Phytochemistry, 1971, Vol. 10, pp. 1857 to 1862. Pergamon Press. Printed in England.

THE COMPOSITION OF TERPENOID HYDROCARBONS FROM *PINUS MONOPHYLLA* WOOD OLEORESIN

EUGENE ZAVARIN,* KAREL SNAJBERK,† CHUNG JEN LEE,‡
MARGARET HENLEY§ and NICHOLAS T. MIROV‖
University of California, Forest Products Laboratory, Richmond, California 94804, U.S.A.

(*Received* 6 *October* 1970)

Abstract—Monoterpenoids and sesquiterpene hydrocarbons of *Pinus monophylla* wood oleoresin have been analyzed by a combination of chromatographic and spectroscopic methods. Three quarters to four fifths of the monoterpenoid hydrocarbons consisted of (+),(±) α-pinene; the rest was sabinene, 3-carene, myrcene, limonene and ocimene, with traces of camphene, β-pinene, β-phellandrene and terpinolene. Variability between trees and differences from *Pinus edulis* were relatively small. Oxygenated monoterpenoids were mainly linalool and bornyl acetate; 0·46% of ethyl caprylate was present compared with 10·5% in *P. edulis*. Sesquiterpenoid hydrocarbons present in *P. monophylla* were α-cubebene, longipinene, cyclosativene, α-copaene, longicyclene, sativene, longifolene, α-guaiene, caryophyllene, β-farnesene, γ-muurolene, α-muurolene, γ-cadinene, δ-cadinene and calamenene.

INTRODUCTION

THE PINES of the subsection Cembroides Engelm. (Sect. Parrya, Mayr., Subgenus *Strobus*, Lemm., = *Haploxylon*, soft pines), or pinyon pines, comprise eight species native to the southwestern quarter of United States and to the northern and central part of Mexico. They are smaller trees than other soft pines and inhabit the usually more arid lower elevations. The U.S. species differ from other soft pines by usually having fewer needles (1–4, vs. 5) in their fascicles. Of the species now recognized, four are entirely Mexican, with *P. culminicola* Andersen and Beaman and *P. maximartinezii* Rzedowski known from a single locality only, and *P. nelsonii* Shaw and *P. pinceana* Gord. known from few scattered localities in eastern part of northern and central Mexico.[1] The other four species are more widely spread. Thus, *P. monophylla* Torr. and Frem. (singleleaf pinyon) is found in southern Idaho, western Utah, northwestern Arizona, most of Nevada and eastern and central California to northern Baja California. In the eastern parts of its range it meets *Pinus edulis* Engelm. *P. edulis* extends through Utah, Colorado, Arizona and New Mexico, with a few populations in southern California, southern Wyoming, eastern Oklahoma and Texas and northern Chihuahua. In the south, the range of *P. monophylla* overlaps that of *P. quadrifolia* Parl., a pine of southern California and northern Baja California, while in parts of Arizona, New Mexico, and Texas close to the Mexican border, the range of *P. edulis* overlaps that of *P. cembroides* Zucc., a pine of northern and central Mexico. With extensive contact existing

* Forest Products Chemist.
† Associate Specialist.
‡ Research Assistant.
§ 1966 National Science Foundation Summer Extension Research Participant.
‖ Research Associate.

[1] W. B. CRITCHFIELD and E. L. LITTLE, JR., *Geographic Distribution of the Pines of the World*, U.S. Department of Agriculture, Forest Service, Misc. Publ. 991 (1966).

between these closely related pine species, natural hybridization could be suspected and was reported for *P. edulis–P. monophylla*[1] and *P. edulis–P. cembroides*[2] pairs.

The present series of investigations has been initiated with the purpose to help clarify certain systematic questions (particularly those of intergradation) regarding the pinyon pine species complex, and uses as criteria the chemical composition of volatile materials from xylem oleoresin. Here, we report on detailed analysis of the monoterpenoids and sesquiterpenoid hydrocarbons, as well as of materials of comparable volatility from the oleoresin of *P. monophylla*. We include also a GLC analysis of monoterpene hydrocarbons from *P. edulis* using the same turpentine sample which was analyzed previously by Mirov and Iloff by preparative methods.[3]

