

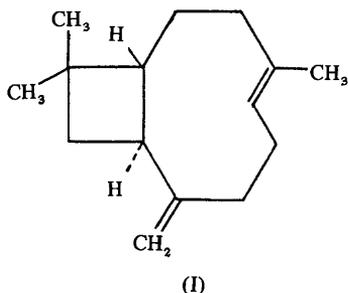
β -Caryophyllene from Black Pepper

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Introduction

Since 1892, it has been known that β -caryophyllene (I) occurred in many essential oils of *Eugenia caryophyllata* Thunb. (cloves), *Oxystigima manii* Harms. (African capaiba), *Syzygium aromaticum* L. (cloves), *Jambrosa caryophyllus* Niedenzu and *Lavendula officinalis* (lavender) in France as a mixture.¹⁾



From *Piper nigrum* L. (black pepper), photo-oxidation products of isocaryophyllene were isolated in 1975.²⁾

In this article, it was reported that β -caryophyllene itself was isolated from black pepper by the preparative gas chromatography, the structure was identified by infrared, ^1H - and ^{13}C -nuclear magnetic resonance spectrometric studies.

Experimental and Results

Dried black pepper fruits from Brazil in 1980 were presented by Takasago Perfumery Co. β -Caryophyllene was finally purified by a Shimadzu preparative gas chromatograph GC 6A, FID, equipped with a Shimadzu The 3rd laboratory of nutrition.

fraction collector APS-5, connected with a 3.0 m \times 0.5 cm stainless steel column packed with OV-17 at 210°C, the carrier gas was helium at 1.6 Kg/cm², injection temperature was 260°C, hydrogen was passed at 0.25 Kg/cm², air was sent at 0.5 Kg/cm².

The ultra violet spectrum was measured with a Hitachi 100-60 spectrophotometer, the infrared spectrum was taken by a JEOL IRA-1 spectrometer, the nuclear magnetic resonance spectrum for ^1H was measured by a Hitachi R-40 90 MHz spectrometer using tetramethylsilane as an internal reference.

The elementary analysis and the nuclear magnetic resonance spectrum for ^{13}C by a JEOL JNM-FX 100 were measured by staffs of Institute of Physical and Chemical Research, Wakoshi, Japan.

All solvents used for the extraction and another were of reagent-grade quality available commercially.

1. Extraction and Purification

From 5 Kg of black pepper fruits, 50.6 g of the residue was obtained after the concentration of the extract in 6 l of chloroform with a rotary evaporator. It was separated roughly to 9 fractions by a silica gel (Kiesel Gel 60, Merck) column (45.0 \times 5.0 cm) chromatography eluted with benzene: ethyl acetate (4:1 v/v), acetone and n-butanol successively. 2.5 g of the third fraction was purified with the preparative gas chromatographic separation using the OV-17 column divided the constituents into two main fractions as shown in Fig. 1.

Each fraction was reanalyzed on the silica gel TLC (Wako gel, B-5), 0.7 g of the first fraction was only detected one spot at $R_f = 0.74$, developed with benzene, but it was changed easily to three spots at R_f

