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COMPOSITIONAL VARIATIONS OF LEAF MONOTERPENES IN *CUPRESSUS MACROCARPA*, *C. PYGMAEA*, *C. GOVENIANA*, *C. ABRAMSIANA* AND *C. SARGENTII**

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Abstract—Two hundred and fifty trees of *Cupressus macrocarpa*, *C. goveniana*, *C. abramsiana*, *C. pygmaea* and *C. sargentii* sampled in 20 localities were analyzed by GLC to determine the monoterpene constituents of their foliage volatile oils. The results separated the species investigated into three distinct groups: *C. sargentii* and *C. abramsiana*, *C. goveniana* and *C. pygmaea*, and *C. macrocarpa*. The southernmost populations of *C. sargentii*, from Zaca Peak (Santa Barbara County) and Chorro Creek (San Luis Obispo County), appeared to be considerably different from the rest. In nearly all oils percentages of α- and γ-terpinene and terpinolene varied in direct proportion to one another which was interpreted by a large similarlty in their mechanisms of formation involving the splitting of α-protons from the 1-*p*-menthene-4-carbonium ion common intermediate.

INTRODUCTION

THE GENUS *Cupressus* consists of about six old world and of eight to 16 new world species distributed in western and southwestern U.S. and in Mexico, the number depending upon the classification used. In the U.S., cypresses rarely occupy extensive land areas and are confined to a series of disjointed groves having little contact with each other. Botanical differences separating individual taxa are subtle, with a resultant indefinite status for many taxa. Although quantitative morphological methods have not been tried for solving the many taxonomic problems, qualitative aspects have been excellently discussed by Wolf[1] and Martinez[2] in their monographs on U.S. and Mexican species of this genus.

The present study represents the continuation of our attempt to contribute to clarification of some systematic problems in *Cupressus* by application of the methodology of chemosystematics, and concerns itself with the interrelationships of five closely related species: *Cupressus macrocarpa* Hartw., *C. pygmaea* (Lemm.) Sarg, *C. goveniana* Gord., *C. abramsiana* C. B. Wolf, and *C. sargentii* Jeps., indigenous to coastal regions of central and northern California. In our earlier publication the taxonomic problems in this group were approached through analysis of the tropolonic fraction of the heartwood extract.[3] Some meaningful results were obtained, but the method was laborious, the analytical procedures were only semiquantitative, and there were difficulties in obtaining enough relatively large heartwood samples. This made it practically impossible to place the work on a

* Part IV in the series "Chemosystematics of *Cupressus*".

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§ Laboratory technician.

[1] C. B. WOLF, *El Aliso* **1**, 1 (1948).

[2] M. MARTÍNEZ, *Las Pináceas Mexicanas*, Ed. Univ. Nac. Auton. de Mexico, pp. 219–288 (1963).

[3] E. ZAVARIN, L. V. SMITH and J. G. BICHO, *Phytochem.* **6**, 1387 (1967).

statistically significant basis, with the result that only very large differences could be considered real. In the present publication our work is based on analysis of monoterpenes from foliage by GLC. The use of essential oils of leaves in chemosystematic studies is now commonplace.[4–10]

We limited ourselves in this work to analysis of monoterpenoid hydrocarbons exclusively, as the higher boiling components were less abundant in this *Cupressus* group and their identity was largely unknown. It is our hope, however, to extend our studies to these compounds as soon as they have been identified. Monoterpene hydrocarbons of *Cupressus* foliage have not been very intensively studied. Guenther[11] summarized the information published by Schimmel & Co. between 1894 and 1913 on *C. sempervirens*, and he mentions the presence of (+)-α-pinene, much (+)-camphene, (+)-sylvestrene (3-carene artifact), some *p*-cymene, and probably fenchene. For oil from shoots of the same species, Mingoia[12] later reported the presence of 80 per cent α-pinene, camphene and a little *p*-cymene. *C. torulosa* foliage was analyzed by Simonsen,[13] who identified (+)-sabinene, α-pinene, (±)-limonene, and a terpinene. Yields in these and a few other species varied from 0·1 to 2·0 per cent. More recently the monoterpenes of *C. lusitanica* foliage have been examined by Sfiras;[14] 70 per cent of the oil represented hydrocarbons composed mainly of α-pinene with some 3-carene, (+)-limonene and a small amount of myrcene, *p*-cymene, camphene, α-terpinene, α- or β-phellandrene and sabinene. Essential oil from *C. macrocarpa* has been thoroughly analyzed by Briggs and Sutherland[15] and contains 40 per cent (−)-α-pinene, 15 per cent sabinene, 3 per cent myrcene, 4 per cent α-phellandrene, probably some α-terpinene, 6 per cent γ-terpinene and terpinolene, and 12 per cent unidentified monoterpenes. Motl and Paknikar[16] reported the presence of α-pinene, β-pinene, camphene, myrcene, limonene, and traces of ocimene and *p*-cymene in the oil from *C. funebris* foliage; Sakhatov and Belova[17] substantiated the presence of α-pinene (mostly), β-pinene, myrcene, and limonene in *C. sempervirens.*

RESULTS AND DISCUSSION

The taxonomic picture of the northern-central group of *Cupressus* species is still quite controversial. Little[18,19] recognizes only three species, *C. macrocarpa*, *C. sargentii* and *C. goveniana* (including *C. abramsiana* and *C. pygmaea*), although the last two are recognized as independent species by Wolf[1]. Wolf does not deny, however, the close relationship

[4] R. T. BAKER and H. G. SMITH, *A Research on the Eucalypts, Especially in Regard to Their Essential Oils*, 2nd Ed., pp. 1–472. Government of the State of New South Wales, Sidney (1920).
[5] R. Z. CALLAHAM, *Forest Sci.* **2**, 101 (1956).
[6] B. M. SAVORY, *Empire Forestry Rev.* **41**, 67 (1962).
[7] E. VON RUDLOFF, *Can. J. Botany* **45**, 1703 (1967).
[8] M. VON SCHANTZ, *Planta Med.* **13**, 369 (1965).
[9] E. VON RUDLOFF, *Can. J. Chem.* **46**, 679 (1968).
[10] F. C. VASEK and R. W. SCORA, *Am. J. Botany* **54**, 781 (1967).
[11] E. GUENTHER, *The Essential Oils*, Vol. VI p. 332, Van Nostrand, New York (1952).
[12] Qu. MINGOIA, *Ann. Chim. Applic.* **24**, 247 (1934).
[13] J. L. SIMONSON, *Indian Forest Records* **10**, 1 (1923); *J. Soc. Chem. Ind.* **42A**, 1099 (1923).
[14] J. SFIRAS, *Roure-Bertrand Fils, Recherches*, **2**, 17 111, (1938); **3**, 115 (1939).
[15] L. H. BRIGGS and M. D. SUTHERLAND, *J. Org. Chem.* **7**, 397 (1942).
[16] O. MOTL and S. K. PAKNIKAR, *Collection Czech. Chem. Commun.* **33**, 1939 (1968).
[17] E. SAKHATOV and N. V. BELOVA, *Farmatsiya, (Moscow)* **17**, 33 (1968).
[18] E. L. LITTLE, JR., *Check List of Native and Naturalized Trees of the United States (including Alaska)*, p. 170, Agr. Handbook No. 41, Forest Service, Washington D.C. (1953).
[19] E. L. LITTLE, JR., *Madroño* **18**, 161 (1966).

