Chemical Studies on the Volatile Constituents of Seaweed IX

On the Volatile Constituents of *Codium fragile*

Teruhisa KATAYAMA

Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama

(Diagram 1; Tables 1-4; Plates 1-2; Text-Fig. 1)

INTRODUCTION

The present series of studies were undertaken in order to elucidate the so-called "tang of the sea" by systematic separation and identification of the volatile components of seaweeds, and to clarify the principle of aromatic components of sea foods by elucidating the aromatic and odoriferous components of seaweeds. Separation and identification of the volatile components of *Ulva pertusa*[1),2),3),4)], *Enteromorpha* sp.[5)], *Porphyra tenera* K.[6)], *Sargassum* sp.[7)], and *Laminaria* sp.[8),9)] have already been reported.

During the course of these studies, it was found that some of the volatile components show anthelmintic effect. By fractionation of the volatile components of *Digenia simplex*[10)], pharmacological activity of the components was determined, and linalool, geraniol and carvone were found to have strong anthelmintic activity[11),12),13)].

In the present series of studies, attempt was made also to classify seaweeds according to their volatile components, since it was found that such volatile components differ with algal species. For this purpose, the volatile components of *Codium fragile* (SURINGAR) HARIOT growing profusely in the Inland Sea of Japan were fractionated, and dimethylsulfide, benzaldehyde, α-methylfurfural, furfural, furfuryl alcohol, 1:8-cineol, linalool, terpinolene, geraniol and eugenol were detected as the aromatic components. As for the odorous components, the presence of formic, propionic, butyric, isovaleric, n-caproic, caprylic, and palmitic acids and p-cresol was detected.

EXPERIMENTAL

Fresh *C. fragile* was collected from the Inland Sea near Fukuyama City, Hiroshima Pref. Fifty kg. of the air-dried material was submitted to steam distillation. Yield of volatile component was approximately 0.034%. The volatile component was fractionated according to the method which was previously described[1),2),3)] and is represented in Diagram 1 of this paper.

A. YIELD OF EACH FRACTION OF VOLATILE COMPONENTS

The yield of each fraction obtained by the above fractionation is given in Table 1.

B. ISOLATION OF VOLATILE COMPONENTS FROM EACH FRACTION

1. Isolation from Distillation Gaseous Phase

a. *Isolation of Dimethylsulfide*: The vapor that did not condense during steam distillation was passed through mercuric chloride, white precipitate being formed. This mercuric

Diagram 1. General scheme for separation of volatile constituents.

