— Research paper —

Screening of deodorizing active compounds from natural materials and deodorizing properties of cineole

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Plant extracts were screened to identify novel deodorizing natural products. Deodorizing activity was detected in an aqueous ethanol extract of cluster mallow leaves by measuring headspace gas by GC (deodorizing activity was defined as percent reduction in headspace odorant concentration following deodorant addition *in vitro*). The deodorant compound was purified using silica gel column chromatography, and the active principle was identified as 1,8-cineole by GC/MS analysis. 1,8-Cineole strongly deodorized (>90%) a relatively wide range of odorous gases, including 2-nonenal, skatole, dimethyl trisulfide, indole, isovaleric acid, and dimethyl disulfide. The compounds also moderately deodorized (>70%) allyl methyl sulfide, n-butyric acid, allyl mercaptan, and dimethyl sulfide. Organoleptic assessment conducted by sniffing headspace gas showed a reduction in odor score of 1.5 to 3. Correlative relationships were observed between 1,8-cineole's deodorizing activity and odorant molecular weight and boiling point. Furthermore, odorants adsorbed onto 1,8-cineole desorbed with increasing temperature, suggesting a physical deodorizing mechanism of cineole. Cineole is known to mask or hide ambient odorants by overwhelming the malodor with pleasant odor. Our study suggests that in addition to odor-masking activity, 1,8-cineole exhibits another deodorizing mechanism in which the gaseous odorant concentration is reduced via adsorption involving physical interaction between 1,8-cineole and odorant molecules. 1,8-Cineole may thus serve as an effective agent for eliminating various unpleasant odors.

1. Introduction

In recent years, large-scale industries such as livestock agriculture, food processing, and wastewater treatment have expanded greatly. These industries often generate numerous types of unpleasant odors, sometimes resulting in complaints from nearby residents. Open-air incineration and restaurants (e.g., beef barbecue restaurants) become sources of odor-related complaints in some cases. In the course of daily life at home, we also often experience unpleasant odors. These odors can arise from a variety of sources, including pet waste, spoiled food (e.g., rotten onions/fish), and raw garbage. Unpleasant breath odor is also experienced at some time in our lives.

Odorous gases have a variety of chemical structures, and include aldehydes, esters, nitrogen-/sulfur-bearing compounds, and short-chain fatty acids. The number of odor compounds is estimated at over 400,000¹⁾. Odor

control methods can be classified into 4 categories, depending on the deodorizing mechanism: (1) physical methods, such as the use of activated carbon or silica gel to absorb malodorous compounds; (2) biological methods, such as the use of sanitizers that inactivate malodor-producing bacteria; (3) chemical methods, which involve chemical inactivation of malodorous compounds; and (4) sensory methods, which involve masking unpleasant odors with fragrances.

Deodorant manufacturers have developed a variety of products employing these mechanisms. For example, green tea, whose primary components are polyphenols (catechins) such as (–)-epigallocatechin gallate, (–)-epigallocatechin, (–)-epicatechin gallate, and gallic acid, exhibits potent deodorizing activity²⁾; thus, deodorant products containing green tea catechins are widely used. Kaki-tannin, the primary ingredient in astringent persimmon fruit, is also widely used in Japan and Southeast Asia for breath odor-neutralizing supple-

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Fig. 1 Structures of 1,8-cineole and 1,4-cineole.

ments, chewing gums, air fresheners, pet deodorants, deodorant lotions, etc.³⁾. The deodorization mechanisms of polyphenolic compounds are reportedly chemical reactions including addition, degradation, and replacement^{4,5)}. Several sulfur-containing gases arising from vegetables of the *Allium* species can be reduced chemically or physicochemically by raw foods such as fruits, vegetables, and mushrooms⁶⁾ (we will cover this point in more detail below). Among the odorous gases associated with breath odor, including garlic breath (i.e., an unpleasant odor released after garlic consumption), hydrogen sulfide (HS) and methyl mercaptan (MM) are almost totally deodorized by green tea *in vitro*, and the levels of these odors are reduced significantly *in vivo*²⁾, while allyl methyl sulfide (AMS) is poorly deodorized compared with HS and MM *in vitro* and *in vivo*⁷⁾. As such, compounds with potent deodorizing activity against AMS have attracted interest as screening targets for new odor-reducing agents.

In our screening programs to isolate active substances exhibiting deodorizing activity against AMS, we identified 1,8-cineole from cluster mallow leaves. Here, we focused upon 1,8-cineole and its isomer, 1,4-cineole, and investigated the deodorizing activity-related properties of these substances (Figure 1).

2. Materials and Methods

Chemicals. 1,8-Cineole (hereafter 1,8-C; purity: 99.8%), 1,4-cineole (hereafter 1,4-C; purity: >95%, Sigma-Aldrich, St. Louis, MO, USA), silica gel (Wakosil C-300), MM, and dimethyl trisulfide (DMTS) were purchased from Wako Pure Chemical Industries (Tokyo, Japan). Finely ground leaves of cluster mallow (Chinese herbal preparation) were obtained from Kahya Co., Ltd. (Osaka, Japan). 2-Nonenal and AMS were obtained

from Alfa Aesar (Ward Hill, MA, USA); skatole, allyl mercaptan (AM), and dimethyl sulfide (DMS) were obtained from Acros Organics (Pittsburgh, PA, USA); indole and butyric acid were obtained from Kishida Chemical Co. (Osaka, Japan); and dimethyl disulfide (DMS), isovaleric acid, and diacetyl were obtained from Tokyo Chemical Industry (Tokyo, Japan).

Preparation of screening samples. A variety of natural materials were purchased from local markets, including fruits (23 types), vegetables (18 types), seeds (12 types), teas/herbal plants (33 types), sea weeds (13 types), and mushrooms (10 types). The natural materials were initially lyophilized and processed by grinding using a mortar. Commercially dried and ground plant materials, such as a variety of teas/herbal plants, including leaves of cluster mallow, were also screened. A total of 109 samples were screened (Table 1).

