column [34, 35] using an internal standard (*n*-tetracosane) after methylation [36] and then silylation with BSA–C_5H_5N [25]. For the estolide waxes (*Picea sitchensis, P. pungens, Chamaecyparis lawsoniana*) determinations were made using the total hydrolysis products (3% methanolic KOH, 3 hr, acidification, Et_2O work up). Positional isomer contents of the various alcohols were obtained by measurement of the TMSi ions [25] from MS recorded at the apices of the corresponding TMSi ether chromatographic peaks. Before this determination the sec. alcohol fractions were first isolated from the whole waxes by PLC [33].

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MONOTERPENE VARIATION IN TWO *ACHILLEA AGERATUM* CHEMOTYPES

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Key Word Index—*Achillea ageratum*; Compositae; irregular monoterpenes; non-head-to-tail terpenes; artemisia ketone; artemisia acetate.

Abstract—Artemisia ketone and artemisia acetate are the main monoterpene components in both the flowers and leaves of *A. ageratum* growing in central Italy, but are replaced by 1,8-cineole in plants growing in Sardinia (Italy).

The flowers and leaves of *Achillea ageratum* growing in northern Sardinia contain the sesquiterpenes agerol [1] and ageratriol [2, 3], whose structures and biosynthesis [4] have already been investigated, and 1,8-cineole, as the virtually only isoleable monoterpene.

The present work is concerned with the terpene content of a sample of *A. ageratum* which grows in the central region of Italy (Emilia) and shows some minor morphological differences. The leaves are more narrow and serrated and the terminal corymbs made by smaller and more dense flowerheads.

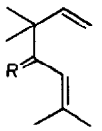
The plants, collected in June, contained agerol, ageratriol and only very small amounts of 1,8-cineole. Additionally this continental sample was found to contain

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