

there is already evidence to indicate that chelated iron is less toxic than ionic iron (4). In regard to subsequent distribution and utilization of iron, it may well be the case that chelation is a necessary requirement, since transport of ionic iron, *per se*, through cellular membranes is questionable. Release of iron from administered chelates to form hemoglobin should present no obstacle because of the known extreme affinity of porphyrins to chelate iron (11).

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Composition of Gum Turpentine of Pines

Pinus nelsonii and *Pinus occidentalis*

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It was found that the turpentine of *Pinus nelsonii* contained 49.60% *l*- α -pinene, 41.50% *l*- β -pinene, 1.02 dipentene, 0.24% camphene, and 0.01% *n*-heptane. The remainder contained 3.74% of oxygenated substances derived mainly from terpenes (including probably some oxygenated sesquiterpenes); about 0.02% of unidentified, low-boiling materials, pot residue, and losses. The possible occurrence in *P. nelsonii* turpentine of the oxygenated materials should be rechecked, using freshly collected material. The identification of camphene and *n*-heptane must be considered as tentative, only. The turpentine of *Pinus occidentalis* contained 63.8% *d*-, *dl*- α -pinene, 22.2% *l*- β -pinene, 7.7% *d*- Δ^3 -carene, 1.8% methyl chavicol, 1.1% dipentene, 0.5% *d*-longifolene, 0.3% linalool, 0.2% camphene, and 0.1% *n*-heptane, with the remaining 2.3% accounted for by unidentified terpenes, pot residue, and losses. The identification of the last four constituents must be considered only tentative.

PINUS NELSONII Shaw is a Mexican piñon or nut pine, growing in a few restricted localities in the desert ranges of Mexican states Tamaulipas, Nuevo Leon, Coahuila, and San Luis Potosí. It is a small bushy tree with long pliant branches, sparse gray-green foliage, and gray bark. Its three cohering needles give the impression of a single, slender needle. A sample of the oleoresin of this pine was received from Aramberri, Nuevo Leon, Mexico. The trees from which the oleoresin was procured grew at an elevation of about 7000 ft. in Valle Hermoso, Miquihuana, Tamaulipas. Herbarium specimens accompanied the oleoresin sample.

Pinus occidentalis Swartz is a pine of the Caribbean area. It grows in eastern Cuba and

on the Island of Hispanola, both in the Dominican Republic and in Haiti. It is closely related to *Pinus caribaea* and also to other pines of Mexico and of the southeastern United States. A generous sample of oleoresin of this pine was collected in August of 1960 at Mare Range section of the Pine Forest, Haiti, courtesy of SHADA.

DISCUSSION AND EXPERIMENTAL

Pinus Nelsonii

The turpentine was distilled under reduced pressure. Toward the end of the operation, the pressure was reduced to 2 mm. and the temperature reached 215°. At the end, small quantities of distillate solidified in the condenser. The pot residue was hard and brittle, showing that all volatile components, including sesquiterpenes and their derivatives, were removed from the oleoresin. Yield of turpentine was 27.8%; its physical characteristics were: density, d_4^{25} , 0.8557; index of refraction, n_D^{25} , 1.4711; and specific rotation, $[\alpha]_{578}^{23}$ -38.5. This was reported by Mirov (1).

A charge of 309.7 Gm. of *P. nelsonii* turpentine was distilled through a 90 cm. long, 2 mm. inside

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TABLE I.—FRACTIONAL DISTILLATION AND PHYSICAL PROPERTIES OF FRACTIONS OF *Pinus nelsonii* TURPENTINE, ATMOSPHERIC PRESSURE

