

to the natural variation of different crops, to the total age of the roots, or to a possible difference in the time of year when they were collected.

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COLLEGE OF PHARMACY,  
UNIVERSITY OF WASHINGTON,  
SEATTLE, WASHINGTON.

## A PHYTOCHEMICAL INVESTIGATION OF THE OLEORESIN OF PINUS MONTICOLA DOUGL.\*

BY P. A. FOOTE<sup>1</sup> AND N. T. MIROV.<sup>2</sup>

Western White Pine (*Pinus monticola* Dougl.) is a five-needle pine growing on the "middle and upper slopes of northwestern mountains from the west side of the continental divide in northern Montana and British Columbia to Washington, Oregon and California" (1). It is a large source of timber in the West. The oleoresin herewith reported on came from trees growing in an environment far from their optimum; so much so that they offer more than ordinary phytochemical interest. Especially will this be true when the oleoresin from other localities has been analyzed. Unlike most spirits of turpentine, which usually consist of terpenes, sesquiterpenes and their oxygenated products, this oil contains about one per cent of a paraffin hydrocarbon, *n*-undecane, C<sub>11</sub>H<sub>24</sub>. This gives our investigation added interest since this paraffin has been identified only once before in the plant kingdom. This was found by Simonsen and Rau (2) in 1922 in the oleoresin of *Pinus excelsa* growing in India. In 1913 Schorger (3) isolated a paraffin hydrocarbon from *Pinus lambertiana*. This had physical properties close to

\* Scientific Section, A. Ph. A., Toronto meeting, 1932.

<sup>1</sup> Prof. of Pharmacy, University of Florida.

<sup>2</sup> Formerly with the U. S. Forest Service.

*n*-undecane but he lacked a sufficient quantity to identify it. Inasmuch as both *Pinus excelsa* and *Pinus lambertiana* are also five-needle pines and thus closely related to *Pinus monticola* it is more than probable that Schorger's paraffin hydrocarbon was *n*-undecane.

So far as we are aware there have been but two other normal paraffin hydrocarbons identified in the volatile oils, *viz.*, *n*-heptane and *n*-pentadecane. The former occurs in the Jeffrey (*Pinus Jeffreyi*) and Digger (*Pinus sabiniana*) pines of the West and in Petroleum Nuts of the Philippines.

The pentadecane occurs in oil of *Kæmpferia galanga*, a plant of the Zingiberacæ used as a condiment in India and the Malay Peninsula.

Why has the tree produced this undecane instead of a terpene or a sesquiterpene? We raise the question as food for thought without being able to answer it. Simonsen and Rau point out that *P. lambertiana* and *P. excelsa* are the only members of the "strobis" (five-leafed) group from which the oil of the oleoresin has been analyzed and that each contains a paraffin hydrocarbon. They speculate that perhaps all members of this group produce paraffins in their oleoresins. Our investigation strengthens this view for Western White Pine belongs in this group. Accepting the theory of the organic origin of petroleum, they also ask the question if the pines have been the source of petroleum, at least in certain areas. These hydrocarbons occur in American petroleum and the Coniferæ have been found in the lower strata.

Have environmental conditions caused the tree to produce an unusual constituent? In the past many investigators have cared little about the environment the plant grew in. They were content to report the botanical classification and their analysis. Hence it is possible for two separate workers to contradict each other and yet each be right. Let us illustrate. It is a well-known fact among large turpentine operators of the Southeast that the same species of *Pinus* will produce different chemical constituents in its oleoresin if produced in different regions. In the West we may cite Western Yellow Pine (*Pinus ponderosa*). Typical Pacific Coast Western Yellow Pine yields a lævo-rotatory oil and consists largely of beta-pinene while the Rocky Mountain form (variety *scopulorum*) has a dextro-rotatory oil and its chief constituent is alpha-pinene. Even on the Pacific Coast the junior author has found that the chemical composition of Western Yellow Pine oleoresin is far from uniform. In view of these facts we consider it essential to report not only the conditions under which these trees are growing but also the time and method of collection of the gum.

