Constituents of Black Pepper. Some Sesquiterpene Hydrocarbons

C. J. Muller and W. G. Jennings

Individual sesquiterpene hydrocarbons were isolated from black pepper oil by column chromatography followed by preparative and capillary gas chromatography. On the basis of matching retentions, infrared and, where applicable, ultraviolet spectra of isolated components, the following were identified as major compounds of the sesquiterpene hydrocarbon fraction: δ-elemene, α-copaene, β-elemene, α-cis-bergamotene, α-santalene, α-trans-bergamotene, β-caryophyllene, α-humulene, β-selinene, α-selinene, β-bisabolene, δ-cadinene, and calamenene.

Previous papers (Hasselstrom et al., 1957; Ikeda et al., 1962; Wrolstad and Jennings, 1965) have considered the monoterpene hydrocarbons of black pepper (Piper nigra) oil. β-Caryophyllene, comprising about 90% of the sesquiterpene hydrocarbon fraction, has also been previously reported (Hasselstrom et al., 1957; Wrolstad and Jennings, 1965). The remaining sesquiterpene hydrocarbons represent a rather complex mixture in which over 25 different peaks can be demonstrated by gas chromatography. This work, a continuation of efforts carried out in this laboratory on the elucidation of volatile constituents of foods, deals primarily with the major constituents of this fraction.

EXPERIMENTAL

Apparatus. Preparative scale gas chromatographic separations were performed in an Aerograph Autoprep modified as described by Wrolstad and Jennings (1965). Modified Beckman Thermotrac units fitted with Hamilton glass-lined injectors were used, with packed columns for intermediate separations, and with capillary columns for final purifications. Trapping of the separated components was accomplished in thin-walled glass capillaries (Jennings et al., 1964). Gas chromatographic conditions are summarized in Table I.

Infrared spectra were taken on thin films between sodium chloride plates on a Beckman IR-8 spectrophotometer with beam condenser. Ultraviolet spectra were determined on a Beckman DB using spectrograde isooctane as solvent.

Materials and Procedure. Steam-distilled Ceylon black pepper oil was obtained from the research department of W. J. Stange Co., Chicago.

The hydrocarbon fraction of black pepper oil was freed from oxygenated materials by passage through short (10 cm.) basic aluminum oxide columns using n-hexane as the eluting solvent. The sesquiterpenes were separated from the monoterpenes by repeated 25-μl. injections on the Triton-X 305 column. Although the column will tolerate injections as large as 250 μl., better preliminary separations were achieved by injecting smaller samples.

When available, sesquiterpene hydrocarbons for use as standards were obtained by isolation and purification from authentic samples of other oils—e.g., celery seed

Department of Food Science and Technology, University of California, Davis, Calif. 95616.

oil—as described above. Retention times relative to β-caryophyllene were calculated on the SF-90(50) capillary column for each purified peak of the standards and for those of black pepper oil (Table II).

Figure 1 shows a chromatogram of 25 μl. of sesquiterpene hydrocarbons from black pepper on Triton-X 305 at 160° C. The 12 peaks indicated were collected in capillary tubes and reinjected successively, using CCl₄ as solvent, on Carbowax 20M and Apiezon-L at 175° and 200° C., respectively. For the final purifications, repetitive 0.1-μl. injections of CCl₄ solutions of purified fractions were required to yield sufficient material for infrared analysis. In spite of the high resolution of the SF96(50) capillary column, 40 to 50 repetitive collections and reinjections were required to free certain peaks from adjacent impurities. None of the peaks originally collected from the Triton-X column was due to a single compound; most, upon injection into the SF96(50) capillary column, were further resolved.

