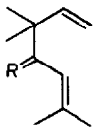
Table 1. Occurrence of mono- and sesqui-terpenes in samples of *A. ageratum* collected in June (% wt)

Locality	agerol	agera-triol	1,8-cineole	1	2	3
Sardinia	0.09	0.30	0.24	trace*	trace*	—
Central Italy	0.10	0.19	trace*	0.032	0.035	0.002

* Detected by GC–MS.

(Table 1) considerable quantities of the non-head-to-tail monoterpenes [5], artemisia ketone (**1**), artemisia acetate (**2**) and its alcohol (**3**), which were separated by a combination of column chromatography and preparative GLC.



1 R = O
2 R = H, AcO
3 R = H, HO

It is of interest to note that the relative amounts of the artemisyl derivatives changed greatly as the young leaves matured, the ratio **2**/**1** continuously rising from about 0.12 in January to about 1.1 in June.

The subsequent finding of trace amounts of **1** and **2** in the plants growing in Sardinia supports the assumption that two chemical races of the *A. ageratum* species have been displayed; they are most likely related to environmental and climatic differences between the two geographic sites.

The occurrence of compounds of the artemisyl group, which have been so far only reported to occur in the *Antemideae* tribe of the Compositae, is not perhaps surprising in *A. ageratum*, a plant which is botanically very close to *Artemisia* species.

EXPERIMENTAL

UV spectra were measured in EtOH. IR spectra were in liquid film. NMR spectra were determined at 60 MHz in CCl_4 for **1** and **2** and in $CDCl_3$ for **3** using TMS as an internal standard. Optical rotations were measured in MeOH. Preparative GLC was accomplished on a 3 m glass column of 20% Apiezon L on chromosorb W. A herbarium voucher of the continental chemotype of *A. ageratum* is deposited in the Botanical Institute of the University of Modena (n. 5891/12).

Extraction and separation of monoterpene components. Fresh flowers (2 kg) and leaves (3 kg) of *A. ageratum*, collected in June in the Emilia region (central Italy), were separately extracted with *n*-hexane (12 and 18 l. resp.) by percolation at room temp. The solvent was evaporated in vacuo to afford a yellow oil (12 g and 22 g resp.). Repeated chromatography over Si gel using a *n*-hexane–Et_2O gradient afforded the isolated monoterpenes. Pure samples were obtained by preparative GLC and distillation.

Artemisia ketone (**1**), (0.52 and 1.04 g)*, bp 181°, λ_{max} 238 nm (ϵ 11.070) [Lit. [6]: λ_{max}^{EtOH} 238 nm (ϵ 11.275)], identified by IR and NMR spectra and by MS fragmentation pattern [6].

Artemisia acetate (**2**), (0.69 and 1.03 g)*, bp 90–92°/10 mm; $[\alpha]_D^{20}$ −29.6° (MeOH; *c* 2.2) [Lit. [7]: $[\alpha]_D^{21}$ −33.9°]; identified by IR, NMR and MS [8].

Artemisia alcohol (**3**), (0.117 g, from both extractions); bp 92–95°/9 mm; $[\alpha]_D^{20}$ −30.1° (MeOH; *c* 2.1) [Lit. [7]: $[\alpha]_D^{20}$ −31.8°]; IR: 3320 cm^{-1}; NMR: δ 1.0 (6H, *s*, Me–C); 1.72 and 1.78 (3H each, *br.s*, Me–C=); 4.1 (1H, *d*, *J* 9 Hz, H–C–O); 5.15 (1H, *br.d*, *J* 9 Hz), H–C=); 4.9–5.4 and 5.7–6.3 δ (3H, *m* (a typical ABC pattern), $CH{=}CH_2$); MS: *m/e* 154 (M^+). $LiAlH_4$ treatment transformed **2** to **3** and $NaBH_4$ converted **1** to racemic **3**.

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* The first value represents yield from flowers and the second one that from leaves.

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QUANTITATIVE SEPARATION OF 1:8 CINEOLE AND β-PHELLANDRENE IN EUCALYPTUS LEAF EXTRACTS

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Key Word Index—*Eucalyptus pauciflora*; terpenes; monoterpenes; essential oils; quantitative separation; 1:8 cineole; β-phellandrene.

Abstract—A method is described for separating 1:8-cineole and β-phellandrene in extracts of eucalyptus leaves on a silica gel column by elution with selective solvents and quantitative analysis of the separates by gas–liquid chromatography.