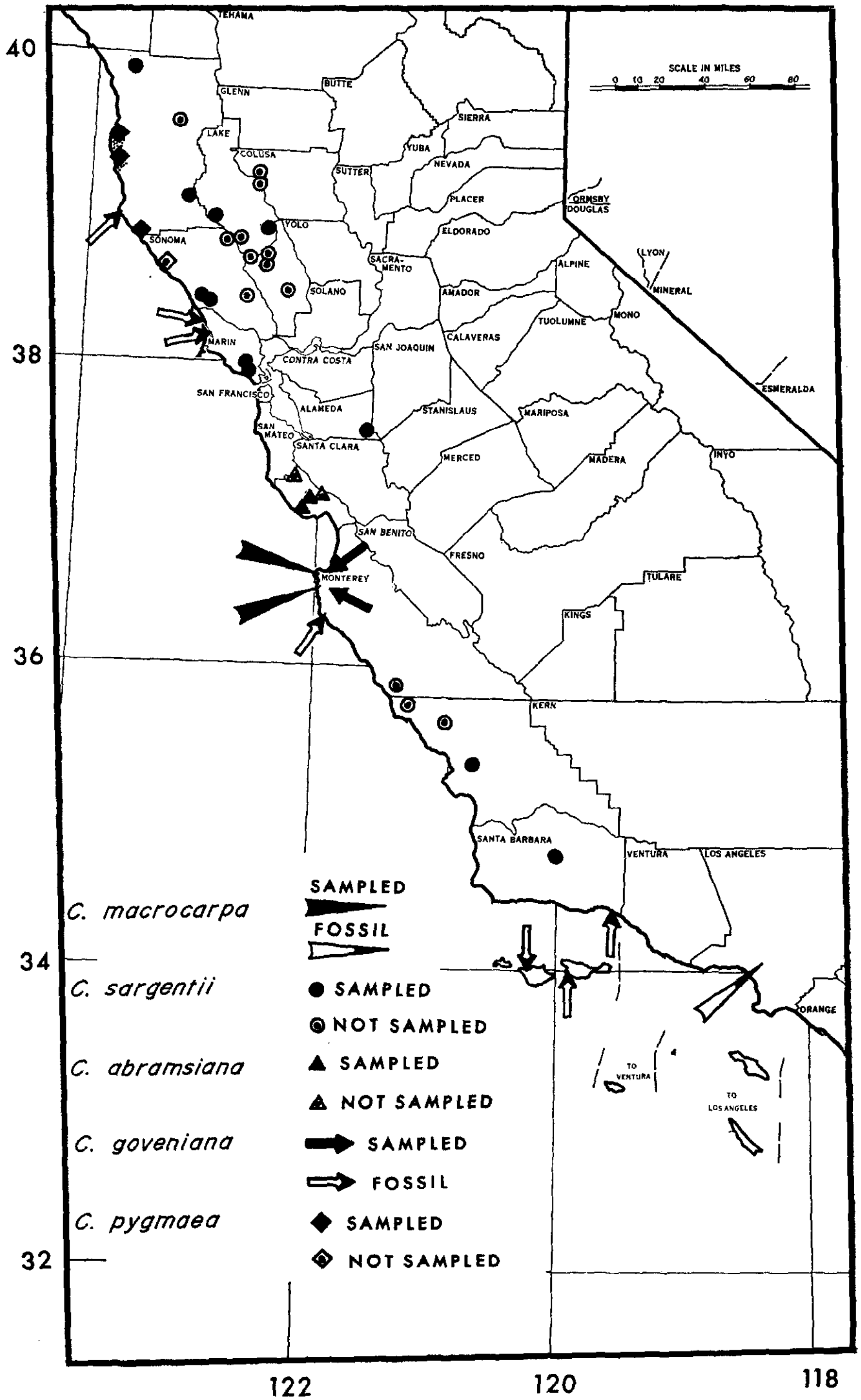


FIG. 1. GEOGRAPHIC DISTRIBUTION OF THE COASTAL *Cupressus* SPECIES.
All locations known are included, with exception of several *C. sargentii* groves, discovered since the work of Wolf, within the northern part of its range.

between *C. goveniana* and *C. pygamaea*, and he places *C. abramsiana* between *C. sargentii* and *C. goveniana* but somewhat closer to the latter.

All five species occur in a series of relatively small, separated groves, spread from northern Mendocino to central Santa Barbara County, a distance of about 500 miles. To gain an insight into the reasons for this characteristic distribution it is expedient to look into the history of this region. The paleobotany of the California coast has been recently discussed by Axelrod[20] and, according to him, from early Pliocene into late Pleistocene (i.e. until shortly after the last glacial [Wisconsin] period) the California coast was covered by a practically continuous strip of the closed-cone pine forest, including species similar to the present day's *P. muricata* and *P. radiata*. Part of the forest vegetation also represented *Cupressus* tree species, and some of these were similar to the five investigated in this work (although they ranged further south than they do now). Thus, a fossil cypress similar to *Cupressus macrocarpa* was uncovered together with closed-cone pines at Rancho La Brea near Los Angeles.[21] *Cupressus goveniana* occurred in association with closed-cone pines on Santa Cruz island about 14,400 years ago,[22] on Santa Rosa Island about 16,000 years ago,[23] near Carpinteria on the mainland over 38,000 years ago[24–26] and near Little Sur, Monterey County (Fig. 1).[27] Today, the southern-most grove of this cypress is located near Monterey, and the southern-most grove of closely related *C. sargentii*, is near Zaca Peak in the San Rafael Mountains. In the north, *C. goveniana* appeared to occupy larger areas than it does now, being identified in several locations along Tomales Bay and near Pt. Arena.[28]

After the last glaciation ended (about 12,000 years ago), a world-wide warming up occurred, and this was followed by moister and cooler conditions of the present day. This warm, so-called xerothermic, period (8000–3000 years ago) fragmented the continued closed-cone pine forest strip into more or less well-separated groves, and modified their composition by elimination of species less tolerant to climate change. As a result, cypresses survived only in a few localities where climatic conditions were particularly favorable and individual stands disconnected themselves from others. The relatively recent separation of the groves of individual species suggests that no great chemical differences between groves of the same species can be expected; this was generally found to be true in the present study.

