Phytochemistry, 1969, Vol. 8, pp 1457 to 1470. Pergamon Press. Printed in England.

COMPOSITION OF OXYGENATED MONOTERPENOIDS AND SESQUITERPENOID HYDROCARBONS FROM THE CORTICAL OLEORESIN OF *ABIES MAGNIFICA* A. MURR.

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(*Received* 4 *February* 1969)

Abstract—Turpentine from cortical oleoresin of *Abies magnifica* A. Murr. was analyzed by a combination of gas–liquid and column chromatography, and the individual materials separated were identified by u.v., i.r., Raman, nuclear magnetic resonance (NMR), and mass spectral (MS) data as well as by the GLC retention data. Nineteen monoterpenoids and twenty-six sesquiterpene hydrocarbons were identified (Tables 1 and 2) including two new compounds, γ-humulene and cyclosativene, whose structures were determined.

INTRODUCTION

EARLIER in the course of our chemosystematic investigations of the genus *Abies*, we reported on the composition of the monoterpenoid hydrocarbons from cortical oleoresins of about twenty species.[1] Later, we investigated the longitudinal compositional variability,[2] the variability on individual level and geographic variability of monoterpenoid hydrocarbons from cortex of U.S. and Canadian species.[3] In these studies, which employed gas–liquid chromatography (GLC) only, little effort has been made to include higher-boiling components of the oleoresins, mainly because extremely little was known on their identity, and the danger of misidentification on the basis of GLC data alone was very great. The present study, which is a reasonably comprehensive analysis of oxygenated monoterpenoids and sesquiterpene hydrocarbons of *A. magnifica* cortical oleoresin, is the beginning of an attempt to fill this gap in our information.

A. magnifica (California red fir) is a majestic tree attaining a height of 200 ft and a girth of 12–25 ft. It is indigenous to southern Oregon and California where it grows at elevations between 6000 and 8000 ft in the north and up to 10,000 ft in its southern range. In its northern range it intergrades with the closely related *A. procera*. Chemically, the only difference now known between the two species is the presence of larger amounts of limonene (roughly 30 per cent) in *A. procera* cortical oleoresin vs. about 4 per cent in *A. magnifica*.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the approximate quantitative composition of monoterpenoidic and sesquiterpenoidic hydrocarbons from *Abies magnifica* cortical oleoresin. Nearly fifty

* Part III in the series "Chemotaxonamy of the genus *Abies*".

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[1] E. ZAVARIN and K. SNAJBERK, *Phytochem.* **4**, 141 (1965).

[2] E. ZAVARIN, *Phytochem.* **7**, 92 (1968).

[3] E. ZAVARIN, W. B. CRITCHFIELD and K. SNAJBERK, to be published.

constituents were identified, and they are arranged in the tables according to the number of rings they contain.

The biosynthesis of monoterpenoids has been discussed by several authors.[4,5] It is now generally believed that it involves the participation of geranyl/neryl pyrophosphates which, through dephosphorylation, oxidation and reduction, yield the known acyclic monoterpenoids. Mono-, bi- and tricyclic constituents are believed to result from a hypothetical 1-*p*-menthene-8-carbonium ion formed by dephosphorylation and cyclization of neryl

TABLE 1. COMPOSITION OF MONOTERPENOIDS FROM CORTICAL OLEORESIN OF *Abies magnifica* A. Murr.*

	Percentage
Acyclic	
Myrcene	3·7
Geraniol	0·3
Geranyl acetate	tr.
Citronellol	1·3
Citronellyl acetate	1·3
Monocyclic	
α-Phellandrene	0·3
β-Phellandrene	48·3
Limonene	3·8
Terpinolene	0·5
α-Terpineol	0·1
Borneol	0·3
Bornyl acetate	0·5
Bicyclic	
α-Pinene	11·3
β-Pinene	23·9
3-Carene	2·9
α-Thujene	0·2
Sabinene	0·6
Camphene	1·0
Tricyclic	
Tricyclene	0·1

* Arranged according to number of rings.

pyrophosphate. One of the several possible routes may involve formation of 1-*p*-menthene-4-carbonium ion through 8/4 hydride shift, and leads to sabinene, α-thujene, α- and γ-terpinene and related monoterpenoids. Another one may go through the formation of a 2-bornane carbonium ion by a second cyclization, and leads to camphene, santene, tricyclene, borneol, bornyl acetate, camphor and similar compounds.

[4] J. H. RICHARDS and J. B. HENDRICKSON, *The Biosynthesis of Steroids, Terpenes and Acetogenins*, pp. 207, 225, 229, W. A. Benjamin, New York (1964).
[5] J. R. HANSON, *Perf. Essent. Oil Rec.* **58**, 787 (1967).

