been observed by the authors. *Previous work.* Benzyl isothiocyanate (BITC) was first identified in the seeds of *Carica papaya* L. [2], and later from other parts of the plant [3]. Gmelin and Kjaer [4] reported that BITC was the only isothiocyanate found in *Carica* and *Jarilla*, suggesting that this compound could be characteristic of the Caricaceae. The concentrations of BITC were examined in macerated seeds of six species of *Carica*, one in *Jarilla* and three in *Jacaratia* [5]. Both *Carica* and *Jarilla* contain high levels of BITC ranging from 1.37 to 1.96% in the macerated embryo and endosperm. However, only 2–4 ppm was found in all three *Jacaratia* species. The striking quantitative differences suggest the possible use of BITC content as a chemotaxonomic criterion.

Present work. Quantitative determination of BITC in seeds of *C. solmsii* was performed according to our previous publication [5], except that BITC in the sample was further confirmed by GC–MS. A Finnigan 3000 Peak Identifier interphased with Varian 1400 Gas Chromatograph was used and the mass spectrum obtained from the sample was identical to that of authentic BITC. Content of BITC was 1.29% of the fr. wt of the embryo and endosperm.

The Caricaceae contains only four genera; we have now surveyed all the genera in this family. *Carica*, *Jarilla* and *Cyclicomorpha* have high levels of BITC in the macerated seeds, but *Jacaratia* has only trace amounts. BITC also appears to be the only isothiocyanate in Caricaceae [4].

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STRUCTURAL DETERMINATION OF SECONDARY ALCOHOLS FROM PLANT EPICUTICULAR WAXES*

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Key Word Index—Angiosperms; gymnosperms; epicuticular waxes; secondary alcohols; TMSi ethers; GC–MS; structural determination; ultrastructure.

In the course of our investigations on the possible relationship between the ultrastructure and chemical composition of plant waxes [1, 2], we needed a rapid method for identifying secondary alcohol constituents. Although the presence of these components in waxes can readily be established by preliminary TLC (Si gel G, C_6H_6, R_f 0.32) and GLC on packed columns used to determine their homologue content, these methods give no information on the position of the OH group. In the past this information has been obtained from purely physical methods [3–6,7 and refs. cited therein] or from chemical degradation after conversion to the corresponding ketone [8–11] but more recently MS of either the free alcohol [12–16] or derived ketone [13, 17–20] has been used. A quantitative assessment of positional isomers is possible from the MS of the ketone [19, 20]. Since our main requirement was for a direct GC–MS method, most of these techniques were not applicable. The free alcohols can be analysed directly by GLC but their MS are complicated by the presence of several fragment ions derived from cleavage of the OH group [21] making reliable quantitative assessment of positional isomers difficult.

In this paper we report the use of TMSi ether derivatives for the facile and unambiguous GC–MS determination of long chain secondary alcohols which occur in plant waxes. Such derivatives have already been employed for locating mid-chain OH groups in other classes of lipid [22–26] and a detailed MS study has recently been published using a series of synthetic secondary alcohol TMSi ethers [21].

The MS of wax secondary alcohol TMSi ethers show the expected ions viz m/e 73 > 75 > 103 > 129 > 89 and at the upper end a weak M^+ (*ca* 1–2% rel. intensity) and a more intense M^+-15 (*ca* 5% rel. intensity) are observed. In the middle of the spectrum only prominent ions corresponding with cleavage α to the TMSi group occur and these fragments thus enable the position of the OH group and isomer content of the original alcohol to be determined (see footnote Table 1). In asymmetrical alcohols the TMSi-containing fragment derived from the shorter chain end is stronger than that from the longer chain end and is the base peak of the spectrum. The spectra are markedly different from those given by primary alcohol TMSi ethers which have the same MW but show an intense M^+-15 (>50% rel. intensity) and ions m/e 75 > 73. In addition silylation produces a marked improvement in peak shape and resolution by GLC compared with the corresponding free alcohols.

The secondary alcohol contents and compositions of

* For the previous paper in this series see Ref. [2].

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Table 1. Secondary alcohol content and composition of some plant epicuticular waxes

