Constituents of Black Pepper

Some Oxygenated Compounds

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The polar compounds of oil of black pepper were separated by column chromatography, and resolved by repetitive gas chromatographic separations. Infrared, mass, and ultraviolet spectroscopic studies on isolated fractions resulted in the identification of linalool, 1-terpinen-4-ol, α-terpineol, cryptone, carvone, *p*-cymene-8-ol, *trans*-carveol, *cis*-carveol, safrole, ar-curcumene, methyl eugenol, nerolidol, and myristicin. Spectral data was used to deduced a probable structure, yet unreported.

A previous paper (Jennings and Wrolstad, 1961) reviewed earlier work on the composition of oil of black pepper *Piper nigrum*, and reported the identification of several new constituents. Subsequent work (Muller and Jennings, 1967; Muller *et al.*, 1968; Russell *et al.*, 1968; Wrolstad and Jennings, 1965) identified a number of mono and sesquiterpene hydrocarbons, and linalool. The present study was directed specifically toward the oxygenated compounds of oil of black pepper.

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, and 60 ml. per minute He flow rate. Secondary separations utilized a modified Beckman Thermotrac with Hamilton glass-lined injectors and Carle micro thermistor detection. The dual columns were 10-foot × 0.125-inch (O.D.) stainless steel, one packed with 5% (w./w.) Carbowax 20M on 60- to 80-mesh AW-DMCS treated Chromosorb G; the other was packed with 5% (w/w.) SF-96(50) admixed with 5% (w./w.) Igepal CO-880 on 60- to 80-mesh AW-DMCS Chromosorb G. Final separations utilized a second Beckman Thermotrac similarly modified, but containing dual 500-foot × 0.03-inch (I.D.) stainless steel open-tubular capillary columns, one coated with SF96(50) admixed with 5% (w./w.) Igepal CO-880, the other with OV-17 admixed with 5% (w./w.) Igepal CO-880. Operating temperatures for the secondary and final separations were varied according to the characteristics of the fractions being separated.

Eluted fractions were trapped in thin-walled glass capillaries, as described previously (Jennings *et al.*, 1964). Infrared spectra were taken on thin films between NaCl plates on a Beckman IR-8 infrared spectrophotometer fitted with a 5× beam condenser. Ultraviolet spectra were determined with a Beckman DB spectrophotometer using spectral grade isooctane except as noted. Mass spectra were obtained with a Varian M66 mass spectrometer by inserting the capillary collection tube directly into the sample inlet system. Accurate mass determinations were made using a Varian V-5560 reference kit.

Materials and Procedure. Steam distilled Ceylon black pepper oil (Lot AC) obtained from the research department of W. J. Stange Co., Chicago, was separated on short (10-cm.) columns of basic alumina (J. T. Baker Co.) following the procedure of Muller and Jennings (1967). The hydrocarbon fraction was removed with *n*-hexane, and the oxygenated materials were then eluted with methanol. The latter fraction was separated into main fractions on the Triton X-305 column by repetitive 50-μl. injections of the MeOH eluent and ballistic programming from 110° to 200° C. and holding the temperature isothermally at 200° C. for the duration of the run. Individual fractions were sealed in capillary tubes and held at −20° C. Each fraction was reinjected on higher efficiency packed columns, and the fractions collected were injected and collected successively on both OV17 and SF96-(50) capillary columns.

