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Constituents of Fruit Oil from Japanese Pepper

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The steam-volatile constituents from ripe fruits of Japanese pepper have been studied using column and gas chromatographic separations, followed by characterization on the basis of infrared, nuclear magnetic resonance, and mass spectrometry. The capillary gas chromatography of the volatile oil from ripe fruits of Japanese pepper revealed the presence of approximately 130 components. Among the components identified were the following: 19 hydrocarbons (α-pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, limonene, β-phellandrene, *p*-cymene, terpinolene, α-copaene, β-caryophyllene, humulene, γ_2-cadinene, α-muurolene, δ-cadinene, γ-cadinene calamenene, and α-calacorene), 15 alcohols (linalool, isopulegol, *trans*- and *cis*-2,8-*p*-menthadien-1-ol, terpinen-4-ol, limonen-4-ol, α-terpineol, 1(7), 8-*p*-menthadien-*trans*-2-ol, citronellol, *trans*- and *cis*-carveol, geraniol, cuminalcohol, β-caryophyllene alcohol, and nerolidol), 9 aldehydes and ketones (citronellal, dihydrocarvone, cryptone, carvone, piperitone, phellandral, cuminaldehyde, perillaldehyde, and piperitenone), 7 esters (isobutyl isovarelate, isobutyl caproate, linalyl acetate, citronellyl acetate, terpinyl acetate, geranyl acetate, and methyl cinnamate), and 6 oxides (1,8-cineol, β-terpinen-3,4-oxide, *trans*- and *cis*-limonen-1,2-oxide, terpinolen-4,8-oxide, and methyl chavicol).

Japanese pepper (*Zanthoxylum piperitum* DC., in Japanese, "Sansho," and its variety *Z. piperitum var. inerme* Makino, "Asakura-sansho") is a popular spicy plant in our country. The young leaves and fruits of Japanese pepper, possessing a pleasant odor and a strong pungent taste, are widely used as spice.

The essential oil from fruits of Japanese pepper have been studied by several authors. Earlier work on the volatile oil has been reviewed by Guenther.[1] Later, Katayama[2] reported the identification of several terpenes on the basis of the R_f value on a chromatostrip. The compounds reported on hitherto were 1,8-cineol, limonene, β-phellandrene, terpinolene, citronellal, linalool, α-terpineol, citronellyl acetate, geraniol, geranyl acetate, and cuminalcohol.

This paper will present the results of our investigation of the volatile oil isolated from ripe fruits of "Asakura-sansho."

Experimental

Apparatus. *Gas Chromatography.* The components were separated on a Varian Aerograph Model 90-P. The columns were : (1) a 25 ft-by-1/4 inch stainless steel column packed with 20 % Triton X 305; (2) a 10 ft-by-3/8 inch aluminum column, 20 % Carbowax 20 M ; and (3) the same column as (2), 20 % SF-96; solid support, 60—80 mesh chromosorb W. These runs were isothermal or programmed. The flow rate of the helium carrier gas was 30—60 m*l*/min. The separated components were trapped in thin-walled glass capillaries following the method developed by Jennings and his co-workers.[3]

For analytical chromatography we used a Hitachi Perkin-Elmer Model F-6D gas chromatograph equipped with a flame-ionization detector. 45 m-by-0.25 mm stainless steel capillary columns coated with HB-2000, with FS-45, and with Q-45 were used. The column temperature was programmed from 70 to 150 °C (HB-2000), and was held isothermally at 80, 130, 150, and 200°C. The nitrogen carrier-gas inlet pressure was 1.0 kg/cm^2.

Infrared Spectroscopy. The infrared spectra were taken as thin films between sodium chloride plates on a Hitachi EPI-G2 spectrometer, equipped with a custom-made beam condenser.

