CH$_2$CCH); mass spectrum m/e 220 (molecular ion), 41, 97, 39, 109, 107.

Peak II (one of the isomers of 9) had the following characteristics: ir (neat) 3.4, 5.76 (saturated five-membered ring C=O), 6.75, 6.85, 7.09 (CH$_2$C=O), 7.19, and 7.25 (CH$_3$CCH$_3$), 7.6, 7.7, 7.8, 7.99, 8.08, 8.35 (strong), 8.59, 8.75, 8.9, 9.05, 9.25, 9.7, 9.85, 9.95, 10.15, 10.75, 11.25, 11.6, 12.2 μ; nmr (CCl$_4$) δ 0.84 and 0.86 (two s, 6 H, CH$_3$CCH$_3$), 1.08, (a shoulder due to CH_3CH) of a singlet at 1.1 (total 6 H, CH$_3$CC=O), 1.93 (m, 3 H, CH$_2$CCH); mass spectrum m/e 220 (molecular ion), 41, 97, 109, 110, 39.

Peak III (dihydro-β-patchoulenone 9) had the following characteristics: ir, nmr, and mass spectral data were superimposable with those of the saturated ketone 9 obtained by the oxidation of alcohol 8.

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California Bay Oil. I. Constituents, Odor Properties

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The steam volatile oil of the leaves of the California "bay" tree (*Umbellularia californica*) was analyzed by capillary and packed column gas chromatography separation with characterization by mass and infrared spectrometry. The characterizations of the major components umbellulone (39%), 1,8-cineole (19%), α-terpineol (7.6%), terpinen-4-ol (6.2%), sabinene (6%), α-pinene (4.7%), and 3,4-dimethoxyallylbenzene (5.4%) were confirmed and 26 additional compounds also characterized. Odor threshold studies indicated that 1,8-cineole was by far the major contributor to the odor of dilute water solutions of the oil. Comparison of the composition and odor properties was made with other well-known "bay" oils from the leaves of Mediterranean bay (*Laurus nobilis*) and West Indian bay (*Pimenta racemosa*).

Deer in certain areas of California are numerous and protected by law. They do significant damage to certain crops. There are some plants that deer generally avoid. One of these is the leaves of the California bay tree (*Umbellularia californica*). It seems likely that the volatile oil of the leaves of California bay contain components responsible for repelling deer.

An increased knowledge of the constituents of the oil of the leaves of *Umbellularia californica* is also of importance for other reasons. The leaves of the tree are used by many Californians as a spice. One major California spice company sells the dried leaves on the retail market.

Toxicity studies of California bay oil and components were carried out in connection with the present work and are reported elsewhere in this journal (MacGregor *et al.*, 1974).

Umbellularia californica leaf oil was analyzed remarkably thoroughly for the time using classical methods early in this century (Powers and Lee, 1904; Semmller, 1908). The structure of the main component umbellulone was elucidated at that time. Other components also characterized were α-pinene, 1,8-cineole, eugenol, safrole, and methyleugenol. Additional studies on the chemical properties of umbellulone were made later by Kiyohiro (1938). In the present study the modern, more thorough methods of capillary gas liquid chromatography (glc)-mass spec-

trometry and infrared spectrometry were used to give a more comprehensive analysis.

EXPERIMENTAL SECTION

Materials. Authentic samples of compounds were obtained from reliable commercial sources or synthesized by well-established methods. They were purified by gas chromatography before use. δ-Terpineol was obtained from commercial α-terpineol using the method described by Mitzner and Lemberg (1966).

California bay oil was obtained from three principal sources. (1) The first source was from the Konocti Bay Corporation, Kelseyville, Calif. This involved direct steam distillation of the chopped, mature leaves in a pilot plant type operation. The leaves were in general at least partly dry and had been picked several days before the steam distillation. The oil was separated from the water layer and then dried over sodium sulfate and filtered. (2) A second method involved using steam distillation–continuous extraction in the laboratory with mature leaves that had been picked fresh (near Hopland, Calif.) and stored at −20°F in polyethylene bags. This was carried out using a 12-l. flask equipped with a steam distillation continuous extraction head of the type described by Nickerson and Likens (1966) at atmospheric pressure and using hexane as solvent. (3) A final source was from fresh mature leaves picked locally (El Cerrito, Calif., hills) using the same procedure as the second source.