To prepare screening samples, natural material prepared as described above (10 g) was weighed in a conical flask, to which 100 mL of 50% aqueous ethanol was then added and stirred for 30 min at room temperature using a magnetic mixer. The sample was then centrifuged for 10 min (1500g), and the supernatant was collected. The sample was re-extracted twice using the method described above. The ethanol was removed from the combined extract using a rotary evaporator. The final concentrated extract solutions were then lyophilized and powdered.

For gas chromatography (GC) analyses for screening, approximately 100 mg of the sample suspended in 1 mL of deionized water was placed in a separate 300-mL conical flask. A variety of phenolic compounds with deodorizing activity, such as catechins, have been isolated from plant extracts and identified to date. To minimize the possibility of isolating known phenolic compounds, we selected samples that gave negative results in a ferric chloride test (ferric chloride test: a few drops of 10% ferric chloride solution are added to the test sample solution; the formation of bluish-black color indicates the presence of phenolic compounds).

For specific analysis of the deodorizing activity against various odorous chemicals, cineole (a colorless liquid) was placed directly inside a 300-mL conical flask just below the neck using a micropipette (cineole volume: 110 μ L, 220 μ L, or 330 μ L), and GC analyses were conducted.

GC and gas detector tube analyses. Screening the natu-

ral materials for deodorizing activity was performed via GC. In each assay, the sample was placed in a 300-mL conical flask as described above, and then 10 μ L of AMS (or other odorous chemical) solution was injected into the flask through the Parafilm[®] covering the flask mouth using a microsyringe and the same method described above for cineole. The mouth of the flask was sealed tightly with Parafilm[®] again (double sealed) immediately after addition of the odorous chemical solution. The flask was left to stand for 60 min at 25°C, and then 2 mL of the headspace gas was analyzed on a Shimadzu GC-2014AF GC apparatus. We assumed that the loss of odorous gases by permeation through the Parafilm and by adsorption onto the glass during 60 min of incubation at 25°C (deodorizing reaction completely or almost completely reached plateau values) was not significantly different between the sample flask and control flask. In the case of DMTS, the loss was 2.4% (average of three measurements). Details regarding GC conditions are shown in **Table 2**. The initial concentration of odorous chemical was 1 ppm in the gas phase, with the following exceptions: for skatole, indole, isovaleric acid, and diacetyl, the initial concentration was 10 ppm, because the GC peaks for these gases at a concentration of 1 ppm were too small under the GC conditions used to allow for quantification. We observed that the deodorizing activity of cineole was unaffected by the initial concentrations of odorous gases tested (**Table 3**).

Deodorizing activity, expressed as percent reduction in odorous gas concentration in the headspace, was calculated using the following formula: deodorizing activity (%) = $(C - S) / C \times 100$, where C represents the peak area for the authentic chemical standard in the headspace gas of the reaction mixture without a test sample (control), and S represents the peak area for the headspace gas of the reaction mixture containing the test sample⁴.

An AP-20 Kitagawa Gas Detector Tube System was used to detect acetic acid, trimethylamine (TMA), acetaldehyde, and ammonia. A tube connected to an aspirating pump was inserted through a slit in the Parafilm covering the sample flask made using a knife, and a 100-mL sample of the headspace gas was withdrawn and analyzed using the gas detector. The odorant concentration (ppm) was determined by direct reading from the scale on the tube. Deodorizing activi-

ty (%) was calculated using the formula described above for GC analyses. For gas detector analyses, the initial concentration of malodorous chemical was 10 ppm.

Solid-phase micro-extraction (SPME) and GC/MS conditions for determination of the active principle. A 2-mg sample was placed in a 2-mL screw-top vial. Collection of volatile compounds was accomplished using a 15-min adsorption period at 60°C, during which the sample was shaken. The SPME fiber introduced into the headspace of the sample vial was an 85- μ m layer of Carboxen/PDMS Stable Flex (Sigma-Aldrich Co.). After SPME, the compounds were analyzed using GC/MS. GC/MS was performed using a Hewlett Packard HP5890 Series II gas chromatograph coupled to a Hewlett Packard HP5872 series mass spectrometer. A DB-WAX capillary column (60 m $\times$ 0.25 mm i.d.; film thickness: 0.25 μ m) was used for separation. High-purity helium was used as the carrier gas at a flow rate of 1.2 mL/min. The column temperature was programmed to increase from 40°C (held for 4 min) to 200°C at 4°C/min. The injection and detection temperatures were 260 and 220°C, respectively. The ionization voltage was set at 70 eV. Chemical components were identified by comparing their mass spectra and retention times to those of commercially available library standards (NIST Chemistry WebBook).

Fractionation of cluster mallow leaf extract using silica gel column chromatography. The 50% aqueous ethanol extract of finely ground leaves of cluster mallow was re-extracted and fractionated as follows. Lyophilized and powdered extract (200 g), prepared as described above, was suspended in water (3.5 L), stirred for 30 min, and then divided into two portions by centrifugation: water soluble and insoluble. After the extraction procedure was repeated twice, 181.4 g of water-soluble material and 11.4 g of insoluble material were obtained. The water-insoluble material was soluble in CHCl_3 and exhibited relatively stronger deodorizing activity compared with the soluble material and a negative ferric chloride test result, which indicated the absence of phenolic compounds such as catechins in the insoluble portion. The silica gel column (silica gel: 170 mL) was prepared using a Komagome pipette with CHCl_3 . The column was 20 cm in length, with a 4-cm internal diameter. The column was loaded with the CHCl_3 -soluble extract material (8.9 g) and eluted with

Table 1 Deodorizing capacities of 109 selected natural material-derived extracts.

