Phytochemistry, 1966, Vol. 5, pp. 97 to 102. Pergamon Press Ltd. Printed in England

CHEMICAL COMPOSITION OF THE TURPENTINES OF SOME EASTERN MEDITERRANEAN PINES IN RELATION TO THEIR CLASSIFICATION

NICHOLAS T. MIROV, EUGENE ZAVARIN and KAREL SNAJBERK

Forest Products Laboratory, University of California, Richmond

(*Received* 13 *April* 1965)

Abstract—The study is concerned with four eastern Mediterranean pines—*Pinus brutia* Ten., *P. eldarica* Medw., *P. pityusa* Steven, and *P. stankewiczii* Suk. Their relation to *P. halepensis* Mill. is indicated and their geographic distribution is discussed. Previous chemical investigations of their turpentines are reviewed and results of more recent original studies are reported. Very little difference was found in turpentine composition of *P. brutia, P. eldarica, P. pityusa*, and *P. stankewiczii*. Turpentines of all four, on the other hand, differed strongly from that of *P. halepensis*, and on this basis it seems that these four pines form a distinctly different group from the latter. The presence of 3-carene in these four pines (other than *P. halepensis*) suggest their possible relationship with *P. sylvestris* L., through its eastern Mediterranean form known as *P. sylvestris* var. *hamata* Sosn. While *P. eldarica, P. brutia*, and *P. pityusa* have levorotatory turpentine, as is that of var. *hamata*, the *P. stankewiczii* turpentine is dextrorotatory as is that of *P. sylvestris* proper.

INTRODUCTION

AMONG the pines of the eastern Mediterranean region there occur four species whose taxonomy is uncertain: *Pinus brutia* Ten., *P. eldarica* Medw., *P. pityusa* Steven and *P. stankewiczii* Suk. Some conservative botanists[1] consider the names of these pines as mere synonyms of *P. halepensis* Mill., a species widely distributed throughout the Mediterranean area; others[2] maintain that all four are separate entities, independent of *P. halepensis*.

Pinus brutia is found in Asia Minor, Iraq, Lebanon, Syria, some islands of eastern Mediterranean, and the Italian province of Calabria (ancient Brutium, whence the name of the pine) (Fig. 1). It is not certain whether *P. brutia* is native in Calabria or was planted there by the ancient inhabitants.[3] Apparently this pine occurs also in the Elburz Mountains, north of Teheran, Iran.[2, 3a] *P. brutia* hybridizes naturally with *P. halepensis*.[4]

Pinus eldarica is a rare pine, found in only one locality—in Azerbaijan, USSR, some 350 km east of the coast of the Black Sea. *Pinus pityusa* occurs in small groups, extending about 400 km along the Black Sea coast of the Caucasus (the ancient Pithyum).[5] *Pinus stankewiczii* grows on the south coast of the peninsula of Crimea in two locations, with a total area of about 450 hectares. Taxonomic difficulties regarding this pine are mentioned by Kolesnikov;[5] some authors consider it an independent species, others believe that it is a

---

[1] G. R. SHAW, *Publ. Arnold Arb.* **5**, 96 (1914).

[2] D. I. SOSNOVSKY, *Vêst. Tiflis. Bot. Sada* **18** (1910).

[3] A. FIORI, *Alpe* **18**, 328 (1931).

[3a] E. BOISSIER, *Flora Orientalis V.* p. 896. Georg, Geneva (1884).

[4] J. PAPAJOANNOU, *Forstwiss. Centr.* **58**, 194 (1936).

[5] A. I. KOLESNIKOV, *Sosna pitsundskaia i blizkie k nei vidy* [*Pitsunda pine and related species*], pp. 12, 58, 71, 142. Goslesbumizdat, Moscow (1963).

variety of *P. pityusa*; while still others think that there are not enough reliable morphological characteristics to warrant any separation from *P. pityusa*.

The turpentine of *P. halepensis* is dextrorotatory and is composed of (+)-α-pinene smaller amounts of other terpenes and some unidentified sesquiterpenes[6–9, 12] (Table 1). From the work of Lacrué,[8] who determined the physical constants of many samples of *P. halepensis* turpentines from Spain, Algeria, Italy, and Greece, it seems that its composition does vary only within rather narrow limits from locality to locality.