Our investigations of *Cupressus* volatile oils indicated that tricyclene, α-pinene, α-thujene, camphene, β-pinene, sabinene, 3-carene, myrcene, α-phellandrene, α-terpinene, limonene, β-phellandrene, *cis*- and *trans*-ocimene, γ-terpinene, terpinolene and *p*-cymene, were present in practically all of the five species investigated, with α-pinene, sabinene, myrcene, limonene, β-phellandrene, and occasionally also 3-carene present in larger amounts. Volatile oil yields

[20] D. I. AXELROD, *Evolution of the Californian Closed-Cone Pine Forest* in *Proc. of the Symposium on the Biology of the California Islands* (edited by R. N. PHILBRICK), Publ. Santa Barbara Botan. Garden, Santa Barbara, California, 1967, pp. 93; Ibid., D. I. AXELROD, *Geologic History of the California Insular Flora*, pp. 267.
[21] H. FROST, *Univ. Calif. (Berkeley) Publ. Botany* **14,** 73 (1927).
[22] R. W. CHANEY and H. L. MASON, *Carnegie Inst. Wash. Publ.* **415,** 1 (1930).
[23] Ph. C. ORR, *Geochronology of Santa Rosa Island, California* in *Proc. of the Symposium on the Biology of the California Islands* (edited by R. N. PHILBRICK) Publ. Santa Barbara Botan. Garden, Santa Barbara, California, 1967, p. 317.
[24] R. W. CHANEY and H. L. MASON, *Carnegie Inst. Wash. Publ.* **415,** 45 (1933).
[25] G. J. FERGUSSON and W. F. LIBBY, *UCLA Radiocarbon Dates* II. *Radiocarbon* **5,** 1 (1963); III, *Radiocarbon* **6,** 318 (1964).
[26] W. S. BROECKER, J. L. KULP and C. S. TUCEK, *Lamont Natural Radiocarbon Measurememts* III. *Science* **124** (3213), 154 (1956).
[27] J. H. LANGENHEIM and J. W. DURHAM, *Madroño* **17,** 33 (1963).
[28] H. L. MASON, *Carnegie Inst. Wash. Publ.* **415,** 81 (1934).

varied around an average of 0·1 per cent on a fresh-leaf weight basis. The amount of oxygenated monoterpenoids and higher boiling materials was relatively low-ranging, from about 2·0 to 23·0 per cent of the total oil, averaging 15 per cent.

Essential oils from all species showed a strong compositional variability from tree to tree. This variability was examined by construction of bar graphs (Figs. 2 and 3) for all species and terpenoids investigated. Definite bimodal distributions were exhibited by α-pinene in *C. sargentii* (the two chemically different southern populations, Chorro Creek

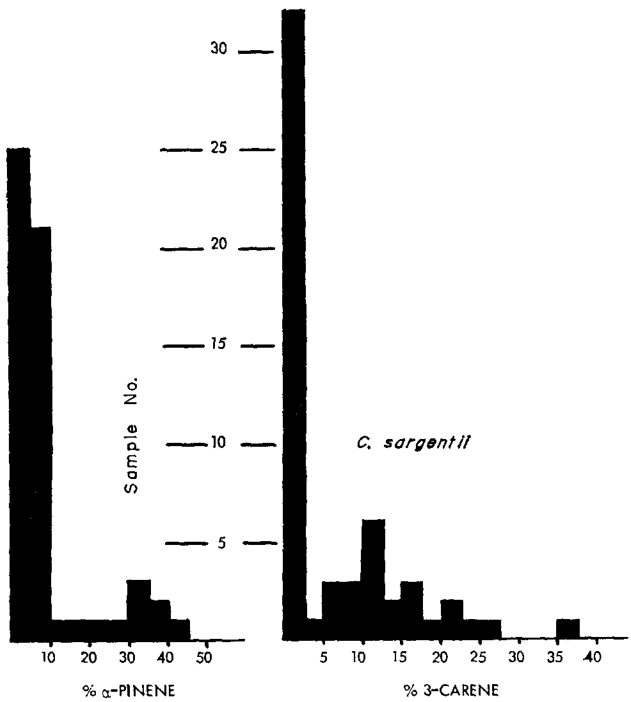


FIG. 2. DISTRIBUTION OF α-PINENE AND 3-CARENE PERCENTAGES IN *C. sargentii*.

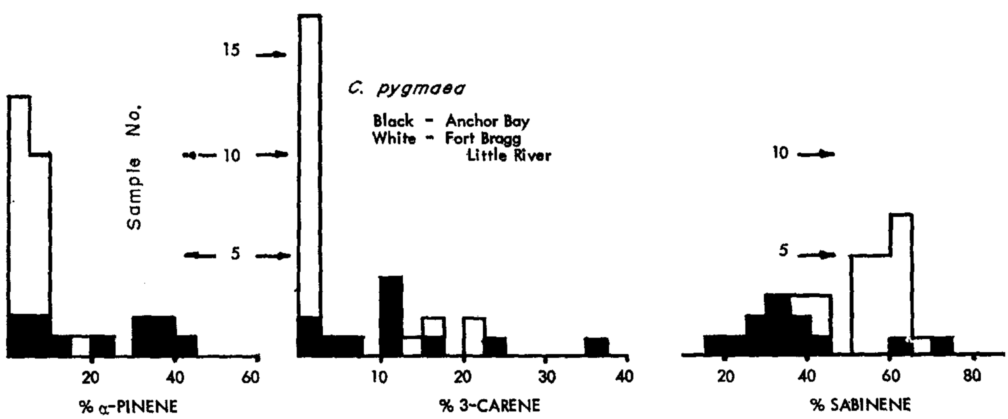


FIG. 3. DISTRIBUTION OF α-PINENE, 3-CARENE AND SABINENE PERCENTAGES IN *C. pygmaea*.

