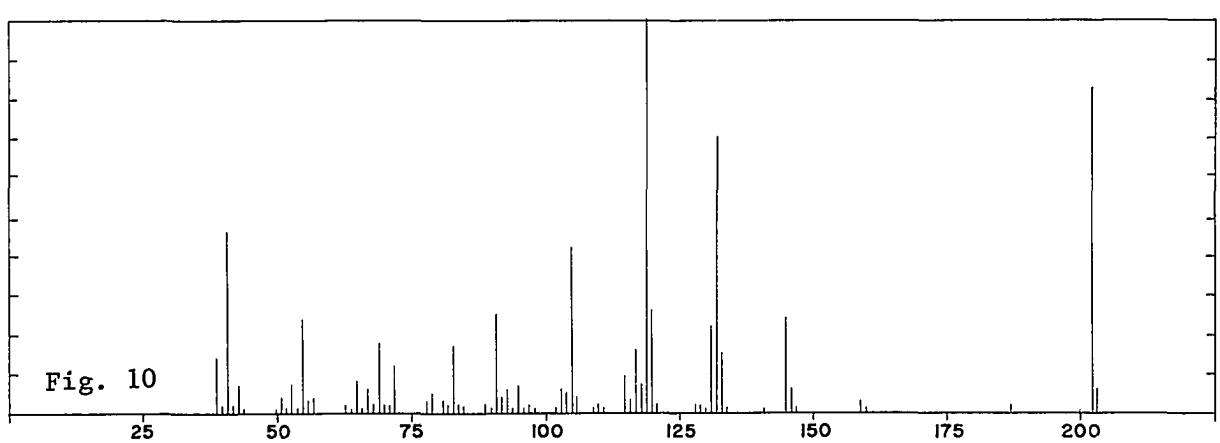
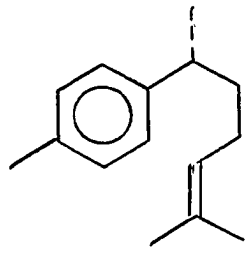


Fig. 10

this hydrocarbon as (-)-α-curcumene (7).



(7)

The nmr (Fig. 9) was consistent with the structure cited by Honwald and Roa[21] for (+)-α-curcumene. The aromatic signal (4H) appeared as a singlet at δ6.94, characteristic of para-dialkyl substituted benzenes. The aryl methyl appeared as a singlet at δ2.25. The vinyl proton appeared as a broad triplet (J = 8 Hz) at δ5.05. Two vinyl methyls appeared as broad singlets at δ1.67 and 1.50, respectively. The benzylic proton at δ2.63 appeared as a sextet due to coupling with the secondary methyl which appeared as a doublet (J = 7 Hz) at δ1.20.

The next component eluted from the column, component M, was closely followed by component O and was obtained as a binary mixture (40% M; 60% O by G.C.). Further elution provided component O in high purity.

Excellent correspondence of the physical properties and IR (Fig. 11) data with that cited in the literature[23] established the identity of component as (+)-α-bisabolene. Elemental and mass spectrographic molecular (204 parent ion) determination confirmed the molecular formula as $C_{15}H_{24}$.

The nmr (Fig. 12) and mass (Fig. 13) spectra were consistent with the structure proposed by Sörm, Pliva, and Herout[23] for α-bisabolene (8).

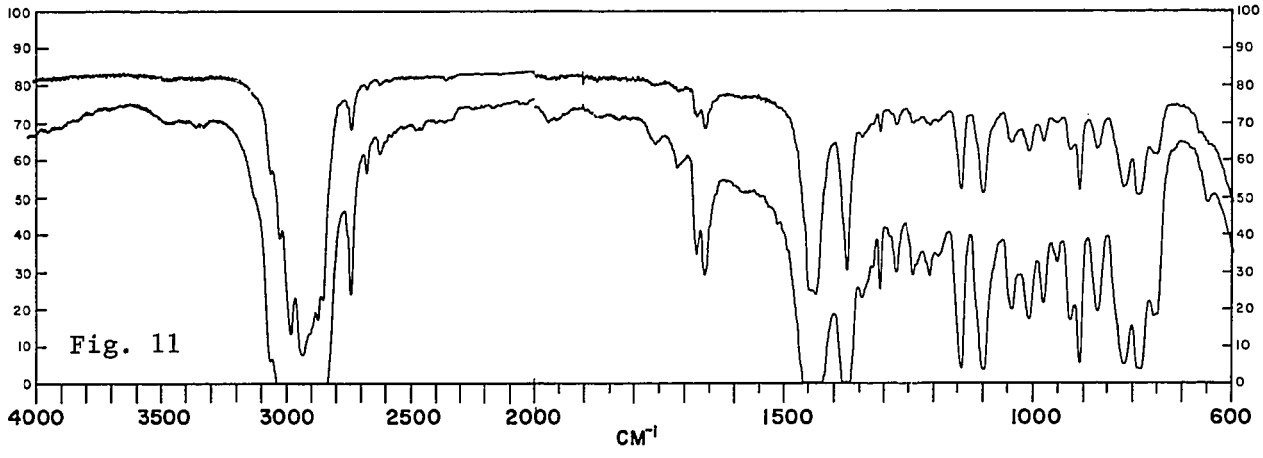
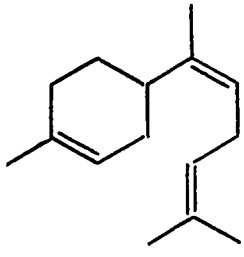


Fig. 11



(+) α-Bisabolene

Fig. 12

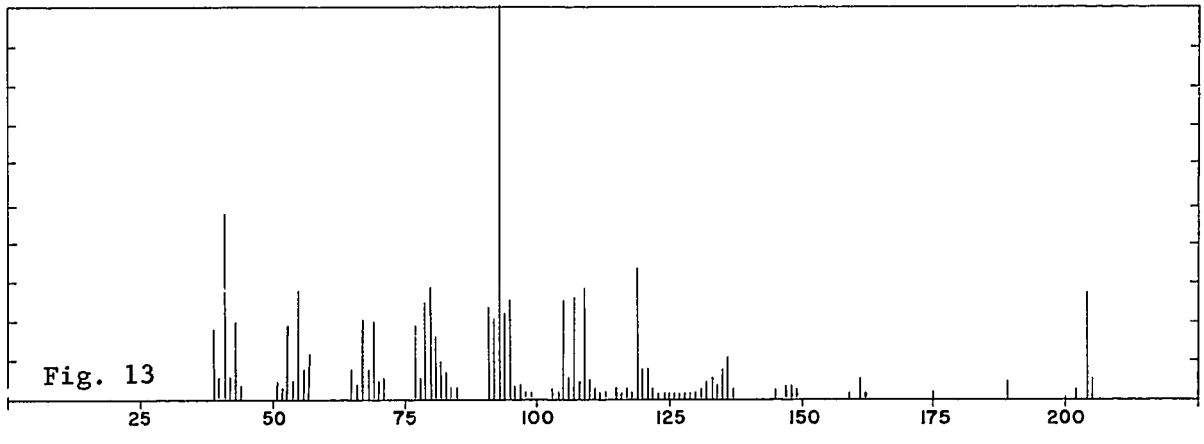
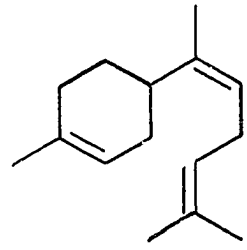


Fig. 13



(8)

The four vinyl methyls (12H) appeared as a broad singlet at δ1.59. One vinyl proton appeared at δ5.35 as a broad singlet while the other two appeared as a miltiplet at δ5.10. The diallylic methylene (2H) appeared as a triplet (J = 7 Hz) at δ2.64.

Further elution provided more (+)-β- bisabolene previously identified.

Rechromatography of the binary mixture containing components M and O on silver nitrate-impregnated silicic acid provided a sample of hydrocarbon identified by G.C. retention time to be component M. Elemental analysis and mass spectrographic molecular weight (204 parent ion) determination indicated a composition of $C_{15}H_{24}$. Hydrogen uptake of three equivalents indicated the compound to be a monocyclic and comparison of the IR spectrum of the hydrogenation product with that shown by Sörm[17] for bisabolane established the skeleton of the compound. The IR spectrum (Fig. 14) of component M exhibited bands characteristic of trisubstituted double bonds.

The nmr spectrum, partially tabulated below, was particularly useful in determining the structure of component M:

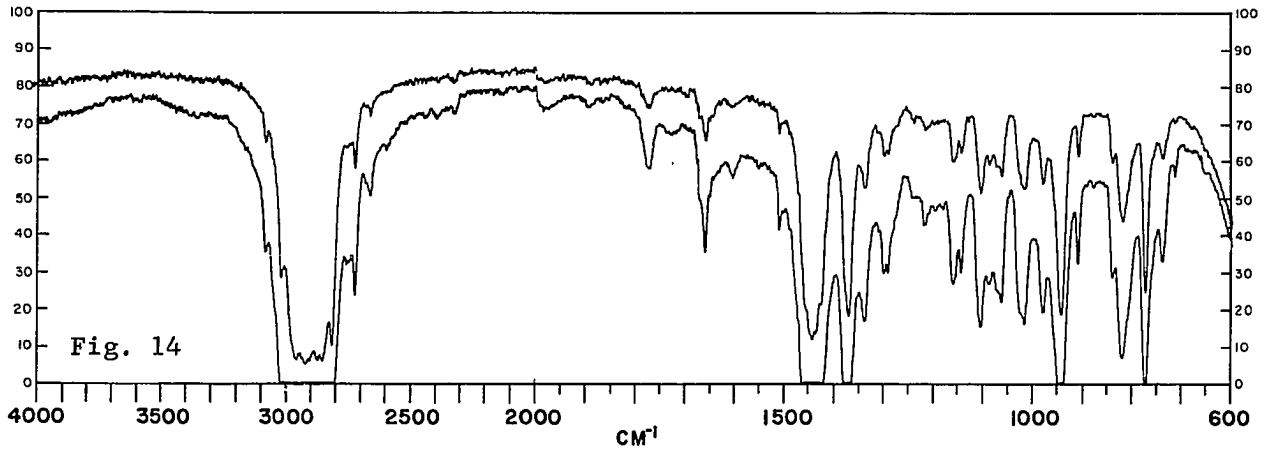
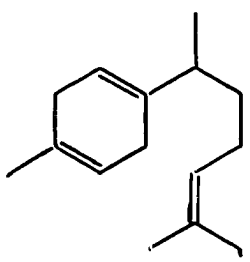


Fig. 14



(+) β-Curcumene

Fig. 15

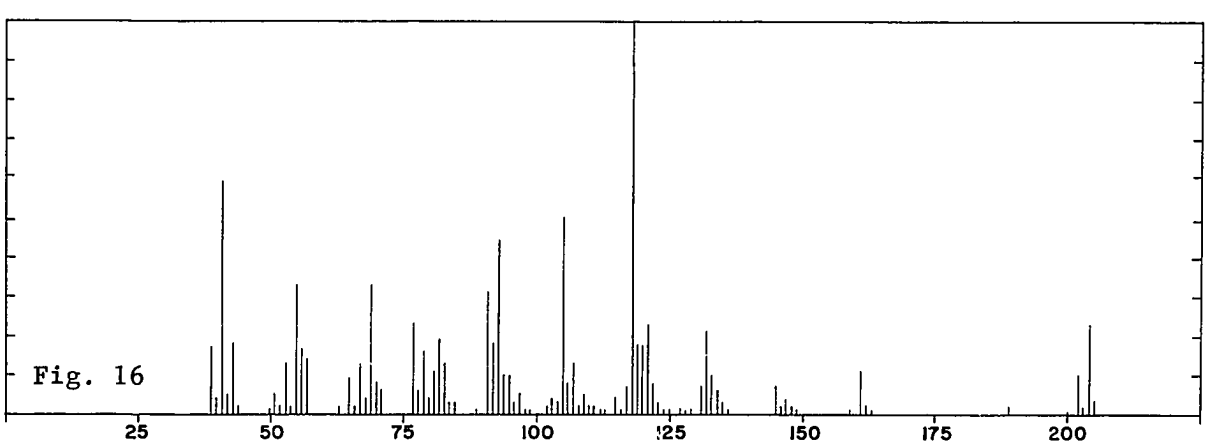
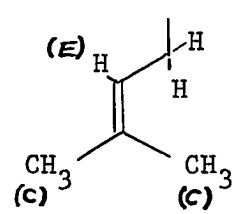


Fig. 16

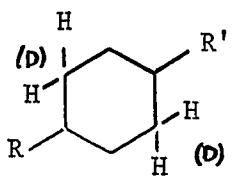
pattern	δ	Multiplicity	J(Hz)	protons	interpretation
A	0.98	d	7	3	sidechain methyl
B	1.57	s		3	vinyl methyl
C	1.64	d	1	6	vinyl methyls (2)
D	2.53	s		4	diallylic methylene (2)
E	5.07	dt	7,1	1	vinyl proton
F	5.37	s		2	vinyl protons

The vinyl proton appearing as a quarteted-triplet centered at δ5.07 could be attributed only to an isopropenyl group adjacent to a methylene. The large coupling constant of the triplet corresponded to splitting by the methylene and the small coupling of the quartet due to allylic splitting by the vinyl methyls.

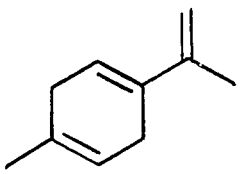


More significant was the pattern D exhibiting a chemical shift usually observed for diallylic methylene. The pattern's appearance as a broad singlet requires that all four protons lie in nearly identical environments, and that coupling constants with adjacent vinyl protons be zero, or nearly zero. Structure (9) was the only one consistent with the established skeleton of component M and nmr data: Excellent cor-

relation of this δ2.53 singlet with nmr data cited in the literature for γ-terpinene[24] (10) confirmed the presence of moiety (9) in component M:

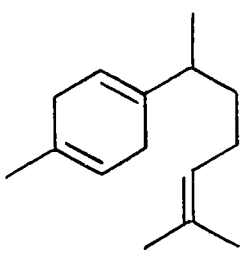


(9)



(10)

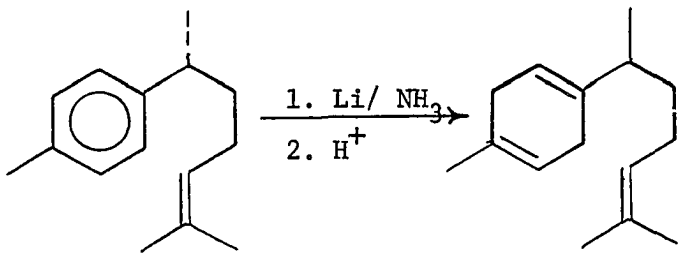
Having determined the locations of the three double bonds on the bisabolane skeleton, component M was identified as β-curcumene[25] (11).



(11)

Since an insufficient quantity of component M was isolated to permit comparison of the physical properties with those cited in the literature, confirmation of the above structure (11) was accomplished by comparison of the nmr and IR spectra of component M with that obtained from the product of a Birch Reduction upon α-curcumene (7). Birch[26] originally reduced this compound using sodium in ammonia but in duplicating this work the procedure was modified[27] using lithium and tetrahydrofuran (THF) as a co-solvent. Additionally, tertiary butyl alcohol was used in place of ethanol as a proton source. The reaction

proceeded as shown below providing the desired hydrocarbon in greater than 99% purity (G.C.).



(7) (11)

Identity of the nmr and IR spectra with those of M confirmed the structure of component M.

Since the starting material was optically active, $[\alpha]_D^{20}$-39.31°, it was anticipated that the synthetic β-curcumene would also possess optical activity. However, the product was optically inactive. Partial reduction of α-curcumene and isolation of the starting material resulted in the recovery of optically active starting material. This indicates that racemization takes place following reduction of the aromatic ring. Insufficient material remained to permit working out conditions to permit the isolation of optically active β-curcumene.

Finally, it is possible to speculate concerning the structure of component L (Fig. 1). A distillation fraction containing components J and K with a trace of L had been examined with UV, and had been found to show an absorption peak at 228 mμ. Since neither components J nor K exhibited such absorptions, this 228 mμ peak must be attributed to component L. Woodward's[28] rules for calculating UV absorption predict that component L would be trisubstituted acylic butadiene. This eliminated all but monocyclic compounds and permits only two possibilities

with the bisabolane skeleton as shown below:

B. Sesquiterpene Hydrocarbons from Pseudoplexaura porosa.

The volatile fraction (0.25% dry weight) was obtained by distillation of the hexane extract from the air dried animal. Chromatography on Florisil separated the sesquiterpene hydrocarbon (STHC) fraction (0.12% dry weight) from the oxygenated volatiles. Gas chromatography (20% Carbowax) indicated that the mixture contained at least nine components, (A -I; Fig. 17).

Prior to beginning isolation of individual components, aliquots of the mixture were subjected to various conditions in order to determine the susceptibility of the mixture to chemical or thermal isomerization. It was observed that components (subsequently identified) having three- and four-membered rings conjugated with olefins readily isomerized under acidic conditions (silicic acid and acidic alumina) and that isomerization was not observed on Florisil. No thermal isomerization was observed.

Distillation of the STHC mixture on a stainless steel spinning band resulted in a significant loss of components A through D (Fig. 17). Chromatography of the receiver's viscous contents on florisil led to the isolation of an aromatic hydrocarbon and a viscous hydrocarbon which moved slower on TLC than the hydrocarbon mixture.

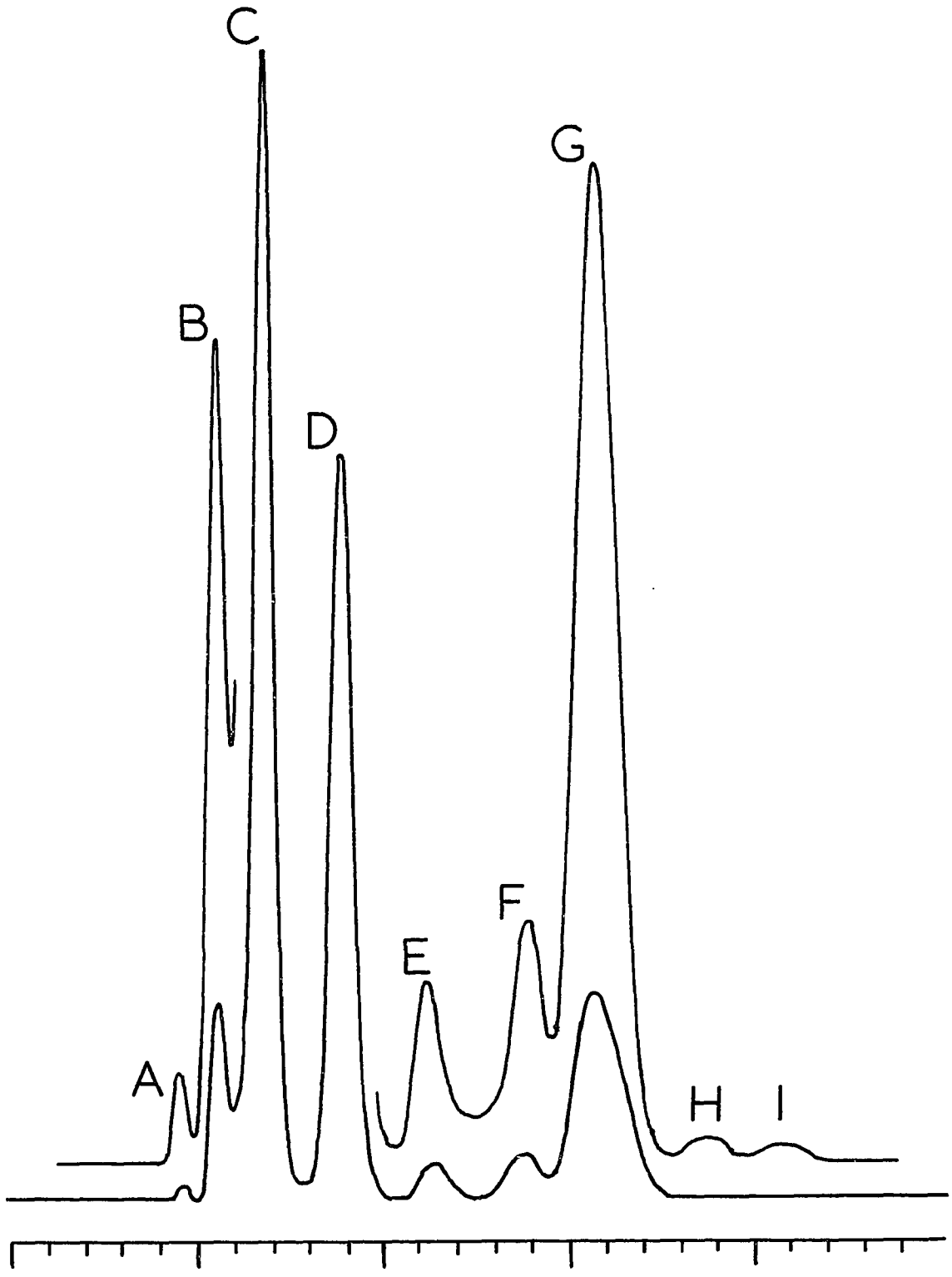
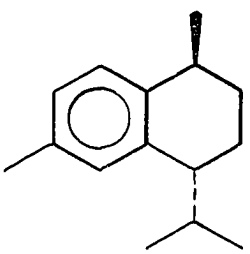


Figure 17

Elemental analysis and mass spectroscopic molecular weight determination (202 parent ion) of the aromatic component indicated a composition of $C_{15}H_{22}$. The excellent correspondence of its physical properties,[29] IR (Fig. 18),[30] nmr (Fig. 19),[30] mass spectra (Fig. 20),[31] and UV spectra[31] with published data established the identity of the hydrocarbon as (+)-calamanene (12).



(12)

Examination of the nmr and IR spectra of undistilled Pseudoplexaura porosa hydrocarbons failed to exhibit bands characteristic of aromatic compounds. Isolations of the STHC's from fresh animal confirmed the lack of aromatic components.

Elemental analysis and mass spectroscopic molecular weight determination (410 parent ion) of the slower moving fraction indicated a composition of $C_{30}H_{50}$. Significant in the IR were bands at 1380 cm^{-1} (gem-dimethyl) and 845 cm^{-1} (trisubstituted olefin). Significant in the nmr spectrum were signals at δ5.38 (vinyl protons); δ1.68 (vinyl methyls); δ1.13 (quaternary methyl); and δ0.91 (isopropyl methyls). Comparison of TLC (hexane) with the STHC mixture demonstrated that this compound also was not in the original mixture.

Distillation of the STHC mixture through a teflon annular still provided a pure sample of component A (Fig. 17), and permitted the isolation of fractions containing two or three components.