As can be seen from Table 1, the largest part (93 per cent) of the monoterpenoids in *A. magnifica* is represented by cyclic compounds with over 48 per cent accounted for by β-phellandrene. The 1-*p*-menthene-4-carbonium ion intermediate seems to be the least

TABLE 2. COMPOSITION OF SESQUITERPENOIDIC HYDROCARBONS FROM CORTICAL OLEORESIN OF *Abies magnifica* A. Murr.*

	Percentage
Acyclic	
Farnesene	0·5
Monocyclic	
β-Elemene	4·5
δ-Elemene	9·2
α-Humulene	1·7
γ-Humulene	4·4
β-Bisabolene	2·4
Bicyclic	
α-Guaiene	0·5
Guaiazulene	tr.
α-Selinene	1·7
β-Selinene	1·9
Selina-4(14),7(11)-diene	tr.
Selina-3,7(11)-diene	tr.
Caryophyllene	11·1
β-Santalene	0·9
β-Bergamotene	0·4
γ-Cadinene	tr.
δ-Cadinene	tr.
γ-Muurolene	4·4
Tricyclic	
α-Cubebene	—
α-Copaene	0·3
β-Copaene	0·3
Sativene	1·7
α-Ylangene	0·4
β-Ylangene	0·5
α-Longipinene	5·8
Longifolene	47·0
Tetracyclic	
Cyclosativene	1·0
Longicyclene	0·6

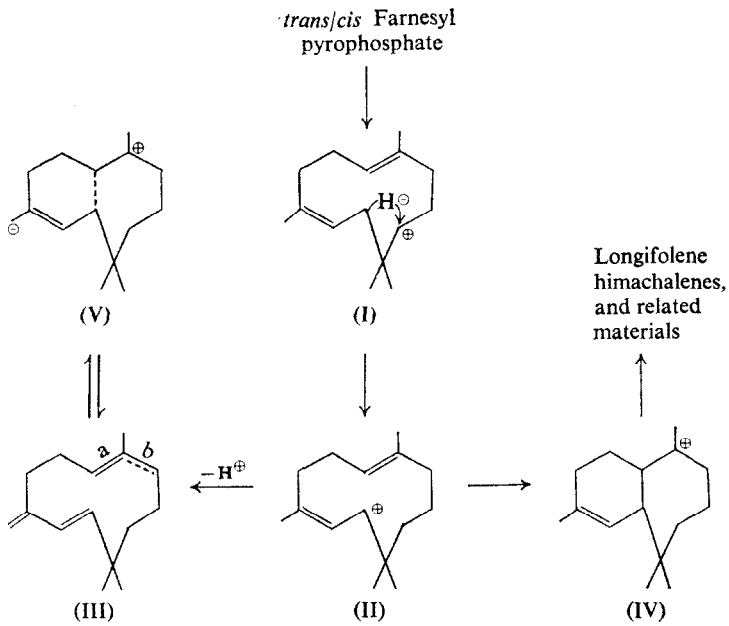
* Arranged according to number of rings.

important with less than 1 per cent of monoterpenoids accounting for it. The 2-bornane carbonium ion route with 2 per cent contributes little, but larger participation (accounting for almost 7 per cent) is exhibited by acyclic materials.

The biosynthesis of sesquiterpenoids, although still in a speculative stage, has been

thoroughly reviewed recently.[4,6] In this case the differentiation into individual compounds is proposed to begin with the formation of *trans/trans* and *trans/cis* farnesyl pyrophosphates. The 1/10 and 1/11 cyclizations of the *trans/trans* and 1/6, 1/7, 1/10 or 1/11 cyclizations of the *trans/cis* compound lead to the six intermediate monocyclic carbonium ions, from which further transformations similar to those of monoterpenoids give the known sesquiterpenoids.

On the basis of these hypothetical biosynthetic transformations, our results—though based only on the hydrocarbon portion of the sesquiterpenoids—indicate that in *A. magnifica* *trans/cis* farnesol routes predominate over *trans/trans* to about 2:1. Within *trans/trans* farnesol compounds both 1/10 (the elemenes, α-guaiazulene and selinenes) and 1/11 (caryophyllene and α-humulene) cyclizations are represented to about an equal extent. However, with *trans/cis* farnesol routes the 1/11 (α-longipinene, longicyclene and longifolene) cyclization accounts for about 80 per cent of the material, chiefly through the occurrence of large



amounts of longifolene. The 1/6 (β-bisabolene, β-santalene and β-bergamotene) and 1/10 cyclizations (the cadinenes, muurolenes, ylangenes, copaenes and sativenes)* account for about only 20 per cent, while 1/7 cyclization is not represented at all.

Many of the sesquiterpenes isolated previously from other families were identified for the first time in Pinaceae. Sativene (VI) (Fig. 1, peak 8) was known so far only from the fungus *Helminthosporum sativum*. It has been hypothesized that helminthosporal, the active material accounting for the heavy wheat losses from *H. sativum* in Canada, forms through oxidation of sativene in this organism.[8] It is possible that a similar mechanism accounts in

* It is also possible that these compounds form through the initial 1/6 cyclization.[7]

[6] W. PARKER, J. S. ROBERT and R. RAMAGE, Sesquiterpene Biosynthesis; *Quarterly Reviews*, pp. 331, 343, The Chemical Society, London, **21**, 331 (1967).

[7] L. WESTFELT, *Svensk Kem. Tidskr.* **79**, 441 (1967).

[8] P. DE MAYO, E. Y. SPENDER and R. W. WHITE, *Can. J. Chem.* **41**, 2996 (1963); P. DE MAYO and R. E. WILLIAMS, *J. Am. Chem. Soc.* **87**, 3275 (1965); P. DE MAYO, J. R. ROBINSON, E. Y. SPENCER and R. W. WHITE, *Experientia* **18**, 359 (1962).

part for the lack of other vegetation in the stands of *A. magnifica*, with helminthosporal being produced by decomposition of sativene in the litter. The possible presence of helminthosporal in leaves and cortex of this fir should not be overlooked.