Nuclear Magnetic Resonance Spectrometry. The NMR spectra were measured on a JEOL Model C-60 spectrometer at 60 MHz in carbon tetrachloride, using tetramethylsilane as an internal reference. The microcell technique was adopted.[4]

Mass Spectrometry. Mass spectra were obtained

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1) E. Guenther, "The Essential Oils," Vol. III, Van Nostrand, New York (1949), p. 376.

2) T. Katayama, *Bull. Japanese Soc. Sci. Fisheries*, **24**, 511 (1958).

3) W. G. Jennings, R. K. Creveling and D. E. Heinz, *J. Food Sci.*, **29**, 730 (1964).

4) S. Ito and I. Miura, This Bulletin, **38**, 2197 (1965).

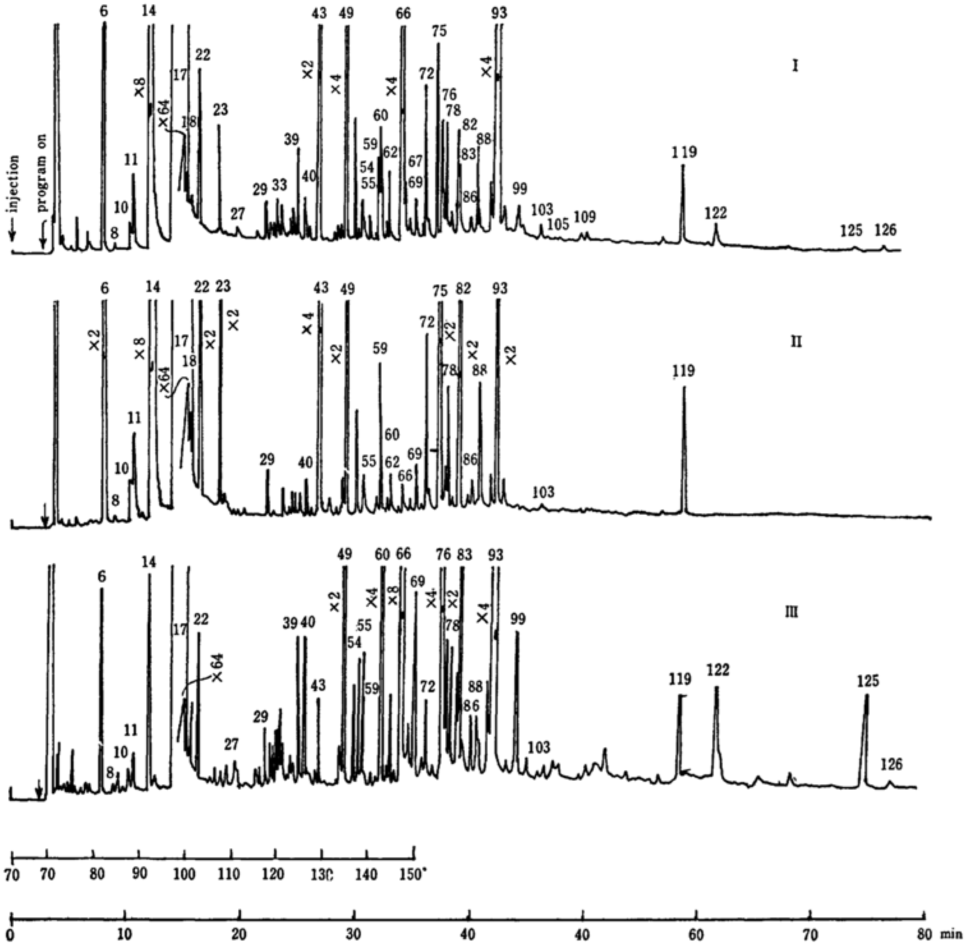

Fig. 1. Gas chromatograms of Japanese pepper fruit oil I. Ripe fruit oil, II. Unripe fruit oil, III. Stored ripe fruit oil 45 m×0.25 mm HB-2000 column; temperature isothermal at 70°C for 3 min then programmed at 2.5°C per minute to 150°C and run isothermal thereafter; sample 1.0—2.0 μl ; range 1×1 except as indicated.

on a Hitachi RMU-6 mass spectrometer. The operating conditions were as follows: ionization voltage, 80 eV; ion-accelerating voltage, 2000 V; temperatures of ionization chamber and sample injection block, 250 and 150°C respectively.

