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TURPENTINE CHEMISTRY AND TAXONOMY OF THREE PINES OF SOUTHEASTERN ASIA

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Abstract—Turpentines of *Pinus khasya** and *Pinus insularis*, and to a lesser degree of *Pinus yunnanensis*, obtained from various localities have been investigated. Data obtained indicate the existence of two chemical races of *P. khasya*: a northern (Assam and Burma) race characterized by high β-pinene, low β-phellandrene and high longifolene content, and a southern (Viet Nam) race characterized by low β-pinene, high β-phellandrene and low longifolene contents. No difference in turpentine composition was found between *Pinus khasya* from Viet Nam and *Pinus insularis* from the Philippines.

3-Carene was identified as a constituent of *P. yunnanensis* oleoresin; this terpene is absent in both *P. khasya* and *P. insularis*. However these findings are based on only one sample of oleoresin collected from a few planted trees, and verification is desirable.

INTRODUCTION

IN THE southeastern part of Asia are found three pine species, *Pinus yunnanensis* Franchet, *P. khasya* Royle, and *P. insularis* Endlicher, whose taxonomic status has not been ascertained. These pines were described by Franchet,[1] Royle,[2] and Endlicher,[3] as independent species, but some botanists[4] now believe that they all belong to the same species.

P. yunnanensis, is a pine of the Chinese province of Yunnan (Fig. 1). It is found to a limited extent in the adjacent provinces of Szechwan and Sikang. In the northeast, *P. yunnanensis* merges into *P. tabulaeformis* Carr., an extremely variable species of northern China and is a subject for special investigation. Shaw[5] considered *P. tabulaeformis* and *P. yunnanensis* to be synonyms of *P. sinensis* Lambert.

In the mountains of Sikang-Yunnan-Szechwan, where *P. yunnanensis* merges into *P. tabulaeformis*, there occurs a pine known as *P. densata* Masters which is believed to be a hybrid between *P. yunnanensis* and *P. tabulaeformis*.[6]

In the southeast, *P. yunnanensis* imperceptibly merges into *P. khasya*. *P. khasya* extends from the mountains of the Khasi states of northeastern India through the Magi hills and toward Yunnan, where it meets *P. yunnanensis*. Southeast of the Khasi country, *P. khasya* extends to Manipur and Upper Burma (Chin Hills, Shan States, and Karenni Country). It occurs also in the northern part of Thailand and in the extreme southeastern part of Laos.

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* Spelled, as are many names in that part of the world, in different ways: khasya, khassiana, kasya, khasia, keseya.

[1] A. FRANCHET, *J. Botan., Paris* 13, 253 (1899).
[2] J. F. ROYLE, *Prodr.* 16, 390 (1868).
[3] I. L. ENDLICHER, *Syn. Conif.* 157 (1847).
[4] CHUNG-LWEN WU, *Acta phytotax. sin.* 5, 131 (1956).
[5] G. R. SHAW, *Publ. Arnold Arb.* 5, 60 (1914).
[6] M. T. MASTERS, *J. Linnean Soc. Zool.* 37, 417 (1906).

In Burma it is known as *P. insularis* (a species to be described later), but in Thailand it goes under the name of *P. khasya*. In the southern Shan States of Burma it forms rather large forests, but in Thailand and Laos it occurs only in a few widely separated patches.

Farther south the altitude is too low for this pine and it appears again in the mountains of southern Viet Nam, where, near Dalat, it occurs in scattered groves over a rather extensive area; here it is known as *P. khasya*.

P. insularis is a Philippine pine. It forms extensive pure forests in the mountain provinces of northern Luzon, and occurs in one or two localities of the Zambales Mountains which extend along the South China Sea coast of the island.