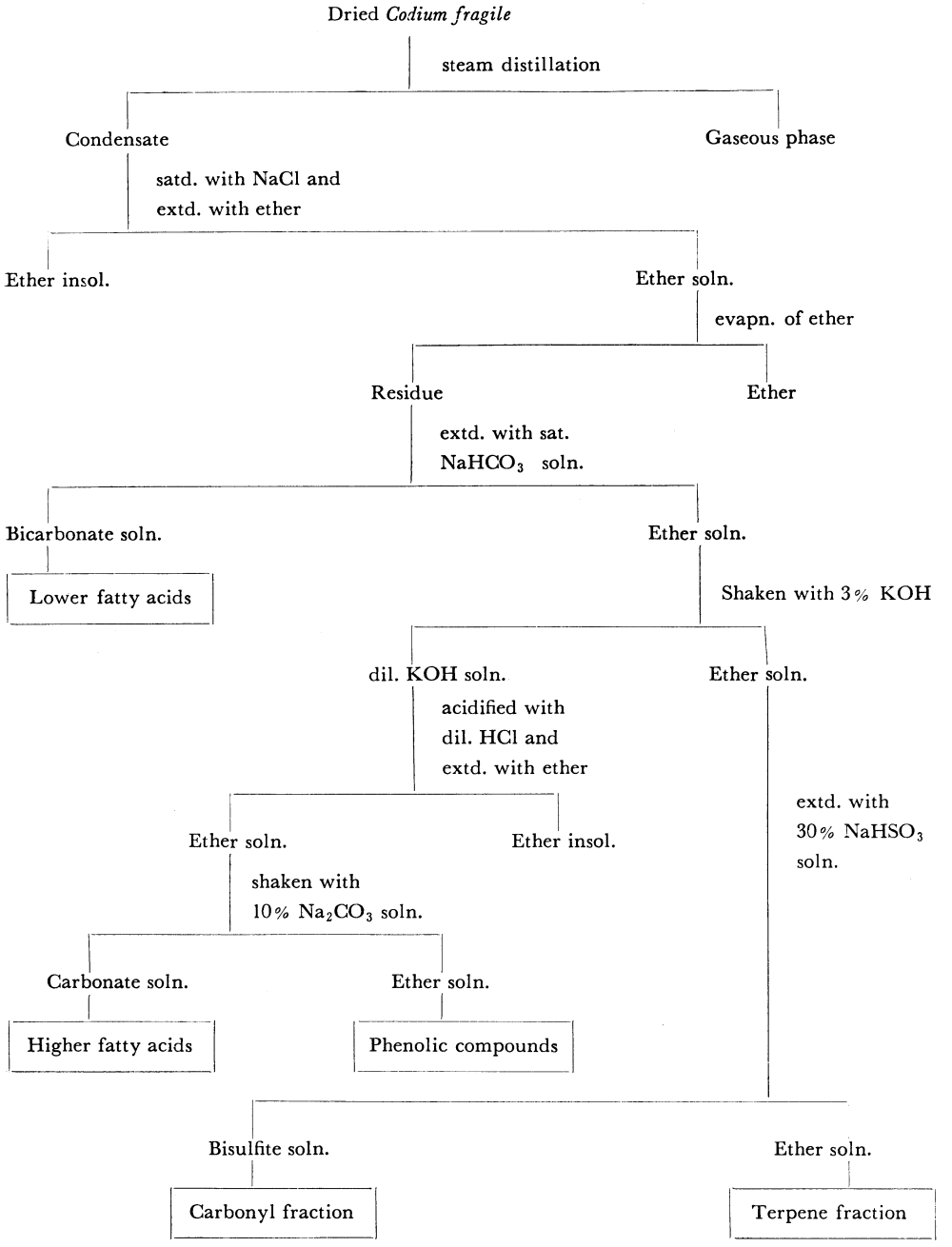


Table 1. Yield of volatile compounds from 50 kg. of air-dried *Codium fragile*.

Fraction	Yield (g)	Odor
1. Dimethylsulfide	6.0*	Aroma
2. $NaHCO_3$-soluble fraction (lower fatty acid fraction)	5.1	Odorous
3. Na_2CO_3-soluble fraction (higher fatty acid fraction)	7.9	Odorous
a. Saturated fatty acid	4.2	——
b. Unsaturated fatty acid	3.1	Odorous
4. Phenol fraction	2.1	Odorous
5. Carbonyl fraction	1.1	Aroma
6. Neutral fraction	6.1	Aroma

* Yield from 100 g. of air-dried *C. fragile*.

chloride double salt melts at 151–152°C, undepressed on being admixed with the corresponding double salt of dimethylsulfide obtained from *Ulva pertusa*[1].

2. Isolation from Condensate Fraction

The condensate fraction was saturated with sodium chloride and extracted with ether. The ether layer was drid over anhydrous sodium sulfate.

a. *Identification of Formic, Acetic, Propionic, Butyric, Isovaleric, n-Caproic, Caprylic Acids from $NaHCO_3$ Soluble Fraction*: The crude oil was repeatedly shaken with sodium bicarbonate solution.

Acidification of the soluble fraction with dilute hydrochloric acid solution made the fraction to separate into a water-insoluble, oily fatty acid fraction and a water-soluble, fatty acid fraction. The former fraction containing oily fatty acids was dissolved in ether, washed three times with saturated sodium chloride solution and dried, and the ether was evaporated (Acid–II). The latter fraction, i.e., the aqueous solution containing water-soluble fatty acids, was saturated with sodium chloride and repeatedly extracted with ether, and the ether was evaporated after drying; the residue was submitted to paper chromatography[2] and the presence of formic (Rf 0.45), acetic (0.52), propionic (0.67), butyric (0.75), and valeric acids was confirmed. Acid–II (oily fatty acid) was extracted with iso-octane and the iso-octane solution was poured over a column (1.5 × 30 cm.) of silica gel[14]. The rate of flow was adjusted to 1 cc. per minute by applying a pressure of 20 cm. Hg of nitrogen gas, and the effluent was collected in 5 cc. fractions. Each fraction was titrated with 0.02N ethanolic potassium hydroxide. The chromatogram of the Acid-II fraction is shown in Text-fig. 1.

The sodium salts of fatty acids separated by column chromatography were character-

ized as their p-bromphenacylesters.

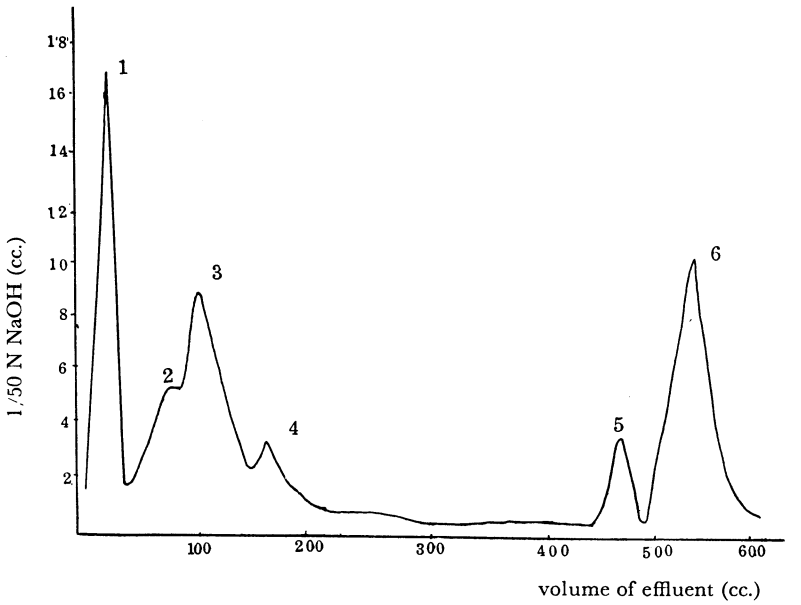
1) Acid II-1: Its p-bromphenacylester, m.p. 71–72°C, showed no depression in the melting point on being admixed with the p-bromphenacylester (m.p. 73–75°C) obtained from *Laminaria* sp.[9].

