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COMPOSITION OF ESSENTIAL OIL FROM LEAVES OF *EUCALYPTUS DELEGATENSIS*

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Key Word Index—*Eucalyptus delegatensis*; Myrtaceae; alpine ash; essential oil; *cis*- and *trans-p*-2-menthen-1-ol; *trans*-piperitol; monoterpenoids; sesquiterpenoids; phenylpropanoids; peppermint aroma; chemotaxonomy

Abstract—The major components of a dichloromethane extract of mature *Eucalyptus delegatensis* leaves were *cis*- and *trans-p*-2-menthen-1-ol, *trans*-piperitol, α- and β-eudesmol, 4-phenyl-2-butanone and methyl cinnamate. The major terpenoid was considered to be *trans*-piperitol, which was responsible for the peppermint aroma of freshly crushed leaves. The steam distillate of the same leaves contained the above compounds together with a number of monoterpene hydrocarbons which were considered to be artefacts. Use of these hydrocarbons as chemotaxonomic markers was considered to be erroneous.

INTRODUCTION

Boland *et al.* [1] recently published their work on the composition of the essential oil from leaves of seedling specimens of *Eucalyptus delegatensis.* We have concluded a similar study of the oil from leaves of a mature specimen of the same species and the results are reported herein.

E. delegatensis R. T. Baker (syn. *E. gigantea* Hook. f.) [2], commonly known as the alpine ash, is a commercially important timber species in Australia. It is a large tree which usually attains 60 m in height, often 90 m. It grows vigorously and is resistant to cold temperatures. As a result of these properties, it is one of a small number of *Eucalyptus* species, mainly of the ash group [3], which were selected by the N.Z. Forest Service for genetic improvement programmes, in order to determine their suitability as a source of hardwood for the South Island of New Zealand and the coldest parts of the North Island [4, 5].

When crushed, the leaves of *E. delegatensis* emit a pleasant peppermint aroma, but this species has never been used as a commercial source of essential oil for the manufacture of synthetic peppermint flavours. Essential oils for that purpose [2, 6] are obtained from *E. radiata* and especially *E. dives*, both of which are members of the peppermint group of Eucalypts [7]. Both these species afford a good yield of an essential oil, some 50 % of which is pipertone. Besides *E. delegatensis*, other *Eucalyptus* species in the ash group also contain oils which are noted for their peppermint aroma, particularly *E. piperita* and *E. andrewsii*.

RESULTS AND DISCUSSION

The yield of the essential oil steam-distilled from the leaves of a mature specimen of *E. delegatensis* (3.9 %) was significantly greater than that obtained from seedling leaves (0.01–0.1 %). Distillation of the leaves of *E. delegatensis* in February (flowering time, summer) and in August (winter) afforded oils in the same yield which had virtually the same optical rotation, refractive index and chemical composition.

Lassak's group investigated the composition of the essential oil which was steam-distilled from the leaves of seedling specimens of *E. delegatensis* [1]. The mean values of their results are listed in column A of Table 1. Column B lists the composition of the essential oil which we obtained by steam-distillation of the leaves of a mature specimen of *E. delegatensis.* The results are the average of two determinations; one carried out on a polar GC column and the other on a non-polar column. Column C lists the composition of the oil which we obtained by extracting the leaves of a mature specimen of *E. delegatensis* with dichloromethane. Column D lists the means by which the components of the oils were identified.

Comparison of the composition of the oils from seedling and adult leaves showed only one major difference, viz. the much greater proportion of *cis*- and *trans-p*-2-menthen-1-ol in the oil from adult leaves. These two isomers, together with piperitol and their dehydration products α- and β-phellandrene, constituted more than half of the weight of the essential oil.

The mass spectral data of the stable *cis-p*-2-menthen-1-ol is listed in Table 2. This alcohol has the same MW and R_t on OV-101 as citronellal, but the two compounds are well separated on a CW-20M column.

