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CHEMICAL STUDIES ON MENTHA

III. On the Odorous Substances of Mint Plants (I)*

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長沢　徹・曾我　治：薄荷属植物の化学的研究
第3報　薄荷植物の臭気成分について（その I）

I INTRODUCTION

Japanese mint[1] (*M. arvensis*, L.)[1][2][3] is the most excellent species[1][4] of mentha, which contains 80～90% of *l-menthol* in the essential oil, but it is a weak point that it has a special bad odor. In recent years, the new excellent superior variety of mentha, named "San-Bi",[5][6] has been discovered in Okayama Prefecture, and bred at the Okayama Agricultural Experiment Station. It has been cultivated in our country, as the mentha has the highest yield of *menthol*. However, "San-Bi" has taken people's attention to the fact that it has a proper disagreable odor.

One of the writers, Nagasawa, has been studied the object of peppermint for many years,[4][7][8][9][10][11][12] especially, the aroma and taste of the Japanese *menthol* crystals, and reported the carbonyl compounds[13] in order to detect the odorous components and bitter substances in the dementholized oils.

The authors have commenced the researches, in view of the fact that the low-boiling and volatile substances were dissolved in the distilled water produced by steam distillation. We have confirmed that the distilled water of "San-Bi" has a strong ammoniacal and fishy odor, which should be related to amines.

The object of this paper is to describe the *nitrogen* compounds dissolved in the distilled water of "San-Bi", and at the same time, to report the *sulfur* compounds, which exist in the first gas generated by the steam distillation.

II CRITICISM ON LITERATURES

We shall discuss some odorous substances of plants as the following.

1. *Sulfur Compounds*

(a) Dimethyl sulfide:—

Dimethyl sulfide has been found in American Peppermint oil[14][15] as one of the odorous substances; it was discovered in the aroma of black tea,[16] green tea,[17] in the various marine plants,[18][19] asparagas,[18] and in the young buds of the brackens.[18] It can be catched with the saturated

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mercuric chloride solution, to a white precipitate.

(b) Hydrogen sulfide:—

Hydrogen sufide was found in the aroma of foods18) in the recent year. Dr. T. Yamanishi17)18)20) found it in the vapors, which generated from the leaves of the various plants by steam distillation, and she presumed that *hydrogen sulfide* shall be generated by decomposition of protein.

(c) Other sulfur compounds:—

*Methyl mercaptane,*16)21) *ethyl mercaptane, propyl mercaptane,*22) and *allylpropyl disulfide*22) have been found in foods.

2. Nitrogen Compounds

As the low-boiling *nitrogen* compounds,23)24) *ammonia, monomethylamine, trimethylamine, iso-butylamine,* and *iso-amylamine* are found in a number of plants, but *dimethylamine* and *ethylamine* are not found so much. Generally, these amines exist in the animal and vegetable kingdom in the form of amino-acids, *betaine, choline,* and alkaloids.

(a) Monomethylamine:—

Monomethylamine is merely an amine which found in the mint plants. It has been expressed that this amine exists in the leaves of "*mentha aquatica*" (water-mint).25) It seems probable that this amine was produced by the result of decomposition of alkaloid, because the leaves of water-mint contain a few alkaloids (0.026-0.034%)26) but a trace of essential oil. This amine24) has been found in the buds of *Mercurialis annua, M. perennis,* in the substerranean stem of Calamus. Moreover, it was found with *trimethylamine* in the products of dry distillation of Pyrethrum flowers. It has been expressed that the mother-bodies of formation of this amine are *choline, betaine, trimethylamine,* and complicated *N-methyl* compounds.24)

(b) Trimethylamine:—

In the kingdom of plants, *trimethylamine*24) is more distributed than *monomethylamine*; it was found in the flowers of *Chenopodiaceae, Rasaceae, Oleaceae, Composite, Liliaceae,* etc., and then in the ergots, lichens, red-seaweeds. In recent years, this amine was found in tangles,27) in flowers of *Heracleum spondylium,*28) and in the fruits of *Phallus impudicus.*28)

(c) Dimethylamine:—

It was found in the fruits of *Russula aurata.*28)

(d) Ethylamine:—

It was found in the flowers of *Sambucus nigra* and *Arum italicum.*28)

(e) iso-Butylamine:—

This amine was found in the flowers28) of *Sambucus nigra, Filipendula ulmaria,* and *Arum italicum.*