#### THE ENVIRONMENT.

The experimental trees are located in the northern part of the Warner Mountains, which is one of the Great Basin Ranges. Warner Mountain ridge is located chiefly in Modoc County, California. R. J. Russell (4) says of this range, "At the North the mountainous unity is very gradually lost in mergence with a high plateau between Albert Lake and Warner Valley. A similar transition at the southern end of the range unites it with the high plateaus just east of Madeline Plains. Goose Lake Valley is located just west of the range with an extensive body of water in its central part. The easterly slope of the range faces the Surprise Valley. The east of this valley raises Hays Canyon Range from the summit of which a broad plateau extends eastward to the Black Rock Desert. The width of the range in its typical portion varies from about eight to twenty miles. Toward the plateaus the range widens rapidly." The trees that were tapped lie strictly in the Great Basin (5).

Russell defines the Great Basin as an area drained by streams which fail to reach the ocean.

The Warner Mountain forests are separated from the other forests of California and Oregon by extensive sage brush plateaus. The wide alkaline Goose Lake Valley separates the Warner Range from other forests of the northern part of California, while a desert extends east of the range. Western White Pine in the Warner Mountains is thus growing isolated by treeless areas from other localities where this species grows.

In order to show the location in Warner Mountains of the Western White Pine stands where experimental tapping was done, and to show the relation of these stands to other vegetation of this region, it appears to be desirable to trace the changes in vegetation from Buck Creek Ranger Station, located on the Westerly slope of the ridge toward Fort Bidwell in the Surprise Valley. In the vicinity of Buck Creek Station the slope is covered by a Western Yellow Pine forest, with occasional junipers (*Juniperus occidentalis* Hooker). In the open places occur wild plum (*Prunus subcordata* Benth.) in mixture with Mountain Mahogany (*Cercocarpus ledifolius* Nutt.). The elevation of this place is 5400'. On the higher slopes of Buck Creek Canyon Incense Cedar (*Libocedrus decurrens* Torrey) is added to Western Yellow Pine, while at 6000' White Fir (*Abies concolor* Parry) appears. Flat places occasionally found at this elevation are swampy and occupied by aspen (*Populus tremuloides* Mich.) groves and a ground cover in which *Veratrum* is conspicuous. At 6200' pure White Fir occupies the ground, although Old Western Yellow Pines go as high as 6750' in open places, otherwise covered with Choke Cherry (*Prunus demissa* Wal.) and Service Berry (*Amelanchier alnifolia* Nutt.). At 7200' among the White Firs, some Western White Pines appear. The top of the ridge lies at 7400'. An extensive flat here reaches eastward and is covered with a park-like forest composed of Western White Pine, Lodgepole Pine (*Pinus contorta* Loud.) and occasional White Firs. Dense stands of pure Lodgepole Pine occupy a swampy depression of the flat and extend up the north slope of Fandango Peak, which rises south of the experimental area. Rocky summits are covered with scrubby Mountain Mahogany. The drier and more elevated portions of the flat are covered with sage brush (*Artemisia tridentata* Nutt.) which occupies also the southern slope, where scrubby Aspens occur in places.

The experimental trees were located in this park-like forest at an elevation of 7400'. This area is exposed to severe winds and the trees growing on the edge of the flat show irregular wind-bitten crowns. When the first trip was made to the top of the ridge on June 8, 1929, the whole plateau was covered with deep snow. On June 16th-18th a severe snow storm took place and interfered with the tapping operation. In shady places snow banks 6' deep were found as late as July 4th. The growing season is very short in this locality and the month of June is a spring month here. Few species of annual plants were flowering during this month but willows (*Salix* sp.) by the stream were blooming. In July *Wyethia mollis* begins to flower and this is the only herbaceous species occupying the ground till the end of the season.

#### OLEORESIN EXTRACTION.

At the beginning of June, 30 cups were set on the trees; 26 on 13 mature trees and one each on four young specimens. Oblong galvanized cups, and aprons attached with nails instead of the conventional ax insertion, were used in this experiment and scarification was performed with a No. O round hack. Oleoresin as it accumulated in the cups was put into friction top tin cans. The trees were tapped first on June 8th. The first sample of oleoresin was collected June 22nd. The last chipping was done July 27th and the last batch of gum was collected August 4th.