Fraction No.	Temperature Range, °C.	% of Total	Density d_4^{25}	Refractive Index n_D^{25}	Specific Rotation $[\alpha]_D^{25}$ Detd. in 1958	Specific Rotation $[\alpha]_D^{25}$ Detd. in 1961	Main Components
1	145-155	1.98	0.8504	1.4622	-53.6	-43.24	α -Pinene
2	155-156	29.20	0.8515	1.4646	-52.1	-39.38	α -Pinene
3	156-157	10.10	0.8523	1.4660	-47.9	-42.11	α -, β -Pinene
4	157-158	7.36	0.8523	1.4672	-44.7	-39.33	α -, β -Pinene
5	158-159	3.55	0.8530	1.4687	-41.6	-39.99	α -, β -Pinene
6	159-160	3.88	0.8534	1.4700	-38.6	-33.20	β -, α -Pinene
7	160-161	6.14	0.8544	1.4713	-35.2	-22.03	β -, α -Pinene
8	161-162	4.58	0.8544	1.4727	-31.2	-19.35	β -, α -Pinene
9	162-163	9.82	0.8545	1.4740	-27.9	-23.11	β -Pinene
10	163-164	13.13	0.8605	1.4756	-25.2	-20.19	β -Pinene
Pot residue	>164	10.26	Redistilled under reduced pressure; see Table II				

TABLE II.—FRACTIONAL DISTILLATION AND PHYSICAL PROPERTIES OF FRACTIONS OF THE POT RESIDUE FROM *Pinus nelsonii* TURPENTINE DISTILLATION (TABLE I) AFTER STORAGE

Fraction No.	Temperature Range, °C.	Pressure, mm.	% of Total	Density d_4^{20}	Refractive Index n_D^{22}	Specific Rotation $[\alpha]_D^{25}$	Main components
11	61-66	22	1.03	0.8667	1.4782	-16.54	β -Pinene and dipentene
12	66-70	22	0.68	0.8671	1.4790	-13.61	β -Pinene and dipentene
13	70-74	22	0.71	0.8868	1.4814	-4.43	β -Pinene and dipentene
14	74-115	22	0.77	0.9261	1.4891	+4.67	Oxygenated terpenes and dipentene
15	115-134	22	1.44	0.9180	1.4942	-15.33	Oxygenated terpenes
16	134(22)-64(2)	22-2	0.90	0.9104	1.4968	-9.71	Oxygenated terpenes
17	64-95	2	0.31	0.9046	1.4972	...	Oxygenated terpenes
18	95-118	2	0.29	0.9571	1.5008	-16.09	Oxygenated terpenes and sesquiterpenes
19	up to 172	1.5	0.26	0.9527	1.5038	...	Oxygenated sesquiterpenes
Pot residue			3.07				Final bath temperature 225° at 1.5 mm. pressure
Loss			0.80				
Total			10.26				

diameter Todd column packed with $\frac{3}{32}$ -in. diam. glass helices. A 5:1 reflux ratio was maintained throughout the operation. The results of distillation and the physical constants of the fractions are shown in Table I.

An unusual feature of the physical properties was very high levo-rotation of the α -pinene (fractions 1 and 2). Never before have we encountered such a high rotation of α -pinene. After distillation, the 10 fractions and the residue were kept in airtight containers for 2½ years at 5°. No doubt some oxidation and other changes in the material occurred during the storage period, as indicated by the rotation figures in columns 6 and 7. Even so, the specific rotation of fraction 1 has remained rather high.

After 2½ years storage, the pot residue of the fractional distillation amounting to 31.8 gm. was distilled under reduced pressure, using a 90 cm. long, 4 mm. inside diameter wire spiral column. A 10:1 reflux ratio was maintained during fractionation. The results are shown in Table II.

Preliminary identification of components and determination of the quantitative composition of all 19 fractions was made by vapor-phase chroma-

tography after the storage period. The Aerograph model A-90-C used was equipped with automatic integrator and with 5 ft. of Silicone 550-20% on chromosorb and 5 ft. Ucon polar 20% on firebrick columns, as recommended by Stanley.¹ Helium was used as the mobile gas phase. The results are shown in Tables III, IV, and VIII. In all cases, samples of known materials were used for comparison of relative retention volumes, which were computed on the basis of the time necessary for α -pinene to appear as equal to 1.0. In quantitative work, the peak areas were assumed to correspond directly to the weight percentages of the individual compounds comprising the fraction under consideration. It was felt that sufficient accuracy was attained as, in most cases, compounds comprising individual fractions did not differ too much structurally. The results are shown in Tables III and IV. In addition to the identified components, unidentified peaks were noted in fractions 1 and 13-19.