The turpentines of *P. brutia* from Cyprus[9, 10*] and from Greece[12] on the other hand were found to contain in addition to α-pinene, substantial amounts of β-pinene and 3-carene. The cyprian turpentine as well as the isolated α-pinene were found to be levorotatory.[9, 10]

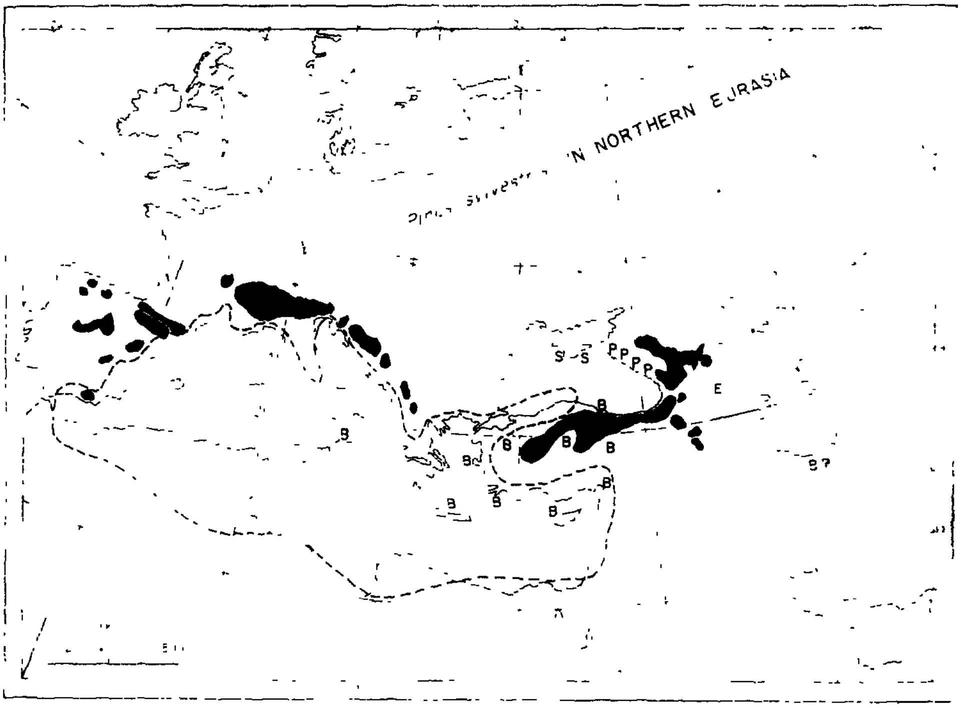

Fig. 1. Geographic distribution of several pines in the Mediterranean region.
Approximate area of: *P. brutia*—(B); *P. stankewiczii*—(S); *P. pityusa*—(P); *P. eldarica*—(E), *P. sylvestris* (Mediterranean region)—(black); *P. sylvestris* (Western part of northern Eurasia)—(····). *P. halpensis*—(– – –).

Arbuzov *et al.*[11] analysed the turpentine of *P. pityusa*. The turpentine had a specific optical rotation of −5·33° and was composed of (−)-α-pinene and 3-carene with a smaller amount of unidentified constituents (Table 1).

---

* The turpentine analysed was "obtained from *P. halepensis* from Cyprus." This would refer to *P. brutia*, because *P. halepensis* does not occur on Cyprus.

[6] M. Vèzes and G. Dupont, *Chim. & Ind. (Paris)* **8**, 318 (1922).

[7] G. Dupont, *Chim. & Ind. (Paris)* **8**, 320 (1922).

[8] M. T. Lacrué, *Inst. Forestal Invest. y Experiencias (Madrid) Bol.* **2**, 116 (1928).

[9] N. T. Mirov and P. M. Iloff, Jr., *J. Am. Pharm. Assoc. Sci. Ed.* **43**, 378 (1955)

[10] Anon. (London), *Imp. Inst. Bull.* **39**, 104 (1939).

[11] B. Arbuzov, L. Bastanova, E. Ivanova *et al. Zh. Prikl. Khim. Leningr.* **5**, 787 (1933)

[12] N. Iconomou, G. Valkanas and J. Büchi, *J. Chromat.* **16**, 29 (1964).