(f) iso-Amylamine:—

*Iso-Amylamine*23) has an ammoniacal odor and has been found in the flowers of *Berberidaceae, Rosaceae, Aceraceae, Asclepiadaceae, Caprifoliaceae,* and ergots of rye malt. In recent years,28) it was found in the flowers of *Sambucus nigra, Filipendula ulmaria,* and in the fruit bodies of *Phallus impudicus, Boletus sanguineus.* Nagasawa (one of the writers) supposed the existence of the odorous substances in the components of the natural perfume and investigated also, on the odorous substances of plants other than mints.*

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* Announced at the meeting of the Chugoku-Shikoku Branch of the Chemical Society of Japan on Jan. 24, 1959 (at Hiroshima University).

III RESULTS AND DISCUSSION

1. Odorous substances in the distillation gas

All mentha, we used, reacted with saturated *mercuric chloride* solution, and formed a white, pale yellow, or dark grey precipitates. "San-Bi" (*M. arvensis*) formed, especially, the remarkable dark grey precipitates. The compounds which may be formed white or dark grey precipitates with saturated *mercuric chloride* solution are shown in the Table 1.

Table 1. Products with saturated mercuric chloride solution

Compounds	Products	Color
Methylamine	$[CH_3N(HgCl)]_2Hg$	white
Ammonia	$Hg(NH_2)Cl$	〃
Methyl mercaptane	$CH_3.S.HgCl$	〃
Dimethyl sulfide	$[(CH_3)_2S]_2+3HgCl_2$	〃
Hydrogen sulfide	$Hg_3Cl_2S_2$	〃
Hydrogen sulfide	HgS	black

We confirmed that *hydrogen sulfide* is contained in the distillation gas, in view of the fact that the test papers of *silver nitrate* and *lead acetate* became brown when they contacted with the gas, which was generated by the steam distillation of all mint plants, of our use. As the result of observation on the products with saturated *mercuric chloride* solution, it can be understood that a large quantity of *hydrogen sulfide* is contained in the gas of "San-Bi". As a large quantity of *carbon dioxide* generates when the mint plants are steam distilled, it can be thought that this *hydrogen sulfide* was produced by the decomposition of protein as Dr. T. Yamanishi has ever suggested.17)18) Further, we presumed that *dimethyl sulfide** may exists in the gas.

2. Nitrogen compounds in distilled water

We found some amines such a large quantity of *ammonia*, a few *trimethylamines*, and two another amines which are supposed as *monomethylamine* and *dimethylamine*, respectively. *Trimethylamine* has a fishy odor and the other amines have that of *ammonia*.

In the first paper of this series,29) we have reported the results of chemical analysis of "San-Bi", which contained much essential oils, and "Aka-Hen", containing a little ones, that "San-Bi" contained a very high percentage of total *nitrogen*, proteinous *nitrogen*, soluble *nitrogen*, and ammoniacal *nitrogen* than "Aka-Hen", in the respective leaves. Especially, proteinous *nitrogen* accounts for 80 per cent of the total *nitrogen*. On the basis of these data, it seemed probable that these amines are decomposed and isolated with a large quantity of *carbon dioxide* by the pyrolysis of protein.

"San-Bi" absorbs a large quantity of nourishment and accumulates essential oil in the bodies vigorously. On the other hand, *nitrogen* compounds in the plant bodies are decomposed and isolated, when they are steam distilled, then an unpleasant odorous substances such as *trimethylamine* dissolved in the distilled water. We assumed therefore, that one of the causes of the unpleasant odor of essential oils depends on the disagreable odorous substances dissolved in the distilled water.

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* T. Nagasawa: Reported at the meeting of Chugoku-Shikoku Branch of the Chemical Society of Japan, on May 5, 1955 (Fukuyama City).