TABLE 1. COMPOSITION OF VOLATILE LEAF

Cupressus sargentii		Tricyclene	α-Pinene L	α-Pinene H	α-Thujene	Camphene	β-Pinene	Sabinene	3-Carene L	3-Carene H
Red Mnt. (Laytonville)	Inner Northern localities	0·6	5·4	m.a.	1·7	1·3	0·1	39·6	—	3·8
			(0·77)		(0·47)			(6·0)		
Red Mnt. (Mayacamas)		0·5	6·5	30·7	2·5	1·3	0·2	36·9	tr	11·2
			(2·2)		(0·88)			(11·8)		(7·4)
Pieta Rd.		0·3	7·1	25·3	1·6	0·7	0·3	39·4	—	8·8
			(2·1)	25·4	(0·6)			(8·7)		8·9
Reiff		0·3	7·0	25·9	1·4	1·3	0·8	34·6	—	11·1
			(3·0)	(4·3)	(0·6)			(10·0)		(3·4)
Monte Rio/ Occidental	Coastal Northern localities	0·1	8·4	19·7	3·5	0·4	0·1	40·8	0·1	16·5
			(3·7)		(1·1)			(9·2)		(5·6)
Tamalpais/ Bolinas Rd.		0·2	6·6	20·2	4·5	0·4	0·1	37·3	tr	16·6
			(2·4)		(1·7)			(8·6)		(5·7)
Cedar Mnt.	Var. Duttonii	0·1	8·7	24·3	3·2	0·3	0·5	42·1	0·4	4·4
			(3·3)	(10·9)	(1·0)			(13·1)		
Chorro Creek	Southern localities	0·2	8·0	m.a.	4·4	0·2	tr	19·7	—	m.a.
			(2·6)		(1·3)			(5·5)		
Zaca Peak		0·2	10·4	18·7	4·9	0·5	0·3	31·4	—	m.a.
			(2·2)		(1·2)			(8·4)		
C. Abramsiana		0·2	8·5	15·5	5·6	0·35	tr	31·6	—	m.a.
			(2·8)		(2·0)			(7·2)		
C. Goveniana		tr	5·2	29·3	2·5	tr	tr	54·0	tr	12·5
			(1·8)	34·5	(0·7)			(8·3)		(6·6)
Cupressus pygmaea										
Ft. Bragg/ Little River		0·3	4·7	16·8	2·0	0·5	tr	†	—	15·6
			(1·6)		(0·5)					(5·9)
Anchor Bay		0·2	6·6	33·9	2·0	0·6	1·1	‡	—	14·5
			(3·4)	(6·7)	(0·5)		(1·2)			(9·7)
C. Macrocarpa		tr	9·5	45·5	2·4	0·3	3·2	40·5	tr	m.a.
			(3·4)	(11·3)	(0·8)		(1·6)	(16·1)		m.a.

* Standard deviations are given in parentheses for three or more trees where average per cent values are higher than 1·0%. With α-pinene (H) and 3-carene (H), the figures refer to individual analyses, if standard deviations are not given. Absence of a mode is indicated by the letters m.a.

and Zaca Peak, were left out to minimize the influence of geographic variability), *C. macrocarpa*, *C. pygmaea* and *C. goveniana*, sabinene in *C. pygmaea*, and 3-carene in *C. sargentii*, *C. pygmaea* and *C. goveniana*. The same bimodality was also discernible where graph construction was limited to single populations.† For terpenoids showing no bimodal

† Strict assignment of specific modality or distribution pattern to a particular terpene is not entirely correct, because with rather close biosynthetic linkages between terpenes any change in concentration of one is likely to be transmitted to others. The same effect can also take place through normalization of the results to 100 per cent, as is commonly done; in *Pinus* and *Abies* this has been demonstrated to play usually a minor role.

OILS FROM COASTAL *Cupressus* SPECIES*

Myrcene	α-Phellandrene	α-Teropinene	Limonene	β-Phellandrene	*cis*-Ocimene	γ-Terpinene	*trans*-Ocimene	Terpinolene	*p*-Cymene
13·5	—	3·4	11·3	11·7	1·1	5·1	—	2·3	0·5
(2·16)		(0·85)	(5·3)	(4·4)		(1·2)		(0·95)	
12·9	0·4	3·4	11·1	9·8	0·9	5·4	—	2·9	0·1
(3·81)		(1·27)	(4·2)	(5·3)		(1·5)		(1·1)	
13·1	—	3·5	10·0	10·2	1·0	5·0	—	2·6	0·1
(1·29)		(1·29)	(2·8)	(5·1)		(2·2)		(0·7)	
11·6	—	2·2	11·3	8·1	0·2	3·2	0·1	2·5	0·2
(2·4)		(1·0)	(7·4)	(4·7)		(0·8)		(0·6)	
10·2	—	4·1	7·9	9·0	0·5	6·6		4·5	1·4
(1·6)		(1·2)	(3·0)	(3·2)		(2·5)		(1·1)	(1·2)
10·2	—	4·2	9·8	12·5	0·3	6·1		4·0	1·6
(2·0)		(0·8)	(2·8)	(3·4)		(1·8)		(1·0)	(1·3)
10·2	0·4	3·6	8·3	5·6	0·7	6·4	—	4·1	0·5
(2·2)		(1·1)	(6·4)	(4·1)		(3·1)		(2·2)	
10·4	—	3·8	27·3	12·8	1·7	5·7	—	4·0	1·6
(2·1)		(1·0)	(10·5)	(4·1)	(0·9)	(1·7)		(1·3)	(0·7)
11·2	—	3·6	18·7	7·1	0·8	5·5	—	3·0	1·5
(2·6)		(0·7)	(7·9)	(1·7)		(1·5)		(0·6)	(1·0)
9·4	0·7	4·8	12·6	14·1	0·4	5·7	—	4·5	0·7
(2·3)		(0·9)	(5·2)	(4·0)		(1·4)		(0·9)	
6·1	0·8	5·1	6·2	0·4	—	8·6	—	2·8	tr
(1·5)		(1·9)	(4·0)			(3·4)		(1·1)	
10·8	—	3·6	5·7	0·4	0·2	7·2	—	2·9	0·5
(3·3)		(1·4)	(2·9)			(3·0)		(0·8)	
7·8	0·1	2·9	4·9	0·7	0·2	5·2	—	2·8	0·1
(1·7)		(1·6)	(3·3)			(3·6)		(0·9)	
6·1	0·7	1·3	1·2	0·6	—	1·8	—	0·8	—
(2·2)		(0·7)	(0·3)			(0·8)			

† Two modes with 3 trees, 41·2% (s = 5·1) and 17 trees, 57·9% (s = 4·9).
‡ Two modes with 9 trees, 30·0% (s = 7·4) and 2 trees with 70·4% and 64·8%.

distribution and occurring in amounts larger than 1 per cent, the goodness of fit into the Gaussian was tested by calculation of the χ^2 statistic,[29] separately for each species with exception of data for chemically indistinguishable *C. goveniana* and *C. pygmaea* which were considered together. Wherever the possibility of interference of geographic variability was suspected the results were checked by repeating the calculations, using data covering more limited geographic distribution ranges. The results suggest that distribution of sabinene

[29] W. J. Dixon and F. J. Massey, Jr, *Introduction to Statistical Analysis*, p. 226, McGraw-Hill, New York (1957).

($\chi^2 = 20{\cdot}5$, df $= 7$) and myrcene ($\chi^2 = 30{\cdot}8$, df $= 3$) in *C. macrocarpa*, limonene ($\chi^2 = 15{\cdot}5$, df $= 6$) in *C. abramsiana*, myrcene ($\chi^2 = 23{\cdot}0$, df $= 8$) in *C. goveniana-C. pygmaea* and myrcene ($\chi^2 = 13{\cdot}9$, df $= 5$) and γ-terpinene ($\chi^2 = 17{\cdot}4$, df $= 8$) in *C. sargentii* substantially deviated from the pure Gaussian. Polymodal and skewed distributions of many terpenoids have been previously noted in Pinacaea[30] and are suggestive of simple inheritance mechanisms, involving control of the terpene levels by a limited number of genes.

