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VOLATILE OIL FROM FOLIAGE OF *SEQUOIADENDRON GIGANTEUM*: CHANGE IN COMPOSITION DURING GROWTH*

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Abstract—The composition of volatile oil from foliage of *Sequoiadendron giganteum* changes considerably during growth of the foliage. The new foliage contains none of the allyl phenylethers safrole, eugenol, *O*-methyl eugenol and elemicin which constitute *ca.* 10% of the oil from the mature foliage. These components appear and increase in concentration in the new foliage as the growing season progresses. In the mature foliage there is a slight decrease in the concentration of these allyl phenylethers at the start of the growing season and a significant increase later on. These data suggest that the allyl phenylethers may be involved in plant metabolism other than as passive metabolic end products. In both the old and new foliage, α-pinene decrease as the growing season progresses and fluctuations in the α-pinene concentrations are accompanied by fluctuations in the opposite direction in α-terpineol concentrations.

INTRODUCTION

RECENTLY we reported an analysis of the volatile oil from foliage of *Sequoiadendron giganteum* (Big Tree).[1] Following completion of that work, several trees from which we had collected foliage were felled and we obtained previously unavailable foliage from the tree tops. Analysis of the oil from the foliage of the tree tops yielded a surprising result. Only a small fraction of the previously reported[1] allyl phenylethers safrole, eugenol, *O*-methyl eugenol and elemicin was found. The starting material used in the earlier work had been collected from lower limbs in December 1966, and January and February 1967. This material was dark green and was the mature foliage accumulated from at least several years' growth. The tree tops, collected in October 1967, were light green and the foliage was obviously new immature growth. The compounds in question are generally believed to be derived from shikimic acid *via* prephenic acid, phenylalanine (or tyrosine) and cinnamic acid (or *p*-coumaric acid).[2] It appeared that a correlation between the concentration of the allyl phenylethers in the oil and the state of maturation of the new foliage might exist.

RESULTS

In order to establish such a correlation the oil from new foliage only on lower limbs was isolated and analyzed periodically through the 1968 growing season. The following year the procedure was repeated and extended to also include separate isolation and analysis of the oil from the old foliage adjacent to new foliage on each branchlet. The study of the 1968 growing season showed that the allyl phenylethers were not present in the new growth but

* Abstracted in part from the Master's Thesis of G. L. L., Portland State University (1969).
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[2] T. A. GEISSMAN and D. H. G. CROUT, *Organic Chemistry of Secondary Plant Metabolism*, pp. 137–166, Freeman, Cooper, San Francisco (1969).

they appeared and increased in concentration as the growing season progressed. The total percent of the safrole, *O*-methyl eugenol, eugenol and elemicin in the mixture increased from 0 to 12% (Fig. 1). At the same time the % of α-pinene decreased from 72 to 61% (Fig. 2). Fluctuations were noted in the other components measured. (See Table 1.)

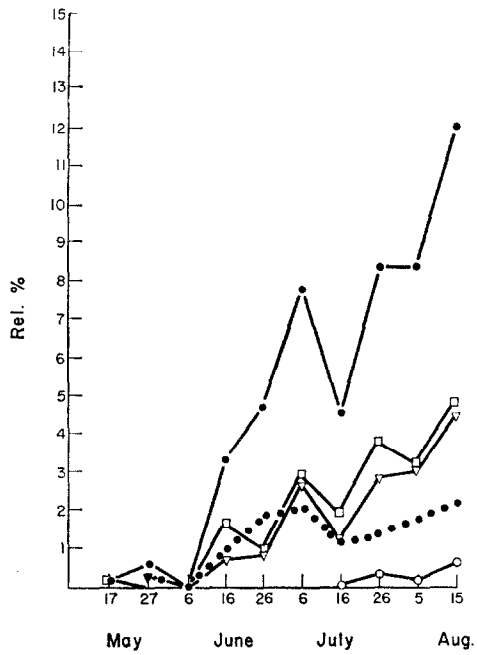

FIG. 1. NEW GROWTH, 1968 ALLYL PHENYLETHERS.
● Total allyl phenylethers; □ safrole; ▽ elemicin; (●●●) eugenol; ○ *O*-methyl eugenol.

