Phytochemistry, 1968, Vol. 7, pp. 135 to 137. Pergamon Press. Printed in England

THE ALTERNATIVE OCCURRENCE OF (−)-KAURENE OR (+)-PHYLLOCLADENE IN THE LEAVES OF *CRYPTOMERIA JAPONICA* D. DON

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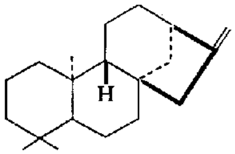
(*Received* 5 *July* 1967)

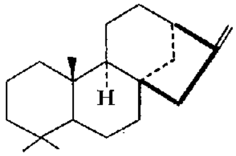
Abstract—Foliage from *Cryptomeria japonica* D. Don was analysed for diterpenoid hydrocarbons. Samples grown from some seed sources furnished (−)-kaurene as the sole product of this type, whilst those from other sources yielded only (+)-phyllocladene. These observations were found to be seasonally independent and it is concluded that *C. japonica* exists in two chemically distinct forms.

INTRODUCTION

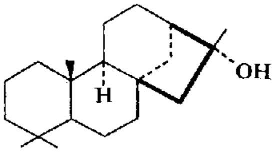
It has been reported that the foliage of *Cryptomeria japonica* contains kaurene (I).[1] We were surprised therefore to obtain phyllocladene* (II) as the only diterpenoid hydrocarbon from the foliage of a very old tree of this species growing on British Forestry Commission land in Argyll, Scotland. Foliage collected later in the year from a plot of young *C. japonica* trees growing in the same district from one seed source contained only kaurene. We therefore decided to enquire more closely into this discrepancy.



I



II



III

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* Phyllocladanol (III) has been previously isolated[2] from a specimen of *C. japonica* heartwood.

[1] M. Takahashi, T. Ito, A. Mizutani and K. Isoi, *J. Pharm. Soc. Japan* **80**, 1488 (1960).

[2] T. Kondo, H. Imamura and M. Suda, *Bull. Agr. Chem. Soc. Japan* **24**, 65 (1960).

RESULTS AND DISCUSSION

There were clearly two possible explanations for our observations: either kaurene and phyllocladene are produced at different times of the year by the same tree or there are two chemically distinct varieties of *C. japonica.* Accordingly since samples from the old tree were no longer available, foliage was collected at monthly intervals for twelve consecutive months from the plot of young trees. The composition of the hydrocarbon fraction was invariant with time, all trees yielding (−)-kaurene (see Table).

TABLE 1.

Location (Scotland)	Seed source	Diterpene isolated
Kilmun, Argyll (old tree)	Unknown	(+)-phyllocladene
Kilmun, Argyll (young trees)	Takahagi, Nr. Tokyo, Japan	(−)-kaurene
Lochgoil, Argyll	Sizokuisi Nat. Forest, Iwate, Japan	(−)-kaurene
Lochgoil, Argyll	Takata Nat. Forest, Niigata, Japan	(+)-phyllocladene
Lochgoil, Argyll	Yanase Nat. Forest, Kooti, Japan)	(−)-kaurene
Lochgoil, Argyll	Nanakura Nat. Forest, Akita, Japan	(−)-kaurene
Lochgoil, Argyll	Sirasawa Nat. Forest, Akita, Japan	(−)-kaurene
Lochgoil, Argyll	Benmore Forest Garden, Argyll, Scotland (ex. Takahagi, Nr. Tokyo, Japan)	(−)-kaurene
C. japonica var. *Sinensis*		
Lochgoil, Argyll	Central Plain of China	(−)-kaurene
C. japonica elegans		
Culzean Castle, Ayrshire	Unknown	(−)-kaurene

In a second experiment, foliage obtained from trees of the same age and near-identical growth environment but derived from different seed sources was examined. The Forestry Commission are carrying out a small provenance experiment at Lochgoil in which trees from seven seed sources (see Table) are being grown, and foliage from each was made available to us. Of these, only one (grown from seed from Takata National Forest, Niigata, Japan) gave phyllocladene.

It is significant that of the ten trees from different sources, eight contained kaurene with no detectable amount of phyllocladene while the other two contained phyllocladene with no detectable amount of kaurene. Thus, from the limited number of trees examined, it appears that so far as the diterpene hydrocarbon fraction of the leaves is concerned, *C. japonica* exists in what might be termed two chemically distinct varieties, one producing (−)-kaurene, the other (+)-phyllocladene.

EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra were obtained with a Varian Associates HA-100 spectrometer.

The GLC experiments were carried out with a Pye "argon" gas chromatograph using a glass column (1·25 m × 3 mm) at 140° packed with 1 per cent SE-30 with an argon carrier-gas flow of 35–40 ml/min.

Gas chromatography-mass spectrometry (GC-MS) experiments were performed 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°, with helium as carrier-gas at a flow rate of 35 ml/min.

Extraction and Identification of Hydrocarbons

Foliage (together with light terminal shoots) of *Cryptomeria japonica* was crushed and extracted with light petroleum (b.p. 60–80°) by stirring at room temperature for 1 hr. The residue, after filtration, was re-extracted with further quantities of solvent until the supernatant was devoid of green pigment. After evaporation *in vacuo*, the residue from the combined extracts was taken up in light petroleum and subjected to column chromatography over neutral alumina (Grade I: Woelm).

Diterpene hydrocarbons were separated from compounds of lower polarity (NMR and GC-MS indicated that these were straight-chain hydrocarbons of low molecular weight) by TLC over $SiO_2/AgNO_3$ (10:1) whilst eluting with light petroleum (b.p. 60–80°).

Diterpenes were identified by comparison with authentic samples of (−)-kaurene and (+)-phyllocladene using NMR, GLC and GC-MS, and by optical rotation studies.

The authentic sample of kaurene had m.p. 51–52° and $[\alpha]_D$ −78° ($CHCl_3$); phyllocladene had m.p. 98–98·5° and $[\alpha]_D$ +13° ($CHCl_3$).

Acknowledgements—We gratefully acknowledge the help given by Messrs. A. B. Lewis, R. Lines and J. R. Mair of the Research Branch, Forestry Commission, Scotland.