THE COMPOSITION OF THE OILS
FROM
SOME CANADIAN CONIFERS

A Thesis

by

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GENERAL INTRODUCTION

During the steps employed in the conversion of the standing tree to wood pulp about two-thirds of the original plant material is discarded, and of the many hundreds of compounds present in the tree only cellulose is utilized to an important extent. Because of the immense volume of timber cut even minor tree constituents are potentially available in large quantities. Thus the volatile oil content of the waste coniferous foliage cut annually in Canada is in excess of ten million pounds. No detailed knowledge of the composition of these oils has hitherto been available. The separation and identification of the constituents of some of these oils was one object of this research.

Most coniferous woods contain two to six percent of fatty and resin acids and their esters. During kraft or soda pulping these compounds are saponified by the alkaline liquor. When the black liquor is concentrated for chemical recovery, these soaps together with smaller amounts of water-insoluble wood constituents separate as crude sulphate soap. By acidification of the sulphate soap, tall oil is obtained.

Although the estimated potential annual production of tall oil in Canada is 35,000 tons, none is actually produced. Commercial utilization of the oil as well as fundamental studies of its composition have been hampered by shortcomings in existing means for separating the constituents. Several techniques for separating tall oil constituents were explored, and are reported in this thesis.

The work on tall oil was carried out at McGill University during 1945 to 1948. The investigation of the volatile oils from coniferous foliage was conducted at the Ontario Research Foundation during 1949 to 1952. The results of the latter investigation have been published, (1, 2, 3, 4, 5).

HISTORICAL INTRODUCTION

Tall Oil

Tall oil is also known as liquid rosin, tallolja, tallol, or talleol. The name is derived from the Swedish "tall" meaning "pine".

In 1893 Klason (6) reported that black liquor contained resin acids, but the first chemical investigation of tall oil was carried out in 1905 by Larssons (7) who distilled the oil in vacuo, and prepared metallic soaps of tall oil acids. He concluded that the product was similar to rosin. In 1909 Fehrion (8) first esterified tall oil with alcohol in the presence of sulphuric acid, and separated the liquid esters from the solid resin acids. He believed the fatty esters to be resin acid derivatives and concluded that tall oil contained one or more resin acids of the formula $C_{20}H_{30}O_2$ dissolved in liquid resin acids. It remained for Bergström in 1910 to recognize the presence of fatty acids of the oleic series, and probably more unsaturated acids (9). The following year he reported (10) the presence in tall oil of palmitic acid, probably oleic and linoleic acids, a mixture of resin acids, and a phytosterol melting at 134°C. Since that time many hundreds of tall oil samples have been analysed for their contents of fatty acids, resin acids and neutral materials, but in comparitively few cases have individual compounds within these broad catagories been isolated, identified or estimated.

Composition of Tall Oil

Jennings (11) in a study of 177 tall oil samples found that seasonal variations in the composition of the oil were small, but that oil from different geographical locations had markedly different compositions. The composition of the oil also depends on the type of wood used (12, 13), the duration of seasoning (14) and the refining procedure (15).

The most complete analytical data available on Canadian tall oils was obtained by Burch (16, 17) and is reproduced in Table I.

Table I

Chemical Analyses of Canadian Tall Oils

Source	Acid No.	Saponification No.	Iodine No.	%Resin Acids	%Neutral Materials	%Fatty Acids
Bathurst	106	118	196	12.1	33.8	54.1
Brown	109	153	231	30.1	18.7	51.2
Canada	100	127	206	22.9	32.3	44.8
Consolidated	133	185	228	35.9	20.0	44.1
Dryden	162	165	260	55.5	11.7	32.8
Pacific	81	102	164	11.8	35.5	52.7

Wafer et al (13) assembled analytical data for several hundred American tall oils from 15 mills. These data are summarized in Table II.

Table II

Chemical Analyses of American Tall Oils

Mill	% Resin Acids	%Unsaponifiable Material	% Fatty Acids
A	45	7.5	49
B	51	8.6	43
C	56	7.0	37
D	38	8.4	54
E	42	6.7	54
F	40		
G	40	7.2	54
H	42	6.7	51
I	42	18.1	39
J	44	7.5	50
K	44	5.6	50
L	39	8.5	52
M	47	7.8	46
N	38	6.9	52
O	58	6.5	36

The data of Edwards (18) summarizing many analyses of Scandinavian and American tall oils are contained in Table III.

Table III

Chemical Analyses of American and Scandinavian Tall Oils

Tall Oil	Acid No.	Saponification No.	% Resin Acids	%Unsaponifiable matter	% Fatty Acids
American	163	173	38.4	8.2	52.0
Swedish (resinous type)	151	174	48.0	10.5	40.8
Swedish (medium type)	160	169	41.4	9.5	48.3
Finnish (resinous type)	156	172	38.7	8.5	51.2
Finnish (medium type)	161	168	32.2	6.8	59.0

These results are sufficient to show that tall oil is an extremely variable product. Variations in composition between products of different mills within one country are usually greater than the difference in average composition between oils of different countries. However the neutral content of Canadian tall oils contrasts sharply with the neutral content of American or Scandinavian oils. Burch (16) suggested that the high neutral content of Canadian tall oils resulted from the high proportion of spruce which is pulped in Canada. In this regard it may be noted that Dryden tall oil - the only oil with analytical characteristics similar to those of foreign oils - was the only oil derived mainly from pine, rather than spruce or fir.

Fatty Acids

Possibly because of their commercial importance or because their chemistry is more completely understood, the fatty acids of tall oil have been much more thoroughly investigated than have other constituents.

In his earliest studies of tall oil, Bergström (10) isolated palmitic acid from a tall oil distillate, and identified oleic acid by the eliadin reaction. He was unable to prepare a hexabromide of linolenic acid from the oil.

Palmitic acid was isolated by Hasselstrom (19, 20) by fractional distillation of the esters followed by separation of the saturated acids as their ether-insoluble lead salts. A trace of hexabromide of linolenic acid was isolated following bromination. Oleic and linoleic acids were identified by their ozonization products.

Dittmer (21) found the lead salts of the fatty acids to be soluble in ether and concluded that saturated acids were absent. He demonstrated the presence of oleic and linoleic acids by permanganate oxidation to the dihydroxy - and tetrahydroxystearic acids, and of traces of linolenic acid by preparation of the hexabromide.

Oleic, linoleic and linolenic acids were also identified by means of their permanganate oxidation products by Pyhälä (22). In addition he reported the possible values. He reported 18% oleic acid, 80% linoleic acid and 1% linolenic acid. No saturated acids were found by the lead salt ether method. The presence of oleic and linoleic acids was confirmed by permanganate oxidation. Linolenic was identified by preparation of the hexabromide and by spectrophotometric means.

Sandström and Sandström (32) also used iodine and thiocyanogen values to calculate the fatty acid composition of a Swedish tall oil. They found 43.4% oleic acid, 50% linoleic acid, and 6.6% linolenic acid. By permanganate oxidation the saturated fatty acid content was found to be 4.8%.

By undisclosed means Alford (33) found 43.6% oleic acid, 19.9% linoleic acid and 36.5% linolenic acid in an American tall oil.

In his study of Canadian tall oils, Burch (16) was unable to effect a useful separation of the fatty acid esters by fractional distillation. However, distillation of hydrogenated fatty esters led to the conclusion that chain lengths other than C_{18} were absent.

The composition of the fatty acid fraction of six Canadian tall oils were estimated by the following procedure: The ultraviolet absorption spectra of the acids were measured after alkali isomerization. The linolenic acid content was calculated from the absorption at 2680 Å, corrected for background absorption. The linoleic acid content was calculated from the absorption at 2340 Å, corrected for absorption due to linolenic acid. Oleic acid was estimated from the iodine number of the mixture after allowance was made for the iodine absorption by the linoleic and linolenic acids. Saturated acids were estimated by difference.

Because of the presence in tall oil of conjugated fatty acids formed during the cooking process, Burch concluded that the above method did not give reliable values for the oleic and saturated acid contents. In the presence of conjugated acids, iodine numbers and consequently oleic acid values were too low. Estimates of saturated acid contents were therefore too high.

The results of Burch's spectrophotometric analyses of the methyl esters of the fatty acid fraction are given in Table IV.

Table IV

Composition of the Fatty Acid Fraction of Canadian Tall Oils

Tall Oil	Linolenic	Linoleic	Oleic	Saturated
Bathurst	1.5,1.5	55.2,52.1	0.0, 0.0	43.3,46.4
Brown	1.1,1.0	61.2,61.0	0.0, 0.0	37.7,38.0
Canada	0.8,0.9	44.2,44.8	2.3, 0.8	52.7,53.5
Consolidated*	0.7,0.9	46.1,47.9	4.5, 0.4	48.7,50.8
Dryden	0.4,0.6	42.0,50.8	36.8,18.4	20.9,30.3
Pacific	1.3,1.4	25.2,27.4	32.2,27.4	41.3,43.8

* Commercial Product

Anderson and Wheeler (34) studied six American tall oils by a spectrophotometric method and by the iodine and thiocyanogen number method. They found the two methods to be in reasonably good agreement. The average values reported by these investigators were 48.4% linoleic acid including 12% conjugated linoleic acid, 44.6% oleic acid, and 7% saturated acids by difference. Saturated acids were also determined by crystallization from acetone at low temperatures. They distilled one sample of esters and concluded that the saturated acids were chiefly palmitic, and that the presence of palmitoleic acid and fatty acids with chain length greater than C 18 was indicated.

Commenting on the numerous investigations of the fatty acid fraction of tall oil, Browning and Calkin (35) in 1946 said, "In general, the methods employed prior to 1927 were inadequate to provide more than a rough qualitative indication of composition and only the relatively few investigations since that time can be taken to have any great degree of reliability. Even the later work has necessarily applied methods which are not adequate to define accurately the composition of the fatty acids and the complete and precise analysis of all the components is a major undertaking which will, ideally, require methods more refined than any which have yet been developed."

Resin Acids

Although the resin acids were the first constituents of tall oil to be recognized (6, 7, 8), the fraction remains the least thoroughly investigated and the least understood. Dittmer (21) in 1926 separated the resin acids from tall oil by selective esterification and obtained yellow crystals from methanol melting at 161 - 166° of composition $C_{20}H_{30}O_2$. He considered the product to be identical with abietic acid. Hasselstrom and Bogart, by the action of glacial acetic acid on material crystallizing from American tall oil, obtained an acid melting at 164.5 - 165.5°C., $[\alpha]_D$ - 53.9°, which was identical with Steele's abietic acid obtained by the action of acetic acid on wood rosin (36). Aschan (37) also reported the presence of abietic acid in tall oil.

However, Fricke (38) in 1927, reported that a methanol solution of the resin acids deposited crystals melting at 138 - 140°C, saponification number 181.3, iodine number 162 which were not identical with abietic acid. He named the material tallic acid. Pollack (39) noted that after removal of abietic acid from the resin acid fraction by crystallization liquid acids remain.

More conflicting evidence was obtained by Pyhälä (22, 40) who obtained resin acids melting over the range 156 - 158° to 175 - 180°C. Aschan and co-workers (37, 41, 42, 43) obtained crystalline material melting at 182 - 183° molecular weight 303, equivalent weight 301 from distilled Finnish tall oil. They concluded that the acid was a sterioisomer of the "Levy abietic acid" melting at 178 - 182° obtained by vacuum distillation and crystallization of American rosin (44) and named it pinabietic acid. However, their product was unable to satisfy the classical criteria of constancy of melting point and rotation and in 1927 they concluded that pinabietic was a mixture (45). Niesen in 1937 apparently still regarded pinabietic acid as a pure compound. He found that the resin acids had Hübl iodine number 160.3, acid number 185.5, Rast molecular weight 301.5, and concluded that the fraction was mainly pinabietic acid (30). He later reported that the ultraviolet absorption spectrum of abietic acid showed a strong shift toward the longer wavelengths when compared to pinabietic acid which he stated had no conjugated system (31).

Hasselstrom and co-workers (46) by a long process of crystallization of pinabietic acid were able to isolate abietic acid melting at 163 - 165°C, $[\alpha]_D - 79.9^\circ$ in ethanol, identical with Steele's abietic acid. They were unable to separate the remaining acids by crystallization but after treating the acids with sulphuric acid they were able to identify a sulphonated dehydroabietic acid and a crystalline lactone formed by hydration of dihydroabietic acid. The presence of dextropimaric acid was considered probable.

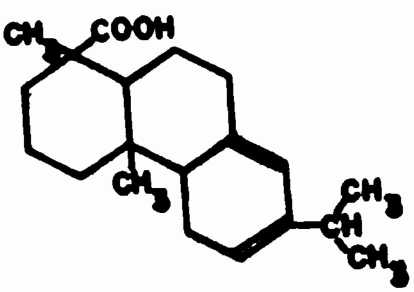
Sandermann (47) from measurements of optical rotation concluded that the resin acids contained 25% dextropimaric acid, 50% abietic acid, 25% pyroabietic acid. By pyroabietic acid he probably meant the mixture of dehydroabietic acid, dihydroabietic acid and tetrahydroabietic acid formed from abietic acid by the action of heat (48, 49).

Ball and Vardell (50) showed by means of ultraviolet absorption spectra that the conjugated acids, abietic acid and neoabietic acid, accounted for about 50 percent of the resin acid fraction.

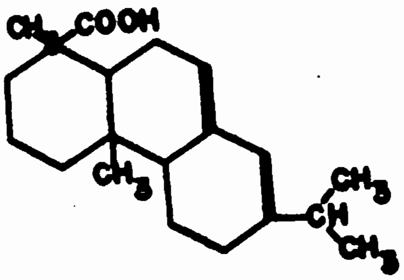
Aschan (39) reported a new series of resin acids termed colophenic acids whose sodium salts are soluble in cold water and stable toward carbon dioxide. These acids were apparently hydroxy acids formed by oxidation of the original acids (51).

The following brief review of the chemistry of the resin acids may assist in the evaluation of the above reports.

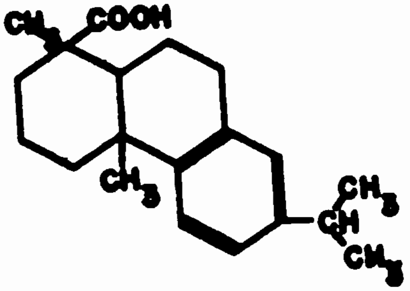
According to Aschan (52) laevopimaric acid (I) is the principal resin acid of tree secretions. This acid is easily isomerized by heat, acids, or alkalies to abietic acid. When this isomerization is carried out under controlled conditions (36) an abietic acid (II) is obtained which satisfies the customary chemical requirements for a pure chemical individual as to constancy of optical rotation and melting point. When abietic acid is heated under more drastic conditions a hydrogen disproportionation and dehydrogenation occurs with the formation of dehydroabietic acid (III) dihydroabietic acid (IV) and tetrahydroabietic acid (V). Abietic acid is also readily oxidized by atmospheric oxygen. Dextropimaric (VI) does not isomerize to abietic acid and is stable to atmospheric oxygen. The accepted structures of these acids are as follows. The figures given below each structure are the percentage of the acid found in wood rosin, according to Harris (53).



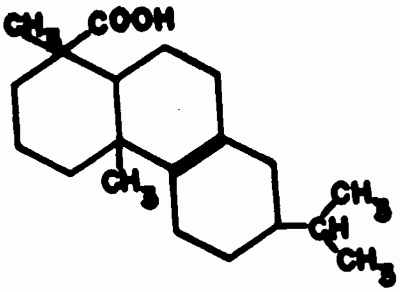
LAEVOPIMARIC ACID
1%



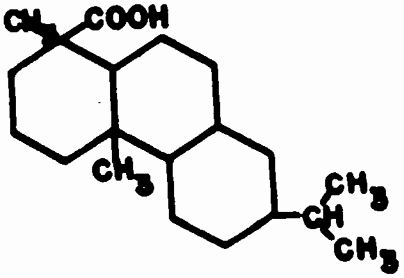
ABIETIC ACID
30 - 40%



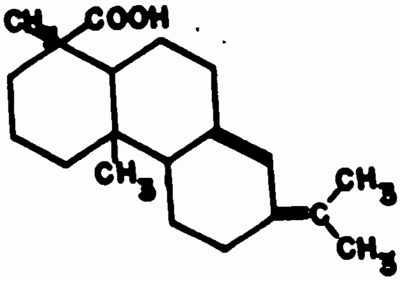
DEHYDROABIETIC ACID
5%



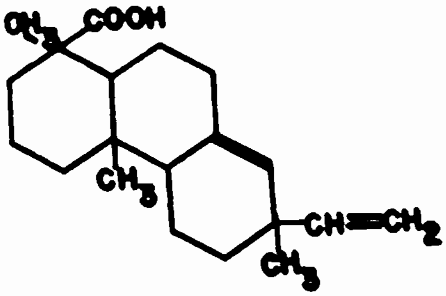
DIHYDROABIETIC ACID
14%



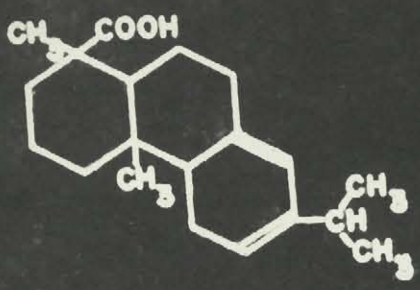
TETRAHYDROABIETIC ACID
14%



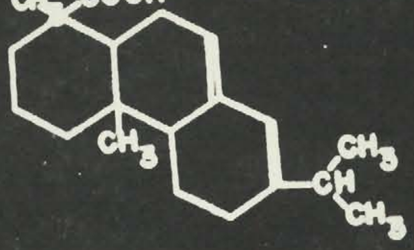
NEOABIETIC ACID
10 - 20%



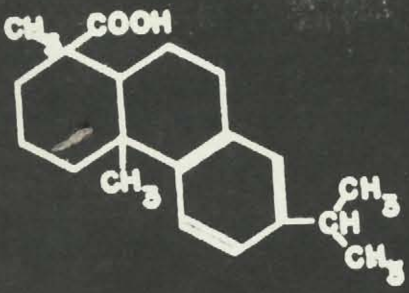
DEXTROPIMARIC ACID
16%



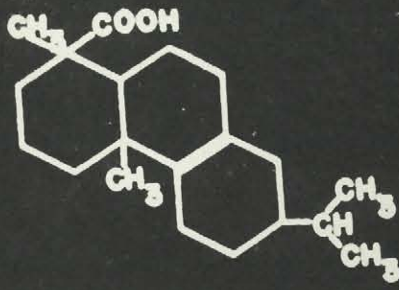
LAEVOPIMARIC ACID
1%



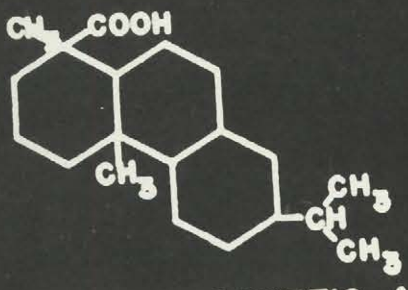
ABIETIC ACID
30 - 40%



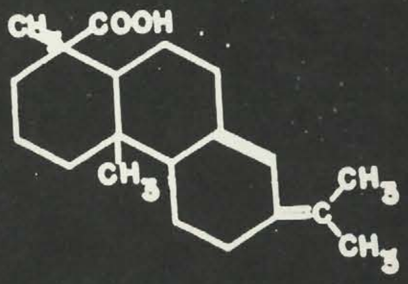
DEHYDROABIETIC ACID
5%



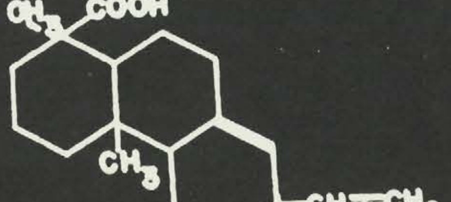
DIHYDROABIETIC ACID
14%



TETRAHYDROABIETIC ACID
14%



NEOABIETIC ACID
10 - 20%



DEXTROPIMARIC ACID
16%

Neutral Material

In 1911 Bergstrom (10) reported the presence of a phytosterol melting at 134°C. in the neutral fraction of tall oil. Sandquist (54) in 1922 again reported the presence of a phytosterol melting at 136-137°C.

Dittmer (21) separated the neutral fraction into a petroleum ether extractable fraction comprising 9.1% of the tall oil, and an ether extractable fraction which made up 3.1% of the oil. By fractional distillation about 40% of the former fraction was obtained as distillate boiling below 235°C. at 2-4 mm. pressure. These fractions contained less than 2% of oxygen on combustion analysis. Dittmer therefore concluded that the fraction was predominantly hydrocarbon.

From the ether-extractable material a crystalline substance giving the phytosterol color reaction with acetic anhydride and sulphuric acid and melting at 135- 136.5°C. was obtained by crystallization from alcohol.

Specific compounds were first identified in the neutral fraction by Sandquist and Hok (55). They separated the neutral materials in 7% yield by ether extraction of the sulphate soap. By fractional crystallization they separated 1.5% of a phytosterol fraction melting at 137-138°C. and a more soluble substance melting at 70.5-71.5°C. The latter compound was subsequently identified (23) as lignoceryl alcohol, $C_{23}H_{47}CH_2OH$.

The phytosterol fraction was submitted to a long series of fractional crystallizations to separate a small amount of a dextrorotatory compound, $[\alpha]_D$ 18.8° in chloroform, and melting at 139.4 - 140.4°C., which they considered to be dihydrositosterol contaminated with 10% of sitosterol. The more soluble fractions yielded a sterol $[\alpha]_D$ - 34.1° melting at 137.4 - 137.8°C., which resembled the β-sitosterol isolated by Anderson and co-workers (56) from corn, wheat and rice.

Hasselstrom (57) obtained sitosterol, dihydrositosterol and lignoceryl alcohol by fractional crystallization of the neutral fraction from an American southern pine tall oil. He concluded that these compounds comprised 63%, 7% and 30% of the solid neutral constituents respectively. From the liquid resene portion by treatment with betaine hydrazide hydrochloride he obtained a ketone boiling at 163 - 167°C. at 2 mm. and amounting to 10% of the fraction. The ketone yielded a semicarbazone melting at 193°C. and a 2, 4 - dinitrophenyl-hydrazone melting at 169.5 - 171°C.

Khaletskiĭ and Solomonick (25) examined the ether soluble portion of crude phytosterols obtained by alkaline pulping of pine wood. By a series of recrystallization they separated three optically inactive alcohols. Their properties are listed in Table V.

Table V

Melting Points of the Aliphatic Alcohols and their Derivatives

	$C_{23}H_{47}CH_2OH$ (°C.)	$C_{22}H_{45}CH_2OH$ (°C.)	$C_{23}H_{47}CH_2OH$ (°C.)
Alcohol	75 - 77	70.5 - 71.5	69 - 70.2
Acetate	...	51 - 53	53 - 53.8
Benzoate	...	43 - 50	50 - 53
	$C_{23}H_{47}CO_2H$	$C_{22}H_{45}COOH$	$C_{23}H_{47}COOH$
Acid(a)	82 - 83	75 - 76.5	72.5 - 73.5
Lead salt of acid	...	115 - 116	114 - 115.5

(a) by oxidation with CrO_3 in acetic acid.

β-sitosterol melting at 135 - 135.5°C. $[\alpha]_D$ - 36° in chloroform was isolated and purified as the benzoate. It was stated that unidentified sterols and a trace of sitostanol were present. Stigmasterol α_1, α_2, α_3, sitosterol and α -sitosterol were absent.

Sherbeck (58) subjected the neutral fraction of a Canadian tall oil to repeated crystallization from alcohol. The least soluble fraction after removal of a less soluble dextrorotatory sterol by crystallization, yielded β-sitosterol melting at 138 - 139.5°C., $[\alpha]_D$ - 33°. The dextrorotatory sterol after removal of unsaturated impurities by treatment with sulphuric acid and acetic anhydride, bromination followed by chromatography yielded, β-sitostanol melting at 139.5 - 140°C, $[\alpha]_D +28°$.

From the more soluble crystalline material lignoceryl alcohol melting at 73.5 - 74°C. was obtained. The acetate melted at 54 - 55°C. The most soluble crystalline material was chromatographed on alumina and the least strongly absorbed fractions purified by chromatography and crystallization. A substance melting at 163 - 165°C, $[\alpha] + 6°$, yielding an acetate melting at 132 - 134°, $[\alpha] + 33°$ was obtained, which Sherbeck considered to be identical with the α'- sitosterol of Wallis and Fernholtz (59)

By fractional distillation Sherbeck separated four optically active oils, the properties of which are given in Table VI.

Table VI

Properties of Optically Active Liquid Alcohols from Tall Oil

Formula or	$C_{13}H_{21}(OH)$ or $C_{14}H_{23}(OH)$	$C_{16}H_{27}(OH)$ or $C_{17}H_{29}(OH)$	$C_{19}H_{31}(OH)$ or $C_{20}H_{33}(OH)$	$C_{15}H_{23}(OH)$ or $C_{16}H_{25}(OH)$
n_D^{25}	1.5054	1.5075	1.5174	1.5241
$d_4^{27.8}$	0.9554	0.9516	0.9674	0.9954
$[\alpha]_D$	$+25°$	$+25°$	$+27°$	$+19°$
	1 double bond	2 double bonds	1 double bond	1 double bond
	2 rings	1 ring	3 rings	3 rings
	tertiary alcohol	tertiary alcohol	tertiary alcohol	secondary alcohol

Less volatile fractions boiling about 210° at 0.020 mm. deposited crystalline material from which was separated 1 - eicosanol $C_{20}H_{43}(OH)$ and 1 - docosanol $C_{22}H_{45}(OH)$ and lignoceryl alcohol. These compounds were identified by their melting point, the melting point of their acetates and urethanes.

