attributed to such a source. Clearly, new methodology is needed to better evaluate the problem of bound residues, and new extractants tried which may result in minimizing readsorption problems.

The results of field studies (Table IV) indicated that over each of the two growing seasons diclofop-methyl had undergone almost complete degradation since no significant amounts of ester or unbound acid residues were recovered from any of the treated soils. In contrast, carryover of unbound ester and acid residues derived from benzoylprop-ethyl and flamprop-methyl were observed both years at all three locations (Table IV). Both chemicals were more persistent in the Melfort silty clay with approximately 30–40% of the applications remaining in the ester and acid forms, while in the heavy clay and sandy loam the figures ranged from 15–23 and 6–25%, respectively. No unbound residues were detected at depths greater than 5 cm, indicating that leaching of these chemicals under field conditions should not be a problem.

In every case, following extraction of unbound residues, reextraction of the soil residua with aqueous sodium hydroxide and boiling triethanolamine solution failed to release detectable amounts of benzoylprop, diclofop, or flamprop acids.

In view of the laboratory fortification studies (Tables II and III) and considering that under field conditions the acid residues (bound) could be present in smaller quantities than those used (2.5 ppm) in the above studies, it was necessary to confirm that the absence of bound carboxylic acid residues was not simply due to inadequate analytical techniques. Hence, samples of untreated soils (20 g) were fortified with 3 μg of either benzoylprop or diclofop acids or 2 μg of flamprop acid. These amounts, equivalent to 0.15 and 0.10 ppm, respectively, were those which could be released from treated field plots (2 kg/plot) if it were assumed that bound acid residues accounted for 5% of the original ester applications. These fortified soils were then extracted using cold aqueous sodium hydroxide and hot triethanolamine solutions as described. Although quantitative analysis was impossible because of interferences on the chromatograms resulting from the high attenuation necessary for detection, peaks due to the respective methyl esters were clearly discernible in all cases, though not on the chromatograms derived from the soil blanks or from the treated field plots. This was taken as confirmation that after a growing season the field soils contained negligible carboxylic residues (less than 5% of the original ester treatments) in a bound form. Since the herbicidal esters are normally applied as postemergence treatments and not directly to the field plots as they were in this study, bound residues derived from benzoylprop, diclofop, and flamprop acids should be insignificant following normal field applicatons.

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Wright, A. N., Mathews, B. L., *Pestic. Sci.* **7**, 339 (1976).

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Essential Oil of *Eucalyptus globulus* in California

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Analysis of the *Eucalyptus globulus* essential oil by using mainly the computerized gas chromatography–mass spectrometry revealed 1,8-cineol, α-, β-, and γ-terpinene, β-pinene, terpinen-4-ol, linalool oxide, α-gurjunene, aromadendrene, alloaromadendrene, globulol (10-hydroxyaromadendrane), and so on. The gas–liquid chromatography comparisons of terpene fractions of the fresh *Eucalyptus* fruits indicated that sesquiterpenes were more significant than monoterpenes. In case of the shoots, monoterpenes such as 1,8-cineol and γ-terpinene were major rather than sesquiterpenes. The plant oil as a renewable source of hydrocarbon-like photosynthetic products was evaluated in connection with the utilization of solar energy.

As a result of decreasing supplies of fossil hydrocarbons it has become necessary to reexamine other sources of raw materials for possible conversion into hydrocarbons (Calvin, 1974, 1976). In connection with the utilization of solar energy for renewable resources, we have reported the constituents of photosynthesizing plants such as rubber tree (*Hevea brasiliensis*), some different species of *Euphorbia*, and other latex-bearing plants which grow naturally in tropical and temperate regions (Nishimura et al., 1977; Nielsen et al., 1977). The results have indicated that those plants contain a large amount of lipids such as

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triglycerides and steroidal triterpenes (about 10 ± 5% of the fresh plant).