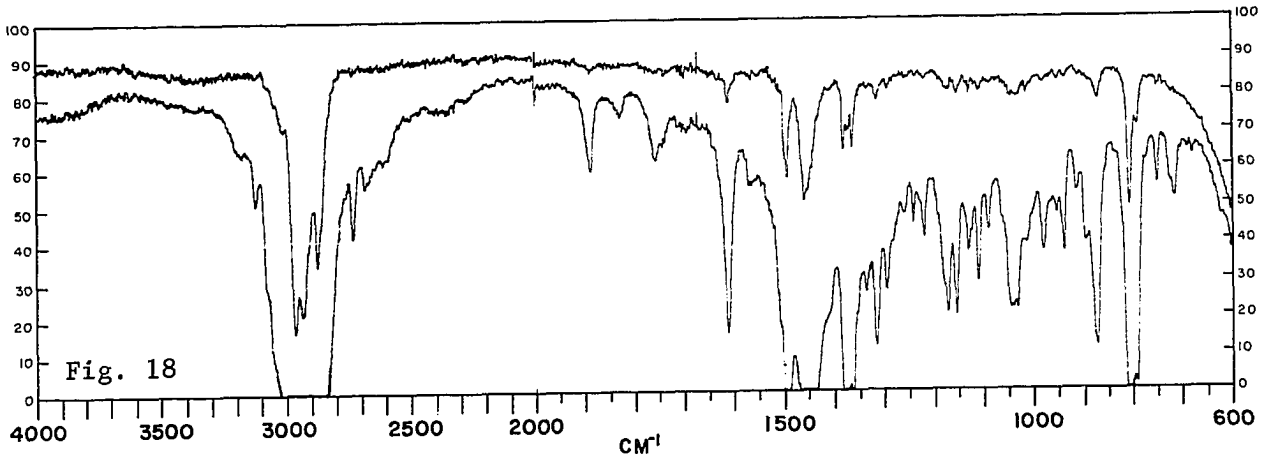
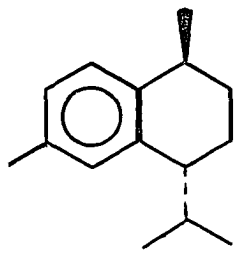


Fig. 18



(+) Calamanene

Fig. 19

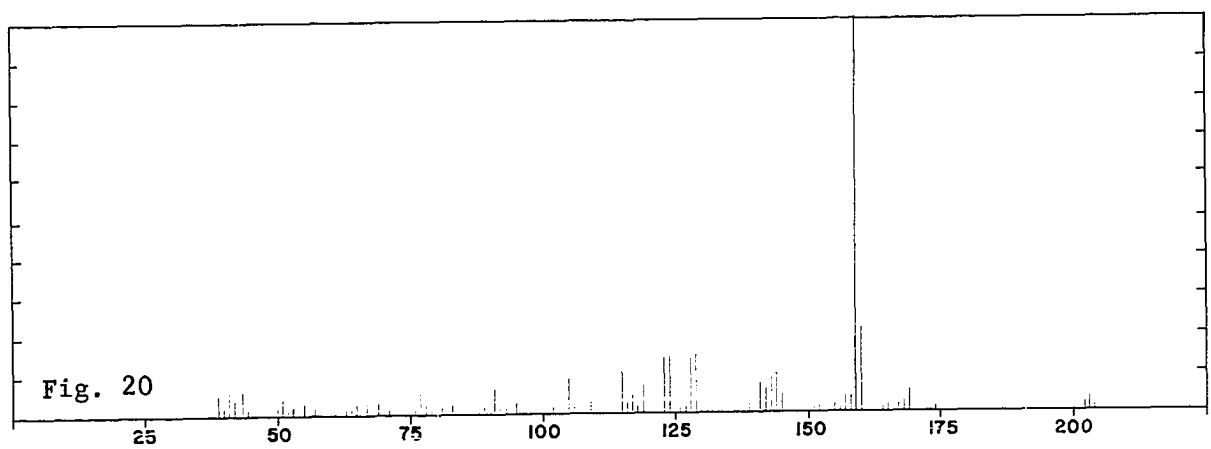
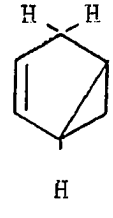


Fig. 20

Elemental analyses and mass spectroscopic molecular weight determination (204 parent ion) indicated a composition of $C_{15}H_{24}$ for component A. Hydrogen uptake of one equivalent indicated the compound to be a tricyclic. The nmr of the hydrogenated compound exhibited signals for two cyclopropane protons at δ0.50.

Significant signals in the nmr spectrum of component A (Fig. 22) were the multiplet at δ4.85, m, 1H (trisubstituted olefin); a doubled triplet centered at δ2.58, 1H; a doubled quartet centered at δ2.10, 1H; and one cyclopropane proton at δ0.24. Since only one of the two cyclopropane protons displayed a normal chemical shift, the second cyclopropane proton could only be shifted due to deshielding by the olefin, and would have to be allylic to the olefin. This was confirmed by the IR spectrum (Fig. 21) for compound A in which the trisubstituted olefin bands showed a shift of approximately 20 cm^{-1} due to conjugation. The doubled triplet and doubled quartet at δ2.58 and δ2.10 respectively displayed general chemical shifts attributable to diallylic protons, and necessarily would be flanked by olefin and the cyclopropane ring (13).



(13)

The difference in chemical shifts of the two diallylic protons arises from the geometry of cyclopropane ring fusion which causes the proton in the deshielding cone of the cyclopropane ring to display a shift down field. Decoupling experiments were consistent with partial structure

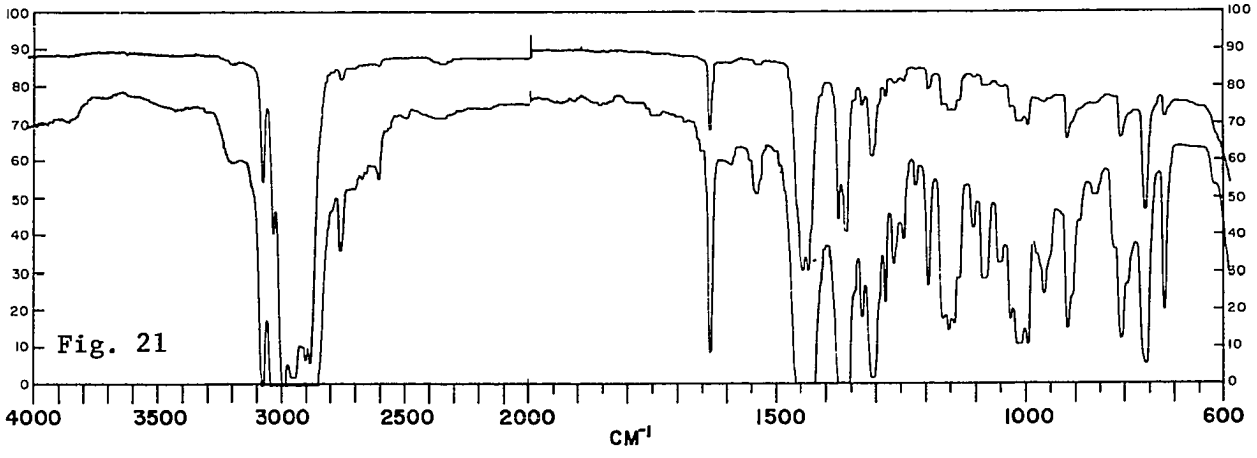
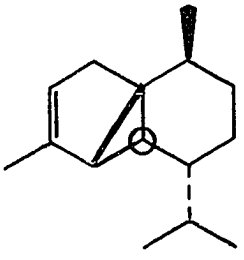


Fig. 21



(+) -Cubebene

Fig. 22

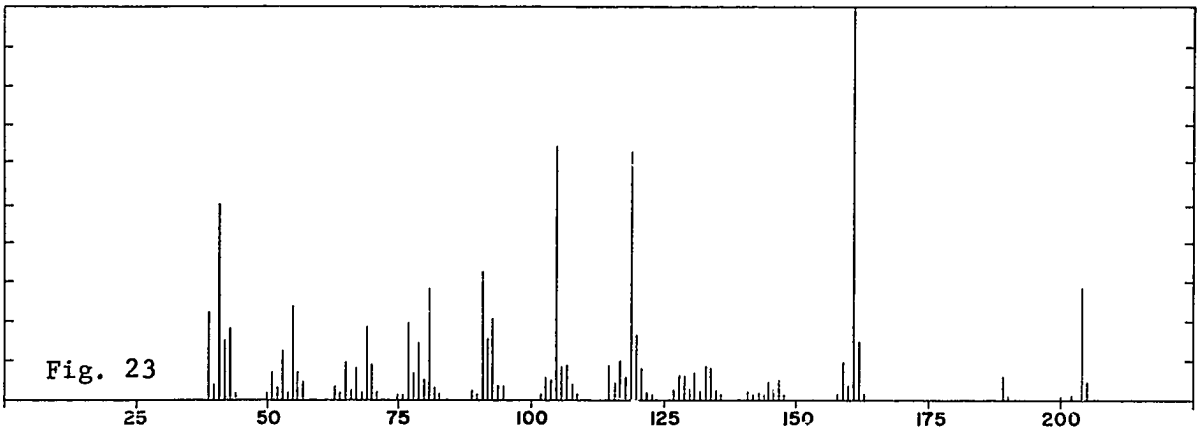
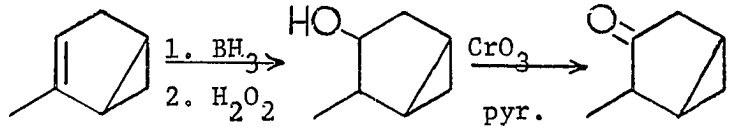


Fig. 23

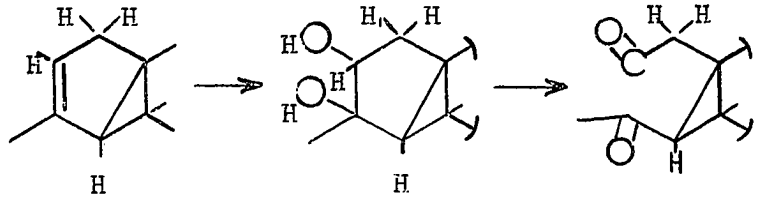
(13) by confirming coupling (17 Hz) between doubled triplet and the doubled quartet, and between both doubled triplet and doubled quartet with vinyl proton (2 Hz).

Location of the vinyl methyl followed from hydroboration of the olefin (14), perioxide oxidation to the secondary alcohol (15), and chromium trioxide oxidation of the secondary alcohol to a ketone (16) which exhibited a normal cyclopentanone absorption band at 1745 cm^{-1} in the IR. This necessarily resulted from introduction of the ketone Beta to the cyclopropane ring since cyclopropyl ketones show shift to lower wave numbers.[57]

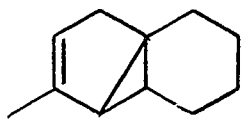


(14) (15) (16)

Oxidation of compound A with osmium tetroxide to the diol (17) and cleavage of viscanol diol with sodium metaperiodate resulted in a keto-aldehyde (18) in which the methylene adjacent to the aldehyde carbon appeared as a doublet (δ2.58, d, J = 1.5 Hz) coupled only with the aldehyde proton appearing at δ9.54, t, J = 1.5. This indicated that the remaining ring was joined adjacent to the methylene group as in a decaline system (19).

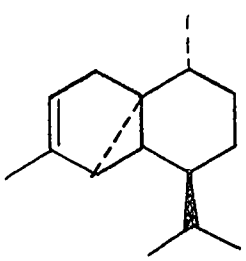


(14) (17) (18)

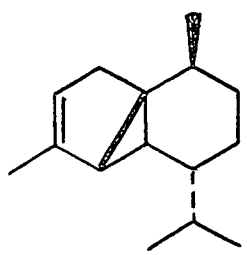


(19)

Confirmation of this partial structure of component A was provided by comparison of physical, IR (Fig. 21), nmr (Fig. 22), and mass (Fig. 23) spectral data with that cited by Ohta[32] who reported the structure of a new tricyclic sesquiterpene hydrocarbon, (-)-α-cubebene (20). Comparison of the nmr and gas chromotography retention times of component A with authentic sample of (-)-α-cubebene supplied by Dr. Ohta established component A as (+)-α-cubenene (21).



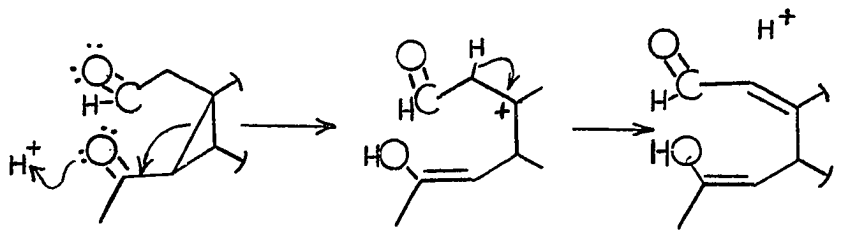
(20)



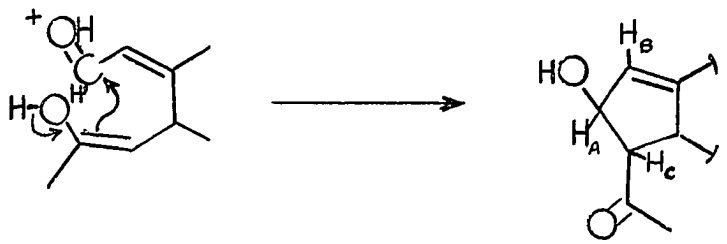
(21)

A chromatography of the keto-aldehyde (22) on silicic acid with ethyl acetate resulted in the recovery of starting material and a new crystalline compound (52% yield) which displayed IR bands characteristic of secondary alcohol, acyclic ketone, and trisubstituted olefin. Significant in the nmr spectrum were signals for a methyl ketone at δ2.25, vinyl proton at δ5.43, a doubled doublet at δ3.03. Significant also was the disappearance of aldehyde signal at δ9.54.

Acid-catalyzed intramolecular condensation of α-cubebeketo-aldehyde (22), outlined below (22) - (23), would result in a compound (20) displaying spectral properties compatible with those of the above compound:

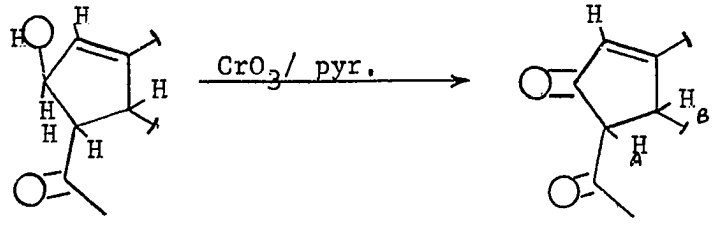


(22)



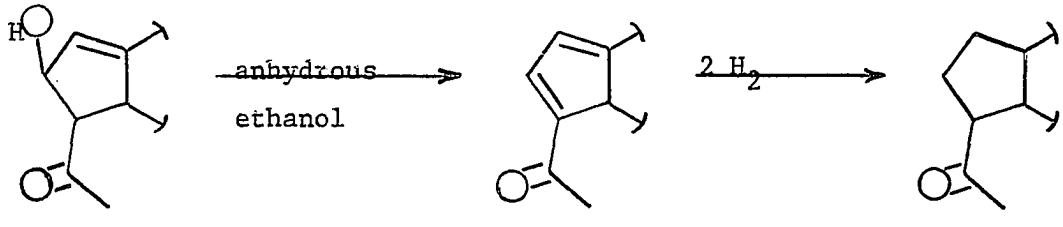
(23)

A signal at δ3.03, dd, J_{AB} = 2 Hz, J_{AC} = 6 Hz, was consistent with the pattern expected for a proton (23_A) on carbon bearing oxygen and coupled with the vinyl proton at δ5.43, d, J_{BA} = 2 Hz (23_B) and a second methine proton (23_C). Oxidation of the alcohol to a ketone resulted in the appearance of a nmr signal at δ3.55, d, J_{AB} = 3Hz (24_A) interpreted as the signal from the methine proton adjacent to both ketones coupled with one proton on adjacent carbon.



(23) (24)

Hydrogenation of the isomerized compound (23) in anhydrous ethanol required two equivalents of hydrogen and resulted in recovery of a colorless liquid sample which displayed a 1706 cm^{-1} absorption (ketone) in the IR spectrum. Mass spectral analysis of this liquid hydrogenation product indicated the parent ion to be m/e 220. In anhydrous ethanol, dehydration of compound (23) followed by hydrogenation of the double bands would require two equivalents and would result in a ketone (25) with m/e 220.

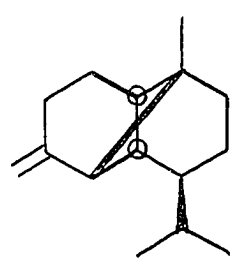


(23) (25)

Attempts to obtain additional isomerized keto-aldehyde by chromatography on silicic acid were unsuccessful. Shaking α-cubebe-keto-aldehyde with methanol containing mineral acid brought about partial isomerization. The initial isomerization upon silicic acid chromatography was in all probability catalyzed by a trace of acid in

the ethyl acetate.

Preparative gas chromatography (20% Carbowax) of a distillation fraction provided a sample of hydrocarbon which was identified by comparative retention times (g.c.) to be component D, (Fig. 15). Elemental analysis and mass spectrographic molecular weight determination (204 parent ion) indicated that component D had a composition of $C_{15}H_{24}$. Excellent correspondence between the physical properties, IR (Fig. 24), nmr (Fig. 25), and mass (Fig. 26) spectra of component D with that shown by Washecheck[12] identified this compound to be the hydrocarbon tentatively reported as (+)-β-ylangene (26). Washecheck's identification was made based upon comparison of IR spectrum of the isolated component with that reported by Hunter[59] for β-ylangene. At that time β-copaene was unreported in the literature. It has been shown[33,30] that corresponding copaene and ylangene compounds are epimeric at the asymmetric center bearing the isopropyl substituent:



(26)

Y. Ohta, K. Ohara, and Y. Hirose[34] in studying the acid catalyzed isomerization of (-)-α-copaene (27) and (+)-α-ylangene (28) have reported that (-)-α-copaene isomerizes to two well known bicyclic hydrocarbons (+)-δ-cadinene (29), [α] + 94°, and (-)-α-muurolene (30), [α] - 66°, while the corresponding (+)-α-ylangene yields compounds (+)-δ-amorphene (31), [α] + 26°, and (-)-α-amorphene (32), [α] - 128°.

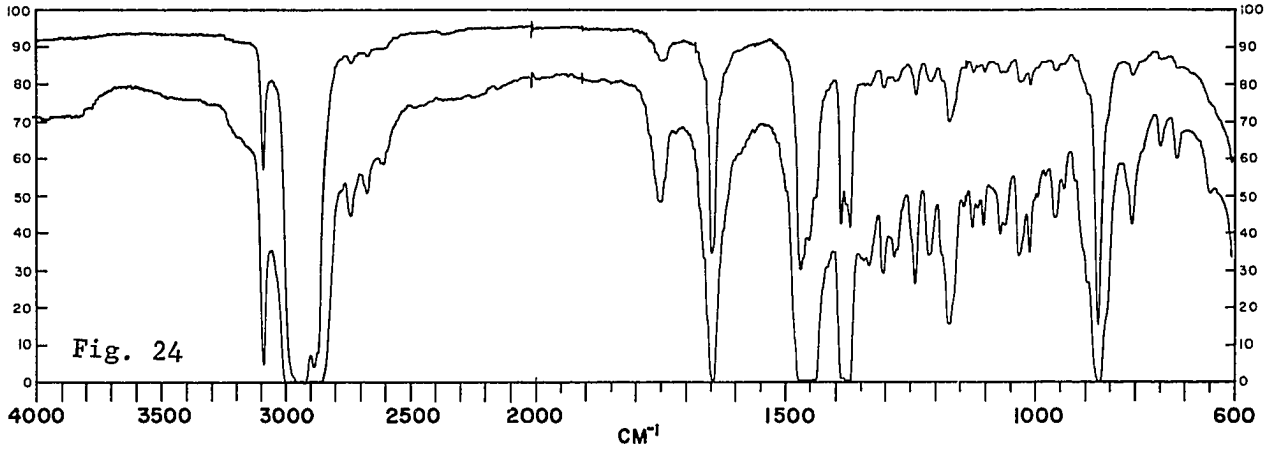
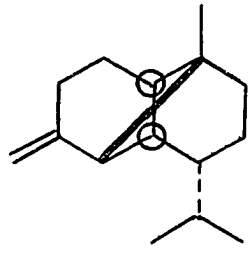


Fig. 24



(+) β-Copaene

Fig. 25

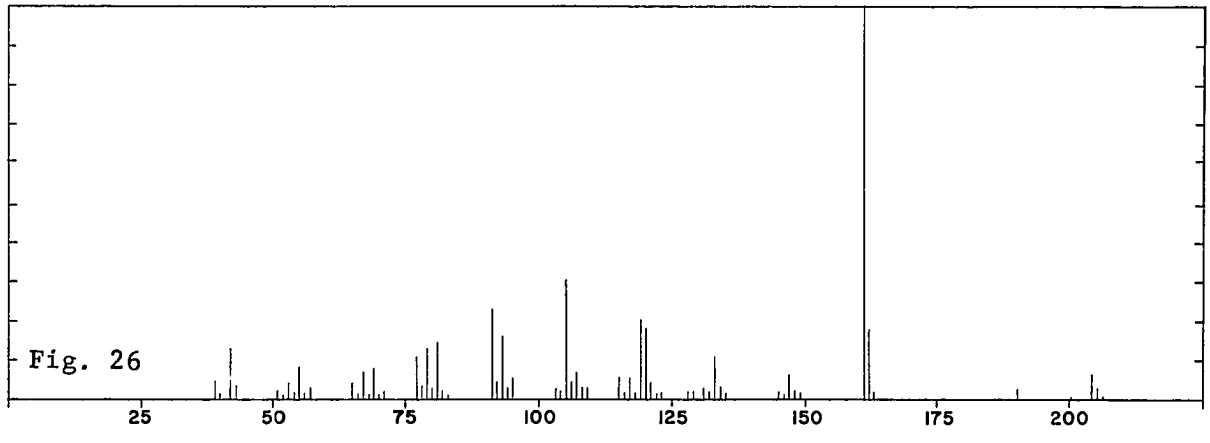
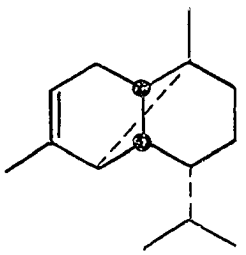
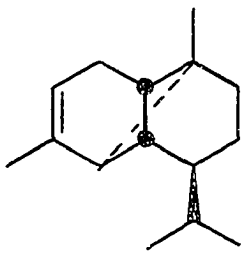


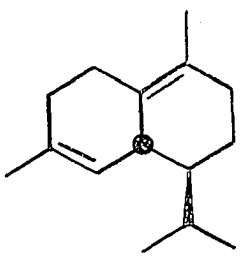
Fig. 26



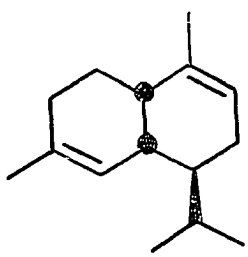
(27)



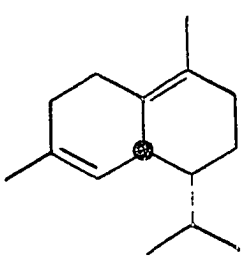
(28)



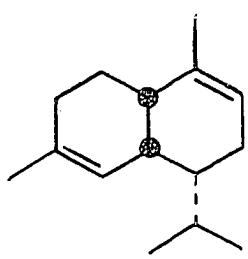
(29)



(30)



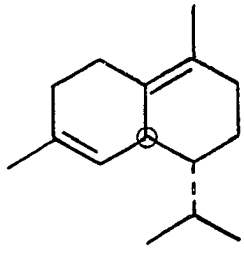
(31)



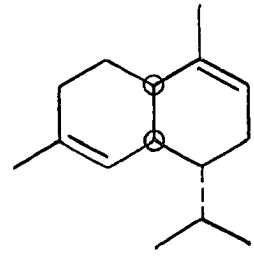
(32)

Since insufficient data is reported in the literature to distinguish between β-copaene and β-ylangene on the basis of physical properties, the identity of component D,(+)-β-copaene, was established by isomerization of component D and identification of the isomerization products. Component D was refluxed in 10% aqueous dioxane with a trace of sulfuric acid for one hour and the acid was removed by washing with saturated bicarbonate solution. Gas chromatography (Carbowax) of the recovered material indicated that component D was no longer present and

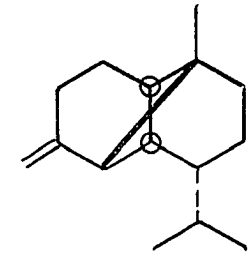
that the mixture contained two components with retention times identical with components F and G (Fig. 17). Preparative gas chromatography permitted the isolation of the two components identified by physical and spectral properties as (-)-δ-cadinene (29) and (+)-α-muurolene (30) shown below, establishing the identity of component D as (+)-β-copaene (31):



(29)



(30)

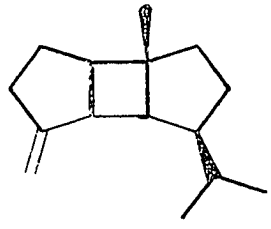


(33)

Parker, Roberts, and Ramage [35] have observed that a decaline type STHC mixture having both cis and trans protons at bridgehead and adjacent isopropyl substituent has not been observed to date. A mixture containing β-copaene (31) and α-cubebene (17) is consistent with this observation whereas β-ylangene and α-cubebene would be an unexpected observation.