The isomeric *trans-p*-menthen-1-ol, which contains an axial hydroxyl group, was unstable and on standing the neat oil partially rearranged to *trans*-piperitol and *p*-1-menthen-4-ol, presumably by way of a non-concerted carbonium ion rearrangement. It also dehydrated readily to α-phellandrene, a property [10] shared with *cis*-piperitol, which also contains an axial hydroxyl group. *Cis*- and *trans-p*-2-menthen-1-ol are not commonly occurring natural products. Lassak suggested that their instability is the reason why they have not been detected more frequently [11].

Comparison of the composition of the oil steam-distilled from the leaves of a mature specimen of *E. delegatensis* with that of the dichloromethane extract

Table 1. Composition (per cent) of essential oils of *E. delegatensis*

	A	B	C	D
3-Methylbutanal	1.2	—	—	—
α-Pinene	1.0	0.3	—	1, 2, 5
α-Thujene	—	2.7	—	1, 2, 3, 5
β-Pinene	0.3	0.1	—	1, 2, 5
Sabinene	—	0.2	—	1, 2, 5
Myrcene	0.9	1.2	—	1, 2, 5
α-Phellandrene	14.3	17.8	0.2	1, 2, 3, 4, 5
α-Terpinene	2.3	1.6	1.1	1, 2, 3, 5
Limonene	0.7	0.4	—	1, 2, 5
1,8-Cineole	0.1	1.7	1.0	1, 2, 3, 5
β-Phellandrene	8.9	5.2	—	1, 2, 3, 4, 5
trans-Ocimene	—	0.4	—	1, 2, 5
γ-Terpinene	3.6	0.3	—	1, 2, 5
p-Cymene	4.4	3.8	0.5	1, 2, 3, 5
Terpinolene	1.9	1.5	—	1, 2, 3, 5
Linalool	0.2	0.7	0.3	1, 2, 3, 5
Citronellal	0.1	—	—	—
trans-p-Menthen-1-ol	1.4	13.8	11.2	3, 4, 5
1-Terpinen-4-ol	1.7	4.2	3.4	1, 2, 3, 5
cis-p-2-Menthen-1-ol	1.5	10.1	10.6	3, 4, 5
β-Caryophyllene	0.9	—	—	—
cis-Piperitol	1.2	0.2	—	5
$C_{10}H_{18}O$	0.5	3.3	2.8	3
α-Terpineol	1.1	1.6	1.0	1, 2, 3, 5
α-Terpinyl acetate	0.3	—	—	—
Piperitone	1.4	0.1	0.2	1, 2, 5
trans-Piperitol	7.6	5.9	15.4	3, 4, 5
Aromadendrene	1.4	—	—	—
Cuminal	1.0	—	—	—
4-Phenyl-2-butanone	4.6	6.2	4.9	1, 2, 3, 4, 5
Unidentified	3.5	—	—	—
Unidentified	1.8	—	—	—
Methyl cinnamate	8.5	8.8	17.0	1, 2, 3, 4, 5
Unidentified	1.2	1.4	—	—
$C_{15}H_{26}O$	1.3	0.6	0.4	3
$C_{15}H_{24}O$	4.2	1.3	2.3	3
γ-Eudesmol	0.3	—	—	—
α-Eudesmol	1.0	1.2	4.9	3, 5
β-Eudesmol	0.6	2.6	10.6	3, 5
Unidentified	0.7	1.0	2.0	—

A = Seedling leaves—steam distillate, mean values [1]; B = mature leaves—steam distillate; C = mature leaves—dichloromethane extract; D = method of identification of components. (1) R_t on OV-101 [8, 9], (2) R_t on Carbowax 20M [8, 9], (3) mass spectrum (GC/MS), (4) ^1H NMR and (5) co-injection with authentic material.

