Composition of Gum Turpentine of Pines XXX*

A Report on *Pinus serotina, Pinus tenuifolia,* and *Pinus yunnanensis*

By N. T. MIROV

Pinus serotina **turpentine was found to consist of *l*-α-pinene, 5 per cent; *l*-limonene, 90 per cent; methyl chavicol, 1 per cent; and 2 or 3 per cent of unidentified sesquiterpenes. *P. tenuifolia* turpentine contained: *d*-α-pinene, 77 per cent; *l*-β-pinene, 6 to 7 per cent; Δ³-carene, 8 per cent; terpinolene, 1 to 2 per cent; methyl chavicol (possibly) less than 1 per cent; linalool, about 1 per cent; sesquiterpene fraction, 1 per cent. *P. yunnanensis* turpentine contained *l*-α-pinene, 87 per cent; possibly small amounts of *l*-β-pinene; oxygenated terpene compounds, 5 or 6 per cent. Apparently there were no sesquiterpenes.**

Pinus Serotina **Mich.**—*Pinus serotina,* or pond pine, sometimes is described as a subspecies (1) or a variety of *Pinus rigida.* Shaw (7) and Little (5) consider this pine as an independent species.

Pinus serotina grows on the coastal plains from southern New Jersey to central and northwestern Florida and Alabama. Its turpentine was analyzed in 1908 by Herty and Dixon (3). The steam distilled turpentine had these physical characteristics: density, $d^{20} = 0.8478$; index of refraction, $n_D^{20} = 1.4734$; specific rotation, $[\alpha]_D^{20} = -105°36'$. The turpentine began to boil at 172°. In the fraction boiling at 175° to 176°, Herty and Dixon identified *l*-limonene by preparation of a tetrabromide. No other terpenes were identified, although a small amount of sesquiterpenes was suspected. Herty and Dixon reported that 90 per cent of the turpentine consisted of *l*-limonene.

In 1954 we received a sample of pond pine oleoresin from the Olustee Experimental Forest, northern Florida. The turpentine was obtained by heating the oleoresin under reduced pressure; at the end of the operation, the pot temperature reached 212°, and the pressure was reduced to 2 mm. The yield of turpentine was 19 per cent of the weight of the oleoresin. The physical characteristics of the turpentine were these: density, $d_4^{25} = 0.8437$; index of refraction, $n_D^{25} = 1.4716$; specific rotation, $[\alpha]_D^{25} = -83.7$.

A 300-Gm. batch of the turpentine was fractionated with a Vigreux-type column, 80 cm. long and 1.5 cm. inside diameter, equipped with a heated jacket. A reflux ratio 10 to 1 was maintained. The pressure was 663 mm. In fractions 1 and 2, α-pinene was identified. A copious precipitate of pinene nitrosochloride was obtained from both fractions when the oil was diluted with glacial acetic acid, chilled, mixed with amyl nitrite, and a 1:1 mixture of hydrochloric and acetic acids was added dropwise. After three recrystallizations from chloroform by addition of cold methanol, the nitrosochloride melted at 104–105°.

Attempts to identify β-pinene in fraction 6 were not successful. Physical characteristics of fractions 8 to 17 (Table I) clearly indicated the presence of large amounts of *l*-limonene. This terpene was identified in fraction 13 by preparation of limonene tetrabromide. After four recrystallizations from ethyl acetate, the tetrabromide possessed a melting point of 105–106°. Fraction 17 was extremely fragrant. It contained a great deal of limonene, to be sure, but also, apparently, an admixture of some oxygenated compounds. This fraction was redistilled to remove most of limonene. The higher boiling part of the fraction (1.3 Gm.) was oxidized to homoanisic acid. [See THIS JOURNAL **43,** 741(1954)]. Recrystallized from hot *n*-hexane, the homoanisic acid possessed a m. p. of 85–86°. The homoanisic acid was further oxidized, by the action of chromic anhydride, to anisic acid. Recrystallized from *n*-hexane, the acid had a melting point of 183.7–184.6°. There was no depression of melting point upon admixture of authentic anisic acid. Thus, presence of methyl chavicol in the turpentine of *P. serotina* was established.[1] Possibly the flask residue contained some sesquiterpenes, but these did not amount to more than 2 or 3% of the total oil.

To sum up, turpentine of *P. serotina* contained: *l*-α-pinene, 5%; *l*-limonene, 90%; methyl chavicol, 1%, and 2 or 3% of unidentified sesquiterpenes.

Pinus Tenuifolia **Benth.**—*Pinus tenuifolia* Benth. was discovered and named in 1839, but later it lost its specific rank and was described by Shaw (7) as a variety of *Pinus pseudostrobus.* Martinez (6) considers it an independent species.