Mean values and standard deviations for terpene percentages of populations investigated are given in Table 1, with geographically close and chemically identical populations pooled. Where bimodality was encountered, the above statistics were computed separately for high (H) and low (L) modes. Variations between individual populations of the same species were essentially of two types. In most instances the differences involved only variations in the number of trees belonging to H or L modes of a particular terpene (Table 2), usually α-pinene or 3-carene—although more basic changes were occasionally encountered.

Cupressus macrocarpa is found in two locations, Point Cypress and Point Lobos, both in Monterey County; distance between the localities is about 3 miles. The difference between the two populations is small—5 out of 7 Point Pobos trees belonged to α-pinene H mode,

TABLE 2. NUMBER OF TREES BELONGING TO LOW AND HIGH α-PINENE AND 3-CARENE MODES, FOUND IN INDIVIDUAL POPULATIONS

		α-PINENE 0–15% L	α-PINENE 15–100% H	3-CARENE 0–3% L	3-CARENE 3–100% H
	Population				
Cupressus sargentii Northern	Laytonville	10	0	9	1
	Red Mountain	11	1	9	3
	Highland	9	2	9	2
	Reiff	6	8	8	6
	Monte Rio	6	1	4	3
	Occidental	11	0	10	1
	Bolinas Road	10	0	8	2
	Tamalpais	20	1	20	1
	Cedar Mountain	15	5	19	1
Southern	Chorro Creek	10	0	10	0
	Zaca Peak	9	1	10	0
C. Abramsiana	Eagle Rock	20	1	21	0
	Bonnie Doon	20	0	20	0
C. Goveniana	Huckleberry Hill	11	2	3	10
	San Jose Creek	12	0	12	0
C. Pygmaea	Anchor Bay	5	6	2	9
	Little River	9	1	6	4
	Ft. Bragg	10	0	9	1
C. Macrocarpa	Pt. Cypress	0	10	10	0
	Pt. Lobos	2	5	7	0

[30] E. ZAVARIN, K. SNAJBERK, TH. REICHERT and E. TSIEN, *Phytochem.* **9**, 377 (1970); E. ZAVARIN and F. W. COBB, JR., *Phytochem.* **9**, 2509 (1970).

while material from Point Cypress belonged exclusively to the H mode. This is in agreement with the botanical studies, in which no morphological differences between the two stands are reported.

Cupressus goveniana is known from two groves inland from the above localities, but the difference between these two populations is more marked, however. With Huckleberry Hill material two trees belonged to the α-pinene H mode and 11 trees belonged to the L mode, while with San Jose Creek trees all 12 samples were of L α-pinene mode. In Huckleberry Hill material only three out of 13 samples of essential oil investigated belonged to 3-carene L mode, while all samples from the San Jose Creek population were practically devoid of 3-carene. Other terpenoids did not show strong differences, however. No morphological differences were reported to exist between the two stands.

Cupressus pygmaea is found in west-central Mendocino County in an area stretching from Fort Bragg down the coast to near Little River, in a small area near Anchor Bay (southern Mendocino County), and in northern Sonoma County, where a grove has been recently discovered.[31] We compared the material from the first two localities, and found substantial differences. The Anchor Bay material (Table 2) included much higher numbers of trees belonging to the H α-pinene and H 3-carene modes than did that from the Little River-Fort Bragg area. In the north, the H mode of sabinene trees was much better represented (17 : 3); for Anchor Bay, the reverse was true (2 : 9). These chemical differences are paralleled by a difference in seed color, mentioned by Wolf.[1] The seeds from the northern, Fort Bragg locality are namely jet-black and shiny, but are brownish to brownish-black from Anchor Bay.

Cupressus abramsiana is found in four localities[1,32,33] three of which (Eagle Rock, Brackenbrae and Bonnie Doon) are in Santz Cruz County, and one (Butano Ridge) in San Mateo County. Wolf does not mention any botanical differences between the stands and considers the species intermediate between *C. goveniana* and *C. sargentii*. Later investigations by McMillan[32] point out the exceptionally high variability between populations in seedling characteristics and germination rates. Surprisingly, in view of McMillan's results, we could detect no significant chemical differences between the Eagle Rock and Bonnie Doon stands, save perhaps for the slightly higher camphene content in the Bonnie Doon material (0–3·6 per cent as compared with traces only in Eagle Rock samples).

Cupressus sargentii. This has by far the greatest distribution range of all coastal *Cupressus* species, growing from Zaca Peak, Santa Barbara County (Lat. 34° 46′) to Red Mountain near Laytonville, northern Mendocino County (Lat. 39° 45′). The Mount Tamalpais-Bolinas Road and Monte Rio-Occidental populations were found by Wolf to be different from the more northern, inland populations on the basis of crown shape (dense and broad), smaller cones with conspicuous umbos and only lightly glaucous seeds; however, these differences were not considered sufficient to separate the plants into subspecies. The Cedar Mountain (Alameda County) population has been designated as *C. sargentii var. duttonii* by Jepson[34] on the basis of larger cones with prominent umbos and leaves mainly lacking dorsal

[31] W. R. Powell. Some vascular plants in Sonoma County. Calif. Division Forestry, Sacramento, p. 8 (1968).
[32] C. McMillan, *Madroño* **12**, 28 (1953).
[33] J. H. Thomas, *Flora of the Santa Cruz Mountains of California*, Stanford Univ. Press, Stanford, Calif., p. 64 (1961).
[34] W. J. Jepson, A Manual of the Flowering Plants of California, Assoc. Students Store, Berkeley, California, p. 58 (1923).

pits. Wolf, however, later concluded that these characteristics were not of sufficient consistency to retain the two taxa. The population on Zaca Peak has been said to represent a different variety; the difference appears essentially to be the presence of dorsal pits (some of which active) on the leaves of the Zaca Peak trees, and their somewhat larger cones having prominent horns. Wolf does not, however, consider these differences sufficiently important to warrant this separation.

We investigated populations covering the entire range of this species on the basis of 136 trees. The central and northern populations showed little difference in composition of their essential oils, and the number of trees belonging to the two α-pinene and 3-carene modes did not vary much (Table 2), although higher percentage of trees belonging to α-pinene and 3-carene H modes in Reiff and Monte Rio populations might have some importance. The Tamalpais-Monte Rio populations were found to be significantly different (t-test, 1 per cent level) from the more northern populations on the basis of higher α-thujene, α-terpinene, γ-terpinene, p-cymene and terpinolene contents as well as somewhat lower myrcene content; this agrees well with the findings of Wolf, mentioned earlier. Cedar Mountain population differed only a little (β-phellandrene) from the Tamalpais-Monte Rio material. The southernmost Zaca Peak and Chorro Creek populations differed from the others in percentages of limonene and sabinene (Fig. 4), as well as in complete absence of the 3-carane H mode.