From the still residues was obtained by distillation, chromatography and crystallization an alcohol melting at 102 - 104°C., $[\alpha]_D + 50°$ which formed an acetate melting at 95 - 96° C., $[\alpha]_D + 54°$. Combination analysis indicated the formula $C_{26}H_{44}O$ or $C_{27}H_{46}O$ and the presence of one double bond and four rings.

Volatile Oils of Canadian Conifers

Oil of Picea Mariana (Mill.) B.S.P.

Picea mariana (Mill.) B.S.P., commonly called black spruce is widely distributed throughout North America from the Yukon Territories south to Alabama, and from the Maritime provinces to the Pacific coast.

Hanson and Babcock (60) reported the preparation of an oil, d_{17} 0.9274, in a yield of 0.57% by steam distillation of foliage from a species designated only as "Picea mariana".

Risi and Brule (61) prepared eleven samples of the oil and reported the properties listed in Table VII.

Table VII

Properties of the Essential Oil of

Picea Mariana (Mill.) B.S.P.

Property	Extreme Limits	Average
d 15	0.9070 - 0.9260	0.9141
$[\alpha]_D$	$-25^{\circ}48^{\circ}$- 41° 48'	-33° 42'
η^{D}_{20}	1.4666 - 1.4722	1.4697
Acid No.	0.99 - 2.44	1.59
Ester No.	57.2 - 133.1	97.0
Saponification No.	59.6 - 135.3	98.6
Boiling Range	155° - 164°	160° to 221°
Solubility in 90% alcohol	in 0.4 to 1.2 volumes	0.7 volumes

Foliage of P. mariana in mixture with foliage of Tsuga canadensis (L.) Carr and Picea glauca (Moench) Voss. is distilled commercially by farmers and small local industry in northeastern United States and eastern Canada. Commercial spruce-hemlock oils examined by Fritzsche Brothers, Inc. (62) had properties varying within the limits:

d_{15}^{15}	0.905 to 0.919
α_D^{15}	$-18^\circ 8'$ to $-26^\circ 10'$
η_D^{20}	1.4673 to 1.4698
Ester Content (calc. as bornyl acetate)	34.3% to 44.8% usually above 37.0%
Solubility in 90% alcohol	soluble in 0.5 to 1.0 volumes.

The chemical composition of the oil has not previously been investigated.

Oil of Tsuga Canadensis (L.) Carr.

Tsuga canadensis or eastern hemlock enjoys a geographical distribution similar to that of black spruce, and the oils of the two species are usually prepared and marketed as a mixture. Cable (63) examined five oils distilled from T. canadensis in Michigan, Wisconsin, New Hamshire, Tennessee and Virginia and reported the properties listed in Table VIII.

The values in Table IX were obtained by Risi and Brule from four samples of T. canadensis grown in Quebec.

Table VIII

Properties of the Essential Oil of Tsuga Canadensis (L.) Carr.

Property	Range
Yield	0.29% to 0.65%
d_{15}^{20}	0.9020 to 0.9234
$[\alpha]_D^{20}$	-14°48' to 21°39'
η_D^{20}	1.4691 to 1.4704
Acid No.	0.33 to 0.70
Ester No.	130.8 to 147.35
Ester No. after acetylation	113.5 to 171.94
Ester content as bornyl acetate	36.33% to 51.57%
Total alcohol content as Borneol	31.24% to 47.41%

Table IX

Properties of the Essential Oil of Tsuga Canadensis (L.) Carr

Property	Extreme Limits	Average
d_{15}	0.9090 to 0.9387	0.9178
$[\alpha]_D$	-22°0' to 24°24'	-23°30'
η_D^{20}	1.4671 to 1.4766	1.4702
Acid No.	0.74 to 2.14	1.31
Ester No.	67.6 to 125.3	105.4
Saponification No.	68.3 to 126.6	106.7
Boiling Range	160° to 165°	162°- 220°
Solubility in 90% alcohol	Sol. in 0.2 to 0.3 volumes	0.3 volumes

Bertram and Walbaum (64) examined an American oil of hemlock and identified l-α-pinene identified as the nitrolbenzylamine, m.p. 121 - 123°, and reported the presence of 35 to 53% l- bornyl acetate and an unidentified sesquiterpene.

Oil of Abies Balsamea (L.) Mill.

Abies balsamea (L.) Mill. or balsam fir is one of the most prolific species in eastern and central Canada. It ranges from the Atlantic coast of Canada westward and northward to northeastern Alberta and is cut extensively for both pulp and lumber.

Risi and Brule (61) distilled fourteen samples of foliage and reported the properties listed in Table X.

Table X

Properties of the Essential Oil of

Abies Balsamea (L.) Mill.

Property	Extreme Limits			Average
d_{15}	0.8800	to	0.8890	0.8828
$[\alpha]_D$	-20°30'	to	26°30'	-23°30'
η_D^{20}	1.4727	to	1.4779	1.4754
Acid No.	0.45	to	0.85	0.60
Ester No.	18.2	to	46.6	29.9
Saponification No.	18.7	to	47.2	30.5
Boiling Range	155°	to	163°	161° to 195°
Solubility in 90% alcohol	Sol. in 4.2	to	7.2 volumes	5.4 volumes

Hunkel (65) found that an oil derived from the twigs and leaves of Abies balsamea contained 17.6% of bornyl acetate and probably l-α- pinene.

More recently an essential oil prepared from Canada balsam (66) was reported to contain l-α-pinene, l-β- pinene, and l-β- phellandrene. However, it is probable that this oil, since it had an ester number of only 2.24, was actually a turpentine oil of balsam fir and that the needle oil of A. balsamea has not previously been examined in detail.

Oil of Thuja Occidentalis L.

Thuja occidentalis L., the eastern arborvitae or eastern white cedar, ranges from the maritime provinces as far westward as Lake Winnipeg, and northward to the southern end of James Bay. Risi and Brule (61) prepared nineteen lots of thuja oil and reported the properties listed in Table XI.

Oil of thuja was examined extensively by Wallach (67, 68, 69, 70). He reported the presence of d-α- pinene, d-α- thujone, l- fenchone, l - borneol free and esterified, as well as carvotanacetone and l - camphor. Wallach later concluded that the latter two compounds were formed during the examination of the oil. Carvatanacetone is formed from thujone by the action of heat; camphor may be formed by oxidation of borneol.

Rutovski and Gusseva found esters of isovaleric acid as well as acetic acid (71).

Table XI

Properties of the Essential Oil of Thuja Occidentalis L.

Property	Extreme Limits	Average
d_{15}	0.9102 to 0.9232	0.9192
$[\alpha]_D$	-5°6' to 16°18'	-12°9'
n_D^{20}	1.4540 to 1.4687	1.4595
Acid No.	0.71 to 1.43	0.96
Ester No.	14.0 to 69.5	30.2
Saponification No.	15.4 to 70.3	31.2
Boiling Range	170° to 215°	177°- 207°
Solubility in 90% alcohol	Sol. in 0.2 to 0.6 volumes	0.4 volumes

DISCUSSION OF RESULTS

Preliminary Separations of Canadian Tall Oils

Sterol Contents of Canadian Tall Oils

These determinations were carried out in order to extend the analytical data assembled by Burch (16) to include sterol contents. The contents of sterols and unsaponifiable material of six Canadian tall oils are reported in Table XII.

Table XII

Sterol Contents of Canadian Tall Oils

Source	Woods Pulped		Unsaponifiable Material 1	Sterols 2
Pacific	Balsam	30%	33.6%, 34.0%	12.70% 12.51%
	Fir	30%		
	Cedar	25%	Av. 33.8%	Av. 12.61%
	Spruce	15%		
Bathurst	Spruce	50%	35.6% 35.7%	11.50% 11.39%
	Fir	40%	Av. 35.7%	Av. 11.45%
	Jack Pine	10%		
Canada	Balsam	45%	32.6% 32.2%	10.35% 10.17%
	Spruce	35%		
	Jack Pine	10%	Av. 32.4%	Av. 10.26%
	Hemlock	10%		

Table XII (Continued)

Sterol Contents of Canadian Tall Oils

Source	Woods Pulped		Unsaponifiable Materials (1)		Sterols (2)	
Brown	Spruce	55%	20.1%	20.6%	9.28%	9.15%
	Balsam	37%				
	Jack Pine	8%	Av. 20.4%		Av. 9.22%	
Consolidated	Spruce	68%	19.6%	19.3%	6.25%	6.32%
	Balsam	24%				
	Jack pine	9%	Av. 19.5%		Av. 6.29%	
Dryden	Jack pine	90%	12.9%	12.9%	3.90%	3.94%
	Spruce	10%	Av. 12.9%		Av. 3.92%	

1. % of original tall oil.
2. calculated as cholesterol and expressed as % of original tall oil.

The digitonin procedure used to determine the sterol content of the oils is known to estimate only sterols carrying a free β hydroxyl in the three position (72). Any sterols not possessing this configuration were therefore not estimated by this procedure. Shebeck (58) subsequently showed that a tall oil prepared from a Brown Corporation sulphate soap contained small amounts of α' - sitosterol. This sterol is not precipitated with digitonin. Some of the material reported as sterol may have been present in the original oil in the form of sterol esters which were saponified during the analytical procedure. Since the saponification numbers of all of the oils examined by Burch were greater than their acid numbers (16) it may be concluded that the oils did contain esters.

The sterol contents of the six tall oils ranged from 3.92% to 12.61%. This variation may be due in part to variations in cooking procedure and plant practice. However the phytosterols would be expected to be stable under cooking conditions and it is difficult to suggest any mechanism by which the oil might be enriched or depleted in sterols during preparation. More probably the varying sterol contents of the oils reflect the varying ratio of sterol contents to fatty and resin acid contents in the woods pulped. Thus Dryden tall oil, prepared from a cook containing a high percentage of Jack pine, contains the smallest percentage of sterols. Bathurst, Canada, and Brown tall oils were prepared from wood mixtures containing a higher percentage of balsam fir than was Consolidated tall oil. It is observed that the three former oils contain significantly more sterols than does the latter oil. From these limited data only tentative conclusions can be drawn, but it appears that balsam fir is richer in phytosterols than is spruce, and that jack pine contributes very little towards the sterol content of the oil.

Most striking, however, is the comparison of the sterols of Canadian tall oils with tall oils of Scandinavia or the United States (55, 57). The Canadian oils contain three to four times as much phytosterol as foreign oils. The high sterol contents of Canadian oils probably result from the high proportion of balsam fir and spruce which is pulped by Canadian mills.

Preparation of Tall Oil

Tall oil is commonly prepared by acidification of a solution of tall oil soap. However as Pollak et al (73) have pointed out, black liquor contains alkali lignin which is precipitated on acidification. It was observed that tall oil prepared by acidification of an aqueous solution of crude sulphate soap was a pasty heterogeneous mass, which could be separated, by vacuum filtration at temperatures above 70°, into a clear dark reddish-brown, oil and a buff coloured powder. The powder was insoluble in common organic solvents but was soluble in dilute sodium hydroxide solution. It may have consisted of acidic material derived from lignin, or from the resin acids by oxidation or polymerization: in any case this insoluble material was not regarded as a proper constituent of tall oil as previously defined, and its investigation was considered to be beyond the scope of this work.

In one experiment a concentrated solution of the soap was separated into a fraction soluble in water at room temperature and a fraction insoluble in water at room temperature and the two fractions acidified separately. The oil derived from the water insoluble soap became partially crystalline on standing. This crystalline material was probably lignoceric acid: subsequent work showed the oil to contain lignoceric acid, and showed lignoceric acid to crystallize readily from fatty acid mixtures and to yield a sparingly soluble sodium salt.

The bulk of the buff-colored powder was associated with the water-soluble fraction of the sulphate soap. Assuming this amorphous powder to be alkali lignin derived from occluded black liquor in the soap, a homogeneous tall oil could probably be prepared from soap which had been purified by repeated salting out from aqueous solution. It was found more convenient, however, to acidify the crude soap solution in the presence of a water-immiscible solvent, to separate and free the organic phase of insoluble solids by filtration, and to remove solvent and traces of water by distillation under reduced pressure. Tall oil used throughout this investigation was prepared in this manner.

Molecular Distillation of Tall Oil

In commercial operation tall oil is often refined by distillation in vacuo (74). Fatty acid, resin acid and unsaponifiable contents quoted for the distilled product do not differ greatly from those of the original oil. Improvement is mainly in color, viscosity and odour. It has been reported.

that the sterols are esterified during distillation and are rejected together with other less volatile constituents as pitch from the still bottoms (75).

The fatty acid methyl esters of tall oil have been studied by fractional distillation in vacuo. The fraction proved to be a complex mixture of compounds of similar boiling point from which individual constituents could not be separated. Burch concluded that only octadecanoic acids were present (16).

Thermal degradation of the distilland has prevented examination of the less volatile portion of tall oil by conventional distillation techniques. The technique of molecular distillation was employed in this study to permit distillation of the least volatile constituents of the oil with minimum thermal hazard.

In 1913 Langmuir (76) showed that in total vacuum a volatile substance evaporates from a surface at a rate predicted by the equation $M = PA \div \sqrt{2\pi MRT}$ where M is moles evaporated per second, P is the vapour pressure in dynes/cm.^{2}, A is area of evaporating surface in cm.^{2}, M is the molecular weight of the evaporating substance, R is the gas constant and T is the temperature in $^\circ$K.

When the distance between evaporating and condensing surfaces is of the same order as the mean free path of the evaporating molecules, evaporation is virtually unobstructed, the Langmuir equation applies, and the process is known as molecular distillation. Brønsted and Hevesy (77) took advantage of the fact that, under these conditions, the rate of distillation is proportional to $\sqrt{1/M}$, to separate the isotopes of mercury. Molecular distillation was applied to organic compounds by Burch (78) in 1929, and has since been used for the separation of vitamins A and D from fish oils (79) for recovery of tocopherols, natural anti-oxidants and sterols from vegetable oils. Hickman states that the technique is particularly well adapted to the separation of sterols from fatty oils (80). It can be seen from the Langmuir equation that under conditions of unobstructed evaporation, distillation takes place whenever a temperature difference exists between evaporating and condensing surfaces; the concept of boiling point then becomes meaningless. Hickman (81) and Embree (82) therefore adopted an arbitrary procedure for conducting molecular distillations which permits comparisons between different substances and different distillations. By this procedure each distillate fraction is collected during unit time, and the temperature of distillation is increased from fraction to fraction according to a predetermined plan. For illustration the quantity of distillate in each fraction may be calculated for the case where, each unit time and therefore each fraction, the temperature is increased in a regular fashion such that $P\sqrt{1/T}$ is doubled every third fraction. For the molecular evaporation of one volatile constituent from a non-volatile solvent,

$M = k\,n\,P\sqrt{1/T}$, where "k" is a constant and "n" is the mole fraction of volatile material in the distilland. If "n" is small, the percent volatile material in the distilland is proportional to "n". If the constants of proportionality are so chosen that the original intrinsic rate of distillation, $kP\sqrt{1/T}$, equals unity, and the original percent volatile material in the distilland equals 100, the values listed in Table XIII result.

Table XIII

Calculated Rate of Distillation

Fraction	Intrinsic Rate of Distillation	% Volatile material in Distilland	Rate of Elimination
1	1.00	100	1.00
2	1.26	99	1.25
3	1.59	97.75	1.55
4	2.00	96.20	1.92
5	2.52	94.28	2.37
6	3.175	91.91	2.91
7	4.00	89.00	3.56
8	5.23	85.44	4.31
9	6.36	81.13	5.17
10	8.00	75.96	6.07
11	10.06	70.00	7.04
12	12.67	62.85	7.95
13	16.00	53.90	8.63

(continued)

Table XIII (Continued)

Calculated Rate of Distillation

Fraction	Intrinsic Rate of Distillation	% Volatile material in Distilland	Rate of Elimination
14	20.15	45.27	9.13
15	25.40	36.14	9.17
16	32.00	26.97	8.64
17	40.70	18.33	7.49
18	50.40	10.84	5.46
19	64.00	4.38	2.80
20	80.70	1.58	1.27

These values may be plotted to give the curve shown in Figure 1.

The maximum of this curve, called the elimination maximum, was used by Hickman and Embree as a measure of the volatility of a compound under conditions of molecular distillation. They found that, for a given design of apparatus and plan of distillation this maximum was reproduceable, and a characteristic property of the compound being distilled.

Tall oil in solution in triölein was distilled in a cyclic, falling-film molecular still. The history of distilation and the weights and properties of the fractions obtained are reported in Table XIV. Fractions 1 to 4 were light yellow oils with a mild fatty odour. Fractions 6 - 9 were yellow to orange solids at room temperature.

TABLE XIV

Molecular Distillation of Tall Oil I

Fraction	Temperature °C	Pressure Hg	Weight g.	% Neutrals	% Resin Acids	% Fatty Acids	Molecular Weight of Fatty Acids	
Original Oil				30.7	24.8	41.4	326,330	Average 328
1	100	8	11.30	34.9	11.4	54.1	310,304	307
2	110	4	15.61	26.5	15.5	55.6	302,301	302
3	120	4-8	17.50	17.5	21.9	61.1	308,310	309
4	130	4-5	22.59	16.8	24.5	59.4	318,311	315
5	140	5-7	20.45	18.5	31.4	50.5	325,333	329
6	150	7-8	15.90	35.8	29.5	32.3	349,347	348
7	160	8-10	9.21	53.5	20.4	25.1	-	
8	170	6	6.04	72.5	10.8	14.2	-	
9	180	6	4.54	81.7	6.6	11.9	-	

FIGURE 1

THEORETICAL ELIMINATION CURVE

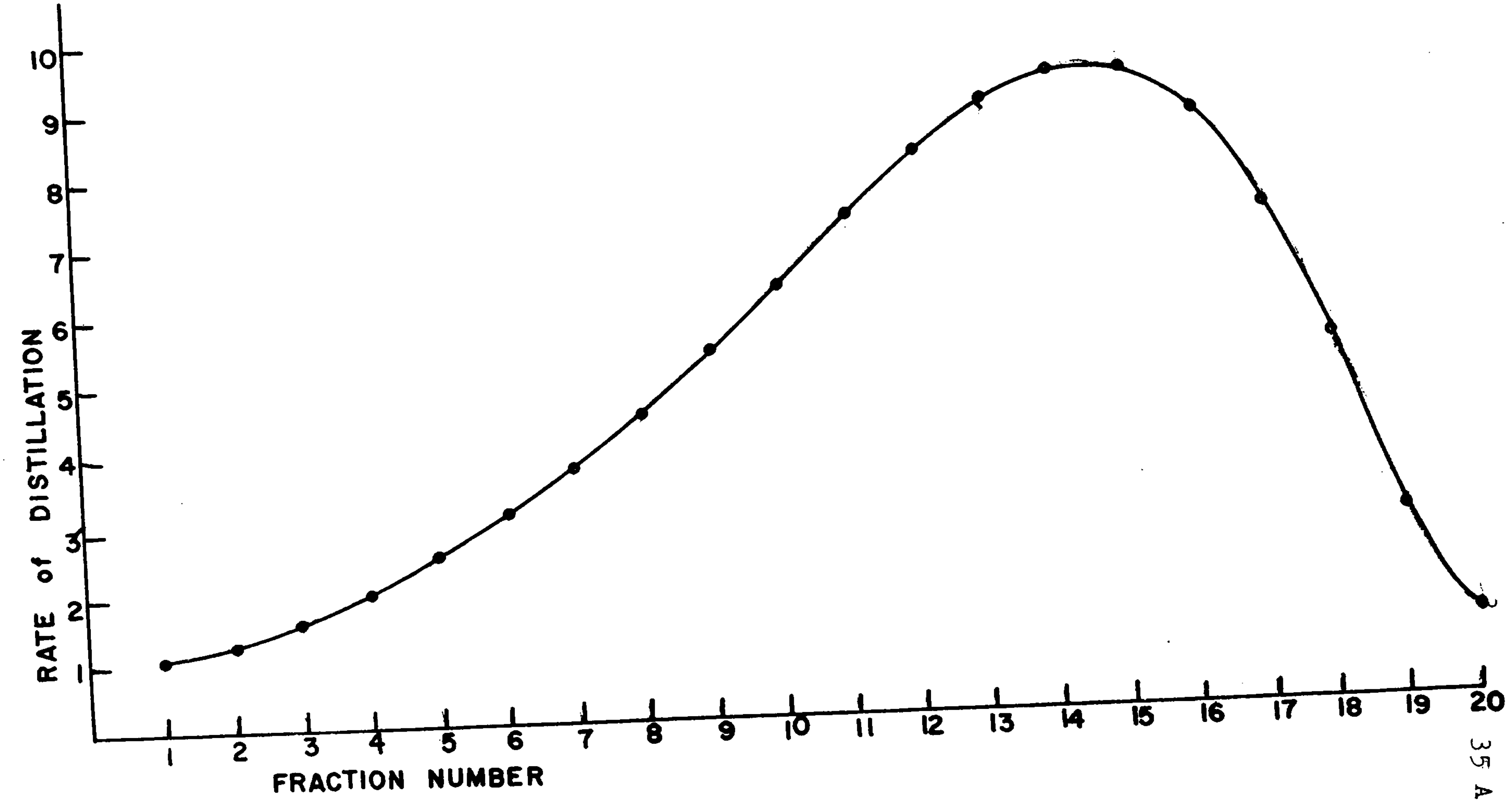


Figure 2 shows the elimination curves for the fatty acids resin acids and neutral material of tall oil obtained by plotting the weight of each component in each fraction against the temperature at which the fraction was distilled.

A second distillation was conducted in such a manner that more fractions were collected. The conditions of distillation and yields of distillates are reported in Table XV. The analytical properties of these fractions are contained in Table XVI. These results were plotted to give the elimination curves of figure 3. The fatty acid methyl esters isolated from fractions 8, 9, 10, and 11 were solid at room temperature η-Tetracosanic acid melting at 80 - 81° was isolated from fraction 9. The methyl ester melted at 56.5 - 57°. Holde and Godbole (83) reported m.p. 80.5 - 81.0° for the free acid and 56.5 - 57° for the methyl ester. Francis and co-workers (84) reported a melting point of 83.5° for synthetic η-tetracosanic acid.

Shape of the Curves - The elimination curve of a pure compound obtained under appropriate conditions is a reasonably symmetrical bell-shaped curve. When a mixture is distilled under similar conditions the distillation of each component is described by an elimination curve whose shape and maximum is independent of the presence of other components. If several chemical species are determined as a single component by the method of analysis this fact may be revealed by irregularities in the elimination curve. Thus,

Table XV

Molecular Distillation of Tall Oil II

Fraction No.	Temperature in °C.	Pressure in of Hg.	Weight of Distillate
1	100 ± 2	17	1.09
2	110 ± 2	5	3.45
3	120 ± 2	4	5.50
4	130 ± 2	4	11.41
5	140 ± 3	5	22.61
6	150 ± 2	3	24.31
7	160 ± 2	3	23.80
8	170 ± 1	3	18.94
9	180 ± 3	3	11.41
10	190 ± 3	5	5.71
11	200 ± 3	3	6.59

TABLE XVI

Properties of Fractions from Molecular Distillation II

Fraction No.	% Fatty Acids	Av.	% Resin Acids	Av.	% Neutrals	Av.	% (a) Sterols	Esterified Acids	Iodine Number of Fatty Acid Methyl Esters	Av.	Molecular Weight of Fatty Acid Methyl Esters	Av.
1	33.9				65.1	65.1						
2	52.8				47.8	47.8						
3	50.1	50.1	10.3	10.3	38.5	38.5			114 113	114	301	301
4	60.0 60.2	60.1	14.0 14.2	14.1	25.6 26.0	25.8			116 113	115	315 307	311
5	62.6 62.3	62.5	19.7 20.0	19.8	17.2 16.9	17.1		25.1	127 129	128	334 328	331
6	55.5 54.8	55.2	25.7 25.3	25.5	18.1 18.6	18.4	16.6	21.7	123 128	126	330 324	327
7	49.7 49.9	49.8	27.9 27.2	27.6	21.7 21.7	21.7	24.3	20.3	126 120	123	354 346	350
8	33.4 34.8	34.1	25.8 25.6	25.7	40.0 39.6	39.8	37.1	22.9	112 111	112	360 353	357
9	22.6 22.8	22.7	18.0 18.3	18.2	58.7 58.8	58.8	42.2	26.5	100 99	100	347 362	355
10	15.5	15.5	8.9	8.9	76.3	76.3	28.5	44.1				
11	10.8	10.8	5.3	5.3	83.6	83.6	16.2	66.1				
Original	41.1 41.8	41.4	24.7 24.9	24.8	30.6 30.7	30.7	35.5		118 121	120	326 330	328

(a) Expressed as % of neutral fraction

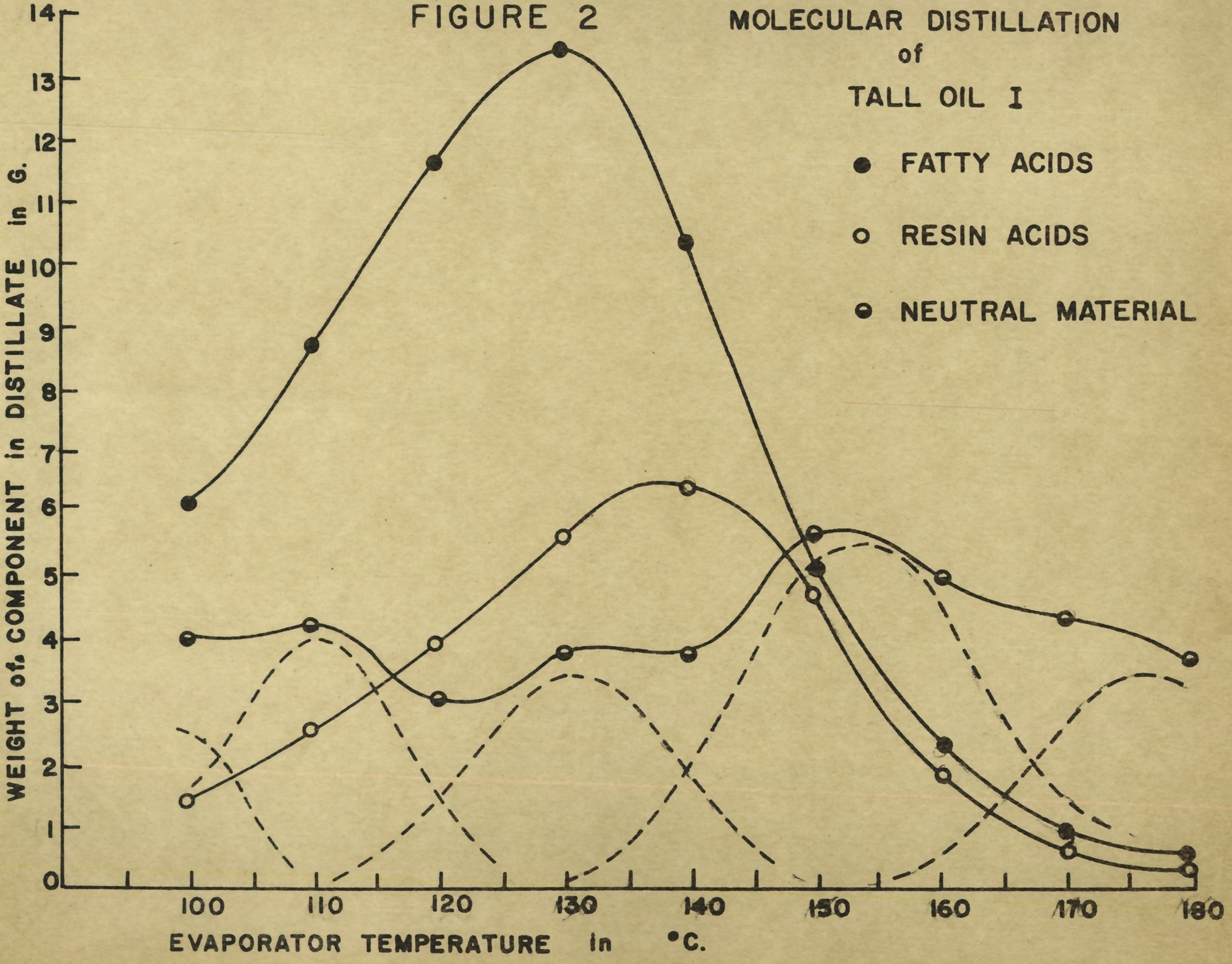


FIGURE 2

MOLECULAR DISTILLATION
of
TALL OIL I

the shape of the elmination curve may be used as a criterion of the chemical homogeniety of a component as estimated by the particular analytical method.