No.	English name	Scientific name ^{a)}	D.A. ^{b)}	No.	English name	Scientific name ^{a)}	D.A. ^{b)}
1	Asparagus	Genus <i>Asparagus</i>	6.0	34	Passion fruit	<i>Passiflora edulis</i>	1.9
2	Bell pepper	<i>Capsicum annuum</i>	0	35	Peach	<i>Amygdalus persica</i>	7.8
3	Bitter melon	<i>Momordica charantia</i>	15.5	36	Pineapple	<i>Ananas comosus</i>	1.9
4	Cabbage	<i>Brassica oleracea</i>	0	37	Plum	Genus <i>Prunus</i>	7.0
5	Carrot	<i>Daucus carota</i>	14.5	38	Prune	<i>Prunus domestica</i>	7.0
6	Cauliflower	<i>Brassica oleracea</i>	28.5	39	Raspberry	Genus <i>Rubus</i>	9.4
7	Cucumber	<i>Cucumis sativus</i>	0	40	Watermelon	<i>Citrullus lanatus</i>	0
8	Crown daisy	<i>Glebionis coronaria</i>	26.3	41	Garden strawberry	<i>Fragaria</i>	9.2
9	Edible burdock	<i>Arctium lappa</i>	5.3	42	Almond	<i>Amygdalus dulcis</i>	13
10	Eggplant	<i>Solanum melongena kouki</i>	52.2	43	Common gardenia	<i>Gardenia jasminoides</i>	12.1
11	Indian spinach	<i>Basella alba</i>	0	44	Common Hazel (Hazelhut)	<i>Corylus avellana</i>	20.4
12	Japanese radish	<i>Raphanus sativus</i>	0	45	Japanese Chestnut	<i>Castanea crenata</i>	7.8
13	Kabocha squash	<i>Cucurbita maxima</i>	18.4	46	Kabocha squash (seed)	Genus <i>Cucurbita</i>	40.7
14	Komatsuna	<i>Brassica rapa</i>	15.8	47	Macadamia nut	<i>Macadamia integrifolia</i>	11.4
15	Okra	<i>Abelmoschus esculentus</i>	0	48	Pecan	<i>Carya illinoensis</i>	29.9
16	Shallot	<i>Allium cepa</i>	0	49	Pine nut	Genus <i>Pinus</i>	36.4
17	Tomato	<i>Solanum lycopersicum</i>	0	50	Pistachio	<i>Pistacia vera</i>	21.6
18	Zucchini	<i>Cucurbita pepo</i>	0	51	Pistachio (hull)	<i>Pistacia vera</i>	25.7
19	Apricot	<i>Prunus armeniaca</i>	5.2	52	Sunflower	<i>Helianthus annuus</i>	17.3
20	Apple	<i>Malus pumila</i>	0	53	Walnut	Genus <i>Juglans</i>	22.2
21	Banana	Genus <i>Musa</i>	7.7	54	Adlay	<i>Coix lacryma-jobi</i>	12.6
22	Blueberry	Genus <i>Vaccinium</i>	11.1	55	Bitter melon (fruit)	<i>Momordica charantia</i>	33.7
23	Cherry	Genus <i>Prunus</i>	6.3	56	Cat's Claw (root, bark)	<i>Uncaria tomentosa</i>	0
24	Dragon fruit	<i>Hylocereus undatus</i>	10.3	57	Cluster mallow (leaf)	<i>Malva verticillata</i>	71.9
25	Fig tree	<i>Ficus carica</i>	6.5	58	Dandelion (leaf)	<i>Taraxacum officinale</i>	39.3
26	Grape (Delaware)	Genus <i>Vitis</i>	10.0	59	Echinacea	<i>Echinacea purpurea</i>	13.9
27	Grapefruit	<i>Citrus × paradisi</i>	11.9	60	Field Horsetail (epigeal stem)	<i>Equisetum arvense</i>	14.1
28	Kiwifruit	<i>Actinidia deliciosa</i>	0	61	Fishwort (leaf)	<i>Houttuynia cordata</i>	46.8
29	Lemon	<i>Citrus limon</i>	5.0	62	Garcinia	<i>Garcinia gummi-gutta</i>	18.6
30	Lime	<i>Citrus aurantiifolia</i>	23.7	63	Guava	<i>Psidium guajava</i>	42.2
31	Lychee	<i>Litchi chinensis</i>	6.7	64	Gymnema (leaf)	<i>Gymnema sylvestre</i>	35.5
32	Nashi Pear	<i>Pyrus pyrifolia</i>	9.6	65	Hibiscus (flower)	<i>Hibiscus sabdariffa</i>	4.0
33	Papaya	<i>Carica papaya</i>	8.0	66	Isodon Herb (Enmeisou)	<i>Rabdosia japonica</i>	57.3

Vegetables

Fruits

Fruits (continued)