Other sesquiterpenoids new for Pinaceae included farnesene, δ-elemene, α-guaiene, guaiazulene, β-santalene, β-bergamotene and the four selinenes mentioned (among other selinenes, γ-selinene has been identified in *A. sibirica* cortex,[9] and sibirene, a selinene, has been identified in *Pinus sibirica* gum oleoresin).[10]

Several new sesquiterpenes were isolated. One, named γ-humulene (III), was found to be a double bond isomer of α-humulene. On catalytic hydrogenation, γ-humulene yielded humulene, identified by a direct comparison with an authentic sample (i.r.). The methyl region in the NMR spectrum of γ-humulene showed the presence of an allylic methyl group (τ 8·59) and a geminal dimethyl group (τ 9·14). Of the remaining high-field protons, six exhibited absorption at τ < 8·20, indicating their α-position in relation to the double bond.

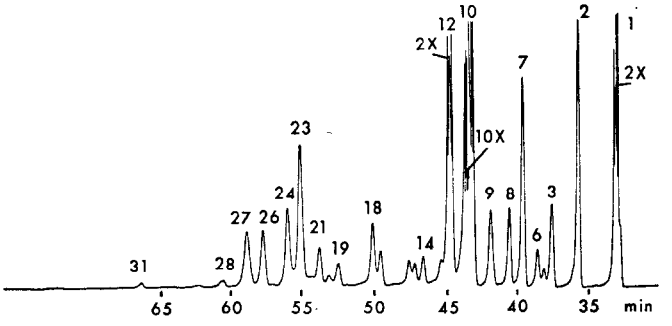


FIG. 1. GAS/LIQUID CHROMATOGRAM OF *A. magnifica* CORTICAL TURPENTINE FRACTION CONTAINING SESQUITERPENOID HYDROCARBONS, COLUMN A (SF96(50)) AT 125°.

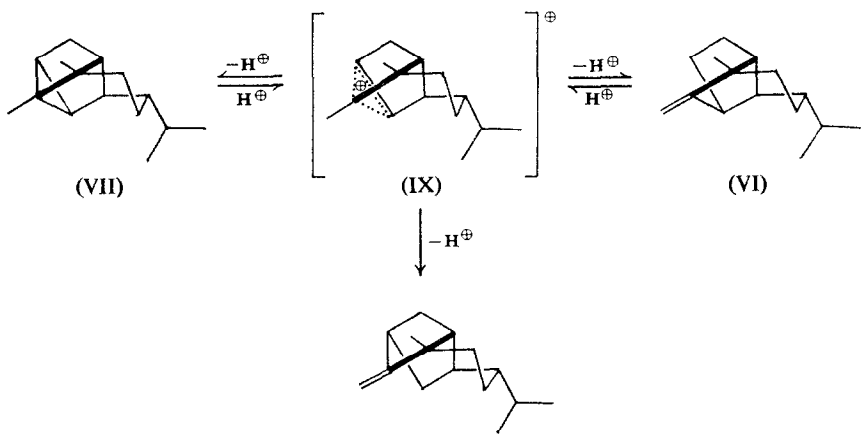
(1) δ-elemene, (2) α-longipinene, (3) cyclosativene overlapping, (4) α-ylangene, (5) α-copaene, (6) longicyclene, (7) β-elemene, (8) sativene, (9) unknown SH X_1, (10) longifolene, (11) β-ylangene, (12) caryophyllene, (13) β-copaene, (14) α-guaiene, (15) β-bergamotene, (16) farnesene, (17) β-santalene, (18) α-humulene, (19) unknown SH X_2, (20) γ-muurolene, (21) unknown SH X_3, (23) γ-humulene, (24) β-selinene, (25) α-muurolene, (26) α-selinene, (27) β-bisabolene, (28) γ-cadinene, (29) δ-cadinene, (30) selina-4(14),7(11)-diene, (31) selina-3,7(11)-diene.

The u.v. max. at 243 nm, the strong i.r. band at 1610 cm^{-1} and the lower field proton absorption in the NMR (see experimental section), strongly favored the proposed conjugated double bond structure (III). The sharp doublets of the *trans*-coupled olefinic protons at τ 4·21 and 4·56 pointed to the absence of protons in the α-position in relation to the double bond. The difference in the chemical shift of the exocyclic methylene group signals (i.r. band at 1650 cm^{-1}) τ 5·19 and 5·27 indicated its presence in a ring or in some other rigid position. The third double bond was trisubstituted and had a more complex splitting pattern, with several small allylic couplings in the triplet resulting from the coupling to a vicinal methylene group. Of the two possible alternatives, III*a* and *b*, the first one seems more attractive. The position of the u.v. absorption maximum, although too low for any conjugated triene, is at the same time too high for the value of 232 nm calculated from the diene rules, and with about the same value (231–232 nm) exhibited by β-phellandrene with

[9] N. A. CHIRKOVA and V. A. PENTEGOVA, *Izv. Sibir. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* 125 (1962).
[10] V. A. PENTEGOVA, N. K. KASHTANOVA, A. I. REZVUKHIN and G. I. KOLIPOVA, *Khim. Prir. Soed.* **2**, 239 (1966), V. A. PENTEGOVA, O. MOTL and V. HEROUT, *Coll. Czech. Chem. Commun.* **26**, 1362 (1961).

the same diene structure.[12] Possibly, this abnormal shift can be attributed to transannular interaction (V) between diene and the double bond at *a*.[17] Structure (V) is equivalent to the deprotonated cation IV and it represents a rather favorable arrangement[4] due to the appropriate steric position of the isolated double bond in the eleven-membered ring, general stability of the six-membered ring formed and the transfer of the positive charge from di- to trisubstituted carbon atom. The distance between double bonds and other structural features make the corresponding resonance interaction much less likely with the double bond at *b*. Biosynthetic considerations are also in agreement with the above, as (assuming no double bond isomerization takes place) the position *a* would be predictable from the location of the corresponding double bond in either *trans/trans* or *trans/cis* farnesyl pyrophosphate precursor.

