

THE TAXONOMIC DISTRIBUTION OF SOME DITERPENE HYDROCARBONS

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and

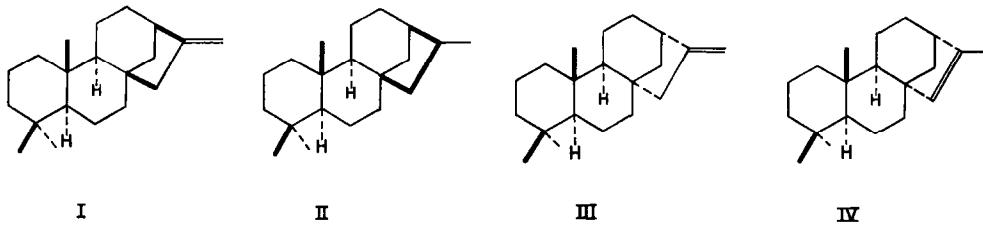
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Abstract—A survey of twenty-eight species of the Podocarpaceae and of nine related gymnosperms for diterpene hydrocarbons has been carried out by gas-liquid chromatography. The results have been used to clarify previously reported occurrences of diterpenes and to assess their taxonomic value.

DITERPENE hydrocarbons have been isolated from the essential oils of many species of the Podocarpaceae endemic to New Zealand and in a few cases from members of related families (see references in Table 2). The hydrocarbons include phyllocladene (I),¹ iso-phyllocladene (II),¹ (+)-kaurene (III),^{2,3*} and (+)-isokaurene (IV)^{2,3} of established structures and configurations, and also rimuene^{4,5} and cupressene⁶ whose structures have still to be determined.



Structural investigation of these latter compounds is hampered by their low yields and by the co-occurrence of one or more of the known hydrocarbons which are separated

* The enantiomer, (−)-kaurene also occurs naturally in some species, e.g. *Podocarpus macrophyllus* and *Agathis australis*.

¹ P. K. GRANT and R. HODGES, *Tetrahedron* **8**, 261 (1960); R. F. CHURCH, R. E. IRELAND and J. A. MARSHALL, *Tetrahedron Letters* No. 17, 1 (1960); R. B. TURNER and K. H. GÄNSHIRT, *Tetrahedron Letters* No. 7, 231 (1961); L. H. BRIGGS, B. F. CAIN, R. C. CAMBIE and B. R. DAVIS, *J. Chem. Soc.* 1840 (1962).

² C. DJERASSI, P. QUITT, E. MOSETTIG, R. C. CAMBIE, P. S. RUTLEDGE and L. H. BRIGGS, *J. Am. Chem. Soc.* **83**, 3720 (1961); H. VORBRÜEGGEN and C. DJERASSI, *J. Am. Chem. Soc.* **84**, 2990 (1962); J. W. AP SIMON and O. E. EDWARDS, *Can. J. Chem.* **40**, 896 (1962); B. E. CROSS, R. H. B. GALT, J. R. HANSON and W. KLYNE, *Tetrahedron Letters* No. 4, 145 (1962); R. A. BELL, R. E. IRELAND and R. A. PARTYKA, *J. Org. Chem.* **27**, 3741 (1962); B. E. CROSS, J. R. HANSON, L. H. BRIGGS, R. C. CAMBIE and P. S. RUTLEDGE, *Proc. Chem. Soc.* 17 (1963).

³ L. H. BRIGGS, B. F. CAIN, R. C. CAMBIE, B. R. DAVIS, P. S. RUTLEDGE and J. K. WILMSHURST, *J. Chem. Soc.* 1345 (1963).

⁴ L. H. BRIGGS, B. F. CAIN and J. K. WILMSHURST, *Chem. & Ind. (London)* 599 (1958); L. H. BRIGGS, B. F. CAIN and R. C. CAMBIE, *Tetrahedron Letters* No. 8, 17 (1959); R. E. IRELAND and P. W. SCHIESS, *Tetrahedron Letters* No. 25, 37 (1960); E. WENKERT and P. BEAK, *J. Am. Chem. Soc.* **83**, 998 (1961).

⁵ R. F. CHURCH and R. E. IRELAND, *Tetrahedron Letters* No. 14, 493 (1961).

