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FOLIAGE SESQUITERPENES AND DITERPENES OF *PODOCARPUS SPICATUS**

STEPHEN D. LORIMER and REX T. WEAVERS

Department of Chemistry, University of Otago, Box 56, Dunedin, New Zealand

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Key Word Index—*Podocarpus spicatus*; Podocarpaceae; infraspecific variation; diterpenes; sesquiterpenes.

Abstract—Investigation of the foliage oils of 49 specimens of *P. spicatus* has shown marked infraspecific variation. Seven sesquiterpene hydrocarbons (δ-elemene, α-copaene, longifolene, germacrene-D, bicyclogermacrene, δ-cadinene and selina-4(14),7(11)-diene), two oxygenated sesquiterpenes (curzerenone and atractylon), ten diterpene hydrocarbons (rimuene, beyerene, sclarene, rosadiene, pimaradiene, sandaracopimaradiene, isopimara-7,15-diene, isopimara-8,15-diene, phyllocladene and kaurene), and two oxygenated diterpenes (8β-hydroxyisopimarene and ferruginol) have been isolated and identified. Of particular interest is the isolation of (+)-germacrene-D and (−)-δ-cadinene, along with the rarely reported curzerenone, atractylon, and (+)-rosadiene. This is the first report of selina-4(14),7(11)-diene in an optically active form. The occurrence of both enantiomers of sandaracopimaradiene is also of interest. The biosynthetic significance of correlations in the levels of the terpenes present is discussed.

INTRODUCTION

Podocarpus spicatus R.Br. *ex* Mirbel (Podocarpaceae), matai, is a robust forest canopy tree with distribution throughout New Zealand [2, 3]. The wood, with its close handsome grain, is exceptionally strong and durable and is used for flooring, weatherboarding and furniture. Other uses of matai have been as a source of food and medicine; the New Zealand Maoris and early European bushmen ate the berries raw. Matai beer, obtained from tapping the trunk, was eagerly sought after by bushmen as a beverage and to check the advance of consumption [4, 5].

De Laubenfels has proposed that matai be reclassified, along with its close relative miro (*Podocarpus ferrugineus*) to the genus *Prumnopitys* [6]. The proposed new taxonomic name is Prumnopitys taxifolia (Sol. ex D. Don) de Laubenfels [7]. This reclassification is also supported on chemotaxonomic grounds by recent work on the distribution of flavanoid glycosides [8].

Monoterpenes previously identified from matai foliage are α-pinene, β-pinene, limonene, myrcene, α-terpinene, β-phellandrene, dipentene, γ-terpinene, *p*-cymene and α-terpineol [9, 10]. Aromadendrene and cadinene have been identified from the sesquiterpene family [9, 10] and the foliage diterpenes which have been reported are kaurene, phyllocladene, isophyllocladene, cupressene and isokaurene [10–13]. Considerable variability in the diterpene composition has been noted [11, 13]. Other studies on foliage extracts have produced evidence of insect moulting hormone activity [14] and insecticidal properties [15]. Allelopathic potential was also discovered, in that the water-soluble leaf extracts were highly toxic towards *Dacrycarpus dacrydioides* seedlings [16].

*Part 5 in the series 'Foliage Components of New Zealand Gymnosperms'. For Part 4 see ref. [1].

RESULTS AND DISCUSSION

General

Foliage samples from 49 trees gathered from five locations throughout New Zealand were extracted and the extracts analysed by capillary GC. Data for the means and ranges of levels for the terpenes identified are given in Table 1. A detailed data description for each tree has been lodged with the Editor.

The extraction techniques used in this study exposed the components to both heat and acid. The foliage/water

Table 1. Terpene levels* in *Podocarpus spicatus* foliage

Compound	Mean	Range
Longifolene (**1**)	0.09	0.00–0.85
Germacrene-D (**2**)	0.14	0.00–0.92
Bicyclogermacrene (**3**)	0.14	0.00–0.56
δ-Cadinene (**4**)	0.04	0.00–0.28
Selinadiene (**5**)	0.03	0.00–0.26
α-Copaene (**6**)	0.04	0.00–0.25
Curzerenone (**8**)	0.03	0.00–0.22
Atractylon (**9**)	0.14	0.00–1.41
Rimuene (**10**)	0.23	0.00–1.93
Beyerene (**11**)	0.38	0.00–1.17
Sclarene (**12**)/Rosadiene (**13**)	0.18	0.00–1.35
Kaurene (**14**)	1.31	0.00–4.08
Sandaracopimaradiene (**15**)/(**16**)	0.09	0.00–0.34
Phyllocladene (**17**)	0.23	0.00–2.21
Pimaradiene (**20**)	0.12	0.00–0.32
Isopimara-7,(15)-diene (**23**)	0.04	0.00–0.18
8β-Hydroxyisopimarene (**25**)	0.63	0.00–3.57

*Expressed relative to octadecane internal standard.

mixture from which steam distillation was conducted typically had a pH of 4. To ensure that the terpenes isolated were not artefacts, cold extractions in the presence of sodium bicarbonate were carried out. No changes in the product compositon were noted by gas chromatography.