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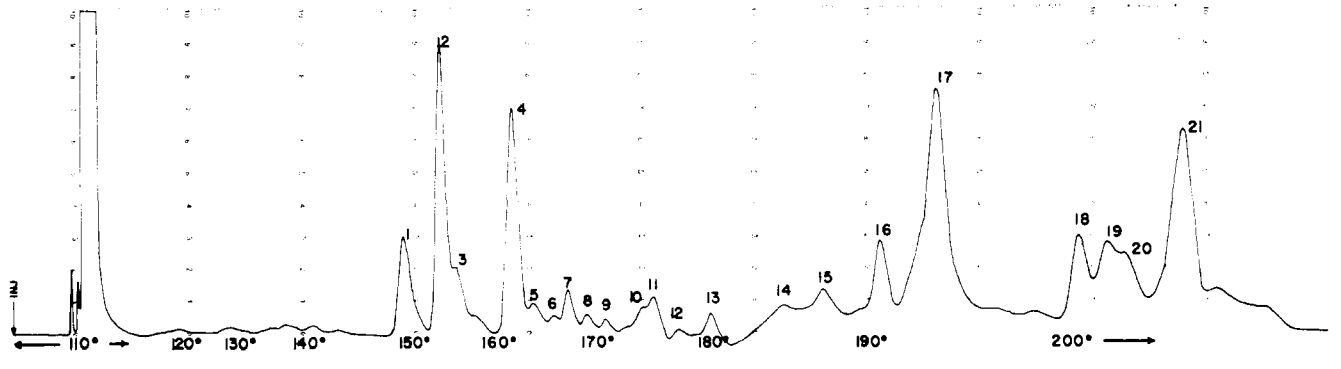


Figure 1. Typical chromatogram

50-μl. injection of methanol eluent of oxygenated fraction of oil of black pepper on Triton X-305 preparative column. Programmed 110° to 200°C.

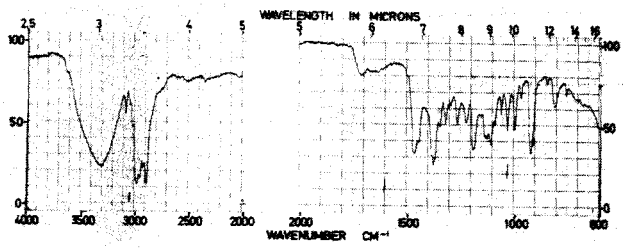


Figure 2. Infrared spectrum of peak 1

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of a 50-μl. injection of the methanol eluent of the oxygenated fraction. After emergence of the solvent peak, the temperature was programmed ballistically from 110° to 200° C. The first broad peaks up to the peak labeled 1 were composed of a large number of more volatile components. Attempts to isolate individual compounds from this section met with only limited success. Any particular cut from this region, when reinjected on a dissimilar column, e.g. SF96(50), yielded a large number of peaks, each of which again refractionated on OV-17 to yield a large number of minute fractions. A great deal of further work will be required to isolate significant quantities of these trace low boilers.

Peak 1 was a solid melting at 60–61° C. The infrared spectrum, taken as a melt between NaCl plates, is shown in Figure 2. Although the sample had been highly purified prior to infrared analysis, subsequent reinjection on a capillary column indicated a trace of impurity had developed. The mass spectrum, Figure 3, shows a 136 peak, which accurate mass determinations showed to be $C_{10}H_{16}$. The parent ion (*m/e* 154) and the strong hydroxyl absorption in the infrared (3400 cm.$^{-1}$) would then give a molecular formula of $C_{10}H_{18}O$. Hasselstrom *et al.* (1957) also reported a solid melting at 61° C. which he identified as epoxydihydrocaryophyllene, whose structure is not compatible with these spectral data and whose presence was not confirmed in the present study.

The prominent P-43 peak in the mass spectrum is indicative of the loss of either a propyl or an isopropyl group; infrared absorptions at 1360 cm.$^{-1}$ and 1380 cm.$^{-1}$ on both sides of the methyl absorption band at 1370 cm.$^{-1}$ support the concept of an isopropyl group. The infrared data also show prominent absorption bands at 3080 cm.$^{-1}$, 990 cm.$^{-1}$, and 910 cm.$^{-1}$, indicative of a terminal vinyl group. The compound exhibited no ultraviolet absorption in iso-octane. The infrared spectrum, when run on a CCl_4 solution, showed the hydroxyl absorption to be centered at 3615 cm.$^{-1}$, indicative of a tertiary alcohol. Comparisons of the mass spectrum with that reported for linalool by von Sydow (1963) and with that obtained from linalool in this study (Figure 5) indicate structural features resembling those of linalool. A major difference is the strong *m/e* 111 peak, absent in linalool, which lacks the isopropyl function and prefers an allylic cleavage.