Seeds

Teas/ Herbal plants

Teas/ Herbal plants (continued)	67	Japanese Honeysuckle	<i>Lonicera japonica</i>	128	Marine algae	87	Wakame	<i>Undaria pinnatifida</i>	29.2
	68	Khat	<i>Catha edulis</i>	0		88	Kombu	<i>Saccharina japonica</i>	6.2
	69	Kothalahimbutu	<i>Salacia reticulata</i>	0		89	Arame	<i>Eisenia bicyclis</i>	56.3
	70	Ku Ding tea (leaf)	<i>Ilex kudingcha</i>	29.1		90	Hijiki (stalk)	<i>Sargassum fusiforme</i>	15.5
	71	Kumazasa (leaf)	<i>Sasa veitchii</i>	12.6		91	Hijiki (bud)	<i>Sargassum fusiforme</i>	16.6
	72	Loquat (leaf)	<i>Eriobotrya japonica</i>	14.6		92	Mozuku	<i>Nemacystus decipiens</i>	9.7
	73	Lotus (flower)	<i>Nelumbo nucifera</i>	19.7		93	Tosakanori	<i>Meristotheca papulosa</i>	11.8
	74	Mate (leaf, twig)	<i>Ilex paraguariensis</i>	26.7		94	Tsunonata	<i>Chondrus ocellatus</i>	38.0
	75	Melilot (Sweet clover)	<i>Melilotus officinalis</i>	13.5		95	Akamoku	<i>Sargassum horneri</i>	12.5
	76	Morning-glory	<i>Ipomoea nil</i>	20.3		96	Kubiretsuta	<i>Caulerpa lentillifera</i>	8.6
	77	Mulberry (leaf)	Genus <i>Morus</i>	53.2		97	Matsumo	<i>Anelipus japonicus</i>	8.6
	78	Olive (leaf)	<i>Olea europaea</i>	25.6		98	Funori	Genus <i>Gloiopeltis</i>	7.3
	79	Persimmon (leaf)	<i>Diospyros kaki</i>	3.4		99	Tengusa (agar)	<i>Gelidium elegans</i>	10.3
	80	Kothalahimbutu	<i>Salacia reticulata</i>	0	Fungi	100	Shiitake mushroom	<i>Lentinula edodes</i>	11.8
	81	Snow tea	<i>Thamnia vermicularis</i>	3.2		101	Enokitake	<i>Flammulina velutipes</i>	0
	82	Taheebo (inner bark)	<i>Tabeuia avellaneda</i>	10.7		102	King trumpet mushroom	<i>Pleurotus eryngii</i>	0
	83	Green tea	<i>Camellia sinensis</i>	20.0		103	Butterscotch mushroom	<i>Pholiota microspora</i>	17.7
	84	Tencha	<i>Rubus suavisissimus</i>	61.7		104	Golden oyster mushroom	<i>Pleurotus cornucopiae</i>	52.5
	85	Tochu (leaf)	<i>Eucommia ulmoides</i>	32.7		105	White mushroom	<i>Agaricus bisporus</i>	11.8
	86	Rafuma (leaf)	<i>Apocynum venetum</i>	0		106	Buna shimeji	<i>Hypsizygus marmoreus</i>	10.9
						107	Maitake	<i>Grifola frondosa</i>	0
						108	Jew's Ear Fungus	<i>Auricularia auricula-judae</i>	6.6
						109	Snow fungus	<i>Tremella fuciformis</i>	6.1

a) Genus and species or genus alone are cited from Wikipedia.

b) D.A.: deodorizing activity

Table 2 Gas chromatography conditions.

Malodorous gas	Column	Detector ^b	Temperature (°C)		Carrier gas flow rate ^a
			injector	oven	
DMDS, DMTS	Unisole F-200 30/60	FPD	120	80	20
MM, AM AMS, DMS	Unisole F-200 30/60	FPD	120	50	20
Isovaleric acid n-Butyric acid 2-Nonenal	Unisole F-200 30/60	FPD	200	120	50
Indole, Skatole	OV-17 60/80	FID	200	120	50
Diacetyl	Sunpak-A 50/80	FID	230	180	50

Notes: ^aNitrogen, mL/min; ^bDetector temperature, 200°C.

Table 3 Deodorizing activity (%) of 1,8-cineole against different initial concentrations of malodorous gases.

Initial concentration (ppm)	1	10	50	100
DMTS	91 ± 0.5	96 ± 0.4	n.d.	95 ± 0.7
Isovaleric acid	n.d.	91 ± 1.9	82 ± 1.2	86 ± 2.6
Indole	n.d.	91 ± 0.7	89 ± 2.1	94 ± 1.3
Skatole	n.d.	85 ± 2.2	94 ± 0.5	94 ± 3.1
DMDS	n.d.	91 ± 1.9	81 ± 1.2	86 ± 2.6

Notes: Concentration of 1,8-cineole, 100 mg/300-mL flask; n.d., not determined.

CHCl₃. The fraction volume was 200 mL, and a total of 20 fractions were collected. For analysis of the deodorizing activity of each fraction, 100 µL of chloroform eluate from each fraction was added to a 300-mL conical flask, and the chloroform was evaporated to dryness by incubation at room temperature.

Desorption analyses. Desorption experiments were conducted using a Shimadzu headspace sampler HS-20 and GCMS-TQ8040 system. A total of 50 mg of 1,8-C and 100 ppm of odorous gas, including 2-nonenal, DMTS, isovaleric acid, or skatole, was placed directly inside a 20-mL HS vial and incubated at 37°C for 1 h. Under these conditions, 99.5–99.7% of each odorous gas was adsorbed to 1,8-C. The HS-20 vial containing 1,8-C with adsorbed odorous gas was left to stand for 60 min at 50, 75, 100, or 150°C, and then the desorbed gas was measured.

Organoleptic measurement (OM). OMs were performed as follows. A 300-mL conical flask containing

50 mg of cineole was incubated with a variety of odorous gases at 25°C for 60 min. To prepare odorous gas solutions, 1:10 or 1:2 dilutions of original odorous solutions were prepared to give a range of concentrations appropriate for organoleptic measurements with a 3–4 malodor intensity level. The volume of odorous gas solution injected into the 300-mL flask was 2–10 µL. The judges sniffed the odorant-containing air inside the odor flask directly after giving oral consent to participate in the study, which was conducted with reference to the “Olfactory Measurement Method Safety Management Manual,” published by the Ministry of the Environment, Government of Japan.

3. Results and Discussion

Identification of the active principle in cluster mallow extract. In this study, we conducted a screening of extracts of a variety of natural materials in an attempt to identify new deodorizing compounds, using an

Table 4 Deodorizing activity, initial concentration, molecular weight, boiling point, solubility, and generation source of odorous gases.