TABLE 1. % COMPOSITION OF NEW FOLIAGE OIL, 1968

Compound	17 May	27 May	6 June	16 June	26 June	6 July	16 July	26 July	5 Aug.	15 Aug.
α-Pinene	72·2	77·8	76·4	68·7	67·3	64·9	71·2	69·3	68·8	61·3
Unknown	0·5	0·2	0·5	0·7	0·6	0·5	0·5	0·4	0·5	0·5
Unknown	0·5	0·6	0·5	0·8	0·4	0·6	0·6	0·6	0·7	0·9
Myrcene	9·0	8·4	7·8	9·4	8·7	9·2	7·6	7·7	8·1	8·7
Limonene	1·4	2·1	4·0	3·3	3·1	2·8	3·5	1·0	2·6	2·5
Terpinolene	0·3	0·4	0·2	0·8	1·1	0·7	1·0	0·7	0·7	0·6
Unknown	—*	—	—	—	—	—	—	—	—	—
l-Terpinen-4-ol	—	—	—	—	—	—	—	—	—	—
α-Terpineol and caryophyllene	4·4	2·9	2·8	3·5	2·2	4·0	2·3	3·8	2·5	3·4
Humulene	9·2	5·8	6·8	8·0	10·4	8·2	7·7	7·0	6·4	8·4
Citronellol	1·7	0·9	1·1	1·0	0·8	1·1	0·8	1·4	1·0	1·1
Unknown	0·6	0·4	0·2	0·4	0·3	0·5	0·3	—	0·3	0·3
Safrole	0·2	—	—	1·7	1·0	3·0	2·0	3·8	3·2	4·8
O-Methyl eugenol	—	—	—	—	—	—	—	0·3	0·2	0·6
Eugenol	—	0·3	—	0·9	1·9	2·0	1·3	1·4	1·7	2·1
Elemicin	—	0·3	—	0·8	1·9	2·7	1·3	2·8	3·1	4·5

* Indicates not detected.

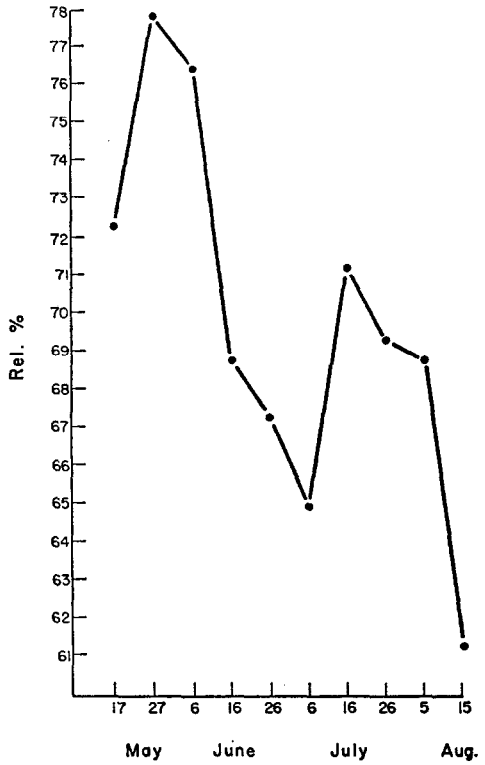

FIG. 2. NEW GROWTH, 1968 α-PINENE.

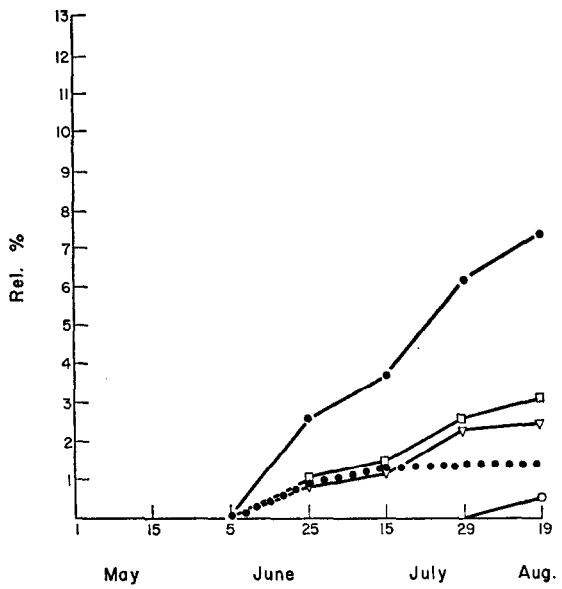

FIG. 3. NEW GROWTH, 1969 ALLYL PHENYLETHERS.
● Total allyl phenylethers; □ safrole; ▽ elemicin; (●●●) eugenol; ○ *O*-methyl eugenol.