Judging by the chemistry of its turpentine, which is much more complicated and quite different from that of *P. pseudostrobus,* we concur with Martinez, and consider *P. tenuifolia* a valid species.

An oleoresin sample of *P. tenuifolia* came to us through the courtesy of Mr. Louis Huguet from the vicinity of Uruapan, Michoacan, Mexico. The turpentine was expelled from the oleoresin under reduced pressure; at the end of the distillation, pot temperature was 180°, and pressure was 2 mm. The yield of turpentine was 27%. The physical characteristics of the turpentine were as follows: density, $d_4^{23.5} = 0.8600$; index of refraction, $n_D^{23.5} = 1.4672$; specific rotation, $[\alpha]_D = +25.6$.

A batch of 964 Gm. of the turpentine was fractionated in the Todd apparatus described in previous articles of this series; 72% of the turpentine was

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Included in this article is a brief summary of the entire project.

[1] Thanks are due to Dr. Gene Kritchevsky for identification of methyl chavicol.

distilled under atmospheric pressure, the rest under a pressure of 9 mm. Hg. The results of the fractional distillation are shown in Table I.

The lower-boiling fractions consisted of *d*-α-pinene; this terpene was identified in fraction 2 by the usual preparation of nitrosochloride, which after several precipitations from chloroform by cold methanol, possessed a melting point of 105 to 106°. Fraction 12 was tested for β-pinene, by the usual preparation of nopinic acid, which, after two recrystallizations from benzene, had a melting point of 127°.

Fraction 16 yielded a nitrosate; after recrystallization from chloroform by addition of cold methanol,

TABLE I.—FRACTIONAL DISTILLATION OF TURPENTINE OF PINUS SEROTINA, P. YUNNANENSIS, AND P. TENUIFOLIA

Fractions	Pressure	Boiling Range, ° C.	Distillate, %	Density	Index of Refraction	Specific Rotation
(1) *Pinus serotina* (308 Gm. used)				d_4^{25}	n_D^{25}	$[\alpha]_D^{25}$
1	663	150–161	1.7	0.8445	1.4617	−42.0
2	663	161–162	4.0	0.8416	1.4681	−49.8
3	663	162–163	1.9			
4	663	163–164	9.5	0.8423	1.4689	−57.9
5	663	164–165	2.2			
6	663	165–166	14.6	0.8426	1.4693	−70.6
7	663	166–167	1.3			
8	663	167–168	2.2	0.8402	1.4702	−88.0
9	663	168–169	16.6	0.8397	1.4706	−100.0
10	663	169–170	4.7			
11	663	170–171	5.5	0.8396	1.4709	−108.4
12	663	171–172	7.8			
13	663	172–173	14.6	0.8396	1.4714	−117.3
14	663	173–174	2.3			
15	663	174–175	1.3	0.8427	1.4725	−111.7
16	663	175–178	1.7			
17	663	178–200	1.9	0.8713	1.4829	−85.0
Residue	...	...	4.9	...	...	...
Losses	...	...	1.3	...	...	...
(2) *Pinus tenuifolia* (964 Gm. used)				d_4^{23}	n_D^{23}	$[\alpha_D^{23}]$
1	760	107–155	0.4			
2	760	155–156	62.0	0.8557	1.4641	+40.4
3	760	156–157	9.7	0.8558	1.4651	+35.1
4	760	38–39	2.6	0.8569	1.4662	+29.9
5	769	39–41	1.1	0.8579	1.4673	+22.8
6	769	41–43	0.4	0.8588	1.4681	+18.9
7	769	43–44	0.8	0.8593	1.4697	+8.2
8	769	44–45	0.5	0.8595	1.4710	−0.2
9	769	45–46	0.8	0.8595	1.4721	−7.0
10	769	46–47	0.9	0.8602	1.4733	−13.7
11	769	47–48	1.0	0.8602	1.4740	−17.0
12	769	48–49	1.2	0.8602	1.4740	−17.0
13	769	49–50	1.3	0.8602	1.4740	−14.0
14	769	50–52	1.4	0.8602	1.4731	−4.6
15	769	52–53	1.2	0.8593	1.4721	+3.5
16	769	53–54	3.5	0.8584	1.4719	+13.5
17	769	54–55	2.3	0.8581	1.4712	+14.5
18	769	55–56	0.5	0.8581	1.4730	+7.4
19	769	56–62	0.7	0.8508	1.4750	−0.9
20	769	62–66	0.6	0.8548	1.4800	−2.1
21	769	66–68	0.5	0.8614	1.4843	−0.9
22	769	68–70	0.3	0.8626	1.4850	−1.2
23	769	70–80	0.4	0.8711	1.4782	−2.5
24	769	80–84	0.6	0.8711	1.4681	−12.1
25	769	84–86	0.5	0.8711	1.4685	−12.0
26	769	86–90	0.3	0.8964	1.4745	−9.4
27	769	90–95	0.8	0.9433	1.4841	−8.9
Residue and losses		...	3.7	...	...	...
(3) *Pinus yunnanensis* (29 Gm. used)				d_4^{25}	n_D^{25}	$[\alpha]_D^{25}$
1	760	153–154	3.8	0.8535	1.4630	−47.3
2	760	154–155	61.3	0.8510	1.4638	−50.5
3	760	155–157	13.1	0.8523	1.4648	−47.7
4	760	157–159	5.9	0.8556	1.4664	−42.1
5	760	159–161	2.4	0.8530	1.4683	−32.2
Residue	...	above 161	7.6	0.8743	1.4823	−6.9
Losses	...	...	5.9	...	...	...