Malodorous gas	Deodorizing activity (%) ^a	Initial conc. (ppm)	MW ^b	Boiling point (°C)	Solubility in water	Generation source (example)
2-Nonenal	95.6 ± 0.6	1	140.22	88–90	— ^d	Body (over middle aged)
Skatole	92.7 ± 1.7	10	131.17	265	— ^d	feces
DMTS	97.3 ± 0.5	1	126.27	170	± ^e	cancer wound
Indole	94.3 ± 0.5	10	117.15	253	± ^e	feces
Isovaleric acid	97.0 ± 1.0	10	102.13	176	± ^e	sole of foot
DMDS	94.6 ± 0.4	1	94.19	110	± ^e	garlic breath
AMS	88.0 ± 4.7	1	88.17	92	± ^e	garlic breath
n-Butyric acid	74.5 ± 7.6	1	88.11	163	± ^e	sole of foot
Diacetyl	40.8 ± 0.2	10	86.09	88	+ ^f	axillae, foot, head
AM	72.4 ± 5.4	1	74.14	67–68	— ^d	garlic breath
DMS	76.8 ± 0.7	1	62.13	37.3	± ^e	rotten meat
MM	69.1 ± 0.7	1	48.11	5.95	± ^e	garlic breath
Acetic acid ^c	17.0 ^h	10	60.05	110	++ ^g	kitchen garbage
TMA ^c	0 ^h	10	59.11	2.9	++ ^g	rotten fish
Acetaldehyde ^c	0 ^h	10	44.05	20	++ ^g	cigarette smoke
Ammonia ^c	0 ^h	10	17.03	– 33.3	++ ^g	livestock waste

Notes: ^a Concentration of cineole, 300 mg/300-mL flask; ^b Molecular weight (g/mol);

^c Deodorizing activity was measured using a gas detector tube system;

^d Insoluble; ^e Slightly soluble; ^f Soluble; ^g Very soluble;

^h Values are expressed as the mean of two independent assays.

AMS-targeted GC-based approach. **Table 1** summarizes the results of our screening of 109 natural material-derived extracts. Among the samples screened, there was a relatively high rate of detection in the tea/herbal plant category and seed category, and cluster mal-low leaves exhibited the highest deodorizing activity. The second highest activity against AMS was detected in a Tencha extract, for which deodorizing activity was reported by Shimizu et al.⁸⁾

Cluster mallow leaves are a good candidate source of compounds for use in deodorant products, exhibiting a deodorizing activity of 70%. The candidate sample was subjected to silica gel column chromatography. Analysis of the water-insoluble/CHCl₃-soluble sample yielded 20 fractions, with peaks exhibiting deodorizing activity against AMS present in fractions 6–9 (a broad peak) and 17. Fractions 6–9 (containing 730 mg of active principle) were combined, and the active principle was further purified by an additional round of chromatography on the same column and following repeated preparative TLC. Based on LC/MS/MS and UV-Vis spectroscopy analyses, the active principle was deduced to be pheophorbide-a (data not shown). As authentic pheophorbide-a exhibited strong deodorizing activity against AMS (to the best of our knowledge,

strong deodorizing activity of pheophorbide-a has not been reported to date; thus, a detailed study will be reported elsewhere), the compound is an interesting candidate odor-reducing agent. However, as pheophorbide-a is reportedly toxic to rats⁹⁾, we did not investigate this compound further.

The chemical constituents of fraction 17 (80 mg) were analyzed by GC/MS, which revealed that 80 components were present (data not shown). Six major components identified by GC/MS analysis included 1,8-C, 2-nonenal, acetic acid, 2-ethyl-1-hexanol, hexanoic acid, and 2-amino-4-methoxyphenol, each of which individually constituted greater than 2% of the total GC peak area and together constituted 30.8% of the total peak area. These compounds were chosen for further analysis. Of the 6 selected compounds, 2-nonenal and acetic acid are typical malodors experienced in daily life. On the other hand, 2-ethyl-1-hexanol, hexanoic acid, and 2-amino-4-methoxyphenol, while less common, are also strongly pungent compounds, in which we are not interested. Furthermore, these 3 compounds exhibited weak deodorizing activities of 18.3%, 0.7%, and 7.7%, respectively, for 300 mg/300-mL flask. The last compound, 1,8-C, exhibited potent deodorizing activity against AMS, which was our screening target (88% for

Table 5 Six major compounds identified by SPME/GC/MS analysis of fraction 17.

Identified compound	Retention time (min)	GC peak area (%)
1,8-Cineole	19.0	13.2
2-Nonenal	24.8	6.6
Acetic acid	26.2	3.8
2-Ethyl-1-hexanol	27.5	2.7
Hexanoic acid	36.7	2.3
2-Amino-4-methoxyphenol	46.9	2.2
Total peak area		30.8

Note: The SPME contaminant, hexamethyl cyclotrisiloxane (6.4%; deodorizing activity: zero) was observed

300 mg of 1,8-C/300-mL flask, **Table 4**, **Table 5**). Thus, we concluded that 1,8-C was the deodorizing active principle in fraction 17.

1,8-C, also known as eucalyptol, is a liquid terpene present in many plants, particularly *Eucalyptus* species¹⁰, and was not included among our 109 screening materials. 1,8-C exhibits a camphor-like odor and is known for its ability to mask offensive odors by its fragrant aroma. We report here for the first time that 1,8-C deodorizes offensive malodors not only by masking them but also by reducing the concentration of odorous gases (Japan Patent 6342382)¹¹. We will discuss the physical mechanism of deodorization in more detail later. Among essential oil components, 1,8-C is generally more useful, as it is easily extractable on a commercial scale. 1,4-C, which is also used as a flavoring and fragrance compound, is found in red wines¹² and plant essential oils¹³. We examined the deodorizing activity of both terpenes using commercially available authentic reagents.