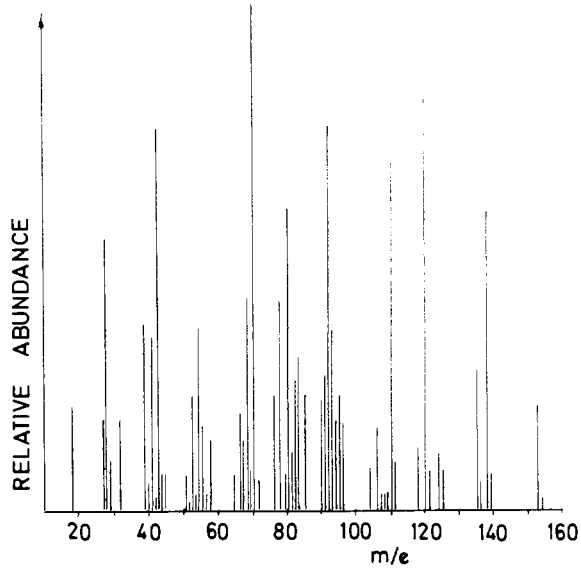
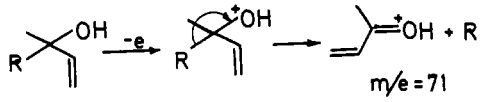
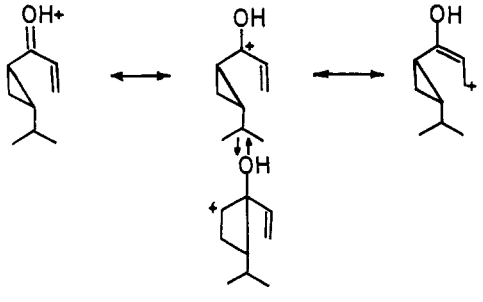


Figure 3. Mass spectrum of peak 1

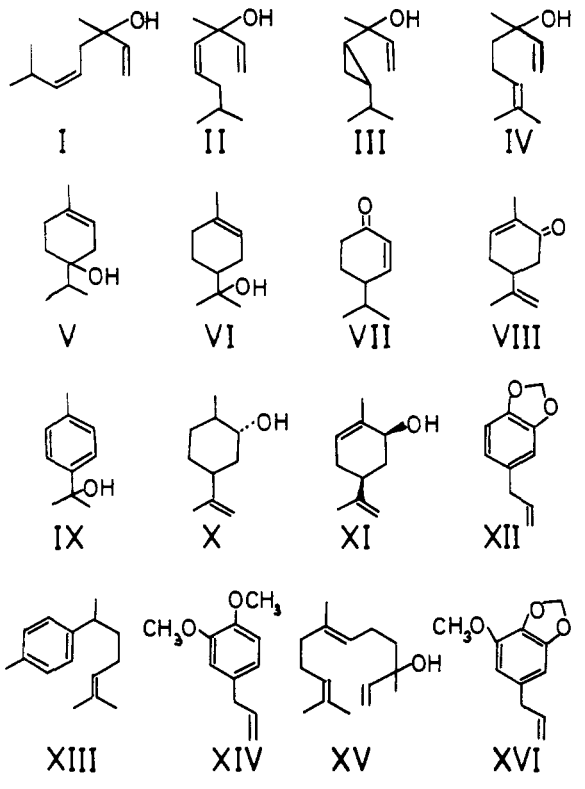
Three structures, represented by I, II, and III, are consistent with these spectral data. The disubstituted double bond in I must be cis as a trans bond in this position would have given strong infrared absorption at 965 cm.$^{-1}$ (Nakanishi, 1962). The position of the tertiary hydroxyl group is indicated by the *m/e* 71 base peak, probably arising from cleavage by the following route:



Another distinctive difference between the mass spectra of peak 1 and linalool is the strong parent ion at *m/e* 154. This weakens support for structure I, as such a compound would be expected to readily lose the tertiary hydroxyl and dehydrate into a triconjugated system. Structure II would also be expected to lose the hydroxyl upon electron impact, and produce an exo-conjugated system. There are also three positions possible for allylic cleavage in structure II, one being allylic to the vinyl group to give the *m/e* 71 peak. The other double bond would be expected to give allylic cleavage for both the isopropyl group and the vinyl group. The fact that there is no P-vinyl peak in the mass spectrum is evidence against structure II. The spectrum of peak 1 has an *m/e* 139 peak *ca.* twice that of the *m/e* 136 peak, indicating that the molecule loses a methyl group more easily than it dehydrates. Structure III would be consistent with this phenomenon, because of the stability of the product formed from loss of the methyl group:



The cyclopropane ring would contribute to a more rigid molecule, and such a hindered alcohol might be expected to be a solid; most monoterpene alcohols (excepting the rigid norbornane-skeleton alcohols and verbanols) are liquids at room temperature. An NMR spectrum would be of great value in confirming this structure, but the amounts of material available precluded high resolution NMR spectroscopy with facilities available.



Peak 2 was identified as linalool (IV), whose infrared spectrum (Figure 4) agrees with that obtained from an authentic sample (Aldrich and Co.). The mass spectrum is compatible with the structure of linalool, and closely matches that reported by von Sydow (1963). Peaks 3, 5, 6, and those emerging after peak 16 (except the major component of peak 18) remain unidentified. A major problem is the lability of these compounds to our techniques of separation. A high degree of degradation and rearrangement is exhibited by many of these compounds, a degree which was not eliminated by using freshly-cleaned glass-lined injectors, relatively cool detectors, and by guarding against acid catalysis. A number of these compounds appear to be terpene alcohols, and while these studies did reveal certain gross structural features for some, a tremendous amount of effort (and technique development?) will be required before their structures can be specified with any degree of certainty.

Peak 4 was identified as 1-terpinen-4-ol (V), whose infrared spectrum agrees closely with that reported by Mitzner *et al.* (1968). The mass spectrum (Figure 5) is compatible with this structure, and an accurate mass determination on the *m/e* 154 peak indicates a molecular formula $C_{10}H_{18}O$.

Peak 7 was shown to be α-terpineol (VI). The infrared spectrum and the mass spectrum (Figure 6) are compatible with this structure. The latter displays a prominent *m/e* 136 peak, and a smaller *m/e* 139 peak. The hydroxyl absorption

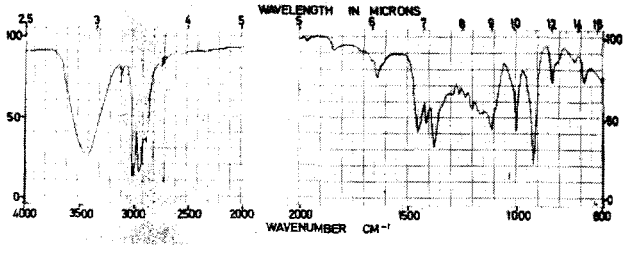


Figure 4. Infrared spectrum of peak 2, agreeing with that of linalool (IV)

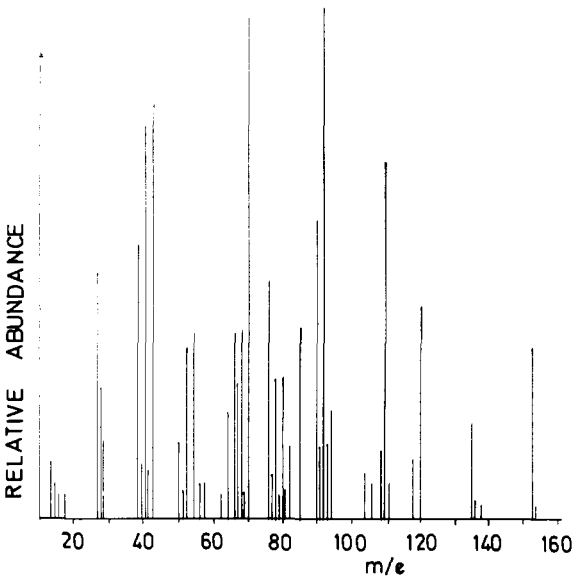


Figure 5. Mass spectrum of peak 4, 1-terpinen-4-ol (V)