The study of new growth in the 1969 growing season gave the same qualitative result as was obtained in 1968 in the variation of the allyl phenylethers and α-pinene (Fig. 3 and Fig. 4). Fluctuations were again noted in the other components as well (Fig. 5 and Table 2). The α-terpineol and caryophyllene peaks were resolved and an inverse relationship between the α-terpineol concentration and the α-pinene concentration was obtained (Fig. 4 and Fig. 5). Furthermore, changes in the concentration of terpinolene parallel those in α-terpineol. The mature growth showed considerable change in essential oil composition as the growing

TABLE 2. % COMPOSITION OF NEW FOLIAGE OIL, 1969

Compound	1 May	15 May	5 June	25 June	15 July	29 July	19 Aug.
α-Pinene	81·2	80·2	78·7	73·9	70·8	75·1	75·1
Unknown	t*	t	t	t	0·3	t	t
Unknown	0·8	0·7	0·7	0·5	0·4	0·7	0·6
Myrcene	6·6	8·1	7·3	6·8	5·8	5·8	6·1
Limonene	1·0	2·4	2·9	3·4	2·2	3·1	2·2
Terpinolene	0·5	0·6	1·0	1·9	2·8	1·1	1·1
Unknown	0·9	0·8	0·8	0·4	0·7	0·6	0·5
1-Terpinen-4-ol	1·8	t	0·5	0·5	t	t	t
Caryophyllene	0·9	1·4	1·4	1·2	1·2	1·6	1·4
α-Terpineol	3·2	2·7	4·3	6·5	9·7	3·2	3·3
Humulene	3·2	2·4	2·6	2·2	2·4	2·7	2·4
Citronellol	t	0·7	t	—	—	t	t
Unknown	t	t	—	—	—	—	t
Safrole	—	—	t	1·0	1·4	2·6	3·0
O-Methyl eugenol	—	—	—	—	—	—	0·4
Eugenol	—	—	t	0·8	1·2	1·4	1·4
Elemicin	—	—	t	0·8	1·1	2·3	2·5

* t Indicates trace amount.

TABLE 3. % COMPOSITION OF OLD FOLIAGE OIL, 1969

Compound	24 April	1 May	15 May	5 June	25 June	15 July	29 July
α-Pinene	77·8	73·4	80·0	75·9	72·7	72·0	69·9
Unknown	0·3	0·5	t	t	t	0·3	t
Unknown	0·8	0·6	0·8	0·6	0·6	0·5	0·6
Myrcene	5·3	5·7	5·8	5·6	5·2	4·8	4·9
Limonene	1·4	1·6	1·4	1·9	2·2	1·8	2·1
Terpinolene	0·4	1·6	0·6	0·6	1·3	1·4	0·7
Unknown	0·8	0·9	0·8	0·7	0·5	0·7	0·9
1-Terpinen-4-ol	t	0·4	t	t	t	t	t
Caryophyllene	1·3	1·0	1·4	1·4	1·1	1·2	1·5
α-Terpineol	0·9	5·5	1·1	2·6	3·5	5·0	2·0
Humulene	2·3	1·9	2·4	2·3	2·0	2·3	2·5
Citronellol	0·4	0·4	t	—	—	t	—
Unknown	—	—	—	—	—	t	t
Safrole	3·8	4·3	3·1	3·6	4·3	4·1	5·7
O-Methyl eugenol	0·6	0·5	0·3	0·3	0·9	—	1·2
Eugenol	1·0	0·7	1·0	1·5	1·8	1·8	2·1
Elemicin	3·3	1·3	1·8	3·0	4·0	4·0	6·2

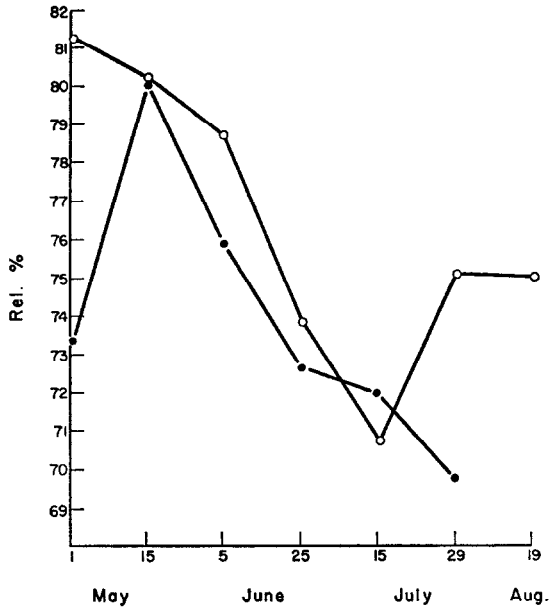

FIG. 4.
● α-Pinene, old growth, 1969; ○ α-pinene, new growth, 1969.

