Some Minor Sesquiterpene Hydrocarbons of Black Pepper

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The volatile compounds of black pepper have been studied further by techniques involving infrared spectroscopy on components isolated and purified by repetitive gas chromatographic separations. α-Cubebene, isocaryophyllene, and γ-muurolene have been identified among the minor constituents, and an additional major constituent has been isolated and identified as β-farnesene. Mechanisms which explain the observed co-occurrence of sesquiterpenes in natural products are discussed.

A previous paper (Muller and Jennings, 1967a) reported the isolation and identification of 13 of the major sesquiterpene hydrocarbons of oil of black pepper (*Piper nigrum*, L.) This paper describes the isolation and characterization of an additional major, and three minor, sesquiterpene hydrocarbon constituents of black pepper oil.

EXPERIMENTAL

Apparatus. Initial gas chromatographic separations utilized a modified Aerograph Autoprep containing an 18-foot × 0.25-inch (O.D.) stainless steel column packed with 10% (w./w.) Triton-X 305 on 60- to 80-mesh HMDS-treated Gas-Chrom Q, operated at 160° C. and a 60 ml. per minute He flow rate. Final purifications were performed on a 500-foot × 0.030-inch (I.D.) stainless steel capillary column coated with SF96(50) admixed with 5% (w./w.) Igepal CO-880 on a modified Beckman Thermotrac fitted with Carle microthermistor detector and Hamilton glass-lined injectors. This was operated isothermally at 175° C. and a 6 ml. per minute He flow rate.

Trapping of the separated components was performed using thin-walled glass capillaries as described previously (Jennings *et al.*, 1964). Infrared spectra were taken neat as thin (about 0.015 mm. thick) films between sodium chloride plates on a Beckman IR-8 infrared spectrophotometer provided with a 5X beam condenser.

Materials and Procedure. The hydrocarbon fraction of Ceylon black pepper oil (Stange and Co.) was obtained by passage through basic alumina followed by preparative gas chromatography, and the sesquiterpene hydrocarbon fraction resolved into 12 major peaks on the Triton-X 305 preparative column as described elsewhere (Muller and Jennings, 1967a). The isolation, purification, and identification of the major constituents of each peak from Triton-X 305 have also been described.

Minor constituents were isolated and purified by a similar procedure [repetitive 0.1- to 0.2-μl. injections of CCl_4 solutions into the SF96(50) capillary column]. Because of the small concentrations involved, and occasionally because of a poorly resolved impurity, as many as 80 to 100 repetitive injections followed by repurifications were required to secure sufficient material (ca. 80 μg.) for infrared analysis.

The isolated compounds were identified by comparison of their infrared spectra with published spectra and with the spectrum of sesquiterpene hydrocarbons isolated from authentic samples of other oils—e.g., cubeb oil. Retention times relative to β-caryophyllene on the SF96(50) capillary column are shown in Table I.

RESULTS AND DISCUSSION

The chromatograms for the initial separation of black pepper oil hydrocarbons on Triton-X 305 have been shown previously (Muller and Jennings, 1967a). Figure 1 (top) shows a chromatogram on the SF96(50) capillary column of peak 7 from Triton-X 305 (tail of peak 6). Figure 1 (bottom) shows a chromatogram under similar conditions of the final purification stages of peak 7–2. The degree of achieved purification illustrated by Figure 1 is typical of the results achieved in the isolation of these minor components.

α-Cubebene (I). The minor component of peak 1 was identified as α-cubebene (peak 1–2) by comparison of its infrared spectrum (Figure 2) with that of α-cubebene isolated from the oil of cubeb (*Piper cubeba* L.). The infrared spectrum of this compound, obtained either from black pepper or from cubeb oil, is similar but not identical

Table I. Retention Times, Relative to β-Caryophyllene, SF96(50) Capillary Column at 175° C.