The major analyses were carried out with the whole oil but, for a more complete study, the oil was divided up into three main fractions by chromatography on activated alumina in the following way. California bay oil (10 ml) in hexane (50 ml) was placed on a hexane-prepared column

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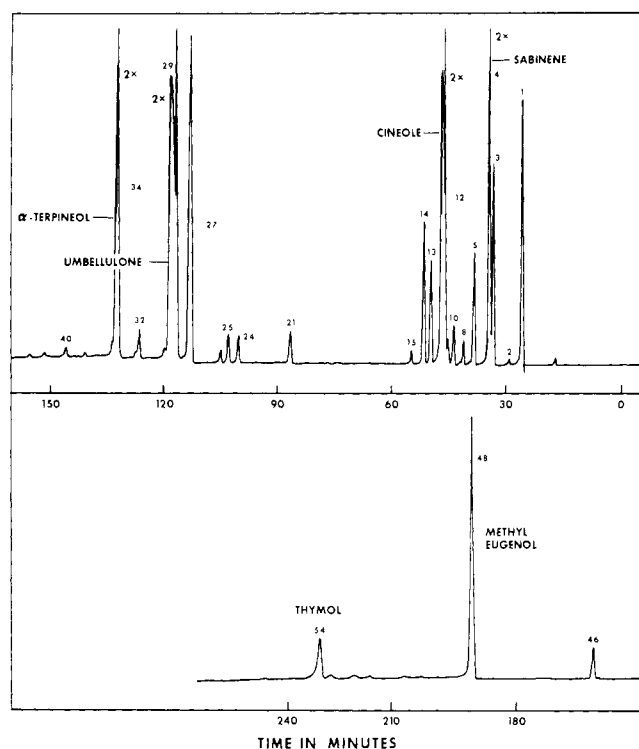
Figure 1. Capillary glc analysis of the steam volatile oil of the leaves of the California bay tree. The capillary column was 500 ft × 0.03 in. i.d., stainless steel coated with Tween-20. The column was temperature programmed from 80 to 170° at 0.5°/min and held at the upper limit.

(310 mm × 35 mm) of activated alumina (Alcoa chromatography F-20; basic). The column was then eluted with 600 ml of hexane. The solvent was removed from this eluent on the steam bath to give the "hydrocarbon" fraction. The column was next eluted with diethyl ether (500 ml) and the solvent removed on the steam bath to give a "nonhydroxylic oxygenated" fraction. Finally, elution with a mixture of ether–methanol (100:30) and removal of solvent gave an "alcohol" fraction.

Capillary Glc–Mass Spectral Analysis. A 500 ft × 0.02 in. i.d. stainless steel capillary column coated with Tween-20 was used. The column was connected to a silicone membrane separator which led into the ionization chamber of a Bendix time-of-flight (Model no. 12-101) mass spectrometer. The column was programmed with a 25-min isothermal period at 75° and then increased at 2°/min to 180°. The separator was held at 100° for 25 min and then programmed at 2°/min to 200°. The ionization voltage was at 70 V and the ion source temperature was at 150°.

Retention time studies were carried out using a 0.03-in. i.d. column similar to that used above, which was programmed from 80 to 170° at 0.5°/min. To determine accurately if an authentic sample had the same glc retention time as a particular peak, it was mixed with a *ca.* 20-fold amount of the bay oil and chromatographed. An appreciable increase in the particular peak would confirm the retention time of the authentic compound used.

Packed Column Glc–Infrared Absorption Spectra Analysis. The major columns used for this work were: (1) a 20 ft × ⅜ in. o.d. aluminum column packed with 20% Carbowax 20-M on 60–80 mesh Chromosorb P; (2) a 5 ft × ⅜ in. o.d. column packed with 60–80 mesh Chromosorb P coated with 10% Tween-20. Components were separated from the whole California bay oil and from the hydrocarbon, nonhydroxylic oxygenated, and the alcohol fractions. Infrared absorption spectra were run on these samples either in the pure form as films between microsalt plates or

in CCl$_4$ solution in an ultramicrocavity cell and using a reflecting beam condensor. The instrument was a Perkin-Elmer Model 237 double beam grating infrared spectrophotometer.