The elimination curve of the resin acids is symmetrical and characteristic of a pure compound. It, therefore, follows that the elimination maxima of the various acids making up the resin acid fraction are very nearly identical, or, less probably, that the resin acid fraction consists of a single resin acid. As a corollary, no fractionation of the resin acids can be achieved by molecular distillation.

Similarly the elimination curves of the sterols were regular and bell-shaped. No fractionation of the sterols can be achieved by molecular distillation.

Under appropriate conditions the elimination curve of the fatty acids showed marked irregularities. The observed curve can be considered as the sum of two bell-shaped curves with maxima at 145° and 165°. These hypothetical curves are shown as dotted lines in Figure 3. Hickman found (85) in a series of aminoanthraquinone dyes, that the elimination maximum increased by 4.5° for each methylene group added to the side chain. If the fatty acids with elimination maximum at 145° are assumed to be C 18 fatty acids, and if the fatty acids with elimination maximum at 165° are assumed to be members of the same homologous series, it may be concluded that the less volatile acids contain about 23 carbon atoms.

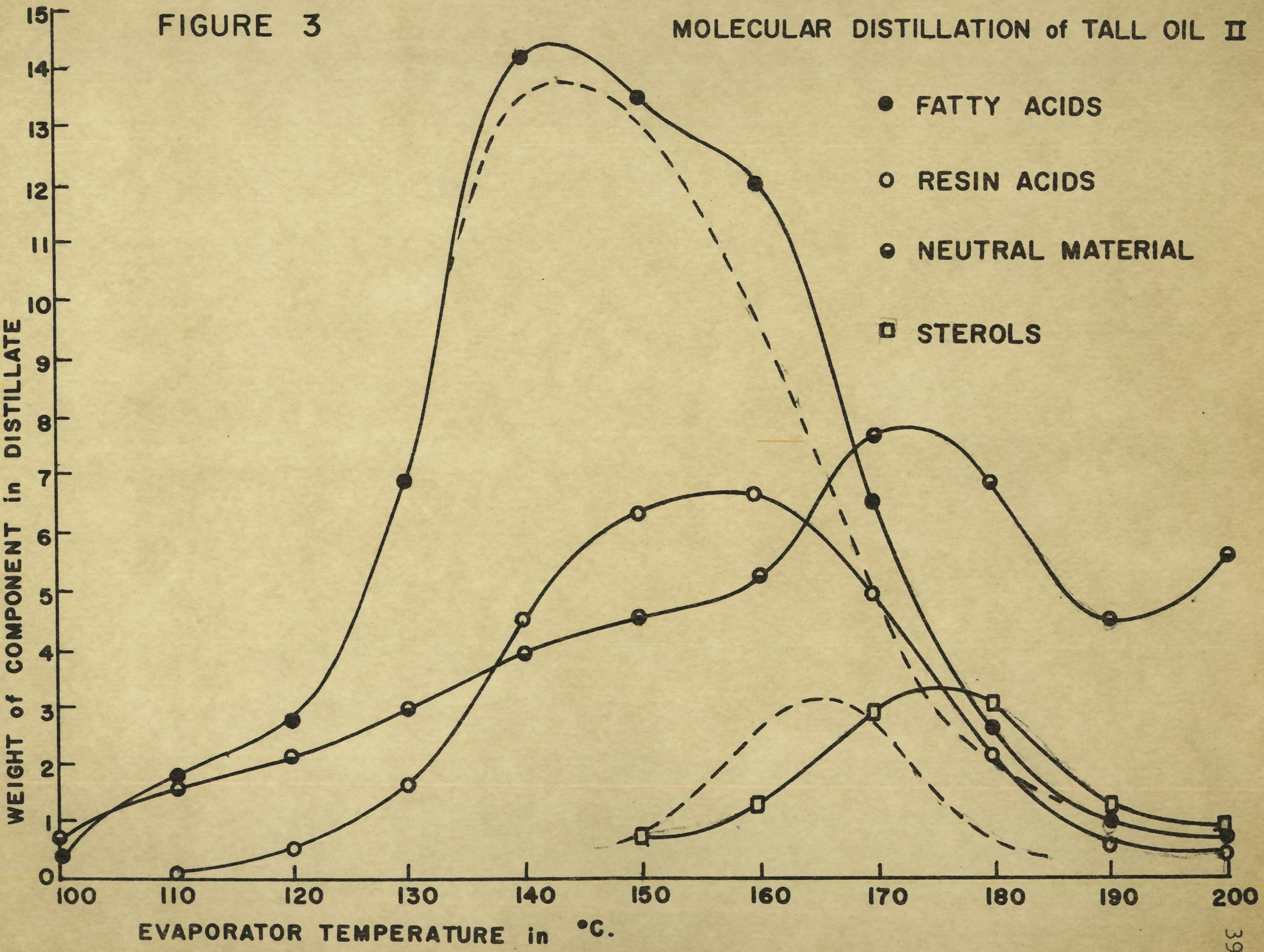


FIGURE 3

MOLECULAR DISTILLATION of TALL OIL II

The elimination curve of the neutral fraction is even more complex. It was found that the curve could be expressed as the sum of five bell-shaped curves. These curves with maxima at 100°, 111°, 131°, 153° and 175° are shown in dotted lines in Figure 2. The construction of these curves is, in a sense, arbitrary. The observed curve might equally well be expressed as the sum of more than five other curves. However, it may be concluded that the neutral fraction contains not less than five major components of widely differing elimination maxima, and that a useful separation is effected by molecular distillation.

Under conditions of distillation I, elimination maxima of about 130° for the fatty acids and 139° for the resin acids were observed. Under conditions of distillation II the following maxima were observed: fatty acids, 145°, and 165°; resin acids, 158°; sterols 175°. A difference of about 20° in elimination maxima is necessary in order to obtain a useful separation of two components in a single pass.

Chemical Properties of the Fractions - The saponification equivalent of the fatty acid methyl esters increased as the temperature of distillation increased. This observation is in agreement with the conclusion that a less volatile acid containing about 23 carbon atoms is present.

The iodine numbers of the fatty acid methyl esters increased, passed through a maximum, then decreased. It is most unlikely that two acids differing only in degree of unsaturation could be separated by molecular distillation. More saturated acids of lower and higher molecular weight than the octadecanoec acids of tall oil must be present to account for this behaviour. The only supporting evidence for the presence of a saturated acid of lower molecular weight was the occurance of a small inflection at about 120° in the fatty acid elimination curve of distillation II. Ample evidence for the presence of a higher molecular weight acid was available, and an acid was isolated from the higher boiling fractions and identified as η-tetracosanic acid by melting point, and by melting point and saponification equivalent of its methyl ester.

Analytical Methods

Burch examined tall oils by the rapid methods of Hastings and Pollak (86). In order that results might be directly comparable attempts were made to use these methods in the present investigation. The methods are in widespread use and are apparently satisfactory for commercial specification of tall oil preparations. For scientific investigations they were seriously lacking in precision, and a gravimetric method of analysis of fatty acids, resin acids, and neutral materials was finally developed. Standard deviations were calculated from the rapid resin acid analyses of seven tall oil samples reported by Burch (16) and from the gravimetric resin acid analyses of fractions from distillation II. Calculations are contained in Table XVII. A standard deviation of 0.26 was found for the gravimetric method as compared to 2.54 for the method of Hastings and Pollak. The standard deviation of the difference between two single analyses is $\sqrt{2}\,\sigma$. If confidence limits are set at twice the standard deviation it follows that differences of less than 7.2% between two resin acid analyses is not significant if the method of Hastings and Pollak is used, but a difference of greater than 0.74% is significant if the gravimetric method is used. The reliability of the average of 100 replicate determinations by the method of Hastings and Pollak is about equal to the reliability of a single determination by the gravimetric method. The gravimetric method has the additional advantage that the various fractions are actually isolated in a form which permits further examination. The sole disadvantage of the method is that it is laborious and time consuming. All the fatty acid, resin acid and neutral meterial contents reported in this thesis were obtained by the gravimetric method.

Table XVII

Precision of two Methods of Resin Acid Analysis

	Method of Hastings and Pollak				Gravimetric Method			
	x	$/x-\bar{x}/$	$/x-\bar{x}/^2$	d.f.	x	$/x-\bar{x}/$	$/x-\bar{x}/^2$	d.f.
Sample 1	32.2	3.7	13.69	3	14.0	0.1	0.01	1
	39.5	3.6	12.96		14.2	0.1	0.01	
	35.9	0.0	0.00					

(Continued)

Table XVII (Continued)

Precision of Two Methods of Resin Acid Analysis

	Method of Hastings and Pollak				Gravimetric Method			
	x	$/x-\bar{x}/$	$/x-\bar{x}/^2$	d.f.	x	$/x-\bar{x}/$	$/x-\bar{x}/^2$	d.f.
Sample 1-	36.1	0.2	0.04		19.7	0.1	0.01	1
Sample 2-	29.9	0.2	0.04	1	20.0	0.2	0.04	
	30.2	0.1	0.01					
Sample 3-	30.9	1.5	2.25	4	25.7	0.2	0.04	1
	33.0	3.6	12.96		25.3	0.2	0.04	
	25.2	4.2	17.64					
	29.1	0.3	0.09		27.9	0.3	0.09	1
	29.2	0.2	0.04		27.2	0.4	0.16	
Sample 4-	55.5	0.0	0.00	2				
	55.3	0.2	0.04		25.8	0.1	0.01	1
	55.8	0.3	0.09		25.6	0.1	0.01	
Sample 5-	22.9	0.0	0.00	2				
	26.5	3.6	12.96		18.0	0.2	0.04	1
	19.2	3.7	13.69		18.3	0.1	0.01	
Sample 6-	9.0	2.8	7.84	2				
	14.0	2.2	4.84		24.7	0.1	0.01	1
	12.5	0.7	0.49		24.9	0.1	0.01	
Sample 7-	11.3	0.8	0.64	2				
	13.5	1.4	1.96					
	11.6	0.5	0.25					
Total -			102.52	16			0.49	7
σ^2		6.43					0.07	
σ		2.54					0.26	

Esterification of Tall Oil Alcohols with Tall Oil Acids

The saponification numbers of all of the tall oils prepared and studied by Burch (16) were much higher than their acid numbers. It is possible that the oils contained esters which had survived the alkaline pulping process, but it is more likely that the esters were formed during preparation and drying of the oil. The presence of esterified acids in the neutral fraction of fractions distilling above 140° from molecular distillation II suggests that esterification had taken place either during preparation or distillation of the oil.

In these experiments, tall oil was prepared from a solution of sulphate soap which had previously been freed of neutral material by ether extraction. The neutral and acid fractions were then combined and heated under anhydrous conditions. Samples periodically withdrawn and analysed gave the results listed in Table XVIII.

In the presence of a large excess of fatty and resin acids the esterification of tall oil alcohols and sterols should be pseudo-unimolecular.

$\mathrm{Ln}\ \frac{Co}{C} = kt$ where "c" = concentration of sterols at time "t".

From the data of Table XVIII, $\mathrm{Ln}\ \frac{Co}{C}$ was calculated and plotted against time. The calculations are recorded in Table XIX and in the graph, Figure 4.

Table XVIII

Esterification of Tall Oil Alcohols

Temperature	Time	%Neutrals	%Acids	%Sterols in Neutrals	%Sterols in Oil
$96 \pm 0.5^\circ$	0	27.2	72.8	29.3, 29.0	7.94
	2.5	28.0	72.0	27.3, 27.5	7.67
	6	29.0	71.0	25.5, 25.1	7.34
	12	29.0	71.0	24.3, 24.4	7.08
$133 \pm 1.0^\circ$	0	25.8	74.2	26.4, 26.3	6.81
	2	29.9	70.1	21.8, 21.5	6.49
	4.5	32.1	67.2	18.8, 19.0	6.07
	10	35.1	64.9	14.1, 14.2	4.98

Table XIX

Esterification of Tall Oil Alcohols

Temperature	Ln $\frac{Co}{C}$	"t" in hr.	k (estimated from figure 4.
96°	0.000	0	0.01 hr.$^{-1}$
	0.0346	2.5	
	0.0786	6	
	0.1146	12	
133°	0.000	0	0.03 hr^{-1}
	0.0481	2	
	0.1150	4.5	
	0.3130	10	

These constants are not true rate constants for the reaction because they contain a factor equal to the esterifiable acid content of the system. Since this factor is not known a true rate constant cannot be calculated.

Significant esterification of the sterols does not take place during preparation of the oil. Assuming the molecular weight of the acids esterified with the sterols to be 328, then the weight of the acid associated with one gram of esterified sterols is $\frac{328 - 18}{386.6}$. In Table XX, are calculated the weights of acids esterified with the sterols. The difference between the increase in weight of the neutral fraction and the weight of acids esterified with sterols is assumed to be the weight of acid esterified with non-sterol alcohols.

Table XX

Esterification of Tall Oil Acids.

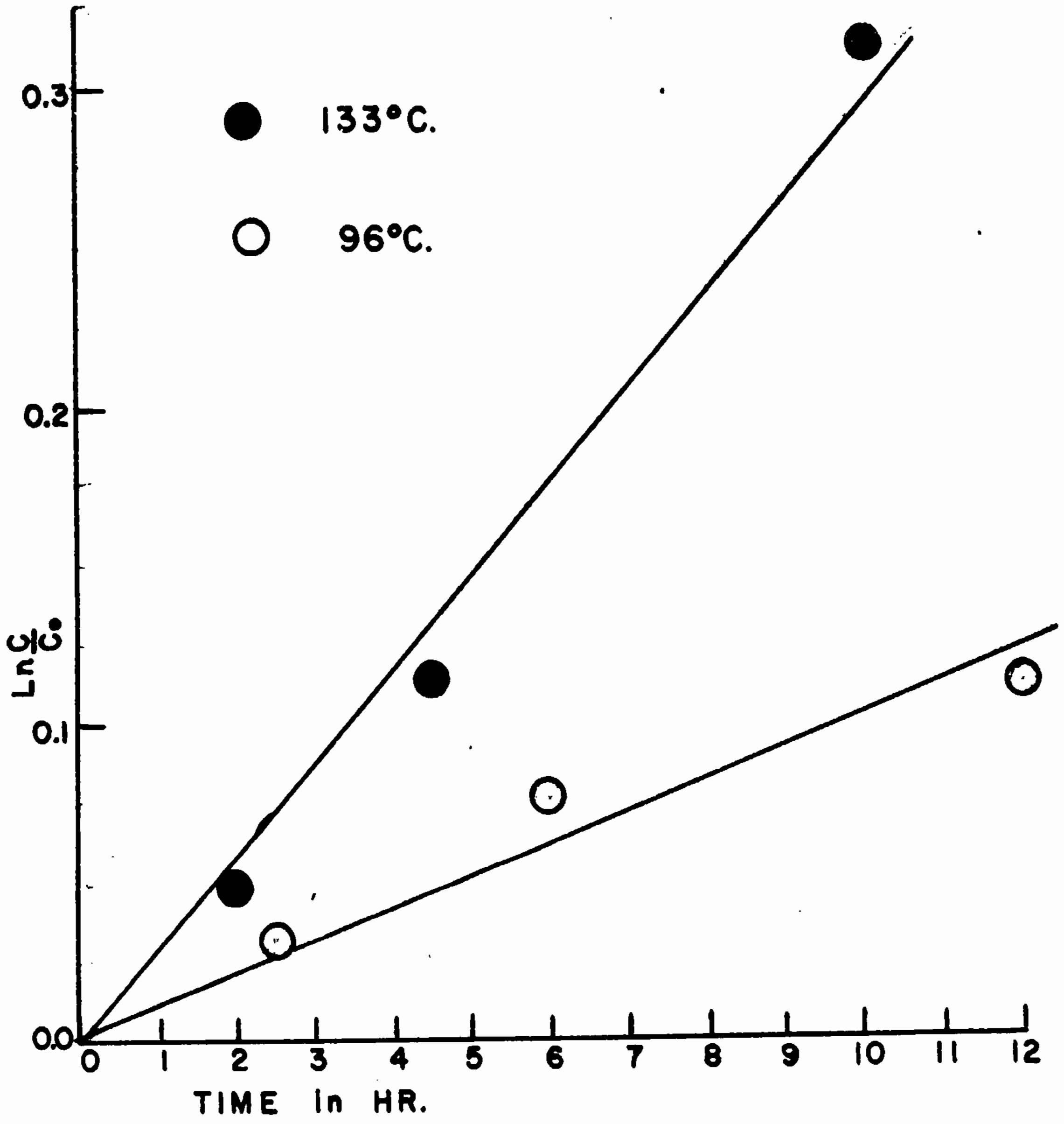
Temperature	Time	Acids Esterified* with Sterols	Increase in Wt* of Neutral Fr^n	Acids in non-* Sterol esters
	0	-	-	-
96°	2.5	0.22	0.8	0.6
	6	0.48	1.8	1.3
	12	0.69	1.8	1.1
	0	-	-	-
133°	2	0.26	4.1	3.8
	4.5	0.60	6.3	5.7
	10	1.47	9.3	7.8

*Percent of oil

FIGURE 4

RATE of ESTERIFICATION of TALL OIL STEROLS

$\ln \frac{C}{C_0}$ vs. T.



These results show that the large increase in weight of the neutral fraction cannot be explained by the esterification of the sterols. A more easily esterified alcohol must be present. Because of its ease of esterification this alcohol is probably primary.

Extraction of Neutral Material

In Table XXI a number of common solvents are compared for their ability to extract neutral material from tall oil soap solution under standard conditions.

Table XXI

Extraction of Tall Oil Soap Solutions

Solvent	Weight of Extract	Weight after Crystallization
Decalin	0.53	0.05
Tetralin	0.42	0.04
Xylene	0.30	0.02
Cyclohexane	0.19	0.00
1,1,2-Trichoroethane	0.40	0.01
1,2-Dichoroethane	0.21	0.06
Cyclohexanol	1.26	0.44
Pentasol	1.02	0.51
Ethyl ether	0.97	0.47
Furfural	0.78	0.31
Cyclohexanone	0.68	0.30

The behavior of the solvents containing oxygen contrasts sharply with the behavior of the hydrocarbons and chlorinated hydrocarbons. With this former class of solvents the possibility exists of hydrogen bonding between the solvent oxygen and the alcoholic hydrogen of the sterols or tall oil alcohols. The fact that the hydrocarbon solvents do not effectively remove these alcoholic constituents, suggests that the alcohols in soap solution are associated with the carboxyl groups of the acids, probably through hydrogen bonds. The superior extractant properties of the oxygen compounds probably lies in their ability to compete with the carboxyl groups of tall oil soaps as electron donors for the formation of hydrogen bonds.

Crystallization of Tall Oil at Low Temperatures

The behaviour of the fatty acids and other constituents was observed when solutions of tall oil in various solvents were cooled to temperatures in the range 0° to -70° C. The results of these experiments are summarized in Tables XXII, XXIII, XXIV, XXV and XXVI.

Crystallization from petroleum ether:- About 70% of the oil remained soluble in petroleum ether even at -70°. The fatty acid, resin acid, and neutral material contents of the fractions soluble in petroleum ether at -70° differed from those of the original oil by less than 2%. Dilution had little effect on the weight of material crystallizing or on the properties of the residue. At higher dilutions proportionately more resin acids were

TABLE XXII

Crystallization from Ten Parts of Petroleum Ether I

Fraction	Temp.	Ppt. % of Oil	% Neutrals	% Sterols in Neutrals	% Fatty Acids	I_2 No. of Fats	Mol Wt. of Fats	% Resin Acids	% Sterols in Precipitate
Original Oil			26.0	35.7	47.5	120	325	26.5	9.3
1	0°	7.26	15.3	19.2	33.6	97	-	51.1	2.9
2	-20°	8.40	25.1	18.6	44.2	84	330	30.7	4.7
3	-40°	3.22	20.7	-	60.0	64	-	19.3	-
4	-60°	3.07	21.7	44.2	67.3	-	288	10.9	9.58
5	-70°	1.56	25.7	-	62.9	-	288	11.4	-
6	Residue	71.44	26.9	30.3	47.5	130	290	28.2	8.1

TABLE XXIII

Crystallization from Five Parts of Petroleum Ether II

Fraction	Temp.	Wt. % of Oil	% Neutral Material	% Resins	% Fatty Acids	I_2 No. of Fatty Acids	Mo. Wt. of Fatty Acids (a)
1	0°	7.00	23.1	28.0	47.1	90	303
2	-30°	9.38	28.3	18.5	51.4	85	363
3	-70°	8.80	26.2	12.5	59.0	71	318
4	Residue	68.46	24.0	27.5	47.5	137.4 140.3	335

(a) Molecular weights were determined with the aid of a new indicator(87) especially recommended for the estimation of saponification equivalents of dark coloured oils. Contrary to claims in the literature, it was found that the colour change was incomplete at the stoichemetric point. In fact, the solutions retained a greenish tinge even after excess acid equivalent to 10 per cent of the soaps had been added. In consequence, little reliance can be placed upon the above values for the molecular weights of the fatty acids.

TABLE XXIV

Crystallization from Two Parts of Petroleum Ether III

Fraction	Temp.	Wt. % of Oil	% Neutrals	% Sterols in Neutrals	% Fatty Acids	I_2 No. of Fatty Acids	% Resin Acids	% Sterols in Fraction
1	0°	Small amount of black material - discarded.						
2	-30°	16.78	26.5	32.6	51.6	68	19.9	8.6
3	-70°	6.82	22.6	42.4	62.2	83	15.2	9.6
4	Residue	73.34	24.2	35.0	45.9	142	28.4	8.5

TABLE XXV

Crystallization from Acetone

Fraction	Description	Wt. % of Oil	% Neutrals	% Fatty Acids	I_2 No. of Fatty Acids	% Resin Acids	% Sterols in Neutrals	% Sterols in Fraction
1	Insoluble at 0°	10.82	44.0	(a)	-	(a)	55.5	24.4
2	Insoluble at -30°	6.80	51.5	37.7	57	10.6	58.1	29.9
3	Soluble at -30°	16.02	28.6	50.6	110	21.6	19.2	5.5
4	Soluble at -70°	55.0	19.7	50.4	139	33.1	11.1	2.18

(a) The sodium soaps of the acids were insoluble in water. Because of mechanical losses, it was not possible to determine the fatty and resin acid contents.