⁶ L. H. BRIGGS and M. D. SUTHERLAND, *J. Org. Chem.* **7**, 397 (1942); L. H. BRIGGS, B. F. CAIN, B. R. DAVIS and J. K. WILMSHURST, *Tetrahedron Letters* No. 8, 8 (1959).

only with difficulty. In order to study these compounds, and to obtain a clearer pattern of their taxonomic distribution, the gas-liquid chromatographic behaviour of the known natural tri- and tetra-cyclic diterpene hydrocarbons has been investigated. The results have been applied to clarify anomalies connected with their occurrence and to determine their taxonomic value in a survey of the diterpene hydrocarbon content of twenty-eight species of the Podocarpaceae and nine related gymnosperms. Erdtman⁷ has already pointed out the possible taxonomic importance of these compounds if it became feasible to distinguish specific patterns of their distribution in the different genera and sub-genera. In view of the difficulties involved in the isolation and separation of the diterpene hydrocarbons by classical methods, the GLC technique appeared to offer the most satisfactory method for investigation of the problem.[†]

Previous investigations of diterpene hydrocarbons by GLC have been carried out by Eglinton and co-workers,^{8b} who examined the behaviour of rimuene, isophyllocladene, and two related derivatives using a column of 4% Apiezon L on Celite 545 at 199°, and by Church and Ireland⁵ who recorded, without details, the relative order of retention of rimuene and five related dienes of the pimaradiene type. In the present study a column of 1% E 301 silicone oil on Gas-chrom P. (Table 1, column 2) has been found to effect

TABLE 1. ABSOLUTE AND RELATIVE RETENTION TIMES

| Diterpene | Column 1 R_t (min.) | Column 1 | | Column 2 | |
|---|--------------------------|-------------|--------------|-------------|--------------|
| | | $R_{t_p}^*$ | R_t (min.) | $R_{t_p}^*$ | R_t (min.) |
| Phyllocladene | 25.0 | 1.00 | 22.0 | 1.00 | |
| Isophyllocladene | 20.9 | 0.84 | 18.5 | 0.84 | |
| Kaurene | 26.3 | 1.05 | 22.9 | 1.04 | |
| Isokaurene | 23.0 | 0.92 | 20.2 | 0.92 | |
| Rimuene | 16.0 | 0.64 | 14.1 | 0.64 | |
| Cupressene | 17.8 | 0.71 | 15.6 | 0.71 | |
| Phyllocladane (α -dihydrophyllocladene) | 25.5 | 1.02 | 22.7 | 1.03 | |
| Kaurane (α -dihydrokaurene) | 27.3 | 1.09 | 24.2 | 1.10 | |
| Dihydrorimuene | 18.5 | 0.74 | 16.3 | 0.74 | |
| Tetrahydromuene | 20.5 | 0.82 | 18.0 | 0.82 | |
| Sandaracopimaradiene | — | — | 18.3 | 0.83 | |

* R_{t_p} = retention time relative to phyllocladene.

excellent separation (see Fig. 1) of the hydrocarbons at temperatures of 120–140°. In earlier studies a column of 3% E 301 on Embacel (column 1) was used at a temperature of 160° but subsequent work was carried out using the former column. The absolute and relative (to phyllocladene as a standard) retention times for some known tri- and tetra-cyclic hydrocarbons are given for both columns in Table 1. The consistency of R_{t_p} values for both columns establishes the validity of applying these values for diagnostic purposes. Accordingly extracts of leaf samples of a number of plants were examined for diterpene hydro-

† Taxonomic studies utilizing gas-liquid chromatography have been previously carried out by Eglinton and co-workers.^{8a}

⁷ H. ERDTMAN, in *Perspectives in Organic Chemistry*, p. 453. Interscience Publishers Inc., New York (1956).

⁸ (a) G. EGLINTON, A. G. GONZALEZ, R. J. HAMILTON and R. A. RAPHAEL, *Phytochemistry*, 1, 89 (1962); G. EGLINTON, R. J. HAMILTON and M. MARTIN-SMITH, *Phytochemistry*, 1, 137 (1962).
(b) G. EGLINTON, R. J. HAMILTON, R. HODGES and R. A. RAPHAEL, *Chem. & Ind. (London)* 955 (1959).

carbons and the results are listed in Table 2. The *Podocarpus* species have been classified according to the revision of this genus by Buchholtz and Gray.⁹ Authoritative names for New Zealand species are those given by Allan¹⁰ in a recent manual of the N.Z. flora while those of other plants are according to Dallimore and Jackson.¹¹