Deodorizing activity of cineole against various malodorous gases in vitro. We investigated the deodorizing effect of cineole on a variety of odorous gases, such as those associated with garlic breath, animal waste, cigarette smoke, and rotten meat (**Table 4**). The deodorizing activity of 1,4-C and 1,8-C was tested against a total of 12 odorous gases using GC.

The review by Iwasaki indicated that the human olfactory sense is generally capable of clearly discriminating differences in odor intensity when there is a 10-fold difference between two odor concentrations and can recognize as little as a 3-fold difference in odor concentration¹⁴. As such, it is preferable for newly developed deodorant products to exhibit deodorizing

activity of approximately 90% against odorous gases. We determined the concentration of cineole necessary to capture >80% of AMS in a 300-mL flask. We also investigated the dose dependence of cineole on deodorizing activity at three doses, 100, 200, and 300 mg/300-mL flask.

In our assay system, 1,8-C exhibited strong deodorizing activity (94–97% for 300 mg of 1,8-C/300-mL flask) against 2-nonenal, skatole, DMTS, indole, isovaleric acid, and DMDS and moderate deodorizing activity (69–88%) against AMS, n-butyric acid, AM, DMS, and MM. In contrast, 1,8-C exhibited only weak deodorizing activity against diacetyl (**Fig. 2A**, **Table 4**).

Compared with 1,8-C, 1,4-C exhibited slightly weaker deodorizing activity, especially against MM and DMS (**Fig. 2B**). It is possible that differences in hydrophobicity (water solubility: 1,8-C, 0.022 g/L and 1,4-C, 0.058 g/L) and boiling point (1,8-C, 176.5°C; 1,4-C, 173.5°C) between 1,8-C and 1,4-C could be responsible for the difference in deodorizing activity. However, whether such moderate differences in physicochemical properties could produce the observed differences in deodorizing activity is unclear. We will discuss the influence of boiling point on deodorizing activity later. Both 1,4- and 1,8-C exhibited dose-dependent deodorizing activity against the odorous gases examined.

The deodorizing activity of 1,8-C against 2-nonenal was investigated in more detail. The activity decreased markedly at a concentration of <50 mg/300-mL flask. The relationship between deodorizing activity and 1,8-C concentration was sigmoidal rather than linear (data not shown).

Table 3 shows that 1,8-C exerts a constant deodorizing effect against a variety of gases over a wide range

Fig. 2 Deodorizing activity of A) 1,8-C and B) 1,4-C against a variety of odorous gases.

Notes: Deodorizing activity was measured at three different concentrations, as described in the "Materials and Methods."

Data represent the mean $\pm$ standard deviation of three independent experiments.

DMTS, dimethyl trisulfide; DMDS, dimethyl disulfide; AMS, allyl methyl sulfide; AM, allyl mercaptan; DMS, dimethyl sulfide; MM, methyl mercaptan.

D.A.: Deodorizing activity.

of concentrations, from 1 to 100 ppm. This means that deodorant products containing 1,8-C could be used to treat ambient malodors exhibiting a wide odor intensity range.

Organoleptic assessment of cineole on various odorous gases. The changes in mean organoleptic scores for the head space of flasks containing 1,8-C incubated with various odorous gases at 25°C for 1 h are shown in Table 6. The organoleptic score was determined by two trained judges who had a national deodorization navigator license. The judges rated the characteristic odor (e.g., greasy or grassy smell for 2-nonenal) of each odorous gas with a score of 0 to 5, where 0 represented the absence of odor, 1 barely noticeable odor, 2 slight malodor, 3 moderate malodor, 4 strong malodor, and 5 severe malodor. The mean odor score was used as the representative score. 1,8-C reduced the organoleptic scores of 2-nonenal, skatole, DMTS, AMS, n-butyric acid, isovaleric acid, and DMDS by more than 2 units.

Greenman et al. investigated the relationship between organoleptic score and the concentrations of pure odor-

ous gases likely responsible for human oral malodor¹⁵⁾. Various concentrations of odorous gases that are constituents of oral malodor were subjectively scored by trained odor judges based on intensity level as 0 (below threshold) to 5 (extremely strong). The authors showed that the organoleptic score was proportional to the log concentration of pure odorous gas. Furthermore, they calculated values indicating the level of increase in gas concentration required to increase the organoleptic score by 1 unit. These values were a 10-fold increase for n-butyric acid and DMDS (in this case, reduction to one-tenth is equivalent to a deodorizing activity of 90%), 42-fold increase for isovaleric acid (deodorizing activity: 97%), 8-fold increase for skatole (deodorizing activity: 87%), and 7.2-fold increase for MM (deodorizing activity: 86%). These data show that deodorizing activities stronger than approximately 90% produce more than a 1-unit reduction in organoleptic score.

A comparison of the organoleptic score data shown in Table 6 with data from Greenman et al. revealed that the reductions in organoleptic scores in this study

Table 6 Changes in organoleptic measurement (OM) score.^a

Odorous gas	Without incubation ^b	With incubation ^c	Reduction of OM score	Deodorizing activity (%) ^d	Deodorizing activity (%) ^e (Greenman et al.)
2-Nonenal	3	0	3	96	—
Skatole	3.5	0.5	3.0	93	87
DMTS	3	0	3	97	—
AMS	3	1.5	1.5	88	—
n-Butyric acid	3	1	2	75	90
Isovaleric acid	3	1	2	97	97
Dimethyl disulfide	3	0	3	95	90

Notes: ^aScores are expressed as the mean of two independent assays conducted by two trained judges.

^bOdorant was incubated at 25°C for 60 min without 1,8-C.

^cOdorant was incubated at 25°C for 60 min with 1,8-C.

^dExcerpted from Table 4.