It is well-known that *Eucalyptus* plants (some 500 species) also produce terpenoidal hydrocarbons (Chippendale, 1973). The essential oils are grouped as medicinal, industrial, and perfumery types, depending on their chemical composition. Furthermore, *Eucalyptus* oil can be regarded as an alternate source of hydrocarbon fuel or an additive to petroleum since it consists of low boiling point compounds, which give high energy when burned. However, nobody knows which species of *Eucalyptus* plants produce the richest energy oil per acre per year. *E. globulus* tree is known to grow very fast (some 1.5 ft high per month in California) and is distributed widely in the world (Hall et al., 1975; Pryor, 1976). As a first step, it is very important to analyze the constituents of *E. globulus* oil and evaluate which part of the tree, fruit, leaf and branch, or shoot is the most valuable. So far only a few mono- and sesquiterpenes such as 1,8-cineol, pinenes, and aromadendrene in the essential oil of *E. globulus* leaves have been reported (Dolejš et al., 1960; Laurent and da Cunha, 1964; Yamashita, 1970; Prakash et al., 1972). The major components of the essential leaf oil of *E. globulus* are definitely monoterpenes, e.g., 1,8-cineol (80.7% of total oil; Yamashita, 1970), but in the case of the fruit oil, we found that the most significant fraction was sesquiterpenes.

The present paper deals with the identification of mono- and sesquiterpenes and the comparison of each terpene fraction from fruits, leaves and branches, and shoots of fresh *E. globulus* in California.

EXPERIMENTAL SECTION

Fruits (29.3 g), leaves and branches (22.6 g), and shoots (23.0 g) of fresh *E. globulus* were collected on June 16, 1977, on the campus of the University of California at Berkeley. Each of them was cut into small pieces and exhaustively extracted with acetone (450 mL) using a Soxhlet extractor for 2 days. Each essential oil was obtained by the steam distillation of the acetone extract. The terpenoid fraction in the steam distillate was extracted with freshly distilled ethyl ether (70 mL × 4). After drying the extract with anhydrous magnesium sulfate, ethyl ether was carefully removed using Vigreux column. Each terpenoid fraction containing ca. 0.5% of solvent (GLC analysis) was obtained from fruit oil, 293 mg; leaf and branch oil, 272 mg; shoot oil, 276 mg.

Gas–liquid chromatography (GLC) was performed on a Varian 2700 GC and a Perkin-Elmer 900 GC equipped with a flame ionization detector (FID) and linear temperature programmer. To isolate the respective components, a Hewlett-Packard 6720A GC equipped with a FID and an effluent splitter with a 1/200 split ratio was used. Glass capillary columns were used, a 40 m long by 0.25 mm i.d. glass capillary coated with silicone OV-101 and a 7.5 m long by 0.7 mm i.d. micropacked glass capillary packed with 100–120 mesh Gas-Chrom Q coated with Dexsil 300 GC (3% by weight of Gas-Chrom Q).

The computerized GC–MS analyses were carried out on a DuPont 492-1 mass spectrometer interfaced with a Varian Aerograph Model No. 204 (GC) equipped with a 7.5 m long by 0.7 mm i.d. micropacked glass capillary column packed with 100–120 mesh Gas-Chrom Q coated with Dexsil 300 GC (3% by weight of Gas-Chrom Q). The MS data were acquired and processed using a DuPont 21-094 data system.

The infrared (IR) spectra were recorded on a Perkin-Elmer Model 567 spectrometer. The proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian T-60, 60 MHz, using CCl_4 as a solvent and Me_4Si as an internal standard.

Table I. Identification of Mono- and Sesquiterpenes Found in *E. globulus* Fruit Oil

peak no.	compound	identification[a]
1	β-pinene	Ms, t_R, S
3	β-terpinene	Ms, t_R, S
5	γ-terpinene	Ms, t_R, S
6	1,8-cineol	Ms, t_R, IR, ^{1}H NMR, S
7	α-terpinene	Ms, t_R, S
8	linalool oxide	Ms, t_R, S
9	terpinen-4-ol	Ms, t_R, IR, S
10	M^+· 152	Ms, T
11	M^+· 152	Ms, T
12	piperitone	Ms, t_R, S
13	sesquiterpene hydrocarbon (M^+· 204)	Ms, T
14	α-gurjunene	Ms, t_R, S
15	aromadendrene	Ms, t_R, IR, ^{1}H NMR, S
16	alloaromadendrene	Ms, T, P
17	eremophilene	Ms, T, P
18	γ-cadinene	Ms, t_R, IR, S
19	sesquiterpene alcohol (M^+· 222)	Ms, T
20	globulol (10-hydroxyaromadendrane)	Ms, IR, ^{1}H NMR