RESULTS AND DISCUSSION

The turpentine composition of the four more northerly species mentioned has been the subject of several investigations, although the analytical methods used were exclusively preparative. The most abundant of the monoterpene hydrocarbons of all these species was (+),(±)-α-pinene (81% in *P. edulis*, 95% in *P. monophylla*, 96% in *P. cembroides* and 94% in *P. quadrifolia*[4]).* Other monoterpenes included myrcene (2%), 3-carene (6%), limonene (1%) and terpinolene (1%) in *P. edulis*, limonene (5%) in *P. monophylla*, limonene (4%) in *P. cembroides*, and ocimene (6%) in *P. quadrifolia*.* Thus, the differences did not appear too promising for the detection of hybridization, in view of the semi-quantitative nature of methodology used. Much less extensive data are available for the higher boiling constituents, with only (+)-longifolene (2%) identified in the oleoresin of *P. cembroides* and ethyl caprylate (10·5%) identified in the oleoresin of *P. edulis*.* Ethyl caprylate has not been reported as a constituent of oleoresin of any other pine; it is likely to represent a promising chemotaxonomic marker for studying oleoresin differences. Sesquiterpenes forming cadinene hydrochloride were reported in a few cases.

Oleoresins of the purely Mexican pines, *P. maximartinezii* and *P. culminicola* have not been studied at all. The oleoresin of *P. pinceana* was found to contain monoterpene hydrocarbons composed of 94% (−)-limonene and 5% (+)-α-pinene; sesquiterpenes amounted to about 5% and were mainly bicyclic.[5]* *P. nelsonii* has been recently investigated using GLC; monoterpenes contained 49·6% of (−)-α-pinene, 41·5% of (−)-β-pinene, 1·0% of limonene and 0·24% of camphene plus 0·01% of *n*-heptane. No sesquiterpenes were reported; instead, 3·7% of indefinite oxygen-containing materials were mentioned—possibly these were turpentine oxidation artifacts.[6] Thus, the turpentine composition of the southern, purely Mexican pinyons seems more diversified and is quite different from that of more northern species.

Table 1 shows the monoterpene hydrocarbon composition of the *P. edulis* turpentine from Fort Defiance, Arizona, and of *P. monophylla* turpentine from Deep Springs, Nevada and from Walkers Pass, southern California. The results indicate the surprising similarity

* Figures recalculated to monoterpene hydrocarbons = 100%.

[2] J. McCORMICK and J. W. ANDERSEN, *Ohio J. Sci.* **63**, 62 (1963)

[3] N. T. MIROV and P. M. ILOFF, JR., *J. Am. Pharm. Assoc. Sci. Ed.* **45**, 629 (1956).

[4] N. T. MIROV, *Composition of the Gum Turpentines of Pines*, U.S. Department of Agriculture, Forest Service, Technical Bulletin 1239 (1961).

[5] N. T. MIROV, *J. Am. Pharm. Assoc. Sci. Ed.* **41**, 673, (1951).

[6] N. T. MIROV, E. ZAVARIN and J. G. BICHO, *J. Pharm. Sci.* **51**, 1131 (1962).

TABLE 1. COMPOSITION OF MONOTERPENE HYDROCARBONS FROM *Pinus edulis* AND *Pinus monophylla*

	Pinus edulis	*Pinus monophylla*	*Pinus monophylla* (Deep Springs)*		
	(Fort Defiance)	(Walker's Pass)	Mean	Stand. Dev.	Range
α-Pinene	76·5	74·5†	82·5	8 0	66·5–90·0
Camphene	0·5	1·0	0·5	0·5	0·0–1·0
β-Pinene	1·0	0·5	1·0	0·5	0·5–1·0
3-Carene	10·5	6·0	0·5	1·0	0 0–3·5
Sabinene	3 0	3·0	3·5	3·5	0 0–12·0
Myrcene	3·0	3·0	6·0	3·5	2·5–13·0
Limonene	0·5	8·0	2·0	4·0	0 0–13·0
β-Phellandrene	—	—	tr	—	0 0– 1·0
Ocimene	3·0	2·5	2·0	0·5	1·0– 4·0
Terpinolene	1·5	1·5	2·0	2·0	0·0– 6·5
Total Monoterpenes	13·7	23·0	19·6	1·5	15·8–22·1

* 14 single trees analyzed. Other analyses are on the bulked material from many trees.