TABLE XXVI

Crystallization from Ethanol

Fraction	Description	Wt. % of Oil	% Neutrals	% Fatty Acids	I_2 No. of Fatty Acids	% Resin Acids	% Sterols in Neutrals	% Sterols in Fraction
1	Insoluble at 0°	8.40	53.3	(b)	-	(b)	64.8	34.5
2	Insoluble at -30°	7.06 (a)	42.5	(b)	-	(b)	54.0	23.0
3	Soluble at -30°	13.64	20.4	59.2	108	21.8	28.3	5.8
4	Soluble at -70°	53.62	20.0	47.2	142	30.4	9.2	1.8

(a) A part of the fraction was lost, by spattering, in the vacuum drying oven.

(b) The sodium soaps of these fractions were insoluble in water. The acids were not determined.

precipitated than at lower dilutions. This selective precipitation of the resin acids appeared to be a function of dilution rather than temperature, The precipiated resin acids were black tarry solids different in appearance from the amber-colored solids isolated from the soluble fractions. Their limited solubility in petroleum ether suggests that they were more polar compounds formed by oxidation of the resin acids of wood. They failed to esterify with methanolic hydrochloric acid under conditions such that esterification of the fatty acids was complete. It is on the basis of this property that these substances are classified as resin acids by the analytical method.

The fatty acids precipitated from solution were of higher average molecular weight and lower average insaturation than the fatty acids remaining in solution. Neutral materials and sterols crystallized in about the same proportions as they occurred in the original oil.

Crystallization from Polar Solvents

Crystallization from acetone and from ethanol gave very different results than did crystallization from petroleum ether. At -70° less material remained in solution in these solvents than remained in solution in petroleum ether: the percentages soluble at -70° were 55.0% and 53.6% from acetone and from ethanol respectively.

The differences in the behavior of the sterols is most striking. Less than 40% of the sterols were removed from petroleum ether solution at -70°C, but almost 90% of the sterols crystallized from ethanol at this temperature. Less resin acids and more fatty acids crystallized from the polar solvents. The insoluble fatty acids were of higher average molecular weight and saturation than were the fatty acids soluble in polar solvent at -70°C.

Composition of the Insoluble Material

The material crystallizing from the polar solvents at -30°C. contained about 50% η-tetracosanic acid, 25% sterols, and a fraction containing / -tetracosanol. η-Tetracosanic acid was isolated as the sparingly soluble sodium salt, freed of neutral material by ether extraction and purified by crystallization from acetone. The free acid melted at 81 - 82°, and the methyl ester melted at 55 - 56°. The melting point of a mixture of this ester with the ester obtained by molecular distillation was 55 - 56.5°. Brigt and Fuch (88) reported melting points of 85°C. for synthetic η-tetracosanic acid and 60° for the ester. Sandquist (23) and co-workers found melting points of 81°C. for the acid and 56° for the ester. The molecular weight of the ester, estimated by saponification, was 384; that calculated for $C_{25}H_{50}O_2$ is 382.64

The sterol content of the ether extract was estimated with digitonin. The filtrate from this determination yielded a small amount of 1-tetracosanol, m.p. 76 - 76.5° from which an acetate m.p. 56° was prepared. Brigl and Fuch (88) reported m.p. 77.5° for synthetic 1-tetracosanol; Sandquist et al (23) reported 76° for the acid, and 56° for the acetate.

Comparison of Results

Foreman and Brown (89) are responsible for a thorough study of the solubilities of pure fatty acids in organic solvents at low temperatures. Their results which are reproduced in Figure 5, show the concentration of linoleic acid remaining in solution at -70° in Skellysolve B, acetone or methanol to be less than 0.5%. The more saturated and higher homologues are even less soluble, and eicosanic acid is practically insoluble at 0°. Foreman and Brown pointed out, however, the solubilities in mixtures will depend to an important extent on the composition of the mixtures, namely, on the presence of other acids in solution.

Burch (16) and others (34) have reported that over 95% of the fatty acids of tall oil are C18 fatty acids containing two double bands or less. However, a 20% solution of tall oil in petroleum ether, acetone or ethanol cooled to -70°, retained fatty acids in concentrations.

6.5%, 5.5% and 5.0% respectively.

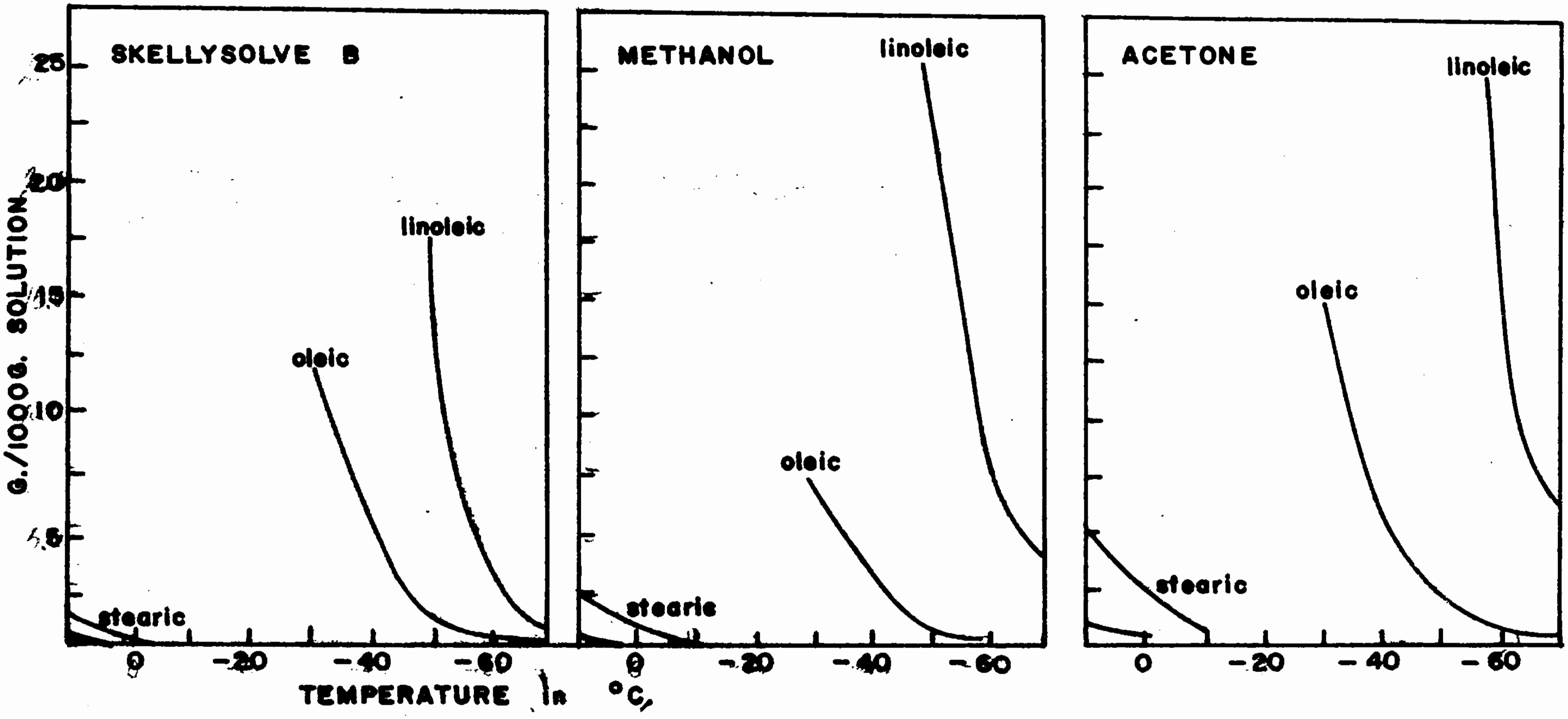
Foreman and Brown found the pure acids to be less soluble in the hydrocarbon solvent than in the polar solvents. The opposite was found to be the case with tall oil. It is known that the solubility of a fatty acid is increased by the presence of another fatty acid in solution, (90). Apparently the solubility of the fatty acids are increased even more by the presence of resin acids in solution. It would be expected that this phenomenon of mutual solubility would be less apparent in solvents of higher dielectric constant. This was found to be the case.

Sterol-Oxalic Acid Addition Compounds

In 1903 Mauthner and Suida (91) described a sparingly soluble addition compound containing two moles of cholesterol and one mole of oxalic acid. Meischer and Kagi (92) showed that many but not all steroids possessing a free trans hydroxyl in the 3 position would yield insoluble addition compounds with oxalic acid. This property has been used to separate cholesterol from wool fat unsaponifiable material on a laboratory (93) and on a commercial scale, (94). The present investigation showed that under appropriate conditions, the tall oil sterols formed sparingly soluble addition compounds with anhydrous oxalic acid. The yield of product was most dependent on

FIGURE 5

SOLUBILITIES of FATTY ACIDS



the choice of solvent in which the reactants were dissolved. Previous workers obtained crystalline addition compound of cholesterol from ethyl ether, ethyl acetate, benzene, and 1,2-dichloroethane solutions. Possibly because of the solvent action of the fatty and resin acids, addition compounds failed to crystallize when tall oil was treated with anhydrous oxalic acid in ethyl ether or ethyl acetate solution. Benzene solution deposited a small amount of granular crystals, but from 1, 2-dichloroethane solution a large mass of well-formed needle-like crystals separated. As is shown in Figure 6, the yield of crystalline material depends on the concentration of tall oil in solution. As the concentration of tall oil is increased, the solubility of the addition compound in the supernatant liquid increases. As the concentration of ethylene dichloride is increased, the solubility of the oxalic acid reactant in the reaction mixture decreases. Optimum yields were obtained from a solution of 1.0 g. of tall oil in 2.0 ml. of 1,2-dichloroethane.

The variation in yield of product with ratio of tall oil to oxalic acid, and tall oil to 1,2-dichloroethane is shown in Table XXVII and in Figure 6 and Figure 7.

The product remaining after decomposition of the addition compound and removal of oxalic acid was a mixture of several compounds. By chromatography on alumina a small amount of n-tetracosanol, and an unidentified material melting up to 163°C. were separated from a larger sterol fraction. The substance melting at 163°C. may have been α'-sitosterol, subsequently identified in tall oil by Sherbeck (58). However, the presence of n-tetracosanol in the product shows that the reaction is not limited to sterols. Probably the oxalic acid crystallizes with an alcohol of crystallization, and any alcohol present which forms a sparingly soluble addition compound will appear in the product.

Table XXVII

Yield of Addition Compound

G.Oxalic Acid / 100G. Tall Oil	Ml.1,2-Dichloroethane / 100G. Tall Oil	G. Addition Compound / 100G. Tall Oil	G. Sterol (a) / 100G. Tall Oil
5.0	50	2.21, 2.34	1.98, 2.10
5.0	100	3.78, 3.86	3.39, 3.46
5.0	200	3.60, 4.58	3.23, 4.10
10.0	50	5.57, 5.40	4.99, 4.85
10.0	100	5.39, 6.68	4.84, 5.98
10.0	200	6.59, 6.76	5.91, 6.06
20.0	50	5.69, 5.83	5.10, 5.22
20.0	100	7.02, 7.00	6.30, 6.28
20.0	200	9.05, 8.33	8.12, 7.43

FIGURE 6

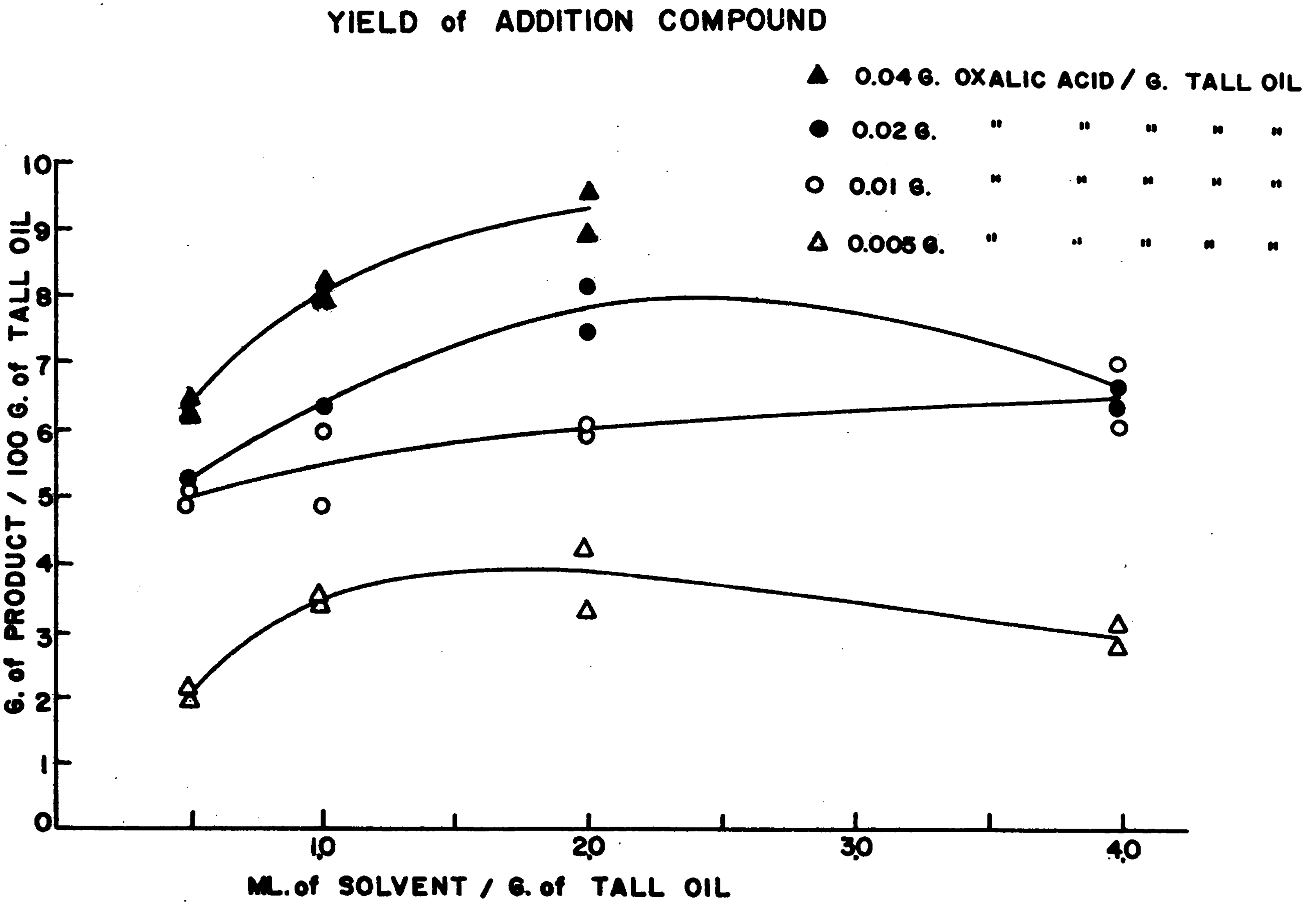

YIELD of ADDITION COMPOUND
▲ 0.04 G. OXALIC ACID / G. TALL OIL
● 0.02 G. " " " " "
○ 0.01 G. " " " " "
△ 0.005 G. " " " " "
G. of PRODUCT / 100 G. of TALL OIL
0
1
2
3
4
5
6
7
8
9
10
1.0
2.0
3.0
4.0
ML. of SOLVENT / G. of TALL OIL

Table XXVII (Continued)

Yield of Addition Compound

G.Oxalic Acid / 100G. Tall Oil	Ml.1,2-Dichloroethane / 100 G. Tall Oil	G.Addition Compound / 100 G. Tall Oil	G.Sterol (a) / 100G. Tall Oil
4.0	50	7.14, 6.90	6.40, 6.19
4.0	100	9.14, 8.84	8.20, 7.93
4.0	200	1.00, 1.06	8.97, 9.51

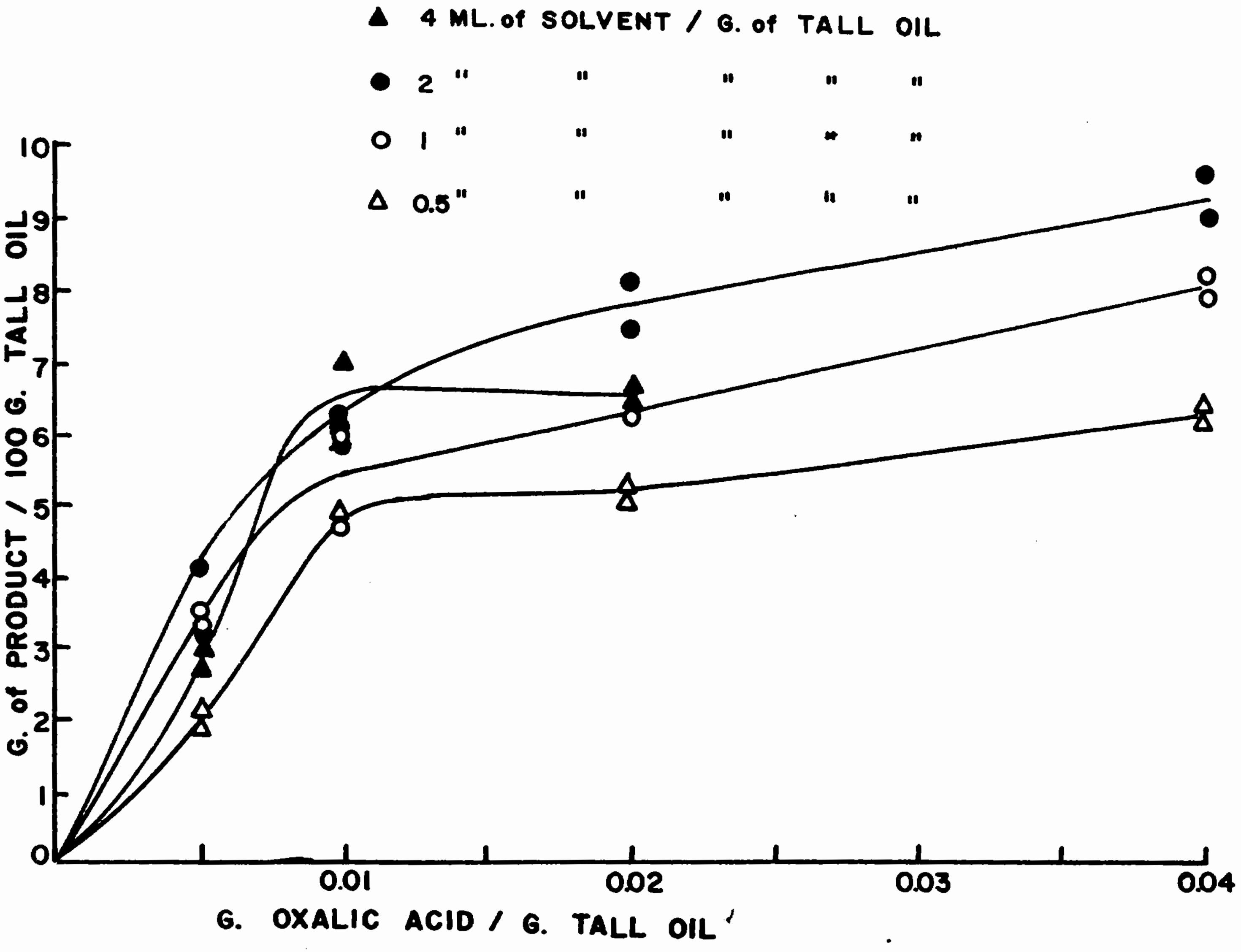
(a) Calculated from the weight of addition compound.

Chromatographic Separation of Tall Oil Alcohols

In a preliminary experiment a petroleum ether solution of tall oil neutral materials was allowed to percolate through a bed of activated alumina and the chromatogram examined under ultraviolet light. At least eighteen, and possibly twenty four fluorescent bands could be counted. The bands were so numerous and so close together that it proved impossible to separate them by sectioning the column in the conventional manner. This experiment showed that while a spacial separation of the neutral constituents could be achieved chromatographically on alumina, such a separation had no practical value unless a mechanical separation of the absorbant corresponding to the zones of absorbed material was possible. Two means were employed to assist in the sectioning of the columns. To reduce the complexity of the chromatograms, a preliminary separation of the neutral material was effected by molecular distilla-

FIGURE 7

YIELD of ADDITION COMPOUND



tion. To render the alcohol constituents visible on the chromatogram, they were esterified with a highly colored acid, azobenzenemonocarboxylic acid, by the method of Ladenburg et al, (95).

The results of the molecular distillation are shown in Table XXVIII and in the elimination curve, Figure 8.

Table XXVIII

Molecular Distillation of
Tall Oil Neutral Materials

Fraction	Distillation Temperature	% of Neutral Material
1	100	5.15
2	110	12.44
3	120	9.01
4	130	12.26
5	140	9.37
6	150	8.83
7	160	11.45
8	170	8.02
9	180	9.68

Fractions 2, 4 and 7, corresponding to the maxima in the elimination curve, were treated with azobenzenemonocarboxyl chloride and chromatograms prepared. In Figure 9 is shown the appearance of these chromatograms and the weights and designation of the fractions obtained therefrom. The group of unresolved bands which constituted fraction 5-3 were separated into six fractions, 5A-1 to 5A-6 in chromatogram 5A. The bands corresponding to 5-1 and 5-2 were washed into the eluate in chromatogram 5A. Since these bands were adequately resolved in chromatogram 5, they were not recovered in chromatogram 5A.

Crystalline products were obtained by saponification of the fractions listed in Table XXIX. The melting points of these products, and the melting points of their acetates are recorded in Table XXIX.

Table XXIX

Melting Points of Tall Oil Alcohols and their Acetates.

Fraction	m.p. of alcohol Crystallization No.			m.p. of acetate Crystallization No.		
	1.	2.	3.	1.	2.	3.
2-2	68-69°					
5-1	68-69°	71.5-72°		55-56.5°	55-56.5°	
5-2	130-136°					
5A-2	69-70°	70-72°	73-73.5°	53.3-54.5°		
5A-4	-	132-134°	136-139°	123.5-125°		
5A-5	-	135-137°	139-140°	125.5-127°		
7-1	71-112°	127-135°				
7-2	136-139°	137-140°		104-115°	122-124°	125-126°

Mixtures of selected pairs of these fractions melted as shown in Table XXX.

The melting point of a mixture of fraction 5-1 with a sample of 1-tetracosanol separated by low temperature crystallization was not depressed.