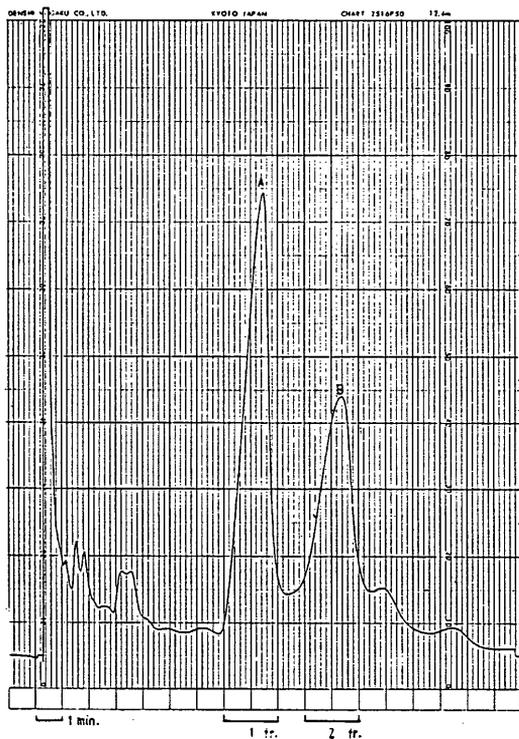


Fig. 1. The preparative gas chromatogram using the OV-17 Column (3.0m x 0.5cm)

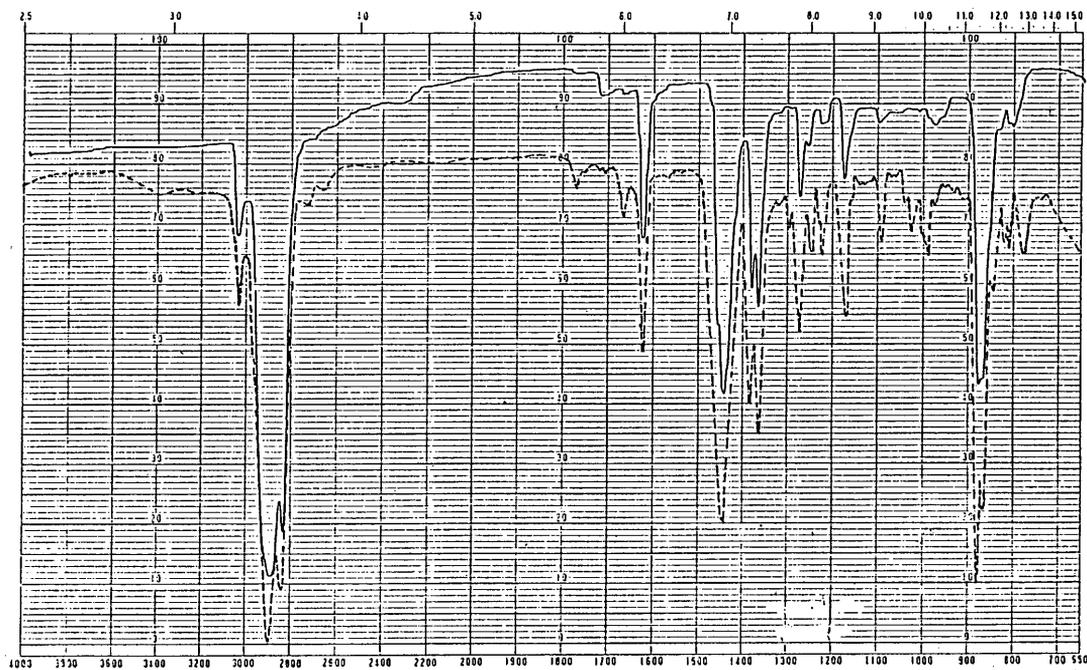


Fig. 2. The infrared spectrum of the oily material of the first fraction (solid line) and the standard spectrum of β -Caryophyllene (broken line)

= 0.74, 0.57, 0.04, developed with acetone: ethyl acetate (1:1 v/v), while it was kept standing at room temperature overnight in open sealed condition, though it was known that the structure was changed easily to isocaryophyllene, caryophyllenols and clovene in acidic condition.^{1a)}

2. Elementary Analysis

The percentage compositions of the elements carbon and hydrogen for the oily material of the first fraction were observed 87.29% and 11.74% respectively, which were taken an average in twice measurements. The total amount of percentage values was 99.03%, then 0.97% could be thought the percentage composition of the element oxygen, but this value was too small to consider the existence of oxygen or another element in the formula. Thus the percentage composition values of carbon and hydrogen were made 88.15% and 11.85% respectively, which were corrected through the deviding each value by 0.9903. Then the formula of $(C_{7.34}H_{11.76})_n$ was obtained.