The demonstration of existence of γ-humulene might be of importance for understanding the biosynthetic pathways involved in formation of longifolene and related sesquiterpenes. It could represent the product of stabilization of the cation II by loss of a proton, with the



(VII) (IX) (VI)

(VIII)

cation II postulated[4] as intermediary, formed by a hydride shift from I, in the sequence *trans/cis* farnesyl pyrophosphate → I → II → IV → longifolene. It might be of significance in this connection that longifolene and related materials represent the main product of the sesquiterpene synthesis in *A. magnifica* cortex. The hydride shift mentioned above seems to be unlikely for sterical reasons in case of the alternative *trans/trans* farnesyl pyrophosphate 1/11 cyclization path utilized by α-humulene.[6] Should the above considerations prove true, it follows that γ-humulene should be regarded as biosynthetically only distantly related to α-humulene.

Another isolated new sesquiterpene was named cyclosativene (VII)[11] because it related to sativene (VI) in the same way as tricyclene related to camphene, longicyclene (XI) to longifolene (XII) and α-santalene to β-santalene. All these, with the exception of α-santalene, have been identified in *A. magnifica* cortical oleoresin.

The spectroscopic data (i.r., Raman, u.v., NMR and mass spectrum) were in agreement with the proposed structure (VII). The presence of a cyclopropane ring was established by

[11] L. SMEDMAN and E. ZAVARIN, *Tetrahedron Letters* 3833 (1968).
[12] H. BOOKER, L. K. EVANS and A. E. GILLAM, Jr. *Chem. Soc.* 1453 (1940).

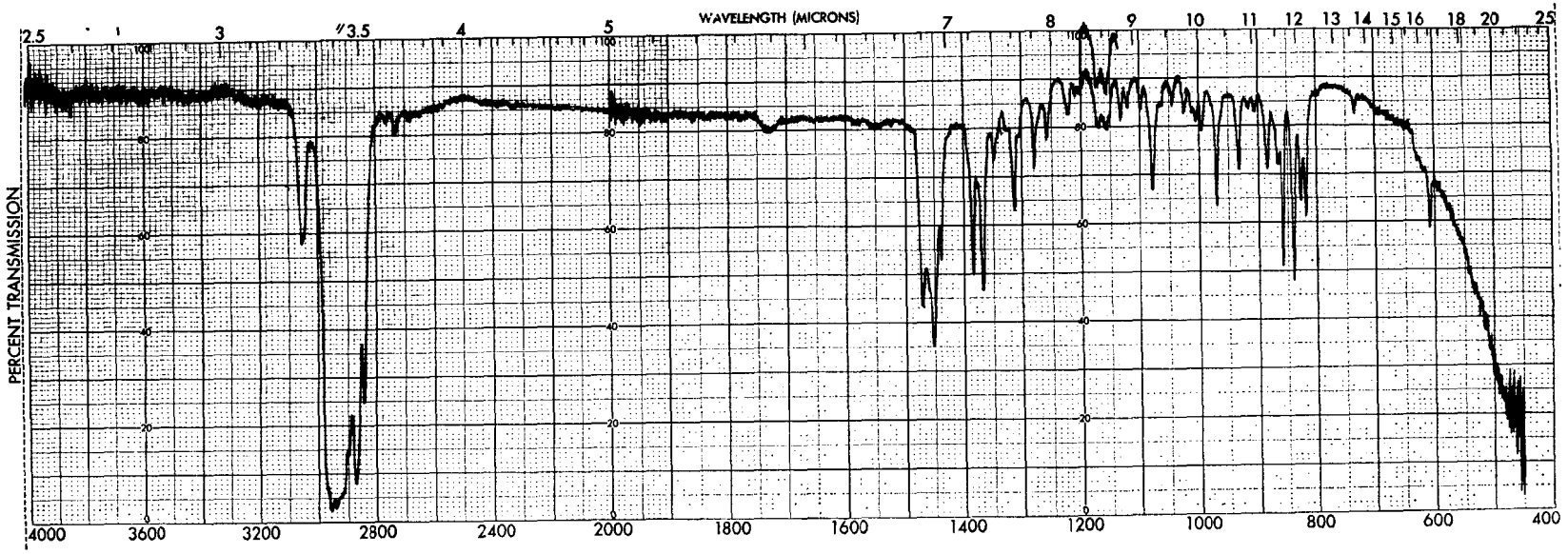


FIG. 2. INFRARED ABSORPTION SPECTRUM OF CYCLOSATINENE.

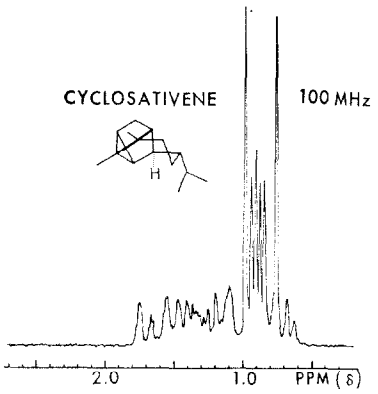


FIG. 3. NMR SPECTRUM OF CYCLOSATIVENE.