RESULTS AND DISCUSSION

δ-**Elemene.** The main component of peak 1 was tentatively identified as δ-elemene (I), by comparison of its infrared spectrum with that published by Gough and Sutherland (1964). Figure 2 shows the infrared spectrum of this compound and that of β-elemene (II), which it resembles. They differ markedly in the relative intensities of the vinyl (906 cm.⁻¹) and vinylidene (895 cm.⁻¹) frequencies. The absorptions centered around 1360 to 1382 cm.⁻¹ in the spectrum of δ-elemene indicate the presence of a gem-dimethyl group (Horák and Plíva, 1960), whereas in β-elemene this group is absent (singlet). δ-Elemene exhibits only end absorption below 2200 A. indicating the absence of conjugation which differentiates it from α-elemene (III).
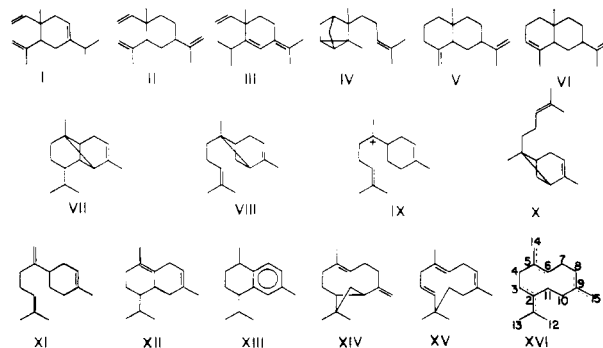
Table I. Gas-Chromatographic Conditions

	Preparative Triton-X 305	Packed Carbowax 20M	Packed Apiezon-L	Capillary SF96(50)
Column length, feet	8	10	10	500
Column diameter, inches	0.25 (O.D.)	0.125 (O.D.)	0.125 (O.D.)	0.030 (I.D.)
Solid support	60–80 Gas Pack F	60–80 Gas Pack F	60–80 Gas Pack F	...
Stationary phase, %	10	5	5	...
Carrier gas flow, ml./min.	60	50	50	6
Temperature, ° C.				
Injector	200	200	200	200
Detector-collector	220	210	210	210
Column	160	175	200	175

α-Copaene. The infrared spectrum of the major component of peak 2 agrees with that of α-copaene (VII), published by DeMayo et al. (1965), and with that of a purified sample of α-copaene isolated from Copaiba balsam oil. Relative retention times on SF96(50) at 175° C. are also in agreement.

β-Elemene. Peak 4 from the Triton-X 305 column was resolved on SF96(50) into four major peaks (Figure 3). The infrared spectrum of the first of these (Figure 2) agrees well with that of β-elemene (II) published by Asselineau and Asselineau (1957) and Hunter and Parks (1964). However, Hunter and Parks suggested that their spectrum was not that of a pure material; similar indications exist in the spectrum shown here—e.g., the presence of the band at 790 cm.⁻¹, and the reduced intensity of the vinylidene band at 895 cm.⁻¹ which, since it corresponds to two vinylidene groups, should be more intense. This compound seems to be very labile, and the possibility of its isomerization on the detector and collector is being investigated. Ultraviolet scanning showed no maxima above 2200 A.

α-trans-Bergamotene and α-cis-Bergamotene. The infrared spectrum of the fourth major peak on SF96(50) (Figure 4, bottom) matches that of α-trans-bergamotene (VIII) by Kovats (1963) and is amazingly similar to that of the second major peak (Figure 4, top). This latter compound is probably α-cis-bergamotene (X).

α-Santalene. The third major peak on SF96(50) is mostly α-santalene (IV). Its infrared spectrum agrees very closely with that of α-santelene by Herout et al. (1957); however, a trace of contamination, shown as a small shoulder on the tail of the major peak, is apparent upon rechromatography of the material used for infrared analysis.

β-Caryophyllene. Peak 6 is mainly β-caryophyllene (XIV), as shown by its infrared spectrum which matches

Table II. Retention Times Relative to β-Caryophyllene on the SF96(50) Capillary Column at 175° C.