2) Acid II-2: *Identification of Caprylic Acid:* Its p-bromphenacylester, m.p. 63.5–64.5°C, showed no depression in the melting point on being admixed with p-bromphenacylester of pure caprylic acid (m.p. 65–66°C).

3) Acid II-3: *Identification of n-Caproic Acid:* Its p-bromphenacylester, m.p. 67.5–68.0°C, showed no depression in the melting point on being admixed with p-bromphenacylester of pure n-caproic acid (m.p. 71–72°C).

4) Acid II-4: *Identification of Isovaleric Acid:* Its p-bromphenacylester, m.p. 65–66°C, showed no depression in the melting point on being admixed with p-bromphenacylester of isovaleric acid (m.p. 66.5–67.5°C).

5) Acid II-5: *Identification of Propionic Acid:* The melting point of its p-bromphenacylester, m.p. 56.0–57.0°C, remained undepressed on being admixed with p-bromphenacylester of propionic acid (m.p. 56.0–56.5°C).



Text-fig. 1. Chromatographic separation of the Acid -II fraction.

6) Acid II-6: *Identification of Acetic Acid:* The melting point of p-bromphenacylester, 82.5–83.5° C, remained undepressed on being admixed with p-bromphenacylester of acetic acid (m.p. 83–84°C).

b. *Fraction Soluble in 3% Potassium Hydroxide and 10% Sodium Carbonate (Higher Fatty Acid Fraction):* The ether solution of the crude oil, left after removal of the bicarbonate-solution portion, was shaken thoroughly with 3% potassium hydroxide and the mixture was allowed to stand overnight in order to break the emulsion. The potas-

sium hydroxide solution was acidified with hydrochloric acid and extracted with ether. The ether extract was shaken further with 10% sodium carbonate solution in order to separate higher fatty acids from phenols. The carbonate-soluble fraction was acidified with dilute hydrochloric acid and extracted with ether, and the ether was evaporated after drying.

The carbonate-soluble fraction contained saturated and unsaturated fatty acids. Their separation was carried out by the method reported in Part I[1] of this series. Recrystallization of saturated fatty acids from ethanol afforded crystals melting at 61–62°C which formed a p-bromphenacylester of m.p. 85.0°C. The ester crystal showed no depression in the melting point on admixture with the corresponding ester of palmitic acid. Thus the presence of palmitic acid was confirmed. Unsaturated fatty acids are being investigated.

c. *Fraction Soluble in 3% Potassium Hydroxide but Insoluble in 10% Sodium Carbonate (Phenol Fraction)*: The fraction soluble in 3% potassium hydroxide solution which was left after removal of the fraction soluble in 10% sodium carbonate, was washed three times with saturated sodium chloride solution and dried, and the ether evaporated. Pale brown, oily substance with cresol-like odor was obtained, which was submitted to chromatostrip[3] with 30% ethyl acetate-hexane mixture as the developer. The papergram was sprayed with 2% aqueous solution of phosphomolybdic acid[16] and a spot of Rf 0.54 was obtained. The spot corresponded to that of p-cresol.

d. *Fraction Soluble in 30% Sodium Bisulfite (Carbonyl Fraction)*: The ether solution of crude oil, left after removal of fatty acids and phenol fraction, was repeatedly shaken with 30% sodium bisulfite solution and the combined bisulfite solution was made alkaline with sodium carbonate. This was subjected to steam distillation; the distillate was saturated with sodium chloride and subsequently extracted with ether. Evaporation of ether after drying left a pale yellow, oily substance which had an aroma of cinnamaldehyde. The oil reduced the Fehling solution and formed a 2,4–dinitrophenylhydrazone. This oil was submitted to chromatostrip[4] and sprayed with a solution of 2,4–dinitrophenylhydrazine sulfite, by which three spots appeared at Rf 0.19 (pink), 0.28 (red), and 0.47 (pink). In order to collect larger amount of each hydrazone separately, a chromato-plate was prepared in the following manner. Silica gel-gypsum mixture was spread over a glass plate, 15 cm. in width and 25 cm. in length, by the same method as described in Part III[3] of this series, and dried for 1.3 hours at 80°C in a reverse-current drier. The carbonyl compound was spotted on a base line drawn 5 cm. away from one end, and this was developed in a tightly topped glass jar measuring 17 cm. in inside diameter. When a solution of 2,4-dinitrophenylhydrazine sulfate was sprayed after development, three bands of 2,4-dinitrophenylhydrazones with Rf values of 0.18 (Band C), 0.27 (Band B) and 0.47 (Band A) were obtained.