When n was 2, the formula became $C_{14.68}H_{23.52}$, then $C_{15}H_{24}$ could be chosen in the molecular

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formula, the percentage compositions of of the elements carbon and hydrogen was calculated from it at 88.16% and 11.85% respectively.

3. Ultra Violet Spectrophotometric Analysis

In the ultra violet spectrum of the oily material of the first fraction, the only one absorption band was shown at 219 nm ($E_{1\%}^{1\text{cm}} = 55.63$; $\epsilon_{\text{max}} = 1137$ on $C_{15}H_{24}$) in ethanol. This band was due to the $\pi \rightarrow \pi^*$ transition (K-band) of the isolated ethylenic chromophore.

4. Infrared Spectrophotometric Analysis

Each band of the spectrum of the oily material of the first fraction was assigned as follows and the unit of figures was cm^{-1} .

A: $\text{CRR}'=\text{CH}_2$;

- 3040(m) CH_2 stretching(?)
- 1770(vw) overtone of 875 cm^{-1}
- 1620(m) $\text{C}=\text{C}$ stretching, non-conjugated
- 1420(m) CH_2 in plane deformation
- 875(s) out-of-plane deformation

B: $\text{CRR}'=\text{CHR}''$; 3040(m) CH stretching

- 1660(vw) $\text{C}=\text{C}$ stretching, non-conjugated
- 865(s) CH out-of plane deformation(?)
- 840-800 absence of a band

C: $\text{CH}_3, \text{CH}_2, \text{CH}$; 2910, 2880, 2840(s) CH stretching

D: $\text{C}-\text{CH}_3$; 1440(s) CH deformation (asymmetrical)

E: $-\text{CH}_2-$; 1460(s) CH deformation

F: $-\text{C}(\text{CH}_3)_2$; 1380(s), 1360(s) CH deformation, approx. equal intensity

G: $-\text{CH}-$; 1330-1310(w) CH deformation

H: $-\text{C}(\text{CH}_3)_2$; 1170(w), 1160(w, sh), 800(w) skeletal vibrations

I: Cyclobutane; 1000-940(w) ring system

The infrared spectrum was shown in Fig. 2. From the above data, it (solid line) was compared with the standard bands of β -caryophyllene (broken line)³⁾, then the isolated compound would be decided β -caryophyllene though slight differences remained between them.

5. ^1H -Nuclear Magnetic Resonance Spectrometric Analysis

In Fig. 3, the peaks at 0.96 and 0.98 ppm were due to methyl groups which were shown two singlets and the integration curve showed 6H atoms, the peaks at

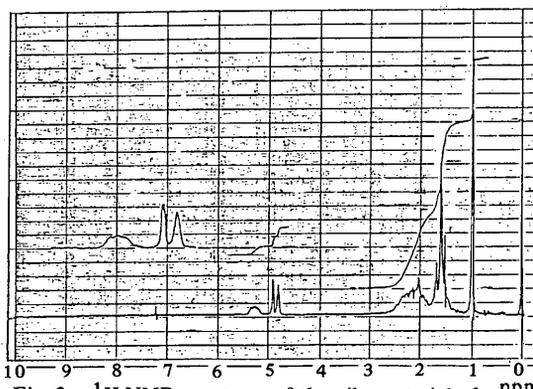


Fig. 3. ^1H -NMR spectrum of the oily material of the first fraction from black pepper in CDCl_3

1.50, 1.60 and 1.68 ppm were shown three singlets and they showed 1H, the methyl group substituted on the double bond and another 3H, and 2H atoms, respectively on the integration curve, the peaks in 1.80-2.50 ppm were due to methylene groups and the integration curve showed 6H atoms, the peaks at 4.81 and 4.91 ppm were shown a singlet and a doublet respectively which were due to the terminal two