Triton-X Peak No.	Compound	RRT
1	δ-Elemene	0.673
2	α-Copaene	0.825
4–1	β-Elemene	0.856
4–2	α-cis-Bergamotene(?)	0.922
4–3	α-Santalene	0.956
4–4	α-trans-Bergamotene	1.01
6	β-Caryophyllene	1.000
8	α-Humulene	1.16
9	β-Bisabolene	1.35
10–1	β-Selinene	1.28
10–2	α-Selinene	1.32
11	δ-Cadinene	1.42
12	Calamenene	1.47

that of a purified sample of commercial (Aldrich Co.) β-caryophyllene, that of the purified compound obtained from Copaiba balsam oil, and that reported by Nigam and Levi (1962). Various other peaks are apparent on inspection of the face and tail of peak 6 (peaks 5 and 7); these are currently under investigation.

α-Humulene. Peak 8 is mainly α-humulene (XV) as indicated by comparison of its infrared spectrum with that by Benesová et al. (1961), and that by Dev (1960).

β- and α-Selinenes. Peak 10 resolved into β-selinene (V) and α-selinene (VI), in addition to other smaller constituents. The selinenes were identified by comparison of their infrared spectra with that of the purified compounds obtained from celery seed oil. In addition, the infrared spectrum of β-selinene agreed with that of β-selinene by Bawdekar and Kelkar (1965). Relative retention times of the authentic compounds also agreed with those from black pepper oil.
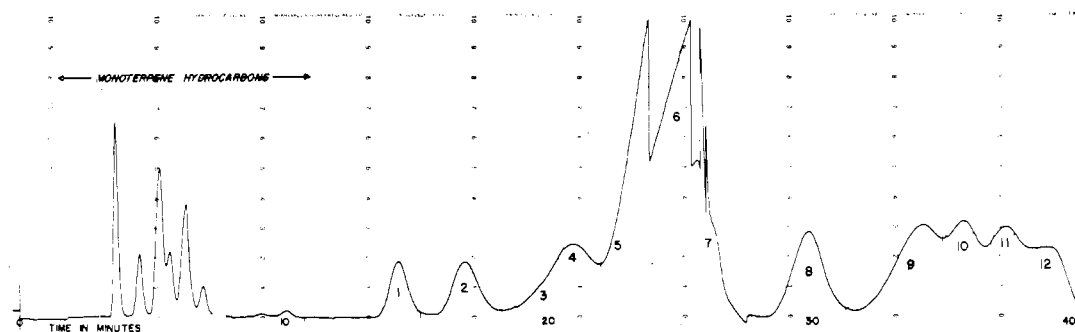