Proton magnetic resonance (pmr) spectra were measured in CDCl$_3$, at 100 MHz, using a Varian HA-100 instrument.

Odor Thresholds. Thresholds of the glc purified compounds were determined as described previously (Guadagni *et al.*, 1963) using Teflon bottles and tubing as containers for the odor solutions.

RESULTS AND DISCUSSION

Figure 1 shows the glc analysis of California bay (*Umbellularia californica*) leaf oil. Table I lists the components characterized using both capillary glc–mass spectrometry and packed column glc–batch infrared spectrometry. Both qualitative and quantitative analyses are listed. Several batches of steam distilled oil from different sources and using different isolation methods were studied. The quantitative analysis shown is from only one of these oils which was felt reasonably representative. There are some differences in the quantitative pattern with the different batches of oil and the quantitative figures shown are only meant to give a general idea of the composition of a typical oil.

Umbellulone is by far the major constituent in California bay oil amounting to almost half of the total oil. A proton magnetic resonance spectrum (pmr) of this compound, isolated by packed column glc, was consistent with the early structure derived by Semmller (1908). Its mass spectrum (two most intense ions every 14 mass units above m/e 20, intensities in parentheses) showed 27 (2.3), 29 (1.4); 41 (11.6), 43 (8.8); 53 (6.0), 55 (4.1); 65 (6.7), 67 (7.0); 77 (14.0), 79 (26.3); 91 (45.8), 94 (11.2); 107 (80.5), 108 (100); 121 (4.7), 122 (6.3); 133 (1.0), 135 (14.0); 149 (0.0), 150 (11.6).

The next most concentrated component was 1,8-cineole at about half the concentration of umbellulone. As we show later, 1,8-cineole is very important to the characteristic odor of California bay oil.

The terpene hydrocarbons are qualitatively very similar to that found in many essential oils. There are, however, only minute traces of sesquiterpenes (β- and α-bisabolenes) present. α-Bisabolene was characterized by comparison of infrared and pmr spectra with published spectra (Nigam and Neville, 1968). The somewhat larger than usual amount of sabinene (6%) may be connected to the structurally related umbellulone. The related α-thujene was also characterized although it did not separate from α-pinene on the Tween-20 capillary. It did separate on a 1000 ft × 0.03 in. i.d. Silicone SF96(50) capillary which was used for its mass spectral characterization.

The commonly occurring α-terpineol (7.6%) and terpinen-4-ol (6.2%) are the major oxygenated terpenes after umbellulone. The small concentration of thymol (1.7%) may arise from rearrangement of umbellulone occurring in the plant or in the isolation of the oil. It has been established (Semmller, 1908; Aries and Kidder, 1947) that umbellulone can be rearranged to thymol in 80% yield if heated at 295° under pressure for 10 hr.

The aromatic compounds 3,4-dimethoxyallylbenzene (methyleugenol), 3,4-dimethoxypropenylbenzene (methylisoeugenol), 4-methoxyallylbenzene (methylchavicol), and safrol are found in many essential oils. The 3,4-dimethoxyallylbenzene proved to have interesting properties in the biological studies which are outlined by MacGregor *et al.* (1974).

Additional trace components tentatively characterized from their mass spectral patterns which are not listed in Table I were cuminaldehyde, 3,4,5-trimethoxyallylbenzene, verbenone, perillen, elemol, geraniol, carvenone, and α-pinene oxide.

Table I. Constituents Characterized in the Steam Volatile Oil of the Leaves of *Umbellularia californica* **(California Bay)**

