COMPOSITION OF GUM TURPENTINES OF *PINUS HALEPENSIS* AND *PINUS BRUTIA* GROWN IN GREECE

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(Received January 27th, 1964)

INTRODUCTION

Variations in the composition of gum turpentine oil (*i.e.* the steam volatile fraction of the oleoresin obtained by wounding a tree of the genus *Pinus*) have been reported for more than 90 well described and characterised species[1]. The detailed analysis of turpentine, which is a mixture of mainly terpenic hydrocarbons, has been hampered by the special difficulties involved in the analysis of this category of compounds. Gas chromatographic analysis is particularly attractive for the identification and characterisation of terpene mixtures. The high separation efficiencies, the inertness of the atmosphere during analysis, and the rapidity of the method, make it a most useful tool for analytical work[2–7]. The analysis of turpentine oil, however, has not been studied to the same extent as the analysis of other essential oils[8–11]. STANLEY AND MIROV[12] were the first to employ gas chromatographic methods in the analysis of American turpentine. The composition of gum turpentine from 22 species of *Pinus* grown in New Zealand has been similarly examined by WILLIAMS AND BANNISTER[10]. The characterisation of a number of turpentine oils of different origin has also been carried out in connection with the requirements of the Pharmacopœa Helvetica V[13].

Little is known about the composition of Greek turpentine. Some early studies are connected only with the determination of physical constants and the qualitative analysis of the mixture, only major components having been detected and characterised[14]. In Greece the production of turpentine oil had already been commercialised in olden times and many centuries of cultivation have resulted in a selection of the genus. The *Pinus halepensis* Mill., which is the main variety abundant on Greek soil, is known to have produced yearly 3–4 kg of oleoresin per tree for over 60 years, the highest reported production in the world[15]. Another variety, *Pinus brutia*, grows in only a few distinct districts of the country and is of minor commercial importance. *Pinus halepensis* grows in regions adjacent to the Mediterranean sea. The composition of this turpentine is reported in early analytical work as 95 % α-*d*-pinene for the low boiling destillate; the higher boiling fraction (tailing), accounting for less than 5 % of the product, being attributed to bornyl acetate (1.4 %) and to higher sesquiterpenes[16]. MIROV[17] has reported a composition of α-*d*-pinene 87 %, myrcene 2 %, sesquiterpenes 4 %, for a product of specific rotation +41.25°. *Pinus brutia* grows in the

Italian province of Calabria (ancient Brutium), Syria, Turkey, Greece and Cyprus. It is reported to give a laevorotatory turpentine, a first analysis of which gave a composition widely different from that of turpentine from *Pinus halepensis* (*l*- and *dl*-α-pinene 62 %, β-pinene 17 %, Δ^3-carene 13 %, terpinolene 2 %, sesquiterpenes 4.6 %), thus supporting the suggestion that the two pines are in fact different species[1]. Some botanists consider *Pinus brutia* to be a variety of *Pinus halepensis*, others believe that the two names are synonyms. *Pinus brutia* crosses naturally with *Pinus halepensis*[18].

EXPERIMENTAL AND RESULTS

To gather information on the change in turpentine oil composition with change in habitat, sampling was carried out in geographically different parts of the country. Samples of oleoresin of *Pinus halepensis* were collected in the districts of Corinth, Attica and Chalkidiki, whereas those of *Pinus brutia* come from Eubœa, where this species is very abundant. The samples were collected from a limited number of trees growing in representative areas of high growth density. Time of sampling and period of collecting were identical in all cases. The turpentine oil was separated by steam distillation on a laboratory scale and stored. Analyses performed at once and after storage indicated no changes in composition.