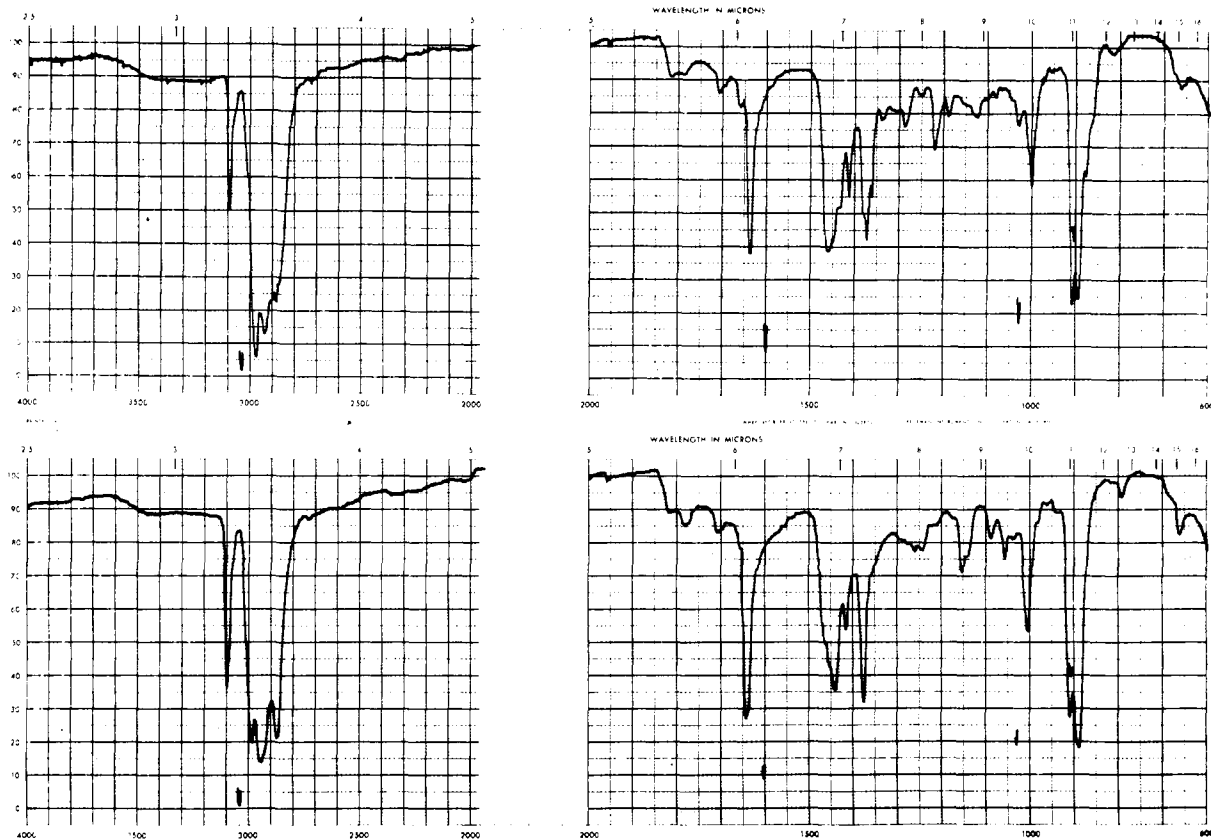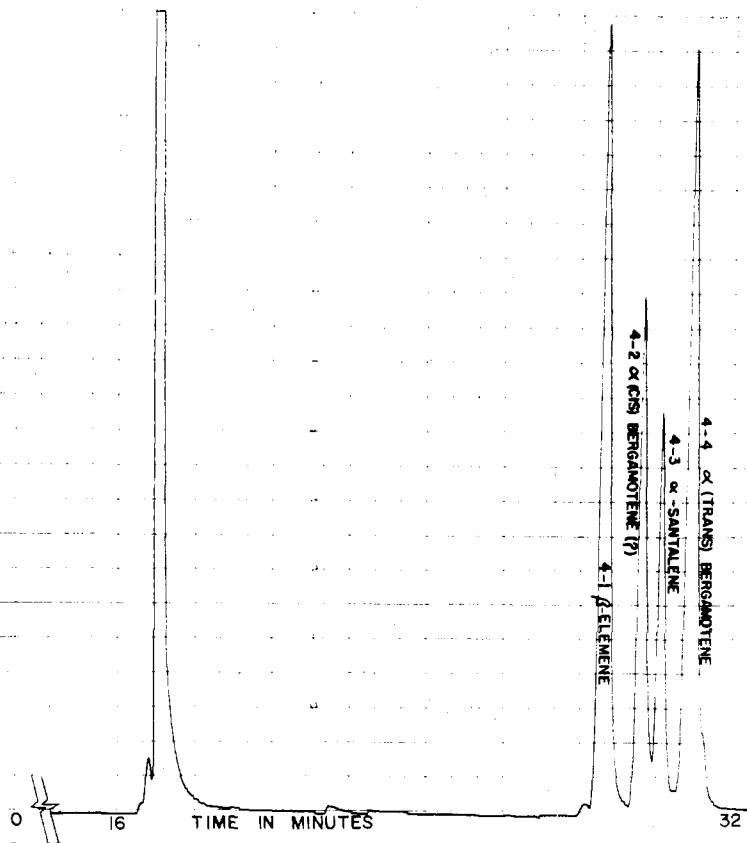Figure 1. Gas chromatogram of 25 μl. of black pepper oil hydrocarbons on the Triton-X 305 preparative column