Compound type	Peak no.	Confirmed identity[a]	Approx rel % in oil
Terpene hydrocarbons, ethers	1	α-Thujene; ms, rt	0.6
	1	α-Pinene; ms, ir, rt	4.1
	2	Camphene; ms, ir, rt	<0.1
	3	β-Pinene; ms, rt	2.7
	4	Sabinene; ms, ir, rt	6.0
	5	Myrcene; ms, ir, rt	1.6
	8	α-Terpinene; ms, rt	0.2
	10	Limonene; ms, rt	0.3
	11	β-Phellandrene; ms, rt	0.1
	12	1,8-Cineole; ms, ir, rt	19
	13	γ-Terpinene; ms, ir, rt	1.6
	14	p-Cymene; ms, ir, rt	1.9
	15	Terpinolene; ms, rt	0.1
Sesquiterpenes	40	β-Bisabolene; ms, ir	0.1
	41	α-Bisabolene; ir, pmr[b]	<0.1
Terpene alcohols	24	Linalool; ms, ir, rt	0.3
	27	Terpinen-4-ol; ms, ir, rt	6.2
	32	δ-Terpineol; ms, ir, pmr, rt	0.2
	34	α-Terpineol; ms, ir, rt	7.6
Terpene esters, ketones	20b	Thujone; ms, rt	0.1
	29	Umbellulone; ms, ir, pmr	39.2
	35	α-Terpinyl acetate; ms, rt	0.1
Aromatic compounds	19	p-Methylanisole; ms, rt	<0.1
	22	Benzaldehyde; ms, rt	<0.1
	30	4-Methoxyallylbenzene; ms, rt	<0.1
	43	p-Cymen-8-ol; ms, rt	<0.1
	46	Safrol; ms, pmr[b]	0.2
	47a	3,4-Dimethoxypropenylbenzene; ms, rt	<0.1
	48	3,4-Dimethoxyallylbenzene; ms, ir, rt (methyleugenol)	5.4
	50	Cuminyl alcohol; ms, rt	0.1
	52	Ethyl cinnamate; ms, rt	0.1
	53	Isoeugenol; ms, rt	0.1
	54	Thymol; ms, ir, rt	1.7

[a] ms, ir, pmr, rt = mass spectral, infrared absorption, proton magnetic resonance spectral, and glc retention evidence, respectively. Evidence cited under this column is consistent with that of an authentic sample unless otherwise indicated (see footnote b). [b] No authentic sample available but spectra consistent with published spectra.

A thorough study of variations in oil composition during the year was not carried out. A complete study of this would probably require sampling over several years. With the few samples that were taken, however, the one sample picked in the winter (February) was considerably lower in 1,8-cineole (4%) and α-terpineol (2%) than those picked during the spring and summer (1,8-cineole, 19%; α-terpineol, 7.6%).

Comparison with Other "Bay" Oils. The leaves of the California bay tree are used as a spice in foods by many Californians in a similar fashion to the commercially available (Mediterranean) Bay Leaves (Laurel). The commercial bay leaves (Laurel) are the dried leaves from the Mediterranean tree *Laurus nobilis.* Steam distillation of the leaves of *Laurus nobilis* gives a commercially available oil, "Oil Laurel Leaf F.C.C. Extra" (Fritzsche-D&O).

A third "bay oil" used fairly widely and available commercially is distilled from the leaves of *Pimenta racemosa* (formally known as *Myrcia acris*), a tree that grows in the West Indies. This oil is sold commercially under the name of "Oil Bay Extra (Oil Myrcia)" (Fritzsche). This tree is apparently completely unrelated (botanically) to either the California bay or the Mediterranean bay.

For comparison with the California bay oil, capillary glc-mass spectra studies were also made on the Mediterranean and West Indian oils. Qualitative and quantitative results are listed in Tables II and III. The constituents of *Laurus nobilis* leaf oil had been fairly thoroughly studied previously using gas chromatography by Pertoldi and Stancher (1967) and other workers. The constituents of West Indian bay (*Pimenta racemosa*) leaf oil have also

been studied by a number of workers, particularly by de Martinez Nadel (1959). Our results for both oils confirm earlier findings and identify some previously unidentified compounds.

It is interesting that there are some similarities between all three oils. They all contain reasonable amounts of 1,8-cineole, *i.e.,* California 19%, West Indian 10%, and Mediterranean 30%. They also contain significant amounts of 3,4-dimethoxyallylbenzene (methyleugenol), *i.e.,* California 5.4% and Mediterranean 4%, or of eugenol, *i.e.,* West Indian 34%. There are also striking differences. The California oil is the only one to contain umbellulone. The West Indian oil has a very large concentration of myrcene (21%). The Mediterranean bay oil contains large amounts of linalool (11%) and α-terpineol acetate (11%).

