Phytochemistry, 1967, Vol. 6, pp. 1019 to 1023. Pergamon Press Ltd. Printed in England

CHEMOTAXONOMIC STUDY OF *PINUS TORREYANA* PARRY TURPENTINE

EUGENE ZAVARIN,* WILHELMINA HATHAWAY,† THOMAS REICHERT‡ and YAN B. LINHART§

University of California, Forest Products Laboratory, Richmond, California

(*Received* 20 *December* 1966)

Abstract—Oleoresins from Torrey Pine (*Pinus torreyana* Parry) from Santa Rosa Island and Torrey Pines State Reserve near Del Mar, California, have been analyzed by gas–liquid chromatography. In each of the two populations the turpentine composition was found to be remarkably similar between individuals. The content of β-phellandrene and limonene differentiated the turpentines from the two stands.

INTRODUCTION

TORREY pine (*Pinus torreyana* Parry) is the rarest of pines, being confined to the north-eastern coast of Santa Rosa Island (Santa Barbara County) and to a short coastal strip south of Del Mar (San Diego County), California, where it is protected in the Torrey Pines State Reserve (the two locations are separated by a distance of about 280 km). It is presently being extensively planted in southern California, New Zealand, and Kenya.

On Santa Rosa Island, the Torrey pines are located on bluffs overlooking the north-east coast and scattered over a series of saddles for a distance of about 1 km prefering the moist north-west-facing slopes. In addition there is a small group of about 10–15 trees located 1 km inland, south-west of the coastal stands. The mature trees are 10–15 m tall with wide, open crowns. Young trees are numerous and the total number of trees on the island is about one thousand.

Near Del Mar, Torrey pines grow in scattered groups along the coast from San Dieguito estuary southward about 8 km. On the exposed cliffs they are dwarfed and prostrate; however, in the sheltered areas they attain a height of 15–18 m and a $\frac{3}{4}$ m dia. The regeneration seems to be poor and the whole population numbers but a few thousand.

Taxonomically, Torrey pine belongs to the group Macrocarpae (big-cone pines) of the sub-genus *Diploxylon*, which includes Digger pine (*P. sabiniana* Dougl.) and Coulter pine (*P. coulteri* D. Don); all three species possessing characteristic heavy cones and long, coarse, grayish needles. Their turpentines are characterized by the presence of varying but sizable amounts of straight-chain saturated hydrocarbons, such as *n*-heptane and *n*-undecane, (5 per cent in Torrey pine, 15 per cent in Coulter pine, and 95 per cent in Digger pine). It was mainly this occurrence of normal paraffins which caused Mirov[1] to include in Macrocarpae the remaining two *Diploxylon* pines with turpentines rich in these compounds—Jeffrey pine

* Associate Forest Products Chemist, Forest Products Laboratory, University of California, Berkeley.

† Riverside City College, California, 1966 National Science Foundation Summer and Winter Extension Research Participant in Forest Products Laboratory of the University of California, Berkeley.

‡ Research Assistant, Work-Study Program, Forest Products Laboratory, University of California, Berkeley.

§ Formerly Assistant Specialist, School of Forestry, and currently a graduate student in Department of Genetics, University of California, Berkeley.

[1] N. T. MIROV, *U.S. Dept. Agr. Forest Serv., Tech. Bull.* No. 1239, (a) p. 62, (b) pp. 4, 5 (1961).

(*P. Jeffreyi* Grev. a. Balf.) (turpentine chiefly composed of *n*-heptane), and *P. oaxacana* Mirov (turpentine containing over 22 per cent of *n*-heptane and *n*-undecane). An up-to-date discussion of the taxonomy of big cone pines was published recently.[2]

The prolonged isolation of the two populations of Torrey pines could be expected to result in the development of genetic differences. Indeed, some differences in the shape of the cones and also in the growth habit have been previously noted.[3] In a recent study Haller[4] investigated the two populations more systematically and came to the conclusion there were genetic differences connected with morphology and color of the needles, cone shape, and growth habit. This prompted us to look into the possible differences in the composition of turpentine between trees from these two populations, since turpentine composition, on a number of occasions, has been shown to be under genetic control.[1b]