The standard Perkin-Elmer vapour fractometer model 116 used was equipped with a thermal conductivity detector and a 2.5 mV Siemens recorder. From a variety

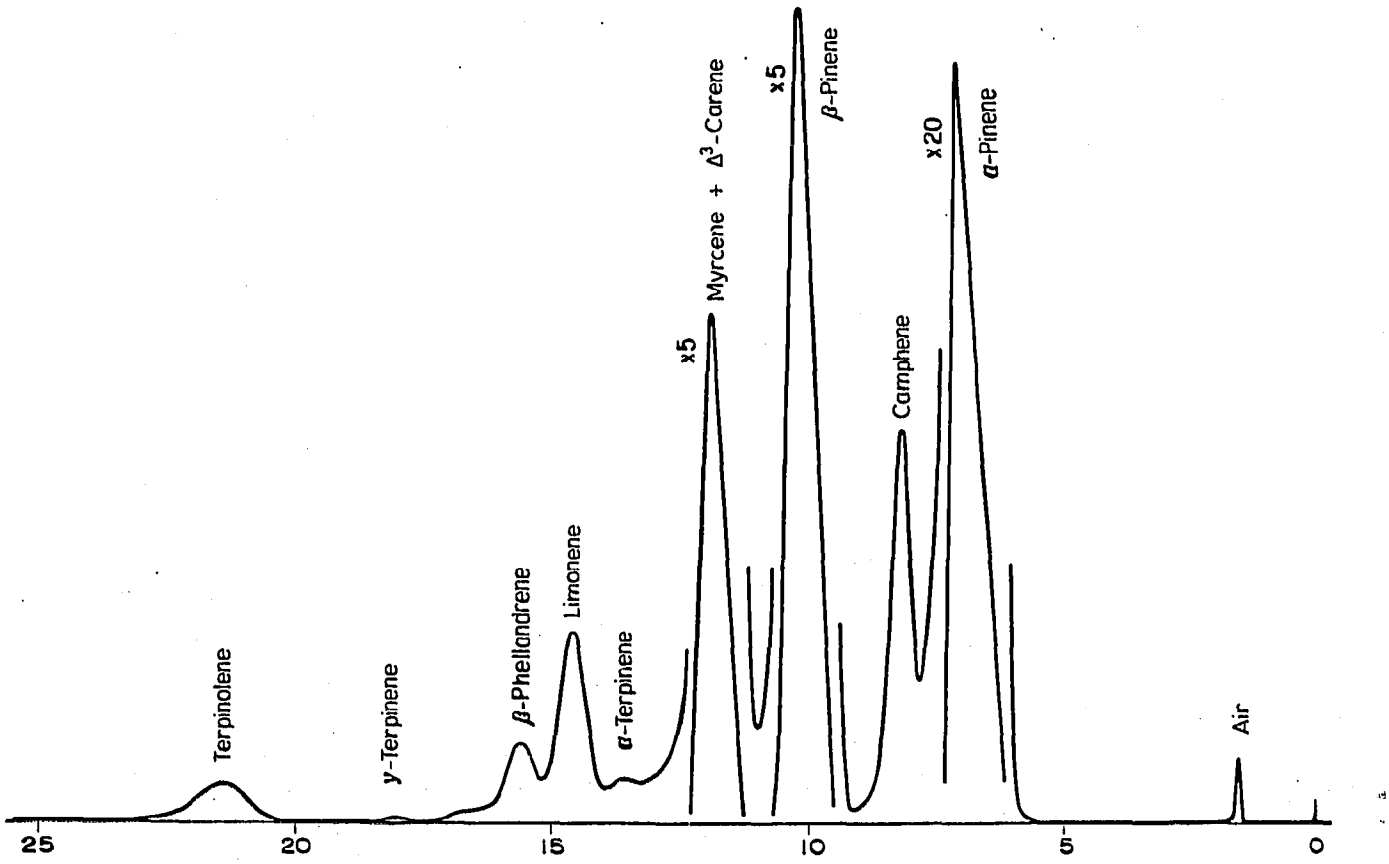

Fig. 1. Chromatogram of turpentine from *Pinus brutia* (Eubœa) on 4 m Carbowax 1500 column at 100° and helium flow rate 84 ml/min.

of columns tested the most satisfactory overall separation of the constituents of the turpentines was obtained with 4 m of Carbowax 1500, 16 % on Chromosorb W, and 4 m diisodecyl phthalate, 20 % on Celite 545, at temperatures of 100° and 162°, and helium flow rates of 84 ml/min and 86 ml/min respectively (Figs. 1 and 2).