1) Isolation of Benzaldehyde: Band A in the above chromatoplate (Rf 0.47) was collected and dissolved in benzene, and the solution was submitted to column chromatography using activated alumina as absorbent[1),17),18)]. One band of pink color was obtained. When benzene was evaporated from this pink band in carbon dioxide atmosphere, a 2,4–dinitrophenylhydrazone sparingly soluble in either methanol or ethanol

was obtained. Recrystallization from ethanol-ethylacetate mixture (1:2) afforded crystals melting at 241–241.5°C, undepressed on admixture with 2,4-dinitrophenylhydrazone of commercial benzaldehyde (m.p. 241–242°C). Their infrared absorption spectra were also in good agreement (Plate 1, Figs. 1 and 2).

2) Isolation of Trans- and Cis-forms of 2,4-Dinitrophenylhydrazone of α-Methylfurfural: The 2,4-dinitrophenylhydrazone with Rf 0.27 (Band B, red color) was dissolved in benzene and submitted to column chromatography with activated alumina as adsorbent, giving two bands, pink (I) and red (II).

Benzene was evaporated from the pink band (I) in carbon dioxide atmosphere under a reduced pressure, and orange needle crystals of m.p. 222.5–223.0°C were obtained. No depression of the melting point occurred on admixture with the corresponding derivative of cis-form of α-methylfurfural (m.p. 223.0°C).[9] Their infrared absorption spectra were in good agreement[9] (Plate 1, Fig. 3).

The 2,4-dinitrophenylhydrazone forming the red band (II) was dissolved in benzene and removal of solvent under a reduced pressure in carbon dioxide atmosphere gave a red 2,4-dinitrophenylhydrazone of m.p. 194–196°C. For further purification, 30 mg. of this hydrazone was dissolved in 20 cc. of chloroform and was submitted to column chromatography using a mixture (2:1) of silica gel (MALLINCRODT) and celeit (JOHNS-MANVILLE) as adsorbent and 2% ethyl ether-petroleum ether as the developing solvent[19),20)]. A brown band and a small amount of pink band were obtained. The brown band was eluted with benzene, benzene was removed and the residue was recrystallized from methanol. Reddish brown needle crystals were obtained. Recrystallization from methanol afforded crystals melting at 202–203°C and repeated recrystallizations failed to raise the melting point. Admixture of the above crystals with the trans-derivative of the 2,4-dinitrophenylhydrazone of α-methylfurfural[9] (m.p. 203–204°C) showed no depression of the melting point. Their infrared absorption spectra were in good agreement[9] (Plate 1, Fig 4).

3) Isolation of Furfural: The 2,4-dinitrophenylhydrazone giving Band C (Rf 0.18, pink) was dissolved in benzene and submitted to column chromatography with activated alumina as adsorbent. A yellow and a red band were obtained, which were respectively designated Band I and II. Benzene was distilled off from the yellow band (I) under a reduced pressure in carbon dioxide atmosphere, and the residue was recrystallized from ethylacetate-methanol mixture (1:2), yielding yellow needle crystals of m.p. 205–206°C. Distilled commercial furfural (b.p. 161°C) was derived to its 2,4-dinitrophenylhydrazone. On submitting it to column chromatography with activated alumina as the adsorbent and benzene as the developer, the cis (m.p. 208°C)- and trans (m.p. 230°C) -derivatives[21)] were separated. Admixture of the above crystals of m.p. 205–206°C with the cis-derivative of the 2,4-dinitrophenylhydrazone of furfural (yellow needles, m.p. 208°C) showed no depression of the melting point. Infrared absorption spectra of the two were also in good agreement[22)] (Plate 2, Fig. 5).

The residue from the red band (II) was recrystallized from methanol-ethylacetate mixture (2:1) to yield red prismatic crystals (m.p.229–229.5°C), which showed no depression in the melting point on admixture with the trans-derivative of the 2,4-

dinitrophenylhydrazone of furfural (red prisms, m.p. 230°C). The infrared spectra of the two were also identical (Plate 2,Figs. 6 and 7).

e. *Terpene Fraction (Neutral Compounds)*

1) Rf Value pf Pure Terpenes in Chromatostrip: Studies on the chromatostrip of terpenes have been carried out by KIRCHNER[23),24),25)], REITSEMA[26)], INDO[27)], FURUKAWA[28),29)] and YAMAMOTO and FURUKAWA[30),31)]. In the present study, Rf values of 31 kinds of pure terpenes were determined, and the results are shown in Table 2.

Table 2. Rf value* of neutral compounds (mainly pure terpenes).