^eDeodorizing activity required to decrease OM score by 1 unit. Adapted from Greenman et al. data (reference 15).

were more than 1 unit greater than those reported by Greenman et al. The effect of odor-masking fragrances reportedly involves different mechanisms. Osada et al. reported that citrus odorants such as citronellal can mask the odor of DMDS via a mechanism other than overwhelming the malodor¹⁶. This suggests that in addition to reducing the odorous gas concentration, a masking activity of 1,8-C contributes in part to its deodorizing effect.

Fungating wounds sometimes occur in patients with advanced cancer. One of the physical symptoms of a fungating wound is malodor caused by bacteria. The primary odorant emanating from fungating wounds of advanced cancer patients was recently identified as DMTS¹⁷.

A popular conception holds that middle-aged persons have a particularly unpleasant odor known as “aging odor”, which is described as an unpleasant greasy or grassy odor emitted from some individuals 40 years of age or older¹⁸. As is the case of aging odor, microorganisms residing on the skin play an important role in the formation of human body odors, including foot odor. Short-chain fatty acids such as isovaleric acid and butyric acid are believed to be the major contributors to foot odor¹⁹. The potent deodorizing activity of 1,8-C against DMTS, 2-neonal, and isovaleric acid suggests that it could be used in products designed to eliminate or reduce these microorganism-associated body malodors.

Possible mechanism of the deodorizing activity of cineole. The general structures of the malodorous com-

pounds deodorized by 1,8-C are quite different. For example, DMTS, DMDS, AMS, AM, DMS, and MM are sulfur-containing compounds, whereas 2-nonenal is an unsaturated aldehyde, isovaleric acid and n-butyric acid are fatty acids, and skatole and indole are aromatic heterocyclic compounds. However, like cineole, which is a cyclic ether and monoterpenoid, all of these compounds are hydrophobic. As shown in Table 4, 2-nonenal, skatole, and AM are insoluble in water, whereas DMTS, DMDS, AMS, DMS, MM, indole, isovaleric acid, and n-butyric acid are slightly soluble in water (solubility: 0.19–6 g/100 mL), and diacetyl is water soluble (solubility: 20 g/100 mL).

Negishi et al. proposed a possible mechanism for the deodorizing effects of foods, including lipids, against diallyl disulfide and diallyl sulfide⁶. The mechanism involves affinity-based physical and chemical interactions between these sulfur-containing compounds and specific food ingredients, each of which has hydrophobic properties.

In another study screening for deodorant compounds, we identified two hydrophobic compounds from plants. One was a high-molecular-weight, non-polar lipid triolein (MW: 885.4 g/mol), and the other was a low-molecular-weight oleic acid (MW: 282.5 g/mol), both of which exhibited potent deodorizing activity against hydrophobic odorous gases (data not shown). This suggests that both high- and low-molecular-weight hydrophobic compounds exhibit deodorizing activity against hydrophobic gases. Taking the above into account, this suggests that 1,8-C, a low-molecular-

Fig. 3 Deodorizing activity of 1,8-cineole as a function of the molecular weight of the odorous gases shown in Table 4.

— Hydrophobic odorous gases shown in Table 4
 --- All odorous gases shown in Table 4

Fig. 4 Deodorizing activity of 1,8-cineole as a function of the boiling point of the odorous gases shown in Table 4.

weight compound (MW: 154.3 g/mol), interacts with hydrophobic odor molecules to draw them together, forming hydrophobic aggregates and thus reducing the intensity of the odor released.

In order to test this hypothesis further, the deodorizing activity of 1,8-C against hydrophilic odorants such as acetic acid, acetaldehyde, TMA, and ammonia was examined. As these odorous compounds could not be easily quantified by GC in the present study, we used a gas detector tube system. We found that 1,8-C exhibited no or weak deodorizing activity against hydrophilic odorants, including acetic acid, acetaldehyde, TMA, and ammonia (solubility: 144 g/100 mL to infinite,

deodorizing activity: 0 to 17% at 300 mg/300-mL flask, **Table 4**). From this observation, it is clear that 1,8-C does not physically interact with hydrophilic odorants. This observation supports the hypothesis that the deodorizing mechanism of cineole involves hydrophobic interactions between the cineole and odorant molecules.

As shown in **Table 4**, the solubility of diacetyl in water is much higher than that of the slightly soluble compounds but much lower than that of the highly soluble compounds. It is interesting to note that diacetyl exhibited much less potent deodorizing activity than the other insoluble or slightly soluble compounds.

Fig 5 Effect of temperature on desorption of odorous gases.

Note: Desorption experiments were conducted as described in the "Materials and Methods."

1,8-Cineole exhibits a deodorizing effect not only by masking odors but also through physical interactions such as van der Waals forces and/or hydrogen bonding between 1,8-cineole and odorants.

Fig. 6 Physical interaction between 1,8-cineole and odorous gases.

The relatively poorer deodorizing activity of diacetyl may thus be due to its intermediate hydrophobicity.

It is well known that adsorption of a water insoluble adsorbate to an adsorbent such as activated carbon occurs primarily via hydrophobic interactions, which are based on van der Waals forces between the adsorbate and adsorbent^{(20), (21)}. Adsorption via van der Waals forces is reportedly affected by molecular properties such as hydrophobicity as well as a variety of other factors, including molecular weight and boiling point. In general, the greater the molecular weight, the stronger the van der Waals forces between the adsorbate and adsorbent, and consequently, the greater the extent of adsorption^{(21), (22)}, which will, in turn, produce a stronger deodorizing effect.

We found a correlation between the deodorizing ac-

tivity of 1,8-C and the molecular weight of the odorous gases tested. 1,8-C tended to deodorize high-molecular-weight (>90 g/mol) odorous gases more effectively than low-molecular-weight (<90 g/mol) gases (Table 4). The coefficient of determination (R^2)⁽²³⁾ between the deodorizing activity of 1,8-C against the odorous gases shown in Table 4 and their molecular weight was high, at 0.6198. When considering only hydrophobic gases, the coefficient of determination was very high, at 0.7027 (Fig. 3). These correlation data suggest that the deodorizing mechanism of cineole is, at least in part, due to adsorption involving van der Waals forces.