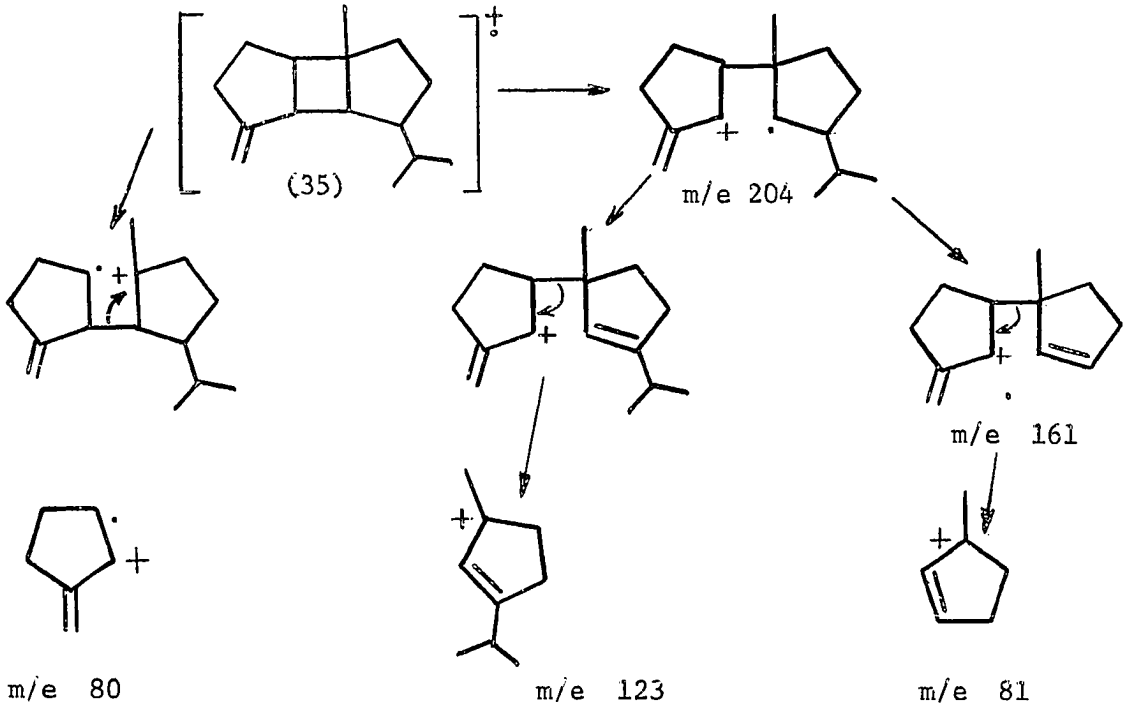
Chromatography of a distillation fraction on silver nitrate impregnated Florisil gave a hydrocarbon mixture which was 88% one component. Continued elution afforded a sample of hydrocarbon which gas-chromatography (LAC-1-R-296 and Carbowax) and silver nitrate TLC indicated was one component. Retention time (g.c.) identified this compound as a component C (Fig. 17), designated (+)-β-isobourbonene. Elemental analysis and mass spectroscopic molecular weight determination (204 parent ion) indicated a composition of $C_{15}H_{24}$. The IR (Fig. 27) and nmr (Fig. 28) spectra showed excellent agreement with spectral data

cited in the literature[36,37] for (-)-β-bourbonene (34). Physical properties observed for component C, however, differed significantly from those reported in the literature for (-)-β-bourbonene and precluded identity of the two compounds.



(34)

A key argument for the bourbonene-like skeleton was obtained from the mass spectrum (Fig. 29) of component C. Fragmentation of β-bourbonene cation radical (35), formally represented below, predicts significant ions of m/e 204 (parent ion), 161, 123, 81, and 80, in excellent agreement with the pattern observed for compound C:



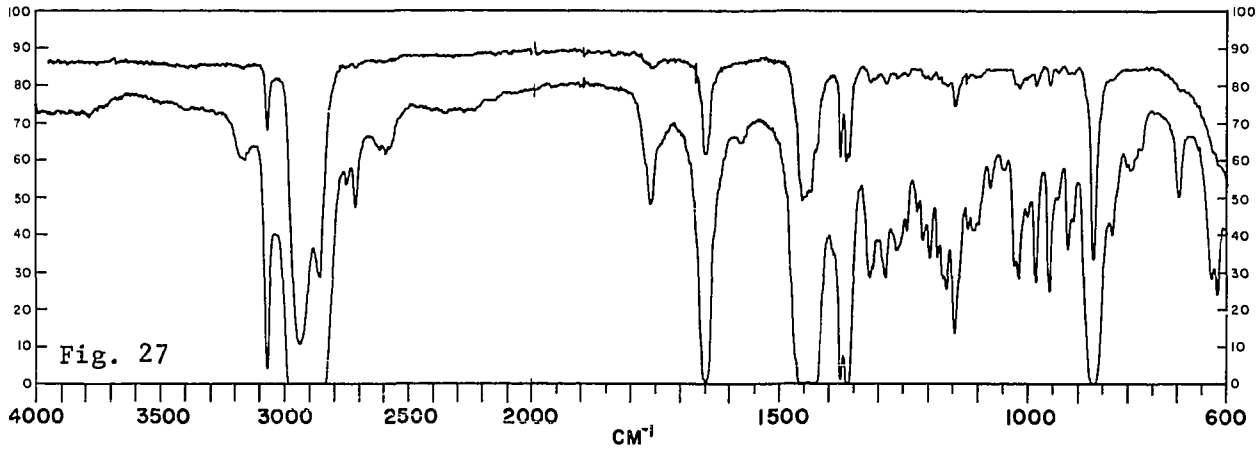
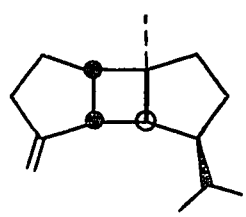


Fig. 27



(+) β-Epibourbonene

Fig. 28

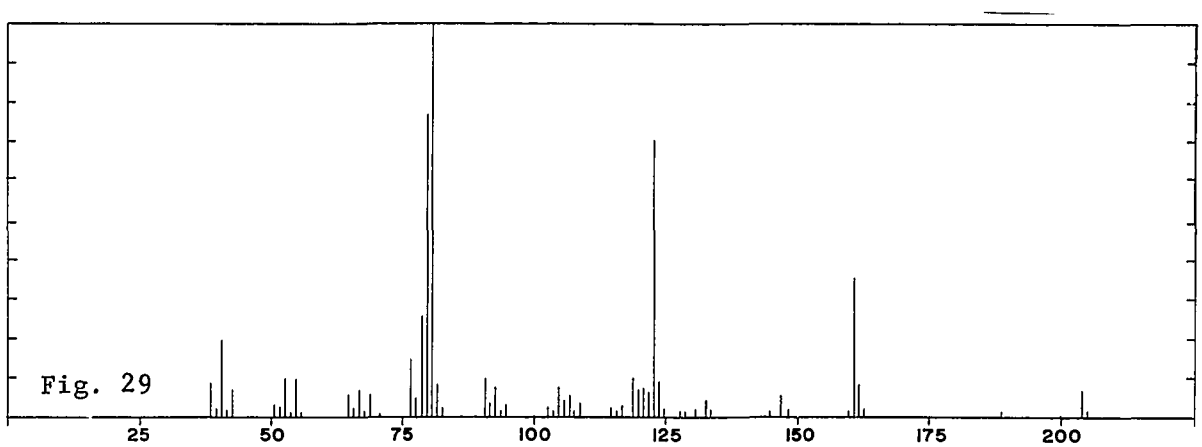
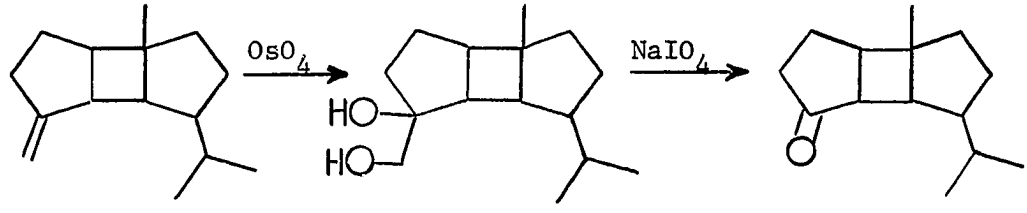


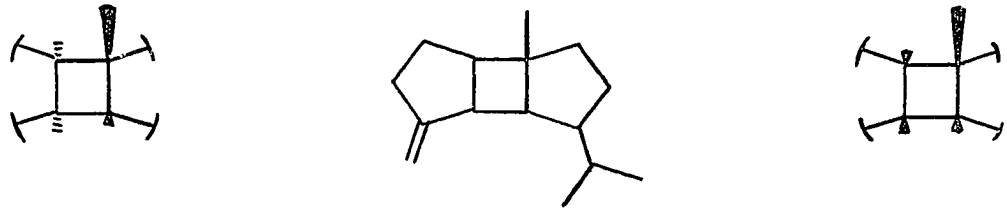
Fig. 29

Oxidation of the olefin (36) with osmium tetroxide and cleavage of the diol (37) with sodium metaperiodate resulted in a ketone (38) whose IR spectrum exhibited a band at 1735^{-1} in excellent agreement with that cited for bourbonone (35).[36-38] Physical properties have not been cited in the literature which precludes comparison of the two ketones. The diol mixture (34) is also unreported.



(36) (37) (38)

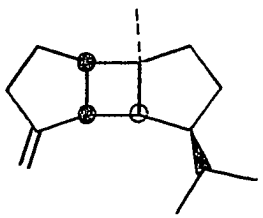
Spectral data of component C and the derived ketone establishes the bourbonene skeleton (36). Steric requirements of a five-membered carbocyclic precludes trans-fusion onto a four-membered ring thus restricting possible isomers to the cis-anti-cis (39) and the cis-syn-cis (40) relationships.



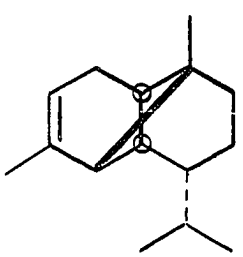
(39) (36) (40)

The extremely close ring proximity required for cis-syn-cis (40) configuration would cause significantly different chemical shifts in the nmr for both quaternary methyl and isopropyl compared with the cis-anti-cis (39) and may be ruled out since the nmr of component C and

its ketone closely agrees with that cited for β-bourbonene (cis-anti-cis). It may be concluded that the difference in physical properties between component C and (-)-β-bourbonene arises from the difference in configurational isomers at the isopropyl substitutient (or the mirror image) shown below:



Rechromatography of an earlier fraction on silver-nitrate-impregnated silicic acid provided a sample of hydrocarbon identified as component B (Fig. 17). Elemental analysis and mass spectroscopic molecular weight determination (204 parent ion) confirmed a composition of $C_{15}H_{24}$. Excellent agreement between observed physical data and the IR (Fig. 30) and nmr (Fig. 31) spectra with that shown in the literature[30,39,40] established the identity of the component B as (+)-α-copaene (41).



(41)

Isolation of Component E (Fig. 17). Chromatography of a distillation on silver-nitrate-impregnated silicic acid provided a sample of

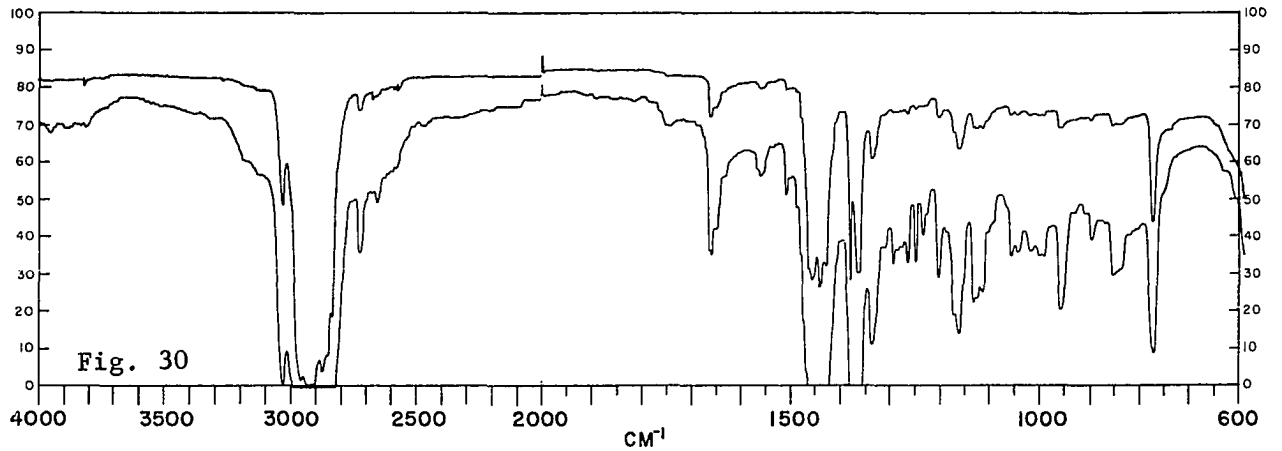
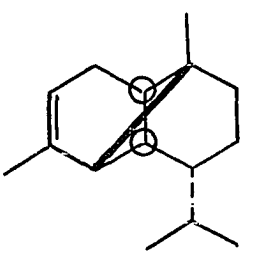


Fig. 30



(+) α-Copaene

Fig. 31

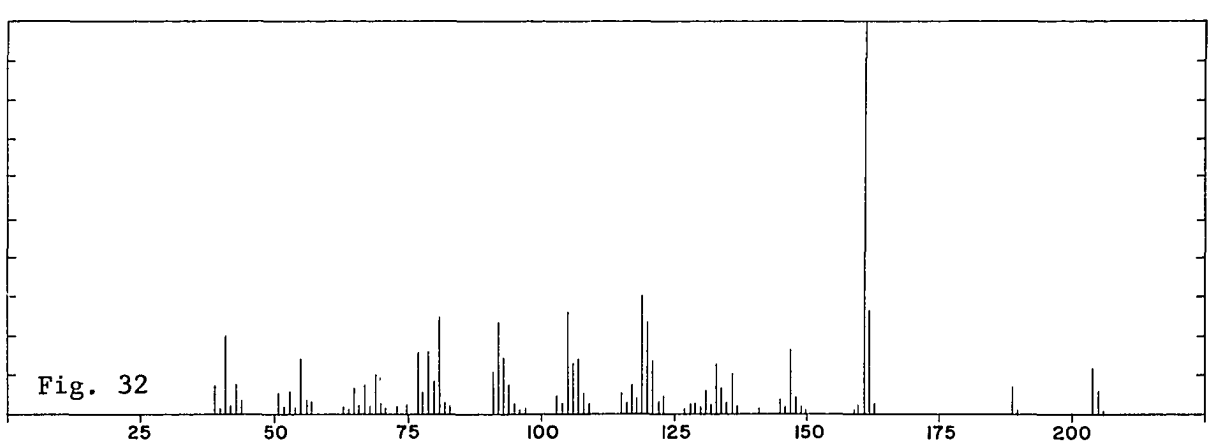
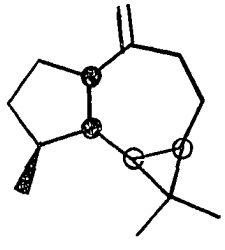


Fig. 32

hydrocarbon identified by retention time (g.c.) to be component E (Fig. 17). Silver nitrate TLC and gas chromatography (Carbowax) indicated that the sample contained a single component. Elemental analysis and mass spectroscopic molecular weight determination (204 parent ion) established the composition as $C_{15}H_{24}$. The IR spectrum (Fig. 33), which was identical in all respects with that shown for (-) alloaromadendrene,[41] displayed significant bands at 3090, 1640, and 884 cm^{-1} (terminal methylene), 1380 singlet, 1215, and 1195 cm^{-1} (gem dimethyl on quaternary carbon). The nmr spectrum (Fig. 34), which was consistent with the structure proposed by Sörm, Herout, Motl, Dolezs, and Soucek[41] for (-) alloaromadendrene (42), displayed signals of terminal methylene (δ4.72), cyclopropane (δ0.50 to 0.10), and quaternary methyl (δ1.04 and δ0.97) protons. The slightly different chemical shifts observed for the quaternary methyls was attributed to the difference in influence of terminal methylene on each of the two methyls.



(42)

Cook and Howard,[42] in examining the sesquiterpene mixture from the plant Illicium anisatum, reported α-cubebene, α and β-copaene, alloaromadendrene, δ-cadinene, and calamanene with gas chromatography relative retention times in close agreement with those shown in Fig. 17. It was reported that alloaromadendrene immediately followed β-copaene, was

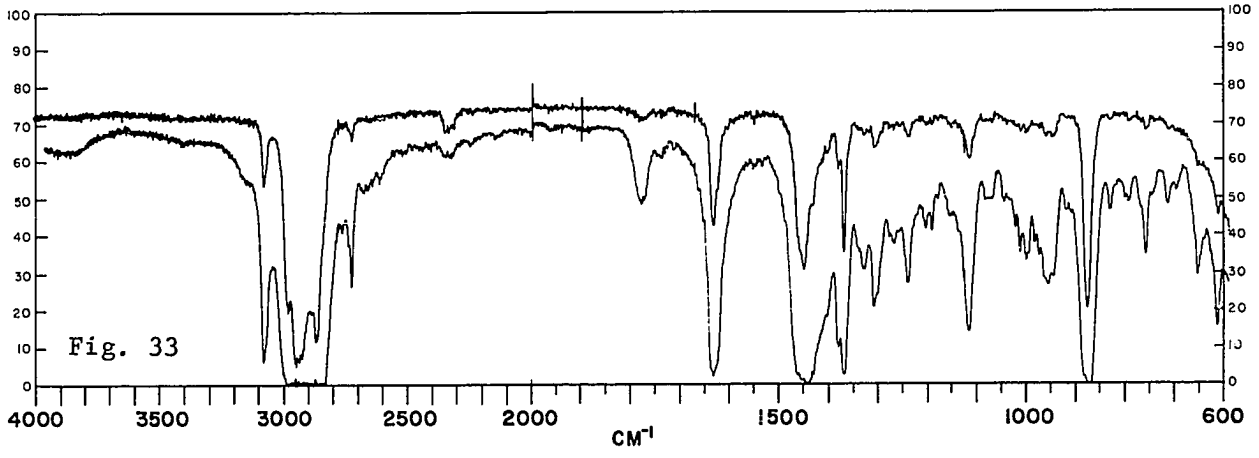
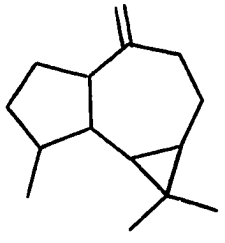


Fig. 33



Component E

Fig. 34

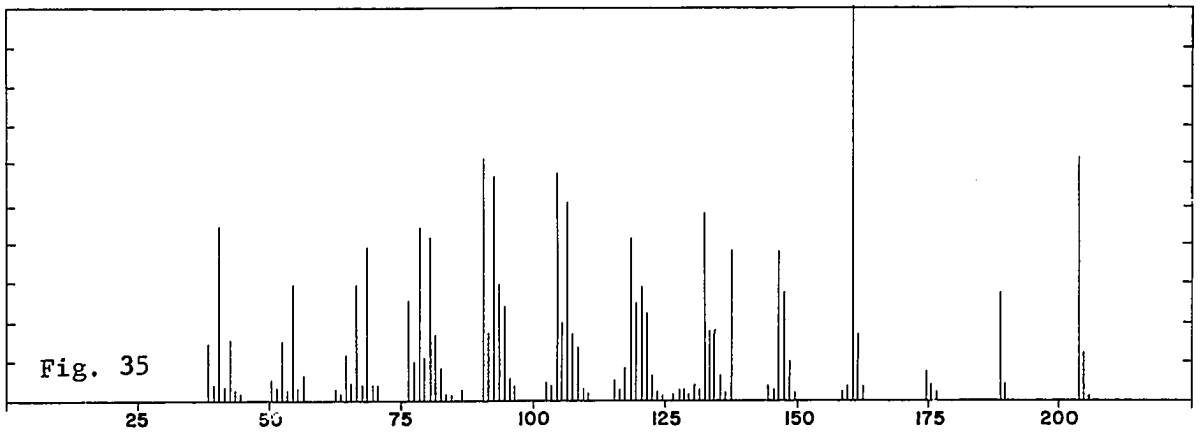
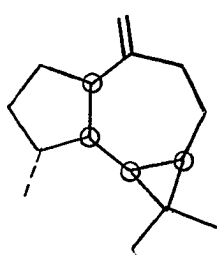


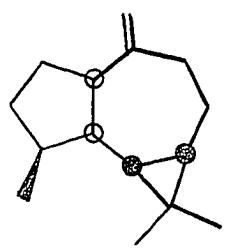
Fig. 35

followed by an unidentified component (α-muurolene in Fig. 17), then δ-cadinene. Physical properties were not obtained for the components, but rather identification was based upon a comparison of spectral data with that obtained from authentic samples.

The significant variation in specific rotation of component E, $[\alpha]_D^{25}$ + 36.40° (neat), compared with that consistently reported[41,43-46] for alloaromadendrene, [α] -21° ±1°, precludes an identity of the two compounds. Since the IR of component E (Fig. 33) is identical with that shown for alloaromadendrene,[41] it may be predicted that "E" has the cis-five-on-seven ring fusion.* Compound E may differ from alloaromadendrene by having the cyclopropane syn- to the five-on-seven fusion (43), or it may simply vary only at the secondary methyl shown below as (+) epi-alloaromadendrene (44).



(43)



(44)

Chromatography of a distillation fraction on silver-nitrate-impregnated silicic acid provided a sample of hydrocarbon which contained components F and G. Preparative gas chromatography provided samples which were greater than 95% pure for each component. Preparative gas chroma-

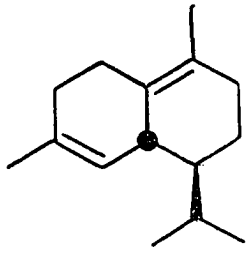
---

* Although (-) alloaromadendrene (42) differs from (-) aromadendrene only by the five-on-seven ring fusion, the published IR spectra of the two compounds differ significantly.[41]

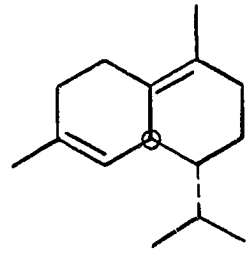
tography of each of the 95% pure samples permitted isolation of components F and G.

Elemental analysis and mass spectroscopic molecular weight determination (204 parent ion) of component F confirmed the composition $C_{15}H_{24}$. Excellent agreement of physical properties obtained from component F with those cited in the literature[14] identified the compound as (+)-δ-muurolene (30). The IR (Fig. 2), nmr (Fig. 3), and mass (Fig. 5) spectra were identical with data obtained previously.

Elemental analysis and mass spectroscopic molecular weight determination (204 parent ion) of component G confirmed a composition of $C_{15}H_{24}$. Excellent agreement between physical properties obtained from component G with those cited in the literature[47-49] established the identity of the compound as (-)-δ-cadinene (46). The IR spectrum (Fig. 36) showed close agreement with that shown in the literature for (+)-δ-cadinene[49] (45). The nmr spectrum (Fig. 37) was consistent with the structure proposed by Büchi, von Wittenau, and White for (+)-δ-cadinene (45). Component G (46) is the mirror image of (45).



