COMPOSITION OF THE VOLATILE OIL FROM THE
FOLIAGE AND TERMINAL BRANCHES OF SIERRA
JUNIPER, JUNIPERUS OCCIDENTALIS, HOOKER

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1954

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Date thesis is presented _May 5, 1954_

Typed by Evelyn Kanzelmeyer

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INTRODUCTION

Sierra Juniper (Juniperus occidentalis, Hooker) is native to altitudes ranging from 500 to 6000 feet in California, Oregon, Washington and western Idaho. It occurs in pure forest form in areas of central and eastern Oregon. Sierra Juniper is a low broadheaded tree 20 to 65 feet high with thick trunk and stout horizontal branches. Mature leaves are scale like, overlapping like tiny shingles but with points of the longest needles standing out from the branch in successive groups of three around the twig. Each leaf is marked on top by a white, resinous exudate. Fruit is berry-like (morphologically a cone) about 1/3 inch in diameter, bluish black with whitish bloom, scattered thickly along the branchlets and maturing at the end of the second season.

The existence of extensive stands of Sierra Juniper in proposed reclamation areas makes utilization of this raw material important. The possible presence of commercially valuable products made it advisable to determine the composition of the volatile oil present in the foliage and terminal branches. (For simplicity the term foliage will be used hereafter in referring to this material). A previous analysis (12,p.613-615) of this oil was performed by Lynn and Fischer on a sample that

had been isolated three years prior to the analytical work. The oil had resinified and required steam distillation to rectify it. Since it is well known that auto-oxidation, polymerization and isomerization readily occur among the members of the terpene series, it seemed desirable to analyze a fresh sample of the oil.

Lynn and Fischer reported that the oil is made up of acetic acid, 0.2 percent; phenols, 0.5 percent; high boiling material and losses, 14 percent; bornyl acetate, 40 percent; free borneol, 11 percent; αphellandrene, para-cymene, and probably camphene, 35 percent. The volatile oil obtained from the wood of this species has been reported (11,p.2204) as being composed of cedrene and cedrol. These two compounds are present in most of the genus juniperus.

COLLECTION AND PREPARATION OF THE
FOLIAGE AND TERMINAL BRANCHES

Foliage samples for this study were obtained
January 24, 1953 and May 9, 1953 in eastern Oregon between
Sisters and Bend, where there are nearly continuous stands
of the Sierra Juniper. The samples consisted of terminal
branches up to 1/4 inch in diameter, and the accompanying
foliage. No attempt was made to separate branches which
had berries from those which did not have them. To insure
a representative sample, the material was obtained from
40 trees, varying from saplings to trees three feet in
diameter. The method of sampling was to cut from five to
ten pounds of material from each tree with pruning shears.
This of necessity limited the height from which the sample
was taken to about ten feet above the ground.

It has been reported (2,pp.882-884) that comminu-
tion and storage have a decided effect on the yield of
oil that may be obtained from material of this type. The
recommended comminution is that obtained from an ensilage
machine. This reduces the material to optimum size for
steam distillation.

Therefore, the sample was comminuted in an ensilage
machine from which half of the knives had been removed.

The reduced material was thoroughly mixed and placed in covered 50 gallon cans. The cans were stored in a constant-humidity, constant-temperature cold room.

OIL CONTENT OF THE FOLIAGE
AND TERMINAL BRANCHES

The quantitative determination of the oil content
was made by steam distillation of 100-125 gram samples in
a Clevenger apparatus (9, vol.1,p.317). The distillation
period was 16-22 hours. Shorter periods did not volatil-
ize all of the oil, while no noticeable increase in the
oil content was observed for distillation periods greater
than 22 hours.

Samples of 100-150 grams were oven-dried for 24
hours at 105°C to determine the moisture content. Lack
of uniformity in the results of these determinations
seemed to be caused by the inclusion of differing propor-
tions of woody material in the samples. The reproduci-
bility could probably have been improved by taking larger
samples; however the bulk of the material limited the
size of the sample that could be handled. Further com-
minution should also improve the results but was undesir-
able because of the accompanying loss of oil.

Results of a series of moisture and oil determina-
tions are shown in Table 1. In most cases the determina-
tions were made on the same date and the value for the
percentage of oil is based on the oven dry-weight of a
sample determined the same day.

TABLE 1

Quantitative Determination of Oil and Moisture Content

Moisture content Percent	Oil content (Based on oven-dry weight) Percent
46.2	2.28
46.6	1.82
	2.03
45.2	2.13
	1.86
	1.79
	2.04
	1.84
35.7	1.53
31.0	1.93
40.5	2.41
47.2	2.72
45.0	2.23
44.7	2.34
45.0	2.04
43.9	1.89
Average 42.7	Average 2.05

RECOVERY OF THE OIL

For the isolation of a volume of oil suitable for
analysis, batch distillation of the foliage was carried
out in a still available at the Chemistry Department of
Oregon State College. The steam was conducted into the
base of the still pot and to a jacket surrounding the
lower portion of the still. Pressure of the steam in the
still could be regulated from 0-20 pounds per square inch
by adjustment of a valve in the condenser system. The
still pot had a capacity of 12-14 pounds of fresh material.

