Pergamon

PII: S0031–9422(97)00203–3

Phytochemistry, Vol. 45, No. 8, pp. 1605–1612, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0031–9422/97 $17.00 + 0.00

ESSENTIAL OILS FROM NEW ZEALAND MANUKA AND KANUKA: CHEMOTAXONOMY OF *KUNZEA*

NIGEL B. PERRY,* JOHN W. VAN KLINK, NERIDA J. BRENNAN, WARWICK HARRIS,† ROSEMARY E. ANDERSON,‡ MALCOLM H. DOUGLAS‡ and BRUCE M. SMALLFIELD‡

Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research Ltd, Department of Chemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand; †Manaaki Whenua, Landcare Research New Zealand Ltd, P.O. Box 69, Lincoln, New Zealand; ‡New Zealand Institute for Crop & Food Research Ltd., Invermay Agricultural Research Centre, Private Bag 50034, Mosgiel, New Zealand

(*Received in revised form* 20 *January* 1997)

Key Word Index—*Kunzea ericoides*; *K. flavescens*; *K. pauciflora*; *K. sinclairii*; *Leptospermum*; Myrtaceae; kanuka; chemotaxonomy; chemotypes; monoterpenes.

Abstract—A standardized analytical GC method has been used to analyse essential oils from selected Australian and New Zealand *Kunzea* species, grown from seed at a single site. The distillation yields and analyses are reported for oils from 26 populations of *K. ericoides* (kanuka) and from single populations of each of *K. flavescens*, *K. pauciflora*, *K. sinclairii* and × *Kunzspermum hirakimata* (a *Kunzea* × *Leptospermum* cross). Principal components analyses of 37 GC peaks in these oils were used to distinguish compositional patterns. Oils from *K. flavescens*, *K. pauciflora* and × *Kunzspermum hirakimata* had chemical compositions distinct from *K. sinclairii* and *K. ericoides*. Oils from New Zealand *K. ericoides* were mainly α-pinene (mean 68%), but some oils had high *p*-cymene contents, particularly oils from one Marlborough provenance (mean 31%). A wild population of *K. ericoides* var. *linearis* gave oils with similar composition to other *K. ericoides* oils. Two *K. ericoides* oils showed weak antifungal activity. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

We are investigating a range of native and introduced plants that could form the basis of an essential oil industry in New Zealand [1]. Two native oil-producing species are manuka, *Leptospermum scoparium* J. R. *et* G. Forst. [2], and kanuka, *Kunzea ericoides* (A. Rich.) J. Thompson [3]. Foliage from both of these myrtaceous species have been used in traditional Maori remedies, including vapour baths and aromatic teas [4].

Kunzea ericoides and *L. scoparium* are small shrubs or trees that grow throughout most of New Zealand. However, *K. ericoides* prefers dry, semi-fertile sites intermediate between warm-temperate and subalpine zones, and is absent in south Westland, Fiordland, Southland and Stewart Island [5]. Previously classified as *L. ericoides* and considered endemic to New Zealand [2], *K. ericoides* as defined by Thompson [3] also occurs in Australia, in eastern regions of southern Queensland, New South Wales, Victoria and South Australia. About 30 other *Kunzea* species are found in Australia [3]. Harris has defined three *Kunzea* taxa in New Zealand by leaf form and geographical distribution: two varieties of *K. ericoides*, named *linearis* and *microflora*, and another *Kunzea* species, *K. sinclairii* (Kirk) W. Harris, which is endemic to New Zealand's Great Barrier Island [6]. Essential oil profiles have been used to distinguish between specimens of *L. scoparium*, *K. ericoides*, *K. sinclairii* and a *Kunzea* × *Leptospermum* cross [7]. The work on the chemotaxonomy of *Kunzea* oils reported here is intended to assist in describing the variability and taxonomic status of *K. ericoides* in New Zealand and Australia.

There is little published work on *Kunzea* chemistry. Steam-distilled oil from *K. ericoides* from northern New Zealand contained α-pinene (77%) and lower amounts of α-terpineol, citral, aromadendrene and the β-triketone, leptospermone [8]. An oil from *K. ericoides* from southern New Zealand contained α-pinene (52%) and lower contents of β-pinene, 1,8-cineole, limonene, β-terpinene, linalol, α-terpineol and aromadendrene [9]. The main sesquiterpene (10%) was not identified but appeared to have the cadinene skeleton [9]. Four antiviral acyl phloroglucinol derivatives, structurally related to leptospermone, have recently been reported in solvent extracts of *K. ericoides* and *K. sinclairii* [10]. Two *K. sinclairii* oils, included in our earlier study on the variation in com-

---

* Author to whom correspondence should be addressed.

position of *Leptospermum* oils, stood out from the *Leptospermum* oils because of their contents of α-pinene (mean 76%) [11].

We now report the analyses of 64 oils distilled from foliage samples of 33 *Kunzea* populations, derived around New Zealand and from Australia.

RESULTS AND DISCUSSION

The foliage samples of *Kunzea* were steam-distilled under the same conditions used for our study on *Leptospermum* chemotaxonomy [11]. Oils were distilled from two plants from 33 different plant populations, growing at a single site, but derived from seed collected from different sites around New Zealand and Australia (Table 1 and Fig. 1). As the plants were raised from seed, the two samples taken from each population represented two genotypes (see ref. [12] for more details).