Foliage sesquiterpenes

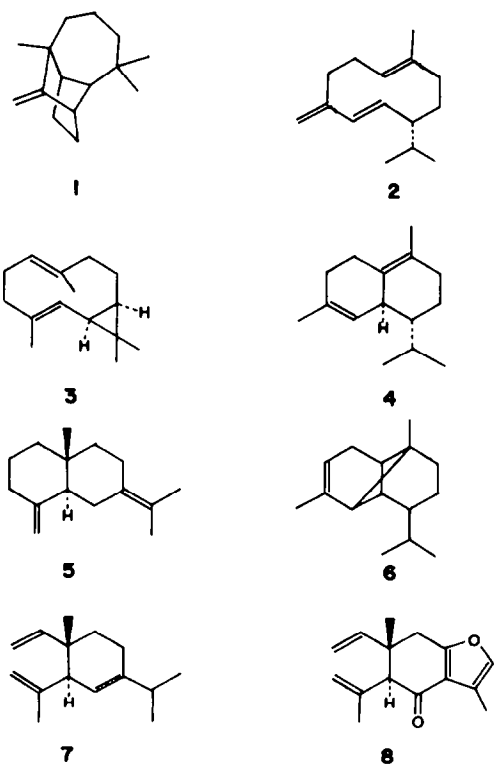
In general, total sesquiterpene levels were low (*ca* 13% of the volatile oil). Preliminary identifications were made on the basis of GC retention data. Subsequently, bulk extractions of appropriate trees were carried out to obtain samples for optical rotation and spectral measurements. Sesquiterpene hydrocarbons identified in this way were (+)-longifolene (**1**), (+)-germacrene-D (**2**), (+)-bicyclogermacrene (**3**), (−)-δ-cadinene (**4**) and (+)-selina-4(14),7(11)-diene (**5**). α-Copaene (**6**) was also obtained, but in insufficient quantity to measure its rotation. δ-Elemene (**7**) was identified from the ^{1}H NMR spectrum of a mixture with longifolene by comparison with that of an authentic sample. Two oxygenated sesquiterpenes, curzerenone (**8**) and atractylon (**9**), were also isolated and characterized. Neither of these compounds exhibited optical activity.

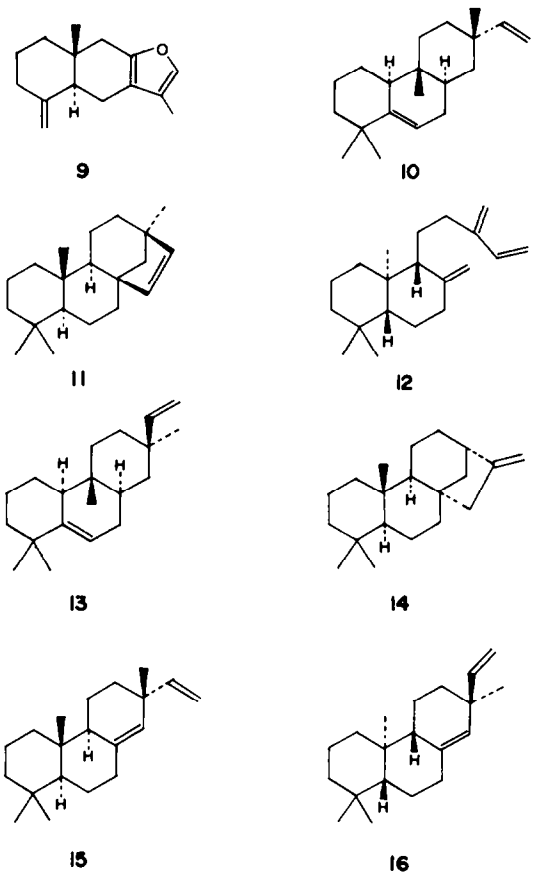
The δ-cadinene (**4**) isolated had the unusual *R* stereochemistry at C-10. (−)-δ-Cadinene has been previously reported from the angiosperm *Dendropanax trifidus* [17], the marine species *Sinularia mayi* [18] and the secretion from the scale insect, *Ceroplastes ceriferus* [19] but the *S* stereochemistry is the most common in nature [20].

Germacrene-D and bicyclogermacrene are not uncommon as components of the essential oils of the Compositae and have now been isolated from two other New Zealand podocarps [1] (Hayman, A. R. and Weavers, R. T., unpublished). However, the (+)-germacrene-D (**2**) found in matai, like the (−)-δ-cadinene (**4**), is enantiomeric to that normally found in the higher plants [18]. (+)-Germacrene-D has been reported from the marine species *Sinularia mayi* [18], from the secretion of the scale insect, *Ceropastes rubens* [19], and from the angiosperms, *Dendropanax trifuidus* [17] and *Solidago altissima* [21]. In the case of *S. altissima* both enantiomers of germacrene-D were isolated and characterized. In two of the four reports of (+)-germacrene-D, (−)-δ-cadinene was also isolated. The (+)-bicyclogermacrene (**3**) isolated from matai is the common stereochemical form.

Selina-4(14),7(11)-diene (**5**) has been isolated from hops [22–24]. It has been proposed that it may be non-enzymatically produced from germacrene-B [25]. If this is true, the selinadiene would be expected to be optically inactive, but the sample isolated from matai had a consistently large and positive specific rotation. In the previous isolation of (**5**) from powdery mildew resistant seedlings of hops, *Humulus lupulus*, other selinane derivatives were co-occurring [24]. No significant levels of other selinanes were found in matai.

A cold water extraction was carried out on a sample of foliage from a tree known to be high in curzereone and atractylon. The liquor from the extract was then subjected to the usual steam distillation procedure. As no significant amounts of volatile materials were noted by GC, the oxygenated terpenoids do not appear to be present as water soluble glycosides.





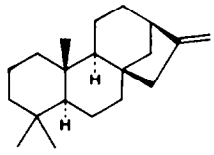
Foliage diterpenes

In like manner, the diterpene hydrocarbons (+)-rimuene (**10**), (−)-beyerene (**11**), (−)-sclarene (**12**), (−)-rosa-5,15-diene (**13**) and (+)-kaurene (**14**) were also isolated and identified.

Sandaracopimaradiene was isolated in the (−)-form (**15**) from one tree and in the (+)-form (**16**) from another. Only on two previous occasions has the occurrence of enantiomers of a diterpene been reported from different specimens of the same species [1, 26]. In *P. spicatus*, both enantiomers of sandaracopimaradiene were isolated from trees containing regular rimuene and rosadiene.

A mixture of kaurene (**14**) and phyllocladene (**17**) from one tree could not be separated, and the mixture was isomerized to isokaurene (**18**) and isophyllocladene (**19**) respectively, by heating with iodine in benzene [27]. Separation, followed by measurement of specific rotations, showed that both the isokaurene and the isophyllocladene were of the normal stereochemical series. This was supported by measurement of the specific rotation of the kaurene/phyllocladene mixture. Isolations of kaurene from trees containing no phyllocladene also gave samples of the normal series.