To determine optimum conditions for the distilla-
tion, the first three runs were carried out with the
steam at different pressures. The first was carried out
at atmospheric pressure, the second at 7-9 pounds per
square inch, and the third at 14-17 pounds per square inch.

At atmospheric pressure 20 liters of distillate had
to be collected to obtain 43 milliliters of oil, a
separatory funnel being used to separate the oil from the
distillate. The oil to water ratio at 7-9 pounds pressure
was 45 milliliters of oil to 16 liters of water, while the
ratio at 14-17 pounds pressure was 46 milliliters of oil
to 12 liters of water. A further increase in pressure
caused the oil to turn a darker yellow than the oil
obtained at the lower pressures and gave it a slightly

different odor. Therefore, conditions adopted for the
remaining distillations were: a pressure of 14-16 pounds,
a distillation rate of 4 liters per hour and collection
of 12 liters of distillate.

Some of the oil was dispersed in the aqueous layer
of the distillate and could not be separated by salting it
out, freezing it out or by allowing the mixture to stand
for 24 hours. Extraction of this oil with petroleum ether
was found to be the only satisfactory method of recovery.
In this manner about 1/4 of the total oil yield was
recovered from the aqueous layer from each distillation.

From 133.2 pounds of foliage, 1075 grams of oil
were recovered, with the yields for individual batches
varying from 1.50 to 2.08 percent and averaging 1.78
percent.

PHYSICAL AND CHEMICAL PROPERTIES
OF THE VOLATILE OIL FROM THE
FOLIAGE AND TERMINAL BRANCH MATERIAL

The oil mechanically separated from the distillate had a light yellow color, while the oil extracted with petroleum ether was a reddish-orange. Both oils had the same typical odor of the foliage.

Density of the oil at $20^{\circ}C$ was 0.9197, refractive index at $25^{\circ}C$ was 1.4783 and the specific rotation was $[\alpha]_D^{27}$ +23.68. Similarly, Lynn and Fischer (12,p.14) reported 0.9215 for the density, 1.4745 for the refractive index and +21.91 for the specific rotation, all measured at $25^{\circ}C$.

The acid number determination resulted in a value of 4.96 as compared to 1.45 reported by Lynn and Fischer. The saponification numbers before and after acetylation were 46.4 and 80.4, whereas Lynn and Fischer reported 110.53 and 149.58 for these determinations.

To determine the aldehyde content, the neutral sulfite method given in the United States Pharmacopoeia and described by Guenther (9,vol. 1,p.283) was employed. This method consists of reacting an exactly measured 10 milliliter sample of oil with a saturated, neutral sulfite solution and measuring the decrease in the volume of oil. Since some of the oil does not separate from the solution,

this is not an exact method. However, by this method the value for the aldehyde content was 6.7 percent.

The iodine number was not determined because Guenther (9,vol. 1, p.305) states that it is of little value since it varies with the size of the sample and the period of contact. In addition the results do not agree with the theoretical values.

To determine the boiling range, a 10 milliliter sample of the oil was distilled at atmospheric pressure. The first distillate had a boiling point of 150°C. When a temperature of 180°C was reached the oil decomposed rapidly. Only 35 percent of the oil distilled between these temperatures.

A second boiling range was determined at reduced pressure. At a pressure of 32 millimeters mercury, 43 percent of the sample distilled between the temperatures of 76° and 109°C. To avoid higher temperatures the pressure was reduced to 20 millimeters mercury and an additional 24 percent of the oil distilled between the temperatures of 107° and 131°C. The pressure was further reduced to 10 millimeters mercury and a final fraction amounting to 24 percent distilled between the temperatures of 120° and 161°C.

CHEMICAL SEPARATION OF THE ALDEHYDES AND ACIDS

Since the acid number and the determination of the aldehyde content did not indicate large amounts of these components in the oil, it seemed best to separate them chemically rather than try to isolate them by fractional distillation. The standard method for the separation of these components was carried out on 280 grams of the oil. The bisulfite extraction yielded 3.0 grams of aldehydes, or 1 percent of the oil. This is much less than the 6.7 percent indicated previously. This difference is attributed to the method employed for the quantitative determination of the aldehydes. The basic extraction yielded 2.3 grams of phenols and 1.6 grams of acids. Since these were minor components of the oil they were put aside to be examined after the major components had been identified.

FRACTIONAL DISTILLATION OF THE NEUTRAL COMPONENTS

For the fractional distillation, a 36 inch Stedman column was used. This column was equipped with a heating jacket and a constant reflux ratio head. A graph supplied by the manufacturer showed the voltage required to maintain the proper temperature gradient along the column. B. L. Bragg (5,p.237-238) studied the efficiency of the column under various conditions and his data were used in an attempt to obtain the best separation.