The New Zealand *K. ericoides* samples gave a mean oil yield of 0.9% (± 0.4) in terms of foliage dry matter, significantly ($P < 0.01$) higher than the mean oil yield from New Zealand *L. scoparium* (0.3%) [11].

The *Kunzea* oils were analysed under the same GC conditions used for *Leptospermum* oils [11]. Thirty seven GC peaks, which reached at least 1% in at least one of the *Kunzea* oils, were selected as chemotaxonomic characters. Most of the monoterpenes previously reported were identified, plus some additional common monoterpenes (Table 2). However, we did not find neral or geranial (i.e. citral) at $>1\%$ in any of the oils: we only detected a peak corresponding with leptospermone at $\sim 1\%$ in one oil (see below). The two main sesquiterpenes in *K. ericoides* oils were *trans*-calamenene and viridiflorol, neither of which has been identified previously in this species. The large number of sesquiterpenes that occur at relatively low concentrations were characterized by their retention indices (Table 2).

The technique of principal components analysis (PCA, for discussion see ref. [11]) was used to pick out patterns of chemical composition from the GC analyses of 64 *Kunzea* oils, using 37 GC peak intensities for each oil. The first two principal components (PCs) accounted for 90% of the variance in the data set. The oil compositions plotted in terms of the first and second PCs are given in Fig. 2. The main contributor to the 1st PC (70% of the variation of the data set) was α-pinene (eigenvector $+0.90$), with β-pinene and *p*-cymene the main contributors to both the 2nd PC (eigenvectors -0.65 and $+0.74$) and 3rd PC (eigenvectors $+0.59$ and $+0.48$).

The two *K. pauciflora* oils (KP, Fig. 2) stand out from all the other *Kunzea* oils on the first and second PCs because of their high β-pinene (mean 47%) and low α-pinene contents (mean 14%). The amounts of both of these compounds in the *K. pauciflora* oils were significantly ($P < 0.01$) different from levels in any of the other *Kunzea* species examined (Table 2). One of the *K. flavescens* oils (KF, Fig. 2) stands out from all the other *Kunzea* oils in the PCA because of its low α-pinene and high sesquiterpene content. Statistical analyses showed significant differences ($P < 0.05$) between the *K. flavescens* oils and the other *Kunzea* oils, particularly in the high proportion (mean 12%) of peak 25, an unidentified sesquiterpene in *K. flavescens* (Table 2). Therefore, oil composition is probably a useful character for distinguishing between *K. pauciflora*, *K. flavescens* and *K. ericoides* in Australia. However, further sampling would be needed to check the range of oil compositions in each species.

The two *K. sinclairii* (KS, Fig. 2) oils fell amongst the *K. ericoides* oils on the PCA plots. The only significant differences ($P < 0.05$) between these two species were in the amounts of two minor sesquiterpene components (Peaks 21 and 22). This contrasts with earlier work which separated *K. sinclairii* from *K. ericoides* by qualitative comparison of profiles from a few oils [7].

The oil from × *K. hirakimata* (KH, Fig. 2) stood out from the *K. ericoides* and *K. sinclairii* oils. Statistical analyses showed that this oil, when compared with *K. ericoides* and *K. sinclairii* oils, had significantly ($P < 0.05$) higher contents of several sesquiterpenes and a peak (at 1.3%) with the retention time of the characteristic *L. scoparium* component leptospermone (Table 2) [11]. These results agree with the previous report that the essential oil profile of × *K. hirakimata* is intermediate between the profiles of the putative parents, *K. sinclairii* and *L. scoparium* [7].

Harris has noted that the Australian *K. ericoides* specimens have a different flowering pattern and morphological appearance to New Zealand *K. ericoides* [12]. However, based on our chemical analysis, the six oils from Australian *K. ericoides* did not cluster separately from the New Zealand *K. ericoides* oils (populations 28, 29 and 30, Fig. 2). Statistical analysis showed some differences in composition between oils from the two countries, but only in the contents of relatively minor components (Table 2, mean $<3\%$). Therefore these analytical results do not support separation of New Zealand and Australian *K. ericoides*. This result contrasts with the analyses of *Leptospermum* oils, which clearly distinguished New Zealand *L. scoparium* from Australian *L. scoparium* [11]. However, the three populations from Australia most likely represent only a small part of the variation of the species in that country. Further sampling and analysis might reveal greater differences in chemical composition.