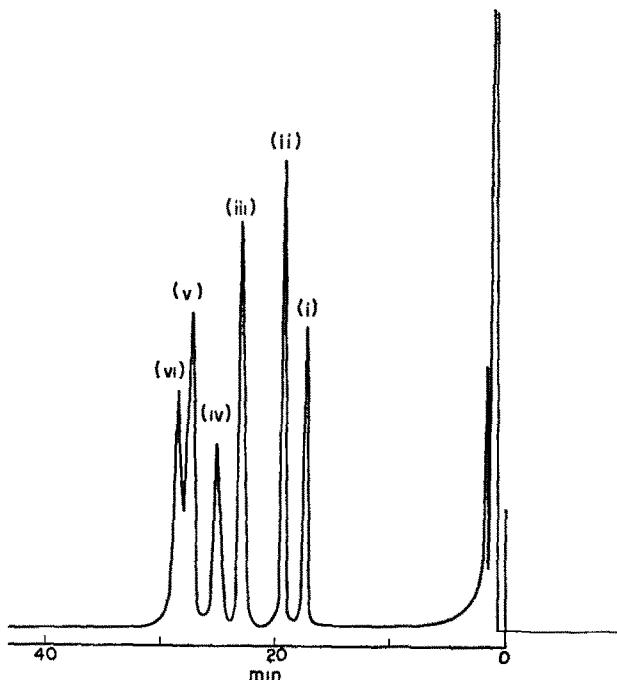


FIG. 1. GAS-LIQUID CHROMATOGRAM
 (i) Rimuene, (ii) Cupressene, (iii) Isophyllocladene, (iv) Isokaurene, (v) Phyllocladene and
 (vi) Kaurene.

The analysis could be readily carried out on 20–30 g of dried leaves, which were continuously extracted with ether and the hydrocarbon fraction isolated in light petroleum eluates by chromatography of the extract on deactivated alumina. Alkanes which accompanied the diterpenes possessed considerably longer retention times at the temperature conditions used for GLC and thus did not interfere, while compounds containing oxygen, were in general, retained on the alumina columns. In view of the diversity of the sources of plant material, and as extracts were examined over a period of 2 yr, no attempt has been made to carry out a comparative quantitative estimation of the diterpenes present. A semi-quantitative estimation of the relative amounts of diterpenes present in each species, however, has been indicated in Table 2. Generally those species whose natural habitat is New Zealand contained large amounts of diterpenes (up to 0.4%) while those from other localities contained much smaller amounts.

⁹ J. T. BUCHHOLTZ and N. E. GRAY, *J. Arnold Arboretum (Harvard Univ.)* **29**, 49 (1948).

¹⁰ H. H. ALLAN, *Flora of New Zealand*, Vol. 1, pp. 104–115. Govt. Printer, Wellington (1961).

¹¹ W. DALLIMORE and A. B. JACKSON, *A Handbook of the Coniferae*, 3rd Edit., Edward Arnold and Co. London (1948).

TABLE 2. NATURAL OCCURRENCE OF SOME DITERPENE HYDROCARBONS

| No. | Species | Natural habitat | Phyllocladene | Isophyllocladene | Kaurene | Isokaurene | Rimuene | Cupressene | References to previous work |
|----------------------|---------------------------------|-----------------|---------------|------------------|---------|------------|---------|------------|-----------------------------|
| PODOCARPACEAE | | | | | | | | | |
| <i>Podocarpus</i> | | | | | | | | | |
| Sect. Afrocarpus | | | | | | | | | |
| 1 | <i>P. falcatus</i> R. Br. | S. Africa | | — | | | | | * |
| 2 | <i>P. gracilior</i> Pilger | E. Africa | — | — | — | — | — | — | * |
| Sect. Dacrycarpus | | | | | | | | | |
| 3 | <i>P. dacrydioides</i> A. Rich | N. Z. | + | + | — | — | — | + | 12, 13, 14, 15 |
| Sect. Eupodocarpus A | | | | | | | | | |
| 4 | <i>P. henkelii</i> Stapf. | S. Africa | ++ | — | — | — | — | — | * |
| 5 | <i>P. latifolius</i> R. Br. | S. Africa | — | — | — | — | — | — | * |
| 6 | <i>P. milanjanus</i> Rendle | Cent. Africa | — | — | — | — | — | — | * |
| Sect. Eupodocarpus B | | | | | | | | | |
| 7 | <i>P. elatus</i> R. Br. | Australia | — | — | — | — | — | — | 16 |
| 8 | <i>P. macrophyllus</i> D. Don | Japan | +++ | +++ | — | — | — | — | 17, 18 |
| Sect. Eupodocarpus C | | | | | | | | | |
| 9 | <i>P. salignus</i> D. Don | Chile | — | — | — | — | — | — | * |
| Sect. Eupodocarpus D | | | | | | | | | |
| 10 | <i>P. neriifolia</i> Kirk | N. Z. | — | — | — | — | — | — | * |
| 11 | <i>P. alpinus</i> R. Br. | Tasmania | +++ | — | — | — | — | — | * |
| 12 | <i>P. hallii</i> Kirk | N. Z. | ++ | — | — | — | — | — | 19 |
| 13 | <i>P. nivalis</i> Hook. | N. Z. | ++ | — | — | — | — | — | 20 |
| 14 | <i>P. nubigenus</i> Lindley | Chile | ++ | — | — | — | — | — | * |
| 15 | <i>P. totara</i> G. Benn. | N. Z. | ++ | — | — | — | — | — | 12, 21 |
| Sect. Nageia | | | | | | | | | |
| 16 | <i>P. nagi</i> Zoll. et Moritz | Japan | — | — | — | — | — | — | * |
| Sect. Stachycarpus | | | | | | | | | |
| 17 | <i>P. andinus</i> Poeppig | Chile | ++ | — | — | — | — | — | * |
| 18 | <i>P. ferrugineus</i> D. Don | N. Z. | ++ | — | — | — | — | — | 22, 23 |
| 19 | <i>P. montanus</i> Loddiges | Cent. America | — | — | — | — | — | — | * |
| 20 | <i>P. spicatus</i> R. Br. | N. Z. | — | — | — | — | — | — | 24, 25, 26* |
| 21 | <i>P. usambarensis</i> Pilger | E. Africa | — | — | — | — | — | — | * |
| <i>Dacrydium</i> | | | | | | | | | |
| 22 | <i>D. bidwillii</i> Hook. | N. Z. | ++ | — | — | — | — | — | 27, 28 |
| 23 | <i>D. colensoi</i> Hook. | N. Z. | ++ | — | — | — | — | — | 14, 28, 29, 30 |
| 24 | <i>D. cupressinum</i> Lamb. | N. Z. | + | — | — | — | — | — | 31 |
| 25 | <i>D. franklini</i> Hook. | Tasmania | ++ | — | — | — | — | — | 28, 32, 33 |
| <i>Phyllocladus</i> | | | | | | | | | |
| 26 | <i>P. alpinus</i> Hook. | N. Z. | +++ | + | — | — | — | — | 28, 32, 33 |
| 27 | <i>P. glaucus</i> Carr. | N. Z. | ++ | — | — | — | — | — | 34 |
| 28 | <i>P. trichomanoides</i> D. Don | N. Z. | — | — | — | — | — | — | 35 |
| ARAUCARIACEAE | | | | | | | | | |
| <i>Agathis</i> | | | | | | | | | |
| 29 | <i>A. australis</i> Salisb. | N. Z. | — | — | ++ | ++ | — | +++ | 18, 36, 37 |
| <i>Araucaria</i> | | | | | | | | | |
| 30 | <i>A. araucana</i> K. Koch | Chile | — | — | — | — | — | — | * |
| 31 | <i>A. bidwillii</i> Hook. | Australia | — | — | — | — | — | — | * |
| 32 | <i>A. excelsa</i> R. Br. | Norfolk Is. | +++ | + | — | — | — | — | 28, 38, 39 |
| CUPRESSACEAE | | | | | | | | | |
| <i>Cupressus</i> | | | | | | | | | |
| 33 | <i>C. macrocarpa</i> Hartweg | California | + | ++ | + | + | — | +++ | 6 |