FIGURE 8

MOLECULAR DISTILLATION of NEUTRAL MATERIAL

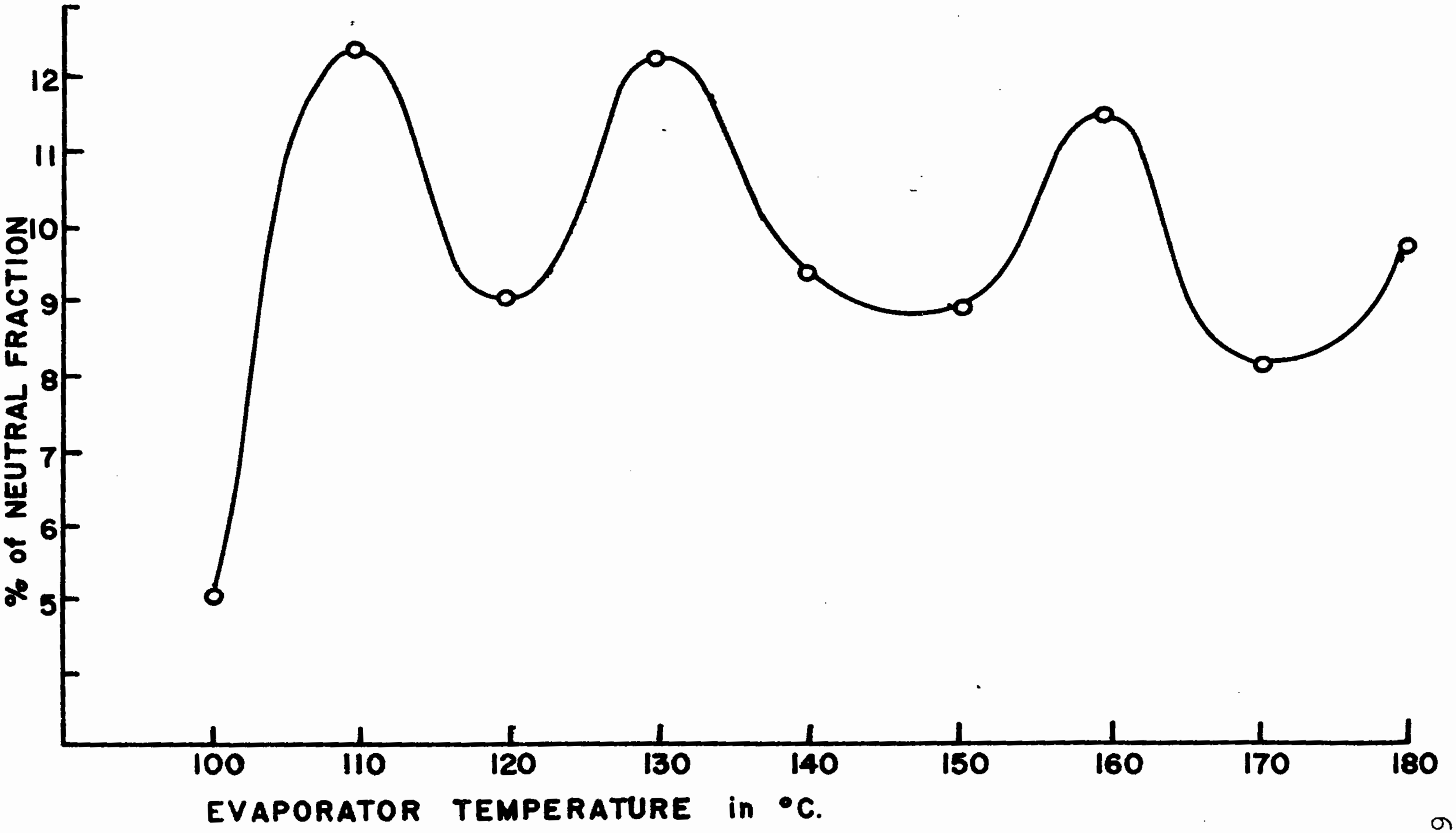


Table XXX

Melting Points of Mixtures

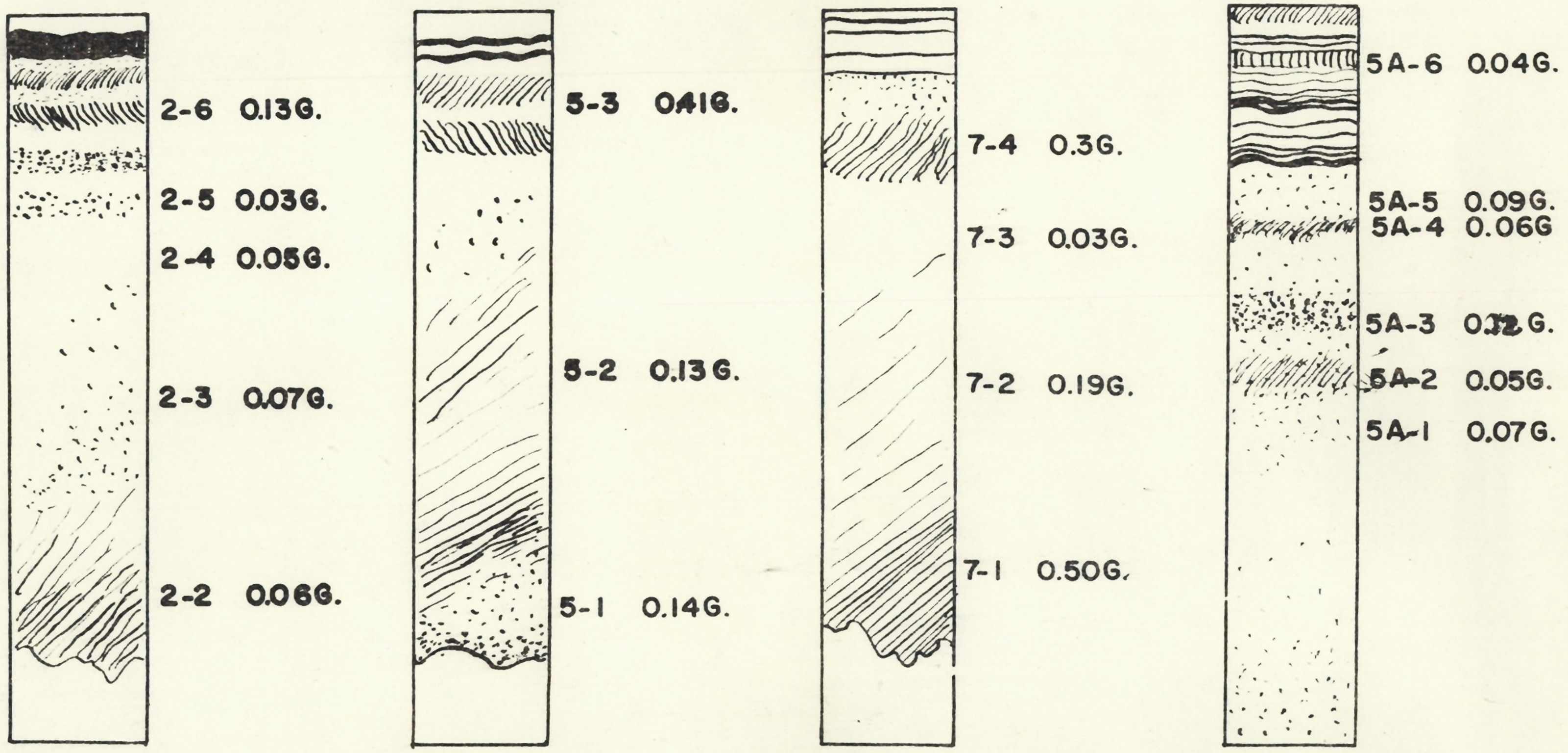
Fraction	Mixed with:	m.p. alcohol	m.p. acetate
5-1	2-2	68 - 70°	
5-1	5A-2	71 - 72°	54 - 56°
5-2	5A-4	134-137 °	
7-2	5A-5	138 - 140°	125-126°
5A-4	5A-5	138 - 139°	125-126°

To summarize, the chromatogram of the esters of distillation fraction 5 were separated into two broad red bands by chromatography on alumina. From the lower half of the bottom band an ester was isolated which on saponification yielded 1-tetracosanol. This material was identified by melting point, and by melting point of its acetate. From the lower half of the upper band the same alcohol was isolated. The melting point of a mixture of these two alcohols or their acetate esters was not depressed.

Similarly from the upper half of both bands, alcohols were isolated whose melting points were not depressed when the two samples were mixed. The melting points of these alcohols increased with successive crystallizations. Sufficient material was not available to continue the crystallizations until constant melting points were attained. Since both samples gave precipitates with digitonin it

FIGURE 9

APPEARANCE of CHROMATOGRAMS



DISTILLATION
FRACTION 2

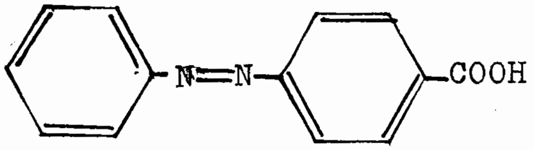
DISTILLATION
FRACTION 5

DISTILLATION
FRACTION 7

DISTILLATION
FRACTION 5-LONG
DEVELOPMENT

is concluded that both fractions are composed of mixtures of the same sterols.

In both cases one chemical species has apparently been separated into two bands on the chromatographic column. Since the alcohol moiety of both bands were identical, the difference in the chromatographic behavior of the esters must have been caused by differences in the acid portion of the molecule. The azo dye was prepared by coupling p-aminobenzoic acid with nitrosobenzene. By this reaction there is no possibility of formation of any azo compound other than azobenzenemonocarboxylic acid with the following structure:



Hartley (96-97) reported the preparation of cis-azobenzenes from the trans isomers by the action of sunlight. He later showed that the two forms exist in equilibrium in the presence of sunlight (98). Cook, (99, 100) separated cis-and trans-azobenzenes by chromatography on alumina with petroleum ether or benzene solvents. Freundlick and Heller (101) found the cis form to be more strongly absorbed on alumina.

In the present study the apparent separation of one compound into two zones must be explained by cis trans isomerism about the nitrogen-nitrogen double bond of the azo dye. Thus the cis and trans isomers of each of the two esters were separated by chromatography on alumina. Because the complexity of the mixture of alcohols is increased by esterification withazobenzenemonocarboxylic acid the subsequent chromatographic examination is hindered rather than aided.

Ladenburg and co-workers (95) reported the separation of cholesterol, stigmasterol and ergosterol esters of azobenzenemonocarboxylic acid on alumina. No anomalous results were reported and no special precautions to prevent isomerization of the azo compound were described. However these workers studied the separation of known binary mixtures. Formation of two bands was taken as evidence of separation. The identity of the constituent of each band was based on the melting point of the ester. All three esters melted in the range 188-201°. Since each ester may exist in two forms, and the melting points of the pure cis and trans forms are not known, and were not determined by Ladenburg et al, their identifications are not convincing.

Essential Oils from Coniferous Foliage

Yields and Properties of the Oils

One to two ton lots of foliage were distilled with steam in a large commercial apparatus. The yields and properties of the oils are shown in Table XXXI.

It was recognized that oil yields obtained with present commercial apparatus by the usual distillation procedure did not necessarily represent the total oil content of the plant material. For that reason laboratory scale distillations of needles and twigs from open grown trees, twenty to fifty years of age, were carried out. Yields and properties of the oils are reported in Table XXXII.

Since the properties of oils prepared in the laboratory and the oils prepared in the commercial still are in reasonable agreement, it appears that no important differences in chemical composition exist between the products of the two methods. However, dramatic differences exist between the yields. Laboratory distillations consistantly gave two to four times as much yield of oil as the large scale distillation, and in the case of white spruce the yield was increased by a factor of twenty. These increased yields may in part be explained by the exclusion of branches and more careful selection of plant

TABLE XXXI

Yields and Properties of Coniferous Oils

Species	Hours Distilled	Yield	d^{20}_{20}	$^{20}_{D}$	α^{30}_{D}	Acid Value	Esters, % as bornyl acetate	Alcohols, % as borneol	Ketones, % as carvone	Aldehydes, % as citral
Balsam fir	6	0.591	0.8825	1.4737	-18.8°	0.23	18.4	18.4	nil	nil
Eastern white cedar	6	0.269	0.9168	1.4612	- 7.8°	0.53	11.8	11.3	50.7	1.4
Hemlock	6	0.249	0.9162	1.4684	-12.9°	0.76	45.7	7.2	2.5	0.5
Black spruce	8	0.461	0.9085	1.4696	-32.1°	0.44	41.7	1.1	1.0	0.3
White spruce	6	0.012	0.8989	1.4761	- 5.8°	3.15	38.4	6.9	3.1	1.1
Jack pine	6	0.047	0.9048	1.4741	-19.8°	2.34	36.3	4.5	nil	1.0
White pine	6	0.147	0.8678	1.4738	-12.0°	0.52	9.3	3.8	2.2	0.6
Red pine	6	0.114	0.8662	1.4741	-10.5°	0.57	7.5	2.4	nil	0.8
Lodgepole pine	6	0.062	0.8659	1.4810	-11.7°	1.23	8.4	6.6	1.4	1.1
Scotch pine	6	0.089	0.8730	1.4757	+ 1.1°	1.57	7.0	9.9	2.2	0.6
Sweet fern	8	0.027	0.8904	1.4887	- 2.9°	1.88	12.5	0.8	1.6	1.1
Hemlock cones	4	0.185	0.8635	1.4751	-10.2°	1.41	11.6	10.2	2.5	1.6
White spruce cones	4	0.154	0.9060	1.4780	-18.4°	1.70	9.1	14.4	1.8	3.1
Scotch pine cones (seeds extracted)	6	0.034	0.8978	1.4877	- 5.8°	3.34	7.2	4.6	6.2	2.1
Scotch pine cones (seeds not extracted)	12	0.083	0.9024	1.4871	- 4.2°	3.93	13.5	7.9	6.5	2.1

Table XXXII

Oil Yields from Laboratory Distillations

Species	Month	Yield %	d_{4}^{20}	η_{D}^{20}
Balsam fir	February	1.70	0.889	1.4736
	April	1.32	0.880	1.4765
White cedar	February	0.86	0.905	1.4495
	April	0.65	0.924	1.4673
Hemlock	February	0.89	0.911	1.4690
	April	0.63	0.920	1.4739
Black spruce	February	0.80	0.926	1.4730
	April	1.74	0.922	1.4765
White spruce	February	0.22	0.898	1.4694
	April	0.39	0.907	1.4695
Jack Pine	March	0.19	0.914	1.4753
White Pine	March	0.14	0.917	1.4780
Scotch pine	March	0.28	0.899	1.4838
Red pine	March	0.36	0.877	1.4786
Sweet fern	May	0.15	0.898	1.4909

material used in laboratory work. However, an increase by a factor of about five is noted in the case of sweet fern, and here the samples distilled by the two methods were very similar. It is not expected that 100 percent of the oil can be recovered economically, but these results do indicate that there is room for improvement in commercial apparatus and operation.

It is commonly believed that oil loss from foliage is continuous from the time of cutting and reaches serious proportions when the foliage becomes dry and brown. If this is true then the yield and quality of the oil will depend on the time lapse between cutting and distillation of the oil. However, the results of experimental distillation of the black spruce foliage reported in Table XXXIII show that no significant loss of oil occurs on drying.

Table XXXIII

Oil Yield from Fresh and Dried Foliage

Material	Days Drying	Yield			Condition
		$\frac{V}{W}$	$\frac{W}{W}$	$\frac{W}{W}$ (Green basis)	
Fresh	0	0.53	0.49	0.49	Fresh green
	2	0.50	0.46	0.46	Fresh green
2 months	7	0.83	0.76	0.44	Air dry green
cold	11[a]	0.80	0.74	0.43	Needles fallen
storage	35	0.90	0.82	0.48	Turning brown

a) The 11 day sample results are weighted average. This sample was separated manually into twigs and needles, and the two fractions distilled separately.

Table XXIV shows that the needles, comprising only one third of the foliage, contain practically all of the essential oil.

Table XXXIV

Oil Content of Twigs and Needles of Black Spruce

	% of Total Weight	Oil Yield ($\frac{W}{W}$)	% of Total Oil
Twigs	65.8	0.20%	17.6
Needles	34.2	1.79%	82.4

To determine whether quality of the oil was affected by the drying period, physical properties of the oils were determined, and the ester contents were estimated by analytical saponification.

Table XXXV

Properties of Oils from Dried Foliage

Days Drying	N_D^{20}	d_4^{20}	Esters (% of Oil)	Esters(% of Green Weight)
2	1.4745	0.9160	30.5	0.140
7	1.4738	0.9177	31.9	0.140
11[a]	-	-	35.3	0.152
35	1.4731	0.9160	30.8	0.148
11 needles	1.4740	0.9292	39.7	-
11 twigs	1.4863	0.9284	14.8	-

a) weighted average.

Time of drying had no detectable effect on oil composition as revealed by physical properties and ester content. The properties of the twig oil has a faint fatty odour, has a low ester content, and to account for the high density and refractive index must contain large amounts of sesquiterpenes and diterpenes. The needle oil has a clean, less complex odour and a high bornyl acetate content.

Chemical Composition of Oils

Oil of Picea Mariana (Mill) B. S. P.

Fractional Distillation

By a series of fractional distillations the oil was separated into 50 fractions. The properties of these fractions are shown in Table XXXVI.

Table XXXVI

Properties of Fractions from Distillation of Black Spruce Oil

Fraction No.	η_D^{20}	d_4^{20}	α_d	b.p.	% Distilled
1	1.4669	0.865	0.00	58° (48 mm)	1.2
2	1.4671	0.867	-0.33	65	2.4
3	1.4634	0.871	-9.17	67	3.7
4	1.4649	0.863	-21.61	69	4.8
5	1.4655	0.863	-22.94	69.5	6.1
6	1.4658	0.862	-26.36	69.5	7.2
7	1.4660	0.863	-34.23	70	8.6
8	1.4669	0.863	-42.15	70	9.7
9	1.4674	0.863	-44.77	71	10.9
10	1.4677	0.864	-47.79	71	12.1
11	1.4682	0.864	-51.82	71	13.3
12	1.4685	0.864	-55.63	70.5	14.5
13	1.4690	0.864	-57.14	70.5	15.7

(Continued)

Table XXXVI (Continued)

Properties of Fractions from Distillation of Black Spruce Oil

Fraction No.	η^{20}_{D}	d^{20}_{4}	αd	b.p.	% Distilled
14	1.4691	0.865	-59.22	70.5	16.9
15	1.4696	0.865	-63.29	70.5	18.1
16	1.4697	0.865	-65.02	70.5	19.3
17	1.4697	0.865	-65.88	71	20.5
18	1.4701	0.866	-66.69	71	21.7
19	1.4702	0.866	-67.58	71	22.9
20	1.4705	-		71	24.2
21	solid			71	30.2
22	1.4785	0.870	-21.08	165.8°(757 mm)	31.4
23	1.4789	0.872	-18.49	166.1	32.6
24	1.4741	0.830	-7.02	166.7	33.8
25	1.4712	0.811	+2.92	168.1	34.9
26	1.4713	0.828	+6.63	170.1	36.1
27	1.4716	0.846	+9.20	170.9	37.2
28	1.4720	0.853	+9.52	171.5	38.4
29	1.4724	0.857	+8.90	171.9	39.7
30	1.4720	0.864	+5.10	173.0	40.9
31	1.4735	0.860	-15.95	176.9	42.0
32	1.4758	0.846	-27.85	178.5	43.2
33	1.4752	0.844	-30.15	178.5	44.5
34	1.4745	0.848	-30.35	178.5	45.6

(Continued)

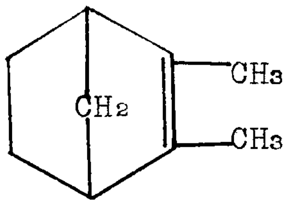
Table XXXVI (Continued)

Properties of Fractions from Distillation of Black Spruce Oil

Fraction No.	n^{20}_{D}	d^{20}_{4}	αd	b.p.	% Distilled
35	1.4753	0.878	-18.9	183.0	48.2
36	1.4820	0.883	-17.7	186.3	48.5
37	1.4856	0.876	-11.5	187.8	48.7
38	1.4875	0.874	- 7.6	188.3	49.0
39	1.4900	0.872	- 4.0	188.8	49.2
40	1.4973	0.876	- 1.7	190.1	49.5
41	1.4806	0.911	+ 1.1	196.4	49.7
42	1.4714	0.934	+ 6.5	203.4	50.3
43	1.4780	0.962	+19.9	209.0	50.6
44	1.4774	0.965	+25.6	209.4	50.8
45	1.4750	0.960	+22.3	211.1	51.4
46	1.4771	0.959	+ 6.2	212.0	51.5
47	1.4640 to 1.4645	0.987	-35.5 to -36.5	-	88.1
48	1.505	0.904	+38°	80-90 (1 mm)	89.0
49	1.495	-	-	90-110	89.4
50	1.510	-	-	110-130	89.6

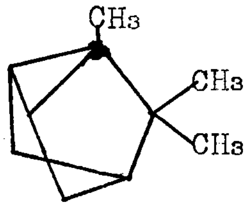
Identification of Constituents

The optically inactive compound of fraction 1 was identified as santene (1) by comparison of its nitrosite with an authentic sample of santene nitrosite prepared from oil of Abies siberica.



I. Santene

The presence of another compound was indicated by the high density of fraction 3. By refractionation a white, optically inactive solid was obtained which was stable to permanganate. This solid was identified as the tricyclic hydrocarbon, tricyclene (II), by comparison of its infra-red absorption spectrum with that of a synthetic sample.



II. Tricyclene

In Table XXXVII are listed the observed maxima.

Table XXXVII

Infra-Red Absorption of Tricyclene

	Optical Density	
Wave length, μ	Natural (46.6% sol'n)	Synethetic (41.4 % sol'n)
3.28	0.26	0.21
3.40	1.1	1.2
3.48	0.91	0.93
7.36	0.21	0.21
7.47	0.39	0.34

Table XXXVII (Continued)

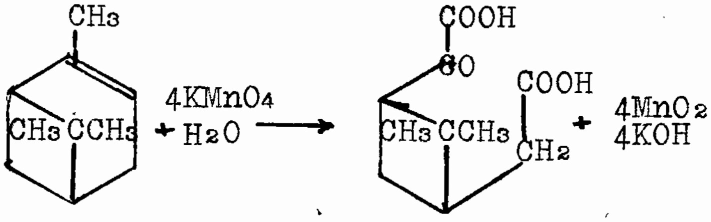
Infra-Red Absorption of Tricyclene

Wave Length μ	Optical Density Natural (46.6% sol'n)	Optical Density Synthetic (41.4% sol'n)
7.75	0.32	0.25
7.91	0.18	0.17
8.00	0.11	0.10
8.16	0.06	0.05
8.32	0.05	0.05
8.52	0.10	0.10
8.61	0.07	0.07
8.71	0.07	0.07
8.80	0.05	0.05
9.02	0.05	0.05
9.31	0.23	0.19
9.70	0.16	0.13
9.88	0.07	0.07
10.34	0.23	0.19
11.10	0.08	0.06
11.56	0.24	0.20
11.78	0.36	0.28
11.94	0.37	0.29
12.36	0.38	0.31

This is the first report of the occurrence of a tricyclic terpene hydrocarbon in nature. Moycho and

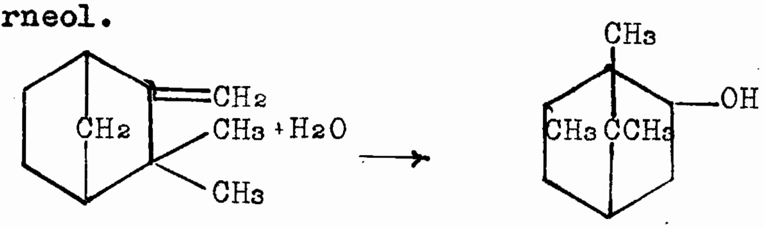
Zienkowski showed that tricyclene is formed in small amounts along with camphene by the dehydration of isoborneol (102). It is possible that both camphene and tricyclene in oil of black spruce were formed by a similar dehydration of borneol in the living plant.

Fractions 4 to 21 were mixtures of two compounds with similar boiling points. The compound with the lower boiling point, refractive index, and optical rotation was identified as l-α-pinene (III) by its permanganate oxidation products. dl-Pinonic acid and l- pinonic acid were formed by oxidation of fractions 6 and 7.



III α-pinene

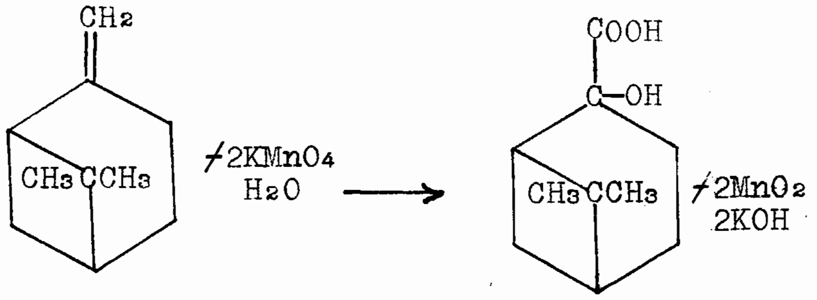
The higher boiling compound was a solid which after crystallization from methanol followed by sublimation melted at 47-48°, $[\alpha]_D$ - 97.5° (C = 10.56 in benzene). This terpene was identified as l-camphene (IV) by hydration to isoborneol.



IV camphene

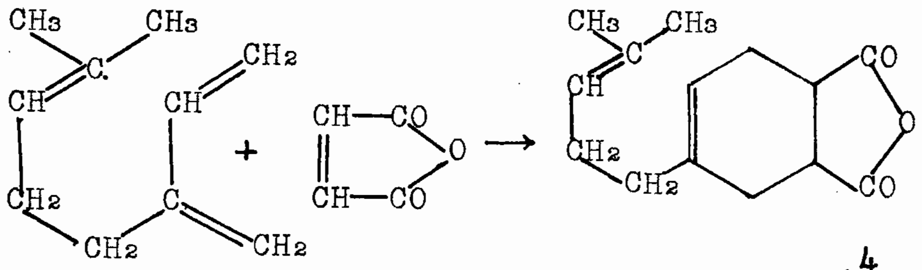
isoborneol

Permanganate oxidation of fractions 22 and 23 gave l-nopinic acid derived from β -pinene (V)



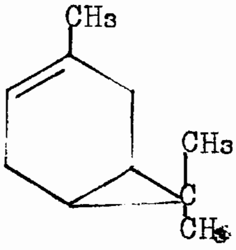
V β -pinene nopinic acid

The low density of fraction 25 suggested the presence of an acyclic terpene. Refractionation gave a liquid with properties η^{20}_{D} 1,4710, d^{20}_{4} 0.800, αD + 0.83 b.p. 167° C. (757 mm) which underwent the diene reaction with maleic anhydride to yield the myrcene adduct.



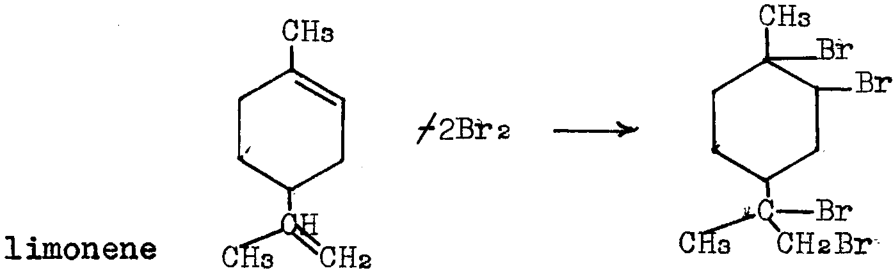
VI myrcene cis -4-isohexenyl -Δ^4 tetrahydrophthalic anhydride

Refractionation of the dextrorotatory fractions 27 and 28 gave a liquid with properties. η^{20}_{D} 1.4729, d^{20}_{4} 0.866, b.p. 172° C. (762 mm) in excellent agreement with those reported by Simmonsen (103) for Δ^3-carene (VII) The liquid formed a nitrosite which melted with decomposition at 147°C., undepressed by an authentic sample of d-Δ^3-carene nitrosate.



VII Δ^3-carene

The laevorotatory compound of fraction 33 was identified as limonene (VIII) by the preparation of dl-limonene tetrabromide. While the optically active tetrabromide was not isolated, the optical rotation of the fraction of about -30° corresponds to a mixture of 76% dl-limonene and 24% of l-limonene with rotation of -125°.



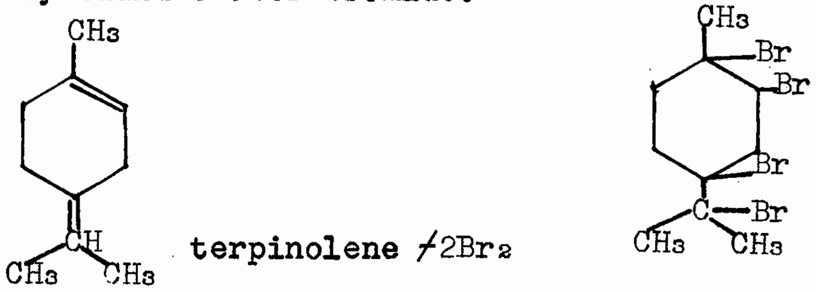
The alcohol and ketone contents of selected fractions were determined and are reported in Table XXXVIII.