The ^{1}H NMR spectrum of the mother liquors from the crystallization of the kaurene/phyllocladene mixtures showed the presence of a component whose resonances matched those of pimara-8(14),15-diene (**20**). GC retention data were also identical. Treatment of the mixture with hydrogen chloride gave a sample which showed a peak in the GC with the same retention time as pimara-8,15-diene (**21**).



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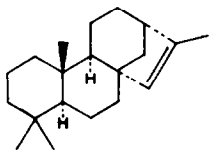

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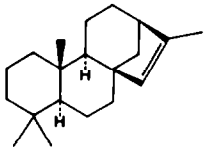

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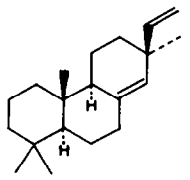

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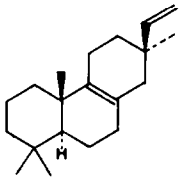

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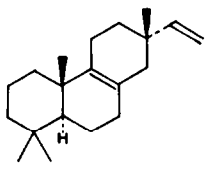

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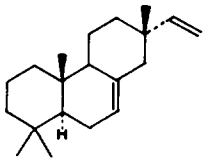

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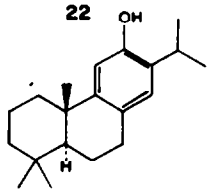

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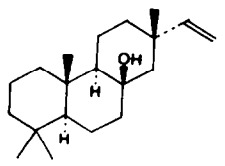

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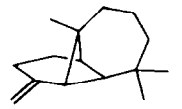

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Small quantities of isopimara-8,15-diene (**22**) and isopimara-7,15-diene (**23**) were also identified by their GC retention data and, in the case of (**23**), by comparison of the ^{1}H NMR spectrum of a mixture with that of the authentic compound. The oxygenated diterpenes, ferruginol (**24**) and (−)-8β-hydroxyisopimarene (**25**) were also isolated and characterized.

Ferruginol (**24**) was isolated from the bark of *Podocarpus ferrugineus* [28], a close relative of *P. spicatus*. To date there has been no reported study of the chemical composition of matai bark, but the ferruginol obtained in this study may well have arisen from bark on the twigs which were extracted.

This is the first reported isolation of normal rosadiene (**13**). *ent*-Rosadiene has been reported in *Dacrydium intermedium* [29] while (±)-rosadiene has been synthesized [30]. Normal sandaracopimaradiene (**15**) has only been reported once before from a natural source, castor bean (*Ricinus communis*) seedlings [31].

Correlation analyses—sesquiterpenes

The level of each terpenoid was calculated relative to an internal standard (octadecane). Correlation analyses were conducted by means of non-parametric statistics as in our study of *D. cupressinum* [32]. Combination of the data for all the samples studied revealed groups of terpenes whose levels were closely inter-related. The statistical significance of the associations, which were not normally distributed, was tested by calculating Kendall rank correlation coefficients [33]. The data are displayed as single-linkage cluster analyses [34] (Fig. 1). For the sesquiterpenes, all the correlations were statistically significant ($p < 0.05$). However, no particularly strong correlations were observed between any of the sesquiterpene hydrocarbons.

The most striking observation is the very low correlation of longifolene (**1**) with any of the other sesquiterpenes. This suggests that the cyclization of an acyclic precursor which leads to longifolene is carried out by a different enzyme than those which induce the cyclizations leading to the other sesquiterpenes. As previous work has found very strong correlation between **1** and α-longipinene (**26**) [35, 36] and a common biogenetic pathway has been proposed, it is at first surprising that no α-longipinene was noted in this study. However, in previous studies, the levels of longifolene have been approximately ten times those for α-longipinene and, in *P. spicatus*, the level of longifolene is low.

Although the correlations are not strong, there is a clustering of germacrene-D (**2**) and bicyclogermacrene (**3**) to which δ-cadinene (**4**) and α-copaene (**6**) are subsequently added. These quantitative correlations between germacrene-D and bicyclogermacrene, and between germacrene-D and δ-cadinene are supported by strong qualitative correlations. Out of 116 literature reports of bicyclogermacrene uncovered in a recent literature search

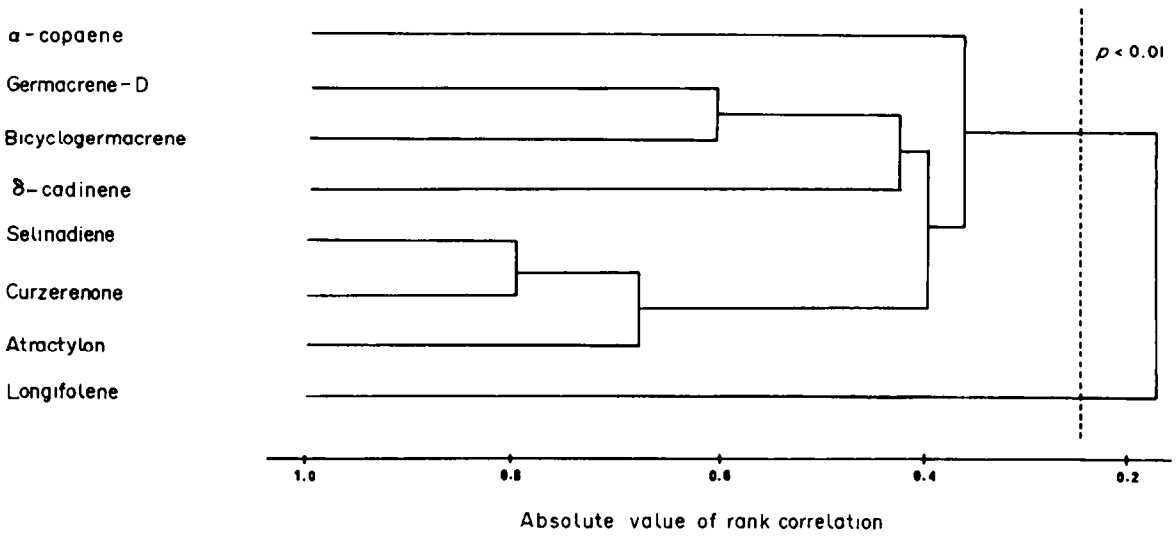

Fig. 1. Cluster analysis of sesquiterpene correlations. Data from 49 matai.