Preparation and Separation of the Volatile Oil. Ripe fruits of "Asakura-sansho," cultivated in the Yoshino district, Nara Prefecture, were crushed and steam-distilled. The yield of the volatile oil was 0.96 per cent of the raw material.

The neutral volatile oil (104 ml), freed from acids and bases, was fractionated into sixteen fractions by distillation under reduced pressure, utilizing a Podbielniak 13 mm-by-24 inch Heli-Grid column. Each fraction was further fractionated by means of silica gel column chromatography and preparative gas chromatography. The individual components were then purified by reinjection into various columns.

Results and Discussion

Figure 1 shows the comparative capillary chromatograms of ripe fruit oil (top of Fig. 1), unripe fruit oil[*1] (middle), and ripe fruit oil stored for a long time at room temperature (bottom). Each chromatogram was obtained by using a flame-ionization detector and by injecting 1.0 μl of the raw volatile oil into a HB-2000 capillary column. The column temperature was held initially at 70℃ for 3

[*1] The oleoresin was obtained by extraction with hexane from unripe fruits of "Asakura-sansho" which were cultivated in Minô, Osaka Prefecture. By steam-distillation of oleoresin, the volatile oil was isolated.

TABLE 1. IDENTIFICATION OF CONSTITUENTS OF VOLATILE OIL FROM RIPE FRUITS OF JAPANESE PEPPER USING CAPILLARY GLC, IR, NMR AND MS SPECTRAL ANALYSES

Peak No. in Fig. 1	Identified component	IR, NMR, MS Lit. Ref.	Authentic sample
6	α-Pinene	IR[a,b] MS[c]	commercial
8	Camphene	IR[a,b] MS[c]	commercial
10	β-Pinene	IR[a,b] MS[c]	commercial
11	Sabinene	IR[a,b] MS[c]	Juniper berry oil[d]
14	Myrcene	IR[a,b] MS[c]	Juniper berry oil[d]
15	α-Phellandrene	IR[a] MS[c]	commercial
17	Limonene	IR[a,b] MS[c]	commercial
	1,8-Cineol	IR[b]	commercial
18	β-Phellandrene	IR[a] MS[c]	not available
22	*p*-Cymene	IR[a,b] MS[c]	commercial
23	Terpinolene	IR[a,b]	dehydration of α-terpineol
27	Isobutyl isovarelate		esterification
29	Isobutyl caproate		esterification
33	β-Terpinen-3,4-oxide		not available
39 40	*trans*- and *cis*-Limonen-1,2-oxide	IR[e]	oxidation of limonene[e]
43	Citronellal	MS[f]	commercial
49	Linalool	IR[b] MS[g]	commercial
54	Terpinolen-4,8-oxide		not available
55	Isopulegol	IR[h] NMR[h] MS[h]	commercial
	trans-2,8-*p*-Menthadien-1-ol	IR[i] NMR[i]	autoxidation of limonene[i]
59	Terpinen-4-ol	IR[b,j] MS[g]	Juniper berry oil[d]
	Linalyl acetate	IR[b]	commercial
60	*cis*-2,8-*p*-Menthadien-1-ol	IR[i] NMR[i]	autoxidation of limonene[i]
	Dihydrocarvone		reduction of carvone
62	α-Copaene	IR[k]	Cubeb oil[l]
66	Cryptone		autoxidation of β-phellandrene[m]
67	Methyl chavicol		oil of *Fagara mantchurica*[n]
69	Limonen-4-ol		not available
72	α-Terpineol	IR[j] MS[g]	commercial
75	β-Caryophyllene	IR[b]	Juniper berry oil[d]
76	Carvone	IR[b] MS[f]	commercial
	Piperitone	MS[f]	Mentha species[o]
	Citronellyl acetate		commercial
78	Phellandral		reduction of perillaldehyde
82	Terpinyl acetate		commercial
83	Cuminaldehyde	MS[f]	commercial
86	Perillaldehyde		commercial
88	Humulene	IR[b]	Juniper berry oil[d]
	1(7),8-*p*-Menthadien-*trans*-2-ol	IR[i] NMR[i]	not available
	Citrodellol	MS[g]	commeircal
90	γ_2-Cadinene	IR[p]	not available
93	Geranyl acetate	MS[q]	commercial
	trans-Carveol	NMR[r]	autoxidation of limonene[i]
99	α-Muurolene	IR[s]	oil of *Mentha piperita*[s]
	cis-Carveol	NMR[r]	autoxidation of limonene[i]
	Geraniol	MS[g]	commercial
103	δ-Cadinene	IR[b]	Juniper berry oil[d]
	γ-Cadinene	IR[b]	Juniper berry oil[d]
105	Piperitenone	IR[b,t] MS[f,t]	Mentha species[o]
109	Calamenene	IR[b] MS[u]	Cubeb oil[l] Yomena oil[u]
118	α-Calacorene	IR[b] MS[u]	Yomena oil[u]
119	Methyl cinnamate		commercial
122	Cuminalcohol	MS[g]	reduction of cuminaldehyde
125	β-Caryophyllene alcohol	mp 96 °C[v]	hydration of β-caryophyllene[v]
126	Nerolidol		commercial