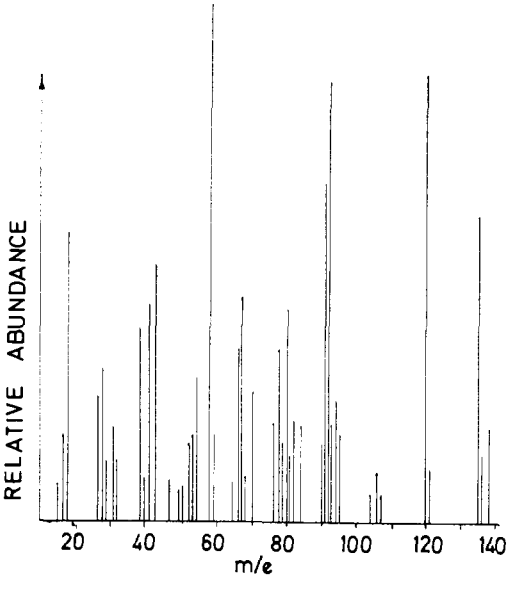


Figure 6. Mass spectrum of peak 7, α-terpineol (VI)

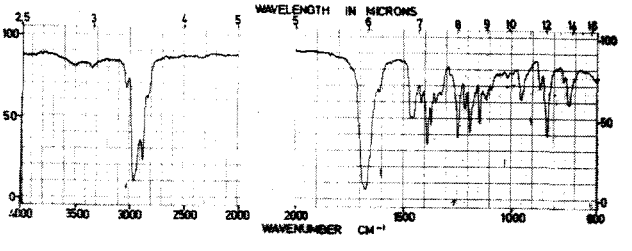


Figure 7. Infrared spectrum of peak 8

exhibited by the infrared spectrum suggests that the *m/e* 136 peak is a P-18 peak, and that the *m/e* 139 peak is a P-15 peak of an alcohol whose molecular formula is $C_{10}H_{18}O$. The infrared spectrum is in close agreement with that of Mitzner *et al.* (1968) for α-terpineol.

Peak 8, whose infrared and mass spectra appear in Figures 7 and 8, respectively, is probably cryptone (VII). The mass spectrum has a parent ion, accurate mass determination

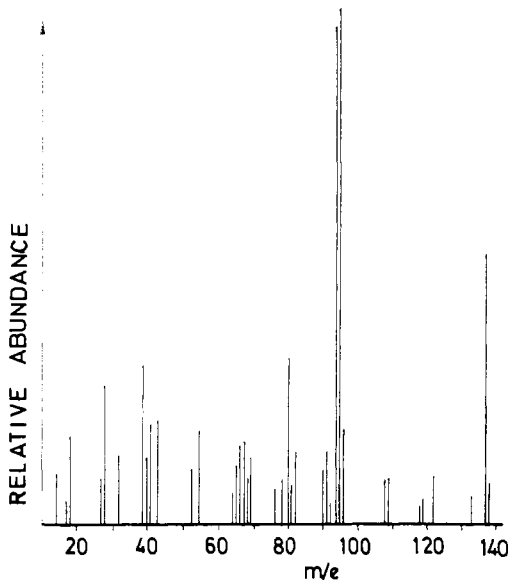


Figure 8. Mass spectrum of peak 8

yielding 138.106. This is in reasonable agreement with the theoretical value of 138.1045 for $C_9H_{14}O$. The carbonyl absorption at 1675 cm.$^{-1}$ indicates a conjugated ketone (Nakanishi, 1962), and the gem dimethyl moiety is supported by the absorptions at 1370 and 1390 cm^{-1}. An ultraviolet spectrum in ethanol showed a λ_{max} at 227 nm., agreeing with that reported by Soffer and Jevnik (1955). The presence of cryptone in pepper oil has been previously reported (Hasselstrom *et al.*, 1957).

The main component of peak 10 was identified as carvone (VIII). The infrared spectrum is identical to that reported in the Sadtler Reference Spectra, and to that of the main fraction of caraway seed, which has been reported to be carvone (Guenther, 1949).