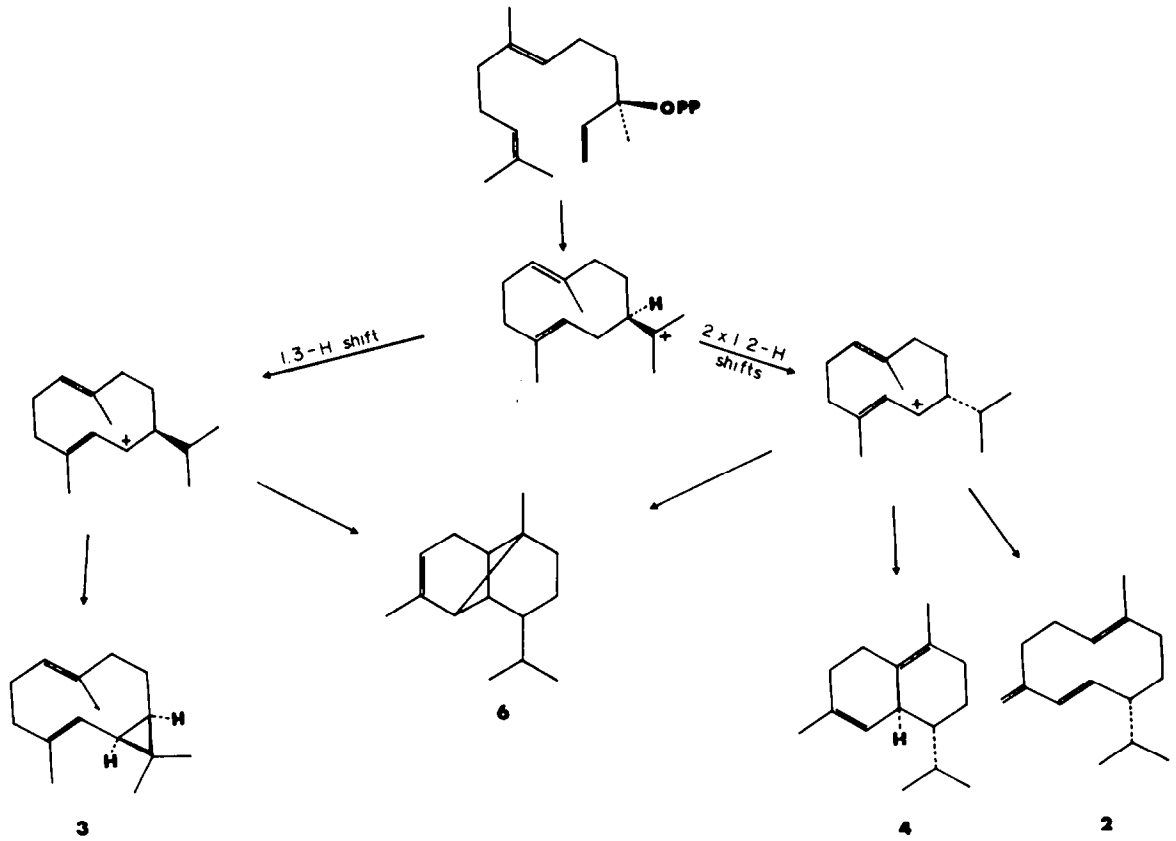

Fig. 2. Synthesis of germacrene related sesquiterpenes.

[37], 86 are in conjunction with germacrene-D. Similarly, for 495 reported findings of δ-cadinene, germacrene-D co-occurs in 103 cases. α-Copaene is also often found in association with the germacrenes. Current biogenetic hypotheses [38] suggest that these sesquiterpenes can all be derived from the same initial cyclization of nerolidyl pyrophosphate, although many steps are involved in proceeding to the sesquiterpenes from the common intermediate (Fig. 2). It is therefore not surprising that the correlations seen here are weak. The fact that the germacrene-D and the bicyclogermacrene have opposite stereochemistries is surprising. The co-occurrence of

germacrene-type sesquiterpenes of opposite stereochemistry has been reported before [17, 19, 21]. This has been explained in terms of enantiomeric germacrenoidal precursors, or alternatively, in terms of a single enantiomer of the precursor from which the two stereochemistries arise through alternative hydride shift pathways as is shown in Fig. 2.

One of the strongest correlations in the sesquiterpene data is between the oxygenated compounds, curzerenone (**8**) and atractylon (**9**). This is not surprising in view of their close structural similarities. The correlation of selina-7(11),4(14)-diene (**5**) with these two furano sesquiterpenes is higher than the correlation of the selinadiene with any of the other sesquiterpene hydrocarbons. Curzerenone, atractylon and the selinadiene all have negative correlations with all the other sesquiterpenes, except for longifolene. This suggests that they are formed by a pathway which is in competition with that which leads to the formation of germacrene-D, bicyclogermacrene, δ-cadinene and α-copaene. A proposal that curzerenone type sesquiterpenes are biosynthesized from germacrene type precursors [39] could explain this substitutional relationship (negative correlation). A possible explanation for the strong correlation between the selinadiene and the furanosesquiterpenes is also given (Fig. 3). In this pathway, the initial folding of farnesyl pyrophosphate leads to a 3*R* enantiomer of nerolidyl pyrophosphate. This, after cyclization, could lead to an intermediate common to atractylon, curzerenone and selina-4(14),7(11)-diene.