† Specific rotation of $[\alpha_D^{20}] = +43{\cdot}80$ indicating presence of 67·7% of (+)-α-pinene and 6·8% of (−)-α-pinene (with $[\alpha_D^{20}]$ of pure α-pinene taken as 53·6%).[6]

in composition between the two species, and the relatively low quantitative variability of the individual compounds in *P. monophylla*. The only significant interspecific difference is limonene present in somewhat larger amounts in *P. monophylla*. Thus, it seems that comparison of the monoterpene hydrocarbon composition does not promise to be of high diagnostic value for the study of *P. edulis–P. monophylla* intergradation, and that other differences must be sought.

Studies of the oxygen-containing monoterpenes and of other compounds of similar volatility, resulted in identification of linalool, bornyl acetate, and ethyl caprylate in *P. monophylla* (Table 2). While bornyl acetate has been so far observed in many oleoresins,[4] linalool has been found so far only in *P. tenuifolia* Benth.,[7] *P. jeffreyi* Grev. and Bal.[8] and *P. cooperi* var. *ornelasi* Martínez.[9] Ethyl caprylate has been identified so far only in the oleoresin of *P. edulis* and its presence in *P. monophylla* is therefore in accord with the close relationship of these two pines. The fact that this compound is present in *P. monophylla* in minute quantities only (0·46%), as against 10·5% in *P. edulis* (monoterpene hydrocarbons = 100%) means it can be used as a distinguishing character for the study of intergradation of these species.

From the structures of monoterpenoids identified, it can be concluded that in *P. monophylla* (as well as in *P. edulis*) the largest part of biosynthetic transformations[10] involve stabilizations of the 1-*p*-menthene-8-carbonium ion not involving interactions with the 1,2-double bond (low (−)-α-pinene, β-pinene, camphene and bornyl acetate). The preferential mechanisms are connected with attacks on saturated carbon atoms by the positively charged C_8 carbon ((+)-α-pinene and 3-carene); a route involving proton split from the

[7] N. T. MIROV, *J. Am. Pharm. Assoc. Sci. Ed.* **47**, 410 (1958).
[8] Schimmel & Co., Report, 1915.
[9] K. SNAJBERK and E. ZAVARIN, unpublished.
[10] L. WESTFELT, *Svensk Kem. Tidsk.* **79**, 441 (1967).

TABLE 2. COMPOSITION OF HIGHER-BOILING TERPENOIDS FROM *Pinus monophylla**

Class		Compound	Per cent*	Relative ret. volume†
		SESQUITERPENES		
Acyclics		β-Farnesene	0 5	1 42
Cyclics, from *trans–cis*- farnesyl pyrophosphate	Cyclization 1/10	α-Muurolene	38·0	1 70
		γ-Muurolene	12 0	1·53
		α-Copaene	tr	0 715
		Sativene	0 3	0 85
		Cyclosativene	tr	0·705
		γ-Cadinene	5 0	2 08
		α-Cubebene	tr	0 62
		δ-Cadinene	2 5	2 20
	Total: 57·9%	Calamenene	0·1	2·54
	Cyclization 1/11	Longifolene	14·5	1 00
		Longicyclene	17·5	0 77
Total: 90·1%	Total: 32 2%	α-Longipinene	0 2	0 67
Cyclics, from *trans–trans*-farnesyl pyrophosphate	Cyclization 1/10	α-Guaiene	2 0	1·07
Total: 4·5%	Cyclization 1/11	Caryophyllene	2 5	1 10
		A	0·3	0 95
		B	tr	1·26
		C	tr	1·31
Total: 5·3%		D	5·0	1 98
		OXYGENATED MATERIALS‡		
		Linalool	0·27	0·76
		Bornyl acetate	0 51	0 95
Total: 1 24%		Ethyl caprylate	0 46	0 52

* Biosynthetic classification is based on the ideas of Parker *et al.*,[11] and identifications on infra-red spectra and relative retention data. For stereo chemistry of α-cubebene see Ref. 12. δ-Cadinene as well as calamenene could belong to muurolene as well as to cadinene series. Retention volumes are given for carbowax column.

† Longifolene = 1·00.

‡ Monoterpene hydrocarbons = 100%.

9- or 10-position (limonene), as well as less usual pathways involving 4–8 hydride shift and leading to sabinene and terpinolene, which are also present but less important. Acyclics (myrcene, ocimene, linalool) occur in secondary although not negligible proportions.