Date.	Oleo Yield, Gm.	Cumulative, Gm.	Yield per Cup per Streak, Oz.	Notes.
June 22	403	403	0.90	30 cups
June 30	1528	1931	1.80	....
July 7	1048	2979	1.20	....
July 10	1092	4071	1.35	27 cups
July 13	812	4883	0.97	30 cups
July 20	1208	6091	1.53	....
July 27	1441	7532	1.82	....
Aug. 4	2267	9799	2.67	28 cups

The foregoing table gives the oleoresin yield throughout the tapping season.

The average yield of 1.53 oz. per cup per streak obtained from Western White Pine in this locality is very small compared with that of other turpentine producing pines. A slight increase in yield may be noticed toward the end of the tapping season, but even the highest amount of oleoresin obtained at the last chipping at the beginning of August, is much smaller than the average yield obtained from Western Yellow Pine in the same region.

It remains to be seen whether a low oleoresin yield is specific to Western White Pine in general, or whether it is due to the fact that this species in the Warner Mountains is growing under unfavorable conditions, being far from its environmental optimum.

#### OLEORESIN EXAMINATION.

Oleoresin obtained from Western White Pine has a honey-like consistence and stringy appearance. It possesses a pungent odor not similar to that of common turpentine. It does not crystallize as readily as Western Yellow Pine oleoresin, but on standing retains its stringy, viscous and homogeneous character. It resembles the oleoresin of Sugar Pine (*Pinus lambertiana*).

Four samples of the oleoresin were subjected to steam distillation for volatile oil separation as soon as they were obtained from the trees. The operation was done in a 1000-cc. round-bottom flask with a Kjeldahl connecting bulb tube placed between the flask and condenser to prevent any rosin from being carried over. After all the oil was removed the remaining rosin was heated in a paraffin bath until all water had been removed. The temperature was not allowed to rise above 145° C. The oleoresin foamed excessively on distillation with steam and the last traces of oil were removed with difficulty. Yield of oil and rosin was as follows:

Sample of Oleoresin.	Per Cent Oil.	Per Cent Rosin.	Per Cent Impurities.
June 22	20.0	78.15	1.85
June 30	15.8	82.20	2.00
July 10	18.0	79.80	2.20
July 30	19.0	79.10	1.90

The average yield of volatile oil of 18.2% might be considered very high, compared with that of other pines growing in California. The volatile oil of Western White Pine has the same pungent odor as the oleoresin, but even more penetrating.

The remainder of the oleoresin was shipped to Gainesville for chemical examination which was begun early in 1930. It had a sp. gr. by hydrometer of 1.002 at 23° C. On steam distillation in the same manner as above 7343.5 Gm. gave 1328 Gm. of oil or 18.08%. This oil was combined with that of the above 4 samples making a total of 1582.6 Gm. for investigation. The hot rosin was strained through cheese-cloth.

#### PROPERTIES OF THE ROSIN.

When compared with standard cubes it graded WG and by immersion in brine solution showed a sp. gr. of 1.045 at 23° C. When a melting point was taken with a Thiele tube it began to soften at 37° C. It melted at 45-49° C. and became completely liquid at 51° C. Its specific rotation in alcohol was -1.58° at 25° C. Acid value 147.3, ester value 14.9, iodine value 19.9.

## ANALYSIS OF THE OIL.

*Preliminary Testing.*—Clear and colorless. Sp. gr.  $\frac{20}{20}$ , 0.8691 (by Westphal). Optical rotation  $\alpha_D +20.59^\circ$  at  $20^\circ$  C. Ind. of ref.  $n_d$ , 1.4646 at  $23^\circ$  C. When cooled to  $5^\circ$  C. over night no separation took place. Acid value 0.0, ester value 0.0. No oxygen or nitrogen compounds.