IV EXPERIMENTAL

1. Apparatus and Manipulation

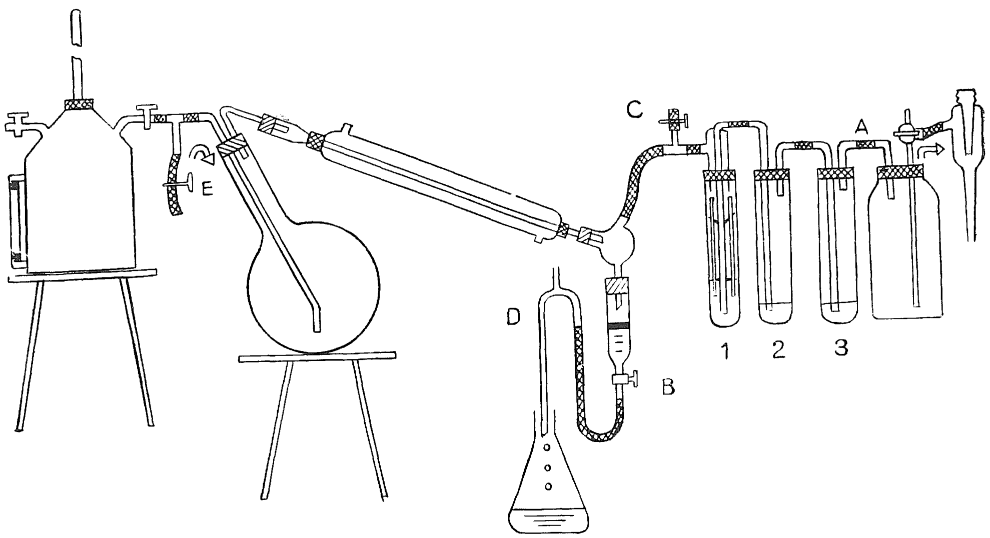

Fig. 1. Distillation Apparatus

Take as a sample 100~150 g. of dry herbs in a round-bottomed 2,000-cc. flask, and connect each apparatus (Fig. 1). Then, shut cock (B) of separating funnel (Cylinder type, vol. 100 cc.), open cock (A) of safety bottle, and then steam distillation was carried out. After distillation was started, the gas was introduced to the absorbents (10~15 cc.) in 100cc. test tube (No. 2. 3), during about 1.5 minutes.

Absorbents as *mercuric chloride* solution (6.5%), distilled water, and *lead acetate* solution, etc. were used. In No. 1 test tube, it was tested that reaction with filter paper dipped in *silver nitrate* solution (1N) or *lead acetate* solution (0.1%). When the generation of gas came to an end, water and the essential oil begins to distil. Then, open the cock (B), pinch cock (C) and these oil and water was received in the separating funnel, the water level of which is controlled by the siphon (D). When dry herbs (100~150 g.) are used, they are steam distilled during 20 minutes. When the steam distillation came to an end, shut the cocks (B) and (C), and open the pinch cock (E) (circuit→). Then, pass the residual gas in the apparatus through absorbents in the test tube by the aspirator during about 30 seconds. On the above mentioned manipulation, the volatile gas is caught by absorbents in the test tube; the water soluble matters with essential oil could be obtained.

Thus, the distilled water obtained by such manipulation was named volatile-water (vol.-water) and the essential oil was named volatile-oil (vol.-oil). Moreover, this vol.-water (700 cc.) was concentrated by cohobation using the same apparatus within 10 minutes for one cohobation.

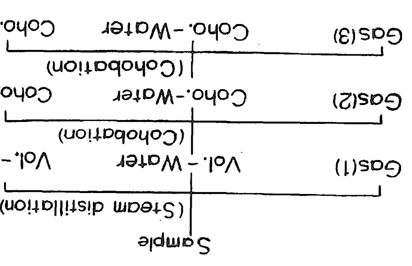

Fig. 2. Outline of Manipulation

The concentrated water was named cohobation-water (coho.-water) and the oil now obtained was named cohobation-oil (coho.-oil). The coho.-oil was kept as alcoholic solution for the use of the other experiments.

As the result of the above-mentioned manipulation, the volatile and water soluble substances were concentrated in the coho.-water; some volatile substances were absorbed in the absorbents of the test tube. A rough outline of the above-mentioned process is shown in Fig. 2.

2. Odorous substances in the gas

Mint plants (lst and 2 nd crop, 1957) were steam distilled with the apparatus shown in Fig. 1. Those experimental results are shown in Table 2. All distilled waters, absorbing gases, have a pickle like odor, which may be estimated as *mercaptane*. This odor was removed by *mercuric chloride* solution. When all mint gases reacted with *mercuric chloride* solution, they produced a white~dirty yellow~dark grey precipitate. The precipitate of the same species do not show a certain color, but has a tendency to differ from the quantity of a sample. "San-Bi" (*M. arvensis*) produced dark grey precipitate distinguishable to the other, but Chirimen-Hakka (*M. viridis*) produced a white or yellowish grey precipitate. Test papers moistened with *silver nitrate* solution, *lead acetate* solution, Nessler's reagent, and Schiff's reagent had a positive reaction. Saturated *barium hydroxide* solution produced a white *barium carbonate* with *carbon dioxide* in the gas.