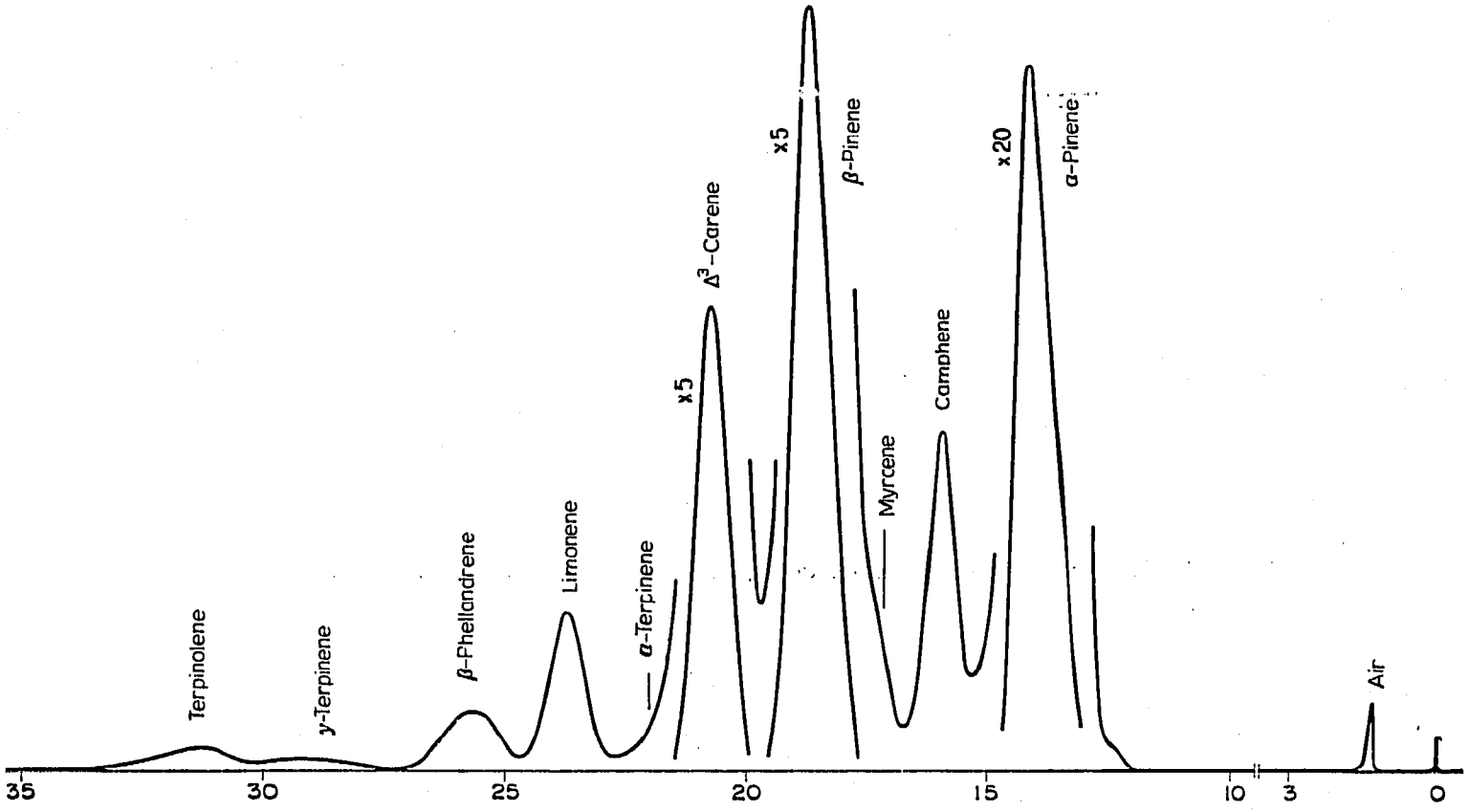

Fig. 2. Chromatogram of turpentine from *Pinus brutia* (Eubœa) on a 4 m diisodecyl phthalate column at 102° and helium flow rate 86 ml/min.