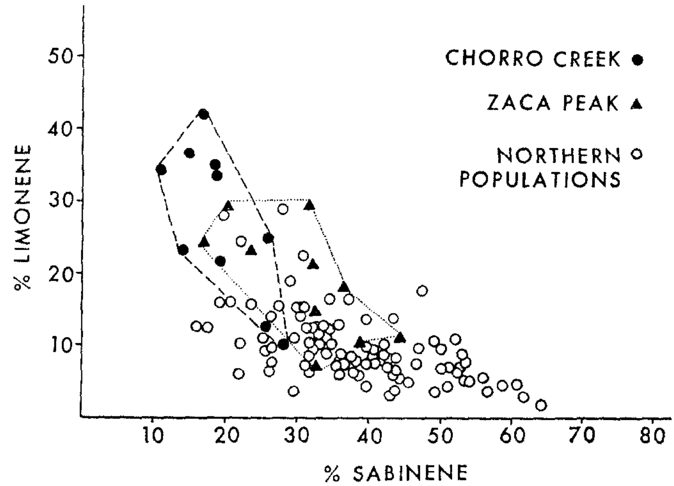


FIG. 4. LIMONENE VS. SABINENE PLOT FOR *C. sargentii* INDICATING SEPARATION OF ZACA PEAK AND CHORRO CREEK POPULATIONS.

Surprisingly, of the two populations Chorro Creek material differed most, with Zaca Peak trees being in between Chorro Creek and the more northern localities. Neither Wolf nor other botanists mention any aberrant behavior connected with the Chorro Creek material.

On the interspecific level the species separated chemically essentially into three groups, including *C. macrocarpa* in one, *C. sargentii* and *C. abramsiana* in the second, and *C. goveniana* and *C. pygmaea* in the third.

C. macrocarpa appeared to be the most distinct of the species, which agrees with earlier morphological conclusions (Table 1). The high proportion of trees belonging to the H α-pinene mode, plus the higher-than-usual percentage of α-pinene in that mode and the relatively high percentages of β-pinene and low amount of γ-terpinene and terpinolene,

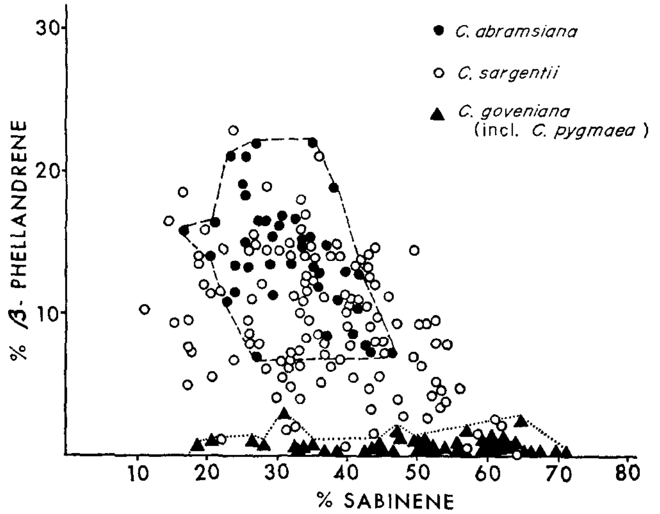


FIG. 5. β-PHELLANDRENE VS. SABINENE PLOT FOR COASTAL *Cupressus* SPECIES, INDICATING SEPARATION OF *C. sargentii* AND *C. abramsiana* FROM *C. goveniana* AND *C. pygmaea*.

distinguishes it from all other species. Low β-phellandrene content also distinguishes it from *C. sargentii* and *C. abramsiana*.

C. goveniana and *C. pygmaea* differ from *C. sargentii* and *C. abramsiana* (Fig. 5) in the lower amount of β-phellandrene of the former, and (less distinctively) their somewhat greater sabinene and γ-terpinene contents. The common occurrence of the 3-carene H mode in some populations distinguished them from central and southern populations of *C. sargentii* and from *C. abramsiana*. No significant difference on the specific level was noticeable between the two species, partly because of high variability between individuals and populations.

C. abramsiana and *C. sargentii* exhibited practically no differences (Fig. 5). Absence of the 3-carene H mode in either of the two populations of *C. abramsiana* and its occasional presence in *C. sargentii* is understandable in view of the tendency of this mode to disappear in *C. sargentii* southern populations. The Butano ridge population of *C. abramsiana* was not investigated as it is closest to *C. sargentii*, according to McMillan. The close placement of these two species partly contradicts the results of Wolf, who placed *C. abramsiana* closer to *C. goveniana*, and also contradicts our previous tropolone analyses which placed *C. abramsiana* with *C. goveniana* and *C. pygmaea* or, if quantities of the isolated tropolones were considered, between these two and *C. sargentii*. However, if the results of the present work are taken together with the results of other studies mentioned, the intermediacy of *C. abramsiana* appears substantiated and the contention is strengthened that it represents an intermediate taxon,[32] possibly a remnant of prexerothermic hybridization of *C. goveniana* and *C. sargentii* in that area.

As in our work on Pinaceae[30] a large number of correlations between the quantities of individual terpenoids were noted and the appropriate regression statistics are summarized in Table 3; the relation of these to biosynthesis has been discussed before.[35] One of the

[35] E. ZAVARIN, *Phytochem.* 9, 1079 (1940).

