COMMUNICATIONS

α-Bergamotenes in Oil of Black Pepper

The presence of both α-*cis*-bergamotene and α-*trans*-bergamotene in oil of black pepper was confirmed and a method for their differentiation is presented. On the basis of retention data and infrared, nuclear magnetic resonance, and mass spectroscopy, both isomers of the α-bergamotenes have been shown to exist in a single natural source.

Recently, Muller and Jennings (1967) reported finding two sesquiterpenes in the oil of black pepper, *Piper nigrum*, whose infrared spectra were extremely similar. That they were indeed two different compounds was shown by their different retention times on capillary columns. One had an infrared spectrum which more closely resembled the spectrum reported by Kovats (1963) and on this evidence it was assigned the structure of α-*trans*-bergamotene (I). The very close similarity of the infrared spectrum of the other compound isolated suggested that the compound might be α-*cis*-bergamotene (II), since these two compounds differ only in the stereochemistry at one carbon atom.

I II

The interest shown in these two compounds prompted us to describe further some properties that would more clearly characterize each of the two isomers.

EXPERIMENTAL

Apparatus. Initial gas chromatographic separations were effected utilizing 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 165° C. and 60 ml. per minute He flow rate. Secondary separations utilized a modified Beckman Thermotrac fitted with a Carle micro thermistor detector and Hamilton glass-lined injectors. The columns in this latter instrument were dual 50-foot × 0.25-inch (O.D.) stainless steel columns packed with 1% (w./w.) SF 96(50) admixed with 5% (w./w.) Igepal Co-880 on 120- to 140-mesh HMDS-treated Chromosorb G, operated at 220° C. and 50 ml. per minute He flow rate. Retention data were gathered on a similar chromatograph containing two 500-foot × 0.030-inch (I.D.) stainless steel open-tubular capillary columns, one of which was 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. The flow rate through each column was 6 ml. per minute.

The components were trapped from the gas chromatographs as described by Jennings *et al.* (1964); infrared spectra were taken neat between sodium chloride plates on a Beckman IR-8 infrared spectrophotometer fitted with a 5× beam condenser. Mass spectra were obtained on a Varian M66 mass spectrometer, inserting the capillary tubes used for collection directly into the sample inlet system. Nuclear magnetic resonance spectra (NMR) were obtained on a Varian A-60 spectrometer essentially following the technique of Flath *et al.* (1967) using tetramethylsilane as an internal standard and $CDCl_3$ as the solvent.

Materials and Procedure. The hydrocarbon fraction of Ceylon black pepper oil (Stange and Co.) was separated following the procedure of Muller *et al.* (1968). This separated on the Triton-X 305 column into 12 main fractions, of which peak 4 was taken for further treatment. Peak 4 from the Triton-X 305 column was then rechromatographed on the 50-foot × 0.25-inch SF96(50) columns at 220° C. to yield four peaks which were individually trapped and checked for purity on the capillary columns. These were labeled 401, 402, 403, and 404 in respective order of elution, the peaks of interest in this study being 402 and 404.

Infrared spectra of collected samples showed that the 50-foot × 0.25-inch SF96(50) column was discharging silicon polymer—i.e., column bleed—and contaminating the trapped fractions. By rechromatographing the trapped fractions once again on Triton-X 305 columns at 160°, this problem of column bleed was minimized. This procedure yielded *ca.* 3 μl. of each component for NMR spectra. While these spectra are of low resolution, they are informa-

Table I. Kovats Indices from Open Tubular Capillary Columns at 175° C.

	α-*trans*-Bergamotene	α-*cis*-Bergamotene
SF 96(50)	1447	1427
OV-17	1529	1515

tive, and are presented with the other spectra to differentiate clearly between the two compounds. Table I presents the retention data from the capillary columns.