6. ^{13}C -Nuclear Magnetic Resonance Spectrometric Analysis

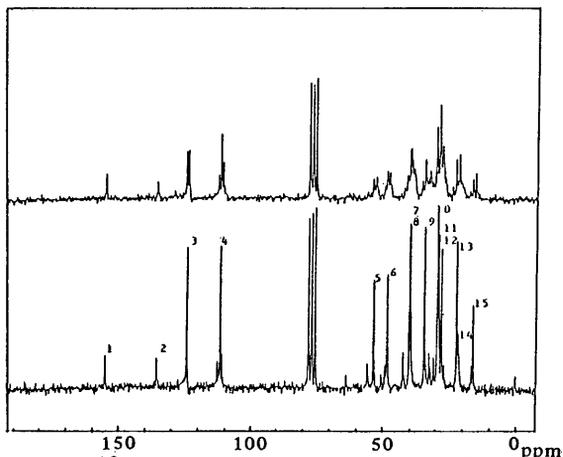


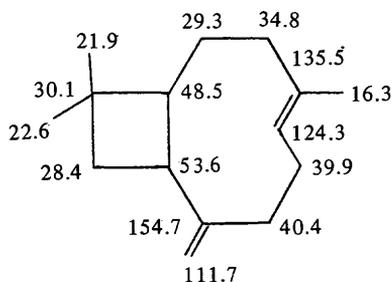
Fig. 4. ^{13}C -NMR Spectrum of the oily material of the first fraction from black pepper with protons completely coupled (above) and protons completely decoupled by broad-band noise (below) in CDCl_3

protons of the asymmetric disubstituted ethylene and the multiplet peaks centered at 5.27 ppm were due to the internal proton of the trisubstituted ethylene.⁴⁾

From Fig. 4 of the ^{13}C -NMR spectrum of the odorous oily material with the protons completely

coupled and the protons completely decoupled by broad-band noise, the peak at 154.7 ppm was assigned to the internal carbon of the asymmetric disubstituted ethylene, the peak at 135.5 ppm to the carbon of the trisubstituted ethylene, substituted by the methyl group the peak at 124.3 ppm to the carbon of the trisubstituted ethylene had a proton, the peak at 111.7 ppm to the terminal carbon of the asymmetric disubstituted ethylene, the peaks at 53.6 and 48.5 ppm to the two methine groups, the peaks at 40.4, 40.0, 34.8, 29.4 and 28.4 ppm to the five methylene groups, the peak at 30.1 ppm to the geminal carbon substituted by the two methyl groups and the peaks at 22.7, 22.0 and 16.3 ppm to the three methyl groups.

From the above ^{13}C chemical shifts and multiplicities in the off-resonance decoupled spectrum, the structure with the assignments of them could be proposed as follows;



Through the series of analyses, we have confirmed that the chemical structure of the odorous oily material from black pepper was not isocaryophyllene but β -caryophyllene.

Acknowledgement

We express our appreciation to Takasago Perfumery Co. for presenting black pepper fruits to us and to staffs of Institute of Physical and Chemical Research for measurements of the elementary analysis and ^{13}C -NMR spectrum.

References

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b. M. Windholz ed.: *The Merck Index 10th Edition*, 1857, Merck & Co., Rahway, N.J., USA.
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In this article, ^1H -NMR spectrum of β -caryophyllene in CCl_4 was shown such as 0.98 and 0.95 (two methyl groups), 1.57(d) (the methyl group substituted on the double bond), 4.76 and 4.88(c) (the terminal methylene group of the double bond), 5.25(c) ppm (the internal methine group of the double bond).

黒コショウからの β -カリオフィレン

山口 功、尾関 幸子

(受理 昭和60年9月17日)

黒コショウから若干刺激臭のある油状物質を得、それを分取型ガスクロで精製単離し、UV, NMR, IR等のスペクトルと元素分析で構造を決定し、それが β -カリオフィレンであると同定した。