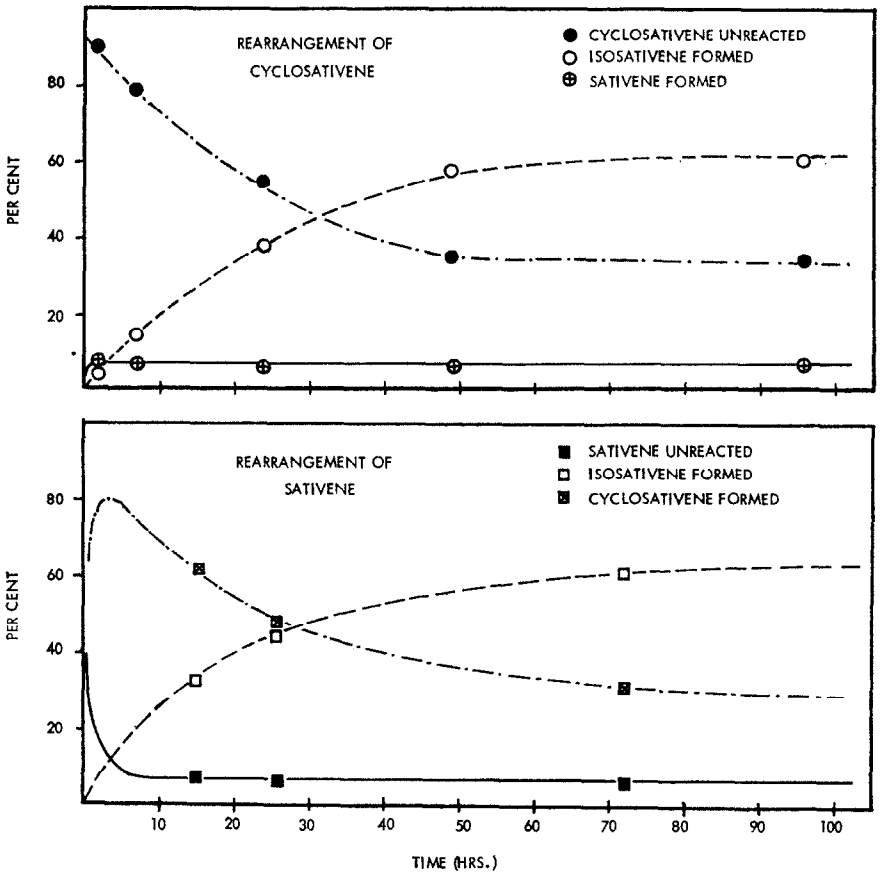
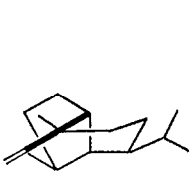
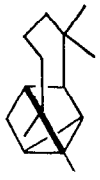


FIG. 4. REARRANGEMENT OF CYCLOSATIVENE AND SATIVENE IN CUPRIC ACETATE IN ACETIC ACID SOLUTION AT 110°.



(X)



(XI)

(XII)

i.r. (3050 and 840 cm^{-1}) and NMR (two protons at τ 9·34 and 9·22). The presence of a tetra-substituted double bond was excluded by Raman spectroscopy, and by the absence of any significant absorption above 200 nm. The i.r. and NMR spectra are presented in Figs. 2 and 3.

Treatment of cyclosativene with cupric acetate in acetic acid at 110° resulted in the formation of a mixture consisting of unreacted starting material, sativene, and another

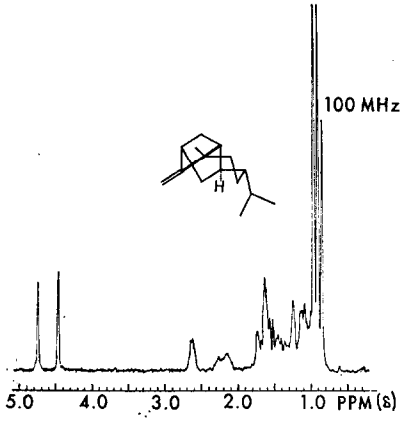


FIG. 5. NMR SPECTRUM OF ISOSATIVENE.

tricyclic sesquiterpene (VIII) (isosativene) (Fig. 4). A similar mixture was also obtained on reacting sativene under the same conditions, thus suggesting carbonium ion (IX) or its classical counterparts as a common intermediate in the rearrangement. The interconversion of cyclosativene and sativene must, therefore, be kinetically controlled, whereas the formation of isosativene (VIII) NMR Fig. 5 is favored thermodynamically (Fig. 5).

The configuration of the isopropyl group in cyclosativene follows from its isomerization to sativene and not to copacamphene (X), and corresponds to that of ylangenes.

Two additional sesquiterpenes were isolated but could not be identified. One of these (SH X_2) showed a great spectroscopic resemblance to caryophyllene.

EXPERIMENTAL

Rotations were determined in $CHCl_3$ at 22°. U.v. spectra were obtained in hexane or ethanol. I.r. spectra were recorded in substantia and in solutions (CCl_4 and CS_2). Raman spectra were determined as described.[13] NMR spectra were recorded at 60 and 100 Hz using $CDCl_3$ solutions and tetramethylsilane as internal standards.

[13] G. F. BAILEY, S. KINT and J. R. SCHERER, *Anal. Chem.* **39**, 1040 (1967).

Collection

The oleoresin was collected near Alpine and Wrights Lakes on State Highway 4 and U.S. Highway 50, respectively, in the Sierra Nevada mountains in eastern California. The resin pockets in the stem cortex were punctured with a needle, and oleoresin was collected in vials to which a small amount of pyrogallol as antioxidant had been added. Some of the vials were not completely filled, and oxygen was replaced by nitrogen. Samples were stored in a freezer.