the nitrosate melted at 146°. Thus the presence of *d*-Δ^3-carene in turpentine of *P. tenuifolia* was established.

Terpinolene was detected in fraction 21. A tetrabromide prepared from the oil of this fraction was recrystallized from chloroform by addition of cold methanol. The melting point of the tetrabromide was 109 to 110°.

Fractions 23 and 24 possessed a fragrant odor, suggesting the presence of an oxygenated terpene compound. Fraction 24 was examined by Dr. Gene Kritchevsky and was found to contain linalool. Its *p*-nitrobenzoate possessed a melting point of 69.0 to 70°. Its phenylurethane melted at 63.6 to 64.2°. The spectral data of fraction 24 suggested the presence of small amounts of methyl chavicol.

Judging from the density of fraction 27, it obviously contained some sesquiterpenes. A hydrochloride was prepared using 3 Gm. of the oil of this fraction, dissolved in 24 cc. of dry ether, and treated with dry HCl gas for thirty minutes at 0°. The ether was then evaporated. Upon standing in a refrigerator for three weeks, hydrochloride crystals were formed, but these melted as soon as the evaporating dish was removed from the refrigerator.

Similar behavior of a hydrochloride melting at low temperature has been observed in *Pinus insularis* and *P. glabra.*

To sum up, turpentine of *P. tenuifolia* contained: *d*-α-pinene, 77%; *l*-β-pinene, 6 to 7%; *d*-Δ^3-carene, 8%; terpinolene, 1 to 2%; methyl chavicol (possibly), less than 1%; linalool, about 1%; sesquiterpene fraction, 1%.

***Pinus yunnanensis* Franchet.**—*Pinus yunnanensis* was described by Franchet in 1899 as an independent species. Shaw (8) considers it as a variety and calls it *Pinus sinensis* var. *yunnanensis.* Lately, however, the name *P. sinensis* has fallen into misuse and to a certain degree has been replaced by the name *P. tabulaeformis. Pinus yunnanensis* has again become in the eyes of some botanists (4) a valid species.

Wu (9), working with herbarium material, arrived at the conclusion that *Pinus yunnanensis* and *Pinus insularis* are the same species. It is seen then, that opinions differ as to the botanical status of *P. yunnanensis.*

This pine grows in all of Yunnan province except the alpine zone and in the southwestern part of Kweichaw province. It also occurs in upper Burma, northern Viet Nam, and in a narrow belt of northern India.

As it was difficult to obtain oleoresin of this pine from its native habitat, the writer tapped a few planted trees growing in the Eddy Arboretum, Institute of Forest Genetics, near Placerville, California. A little more than 30 Gm. of turpentine was obtained by heating the oleoresin under reduced pressure. At the end of the distillation, temperature was raised to 180°, and the pressure was reduced to 1 mm.

The yield of turpentine was 22.6%; its characteristics were as follows: density, $d_4^{21.5} = 0.8591$; index of refraction, $n_D^{23} = 1.4663$; specific rotation, $[\alpha]_D^{23} = -44.3$.

A batch of 29 Gm. was fractionated in a Todd column fitted with a Vigreux-type tube 80 cm. long and 1.5 cm. inside diameter. The reflux ratio was 10 to 1 throughout the distillation. Results of the fractionation are presented in Table I.

Judging from the physical characteristics of the fractions, *P. yunnanensis* turpentine consisted of at least 87% *l*-α-pinene. A pinene nitrosochloride was prepared from fraction 2. The nitrosochloride, recrystallized from chloroform by addition of cold methanol, possessed a melting point of 106–107°. Addition of authentic pinene nitrosochloride did not lower the melting point.

A slight drop in density of fraction 5 suggested the presence of another terpene, but there was not enough material to identify it.

Characteristics of the residue (which was not "oily" and was very fragrant) do not point to any appreciable amounts of sesquiterpenes, but they suggest the presence of a dextrorotatory oxygenated compound.