Table 2. Mass spectral data (GC/MS) of *cis-p*-2-menthen-1-ol (at 70 eV) (significant ions > 30% of the base peak)

m/z	% Relative intensity	Assignment
41	35.5	$[C_3H_5]^+$
43	100.0	$[C_3H_7]^+$
69	41.0	$[C_5H_9]^+$
93	83.7	$[M-(C_3H_7+H_2O)]^+$
111	34.9	$[M-C_3H_7]^+$
121	36.4	$[M-(H_2O+Me)]^+$
136	38.5	$[M-H_2O]^{+\cdot}$
139	70.3	$[M-Me]^+$
154	17.7	$M^{+\cdot}$

showed some significant differences. The most obvious was the almost total absence of monoterpene hydrocarbons from the solvent extract. Steam-distillation is known to promote the dehydration of labile alcohols such as piperitol and 2-menthen-1-ol [12, 13]. As a result, the monoterpene hydrocarbons in the steam-distillate were probably artefacts.

Compared with those in the steam-distillate, the major constituents of the leaves which were less volatile than the monoterpenoids, viz. methyl cinnamate and the sesquiterpenoids α- and β-eudesmol, were more abundant in the solvent extract as expected. These three products constituted one-third of the total solvent extract.

All the other products in the solvent extract occurred in proportions which were comparable with those of the same compounds in the steam-distillate, except for *trans*-piperitol which was the dominant terpenoid in the solvent extract. The maximum concentration of *trans*-piperitol recorded in the steam-distillates of seedling leaves of *E. delegatensis* exceeded those of all the other volatile products except for that of α-phellandrene [1]. These data suggested that the major terpenoid in the leaves was *trans*-piperitol.

Because the two *p*-2-menthen-1-ol isomers are related to piperitol by a simple rearrangement, they might also be expected to occur together with piperitol in the oil glands of the leaves. These three terpenoids constituted more than one-third of the total weight of the solvent extract. The preponderance of piperitol in the oil glands is undoubtedly responsible for the peppermint-like aroma of freshly crushed leaves of *E. delegatensis*.

The alpine ash is taxonomically classified in a series [3] of the subgenus *Monocalyptus* of *Eucalyptus* along with species, the oils of which were examined by Lassak and Southwell [11]. The essential oils of all the species within this series, which have so far been examined, contain *cis*- and *trans-p*-2-menthen-1-ol at relatively high concentrations. Whereas the oil of *E. delegatensis* has only a moderate proportion of *p*-cymene, the oils of all the other species have large amounts. On the other hand, the oils of three of the species examined by Lassak contain piperitone at low concentrations while the oils of the other six species contain large proportions of piperitone.

E. stenostoma, like *E. delegatensis*, is one of the species, the oil of which has a low piperitone content. However, *E. stenostoma* has been placed in the *sieberi* group of this series [3] while *E. delegatensis* has been placed in the *obliqua* group of the series along with the other species, the oils of which have a high *p*-cymene content.

Chemotaxonomic arguments for the placement of a species within these sub-series therefore depend on which terpenoid is chosen as a genetic marker. Banthorpe *et al.* believe that the major components of an essential oil are the most appropriate compounds to use as taxonomic characters [14] since they are probably at the end of a biogenetic pathway in that species.

The monoterpene hydrocarbons in the essential oil of *E. delegatensis* were considered above to be artefacts and, furthermore, since *p*-cymene is a product of the aerial oxidation of *p*-menthadienes [15], then *p*-cymene should not be used as a phylogenetic marker. Piperitone might possibly be a better product to use in these cases, since it is unchanged during steam-distillation and is probably the end-product of menthane biogenesis in these Eucalypts. Alternatively, a more accurate placement of the species might be obtained by examining the major volatile compounds in a solvent extract.

Since *p*-2-menthen-1-ol dehydrates readily to α-phellandrene and since the latter product is a major constituent of the steam-distillates of a large number of *Eucalyptus* species [2], then examination of the solvent extracts of the leaves of these species might reveal that *p*-2-menthen-1-ol is an important constituent of the *Eucalyptus* genus in general.