A preliminary fractional distillation was carried out using 290 grams of the oil remaining after separation of acids and aldehydes (oil from the aldehyde determination was included to make it 290 grams). The distillation was divided into three phases. The distillation was started at a pressure of 28.2 milliliters mercury. When the temperature of the distilling oil reached 100°C the pressure was reduced to 9.5 milliliters. The column was put on total reflux and after equilibrium conditions were again established the distillation was continued until the temperature of the distilling fraction reached 100°C again. The pressure was then reduced to 1.0 millimeter mercury for the remainder of the fractionation. This was done to prevent polymerization and decomposition. After collection of 62 fractions the residue in the still was

not sufficient for efficient fractionation in the Stedman column. Therefore, the residue was transferred to a smaller flask equipped with an eight inch, insulated Vigreux type column and the distillation was completed at 1.0 millimeter mercury.

Results of the fractionation along with some of the physical properties of the fractions are reported in Table 2. To improve the separation, fractions 1 through 4 were redistilled using an insulated, 14 inch Vigreux column at a reflux ratio of 10:1 and a pressure of 53 millimeters mercury. The refractive indices of the new fractions were nearly the same as before the redistillation, so no additional fractions were distilled a second time.

Attempts were made to identify the fractions in order that sharper cuts might be made in taking fractions during the second fractionation. This preliminary examination indicated the presence of a borneol ester. Since borneol is a high melting solid, it was thought that saponification and the subsequent removal of the crystalline borneol would make the fractionation more efficient.

With this in mind the remainder of the oil was divided into two portions for ease in handling and saponified. A 220 gram sample was saponified by refluxing it for two hours in an alcoholic sodium hydroxide solution.

TABLE 2

Fractionation of the Neutrals, and Properties of the Fractions Obtained

Fraction Number	Weight grams	Cumulative Yield percent	Pressure millimeters mercury	Distillation Temperature °C	Boiling Point at Atmospheric Pressure °C	Specific Rotation $[\alpha]_D^{27}$	Refractive Index n_D^{22}
1	9.5	3.27	28.2	61-61.5	154-156	26.2	1.4617
2	4.1	4.6	28.2	61.5-62.5			1.4615
3	4.4	6.2	28.2	62.5-63.5			1.4620
4	4.3	7.7	28.2	63.5-64			1.4628
5	2.2	8.4	28.2	64-65			1.4638
6	3.0	9.5	28.2	65-66.4	159	34.8	1.4644
7	2.6	10.4	28.2	66.5-68			1.4651
8	4.7	11.9	28.2	68-70			1.4659
9	2.7	12.9	28.2	70-71.5	169	86.5	1.4686
10	3.8	14.1	28.2	71.5-73			1.4701
11	4.7	15.8	28.2	73-75			1.4718
12	3.2	16.9	28.2	75-76			1.4762
13	3.8	18.3	28.2	76-77			1.4764
14	7.6	20.9	28.2	77-77.8	169	7.7	1.4793
15	7.6	23.5	28.2	77.8-78.5			1.4811
16	7.6	26.2	28.2	78.5-79			1.4820
17	5.4	28.0	28.2	79-80	169	7.7	1.4809
18	3.9	29.4	27.4	80-81			1.4795
19	2.3	30.1	27.4	81-82			1.4780
20	2.6	31.0	27.4	82-84.5	174-177	7.6	1.4790
21	3.0	32.1	27.4	84.5-86			1.4789
22	1.8	32.7	27.4	86-89			1.4804
23	3.0	33.7	27.4	89.8-100.8	182		1.4774
24	1.5	34.2	9.5	80-83			1.4745
25	0.9	34.5	9.5	83-85.5	193	16.7	1.4730
26	1.7	35.2	9.5	85.5-87			1.4740
27	5.1	37.9	9.5	87-88	197-200		1.4760
28	3.1	38.1	9.5	88-88.5			1.4768
29	6.5	40.2	9.5	88.5-89			1.4776
30	3.2	41.3	9.5	89-90	200-203		1.4773
31	7.5	43.9	9.5	90-91			1.4771
32	5.8	45.9	9.5	91-92	205-209	18.0	1.4744
33	1.9	46.4	9.5	92-93			1.4720
34	3.4	47.8	9.5	93-95	210		1.4704
35	6.1	49.8	9.4	95-96			1.4690
36	2.5	50.7	9.4	96-97	209-210		1.4680
37	3.3	51.9	9.4	97-98			1.4689
38	2.5	52.6	9.4	98-99	209-210	35.4	1.4699
39	3.2	53.8	1.0	46-47			1.4704
40	6.4	56.0	1.0	47-48	212		1.4743
41	2.2	56.8	1.0	48-49.5			1.4730
42	1.6	57.4	1.0	49.5-52	*		1.4799
43	3.5	58.5	1.0	52-53		10.2	1.4788
44	6.2	60.7	1.0	53			1.4780
45	3.1	61.8	1.0	53-54.5			1.4810
46	2.5	62.7	1.0	54.5-56			1.4860
47	2.1	63.3	1.0	56-58.5			1.4920
48	0.4	63.4	1.0	60-64			1.4955
49	5.8	65.5	1.0	64-65			1.5042
50	2.0	66.0	1.0	65-67		11.07	1.5060
51	3.3	67.2	1.0	70-72			1.5067
52	4.6	68.7	1.0	72			1.5060
53	2.4	69.7	1.0	72-76			1.5060
54	3.6	70.7	1.0	76-78			1.5051
55	1.0	71.0	1.0	78-80			1.5047
56	1.8	71.4	1.0	80-83			1.5045
57	4.2	73.1	1.0	83-86		10.8	1.5026
58	3.0	74.9	1.0	86-89			1.5017
59	2.1	75.5	1.0	89-91			1.5020
60	2.0	76.2	1.0	91-93			1.5016
61	2.0	76.5	1.0	93-94			1.5027
62	0.86	76.7	1.0	94-96		-0.5	1.4986
63	1.8	77.2	1.0	96-100			1.5027
64	1.0	77.5	1.0	100-102			1.5048
65	2.4	77.9	1.0	122-129		-5.2	1.5080
66	6.7	79.6	1.0	129-131			1.5135
67	1.8	80.3	1.0	129-131			1.5147
68	1.8	81.1	1.0	131-152			1.5175
Residue	17.1	87.4					
Losses	38.8	100.0					