INTRODUCTION

In analyzing monoterpene composition of pentane extracts of leaves of several species of eucalyptus by gas–liquid chromatography (GLC) with a carbowax column, Shimizu [1] noted that β-phellandrene and 1:8-cineole had identical retention times. Hence, he reported peaks

obtained for these as "β-phellandrene and/or 1:8-cineole." von Rudloff [2] found that 1:8-cineole can be separated from terpene hydrocarbons on highly polar GLC columns, such as β,β-oxydipropionitrile. The procedure presented herein employs prefractionation on a silica gel column, followed by GLC with a carbowax column, which has some advantages over highly polar columns when analyzing monoterpenes. The method may be especially valuable where 1:8-cineole is of commercial importance.

RESULTS AND DISCUSSION

We first verified that β-phellandrene and 1:8-cineole could be separated by elution chromatography on a silica gel column. Samples of the two compounds were run through the column individually and the eluates were analyzed by GLC. β-Phellandrene was eluted by petrol, while 1:8-cineole was eluted by ethyl ether–petrol (1:9).

GLC of the pentane extract of *Eucalyptus pauciflora* Sieb. ex Spreng. leaves revealed 0.19 μl β-phellandrene and/or 1:8-cineole. To determine the relative amounts of the two monoterpenes, a sample of the extract was separated on the silica gel column. Using *p*-cymene as a standard, we estimated by GLC that the first eluate contained 0.06 μl β-phellandrene and the second 0.11 μl 1:8-cineole. The sum of these, 0.17 μl, was less than the total occurring in the original sample (0.19 μl), indicating a moderate loss in the silica gel chromatographic process. Additional trials with standards likewise showed small losses, but the relative amounts of β-phellandrene and 1:8-cineole recovered in the eluates were approximately the same as in the original sample.

For approximate analyses the procedure can be simplified to (1) running a GLC analysis of the original sample and (2) eluting with petrol from a silica gel column and examining the eluate by GLC. If the samples contain monoterpene hydrocarbons other than β-phellandrene, these can be used to adjust peak sizes for effects of fluctuating operating conditions, eliminating the need for internal standards. The procedure is exemplified below on the peak area data for an original sample and the petrol eluate.

1. Area of combined β-phellandrene-1:8-cineole peak 12.8
2. Total area of all other monoterpene hydrocarbon peaks in original sample (32.7 + 0.9 + 1.0) 34.6
3. Area of β-phellandrene peak in petrol eluate 4.8
4. Total area of all other monoterpene hydrocarbon peaks in petrol eluate (36.8 + 1.2 + 1.4) 39.4
5. Adjusted area of β-phellandrene peak in petrol eluate = 34.6(4.8)/39.4 4.2
6. Area of combined peak (12.8) is then judged to be composed of β-phellandrene (4.2) and 1:8-cineole (12.8 − 4.2 = 8.6). From these peak areas and areas of other peaks in the original sample, percentage composition is determined in the usual manner as tabulated below. The percentage composition obtained here is approximately the same as that obtained when a standard was employed.

	Area	*Percent*
α-pinene	32.7	44
β-pinene	0.9	1
Limonene	1.0	1
β-phellandrene	4.2	6
1:8-cineole	8.6	12
Unknown	27.3	36
	74.7	100

Using the simplified procedure outlined, we sampled five *E. camaldulensis* Dehnh. trees from families studied by Shimizu [1]. The relative amounts of β-phellandrene and 1:8-cineole varied greatly (Table 1).

The observation that β-phellandrene and 1:8-cineole have identical retention times under our GLC procedures may raise questions as to the reliability of previous analyses of pine oleoresin where β-phellandrene was indicated as a major component. We reanalyzed cortical oleoresin of two *Pinus elliottii* Engelm. trees previously reported as having high β-phellandrene by the methods outlined here and found no 1:8-cineole [3].

EXPERIMENTAL

Sample collection. Eucalyptus leaf samples were prepared by cutting the leaves into 1/8″ strips and filling a 20 ml vial. *n*-Pentane was added to cover the leaf material; the vial was then capped and extraction continued for 24 hr at room temperature (24°). The liquid sample was then decanted and concentrated by evaporation [4].