Table 2. Reaction with Mercuric chloride solution (6.5%)

Species of Mints*		Time of Crop 1957(I) ~ (II)	Weights of Fresh Herbs	Oil Yields to Fresh Herbs	Reaction with 6.5% $HgCl_2$ sol.	
		Month-Day	(g)	(%)	Precipitate	Color
M.arvensis	San-Bi	6.21	413	0.29	+	grey
	Aka-Kuki	10.22(1956)	3,750	0.28	+	white
	Kakô	6.22	1,418	0.17		〃
M.piperita	English white hair	6.14	1,069	0.22	+	〃
	American white	6.13	2,918	0.12	+	pale yellow
M.viridis	Chirimen white hair	5.30	1,410	0.17	+	white
	German crape	6.12	2,614	0.13	+	〃
M.arvensis	San-Bi	8.6	12,368	0.51	+	grey
	Aka-kuki	〃	1,650	0.47	+	white
	Brazil	〃	2,475	0.43	+	gray
	Inu	〃	1,613	0.38	+	white
	Kakô	8.14	3,125	0.38	+	〃
M.piperita	American white	8.26	1,313	0.20	+	grey
	English white	8.15	605	0.19	+	white
M.viridis	Chirimen white hair	8.22	1,313	0.34	+	〃
	Chirimen red stem	8.13	10,256	0.22	+	pale yellow

* Cultivated at Matsue City

3. Odor and PH of Volatile-water

Volatile-water has a special odor in compliance with the species. By means of cohobation, PH of the volatile-water became basic, however, the water which absorbed the gases (gas-absorb-water) was still showed a weak acidic property. An example is shown in Table 3. Since vol.-water becomes basic remarkably, it is probable to assume as that a large quantity of basic substances are dissolved in the water. Further, in view of the fact that a sharp precipitate was formed by Nessler's reagent, the presence of basic substances was shown.

Table 3. PH of Volatile-water and Gas-absorb-water

Distillation No.	Volatile-water		Gas-absorb-water	
	Volume (cc.)	PH	Volume (cc.)	PH
1	720	4.70	70	5.05
2	↓ 150	7.60	70	5.95
3	↓ 82	8.10	70	6.00

N.B. Sample : English white hair (M.piperita)
Fresh herbs…………1,069g. (1957, 6. 14)

4. Nitrogen compounds in the volatile-water of "San-Bi" *(M. arvensis)*

The dry herbs (5, 115 g.) of "San-Bi" were steam distilled and 2,400 cc. of volatile water were obtained, and transformed to 100 cc. of coho.-water (PH 8.8~9.0). To the coho.-water (100 cc.) added 6N *HCl* and concentrated to 5cc. of brown solution under reduced pressure. When it becomes alkaline by adding some *sodium hydroxide* solution, emerged a queer strong ammoniacal and fishy odor.

At the preliminary experiment, the following test was carried out. About same volume of sample to be tested and 10 per cent solution of *ammonium chloride* (Kantôkagaku-make), *hydrochloride* salts (Merck-make) of *monomethylamine* (MMA), *dimethylamine* (DMA) and *trimethylamine* (TMA), were applied from capillary tube on Tôyô-No. 50 filter paper, and then carried out the procedures of Obata as follows.[30] —

a) Spray with Nessler's reagent.

b) Spray with 0.1 per cent *ninhydrin-butanol* solution, and then heat to 95°C for 5 minutes.

c) Spray with 0.5 per cent *p-quinone-ethanol* solution, and dry, then spraying with 0.5 per cent caustic *potash-ethanol* solution.

d) Expose on *iodine* vapour.

These experimental results are shown in Table 4.

Table 4. Reaction of Nitrogen compounds with Reagents

Reagents	MMA		DMA		TMA		Ammonia		Sample	
	R.	Color	R.	Color	R.	Color	R.	Color	R.	Color
Nessler's R.	—	—	—	—	—	—	+++	yellow	+	yellow
Ninhydrin	+++	purple	++	purple	+	purple	+	orange	+	purple
p-Quinone	++	pink	+++	pink	+	white*	+	white*	+	white*
Iodine	+	yellow	++	yellow	+++	yellow	++	yellow	+	yellow

N. B. R. : Reaction
* White spot in red-brown spot

(1) The reagents which colored clearly these spots were as follows. *Ammonia* . . . . . Nessler's reagent; *Monomethylamine* . . . . . *Ninhydrin*; *Dimethylamine* . . . . . *p-quinone*; *Trimethylamine* . . . . . *Iodine.*

(2) The best reagent, being able to detect all spots, was *ninhydrin-butanol* solution.