* Decomposed on boiling at atmospheric pressure.

The alcohol was replaced with water, and the aqueous layer was separated from the oil. The oil was washed with distilled water, dried over anhydrous sodium sulfate and placed in the refrigerator to allow the borneol to crystallize. A second sample of the oil was treated in similar fashion. The aqueous layers on acidification and extraction with ether yielded 38.07 grams of acidic material which was further separated into 18.87 grams of phenols and 19.20 grams of acids.

The borneol did not precipitate from the oil after standing for one week in the refrigerator, nor did it crystallize when the mixture was dissolved in hexane and cooled in a dry ice-acetone bath.

Without further attempt to remove the borneol, the oil was fractionated again with the Stedman column. During this distillation the "take off" stopcock was manually operated at times when the preliminary analysis indicated that a mixture was reaching the still head. This was done to increase the reflux ratio so that a better separation might be obtained. The procedure followed, was to alternately leave the stopcock open for one minute and closed for periods of one to five minutes depending on the boiling points of the suspected components.

After removal of fraction 23, the distillate reaching the head of the column solidified and would not

drain off in the conventional manner. When the still head was heated slightly the material sublimed, instead of melting and draining from the still. The distillation had to be discontinued so that the column could be washed out with hot hexane, which on cooling precipitated the borneol. Again unsuccessful attempts were made to crystallize the borneol from the still residue. The still pot was replaced and the distillation was continued until the still head was again filled with solid borneol. The borneol was removed from the column with hexane and the distillation was continued without a column, using a condenser filled with warm water.

After removal of the borneol, the residue was not sufficient for fractionation in the Stedman column so distillation was completed using an eight inch, insulated Vigreux column. The results of the fractionation along with some physical properties of the fractions are shown in Table 3.

To remove any traces of oxygenated compounds, the first eleven fractions were distilled over metallic sodium at atmospheric pressure. (Preliminary work on the low boiling fractions indicated that they were predominately terpene hydrocarbons). Previous to distillation over sodium these fractions were pale yellow, while after this treatment all were colorless. No fractions beyond the

TABLE 3

Fractionation of Saponified Oil and Properties of the Fractions

Fraction Number	Weight grams	Cumulative Yield percent	Pressure millimeters mercury	Distilling Temperature °C	Boiling Point at Atmospheric Pressure °C	Specific Rotation $[\alpha]_D^{27}$	Refractive Index n_D^{26}	Density at 20°C
1	7.36	1.78	29.1	60.5	154		1.4612	0.857
2	7.45	3.58	29.1	60.5-62	154		1.4633	0.851
3	5.90	5.00	29.1	62.8-65.7	155-157	57.1°	1.4650	0.845
4	6.85	6.65	29.1	66.0-67.5	160-161		1.4659	0.844
5	5.57	8.00	29.1	67.5-68.5	161-162	85.2°	1.4664	0.839
6	7.10	9.71	29.1	68.5-70	160		1.4664	0.838
7	6.25	11.22	29.1	70-71.8	164-165		1.4682	0.843
8	5.78	12.62	29.1	72.5-74.8	166-167	52.7°	1.4719	0.840
9	8.54	14.68	29.1	74.8-76.2	168-170		1.4727	0.851
10	7.01	16.38	29.1	76.2-77.5	170-171	9.93°	1.4795	0.880
11	4.72	17.52	28.0	77.5-78.5	175		1.4838	0.876
12	14.56	21.04	28.0	78.5-79	175	8.57°	1.4842	0.863
13	8.50	23.09	28.0	79	175		1.4849	0.864
14	9.24	25.32	28.0	79-80	175-177	7.23°	1.4831	0.876
15	4.84	26.44	28.0	80	176		1.4802	0.850
16	6.87	28.10	27.0	80.9-82	177-178		1.4784	0.849
17	5.52	29.43	27.0	82-84	179-180	2.80°	1.4775	0.849
18	5.56	30.77	27.0	84-87	181		1.4792	0.878
19	3.40	31.59	27.0	87-89	189	3.08°	1.4828	0.902
20	4.33	34.26	9.4	77.9-85	197		1.4786	0.942
21	13.67	37.56	9.4	85.5-87	206-207	14.7°	1.4777	0.947
22	15.30	41.26	9.4	87-88	206		1.4798	0.953
23	10.26	43.76	9.4	88	207	19.8°	1.4801	0.949
	50.84	56.05	9.4	*				
24	22.00	61.35	1.2	60	216		1.4802	0.952
25	13.00	64.49	1.2	78-81		11.07°	1.4950	0.947
26	6.45	66.05	1.2	81-86	**		1.4971	0.952
27	12.80	69.15	1.2	86-91		10.80°	1.5020	0.956
28	18.33	73.58	1.2	91-96			1.5045	0.960
29	20.85	78.63	1.2	96-106		-0.58°	1.5055	0.968
30	20.87	83.63	1.2	106-120		-13.7°	1.5069	0.966
Residue	35.50	92.21						
Losses	32.20	100.00						

