Phytochemistry, 1975, Vol. 14, pp. 211–212. Pergamon Press. Printed in England.

MONOTERPENES OF YOUNG CORTICAL TISSUE OF *PINUS RADIATA*

J. A. ZABKIEWICZ and P. A. ALLAN

New Zealand Forest Service, Forest Research Institute, Rotorua, New Zealand

(*Received* 12 *March* 1974)

Key Word Index—*Pinus radiata*; cortical monoterpenes; toluene; chemotaxonomy.

Pinus radiata D. Don, in all areas where it occurs, is highly variable in its morphological characteristics and this was reflected in early beliefs that the stands were mixtures of more than one species [1]. The wide range of morphological variants has, however, now been grouped as a single species, and chemotaxonomic methods (using monoterpene analysis of wood oleoresin) have reinforced this classification [2].

Despite some relative variation, the principal monoterpenes had been identified as (±)-α-pinene and (−)-β-pinene [3]. These have been described as constituting 98% of the monoterpene fraction, with α-pinene concentration varying from 12·6% to 58%, together with trace amounts of camphene, limonene, and β-phellandrene, as well as a further unknown [4]. These analyses were of the steam distillate from oleoresin collected at breast height level from mature trees. More recent analyses showed that in younger trees—as well as in different tissues—more variation and more components were evident. Limonene appeared as a major constituent (as well as α- and β-pinenes), together with variable quantities of β-phellandrene, Δ^3-carene, camphene, sabinene, and myrcene [5].

Preliminary examination of the monoterpene fraction of *P. radiata* cortical resin revealed even more extensive variation. This was found to be due to our sampling the resin from young growing tissues, and not from older suberized regions.

Accordingly we wish to report the identification of further monoterpenes in *P. radiata*, and the variation in composition in oleoresin samples from resin canals of young cortical tissues. The results (and properties of compounds actually isolated) are summarized in Table 1. The data are based on the analysis of the cortical oleoresin (first-year upper laterals) from 316 trees from populations originating at Kaingaroa, Nelson (both in New Zealand), Ano Nuevo, Monterey, and Cambria (all in the U.S.A.), and Guadalupe (in Mexico).

It is obvious that in oleoresin from the apical region of this pine species the number of monoterpene constituents is both greater than, and in differing proportions from, that existing in wood oleoresin. It is confirmed that in the oleoresin of these younger tissues α- and β-pinenes do not predominate; instead Δ^3-carene, limonene, β-phellandrene, and terpinolene were found to be the major constituents. However, analysis of the resin blister oleoresin from the same trees showed, as expected from previous reports, that α- and β-pinenes are the main components. It may be concluded that with *P. radiata* the sampling position is all-important for consistent and valid comparisons of monoterpene composition. Toluene was also identified in certain extracts; its presence was clone dependent and analysis on glass or metal columns did not affect its relative proportion. It was possibly formed by decomposition of non-monoterpene components of the oleoresin.

EXPERIMENTAL

Analyses. Analytical GLC was carried out on samples of oleoresin collected from both the apical regions and at breast height. The resin samples were dissolved directly in diethyl ether prior to analysis, and no attempt was made to determine absolute masses, so that all results are as relative percentage composition of the volatile fraction. Peak areas were obtained with a Kent Chromalog II integrator, and as detector responses were almost identical for all substances tested no relative response corrections were necessary. Various packed columns were used (ApL, Carbowax 1540, Carbowax 400/Porasil C, β, β-oxydipropionitrile,1,2,3-Tris(2-cyanoethoxy)propane, FFAP, or a 1:1 mixture of Carbowax and FFAP), but a capillary Carbowax 1540 or SCOT FFAP capillary was essential for best resolution

Table 1. *P. radiata* cortical oleoresin compositions (volatile fraction) and optical rotations of major components

Compound	Abundance (%) range	Main peak frequency (%)‡	Optical rotation Observed	Optical rotation Literature [6]
α-Pinene	3–77	33·5	$[\alpha]_D + 21{\cdot}6$	$[\alpha]_D + 51$
Toluene	0–23†			
Camphene	0–29			
β-Pinene	0–49	8·5	$[\alpha]_D - 24{\cdot}3$	$[\alpha]_D - 21$
Sabinene	trace*			
Myrcene	0–17*			
Δ^3-Carene	0–72	16·5	$[\alpha]_D + 9{\cdot}3$	$[\alpha]_D + 7$
α-Phellandrene	0–7			
α-Terpinene	0–3			
Limonene	0–83	31·6	$[\alpha]_D - 121{\cdot}6$	$[\alpha]_D - 123$
β-Phellandrene	0–48	4·8		
γ-Terpinene	0–13			
p-Cymene	trace*			
Terpinolene	0–57	5·1		

* Routine analytical conditions precluded separation; these data obtained for other samples analysed under different conditions.

† We wish to thank Dr. G. Leary, Chemistry Division, DSIR, Wellington, for assistance with the GC/MS facilities used to identify this compound.

‡ These data are for the oleoresin from the young cortical tissue: breast height analyses of samples from resin blisters of the same trees gave the following major peak frequency: α-pinene 70·3%, β-pinene 28·5%, limonene 0·9%, and terpinolene 0·3%. Even these data may not truly represent "mature" material, as at the time of sampling (Jan–March 1971) the trees were 7·5 years old.

of the complete naturally occurring mixture. The following conditions were used to obtain the data in the table: analytical conditions; Pye 104 with FID, 1·5 m × 6 mm glass column, Carbowax 400/Porasil C, 125°, 35 ml/min N_2.

Bulk extraction. Young, defoliated twigs and stems (5 kg) from trees of a New Zealand population were chopped and extracted overnight with diethyl ether, the solvent decanted off, and the tissues re-extracted. The combined extracts were concentrated and then steam-distilled to yield an oil (13·93 g), of which the principal monoterpene constituents were isolated by preparative GLC (Pye 105, 3 m × 9·5 mm glass columns, 20% Carbowax 1540 on GC P 100–120 mesh, 120°, 40 ml/min N_2). A similar procedure was followed using extracts from a tree known to be high in β-phellandrene, so its identity could be verified by IR spectroscopy.

REFERENCES

1. Forde, M. B. (1964) *N.Z. J. Bot.* **2**, 486.
2. Bannister, M. H., personal communication.
3. Mirov, N. T. (1961) *U.S. Dept. Agric., For. Serv. Tech. Bull. No.* 1239.
4. Bannister, M. H., Williams, A. L. and McDonald, I. R. C. (1962) *N.Z. J. Sci.* **5**, 486.
5. Valenzuela, P., Cori, O. and Yudelevich, A. (1966) *Phytochemistry* **5**, 1005.
6. Devon, T. K. and Scott, A. I. (1972) *Handbook of Naturally Occurring Compounds* Vol. 2, Academic Press, New York.