Table XXXVIII

Alcohol and Ketone Contents of Black Spruce Fractions

Fraction	% Alcohol	% Ketone
36	10.0	5.15
38	-	4.5
39	9.3	-
40	12.5	10.3
41	43.0	45.2
42	54.6	42.6
43	61.0	-
44	-	10.3
45	70.5	8.6

The low density, alcohol and ketone contents of fractions 35 to 40 suggests the presence of a high boiling hydrocarbon of high refractive index. The presence of terpinolene was demonstrated by preparation of the crystalline tetrabromide.



Fractions 41 to 46 were mainly mixtures of alcohols and ketones, with alcohols predominating in the higher boiling fractions. Following collection of fraction 46, solid l-borneol appeared in the distillate.

From fraction 42 a mixture of semicarbazones was prepared in 15% yield, and from this mixture was separated in 7% yield a semicarbazone from which camphor with $[\alpha]_D + 6^\circ$ was regenerated.

The dextrorotation of fraction 44 cannot be explained by the presence of a d-camphor or l-borneol. In view of the low yield of camphor semicarbazone obtained, it is possible that other ketones may be present. The ketone components required more than a week to react completely with the hydroxylamine hydrochloride solution used in their analysis. Any other ketone present must, therefore, be very unreactive.

An alcohol with properties n_D^{20} 1.4842, d_4^{20} 0.955

$\alpha_D + 13.2^\circ$ was isolated from fractions 43, 44 and 45 by means of the borate ester. It may be noted that the process of isolation resulted in a product with a decreased dextrorotation. Since transesterification with tributyl borate is unlikely to result in racemization, the decreased dextro-rotation of the regenerated product suggests that this material is contaminated with a more reactive, levorotatory alcohol, probably borneol. The dextrorotatory alcohol is probably tertiary since it failed to form a urethane or a p-nitrobenzoyl ester.

The main constituent of oil of Picea mariana was identified in fraction 47 as l-bornyl acetate.

It is unlikely that the dextrorotatory sesquiterpene of fraction 48 is cadinene in spite of the fact that the dihydrochloride of l-cadinene was prepared from it in good yield. Probably this derivative was formed by the action of hydrogen chloride on some related sesquiterpene. Dextrorotatory copaene yields the l-cadiene derivative when treated with hydrogen chloride, but the high refractive index of fraction 48 is inconsistant with the presence of a high percentage of copaene. Briggs and Taylor (104) isolated a sesquiterpene from oil of Dacrydium kirkii with properties η_D^{25} 1.5055, d_4^{25} 0.9239, $\alpha_D^{30} + 60^\circ$, from which l-cadinene hydrochloride was prepared in 60% yield. They considered the fraction to consist mainly of cadinene.

Oil of Tsuga Canadensis (L.) Carr

Fractional Distillation

By a series of fractional distillations the oil was separated into fractions with properties listed in Table XXXIX.

Table XXXIX

Properties of Fractions from Distillation of Hemlock Oil

Fraction	η_D^{20}	d_4^{20}	α_D	b.p.	% Distilled
1	1.4581	0.877	-1.18	143.2/755°	0.2
2	1.4599	0.875	+1.69	151.8	1.7
3	1.4615	0.869	+5.14	154.0	2.9
4	1.4632	0.863	+8.99	154.4	4.2
5	1.4639	0.863	+10.03	155.0	5.4
6	1.4640	0.861	+10.11	155.3	6.6
7	1.4642	0.859	+9.93	155.3	7.8
8	1.4644	0.860	+9.37	155.4	9.1
9	1.4647	0.860	+8.54	155.4	10.3
10	1.4649	0.861	+7.08	155.5	11.6
11	1.4639	0.864	+8.29	155.5	12.8
12	1.4651	0.860	+8.20	155.6	14.0
13	1.4655	0.861	+5.36	155.7	15.3

(Continued)

Table XXXIX (Continued)

Properties of Fractions from Distillation of Hemlock Oil

Fraction	η^{20}_{D}	d^{20}_{4}	α_D	b.p.	%Distilled
14	1.4665	0.862	-4.43	156.0	16.5
15	1.4673	0.863	-13.27	156.3	17.7
16	1.4680	0.864	-21.38	157.0	19.0
17	1.4684	0.865	-25.56	157.0	20.2
18	1.4691	0.866	-34.45	157.5	21.5
19	1.4696	0.866	-39.66	157.5	22.7
20	1.4699	0.867	-45.37	157.8	24.0
21	1.4701	0.867	-48.37	157.9	24.7
22	solid				25.6
23	solid				26.1
24	solid	m.p. 29.2°			26.8
25	solid	m.p. 39.0°			28.2
26	solid	m.p. 43.2°		160.1/746	31.4
27	1.4755	0.871	-26.95	164.0	32.6
28	1.4776	0.871	-14.07	165.8	33.8
29	1.4745	0.854	-9.48	166.3	34.9
30	1.4700	0.799	+1.53	168.0	36.1
31	1.4701	0.809	+29.14	169.3	37.2
32	1.4723	0.843	+54.63	172.8	38.4
33	1.4789	0.848	-21.97	177.0	39.6
34	1.4788	0.848	-30.67	177.3	40.7
35	1.4784	0.847	-35.33	177.3	42.0

(Continued)

Table XXXIX (Continued)

Properties of Fractions from Distillation of Hemlock Oil

Fraction	η_D^{20}	d_4^{20}	α D	b.p.	%Distilled
Residue and loss					44.3
36	1.4760	0.851	-29.54	174	45.5
37	1.4754	0.863	-28.40	179	46.8
38	1.4634	0.908	- 5.44	193	48.0
39	1.4750	0.955	- 0.06	207	49.4
40	1.4656	0.979	-17.88	223	50.8
41	1.4647	0.982	-18.15		52.3
42	1.4642	0.984	-18.39		53.6
43	1.4640	0.984	-18.40		55.1
44	1.4640	0.984	-18.51		56.5
45	1.4640	0.985	-18.55		57.9
46	1.4638	0.985	-18.65		59.3
47	1.4637	0.985	-18.65		60.8
48	1.4637	0.985	-18.68		62.2
49	1.4638	0.985	-18.68		63.7
50	1.4635	0.985	-18.68		65.1
51	1.4650			97/10mm	68.3
52	1.4650			97	71.5
53	1.4650			97	74.8
54	1.4650			97	77.9

(Continued)

Table XXXIX (Continued)

Properties of Fractions from Distillation of Hemlock Oil

Fraction	n_D^{20}	d_4^{20}	α_D	b.p.	%Distilled
55	1.4651			97	81.0
56	1.4652			97	84.3
57	1.4658			98	87.4
58	1.4667			99	90.5
59	1.4748			100-104	92.8
Residue and loss					93.7
Sesquiterpene fraction				43-70/0.4 mm	97.6
Higher boiling compounds				70-140/0.4 mm	97.9

Sesquiterpenes and higher boiling compounds were redistilled to give 40 fractions roughly equal volume with properties shown in Table XL.

Table XL

Properties of Sesquiterpene Fractions

Fraction	n_D^{20}	d_4^{20}	α_D	b.p./4 mm
60	1.4750	0.952	-	
61	1.4665	0.977	-16.47	
62	1.4690	0.981	-	

(Continued)

Table XL (Continued)

Properties of Sesquiterpene Fractions

Fractions	η^{20}_{D}	d^{20}_{4}	α D	b.p./4 mm
63	1.4800	0.967	-19.50	
64	1.4918	0.950	-15.33	91.5
65	1.4952	0.948	-13.41	92.0
66	1.4968	0.948	-13.20	92.0
67	1.4973	0.949	-12.60	92.2
68	1.4972	0.947	-11.02	93.1
69	1.4964	0.944	-	94.0
70	1.4950	0.937	- 6.53	95.0
71	1.4940	0.932	-	95.7
72	1.4938	0.929	- 0.97	96.5
73	1.4930	0.927	-	97.5
74	1.4931	0.923	- 0.60	97.5
75	1.4947	0.929	- 0.71	100
76	1.4963	0.934	-	102
77	1.4970	0.938	+ 7.13	103
78	1.4970	0.937	-	105
79	1.4968	0.936	+25.32	107
80	1.4960	0.931	+31.73	108.5
81	1.4954	0.928	+33.73	109
82	1.4949	0.926	+26.79	112
83	1.4932	0.935	+ 9.72	114

(Continued)

Table XL (Continued)

Properties of Sesquiterpene Fractions

Fraction	η_D^{20}	d_4^{20}	α_D	b.p./4 mm
84	1.4906	0.945	-	116
85	1.4892	0.951	- 9.00	117.5
86	1.4890	0.948	- 6.82	120.0
87	1.4890	0.943	-	122.5
88	1.4888	0.936	+ 0.92	124.0
89	1.4890	0.934	-	125.0
90	1.4915	0.943	- 2.9	126.0
91	1.4922	0.950	- 6	128.1
92	1.4940	0.958	- 12	131.5
93	1.4945	0.953	-14.2	133.5
94	1.4949	0.949	- 16	134.9
95	1.4965	0.952		137.8
96	1.5003	0.962		141.0
97	1.5044	0.970		145.5
98	1.5082	0.972		151.5
99	1.5125	0.963		153

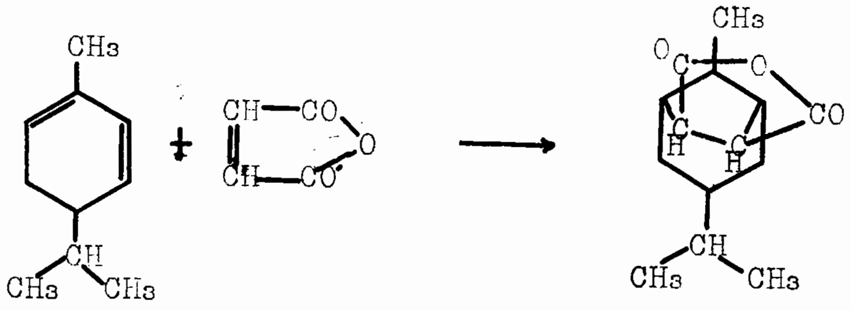
Identification of Constituents

Fractions 1 and 2 were mainly tricyclene. It appears that this compound may have widespread occurrence among coniferous oils, but has not been recognized because it is not easily separated from α-pinene by distillation.

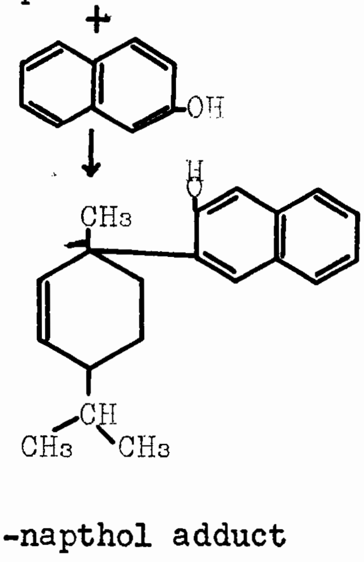
Fractions 4 to 12 consisted mainly of d-α-pinene and fraction 28 of l-β- pinene, since on permanganate oxidation they yielded dl- and d-pinonic acid and l-nopinic acid respectively.

l-Camphene was identified in the solid fractions 22 to 26 by hydration to isoborneol.

Both fractions 30 and 32 underwent the diene reaction with maleic anhydride, to give adducts of myrcene and d-α-phellandrene respectively. An α-phellandrene adduct was also formed when fraction 32 was heated with β -naphthol.



α -phellandrene maleic anhydride adduct



β -napthol adduct

Fraction 35 contained limonene since dl- and l-limonene tetrabromides were separated from the products of bromination.

The presence of oxygen compounds in fractions distilling after fraction 36 was indicated by the increase in density. Chemical analyses of selected fractions are contained in Table XLI.

Table XLI

Analyses of Hemlock Fractions

Fraction	% Esters[a]	% Alcohols[b]	% Carbonyl[c]
36	-	6.5	1.9
37	-	4.0	-
38	-	8.3	61.1
39	20.1	46.7	25.1
40	67.9	-	3.0
41	94.9	-	-
42	96.1	-	-
43	98.3	-	-

a) as bornyl acetate by saponification.

b) as borneol by acetytation and saponification.

c) as thujone by reaction with hydroxylamine.

Fractions 44 - 50 contained 97.6 to 99.0% ester as bornyl acetate.

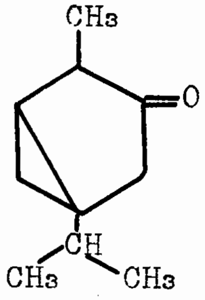
From fraction 38 a mixture of four semicarbazones was obtained, three of which were decomposed by oxalic acid to give, in each case, a ketone with a low refractive index η_D^{20} 1.450. The properties of these semicarbazones are summarized in Table XLII.

Table XLII

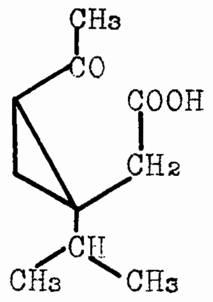
Properties of Semicarbazones

Semicarbazone	Solubility	m.p.	Regenerated ketone, η_D^{20}
1	insoluble in ethanol crystallized from water	253°	-
2	crystallized from methanol	193°	1.4496
3	soluble in cold methanol crystallized from methanol and water	176°	1.4502
4	soluble in cold methanol crystallized from methanol and water	128°	1.4509

The regenerated ketones yielded α-thujaketonic acid on permanganate oxidation and were, therefore, either ℓ- thujone or d-isothujone.



thujone or isothujone



α-thujaketonic acid

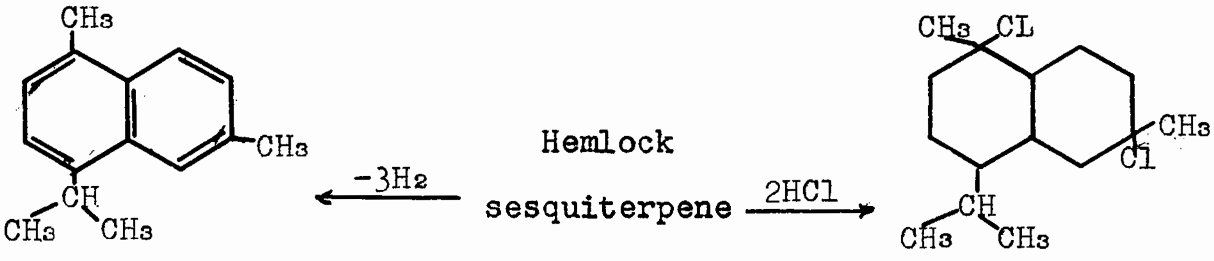
Probably semicarbazone 2 was 1-thujone semicarbazone, m.p. 197- 199°, and semicarbazone 3 was d-isothujone semicarbazone, m.p. 172° according to Short and Read (105). The ketone fraction of oil of hemlock therefore consists of 1-thujone and d-isothujone.

The alcohol of fraction 39 was isolated by means of the borate ester. It failed to give a urethane with α-napthyl isocyanate, and with p-nitrobenzoyl chloride gave only a trace of dl-bornyl p-nitrobenzoate. The sodium alcoholate of the compound reacted with p-nitrobenzoyl chloride to give an ester m.p. 55.5 - 57° which has not been previously described.

Fractions 40 - 50 consisted of an ester with properties in good agreement with those of bornyl acetate (106). Following saponification a solid alcohol and acetic acid (as the anilide) were isolated. With p-nitrobenzoyl chloride the alcohol gave an ester m.p. 152 - 154°C. which is not described in the literature. This ester must have been dl-bornyl p-nitrobenzoate since racemic borneol was obtained from it by saponification. A small amount of 1-bornyl p-nitrobenzoate was isolated from the mother liquors.

Fractions 61 to 100 contained at least eight compounds each comprising 0.2 to 0.3% of the oil. On dehydrogenation only the dextrorotatory compound of fraction

80 to 83 yielded cadalene. From this same material, 1-cadinene hydrochloride was prepared in 24.5% yield.



cadalene

cadinene hydrochloride

However the dextrorotation of this fraction is not consistant with the presence of a large percentage of 1-cadinene.

Fractions 97 to 100 were green in color. Since this green coloration was extracted with 85% phosphoric acid, the presence is indicated of an azulene compound (107) in amounts too minute to permit isolation or identification.

Oil of Abies Balsamea (L.) Mill

Fractional Distillation of the Oil

Fractional distillation of the oil yielded a series of fractions described in Table XLIII and a fraction containing sesquiterpenes and higher boiling compounds. This latter fraction was separated into the fractions, each comprising about 0.1% of the original oil, listed in Table XLIV.

Table XLIII

Properties of Fractions from Distillation of Balsam Oil.

Fraction	n_D^{20}	d_4^{20}	α_D	b.p.	% Distilled
1	1.4626	0.859	+ 0.08		0.5
2	1.4662	0.863	+ 0.08		1.6
3	1.4625	0.860	- 2.03		2.3
4	1.4640	0.857	- 4.23		3.5
5	1.4644	0.855	- 5.12		4.8
6	1.4648	0.855	- 6.03		5.9
7	1.4648	0.855	- 7.00		7.1
8	1.4651	0.855	-10.06		8.2
9	1.4660	0.855	-19.09		9.4
10	1.4654	0.855	-12.56		10.5
11	1.4664	0.856	-21.28		11.6
12	1.4690	0.852	-51.11		12.5
13		solid			17.9
Loss					18.6
14	1.4783	0.870	-19.54	166.9°/ 758 mm	46.0
15	1.4775	0.872	-24.39	165.3°/ 751 mm	47.2
16	1.4782	0.872	-20.00	166.1	48.4
17	1.4780	0.871	-19.71		49.7
18	1.4781	0.871	-19.48		50.7
19	1.4780	0.872	-19.48		51.7

(Continued)

Table XLIII (Continued)

Properties of Fractions from Distillation of Balsam Oil

Fraction	η_D^{20}	d_4^{20}	α_D	b.p.	% Distilled
20	1.4781	0.873	-19.35		53.0
21	1.4780	0.873	-18.94	166.3	54.1
22	1.4733	0.842	- 5.65	167.8	55.2
23	1.4718	0.855	+ 8.99	170.9	56.4
24	1.4718	0.864	+11.96	171.5	57.5
25	1.4717	0.865	+12.84	171.7	58.6
26	1.4715	0.865	+13.38	171.7	59.8
27	1.4715	0.865	+13.66		61.0
28	1.4715	0.865	+13.88		62.2
29	1.4715	0.865	+14.00		63.3
30	1.4715	0.866	+14.01		64.6
31	1.4715	0.865	+13.37	172.0	65.9
32	1.4738	0.863	- 3.03	173.7	67.0
33	1.4774	0.849	-36.58	177.0	68.1
34	1.4774	0.846	-43.70		69.2
35	1.4778	0.846	-45.13	177.9	70.4
36	1.4776	0.846	-49.76	177.9	71.4
37	1.4775	0.844	-51.33		72.6
38	1.4770	0.844	-52.53	180.0	73.5
39	1.4773	0.840	-49.49		74.6

(Continued)

Table XLIII (Continued)

Properties of Fractions from Distillation of Balsam Oil

Fraction	η^{20}_{D}	d^{20}_{4}	α_{D}	b.p.	% Distilled
40	1.4758	0.842	-57.02		75.6
Residue and Loss					78.4
41	1.4770	0.842	-43.55		79.5
42	1.4783	0.878	-19.23		80.6
43	1.4819	0.900	- 7.97		81.8
44	1.4725	0.939	+ 0.52		82.1
45	1.4806	0.956	+18.67		82.4
46	1.4830	0.951	+ 0.98		82.6
47	1.4793	0.962	-27.07		82.9
48	1.4725	0.969	-38.73		83.2
49	1.4683	0.973	-41.61		83.5
50	1.4673	0.976	-42.22		83.8
51	1.4665	0.978	-42.45		84.1
52	1.4660	0.977	-42.86		84.5
53	1.4656	0.978	-43.22		84.7
54	1.4653	0.979	-43.60		85.0
55	1.4651	0.979	-43.78		85.3
56	1.4649	0.980	-43.98		85.6
57	1.4641	0.980	-43.96		88.1

(Continued)

Table XLIII (Continued)

Properties of Fractions From Distillation of Balsam Oil

Fraction	η_D^{20}	d_4^{20}	α_D	b.p.	%Distilled
58	1.4637	0.980	-43.67		89.0
59	1.4632	0.980	-43.14		92.7
60	1.4632	0.980	-42.74		93.6
61					95.0
Residue					97.3
Sesquiterpenes and higher boiling compounds					99.4

Table XLIV

Properties of Sesquiterpene Fractions

Fraction	η_D^{20}	d_4^{20}	α_D	b.p./10 mm
62	1.4698	0.966	-32.24	102
63	1.4900	0.953		102-104.5
64	1.4980	0.946	- 0.79	106-106.5
65	1.4981	0.943	- 4.88	106.5-108
66	1.4961	0.942	-10.46	108.5-110
67	1.4938	0.941	-13.07	112.5-113
68	1.4927	0.938	-10.84	113.5-114

(Continued)

Table XLIV (Continued)

Properties of Sesquiterpene Fractions

Fraction	η_D^{20}	d_4^{20}	α_D	b.p./10 mm.
69	1.4919	0.935	- 7.52	116.3-118
70	1.4931	0.935	- 8.87	119.7-119.9
71	1.4937	0.938	- 5.99	120.2-120.3
72	1.4903	0.929	- 1.34	122.5-125.5
73	1.4884	0.916	- 0.58	
74	1.4910	0.921	- 5.24	112.8-127
75	1.4902	0.911	+ 6.93	127-128.8
76	1.4903	0.907	+ 7.33	130-131
77	1.4913	0.908	+15.11	131-131.5
78	1.4916	0.909	+20.74	131.5
79	1.4804	0.922	+ 5.25	131.3-140.5
80	1.4798	0.921	- 0.05	140.5-143.7
81	1.4816	0.921	- 3.65	143.7-146
82	1.4849	0.939	- 7.30	146

Identification of Constituents

The high density of fraction 2 suggested the presence of tricyclene in admixture with another optically inactive compound. Redistillation of fractions 1, 2 and 3 separated two optically inactive compounds b.p. 139.5°C., η_D^{20} 1.4664,

d_4^{20} 0.862, and b.p. 151-153.0° C. η_D^{20} 1.4610, d_4^{20} 0.866. The former compound was identified as santene by preparation of the characteristic blue nitrosite. The latter liquid after removal of unsaturated impurities by permanganate oxidation yielded pure crystalline tricyclene. It now appears that tricyclene has widespread occurrence in coniferous oils. It has not been detected previously because it is separated from α-pinene only by most careful fractional distillation. In these investigations it was first detected by the almost imperceptible increase in the density of the fraction distilling immediately before α-pinene fraction.

It may be significant that all of the oils found to contain tricyclene to date, also contain camphene and borneol or bornyl acetate.

The three structures are closely related: hydration of camphene yields isoborneol - the geometric isomer of borneol - and dehydration of isoborneol yields camphene and a small amount of tricyclene (102). The biological synthesis of these compounds may procede through similar hydration and dehydration reactions in the plant.

Fraction 6 consisted of dl-α-pinene and fractions 14 to 21 of l-β-pinene since on permanganate oxidation they yielded dl-pinonic acid and l-nopinic acid respectively. The β-pinene content of the oil of 35% is the highest recorded for any oil. Fraction 18 is believed to be one of the purest samples of β-pinene ever isolated and the reported constants are, therefore, believed to be the most reliable available for this terpene.

The solid constituent of fraction 13 was *l*-camphene since it was converted by hydration to isoborneol.

The presence of an acyclic terpene was indicated by the low density of fraction 22. The presence of a conjugated system of double bonds was demonstrated by reaction with maleic anhydride (108). The Diels-Alder adduct was identical with the adduct of myrcene.

If β-phellandrene were present as might be expected from the work of Smith and West (109), it would be found in fraction 29. This fraction, however, failed to form a solid nitrosite when treated with cold nitrous acid, indicating the absence of significant amounts of α or β -phellandrene (110). It is remarkable that β-phellandrene should be found in the volatile oil obtained from the oleoresin but not in the oil from the foliage. The dextrorotatory compound of fraction 29 was shown to be d-Δ^3-carene, by preparation of the characteristic nitrosate.

dl- and *l* -Limonene tetrabromides were isolated from the bromination products of fraction 37.

Fractions 49 to 60 distilled with fairly constant physical properties, but chemical analyses reported in Table XLV indicate a low degree of purity.

Table XLV

Chemical Analyses of Balsam Fractions.

Fraction	% Esters[a]	% Esters after Acetylation[a]	% Carbonyl[b]
42	0.0	0.0	9.3
43	0.0	38.9	21.1
44	0.0	39.0	22.2
45	12.8	49.0	15.1
46	20.1	39.7	14.0
47	25.7	61.8	17.8
48	57.3	74.2	6.9
49	69.9	83.6	4.5
50	73.5	87.6	3.9
51	76.4	87.0	-
52	75.6	86.5	-
53	79.1	87.5	3.6
54	79.8	89.3	-
55	81.9	89.7	-
56	81.7	89.5	3.0

a) as bornyl acetate

b) as carvone

The difficulties experienced in separating these more polar compounds may have been due to azeotrope formation.

The esters of fractions 48 to 56 were acetates exclusively, as is shown by comparison of the Duclaux constants of the acid obtained by saponification of these fractions with constants of known acids.