The GC data set was reduced to just 51 New Zealand *K. ericoides* oils and subjected to PCA to search for compositional differences (chemotypes). The first two PCs accounted for 92% of the variance in the data set. The oil compositions plotted in terms of these PCs are given in Fig. 3. The main contributors to both the 1st PC and the 2nd PC were α-pinene (eigenvectors $+0.80$ and $+0.53$, respectively) and *p*-cymene (eigenvectors -0.59 and $+0.67$, respectively). The two oils of plants from Okiwi Bay, Marlborough stand

Table 1. Details of *Kunzea* samples and oil yields

Population number	Species	CHR Number	Site of origin	Latitude south	Oil yield*
1	*K. sinclairii*	496712	Mt Hobson, Great Barrier Island, N. Auckland	36°11′	1.12
2	*K. ericoides*	499656	Okahukura, Kaipara Harbour, N. Auckland	36°21′	1.08
3	*K. ericoides*	499666	Cape Colville, Coromandel Peninsula, S. Auckland	36°28′	1.13
4	*K. ericoides*	499649	Whangapoua Rd, Coromandel Peninsula, S. Auckland	36°44′	1.41
5	*K. ericoides*	499658	Mangakirikiri River, Gisborne	38°01′	0.85
6	*K. ericoides*	499665	Kuirau Park, Rotorua	38°03′	1.13
7	*K. ericoides* var. *microflora*	499655	Rainbow Mountain, Rotorua	38°19′	0.93
8	*K. ericoides*	499670	Waitoha, Picton, Marlborough	41°17′	0.73
9	*K. ericoides*	499654	Wither Hills, Marlborough	41°33′	0.95
10	*K. ericoides*	499667	Okiwi Bay, Marlborough	42°13′	0.51
11	*K. ericoides*	499662	Nelson Creek, Westland	42°25′	0.70
12	*K. ericoides*	499664	Jacks Pass, Hanmer, Canterbury	42°29′	0.83†
13	*K. ericoides*	499660	Lottery River, Canterbury	42°37′	0.84
14	*K. ericoides*	499661	Eyrewell Science Reserve, Canterbury	43°26′	1.88
15	*K. ericoides*	499659	Quail Island, Banks Peninsula, Canterbury	43°38′	0.51
16	*K. ericoides*	499663	Charteris Bay, Banks Peninsula, Canterbury	43°39′	0.99
17	*K. ericoides*	499652	Bankside Scenic Reserve, Canterbury	43°42′	1.68
18	*K. ericoides*	499669	Kaituna Valley, Banks Peninsula, Canterbury	43°47′	0.42
19	*K. ericoides*	499657	Peel Forest, Canterbury	43°55′	0.98
20	*K. ericoides*	499668	Wanaka, Otago	44°40′	0.90
21	*K. ericoides*	499644	Dickson Hill, Otago Peninsula	45°51′	0.42
22	*K. ericoides*	499651	Pupu Springs, Nelson	40°51′	0.78
23	*K. ericoides*	499647	Takaka Hill, Nelson	41°02′	0.80
24	*K. ericoides*	499653	Matai Valley, Nelson	41°16′	0.90
25	*K. ericoides*	499646	Berlins, Lower Buller Valley, Buller	41°52′	0.78
26	*K. ericoides*	499650	Boundary Stream, Poulter River, Canterbury	43°03′	0.94
27	*K. ericoides*	499654	Wither Hills, Marlborough‡	41°33′	0.95
28	*K. ericoides*	499648	Bemm River, Victoria, Australia	37°41′	1.15
29	*K. ericoides*	499643	Nethercote, NSW, Australia	37°01′	2.03
30	*K. ericoides*	499641	Seed sample, Australia	—	1.05
31	× *Kunzspermum hirakimata*	499645	Mt Hobson, Great Barrier Island	36°11′	1.12†
32	*K. pauciflora*	499640	Seed sample, Australia	—	1.51
33	*K. flavescens*	499642	Seed sample, Australia	—	0.65

* In ml $100\ g^{-1}$ dry matter; mean of two values unless noted otherwise.

† Only one oil analysed.

‡ Same site of origin as population 9.

out for their high *p*-cymene (mean 31%) and low α-pinene contents (mean 41%). This same location yielded *L. scoparium* plants with unusual oils, high in *trans*-methyl cinnamate [11]. Other oils also stand out from the main group of *Kunzea* oils on the basis of their high *p*-cymene (Peak 5) and low α-pinene (Peak 2) contents. There was a strong negative correlation between *p*-cymene and α-pinene concentrations ($R^2 = 0.8$), as is inevitable for the two main components in a set of normalized GC data. Therefore, there is a high *p*-cymene (>5%) chemotype of *K. ericoides*, but not all pairs of plants from the same site were of the same chemotype (see Fig. 3). For example, the two oils from the *K. ericoides* var. *microflora* plants