a) B.M. Mitzner, E.T. Theimer, J.K. Freeman, *Appl. Spectroscopy*, **19**, 169 (1965). b) J.Pliva, M. Horak, V. Herout, F.Sorm, Terpenspektren, Sammlung der Spektren und PhysikalishenKonstanten, Bd. I and II. Akademie Verlag, Berlin1960 and1963. c) R.Ryhage, E. von Sydow, *Acta Chem. Scand.*,**17**, 2025 (1963). d) K. Nishimura, Y. Hirose, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan, Pure Chem. Sect.*), **83**, 350 (1962). e) W.F. Newhall, *J. Org. Chem.*, **29**, 185 (1964). f) E. von Sydow, *Acta Chem. Scand.*, **18**, 1099 (1964). g) E.von Sydow, *ibid.*, **17**, 2504 (1963). h) R.Teranishi, "Flavor Chemistry," Am. Chem. Soc., Washington, D.C. (1966). i) C.S.Foote, S.Wexler, W.Ando, *Tetrahedron Letters*, **1965**, 4111. j) B.M.Mitzner, S.Lemberg, *Am. Perf. & Cosm.*, **81**, March 25; May 27 (1965). k) P. de Mayo, R.E. Williams, G. Büchi, S.H.Feairheller, *Tetrahedron*, **21**, 619 (1965). l) Y.Ohta, T. Sakai, Y. Hirose, unpublished m) O. Wallach, *Ann.*, **343**, 28 (1905). n) Y. Ohta, Y. Hirose, unpublished o) S.Shimizu, *Perf. Essen. Oil Rec.*, **56**, 212 (1965). p) C.C.Karta, P.S.Kalsi, A.M. Shaligram, K. K. Chakravarti, S. C. Bhattacharyya, *Tetrahedron*, **19**, 241 (1963). q) E. von Sydow, *Acta Chem. Scand.*, **19**, 2083 (1965). r) S.H.Schroeter, E.L.Eliel, *J.Org. Chem.*, **30**, 1 (1963). s) R. Vlahov, M. Holub, I. Ognjanov, V. Herout, *Coll. Czech. Chem. Commun.*, **31**, 808 (1966). t) M.G. Moshonas, *J. Food Sci.*, **32**, 206 (1967). u) N. Tsubaki, K. Nishimura, Y. Hirose, This Bulletin, **39**, 312 (1966). v) Y. Asahina, T. Tsukamoto, *J. Pharm. Soc. Japan*, **484**, 463 (1922).