[a] Ms, mass spectrometry; t_R, gas chromatographic retention time; IR, infrared spectrum; ^{1}H NMR, proton magnetic resonance spectrum; S, standard compound, GLC co-injection; P, published data; T, tentative.

The components were identified from the comparisons of GLC retention time (t_R), mass spectrometric fragmentation (MS), IR and ^{1}H NMR with those of standard compounds or published data (Yukawa and Ito, 1973; Stenhagen et al., 1974).

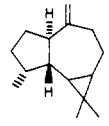
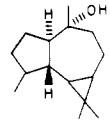
RESULTS AND DISCUSSION

The *E. globulus* fruit oil obtained by the steam distillation was further separated using capillary gas–liquid chromatography (3% Dexsil 300 GC on 100–120 mesh Gas-Chrom Q) as shown in Figure 1. At least 20 peaks were detected on the GLC trace. When GLC liquid phase OV-101 was used, a part of monoterpenes (peaks 3 to 6) could not be separated. For the GLC of terpenoid compounds, particularly triterpenoid (Nielsen et al., 1977), a semipolar liquid phase such as Dexsil 300 GC or OV-17 should be better.

The components identified from GLC co-injection of standard compounds, interpretation of MS, and direct comparisons of MS with that of standard compounds are presented in Table I. The peak numbers correspond to those of Figure 1. Each chemical structure of peaks 16 and 17 was presumed from the comparisons of published MS data (Stenhagen et al., 1974). The most significant components are aromadendrene (peak 15) and peak 20 (mp 86 °C, $[\alpha]_D$ −40.1° in $CHCl_3$), whose MS, IR, and ^{1}H NMR data are shown in Table II. The optical rotation $[\alpha]_D$ and IR spectrum of peak 20 were identical with globulol which has been reported by Dolejš et al. (1960).

Furthermore, terpene fractions from fruits, leaves and branches, and shoots of fresh *E. globulus* were compared.

Table II. MS, IR, and ^{1}H NMR Data of Aromadendrene (Peak 15) and Globulol (Peak 20)

compound	MS (rel intensity)	IR (ν_{max}),[a] cm^{-1}	^{1}H NMR (δ)
 aromadendrene (peak 15)	204 (M^+·, 45.0), 189 (27.9), 175 (8.2), 161 (87.3), 147 (27.1), 133 (56.0), 121 (59.2), 119 (79.9), 107 (80.2), 105 (73.8), 93 (68.5), 91 (51.7), 81 (80.0), 79 (47.5) 69 (100), 67 (72.2), 55 (49.1), 43 (19.0)	3080 (w), 2950 (s), 2860 (s), 1633 (m), 1455 (s), 1375 (m), 1248 (w), 972 (w), 951 (w), 920 (w), 885 (s), 764 (w)	0.45-0.75 (m, 2 H) 0.95 (d, 3 H), 0.98 (s, 3H), 1.04 (s, 3 H), 1.18-2.52 (m, 11 H), 4.43 (m, *exo*-methylene)
 globulol (peak 20)	222 (M^+·, 2.5), 204 (28.3), 189 (33.2), 175 (8.7), 161 (62.7), 147 (36.5), 135 (43.4), 133 (43.0), 121 (48.5), 119 (53.0), 107 (65.3), 105 (56.3), 93 (68.2), 91 (58.1), 81 (75.7), 79 (56.9), 69 (68.9), 67 (62.5), 43 (100)	3380 (s), 2950 (s), 2862 (s), 1456 (s), 1375 (s), 1249 (w), 1110 (w), 983 (m), 886 (m), 755 (w)	0.45-0.70 (m, 2 H), 0.95 (d, 3 H), 0.98 (s, 3 H), 1.03 (s, 3H), 1.04 (s, 3H), 1.18-2.20 (m, 11 H)

[a] Infrared spectra are reported with the size of maxima abbreviated as s meaning strong, m for medium, and w for weak.