Correlation analyses—diterpenes

The most studied biosynthesis in the diterpenes has been that of kaurene (**14**) [40]. Two classes of enzymic activity have been noted. One, so called a activity, is associated with cyclization of geranylgeranyl pyrophosphate to bicyclic species, and the other (*b* activity) involves subsequent modification of the carbon skeleton. In

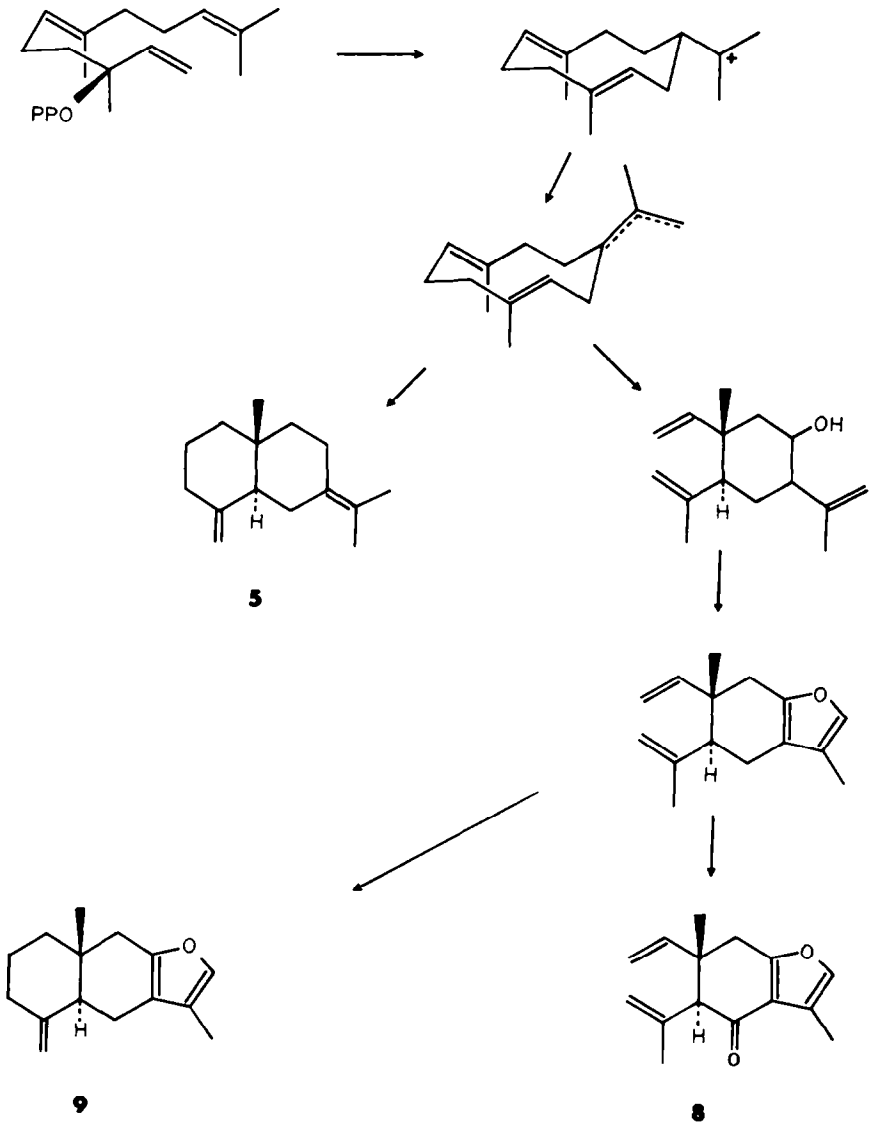

Fig. 3. Synthesis of furanosesquiterpenes.

Dacrydium intermedium, *a* activities of two types were noted, leading to either normal or *ent* diterpenes [29]. Associated with each *a* activity were various *b* activities which lead to tricyclic and tetracyclic species.

In matai, diterpenes from both the *ent* and normal stereochemical series are once again present. The *ent* series is represented by *ent*-sclarene (**12**) and *ent*-sandaracopimaradiene (**16**), while the normal series is more predominant with rimuene (**10**), beyerene (**11**), rosadiene (**13**), kaurene (**14**), sandaracopimaradiene (**15**), phyllocladene (**17**), pimara-8(14),15-diene (**20**), the isopimaradienes (**22**) and (**23**) and 8β-hydroxypimarene (**25**). There is evidence of only one *b* activity in the *ent* series, that leading to **16**, but examples of several different *b* activities can be seen in the normal series (Fig. 4). Added to this is the occurrence of two distinct sets within the normal series; those with or derived from the C-13 *S* stereochemistry and those derived from a C-13 *R* stereochemistry. These two sets are present for each *b* activity except that there is no C-13 *S* analogue for beyerene.

When the data for diterpene levels was submitted to cluster analysis, significant correlations were observed between all the diterpenes. A dendrogram representation of the results is given in Fig. 5.

The strongest clustering is found between kaurene (**14**) and beyerene (**11**), to which pimara-8(14),15-diene (**20**) then joins. This is consistent with the biogenetic scheme proposed in Fig. 4, as all three are derived from the common intermediate (**27**). Beyerene and kaurene diverge at (**28**) and are therefore more strongly correlated. In a similar manner, rosa-5,15-diene (**13**) would be expected to cluster into the kaurene, beyerene, pimaradiene cluster. However, under the conditions of the initial investigation, it did not prove possible to separate rosadiene from sclarene. Thus, in the correlation analysis these two diterpenes appear as one variable. Furthermore, the levels of sclarene were later shown to be high relative to those of rosadiene and the latter peak was seldom very significant.

The clustering of sandaracopimaradiene (**15**) and (**16**) and 8β-hydroxyisopimarene (**25**) is also significant. This supports the contention, as in Fig. 4, that these two diterpenes are derived from a common intermediate (**29**). The level of correlation of the sandaracopimaradienes with alcohol (**25**) may be higher than is reported here, as the GC analyses could not distinguish between the enantiomers (**15**) and (**16**), and (**25**) is presumably only correlated with the normal enantiomer (**15**). Sandaracopimaradiene and 8β-hydroxyisopimarene have previously been found together in *Dacrydium colensoi* [41] and in *Senecio subrubriflorus* [42].