(45)



(46)

Since it was shown that the compound (+)-calamanene obtained from residues of distillation of the hydrocarbon mixture on a stainless steel band, some speculations as to its origin are in order. (+)-

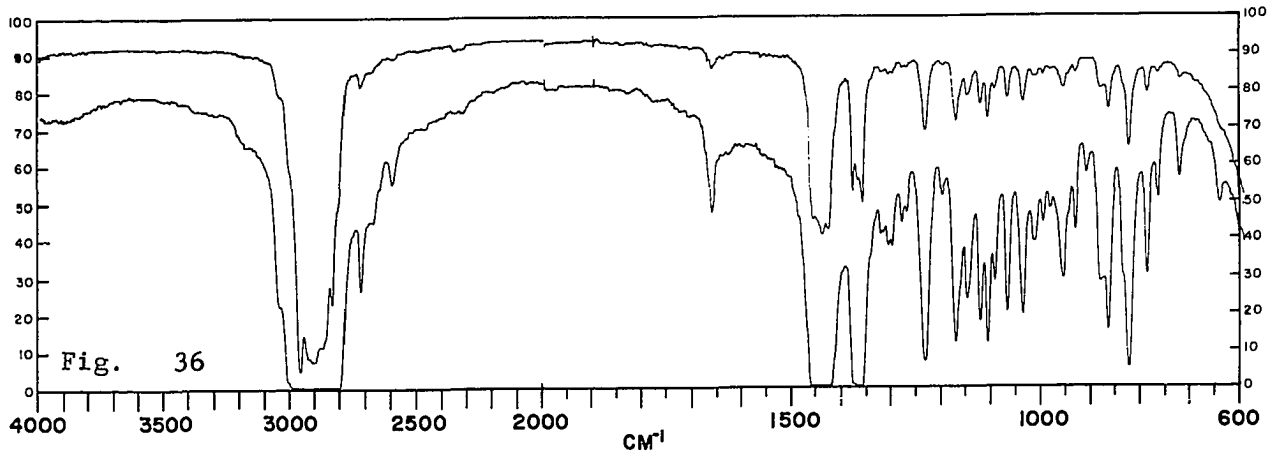
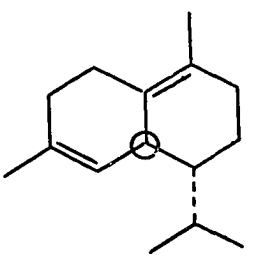


Fig. 36



(-) δ-Cadinene

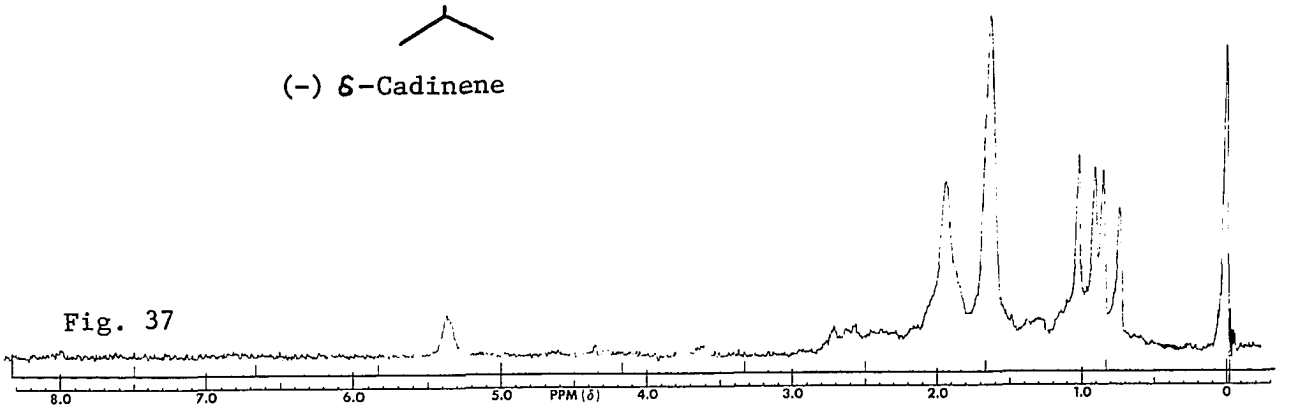


Fig. 37

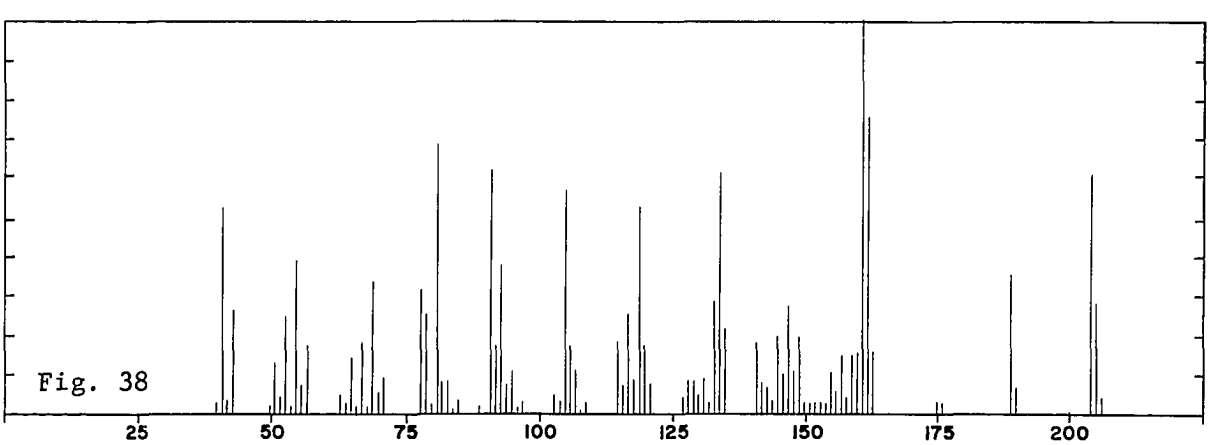
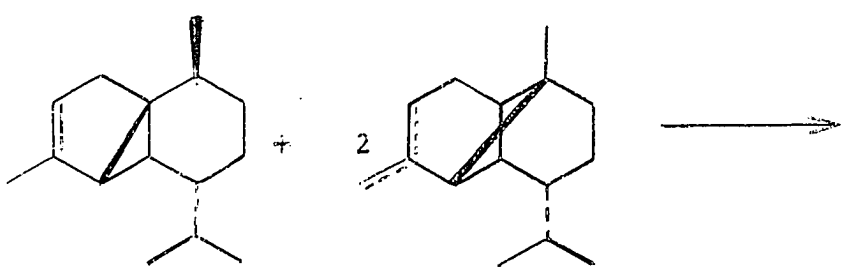
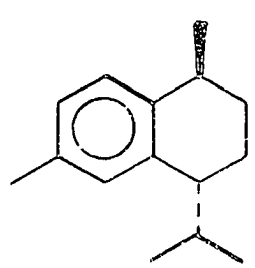


Fig. 38

Calamanene, a dehydro-sesquiterpene hydrocarbon, was obtained with a dihydro-sesquiterpene dimer suggesting a disproportonation reaction. Significant in the mass spectrum of the dihydrodimer were the parent ion of 410 and a base peak of 205. This parent ion suggests that the dimerization results from a pairing of two $C_{15}H_{25}$ radicals to form a single bond between the two sesquiterpene moieties. If the dihydrodimer were formed by a two plus two cycloaddition of two sesquiterpenes followed by hydrogenation of a double bond, the ions of 204 and 206 would be anticipated in the mass spectrum. Since (+)-α-cubebene possesses two degrees of unsaturation in one ring, aromatization of this compound could be expected and it is significant that (+) calamanene (46) has identical stereochemistry with (+)-α-cubebene (47). Since the total amounts of (+)-α-cubebene, (+)-α- and (+)-β-copaene were observed to diminish during distillation, it seems most likely that the disproportonation reaction involved three sesquiterpene hydrocarbons as shown below:



(47)

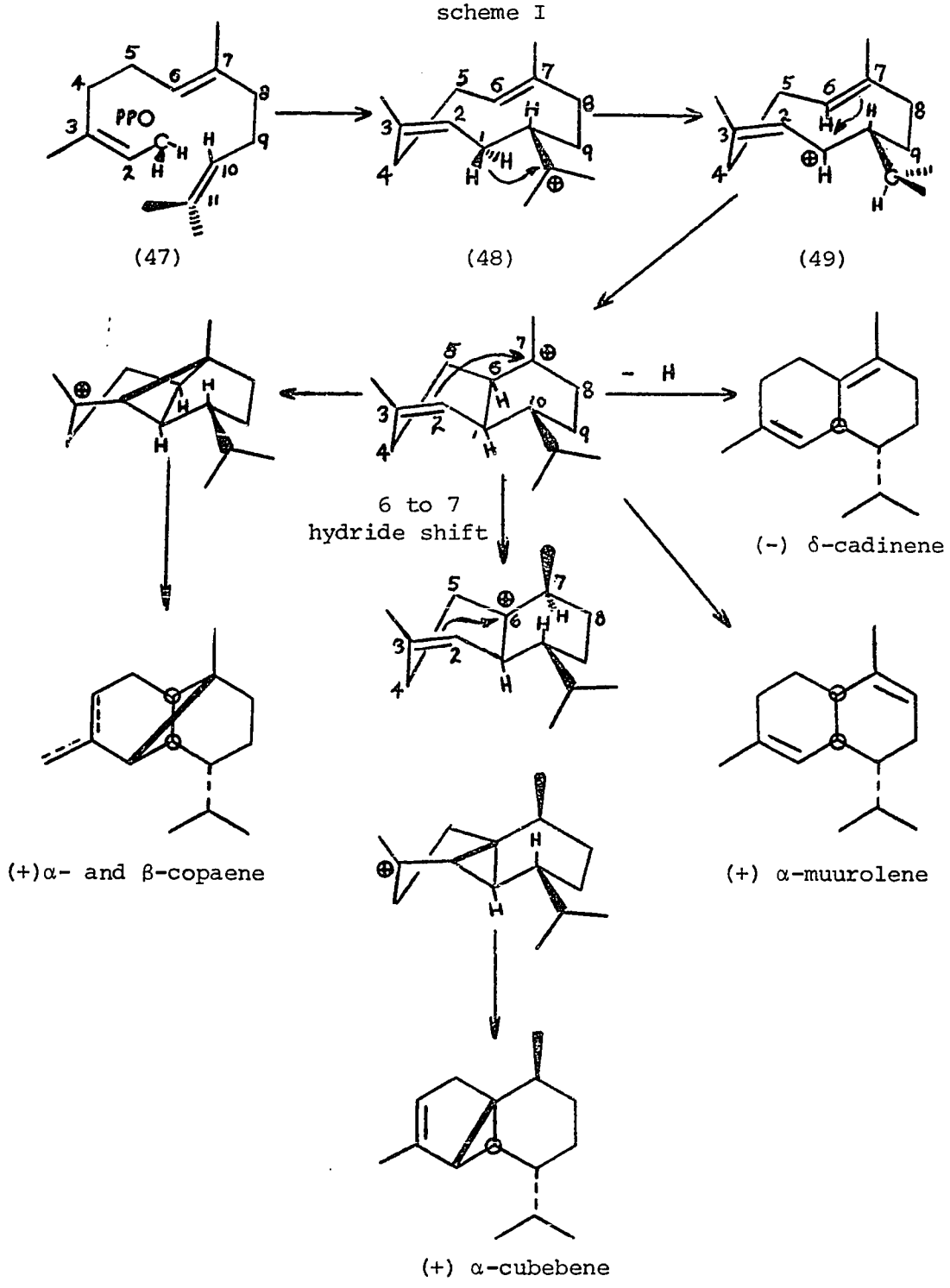


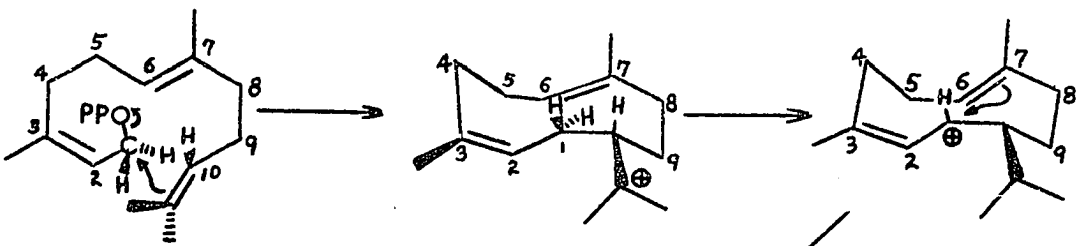
+ $C_{30}H_{50}$

(46)

Consideration of biosynthetic pathways presents one interesting observation. Components A, B, D, F, and G (Fig. 17) are believed to arise from common cation (50) resulting from cyclization of cis-farnesyl pyrophosphate[35] (47) outlined below in scheme I. However, both aromadendrene and bourbonane skeletons are considered to arise via different routes from cyclization of trans- farnesyl pyrophosphate.[35] Although the appearance of a hydrocarbon mixture from both cis- and trans- farnesyl pyrophosphate should not be considered impossible, it would indeed be an atypical phenomenon. The appearance of a bourbonane-type skeleton may be envisioned by examining a conformer (51) of cation (49) as shown in scheme II. Cation (51) undergoes cyclisation with both C_6 and C_1 protons on the same side of the ring as the isopropyl group (scheme II). If the C_1 proton is trans- to the C_6 proton and isopropyl substitutient, it may be shown using Drieding models that the decalin ring is distorted sufficiently to bring the C_1 cationic center closer to the C_7 carbon. Closure from C_7 to C_1 would lead to cation (52) which fillowing a second ring closure (53), could afford a bourbonane-type skeleton. The product of this scheme would be predicted to be the geometric isomer of (-)-β-bourbonene at the isopropyl substitutient. The foregoing scheme (Scheme II) would predict that "(+)-β-epibourbonene" would have the same geometry as (-)-β-bourbonene, with exception of isopropyl substitutient. Intuition would assign (+)-β-epibourbonene with geometry identical with (+)-β-bourbonene, with the exception of the isopropyl substitutient. It is interesting to note that α-copaene (54) and α-ylangene (55) differing only by the geometry of the isopropyl substitutient show opposite signs of rotation.[30,33]

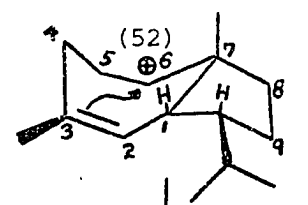
scheme I

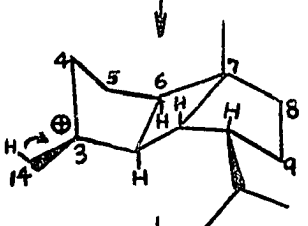

PPO
(47)
(48)
(49)
- H
6 to 7
hydride shift
(-) δ-cadinene
(+)α- and β-copaene
(+) α-muurolene
(+) α-cubebene

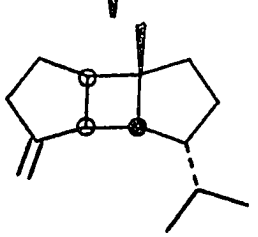


(47)

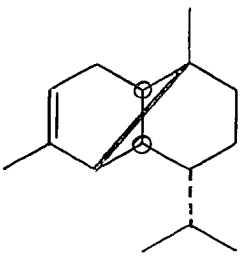
(51)



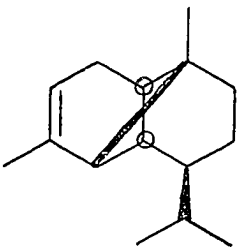




epibourbonene

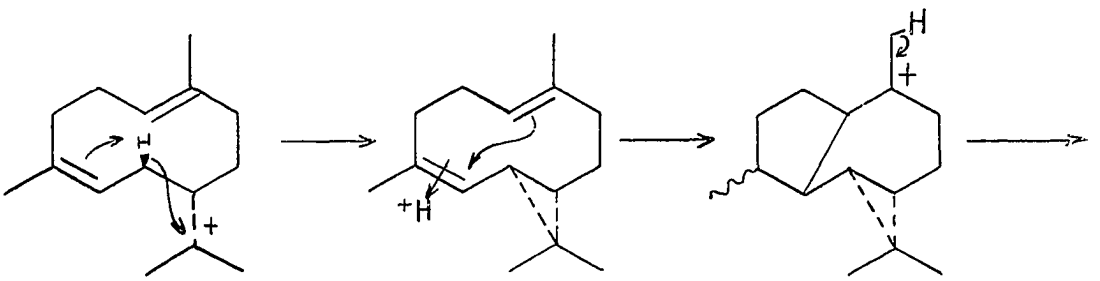


(54)

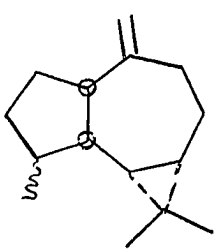


(55)

The appearance of an aromadendrane type skeleton may be envisioned by isomerization of cation (56) as indicated below:



(56) (57) (58)



Cation (57) could cyclize as shown to afford a five on seven type skeleton. Loss of a methyl proton from cation (58) would provide the alloaromadendrene-like hydrocarbon. The scheme proposed would predict that the cyclopropane ring and the C_6 proton would like on the same side of the ring which is required by the IR spectrum of component E.

Specimens of Pseudoplexaura porosa obtained from Bimini; Jamaica; Puerto Rico; Miami, Florida; and Bermuda were individually ground and extracted with hexane. Following removal of the solvent, the residue was chromatographed on Florisil and the hydrocarbon fraction of each specimen isolated. IR and nmr spectra and gas-chromatography (Carbowax) fingerprints were obtained for each hydrocarbon fraction. Comparison of spectra and gas-chromatographic analyses failed to indicate variation in the number or types of components, and less than 2% variation in relative concentrations.

C. Eunicea (Eunicea) palmeri

The sesquiterpene hydrocarbon fraction from Eunicea palmeri (0.12% total weight) was isolated by chromatography (Florisil) of the volatile components of the hexane extract. Chromatography of the mixture on silver-nitrate-impregnated silicic acid with 10% benzene afforded a sample of hydrocarbon which was 90% one component, and a sample of a second which was shown by gas chromatography and silver nitrate TLC to be a single component.

Elemental analysis and mass spectrographic molecular weight determination (204 parent ion) confirmed the composition of the pure component as $C_{15}H_{24}$. Comparison of physical properties with that cited in the literature[12] and determined from Pseudoplexaura porasa established the identity of the compound as (+)-β-copaene. The IR (Fig. 24), nmr (Fig. 25), and mass (Fig. 26) spectra were identical with that obtained from (+)-β-copaene from Pseudoplexaura porosa.

Chromatography of the fraction which was 90% one component on silver-nitrate-impregnated silicic acid with 5% benzene in hexane provided a sample of hydrocarbon identified as (+)-α-muurolene. Elemental analysis and mass spectrographic molecular weight determination (204 parent ion) confirmed a composition of $C_{15}H_{24}$. Comparison of physical properties with that cited in the literature[14] established the identity of the compound. IR (Fig. 2) and nmr (Fig. 3) spectra were identical with spectra obtained from (+)-α-muurolene isolated from Plexaurella dichotoma and Pseudoplexaura porosa.

EXPERIMENTAL

All melting points are uncorrected. All solvents were redistilled before use. Hexane used for silver nitrate chromatography was distilled from calcium hydride. "Wet" hexane was simply redistilled. Methylene chloride was passed through Linde molecular sieve 4A, pyridine was distilled from barium oxide, and tetrahydrofuran and monoglyme were distilled from lithium aluminum hydride. Chromatographic supports were Florisil (Floridin Co., 100-200 mesh), SilicAR CC-7 (Mallinckrodt Chemicals, AR 100 mesh), and silicic acid (Mallinckrodt Chemicals, 100 mesh). Silver-nitrate-impregnated silicic acid and silver-nitrate-impregnated Florisil were prepared using a modification of the procedure of Norin and Westfelt.[56] Support material (silicic acid pH 4 or Florisil; 300 g) was slurried into an aluminum-foil-covered boiling flask (2 liter) containing a solution of silver nitrate (100 g) in water (500 ml). As much water as possible was removed by water aspiration on a rotary evaporator and the support was then activated by heating at 110° under vacuum for 24 hours. Oxidative degradation of the hydrocarbons during chromatography on the silver-nitrate-impregnated supports could be largely avoided by use of high flow rates (e.g., 20 ml/min) and very dry solvents (dried on calcium hydride).

Thin layer chromatography was performed on 5 x 20 cm. glass plates coated with Merck (Darmstadt) silica gel H or silica gel $PF_{254+366}$

containing 20% by weight silver nitrate. The plates were placed in an iodine vapor tank for visualization of the chromatogram. Thick layer preparative chromatography was performed on either 20 x 20 cm or 10 x 20 cm. glass plates coated with Merck (Darmstadt) silica gel $PF_{254+366}$. The silica gel PF plates were visualized under UV light (254 or 366 nm).

Gas chromatographic analyses were performed on a Hewlett-Packard (F&M), Model 402, using 4 foot by 8 mm. columns containing either 5% or 20% Carbowax on Gaschrom Z support (80-100 mesh). Carrier gas (helium) flow was maintained at 10 ml. per minute. Flash and detector heaters were maintained at 190° and the oven temperature was maintained at either 100° or 140°. Preparative gas chromatography was performed either on an Aerograph Autoprep, Model A-700 using 20 foot by 3/8 inch columns containing 20% LAC 1-R-296 DEGA on Chrom P (45-60 mesh), or on an all glass preparative gas chromatograph utilizing a glass splitter connected to an analytical thermal conductivity cell (3-5% sample passing to detector) which was fabricated with help from shop and electronic personnel from the Chemistry Department. Columns used were either a 24 foot by 3/8 inch or a 10 foot by 3/8 inch glass column containing 5% Carbowax on Gas-Chrom Z (80-100 mesh).

Infrared absorption (IR) spectra were recorded on a Beckman IR-8 spectrophotometer. The IR spectra were run as a thin film between two salt discs, as a thick film in a 0.1 mm. cell, as a solid solution in anhydrous potassium bromide, or as a solution in varying concentration in chloroform or carbon tetrachloride.

Nuclear magnetic resonance (nmr) spectra were taken on a Varian A-60 spectrometer using tetramethylsilane (TMS) as an internal

reference. Samples were run as neat liquids, or as solutions of varying concentrations in carbon tetrachloride, benzene, and chloroform. Chemical shifts were reported in δ-values (ppm from TMS), and are followed by the multiplicity of the signals, and the corresponding coupling constants. The multiplicities are denoted by the symbols: s, singlet; d, doublet; dd, doubled doublet; t, triplet; q, quartet; m, multiplet. Coupling constants are reported in Hz.

Analyses were carried out by the Alfred Bernhardt Laboratories Mülheim, West Germany, and by mass spectra kindly performed by the Mass Spectroscopy Center, Purdue University, Lafayette, Indiana. Digital data reduction of the mass spectral data was performed by the University of Oklahoma Computer Facility utilizing the IBM 360 System.

A. Plexaurella dichotoma

Isolation of Sesquiterpene Hydrocarbon (STHC) Fraction. Air dried Plexaurella dichotoma (21.5 k) collected at Miami, Florida, in June 1966 and June 1967 was batch leached twice with hexane (1.5 liters per kg weight; then 1.0 liter per kg weight). The solution was filtered (Whatman No. 1) and the solvent was removed first by aspiration on a rotary evaporator at steam bath temperature then by evacuation to 0.1 mm. for 12-14 hours at room temperature. The black tarry residue (880 g) was distilled through a falling film molecular still (Nester-Faust) at 80°/0.07 mm. The yellow-orange distillate (143 g) was chromatographed in hexane through Florisil (1000 g) yielding 63.4 g (0.30% of dry weight) of colorless liquid hydrocarbons. Gas chromatography of the mixture (5% Carbowax) revealed a mixture of at least

fourteen components (Figure 1).

Distillation of the STHC Fraction. The STHC mixture from above (40 g) was placed in a teflon annular still (Nester-Faust, Model NFT-60; 127 theoretical plates), and the system was evacuated to 0.85 mm. pressure. The components of lower concentration (A through I, Fig. 1) were distilled from 60° to 71° without attempting to obtain separation (reflux ratio 50:1; reflux valve throw 3 mm.). Distillate samples were removed from the drip tip at regular intervals (20 min.) and injected into the gas-chromatograph (20% Carbowax). After approximately 5.0 g of STHC had been removed, components J and K (believed then to be a single component) were detected in the distillate. The reflux ratio was adjusted to total reflux and pot and jacket controls were adjusted until the jacket temperature was 1-2 degrees below the head temperature. By alternating reflux ratio between total reflux and 100:1 (reflux valve throw 1 mm.) at 1 hour intervals, the remaining traces of components G, H, and I were removed. Components A through I were combined (Fraction 1).

Fraction 2 (1.3 g) was removed at 71°/0.85 mm. (reflux ratio 100:1; valve throw 1 mm), and appeared to contain a single component (J and K in Fig. 1) by gas chromatographic analysis.

Fraction 3 (4.4 g) was removed at 71°/0.85 mm. (reflux ratio 100:1; valve throw 1 mm.). Gas-chromatography of fraction 3 indicated that J, K, and traces of component L (estimated by g.c. to be less than 1%) were present in the distillate. The UV spectrum of fraction 3 in hexane showed a maximum at 228 mμ. Since neither components J or K (subsequently isolated) displayed a maximum at 228 mμ, this absorption was attributed to component L.

The distillation was interrupted and the contents of the pot chromatographed on Florisil (100 g) with hexane. Gas-chromatography of the recovered STHC (20 g) indicated that component P was no longer present and that the concentration of component M had been reduced by approximately 50% relative to components N and O. The distillation was not resumed and the recovered STHC mixture was designated fraction 4.

Isolation of (+)-α-Muurolene and (+)-β-Bisabolene. The nmr spectrum of fraction 2 revealed that the liquid was not a single component. Based upon an exo-methylene peak at δ4.73, s, 2H, the total integral exceeded forty-five protons.