Table III. Yields of Various Fractions From Fruits, Leaves, Branches, and Shoots of Fresh *Eucalyptus globulus*[a]

	acetone–ether-soluble components		water and water-soluble components, %[b]	residue %[b]
	volatiles, %[b]	nonvolatiles, %[b]		
fruits	1.0	3.3	49.8	45.9
leaves and branches	1.2	5.1	57.3	36.4
shoots	1.2	4.7	64.9	29.2

[a] Samples were collected on June 16, 1977, on the campus of the University of California at Berkeley. [b] Percent of fresh sample (w/w).

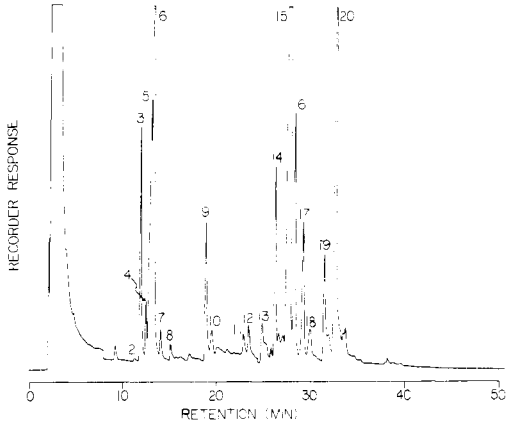


Figure 1. Gas–liquid chromatogram of the essential oil of *E. globulus* fruits. Column: 7.5 m × 0.7 mm i.d. micropacked glass capillary, packed with 3% Dexsil 300 GC on Gas-Chrom Q (100–120 mesh), linearly temperature programmed from 40–250 °C at 4 °C/min, injection port temperature 320 °C, flow rate (He carrier gas) 8 mL/min, H_2 flow rate 20 mL/min, air flow rate 250 mL/min.

The yields of various fractions were summarized in Table III. Although the most significant fractions of all in terms of renewable fuel are volatiles (i.e., mono- and sesquiterpenes; fruits, 1.0% (w/w); leaves and branches, 1.2%; shoots, 1.2%), the nonvolatile fraction which can be dissolved into organic solvent must be important as an energy source to some extent. Gas–liquid chromatograms of volatile fractions from fruits, leaves and branches, and shoots of fresh *E. globulus* were compared in Figure 2. The result indicated that the major components were quite the same except for the difference of the amounts of each

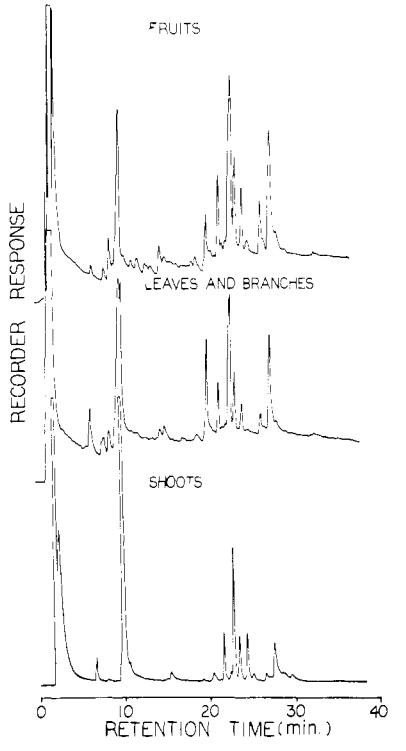


Figure 2. Gas–liquid chromatograms of volatile fractions from fruits, leaves and branches, and shoots of fresh *E. globulus*. Column: 7.5 m × 0.7 mm i.d. micropacked glass capillary, packed with 3% Dexsil 300 GC on Gas-Chrom Q (100–120 mesh), linearly temperature programmed from 80 to 290 °C at 4 °C/min, injection port temperature 320 °C, flow rate (He carrier gas) 8 mL/min, H_2 flow rate 20 mL/min, air flow rate 250 mL/min.