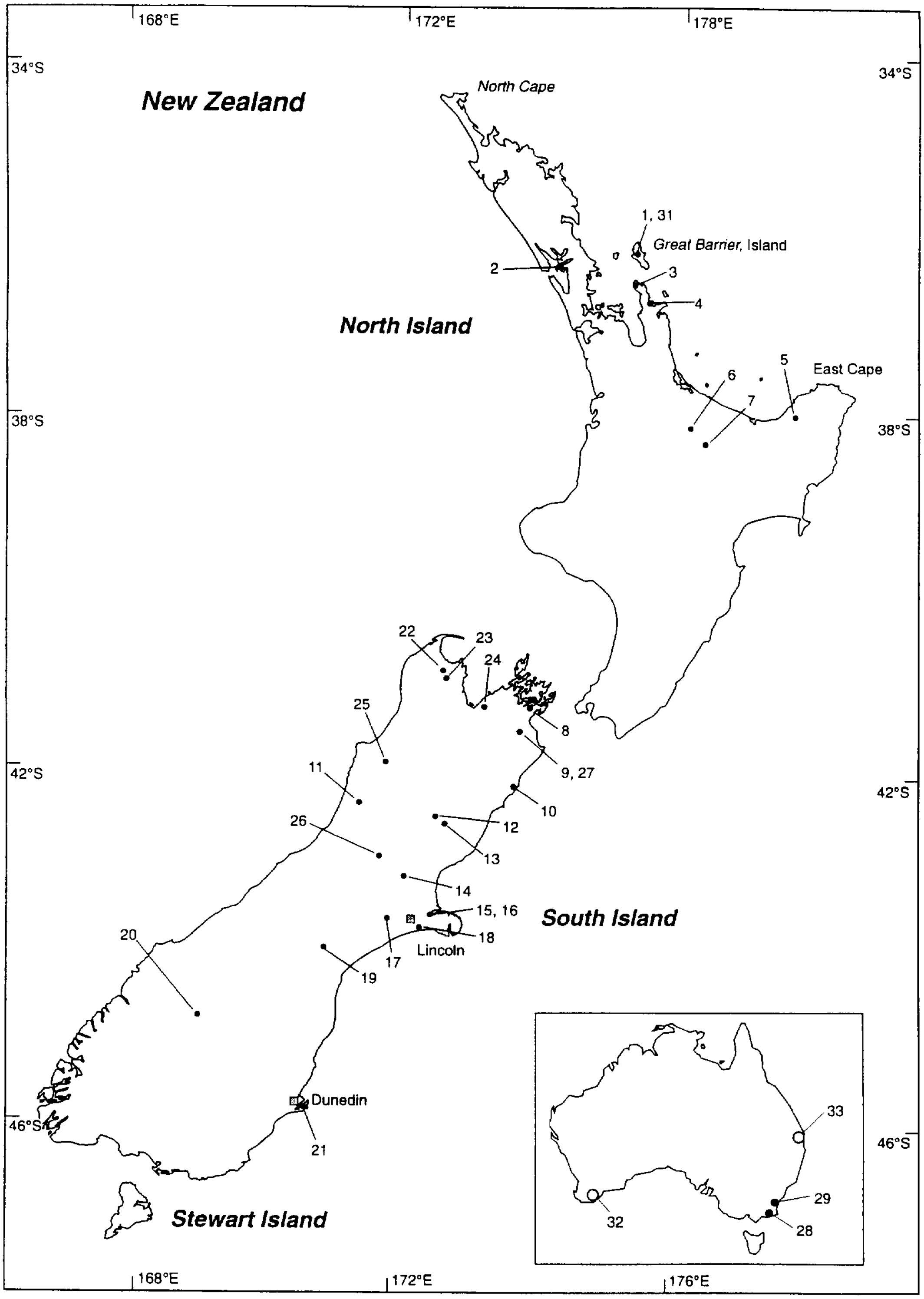

Fig. 1. Sites of origin of *K. ericoides* and *K. sinclairii* populations in New Zealand and Australia (●) and centres of natural distribution of *K. pauciflora* and *K. flavescens* in Australia (○). Site details are given in Table 1.

(population 7) had *p*-cymene contents of 0.2 and 19.8%. Most of the New Zealand *K. ericoides* oils with high *p*-cymene contents also contained relatively high amounts of γ-terpinene. *p*-Cymene and γ-terpinene are closely linked biosynthetically in *Thymus vulgaris* L. [13]; this is probably also the case in *K. ericoides*. The occurrence of the high *p*-cymene chemotype does not seem to correlate closely with geographical origin or morphological features, although it is interesting that five of the six Australian *K. eri-*