In view of the relatively small number of samples run, statistical investigation of individual variability did not yield much information, with only the sabinene–terpinolene pair exhibiting a very high correlation coefficient, $r = +0{\cdot}924$ (with sabinene as independent variable, $b = 0{\cdot}510$ and $a = 0{\cdot}093$, i.e. proportionality relationship), and α-pinene–sabinene pair, exhibiting a highly significant substitution relationship ($r = -0{\cdot}728$, $b = -0{\cdot}321$, $a = 29{\cdot}74$; with 14 samples, $df = 12$, $r_{5\%} = 0{\cdot}532$ and $r_{1\%} = 0{\cdot}661$). Other correlations were not significant statistically. The positive correlation between sabinene–terpinolene, taken together with the negative correlation between sabinene and α-pinene, suggests a particularly close biosynthetic tie between the first pair and is consistent with the

[11] W. PARKER, J. S. ROBERTS and R. RAMAGE, *Sesquiterpene Biosynthesis*, *Quart. Rev.* **21**, 331 (1967).
[12] A. TANAKA, H. UDA and A. YOSHIKOSHI, *Chem. Commun.* 308 (1969).

formation of both through 1-*p*-methene-4-carbonium ion as a common intermediate. This was found true earlier in case of *P. muricata* and was discussed in our previous publication.[13]

Table 2 lists the 4·6% sesquiterpene hydrocarbons contained in the oleoresin of *P. monophylla*. Because information on such compounds for the other pinyons is wanting, these data cannot be evaluated as to the potential value of sesquiterpene hydrocarbons for the investigation of the introgradation of *P. monophylla* and *P. edulis* or for other Cembroides. Of the fifteen sesquiterpenes identified, α-cubebene, sativene, cyclosativene, α-guaiene, and β-farnesene are new to *Pinus* xylem oleoresins. They have been previously identified in *Abies magnifica* cortical oleoresin. Two additional unknown sesquiterpene hydrocarbons were isolated. Their i.r. spectra showed the presence of a $CH_2 = CR_2$ group, of an additional di- or trisubstituted double bond or cyclopropane ring, and of two geminal methyls on saturated carbon atom, in each case. The identification of these materials is under investigation.

From the point of view of biosynthesis, analysis of the sesquiterpenes suggests that most *P. monophylla* sesquiterpene hydrocarbons are formed through *trans–cis* farnesyl pyrophosphate (95%), with the *trans–trans* compound accounting for only 4·5% and the rest falling on acyclic β-farnesene, which could form from either precursor. Within the *trans–cis* group 1–10 cyclization appears favored (58%), although 1/11 cyclization is also appreciable (32%), while other types of cyclizations are absent. Within the *trans–trans* route, 1–10 and 1–11 cyclization pathways take place roughly to the same degree. The lesser participation of the *trans–trans* farnesyl pyrophosphate in the formation of sesquiterpenoids in *P. monophylla* wood oleoresin is in accord with the data on other pines,[10,14] and is in contrast with data for *Abies* cortical oleoresins, where *trans–trans* sesquiterpenoids occasionally assume high values.[15] It is impossible to say whether this difference is taxonomic, or whether it reflects the difference between xylem and cortical resin systems, as nothing is known of cortical sesquiterpenoids of *Pinus; Abies*' wood does not produce oleoresin for all practical purposes.

EXPERIMENTAL

Fourteen samples for individual *P. monophylla* trees were collected near Deep Springs, northern Inyo County, California, while the bulked material, amounting to 1730 g was obtained about 3 miles west from Walker's pass, near Hw. 178, northeastern Kern County, California, by the methods previously described,[16] using 2- and 15-g vials, respectively.

Turpentine was separated from the rosin of the bulked samples by distillation at 10–50 mm until the boiling point of the distillate reached 200°. The total distillate from 1730 g of oleoresin amounted to 425 g (24·5%). The turpentine obtained was subsequently fractionated (using a Todd fractionation assembly) into fractions boiling 1·5° apart and spanning the temperature range from 38° at 50 mm to 125° at 6 mm pressure; the fractions compositionally close were combined. Further analysis was performed on several representative fractions from the series obtained.