* Borneol solidified in the still column and head.

** Decomposed on being heated at atmospheric pressure.

eleventh were purified in this manner since some decompo-
sition was noted during the distillation of that fraction.
Table 4 is a tabulation of the results of these distilla-
tions.

TABLE 4

Distillation of the First Eleven Fractions
Over Metallic Sodium

Fraction Number	Weight grams	Distilling Temperature degrees centigrade	Weight Recovered grams	Refractive Index n_D^{25}
1	7.36	149-151	4.5	1.4636
2	7.45	149-152	6.9	1.4642
3	5.90	152-155	5.0-	1.4663
4	6.85	152-156	2.0	1.4671
5	5.57	156-159	9.65	1.4679
6	7.10	160-161	8.00	1.4679
7	6.25	164	2.0	1.4686
8	5.78	164	4.3	1.4689
9	8.54	168-170	8.0	1.4745
10	7.01	168-172	5.0	1.4800
11	4.72	172-174	4.5	1.4860

CHEMICAL EXAMINATION OF THE FRACTIONS

Identification of Fractions Distilling Between 60
and 68°C at 29 Millimeters Mercury. The physical proper-
ties of these fractions indicated the presence of

  pinene. Treatment of 2.0 grams of oil from these frac-
tions according to Wallach's method for the formation of
a nitrosochloride derivative (24,p.25) produced 0.1908
gram of crude nitrosochloride which melted at 105-108°C.
After recrystallization from a methanol-chloroform (2:1)
solution the melting point was 113-114°C while the report-
ed value for the melting point of ∝pinene nitrosochloride
is 115°C. Simonsen points out however (19,p.179), that
the melting point for this derivative is frequently
reported as 105-108°C and is dependent on the rate of
heating since it decomposes at the melting point. To
confirm that this was pinene nitrosochloride it was con-
verted to the nitrolpiperidine according to Wallach's
procedure (23,p.181). The melting point of this deriva-
tive was 119°C as compared to the literature value of
118-119°C.

  These fractions made up 10-12 percent of the total
oil. From measurements of the optical rotation of these
fractions, they must consist of a mixture of d and dl
∝pinene. Tests were made for the presence of fenchene

and thujene in these fractions with negative results.

Identification of Fractions Distilling Between 68 and 75°C at 29 Millimeters Mercury. The boiling range for these fractions at atmospheric pressure was 160-163°C. The terpenes included in this boiling range are camphene, βpinene and sabinene. Camphene is a solid at room temperatures (melting point 50°C) while these fractions were liquid and would not crystallize on being cooled to -10°C. Further evidence for the absence of camphene was provided when no hydrochloride could be formed from these fractions.

Neither βpinene nor sabinene form crystalline addition products. The best method for the identification of either of these compounds is by conversion to its oxidation product. Wallach's procedure (25,p.270) was employed for the oxidation, with some slight modification in the final isolation of the product.

To a solution of 15 grams of potassium permanganate and 3.25 grams of sodium hydroxide in 100 grams of water, were added 100 grams of crushed ice and 6 grams of oil from fractions 6 and 7 of the second series of fractions. The mixture was shaken for one hour, after which the unreacted oil was removed by steam distillation. Only traces of unreacted oil were recovered. The manganese dioxide was removed by filtration and the solution was

evaporated under reduced pressure to a volume of 100 milliliters. Any non-volatile neutrals were removed by ether extraction and the solution was acidified with dilute sulphuric acid. The free acids were extracted with ether. Hexane was added to the ether solution until it became cloudy. Slow evaporation of this solution caused the acid to precipitate. The melting point of this acid after three recrystallizations from an ether-hexane solution was 59-60°C. The value for the neutralization equivalent was 187. The reported melting point of sabinenic acid is 57°C and its molecular weight is 184. Therefore it was concluded that sabinene is present in these fractions, which make up 4 percent of the oil.