Compounds	Rf-value	Compounds	Rf-value
Hydrocarbons		benzaldehyde	0.46
α-pinene	0.88	propionaldehyde	0.58
β-pinene	0.82	vanilline	0.074
terpinolene	0.75	isovanilline	0.054
cadinene	0.83	cinnamaldehyde	0.34
p-cymene	0.61	*Alcohols and phenolic compounds*	
d-limonene	0.70	geraniol	0.20
β-phellandrene	0.82	citronellol	0.01
Esters		eugenol	0.32
linalylacetate	0.53	carvacrol	0.34
geranylacetate	0.50	terpineol	0.24
terpenylacetate	0.51	isoborneol	0.32
Carbonyl compounds		isoeugenol	0.28
pseudoionone	0.34	menthol	0.31
fenchone	0.51	thymol	0.39
ionone	0.59	linalool	0.34
carvone	0.40	borneol	0.28
citronellal	0.49	anethol	0.02
citral	0.37	furfuryl alcohol	0.13
anisaldehyde	0.22	*Oxides*	
furfural	0.29	1:8-cineol	0.49
perillaldehyde	0.41	ascaridol	0.45

* Developing temperature, 25°C; developing solvent, 15% ethyl acetate in n-hexane; adsorbent, silica gel.

2) Terpene Fraction from *C. fragile:* The neutral oil left after removal of free and higher fatty acid, phenol and carbonyl fractions was fractionated with the WIDMER micro-fractionation apparatus improved by the author[3)]. The result of the frationation is listed in Table 3.

Each of these fractions was submitted to chromatostrip[3),23),24),25),28),29)] with the results shown in Table 4.

Table 3. Result of fractional distillation.

Fraction	Pressure (mm)	b. p. (°C)	Yield (g)	N_D^{25}	Color
I	15	-50	0.5	1.4719	light yellow
II	〃	51-60	0.2	1.4723	〃
III	10	-50	0.4	1.4762	〃
IV	〃	51-60	0.3	1.4775	〃
V	5	-50	0.2	1.4821	〃
VI	〃	51-60	0.4	1.4830	〃
VII	3	50-60	0.5	1.4886	〃
VIII	〃	61-70	1.2	1.4896	〃
IX	〃	71-80	0.8	1.4890	〃
X	〃	81-90	0.5	1.4823	brown
XI	〃	91-100	0.7	1.4812	〃
XII	〃	101-110	0.1	1.4782	〃
XIII	〃	111-120	0.5	1.4776	〃
XIV	〃	121-130	0.4	1.4835	〃
XV	〃	131-140	0.7	1.4885	〃
XVI	〃	141-150	0.8	1.4892	〃

On the basis of the Rf value, coloration, and pressure-boiling point curve[32),3)], the presence of furfuryl alcohol, terpinolene, 1:8-cineol, linalool, geraniol and eugenol was revealed.

SUMMARY

1. The presence of dimethylsulfide was confirmed in the gaseous phase which did not condense during steam distillation.

2. The fraction soluble in sodium bicarbonate solution was separated into two fractions: the water-soluble fatty acids and the water-insoluble fatty acids. The presence of formic, acetic, propionic, butylic, valeric acids in the first fraction was confirmed by paper chromatography. The second fraction was submitted to silica gel column chromatography with methanol as the stationary phase and iso-octane as the mobile phase under a pressure of nitrogen gas, and each of the fatty acids thus separated was characterized as its p-bromphenacylester; the presence of isovaleric, n-caproic, and caprylic acids was confirmed.

3. Phenol fraction was submitted to chromatostrip and presence of p-cresol was confirmed.

4. Carbonyl fraction was fractionated by a chromatoplate and subsequently treated with 2,4-dinitrophenylhydrazine. The benzene extract of each hyrazone band was column-chromatographed. The presence of benzaldehyde, α-methylfurfural, and furfural was confirmed.

5. Terpene fraction was fractionated with a micro-fractionation apparatus and each of the obtained fractions was submitted to chromatostrip. From their Rf values, color and pressure-boiling point curves, the presence of furfuryl alcohol, terpinolene, 1:8-

Table 4. Fractionation of neutral compounds with chromatostrip.

No.	b.p. (°C)	Pressure (mm)	Rf values															
I	-50	15	furfuryl alcohol 0.13		0.23				0.42	0.51						pink 0.74	0.81	
II	51-60	〃	0.12 purple		0.22				0.43	0.51	1:8-cineol 0.52					0.75	0.81	
III	-50	10			0.23				0.43							0.74	pink	
IV	51-60	〃			light-blue				0.43		0.53					terpinolene		
V	-50	5							0.43		pink							
VI	51-60	〃					linalool		0.43						0.71			
VII	50-60	3					0.33		pink						0.72			
VIII	61-70	〃					0.34					0.56			0.73	0.73		
IX	71-80	〃					0.33					0.56			pink	0.74		
X	81-90	〃		geraniol 0.20			0.33					pink		0.67		pink		0.87
XI	91-100	〃		0.19			pink	eugenol					0.61	0.67				0.87
XII	101-110	〃		pink		0.29		0.34					0.60	0.68			0.80	pink
XIII	111-120	〃				0.29		0.35					0.60	0.67	0.70		0.80	
XIV	121-130	〃				pink		0.35					0.60	pink	0.70		0.80	
XV	131-140	〃						pink					pink		0.70		0.80	
XVI	141-150	〃													pink		0.81 pink	

cineol, linalool, geraniol and eugenol was revealed.