In herbaria, *P. yunnanensis*, *P. khasya*, and *P. insularis* look alike; in their natural habitats and in plantations these three pines display some morphological variations.[7,8]

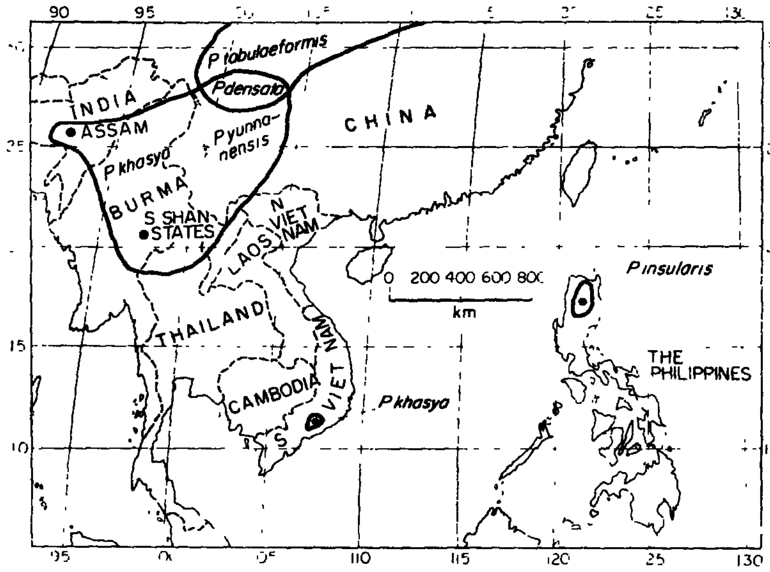

FIG. 1. DISTRIBUTION OF PINES IN SOUTHEASTERN ASIA.

The chemical composition of *P. tabulaeformis* and *P. densata* turpentines as well as of Viet Namese *P. khasya* turpentine apparently has never been satisfactorily investigated. *P. yunnanensis* turpentine obtained from several trees planted in California was examined by Mirov[9] and found to be composed chiefly of (−)-α-pinene. There was not enough material available to identify other terpenes. *P. khasya* turpentine from Assam was found by Simonsen and Rau in 1922 to be dextrorotatory and to consist of 70% α-pinene, 10% β-pinene, and 10% longifolene.[10] The *P. khasya* turpentine from Burma was later found to be similar in composition except for the sesquiterpenes which apparently were not investigated[11] (Table 2).

The turpentine from *P. insularis* on the other hand was found by Iloff and Mirov to be composed of 74% (+)-α-pinene, 3·0% (−)-β-pinene, 7% l-β-phellandrene, 7·0% tails

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[7] C. J. A. SHELBOURNE, *Commonwealth Forestry Rev.* **42**, No. 114, 334 (1963).

[8] B. M. SAVORY, *Empire Forestry Rev.* **41**, No. 107, 67 (1962).

[9] N. T. MIROV, *J. Am. Pharm. Assoc. Sci. Ed.* **47**, 410 (1958).

[10] J. L. SIMONSEN and M. G. RAU, *Indian Forest Records* **9**, 112 (1922).

[11] G. S. FISHER, *Chem. & Ind. (London)* **44**, 1761 (1963).

(including a sesquiterpene different from longifolene) and 5·0% pot residue.[12] Thus, it contained more β-phellandrene but apparently no longifolene.

The foregoing indicates that taxonomic relationship of the three south-western Asiatic pines, *P. yunnanensis*, *P. khasya*, and *P. insularis*, based chiefly on morphology of herbarium specimens has not been satisfactorily settled. Chemical studies of these three pines have been so limited that they could not clarify the situation. Accordingly, we analysed seven additional samples of oleoresin from several populations of these pines (Table 1).