Chromatography of this fraction on silver-nitrate-impregnated silicic acid (33 g) with 8% benzene in hexane provided, in fractions 2-20 (100 ml each; flow rate 20 ml per minute) a sample of hydrocarbon (0.620 g) identified as (+)-α-muurolene, n_D^{25} 1.5052, d_4^{25} 0.9211, $[\alpha]_D^{25}$ +67.26° (neat); Lit[14]: n_D^{20} 1.5074, d_4^{20} 0.9190, $[\alpha]_D^{20}$ -66.0° (neat). The IR spectrum (film) which agreed with that shown in the literature,[14] showed significant bands at 1680, 880, 845, 827, and 800 cm^{-1} (trisubstituted double bonds), a doublet at 1380 cm^{-1} (gem dimethyl). The nmr spectrum (neat) exhibited signals at δ5.36, broad m, 2H (vinyl protons); δ1.88, broad s, 2H (allylic methine protons); δ1.67, s, 6H (vinyl methyls); δ0.88, d, J = 7 Hz, 3H and δ0.82, d, J = 7 Hz, 3H (isopropyl methyls). The mass spectrum displayed significant peaks at m/e 204 (parent ion) and at 161 (base peak; $M-C_3H_7$).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.84%. Found: C, 87.96%; H, 11.99%.

On continued elution with 25% benzene in hexane, fractions

47-60 (100 ml each; flow rate 20 ml per min.), provided a sample of hydrocarbon (0.684 g) identified as (+)-β-bisabolene, n_D^{25} 1.4884, d_4^{25} 0.8664, $[\alpha]_D^{25}$ +83.06° (neat). Lit.[18]: n_D^{20} 1.4880, d_4^{20} 0.8673, $[\alpha]_D^{20}$ -84.0° (neat). The IR spectrum (film) which agreed with that shown in the literature,[19] showed significant bands at 1645 and 890 cm^{-1} (terminal vinyl), 1680, 830, and 789 cm^{-1} (trisubstituted double bond), and a doublet at 1380 cm^{-1} (gem dimethyl). The nmr spectrum (neat) exhibited signals at δ5.43, m, 1H (vinyl proton); δ5.22, m, 1H (vinyl proton); δ4.84, s, 2H (exo-methylene); δ2.11, s, 6H (allylic protons); δ1.95, broad s, 3H (allylic protons); δ1.65, broad s, 6H (vinyl methyls); δ1.61, s, 3H (vinyl methyl). The mass spectrum displayed significant peaks at m/e 204 (parent ion) and at 93 (base peak).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.84%. Found: C, 87.91%; H, 11.79%.

Hydrogenation of (+)-β-Bisabolene. (+)-β-Bisabolene (160 mg, 0.79 mmoles) was hydrogenated at atmospheric pressure in anhydrous ethanol (10 ml) over 10% palladium on carbon (19 mg). The sample absorbed 2.77 molar equivalents of hydrogen (92% of theoretical). The ethanol was removed on a rotary evaporator under reduced pressure, during which time drying of the catalyst was prevented by periodically adding heptane to the flask. Chromatography of the residue on silver-nitrate-impregnated silicic acid (5 g) provided in the first 100 ml of eluent, a sample of hydrocarbon (160 mg; 97% of theoretical) whose IR spectrum was identical with that shown for bisabolane.[17]

Anal. calcd for $C_{15}H_{30}$: C, 85.62%; H, 14.38%. Found: C, 85.63%; H, 14.37%. M.w. 210 (m.s.).

Hydrogenation of (+)-β-Bisabolene. (+)-β-Bisabolene (160 mg, 0.79 mmoles) was hydrogenated at atmospheric pressure in anhydrous ethanol (10 ml) over 10% palladium on carbon (19 mg). The sample absorbed 2.77 molar equivalents of hydrogen (92% of theoretical). The ethanol was removed on a rotary evaporator under reduced pressure, during which time drying of the catalyst was prevented by periodically adding heptane to the flask. Chromatography of the residue on silver-nitrate-impregnated silicic acid (5 g) provided in the first 100 ml of eluent, a sample of hydrocarbon (160 mg; 97% of theoretical) whose IR spectrum was identical with that shown for bisabolane.[17]

Anal. calcd for $C_{15}H_{30}$: C, 85.62%; H, 14.38%. Found: C, 85.63%; H, 14.37%. M.w. 210 (m.s.).

Isolation of (-)-α-Curcumene and (+)-α-Bisabolene. Fraction 4 from above (2.0 g) was chromatographed on silver nitrate impregnated silicic acid (240 g). Elution with 5% benzene in hexane provided, in fractions 7-12 (100 ml per fraction; flow rate 3.3 ml per minute), the hydrocarbon previously identified as (+)-α-muurolene. Continuing elution with 8% benzene in hexane provided, in fractions 18-25, a sample of hydrocarbon identified as (-)-α-curcumene (component N, Fig. 1), n_D^{20} 1.5015, d_4^{20} 0.8777 $[\alpha]_D^{20}$ -39.31° (neat), m.w. 202 (m.s.). Lit[18]: n_D^{24} 1.5020, d_4^{24} 0.8749, $[\alpha]_D^{24}$ +40.0° (c. 3.3; $CHCl_3$). The IR spectrum, which agreed with that cited in the literature[19] showed significant bands at 1900 and 1793 cm^{-1} (para-disubstituted benzene), 1515 cm^{-1} (aromatic), 1670 and 810 cm^{-1} (trisubstituted double bond). The nmr spectrum (neat) displayed signals at δ6.94, s, 4H (para-disubstituted benzene); δ5.05, t, J = 8 Hz, 1H (vinyl proton); δ2.63, q, J = 7 Hz, 1H (benzylic proton);

δ2.25, s, 3H (aryl methyl); δ1.67, broad s, 3H (vinyl methyl); δ1.50, broad s, 3H (vihyl methyl); δ1.20, d, J = 7 Hz, 3H (secondary methyl). The UV spectrum (in hexane) agreed with that shown for α-curcumene,[19] having maxima at λ = 273, 368, 266, and 260 nm. The mass spectrum agreed closely with that shown in the literature,[19] exhibiting intense peaks at m/e = 202 (molecular ion), 145, 132 (base peak), 119, 105, and 92.

Anal. calcd for $C_{15}H_{22}$: C, 89.03%; H, 10.97%. Found: C, 89.17%; H, 10.90%.

Continuing elution (8% benzene in hexane) provided, in fractions 38-41, a mixture of hydrocarbons (0.093 g) consisting of components M, Fig. 1 (40% by g.c.) and component O, Fig. 1 (60% by g.c.).

Continuing elution with 15% benzene provided, in fractions 48-56, a sample of hydrocarbon (0.320 g) identified as (+)-α-bisabolene (component O, Fig. 1), n_D^{20} 1.4955, d_4^{20} 0.8728, $[\alpha]_D^{20}$ +63.5° (c. 12.75, $CHCl_3$), (m.s.). Lit[23]: n_D^{20} 1.4879, d_4^{20} 0.8730, $[\alpha]_D^{20}$ +75°. The IR spectrum, which agreed with that shown in the literature,[23] displayed significant bands at 1680, 1660, 820, and 790 cm^{-1} (trisubstituted double bonds). The nmr spectrum showed signals at δ5.35, broad s, 1H (vinyl proton); δ5.10, m, 2H (vinyl protons); δ2.64, t, J = 7 Hz, 2H (diallylic methylene); δ1.92, crude d, 5H (allylic methylene and methine protons); δ1.59, broad s, 9H (vinyl methyls). The mass spectrum displayed significant peaks at m/e = 204 (molecular ion) and 93 (base peak).

Continued elution with benzene provided (+)-β-bisabolene identified above.

Isolation of α-Curcumene. Fraction 4 from above (13.5 g) was chromatographed on silver nitrate impregnated silicic acid (250 g). Elution with 8% benzene in dry hexane provided in cuts 41-55 (100 ml each; flow rate 3.3 ml per minute), a sample of hydrocarbon (0.240 g) which was approximately 90% component M, Fig. 1 (estimated from g.c.).

Chromatography of this mixture on silver-nitrate-impregnated silicic acid (24 g) with dry hexane provided, in fractions 60-70 (100 ml per fraction: flow rate 10 ml per minute), a sample of the main component M (0.140 g) identified as α-curcumene. The IR spectrum showed significant bands at 1665, 950, 825, and 790 cm^{-1} (trisubstituted double bonds). The nmr spectrum displayed signals at δ5.37, broad s, 2H (vinyl protons); δ5.07, dt, J_{AB} = 7 Hz, J_{AC} = 1 Hz, 1H (vinyl proton); δ2.53, s, 4H (diallylic methylenes); δ1.64, d, J = 1 Hz, 6H (vinyl methyls); δ0.98, d, J = 7 Hz, 3H (sidechain methyl). The mass spectrum displayed significant peaks at m/e 202 (molecular ion), 133, and 119 (base peak).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.85%. Found: C, 88.32%; H, 11.85%.

Hydrogenation of β-Curcumene. β-Curcumene (117 gm; 0.57 mmoles) was hydrogenated at atmospheric pressure in anhydrous ethanol (10 ml) over palladium on carbon (11 mg). The sample absorbed 2.83 molar equivalents of hydrogen (94% of theoretical). The produce was isolated using the same procedure utilized to recover the hydrogenation product of (+)-β-bisabolene. The IR spectrum compared favorably with the spectrum shown for bisabolane.[17]

Anal. calcd for $C_{15}H_{30}$: C, 85.62%; H, 14.38%. Found: C, 85.64%; H, 14.18%. M.W. 210 (m.s.).

Reduction of (-)-β-Curcumene to β-Curcumene with Lithium and Alcohol in Liquid Ammonia (Birch Reduction). β-Curcumene was synthesized from α-curcumene by Birch[26] who used sodium, ethanol, and liquid ammonia. In performing this reaction, the modification of Cella, Brown, and Burtner[27] was employed.

(-)-α-Curcumene (0.490 g; 2.43 mmoles) dissolved in tetrahydrofuran (10 ml) and tertiary butanol (10 ml) were placed in a 100 ml three-neck flask fitted with dry ice-acetone condenser and magnetic stirrer, and immersed in a dry ice-acetone bath. Ammonia (50 ml) was doubly distilled into the reaction vessel and the solution was stirred for ten minutes. Lithium (100 mg; 14.29 mmoles) was added to the stirred mixture at the rate of 10 mg per five minutes. The mixture was stirred for an additional forty-five minutes during which time the blue color disappeared after which time the ethanol (10 ml) was added. Following evaporation of the ammonia, the residue was added to a solution of saturated ammonium chloride (20 ml), and the aqueous phase was extracted with hexane (3 washings; 50 ml each). The hexane was evaporated on a rotary evaporator under reduced pressure following drying over sodium sulfate, to yield a sample of hydrocarbon (0.504 g) contaminated with tertiary butyl alcohol and less than 1% starting material (estimated from g.c.).

Chromatography of the above mixture on silver-nitrate-impregnated silicic acid (10 g) with 1% benzene in dry hexane provided a sample of hydrocarbon (0.480 g; 97% yield) identified as β-curcumene, n_D^{20} 1.4912, d_4^{20} 0.8738, $[\alpha]_D^{20}$ 0.0. Lit[52]: n_D^{20} 1.4975, d_4^{20} 0.8810, $[\alpha]_D^{20}$ +32 (neat). The IR and nmr spectra were identical with those of β-curcumene (component M, Fig. 1) described above.

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.85%. Found: C, 87.85%; H, 11.96%. M.W. 204 (m.s.).

Partial Reduction of (-)-α-Curcumene with Lithium and Alcohol in Liquid Ammonia. (-)-α-Curcumene (0.236 g; 1.17 mmoles) dissolved in tetrahydrofuran (5 ml) and tertiary butyl alcohol (5 ml) was placed in a 100 ml three-neck flask fitted with dry ice-acetone condenser and magnetic stirrer, and immersed in a dry ice-acetone bath. Ammonia (25 ml) was doubly distilled into the reaction flask and the solution stirred for ten minutes. Lithium (24 mg; 3.43 mmoles) was added to the stirred mixture. Following the discharge of the deep blue color (approximately 1 minute) ethanol (5 ml) was added and the ammonia was evaporated. The residue was added to a saturated solution of ammonium chloride (10 ml) and the aqueous phase was extracted with hexane (two washings of 50 ml each). The hexane was evaporated on a rotary evaporator under reduced pressure following drying over sodium sulfate, to yield a sample of hydrocarbon (0.233 g) which was identified as predominantly starting material.

Chromatography of Partially Reduced (-)-α-Curcumene. The partially reduced (-)-α-curcumene from above (0.233 g) was chromatographed on silver-nitrate-impregnated silicic acid (25 g). Elution with 5% benzene in dry hexane provided in fractions 14-20 (100 ml each; flow rate 3.3 ml per minute) a sample of hydrocarbon identified as (-)-α-curcumene (0.187 g), $[\alpha]_D^{20}$ -30° (c = 17.5; $CHCl_3$).

B. Pseudoplexaura porosa

Isolation of the Sesquiterpene Hydrocarbon (STHC) Fraction and Volatile Oxygenated Fractions. Air dried Pseudoplexaura porosa (9.5 kg)

collected at Bermuda in June 1968 was batch leached twice with hexane (1.5 liter per kg wt; then 1.0 liter per kg). The solution was filtered (Whatman No. 1) and the solution was concentrated to approximately 4 liters. Following storage at zero degrees for one week, the solid material (27 g) was filtered (Whatman No. 1) and the solvent was removed first by aspiration on a rotary evaporator then by evacuation to 0.1 mm for 8-10 hours. The chocolate tarry residue (700 g; 7.5% total dry weight) was distilled through a falling film molecular still (Nester-Faust) at 80°, 100°, and 120° at 0.07 mm. The distillates, varying in color from light yellow to deep orange, were separately chromatographed on Florisil (10 to 1 ratio) starting with hexane, then benzene, then ethylacetate. The colorless hydrocarbon fractions (Fraction 1.1) were eluted with hexane. The volatile oxygenated materials (Fractions 1.2 and 1.3) were eluted by the more polar solvents. Results of the distillation and chromatography appear below in Table 1. Gas chromatography (5% Carbowax) of the STHC mixture revealed at least nine components (Figure 17).

TABLE 1

Chromatography of Pseudoplexaura porosa Volatile Material

Distillation Cut		Weight (g) Material Eluted by:			
Temp.	Weight (g)	Hexane	Benzene	Ethylacetate	Lost
80°	81.0	61.0	7.7	10.5	0
100°	134.0	52.0	13.7	65.9	3.4
120°	31.7	0.9	2.0	18.5	1.0
totals	246.7	113.9	23.4	95.9	4.4
Fract.		1.1	1.2	1.3	

Isomerization of the STHC Mixture. To the STHC mixture (fraction 1.1) from above (0.5 g) dissolved in hexane (10 ml) was added three grams of silicic acid (SilicAR 100; pH = 4). The slurry was stirred for 12 hours in a nitrogen atmosphere. Samples were removed at three-hour intervals and injected into an analytical gas chromatograph (5% Carbowax). Components A and B disappeared, the concentration of C and D significantly decreased, and components F and G increased. The relative rates of disappearance were $B > A > D \approx C$.

The above experiment was repeated using Florisil and acidic, basic, and neutral aluminas. Isomerization of the STHC mixture was observed for all supports with exception of Florisil. Isomerization was only slight on neutral alumina.

The STHC mixture (1 g) was heated to 120° for 12 hours under a nitrogen atmosphere. Gas chromatography of samples prior to and

following heating indicated that thermal isomerization was not significant.

Spinning Band Distillation of the STHC Mixture (Fraction 1.1). The STHC mixture (50 g) was placed in the receiver of an 18 in. Nester-Faust spinning band column rated at 23 theoretical plates, the system was evacuated to 0.08 mm and the temperature of the pot was gradually raised until the liquid refluxed from the condenser drip tip (60-70 drops per minute). Following total reflux for three hours, the reflux ratio was set at 75:1 and the distillation was begun. The distillation was followed by periodically removing samples from the distillate drip tip and injecting into an analytical gas chromatograph (5% Carbowax). The results of the distillation are given in Table 2.

TABLE 2

Spinning Band Distillation of Pseudoplexaura porosa Hydrocarbons

Fraction	Wt (g)	Temperatures			Composition (estimated from g.c.)
		Head	Jacket	Pot	
2.1	4.642	46.5°	34°	120°	10%A; 60% B; 30% C.
2.2	1.733	47-48°	34°	124°	trace A; 85% B; 15% C; trace D.
2.3	2.112	48-49°	35°	135°	49% B; 49% C; 2% D.
2.4	3.683	49-49°	35°	140°	15% C; 50% D; 40% E; 10% F.
2.5	37.8	residue			

During the distillation both the viscosity of the pot material and the temperature of the pot significantly increased. Following

removal of fraction 2.4, the distillation was concluded.

Isolation of (+) Calamanene and a Sesquiterpene Dimer. Thin layer chromatography of the receiver residue (fraction 2.5) in hexane displayed a band (rf = 0.5 to 0.6) not observed in the original STHC mixture. NMR of the residue (CCl_4) displayed signals at δ7.0, m, (aromatic protons) not observed in the undistilled STHC mixture.

Chromatography of the fraction 2.5 (5 g) on Florisil (20 g) in hexane yielded in fractions 5-10 (50 ml per fraction; flow rate 3 ml per minute) a sample of hydrocarbon (2.8 g) whose movement on TLC was identical with that shown by the STHC mixture. Following the addition of 10 ml diborane in THF (2.4 molar), the reaction mixture was again chromatographed on Florisil (10 g) with hexane to yield a sample of hydrocarbon (0.835 g) identified as (+) calamanene, n_D^{25} 1.5164, d_4^{25} 0.9309; $[\alpha]_D^{25}$ +66.35 (neat), m.w. 202 (m.s.). Lit[29]: n_D^{28} 1.5155, d_4^{28} 0.9276, $[\alpha]_D^{28}$ +82° (neat).[50] The IR spectrum, which agreed with that cited in the literature for (-) calamanene,[30] showed significant bands at 3030 and 1616 cm^{-1} (aromatic); 1175, 1160, 870, and 805 cm^{-1} (1,2,4 substituted phenyl); 1380 cm^{-1} doublet (gem dimethyl). The nmr spectrum (neat), which agreed with that shown in the literature[30] for (-) calamanene, exhibited signals at δ7.0, m, 3H (aromatic protons); δ2.26, s, 3H (aryl methyl); δ1.24, d, J = 7 Hz, 3H (isopropyl methyl). The mass spectrum, which agree with that shown for (+) calamanene,[31] displayed significant peaks at 202 (molecular ion) and 159 (m - C_3H_7; base peak).

Anal. calcd for $C_{15}H_{22}$: C, 89.03%; H, 10.97%. Found: C, 88.73%; H, 10.70%.

Continued elution with hexane provided, in fractions 12-18 (50 ml per fraction; flow rate 3 ml per minute), a sample of hydrocarbon (2.18 g) which was an extremely viscous colorless liquid after removal of all solvent. The IR spectrum showed significant bands at 1380 cm^{-1} (gem dimethyl); 845 cm^{-1} (trisubstituted double bonds). The nmr spectrum (CCl_4) displayed signals at δ5.38, m, 2H (vinyl protons); δ1.68, s, 6H (vinyl methyl); δ1.13, s, 6H (quaternary methyl); δ0.91, d, J = 7 Hz, 6H (isopropyl methyls); δ0.84, d, J = 7 Hz, 6H (isopropyl methyls). Significant in the mass spectrum were peaks of m/e = 410 (parent ion), 367, and 205 (base peak).

Calcd for $C_{30}H_{50}$: C, 87.73%; H, 12.29%. Found: C, 88.02%; H, 12.00%.

Examination of Pseudoplexaura porosa for (+) Calamanene. Unextracted Pseudoplexaura porosa (300 g) was ground in a Waring blender and extracted with hexane (four washings; 300 ml each). The extract was concentrated on a rotary evaporator and the residue was chromatographed on Florisil (200 g) with hexane. The hexane eluent was evaporated on a rotary evaporator under reduced pressure. Diborane in THF (2.4 molar; 10 ml) was added to the residue and the reaction mixture was chromatographed on Florisil (200 g) with hexane. Removal of the hexane solvent under reduced pressure on a rotary evaporator left no apparent residue. The boiling flask was washed with spectral grade hexane (3 ml) and examined on a UV spectrophotometer (Beckman DK 1). The sample failed to exhibit any bands reported in the literature[31] for (-) calamanene.

Annular Still Distillation of the STHC Mixture (Fraction 1.1).

The receiver of a teflon annular still (Nester-Faust, Model NFT-60; 127 theoretical plates) was charged with the STHC mixture (55 g) and the system was evacuated to 0.07 mm. The pot, jacket, and heat temperature controls were adjusted until, with the top of the column 2° lower than the head temperature, any further reduction of the head temperature resulted in loss of condensation of liquid at the condenser drip tip (10 drops per minute). The system was equilibrated at total reflux for five hours before setting the reflux ratio at 100 to 1 and the reflux valve throw to 1 mm. As the distillation progressed, pot, jacket, and head controls were adjusted to maintain the conditions described above. Samples were removed from the drip tip at 20-minute intervals and were injected into an analytical gas chromatograph (5% Carbowax) to determine their composition. Results of the distillation are given below in Table 3.

TABLE 3

Annular Still Distillation of *Pseudoplexaura porosa* Hydrocarbons

Fraction	Wt	Head Temp	Pressure	Composition
3.1	1.157	63°	0.70 mm	100% A
3.2	0.920	63°-64°	0.70 mm	25% A; 75% B
3.3	3.548	64°	0.70 mm	85% B; 15% C
3.4	0.235	64°-65°	0.70 mm	60% B; 40% C
3.5	0.179	65°	0.70 mm	50% B; 50% C
3.6	0.929	65°	0.70 mm	25% B; 40% C; 35% D
		Distillation interrupted; System Re-equilibrated		
3.7	0.455	62°-63°	0.50 mm	40% C; 60% D
3.8	9.838	63°-64°	0.50 mm	10% C; 80% D; 10% E
3.9	3.218	64°-65°	0.50 mm	70% D; 25% E
3.10	3.210	65°	0.50 mm	40% D; 60% E
3.11	11.716	65°-66°	0.50 mm	13% E; 60% F; 15% G; 6% H; 6% I
3.12	17.025	pot residue		

Properties of (+)-α-Cubebene. Gas chromatography (5% Carbowax and 5% LAC-1-R-296) indicated fraction 3.1 (1.157 g) to be a single component (Component A; Fig. 1) identified as (+)-α-cubebene, n_D^{25} 1.4792, d_4^{25} 0.8731, $[\alpha]_D^{25}$ +24.06 (neat). Lit[32]: $[\alpha]_D^{30}$ -20.0 (0.825 c; CCl_4). The IR spectrum (neat), which agreed with that cited in the literature,[32] exhibited significant bands at 1640, 820, and 770 cm^{-1} (cyclic tri-

substituted conjugated olefin), 3040 cm^{-1} (olefin), 1445 cm^{-1} (cyclopentane methylene), 1380 doublet, 1170, and 1145 cm^{-1} (isopropyl methyls). The nmr spectrum which was identical with the spectrum of authentic (-)-α-cubebene provided by Dr. Yashimoto Ohta, displayed signals at δ4.85, m, 1H (trisubstituted olefin); δ2.58, dt, $J_{AA'}$ = 17 hz, J_{AB} = 2 Hz, 1H and 2.10, dq, $J_{AA;}$ = 17 Hz, $J_{AB} = J_{AC}$ = 2 Hz, 1H (diallylic methylene); δ1.74, t, J = 2 Hz, 3H (vinyl methyl); δ0.95, d, J = 7 Hz, 6H (isopropyl methyl); δ0.91, d, J = 7 Hz, 3H (secondary methyl); 0.24, m, 1H (cyclopropane proton). The mass spectrum displayed significant peaks at m/e = 204 (molecular ion), 161 (base peak), 119, and 105.

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.26%; H, 11.73%.

NMR Decoupling of (+)-α-Cubebene. The vinyl hydrogen, appearing as a multiplet at δ4.85, was shown to be coupled with both diallylic protons appearing at δ2.58 and δ2.10, and with the vinyl methyl appearing at δ1.74. Irradiation at δ5.19 caused the doubled triplet centered around δ2.58 to collapse to a doubled doublet, the doubled quartet centered around δ2.10 to collapse to a doubled triplet, and the triplet at δ1.74 to collapse to a crude singlet. The diallylic proton centered around δ2.58 was shown to be coupled with the diallylic proton centered around δ2.10. Irradiation at δ2.58 caused the doubled quartet centered around δ2.10 to collapse to a crude quartet. Irradiation at δ2.10 caused the doubled triplet centered around δ2.58 to collapse to a crude triplet.