6. The principle of aromatic components of *Codium fragile* is dimethylsulphide, benzaldehyde, α-metylfurfural, furfural, furfuryl alcohol, terpinolene, 1 : 8-cineol, linalool, geraniol and eugenol. The odorous components of *C. fragile* are formic, acetic, propio nic, butyric, isovaleric, n-caproic, caprylic acids and p-cresol.

The author expresses his deep gratitude to Prof. T. TOMIYAMA of Kyushu University for his kind guidance throughout the course of this work and to Prof. T. MATSUURA, Prof. T. YAMAMOTO and Mr. T. FURUKAWA of Hiroshima University for their valuable advice concerning the chromatostrip of terpene.

REFERENCES

(1) KATAYAMA, T. & TOMIYAMA, T. 1952. Bull. Jap. Soc. Sci. Fish., **17**:132.
(2) KATAYAMA, T. 1953. Ibid., **19**: 793.
(3) KATAYAMA, T. 1955. Ibid., **21**: 412.
(4) KATAYAMA, T. 1955. Ibid., **21**: 416.
(5) KATAYAMA, T. 1955. Ibid., **21**: 420.
(6) KATAYAMA, T. 1956. Ibid., **22**: 244.
(7) KATAYAMA, T. 1955. Ibid., **21**: 425.
(8) KATAYAMA, T. 1958. Ibid., **24**: 346.
(9) KATAYAMA, T. 1958. Ibid., in press.
(10) KATAYAMA, T. 1958. Ibid., **24**: 205.
(11) BANDO, T. & KATAYAMA, T. 1955. Folia Pharmacologica Japonica, **51**: 40.
(12) BANDO. T. & KATAYAMA, T. 1955. Ibid., **51**: 112.
(13) BANDO, T. & KATAYAMA, T. 1958. Ibid., in press.
(14) VANDENHEUVEL, F. A. & HAYES, E.R. 1952. Anal. Chem., **24**: 960.
(15) JUDEFEIND, W.L. & REID, E.E. 1920. J. Amer. Chem. Soc., **42**: 960.
(16) RALP, L.H. 1951. Ibid., **73**: 852.
(17) BUCHMAN, E.R., SCHLATTER, M.J. & REIMS, A.O. 1942. Ibid., **64**: 2701.
(18) JONATHAN, W.W. 1948. Anal. Chem., **20**: 726.
(19) GORDON, B.E., WOPAT, F., BURNHAM, H.D. & JOHNES, L.C. 1951. Ibid., **23**: 1754.
(20) ROBERTS, J.D. & GREEN, C. 1946. Ibid., **18**: 335.
(21) BRADDOCK, L.I., GARLOW, K.I., GRIM., L.I., KIRKPATRICK, A.F., PEASE, S.W., POLLARD, A.J., PRICE, E.E. REISMANN, T.L., ROSE, H.H. & WILLARD, M.L. 1951. Ibid., **23**: 420.
(22) ONISHI, I. 1958. Studies of Essential Oils of Tobacco Leaves. Central Research Institute, Japan Monopoly Corporation, Tokyo.
(23) KIRCHNER, J.G., MILLER J. M. & KELLER J.G. 1951. Anal. Chem., **23**: 420.
(24) MILLER, J.M. & KIRCHNER, J. G. 1951. Ibid., **23**: 428.
(25) MILLER, J.M. & KIRCHNER, J. G. 1953. Ibid., **25**: 1107.
(26) REITSEMA, R. H. 1954. Ibid., **26**: 960.
(27) INDO, G. & OZAWA, T. 1953. Reports Asssoc. Camphor Ind. Eng. Jap., **18**: 29.
(28) FURUKAWA, T. 1955. J. Fac. Education Hiroshima Univ., **3**: 53.
(29) FURUKAWA, T. 1956. Ibid., **4**: 37.
(30) YAMAMOTO, T. & FURUKAWA, T. 1957. Ibid., **5**: 67.
(31) FURUKAWA, T. 1958. J. Sci. Hiroshima Univ., **21**: 285.
(32) DANIEL, R. S. 1947. Ind. Eng. Chem., **39**: 517.