TABLE 1. ANALYSIS OF TURPENTINES OF SOME MEDITERRANEAN PINES[a]

Pine	Specific rotation of turpentine ($[\alpha]_D^{25}$)	Specific rotation of α-pinene ($[\alpha]_D^{25}$)	Percentages of components													
			α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene	Longifolene	Sesquiterpene U-1·12	Sesquiterpene U-1·50	Higher boiling materials and loss
P. eldarica (USSR)	−13·7[f]	(−)[f]	56·0	0·5	4·0	29·0	0·5	1·5	0·5	1·0	0·5	2·5	0·5	3·5	0·5	—
P. stankewiczii (USSR)	+24·2	+32·83	57·0	tr	6·5	26·5	1·0	1·0	0·5	tr	tr	2·5	0·5	4·0	0·5	—
P. brutia (Iran)	−32·0	(−)	79·5	1·0	6·0	9·0	—	tr	1·5	0·5	—	—	0·2	2·0	0·3	—
P. brutia (Syria)	−17·2	(−)	73·5	1·0	8·5	8·5	—	0·5	3·5	2·0	—	—	1·0	1·5	tr	—
P. brutia (Turkey)	−32·8	(−)	71·5	0·5	11·5	12·5	—	1·0	0·5	0·5	tr	1·0	0·2	0·8	tr	—
P. brutia (Turkey)	−33·7	(−)	71·5	1·0	13·0	10·5	tr	1·0	1·0	0·5	tr	0·5	0·4	0·6	tr	—
P. brutia (Cyprus)[9,b]	−28·7	−44·9	63·4	—	17·0	13·0	—	—	—	—	—	2·0	—	—	—	4·6
P. brutia (Greece)[12,e]	—	—	68·1	0·7	16·6	11·6	—	0·9	0·8	0·5	tr	0·7	Not investigated			—
P. pityusa (USSR)[11]	−5·33	−13·85	69·8	—	—	23·6	—	—	—	—	—	—	—	—	—	6·6
P. halepensis (France)[6,7,c]	+48·55	+48·13	95·0	—	—	—	—	—	—	—	—	—	—	—	—	3·86
P. halepensis (Israel)[9,b]	+41·25	+49·6	94·0	—	—	—	—	2·0	—	—	—	—	—	—	—	4·0
P. halepensis (Greece)[12,d]	—	—	96·2	0·7	0·8	0·2	—	0·7	1·2	0·1	—	0·1	Not investigated			—
P. sylvestris (France)[13]	+4·61	+13·0[18]	60·6	—	17·4	16·9	—	—	—	—	—	—	—	—	—	5·1

[a] Analyses for the last seven turpentines were taken from literature. Unknown trace constituents are not reported.

[b] The figures have been corrected to exclude fractionation residue.

[c] Contained 1·14% of bornyl acetate; specific rotation represents average, calculated from the data given.

[d] Average of 3 localities. Contained also traces of *p*-cymene.

[e] Contained also α-terpinene (0·1%) and traces of *p*-cymene.

[f] In cases where specific rotations were determined at conditions other than stated above, the corresponding corrections are made following each figure. Parentheses designate that the negative rotation of the total turpentine could be only explained by the (−)-α-pinene present and not by the presence of other (−)-components even if highest rotation values reported in literature are used in calculation.

Thus, preceding reports show that *Pinus halepensis* is devoid of 3-carene, whereas both *P. brutia* and *P. pityusa* turpentines contain considerable quantities of this terpene. In addition α-pinene contained in *P. halepensis* turpentine is chiefly dextrorotatory while that of *P. brutia* and *P. pityusa* is levorotatory. Therefore it appeared to us desirable to investigate further the problem of turpentine chemistry and its relation to the taxonomy of the eastern Mediterranean pines, still often lumped together under the name of *P. halepensis*.

DISCUSSION

Earlier investigations indicated that *Pinus halepensis* turpentine does not contain 3-carene but that turpentines of *P. brutia* and *P. pityusa* contain appreciable amounts of this terpene. The present analysis by GLC verifies the occurrence of large quantities of 3-carene in *P. brutia* turpentine from three new localities, Turkey, Syria, and Northern Iran, and also shows its occurrence in *P. eldarica* and *P. stankewiczii* turpentines (Table 1).