Isolation of Sesquiterpenic Hydrocarbons

The oleoresin (563 g) was dissolved in Et_2O (distilled over CaH_2) and exhaustively extracted in the usual way with a cold solution of 1% NaOH. The etheral solution was washed once with a few ml of 0·01 N H_2SO_4 maintaining the pH above 8, and several times with water; the solution was finally dried with Na_2SO_4 and Et_2O removed by distillation to produce an acid-free fraction (52 per cent) which could be divided into hydrocarbons (30 per cent of the total) and oxygenated terpenoids (22 per cent) by chromatography on deactivated Al_2O_3, using light petroleum (b.p. 30–60°) followed by Et_2O as eluents. The hydrocarbon fraction was distilled at reduced pressure yielding mono- (124 g, 22 per cent) and sesquiterpenes (33 g, 6 per cent) as two different fractions, while the diterpenes remained as a residue. The sesquiterpenes were distilled at 8 mm pressure on a Nester/Faust Annular Teflon Spinning Band Distillation Column giving twelve fractions (Table 3).

TABLE 3. DISTILLATION OF SESQUITERPENIC HYDROCARBONS

Fraction	Weight (g)	B.p. (°C/8 mm)
1	2·7	92–93
2	1·4	93–95
3	0·7	95–97
4	1·7	97–100
5	2·5	100–102
6	3·2	102–104
7	5·7	104–105
8	5·5	105–106
9	4·5	105–106
10	0·2	106–108
11	4·0	108–111
12	0·2	111–116

From these fractions pure hydrocarbons were obtained by combinations of gas–liquid chromatography and $AgNO_3$–silica gel chromatography,[14] referred to below simply as chromatography.

TABLE 4. COLUMNS FOR GLC

Column	Coating	Temperature (°C)	Dimensions (ft × in.)
A	SF 96 (50)	150	500 × 0·02
B	Apeizon L	150	500 × 0·02
C	Carbowax 20M	125	500 × 0·02
D	SF 96(50), 4 per cent	150	20 × 0·5
E	Carbowax 20M, 1 per cent	150	53 × 0·2
F	SF 96(50)	160	900 × 0·03
G	Apeizon L	160	900 × 0·03
H	Carbowax 20M	150	900 × 0·03

[14] T. NORIN and L. WESTFELT, *Acta Chem. Scand.* **18**, 572 (1964).

Table 4 summarizes columns used in GLC work; Columns A–C were employed for qualitative separations, and columns D–H for preparative purposes. With columns D and E, acid-washed and silanized chromosorb G, 100/120 mesh, was used as the support; the remaining columns were capillary.

The hydrocarbons used in spectroscopic examination were at least 98 per cent pure if not otherwise stated.

For complete identification by optical rotation, i.r., Raman, NMR and mass spectrum 5 to 10 μl was required. When less than 5 μl was available usually only i.r. and/or NMR analyses were performed. Figure 4 depicts the chromatogram of the total sesquiterpene fraction with individual components indicated.

δ-Elemene

Distillation fractions 1 and 2 were mainly mixtures of α-longipinene and δ-elemene with small amounts of longicyclene, cyclosativene, α-ylangene, and α-copaene. Chromatography starting with light petroleum and with continuous addition of an increasing percentage of ether to about 20 per cent yielded δ-elemene in the last fractions. Small impurities were removed by GLC column D. Identification was by i.r., Raman, NMR and mass spectrum, $\alpha_D - 0{\cdot}8°$, $c = 2{\cdot}1$.

α-Longipinene

In the chromatographic separation of distillation fractions 1 and 2, α-longipinene was eluted with light petroleum containing less than 0·5 per cent ether. GLC with column E gave α-longipinene, $\alpha_D + 40{\cdot}1$, $c = 1{\cdot}1$. Identification was by i.r., Raman, NMR and mass spectrum.

Cyclosativene

In the argentative chromatography of distillation fractions 1 and 2, two hydrocarbons were eluted with the front, indicating absence of double bonds. Further separation by GLC columns F and H gave cyclosativene. Spectral data: $\alpha_D + 94{\cdot}1°$, $c = 0{\cdot}3$; i.r. maxima at 3050, 1380, 1365, 859 and 840 cm^{-1}; NMR τ 9·01 (s, 3) quarternary methyl, 9·23 (s, 3) quarternary methyl 9·12 (d, 3, J6 Hz), 9·09 (d, 3, J6 Hz) isopropyl, 9·34 (d, 1, J5·5 Hz) cyclopropane proton and 9·22 (partly unresolved, 1); mass spectrum (70 eV) m/e (rel. intensity) 204 (100), 189 (16), 161 (61), 119 (35), 105 (65), 91 (82), 79 (48), and 41 (58).

α-Ylangene and α-Copaene

In the purification of α-longipinene on column E, α-ylangene and α-copaene were obtained in small amounts as a mixture which could be partly separated on columns F and G yielding α-ylangene and α-copaene. Identification was by i.r.

Longicyclene

This compound was present together with cyclosativene and in initial chromatography fraction of distillation fractions 1 and 2. Separation on column F gave longicyclene $\alpha_D + 34{\cdot}3°$, $c = 0{\cdot}5$, identified by i.r., Raman, and mass spectrum.

β-Elemene

Column chromatography of distillation fractions 3 and 4, using 20% Et_2O in light petroleum, gave β-elemene and small amounts of δ-elemene. Further purification by GLC on column E yielded pure β-elemene $\alpha_D - 13{\cdot}8°$, $c = 0{\cdot}7$. Identification was by i.r., Raman, NMR and mass spectrum.

