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THE DITERPENES FROM THE LEAVES OF *CRYPTOMERIA JAPONICA*

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Abstract—*Cryptomeria japonica* trees can be sub-divided into four chemically distinct varieties on the basis of the diterpene hydrocarbon content of their leaves. Seven other species from the Taxodiaceae have been analysed for diterpenes.

WE HAVE extended our previous[1] investigations into the hydrocarbon components obtained from the leaves of *Cryptomeria japonica* D. Don. The results that emerge are somewhat more complex than appeared from our earlier, more limited, enquiry.

In our new experiments we have examined the hydrocarbons from (*a*) single trees grown from the same seed sources as before but in a different locality and (*b*) six trees chosen at random from a plot raised from the same seed source in each of the two localities. The compositions of the hydrocarbon fractions are summarized in Table 1. The results already published[1] are included in the table for the purpose of comparison.

TABLE 1. DITERPENE ANALYSES OF DIFFERENT *Cryptomeria japonica* TREES

Forestry Commission identification number†	Diterpenes present in the leaves			
	Lochgoil		Glynn	
	1 tree/plot	6 trees/plot	1 tree/plot	6 trees/plot
55/106			K	
55/108			K	
55/114	K*	6K	K	
55/115	P*	3K + 3P	K	3K + 2P + 1S
55/116	K*	6K	K	
55/117			S	2K + 4S*
55/118			K	
55/119	K*	5K + 1P	K	
55/120	K*	6K	K	
53/210	K*	6K		
56/227	K*	6K	S	5K* + 1(K + P)

Location of other trees examined		Location of other trees examined	
Kilmun, Argyll (parent of 53/210)	K*	Culzean Castle, Ayrshire (*C. japonica elegans*)	K*
Kilmun, Argyll	P*	Corsehill Gardens, Ayr (*C. japonica elegans*)	K
Loch Lomond Park	P*	Botanic Gardens, University of Liege (*C. japonica* var. Lobbi Carriere?)	(K + P*)
Auchincruive, Ayr	K*		

Key: K = kaurene, P = phyllocladene, S = sclarene.

* These diterpenes were isolated by chromatography.

† All seeds (except 56/227, from Royal Botanic Gardens, Edinburgh) were supplied to the Forestry Commission by Professor B. Lindquist, Goteborg, Sweden.

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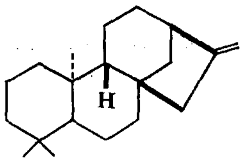
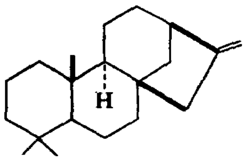
[1] R. A. APPLETON, R. McCRINDLE and K. H. OVERTON, *Phytochem.* 7, 135 (1968).

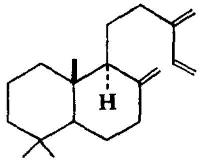
Several points deserve mention; four of these are apparent from the table. (1) The enlarged sample underlines the fact that, based on the trees examined, *C. japonica* is predominantly a (–)-kaurene (I) producer (sixty-six out of eighty-four trees). Where we have been able to isolate sufficient material for the measurement of optical rotation, this has shown that in every case hydrocarbons belonging to one antipodal series only occurred, i.e. (–)-kaurene (fourteen out of sixty-six trees), (+)-phyllocladene (II) (three out of nine trees) and (+)-sclarene[2] (III) (four out of seven trees) even where kaurene and sclarene co-occur. (2) Trees from the same seed source are *not*, as we had previously supposed, uniform in their hydrocarbon production (see Trees 55/115, 55/117, 55/119 and 56/227). (3) We have now detected sclarene as the major component alternative to (–)-kaurene and (+)-phyllocladene in trees from three seed sources (55/115, 55/117 and 56/227). We also find, by use of more sensitive GLC equipment, that the previously examined hydrocarbon fractions which we had thought to be homogeneous and to consist of either (–)-kaurene or (+)-phyllocladene are in fact contaminated by about 1 % of material which has the same GLC retention time as the other hydrocarbon. (4) In only two trees out of eighty-four are there mixtures of kaurene and phyllocladene in comparable amounts (4:3). One of these trees was a cultivar.