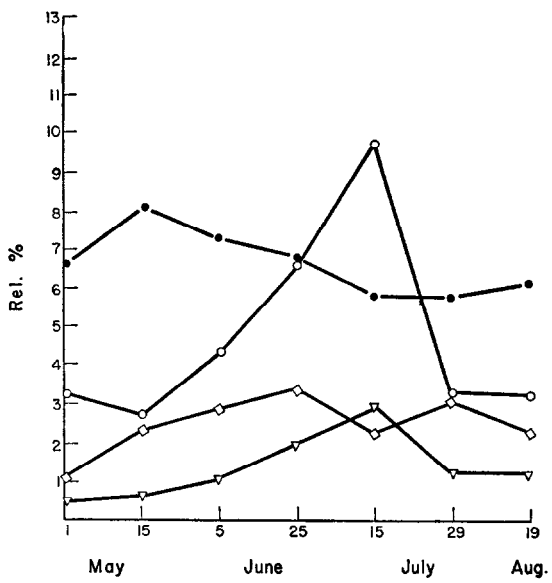

FIG. 5. NEW GROWTH, 1969 MONOTERPENES (EXCEPT α-PINENE).
● Myrcene; ○ α-terpineol; ◇ limonene; ▽ terpinolene.

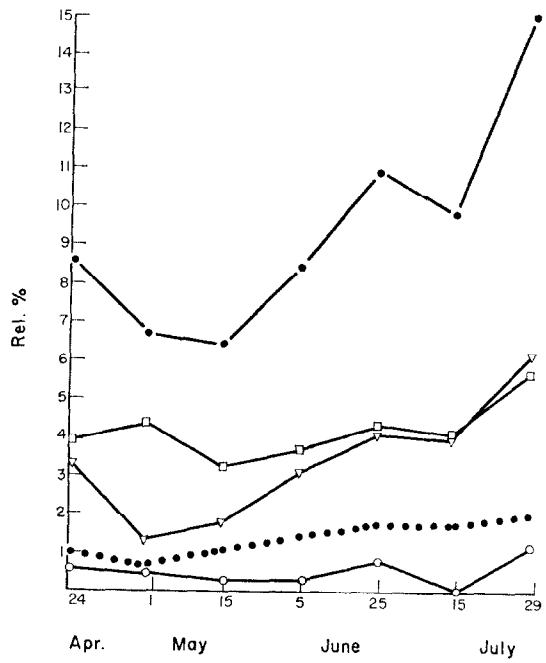

FIG. 6. OLD GROWTH, 1969 ALLYL PHENYLETHERS.
● Total allyl phenylethers; □ safrole; ▽ elemicin; (●●●) eugenol; ○ *O*-methyl eugenol.

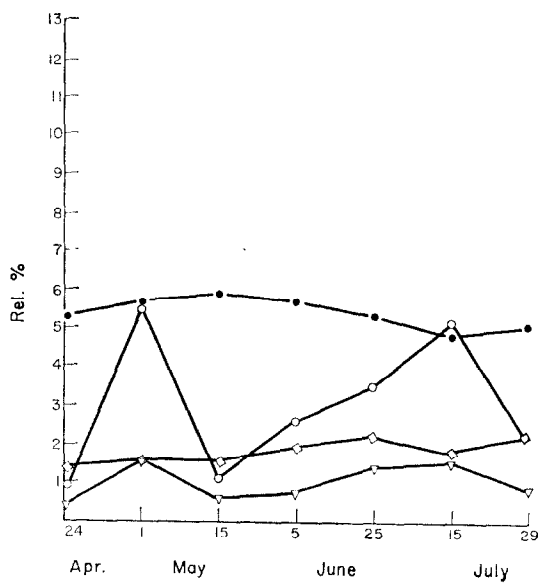

FIG. 7. OLD GROWTH, 1969 MONOTERPENES (EXCEPT α-PINENE).
● Myrcene; ○ α-terpineol; ◇ limonene; ▽ terpinolene.

season progressed. The allyl phenylethers individually fluctuated in concentration. The total allyl phenylether percentage decreased slightly as new growth began to appear at the beginning of the growing season and increased substantially toward the end of the growing season (Fig. 6). Again an inverse relationship between the α-terpineol concentration and the α-pinene concentration was indicated (Fig. 7, Fig. 4 and Table 3).