The identifications of α -pinene, β -pinene, and dipentene were substantiated by preparation of

¹ Dr. R. G. Stanley, U. S. Forest Service, personal communication.

crystalline derivatives, as given in Table V. *n*-Heptane and camphene were identified only on the basis of relative retention volumes and boiling points, and for this reason their presence must be regarded as established only tentatively.

Of the rather large number of unknown peaks encountered, five were singled out because of either their intensity or their characteristic position in vapor-phase chromatographic experiments.

Peaks A and B from fraction 1 (relative retention volumes of 0.30 and 0.34; Ucon polar column; T = 130°; 29 ml./min.) appear near *n*-heptane and could easily correspond to its homologs.

Peak C from fractions 14 and 15 appeared as a

singlet at 210° (17.6 ml./min., Silicone 550), with relative retention volume of 2.13 and as a doublet at 152° (22.0 ml./min., Silicone 550), with relative retention volumes of 3.1 and 3.5. Its infrared spectrum obtained on a smear from the vapor-phase-chromatography condensate indicated, among other groups, the presence of a hydroxyl group (band at 3450 cm.⁻¹), a CH₂ = CR₂ group (bands at 3100, 1417, and 890 cm.⁻¹), a carbonyl group (band at 1715 cm.⁻¹, the position agreeing best with a non-conjugated keto group either in straight chain or in six-membered ring), and probably an isopropyl group (doublet at 1390 and at 1374 cm.⁻¹). Apparently the material represented a mixture of an alcohol and a ketone. *p*-Nitrobenzoylation of fraction 4 yielded a small amount of *p*-nitrobenzoate, which melted after recrystallization from ethanol at 53–54°. 2,4-Dinitrophenylhydrazone was formed from the same fraction, but in an amount too small for any further work. Examination of all the data obtained indicated that the alcohol in fraction 4 was not identical with any terpene alcohol recorded in pine turpentine.

Peak D, present in fractions 15–18, appeared at a relative retention volume of 4.03 (Silicone 550, 210°, 17.6 ml./min.). The infrared spectrum indicated the presence of a hydroxyl group (band at 3420 cm.⁻¹), a double bond (bands at 3050 and 1650–1690 cm.⁻¹), and probably an isopropyl group (doublet at 1375 and at 1390 cm.⁻¹).

Peak E, present in the same fractions, appeared at the relative retention volume of 5.09 when run under the same conditions. The infrared spectrum indicated the presence of a hydroxyl group (band at

TABLE III.—VAPOR-PHASE CHROMATOGRAPHIC ANALYSIS OF THE TURPENTINE OF *Pinus nelsonii* EXCLUDING POT RESIDUE FROM TABLE I

Fraction No.	% of Total	α -Pinene, %	Camphene, %	β -Pinene, %
1 ^a	1.98	85.0	0.6	12.5
2	29.20	91.8	0.2	8.0
3	10.10	78.8	0.2	21.0
4	7.36	68.8	0.2	31.0
5	3.55	56.0	1.0	43.0
6	3.88	44.7	0.7	54.6
7	6.14	35.5	0.5	64.0
8	4.58	23.2	0.4	76.4
9	9.82	8.7	tr.	91.3
10 ^b	13.13	1.3	tr.	97.5
Pot residue	10.26	see Table IV		

^a *n*-Heptane, 0.6%; peak A, 0.1%; peak B, 1.2%. ^b Dipentene, 1.2%.