Table 1. Physical properties of oleoresins

Sample No.	Species and source	Weight of oleoresin (g)	Turpentine yield (%)	Density (d_4^{25})	Index of refraction (n_D^{25})	Specific rotation ($[\alpha]_D^{25}$)
B–1	*P. khasya* Forest Reserve at Kalaw, southern Shan States, Burma	353	26·6	0·8612	1·4696	+26·92°
B–2	*P. khasya* From a single tree near Kingswood School, Kalaw, southern Shan States, Burma	65	27·0	0·8549	1·4640	+47·67°
VN–1	*P. khasya* Vicinity of Dalat, Viet Nam	111	26·7	0·8487	1·4692	+32·46°
A–1	*P. khasya* Shillong Range District, Assam, India	64	24·5	0·8560	1·4747	−4·22°
A–2	*P. khasya* Shillong Range District, Assam, India	250	15·0	0·8720	1·4751	−0·43°
Y–1	*P. yunnanensis* Eddy Arboratum, Institute of Forest Genetics, Placerville, California	13	20·4	0·8584	1·4658	−43·92°
Ph–1	*P. insularis* Luzon, Philippine Islands, grown in Zambia (Africa)	5	—	—	—	—

DISCUSSION

The data presented herein indicate that *P. khasya* from Viet Nam and *P. insularis* are nearly identical in the composition of their turpentines. It is also evident that *P. khasya* has two different chemical races, the "physiological forms" of Penfold[13]—a northern race, occurring in Assam and possibly in northeastern Burma and extending to the adjacent areas of Yunnan, and a southern race which occurs in southern Viet Nam and which also includes *P. insularis* of the Philippines. The northern race may be characterized by a high percentage of β-pinene, a low percentage of β-phellandrene, and a generally high percentage of longifolene; the southern race has a low percentage of β-pinene, a high percentage of β-phellandrene, and a low percentage of longifolene.

[12] P. M. Iloff, Jr. and N. T. Mirov, *J. Am. Pharm. Assoc. Sci. Ed.* **43**, 742 (1954).

[13] A. R. Penfold, *Australasian J. Pharm.* **16**, 168 (1935).

In the southern Shan States of Burma (and possibly in adjacent parts of Thailand extending into Laos) the two races seem to meet. The chemical composition of turpentine from that area (sample analysed by Fisher, and our composite sample B-1) is that of the northern race of trees. Our sample B-2 from the same area was anomalous inasmuch as it contained only a small amount of β-pinene, this should not be too significant, however, as the sample was secured from a single tree. To determine the taxonomic status of *P. khasya* in the Karenni country, in the southern Shan States, and in Thailand and Laos, samples from all of these places should be examined. In these localities, however, this pine occurs in widely separated, often inaccessible places which presently are hard to visit.

Our data indicate that turpentine of *P. yunnanensis* contains 3-carene, a terpene absent in both *P. khasya* and *P. insularis*. The consistency of the presence of this terpene in *P. yunnanensis* is a subject for a more thorough investigation.

EXPERIMENTAL

Materials

All oleoresin samples investigated (Table 1) were obtained from a large number of trees, except for the sample B-2 collected from a single tree, and the sample Ph-1 collected from two trees. To separate turpentine from rosin, oleoresin was heated at 25 mm pressure and the temperature was slowly raised to 180°. Distilled turpentine was removed and the residue heated again at 215° and 2 mm pressure; combined distillates were subsequently used for investigations. Before heating, each oleoresin was checked by GLC for the presence of low-boiling constituents such as *n*-heptane which could easily be lost under the distillation conditions employed. Table 1 lists the yields and physical constants of the turpentines obtained.

Fractionation of Turpentines

The turpentine samples B-1 (80·0 g) and VN-1 (29·6 g) were fractionated under reduced pressure in a 90 cm long, 25 mm inside-diameter Stedman column at a 10:1 reflux ratio. Physical constants of the fractions obtained were determined and the fractions analysed by GLC methods. From the specific rotations of the fractions obtained and their compositions it followed that α-pinene in Burmese B-1 turpentine existed mainly as (+), and β-pinene as (−) optical isomer; similarly, α-pinene from Viet Namese turpentine VN-1 was found to be composed mainly of (+), and β-phellandrene of (−) form. For determination of the specific rotations of α- and β-pinenes from Assamese A-2 oleoresin, a 4·5 g sample of the corresponding turpentine was separated by fractionation into 6 fractions. Specific rotations for pure terpenes were computed from the rotations of first and last fraction, and the corresponding analyses by GLC to +40·55 for α-pinene and −27·10 for β-pinene. Since this oleoresin stemmed from the same general area as did that used in the work of Simonsen, this seems to dispose of the question of the rotation sign of β-pinene from *P. khasya* of Assam.[11]