The turpentine from a composite sample of oleoresin obtained from Torrey pine trees planted in Golden Gate Park in San Francisco was investigated by Haagen-Smit *et al.*[5] who found it was composed of 75 per cent of limonene, 5 per cent of *n*-undecane, 4 per cent of longifolene, 1·1 per cent of *n*-decyl aldehyde,* 0·2 per cent of lauraldehyde, 0·2 per cent of an unidentified carbonyl compound and of less than 0·1 per cent each of *n*-nonane and *n*-heptane. Later, Williams and Bannister[6] used GLC to analyze a sample of turpentine from a Torrey pine of unidentified provenance growing in the New Zealand Forest Service Arboretum at Whakarewarewa, near Rotorua. The sample contained 81 per cent of limonene, 2·0 per cent of *n*-heptane, 2·0 per cent of *n*-nonane, 2·5 per cent of α-pinene, traces of camphene, 8·5 per cent of *n*-undecane, traces of β-pinene, and 3·5 per cent of myrcene. The technique did not permit analysis of higher boiling turpentine components such as sesquiterpenes and straight-chain aldehydes. An analysis of Torrey pine turpentine (exclusive of higher-boiling materials) from a single tree has been also reported by Critchfield[2] (3·2 per cent of *n*-heptane, 2·4 per cent of *n*-nonane, 1·8 per cent of α-pinene, 7·7 per cent of *n*-undecane, traces of β-pinene, 2·7 per cent of myrcene, and 82·2 per cent of limonene).

DISCUSSION

Compounds were identified using GLC by their relative retention volumes shown in Table 1.

As can be seen from Tables 2 and 3, the trees from each of the two provenances show a remarkable constancy in the composition of their turpentines. This is not surprising in view of the extremely limited range of both populations and of the long isolation of the respective stands. Comparison of the material from Santa Rosa population with that from Del Mar reveals consistent differences which, although small by comparison with what one occasionally finds with other species, are definitely significant, particularly in view of the low variability on individual level. The main differences are in the amount of limonene (73·4 vs. 84·2 per cent) and β-phellandrene (8·7 vs. 0·1 per cent). Smaller differences include higher per cent of *n*-hydrocarbons in Santa Rosa population (13·4 vs. 10·0 per cent), as well as lower per cent of

* In the original paper authors reported 10 per cent of *n*-decyl aldehyde in turpentine due to an error in calculation.

[2] W. B. CRITCHFIELD, *U.S. Forest Serv. Res. Paper* NC-6. N. Central Forest Exp. Sta., St. Paul, Minn. (1966).
[3] A. D. LINDSAY, Torrey Pine (*Pinus torreyana* Parry) Commonwealth Forestry Bureau Leaflet No. 23 (1932). L. F. Johnston, Commonwealth government printer, Canberra.
[4] J. R. HALLER, In *Symposium on the Biology of the California Channel Islands.* University of California, Santa Barbara. In press (1967).
[5] A. J. HAAGEN-SMIT, C. T. REDEMANN and N. T. MIROV, *J. Am. Chem. Soc.* **69**, 2014 (1947).
[6] A. L. WILLIAMS and M. H. BANNISTER, *J. Pharm. Sci.* **51**, 970 (1962).

each myrcene, longifolene and peak 0·65. Also, cineole and *n*-decyl aldehyde seem to be either absent or present in amounts escaping detection in Santa Rosa oleoresins. The results for Del Mar material agree well with the data reported earlier by Haagen-Smit *et al.*,[5] Williams and Bannister,[6] and Critchfield[2] thus indicating that the corresponding seeds always originated from this particular provenance. The markedly lower content on *n*-heptane and *n*-nonane observed by Haagen-Smit *et al.*[5] was probably due to mechanical losses during distillation of these relatively volatile materials.

Of the remaining four pines of *Macrocarpae sensu lato*, *Pinus jeffreyi* and *P. sabiniana*, are essentially *n*-heptane pines. As mentioned, the presence of this normal hydrocarbon in their turpentines links these pines chemically to *P. torreyana*. While the first of the two does not cross with *P. torreyana*, hybrids between *P. torreyana* and *P. sabiniana* have been obtained.[2] In addition to normal paraffins *P. coulteri* contains 16–35 per cent of β-phellandrene and

TABLE 1. RELATIVE RETENTION VOLUMES OF SOME MATERIALS ENCOUNTERED

β,β-Oxydipropionitrile column, 65°		Carbowax 20 M column, 142°	
Compound	Rel. ret. volume	Compound	Rel. ret. volume
n-Hexane	0·19	*n*-Octyl aldehyde	0·34
n-Heptane	0·27	*n*-Decyl aldehyde	0·69
n-Octane	0·36	*n*-Dodecyl aldehyde	1·60
n-Nonane	0·57	Longifolene†	1·00
n-Decane	0·95	Peak 0·65	0·65
α-Pinene*	1·00		
n-Undecane	1·73	DEGS column, 109°	
β-Phellandrene	3·52	Limonene	0·26
n-Dodecane	3·36	β-Phellandrene	0·28
Terpinolene	4·25	Cineole	0·31
Cineole	4·85	Peak 0·65	0·62
		Decyl aldehyde	0·91
		Longifolene	1·0‡

* Appeared under these conditions after 5·8 min.
† Appeared under these conditions after 12·8 min.
‡ Appeared under these conditions after 22·8 min.