Aroma Properties. Table IV lists the odor thresholds (*T*) found in water solution for most of the identified California bay oil components. Also listed is the ratio of components concentration (*C*) in the oil (parts per billion) to components threshold (*T*) concentration (parts per billion). This ratio has been used by some of the authors in work on other oils (Guadagni *et al.,* 1966) and has been termed "odor units" (U). Near the threshold of a mixture these odor units appear to be additive (Guadagni *et al.,* 1966). Therefore, the "odor unit" value for the whole oil was calculated and the percentage contribution for each component was calculated as equal to 100 × components odor units/whole oil odor units. While the additive relation does not hold at very high concentrations, these calculations give us an idea of the relative aroma significance of the components, especially at low concentrations.

Table II. Constituents Characterized in the Steam Volatile Oil of *Laurus nobilis* (Mediterranean Bay) Leaves

Compound type	Peak no.	Confirmed identity	Approx rel % in oil
Terpene hydrocarbons, ethers	1	α-Pinene; ms, rt	12
	2	Camphene; ms, rt	2
	3	β-Pinene; ms, rt	6
	4	Sabinene; ms, rt	5
	5	Myrcene; ms, rt	1
	5m	α-Phellandrene; ms	0.5
	8	α-Terpinene; ms, rt	0.5
	10	Limonene; ms, rt	4
	12	1,8-Cineole; ms	30
	13	γ-Terpinene; ms, rt	1
	14	p-Cymene; ms, rt	2
	15	Terpinolene; ms, rt	0.3
Terpene alcohols	24	Linalool; ms, rt	11
	27	Terpinen-4-ol; ms, rt	3
	34	α-Terpineol; ms, rt	6
Terpene esters	34m	α-Terpineol acetate; ms, rt	10
Aromatic compounds	48	3,4-Dimethoxyallylbenzene; ms, rt	4
	48m	Eugenol; ms, rt	2

Table III. Constituents Characterized in the Steam Volatile Oil of *Pimenta racemosa* (West Indian Bay Formally Called *Myrcia acris*) Leaves

Compound type	Peak no.	Confirmed identity	Approx rel % in oil
Terpene hydrocarbons, ethers	1	α-Thujene; ms, rt	1.9
	1	α-Pinene; ms, rt	1.9
	3	β-Pinene; ms, rt	0.4
	4	Myrcene; ms, ir, rt	21.3
	10	Limonene; ms, ir, rt	6.0
	11	β-Phellandrene; ms, rt	1.1
	12	1,8-Cineole; ms, ir, rt	9.7
	13a	Ocimene; ms, rt	2.3
	14	p-Cymene; ms, ir, rt	1.7
Terpene alcohols	24	Linalool; ms, rt	3.2
	27	Terpinen-4-ol; ms, ir, rt	0.9
	34	α-Terpineol; ms, ir, rt	2.3
Aromatic compounds	48	3,4-Dimethoxyallylbenzene; ms, ir, rt	0.2
	53	Isoeugenol; ms ⎱ Eugenol; ms, ir, rt ⎰	33.8
	59a	4-Hydroxyallylbenzene; ms, ir, rt (chavicol)	8.9
Aliphatic compounds	12b	Octan-3-one; ms	0.8
	17a	Octan-3-ol; ms	0.3
	20a	Oct-1-en-3-ol; ms, ir	1.6

Table IV. Odor Thresholds in Water Solution and Estimated Odor Contribution of Components of *Umbellularia californica* (California Bay) Leaf Oil

Compound	Odor threshold in water (T) in parts per 10^9	Odor units ($C/T \times 10^{-6}$, U)	% odor units of whole oil
Whole oil	6.5	154	100
α-Pinene	6	8.0	5.2
β-Pinene	140	0.2	0.1
Sabinene	75	0.8	0.5
Myrcene	13	1.0	0.6
Limonene	10	0.3	0.2
1,8-Cineole	1.3	146	95
p-Cymene	150	0.1	0.05
Linalool	6	0.5	0.3
Terpinen-4-ol	340	0.2	0.1
α-Terpineol	350	0.2	0.1
Umbellulone	750	0.5	0.3
Methyleugenol	68	0.8	0.5
Thymol	86	0.2	0.1
Total for constituents			103.1

Table V. Estimated Odor Contribution of Components of *Laurus nobilis* (Mediterranean Bay) Leaf Oil in Water Solution

Compound	Odor units ($C/T \times 10^{-6}$)	% odor units of whole oil
Whole oil ($T = 2.5$ ppb)	400	100
α-Pinene	20	5
β-Pinene	0.1	0.03
Sabinene	0.7	0.2
Myrcene	0.8	0.2
Limonene	4.0	1.0
1,8-Cineole	230	58
Linalool	18	4.5
Terpinen-4-ol	0.1	0.03
α-Terpineol	0.2	0.06
Methyleugenol	0.6	0.1
Eugenol ($T = 6$ ppb)	3.0	0.75
Total for constituents		69.8%

"Odor units" have also been calculated for the Mediterranean and West Indian bay oils and these are listed in Tables V and VI.