*Distillation.*—1582.6 Gm. of oil was distilled at 2–4 mm. making  $5^\circ$  cuts for the first distillation. This was then fractionated with a Vigreux column, making the cuts as near one-degree intervals as possible. After a total of four distillations the fractions were as follows:

B. P.	Sp. Gr. $\frac{15}{15}$	Ref. Ind. $n_d$ at $26^\circ$ C.	Opt. Rot. $26^\circ$ C.	Wt., Gm.
155–156 (765 mm.)	0.8734	1.4658	+13.30	48.0
156–157 .....	0.8654	1.4638	+13.30	230.0
157–158 .....	0.8656	1.4642	+13.30	279.3
158–159 .....	0.8646	1.4640	+12.70	174.1
159–160 .....	0.8674	1.4649	+11.06	98.7
160–161 .....	0.8713	1.4667	+ 8.80	20.4
161–162 .....	0.8817	1.4689	+ 6.95	11.2
162–163 .....	0.8693	1.4672	+ 3.62	7.7
163–164 .....	0.8729	1.4677	+ 0.16	8.2
43–44 (5 mm.)	0.8656	1.4641	+ 9.85	151.8
44–45 .....	0.8610	1.4634	+ 8.38	108.3
45–46 .....	0.8702	1.4657	+ 6.12	53.5
46–47 .....	0.8705	1.4659	+ 4.20	18.7
47–48 .....	0.8830	1.4681	+ 3.00	16.4
48–50 .....	0.8830	1.4658	+ 2.25	14.8
50–51 .....	0.8679	1.4644	+ 3.25	8.8
51–52 .....	0.8808	1.4664	.....	3.1
52–54 .....	0.8579	1.4624	.....	3.5
54–55 .....	0.8928	1.4681	+ 3.25	13.3
55–61 .....	0.8499	1.4553	.....	3.1
61–68 .....	0.8816	1.4603	.....	6.1
68–75 .....	0.8390	1.4475	– 2.20	26.0
75–85 .....	0.8719	1.4538	+ 5.40	7.1
85–90 .....	0.8965	1.4668	+10.05	11.4
90–95 .....	0.9375	1.4797	+17.68	13.0
95–100 .....	0.9659	1.4888	+22.40	6.6
100–105 .....	0.9615	1.4938	+19.50	10.8
Near 105 .....	0.8829	1.4554	– 1.50	10.8
Residue .....	.....	1.5001	.....	23.1
Total				1387.8

While distilling over the last fraction (near 105) the contents of the flask exploded which might be attributed to sudden polymerization. As indicated by the sp. gr. not running uniform, the oil appears to have one or more terpenes that polymerize easily (phellandrene?). Because of this it would be difficult to estimate the % of sesquiterpenes which we would expect in the last four fractions.

The sp. gr. was taken by a Mohr-Westphal balance or a 4-cc. pycnometer when the quantity was not sufficient. Determinations were taken at  $25^\circ$  C. and corrected to  $\frac{15}{15}$ , using a correction of 0.00075 per degree.

## IDENTIFICATION OF CONSTITUENTS.

*d- $\alpha$ -Pinene*.—The physical properties of the fraction boiling at 156–157° C. and the nitrosyl chloride derivative proved the presence of *d- $\alpha$ -pinene*. The pinene nitrosyl chloride was prepared by the method of Wallach. It formed with ease and abundance and melted at 108° C. Taking the first five fractions as *d- $\alpha$ -pinene* the oil contains approx. 59.8% of this constituent.

*$\beta$ -Pinene*.—Using 12 cc. of the fraction 43–44° C. (5 mm.), nopinic acid was prepared according to the method of Wallach. It melted at 112° C. but recrystallization raised it to 126° C. The physical properties are in close accordance with those recorded for  *$\beta$ -pinene*. Taking the seven fractions from 160–161° C. to 45–46° C. (5 mm.) as  *$\beta$ -pinene* the oil contains approx. 26% of it.

*Limonene*.—Before fractionation the oil had an odor reminding one of limonene. The fractions boiling between 50° C. and 75° C. (at 5 mm.) had a more distinctive odor of it, but its tetrabromide could not be obtained. It is possible that the decrease in rotation changing to *lævo* and then to *dextro* again is due to *l-limonene*.

The fractions boiling between 46° C. and 55° C. (5 mm.) were treated with dry HCl gas for sylvestrene and N<sub>2</sub>O<sub>3</sub> for the nitrosite of phellandrene but the results were negative.

The remaining fractions stood for 12 months before the investigation could be continued. They were kept in well-filled test-tubes protected from light.