Triton-X 305 Peak No.	Compound	Relative Retention	
1–1	δ-Elemene	0.673	(1)
1–2	α-Cubebene	0.715	(2)
2	α-Copaene	0.825	(1)
4–1	β-Elemene	0.856	(1)
4–2	α-(*cis*)-Bergamotene	0.922	(1)
4–3	α-Santalene	0.956	(1)
4–4	α-(*trans*)-Bergamotene	1.01	(1)
5–1	Isocaryophyllene	0.946	(2)
5–2	Cedrene (?)	0.986	(3)
6	β-Caryophyllene	1.000	(1)
7–1	β-Caryophyllene	1.000	(1)
7–2	β-Farnesene	1.03	(2)
7–3	Thujopsene (?)	1.05	(3)
8–2	α-Humulene	1.16	(1)
8–4	γ-Muurolene	1.21	(2)
9	β-Bisabolene	1.35	(1)
10–1	β-Selinene	1.28	(1)
10–2	α-Selinene	1.32	(1)
11	δ-Cadinene	1.42	(1)
12	Calamenene	1.47	(1)

(1) Compounds previously isolated from black pepper oil (Muller and Jennings, 1967a).
(2) Compounds isolated and identified in this study.
(3) Compounds whose presence is suspected (only retention data).

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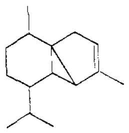
to that of α-cubebene obtained by Ohta *et al.* (1966). The discrepancy is believed to be a slight contamination (906 cm.$^{-1}$) apparent in the spectrum published by Ohta *et al.* (1966). This 906-cm.$^{-1}$ band might be due to the vinyl frequency of δ-elemene (II), the compound which precedes α-cubebene in both cubeb and black pepper oils. In the former oil, α-cubebene predominates, while in the latter, δ-elemene is the major component. The relative retention times of α-cubebene from both sources agree.

Isocaryophyllene (III). The infrared spectrum (Figure 3) of a minor component of peak 5 (face of peak 6) agrees with the infrared spectrum of isocaryophyllene published by Pliva *et al.* (1960). The same compound also was isolated from a commercial sample (Aldrich and Co.) of β-caryophyllene. Relative retention times of both compounds agree on the SF96(50) capillary column at 175° C.

β-Farnesene (IVa). Peak 7 is composed largely of β-caryophyllene, from which a minor component, peak 7–2, was separated with difficulty. This was identified as β-farnesene by comparison of its infrared spectrum with that by Pliva *et al.* (1960) and with the infrared data of Naves (1966) and Bhati (1963). The spectrum is amazingly similar to that of myrcene (IVb) by Mitzner *et al.* (1965) and Wrolstad and Jennings (1964). It differs in the split —CH_3 frequencies (1376–1383) owing to the additional methyl in β-farnesene as compared with myrcene.

γ-Muurolene (V). Peak 8–4, the second peak following α-humulene on the SF96(50) capillary column, was identified as γ-muurolene by comparison of its infrared spectrum with that by Pliva *et al.* (1960) and a spectrum of γ-muurolene by Westfelt (1966).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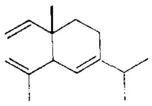
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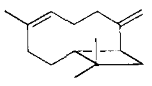
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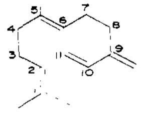

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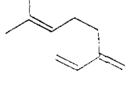
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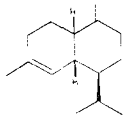

V

The numbering system for sesquiterpene hydrocarbons employed by Minyard *et al.* (1966) was used here because of its wide applicability to hydrocarbons, including those which at first appear to violate the isoprene rule. It also permits examination of biogenetic relationships, as in the case of thujopsene (XVII).