DISCUSSION

It has been established that an increase in the concentration of the allyl phenylethers safrole, eugenol, *O*-methyl eugenol and elemicin occurs in the foliage of *Sequoiadendron giganteum* during the growing season in both mature and new foliage. A comparable result (safrole only) with the essential oil of Ho leaf has recently been reported.[3]

The maturation process in *Sequoiadendron giganteum* is characterized by development of wood from the soft light green new growth. This presumably involves lignin formation and it should be noted that lignin arises from the same metabolic precursors as the allyl phenyl ethers.[2] In growing spruce tips, for instance, it has been found that lignin is not present in 2·5–3-week-old foliage but is present after 3·5–4 months.[4] Therefore, it is possible to hypothesize that production of the allyl phenylethers safrole, eugenol, *O*-methyl eugenol and elemicin in the new foliage occurs when lignin is also being produced. The allyl phenyl ethers may thus be merely part of a route for handling excess lignin precursors or they may be more directly involved in lignification. The latter possibility could occur *via* conversion of the allyl phenylethers back into lignin precursors or by their exerting some control over the metabolic processes. Eugenol itself has already been considered as a possible precursor.[5] Although it does form lignin-like polymer with enzyme-containing plant material, there were qualitative and quantitative differences between this polymer and lignin.[6] These data do not, however, rule out possible conversion of eugenol and the other allyl phenylethers into lignin precursors. The changes in their concentration in old foliage are not inconsistent with this hypothesis. The decrease in concentration followed by an increase suggests active metabolism in this pool of compounds rather than passive storage.

The presence of allyl phenylethers in oils from red juniper,[7] Rocky Mountain juniper,[8] and probably savin juniper,[9] has been reported. Variations in their concentrations were reported from specimen to specimen but seasonal data was not obtained. Such data would be of interest in assessing the generality of the observation made in *Sequoiadendron giganteum.*

The fluctuations in the terpene components during the growing season show an inverse relationship between α-terpineol and α-pinene. This is consistent with the suggestion by Ruzicka[10] that α-pinene is formed from α-terpineol.

EXPERIMENTAL

GLC was performed with an Aerograph Autoprep Model 700 equipped with a thermal conductivity detector and a non-linear temperature programmer. The spinning bond distillation apparatus was Nester Faust Model 65 TB-60 cm.

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[7] A. R. VINUTHA and E. VON RUDLOFF, *Can. J. Chem.* **46**, 3743 (1968).
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[9] E. VON RUDLOFF, *Can. J. Chem.* **41**, 2876 (1963).
[10] L. RUZICKA, *Experientia* **9**, 357 (1953).

1968

New growth was collected from approximately 15 *ca.* 30-yr-old *Sequoiadendron giganteum* on the west shoulder of McLoughlin Boulevard, Milwaukee, Oregon. Collections were made at 10-day intervals beginning with the appearance of new growth on 17 May. Samples of approximately 250 g each of new growth only were macerated and steam distilled under a continuous distillation–extraction head for 24 hr as previously described.[1] The final samples were isolated as previously described and were analyzed by GLC with a 1·5 m × 6 mm o.d. aluminum column having 20% polyethylene glycol (Carbowax 20M) as liquid phase on Chromosorb W (60–80 mesh). Temperature was increased from 80° to 210°. The peaks were previously identified[1] and several were confirmed by the peak-enhancement technique. Peak areas were determined by height × half-width and the data is presented in Table 1.

1969

Foliage was collected every 2–3 weeks beginning 24 April at the same location. The samples consisted of the last 15–20 cm of the lower branch tips from all sides of each tree. New foliage was separated from old growth and treated as before. The cyclohexane was removed using a spinning bond column. The oil was analyzed by GLC with both the previously described column and with a 3 m × 9 mm column with the same packing. The identities of all the known peaks were confirmed by peak enhancement. Areas were determined by height × half-width. Data presented in Tables 2 and 3 are average of two separate determinations. The two determinations agreed within 1% for all peaks representing *ca.* 10% or more of the total. Smaller peaks agreed within 5%.

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