To sum up, *P. yunnanensis* turpentine contains *l*-α-pinene, 87%; possible *l*-β-pinene (small amounts), and oxygenated terpene compounds 5 or 6%. Apparently there were no sesquiterpenes.

ENTIRE PROJECT SUMMARY

This paper completes the project of systematic investigation of chemical composition of gum turpentines of the species of genus *Pinus.* Turpentines of 66 pines have been investigated or, in some instances, re-examined.

Some of the results of our inquiry are given.

We have found ethyl caprylate in the turpentine of *Pinus edulis.* Previously this ester was positively identified only in fusel oil of grape brandy.

A sesquiterpene, albicaulene, a corresponding sesquiterpene alcohol, albicaulol, and a diterpene, cembrene, were all three found in *Pinus albicaulis* turpentine and named. These are apparently new substances, not reported previously in any plants. Later we found albicaulene and albicaulol in several more pines.

A bicyclic sesquiterpene which was found in *Pinus pinceana* turpentine and which was named maderene is also probably a new sesquiterpene. A diterpene occurring in *P. koraiensis* turpentine which formed maleic anhydride adduct, is probably a newly found hydrocarbon.

Longifolene was reported by us in turpentines of many American and Mexican pines, often in large quantities.

We have found methyl chavicol in gum turpentines of several pines.

Linalool was identified in turpentine of *Pinus tenuifolia.*

We have found that paraffin hydrocarbons are not so rare as ingredients of pine gum turpentines as had been previously supposed; *n*-heptane

was found in turpentines of 8 pines; *n*-undecane in 7.

Myrcene was found in turpentines of 5 pines and ocimene in 1. Terpinolene was detected in turpentines of 5 pines.

A bicyclic terpene, Δ^3-carene, proved to be a rather common terpene in American pines. Some varieties of *Pinus ponderosa* contained more than 50 per cent of this terpene.

Besides contributing to knowledge of turpentine chemistry, information reported in the publications of this series has served as a foundation for studying the distribution, variability, and inheritance of terpenes and associated nonterpene compounds of the species of the genus *Pinus*.

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Concentration of an Alfalfa Growth Factor for *Neurospora sitophila* and Its Use in the Microbiological Assay of Pyridoxine*

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Dehydrated alfalfa leaf meal contains an unidentified factor(s) necessary for maximum growth of *Neurospora sitophila* in Difco Bacto Pyridoxine Assay Medium. A procedure for one-thousand-fold concentration of this factor was developed which involved extraction of dehydrated alfalfa leaf meal with dilute hydrochloric acid, precipitation of inert material with ethanol, removal of impurities soluble in diethyl ether, chloroform, and benzene, followed by chromatographic adsorption of the active substance on fuller's earth, and then elution with hydrochloric acid. Supplementation of 100 Gm. of Difco Bacto Pyridoxine Assay Medium with approximately 0.5 Gm. of concentrate will improve use of the medium for the microbiological assay of pyridoxine in vitamin products containing alfalfa concentrates.

IN A PREVIOUS PUBLICATION (1) in 1953, it was shown that the addition of alfalfa extracts caused excessive growth of *Neurospora sitophila* in a medium considered complete for the microbiological assay of pyridoxine. This method cannot be employed for accurate pyridoxine assays of vitamin products containing alfalfa concentrates because they contain another factor necessary for maximum growth of the test organism. The nature of this substance was studied.

EXPERIMENTAL

Dehydrated alfalfa leaf meal was used as the source of all fractions discussed in this investigation.

Difco Bacto Pyridoxine Assay Medium (Formula revised July 1954) (2), patterned after that described by Stokes, *et al.* (3), and modified by Barton-Wright (4), was employed because it is highly standardized and results can be duplicated reasonably. The fact that this commercial product is deficient in an unidentified factor(s) present in alfalfa, but which is required for maximum growth of the assay organism *Neurospora sitophila* 299 ATTC 9276, is the basis for this investigation. Any significant growth response by this organism above that obtained with the basal medium, and caused by supplementation with alfalfa or its fractionation products, was a verification of the presence of an essential substance in the fraction being tested.

Difco Bacto Neurospora Culture Agar (2) was used for maintaining a stock culture of the organism, and for preparing the inoculum. This culture was transferred at fifteen-day intervals and stored under refrigeration at 0°. To prepare an inoculum of *Neurospora sitophila*, a transfer was made forty-eight hours prior to the assaying period.

Pyridoxine stock solution was prepared by dissolving 20 mg. of pyridoxine in 200 ml. of distilled water. This standard solution was discarded after one month, and a new solution prepared. It was stored under refrigeration in an amber bottle to prevent decomposition or inactivation. The concentration of pyridoxine used per assay flask (volume 10 ml.) was 0.1 mg.

To rehydrate the assay medium, 5 Gm. Difco

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