Table 2. Composition of *Kunzea* oils

				Mean composition, % of total peaks (s.d.)					
	Peak DB-1	*RI* DB-WAX	Identity	*Kunzea ericoides* NZ, $n = 51$	*Kunzea ericoides* Aus, $n = 6$	*Kunzea flavescens* $n = 2$	*Kunzea pauciflora* $n = 2$	*Kunzea sinclairii* $n = 2$	× *Kunzspermum hirakimata* $n = 1$
1	922	942		0.6 (0.6)	0.9 (0.5)	0.1 (0.1)	0.3 (0.0)	0.3 (0.0)	0.3
2	930	1015	α-Pinene*,†	67.6 (10.8)	59.7 (10.1)	49.3 (25.0)	14.3 (1.2)	73.9 (3.0)	49.9
3	960		Camphene†	0.0 (0.0)	0.1 (0.0)	0.1 (0.0)	3.1 (0.5)	0.1 (0.1)	0.1
4	969	1077	β-Pinene*,†	0.6 (0.2)	0.6 (0.3)	0.2 (0.0)	47.4 (2.2)	0.7 (0.0)	1.2
5	1008	1245	*p*-Cymene*,†	5.8 (8.3)	12.6 (7.5)	0.5 (0.1)	0.0 (0.0)	0.1 (0.2)	0.5
6	1014	1186	1,8-Cineole†,‡	4.3 (2.5)	6.2 (1.2)	0.8 (0.3)	1.6 (0.0)	4.2 (4.0)	8.4
7	1038	1226	γ-Terpinene*,†	1.6 (2.5)	2.0 (1.9)	0.1 (0.1)	1.0 (0.1)	0.2 (0.1)	0.5
8	1060	1255		0.4 (0.5)	0.6 (0.5)	0.1 (0.1)	0.4 (0.0)	0.1 (0.0)	0.2
9	1062	1553	Linalool*	1.7 (1.1)	1.2 (0.5)	0.5 (0.2)	1.8 (0.5)	1.9 (0.1)	3.0
10	1069			0.2 (0.3)	0.2 (0.1)	0.5 (0.2)	0.0 (0.0)	0.2 (0.1)	0.2
11	1086			0.8 (0.6)	1.1 (0.7)	0.4 (0.1)	0.2 (0.0)	0.1 (0.1)	0.2
12	1104			0.3 (0.2)	0.4 (0.2)	0.0 (0.0)	2.7 (0.4)	0.0 (0.0)	0.1
13	1115			0.2 (0.2)	0.2 (0.1)	0.1 (0.0)	2.3 (0.4)	0.0 (0.0)	0.0
14	1151			0.0 (0.0)	0.1 (0.0)	0.1 (0.0)	2.5 (0.3)	0.0 (0.0)	0.0
15	1161		α-Terpineol†	0.7 (0.4)	0.8 (0.1)	0.0 (0.0)	0.4 (0.1)	0.8 (0.5)	1.6
16	1166			0.1 (0.1)	0.2 (0.1)	0.1 (0.2)	1.9 (0.3)	0.0 (0.0)	0.1
17	1200	1200	*n*-C_{12} Internal std						
18	1391			0.2 (0.2)	0.1 (0.1)	0.4 (0.3)	0.0 (0.0)	0.5 (0.2)	0.9
19	1398		β-Caryophyllene†	0.2 (0.4)	0.1 (0.1)	3.4 (0.0)	0.0 (0.0)	0.4 (0.1)	1.8
20	1419			0.2 (0.2)	0.4 (0.1)	1.7 (1.2)	0.0 (0.0)	0.2 (0.0)	1.0
21	1425			0.2 (0.4)	0.0 (0.1)	0.3 (0.1)	0.1 (0.0)	0.9 (0.1)	2.8
22	1441			0.2 (0.2)	0.3 (0.1)	0.8 (0.4)	0.3 (0.2)	0.0 (0.0)	0.1
23	1449			0.1 (0.1)	0.2 (0.2)	1.3 (0.6)	0.1 (0.0)	0.1 (0.0)	0.2
24	1458			0.1 (0.1)	0.1 (0.1)	0.2 (0.3)	0.0 (0.1)	0.0 (0.0)	1.7
25	1469	1696		0.8 (0.9)	2.0 (1.1)	12.2 (4.9)	0.9 (0.9)	1.4 (0.2)	1.4
26	1478			0.1 (0.1)	0.1 (0.1)	1.0 (1.0)	0.4 (0.3)	0.2 (0.0)	0.3
27	1485			0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.1 (0.1)	0.0 (0.1)	1.4
28	1489		*trans*-Calamenene*,†	1.7 (1.0)	0.4 (0.6)	0.2 (0.1)	0.3 (0.4)	2.4 (1.0)	3.5
29	1497	1726		0.3 (0.2)	0.1 (0.1)	2.6 (2.7)	1.2 (0.7)	0.5 (0.1)	1.3
30	1503			0.5 (0.5)	0.1 (0.3)	0.2 (0.2)	0.2 (0.2)	1.0 (0.2)	2.0
31	1536			0.7 (0.5)	0.8 (0.5)	2.4 (0.8)	4.3 (1.3)	0.9 (0.2)	0.3
32	1543			0.3 (0.6)	0.0 (0.0)	0.8 (0.2)	0.0 (0.0)	0.0 (0.0)	0.2
33	1546			0.2 (0.3)	1.0 (0.9)	1.2 (0.6)	0.6 (0.4)	0.3 (0.0)	0.1
34	1553	2062	Viridiflorol*,†	2.8 (1.7)	1.0 (0.4)	0.8 (0.7)	0.1 (0.1)	1.3 (0.1)	2.3
35	1563			0.9 (0.5)	0.4 (0.1)	0.9 (0.8)	0.2 (0.1)	0.5 (0.0)	0.8
36	1596		Leptospermone†	0.4 (0.0)	0.3 (0.1)	0.9 (0.0)	0.1 (0.0)	0.2 (0.0)	1.3
37	1606			0.0 (0.0)	0.0 (0.0)	1.7 (1.9)	0.7 (0.5)	0.2 (0.0)	0.0
38	1617			0.0 (0.0)	0.0 (0.0)	1.6 (1.7)	0.7 (0.3)	0.0 (0.0)	0.4
39	1800	1800	*n*-C_{18} Internal std						

* Identification by ^{1}H NMR (200 MHz) on an oil with a high level of this component.

† Identification by coinjection on DB-1.

‡ 1,8-Cineole and limonene co-elute on DB1, but ^{1}H NMR showed no limonene signals.

coides were high *p*-cymene plants. By contrast, chemotypes in *L. scoparium* did relate to geographical origin and morphological features [11].