TABLE 2—continued

| No. | Species | Natural habitat | Phyllocladene | Isophyllocladene | Kaurene | Isokaurene | Rimuene | Cupressene | References to previous work |
|-----------------|---|-----------------|---------------|------------------|---------|------------|---------|------------|-----------------------------|
| 34 | <i>Libocedrus</i> <i>L. bidwillii</i> Hook. | N. Z. | + | + | — | (+) | — | — | 40, 41 |
| 35 | <i>L. plumosa</i> Sargent | N. Z. | — | + | +++ | — | — | — | 42, 43 |
| SCIADOPITYACEAE | | | | | | | | | |
| 36 | <i>Sciadopitys</i> <i>S. verticillata</i> Sieb. et Zucc. | Japan | — | + | +++ | — | — | — | 42, 43 |
| TAXODIACEAE | | | | | | | | | |
| 37 | <i>Cryptomeria</i> <i>C. japonica</i> D. Don | Japan | — | + | +++ | + | — | — | 44 |

* Species not previously investigated.

† Sample from Royal Kew Botanic Gardens.

Peak areas are classified as small (+), medium (++) and large (+++).

Samples 1, 2, 5, 6, 7, 9, 11, 17, 24, 25, 30 and 31 were obtained from authenticated species grown in the Oxford Botanic Gardens; samples 3, 4, 10, 11, 14, 18, 19, 20 and 21 from the Royal Kew Botanic Gardens; samples 8, 16, 36 and 37 from the Government Forest Experiment Station, Meguro, Tokyo; samples 12, 13 and 15 from the New Zealand Forest Survey. Samples 22, 23, 26, 27, 28, 29, 32, 33, 34, 35 and additional samples of 3, 7, 10, 12, 13, 15, 18, 20 and 24, were obtained from the Auckland and Central areas of the North Island, N.Z., through the kind assistance of Dr. B. F. Cain and Dr. R. C. Cooper. Additional samples of 36 and 37 were obtained from Bagley Wood, Oxford, through the kind assistance of Dr. W. R. H. Handley, Forestry Institute, University of Oxford. Except in the case of 20, duplicate samples gave similar results to those in the table.