TABLE IV.—VAPOR-PHASE CHROMATOGRAPHIC ANALYSIS OF THE TURPENTINE OF *Pinus nelsonii*, POT RESIDUE FROM TABLE I, AFTER STORAGE^a

Fraction No.	% of Total	α -Pinene, %	β -Pinene, %	Dipentene, %	Peak C, %	Peak D, %	Peak E, %	Sum of Other Peaks, %
11	1.03	1.0	84.3	14.7
12	0.68	0.2	75.6	24.2
13	0.71	...	47.8	45.7	6.5
14	0.77	...	7.3	24.1	54.1	14.5
15	1.44	2.8	14.8	31.1	45.7	5.6
16	0.90	19.9	56.0	24.1
17	0.31	13.5	55.0	31.5
18	0.29	2.5	30.6	66.9
19	0.26	100.0

^a The composition of *P. nelsonii* turpentine, calculated from Tables III and IV, is as follows: 0.01% *n*-heptane; 49.60% α -pinene; 41.50% β -pinene; 0.24% camphene; 1.02% dipentene; traces peak A; 0.02% peak B; 0.63% peak C; 0.68% peak D; 1.41% peak E; 1.02% sum of other high-boiling materials; 3.07% pot residue; 0.80% loss.

TABLE V.—IDENTIFICATION OF THE COMPONENTS OF TURPENTINE FROM *Pinus nelsonii*

Terpene	Derivative	Prepared from Fraction No. ^c	M.p., °C.	Mixed M.p.	Other Identification ^a
<i>n</i> -Heptane ^b Bp, VP
α -Pinene	Piononic acid semi-carbazone	2	204–205 Bp, n, d, α , VP
Camphene ^b Bp, VP
β -Pinene	Nopinonic acid	10	127.8–128.8	no depression	Bp, n, d, α , VP
Dipentene	Tetrabromide	13	121–122	no depression	Bp, n, d, α , VP

^a Bp = boiling point, n = refractive index, d = density, α = specific rotation [α]_D²⁵, VP = vapor-phase chromatography. ^b *n*-Heptane and camphene were identified only on the basis of relative retention volumes and boiling points; thus their presence must be regarded as established only tentatively. ^c See Tables I, II and IX.

3400 cm.^{-1}), a $\text{CH}_2=\text{CR}_2$ nonconjugated double bond (bands at 3090, 1635, and 885 cm.^{-1}), and possibly an isopropyl group (doublet at 1387 and at 1373 cm.^{-1}).

Preparation of hydrochloride from either fraction 15 or fraction 16 resulted only in noncrystallizable oils. Fraction 19 gave a mixture of hydrochlorides melting indefinitely between 99 and 110°, even after three recrystallizations. Fraction 16 gave a fairly good yield of *p*-nitrobenzoate which, after recrystallization from alcohol and following purification with charcoal, sublimed between 190 and 232°, without melting. As the ester would be expected to have a vapor pressure too high for sublimation, it is possible that *p*-nitrobenzoic acid was eliminated and sublimed during the melting. The ester obtained probably was derived from the compound of Peak E, as the latter is the main constituent of fraction 16.

The infrared spectra of the remaining fractions 17, 18, and 19, all showed the presence of hydroxyl compounds.

As considerable time elapsed after fractionation and the determination of physical constants on the fractions before the vapor-phase chromatographic analysis and characterization of the constituents were performed, the specific rotations of the 10 fractions were redetermined. In all cases, a considerable drop in rotation values was noted. Shaking fractions 2 and 10 with potassium iodide solution liberated iodine. This suggested that air oxidation had taken place during the storage period and cast doubt as to whether the oxygenated terpenes of fractions 14–19 were present in the original tur-

pentine. As no fresh *Pinus nelsonii* turpentine was available to check this point, the work on these materials was abandoned.

The composition of *Pinus nelsonii* turpentine differs considerably from that of the other five piñon pines in that this species contains a large percentage of *l*- β -pinene (1). Its α -pinene possesses unusually high rotatory power. It would be interesting to compare the turpentine of this piñon with the turpentine of the new piñon pine discovered in high mountains of Nuevo Leon, Mexico (2).

Pinus occidentalis

The turpentine was obtained by heating 3.5 Kg. of the oleoresin under reduced pressure, so that at the end of the operation the temperature of the charge reached 190° while the pressure was decreased to 3.4 mm. of mercury.