As *b* activities are observed in both the normal and enantiomeric series, it is not clear whether the *b* enzymes have low substrate specificities, or whether there are pairs of stereospecific enzymes. The isolation of only one diterpene derived from *b* activity in the *ent* series suggests the latter. A test of the specificity of the processes occurring within the normal series was made by making the assumption that the *b* enzymes have low substrate specificity. If this is so, the relative levels of the two sets of compounds derived from the two different C-13 stereo-

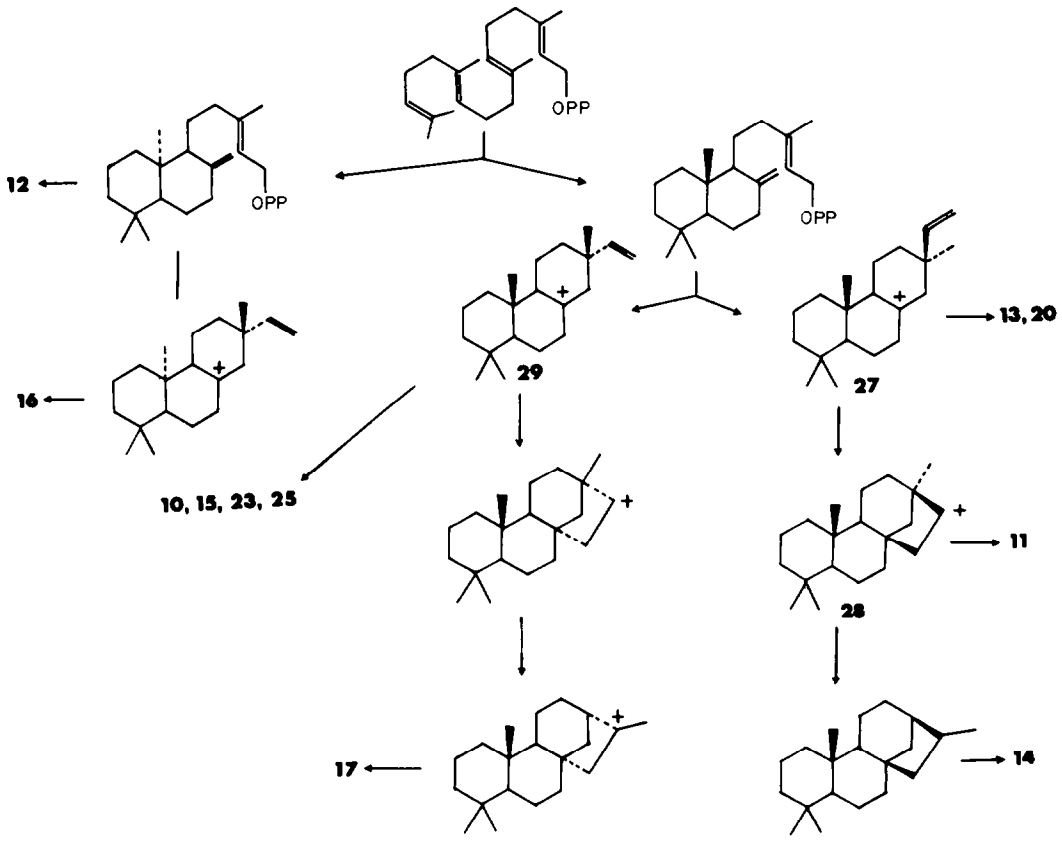

Fig. 4. Synthesis of diterpenes in *Podocarpus spicatus*.

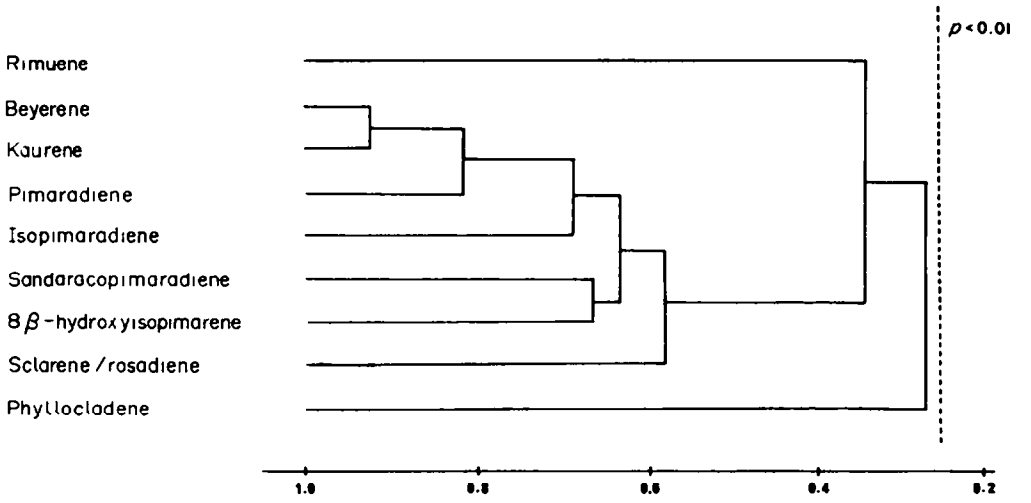

Fig. 5.

chemistries should be the same. This assumption was tested using the Wilcoxon statistical test, a non parametric test to ascertain whether the means of two populations are the same, assuming they both have the same distribution [33]. The levels of the diterpenes comprising each group were added together to give a group statistic for each tree. These group statistics were then compared throughout the populations. As the test proved to be highly significant, ($p < 0.01$) the levels of the two sets of normal diterpenes are different. Thus it seems probable that there are different enzymes synthesizing the two sets.

Geographic variations

The Kruskal–Wallis one-way analysis of variance test [33, 34] was used to test the samples from various regions of New Zealand for geographical variation. All the samples were ranked according to the value of the variable in question, and then the rank sums of the different groups were tested for significance.

Analysis of the matai data showed highly significant ($p < 0.01$) differences between the populations for all the named terpenes, with the exception of longifolene and bicyclogermacrene.

The medians of the individual terpene levels for each population, for asymmetric distributions, provide a more representative measure of location than the means. For example, Zavarin *et al.* used the medians of monoterpene levels in the populations of *Abies concolor* (Pinaceae) to derive similarity indices, and found two clusters corresponding to two geographic areas [43]. The population medians for the named terpenes found in matai were calculated, and the resultant matrices of distances for sesqui- and diterpenes are given in Table 2. In the diterpene case, the Motueka sample is separated from the other samples, while, in the sesquiterpene case it is the Hokitika sample that is separated. This is not surprising, as different enzyme systems are involved in sesqui- and diterpene biosynthesis. These could evolve separately.