Species	% total wax	Homologue and isomer content (%)				
		C_{27}*	C_{28}	C_{29}*	C_{30}	C_{31}*
Brassica oleracea var. *gemmifera*	12.5	0.5	0.9	13-ol 0.9 14-ol 36.3 15-ol 60.1	0.4	0.9
Pisum sativum	5.5	1.0	nd	13-ol 0.1 14-ol 2.0 15-ol 3.1	1.0	14-ol 2.4 15-ol 37.1 16-ol 51.8
Clarkia elegans	3.0	tr	nd	13-ol 1.0 14-ol 16.0 15-ol 83.0	tr	nd
Papaver somniferum	65.7	10-ol 0.6	tr	10-ol 99.4	nd	tr
Chelidonium majus	66.0	10-ol 2.7	tr	10-ol 95.3	tr	2.0
Exochorda racemosa	30.1	7-ol 0.1 8-ol 0.4 9-ol 0.4 10-ol 0.2	8-ol tr 9-ol 0.2 10-ol 0.1	9-ol 2.3 10-ol 93.0 11-ol 1.0	0.4	9-ol tr 10-ol 1.0 11-ol 0.9
Prunus domestica	48.3	7-ol 0.1 8-ol 0.8 9-ol 0.5 10-ol 0.3	0.5	9-ol 2.9 10-ol 92.7 11-ol 1.0	0.6	0.6
Aquilegia alpinum	57.0	0.7	tr	10-ol 98.3	tr	tr
Rhus cotinus atropurpurea	38.3	0.4	0.8	9-ol 2.4 10-ol 93.2 11-ol 2.8	0.4	tr
Tropaeolum majus	47.5	8-ol 0.2 9-ol 0.5 10-ol 0.3	0.5	9-ol 3.0 10-ol 92.5 11-ol 3.0	tr	tr
Tulipa gesneriana	37.8	2.7	1.0	9-ol 2.9 10-ol 92.4 11-ol 1.0	nd	tr
Picea sitchensis	19.7	1.0	nd	10-ol 98.0	nd	1.0
Picea pungens	10.0	0.6	tr	10-ol 98.8	tr	0.6
Chamaecyparis lawsoniana	28.6	tr	nd	10-ol 100	nd	tr
Agathis australis	27.8	0.8	tr	10-ol 98.0	tr	1.2
Ginkgo biloba	48.9	10-ol 1.1	tr	10-ol 98.9	tr	tr

* Key fragment ions: C_{27} sec. alcohol TMSi ether (*m/e*) M^+-15 (453), 7-ol (187 + 383), 8-ol (201 + 369), 9-ol (215 + 355), 10-ol (229 + 341); C_{29} sec. alcohol TMSi ether (*m/e*) M^+-15 (481), 9-ol (215 + 383), 10-ol (229 + 369), 11-ol (243 + 355), 13-ol (271 + 327), 14-ol (285 + 313), 15-ol (299); C_{31} sec. alcohol TMSi ether (*m/e*) M^+-15 (509), 9-ol (215 + 411), 10-ol (229 + 397), 11-ol (243 + 383), 14-ol (285 + 341), 15-ol (299 + 327), 16-ol (313).

a number of plant epicuticular waxes are shown in Table 1. These waxes were originally selected because they exhibited a well defined crystalline ultrastructure in their natural state on the plant surface [2]. In all of the waxes examined the secondary alcohol fraction comprised one major homologue (C_{29}, C_{31} only in *Pisum*) and 3 types of hydroxylation were found: (a) asymmetrical (e.g. C_{29}-10-ol) with no other isomers detectable, found in *Papaver*, *Chelidonium*, *Aquilegia* and gymmosperms; (b) asymmetrical (e.g. C_{29}-10-ol) with small amounts of other isomers, found in *Exochorda*, *Rhus*, *Tropaeolum*, *Tulipa* and *Prunus*; (c) mainly symmetrical (e.g. C_{29}-15-ol) with substantial proportions of other isomers, found in *Brassica*, *Pisum* and *Clarkia*. In waxes from *Papaver*, *Chelidonium*, *Prunus*, *Aquilegia* and *Tropaeolum* secondary alcohols were the major lipid class; this type of wax has not been previously recognized. We have confirmed using the TMSi method the structural assignments already made for the secondary alcohols of *Brassica* [4, 11, 19, 20], *Pisum* [10, 13], *Chelidonium* [8], *Prunus* [14] and the gymmosperms [7] but the occurrence of secondary alcohols in the other species (Table 1) has not been reported before.

In the plants studied there was a definite correlation between the presence of nonacosan-10-ol and the occurrence of small crystalline wax tubes on the plant surface. This finding provides another example of the close association between particular compounds or classes of compound and specific ultrastructural forms of epicuticular waxes. Similar relationships have previously been demonstrated between plate-type structures and primary alcohols [27, 28] and between long thin tubes and β-diketones [28–32]. All the ultrastructural aspects of this work and further confirmation by recrystallization of secondary alcohols in a model system will be dealt with in another publication.

EXPERIMENTAL

Epicuticular waxes were isolated using brief immersion in $CHCl_3$ from mature leaves of the plants listed (Table 1), except that from *Prunus domestica* (cv. Yellow Egg) which was obtained from ripe fruits. Chromatographic analysis was carried out as described in [33] and GC–MS on a 1 m × 2 mm i.d. stainless steel column packed with 1% Dexsil 300 using the operating conditions of [25]. The amounts of sec. alcohols were determined by GLC of the total wax on a Dexsil 300

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