Separation of oxygenated terpenoids from hydrocarbons, as well as separation of esters from alcohols, was made by chromatography on active neutral Merck Al_2O_3 (1:50, absorbent to turpentine) with light petroleum–benzene–ether mixtures of varying composition as eluent. Separation of hydrocarbons from each other was achieved by chromatography on silicagel (Merck, mesh 30–70)–silver nitrate mixture 1:50,[17] using same eluents as above.

Final separation and purification of compounds was achieved by preparative GLC, using a Varian Aerograph Instrument Model A-90-C with 1% Carbowax 20 M on Chromosorb G 100/120, 8 mm × 6 m

[13] E. ZAVARIN, *Phytochem.* **9**, 1049 (1970).
[14] V. A. PENTEGOVA, ZH. V. DUBOVENKO, L. N. VOLSKII, S. M. VASILIUK, M. A. CHIRKOVA and E. N. SHMIDT, *Izv. Sib. Otd. Akad. Nauk SSSR; Ser. Khim. Nauk* **139**, 114 (1968).
[15] L. A. SMEDMAN, K. SNAJBERK, E. ZAVARIN and T. R. MON, *Phytochem.* **8**, 1471 (1969).
[16] N. T. MIROV, E. ZAVARIN, K. SNAJBERK and K. COSTELLO, *Phytochem.* **5**, 343 (1966).
[17] T. NORIN and L. WESTFELT, *Acta Chem. Scand.* **18**, 572 (1964).

column, flow 50 ml/min, T = 125°, thermal conductivity detector, Sargent Model SR recorder, and using 1 mm dia. Teflon tube as a collector.

Fractionation in all cases was monitored by (a) TLC analysis, using 2·5 × 7 5 cm plates covered with a 0·2 mm layer of Al_2O_3,G, Merck, developing with light petroleum and using a 5% solution of isovanillin in conc. H_2SO_4 as a chromogenic spray—this gave brighter and more characteristic colors than did the commonly used vanillin;[18] and (b) GLC using a Varian Aerograph Model 1200 instrument with flame conductivity detector in combination with the Sargent Model SR recorder and SF96 or Carbowax 20 M, 1% on Chromosorb G, 100–120, 3 mm × 6·5 m columns, T = 125°, flow rate, 15 ml/min, for sesquiterpenoids and oxygenated materials. Methods used in the quantitative analysis of monoterpenoids by GLC were described previously.[19]

To determine if any artifacts were formed during preparative isolation, quantitative composition of the turpentine was examined by analytical GLC in a separate experiment, using fresh oleoresin. The results indicated that all compounds identified were originally present. The only difference included absence of the sesquiterpene hydrocarbon peak relative retention volume 1 98 in the preparatively separated material. Integration of the peak areas was performed with a Varian Aerograph digital Model 470 integrator.

I.r. spectra were obtained with a Perkin–Elmer 457 spectrophotometer in combination with a beam condenser and a 0 5 mm Barnes micro liquid cell Identification of the compounds isolated was performed on the basis of 30–50, mostly 40–50 well-resolved peaks using either literature data[20] or i.r. spectra previously obtained on authentic substances. Bands (in cm^{-1}) assigned to specific structural features of the two unidentified sesquiterpene hydrocarbons included: relative retention volume 0·85: 3075, *S*; 3030, *M*; 1755, *M*, 1660, *S*; 1380, *S*; 1373, *S*; 1368, *S*; 875, *S*. Relative retention volume 1·31: 3095, *S*; 3050, *M*, 1800, *W*; 1750, *W*; 1670, *W*; 1634, *S*, 1387, *S*, 1378, *S*; 1369, *S*, 898, *S*.

Acknowledgements—We would like to thank Dr. John Mawby and Mr. David Mossner, Deep Springs College, Deep Springs, California, for the collection of oleoresin, and Dr. W. B. Critchfield, Pacific Southwest Forest and Range Experiment Station, for manuscript review

[18] E. TYIHÁK, D. VAGUJFALVI and P. L. HAGONY, *J. Chromatog.* **11**, 45 (1963).
[19] E. ZAVARIN, W. HATHWAY, T. REICHERT and Y. LINHART, *Phytochem.* **6**, 1019 (1967).
[20] J. A. WENNINGER, R. G. YATES and M. DOLINSKY, *J. Assoc. Offic. Analyt. Chemists* **50**, 1313 (1967).