We found very little difference, if any, in turpentine composition of *P. brutia, P. eldarica, P. pityusa,* and *P. stankewiczii.* The presence of small amounts of camphene, sabinene, limonene, γ-terpinene, terpinolene, myrcene, and β-phellandrene is of minor importance for the taxonomic differentiation of these pines.

Some botanists consider the five pines as independent species, while others lump four of them as *P. brutia*, or all five as *P. halepensis*. This is a matter of personal preference.[14] The unescapable conclusion based on our investigations is that *P. brutia, P. eldarica, P. pityusa,* and *P. stankewiczii* compose one group, characterized by large amounts of 3-carene in their oleoresin, whereas *P. halepensis* forms another, monospecific group, containing but traces, if any, of 3-carene. As *P. brutia* crosses naturally with *P. halepensis*, the hybrids between these pines no doubt will contain 3-carene, but this will not invalidate our contention that 3-carene is essentially a terpene of the *P. brutia* group and not of *P. halepensis*.

There is one more pine in the Mediterranean region that contains 3-carene in its turpentine. It is *Pinus sylvestris* L.[15] whose main range is in northern Eurasia—from Scotland almost to the Siberian coast of the Pacific (Fig. 1). In the Mediterranean area it occurs as a Tertiary relic in Spain, southern France, and northern Italy, and also in the Balkans, the Caucasus, and Asia Minor. In the Caucasian Mountains it is known as *P. sylvestris* var. *hamata* Sosn., and in Transcaucasia it includes several varieties whose taxonomy is uncertain and whose chemistry is not known. Apparently *P. sylvestris* of the eastern Mediterranean does not cross naturally with *P. brutia* at present. The relationship of the Tertiary *P. sylvestris* to pines of the *P. brutia* group is not known. Presence of 3-carene in both seems to indicate that they are closely related. Perhaps they were even more closely related during the Tertiary Period. As seen in Table 1 the turpentine of *P. stankewiczii* has a positive, while turpentines of other members of *P. brutia* group have negative specific rotations. Apparently this is connected with the presence of (+)-α-pinene in the first and of (−)-α-pinene in the second case. Similarly while the turpentine of *P. sylvestris* proper has a negative specific rotation ((−)-α-pinene) that of its eastern Mediterranean form, known as *P. hamata* Sosn., is positively rotating.[16] This makes the problem of the relationship of these pines even more alluring. A thorough investigation of the geographic occurrence of 3-carene and (+)-(or

---

[13] G. DUPONT and M. BARRAUD, *Chim. & Ind. (Paris)* **19**, Special Number 4$^{\text{bis}}$, 559 (1928).
[14] N. T. MIROV, *Lloydia* **26**, 117 (1963).
[15] N. T. MIROV, *U.S. Dept. Agr. Forest Serv. Tech. Bull.* **1239**, 74 (1961).
[16] V. I. LIPSKY, *Trudy Tiflis. Bot. Sada*, **4**, 1 (1899).

(−)-) α-pinene in *P. sylvestris* of the Caucasus and in the related pines of Transcaucasia and Asia Minor may shed some light on the taxonomic relationship of the pines of the eastern Mediterranean area and even, possibly, on the tertiary migration routes of pines there.

EXPERIMENTAL

Materials

The two samples of *P. brutia* oleoresin from Turkey were collected in 1960 in the forest region of Mugla, southwestern part of the Asia Minor. The oleoresin samples of *P. brutia* from Syria, *P. eldarica* from Iran, *P. eldarica* and *P. stankewiczii* were supplied in 1962 by Mr. Ibrahim Nahal, Institut Botanique, Montpellier, from the USSR. More accurate descriptions of the localities were not furnished, but since these pines occur in well-known restricted areas there is no cause for confusion except with *P. eldarica* from Iran. This species has been studied intensively by Russian investigators, and they all agree that the only place where it grows naturally is in the northwestern part of the Soviet Azerbaijan, on the slopes of the ËldárOuk̈hi ("Guardian of the People") along the southwestern side of the Lori Valley.[5] Most probably the Iranian sample called *P. eldarica* came actually from *P. brutia*, a species indicated for northern Iran by Boissier[13] and commented upon by Sosnovsky.[2]

Isolation of Oleoresin

In each case the volatile part of the oleoresin was separated by distillation under reduced pressure, and the physical constants of the turpentines obtained were determined. The values for the specific rotations are listed in Table 1.