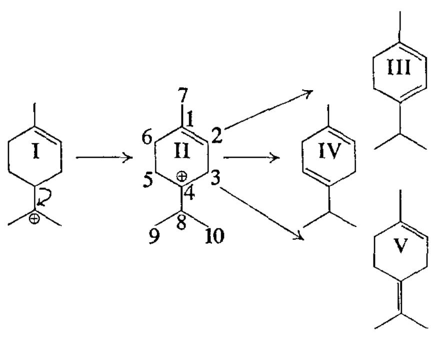
TABLE 3. LINEAR REGRESSION ANALYSIS FOR THE MORE IMPORTANT TERPENOID PAIRS

Independent variable	Dependent variable	Constant A	Slope B	Corr. coeff. R	Std. error of estim.
C. macrocarpa $r_{5\%} = 0{\cdot}482$; $r_{1\%} = 0{\cdot}606$					
γ-Terpinene	Terpinolene	0·127	0·383	0·614	0·421
Sabinene	γ-Terpinene	0·63	0·028	0·544	0·72
α-Pinene	β-Pinene	−0·46	0·088	0·907	0·68
α-Pinene	Sabinene	80·8	−0·98	−0·968	4·15
	$r_{5\%} = 0{\cdot}308$; $r_{1\%} = 0{\cdot}959$				
α-Terpinene	γ-Terpinene	−0·27	1·74	0·967	0·21
C. abramsiana $r_{5\%} = 0{\cdot}308$; $r_{1\%} = 0{\cdot}398$					
γ-Terpinene	Terpinolene	3·51	0·181	0·305	0·826
α-Terpinene	γ-Terpinene	0·334	0·800	0·834	0·473
α-Terpinene	Terpinolene	0·563	0·978	0·636	1·06
α-Pinene	Sabinene	39·5	−0·927	−0·362	6·75
Sabinene	β-Phellandrene	22·6	−0·266	−0·472	3·60
Sabinene	Limonene	22·7	−0·326	−0·403	4·87
C. goveniana/*C. pygmaea* $r_{5\%} = 0{\cdot}264$; $r_{1\%} = 0{\cdot}342$					
α-Terpinene	γ-Terpinene	0·869	1·62	0·871	1·706
α-Terpinene	Terpinolene	1·89	0·243	0·502	0·81
γ-Terpinene	Terpinolene	1·60	0·172	0·636	0·72
α-Pinene	Sabinene	59·4	−0·929	−0·759	8·22
Sabinene	3-Carene	26·4	−0·398	−0·577	7·10
C. sargentii (excluding Chorro Creek and Zaca Peak populations) $r_{5\%} = 0{\cdot}187$; $r_{1\%} = 0{\cdot}244$					
α-Terpinene	Terpinolene	1·61	0·498	0·522	1·15
α-Terpinene	γ-Terpinene	2·16	0·926	0·608	1·71
γ-Terpinene	Terpinolene	0·98	0·442	0·706	0·96
Sabinene	Terpinolene	4·96	−0·040	−0·300	1·29
Sabinene	3-Carane	7·33	−0·135	−0·266	4·97
β-Phellandrene	Limonene	7·72	0·218	0·214	4·76
β-Phellandrene	β-Pinene	0·60	−0·036	−0·397	0·39
	$r_{5\%} = 0{\cdot}193$; $r_{1\%} = 0{\cdot}252$				
α-Pinene	Myrcene	11·92	−0·0785	−0·193	2·88
Sabinene	α-Pinene	16·73	−0·185	−0·260	6·99
Sabinene	Myrcene	10·8	0·0091	0·033	2·82
	Same, populations 12–16 only; $r_{5\%} = 0{\cdot}252$; $r_{1\%} = 0{\cdot}328$				
Sabinene	Terpinolene	6·49	−0·0624	−0·501	1·12
Sabinene	α-Terpinene	5·83	−0·0421	−0·376	1·08
Sabinene	γ-Terpinene	8·95	−0·0709	−0·339	2·05
α-Pinene	Myrcene	11·04	−0·115	−0·327	2·17
β-Phellandrene	β-Pinene	0·619	−0·0378	−0·404	0·406
β-Phellandrene	Limonene	4·70	−0·429	−0·474	3·70
α-Terpinene	Terpinolene	0·726	0·789	0·710	0·915
α-Terpinene	γ-Terpinene	−0·028	1·483	0·795	1·32

problems involved is the geographic variability of the data. Thus, correlations between terpenes synthesized quite independently by plants could be introduced and/or existing correlations could be obscured if several populations differing in terpenoid composition are pooled for statistical computations. Therefore, when this was suspected we checked the relationships obtained using either a single population or several geographically close populations not differing much in mean values for individual terpenes. All correlations not significant on the basis of reduced sample space were then eliminated from consideration; such correlations were few, and relatively small.

Of the correlations encountered, exceptionally strong positive correlations approaching proportionality between α-terpinene, γ-terpinene and terpinolene in nearly all species, and between (—)-α- and β-pinene in *C. macrocarpa*, were most striking. According to Rule II discussed in our previous publication,[35] this suggests that these compounds are closer biosynthetically to each other than to other compounds to which they are correlated in different manner. The close relationship between (—)-α- and β-pinene* has been encountered before and is easy to understand as it involves in both cases interaction of the positively charged C_8 of 1-*p*-menthene-8-carbonium ion (I) with the same double bond at C_1.[30] This is also true for the routes to α- and γ-terpinene (III and IV), which differ only in the loss of the protons at C_3 vs. C_5 respectively from the 1-*p*-menthene-4-carbonium ion (II). Apparently, formation of terpinolene (V) also goes through the same carbonium ion, i.e. it involves 4–8 hydride shift in 1-*p*-menthene-8-carbonium ion followed by loss of proton at C_8, rather than by direct formation from 1-*p*-menthene-8-carbonium ion, by loss of proton at C_4 due to strong correlation between terpinolene and the terpinenes. This has also been found in the case of *P. muricata*.[35] Unlike results obtained with *P. muricata*, however, the correlations between terpinenes and other terpenes synthesized though 1-*p*-menthene-4-carbonium ion were not definite, and there was only one case of sabinene/γ-terpinene positive correlation. Apparently the enzymatic systems in *Cupressus* are, contrary to those in *Pinus*, able to control the rate of material flow through terpinolene and terpinenes on one side, and sabinene and α-thujene on the other. This is understandable, as in the latter case the necessary transformations should be more elaborate than mere expulsion of α-protons.

The independent biosynthesis of two terpenoids, (large constant A, combined with very small slope B, and small standard error of estimate) was only found in the case of sabinene and myrcene in *C. sargentii*. However, it is significant because it points to the larger difference between the formation mechanisms of these two compounds, and it is in line with present-day biosynthetic ideas connected with the biogenesis of cyclic versus acylic monoterpenoids.[36]



EXPERIMENTAL

Collection of Samples

Table 4 gives the populations sampled, together with geographic and other pertinent data. About a pound of foliage was collected from different branches of each tree, wrapped in aluminium and polyethylene foils, shipped to the laboratory as soon as possible and stored at about −15°. The possible effect on volatile

* Practically always (−).

[36] W. D. Loomis, *Biosynthesis and Metabolism of Monoterpenes* in *Terpenoids in Plants* (edited by J. D. Pridham), p. 62, Acadamic Press, New York (1967).

TABLE 4. *Cupressus* POPULATIONS SAMPLED*

	Population no.	Elevation	Latitude	Longitude
C. macrocarpa				
Point Cypress	1	50′	36° 35′	121° 59′
Point Lobos	2	100′	36° 31′	121° 57′
C. goveniana				
Huckleberry Hill	3	400′	36° 35′	121° 56′
San Jose Creek	4	400′	36° 31′	121° 55′
C. pygmaea				
Anchor Bay	5	1000′	38° 48′	123° 30′
Little River	6	200′	39° 17′	123° 48′
Ft. Bragg	7	500′	39° 27·3′	123° 48′
C. abramsiana				
Eagle Rock	8	2500′	37° 4′	122° 11′
Bonnie Doon	9	1600′	36° 58′	122° 10′
C. sargentii				
Zaca Peak	10	3000′	34° 46′	120° 1′
Chorro Creek†	11	1520′	35° 29′	120° 31′
Cedar Mountain	12	2500–3000′	37° 33′	121° 37′
Mount Tamalpais	13	2500′	37° 55′	122° 37′
Bolinas Road	14	1800′	37° 58′	122° 38′
Occidental-Camp Meeker	15	300′	38° 23′	122° 56′
Monte Rio	16	2200′	38° 26′	122° 59′
Reiff	17	2000′	38° 53′	122° 29′
Pieta Road	18	2000′	38° 57′	122° 58′
Red Mountain (Maycamas)	19	3000′	39° 5′	123° 8′
Red Mountain (Laytonville)†	20	3000′	39° 56′	123° 42·5′

* Populations designated in accordance with Wolf. Little River is southern part of Wolf's Fort Bragg locality; Bolinas Road belongs to Mount Tamalpais and Monte Rio to Occidental/Camp Meeker Wolf's locations.