Table XLVI

Duclaux Constants of Organic Acids

Constants	A	B	C
Observed	7.0	7.3	7.7
Formic Acid	3.95	4.40	4.55
Acetic Acid	6.8	7.1	7.4
Propionic Acid	11.9	11.7	11.3

Borneol was isolated from the saponification mixture. The ester fraction could not be freed of alcohol and carbonyl impurities by distillation but by crystallization a pure sample of crystalline bornyl acetate was prepared.

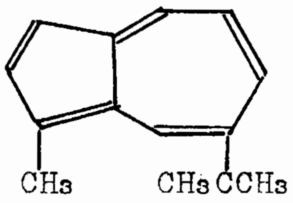
Fractions 43 to 48 were separated by chemical methods. Carbonyl compounds were removed as the semicarbazones. Alcohols were separated from the remaining non-carbonyl material as the borate esters. The semicarbazones of fractions 43 and 44 yielded l-camphor on hydrolysis. The semicarbazones of fractions 45 to 48 were stable to hydrolysing agents.

An alcohol boiling at 82-83°C., (10 mm) η^{20}_{D} 1.4743 was isolated from fractions 43 and 44. It formed a p-nitrobenzoate, m.p. 105-107° C., $[\alpha]$ $+21.02^{\circ}$ in low yield. Another alcohol fraction b.p. 91.5-96° C. (12 mm), η^{20}_{D} 1.4848, d^{20}_{4} 0.952, α D-12.34° was obtained from fraction 45 and 46. This fraction gave low yields of borneol derivatives with p-nitrobenzoyl chloride, α-naphthyl isocyanate or phenylisocyanate and failed to form a solid hydrochloride. The main constituent appears to be an unreactive alcohol, probably a tertiary alcohol.

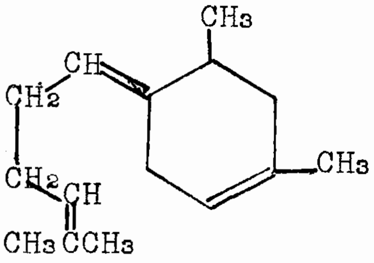
The isolation of borneol derivatives does not prove that free borneol was present in the oil. Bornyl borate may be formed by transesterification of the ester with butyl borate.

Identifiable dehydrogenation products were obtained from fractions 73, 78 and 80. The former two fractions yielded cadalene. S-Guiaiazulene was isolated from the dehydrogenation products of the latter.

l-cadinene hydrochloride, and bisabolene hydrochloride were formed by the action of hydrogen chloride on fraction 77. Thus oil of Abies balsamea resembled oils of Picea mariana and Tsuga canadensis in that the fractions which yield the hydrochloride of L-cadinene are dextrorotatory.



S - Guiaiazulene



Bisabolene

Oil of Thuja Occidentalis L.

Fractional Distillation

The oil was separated by fractional distillation into 72 terpene fractions described in Table XLVII and a sesquiterpene and higher boiling fraction. This latter fraction was separated into five fractions listed in Table XLVIII.

Table XLVII

Properties of Fractions from Distillation of Cedar Oil

Fraction	$^{20}_{D}$	d^{20}_{4}	α_D	b.p.	% Distilled
1	1.4484	0.846	- 1.55°	140.8°/ 760 mm.	0.55
2	1.4600	0.842	-10.79	155.0	1.17
3	1.4623	0.849	- 7.36	156.0	1.80
4	1.4641	0.850	- 5.47	156.5	2.43

Table XLVII (Continued)

Properties of Fractions from Distillation of Cedar Oil

Fraction	η_D^{20}	d_4^{20}	α_D	b.p.	% Distilled.
5	1.4655	0.855	- 5.95	156.9	3.05
6	1.4676	0.855	-10.04	157.7	3.68
7	1.4692	0.857	-17.45	148.3	4.31
8	1.4708	0.860	-28.95	159.2	4.92
9	1.4713	0.863	-40.86	159.6	5.42
10	1.4709	0.857	- 6.88	161.7	6.05
11	1.4703	0.846	+55.53	164.5	6.75
12	1.4705	0.845	+55.47	165.2	7.36
13	1.4710	0.848	+51.25	165.3	7.98
14	1.4717	0.848	+43.95	165.5	8.69
15	1.4722	0.844	+31.07	165.8	9.32
16	1.4703	0.810	+ 6.83	168.0	9.89
17	1.4705	0.816	+ 5.14	170.5	10.46
18	1.4730	0.837	+ 3.54	171.5	11.02
19	1.4773	0.838	+ 4.51	176.0	11.65
20	1.4781	0.840	- 9.72	177.4	12.24
21	1.4785	0.840	-11.34	177.8	12.75
22	1.4698	0.884	-22.43	178.0	14.72
Nonvolatile residue					14.95
Loss					
23	1.4556	0.922	-32.44°	105.5°/ 50 mm.	38.54

Table XLVII (Continued)

Properties of Fractions from Distillation of Cedar Oil

Fraction	η_D^{20}	d_4^{20}	α_D	b.p.	% Distilled
24	1.4535	0.923	-32.74	106	39.18
25	1.4511	0.918	-26.01	109	41.82
26	1.4505	0.916	-22.62	109	42.55
27	1.4503	0.915	-21.78	109	45.18
28	1.4497	0.914	-18.26		45.87
29	1.4493	0.913	-16.89	90°/20 mm.	46.60
30	1.4493	0.912	-17.27		49.24
31	1.4490	0.912	-16.67		54.22
32	1.4488	0.912	-14.09		62.14
33	1.4490	0.913	- 7.23		62.81
34	1.4490	0.913	- 6.33		63.45
35	1.4490	-	- 6.33		64.12
36	1.4486	0.912	- 5.46		64.81
37	1.4489	0.913	- 9.12	90°/20 mm.	65.49
38	1.4488	0.913	-10.00		66.14
39	1.4487	-	- 6.97		66.81
40	1.4488	0.914	- 2.86		67.45
41	1.4490	-	+ 1.51		68.12
42	1.4490	-	+ 4.53		68.75
43	1.4490	0.914	+ 6.89		69.42
44	1.4490	-	+ 9.94		70.07

(Continued)

Table XLVII (Continued)

Properties of Fractions from Distillation of Cedar Oil

Fraction	η^{20}_{D}	d^{20}_{4}	α_D	b.p.	% Distilled
45	1.4490		+13.58		70.74
46	1.4490	0.914	+17.03		71.38
47	1.4490	0.915	+10.44		72.08
48	1.4492	0.915	+19.23		72.73
49	1.4494	-	+29.07		73.42
50	1.4494	0.914	+33.81		74.13
51	1.4494	-	+40.14		74.77
52	1.4494	-	+44.68		75.41
53	1.4496	0.915	+48.81		76.10
54	1.4495	0.914	+32.78		76.76
55	1.4495	0.917	+55.45		77.40
56	1.4495	0.917	+61.85		78.06
57	1.4494	0.916	+65.00		78.70
58	1.4504	0.913	+65.52	91°/20mm.	79.37
59	1.4568	0.934	+22.70		79.95
60	Solid, m.p. 90-110°				81.78
61	1.4726	0.949	+ 5.51°	102°/20 mm.	82.44
62	1.4740	0.940	+22.91	102	83.12
63	1.4750	0.939	+19.40	102	83.69
64	1.4760	0.944	+ 8.39	108.5	84.42
65	1.4693	0.958	- 3.30	110	85.08

(Continued)

Table XLVII (Continued)

Properties of Fractions from Distillation of Cedar Oil

Fraction	η^{20}_{D}	d^{20}_{4}	α_D	b.p.	% Distilled
66	1.4634	0.967	-11.83	110	85.79
67	1.4634	0.967	-18.02	110	86.43
68	1.4632	0.968	-22.32	110	87.13
69	1.4632	0.969	-27.38	110	87.81
70	1.4650	0.961	-26.44	98°/10 mm.	88.52
71	1.4650	0.960	-26.34	102	89.27
72	1.4659	0.958	-25.71	104	89.95
Nonvolatile residue					90.52
Sesquiterpenes and high boiling compounds					98.42

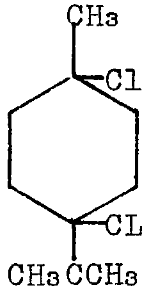
Table XLVIII

Properties of Sesquiterpene Fractions

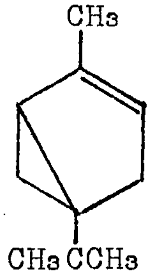
Fraction	η^{20}_{D}	d^{20}_{4}	α_D	Boiling range °C.	% of original oil
A	1.4655	0.962	-21.84°	96.4-98.2°/ 10 mm.	0.5
B	1.4645	0.962	-38.91	105.8-109.4/ 10 mm.	0.6
C	1.4955	0.968	+17.7	116-124/10 mm.	0.4
D	1.4888	0.983	-35.4	115.3-116.2/ 2 mm.	0.1
E	1.5033	1.026	-	159.3-161.0/ 1 mm.	0.1

Identification of Constituents

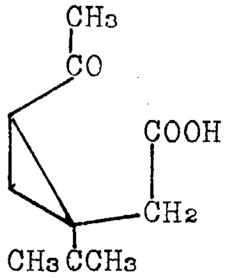
By redistillation of fraction 1, unidentified low boiling fraction b.p. 96-133°C., η_D^{20} 1.4265, α_D+ 1.160°, and a levo compound, b.p. 150.5 - 152.5°C., η_D^{20} 1.4559, α_D -14.76° were separated. The latter compound was l-thujene since it yielded terpinene hydrochloride when treated with hydrogen chloride, and was oxidized by permanganate to *l*- α-thujaketonic acid.



terpinene
dihydrochloride



α-thujene



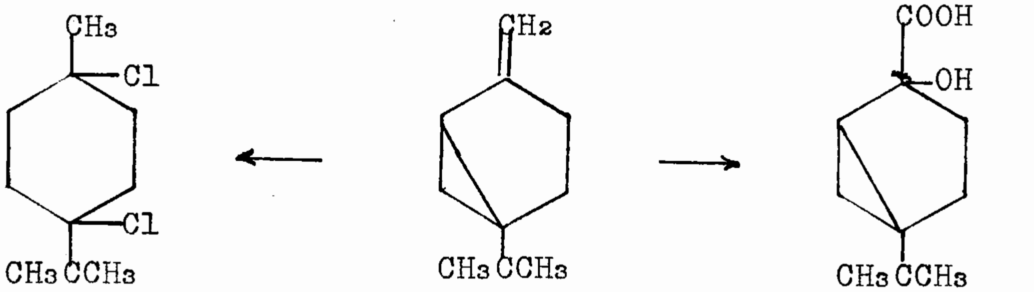
α- thujaketonic
acid

Fraction 5 was oxidized by permanaganate to yield dl-pinonic acid proving the presence of dl-α-pinene.

Although no solid terpene was isolated, the high negative rotation of fraction 9 suggested the presence of a small amount of camphene. Isoborneol was identified in the hydration products of this fraction.

Fraction 11 gave terpinene hydrochloride when treated with hydrogen chloride. Terpinene hydrochloride may be formed by the action of hydrogen chloride on α-thujene, sabinene, α-, β-, or γ-terpinene, as well as terpinen-4-ol and terpinen-1-ol (111).

When fraction 11 was oxidized with permanganate, the chief product of reaction was nopinic acid. This acid could only derive from β-pinene. However β-pinene cannot yield terpinene hydrochloride. Despite the fact that fractions 11 to 14 distilled with nearly constant properties the fraction must be a mixture of two terpenes with nearly identical boiling points. From the mother liquors from crystallization of nopinic acid a few milligrams of sabinenic acid were isolated. Separation of nopinic and sabinenic acids by fractional crystallization was very difficult: both acids are α-hydroxy acids forming sparingly soluble sodium salts; nopinic acid is the least soluble. For these reasons it is felt that the very low yield of sabinenic acid is still adequate for the characterization of sabinene. In addition, of all the terpenes capable of yielding terpinene hydrochloride, only sabinene has a boiling point very nearly identical with that of β-pinene. Simmonsen (112) reported a boiling point of 163-165°C. for d-sabinene.



Terpinene hydrochloride

Sabinene

Sabinenic acid

Fraction 16 reacted violently with maleic anhydride to give the adduct of myrcene.

dl-Limonene tetrabromide was prepared by bromination of fraction 21.

The ketone of fraction 23 was very unreactive. Impurities were removed by oxidation with hot nitric acid leaving pure fenchone m.p. 5.0°C., identified by preparation of the oxime.

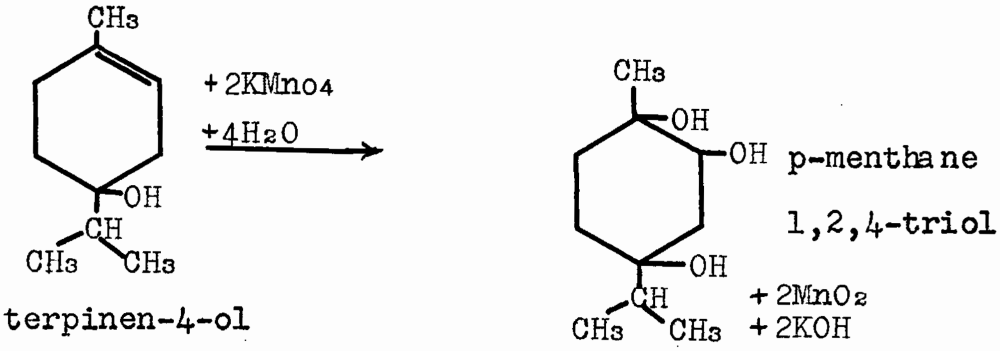
Fractions 26 to 58 were mixtures of two compounds difficultly separable by fractional distillation. Fraction 53 yielded a semicarbazone, m.p. 170-175°C., $[\alpha] + 180^\circ$ (C = 2.49 in chloroform), which must have been d-isothujone semicarbazone since it was hydrolysed to yield an oil, η^{20}_{D} 1.4494 d^{20}_{4} 0.916, $\alpha_D + 66.3$ which was oxidized by permanganate to α-thujaketonic acid.

For d-isothujone semicarbazone Short and Read (105) reported m.p. 172°C., $[\alpha] + 222^\circ$ (C = 1, methanol).

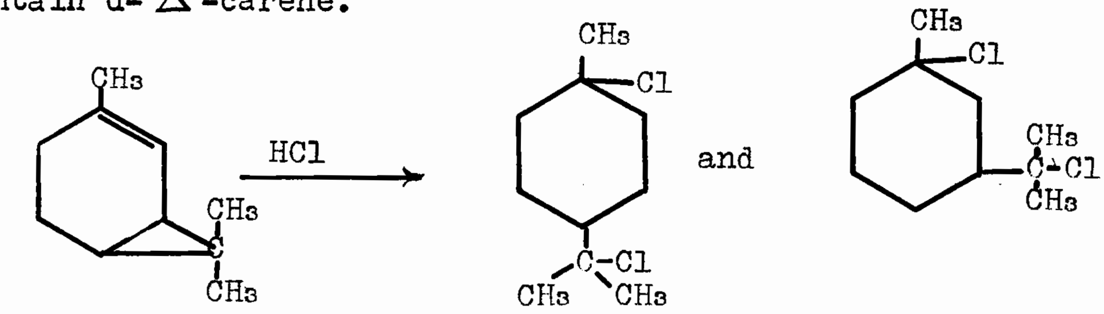
l - Thujone semicarbazone, m.p. 197-199°C., was obtained from fraction 32. Hydrolysis gave an oil, η^{20}_{D} 1.4490 which yielded α-thujaketonic acid on permanganate oxidation.

The solid fraction 60 was l-camphor, identified as the 2,4-dinitrophenyl hydrazone.

When fraction 62 was treated with hydrogen chloride a small amount of terpinene hydrochloride was obtained. This derivative could have been obtained from terpinen-4-ol, terpinen-1-ol or possibly γ-terpineol. When the compound was treated with the calculated amount of permanganate to form the glycerol, a compound was obtained which was soluble in water and separated as a gelatinous precipitate from benzene. Even after repeated crystallization from chloroform the product did not melt sharply but rectangular anisotropic needles melting up to 128°C. were obtained. p-Menthane 1,2,4-triol melts at 128-129°C. The glycerols derived from terpinen-1-ol and γ-terpineol melt at 120-121°C.,and 110-112 °C.respectively. (113)



The ester of fraction 69 was bornyl acetate. The dextrorotation of fractions 18 and 19 cannot be due to contamination with d-sabinene, since their boiling points differ from that of d-sabinene by more than 6° C. Fraction 19 gave dl-limonene hydrochloride when treated with hydrogen chloride. The hydrochloride of fraction 18 melted at 71.5-72°C. and may have been sylvestrene hydrochloride, m.p. 72°C. No authentic sample was available. Both fractions failed to form crystalline nitrosites or nitrosates indicating the absence of Δ^3-carene, α- or β-phellandrene. These fractions may contain d-Δ^4-carene.



Δ^4-carene

limonene hydrochloride

sylvestrene hydrochloride

The five sesquiterpene fractions were of questionable purity. Fraction A was mainly l-bornyl acetate. Fraction B was an ester which on saponification yielded acetic acid and an alcohol with properties n^{20}_{D} 1.4748, d^{20}_{4} 0.935, α_D -49.62°. The alcohol formed a p-nitrobenzoate m.p. 140-141.3°C., $[\alpha]_D$-38.1° (C = 5.67, chloroform) which does not appear to have been previously described. It failed to form a urethane with phenyl isocyanate, and gave only oily products with hydrogen chloride.

Fraction C when dehydrogenated with palladium-charcoal (114) gave a small amount of cadalene picrate.

At 100°C. the fraction reacted violently with sodium, but below 70°C. the reaction proceeded sluggishly or not at all. It failed to yield crystalline products with p-nitrobenzoyl chloride or phenyl isocyanate. The fraction may have contained an oxygenated derivative of a sesquiterpene related to cadalene. However, the yield of cadalene was low and the purity of the fraction questionable.

Fractions D and E yielded no aromatic products on dehydrogenation. Too little material was available to permit more detailed examination of these fractions.

Percent Composition of the Oils

From the distillation data for each oil a plot of properties against percent distilled was made, and the inflection points in the curve representing each property were marked. The percent occurrence of each compound was given by the distance between the inflection points of the corresponding plateau. The percentage compositions of the oils are summarized in Table XLVIX.

Table XLVIX

Present Composition of the Oils

Species	Picea Mariana	Tsuga canadensis	Abies balsamea	Thuja occidentalis
α-pinene	16	17.6	8.4	2.6
camphene	10	11.5	6.8	2.0

(Continued)

Table XLVIX (Continued)

Present Composition of the Oils

Species	Picea mariana	Tsuga canadensis	Abies balsamea	Thuja occidentalis
β-pinene	6.5	2.9	36.1)	3.5
sabinene	-	-	-)	
limonene	6.5	5.9	11.1	3.6
myrcene	3.5	2.4	2.3	1.3
tricyclene	1	2.2	1.3	-
santene	2.5	-	1.3	-
Δ^3-carene	5	-	11.1	-
α-phellandrene	-	1.8	-	-
α-thujene	-	-	-	0.8
terpinolene	1	-	0.05	-
bornyl acetate	37	43.4	14.6	5.9
camphor	1	-	0.2	2.5
thujone	-	1.3	-	56.7
borneol	1	-	0.1	-
fenchone	-	-	-	7.8
terpinen-4-ol	-	-	-	2.7
sesquiterpene - related to cadinene	1	0.2	0.1	-
bisabolene	-	-	0.1	-
unidentified esters	0.5	-	-	0.6
unidentified alcohols	-	1,3	0.4	-
unidentified ketones	-	-	0.2	-

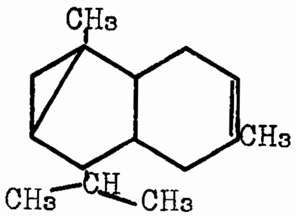
The Dextrorotatory Sesquiterpenes

The hydrochloride of l-cadinene was prepared from fractions of oil of Picea mariana, Tsuga canadensis, and Abies balsamea despite the fact that these fractions had dextrorotations of +25.6°, +26.8° and + 22.8° respectively. According to Campbell and Soffer (115) pure l-cadinene prepared by dehydrohalogenation of the crystalline hydrochloride has properties n_D^{20} 1,5071, d_4^{20} 0.9199, b.p. 136°-138° C./11 mm. α_D^{25} -113.0°. In the absence of a reliable estimate of homogeneity it might be concluded that the sesquiterpene fractions contain l-cadinene and a second component responsible for the dextrorotation of the fraction. However, cadalene was obtained in a yield of 32.5% from the sesquiterpene of Abies balsamea. Linstead and co-workers obtained cadalene in 50% yield by dehydrogenation of pure l-cadinene under comparable conditions (116)

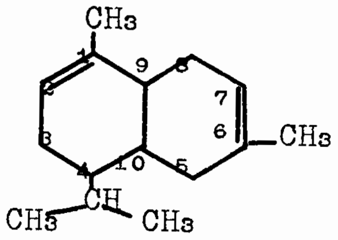
Along with a small amount of bisabolene hydrochloride l-cadinene hydrochloride was obtained in 33.7% of theoretical yield from the balsam sesquiterpene. Under the same conditions a 64.7% yield of hydrochloride was obtained from pure l-cadinene regenerated from the hydrochloride. Thus if l-cadinene were the precursor of these derivatives it would consitute, at a minimum, 60% of the fraction. Since bisabolene, which is optically inactive, is present in detectable quantities, the consituent responsible for the dextrorotation of the fraction could not amount to more than about 30% of the fraction. Accepting the value of D - 113.0° for 1-cadinene, the relation of the dextrorotatory compound would have to be about +300° to account for the observed rotation of +22.8° of the balsam fraction . No sesquiterpene with a rotation even approaching +300° is known. More probably the dextrorotation is associated with the precursor of the cadalene and 1-cadinene hydrochloride.

Deussen (117) was first to isolate a dextrorotatory sesquiterpene, from West Indian sandlewood oil, which gave the hydrochloride of 1-cadinene. Later in a study of African copaiba balsam he found that as the optical rotation rose with the purification of the fraction, the yield of hydrochloride increased (118). The fraction with a rotation of +51° gave the hydrochloride in 50% yield. These sesquiterpenes he called "d-cadinene".

Later it was shown that African copaiba balsam contains a tricyclic sesquiterpene, copaene, which is converted to 1-cadinene hydrochloride by the action of hydrogen chloride (119).



Copaene



1-Cadinene

Wallach first suggested the dehydrohalogenation of the crystalline hydrochloride as a means of preparing pure cadinene (120). The accepted structure of cadinene is based on studies of a sesquiterpene prepared in this way. (115). However it has never been shown that the regenerated sesquiterpene is identical with the original sesquiterpene.

Fraction 48 of oil of Picea mariana was subjected to careful fractional distillation to separate a lower boiling levorotatory fraction and the sesquiterpene fractions listed in Table L.

Table L

Redistillation of Black Spruce Sesquiterpene

Fraction	n_D^{20}	α_D	Fraction	n_D^{20}	α_D
1	1.5013	+31.2°	10	1.5082	+69.5°
2	1.5035	+40.6	11	1.5088	+71.9
3	1.5048	+46.3	12	1.5091	+73.2
4	1.5058	+51.3	13	1.5093	+74.0
5	1.5061	+56.1	14	1.5093	+74.7
6	1.5065	+58.8	15	1.5095	+75.0
7	1.5070	+61.9	16	1.5100	+73.5
8	1.5076	+65.2	17	1.5102	+65.5
9	1.5080	-	18	1.5096	-

Fraction 15 gave the hydrochloride in 43.7% yield.

The infra-red absorption spectrum of fraction 15 was compared with the spectrum of l-cadinene prepared from the hydrochloride*. Both spectra showed similar functional group absorption bands, but l-cadinene showed an additional weak band at 1740 cm.$^{-1}$. In the characteristic absorption band region l-cadinene had bands of moderate intensity at 911, 823, 790 and 760 cm.$^{-1}$, which were not present in the spectrum of the spruce sesquiterpene. It is therefore concluded that the spruce sesquiterpene does not contain detectable amounts of the compound produced by the dehydrohalogenation of l-cadinene hydrochloride.

The rate of oxidation of fraction 15 with perbenzoic acid is shown in Table LI.

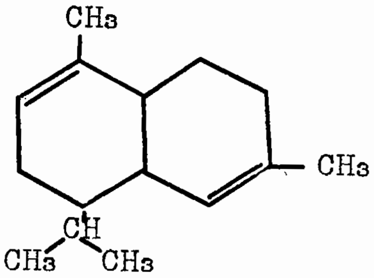
* The writer is indebted to Miss E.M. Kirby who recorded the spectra of these compounds, and assisted in their interpretation.