GLC and elution chromatography. The Si gel column was prepared by pouring 2 g of Si gel (100–200 mesh) into a glass pipette (approx 3/8″ o.d., 6″ long) with a small amount of glass wool below and above the Si gel. (In a personal communication, E. von Rudloff recommended use of PEG deactivated silica as described by Kugler [5]). The column was then saturated with *n*-pentane. The leaf extract (2 μl) was combined with 25 μl of 2.5% paracymene standard in *n*-pentane and analyzed by GLC. The sample was then separated on the Si gel column. The monoterpene hydrocarbons were eluted from the column with 2 ml petrol and 1:8-cineole was eluted with 2 ml of a mixture of ethyl ether and petrol (1:9). 2.5% *p*-Cymene standard (25 μl) was added to the latter eluate because the *p*-cymene in the original sample had eluted in the petrol fraction. The two eluates were then analyzed by GLC. The gas

Table 1. Monoterpene composition (percent) of pentane extracts of leaves of 5 *Eucalyptus camaldulensis* trees

Tree No.	α-pinene	β-pinene	Myrcene	α-phellandrene	Limonene	β-phellandrene	1:8-Cineole	γ-terpinene	*p*-cymene
9–72	5	3	2	20	0	49	0	0	21
61–72	2	2	1	10	3	26	37	10	9
343–74	9	0	0	0	4	0	70	10	7
345–74*	0	0	0	0	1	0	69	16	14
354–74*	7	3	2	18	3	55	0	4	8

* Species identity uncertain.

chromatograph was equipped with a 4.8 × 610 mm copper column packed with Carbowax 20M on a Chromosorb W, 60–80 mesh support and a FID and operated at 120°. All steps were repeated on triplicate samples and resulting data were averaged.

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IDENTIFICATION OF VOLATILE CONSTITUENTS OF *SASSAFRAS ALBIDUM* ROOT OIL*

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Key Word Index—*Sassafras albidum*; Lauraceae; root oil; monoterpenes; sesquiterpenes; allylbenzenes; propenylbenzenes; aromatic aldehydes.

Abstract—Sassafras root bark oil was examined by GC–MS for the possible natural occurrence of 1′-hydroxysafrole, a potent hepatocarcinogenic mammalian metabolite of safrole. Six monoterpenes, 2 sesquiterpenes, 6 allylbenzenes, 2 propenylbenzenes, 2 acroleins and 1 benzaldehyde derivative were identified. Eleven out of these 19 sassafras constituents are reported for the first time. However, 1′-hydroxysafrole was not detected.

INTRODUCTION

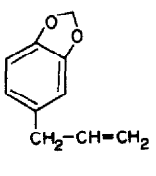
Safrole (**1**) is the major constituent (about 80%) of sassafras oil obtained from the root bark of *Sassafras albidum* (Nutall) Nees (Lauraceae) by steam distillation. Prior to 1960, both sassafras oil and safrole were used extensively as flavors in confections, soft drinks such as root beer, and in pharmaceutical preparation. In 1960, the United States Food and Drug Administration banned the use of safrole as a food additive when it was found to be hepatocarcinogenic in the rat [1–4].

Recent metabolism studies showed that 1′-hydroxysafrole (**2**) was a proximate carcinogenic metabolite of safrole in several experimental animals [5]. The finding that the hydroxy metabolite (**2**) was a more potent hepatocarcinogen than safrole [6] prompted the present investigation of sassafras root bark for the possible natural occurrence of (**2**) and related compounds. A detailed phytochemical study of this plant material was also of interest in our current program of isolation, structure elucidation and bioassay of potentially carcinogenic natural products [7–10].

RESULTS AND DISCUSSION

Table 1 lists the nineteen compounds identified by GC–MS in the sassafras root bark oil fractions A and B. These include six monoterpenes (**3–8**), two sesquiterpenes (**10** and **11**), six allylbenzenes (**1**, **12**, **13**, **15**, **17**, and **20**), two propenylbenzenes (**9** and **16**), two acrolein derivatives (**14** and **18**), and one benzaldehyde derivative (**19**). Among the nineteen compounds reported here, compounds **1**, **3–6**, **12**, **14**, and **18** were identified earlier in the sassafras root [20,23,24].

Safrole (**1**) was the major volatile constituent (about 90%) in oil A, while oil B contained 5-methoxyeugenol (**17**, 30%), asaron (**16**, 18%), piperonylacrolein (**18**, 11%), coniferaldehyde (**14**, 7%), safrole (**1**, 6%), and camphor



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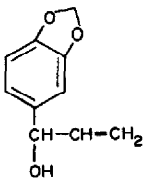

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* Part 5 in the series "Potential carcinogens"; for Part 4 see ref. [10].

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