A Carbowax 20M column resolved peak 11 into two major fractions. The first (11-A) was identified as *p*-cymene-8-ol (IX). The infrared spectrum matches that reported by Mitzner *et al.* (1968) and that reported by Andersson and von Sydow (1964). The mass spectrum (Figure 9) shows a strong

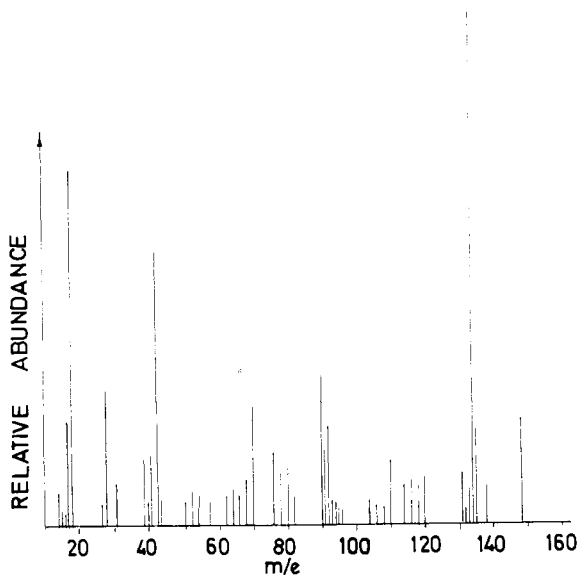


Figure 9. Mass spectrum of peak 11A, *p*-cymene-8-ol (IX)

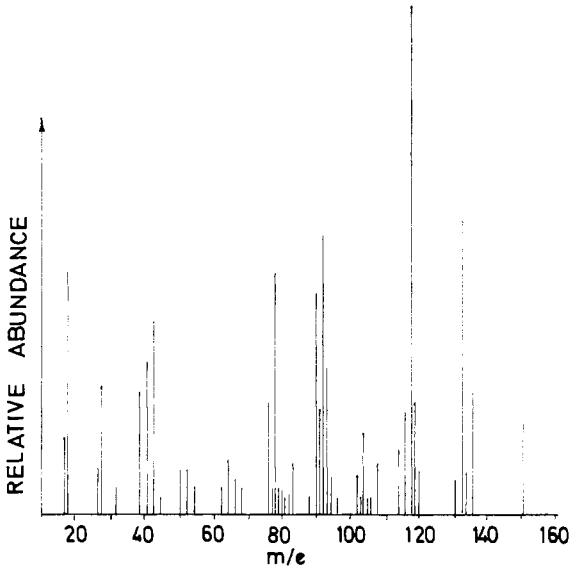


Figure 10. Mass spectrum of peak 12, *cis*-carveol (XI)

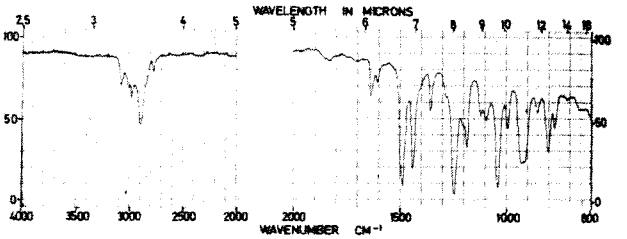


Figure 11. Infrared spectrum of peak 13, safrole (XII)

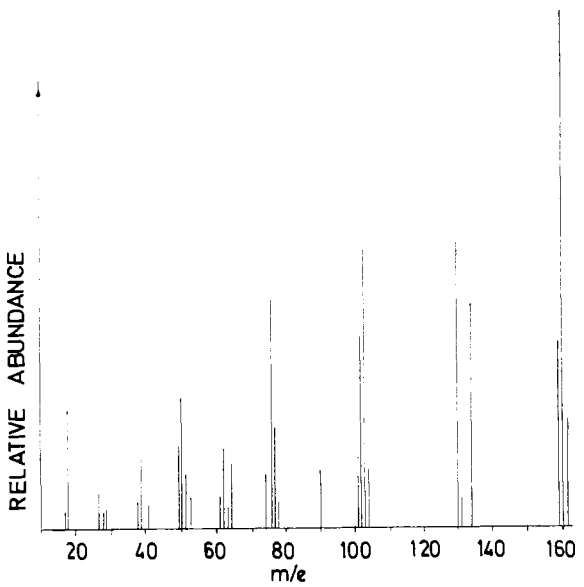


Figure 12. Mass spectrum of peak 13, safrole (XII)