Table 2. Distances between matai populations

	Berwick	Hokitika	Pureora	Motueka	Tuatapere
(a) Based on sesquiterpene levels					
Berwick	0.00				
Hokitika	0.35	0.00			
Pureora	0.08	0.36	0.00		
Motueka	0.12	0.28	0.13	0.00	
Tuatapere	0.16	0.31	0.19	0.11	0.00
(b) Based on diterpene levels					
Berwick	0.00				
Hokitika	0.56	0.00			
Pureora	0.83	0.61	0.00		
Motueka	2.24	2.48	3.06	0.00	
Tuatapere	0.54	0.79	1.27	1.88	0.00

CONCLUSIONS

We have reported here the identification of seven sesquiterpene hydrocarbons, included in which are representatives of germacrene type sesquiterpenes belonging to both C-10 stereochemical series. Also isolated were two furano-sesquiterpenes, nine diterpene hydrocarbons and two oxygenated diterpenes. Of the diterpenes only phyllocladene (**17**) and kaurene (**14**) have been positively identified in previous examinations of *P. spicatus*. The GC peak which was previously attributed to 'cupressene' [13], may have had contributions from beyerene (**11**), sclarene (**12**) and rosadiene (**13**), as these compounds may not have been resolved under packed column gas chromatographic conditions. The previously reported isophyllocladene and isokaurene were not observed.

EXPERIMENTAL

General. Sampling, extraction and data handling methods are as described in reference [32]. Specific rotations were recorded as

$CHCl_3$ solutions. NMR spectra were recorded as $CDCl_3$ solutions.

Bulk extractions. Individual terpenes were isolated by bulk extraction of foliage which had been shown to have a high level of the desired component. The following example illustrates the general procedure. Foliage (240 g) was ground in a Wiley mill and the solids were slurried with H_2O (*ca* 750 ml) containing a small amount of silicone anti-foam agent. Steam distillation with concurrent extraction into hexane (50 ml) for four days gave, after solvent removal, a green oil (2.38 g). The extract was eluted through alumina (66 g) with hexane (100 ml) followed by Et_2O (2 × 100 ml). The ether fraction (0.230 g) was chromatographed on silica (15% ether/hexane) and gave: (a) at high R_f, 8β-hydroxyisopimarene (**25**) (0.078 g), identified by comparison with an authentic sample (IR, ^{1}H NMR and GC); ^{13}C NMR δ15.7 (*q*, C-20), 17.1 (*t*, C-11), 17.9 (*t*, C-2), 18.5 (*t*, C-6), 21.7 (*q*, C-19), 24.4 (*q*, C-17), 33.5 (*q*, C-18), 33.5 (*s*, C-4), 36.4 (*s*, C-10), 37.2 (*s*, C-13), 38.2 (*t*, C-12), 39.5, (*t*, C-1), 42.2 (*t*, C-3), 43.6 (*t*, C-7), 51.7 (*t*, C-14), 56.6 (*d*, C-9), 57.1 (*d*, C-5), 72.5 (*s*, C-8), 108.5 (*t*, C-16), 151.6 (*d*, C-15); $[\alpha]_D^{20}$ −8° ($CHCl_3$; *c* 1.0) (lit. −7° [41]); retention indices on SE-30: 2077 (170°); 2105 (190°); (b) at medium R_f, ferruginol (**24**) (0.031 g) identified by comparison with an authentic sample (IR, ^{1}H NMR, GC and MS).

Curzerenone (**8**) was isolated similarly from an ether eluent; UV λ_{max} nm (log ε) 272 (3.3); IR ν_{max} cm^{-1} 1674, 910, 734; ^{1}H NMR (90 MHz) δ1.20 (3H, *s*), 1.81 (3H, *s*), 2.15 (3H, *s*), 2.91 (1H, *d*, *J* = 17 Hz), 2.74 (1H, *d*, *J* = 17 Hz), 3.00 (1H, *s*), 4.71 (1H, *d*, *J* = 10 Hz), 4.98 (1H, *d*, *J* = 10 Hz), 4.92 (1H, *m*, *J* = −0.2, 17.9 Hz), 4.93 (1H, *m*, *J* = −0.2, 9.3 Hz), 5.78 (1H, *dd*, *J* = 17.9, 9.3 Hz), 7.05 (1H, *s*); EIMS (probe) *m/z* 230; retention indices on OV-1: 1567 (130°); on BP-20: 2196 (155°); 2208 (165°).

Non polar components. The hexane eluent from the alumina column, after solvent removal, was fractionally distilled on a Kugelrohr apparatus. This gave rough separation into mono-, sesqui- and diterpene fractions. The individual terpene fractions were then separated via CC on $AgNO_3$-silica gel (hexane–benzene). These fractions were further purified by $AgNO_3$–silica gel prep. TLC (hexane–benzene, 7:3).

Longifolene (**1**) *and δ-cadinene* (**4**) were identified by comparison with authentic samples (IR, ^{1}H NMR and GC). Longifolene (**1**) had $[\alpha]_D^{20}$ +42° ($CHCl_3$; *c*5.0) (lit. +42° [44]); retention indices on OV-1: 1409 (130°); on BP-20: 1640 (155°); 1654 (165°). δ-Cadinene (**4**) had $[\alpha]_D^{20}$ −44° ($CHCl_3$; *c* 2.2) (lit. −68° [18]); retention indices on OV-1: 1510 (130°); on BP-20: 1785 (155°); 1794 (165°).

Selina-4(14),7(11)-diene (**5**) *and α-copaene* (**6**) were identified by comparison of their IR and ^{1}H NMR spectra with published data [22, 23, 45]. **5** had $[\alpha]_D^{20}$ +82° ($CHCl_3$; *c*2.9), +76° (*c*1.5), +77° (*c*0.6); retention indices on OV-1: 1522 (130°); on BP-20: 1816 (155°); 1828 (165°). **6** had $[\alpha]_D^{20}$ 0° (lit. −6.3° [45]); retention indices on OV-1: 1380 (130°); on BP-20: 1544 (150°); 1550 (155°); 1558 (165°).

δ-Elemene (**7**) was identified by comparison of the ^{1}H NMR spectrum of a mixture with longifolene with that of an authentic sample, and by its GC retention behaviour; retention indices on OV-1: 1340 (130°); 1531 (155°).