It is also known that van der Waals forces are affected by the boiling point of adsorbates^{(22), (24), (25)}. The higher the boiling point, the stronger the van der Waals forces. We found a correlation between the deodorizing

activity of 1,8-C and the boiling point of the odorous gases tested (Fig. 4), although there were several exceptions, including 2-nonenal. The coefficient of determination (R^2) between the deodorizing activity of 1,8-C against the odorous gases and boiling points of the gases was 0.3286, indicating a moderate correlation.

The van der Waals forces between 1,8-C and 2-nonenal may be governed by the combined effects of their molecular properties. The number of carbons on hydrophobic molecules is important because the greater the number of carbons, the stronger the hydrophobic interaction. Linear carbon chain molecules can produce stronger hydrophobic interactions than branched molecules, because carbon branches produce steric hindrance. The fact that 2-nonenal has a long thin structure may explain its stronger deodorizing activity, although the boiling point of 2-nonenal is not higher than that of the other high-molecular-weight gases tested (Table 4).

As van der Waals forces are relatively weak, physical adsorption is a readily reversible phenomenon. Because of their hydrophobicity, by raising the temperature, adsorbed hydrophobic gas molecules would be desorbed from 1,8-C, similar to the case of thermal desorption of an adsorbate from activated carbon surfaces^{24), 26)}. In view of the temperature dependence of adsorbate desorption, a desorption experiment was conducted at 50, 75, 100, and 150°C at a constant 1,8-C dose of 50 mg/35-mL vial. The effect of temperature on the desorption of gases (including DMTS, 2-nonenal, isovaleric acid, and skatole) from 1,8-C is shown in Figure 5. The desorption of gases from 1,8-C was proportional to the incubation temperature. DMTS and 2-nonenal reached 53.3 ppm and 38.6 ppm at 150°C, respectively, suggesting that the adsorption process involves a physical mechanism associated with van der Waals forces. The desorbed concentrations of isovaleric acid and skatole (21.8 ppm and 4.1 ppm, respectively) were lower than those of 2-nonenal and DMTS.

Deodorization of isovaleric acid and skatole could involve additional mechanisms. In addition to van der Waals forces, hydrogen bonding is another type of force in physical adsorption^{20), 21)}. Isovaleric acid and skatole could form hydrogen bonds with the oxygen atom in 1,8-C (Fig. 6). From the above, the lower level of thermal desorption could be due to the formation of hydrogen bonds between those gases and 1,8-C. Addi-

tional investigations, such as adsorption isotherm measurements, may be necessary to clarify the adsorption mechanism of 1,8-C in more detail.

Concluding remarks

The aim of this work was to discover novel natural products exhibiting deodorizing activity from natural material extracts. The screening target was AMS, which is a major component of garlic breath, and 1,8-C was identified from cluster mallow leaves as active against AMS. 1,8-C exhibited potent deodorizing activity against not only AMS but also other odorous gases that are components of diet- and disease-associated malodors, such as foot odor, aging-associated odor, and malodors emitted from fungating cancer wounds and feces. Our data indicate that 1,8-C could be a particularly useful odor-reducing agent. The possible deodorizing mechanism of cineole is suggested to involve van der Waals forces, and to a lesser extent, hydrogen bonding.

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Our interest in the topic of hydrophobic interactions between cineole and odorous molecules was inspired and complemented by communications with Zenzaburo Tozuka (Osaka University).

Key words : cineole, malodor, deodorizing activity, hydrophobic interaction

Abbreviations: GC, gas chromatography; MS, mass spectrometry; DMTS, dimethyl trisulfide; DMDS, dimethyl disulfide; AMS, allyl methyl sulfide; DMS, dimethyl sulfide; AM, allyl mercaptan; MM, methyl mercaptan; TMA, trimethylamine, SPME, solid-phase micro-extraction.

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天然物からの消臭活性物質の探索とシネオールの消臭作用の特性

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要旨: 天然物由来の新しい消臭物質の発見を目的として, 活性の測定にガスクロマトグラフィー (GC) を用いて, 植物抽出物からのスクリーニングを試みた. その結果, フユアオイの 50% エタノール水による抽出物に消臭活性を検出した. 活性の本体をシリカゲルカラムクロマトグラフィーにより精製し, 1,8-シネオールと同定した. 1,8-シネオールは, 比較的広い範囲の悪臭ガス (2-ノネナール, スカトール, インドール, ジメチルトリスルフィド, ジメチルジスルフィド, イソ吉草酸) に対して, 消臭率 90% 以上の強い活性を示した. また, アリルメチルスルフィド, 酪酸, アリルメルカプタン, ジメチルスルフィドに対し 70% 以上の消臭率を示した. なお, 消臭活性の強さを表す消臭率は, 消臭物質を含む *in vitro* の測定系に悪臭ガスを添加して反応させ, その際に消失したガス量の原ガス量に対する割合 (%) である. 官能試験 (6 段階評価) では, 1,8-シネオールは臭気強度を 1.5~3 段階低減させた. 1,8-シネオールには, 芳香によって臭気強度を低下させるマスキング効果が知られている. 一方, 1,8-シネオールは消臭活性の強さと分子量, または消臭活性と沸点との間に相関関係が認められた. また 1,8-シネオールに一旦吸着された悪臭ガスは高温で物理的に脱着した. これらの知見から, 1,8-シネオールにはマスキング以外に, 悪臭ガスを吸着することにより, その濃度を低下させる物理的機序の存在が推定された.

キーワード: シネオール, 悪臭, 消臭活性, 疎水性相互作用

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