This operation was performed by the personnel of the Pacific Southwest Forest Experiment Station at Placerville, Calif.

Yield of turpentine was 20.0%; the flask residue (*i.e.*, rosin) was hard and brittle.

The turpentine possessed the following characteristics: density, d_4^{25} , 0.859; index of refraction, n_D^{25} , 1.4688; and specific rotation, $[\alpha]_D^{25} + 10.88$

A charge of 425 Gm. of the turpentine was fractionated in a 90 cm. long, 25 mm. inside diameter Stedman column. A 10:1 reflux ratio was maintained during fractionation. The results are shown in Table VI.

The preliminary identification of components and determination of the quantitative composition of

TABLE VI.—FRACTIONAL DISTILLATION AND PHYSICAL PROPERTIES OF THE FRACTIONS OF *Pinus occidentalis* TURPENTINE

Fraction No.	Temperature Range, °C.	Pressure, mm.	% of Total	Density, d_4^{25}	Refr. Index n_D^{25}	Specific Rotation $[\alpha]_D^{25}$	Main components
1	100.0–102.0	154.0	1.15	0.856	1.4597	+20.53	α -Pinene
2	102.0–102.0	154.0	26.25	0.857	1.4637	+21.26	α -Pinene
3	72.5–72.5	51.5	11.30	0.857	1.4637	+21.20	α -Pinene
4	72.5–72.7	51.5	12.70	0.853	1.4640	+20.89	α -Pinene
5	72.7–74.5	51.5	6.60	0.855	1.4653	+16.82	α -Pinene
6	74.5–77.0	51.5	5.20	0.860	1.4691	+ 4.57	α -Pinene and β -pinene
7	77.0–78.8	51.5	7.55	0.861	1.4729	– 8.46	β -Pinene and α -pinene
8	78.8–79.8	51.5	10.25	0.861	1.4757	–17.50	β -Pinene
9	79.8–81.3	51.5	2.70	0.862	1.4756	–14.18	β -Pinene and Δ^3 -carene
10	81.3–83.0	51.5	1.30	0.862	1.4744	– 7.11	β -Pinene and Δ^3 -carene
11	83.0–84.5	51.5	5.50	0.860	1.4716	+ 8.97	Δ^3 -Carene and β -pinene
12	66.5–66.5	20.5	2.35	0.856	1.4716	+11.54	Δ^3 -Carene and dipentene
13	66.5–68.0	20.5	1.12	0.851	1.4764	– 1.70	Dipentene, Δ^3 -carene, and linalool
14	up to 125	4.0	2.57	0.951	1.5009	+ 6.69	Methyl chavicol and longifolene
Trap	(dry ice-acetone)	...	1.30	0.858	1.4686	+ 8.79	α -Pinene, β -pinene, and Δ^3 -carene
Pot residue	1.25	Heated up to 215° at 4 mm.
Loss	0.77	

TABLE VII.—VAPOR-PHASE CHROMATOGRAPHIC ANALYSIS OF THE TURPENTINE OF *Pinus occidentalis*^a

Fraction No.	% of Total	<i>n</i> -Heptane, %	α -Pinene, %	Camphene, %	β -Pinene, %	Δ^3 -Carene, %	Di-pentene, %	Linalool, %	Methyl Chavicol, %	Longifolene, %	Un-identified, %
1	1.5	...	100.0
2	26.25	...	100.0
3	11.30	...	100.0
4	12.70	...	98.0	0.5	1.5
5	6.60	...	88.5	1.5	10.5
6	5.20	...	59.0	0.5	40.5
7	7.55	...	29.75	0.25	70.0
8	10.25	...	7.0	...	93.0
9	2.70	81.0	19.0
10	1.30	67.0	33.0
11	5.50	21.0	79.0
12	2.35	84.0	16.0
13	1.12	18.0	61.0	15.0	6.0
14	2.57	0.5	1.0	3.0	71.5	17.5	6.5
Trap	1.30	6.5	56.0	...	20.0	16.5	1.0

^a The composition of *P. occidentalis* turpentine, calculated from the above analysis, is as follows: 0.1% *n*-heptane; 63.8% α -pinene; 0.2% camphene; 22.2% β -pinene; 7.7% Δ^3 -carene; 1.1% dipentene; 0.3% linalool; 1.8% methyl chavicol; 0.5% longifolene; 0.2% unidentified; 1.3% pot residue; 0.8% loss.