peak. In the case of the *Eucalyptus* fruits, sesquiterpenes are more significant than monoterpenes. On the other hand, in the shoots, monoterpenes such as 1,8-cineol and γ-terpinene are major components rather than sesquiterpenes. Further investigation is necessary to elucidate which species of *Eucalyptus* plants produce the largest amount of oils and how many gallons of hydrocarbon per acre per year are produced.

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Carbon-13 Nuclear Magnetic Resonance Studies of Lipids and Starch Digestion in Intact Seeds

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The carbon-13 pulse Fourier transform nuclear magnetic resonance (NMR) technique for measurement of intact plant tissue has been used for the characterization and estimation of fatty acid composition in seeds of *Leucas cephalotes*, *Stocksia brahuica*, and *Avena fatua*. The carbon-13 NMR analysis of monoenoic and dienoic fatty acid concentrations was compared with destructive gas chromatographic analysis. The presence of laballenic esters in *L. cephalotes* and cyanolipids in *S. brahuica* can be detected by carbon-13 NMR spectroscopy. The isoprenoid hydroxynitrile moiety of the cyanolipid was shown to have the trans configuration. Carbohydrate digestion in the excised endosperm of the postgerminated *A. fatua* was observed by the carbon-13 NMR technique.

Isolation of pure substances through a multistep purification procedure has been a standard method for the characterization and recognition of the presence of certain chemical constituents in a biological system. It is not always certain, however, whether a compound so obtained does truly reflect its structure in an intact biological environment. Until recently there has been a lack of a specific technique for the direct observation and determination of chemical constituents in certain living matter, such as viable plant tissue, despite the fact that such a capability could provide a great deal of information. A fast and nondestructive method of chemical analysis of plant tissue has now become feasible as a result of spectacular advances in pulse Fourier transform NMR (FT NMR) in the past decade.

Compared with proton wide-line NMR, the ^{13}C FT NMR provides both improved sensitivity gain and resolution necessary for the study of heterogeneous material. This relatively new, sophisticated technique has already afforded an efficient analytical method for seed selection in a plant breeding program which requires the nondestructive selection of seeds on the basis of composition (Schaefer and Stejskal, 1974). More recently, the application of ^{13}C NMR analysis to intact plant tissues has been extended to the study of fruit endocarp (Kainosho, 1976), as well as seed coat (Kainosho and Konishi, 1976). High-resolution, natural abundance ^{13}C NMR spectra of many constituents in the plant tissues can be obtained by the Fourier transform technique in a reasonably short time. Sharp NMR spectra are observed from these heterogeneous materials because of local mobility in the cytoplasm and in the intercellular fluid, even though they may be confined in a lignified tissue.

In the light of the rapid developments in this fascinating technique for the study of chemistry *in the gross*, ^{13}C NMR measurements of some seeds which are related to a number of research interests in this laboratory were undertaken. It has been meticulously demonstrated that solid polymers give well-resolved ^{13}C spectra by using cross-polarization experiments with magic angle spinning (Schaefer and Stejskal, 1976). However, there are limitations in routine experiments in that NMR signals of minor constituents or rigidly bound components in a complex biological matrix cannot be elicited without an extensive acquisition of pulsed transients or without some means of modification of the raw materials (Kainosho and Ajisaka, 1978). Nevertheless, highly abundant cellular components could be easily identified by repetitive technique with overnight acquisitions (60K). Thus ^{13}C NMR may be used as a simple and effective method for the direct characterization of fatty acids and sugars in seeds. Studies on seeds of *Leucas cephalotes* (Labiatae), *Stocksia brahuica* (Sapindaceae), and *Avena fatua* (wild oats) are reported herein.

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EXPERIMENTAL SECTION

Detailed experimental procedure for the standard pulse

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