- ¹² H. A. A. AITKEN, *J. Soc. Chem. Ind.* **48**, 344T (1929).
- ¹³ G. J. E. HUNTER, *J. Soc. Chem. Ind.* **51**, 394T (1932).
- ¹⁴ L. H. BRIGGS, *Rept. Australian New Zealand Assoc. Advance Sci.* **23**, 45 (1937).
- ¹⁵ L. H. BRIGGS, *J. New Zealand Inst. Chem.* **23**, 92 (1959).
- ¹⁶ L. H. BRIGGS, unpublished results.
- ¹⁷ K. NISHIDA and H. UOTA, *Bull. Agric. Chem. Soc. Japan* **6**, 82 (1930); *J. Agric. Chem. Soc. Japan* **6**, 1078 (1930), 7, 157, 957 (1931).
- ¹⁸ L. H. BRIGGS and R. W. CAWLEY, *J. Chem. Soc.* 1888 (1948).
- ¹⁹ L. H. BRIGGS, *Trans. Roy. Soc. New Zealand* **70**, 173 (1940).
- ²⁰ J. MURRAY, *J. Appl. Chem.* **10**, 366 (1960).
- ²¹ G. B. BEATH, *J. Soc. Chem. Ind.* **52**, 338T (1933).
- ²² J. R. HOSKING and W. F. SHORT, *Rec. trav. chim.* **47**, 834 (1928).
- ²³ L. H. BRIGGS, R. W. CAWLEY, J. A. LOE and W. I. TAYLOR, *J. Chem. Soc.* 956 (1950).
- ²⁴ J. M. BUTLER and J. T. HOLLOWAY, *J. Soc. Chem. Ind.* **58**, 223 (1939).
- ²⁵ L. H. BRIGGS and J. A. LOE, *J. Chem. Soc.* 958 (1950).
- ²⁶ J. R. McGIMPSEY and J. MURRAY, *J. Appl. Chem.* **10**, 340 (1960).
- ²⁷ W. J. BLACKIE, *J. Soc. Chem. Ind.* **48**, 357T (1929); **49**, 26T (1930).
- ²⁸ L. H. BRIGGS, *J. Chem. Soc.* 79 (1937).
- ²⁹ F. H. McDOWALL and H. J. FINLAY, *J. Soc. Chem. Ind.* **44**, 42T (1925).
- ³⁰ M. S. CARRIE, *J. Soc. Chem. Ind.* **51**, 367T (1932).
- ³¹ A. R. PENFOLD and J. L. SIMONSEN, *J. Proc. Roy. Soc. N. S. Wales* **63**, 95 (1929).
- ³² L. H. BRIGGS, *J. Soc. Chem. Ind.* **56**, 137T (1937).
- ³³ C. W. BRANDT, *New Zealand J. Sci. and Technol.* **20**, 88 (1938).
- ³⁴ E. G. BROOKER, *New Zealand J. Sci.* **2**, 212 (1959).
- ³⁵ L. H. BRIGGS and M. D. SUTHERLAND, *J. Org. Chem.* **13**, 1 (1948).
- ³⁶ J. R. HOSKING, *Rec. trav. chim.* **47**, 578 (1928); **49**, 1036 (1930).
- ³⁷ L. H. BRIGGS and W. I. TAYLOR, *J. Chem. Soc.* 407 (1950).
- ³⁸ L. H. BRIGGS, *J. Soc. Chem. Ind.* **60**, 222 (1941).
- ³⁹ L. H. BRIGGS and W. I. TAYLOR, *J. Soc. Chem. Ind.* **66**, 168 (1947).
- ⁴⁰ B. H. GOUDIE, *J. Soc. Chem. Ind.* **42**, 350T (1923).
- ⁴¹ K. S. BIRRELL, *J. Soc. Chem. Ind.* **51**, 397T (1932).
- ⁴² J. KAWAMURA, *Bull. Imp. Forestry Expt. Stat. Tokyo* No. **31**, 93 (1931).
- ⁴³ K. NISHIDA and H. UOTA, *J. Agr. Chem. Soc. Japan* **11**, 489 (1935); **12**, 308 (1936).
- ⁴⁴ S. UCHIDA, *J. Am. Chem. Soc.* **38**, 687 (1916).

The plants listed in Table 2 cover all the previously investigated species of the Podocarpaceae with the exception of *Phyllocladus rhomboidalis* A. Rich.⁴⁵ (Tasmania), *Dacrydium biforme* Pilger^{14,28,46,47} (N.Z.), *D. kirkii* F. Muell⁴⁸ (N.Z.), and *D. laxifolium* Hook.²⁰ (N.Z.) each of which has been shown to contain phyllocladene in their essential oils. Although all other plants now investigated contained one or more of the known diterpenes, unexpectedly, no diterpenes could be discerned in the extracts of five previously unexamined species *viz.* *Podocarpus falcatus*, *P. gracilior*, *P. salignus*, *P. usambarensis*, and *P. elatus*.*