RESULTS AND DISCUSSION

The mass spectra shown are both compatible with the assigned structures for the α-bergamotenes. The similarity of both the mass and infrared spectra (Figures 1 and 2) also indicates that these two compounds are closely related isomers. In each case, the parent peak at *m/e* 204 is consistent with the assignment of the empirical formula $C_{15}H_{24}$. Both contain a large *m/e* 93 peak second only to the *m/e* 119 base peak. Since the base peak of *m/e* 93 for the pinenes is ascribed to the loss of the disubstituted carbon bridge (Reed, 1966) the large *m/e* 93 peak evidenced by these two isomers provides additional evidence for the

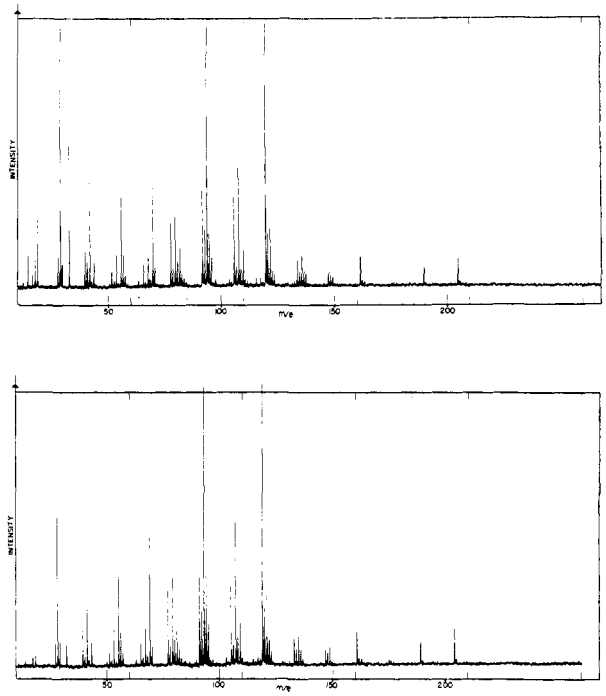

Figure 2. Top, mass spectrum of peak 404 (α-*trans*-bergamotene, I). Bottom, mass spectrum of peak 402 (α-*cis*-bergamotene, II)

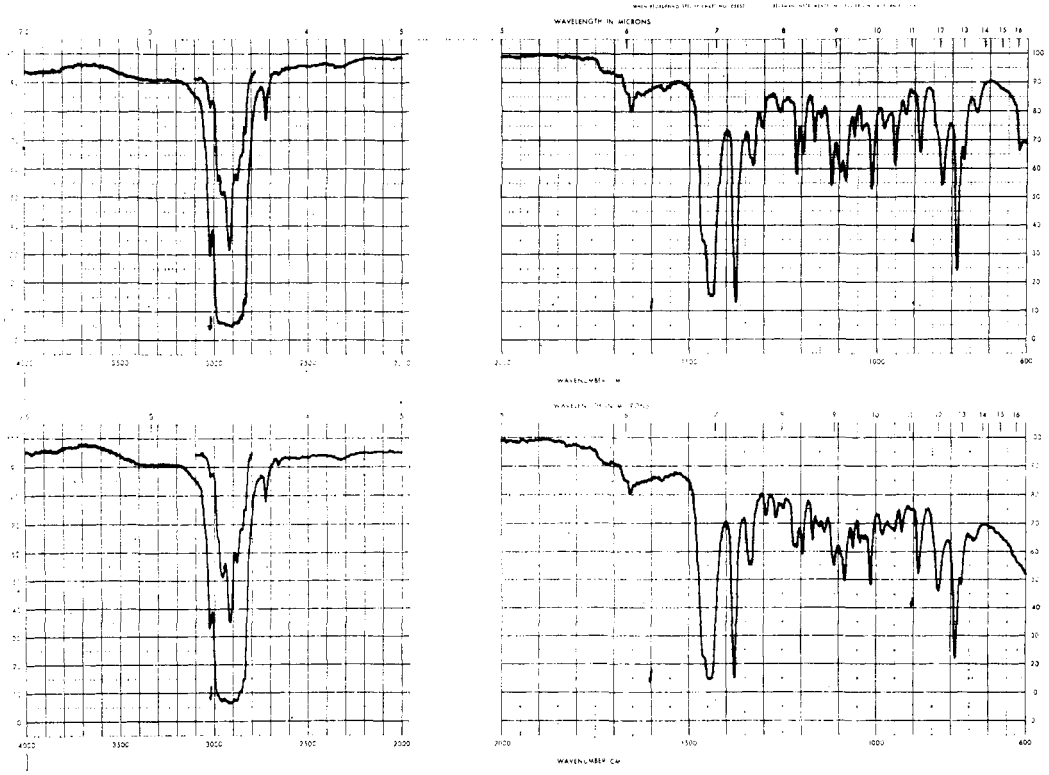

Figure 1. Top, infrared spectrum of peak 404 (α-*trans*-bergamotene, I). Bottom, infrared spectrum of peak 402 (α-*cis*-bergamotene, II)