Examination of the New Zealand *K. ericoides* GC data for other components with bi- or polymodal distributions of contents did find other cases, but it would seem pointless to try to define a large number of chemotypes. Harris has noted that populations of *K. ericoides* south of *ca* latitude 38° in New Zealand have distinctly smaller leaves than populations further north [12]. Statistical analyses were performed to see if composition and yield differed significantly between the northern (populations 2, 3, and 4) and southern groups (populations 6 to 27). Population 5, from the East Cape of the North Island, was excluded as it has features intermediate between the two groups. Peaks 9 (linalol, mean 3.2% north, 1.5% south), 15 (α-terpineol), 22, 25 and 26 had significantly ($P < 0.05$) higher contents in the northern populations, while peak 36 (leptospermone, mean 0.2% north, 0.4% south) was the only one that was significantly ($P < 0.01$) lower in the northern populations. There was also a significant ($P < 0.05$) difference between the oil yield for northern (mean 1.2%) populations

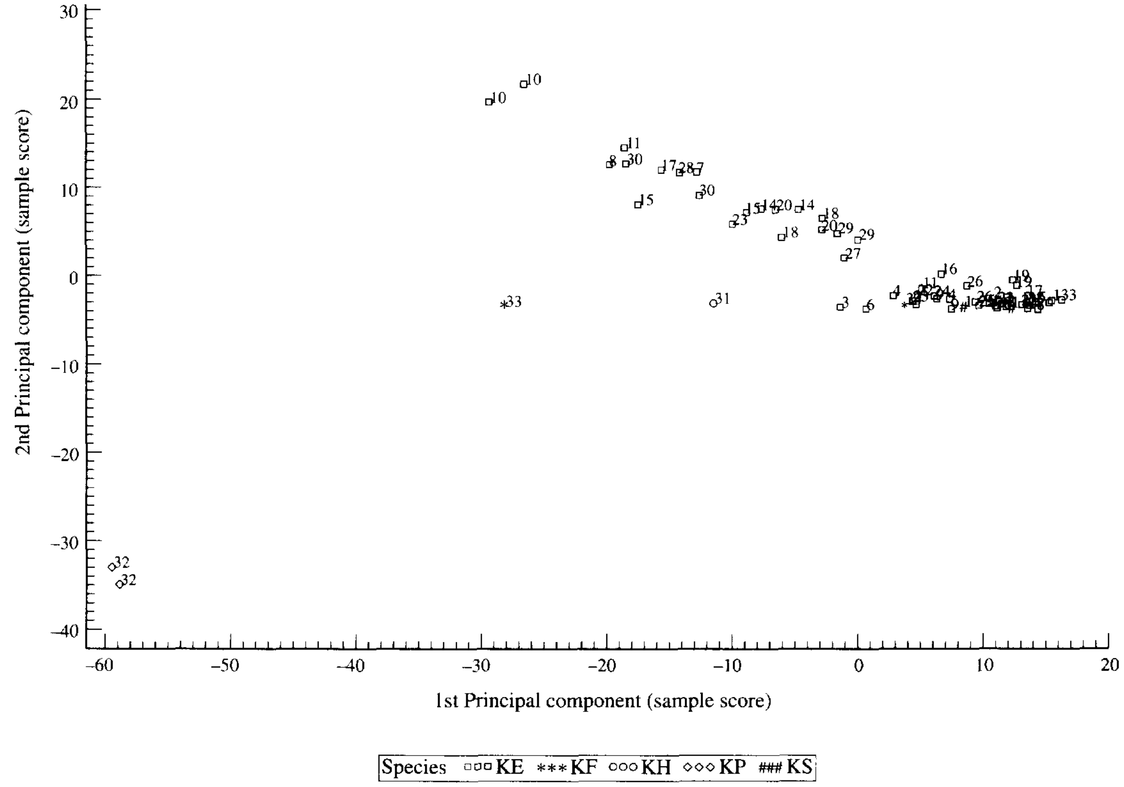

Fig. 2. Compositions of *Kunzea* oils in terms of the first and second PCs; species are identified by different symbols and labelled with population numbers (Table 1).