3·0–3·9 per cent of limonene and *P. oaxacana* 15–16 per cent of limonene in its turpentine. Thus, of the two provenances of *P. torreyana* investigated, the Santa Rosa population seems to be chemically slightly closer to Coulter pine due to the presence of β-phellandrene. While crossing of the Del Mar *P. torreyana* with *P. coulteri*, yielded no germinable seed[2] it would be interesting in view of these results to repeat this experiment using Santa Rosa material.

In regard to *P. oaxacana*, although the presence of limonene in its turpentine links it chemically to *P. torreyana* little can be said as to which of the two populations shows more affinity to that pine, particularly as the turpentine of *P. oaxacana* has not been analyzed by GLC methods, and β-phellandrene could have been overlooked in the only analysis available.

The oleoresin for our study was obtained from seven Torrey pine trees growing on Santa Rosa Island and from thirteen trees growing at the Torrey Pines State Reserve by the method described in our earlier publication[7] and the wounds were corked after oleoresin extraction. On Santa Rosa Island the sampled trees were scattered throughout the whole population and

[7] N. T. MIROV, E. ZAVARIN, K. SNAJBERK and K. COSTELLO, *Phytochem.* **5**, 343 (1966).

were 100–200 m apart, while at Del Mar the sampled area was about 1·5 km long with sampled trees 5–150 m apart.

TABLE 2. TURPENTINE ANALYSIS OF *Pinus torreyana* FROM SANTA ROSA ISLAND*

Sample No.	*n*-Heptane	*n*-Nonane	α-Pinene	*n*-Undecane	β-Pinene	Myrcene	Limonene	β-Phellandrene	Cineole	Peak 0·65	*n*-Decyl aldehyde	Longifolene	Total terpenes
195	2·5	2·0	3·0	7·0	0·5	1·5	70·5	12·5	—	tr	—	1·3	20·7
196	4·5	2·5	3·0	9·0	tr	1·5	71·0	9·0	—	0·3	—	3·0	18·5
197	3·5	2·5	2·5	6·0	tr	1·5	77·5	6·5	—	tr	—	2·5	25·5
198	3·5	2·5	3·0	7·5	tr	1·5	74·0	8·5	—	tr	—	3·0	20·5
199	3·5	3·0	3·0	8·5	tr	1·5	72·5	8·0	—	0·4	—	3·5	20·5
200	6·0	1·5	2·5	6·0	0·5	2·5	72·0	9·0	—	0·3	—	3·5	—
201	1·5	2·5	2·5	8·0	tr	2·0	76·0	7·5	—	tr	—	2·5	23·0
Mean	3·6	2·4	2·8	7·4	0·1	1·7	73·4	8·7	—	0·1	—	2·8	21·5
Mean dev.	1·0	0·3	0·2	0·9	0·2	0·3	2·1	1·2	—	0·2	—	0·6	1·6

* In per cent of total terpenes. Last column in per cent of total oleoresin.