Figure 2. Infrared spectrum of δ-elemene (I) (top). Infrared spectrum of β-elemene (II) (bottom)

Figure 3. Gas chromatogram of 0.2 μl. of peak 4 from Triton-X 305 on the SF96(50) capillary column at 175° C. (CCl₄ solvent)
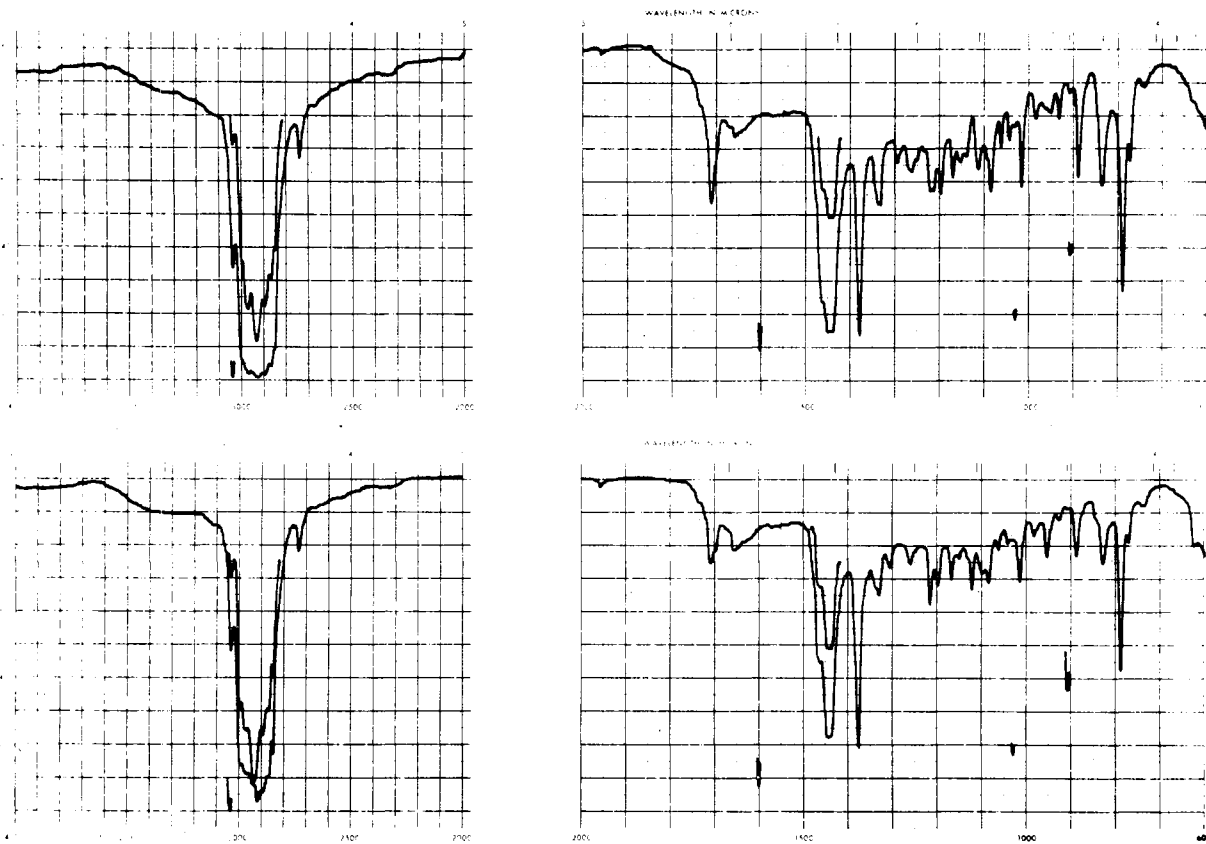
Figure 4.   Infrared spectrum of peak 4-2 [α-*cis*-bergamotene (?), X] (top).   Infrared spectrum of peak 4-4 (α-*trans*-bergamotene, VIII) (bottom)

β-Bisabolene. Of various peaks present in fraction 9, β-bisabolene (XI), was tentatively identified by comparison of its infrared spectrum with that in the literature (Plíva *et al.*, 1951).