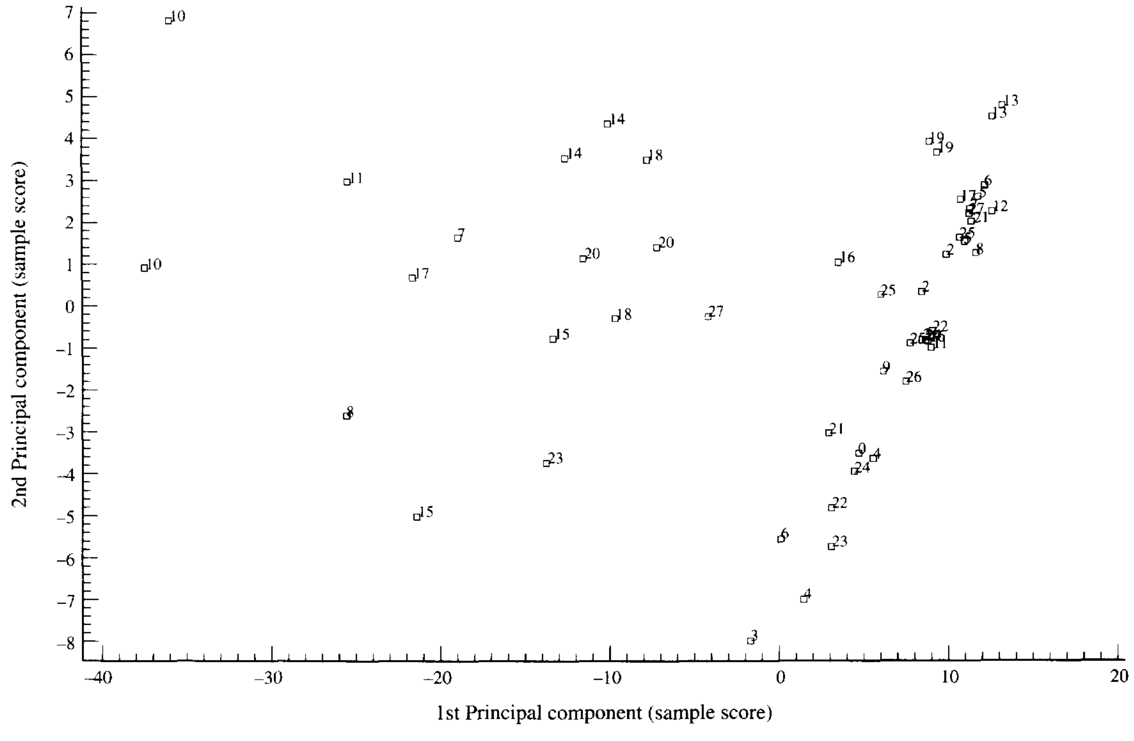

Fig. 3. Compositions of New Zealand *Kunzea ericoides* oils in terms of the first and second PCs, labelled with population numbers (Table 1).

Table 3. Antimicrobial assays* of selected Myrtaceae oils: *K. ericoides*, *L. scoparium* and Australian tea-tree oil

	Bacillus subtilis†				*Trichophyton mentagrophytes*‡			
Dose§	North¶	South**	manuka††	Tea-tree‡‡	North¶	South**	manuka††	Tea-tree‡‡
1200	0	0	8	1	1	4	5	0
600	0	0	5	0	1	1	3	0
300	0	0	3	0	1	1	3	0

* Results are width of inhibition zone, in mm.

† American Type Culture Collection number 19659.

‡ American Type Culture Collection number 28185.

§ Dose of oil in μg per disk.

¶ Northern *K. ericoides* oil, population 2.

** Southern *K. ericoides* oil, population 10.

†† East Cape *L. scoparium* oil.

‡‡ *Melaleuca alternifolia* oil.

and those in the south (mean 0.9%). Although these differences are significant, they involve minor components, so that these features could not be reliably used to distinguish taxa without further inter- and intra-population sampling.

Only one of the two recognized morphological varieties of *K. ericoides* was included in the samples from the experimental garden. One plant of *K. ericoides* var. *mircroflora* was typical of the high α-pinene chemotype, the other was of the high *p*-cymene chemotype (population 7, see Fig. 3). Two oils from a wild population of *K. ericoides* var. *linearis* were similar to other kanuka oils, apart from the relatively high 1,8-cineole/limonene (peak 6) concentration (mean 9.5%), though this may have been due to environmental or seasonal effects.

Our previous study on the antibacterial and antifungal properties of *L. scoparium* oils, found that oils from an East Cape population was the most active [11]. Oils from northern and southern populations of *K. ericoides* were tested for antimicrobial activity, alongside an East Cape *L. scoparium* oil and a commercial Australian tea tree (*Melaleuca alternifolia* Cheel) oil, rich in terpinen-4-ol (Table 3). Neither of the oils from *K. ericoides* showed an antibacterial activity at the concentrations tested. Oils from both populations showed mild antifungal activity, with the southern oil showing higher activity at the largest dose. The East Cape *L. scoparium* oil showed higher activity than the two *K. ericoides* oils. It is important that the difference in antimicrobial activity between these two species is recognized, because *K. ericoides* and *L. scoparium* often grow together and close examination is needed to distinguish their foliage.

Kunzea ericoides oils are obtained in higher yields than *L. scoparium* oils [11] and contain predominantly monoterpenes. We found a clear distinction between two other *Kunzea* species and *K. ericoides* in the amounts of major oil components. The greater separation of the *K. pauciflora* oils than those of *K. flavescens* from the *K. ericoides sensu lato* (i.e., including Australian *K. ericoides*) oils is in conformity with the morphological differences between these species. *Kunzea pauciflora*, with its purple-mauve flowers, has the appearance of being a distant relative of the white-flowered *K. flavescens* and *K. ericoides*. Our study has distinguished two chemotypes within *K. ericoides sensu lato*, the most common one with low *p*-cymene, the less common one with high (>5%) *p*-cymene. These chemotypes co-occur within populations as is the case with other oil-rich plants, such as *Thymus vulgaris* [13]. More comprehensive sampling would be needed in order to determine whether the frequency of these chemotypes fitted with taxonomic or geographical groupings.