EXPERIMENTAL

Isolation of essential oil. Fresh leaves (100 g) were picked in February and cut into pieces 3–5 cm long. The essential oil was then isolated by simultaneous cohobation in H_2O (800 ml) and extraction into Et_2O (20 ml) over a period of 4 hr in a Likens–Nickerson apparatus. The colourless oil (yield 3.9 %) had $[\alpha]^{20}_{D}$ −53° (*c* 0.47; $CHCl_3$) and n^{20}_{D} 1 4821. Leaves picked in August and extracted likewise into CH_2Cl_2 afforded an oil, the yield, physical constants and composition of which were virtually the same as those above.

Solvent extraction of leaves. Whole leaves (35 g), freshly picked in August, were shaken for 10 hr in CH_2Cl_2(230 ml) and then allowed to stand at ambient temp for 2 days. Insoluble and precipitated material was then filtered off and the solvent evapd. The residue was dissolved in EtOH (99 %, 100 ml) and the mixture was left to stand at 0–5° for 2 days. Precipitated waxes were filtered off and the solvent was evapd to leave a green oil (2.35 g, 6.6 %).

Preparation of cis- *and* trans-*piperitol.* A (1 : 2) mixture of these two alcohols was prepared by the reduction of piperitone with $NaBH_4$.

Preparation of cis- *and* trans-p-2-*menthen*-1-*ol* These two alcohols were prepared by the method of ref. [16].

Analytical GC was performed on an FID instrument using H_2 as carrier gas, with a split ratio of 75 : 1. Two columns were used for this work. (i) A 55 m × 0.5 mm i.d. SGE soda glass SCOT column with OV-101 as the stationary phase, $N_{\text{eff}} = 51\,300$, linear gas flow rate 30 cm/sec. Injector temp. 250°, detector temp. 320°, initial oven temp. 70°, final oven temp. 250°. The oven temp. was held for 5 min before and after programming at a rate of 4° per min. Injections of 1 μl of a soln of the essential oil in Et_2O (10 mg/ml) were used. (ii) A 50 m × 0.2 mm i.d. SGE vitreous silica WCOT column with Carbowax CW-20M as stationary phase, $N_{\text{eff}} = 123\,900$, linear gas flow rate 35 cm/sec. Injector temp. 250°, detector temp. 240°, initial oven temp 50°, final oven temp 200°. The oven temp. was held for 5 min before and after programming at a rate of 4° per min. Injections were as for (i).

R_I values were obtained by adding together equal vols. of (a) a soln of the essential oil in Et_2O (10 mg/ml) and (b) a soln of each *n*-alkane (30 mg) from hexane to eicosane in pentane (25 ml) and chromatography of 1 μl of the combined mixture of (a) and (b) as in both (i) and (ii).

Identification of individual components of the essential oil was carried out by addition of 2–5 mg of the pure compounds, obtained either from above or from commercial sources, to the soln of the essential oil in Et_2O (10 mg/ml), and chromatography as in (i) and (ii). Peak enhancement on both columns was then regarded as confirmation of the identity of the components.

Preparative GC. Chromatography was performed on a TCD instrument and He was used as carrier gas at a flow rate of 20 ml/min. A glass column 2 m × 4 mm i.d. packed with 5 % OV-101 on AWS Gas Chrom Q was employed to effect separation of the components from a 100 μl injection of a soln of the essential oil in Et_2O (20 mg/ml). Injector temp. 225°, detector temp. 265°, initial oven temp. 70°, final oven temp. 250°. The oven temp. was held for 5 min before and after programming at a rate of 2° per min. Fractions were collected in open (hard) glass capillary tubes 10 cm × 1 mm i.d. which were cooled with solid CO_2. $CDCl_3$ (25 μl) was then added to the tubes and the ends of the tubes were sealed

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