Hydrogenation of (+)-α-Cubebene. (+)-α-Cubebene (292 mg; 1.43 mmoles) was hydrogenated at atmospheric pressure in anhydrous ethanol

(20 ml) using 5% palladium on carbon (30 mg). The sample took up 1.47 mmoles (1.02 equivalents). After filtering the mixture, the solvent was removed on a rotary evaporator under vacuum to yield dihydro-(+)-α-cubebene, n_D^{25} 1.4689. Significant in the nmr was a signal displayed at 0.59, m, 2H (cyclopropane hydrogens).

Anal. calcd for $C_{15}H_{26}$: C, 87.30%; H, 12.69%. Found: C, 87.30%; H, 12.93%.

Osmium Tetroxide Oxidation of (+)-α-Cubebene. The procedure followed was that of Büchi.[53] (+)-α-Cubebene (408 mg; 2 mmoles) was dissolved in anhydrous pyridine (1 ml) contained in a 100 ml boiling flask wrapped with foil to exclude sunlight and cooled to 0° in an ice bath. Osmium tetroxide (500 mg; 2.9 mmoles), dissolved in anhydrous pyridine (5 ml) cooled to 0°, was added dropwise to the olefin solution. The reaction mixture was allowed to stand at room temperature for forty-eight hours and then was refluxed for four hours with a mixture of mannitol (3.5 g), potassium hydroxide (3.5 g), water, benzene, and ethanol (10 ml each). Following the addition of water (100 ml), the solution was extracted with ether (three washings; 75 ml each). The ethereal solution was washed with 6 N HCl to remove pyridine (three washings, 20 ml each) and the ether phase was dried over anhydrous sodium sulfate. Removal of the solvent on a rotary evaporator under reduced pressure yielded a crystalline solid (395 mg; 1.66 mmoles) which showed one component on TLC (R_f = 0.20 in 20% ethyl acetate in benzene), gave a positive diol test, and was designated (+)-α-cubebediol, m.p. 67-68°, $[\alpha]_D^{25}$ +4.24° (c = 10.15; $CHCl_3$). The IR spectrum ($CHCl_3$) exhibited significant bands at 3600 to 3500 cm^{-1} (polyhydroxyl alcohol),

1075 cm^{-1} (secondary alcohol with α-branching). The nmr spectrum ($CDCl_3$) displayed signals at 3.38, t, J = 8 Hz, 1H (hydrogen on carbon bearing oxygen); δ2.84, broad m, 2H (proton on oxygen) δ1.82, d, J = 8 Hz, 2H (methylene adjacent cyclopropane); δ1.28, s, 3H (quaternary methyl); δ1.00, d, J = 7 Hz, 3H and δ0.91, d, J = 7 Hz, 3H (isopropyl methyls); δ0.43, m, 2H (cyclopropane protons).

Anal. calcd for $C_{15}H_{26}O_2$: C, 75.58%; H, 10.99%; O, 13.42%. Found: C, 75.41%; H, 10.74%; O, 13.42%.

Oxidation of (+)-α-Cubebediol with Sodium Metaperiodate. (+)-α-Cubebediol (395 mg; 1.66 mmoles) was dissolved in 50% aqueous methanol (50 ml). Excess sodium metaperiodate (845 mg; 3.95 mmoles) was added and the reaction mixture was stirred for one and one-half hours, during which time the solution became cloudy as sodium iodate formed and precipitated. The reaction mixture was extracted with hexane (three washings; 100 ml each) and the organic phase was dried over anhydrous sodium sulfate. Removal of solvent on a rotary evaporator under vacuum left the ketoaldehyde as a clear colorless liquid. The IR spectrum (film) exhibited significant bands at 2820, 2720, and 1725 cm^{-1} (aldehyde), 1685 cm^{-1} (conjugated ketone), 1170 cm^{-1} (ketone), 1380 cm^{-1} (gem dimethyl). The nmr spectrum ($CDCl_3$) displayed signals at δ9.54, t, J = 1.5 Hz, 1H (aldehyde proton); δ2.58, d, J = 1.5 Hz, 2H (methylene protons adjacent to aldehyde); δ2.26, s, 3H (methyl ketone); δ1.91, d, J = 5.5 Hz, 1H (proton adjacent to ketone); δ0.99, d, J = 4.5 Hz, 3H (secondary methyl); δ0.93, d, J = 5.5 Hz, 6H (isopropyl methyls). M.W. 236 (m.s.).

Anal. calcd for $C_{15}H_{24}O_2$: C, 76.23%; H, 10.23%; O, 13.54%. Found: C, 76.26%; H, 10.32%; O, 13.21%.

Isomerization of Ketoaldehyde on Silicic Acid. A sample of ketoaldehyde (350 mg) was chromatographed on silicic acid (50 g) with 30% ethyl acetate in benzene. Fractions 7-17 (50 ml per fraction; flow rate 100 ml per hour) afforded a sample of solid (182 mg; 52% yield), m.p. 74-76°. The IR spectrum (KBr) showed significant bands at 3600, 1100 cm^{-1} (secondary alcohol), 1715, 1100 cm^{-1} (normal aliphatic ketone), 1650 and 840 cm^{-1} (trisubstituted olefin), 1380 and 1170 cm^{-1} (gem dimethyl). The nmr spectrum ($CDCl_3$) displayed signals δ5.43, d, J = 2 Hz, 1H (allylic proton on carbon bearing oxygen); δ4.95, m, 1H (vinyl proton); δ3.03, dd, J_{AB} = 2 Hz, J_{AC} = 6 Hz, 1H (proton on carbon bearing oxygen); δ2.25, s, 3H (methyl ketone); δ1.12, d, J = 6 Hz, 3H (secondary methyl); δ0.91, d, J = 6.5 Hz, 3H and δ0.78, d, J = 6.5 Hz, 3H (isopropyl methyls). No cyclopropane proton signals were observed.

Anal. calcd for $C_{15}H_{24}O_2$: C, 76.23%; H, 10.25%; O, 13.54%. Found: C, 76.36%; H, 10.32%; O, 13.32%.

Oxidation of Isomerized (+)-α-Cubebeketo-Aldehyde with Chromium Trioxide.[54] Isomerized ketoaldehyde (50 mg; 0.21 mmole) dissolved in anhydrous pyridine (5 ml) was added dropwise to a solution of chromium trioxide (40 mg; 0.4 mmole) in anhydrous pyridine (4 ml) cooled to 0°. The reaction mixture was allowed to stand at room temperature for twelve hours. The reaction mixture was dissolved in ether (200 ml), washed with 6 N aqueous hydrochloric acid (four washings; 20 ml each), and the ethereal solution dried over anhydrous sodium sulfate. Removal of solvent on a rotary evaporator under vacuum afforded a colorless liquid which exhibited significant bands in the IR spectrum ($CHCl_3$)

at 1715 cm^{-1} (ketone), 1615 cm^{-1} (intromolecular H-bonded β-diketone) 1380 cm^{-1} (gem dimethyl). The nmr spectrum ($CDCl_3$) displayed signals at δ5.72, t, J = 1.5 Hz, 1H (vinyl proton to carbonyl); δ3.55, d, J = 3 Hz, 1H (diketone proton); δ2.44, s, 3H (methyl ketone); δ1.00, d, J = 6.5 Hz, 3H (secondary methyl); δ0.86, d, J = 6.5 Hz, 3H and δ0.77, d, J = 6.5 Hz, 3H (isopropyl methyls).

No satisfactory analysis was obtained for the compound.

Hydrogenation of Isomerized Ketoaldehyde. Isomerized ketoaldehyde (50 mg; 0.212 mmole) was hydrogenated at atmospheric pressure in anhydrous ethanol (17 ml) with 5% palladium on carbon (11 mg). The sample took up 0.426 mmole hydrogen (2.04 equivalents). The solvent was removed on a rotary evaporator under reduced pressure to afford a liquid sample which showed significant bands in the IR (film) at 1710 cm^{-1} (ketone), 1380 cm^{-1} doublet (gem dimethyl). The nmr displayed signals at δ2.18, s, 3H (methyl ketone); δ1.08, d, J = 6 Hz, (ring methylenes); δ0.93, d, J = 6 Hz, 3H (secondary methyl); δ0.92, d, J = 6 Hz, 3H (secondary methyl); δ0.70, d, J = 6 Hz, 3H (secondary methyl). Molecular weight determination (m/e = 220 parent ion) indicated a composition of $C_{15}H_{24}O$.

Hydroboration of (+)-α-Cubebene. The procedure followed was according to Brown.[55] To (+)-α-cubebene (256 mg; 1.25 mmoles) dissolved in anhydrous ether (8 ml) cooled to 4° was added 1 M diborane in THF solution (5 ml) and the reaction mixture stirred for 10 hours. Following the addition of ethanol (1 ml) to destroy unreacted diborane, the mixture was made basic with the addition of 3M sodium hydroxide (0.14 ml; 0.42 mmole). A 30% hydrogen perioxide solution (0.15 ml;

1.5 mmoles) was added slowly with a syringe and the mixture was heated for two hours on a steam bath. The solution was diluted with ether (150 ml), washed with water (5 ml per washing) until the pH of the water was less than eight, and then dried over anhydrous sodium sulfate. Removal of the solvent on a rotary evaporator under vacuum afforded a white solid residue. TLC of this residue (20% ethyl acetate in benzene) indicated one trace component (R_f = 0.5), one major (R_f = 0.45) and origin material.

Chromatography of this mixture on silicic acid (50 g) using 5% ethyl acetate in hexane provided in fractions 12-26 (35 ml per fraction; flow rate 70 ml per hour) crystalline material (161.3 mg) which melted at 89-90°, designated α-cubebol. The IR spectrum of this material showed significant bands at 3620 and 1100 (secondary alcohol). The nmr spectrum ($CDCl_3$) displayed signals at δ3.66, q, J = 8Hz, 1H (secondary alcohol); δ2.37, d, J = 20.5 Hz, 1H (allylic proton adjacent carbon bearing oxygen); δ1.75, dd, J_{AB} = 8 Hz, 1H (allylic proton adjacent carbon bearing oxygen); δ1.17, d, J = 6.5 Hz, 3H (secondary methyl); δ0.98, d, J = 6 Hz, 3H (secondary methyl); δ0.93, d, J = 6 Hz, 3H (secondary methyl); δ0.88, d, J = 6 Hz, 3H (secondary methyl); 0.62, m, 1H and δ0.43, t, J = 3 Hz, 1H (cyclopropane protons).

Anal. calcd for $C_{15}H_{26}O$: C, 81.02%; H, 11.76%; O, 7.20%. Found: C, 81.00%; H, 11.75%; O, 7.25%.

Chromium Trioxide Oxidation of (+)-α-Cubebol.[54] (+)-α-cubebol (161 mg; 0.73 mmole) dissolved in anhydrous pyridine (2 ml) was added dropwise to a stirred solution of chromium trioxide (77 mg; 0.77 mmole) in pyridine (4 ml) cooled to 4°. The reaction proceeded at 4° for four-

teen hours before isopropyl alcohol (0.25 ml) was added to reduce unreacted chromium trioxide. The mixture was added to 5% aqueous sulfuric acid (50 ml) and extracted with ether (three washings; 50 ml each). Following drying over anhydrous sodium sulfate, removal of solvent on a rotary evaporator under vacuum afforded a colorless liquid (141 mg; 0.64 mmole) designated α-cubebeone. The IR spectrum showed significant bands at 1745 cm^{-1} (cyclopentanone), 1380 doublet, 1170 and 1145 cm^{-1} (isopropyl methyls). The nmr spectrum ($CDCl_3$) displayed signals at δ2.26, dd, J_{AB} = 19 Hz, J_{AC} = 2 Hz, 1H (allylic proton adjacent to carbonyl); δ2.04; d, J = 19 Hz, 1H (allylic proton adjacent carbonyl); δ1.08, d, J = 7 Hz, 3H (secondary methyl); δ1.01, d, J = 6.5 Hz, 3H (secondary methyl); δ0.93, d, J = 6.5 Hz, 3H (secondary methyl); δ0.88, d, J = 6.5 Hz, 3H (secondary methyl); δ0.72, t, J = 2 Hz, 1H (cyclopropane proton); δ0.19, m, 1H (cyclopropane proton).

Anal. calcd for $C_{15}H_{24}O$: C, 81.76%; H, 10.98%; O, 7.26%. Found: C, 81.94%; H, 10.62%; O, 7.43%.

Isolation of (+)-β-Copaene. Preparative gas chromatography of fraction 3.8 (2.5 g) on a 20' x 3/8" 20% Carbowax on Chromsorb P column and collection of the center of the component D (Fig. 17) peak provided a sample of hydrocarbon (1.8 g) identified as the compound initially reported by Washecheck as (+)-β-ylangene but now recognized as β-copaene, n_D^{25} 1.4988, d_4^{25} 0.9220, $[\alpha]_D^{25}$ +9.17 (neat). Lit[12]: n_D^{25} 1.4983, d_4^{25} 0.9205, $[\alpha]_D^{25}$ +10.6 (neat). The IR spectrum (film), which was identical with that shown by Washecheck[12] for β-ylangene (= β-copaene) showed significant bands at 3040, 1643, and 872 cm^{-1} (terminal methylene), 1380 doublet, 1170 and 1145 cm^{-1} (isopropyl methyls). The

nmr spectrum (neat), which was identical with that shown by Washecheck,[12] exhibited signals at δ4.58, m, 2H (terminal methylene); δ0.85, d, J = 5.5 Hz, 6H (isopropyl methyls); δ0.68, s, 3H (quarternary methyl). The mass spectrum displayed significant peaks at m/e 204 (parent ion), and 161 (base peak; m - C_3H_7).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.84%. Found: C, 88.18%; H, 11.88%.

Isomerization of β-Copaene to α-Muurolene and δ-Cadinene. The procedure used was a modification of that reported by Y. Ohta[34] for the isomerization of α-copaene. β-Copaene (725 mg) was added to a solution of dioxane (9 ml), water (1 ml) and sulfuric acid (20 mg) and the solution was refluxed for one hour in a nitrogen atmosphere. The reaction mixture was diluted with hexane (200 ml) and washed with saturated sodium bicarbonate solution (three washings; 50 ml each) and the organic phase was dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator under reduced pressure and a sample of the colorless liquid residue was injected into a gas chromatograph. Retention times indicated that the β-copaene had isomerized completely to two compounds with retention times identical to those of components F and G (Fig. 17).

Preparative gas chromatography of the mixture on a 20' x 3/8" 20% Carbowax on Chromsorb P column (gas flow 120 ml per minute; column temperature 160°) gave in 52 to 58 minutes a sample of hydrocarbon identified as (-)-α-cadinene, $[\alpha]_D^{25}$ -80° (Lit[49]: +94.3) and in 61 to 66 minutes a sample of hydrocarbon identified as (+)-α-muurolene, $[\alpha]_D^{25}$ +58.2° (Lit[14]: -66°). The IR and nmr spectrum were identical with those cited below for (-)-α-cadinene and (+)-α-muurolene.

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found for δ-cadinene: C, 88.29%; H, 11.71%. Found for α-muurolene: C, 88.32%; H, 11.67%.

Isolation of (+)-β-Epibourbonene. Chromatography of distillation fraction 3.3 (3.55 g) on 25% silver-nitrate-impregnated Florisil (178 g) with 4% benzene in dry hexane provided in fractions 8-26 (50 ml per fraction; flow rate 150 ml perminute) a sample of a hydrocarbon mixture designated fraction 3.1.1 (2.114 g) whose retention time on 5% Carbowax was identical with component B (Fig. 17). The nmr spectrum (CCl_4) displayed vinyl proton signals at δ5.22, δ5.03, and δ4.72 in the ratio 15:1:1.

Continued elution with benzene provided in fractions 30-40 a sample of hydrocarbon (0.730 g) which gas chromatography (5% Carbowax and LAC-1-R-296) and silver nitrate TLC indicated was a single component. Retention time on gas chromatography identified this compound, designated (+)-β-epibourbonene, as compound C (Fig. 17), n_D^{25} 1.4876, d_4^{25} 0.9069, $[\alpha]_D^{25}$ +68.27° (neat), M.W. 204 (m.s.). Lit[36,37] for β-bourbonene: n_D^{22} 1.4896, d_4^{20} 0.8948, $[\alpha]_D^{25}$ -92.12°. The IR spectrum, which agreed with that cited in the literature for β-bourbonene,[36] exhibited significant bands at 3090, 1665, and 885 cm^{-1} (terminal methylene), 1380 doublet, 1170, and 1145 cm^{-1} (isopropyl methyls). The nmr spectrum, which also closely resembled that cited for β-bourbonene,[36] exhibited signals at δ4.67, broad s, 2H (exomethylene); δ2.38, broad s, 2H (cyclobutane protons); δ2.28, d, J = 1.5 Hz, 1H (cyclobutane proton); δ1.3 to 1.8, m, 8H (allylic and pseudoallylic protons); δ0.95, s, 3H (quaternary methyl); δ0.87, d, J = 4.5 Hz, 6H (isopropyl methyls).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.21%; H, 11.89%.

Osmium Tetroxide Oxidation of (+)-β-Epibourbonene. The procedure followed was that of Büchi.[53] (+)-β-Epibourbonene (512 mg; 2.5 mmoles) was dissolved in anhydrous pyridine (2 ml) contained in a 100 ml boiling flask wrapped in foil to exclude sunlight and cooled to 0° in an ice bath. Osmium tetroxide (500 mg; 2.9 mmoles), dissolved in anhydrous pyridine (5 ml) cooled to 0°, was added dropwise to the olefin solution. The reaction mixture was allowed to stand at room temperature for forty-eight hours and then was refluxed for five hours with a mixture of mannitol (3.5 g), potassium hydroxide (3.5 g), water, ethanol, and benzene (10 ml each). Following the addition of water (100 ml), the solution was extracted with ether (three washings; 100 ml each). The ethereal solution was washed with 5% sulfuric acid to remove pyridine (four washings; 20 ml each) and the ethereal phase was dried over anhydrous sodium sulfate. Removal of the solvent on a rotary evaporator under reduced pressure yielded a crystalline solid (575 mg; 2.42 mmoles) which showed one component (R_f = 0.18 with 20% ethylacetate in benzene) and gave a positive diol test. The compound was designated (+)-β-epibourbonene-diol, m.p. 66-68°, $[\alpha]_D^{25}$ +6.83 (10.7 c; $CHCl_3$). The IR spectrum (KBr) exhibited significant bands at 3400 to 3200 cm^{-1} (polyvalent alcohol). The nmr spectrum ($CDCl_3$) displayed signals at δ3.65, s, 2H (-C-CH_2-O-; 2 -OH)*, δ0.92, s, 3H (quaternary methyl); δ0.85, d,

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*The concurrence of both -C-CH_2-O- and -OH signals as a single peak was demonstrated in two ways: Addition of D_2O caused the integral of the δ3.65 peak to decrease by one-half. Cooling of the original sample caused the δ3.65 peak to appear as two singlets, δ3.65 and δ3.60.

J = 4 Hz, 6H (isopropyl methyls).

Anal. calcd for $C_{15}H_{26}O_2$: C, 75,58%; H, 10.99%; O, 13.42%. Found: C, 75.52%; H, 11.10%; O, 13.38%.

Oxidation of (+)-β-Epibourbodiol with Sodium Metaperiodate. (+)-β-Epibourbodiol (200 mg; 0.84 mmole) was dissolved in 50% aqueous methanol (25 ml). Sodium metaperiodate (210 mg; 0.98 mmole) was added and the reaction mixture was stirred for two hours, during which time the solution became cloudy as sodium iodate precipitated. The reaction mixture was extracted with ether (three washings; 75 ml each) and the combined organic phase dried over anhydrous sodium sulfate. Removal of the solvent on a rotary evaporator under vacuum left a clear liquid residue, $[\alpha]_D^{25}$ +19.2° (c = 7.5; $CHCl_3$) which TLC indicated contained one component (R_f = 0.30 in benzene). Physical properties of authentic bourbonone were not available for comparison. The IR spectrum (film), which agreed with that cited for bourbonone,[37] exhibited significant bands at 1735 cm^{-1} (cyclopentanone conjugated with cyclobutane ring), 1380 doublet, 1170 and 1145 cm^{-1} (isopropyl methyls). The nmr spectrum ($CDCl_3$), which agreed with that cited for bourbonone, displayed signals at δ0.87, d, J = 5 Hz, 6H (isopropyl methyls); δ1.14, s, 3H (quaternary methyl); from δ1.5 to δ2.80, m, 13H (cyclic methylene and methine protons).

Anal. calcd for $C_{14}H_{22}O$: C, 81.56%; H, 10.68%; O, 7.76%. Found: C, 81.89%; H, 10.75%; O, 7.36%.

Isolation of (+)-α-Copaene. Chromatography of fraction 3.1.1 from above on silver-nitrate-impregnated silicic acid (20 gm) with 1% benzene in dry hexane provided in fractions 6-12 (10 ml per fraction;

100 ml per minute) a sample of hydrocarbon (1.42 gm) shown by gas chromatography (Carbowax) to contain one component identified as (+)-α-copaene, n_D^{25} 1.4899, d_4^{25} 0.9061, $[\alpha]_D^{25}$ +6.93°. Lit[30,39,40]: n_D^{26} 1.4885, d_4^{26} 0.9055, $[\alpha]_D^{30}$ -6.53°. The IR spectrum which agreed with that cited[30] for (-)-α-copaene exhibited significant bands at 3030, 1670, 850, and 780 cm^{-1} (trisubstituted cyclic olefin), 1380 doublet, 1170 and 1145 cm^{-1} (isopropyl methyls) and 1390 singlet (quaternary methyl). The nmr spectrum, which agreed with that shown[30] for (-)-α-copaene, displayed signals at δ5.18, m, 1H (vinyl proton); δ2.18, m, 3H (bridgehead protons); δ1.63, m, 7H (vinyl methyl, allylic protons); δ0.83, d, J = 5 Hz, 6H (isopropyl methyls); δ0.74, s, 3H (quaternary methyl). Significant in the mass spectrum were peaks of m/e 204 (molecular ion) and 161 (base peak; m - C_3H_7).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.84%. Found: C, 88.37%; H, 11.89%.

Continued elution provided in fractions 20-25 a sample of hydrocarbons (0.582 gm) which silver nitrate TLC indicated was a mixture of two components. Significant in the IR of the mixture was a band at 860 cm^{-1} (conjugated exomethylene) and 1390 singlet (quaternary methyl). The nmr spectrum (neat) displayed signals at δ4.90 to δ5.10, m, (vinyl protons); δ1.65, m, (vinyl methyl); δ0.95, s, (quaternary methyl); δ0.75, d, (isopropyl methyls).

Isolation of Component E (Fig. 17). Distillation fraction 3.9 (2.0 g) was chromatographed on 20% silver-nitrate-impregnated silicic (90 g) starting with 2% dry benzene in hexane. Fractions 4-10 (50 ml per fraction; flow rate 100 ml per hour) contained trace amounts of

components F and G (Fig. 17).

Continued elution with 6% dry benzene in hexane provided in fractions 11-15 a sample of hydrocarbon (1.09 g) previously identified as β-copaene.

Following fraction 19, the eluent was changed to dry benzene; and in fractions 22-28, a sample of hydrocarbon (0.893 g) was obtained which silver nitrate TLC and gas chromatography (Carbowax) indicated contained one component identified as component E (Fig. 17), n_D^{25} 1.5002, d_4^{25} 0.9255, $[\alpha]_D^{25}$ +36.40° (neat). The IR spectrum (film) exhibited significant bands at 3090, 1645, and 885 cm^{-1} (terminal methylene), 1380 singlet, 1215, and 1195 cm^{-1} (gem dimethyl on quaternary carbon). The nmr spectrum (neat) displayed signals at δ4.72, s, 2H (terminal methylene); δ1.04, s, 3H (quaternary methyl); δ0.97, s, 3H (quaternary methyl); δ0.96, d, J = 7 Hz, 3H (secondary methyl); δ0.50 to 0.10, m, 2H (cyclopropane protons). The UV spectrum (hexane) exhibited no absorption peaks above 200 mμ. Significant in the mass spectrum were peaks at m/e 204 (molecular ion) and 161 (base peak; m - C_3H_7).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 87.77%; H, 11.87%.

Isolation of (+)-α-Muurolene and (-)-δ-Cadinene. Chromatography of distillation fraction 3.11 (5 g) on 25% silver-nitrate-impregnated silicic acid (500 g) starting with 2% dry benzene in hexane provided in fractions 22-27 (50 ml per fraction; flow rate 100 ml per hour) a sample of hydrocarbon (531 mg which was 85% (+) calamamene (by g.c.) previously identified.

Continued elution provided in fractions 30-44 a sample of

hydrocarbon (2.073 g) which was a mixture of components F and G. The relative concentrations of the two components varied from fraction 30 through 44; however, no distinct separation was obtained. All fractions containing only components F and G were combined.