Previous investigations of the *Dacrycarpus* species, *P. dacrydioides*, by Aitken¹² and Hunter¹³ did not reveal the presence of diterpenes, although Briggs¹⁴ suggested that the compounds with melting points 86° and 117–118° isolated by Hunter were of this type. Later, however, Briggs¹⁵ reported that Murray had failed to find diterpenes when the essential oil was reinvestigated. In the present work small amounts of phyllocladene, isophyllocladene, and cupressene have been shown to be present in the high boiling residues from Murray's extract, kindly provided by Dr. R. E. Corbett. In a further extract of a sample of the leaves of *P. dacrydioides* grown in the North Island of N. Z. the quantities of these diterpenes were barely discernable. From its melting point it would appear that Hunter's material, m.p. 86°, was impure phyllocladene and that Murray overlooked the comparatively small amounts present.

Surprisingly, phyllocladene and isophyllocladene were the major diterpenes found in *P. macrophyllus* during the present survey. Nishida and Uota¹⁷ had previously reported the presence of α -, β -, and γ -podocarpanes. α -Podocarpane was subsequently identified as (–)-kaurene¹⁸ while γ -podocarpane, now known to be (–)-kauran-16-ol,³ is probably an artefact of the isolation procedure. The oil, β -podocarpane, has recently been suggested to be impure (–)-kaurene.³ The presence of kaurene has been confirmed in the present work while one of a further two probable diterpenes in the leaves possessed an R_{t_p} value identical to that of cupressene.

The species *P. alpinus*, *P. hallii*, *P. nivalis* and *P. nubigenus* of the section *Eupodocarpus* D all contained large amounts of phyllocladene while *P. acutifolius* and *P. totara* were found to contain rimuene as the major component. Rimuene also occurred in *P. hallii*. The presence of phyllocladene in the essential oils of *P. hallii*¹⁹ and *P. nivalis*²⁰ has been previously reported while a diterpene, "totarene", isolated by Aitken¹² from *P. totara* was subsequently identified as rimuene by Beath.²¹ Murray,²⁰ however, reported that some samples of *P. totara* which he examined contained mainly phyllocladene and in the light of the failure to find any phyllocladene in the present sample of *P. totara* it appears that considerable variation of the hydrocarbon constituents within a single species can occur.

The difficulty of obtaining pure compounds in the diterpene hydrocarbon field and of rigorously determining the purity of the compounds prior to the advent of GLC is well illustrated by the previous work with the *Stachycarpus* species, *P. ferrugineus*. Hosking and Short²² initially reported the occurrence of a diterpene, "mirene", from this source. In a later investigation Briggs and co-workers²³ isolated phyllocladene, and (–)-kaurene from the same tree and showed that Hosking and Short's "mirene" was a mixture of these two compounds, the name "mirene" being transferred to a further diterpene not isolated by

* Professor L. H. Briggs has informed us that working on a larger scale he also failed to isolate diterpene hydrocarbons from the essential oil of *P. elatus*.

⁴⁵ R. T. BAKER and H. G. SMITH, *Pines of Australia* p. 419. Technological Museum, Sydney (1910).

⁴⁶ B. H. GOUDIE, *J. Soc. Chem. Ind.* **42**, 357T (1923).

⁴⁷ H. A. A. AITKEN, *J. Soc. Chem. Ind.* **47**, 223T (1928).

⁴⁸ L. H. BRIGGS and W. I. TAYLOR, *J. Org. Chem.* **12**, 551 (1947).

the former workers. This latter "mirene" has in turn⁴⁹ been shown from NMR data to be a mixture of phyllocladene and (+)-kaurene despite its apparent homogeneity according to usual criteria of purity. The non-homogeneity of a sample of "mirene" kindly provided by Professor L. H. Briggs was readily shown by GLC during the present investigation. In addition to phyllocladene and kaurene, the suspected²³ isophyllocladene, rimuene and cupressene have now been found to be present in *P. ferrugineus*.

The apparent variation within a species is exemplified by the previous work with *P. spicatus*. Butler and Holloway,²⁴ working with a sample of the South Island-grown tree, recorded the isolation of (+)-kaurene as 25 per cent of the essential oil whereas Briggs and Loe²⁵ found that the North Island-grown *P. spicatus* contained small amounts of phyllocladene. The presence of (+)-kaurene in a South Island-grown tree has been confirmed by Murray and McGimpsey.²⁶ A sample of a North Island-grown tree investigated in the present work was found to contain kaurene and a further hydrocarbon with R_{f_2} 0.74 but no trace of phyllocladene. However, a sample of *P. spicatus* obtained from Kew contained phyllocladene as the major diterpene and only a trace of kaurene. It appears that variation within this species can occur, regardless of geographical location of the source.