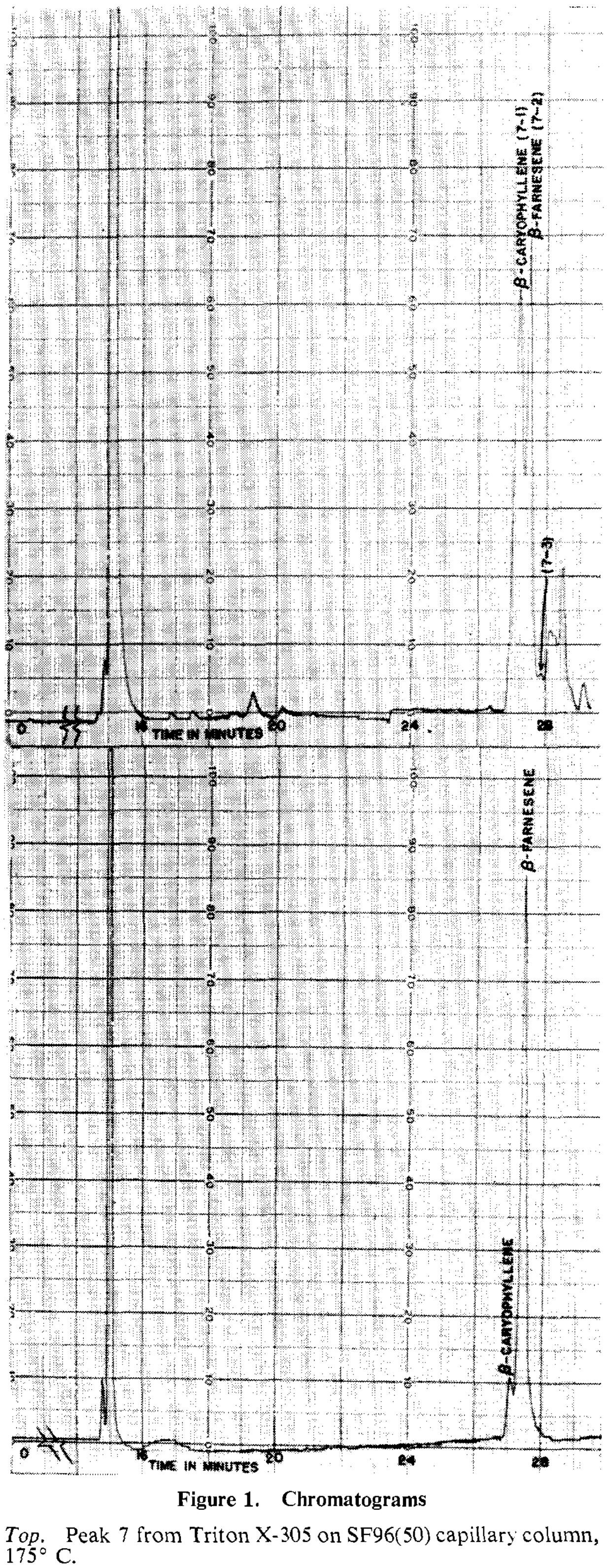


Figure 1. Chromatograms

Top. Peak 7 from Triton X-305 on SF96(50) capillary column, 175° C.

Bottom. Final purification of peak 7–2 from above; SF96(50) at 175° C.

Advanced techniques of isolation, which permit characterization of trace constituents, show that various sesquiterpenes, whose occurrence has previously been ascribed to a single species, are in fact, widespread. Among botanical relatives—e.g., *Piper cubeba* L. (cubeb) and *Piper nigrum* L. (black pepper)—striking similarities in over-all composition do exist, although the proportions of the individual compounds may vary. δ-Elemene (II), α-cubebene (I), α-copaene (the C_2 epimer of α-ylangene, XIIb), and β-caryophyllene have been found to occur in both black pepper and cubeb oil, and cedrene and thujopsene (XVII) also have been isolated from cubeb oil (Muller and Jennings, 1967, a and b). These latter two