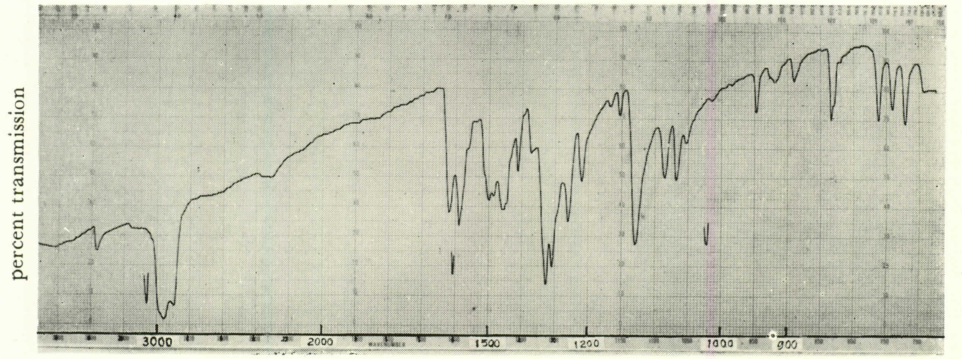
EXPLANATION OF PLATES

Plate 1

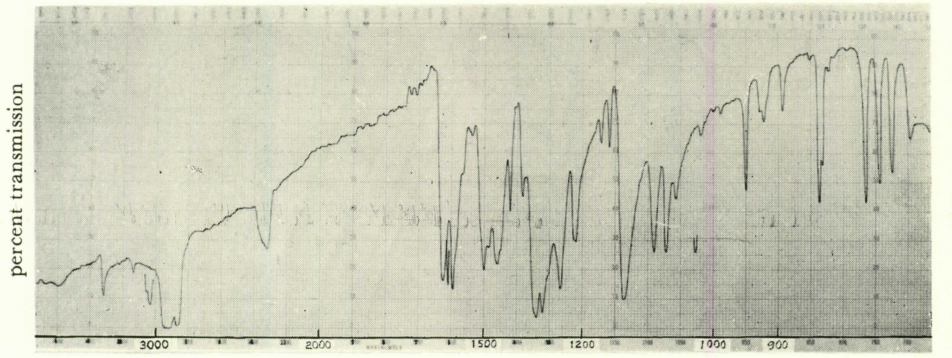
Fig. 1. The infrared absorption spectra of a 2,4-dinitrophenylhydrazone (m.p. 241-241.5°C).
Fig. 2. The infrared absorption spectra of 2,4-dinitrophenylhydrazone of benzaldehyde (m.p. 241-242°C).
Fig. 3. The infrared absorption spectra of 2,4-dinitrophenylhybrazone (m.p. 222.5-223.0°C).
Fig. 4. The infrared absorption spectra of a 2,4-dinitrophenylhydrazone (m.p. 202-203°C).

Plate 2

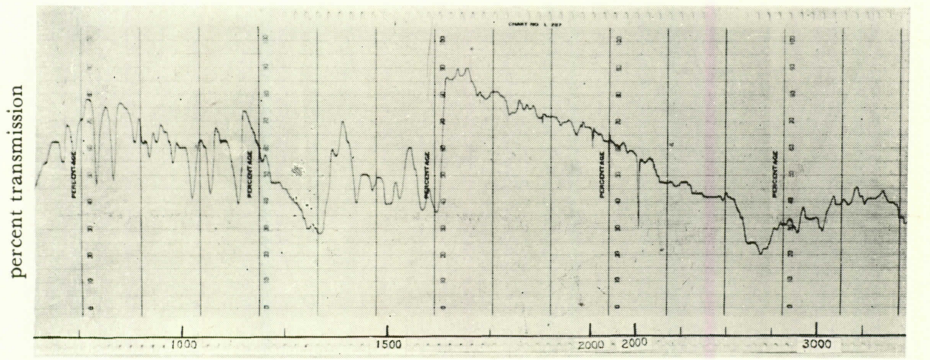
Fig. 5. The infrared absorption spectra of a 2,4-dinitrophenylhydrazone (m.p. 205-206°C).
Fig. 6. The infrared absorption spectra of a 2,4-dinitrophenylhydrazone of m.p. 229-229.5°C.
Fig. 7. The infrared absorption spectra of the trans-derivative of 2,4-dinitrophenylhydrazone of furfural (m.p. 230°C).