TABLE 3. TURPENTINE ANALYSIS OF *Pinus torreyana* FROM NEAR DEL MAR*

Sample No.	*n*-Heptane	*n*-Nonane	α-Pinene	*n*-Undecane	β-pinene	Myrcene	Limonene	β-Phellandrene	Cineole	Peak 0·65	*n*-Decyl aldehyde	Longifolene	Total terpenes
239	2·0	0·5	2·5	6·0	0·5	2·0	85·5	—	2·0	0·5	tr	3·5	24·0
240	2·5	1·5	3·0	6·5	tr	2·5	84·0	tr	1·0	0·5	0·5	3·5	24·5
241	2·5	1·5	2·5	5·0	tr	4·0	84·0	0·5	0·5	1·0	1·0	4·0	26·5
242	3·0	2·0	2·5	6·0	0·5	3·5	82·0	0·5	0·5	0·5	0·5	4·5	25·0
243	1·0	1·5	2·5	7·0	tr	2·0	86·0	tr	1·0	0·5	0·5	4·0	24·0
244	2·5	1·5	2·5	6·0	tr	3·0	85·5	—	2·5	0·5	0·5	3·5	25·5
245	2·0	1·5	3·0	6·0	tr	4·0	83·0	0·5	tr	tr	tr	2·0	26·0
246	2·5	1·5	3·0	7·0	tr	4·0	82·0	tr	1·0	0·5	0·5	4·0	24·5
247	2·0	1·5	2·5	6·0	0·5	2·0	85·5	—	2·0	0·5	tr	3·0	24·0
248	2·5	2·0	2·5	6·0	0·5	3·0	83·5	—	tr	0·5	0·5	5·0	26·0
249	2·5	1·5	3·0	5·5	tr	2·5	85·0	—	0·5	0·5	0·5	2·0	25·0
250	2·5	2·0	3·0	6·0	tr	2·5	84·0	tr	0·5	0·5	0·5	4·5	24·5
251	1·5	2·0	3·0	6·0	0·5	2·5	84·5	—	0·5	0·5	0·5	5·5	21·5
Mean	2·2	1·7	2·7	6·1	0·2	2·9	84·2	0·1	0·9	0·5	0·4	3·8	24·7
Mean dev.	0·4	0·2	0·2	0·4	0·25	0·6	1·1	0·15	0·5	0·1	0·2	0·8	0·9

* In per cent of total terpenes. Last column per cent of total oleoresin.

EXPERIMENTAL

For analysis, an Aerograph Hy-Fi Model 600 C gas–liquid chromatographic instrument in combination with Brown-Honeywell "Electronik" recorder was used. Quantities of individual compounds were calculated from the integrated peak areas, using a disk chart integrator, Model 201 B. The oleoresin was injected directly (about 0·2 λ) after diluting it 1:15 with carbon disulfide; this diluent gives an exceptionally weak signal with the flame conductivity detector, thus allowing analysis of low-boiling materials such as *n*-heptane appearing early on the chromatogram. The commercial carbon disulfide usually contains an impurity which gives rise to a small peak at relative retention volume of 1·19 (relative to α-pinene). This rarely interferes with the analysis because of its low intensity and good separation from the terpenes. However, prolonged use of carbon disulfide results in an appreciably faster rate of corrosion of the metallic parts of the flame detector.

For the monoterpene analysis, a 10 ft by $\frac{1}{16}$ in. i.-d. column at 65° (flow rates 10 ml/min for both H_2 and N_2) filled with β, β-oxydipropionitrile 10 per cent on acid-washed Chromosorb W 60/80 was used. The relative retention volumes of terpenes encountered were published earlier.[8] Table 1 lists relative retention volumes of normal paraffins, most of which were identified in this study, together with some terpenes for comparison.

For analysis of higher-boiling materials, an 8 ft by $\frac{1}{4}$ in. i.-d. column filled with Carbowax 20 M, 10 per cent on Chromosorb W 60/80 acid-washed at 142° with flow rates of 10 ml/min for both H_2 and N_2 was used. Table 1 gives relative retention volumes for the various materials encountered.

The total terpene content of each oleoresin sample was determined by the internal standard method, using a 15 per cent solution of isopropylbenzene in mineral oil. Isopropylbenzene with a relative retention volume of 4·4 (α-pinene) appears right after β-phellandrene on the chromatogram. The content of the terpene and paraffin hydrocarbons was set as 100 per cent, and the per cent of oxygenated materials and sesquiterpenes was calculated on this basis. Tables 2 and 3 give the results.

In addition to the compounds mentioned, *n*-hexane, *n*-octane, camphene, 3-carene, sabinene, and terpinolene were often encountered in trace amounts in either of the two populations; Del Mar material also contained *n*-dodecyl aldehyde in small amounts. The identification of β-phellandrene, cineole, and decyl aldehyde has been substantiated using DEGS column (10 per cent DEGS on Chromosorb W 60/80 acid-washed, 15 ft × $\frac{1}{16}$ in. i.d. column, $T=109°$, flows 10 ml/min for both H_2 and N_2)—Table 1. Because materials encountered in substantial amounts were previously identified by preparative methods, all identifications were performed by comparison of relative retention volumes only.

Acknowledgements—The authors extend their appreciation to Frederick A. Meyer, Supervisor, Park Resource Management, Dept. of Parks and Recreation, Division of Beaches and Parks, State of California, Sacramento, for granting the permission to sample the trees in the Reserve, and to Messrs. N. R. Vail and A. L. Vail for permission to turpentine trees on their Santa Rosa Island property.

[8] N. T. Mirov, E. Zavarin and K. Snajberk, *Phytochem.* **5**, 97 (1966)