Rimuene (**10**), *beyerene* (**11**), ent-*sclarene* (**12**), *rosa-5,15-diene* (**13**), *kaurene* (**14**), *and sandaracopimaradienes* (**15**) *and* (**16**). These were identified by comparison with authentic samples (IR, ^{1}H NMR and GC retention indices on OV-1 and CW-20 M columns at 170 and 190° [46]). **10** had $[\alpha]_D^{20}$ +54° (lit. +53° [44]); retention indices on CW-20M: 2159 (170°); 2192 (190°); on SE-30: 1886 (170°); 1907 (190°). **11** had $[\alpha]_D^{20}$ −32° (lit. −50° [44]); retention indices on CW-20M: 2203 (170°); 2245 (190°); on SE-30: 1915 (170°); 1944 (190°). **12** had $[\alpha]_D^{20}$ −26° (lit. −22° [29]); retention indices on CW-20M: 2235 (170°); 2264 (190°); on SE-30: 1934 (190°). **13** had $[\alpha]_D^{20}$ +43° (lit. −40° [29]); retention indices on CW-20M: 2200 (170°); 2232 (190°); on SE-30: 1913 (170°); 1935 (190°). **14** had $[\alpha]_D^{20}$ +73° (lit. +101° [44]); retention indices on CW-20M: 2350 (170°); 2393 (190°); on SE-30: 2006 (170°); 2035 (190°). **15** had $[\alpha]_D^{20}$ −8° and the enantiomer **16** had $[\alpha]_D^{20}$ +14° (lit. −12° [44]); retention indices on CW-20M: 2245 (170°); 2283 (190°); on SE-30: 1945 (170°); 1971 (190°).

Pimara-8(14),15-diene (**20**) was identified as a component of a mixture with kaurene by comparison with an authentic sample (^{1}H NMR and GC); retention indices on CW-20M: 2216 (170°); 2249 (190°); on SE-30: 1927 (170°); 1956 (190°). Further confirmation was by conversion to pimara-8,15-diene (**21**) (see below).

Isopimara-8(15)-diene (**22**) was identified as a minor component by GC comparison with an authentic sample; retention indices on CW-20M: 2176 (170°); 2208 (190°); on SE-30: 1898 (170°); 1921 (190°).

Isopimara-7,15-diene (**23**) was identified as a component of a mixture with beyerene by comparison with an authentic sample (^{1}H NMR and GC); retention indices on CW-20M: 2297 (170°); 2331 (190°); on SE-30: 1968 (170°); 1994 (190°).

Germacrene-D (**2**), *bicyclogermacrene* (**3**) *and atractylon* (**9**). These components did not survive Kugelrohr distillation and were isolated from the hexane eluant of the alumina column by prep. TLC on silica (dry pentane elution). **2** and **3** were identified by comparison with authentic samples (IR, ^{1}H NMR and GC). **2** had $[\alpha]_D^{20}$ +210° ($CHCl_3$; *c*3.2) (lit. +190° [18]); retention indices on OV-1: 1473 (130°); on BP-20: 1752 (155°). **3** had $[\alpha]_D^{20}$ +66° ($CHCl_3$; *c* 3.0) (lit. +61° [44]); retention indices on OV-1: 1487 (130°); on BP-20: 1774 (155°); 1785 (165°). Atractylon (**9**) was identified by IR, ^{1}H NMR and MS [47, 48].

Pimara-8,15-diene (**21**). HCl was bubbled through a solution of the mixture of kaurene and pimara-8(14),15-diene (0.070 g) in $CHCl_3$ (5 ml) for 45 min. The solution was washed with satd $NaHCO_3$ and water and the solvent was removed to give an oil (0.089 g). The presence of **21** in the ensuing mixture was confirmed by comparison (^{1}H NMR and GC retention indices on OV-1 and CW-20M columns at 170° and 190°) with an authentic sample produced from manool [49].

Phyllocladene (**17**) was obtained as a mixture with kaurene (45% phyllocladene by GC). This had $[\alpha]_D^{20}$ +44° ($CHCl_3$; *c* 4.0). A solution of this mixture (0.090 g) and iodine (0.120 g) in dry benzene (20 ml) was heated under reflux for 9 hr. The cooled mixture was washed with aq. $Na_2S_2O_3$ soln (2 × 15 ml) and H_2O (2 × 15 ml), dried over dry $MgSO_4$, and evapd to give a yellow oil (0.090 g) which on prep. TLC on $AgNO_3$–silica gel (hexane–benzene, 7:3) gave (a) isokaurene (**18**) (0.020 g); $[\alpha]_D^{20}$ +16° ($CHCl_3$; *c* 2.0) (lit. +30° [50]); retention indices on CW-20M: 2282 (170°); 2324 (190°); on SE-30: 1970 (170°); 2000 (190°); and (b) at slightly higher R_f, isophyllocladene (**19**) (0.015 g); $[\alpha]_D^{20}$ +14° ($CHCl_3$; *c* 1.4) (lit. +16° [44]); retention indices on CW-20M: 2245 (170°); 2283 (190°); on SE-30: 1945 (170°); 1971 (170°). These substances were identified by comparison with authentic samples (^{1}H NMR and GC).

Cold pentane extractions. Foliage samples from three trees known to have high levels of germacrene-D, bicyclogermacrene, selina-4(14),7(11)-diene, curzerenone and atractylon were ground up in liquid N_2. The resulting material was divided into two approximately 5 g portions. To one was added aq. Na_2CO_3 and pentane, and the solution was stirred for 3 hr. The pentane layer was then decanted and the solution was analysed by GC. The second sample was treated as in the initial investigation. Comparison of the GC traces from the two extractions showed no significant differences in composition.

Cold water extraction. Foliage from a tree known to be high in curzerenone and atractylon was ground up in liquid N_2. The resulting material was added to water and the mixture was stirred

for 24 hr. The mixture was filtered and the filtrate, which had a pH of 4, was steam distilled and solvent extracted as for the initial investigation. Analysis by GC showed no significant levels of terpenes.

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