Continued elution afforded a mixture of components F and G with small amounts of components C, D, and E (Fig. 17).

Preparative gas chromatography on a 20' x 3/8" LAC-1-R-296 on Chromsorb P (60/80 mesh) column provided samples which were approximately 95% (analytical g.c.) either component F or component G. Rechromatography of the sample which was 95% F, collecting only the leading half of the effluent, afforded a sample of hydrocarbon identified as (+)-α-muurolene, n_D^{25} 1.5052, d_4^{25} 0.9211, $[\alpha]_D^{25}$ +67.26° (neat), m.w. 204 (m.s.). Lit[14]: n_D^{20} 1.5074, d_4^{20} 0.9190, $[\alpha]_D^{20}$ -66.0° (neat). The IR spectrum (film), which agreed with that shown in the literature[14] for (-)-α-muurolene, showed significant bands at 1680, 880, 845, 827, and 800 cm^{-1} (trisubstituted double bonds), a doublet at 1380, 1170, and 1145 cm^{-1} (isopropyl methyls). The nmr spectrum (neat) exhibited signals at δ5.36, broad m, 2H (vinyl protons); δ1.88, broad s, 2H (allylic methine protons); δ1.67, s, 6H (vinyl methyls); δ0.88, d, J = 7 Hz, 3H and δ0.82, d, J = 7 Hz, 3H (isopropyl methyls).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.23%; H, 11.73%.

Repeated preparative gas chromatography of the fraction 95% component G, collecting only the latter half of the effluent, provided a sample of hydrocarbon identified as (-)-δ-cadinene, n_D^{25} 1.5070, d_4^{25} 0.9170, $[\alpha]_D^{25}$ -90.5° (neat). Lit[47-49]: n_D^{20} 1.5083, d_4^{25} 0.9217, $[\alpha]_D^{20}$

+94°. The IR spectrum (film), which agreed with that shown in the literature for (+)-δ-cadinene, showed significant bands at 1650 and 835 cm^{-1} (trisubstituted olefin), 1380 doublet, 1170, and 1145 cm^{-1} (isopropyl methyls). The nmr spectrum (neat) displayed signals at δ5.37, m, 1H (vinyl proton); δ2.60, m, 1H (diallylic proton); δ1.91, broad s, 6H (allylic protons); δ1.62, s, 6H (vinyl methyls); δ0.96, d, J = 7 Hz, 3H and δ0.82, d, J = 7 Hz, 3H (isopropyl methyls). Significant in the mass spectrum were peaks at m/e 204 (molecular ion) and 161 (base peak; m - C_3H_7).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.37%; H, 11.73%.

C. Eunicea palmeri

Isolation of the Sesquiterpene Hydrocarbon (STHC) Fraction. Air dried Eunicea palmeri (4.3 kg) collected at Miami, Florida, in June 1967 was batch leached twice with hexane (2 liters per kg weight; then 1 liter per kg) and the extract was concentrated to one liter. Following refrigeration for two weeks, the solution was filtered (Whatman No. 1) and the solvent was removed on a rotary evaporator under vacuum to yield a black tarry reside (356 g; 8.3%). Distillation of this residue through a falling film molecular still (Nester-Faust) at 70°/0.005 mm; 85°/0.05 mm; 100°/0.05 mm; and 120°/0.05 mm yielded respectively 11.0 g; 25.3 g; 40.5 g; and 56.4 g. TLC in hexane indicated that only the 70° and 85° fractions contained components having an R_f greater than 0.8. Chromatography of the combined 70° and 85° fractions (36.3 g) on Florisil (180 g) with hexane yielded 5.17 g of colorless hydrocarbons (0.12% total weight).

Isolation of (+)-β-Copaene. Chromatography of the hydrocarbon fraction from Eunicea palmeri (2.71 g) on silver-nitrate-impregnated silicic acid (500 g) starting with 10% benzene in hexane provided in fractions 19-22 (50 ml per fraction; flow rate 100 ml per hour) a sample of hydrocarbon (0.348 g) which was approximately 90% one component (g.c.).

Continued elution provided in fractions 31-40 a sample of hydrocarbon (290 mg) which silver nitrate TLC and gas chromatography (30% SE-30) indicated was a single component, n_D^{25} 1.4986, $[\alpha]_D^{25}$ +9.60, M.W. 204 (m.s.) whose physical properties were in close agreement with those determined above for (+)-β-copaene, n_D^{25} 1.4983, $[\alpha]_D^{25}$ +9.17 (neat). The IR spectrum (film), which was identical with that obtained for (+)-β-copaene from Pseudoplexaura porosa, significant bands at 3040, 1643, and 872 cm^{-1} (terminal methylene), 1380 doublet, 1170 and 1145 cm^{-1} (isopropyl methyls). The nmr spectrum (neat) was identical with that found above for (+)-β-copaene, displaying signals at δ4.58, m, 2H (terminal methylene); δ0.85, d, J = 5.5 Hz, 6H (isopropyl methyls); δ0.68, s, 3H (quaternary methyl), M.W. 204 (m.s.).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.18%; H, 11.88%.

Isolation of (+)-α-Muurolene. Re-chromatography of fractions 19-22 from above (348 mg) on silver-nitrate-impregnated silicic acid (25 g) starting with 4% benzene in hexane yielded in fractions 8-15 (50 ml per fraction; 150 ml per hour) a sample of hydrocarbon identified as (+)-α-muurolene, n_D^{25} 1.5055, d_4^{25} 0.8678, $[\alpha]_D^{25}$ +63.06° (neat), M.W. 204 (m.s.). Lit[14]: n_D^{20} 1.5074, d_4^{20} 0.9190, $[\alpha]_D^{20}$ -66.60° (neat). The IR spectrum was identical with that obtained above for (+)-α-muurolene

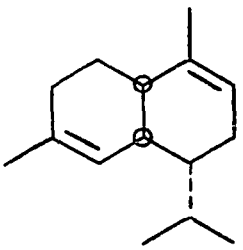
and showed significant bands at 1680, 880, 845, 827, and 800 cm^{-1} (trisubstituted olefins), a doublet at 1380, 1170, and 1145 cm^{-1} (isopropyl methyls). The nmr (neat) was identical with that of (+)-α-muurolene obtained above displaying signals at δ5.36, broad m, 2H (vinyl protons); δ1.88, broad s, 2H (allylic methine protons); δ1.67, s, 6H (vinyl methyls); δ0.88, d, J = 7 Hz, 3H and δ0.82, d, J = 7 Hz, 3H (isopropyl methyls).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.28%; H, 11.58%.

SUMMARY

Twelve sesquiterpene hydrocarbons have been isolated from three species of gorgonianas. (+)-α-Muurolene (1) was obtained from Eunicea palmeri, Pseudoplexaura porosa, and Plexaurella dichotoma. (+)-β-Copaene (2) previously reported by Washecheck as (+)-β-ylangene, was reisolated from Pseudoplexaura porosa and its identity was established by acid isomerization and identification of the isomerization products. It (2) was also obtained from Eunicea palmeri. Other components obtained from Plexaurella dichotoma were (+)-β-bisabolene (3), (+)-α-bisabolene (4), (-)-α-curcumene (5), and β-curcumene (6). β-Curcumene was identified by reduction of (-)-α-curcumene and comparison of spectral properties. Other compounds obtained from Pseudoplexaura porosa were (+)-α-cubebene (7), (+)-α-copaene (8), (-)-δ-cadinene (9), and (+) calamanene (10). Calamanene was shown to be an artifact arising from a disproportionation reaction. Two unreported compounds were obtained from Pseudoplexaura porosa. Component C was shown to have a β-bourbonene-like skeleton and probably is a configurational isomer at the isopropyl substitutient, (+)-β-epibourbonene (11). The second unreported compound was shown to have an alloaromadendrene skeleton (12).

All compounds, with exception of the monocyclic hydrocarbons, exhibit the opposite rotation reported in the literature for corresponding compounds isolated from terrestrial sources.



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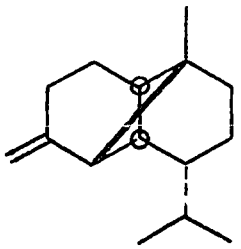

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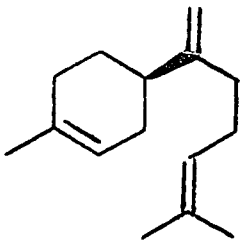

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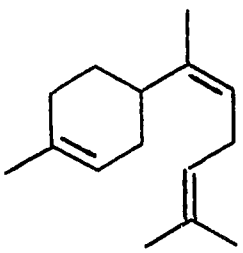

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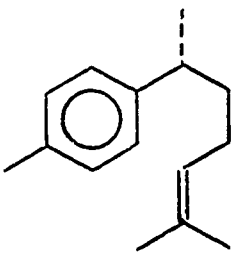

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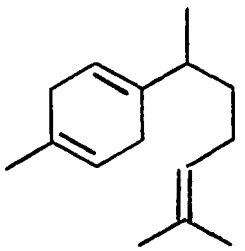

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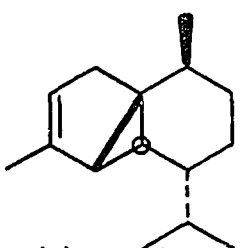

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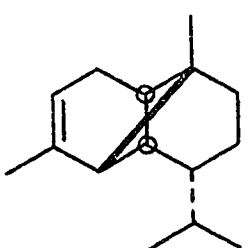

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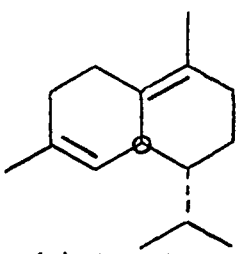

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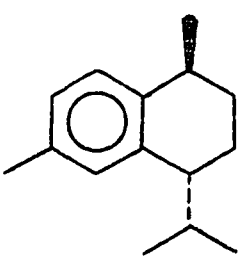

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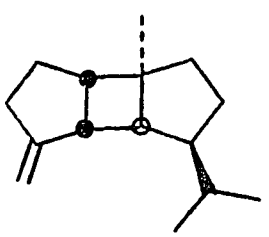
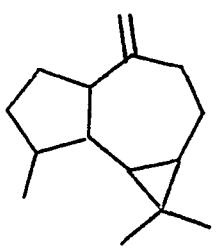


(+) β-Epibourbonene



Alloaromadendrene

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II. ALKYL DISULFIDES FROM PSEUDOPLEXAURA POROSA

INTRODUCTION

The occurrence of either C_{16} or C_{18} alkyl disulfides in living organisms has not been previously reported.

A lipid fraction encountered in residues from anaerobic decomposition of the gorgonian Pseudoplexaura porosa has been shown to contain a mixture of alkyl disulfides.

RESULTS AND DISCUSSION

The disulfide containing fraction was isolated from the anaerobic decomposition residue of Pseudoplexaura porosa by Leon Ciereszko who had previously reported the isolation of dehydrogorgosterol from the same material.[1]

Chromatography of the fraction on SilicAR CC-7 isolated the disulfide mixture. The nmr spectrum displayed signals which were similar to those of long chain fatty alcohols. The methyl region, δ0.98, t, J = 5 Hz, 3H ($-CH_3$) indicated that branching was not present. The IR absorption spectrum failed to exhibit bands characteristic of alcohols, and showed only bands characteristic of carbon-hydrogen bonds.

Mass spectral data on the disulfide (Fig. 39) indicated the material to be a homologous series of three components with parent ions of m/e 514, 542, and 570. These data coupled with chemical analysis indicated that the compounds were disulfides with saturated n-alkyl substituents, with molecular formulas of $C_{32}H_{66}S_2$, $C_{34}H_{70}S_2$, and $C_{36}H_{74}S_2$.

Utilizing mass spectroscopy parent ion data (Table 1) obtained at low voltage (8 eV), the average molecular weight (AvgMW) of the mixture was determined from equation 1:

$$(1)\quad \text{AvgMW} = \frac{\Sigma(m/e)_i(\text{intensity})_i}{\Sigma(\text{intensity})_i}$$

$$= 538.579 \text{ mass units}$$

m/e	0	1	2	3	4	5	6	7	8	9	10	11	12	13	m/e
30										3		33	6	80	30
44	4	2		3						2		57	11	100	44
58	5	2	4	5							6	43	6	58	58
72	4	2	1	1	1	1	1	2	1	10	6	29	6	39	72
86	3	29	3	4	1	1	1	2	1	8	4	19	3	11	86
100	1	15	2	2	1	2	1	1	4	1	7	2	6	1	100
114	6	1	1		1		1		2	1	3	1	4	1	114
128	4	1	8				1		1	1	1	1	2		128
142	2							1		1	1	1	1	2	142
156		2								1	1	1		1	156
170		2												1	170
184		2													184
198		2						3	1				1		198
212		2											1	1	212
226	1	1	1	1											226
240		1		1									1		240
254		6	2	7	2	1			1						254
268									1						268
282		4	1	5	2	2			10	1	1				282
296															296
310									7	2	1				310
324															324
338															338
352															352
366															366
380															380
394															394
408															408
422															422
436															436
450									1						450
464															464
478									4	2	1				478
492									4	2	1				492
506									35	13	6	2			506
520									5	3	1				520
534									53	22	10	3			534
548															548
562									18	8	3	1			562

Figure 39: Mass Spectrum of Pseudoplexaura porosa Disulfides. Relative Intensity at 75 eV versus m/e.

TABLE 1

m/e Versus Relative Intensity

Mass (m)	m	m+1	m+2	m+3
514	34.52	13.27	5.66	1.54
542	52.73	21.91	9.57	2.67
570	18.42	7.77	3.14	0.88

The above calculations assume both linear relationship between parent ion concentration and ion vapor pressure and virtually no rearrangement or decomposition of parent ions. These assumptions are reasonable in this case since, first, the parent ions differ in weight by only 5% and differences of partial vapor pressures would be anticipated to be small; and second, the low energy (8 eV) used to ionize the sample would be expected to bring about little or no "cracking of n-alkyl disulfides.

Since each component contained two sulfur atoms (64.132 mass units) per molecule, the average carbon (AvgC) and average hydrogen (AvgH) composition was determined from the average molecular weight and the general empirical formula for acyclic hydrocarbons (2) as shown be low, (3) - (5):

(2) mole. wt. = C_nH_{2n+2}

(3) $(AvgMW - 64.132) = (12.011)n_{avg} + (1.008)(2n_{avg} + 2)$

(4) $AvgC = (12.011)(n_{avg})$

(5) $AvgH = (1.008)(2n_{avg} + 2)$

Finally, the percent elemental composition is expressed in equation (6):

$$(6)\quad \text{percent composition} = \frac{(\text{wt. element})\ (100)}{\text{AvgMW}}$$

Calculated from mass spectral data: C, 75.53%; H, 12.59%; S, 11.88%. Found by combustion: C, 75.56%; H, 12.61%; S, 11.84%.

Cleavage of the sulfur-sulfur bond with lithium aluminum hydride in ether and pentane provided a sample of liquid which upon mass spectral analysis gave two parent ions of m/e 258 and 286 corresponding to: $C_{16}H_{33}SH$ and $C_{18}H_{37}SH$.

Desulfurization with Raney nickel and alcohol afforded a sample of hydrocarbon which mass spectral analysis indicated contained two parent compounds of m/e 226 and 254 which corresponded to the C_{16} and C_{18} alkyl chains observed in both the parent disulfides and in the corresponding thiols.

Attempts to isolate the disulfide from undecomposed residues from Pseudoplexaura porosa failed to produce the desired material. Since the ratios of R-S-S-R, R-S-S-R', and R'-S-S-R' (R = $C_{16}H_{33}$; R' = ($C_{18}H_{37}$) in the disulfide mixture (Table 1) resemble a statistical distribution for two arguments, R and R', it may be speculated that under the anaerobic decomposition conditions, the corresponding alkyl sulfides acted as reducing agents:

$$\text{R-SH} + \text{R'-SH} \longrightarrow \text{R-S-S-R'} + 2\ e^- + 2\ H^+$$

This speculation is given additional support with the reported isolation of dihydrogogosterol from the same decomposition residue.[1]

$$2\ \text{R-SH} + \text{gogosterol} \longrightarrow \text{R-S-S-R} + \text{dehydrogogosterol}$$

The presence of long chain alkyl sulfides in living organisms has not been reported to date.

EXPERIMENTAL

All melting points are uncorrected. All solvents were redistilled before use. SilicAR CC-7 (100-200 mesh; Mallinckrodt Chemical) was used as preparative chromatographic support. Thin-layer chromatography (TLC) plates were prepared by spreading a slurry of Silica Gel H (30 g) in water (70 ml) onto 5 x 20 mm glass plates. Following evaporation of water at room temperature the plates were activated at 110° for three hours. Lithium aluminum hydride (LAH) was obtained from Metal Hydrides Inc., Beverly, Massachusetts. Raney Nickel No. 28 was obtained from W. R. Grace and Company, Pittsburg, Tennessee.

IR spectra were recorded on a Beckman IR-8 Spectrophotometer. The IR spectra were run as solid solutions in anhydrous potassium bromide, or as thin films between two salt discs.

NMR spectra were taken on a Varian A-60 spectrometer using tetramethylsilane (TMS) as an internal reference. Samples were dissolved in deuterochloroform.

Analyses were carried out by Alfred Bernhardt Laboratories, Mülheim, West Germany. Mass spectra were kindly performed by the Mass Spectroscopy Center, Purdue University, Lafayette, Indiana. Digital data reduction of the mass spectral data was performed at the University of Oklahoma computer center utilizing the IBM 360 system.

The sample of disulfide, isolated from the anaerobic decomposition of the animal Pseudoplexaura porosa, was obtained and kindly donated by Professor Leon S. Ciereszko, the University of Oklahoma.

Isolation of the Pseudoplexaura Porosa Disulfide Fraction.* Freshly obtained Pseudoplexaura porosa were placed in a trough of slowly running sea water and the animals permitted to decompose for three weeks. Following decomposition the residue was collected from the bottom of the tank by centrifugation and dried. Hexane extracts of the residue were chromatographed on a large Florisil column. Removal of hexane from early eluent provided a white solid which contained the disulfide.

Chromatography of the Pseudoplexaura Porosa Disulfide. Thin-layer chromatography (TLC) in hexane of the white solid obtained by Professor Ciereszko indicated one major component (r_f = 0.35) and one trace component (r_f = 0.24) identified by r_f as sterols. Chromatography of the sample (0.520 g) on SilicAR (52 g) in hexane provided in cuts 3-7 (flow rate 8 ml per minute; 40 ml per cut) a sample of white solid (0.436 g) identified by TLC as the major component, m.p. 50-52°. The IR spectra (KBr) showed significant bands at 2925, 2850, 1470 cm^{-1} (aliphatic methylene), and 2960, 1460, and 1380 cm^{-1} (aliphatic methyl). The nmr spectra ($CDCl_3$) displayed signals at δ2.67, t, J = 7 Hz, 2H (methylene under sulfur); δ1.25, s, 14H (aliphatic methylenes); and δ0.89, t, J = 5 Hz, 3H (terminal methyl).

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*The experiment was performed by Leon S. Ciereszko, of the University of Oklahoma, at the Bermuda Biological Station, in August 1963.

Anal. found by combustion: C, 75.56%; H, 12.61%; S, 11.84%.

Cleavage of the Sulfur-sulfur Bond with Lithium Aluminum Hydride.[2] To Pseudoplexaura porosa disulfide (66.7 gm), dissolved in anhydrous ether (2 ml) and pentane (2 ml) in a 25 ml round bottom flask fitted with drying tube and immersed in an ice bath, was added excess LAH and the mixture was allowed to stand overnight. Following addition of 2N HCl (5 ml) the organic layer was separated and the aqueous phase was extracted twice with ether (20 ml each). The combined organic phases were dried over anhydrous sodium sulfate. Removal of solvent on a rotary evaporator under vacuum left a clear colorless liquid (67 mg) having a faint odor of hydrogen sulfide. Mass spectral analysis of this material indicated two parent ions at m/e 258 and 286 consistent with structures $C_{16}H_{33}SH$ and $C_{18}H_{37}SH$.

Desulfurization of Pseudoplexaura porosa Disulfide with Raney Nickel (RANi). The procedure used was that of Gregg, Iddles, and Stearns.[3] To Pseudoplexaura porosa disulfide (22.4 mg) dissolved in absolute alcohol (10 ml) in a 50 ml boiling flask fitted with condenser was added RANi (1.0 g) and the slurry refluxed for eighteen hours. The product was isolated by diluting the reaction slurry with water (5 ml) and extracting with hexane (five washings; 10 ml each). After drying over anhydrous sodium sulfate, the solvent was removed on a rotary evaporator under vacuum to yield a clear colorless liquid (19 mg). Mass spectral analysis indicated that the liquid contained compounds with parent ions of m/e 226 and 254 corresponding to $C_{16}H_{34}$ and $C_{18}H_{36}$.

Attempted Isolation of Pseudoplexaura porosa Disulfide from Molecular Distillation Residues. The residue (100 g) from the falling-

film molecular distillation (Nester-Faust) of undecomposed Pseudoplexaura porosa collected in June 1968 at Bermuda was chromatographed on Florisil (300 g) with hexane. All fractions which showed a TLC r_f greater than 0.24 (sterols) were combined. Chemical analysis showed this material to contain no sulfur.

SUMMARY

A homologous series of n-octadecyl and n-hexadecyl disulfides has been identified in decomposition residues of Pseudoplexaura porosa.. Failure to isolate the disulfide mixture from fresh animal indicates that the disulfide arises in the reductive decomposition of the animal. This is the first report of the occurrence of long chain n-alkyl disulfides in material derived from recent organisms.

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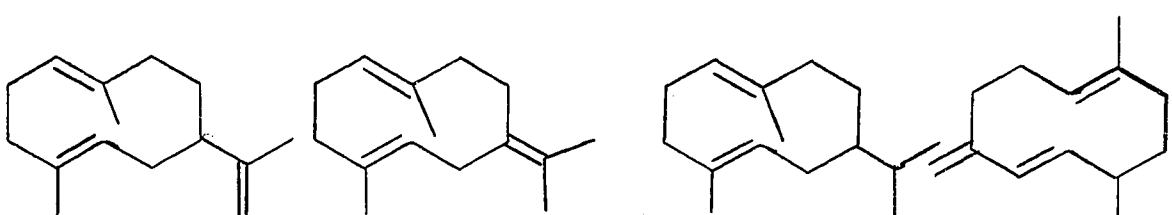
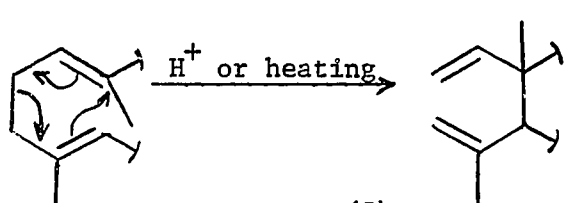
III. GERMACRENE A: AN ELUSIVE SESQUITERPENE HYDROCARBON

INTRODUCTION

The germacrenes, (1) - (4), are a class of isoprenoid monocyclic sesquiterpene hydrocarbons having common structural features of a ten-membered ring-containing two double bonds, an isopropyl or isopropyl-derived substitutent, and two methyl substitutents.[4] Germacrenes A (1), B (2), and C (3) have 1,5-trans-ring olefins which may undergo a Cope Rearrangement (5) to corresponding elemenes. The ease with which this rearrangement may be brought about, either by exposure to acid (silica gel) or by subjecting samples to elevated temperatures, has greatly hindered the isolation of these compounds from natural sources.[1-4,7]

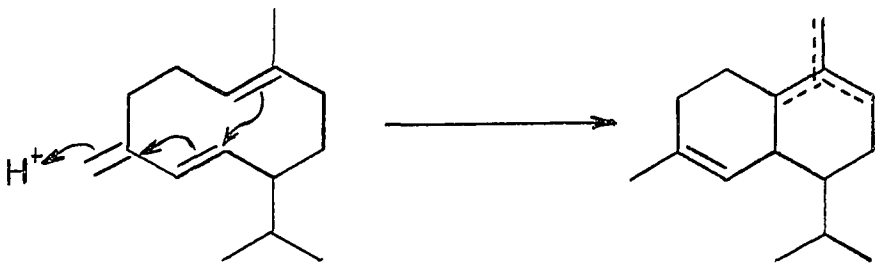


(1) (2) (3) (4)



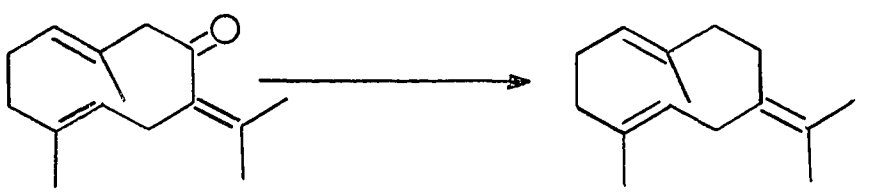
(5)

Germacrene D (4) undergoes a similar rearrangement in the presence of silica gel to yield bicyclics of the muurolene, amorphene, and cadinene classes (6).[5]



(4) (6)

Germacrene B was prepared by Sörm, et. al. from naturally occurring germacrone (7), but has not been isolated from natural sources.[3]



(7) (2)

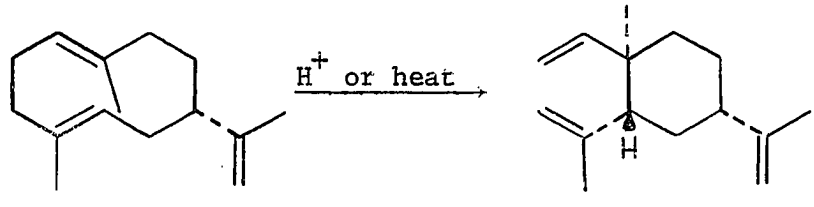
Recently, Japanese workers have reported the isolation of germacrenes C (3) and D (4) from natural sources.[4,5]

Washecheck examined the sesquiterpene hydrocarbon fraction obtained by molecular distillation or residues from Eunicea mammosa and reported the fraction was virtually one component identified as (+)-β-elemene.[6] Reexamination of a sample of hydrocarbons obtained without distillation indicated that (+)-β-elemene represented only approximately forty percent of the total mixture and that a second component was present which apparently isomerized to (+)-β-elemene upon molecular distillation.[7]

In this present work, the sesquiterpene hydrocarbon fraction from Eunicea mammosa has been reexamined and the second component of the mixture isolated and identified as the unreported germacrene A. Spectral data, rotation, and refractive index have been obtained and the thermal and acid isomerization of this compound to (+)-β-elemene verified.