parent peak at *m/e* 150 and a P-15 base peak similar to that reported by Andersson and von Sydow (1964).

The second component (peak 11-B) was *trans*-carveol (X); the infrared spectrum was a precise match for that reported by Mitzner *et al.* (1968).

Peak 12 was identified as *cis*-carveol (XI), whose infrared spectrum agrees with that published by Mitzner *et al.* (1968). The mass spectrum (Figure 10) is compatible with the assigned structure, and exhibits a parent peak at *m/e* 152.

Peak 13 was safrole (XII). Figures 11 and 12 show the in-

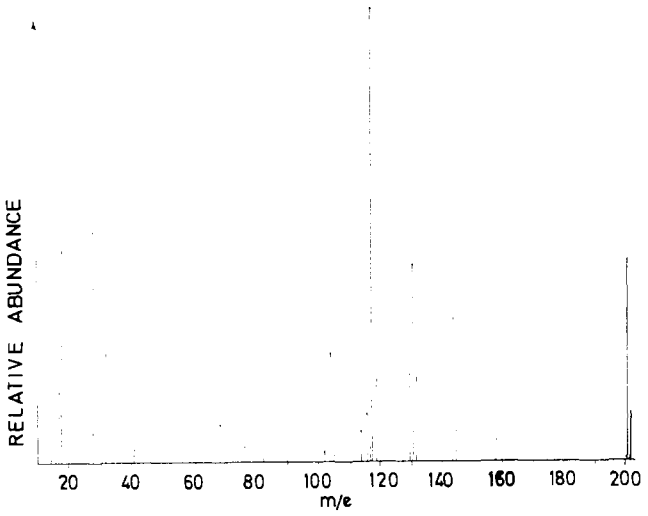


Figure 13. Mass spectrum of peak 14, ar-curcumene (XIII)

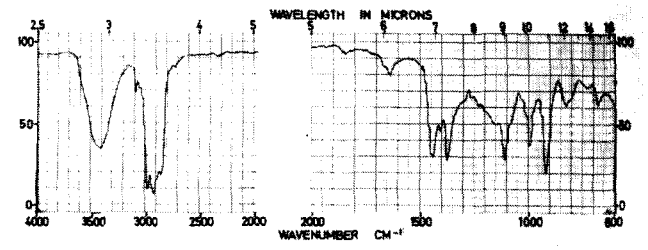


Figure 14. Infrared spectrum of peak 16, nerolidol (XV)

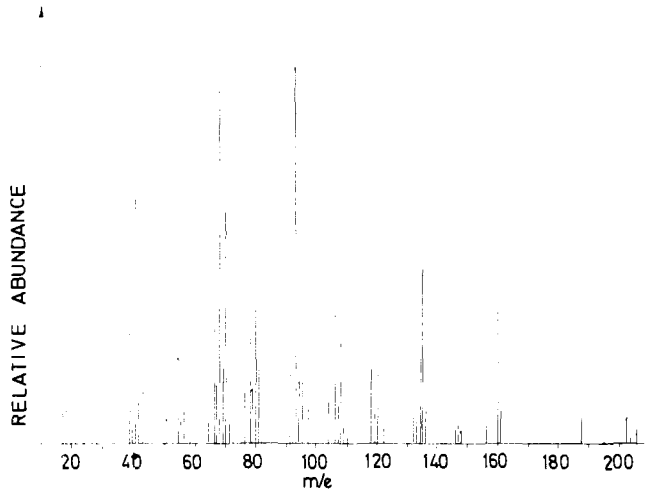


Figure 15. Mass spectrum of peak 16, nerolidol (XV)

frared and mass spectra, respectively. The latter displays a strong parent ion whose accurate mass was *m/e* 162.067, in good agreement with the value of 162.0681 calculated for $C_{10}H_{10}O_2$. The infrared spectrum matches that of a commercial sample (MC and B) and retention data for the two agree.