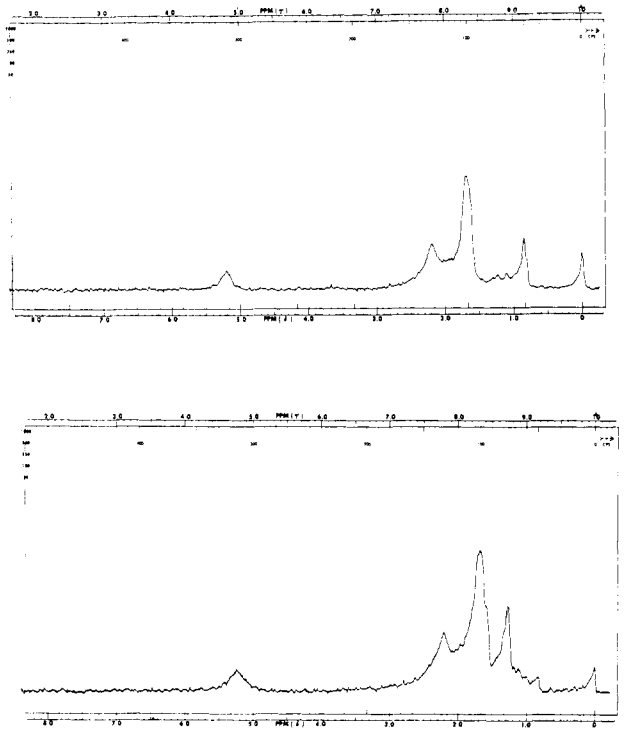

Figure 3. Top, NMR spectrum of peak 404 (α-*trans*-bergamotene, I). Bottom, NMR spectrum of peak 402 (α-*cis*-bergamotene, II)

assignment of their basic structures to that of the bergamotenes.

The NMR spectrum (Figure 3) of each isomer is consistent with the structure of the α-bergamotenes and provides sufficient data to differentiate the structures. The broad signal at 5.25 δ shows two vinyl protons. This rules out the beta isomers as the spectra would then be expected to have signals at 5.06 δ (1H, vinyl proton), 4.64 δ and 4.57 δ (2H, exocyclic double bond) as reported by Gibson and Ermaen (1967). The methyl signals at 1.27 δ and 0.85 δ in the two spectra are the only features that are significantly different. Jackman (1959) has shown that a methyl proton which lies over the double bond in the pinenes is more shielded than the other methyl protons that are not so shielded. The compound whose spectrum displayed a methyl signal at 0.85 δ—i.e., fraction 404—was therefore assigned structure I and the other whose spectrum shows a methyl signal at 1.27 δ was assigned structure II.

To the authors' knowledge, both isomers of α-bergamotene had not been previously reported in other natural products. This may have been because the similarity of their infrared spectra and other properties led to the conclusion that they were a single compound.

ACKNOWLEDGMENT

The authors are grateful to U. S. Kern, Chemistry Department, University of California, Davis, for assistance in obtaining the NMR spectra.

LITERATURE CITED

Flath, R. A., Henderson, N., Lundin, R. E., Teranishi, R., *Appl. Spectry.* **21,** 183 (1967).
Gibson, T. W., Erman, W. F., *Tetrahedron Letters* **1967,** 905.
Jackman, L. M., "Nuclear Magnetic Resonance Spectroscopy," p. 129, Pergamon Press, London, 1959.
Jennings, W. G., Creveling, R. K., Heinz, D. E., *J. Food Sci.* **29,** 730 (1964).
Kovats, E., *Helv. Chim. Acta* **46,** 2705 (1963).
Muller, C. J., Jennings, W. G., J. AGR. FOOD CHEM. **15,** 762 (1967).
Muller, C. J., Jennings, W. G., Creveling, R. K., J. AGR. FOOD CHEM. **16,** 113 (1968).
Reed, R. I., "Applications of Mass Spectrometry to Organic Chemistry," p. 99, Academic Press, London, 1966.

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Received for review May 3, 1968. Accepted August 5, 1968. This project was supported by Research Grant UI00267 from the National Center for Urban & Industrial Health, U. S. Public Health Service.