(3) In this experiment, we found that *trimethylamine* is colored by *ninhydrin-butanol* solution.[31]

Then, paper chromatography was carried out using one-dimensional ascending method. A proper quantity of the sample was spotted from a capillary tube to the original point (5 cm from the lower edge) of the Tôyô No. 50 filter paper (2×40 cm) strip. The filter paper strip was developed during 15 hours at 24~26° (room temperature) with a solvent of *n-butanol-ethanol-water* (4 : 1 : 1). Sample was developed with pure *nitrogen* compounds, and then these spots were made visible with the above-mentioned reagents. The results of examination are as follows:–(Fig. 3, Table 5)

Table 5. Rf values of Nitrogen Compounds

Base	Nessler's R.			Ninhydrin			Iodine		
	Rf			Rf			Rf		
	Pure	Sample	Color	Pure	Sample	Color	Pure	Sample	Color
Ammonia	0.18	0.18	yellow	0.18	0.18	yellow	0.18	0.18	yellow
Monomethylamine	—	—	—	0.20	—	purple		—	yellow
Dimethylamine	—	—	—	0.23	—	purple		—	yellow
Trimethylamine	—	—	—	0.25	0.25	purple	0.25	0.25	yellow
					0.11	purple			
					0.06	purple			

Fig. 3. Paper chromatogram of Nitrogen Compounds in the Vol.-water of "San-Bi"
Rf: 0.06, 0.11, 0.18, 0.25

(1) It was confirmed that both *ammonia* (Rf: 0.18) and *trimethylamine* (Rf: 0.25) were contained in the volatile-water.

(2) It seems doubtless that these spots (Rf: 0.11, 0.06) of *dimethylamine* and *monomethylamine* were disturbed by the existence of a large quantity of *ammonia*.

(3) These amounts of nitrogen compounds in the volatile water existed in the following order.

ammonia>*trimethylamine*>*dimethylamine*?>*monomethylamine*?
Rf: 0.18 0.25 0.11 0.06

V CONCLUSION

1) The writers found that *hydrogen sulfide*, ammoniacal compounds and carbonyl compounds exist in the gas produced by the steam distillation of various Mint plants. "San-Bi", especially, contained a large quantity of *hydrogen sulfide*. Besides, we assumed the existence of *dimethyl sulfide*.

2) It was found that both *ammonia* and *trimethylamine* existed in the volatile-water of "San-Bi", while the presence of *dimethylamine* and *monomethylamine* is almost probable.

3) One of the unpleasant odor of "San-Bi" is due to the presence of the ammoniacal compounds as *trimethylamine*.

REFERENCES

1) T. Nagasawa: "Peppermint and Pyrethrum", (**1942**).

2) E. Gildemeister: "Die Ätherischen Öle", 3 Aufl. I (**1928**), p. 560, 581.

3) E. Guenther: "The Essential Oils", 3d Ed. II (**1957**), p. 396, 400; 2d Ed. III (**1952**), p. 640~663.

4) T. Nagasawa: "Science of Mentha", (**1950**).

5) T. Nagasawa: Treatise on Beni-Hakka (Crimson-mint), *Bull. Okayama Agr. Reform*, No. **59**, 24~31 (1953).

6) T. Nagasawa: Studies on the Progress of Japanese Genus Mentha (V), Researches on Beni-Hakka ("San-Bi"), *Special Bull. Okayama Agr. Expt. Sta.*, No. **50**, 1~34 (1954).

7) E. Shinosaki, T. Nagasawa: Researches on the Japanese Peppermint Oils (IV), *Repts. Imp. Ind. Research Inst., Osaka, Japan*, **10** (15), 1~60 (1929); *J. Soc. Chem. Ind. Japan*, **32**, 582~587 (1929).

8) T. Nagasawa: Properties of Japanese Mint Oils, *Repts. Peppermint-Pyrethrum Inst., Agr. Govern., Japan.* No. **1**, 60~64 (1943).