RESULTS AND DISCUSSION

The sesquiterpene hydrocarbon fraction from Eunicea mammosa Lamouroux (0.8% dry weight) was isolated by chromatography (Florisil) of the hexane extracted residue. Gas chromatography of the mixture (5% Carbowax) indicated that the mixture contained two major components (B and D, Fig. 40) and two minor constituents (A and C, Fig. 40). Aliquots of the mixture subjected to high temperature (120°) and to acidic chromatography supports (SilicAR CC-7; pH 7) readily demonstrated the ease with which germacrene A (8) isomerized to (+)-β-elemene (9).

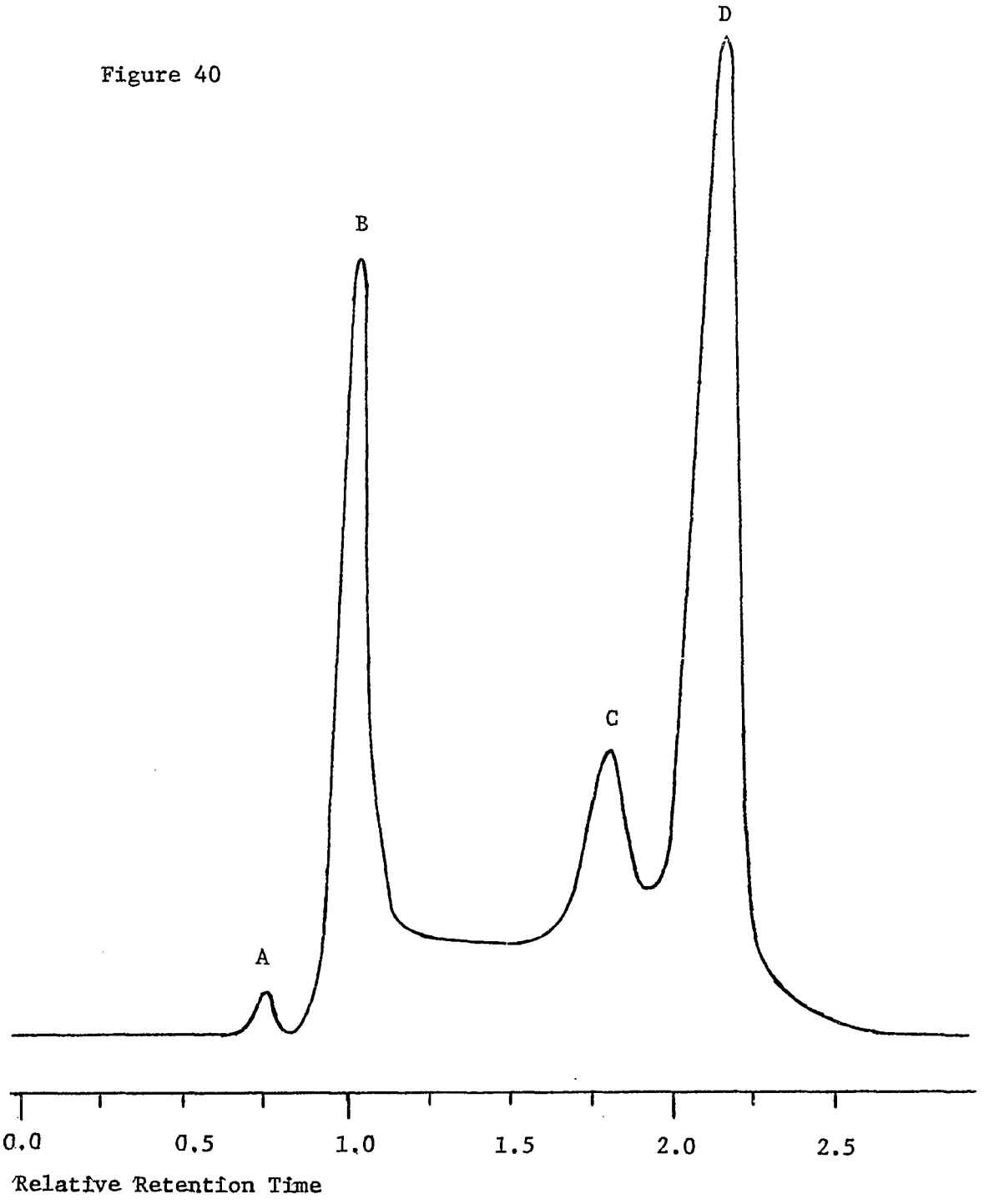


(8) (9)

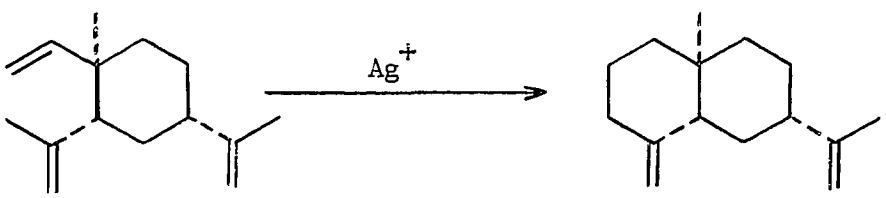
Attempts to isolate germacrene A by preparative gas chromatography (Carbowax) resulted in total thermal isomerization of germacrene A to (+)-β-elemene.

Since it had been demonstrated that acidic supports (silica gel, SilicAR) catalyzed the isomerization of germacrene A, the isolation and purification of germacrene A was attempted on Florisil (basic) impregnated with silver nitrate. Chromatography of the STHC mixture failed to bring about significant isomerization of germacrene A, but quantita-

Figure 40



tively isomerized the slow moving (+)-β-elemene to the faster moving β-selinene (10), which was eluted from the column at a constant rate and prohibited the isolation of pure germacrene A. The silver ion catalyzed isomerization of (+)-β-elemene was observed and discussed by Washecheck.[6]



(9) (10)

Since conventional techniques employed in separating hydrocarbon mixtures had been shown inapplicable for isolation of germacrene A, chromatography of germacrene A was attempted on sugar. A pilot trial run using confectioners sugar mixed with Florisil (Florisil required to avoid tight packing of the sugar) indicated that isolation of germacrene A could be achieved with this combination.

Chromatography on sugar-impregnated Florisil, prepared identically to silver-nitrate-impregnated supports, was attempted using a 120 to 1 ratio of support to STHC material ratio. The results of this chromatography afforded absolutely no separation of the four components. However, the STHC material was recovered intact (G.C.).

A sugar-Florisil adsorbent prepared by blending solid confectioners sugar with Florisil (1 to 2 ratio) in hexane permitted successful isolation of the elusive germacrene A in a pure state. Spectral properties, refractive index, and rotation of the unreported compound were recorded. The IR spectrum (Fig. 44) displayed bands characteristic

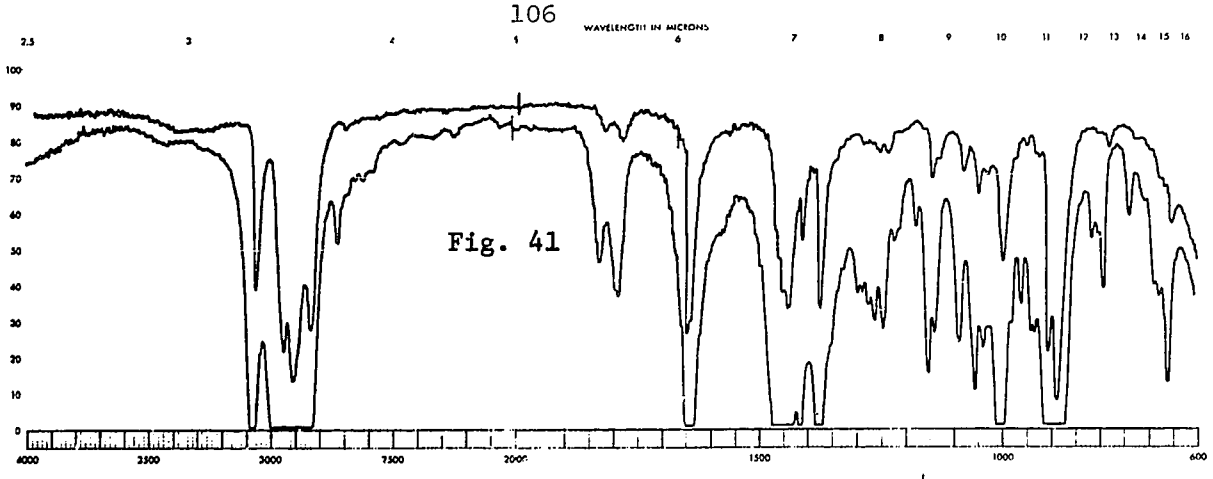
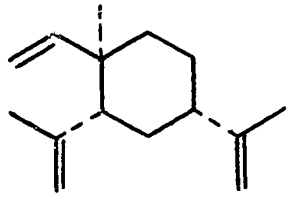
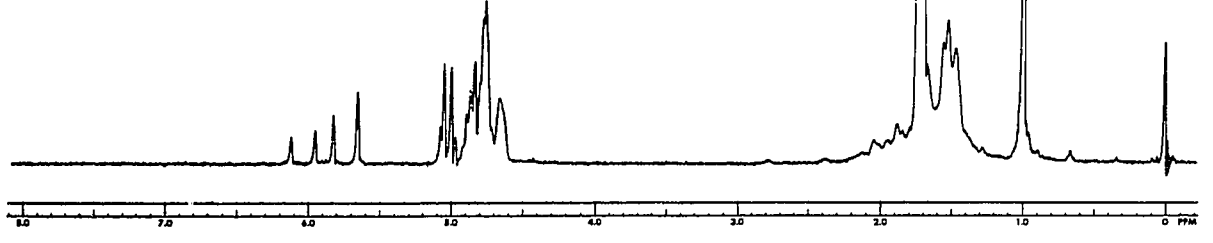


Fig. 41



(+)-β-Elemene

Fig. 42



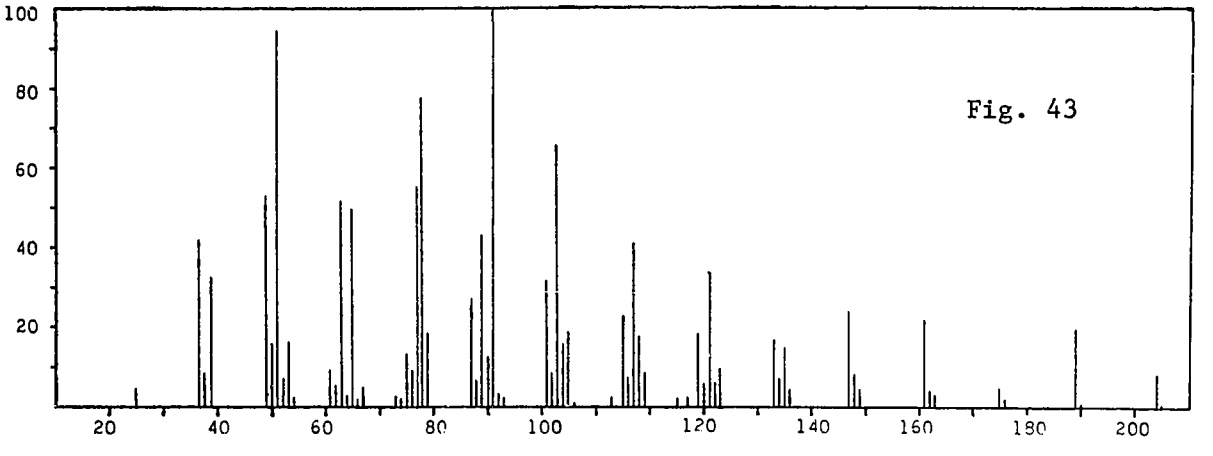


Fig. 43

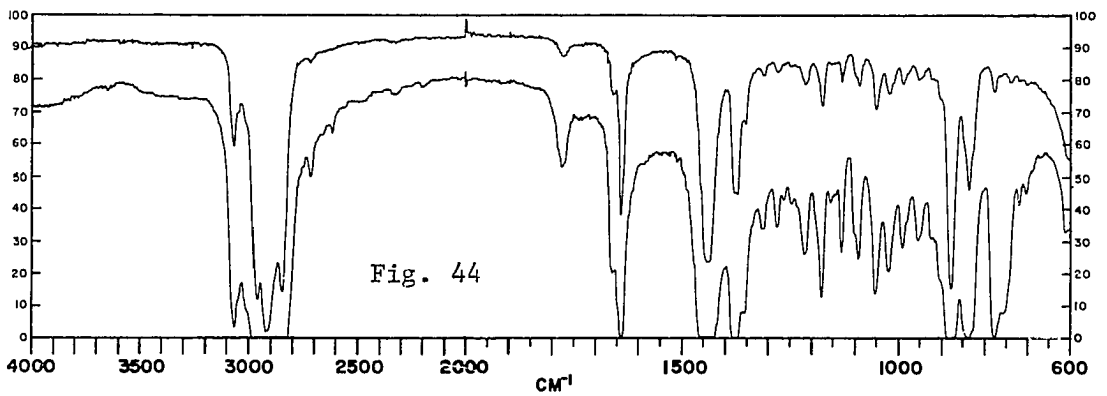
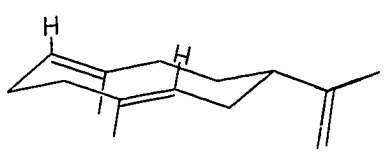


Fig. 44



(-)-germacrene-A

Fig. 45

of terminal methylene and trisubstituted double bonds. The nmr spectrum (Fig. 45) was compatible with the structure proposed for germacrene A (8) displaying a broad signal from δ5.30 to 4.80, 2H (vinyl protons of two trisubstituted double bonds), an exomethylene doublet at δ4.58, and vinyl methyls at δ1.68 and 1.45.

Isomerization of germacrene A was demonstrated by injecting a sample of the compound into an analytical gas chromatogram and observing a single peak having a retention time identical with elemenes, and by heating a sample of germacrene A in an nmr tube to obtain a spectrum identical with that shown for (+)-β-elemene (Fig. 42).

EXPERIMENTAL

All solvents were redistilled from calcium hydride before use. Chromatographic supports were Florisil (Floridin Co., 100-200 mesh) and SilicAR CC-7 (Mallinkcrodt Chemicals, AR-100 mesh). Silver-nitrate-impregnated Florisil was prepared using a modification of the procedure of Norin and Westfelt[56] for preparation of silver-nitrate-impregnated silicic acid. Florisil (300 g) was slurried into an aluminum-foil-covered boiling flask (2 liters) containing a solution of silver nitrate (100 g) in water (500 ml). As much water as possible was removed by water aspiration on a rotary evaporator and the support was activated by heating to 110° under vacuum (0.1 mm) for twenty-four hours. "Nonactive" sugar Florisil adsorbent was prepared using the procedure for the preparation of silver-nitrated Florisil. Florisil (200 g) was added to a solution of confectioners sugar (100 g in 300 ml water) and the water evaporated on a rotary evaporator. The adsorbent was dried at 110° under vacuum (0.1 mm) for twenty-four hours. "Active" sugar-Florisil adsorbent was prepared by blending Florisil (120 g), confectioners sugar (Colonial Sugars Co., 60 g), and hexane (500 ml) in a Waring Blender for six minutes. The sugar-Florisil slurry was poured into the chromatography column and occasionally stirred to prevent cracking of the support during packing. Sugar-Florisil support required nitrogen pressure (2.5 psi) to insure adequate flow rates during chromatography.

Gas chromatographic analyses were performed on a Hewlett-Packard (F&M), Model 402, using a four foot by eight millimeter glass column containing 5% Carbowax on Gaschrom Z support (80-100 mesh). Carrier gas flow (helium) was maintained at 10 ml per minute. The detector temperature was maintained at 190° and the oven temperature was maintained at 110°. The flash heater was not utilized.

Infrared (IR) absorption spectra were recorded on a Beckman IR-8 spectrophotometer. The IR spectra were run on a thin film between two salt discs, or as a thick film in a 0.1 mm cell.

The nuclear magnetic resonance (nmr) spectra were taken on a Varian A-60 spectrometer using tetramethylsilane (TMS) as internal reference. Samples were run as solutions (approximately 40%) in carbon tetra-chloride. Chemical shifts are reported in δ-values (ppm from TMS), and are followed by the multiplicity of the signals, and the corresponding coupling constants. The multiplicities are denoted by the symbols: s, singlet; d, doublet; and m, multiplet. Coupling constants are reported in Hz.

Isolation of the Sesquiterpene Hydrocarbon (STHC) Fraction from Eunicea mammosa Lamouroux. Air dried Eunicea mammosa Lamouroux (1.5 kg) collected in Bimini in June 1969 was leached twice with hexane (1.5 liters per kg weight; then 1.0 liter per kg weight). The solution was filtered (Whatman No. 1) and the solute was concentrated to approximately 75 ml under reduced pressure on a rotary evaporator. Care was taken to maintain the temperature of the solution under 35°. The residue in hexane was chromatographed on Florisil (300 g) with hexane, combining all eluent prior to the first yellow pigment band. Removal of the sol-

vent at 28° under reduced pressure on a rotary evaporatory yielded a sample of colorless liquid (12 g; 0.8% dry weight) identified by nmr and IR as the hydrocarbon fraction. Gas chromatography of the mixture (5% Carbowax) revealed a mixture of a minimum of four components. Components A and C represented less than 2% of the total hydrocarbon mixture. The STHC mixture was diluted with 250 ml of carbon tetrachloride and stored in a Dewar Flask containing dry ice and acetone.

Thermal Isomerization of Germacrene A. The STHC mixture from above (0.20 g) was placed in a boiling flask fitted with condenser and the flask was immersed in an oil bath maintained at 120°. The system was maintained under nitrogen atmosphere. After three hours gas chromatography of the mixture indicated that germacrene A (component D, Fig. 40) was no longer present and that the relative concentration of β-elemene (component B, Fig. 40) had more than doubled as judged by the peak heights of components A and C (Fig. 40), whose relative concentrations had remained constant.

Chemical Isomerization of Germacrene A. The STHC mixture from above (0.1 g) was placed in a boiling flask with SilicAR CC-7 (2 g) and hexane (5 ml) and stirred under a nitrogen atmosphere at room temperature. After three hours gas chromatography of the hydrocarbon solution indicated that germacrene A was no longer present and that β-elemene had more than doubled its relative concentration, again by comparison to components A and C.

Repetition of the experiment on a second aliquot of the STHC mixture (0.1 g) using Florisil (2 g) in place of the SilicAR CC-7 failed to show any significant (5% or more) isomerization of the germacrene A.

Chromatography of Germacrene A on Silver-Nitrate-Impregnated Florisil: Isolation of β-Selinene. The STHC mixture from above (2.0 g) was chromatographed on 30% silver-nitrate-impregnated Florisil with 2% dry benzene in dry hexane. Fractions 20-28 (20 ml per fraction; 3 ml per minute) afforded a sample of hydrocarbon (0.105 g) which was shown by gas chromatography (Carbowax) and silver nitrate TLC to be a single component identified as component C (Fig. 40). The IR spectrum (film) was identical in all respects with that shown by Sörm for β-selinene.

Continuing the elution afforded in fractions 30-60 a mixture varying in concentration from 90% component C, 10% component D to 40% component C, 60% component D (1.8 g). No β-elemene was obtained.

Chromatography of Germacrene A on Sugar-Impregnated Florisil. The STHC mixture (4.0 g) was chromatographed on (33%) sugar-impregnated Florisil (700 g) in hexane. The chromatography column was fitted with a water jacket and chilled water (5°) was circulated during the chromatography. Virtually all of the starting mixture was recovered in the early fractions, 10-30 (50 ml per fraction; 5 ml per minute). Gas chromatography of individual fractions showed no significant changes in the relative amounts of the components.

Isolation of Germacrene A. The STHC mixture (4.0 g) was chromatographed on a sugar-Florisil support prepared by simply blending the two dry materials (540 g) in hexane (1500 ml). During the chromatography the support was maintained at 5°-7° by circulating chilled water through the water jacket surrounding the column. Elution with 2% dry benzene in dry hexane provided in fractions 50-65 (nitrogen pressure 2.5 psi; 25 ml per fraction; 5 ml per minute) a sample of

hydrocarbon (0.385 g) identified by gas chromatography to be component D (Fig. 40),* n_D^{25} 1.5089, $[\alpha]_4^{25}$ -3.2° (c = 14.39; CCl_4); -2.8 (c = 6.15; CCl_4). The IR spectrum (film) showed significant bands at 3060, 1640, and 880 cm^{-1} (terminal methylene), 1660, and 840 cm^{-1} (trisubstituted double bonds). The nmr spectrum (CCl_4) displayed signals at δ5.30-4.80, m, 2H (trisubstituted double bond protons); δ4.58, d, J = 4.5 Hz, 2H (terminal methylene); δ2.12, m, 9H (allylic methine and methylene protons); δ1.68, s, 3H (vinyl methyl); δ1.45, s, 6H (vinyl methyls).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.23%; H, 11.74%.

Gas chromatography (5% Carbowax) of this sample with the injection port maintained at 240° showed a single component with retention time identical with that observed for β-elemene.

Heating of germacrene A (20 mg) in an nmr tube to 120° for three hours prior to determining the spectrum (CCl_4) afforded a spectrum which was identical with that shown by Washecheck for elemene (Fig. 42).

Anal. calcd for $C_{15}H_{24}$: C, 88.16%; H, 11.83%. Found: C, 88.39%; H, 11.85%.

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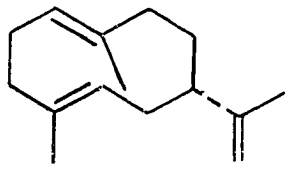
*Gas chromatography of this sample displayed a positive change of base line beginning sharply at the point corresponding to the retention time of elemene. This remained constant until the germacrene A peak came off, and then dropped back to the original base line position. It was assumed that this phenomenon represented the continual thermal isomerization of germacrene A. This received verification when it was shown that the magnitude of the base line change was proportional to the column temperature; i.e., the rate of thermal isomerization of germacrene A.

SUMMARY

Germacrene A, shown below, a previously unreported sesquiterpene hydrocarbon, has been isolated from the gorgonian Eunicea mammosa and its spectral properties, rotation, and refractive index has been obtained. Isolation of germacrene A was accomplished using chromatographic adsorbent prepared by blending Florisil and confectioners sugar, a technique novel to sesquiterpene hydrocarbon chemistry.



(-) Germacrene A

Structural confirmation of germacrene A was demonstrated by thermal isomerization to the predicted product, (+)-β-elemene.

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