min, then programmed at 2.5℃ per minute to 150℃, and run isothermally at 150℃ until all the components had emerged. For the determination of the location of a particular peak, an authentic sample or a purified component was mixed with the original oil and chromatographed using the same analytical conditions as above. The location was confirmed by an appreciable increase in the particular peak.

Among the more than 130 component peaks observed in the chromatogram of fruits oils, fifty-six compounds were isolated and determined. Table 1 lists the components which were confirmed by a comparison of their infrared, nuclear magnetic resonance, and mass spectra with those of authentic samples and the literature data. Authentic samples were obtained from reliable commercial sources or synthesized by well-established methods, and were purified by gas chromatographic separation before use. In a few cases authentic samples were not available.

The mono- and sesqui-terpenic hydrocarbons were identified by direct comparison with authentic samples, except for β-phellandrene and γ_2-cadinene. The main components were limonene and β-phellandrene. Most of the hydrocarbons in the fruit oil of Japanese pepper are commonly found also in other oils of the same family (*Rutaceae*)-citrus oils, orange oil,[5,6] lemon oil[7,8] and natsudaidai oil.[9]

Among the oxygenated components, geranyl acetate, linalool, cryptone, and citronellal were present in quite large amounts. The two isobutyl esters were supposed to be related by a poignant principle, sanshool.[10]

It is most interesting that almost all of the auto- and photo-oxidation products from limonene[11-13] were isolated from fruits of Japanese pepper. These were *trans*- and *cis*-limonen-1,2-oxide, *trans*- and *cis*-2,8-*p*-menthadien-1-ol, *trans*- and *cis*-carveol, 1(7),8-*p*-menthadien-*trans*-2-ol, carvone, and perillaldehyde. Some of them were also found in citrus oils, orange oil,[5,14,15] and grapefruit oil.[16] Cryptone was also derived from the autoxidation of β-phellandrene.[17] These facts are indicated in Fig. 1.

The three monoterpenic oxygenated compounds, β-terpinen-3,4-oxide, terpinolen-4,8-oxide, and limonen-4-ol, had not previously been found in natural products. Besides, it seems that this is the first report on the isolation of β-caryophyllene alcohol from natural sources.

β-Terpinen-3,4-oxide. The IR (Fig. 2) and NMR spectra of this substance showed the presence of an isopropyl group ($\nu_{\max}^{\text{film}}$ 1380, 1360 cm^{-1}; δ 0.92, 0.94 ppm, each 3H d. J 7 Hz), an exocyclic methylene group ($\nu_{\max}^{\text{film}}$ 3060, 1640, 880 cm^{-1}; δ 4.62 ppm, 2H s.), and a proton linked to an epoxide ring ($\nu_{\max}^{\text{film}}$ 850 cm^{-1}, 1,2-epoxide; δ 2.87 ppm, 1H unresolved). Its mass spectrum showed a molecular ion peak at m/e

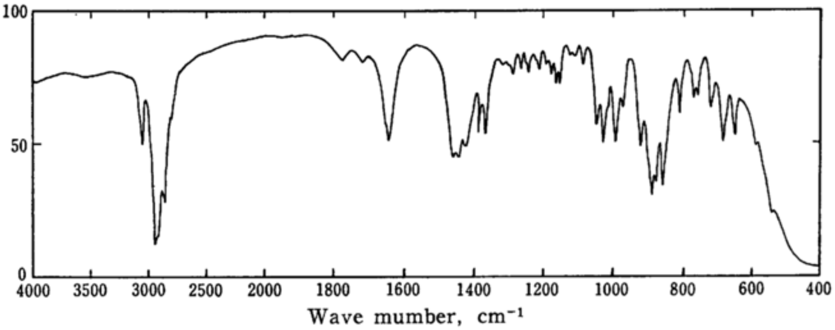

Fig. 2. IR spectrum of β-terpinen-3,4-oxide.