EXPERIMENTAL

Plant material. The populations studied (see Table 1 for species and site of origin) were all grown from seed at Landcare Research, Lincoln (43°38′ S, 172°30′ E) from four plantings. Populations 1–21 were sown in September 1983, populations 22–29 in November 1984; and populations 30, 32 and 33 in November 1986 from seed purchased from Nindethana Seed Services, Western Australia. × *Kunzspermum hirakimata* (population 31), an intergenic hybrid [7], was discovered in population 1, and material was obtained from a plant raised from a cutting of this original plant [12]. Voucher specimens are deposited in the Landcare Research Herbarium (CHR), Lincoln (voucher numbers in Table 1). Leaf and twig samples were taken from two individuals of most of the *Kunzea* provenances on April 15, 1994. Two *K. sinclairii* samples were taken on March 15, 1994. All samples were sorted to remove dead material and woody stems (diameter > 5 mm) and stored at 3° and 18% relative humidity for at least 7 days. Dried plant material was chopped into 2.5 cm lengths immediately before steam-distillations. Sub-samples were taken immediately prior to steam-distillation for the determination of dry matter content, by drying to constant weight at 80°; values ranged from 86 to 95%. Fresh samples had dry matter contents typically around 50–55%.

Foliage from two individuals in a wild population of *K. ericoides* var. *linearis* growing at Tom Bowling

Bay, near North Cape, North Auckland (34°26′ S, 172°58′ E) was collected in March 1995, treated and distilled as described above. A voucher specimen is retained (CHR 485682). Data from the oils of these samples were not included in the main data set.

Steam distillation. Two laboratory-scale stills were used, one glass (600 × 100 mm), and one stainless steel (500 × 100 mm), using the same methods as previously described [11].

GC analyses and component identification. Kunzea oils were analysed as 1% solns in hexane containing two ref. compounds (0.5% *n*-dodecane and 0.5% *n*-octadecane). Samples were analysed on a 10 m J&W DB-1 (0.25 mm id, 0.25 μm film) column with H_2 carrier gas (linear velocity 50 cm s^{-1}) with a temp. programme of 80 to 160° at 5° min^{-1}. Injections (0.5 μl) were made into a split (100:1) injector set at 260°. The FID was set at 350°. The software was set up to recognize 37 peaks, after using the two int. ref. compounds to correct for R_t variations between runs. Peak areas of C12 and C18 were excluded from peak quantitation data and the remaining peak areas were normalized. GC data on the 64 oils in this study are available as Supplementary Material. ASCII files of these GC results were subjected to statistical processing using SAS software. Statistical analyses were performed using SAS Institute software: linear correlations and Principal Components Analyses (PCA) were run on unscaled peak data; significance of differences were tested by a General Linear Model (GLM) procedure.

DB-1 Kovats retention indices (RIs) of the 37 peaks were measured by conjection of a *K. ericoides* oil with *n*-alkanes (C_8–C_{24}) using the standard GC method above. RIs were calcd by linear interpolation between the uncorrected R_ts of the *n*-alkanes bracketing the peak in question (see Table 2). RIs for the larger peaks were also measured on a 10 m J&W DB-Wax column with H_2 carrier gas using a temp. programme of 50 to 250° at 5° min^{-1} and the same *n*-alkanes.

Major components in selected oils were identified by comparing their 200 MHz ^{1}H NMR spectra (in $CDCl_3$) with the spectra of ref. compounds. Some components were identified by coinjection with ref. compounds, others by comparison with identified components in the GC trace of a *L. scoparium* oil sample [11].

Biological assays. Antimicrobial assays were performed at the University of Canterbury as previously described [11].

Acknowledgements—We thank G. Barns, Chemistry Department, University of Canterbury, for antimicrobial assays. This research was supported by the New Zealand Foundation for Research, Science and Technology.

REFERENCES

1. Perry, N. B., Douglas, M. H. and Porter, N. G., *Perfumer and Flavorist*, 1993, **18**, 25.
2. Allan, H. H., *Flora of New Zealand. Indigenous Tracheophyta. Psilopsida, Lycopsida, Filicopsida, Gymnospermae, Dicotyledones*, Vol. 1. DSIR, Wellington, 1961, p. 320.
3. Thompson, J., *Telopea*, 1983, **2**, 379.
4. Brooker, S. G., Cambie, R. C. and Cooper, R. C., *New Zealand Medicinal Plants*. Heinemann, Auckland, 1987, p. 170.
5. Wardle, P., *Vegetation of New Zealand*. Cambridge University Press, Cambridge, 1991.
6. Conner, H. E. and Edgar, E., *New Zealand Journal of Botany*, 1987, **25**, 115.
7. Harris, W., Porter, N. G. and Dawson, M. I., *New Zealand Journal of Botany*, 1992, **30**, 213.
8. Short, W. F. and Johnson, D., *Report of the Australasian Association for Advances in Science*, 1926, **16**, 220.
9. Corbett, R. E. and Gibson, M. G. C., *Journal of Science, Food and Agriculture*, 1959, **10**, 198.
10. Bloor, S. J., *Journal of Natural Products*, 1992, **55**, 43.
11. Perry, N. B., Brennan, N. J., van Klink, J. W., Harris, W., Douglas, M. H., McGimpsey, J. A., Smallfield, B. M. and Anderson, R. E., *Phytochemistry* 1997, **44**, 1485.
12. Harris, W., *New Zealand Journal of Botany*, 1996, **34**, 333.
13. Stahl-Biskup, E., *Journal of Essential Oil Research*, 1991, **3**, 61.