Sativene (III)

In the chromatography of distillation fractions 3 and 4, sativene was eluted together with α-longipinene, α-ylangene, α-copaene and longifolene, which was the major component. Separation by GLC on columns D, E and F gave sativene (95 per cent pure) $\alpha_D + 169{\cdot}3°$, $c = 0{\cdot}3$. Identification was by i.r., Raman, NMR and mass spectrum.

Longifolene

Distillation fractions 5–8 were mainly mixtures of longifolene and caryophyllene (5:1) with small amounts of β-copaene and β-ylangene. A small part of these fractions were partly separated by GLC column D. Further GLC separation with column E gave pure longifolene $\alpha_D + 48{\cdot}5$, $c = 1{\cdot}2$. Identification was by i.r., Raman, NMR, and mass spectra.

β-Ylangene and β-Copaene

Small amounts of β-ylangene and β-copaene occurred together with longifolene. However, only β-copaene could be isolated by column chromatography and by GLC columns D, E and F. β-Ylangene could be identified only by its retention volume in GLC columns A, B and C.

Caryophyllene

Distillation fractions 5–8 yielded pure caryophyllene $\alpha_D - 8{\cdot}9°$, $c = 3{\cdot}8$ by chromatography or by GLC columns D and E. Identification was by i.r. and Raman.

α-Guaiene

Distillation fractions 9 and 10 were mainly mixtures of longifolene and caryophyllene together with other slightly higher boiling sesquiterpenes. GLC on column D removed the major portion of the longifolene and caryophyllene yielding three more fractions, the first of which was a mixture of α-guaiene, β-bergamotene and farnesene. Repeated GLC on column F gave α-guaiene (90 per cent pure). Identification was by i.r.

β-Bergamotene

β-Bergamotene could be obtained only about 80 per cent pure by the same procedures as described for α-guaiene. I.r. indicated the presence of β-bergamotene which, however, could not be verified by other methods due to insufficient material.

Farnesene

The presence of farnesene was strongly indicated by GLC on columns A, B and C, and also by TLC on $AgNO_3$-impregnated silica gel.

β-Santalene

The third fraction which was obtained from the separation of distillation fractions 9 to 10 was a mixture of α-humulene and β-santalene. Column G yielded β-santalene as a mixture with α-humulene (8:2). The i.r. spectrum with strong bands at 1650, 1375, 1100 and 880 cm^{-1} was indistinguishable from one expected of the α-humulene/β-santalene mixture of the above composition. Furthermore, the retention times of the compound isolated were identical with those of authentic β-santalene, using columns A and B.

α-Humulene

α-Humulene (at least 95 per cent pure) was obtained by the chromatographic procedure described for β-santalene. Identification was by i.r.

Sesquiterpenic Hydrocarbons X_2 (SH X_2)

The last fraction from the GLC separation of distillation fractions 9 to 10 was a mixture of SH X_2, γ-muurolene, and SH X_3. SH X_2 was obtained in pure form but poor yield by chromatography with 10 per cent ether in light petroleum followed by GLC on columns G and H. SH X_2 crystallized at 0° and melted at 22–23°; it exhibited i.r. bands at 1670, 1635, 1380, 1372, 1365, 890 and 835 cm^{-1}. Between 1300–1100 cm^{-1} spectrum greatly resembled caryophyllene. The NMR demonstrated the presence of three methyl groups, τ 9·10 (s, 3), 8·80 (s, 3), and 8·48 (br s, 3). The double bond region included signals at 4·96 (br q, 1, *J* 3 and 12 Hz), and an exocyclic methylene group at 5·21 and 5·27 (s, 2). An octet (o, 1, *J* 2, 12 and 19 Hz) was centered at 7·14. The NMR also indicates approximately seven protons in α-position to a double bond (i.e. $\tau < 8{\cdot}20$). Mass spectrum (70 eV) showed the following major peaks: *m/e* (rel. intensity) 204 (13), 91 (61), 81 (43), 79 (65), 69 (199), 41 (83). The data suggested a sesquiterpene of the caryophyllene type.

γ-Muurolene

In the chromatographic separation of SH X_2, γ-muurolene was obtained in a rather crude form when eluting the column with approximately 2% Et_2O in light petroleum. Due to insufficient material further purification by GLC was not possible, but the presence of γ-muurolene could be established by retention volume on GLC columns A, B and C.

Sesquiterpenic Hydrocarbon X_3 (SH X_3)

SH X_3 appeared in the chromatographic separation when using 0·5 per cent ether in light petroleum. Separation by GLC on columns D, E and F gave: 90 per cent pure SH X_3. Spectral data: i.r. maxima at 1665, 1635, 1380, 1360, 880 and 835 cm^{-1}; NMR τ 9·04 (d, 6, *J* 6·5 Hz, isopropyl group), 9·08 (d, 3, *J* 6 Hz, secondary methyl group), 4·29 (br s, 1, olefinic on trisubstituted double bond, and 5·19 (br s, 2, exocyclic methylene); eight protons had $\tau < 8{\cdot}20$.