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6) K. Maekawa, M. Kodama, M. Kushii and M. Mitamura, *Agr. Biol. Chem.*, **31**, 373 (1967).

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13) C. S. Foote, S. Wexler and W. Ando, *Tetrahedron Letters*, **1965**, 4111.

14) G. L. K. Hunter and M. G. Moshonas, *Anal. Chem.*, **37**, 378 (1965).

15) T. H. Schultz, R. Teranishi, W. H. Mc Fadden, P.W. Kilpatrick and J. Corse, *J. Food Sci.*, **29**, 790 (1964).

16) G.L.K. Hunter and M. G. Moshonas, *ibid.*, **31**, 167 (1966).

17) O. Wallach, *Ann.*, **28**, 340 (1904).

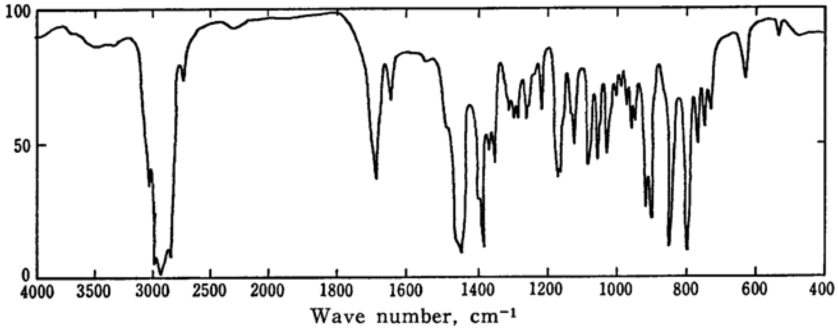


Fig. 3. IR spctrum of terpinolen-4,8-oxide.

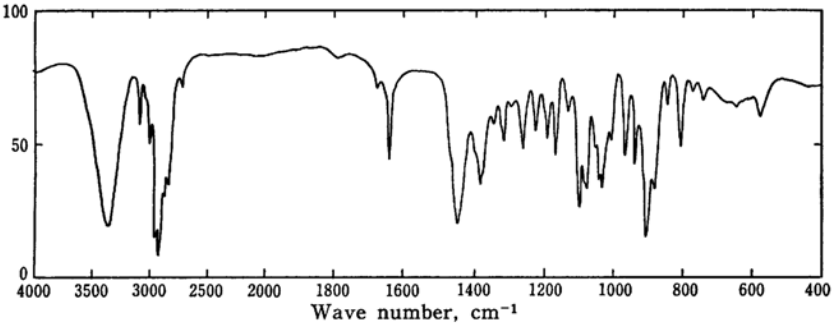
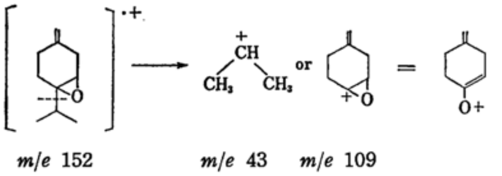
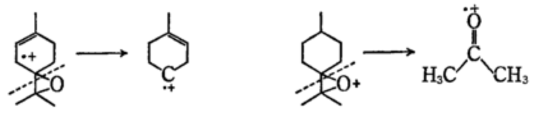


Fig. 4. IR spectrum of limonen-4-ol.

152 ($C_{10}H_{16}O$, 6%), a base peak at *m/e* 43, and conspicuous peaks at *m/e* 109 (48 %), and 81 (83%).