δ-Cadinene. Peak 11 appeared to be a rather complex mixture of which δ-cadinene (XII), one of the last peaks on SF96(50), was the main component. Its infrared spectrum shows some similarity to those in the literature (Plíva *et al.*, 1953) but agrees much more closely with δ-cadinene obtained from Copaiba balsam oil. Owing to the small amount of material available, its optical rotation was not determined; thus, the presence of an optical isomer is entirely possible.

Calamenene. Peak 12 was also a complex mixture. Calamenene (XIII) was identified by its infrared spectrum which matched that in the literature (Šorm *et al.*, 1953; Tyagi *et al.*, 1963), and that of the purified compound obtained from cubeb oil (Vonášek *et al.*, 1960). Relative retention times on SF96(50) at 175° C. agreed for both compounds.

CONCLUSIONS

The numbering system used here is that of Minyard *et al.* (1966), which seems best suited for sesquiterpene nomenclature, since it can easily be applied to any of the sesquiterpenes.

The extreme similarity of the infrared spectra of what is believed to be α-*cis*-bergamotene (peak 4-2) and α-*trans*-bergamotene (Peak 4-4) is truly remarkable. Except for

the fact that both peaks (4-2 and 4-4) were resolved on the capillary SF96(50) from the same original Triton-X 305 fraction and so obviously had different retention times, they might have been regarded as the same compound. On SF96(50), the trans isomer emerged after β-caryophyllene, while the cis (?) isomer emerges before (Table II). Both compounds emerge before β-caryophyllene on Triton-X 305 and on Carbowax 20M. Nigam and Levi (1966) report a relative retention time longer than β-caryophyllene on SE-30 for an α-bergamotene. In this respect, and judging by the similarity of behavior between Apiezon-L and SF96(50) columns, there seems to be a discrepancy between our results and those of Minyard *et al.* (1966) who found an α-bergamotene which emerges before β-caryophyllene on both Apiezon-L and Carbowax 4000 columns. They assigned the trans configuration to this compound on the basis of comparison of its PMR spectra with that of Kovats (1963). In the work reported here, the compound which emerges after β-caryophyllene on SF96(50) matches the infrared spectra of the trans isomer by Kovats (1963) and seems to indicate that either Apiezon-L and SF96(50) do not behave similarly with respect to these compounds, or that some clarification is needed on this point.

If the identity of compound X (tentatively named cis here) holds true, its presence and that of the trans isomer seem to indicate that a precursor such as IX as suggested by Kulkarni *et al.* (1963, 1966) might be involved. The fact that β-bisabolene and α-santalene are present in black

pepper oil seems to substantiate their assumption and that of Ruzicka (1959). Further investigation of the sesquiterpene hydrocarbons present in lesser quantities in this oil might reveal the presence of both β isomers of bergamotene as well.

The presence of various compounds belonging to the cadinene and selinene series in the oil seems to indicate that perhaps a 10-carbon ring compound such as a germacrene (XVI), capable of forming carbonium ions which may close across C_6 and C_{11} (to give rise to cadinenes) or across C_5 and C_{10} (to give selinenes), might be a precursor of both series. Such a compound might also give rise to compounds of the guaiene series by closure across C_6 and C_{10}. Examination of models indicates that this is feasible. Recent reports on the isolation of germacrenelike lactones (Joshi *et al.*, 1967) seem to offer support for this hypothesis, but much more information on the constituents of this and other oils is required.

ACKNOWLEDGMENT

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