Identification of Fractions Distilling Between 75 and 80°C at 28 Millimeters Mercury. Compounds included in the boiling range of these fractions are: the terpinenes, the phellandrenes, limonene, Δ^3carene and para cymene. The absence of the terpinenes was indicated when no crystalline hydrochloride could be formed from these fractions. A test for the presence of Δ^3carene through nitrosate formation was negative. A color reaction to determine the presence of this compound was also negative.

Employing Gaponenkov's procedure (7), 20 milligrams of a nitrosite derivative were formed from 2.0 grams of fraction 15 (first series of fractions). After

recrystallization the nitrosite melted at 108-110°C with decomposition. A survey of the terpene nitrosites excluded all but the phellandrene nitrosites. The melting point of this nitrosite corresponded best to the literature value for the melting point of the αnitrosite of dl αphellandrene (melting point ∼113°C). It should be pointed out that there are six isomeric αphellandrene nitrosites and three isomeric βphellandrene nitrosites, hence the nitrosites are not good derivatives to differentiate αand βphellandrene. However, the nitrosites are good derivatives for proving the presence of phellandrenes.

Crystalline nitrosites were also obtained from fractions 17 and 20 from the first series of fractions. These nitrosites melted at 105-107°C. From the second series of fractions, nitrosites were formed from fractions 9 and 14, in yields of 0.2 and 0.1 grams respectively. The melting point of these nitrosites was 98-100°C. This corresponds to the melting point for βphellandrene βnitrosite, (melting point 97-98°C), or αphellandrene d βnitrosite (melting point 100°C).

To differentiate between the isomers, 1.5 grams of fraction 9, of the second series of fractions, and 0.75 gram of maleic anhydride were dissolved in ether and the solution was refluxed for thirty minutes. Cooling the solution did not separate an adduct, nor did evaporation

of the ether. This indicates the absence of α phellan-
drene, since the conditions above have been recommended
for the formation of the maleic anhydride adduct of
α phellandrene when both of the phellandrene isomers are
present. A second attempt to form a maleic anhydride
adduct was made on 2 grams of fraction 12, of the second
series of fractions. The method described above again
produced no derivative.

Bromination serves as another method for distin-
guishing the phellandrene isomers. β phellandrene forms
a crystalline bromide while the α isomer forms only a
liquid product. Therefore 1.0 gram of fraction 13
(second series of fractions) was brominated according to
the method of Baeyer (1,p.448). An oily bromide separated
which crystallized after standing for three days in the
refrigerator. The melting point of this bromide could not
be raised above 116-118°C. The literature value for the
melting point of β phellandrene tetrabromide is 118-119°C.
Therefore β phellandrene must be the isomer present in
this fraction.

In attempts to form β phellandrene tetrabromide
from fraction 16 (first series of fractions), a bromide
with the melting point 122-123°C was isolated. It seems
that this must be dipentene or dl-limonene tetrabromide
which melts at 124°C. The yield was only 50 milligrams

from 2.0 grams of fraction 16, so after two recrystalli-
zations there was insufficient derivative left for further
purification.

The ether solution of fraction 12 and maleic
anhydride described above was evaporated to dryness. The
residue was taken up in 10 milliliters benzene and the
solution was refluxed for one hour. When the solution
cooled 0.1 gram of adduct was removed. After recrystalli-
zation the adduct melted at 147°C, which is the literature
value for the melting point of the limonene-maleic anhy-
dride adduct. From 2.0 grams of fraction 14 (second
series of fractions), treatment with maleic anhydride pro-
duced 0.5 gram of the same adduct. This confirms the
presence of dl-limonene in these fractions.

The presence of para-cymene was indicated in frac-
tions 12 through 14 (second series of fractions) by
products obtained from the alkaline permanganate oxidation
of a 5 gram sample of oil from these fractions. The oxi-
dation was carried out according to Wallach's method for
terpenes (26,p.297). An acid with a neutralization equiva-
lent of 183 was isolated from the oxidation mixture. The
acid melted at 156-157°C as compared to the literature
value of 156-157°C for para hydroxyisopropyl benzoic acid,
(calculated neutralization equivalent 180). Twenty milli-
grams of this acid were heated with dilute hydrochloric

acid at the boiling point for ten minutes. The acid was filtered from the mixture and recrystallized from water. The acid now melted at 160-161°C, agreeing with the literature value for para-isopropyl benzoic acid. The above acids are the expected products from oxidation of para-cymene under the above conditions.

The fractions distilling between 75 and 80°C at 28 millimeters mercury amounted to 10-12 percent of the oil and consisted of βphellandrene, dl-limonene, and para-cymene. In addition there is probably some αphellandrene present in these fractions.

Identification of Fractions Distilling Between 81 and 89°C at 28 Millimeters Mercury. The properties of these fractions corresponded to the properties of terpinolene and γterpinene. Normally γterpinene occurs in small amounts when the αisomer is present. Since αterpinene had not been found in the lower boiling fractions, it did not seem likely that γterpinene would be present in these fractions. To determine if terpinolene was the component making up these fractions, the method of Baeyer (1,p.448) was employed to brominate 2.0 grams of fraction 21 (first series of fractions). From the bromination mixture 0.71 gram of bromide was produced. After recrystallization from ethyl acetate the melting point was 115-116°C. The literature value for the

melting point of terpinolene tetrabromide is 116°C.