† Collected by E. Zavarin. The rest were collected by L. Lawrence.

oil composition of storage in the field was checked by storing at room temp. the foliage of *C. macrocarpa* wrapped in aluminium/polyethylene and analyzing it at three equally-spaced intervals within 15 days time. No significant differences were noticeable during that time in composition or in volatile oil content.

The oil to be analyzed was separated from the foliage by steam distillation of the Waring blendor macerated material. The maceration was made under ice to avoid volatilization of the oil due to heat generated. To avoid any possibility of contamination by the terpenes of wood or bark, the foliage was carefully separated from branchelets before maceration. Steam distillation was conducted for 15 min, using circulating trap distillation apparatus although no visual increase in oil content was noticeable after 5 min. The oil resulting was separated with pipette and stored at −15°; a trace of pyrogallol was added as antioxidant.

The effect of seasonal variation on the chemical composition of oil was checked by sampling a *C. macrocarpa* tree growing on the premises of the San Francisco College for Women at various times between April and October. A further check was made by analyzing Cedar Mountain and Mount Tamalpais *C. sargentii*, and Eagle Rock and Bonnie Doon populations of *C. abramisiana*, at two different times of a year. Results of both of these tests, summarized in Table 5, indicate that only few differences of significant magnitude (*t*-test of significance) were encountered. No significant variations in analyses were found in foliage produced in different years.

Analysis

Analysis of the volatile oils was performed using Varian Aerograph GLC instrument Model 1200, in conjunction with Sargeant Model SR recorder equipped with Disc integrator Model 204. The columns used included (a), β,β-oxydipropionitrile 10% on Chromosorb 60/80 acid washed and silanized, copper, 6 ft × $\frac{1}{8}$ in. o.d., H_2/N_2, flows of 15 ml/min., (b) Carbowax 20M, otherwise as above. Column b was used to determine the amounts of individual compounds in limonene/α-terpinene and α-pinene/α-thujene pairs, not separable or difficult to separate using column (a).

TABLE 5. SEASONAL VARIABILITY OF THE VOLATILE OIL COMPOSITION*

<table>
<tr><th></th><th>α-Pinene</th><th>α-Thujene</th><th>β-Pinene</th><th>Sabinene</th><th>Myrcene</th><th>α-Terpinene</th><th>Limonene</th><th>β-Phellandrene</th><th>γ-Terpinene</th><th>Terpinolene</th></tr>
<tr><td>C. sargentii, Mnt. Tamalpais</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Average for June 11, 1966</td><td>4·6‡</td><td>3·6‡</td><td>0·1</td><td>32·0</td><td>9·9</td><td>4·7</td><td>13·7‡</td><td>13·7</td><td>7·5</td><td>5·1§</td></tr>
<tr><td>Average for April 20, 1967</td><td>6·2</td><td>5·0</td><td>tr</td><td>38·0</td><td>11·9</td><td>4·3</td><td>10·0</td><td>12·8</td><td>6·4</td><td>3·6</td></tr>
<tr><td>C. sargentii, Cedar Mnt.</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Average for July 9, 1966</td><td>8·6</td><td>3·0</td><td>0·8</td><td>39·1</td><td>9·0</td><td>4·3</td><td>7·2</td><td>4·9</td><td>8·3‡</td><td>5·2</td></tr>
<tr><td>Average for March 2, 1968</td><td>8·8</td><td>3·4</td><td>0·3</td><td>44·4</td><td>10·1</td><td>3·2</td><td>8·9</td><td>6·2</td><td>4·8</td><td>3·2</td></tr>
<tr><td>C. abramsiana, Eagle Rock</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Average for August 27, 1966</td><td>7·4</td><td>6·8</td><td>tr</td><td>30·7</td><td>7·7§</td><td colspan="2">18·9</td><td>16·8</td><td>5·1</td><td>5·2‡</td></tr>
<tr><td>Average for March 2, 1968</td><td>9·6</td><td>6·9</td><td>0·2</td><td>30·6</td><td>11·4</td><td colspan="2">14·8</td><td>15·6</td><td>4·9</td><td>4·5</td></tr>
<tr><td>C. abramsiana, Bonnie Doon</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Average for August 13, 1966</td><td>7·2</td><td>4·1</td><td>0·1</td><td>31·5</td><td>10·9§</td><td colspan="2">20·1</td><td>12·2</td><td>5·7‡</td><td>3·9</td></tr>
<tr><td>Average for March 9, 1968</td><td>9·2</td><td>4·3</td><td>tr</td><td>31·8</td><td>7·9</td><td colspan="2">22·4</td><td>11·5</td><td>7·1</td><td>4·4</td></tr>
<tr><td>C. macrocarpa, single tree†</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>April 8</td><td colspan="2">53·6</td><td>3·2</td><td>31·4</td><td>4·2</td><td>1·2</td><td>1·1</td><td>0·8</td><td>2·0</td><td>0·8</td></tr>
<tr><td>April 29</td><td colspan="2">51·3</td><td>2·9</td><td>34·4</td><td>4·7</td><td>1·2</td><td>1·1</td><td>0·8</td><td>1·8</td><td>0·9</td></tr>
<tr><td>June 25</td><td colspan="2">52·5</td><td>3·3</td><td>32·5</td><td colspan="3">8·0</td><td>0·9</td><td>1·4</td><td>0·3</td></tr>
<tr><td>October 5</td><td colspan="2">50·6</td><td>2·8</td><td>36·2</td><td>4·5</td><td colspan="2">2·2</td><td>0·5</td><td>2·0</td><td>0·6</td></tr>
</table>

* Given for more important monoterpenes, only. No attempt was made to sample the same set of trees in *C. sargentii* and *C. abramsiana* experiments. Symbols‡ indicate significant differences on 5%, and symbols§ on 1% level in *t*-test of significance.

† α-Thujene was about 1·5% only.

The analysis results were reported, normalized to 100%, and included only monoterpene hydrocarbons. Oxygenated monoterpenes and sesquiterpenes appeared to be quantitatively secondary as determined by the internal standard methodology,[37] amounting to less than 25% and as an average, 15%. Correlation analysis was performed, using the CDC 6400 computer of the University of California.

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[37] E. ZAVARIN, W. HATHWAY, Th. REICHERT and Y. LINHART, *Phytochem.* 6, 1019 (1967).