The four members of the *Dacrydium* genus in Table 2 were all found to contain considerable amounts of phyllocladene and lesser amounts of isophyllocladene. The former has previously been reported as a constituent of *D. colensoi*,^{14,27,28} *D. cupressinum*,^{14,29} and also a further two species *D. kirkii*⁴⁸ and *D. biforme*^{14,28} samples of which were not available during the present work. *D. cupressinum* also contains larger amounts of rimuene^{29,30} and is the present source for the isolation of the crystalline compound for structural studies. Small amounts of rimuene have now been shown to be present in *D. colensoi*, while kaurene together with two further major compounds with R_{f_2} values of 0.74 and 1.50, respectively, are additional constituents of the Tasmanian species, *D. franklini*. Carman and Grant⁵⁰ have reported the isolation of a bicyclic diterpene, biformene, from the heartwood of *D. biforme* and also its formation by the acid dehydration of its congener manoöl (cf.⁵¹). As it appeared possible that biformene might also occur in the leaves of *Dacrydium* species, an attempt was made to prepare it from manoöl for use as a reference sample. However, repetition of Carman and Grant's experiment using manoöl (which gave a single peak on GLC with no tailing (cf.⁸) for periods of 30, 45 and 60 min all gave mixtures of at least eleven compounds. In view of the difficulty in choosing which of the peaks corresponded to biformene it has not been possible to examine leaf samples for this hydrocarbon.

The three New Zealand *Phyllocladus* species, *P. alpinus*,^{28,32,33} *P. glaucus*,³⁴ and *P. trichomanoides*³⁵ have all been previously reported to contain phyllocladene while isophyllocladene has been isolated as an additional constituent of the latter tree. A further diterpene, b.p. 156–157/1 mm, also reported³⁵ from this latter source would appear to be either kaurene or rimuene, both of which have now been shown to occur in the leaves. The present work shows that, in addition to phyllocladene, isophyllocladene is common to all three species while rimuene is also present in small amount in *P. glaucus*.

In addition to members of the Podocarpaceae, a further nine related species from which diterpenes had been reported previously or in which diterpenes could be expected to be present were examined. These included four species belonging to the family Araucariaceae.

⁴⁹ L. H. BRIGGS, B. F. CAIN, R. C. CAMBIE, B. R. DAVIS and P. S. RUTLEDGE, *J. Chem. Soc.* 1850 (1962).

⁵⁰ R. M. CARMAN and P. K. GRANT, *J. Chem. Soc.* 2187 (1961).

⁵¹ S. BORY and C. ASSELINÉAU, *Bull. Soc. Chim. France* 7, 1355 (1961).

The New Zealand "kauri" *Agathis australis*^{18,36,37} contained, as expected, (—)-kaurene and (—)-isokaurene, but also considerable amounts of cupressene. The same compound was also found to be present in two *Araucaria* species, *A. bidwillii* and *A. araucana*, co-occurring with kaurene in the latter. The remaining member *A. excelsa* has previously been reported^{38,39} to contain phyllocladene and isophyllocladene both of which were again shown to be present.

Three members of the Cupressaceae were examined. *Cupressus macrocarpa* has been reported⁶ to contain cupressene and isophyllocladene. The present work showed that it also contained phyllocladene, kaurene, isokaurene, and two further unidentified hydrocarbons but with cupressene as the major constituent. During the investigation an original sample of cupressene isolated from *Cupressus macrocarpa*⁶ was shown to be a mixture of phyllocladene, isophyllocladene, and a major diterpene hydrocarbon (R_{t_p} 0.71) for which the name cupressene is retained. The same compound as ascertained by identical R_{t_p} value was also found to occur in comparatively large amounts in *Podocarpus ferrugineus*. Fractional crystallization of solid diterpene mixtures from large scale extracts of this latter source and GLC investigation showed that cupressene could be obtained in *ca.* 80 per cent purity by this method, but that further purification is then more readily effected by preparative GLC.

The two New Zealand *Libocedrus* species, *L. bidwillii* and *L. plumosa* both contained small amounts of isophyllocladene but the latter source also contained small amounts of phyllocladene, kaurene, isokaurene, and rimuene. A possible diterpene, m.p. 53–55°, which is not identical with rimuene of the same melting point, has been isolated by Birrell⁴¹ from *L. bidwillii*. The major constituent of the present extract of *L. bidwillii* has an R_{t_p} value of 1.18 and is presumably a diterpene while another compound with R_{t_p} 1.22 was present in *L. plumosa*.