TABLE VIII.—RELATIVE RETENTION VOLUMES OBTAINED BY VAPOR-PHASE CHROMATOGRAPHY OF COMPONENTS OF TURPENTINES FROM *Pinus nelsonii* AND *P. occidentalis*

Terpene	Silicone 550 20% on Chromosorb; 5 ft. Col., (—20 ml./min.—)		Ucon Polar 20% on Firebrick; 5 ft. Col., (—30 ml./min.—)	
	R.R.V. ^a	Temp., °C.	R.R.V. ^a	Temp., °C.
<i>n</i> -Heptane	0.31	130	0.24120	
α -Pinene	1.00	130	1.00120	
Camphene	1.15	130	1.21	120
β -Pinene	1.35	130	1.44	120
Δ^3 -Carene	1.48	130	1.70	120
Dipentene	1.67	130	2.09	120
Linalool	2.34	130	2.98	120
Methyl chavicol	2.94	200	4.75	200
Longifolene	5.88	200	7.66	200

^a Relative retention values.

the fractions obtained was determined by vapor-phase chromatography, as described above. The results are shown in Tables VII and VIII. In addition to identified components, several small unidentified peaks were noted in fractions 13 and 14.

The specific rotations of individual terpenes were computed from the rotation data in Table VI and from vapor-phase chromatography results, considering also the melting points of the prepared deriva-

tives. The compounds were then designated as *d* or *l*, if the optically active material represented at least 80% of the total, and as *d*, *dl* or *l*, *dl* if it was less than 80% and more than 0% of the total. The maximum rotation values for individual terpenes, given in the literature, were used in this computation.

The identification of *d*, *dl*- α -pinene, *l*- β -pinene, *d*- Δ^3 -carene, dipentene, and methyl chavicol was substantiated by preparation of crystalline derivatives, as given in Table IX. Because of their small amounts, *n*-heptane, camphene, and linalool were identified only on the basis of relative retention volumes and, where possible, of constants from Table VI. For this reason, their identification must be regarded as only tentative. In the case of longifolene, the material was identified by physical constants and relative retention volumes and also by infrared spectrum on the sample collected by condensation of the corresponding fraction separated by vapor-phase chromatography.

The close relationship of *Pinus occidentalis* to *P. caribaea* is manifested by the presence of Δ^3 -carene in both pines (1).

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TABLE IX.—IDENTIFICATION OF THE COMPONENTS OF *Pinus occidentalis* TURPENTINE

Terpene	Derivative	Fraction	M.p., °C.	Other Criteria ^a
<i>n</i> -Heptane	...	Trap	...	Bp, VP
α -Pinene	Nitroschloride	2	102.8 (decompn.) ^b	Bp, n, d, α , VP
Camphene	...	4, 5, 6, 7	...	Bp, VP
β -Pinene	Nopinic acid	8	124.0–125.8 ^b	Bp, n, d, α , VP
Δ^3 -Carene	Nitrosate	11	146.2 (decompn.) ^b	Bp, n, d, α , VP
Dipentene	Tetrabromide	13	121.8–122.6 ^b	Bp, n, d, α , VP
Linalool	...	13	...	Vp, Bp
Methyl chavicol	Anisic acid	14	181.8–183.0 ^b	Bp, n, d, α , VP
Longifolene	...	14	...	Vp, Bp, α , IR

^a Bp = boiling point, n = refractive index, d = density, α = specific rotation $[\alpha]_D^{25}$; VP = vapor-phase chromatography, IR = infrared spectroscopy. ^b Mixed melting point, no depression.