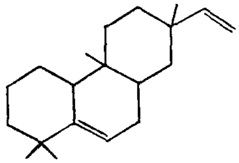
(–)-Kaurene (I)



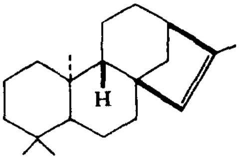
(+)-Phyllocladene (II)



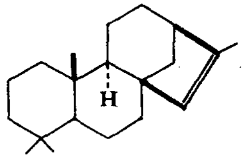
(+)-Sclarene (III)



Rimuene (IV)



Isokaurene (V)



Isophyllocladene (VI)

We have also examined seven other species representing seven of the ten genera which make up the Taxodiaceae.[3] Of the three major components found in *C. japonica* (kaurene, phyllocladene and sclarene) only *Sciadopitys verticillata* (two trees) contains (–)-kaurene (and no phyllocladene). There are two reports[4] in the literature concerning the hydrocarbon fractions from *S. verticillata*; in one case kaurene was found, in the other phyllocladene. *Athrotaxis selaginoides* contains rimuene (IV) as the major hydrocarbon. *Sequoia sempervirens*, *Sequoiadendron giganteum*, *Metasequoia glyptostroboides*, *Taxodium distichum* and *Cunninghamia lanceolata* do not contain kaurene, isokaurene (V),* phyllocladene, isophyllocladene (VI)* or sclarene in amounts detectable by the analytical methods employed.

* If fresh extracts of *C. japonica* in light petroleum are left in contact with the crushed twigs for 2–3 hr then appreciable isomerization of kaurene into isokaurene and phyllocladene into isophyllacladene occurs.

[2] A. Ahond and B. Gastambide, *Bull. Soc. chim. France* 4533 (1967).

[3] R. Hegnauer, *Chemotaxonomie der Pflanzen*, Vol. 1, p. 421, Birkhauser Verlag.

[4] K. Nishida and H. Uota, *J. Agric. Chem. Soc. Japan* **12**, 308 (1936); L. H. Briggs and R. W. Cawley, *J. Chem. Soc.* 1888 (1948).

EXPERIMENTAL

NMR spectra were determined in $CDCl_3$ on a Varian Associates HA 100 with T.M.S. as internal standard. GLC experiments were carried out with a Perkin–Elmer F11 gas chromatograph using a stainless-steel column (4 m × $\frac{1}{16}$ in. o.d.) at 180° packed with 2·5 per cent SE-30 and GC-MS experiments with an L.K.B. 9000 A gas chromatograph-mass spectrometer using a glass column (3 m × 6 mm) with a 1 per cent SE-30 packing at 150°.

General Extraction and Identification of Hydrocarbons

The foliage was air-dried, crushed and extracted with light petroleum (b.p. 40–60°) by stirring for 10 min at 20°. The plant material was filtered off and the solvent evaporated *in vacuo*. The resulting green wax was redissolved in light petroleum–ether (20:1) and chromatographed over neutral alumina (Woelm: Grade I). The eluates were evaporated to small bulk and the identity of the extracted diterpene hydrocarbons established by GLC (see Table 1).

Isolation of Diterpenes

When sufficient material was available (see Table 1*) the diterpenes recovered from chromatography over alumina were purified further by either column or TLC over $SiO_2/AgNO_3$ (10:1) and elution with light petroleum. The diterpenes obtained, (−)-kaurene, (+)-phyllocladene and (+)-sclarene were identical (NMR, GLC, GC-MS and $[\alpha]_D$) with authentic samples. The authentic sample of kaurene had m.p. 51–52° and $[\alpha]_D - 78°$ ($CHCl_3$); phyllocladene had m.p. 98–99° and $[\alpha]_D + 13°$ ($CHCl_3$); sclarene (obtained as an oil by dehydration[2] of manool) had $[\alpha]_D + 31°$; NMR resonances at τ3·62 (*q*; $J = 18, 11$ Hz; 1H-14), 4·77 (*d*; $J = 18$ Hz; 1H-15), 4·95 (*d*; $J = 11$ Hz; 1H-15), 5·00 (*s*; 2H-16), 5·14 (*bs*; 1H-17), 5·43 (*bs*; 1H-17), 9·12, 9·19 and 9·31 (all *s*;—CH_3's). Rimuene was identified by its GLC retention time and mass spectral fragmentation pattern on GC-MS.

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