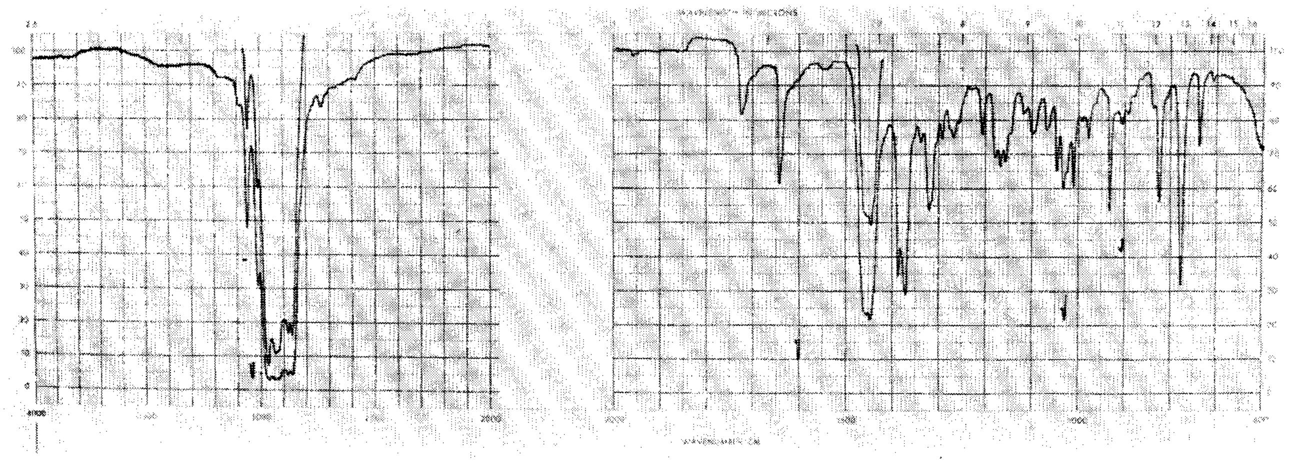


Figure 2. Infrared spectrum of α-cubebene

Polystyrene calibration marks. 3026.27, 1601.0, 1027.7, and 906.5 cm.$^{-1}$

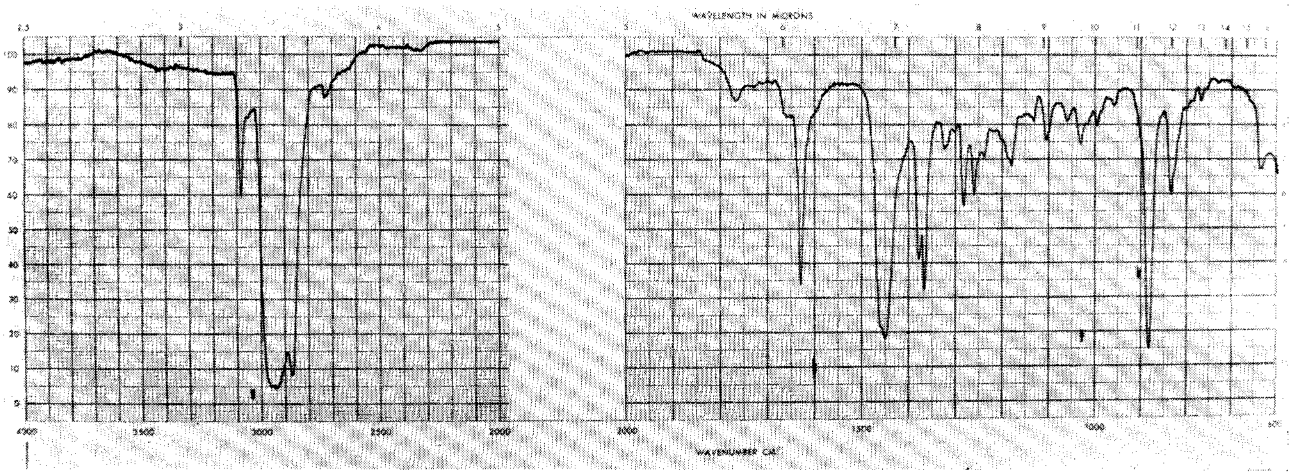


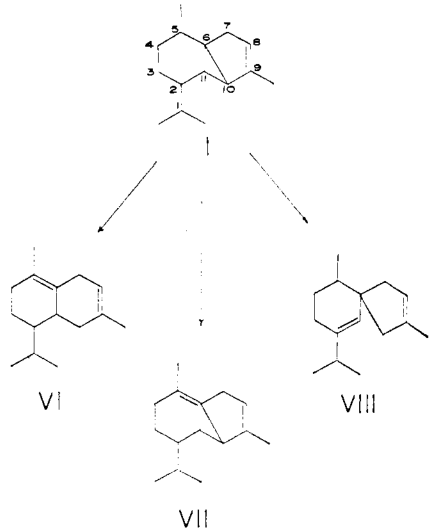
Figure 3. Infrared spectrum of isocaryophyllene

Polystyrene calibration marks. 3026.27, 1601.0, 1027.7, and 906.5 cm.$^{-1}$

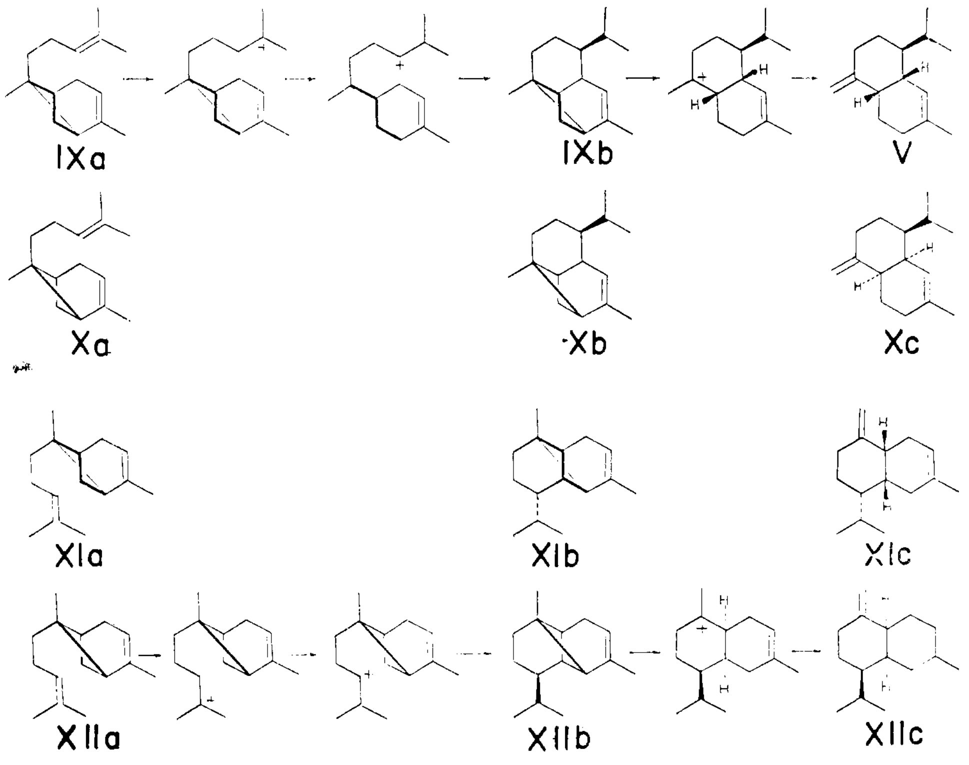
may also exist in black pepper oil, the former as a minor component of peak 5 (peak 5–2), and the latter as peak 7–3 from Triton-X 305 (Figure 1, top). Relative retention times of these peaks on the SF96(50) capillary column agree for cedrene and for thujopsene, respectively, as isolated from Texas cedar oil (International Flavors and Fragrances, New York).

The biogenesis of cedrene has been postulated as resulting from rearrangement of bisabolene (Stork and Clarke, 1955). Isocaryophyllene (III) might be considered as derived from β-caryophyllene by trans-cis isomerization of the C_5-C_6 double bond. This might occur during extraction of the oil, in which case isocaryophyllene might be considered an artifact.