The previous literature concerning the diterpene hydrocarbon content of the Japanese species *Sciadopitys verticillata*, which is now considered to belong to a separate family of its own, is confusing. Nishida and Uota⁴³ reported the occurrence of sciadopitene which was subsequently shown by Briggs^{14,28} to be identical with (+)-phyllocladene. Briggs also suggested that a further hydrocarbon isolated from the same source by Kawamura⁴² was probably the enantiomer of the usual (—)-isophyllocladene. It should be pointed out however, that it is unlikely that (+)-phyllocladene should occur with (—)-isophyllocladene in the same leaves. The position is further complicated by the work of Kawamura who also isolated (—)-kaurene¹⁸ (α -podocarpane⁴²) and a further isomer, δ -podocarpane which is probably identical with (—)-isokaurene.¹⁸ The present work showed that kaurene is the major diterpene constituent while a smaller amount of isophyllocladene is also present. No trace of phyllocladene or isokaurene could be found in the leaf extracts of two samples of the plant, one originating from Japan and the other from Oxford, but two further constituents with R_{t_p} values of 0.74 and 1.34, respectively, were present in each sample. The absence of isokaurene can be readily explained since this compound could be formed under the conditions of Kawamura's isolation procedure. Nishida and Uota's isolation of (+)-phyllocladene from this source, however, is inexplicable on the basis of the present results unless variation of hydrocarbon constituents occurs in the plant and moreover, unless (+)-phyllocladene and (—)-kaurene can co-occur in the same leaves. It is perhaps noteworthy, that if Nishida and Uota's results for *S. verticillata* and *P. macrophyllus* (see earlier) were interchanged no anomalies would exist.

A single species of the Taxodiaceae, *viz.* *Cryptomeria japonica*, has been previously reported to contain diterpenes. Uchida⁴⁴ isolated " α -cryptomerene" which has now been shown³ to be identical with (—)-isokaurene, while it has been suggested³ that β -cryptomerene, isolated as a by-product in the preparation of " α -cryptomerene hydrochloride" by Uchida is (—)-kauran-16-ol. Kaurene, isokaurene, isophyllocladene, and the hydrocarbon with R_{f_p} 0.74, were all shown to be present in the current work.

Although only a limited number of species of the Podocarpaceae* have been examined it would appear from the present survey that the diterpene hydrocarbons are of doubtful taxonomic value. From the observation that these compounds were absent in five species distributed over three of the sub-sections of the genus *Podocarpus* and whose natural habitats are quite distinct, the presence of these compounds is not even diagnostic of the family. Moreover no clear patterns of characteristic constituents emerge for the different genera or sub-sections of the family. Nevertheless, a few comments can be made. From the results in Table 2 it is apparent that small amounts of isophyllocladene normally accompany the occurrence of phyllocladene but the same does not necessarily hold in the case of kaurene and isokaurene. While it is known that phyllocladene and kaurene can be partially isomerized to isophyllocladene and isokaurene, respectively, and that the reverse can occur on activated alumina the same does not normally occur on alumina which has been deactivated. However, while care has been taken to avoid conditions leading to isomerization of the diterpenes the possibility of isomerization during the extraction and subsequent procedures cannot be overlooked.

Without exception all New Zealand species of the Podocarpaceae contain diterpenes which are usually present in considerable amount. Of these species all contained either phyllocladene or isophyllocladene with the exception of *P. acutifolius* and possibly *P. spicatus* (see²⁸) but this may be merely a factor of local variation. Rimuene and especially cupressene are of much wider distribution than previously thought and it is clear from the presence of unidentified peaks in GLC examination that further diterpenes, not identical with any of the known compounds, are present in certain species.

EXPERIMENTAL

Gas-liquid chromatography

GLC investigation of pure diterpene hydrocarbon standards was carried out on:

Column 1—a column (350 cm) of Embacel coated with 3% E 301 silicone oil (supplied by I.C.I., Nobel Division, Glasgow); column temp. 160°, inlet temp. 180°; inlet press. 13 lb/in²; carrier gas, hydrogen; flame ionization detector.

Column 2—a presiliconized† column (120 cm) of Gas-chrom P (100–120 mesh) coated with 1% E 301 silicone oil; column temp. 140°, inlet temp. 160°, inlet press 7 lb/in²; carrier gas, argon; Pye "Argon" Chromatograph.

Samples were injected (0.063 μ l.) in 1% chloroform solution.

To assess the linearity of the β -ray ionization system, standard solutions were run using both detection systems. The peak areas were found to be reproducible to $\pm 5\%$ with the β -ray ionization detector (detector voltage 1250, sensitivity $\times 10$).

* The family embraces approximately 100 species.

† The column was prepared according to unpublished directions kindly provided by Dr. E. C. Horning.

Preparation of leaf extracts

In general the leaves of the plant to be examined were air-dried, finely ground and a 20–40 g sample continuously extracted (Soxhlet) with ether for 12–18 hr. The extract was concentrated to dryness, the residue dissolved in the minimum volume of benzene, and chromatographed on alumina (P. Spence grade H) (deactivated with 5 per cent of 10% acetic acid) packed in light petroleum (b.p. 60–80°). Initial fractions eluted with light petroleum were examined for the presence of diterpene hydrocarbons by GLC comparison with authentic samples. Samples of all leaf extracts and specimens of all leaves used have been retained in the Department of Chemistry, University of Auckland.

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