To confirm the presence of terpinolene, 4.0 grams of oil from fractions 16 and 17 (second series of fractions) were oxidized in alkaline permanganate solution, according to the method of Wallach (26,p.297). Two products were isolated from the oxidation. Both were neutral products but were easily separated because of differences in solubility in ethanol. The first product, which was quite soluble in alcohol, was recrystallized from ethyl acetate and melted at 148-149°C with gas evolution. This is the reported value for para menthane 1,2,4,8,tetrol, the product terpinolene should form under conditions of this oxidation. Three-tenths gram of this compound was isolated from the oxidation mixture. The second product isolated from the oxidation mixture was sparingly soluble in alcohol and amounted to 0.5 gram. This substance melted at 239-240°C after recrystallization from a mixture of alcohol and water. Of the oxidation products that may be obtained from terpenes with boiling points similar to those of fractions 16 and 17, only para menthane 1,2,4,5 tetrol has a melting point near this value, (melting point 237-238°C). Para menthane 1,2,4,5 tetrol is the compound that is obtained from oxidation of γterpinene by alkaline permanganate. No other derivative for this terpene could be formed from

these fractions. The above fractions amount to 3-5 per-
cent of the oil. Terpinolene was identified and the
presence of ɣterpinene was also indicated in these
fractions.

Identification of Fractions Distilling Between 85
and 90°C at 9.5 Millimeters Mercury. The properties of
these fractions indicated the presence of terpene alco-
hols. The fractions reacted slowly with metallic sodium
and gave a positive hydroxamate test for alcohols. Elze's
phthalic anhydride method, described by Guenther (9,vol.2,
p.794), for the separation of primary and secondary
alcohols from mixtures of terpene alcohols was applied to
these fractions. From 4.0 grams of fractions 28, 29 and
30 (first series of fractions), 2.0 grams of an acid
phthalate were obtained. The ester melted at 160-163°C
without purification (melting point of bornyl acid
phthalate 164-165°C). The ester was saponified in an
alcoholic solution of sodium hydroxide, which on dilution
with water separated the crystalline borneol, melting
point 202-203°C. The literature value for the melting
point of borneol is 203-204°C.

Two grams of fraction 22 (second series of frac-
tions) were treated with phenyl isocyanate as described
by Palkin and associates (15,p.22). The reaction mixture
yielded 0.4 gram of a urethane which melted at 135-137°C

after recrystallization. The melting point of d-bornyl phenyl urethane is 138°C.

Terpinene 4-ol, a tertiary alcohol, is frequently found in oil mixtures which contain sabinene. The reason that both of these compounds often occur in the same oil is that hydration of sabinene converts this terpene to terpinen 4-ol. To show that terpinen 4-ol was present 2.0 grams of fraction 22 (second series of fractions) was treated according to Wallach's procedure for the formation of nitrosochlorides. After six days in the refrigerator 0.12 gram of white crystals formed. The nitrosochloride melted at 115-116°C, agreeing with the value Penfold (16,p.313) reported for the melting point of terpinen 4-ol nitrosochloride. The nitrosochloride was converted to the nitrolpiperidine which melted at 171-173°C. The literature value for the melting point of terpinen 4-ol nitrolpiperidine is 172-174°C. These fractions amounted to about 10 percent of the oil and consisted of d-borneol and terpinen 4-ol.

Identification of Fractions Distilling Between 92 and 99°C at 9.5 Millimeters Mercury. At this point in the analysis the character of the fractions collected from the two distillations was quite different.

As may be noted from Table 3, 50.84 grams of solid borneol were collected from the second fractionation. An

additional 18 grams of solid borneol were crystallized from fraction 24 of this series of fractions. The identity of the borneol was determined from its melting point and through formation of its 3,5-dinitro benzoate, melting point 155-156°C.

From the first series of fractions no solid borneol was isolated. Fractions 33 through 44 correspond to the above boiling range and remained liquid when cooled to -10°C. These fractions reacted slowly with metallic sodium and gave a positive hydroxamate test for esters. Saponification of 2.5 grams of fraction 35 in alcoholic sodium hydroxide followed by dilution of the reaction mixture with three volumes of water caused separation of 1.5 grams of crystalline solid, which melted at 201-203°C after recrystallization from hexane. The compound formed a 3,5-dinitrobenzoate melting at 155-156°C, which confirmed its identity as borneol.

The remainder of these fractions were saponified. To identify the acid portion of the ester, the acid was isolated as the sodium salt. An attempt to form the anilide from this salt was unsuccessful because the reaction flask was broken and its contents were lost. From later work on the acids recovered from the saponification of the oil sample for the second distillation it was shown that the ester was borneol acetate.

Identification of the High Boiling Fractions Dis-
tilling Above 70°C at 1.0 Millimeter Mercury. The boiling
range of these fractions was in the range of sesqui-
terpenes and their alcohol derivatives. Tests for alco-
hols were negative.

The sesquiterpene, cedrene is one of the major
components of Sierra Juniper wood oil. A color test per-
formed on these fractions to indicate its presence was
negative.