α-Cubebene appears somewhat heat-labile; its cyclopropane ring might open in any one of three positions to produce δ-cadinene (VI) (C_6-C_{10} opening), a guaiene (VII) (C_6-C_{11} opening), or a spiro compound possessing the acoranene skeleton (VIII) (C_{10}-C_{11} opening) (Dauben, 1967). This may explain the production of a blue liquid (probably an azulene) when α-cubebene is heated above 220° C.



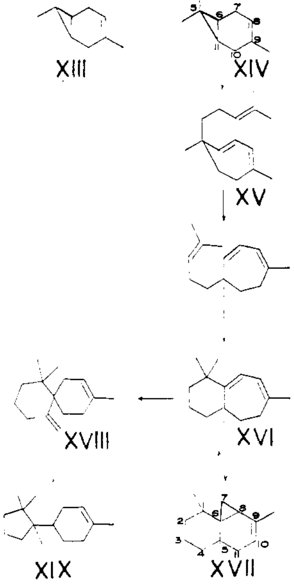
γ-Muurolene (V) could be derived from an α-(*cis*)-bergamotene (IXa) (*cis* refers here to the configuration of the isoprene tail with respect to the ring) by a mechanism in which a carbonium ion, initially formed at C_1, forms through a hydride shift a second carbonium ion at C_2, which then cyclizes across C_7 to form intermediate IXb. The cyclobutane ring of this latter might then open between C_5-C_{10} to produce, among other products, γ-muurolene (V). The four-membered ring C_5-C_6-C_{11}-C_{10} fixes the structure so that ring closure from C_2 to C_7 will produce a cis-locked Decalin system. The double bond between C_8 and C_9 takes no part in the closure or in the subsequent formation of the double bond at C_5; therefore, if the closure occurs via a cis chain isomer such as IXa, the product obtained will be V (γ-muurolene). If closure occurs via a trans chain isomer such as XIIa, one of the products formed will be XIIc ($γ_2$-amorphene).



Amorphenes and muurolenes are C_2 epimers (Motl *et al.*, 1965, 1966; Westfelt, 1966); which isomer is formed depends on whether the attack on the carbonium ion at closure is effected axially or equatorially. Closure of the ion from XIIa will lead to two epimeric compounds: α-ylangene (XIIb) and α-copaene (Motl *et al.*, 1966; Westfelt, 1967).

In view of the above, possibly the α-(*cis*)-bergamotene recently isolated from black pepper oil (Muller and Jennings, 1967a) is the isomer IXa instead of Xa. The presence of γ-muurolene gives some weight to this idea. Possibly also both α-copaene and α-ylangene might be present in black pepper. While these should have slightly different retention times on the SF96(50) capillary column (Teranishi, 1967), the similarity of their infrared spectra (Hunter and Brogden, 1964; Westfelt, 1967) might cause confusion.

Several compounds such as cuparene (Enzell and Erdtman, 1958), cuprenenes (Dauben and Oberhansli, 1966), chamigrene (Ito *et al.*, 1967), and, recently, longifolene have been related to thujopsene through widdrol (Dauben and Friedrich, 1967). The biogenesis of thujopsene (XVII) is still somewhat obscure. The precursor of thujopsene might be envisioned as compound XIV [α-(*cis*)-carenene (?)—the isoprenolog of 3-carene, XIII—] whose cyclopropane ring could open between C_6 and C_{11} to produce compound XV, which could then close from C_1 to C_6 to produce XVI. This intermediate (XVI), the hydrocarbon corresponding to widdrol, could then produce thujopsene (XVII) or chamigrene (XVIII), the spiro compound which in turn could give rise to cuparenes (XIX)



In black pepper oil, several of the isoprenologs of the monoterpenes have been found (Muller and Jennings, 1967a); these results are consistent with the postulate of Minyard *et al.* (1966) that similar biochemical mechanisms exist for the production of monoterpenes and their sesquiterpene analogs. Thus, it is reasonable to expect the presence of α-(*cis*)carenene in black pepper oil where 3-carene (XIII) has been found in large amounts by Wrolstad and Jennings (1965).

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