percent transmission
3000
2000
1500
1200
1000
900
wave number, cm⁻¹

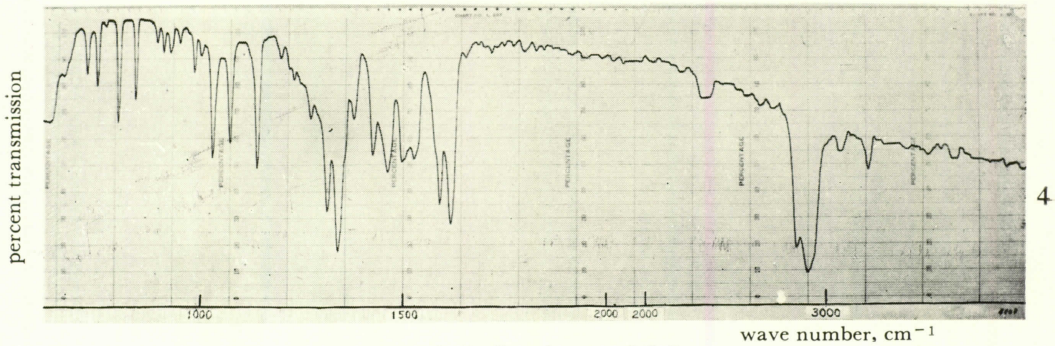
1


percent transmission
3000
2000
1500
1200
1000
900
wave number, cm⁻¹

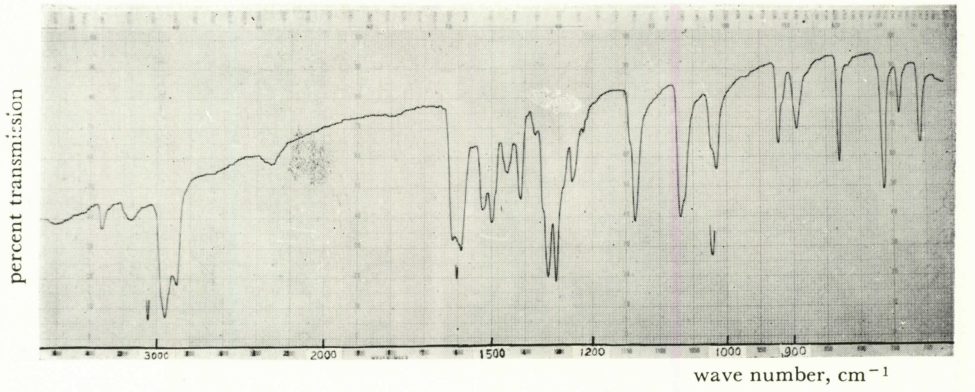
2


percent transmission
1000
1500
2000
2000
3000
wave number, cm⁻¹

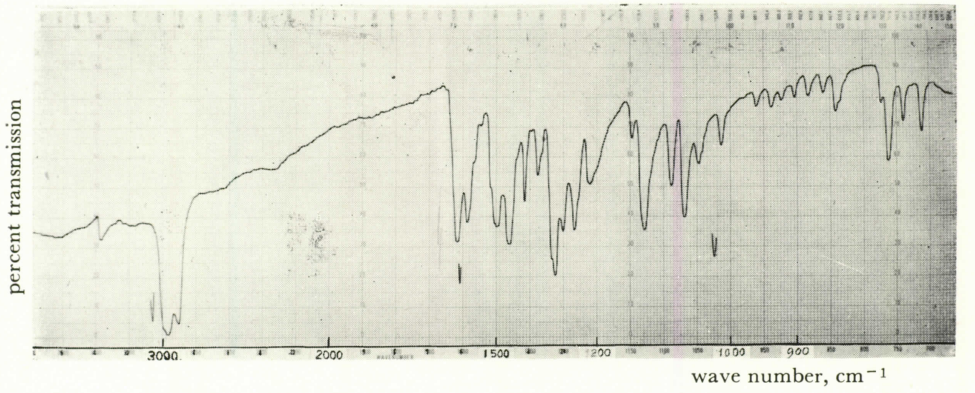
3


percent transmission
1000
1500
2000
2000
3000
wave number, cm⁻¹

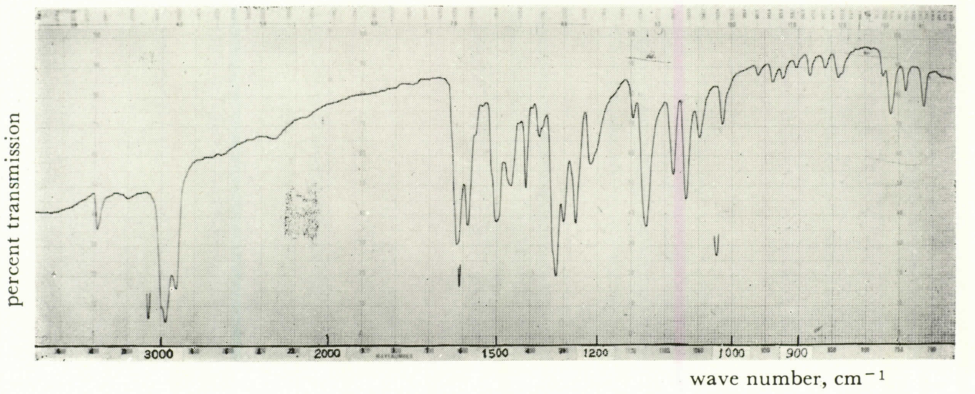
4


percent transmission
3000
2000
1500
1200
1000
900
wave number, cm⁻¹

5


percent transmission
3000
2000
1500
1200
1000
900
wave number, cm⁻¹

6


percent transmission
3000
2000
1500
1200
1000
900
wave number, cm⁻¹

7