*N-Undecane*.—The fraction 68–75° C. (5 mm.) weighing 26.0 Gm. was distilled over metallic sodium at 766.6 mm. giving the following fractions:

B. P.	Sp. Gr. $\frac{15}{16}$	Ref. Ind. $nd$ at 27° C.	Wt. in Gm.
160–170	...	1.4490	2.0
170–175	...	1.4475	2.4
175–180	0.8174	1.4452	4.5
180–185	0.8063	1.4409	3.6
185–190	0.7803	1.4405	9.0

The last fraction (185–190) showed such a low sp. gr. and index of refraction that a paraffin hydrocarbon was suspected. 5 cc. of it was treated with fuming H<sub>2</sub>SO<sub>4</sub> in accordance with the U. S. P. X test for kerosene in oil of turpentine. After centrifuging, 2.5 cc. remained as a clear top layer. In order to get more of this hydrocarbon for identification the remaining fractions between 60° C. and 90° C. (5 mm.) were treated in a similar manner and combined giving a total of 14.5 cc. In each case the H<sub>2</sub>SO<sub>4</sub> treatment was continued until the oil no longer imparted a color to the acid. The hydrocarbon was washed with aqueous K<sub>2</sub>CO<sub>3</sub> and dried over metallic sodium.

The boiling point was determined by distilling 10 cc. in a 15-cc. distilling flask, using an asbestos heating board, asbestos wrapping around the neck of the flask, a direct flame and a standardized Anschutz thermometer. The liquid was brought to boiling with the thermometer immersed in it. In this way the thermometer registered approx. 195° C. This was continued for several minutes and then the heat increased to such a point that the liquid would distil over at the rate of 30 drops per minute. The thermometer was then raised so that the center of the bulb was opposite the bottom of the side exit tube. 9 cc. distilled over between 196.3

and 197.9° C. (corr.) at 761.4 mm. The distillation was stopped at this point to prevent superheating. The residue in the flask showed no discoloration.

The sp. gr. was taken (before the b. p.) in an 8.5-cc. Sprengel tube giving the following results:

at  $\frac{15}{16}$ , 0.7457; at  $\frac{15}{4}$ , 0.7451; at  $\frac{20}{20}$ , 0.7432; at  $\frac{20}{4}$ , 0.7422; at  $\frac{25}{25}$ , 0.7407.

The melting point of the paraffin compound was taken as follows: 13 cc. in a  $\frac{1}{2}$ -oz. prescription bottle was frozen by solid CO<sub>2</sub> and alcohol. A pentane thermometer was immersed in it during freezing. It solidified at approx. -26° C. The temperature of the bath was allowed to raise slowly. So determined, the m. p. was -26.1 to -25.8° C.

The index of refraction was taken at three different temperatures. The average of five readings at each temperature follows:  $n_d$  at 15° C., 1.4210;  $n_d$  at 20° C., 1.4193;  $n_d$  at 25° C., 1.4171.

The rotation in a 10-mm. tube was zero.

A cryoscopic molecular weight determination in benzol gave 155.1. Required for C<sub>11</sub>H<sub>24</sub> 156.2.

Of the paraffin hydrocarbons having this empirical formula only one corresponds in above physical properties, *viz.*, *n*-undecane. Branched compounds would naturally have a lower b. p. So far as we are aware *n*-undecane has heretofore been identified only in the oleoresin of *Pinus excelsa* (2) and Pennsylvania petroleum (6).

The physical constants of this compound were recently determined by Shepard, Henne and Midgley (7). The source of their compound was petroleum. Inasmuch as it is exceedingly difficult to separate and purify the constituents of petroleum, *Pinus monticola* would seem to offer a good source of *n*-undecane for such a study.

*Sesquiterpenes*.—Present but none identified. Polymerization prevents a determination of their %.

#### SUMMARY.

The oleoresin of *Pinus monticola* Dougl., obtained from trees growing under unfavorable conditions in the Warner Mountains of California, has been investigated. The environment of the trees is described. Details of the oleoresin extraction are given, supplemented by tables of yield for oleoresin, oil and rosin. The properties of each are described. The oil contains *d*- $\alpha$ -pinene, 60%;  $\beta$ -pinene, 26%; *n*-undecane, 1-2%; and sesquiterpenes. Limonene is perhaps present.

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COLLEGE OF PHARMACY,  
UNIVERSITY OF FLORIDA,  
GAINESVILLE, FLA.