9) T. Nagasawa: Essential oil of "Hokushin" mint in Hokkaido, *Repts. Peppermint-Pyrethrum Inst., Agr. Govern., Japan.* No. **1**, 65∼73 (1943).

10) T. Nagasawa: Aroma and Odor of Japanese Mentha, *Koyo*, No. **7**, 30 (1949).

11) T. Nagasawa: A treatise on "Inu-Hakka" (Dog-mint), *Rapid Bull. Okayama Agr. Reform*, No. **16**, 35 (1950).

12) T. Nagasawa: Diagnosis of Mentha (Studies on the Progress of Japanese Genus Mentha (I)), *Koryo*, No. **13**, 17∼19 (1950).

13) T. Nagasawa: Chemical Studies on mentha (II), On the odorous Components of the Japanese Peppermint Oils (1), *Bull. Shimane Univ.* (*Natural Sci.*), No. **9**, 30∼43 (1959).

14) E. Guenther: "The Essential Oils", Vol. 1 (**1949**), p. 311 . . . . U S P, XIII, p. 390.

15) T. Nagasawa: On the Progress of Japanese Genus Mentha, *Coll. Ess. Comm. Shimane Univ.* (*Natural Sci.*), 12∼33 (**1960**).

16) R. Yamamoto, K. Itô and H. Tin: Studies on the Essential Oil of Formosan Black Tea (V), *J. Agr. Chem. Soc. Japan*, **16**, 791∼799 (1940).

17) M. Tsujimura, T. Yamanishi, R. Akiyama and S. Tanaka: Studies on the Flavoring Substances of Green Tea (1), On Sulfur Compounds, *J. Agr. Chem. Soc. Japan*, **29**, 145∼148 (1955).

18) T. Yamanishi: Flavors and Sulfur Compounds, *Chem. and Chem. Ind.* (Chem. Soc. Japan) **10**, 220∼222 (1957).

19) Y. Obata and K. Matano: *Bull. Japanese Soc. Sci. Fish.*, **17**, 60 (1952).

20) T. Yamanishi, J. Takagaki and M. Tsujimura: *Bull. Agr. Chem. Soc. Japan*, **20**, 127∼130 (1956).

21) T. Katayama: Chemical Studies on Volatile Constituents of Sea-weed, On Volatile Constituents of *Digenia simplex*, *Bull. Japanese Soc. Sci. Fish.*, **24**, 205∼208 (1958).

22) T. Yamanishi and K. Orioka: Chemical Studies on the Change of Flavor and Taste of Onion by Boiling, *J. Home Economics*, **6**, 45∼47 (1955).

23) E.H. Rood: "Chemistry of Carbon Compounds", I-A (**1951**), p. 388∼399.

24) M. Kotake et al: "大有機化学", Vol. IV, p. 52∼70 (**1959**).

25) Merck Co.: "The Merck Index of Chemicals and Drugs", 6th Ed. (**1952**).

26) A. I. Karaev, R.K. Aliev, and P.A. Yvzbashinskayu: The chemical composition of the wooly hedge-nettle grass and water-mint leaves and effect of its compounds on the contracting properties of uterus muscles, *Doklady Akad. Nauk Azerbaidzhan. S.S.R.* **II**, No. 3 187∼93 (1955); *C.A.*, **51**, 3924f (1957).

27) T. Katayama: Chemical Studies on Volatile Constituents of Seaweed (XIII), On Volatile Constituents of *Laminaria* sp., *Bull. Japanese Soc. Sci. Fish.*, **24**, 346∼354 (1958).

28) M. Steiner, E. Stein, V. Kamienski: Paper chromatographic detection of primary, secondary and tertiary amines in plants, *Naturwissenschaften*, **40**, 483 (1953); *C.A.*, **48**, 8333f (1954).

29) T. Nagasawa, O. Soga: Chemical Studies on Mentha (I), On the Chemical Compositions in the Plants of the "San-Bi" (*M. arvensis*, L.) and the Transmuted "Aka-Kuki", *Bull. Shimane Univ.* (*Natural Sci.*), No. **7**, 53∼76 (1957).

30) Y. Obata, K. Matano: Studies of the Flavors of Katsuobushi (II), Identification of Volatile Substances, *J. Agr. Chem. Soc. Japan*, **26**, 184∼185 (1951).

31) S. Miyahara: Separation and Determination of Methylamines in Fishes. (1), *J. Chem. Soc. Japan*, **81**, 1158∼1163 (1960).