γ-Humulene (I)

Fraction 11 from the distillation was separated by chromatography into six fractions starting with 1 per cent and finishing with 20 per cent ether in light petroleum. The middle fraction contained a mixture of β-selinene and γ-humulene which could be separated chromatographically using 4 per cent ether in light

petroleum. The last impurities were removed by GLC column G: Spectral data: i.r. maxima at 1670, 1650, 1610, 1380, 1360, 970, 855 and 870 cm^{-1}; NMR τ 9·14 (s, 6, geminal dimethyl group), 8·59 (br d, 3, J1·5 Hz, allylic methyl group), 4·21 (d, 1, J16·0 Hz, olefinic with *trans*-coupling), 4·54 (d, 1, J16·0 Hz, olefinic with *trans*-coupling), 4·79 (br t, 1, J7·5 Hz, olefinic coupled to methylene), 5·19 (d, 1, J2 Hz, one of two exocyclic protons), and 5·27 (d, 1, J2 Hz, one of two exocyclic protons); six saturated protons had $\tau < 8{\cdot}20$; u.v. maxima at 243 nm – log $\epsilon = 3{\cdot}4$. mass spectrum (70 eV) *m/e* (rel. intensity) 204 (56), 189 (23), 161 (48), 133(61), 107 (52), 93 (100), 91 (62), 79 (52) and 41 (58).

β-Selinene

β-Selinene was obtained from distillation fraction 11 by column chromatography with 5–15 per cent ether in light petroleum. Small impurities were removed by GLC column D yielding pure β-selinene $\alpha_D + 59{\cdot}8$, $c = 1{\cdot}2$. Identification was by i.r., Raman, NMR, and mass spectrum.

α-Muurolene

The presence of α-muurolene could be demonstrated only by GLC columns A, B and C, using total sesquiterpene material.

β-Bisabolene

Chromatography of distillation fraction 11 gave material which, after purification by GLC (columns C and E), yielded pure β-bisabolene, identified by i.r., NMR, and mass spectra.

α-Selinene

Upon chromatography with 2 to 5 per cent ether in light petroleum, distillation fraction 11 gave a fraction enriched in α-selinene which was further purified by GLC on columns D and E. Identification was by i.r., Raman, NMR, and mass spectrum.

γ-Cadinene

Identification was by GLC columns A, B and C using total sesquiterpene material.

δ-Cadinene

This compound could be identified by i.r. in the distillation fraction 11 after column chromatography with 5 per cent ether in light petroleum and GLC using columns E and F.

Selina-4(14), 7(11)-diene and Selina-3, 7(11)-diene

These two compounds were tentatively identified by GLC columns A, B and C by comparison with respective constituents of hop oil.[15]

Guaiazulene

After distillation of the sesquiterpene was stopped, the blue principle of the oleoresin was concentrated at the top of the column and removed with light petroleum. The crude product was further purified on deactivated Al_2O_3 plates with light petroleum as solvent yielding pure guaiazulene (less than 0·1 per cent of the total oleoresin). Identification was by u.v. and i.r.

Isolation of Oxygenated Monoterpenes

The fraction containing the oxygenated terpenoids was distilled, and distillate (2 g, 0·36 per cent) was separated on deactivated aluminum oxide with light petroleum followed by benzene and ether to give monoterpene alcohols and monoterpene acetates in different fractions. Using GLC on 10 per cent DEGS (20 ft × ¼ in., 120°), most compounds could be obtained in rather pure form (better than 90 per cent pure). The following compounds were isolated and identified by i.r.: bornyl acetate, citronellyl acetate, geranyl acetate, borneol, α-terpineol, citronellol, and geraniol.

Monoterpenoid Hydrocarbons

The respective fraction was analyzed by GLC using 10 per cent β,β-oxydipropionitrile on acid-washed and silanized chromosorb P, 60/80.

[15] R. BUTTERY, R. LUNDIN and L. LING, *Agr. Food Chem.* **15**, 58 (1967).

Check of Artifact Formation

A total oleoresin injection with a Hamilton injection block[16] showed that no artifacts were introduced during the work-up. Furthermore, no appreciable change in percentage composition was indicated.

Rearrangement of Cyclosativene

1–10 μl of cyclosativene (or sativene) was dissolved in 0·5 to 1·0 ml of 0·01 M $CuAc_2$ in acetic acid solution. The reaction took place in sealed glass ampoules at 110°. Samples from the reaction mixture were taken in intervals of 0·5 hr and pipetted into H_2O and then extracted with light petroleum. The extracts were analyzed by GLC on Carbowax 20M column (1 per cent on Chromosorb G 100–200 mesh, 15 in. × $\frac{1}{8}$ in., 120°). Reaction products were isolated by GLC column G and identified by i.r. and mass spectrum. Compound IV (isosativene) was also characterized by NMR and i.r. giving the following spectral data: i.r. 1660, 1385, 1370 and 880 cm^{-1}; NMR τ 5·54 (s, 1), 5·26 (s, 1) (exocyclic methylene), 9·01 (s, 3) (quarternary methyl), 9·11 (broad d, 6, J 6 Hz) (isopropyl) and 7·38 (broad d, 1) (allylic proton); mass spectrum (70 eV) m/e (rel. intensity) 204 (62), 189 (24), 161 (64), 105 (100), 103 (44), 93 (39), 91 (41) and 79 (26).

Acknowledgement—We gratefully acknowledge the Grant No. GB3954 from National Science Foundation, Division of Biological and Medical Sciences, supporting in part the present work.

[16] L. SMEDMAN, K. SNAJBERK, E. ZAVARIN and D. MON, *Phytochem.* **8**, 1471 (1969).

[17] Transannular electronic transitions leading to a bond in the excited state are known. E.g. such a transition has been demonstrated in the *trans*-5-cyclodecenone by E. M. KOSOWER, W. D. CLOSSON, H. L. GOERING and Y. C. GROSS, *J. Am. Chem. Soc.* **83**, 2013 (1961).