Chromatography

Since all of the turpentines examined stemmed either from various populations of *Pinus brutia* or from species very closely related to this pine, and since the main constituents of the turpentine of this pine have been preparatively identified before,[9] the identification of the individual terpenes in the present work was made chiefly by GLC methods. In this analysis Aerograph Hy-Fi Model 600 C gas–liquid chromatographic instrument, equipped with 6 ft by $\frac{1}{8}$ in. size columns in combination with Brown-Honeywell "Electronik" recorder, was used. The quantities of individual terpenes were calculated from the integrated peak areas, using disk chart integrator, Model 201 B. The oleoresin was injected directly (about 0·2 λ sample) after diluting it 1:15 with ethyl ether, which was found to give much sharper peaks. Merck's Reagent Anhydrous ether was used as this, unlike other samples contained only traces of an impurity running like camphene. The impurity does not seem to be connected with peroxide formation, as methods recommended for removal of peroxides from ether were found to be ineffective in removing it.

The stationary phases used for separation of terpenes included Silicone 550 20 per cent on Chromosorb 60/80 at 130° and β,β-oxydipropionitrile 10 per cent on the same support at 65° with the H_2 and N_2 flow of 15 ml/min each in either case. For separation of sesquiterpenes the same Silicone column at 180° (H_2 and N_2 flow 20 ml/min each) and Ucon polar 20 per cent on firebrick at 134° (H_2 and N_2 flow 15 ml/min each) were used. The relative retention volumes obtained were identical to those of authentic samples run under the same conditions and were, as a rule, close to those published previously;[17, 18] they are listed for terpenes in

---

[17] N. T. Mirov, E. Zavarin and J. G. Bicho, *J. Pharm. Sci.* **51**, 1131 (1962).

[18] M. H. Klouwen and R. ter Heide, *J. Chromat.* 7, 297 (1962).

Table 2. In the case of sesquiterpenes longifolene appeared after 13·5 min with Silicone and after 16·8 min with Ucon column. Two additional, unidentified, strong peaks appeared at relative retention volumes of 1·12 and 1·50 with Ucon (longifolene = 1·0); with Silicone, peak 1·12 merged with longifolene and peak 1·50 appeared at the relative retention volume of 1·16. Unidentified trace components were neglected.

TABLE 2. RELATIVE RETENTION TIMES FOR VARIOUS TERPENES

Terpene	Silicone 550, 130 α-Pinene = 4·3 min.	β,β-Oxydipropionitrile 65° α-Pinene = 5·1 min.
Santene	0·77	0·86
Tricyclene	1·32	0·93
α-Pinene	1·00	1·00
Thujene	0·91	1·17
Camphene	1·12	1·53
β-Pinene	1·32	1·90
3-Carene	1·56	2·33
Sabinene	1·23	2·56
α-Phellandrene	1·51	3·02
Myrcene	1·26	3·33
Limonene	1·72	3·57
β-Phellandrene	1·79	4·09
γ-Terpinene	1·98	4·95
Terpinolene	2·37	6·00
p-Cymene	1·72	8·47

α-Pinene from the turpentine of *P. stankewiczii* was additionally identified by its i.r. spectrum. The terpene was separated using Wilkens Autoprep A–700 gas–liquid chromatographic instrument in combination with $\frac{3}{8}$ in. by 20 ft column with Silicone SE–30 10 per cent on Chromosorb 60/80 as stationary phase, and at 250 ml per minute helium flow rate and 130 temp. The α-pinene obtained exhibited a specific rotation of $[\alpha]_{D}^{25} = +32{\cdot}83$.

Acknowledgements—The authors extend their appreciation to Mr. Ibrahim Nahal, Institut Botanique, Montpellier, France and to the Directorate-General of Forestry, Ministry of Agriculture, Turkey for procurement of oleoresin samples used in this investigation.