It can be seen that 1,8-cineole with 95% of the odor units is by far the most important aroma component of

Table VI. Estimated Odor Contribution of Components of *Pimenta racemosa* (West Indian Bay Formally Called *Myrcia acris*) Leaf Oil

Compound	Odor units $(C/T \times 10^{-6})$	% of odor units of whole oil
Whole oil (T = 3.5 ppb)	286	100
α-Pinene	3.1	1.1
β-Pinene	0.02	0.1
Myrcene	16	5.7
Limonene	6.0	2.2
1,8-Cineole	75	26
Linalool	5.3	1.9
Terpinen-4-ol	0.03	0.1
α-Terpineol	0.06	0.2
Eugenol	56	20
Oct-1-en-3-ol (T = 1.4 ppb)	12	4.2
Total for constituents		61.5

California bay oil. A number of panelists familiar with the odor of California bay leaves also indicated that dilute solutions of 1,8-cineole had an odor very similar to that of California bay leaves. Although it comprises 39% of the California bay oil by weight, umbellulone only contributes 0.3% of the "odor units" near threshold concentrations.

With a contribution of 58% of the odor units, 1,8-cineole also is the major aroma component of Mediterranean bay oil. This is probably one of the reasons California bay leaves have been used as a spice in a similar way to the Mediterranean bay leaves. However, the high concentration of linalool in Mediterranean bay oil and its reasonable (4.5%) contribution to the "odor units" probably tends to soften the aroma impact of the 1,8-cineole somewhat. Approximately 30-40% of the odor of the Mediterranean and West Indian bay oils is still unaccounted for and

may be due to some relatively minor components which have not been characterized.

The concentration of 1,8-cineole in West Indian bay is much smaller than the other oils at only 10%, but it still contributes substantially (26%) to the total "odor units" near threshold concentrations. Eugenol and myrcene with contributions of 19.6 and 5.7% also contribute considerably, and the odor of West Indian bay is quite different from that of both California and Mediterranean bay oils.

ACKNOWLEDGMENT

The authors thank Robert E. Lundin for pmr spectra and James A. Rogers of Fritzche-D&O for authentic samples of Mediterranean and West Indian Bay Oils.

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California Bay Oil. II. Biological Effects of Constituents

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The acute oral toxicity of California bay (*Umbellularia californica*) oil was greater than that of Mediterranean (*Laurus nobilis*) or West Indian (*Pimenta racemosa*) bay oil. The toxicity of California bay oil was due primarily, but not entirely, to umbellulone, a component not present in Mediterranean or West Indian bay oil. 3,4-Dimethoxyallylbenzene (DMAB), a constituent of California and Mediterranean bay oils, produced sedation in mice at low doses and a reversible narcosis at higher doses. A reversible narcosis was also observed in stickleback fish exposed to DMAB. DMAB prevented the death of mice treated with lethal convulsant doses of strychnine. The effects of DMAB suggest it may have some relatively specific central nervous or myoneural effects and indicate a potential clinical utility of this material as a drug.

A knowledge of the biological effects of the constituents of California bay (*Umbellularia californica*) oil is of interest for several reasons. It is known that certain plants produce a variety of chemicals which serve as protectants against insect predators and herbivores (Martin-Smith and Sneader, 1969; Whittaker and Feeny, 1971). Several

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simple terpenes have been shown to function as insect defensive agents, pheromones, and even as attractants for cats (Martin-Smith and Sneader, 1969; Martin-Smith and Khatoon, 1963). Many mammals, including deer, have highly developed olfactory senses and use chemical signals in social communication (Ralls, 1971). In the Department of Agriculture, investigations of the ability of volatile components present in California bay leaves to repel deer, which are a significant hazard to crops in some areas (Buttery et al., 1974), and to attract insects (USDA Bulletin No. 351) have been initiated. The composition of Cali-