To identify the peaks obtained in the chromatograms, retention data were compared with those of authentic samples taken under the same conditions. Further proofs of identity were obtained by correlating our data with literature results, as described in another paper[19]. A Carbowax 1500 column gave better shaped peaks, more suitable for quantitative analysis, while a diisodecyl phthalate column gave a better separation. Myrcene and Δ^3-carene, which appear together on the first column, were well resolved on the second column, where myrcene precedes β-pinene. The results of the quantitative analyses, calculated by the methods already described[9], are identical for both columns within the limits of experimental error (Table I). The different temperatures employed show that there is no isomerisation under these analytical conditions; this has also been established in other studies[7].

There are practically no differences in the composition of the *Pinus halepensis* samples, which indicates that this variety on Greek soil does not show variation of turpentine oil composition with change in habitat. Since turpentine oil composition is connected with the tree physiology[20] this would infer that the *Pinus halepensis* variety in Greece is of unique genus. The composition of *Pinus brutia* turpentine differs

TABLE I

GAS CHROMATOGRAPHIC ANALYSIS OF TURPENTINES OF *Pinus halepensis* AND *Pinus brutia*

Compound	*Pinus halepensis*						*Pinus brutia* Eubœa	
	Attica		*Corinth*		*Chalkidiki*			
	Carbowax 1500	*Diisodecyl phthalate*	*Carbowax 1500*	*Diisodecyl phthalate*	*Carbowax 1500*	*Diisodecyl phthalate*	*Carbowax 1500*	*Diisodecyl phthalate*
α-Pinene, %	96.2	96.3	96.1	96.2	96.0	96.1	68.1	67.1
Camphene, %	0.7	0.65	0.7	0.7	0.8	0.7	0.7	0.9
β-Pinene, %	0.9	0.9	0.8	0.8	0.7	0.6	16.6	16.9
Unidentified	traces		traces		traces		traces	
Myrcene, %	1.05	0.75	1.1	0.8	0.8	0.5	12.5	0.9
Δ^3-Carene, %		0.25		0.3		0.2		11.6
α-Terpinene, %	—	—	—	—	—	—	0.1	0.1
Limonene, %	1.0	1.0	1.1	1.0	1.45	1.5	0.8	0.7
β-Phellandrene, %	0.05	0.05	0.1	0.05	0.05	0.1	0.5	0.5
p-Cymene, %	traces		traces		traces		traces	
γ-Terpinene, %	—	—	—	—	traces		traces	
Terpinolene, %	0.1	0.1	0.1	0.15	0.2	0.25	0.7	0.7

greatly from that of *Pinus halepensis* in having a high content of β-pinene and Δ^3-carene. This supports the theory that the two varieties are different species[1].

The relative retention times of components of turpentines from *Pinus halepensis* and *Pinus brutia* are shown in Table II.

TABLE II

RELATIVE RETENTION TIMES OF TERPENE HYDROCARBONS OF TURPENTINES FROM *Pinus halepensis* AND *Pinus brutia* COMPUTED FROM CHROMATOGRAMS 1 AND 2

(α-Pinene = 1.00)

Compound	*Stationary phase*	
	Carbowax 1500	*Diisodecyl phthalate*
α-Pinene	1.00	1.00
Camphene	1.32	1.15
β-Pinene	1.67	1.36
Myrcene	2.04	1.28
Δ^3-Carene	2.05	1.53
α-Terpinene	2.42	1.67
Limonene	2.67	1.79
β-Phellandrene	2.86	1.95
γ-Terpinene	3.4	2.08
Terpinolene	4.16	2.46

SUMMARY

Separate samples of gum turpentine *Pinus halepensis* and *Pinus brutia* grown in Greece were analysed by gas–liquid partition chromatography using two stationary phases of different polarity. It was found that the turpentine of *Pinus halepensis* consisted mainly of *d*-α-pinene (about 96 %), while that of *Pinus brutia* contained less

d-α-pinene (about 68 %) and substantially amounts of β-pinene (about 16 %) and Δ^3-carene (about 12 %). Other identified compounds were: camphene, myrcene, α-terpinene, limonene, β-phellandrene, *p*-cymene, γ-terpinene and terpinolene.

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