Peak 14 was identified as ar-curcumene (XIII). The infrared spectrum shows no prominent absorptions attributable to functional groups other than C and H, and compares favorably with the spectrum published by Wenninger *et al.* (1967) for ar-curcumene. The mass spectrum (Figure 13) displays a strong parent ion whose accurate mass determination yielded 202.170; the theoretical value for $C_{15}H_{22}$ 202.1721.

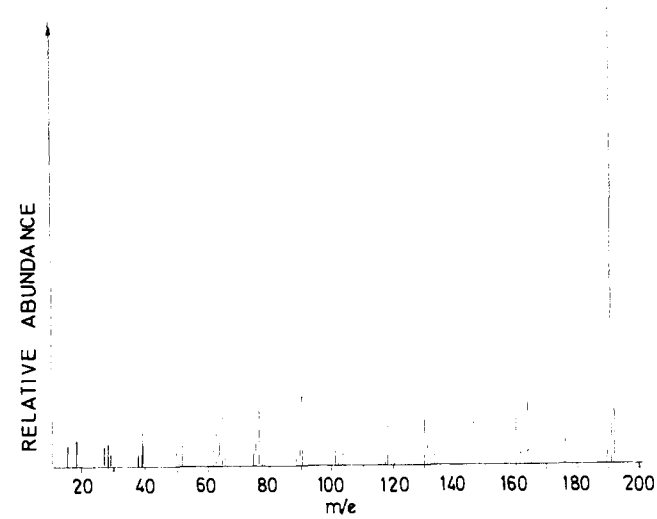


Figure 16. Mass spectrum of peak 18, myristicin (XVI)

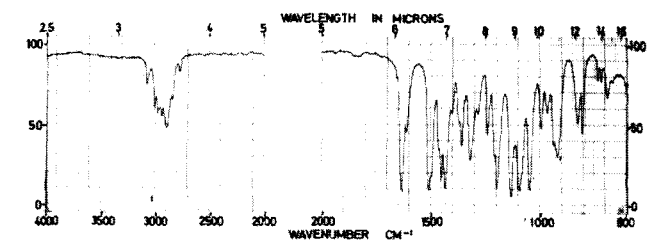


Figure 17. Infrared spectrum of peak 18, myristicin (XVI)

This hydrocarbon is of sufficient polarity that it was separated cleanly from the initial hydrocarbon fractions. As no evidence was found for its presence in the hydrocarbon fraction (Muller *et al.*, 1968), this compound is probably not due to artifact formation.

Peak 15 was found to be methyl eugenol (XIV). Mass and infrared spectra and retention data agreed with those obtained from a commercial sample of methyl eugenol (MC and B). Close examination for *cis* and *trans*-methyl isoeugenol failed to reveal their presence.

Peak 16 was nerolidol (XV). The infrared spectrum (Figure 14) is very similar to that of linalool (Figure 4), but it agrees more closely with the nerolidol spectrum in the Sadtler Reference Spectra. Mass spectral data (Figure 15) show a prominent peak, probably P-18, whose accurate mass determination yielded *m/e* 204.188, indicative of an ion $C_{15}H_{24}$ (theoretical value 204.1787). The strong hydroxyl absorption in the infrared and the small 207 peak in the mass spectrum point to a molecular formula $C_{15}H_{26}O$—an alcohol whose parent ion does not show in the mass spectrum.

Peak 18 was identified as myristicin (XVI). The mass spectrum, Figure 16, shows a strong parent and base peak (indicative of aromaticity) at *m/e* 192. Accurate mass determination yielded a formula $C_{11}H_{12}O_3$. Comparison of the infrared spectrum (Figure 17) with that of an authentic sample supplied from work of Shulgin *et al.* (1967) showed the two spectra to be identical.

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