β-Phellandrene from turpentine B-1 (fraction 6) Table 2, was identified by preparation of nitrosite, m.p. 96–97°; α-pinene from turpentine VN-1 (fraction 1) was characterized as nitrosochloride, m.p. 97·0–98·5°, and β-phellandrene from the same turpentine (fraction 3) as nitrosite, m.p. 98·5–99·5°. Neither of the derivatives showed a melting point depression when admixed with an authentic sample. No attempt was made to identify by preparation of the derivatives the other terpenoids present in these two or other turpentines investigated—partly because some of these components have been previously identified by other workers,

TABLE 2. RESULTS OF GLC ANALYSES OF VARIOUS OLEORESIN SAMPLES OF THREE PINE SPECIES OF SOUTHEASTERN ASIA

Oleoresin	Components of turpentine (%)												
	α-Pinene	Camphene	β-Pinene	3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene	*P*-Cymene	Longifolene	Unknown sesquiterpene A	Unknown sesquiterpene B
P. yunnanensis (Y-1)	90·1	1·0	4·5	2·0	—	0·5	0·5	0·5	—	—	0·1	0·1	0·3
P. khasya, Assam[10]	70·0	—	10·0	—	—	—	—	—	—	—	10·0	—	—
P. khasya, Assam (A-1)	46·5	0·5	47·0	—	—	0·5	1·0	2·5	—	—	1·0	1·0	tr
P. khasya, Assam (A-2)	39·0	0·5	52·0	—	—	0·5	1·0	1·0	tr	—	5·0	0·5	0·5
P. khasya, Burma[11]	75·3	0·6	20·0	—	—	0·4	0·4	2·4	—	—		no data	
P. khasya, Burma (B-1)	68·5	0·5	23·5	—	—	0·5	1·0	2·0	tr	tr	4·0	tr	tr
P. khasya, Burma (B-2)	90·5	1·5	1·5	—	—	1·0	1·0	0·5	—	—	4·0	tr	tr
P. khasya, Viet Nam (VN-1)	78·5	tr	1·5	—	0·5	1·2	0·5	17·0	0·5	—	0·1	0·2	tr
P. insularis, Philippines[12]	74·0	—	3·0	—	—	—	—	over 7·0	—	—	—	about 2·0	—
P. insularis, (Ph-1) Philippines	82·0	0·5	1·0	—	—	1·5	1·0	11·5	0·5	—	1·0	1·0	tr

and partly because some occurred in amounts too small for a convenient preparative identification. In these cases identification was accomplished by GLC data alone, as described below.

All oleoresins obtained were analysed for the composition of their turpentines by GLC methods, using a Model 600 C flame ionization detector equipped Aerograph Hy-Fi, in combination with a Brown-Honeywell "Electronik" recorder equipped with a disc chart integrator, Model 201B. Table 2 gives the results. For terpene analysis a β,β-oxydipropionitrile column (10 per cent on chromosorb W acid washed 60/80 at 65°) and silicone 550 column (20 per cent on the same support at 130°) were used. For sesquiterpene analysis the same silicone column was used at 180° as well as a Ucon polar column (20 per cent on the same support) at 130°. The oleoresin was diluted with ethyl ether prior to its introduction in the instrument. Details of the procedures used, and relative retention volumes obtained, either have been published,[14, 15] or will appear in connection with our study of Mediterranean pines. The unknown "sesquiterpenes" A and B had relative retention volumes of 1·12 and 1·50 with Ucon procedure, and 1·00 and 1·35 with silicone procedure (longifolene = 1·00). The terpenoids separated were identified by comparing their relative retention volumes with the relative retention volumes of authentic samples run under the same conditions, compositions of turpentines were computed from the integrated peak areas.

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

[15] M. H. KLOUWEN and R. TER HEIDE, *J. Chromat.* **7**, 297 (1962).