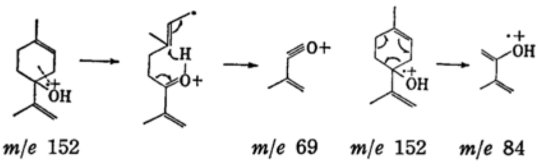


Terpinolen-4,8-oxide. The mass spectrum of this substance showed a molecular ion peak at *m/e* 152 ($C_{10}H_{16}O$, 3%) and a base peak at *m/e* 94 (M^+–CH_3COCH_3). The mass spectrum of the dihydro-oxide showed a molecular ion peak at *m/e* 154 and a base peak at *m/e* 58 (CH_3COCH_3). According to the NMR spectrum, two methyl groups were assigned to the *gem*-dimethyl group attached to a carbon-bearing oxygen (δ 1.20 ppm, 6 H s.) and one methyl group to –CH=C–CH_3 (ν_{max}^{film} 1685, 790 cm^{-1}; δ 1.62 ppm, 3H s., 5.34, 1H unresolved). Its IR spectrum (Fig. 3) showed an absorption peak at 840 cm^{-1} due to an 1,2-epoxide ring.



Limonen-4-ol. From the IR (Fig. 4) and NMR spectra of this substance the presence of a hydroxyl group (ν_{max}^{film} 3370 cm^{-1}), an isopropenyl group (ν_{max}^{film} 3100, 1640, 900 cm^{-1}; δ 1.75 ppm, 3H s., 4.74, 4.92, each 1H broad s.), and a methyl group attached to a trisubstituted double bond (δ 1.65 ppm, 3H s., 5.20, 1H unresolved) were inferred. This alcohol was hydrogenated with platinium oxide in methanol to give a tetrahydro-derivative, which was identified with the known tetrahydroterpinen-4-ol.[18] Its mass spectrum showed a molecular ion peak at *m/e* 152 ($C_{10}H_{16}O$, 11%), a base peak at *m/e* 69, and strong peaks at *m/e* 84 (85 %) and 41 (60 %), which were explained by the following fission mode:

From these facts the structure of this alcohol was deduced to be 1,8-*p*-menthadien-4-ol.



As is shown in Fig. 1, the amounts of citronellal and β-phellandrene decreased when the fruit oil stood at room temperature. On the contrary, the amounts of isopulegol and cryptone increased. Citronellal was also isomerized to isopulegol by distillation and by column

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[18] B.M. Mitzner and S. Lemberg, *Amer. Perf. & Cosm.*, **81**, June 80 (1966).

chromatographic separation. Schulte-Elte and Ohloff[19] reported that the thermal isomerization of citronellal yielded a mixture of isopulegol and neoisopulegol.

During the study of the chemical characteristics of the fruit oil, we attempted to identify the principal flavor components other than citronellal in Japanese pepper, but we were not able to reach any conclusions. Its seems that the characteristic aroma of Japanese pepper is chiefly attributable to citronellal after all.

A comparison of the constituents of volatile oils of unripe fruit oil and ripe fruit oil stored for a long time is in progress. Our results will be reported later.

The authors thank Dr. C. S. Foote, University of California, Los Angels, for supplying authentic samples and IR and NMR spectra of 1(7),8-*p*-menthadien-*trans*-2-ol, *trans*- and *cis*-2,8-*p*-menthadien-1-ol, *etc.*, which led to its identification. The authors are also grateful to Dr.S. Shimizu, The Shinshu Uniersity, Ina, for samples of piperitone and piperitenone, and to Dr. M. Romanuk, Czechoslovak Academy of Science, Prague, for authentic α-muurolene. Thanks are also due to Mr. M. Shiga, Shiono Koryo Kaisha Ltd., Osaka, who kindly provided oleoresin from unripe fruits of "Asakura-sansho."

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[19] K. H. Schulte-Elte and G. Ohloff, *Helv. Chim. Acta*, **50**, 153 (1967).