Ether solutions of 2.0 gram samples from fractions
51, 58 and 66 (first series of fractions) were cooled in
an ice-bath and dry hydrogen chloride was passed into the
solutions until they were saturated. After standing for
three days at 6°C, 0.1 and 0.05 grams of crystalline
hydrochlorides were separated from fractions 51 and 58
respectively. After recrystallization the hydrochlorides
from both of these fractions melted at 116-117°C. The
reported melting point for cadinene dihydrochloride is
117-118°C.

Treatment of 1.0 gram samples of fractions 25, 27
and 29 (second series of fractions) with a cold, saturated
solution of hydrogen chloride in glacial acetic acid
produced 0.10, 0.20 and 0.15 grams of this same dihydro-
chloride.

Further evidence for the presence of cadinene was supplied by the sulphur dehydrogenation of a 15 gram sample of fraction 29. Ruzieka's procedure was employed with a 30 percent yield of cadalene. The cadalene was identified through formation of its picrate (melting point 114-114.5°C). The reported value for the melting point of cadalene picrate is 115°C. Cadinene has been identified in the high boiling fractions which make up 20-25 percent of the oil.

Identification of the Acidic Components. Distillation of the acids separated from the saponification mixture in the preparation of the second oil sample for fractionation yielded 6 grams of ether, 5.0 grams of acetic acid, 0.8 gram of a pale yellow oil, 1.4 grams of a red-orange oil and 4.5 grams of residue.

The acetic acid was identified through a positive ferric chloride color reaction, formation of the para-bromo-phenacyl ester and the potassium salt. The para-bromo-phenacyl ester melted at 83.5-84.5°C, as compared to the literature value of 85°C. The potassium salt was obtained by steam distilling the aqueous layer remaining after the saponification and removal of the acids. The condensate was neutralized and evaporated to dryness. A total of 14.6 grams of the potassium salt were obtained by this method. The salt was recrystallized from methanol-

ether solution and melted at 289-290°C. The literature value for the melting point of potassium acetate is 292°C. Attempts to determine the neutralization equivalent of the red-orange oil showed that it was not completely soluble in basic solution, so no further work was done on this component.

The phenolic fraction separated 1.1 grams of crystalline material which melted at 192-194°C after recrystallization from acetone. This compound was not identified.

The remainder of the phenolic fraction was distilled yielding 12.1 grams of pale yellow oil and 5.6 grams of residue. The boiling range of the phenols at atmospheric pressure was 184-234°C. The refractive index for the phenol mixture was 1.5330 at 25°C. The mixture was a pale yellow color and would not crystallize at -10°C. An alcoholic solution of the phenols gave a red-orange color with ferric chloride while an aqueous solution gave a purple-violet color with this reagent. The phenolic portion of the oil was not characterized further.

SUMMARY AND DISCUSSION

The volatile oil obtained by steam distillation of the foliage and terminal branches of Sierra Juniper is composed of αpinene 10-12 percent; a mixture of βphellandrene (and probably some α phellandrene), dl-limonene and para-cymene 10-12 percent; sabinene 4 percent; terpinolene 3-5 percent; terpinene 4-ol 6-8 percent; d-borneol free and combined as bornyl acetate 22-25 percent; cadinene 20-25 percent; acetic acid free and combined as bornyl acetate 4-5 percent; phenols 1.0 percent; traces of aldehydes, residue and losses 16-18 percent. Possibly γterpinene is present in this oil. However, the only derivative obtained to indicate its presence was an oxidation product. The fact that no other derivative could be formed is not surprising in view of the fact that a number of instances have been reported where a crystalline addition product could not be obtained because of the presence of other components which inhibit crystallization, or form a similar product causing separation of an oily eutectic. Penfold (16,p.311) cites specifically that the nitrosites of the terpinenes are noted for not crystallizing when other components are present.

It is nearly impossible to obtain good separation of some of the mixtures in essential oils. To illustrate

the difficulty, a mixture of the phellandrenes, limonene, para-cymene, terpinene and terpinolene would have a boiling range of 172-183°C.

In addition to cadinene there might be other sesquiterpenes present in the higher boiling fractions. A large number of the sesquiterpenes form hydrochlorides. However, only cadinene dihydrochloride was isolated. Since the volatile oil from the wood of Sierra Juniper contained the sesquiterpene, cedrene, appropriate fractions were tested for the presence of this compound with negative results.

About half of the amount reported as losses and residue was a dark-sticky mass of residue, which was probably derived from some of the more easily polymerized terpenes such as the phellandrenes.

Comparison of the composition of the fresh oil sample in this investigation with the one analyzed by Lynn and Fischer which had partially resinified, showed that the fresh sample contained αpinene, sabinene, terpinolene, terpinene 4-ol, limonene, cadinene, and possibly γterpinene in addition to the components reported present in the resinified oil. The presence of camphene, which Lynn and Fischer thought to be present but were unable to identify because of insufficient

material, could not be confirmed. It seems that the fraction which the above authors thought to be camphene was actually sabinene.

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