Table LI

Oxidation of Sesquiterpene with Perbenzoic Acid

Time	Equivalents oxygen / mole sesquiterpene
5 min.	1.25
30 min.	1.64
60 hr.	1.79
96 hr.	1.86

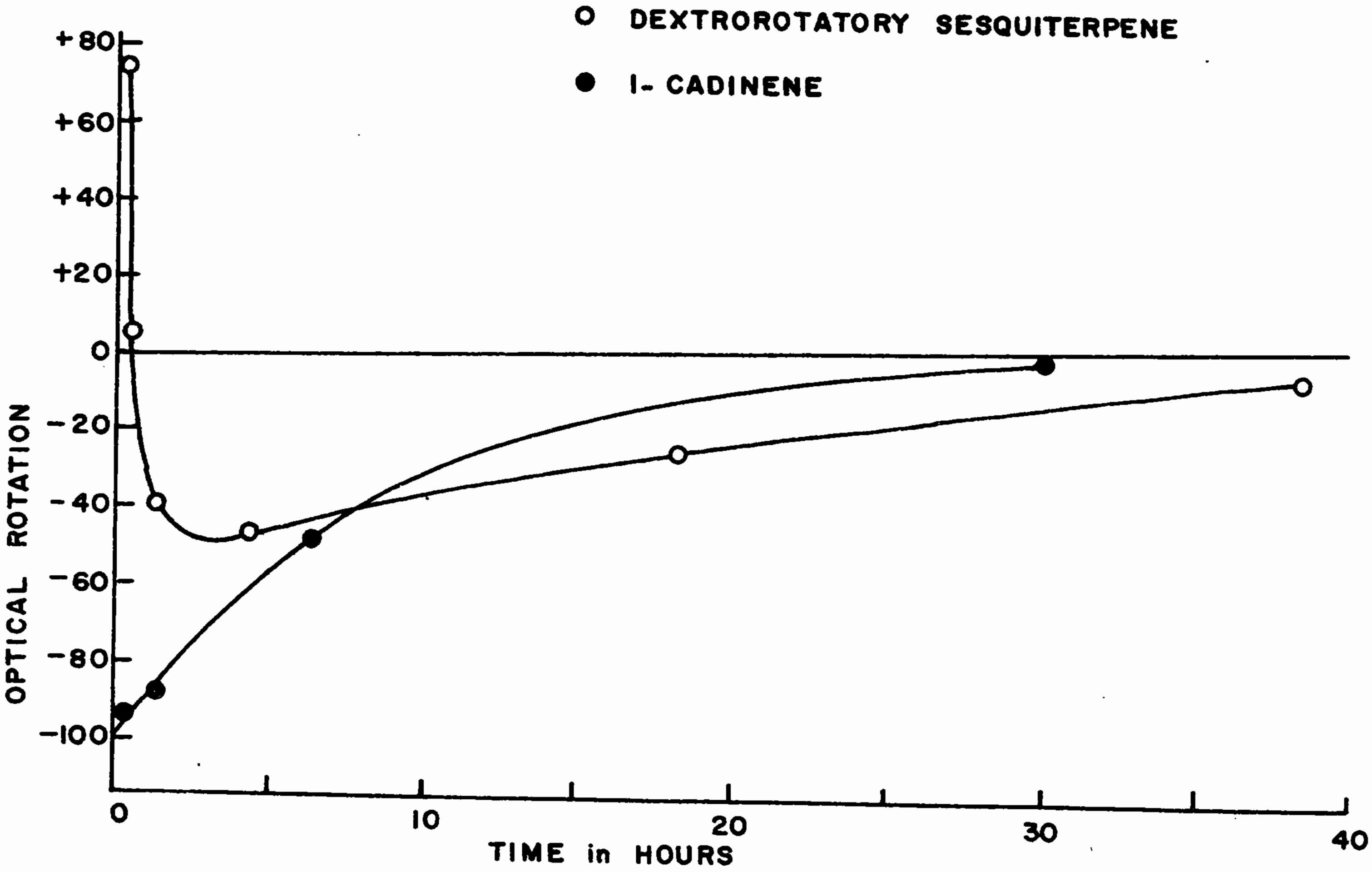
The cyclopropane ring is stable to perbenzoic acid. The spruce sesquiterpene is therefore doubly unsaturated, and not a tricyclic sesquiterpene similar to copaene. It must have the same steric configurations as l-cadinene about the asymmetric carbon atoms in the 4, 9, and 10 positions. If the structure of Campbell and Soffer for l-cadinene is accepted and it is assumed that no migration of the double bonds of the spruce sesquiterpene occur under the influence of acid, then the structure of the spruce sesquiterpene must be



However, Figure 10 shows that when the spruce sesquiterpene is heated with dilute sulphuric acid, it undergoes a rapid inversion followed by a slow racemization. When l-cadinene is heated under similar conditions a slow racemization is observed. The product of inversion is probably l-cadinene. It was observed that the product of maximum levorotation isolated after three hours isomerization still gave the hydrochloride in a yield of 28% of theoretical. The decreased yield of hydrochloride is explained by the formation of racemate; the almost completely racemized product formed by thirty-eight hours isomerization yielded the hydrochloride in a yield of only 3.4%.

It is concluded therefore that the black spruce sesquiterpene has the same steric configuration as l-cadinene about carbon atoms 4, 9, and 10 and has a system of double bonds which migrate under the influence of acid to the 1, 2 and 6, 7 position. The most probable positions are the 1, 2 and 5, 6; the 2, 3 and 7, 8 positions are possible alternate locations of the double bonds.

FIGURE 10 ISOMERIZATION of SESQUITERPENES



EXPERIMENTAL

Preliminary Separations of Canadian Tall Oils

Preparation of Tall Oil

Tall oil soap supplied by the Brown Corporation, LaTuque, Quebec, was used in these and all subsequent preparations.

Method A - acidification of the whole soap: To 10 g. of tall oil soap in a 250-ml centrifuge tube was added 50 ml. of boiling water and the mixture was stirred vigorously. A buff colored suspension resulted. The mixture was acidified to pH 4 with 50% sulphuric acid, then centrifuged for fifteen minutes and the lower aqueous layer withdrawn. The oil was washed in the centrifuge tube with 50 ml. of hot water then transferred to a tarred 50-ml flask using 50 ml. of benzene. Solvent and residual water were removed by distillation and the residue dried at 50°C. in a vaccum drying oven. The product was a black, heterogeneous, semi-solid mass weighing 6.64 g.

Method B - acidification of the soap in the presence of ethylene dichloride:- To 50 ml. of a 20% aqueous solution of the soap at 70°C. was added 10 ml. of ethylene dichloride and 2 g. of a Celite filter-aid. The mixture was acidified to pH 4 with vigorous stirring, then allowed to settle. The upper aqueous layer was decanted and the lower layer filtered under suction. The filter cake was washed with 10 ml. of warm ethylene dichloride. The filtrate was washed with water until the washings were neutral to litmus. After removal of the solvent, a viscous reddish black oil weighing 5.72 g. was obtained. The oil was completely soluble in methanol, acetone or benzene: the brown amorphous residue on the Buchner funnel was insoluble in these solvents.

The sterol determinations reported in Table XII were carried out on tall oils prepared by Burch by a method similar to method A. In the remainder of this thesis the term "tall oil" will be understood to mean that oil which is produced when the tall oil soap of Brown Corporation is treated according to method B.

Methods of Analysis

Unsaponifiable material:- A 5.00 g. sample of tall oil was boiled under reflux for ninety minutes with 12.5 ml. of 2N potassium hydroxide in methanol. The mixture was diluted with 50 ml. of water and extracted with four 30-ml. volumes of diethyl ether. The combined ether extracts were gently swirled with 20 ml.

of water, then shaken successively with 2, 5 and 20 ml. of water. Ether was removed by distillation and the residue, consisting of unsaponifiable material, was dried at 50°C. in a vacuum drying oven, cooled and weighed

$$\% \text{ unsaponifiable matter} = \frac{\text{weight of residue} \times 100}{\text{weight of sample}}$$

Neutral material:- A solution of 5.00 g. of tall oil in 10 ml. of methanol was shaken with 50 ml. of 0.5 N aqueous potassium hydroxide. The resulting soap was extracted with four 30-ml. volumes of ether. The extracts were washed and the neutral material recovered exactly as in the determination of unsaponifiable material.

Fatty acids:- The aqueous extracts and wash waters from the determination of unsaponifiable material or neutral material were combined, acidified, and extracted with four 10-ml. volumes of diethyl ether. The ether was removed by distillation and the weight of the residue estimated to the nearest gram. For each gram of residue 10 ml. of methanol and 0.2 ml. of concentrated hydrochloric acid was added and the mixture boiled under reflux for three hours. The bulk of the methanol was distilled and the residue transferred to a separatory funnel with 50 ml. of diethyl ether. The ether solution was shaken repeatedly with

10-ml. volumes of 2% aqueous potassium hydroxide solution, until a clear colorless extract was obtained. The combined aqueous extracts were back extracted with three 10-ml. volumes of ether. The combined, washed ether extracts were drained into a tarred flask and ether removed by distillation. Any droplets of water remaining with the esters were removed as an azeotrope with benzene. Traces of benzene were removed at 50°C. in a vacuum drying oven.

$$\% \text{ fatty acids as oleic acid} = \frac{\text{weight of residue} \times 282.5}{\text{weight of sample} \times 2.965}$$

Resin acids:- The combined aqueous extracts and wash waters from the determination of fatty acids were acidified and extracted with three 10-ml volumes of diethyl ether. The extracts were washed with water and the ether distilled. Traces of water were removed as an azeotrope with benzene. The acids were then dried at 50°C. in a vacuum drying oven and weighed.

$$\% \text{ resin acids} = \frac{\text{weight of residue} \times 100}{\text{weight of sample}}$$

Sterol content:- Unsaponifiable material isolated as previously described, was dissolved in about 100 parts of ethanol and an aliquot withdrawn. The size of the aliquot was calculated to give a sample containing about 0.03 g. of sterol. Alcohol was removed by heating at 100°C. for one hour. The weight of the sample was then accurately determined. This procedure was necessary to ensure uniform sampling of the heterogeneous fraction.

To the sample dissolved in 15 ml. of 90% ethanol was added 40 ml. of a 0.5% solution of digitonin in 90% ethanol. The solution was warmed to 60°C. and allowed to stand one hour. The precipitate was collected on a Gooch crucible, washed with ethanol and dried at 110°C.

% sterols in unsaponifiable material

$$= \frac{\text{weight of precipitate} \times 23.9}{\text{weight of sample}} \quad (121)$$

Molecular weight of the fatty acids:- Fatty acid methyl esters (2 g.) isolated during gravimetric analysis of the oil were boiled under reflux for one hour with 35.00 ml. of 0.5 N potassium hydroxide in ethanol in a 250-ml. Kimble glass flask. Excess alkali was titrated with standardized 0.5 N sulphuric acid using phenolphthalien indicator. A blank determination was carried out. The molecular weight of fatty acid was calculated from the equation

$$\text{molecular weight} = \frac{100W}{(A-B)N} - 14.01$$

W = sample weight in g.

A = ml. of standard acid consumed by the blank

B = ml. of standard acid used in titration of sample

N = normality of standard acid

Iodine number of the fatty acid esters:- To an accurately weighed 200- to 300-mg. sample of fatty acid methyl esters in a 250-ml. glass stopped conical flask was added about 15 ml. of chloroform and exactly 30.00 ml. of Wijs reagent (iodine monochloride in glacial acetic acid) and 10 ml. of mercuric acetate in glacial acetic acid. The flask was stoppered and allowed to stand in the dark. After three minutes 15 ml. of 15% aqueous potassium iodide and 75 ml. of water was added and excess iodine titrated with O.I.N sodium thiosulphate using a starch indicator. The iodine number was calculated from the equation

$$\text{Iodine number} = \frac{1.269\ (A-B)}{NW}$$

A = ml. of thiosulphate consumbed by the blank.

B = ml. of thiosulphate used in titration of sample.

W = sample weight in g.

N = normality of sodium thiosulphate solution.

This is essentially the Wijs procedure as modified by Hoffman and Green (122).

Molecular Distillation of Tall Oil

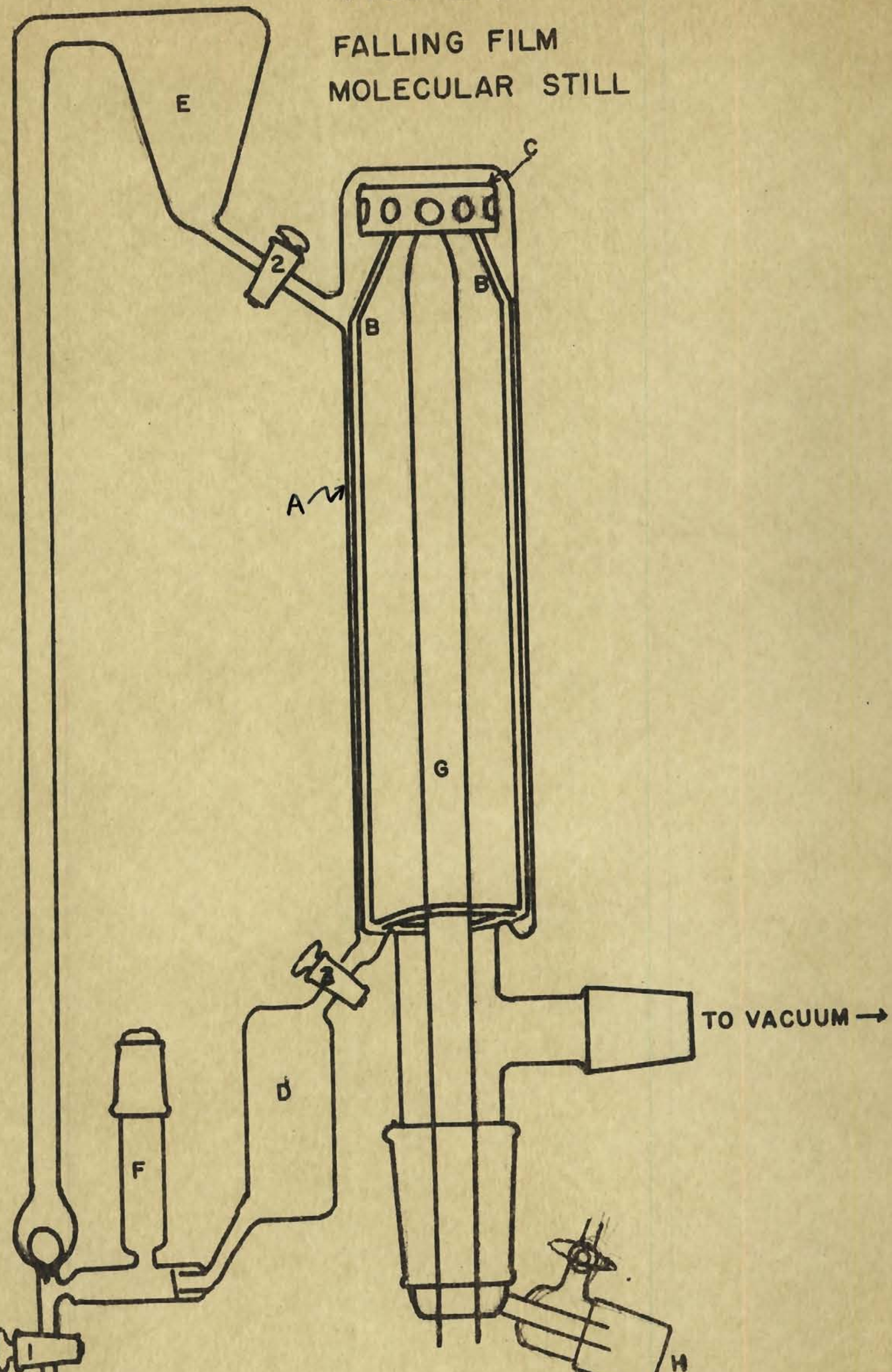
Apparatus:- A cyclic, falling-film molecular still of the type described by Quackenbush and Steenbuck (123) was constructed.

The circulating pump described by these workers was found to be inadequate and was replaced by a magnetic circulating pump of the type described by Oliver(124). Vacuum was maintained by a mercury diffusion pump (Ace Glass catalogue No. 8705) backed by a Duoseal mechanical pump. Pressure was measured with a Pirani gauge connected to the apparatus at a point between the Dry Ice trap and the pumping system. The apparatus is illustrated in Figure 11. The evaporating surface, A, was constructed of a Pyrex tube 50 mm. in diameter and 450 cm. long. It was wound with nichrome ribbon and heated electrically. The temperature was measured by means of a copper-constantan thermocouple in intimate contact with the external surface of the tube and located near the middle of the tube and equidistant between the two turns of the nichrome ribbon. The entire tube was insulated with about 5 cm. of asbestos. Temperature was controlled with a rheostat.

Oil was distributed over the evaporating surface in a "windshield wiper fashion" by three longitudinal glass rods, B, suspended from the rotating disc, C. Alnico bar magnets were embedded in the disc and a corresponding set of external magnets were motor driven at a rate of 20 to 40 revolutions per minute to provide motive power. Distilland was collected in the annulus at the bottom of the evaporating surface, drained to the

FIGURE 11

FALLING FILM
MOLECULAR STILL



lower reservoir, D, and finally returned to the upper reservoir, E, by means of the magnetically operated circulating pump F. The distillate which condensed on the water cooled condensor, G, drained and was collected in the receiver, H.

Procedure:- The still was evacuated and the oil charge admitted through stopcock 1. Stopcock 2 was closed and the oil was pumped to the upper reservoir E. The temperature of the evaporating surface was raised to 60°C. Oil was allowed to drip at about two drops per second through stopcock 2 and spread over the warm surface. As soon as the upper chamber had emptied the entire oil charge was returned to it by means of the circulating pump. The oil was cycled through the apparatus in this manner until the pressure within the system fell below 10 microns of mercury.

The evaporating surface was then raised to the first distillation temperature and the oil fed from the upper reservoir at 4 to 8 drops per second throughout the duration of the cycle. At the conclusion of the cycle stopcock 2 was closed and the surfaces allowed to cool and drain. Stopcock 3 was closed and air admitted to the remainder of the apparatus. The condenser was removed at the standard taper joint and adhering distillate washed into the receiver with diethyl ether.

The apparatus was then reassembled, the vacuum again established, the distilling surface heated to a new, higher temperature, and the second cycle carried out as before.

Ether was removed from the distillates by distillation and by heating at 50°C. in the vacuum drying oven. Distillate samples were then weighed and analysed for fatty acids, resin acids, neutral material and sterols. The iodine numbers and molecular weights of selected fatty acid esters were determined.

Preparation of triölein:- A non-volatile diluent and carrier for tall oil was required during some distillations. Triölein was selected as the best meeting the requirements of such a diluent. It has sufficiently low viscosity to permit easy circulation through the still; is substancially non-volatile in the molecular still at temperatures below 180°C., it is a good solvent for tall oil and tall oil constituents. The sole disadvantage of the triölein diluent is its chemical similarity to some of the tall oil constituents.

Triölein was prepared by the method of Baxter, Gray and Fischer (125). A mixture of 474 g. of oleic acid(C.P., General Chemical Corporation), and 50 g. of glycerine in a three-necked flask was heated at 120°C.

for four hours with vigorous stirring. The flask was then fitted with a 12-in. distilling column and water trap as shown in Figure 12, and 100 ml. of chlorobenzene was added. The mixture was slowly distilled, the pot temperature being maintained at 170 - 175°C. After eighteen hours the reaction mixture had ceased to evolve water. Chlorobenzene was removed by distilling at about 20 mm. pressure until the pot temperature reached 175°C., The product was mixed with one-half volume of ethanol, decolorized with carbon and neutralized to pH7with potassium hydroxide. One volume of water was added and the ester extracted with ether. The product was heated at 50°C. and 1 mm. pressure for sixteen hours to remove as much volatile material as possible. Triölein was obtained in a yield of 460 g.

<u>Molecular distillation I</u>:- A mixture of 150 g. of tall oil and 150 g. of triólein was circulated through the still for two hours to remove dissolved gasses and volatile solvents. The first fraction was distilled at 100°C. The evaporator temperature was increased in 10°C. increments each forty minute cycle. From the weights and analyses of the charge and the distillates the following recoveries were calculated:

Weights Charged			Weights Recovered		
Tall Oil	-	150 g.	Distillate	-	123.3 g.
Fatty Acids	-	62.1 g.	Fatty Acids	-	58.8 g.
Resin Acids	-	37.2 g.	Resin Acids	-	27.0 g.
Neutrals	-	46.1 g.	Neutrals	-	37.4 g.
		145.4 g.			123.2 g.

Molecular distillation II: - A mixture of 150 g. of tall oil and 150 g. of triolein with cycle times of thirty minutes and temperature increments between fractions of 10°C. In addition to analyses for fatty acids, resin acids, neutrals and sterols, the esterified acid content of the neutral fraction was determined by saponifying the neutral material and determining its unsaponifiable contents and acid contents by the gravimetric procedures described under "Methods of Analysis".

The following recoveries were obtained:

Weight Charged			Weights Recovered		
Tall Oil	-	150 g.	Distillate	-	134.0 g.
Fatty Acids	-	62.1 g.	Fatty Acids	-	61.8 g.
Resin Acids	-	37.2 g.	Resin Acids	-	27.2 g.
Neutrals	-	46.1 g.	Neutrals	-	45.0 g.

Isolation of n-Tetracosanic acid:- The fatty acid methyl esters of fractions 8, 9, 10, and 11 from molecular distillation II were solid at room temperature. The free fatty acids derived from fraction 9 were recovered by acidifying the aqueous solution from the analytical saponification and extracting with ether. The crude acids remaining after evaporation of the ether were crystallized three times from methanol. The product weighed 0.34 g. and melted at 80-81°C.

This acid (0.26 g.) was boiled under reflux for three hours with 5 ml. of methanol containing 0.1 ml. of concentrated hydrochloric-acid. The mixture was poured into water and the precipitated ester separated and recrystallized from methanol. The ester formed small colorless platelets, m.p. 56.5 - 57°C.

Esterification of Tall Oil Alcohols with Tall Oil Acids

Extraction of neutral material:- A solution of 250 g. of tall oil soap in 2000 ml. of water was exhaustively extracted with diethyl ether in a continuous apparatus. Tall oil was then prepared from the extracted soap solution. Yields of neutral material and extracted tall oil were 42 g. and 129 g. respectively.

Esterification at 96°C.:- Neutral material (20.2 g.) and extracted tall oil (62.0 g.) in about 60 ml. of benzene were introduced into the esterification apparatus shown in Figure 12 but fitted with a water trap for use with solvents lighter than water. Heat input was adjusted to maintain distillation of the benzene at about two drops per second. Temperature of the reactants remained at 96 ± 0.5°C. throughout the experiment. Samples were withdrawn at intervals and without prior removal of solvent were separated into neutral and acid fractions. The sterol contents of the neutral fractions were determined by precipitation with digitonin.

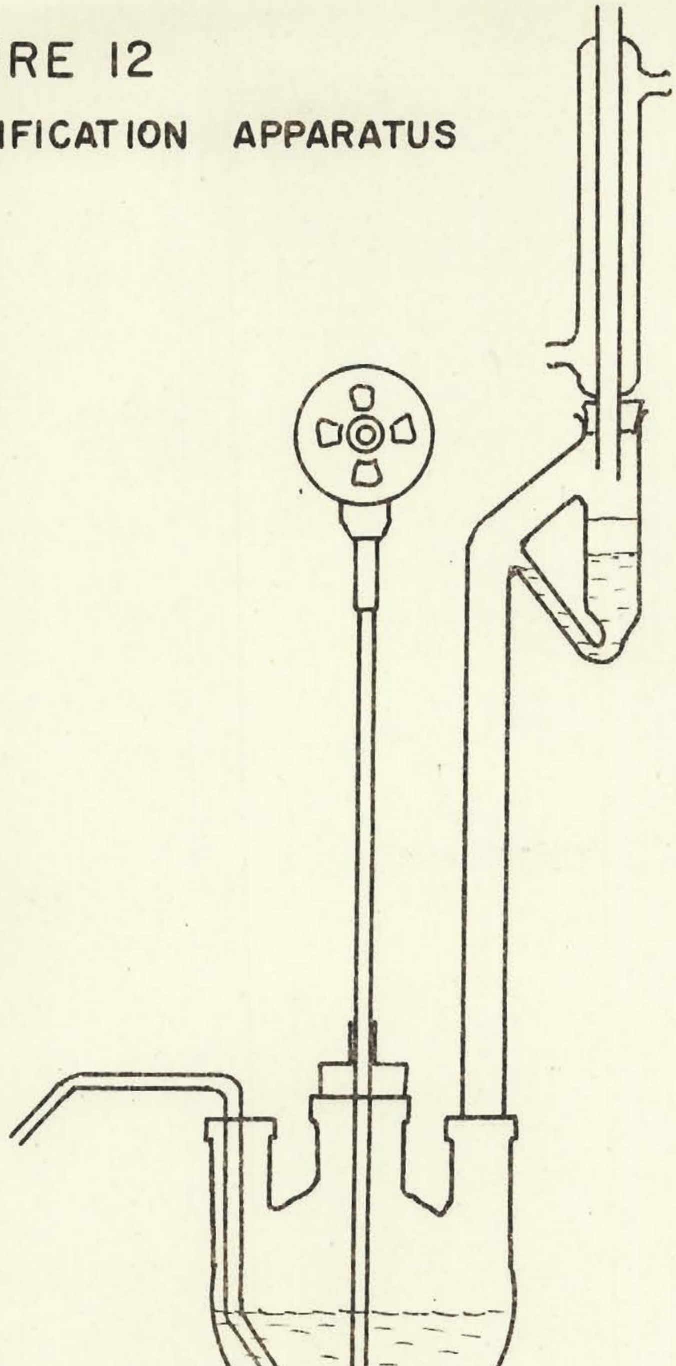
Esterification at 133°C.:- A solution of 17.7 g. of neutral material and 62.1 g. of extracted tall oil in 60 ml. of toluene were slowly distilled in the esterification apparatus. The temperature of the reactants remained at 133 ± 1°C. Samples were withdrawn periodically and analysed for neutral material, unesterified sterols and acids.

Crystallization of Tall Oil at Low Temperatures

Apparatus:- The tank in which the tall oil solutions were cooled and filtered was an adaptation of a design by Quackenbush and Steenbock (126). The apparatus is illustrated in Figure 13. It consisted of a metal tank insulated on the bottom and sides with three inches of corrugated cardboard and asbestos fibre. The tank was contained in a wooden box fitted with an insulated lid. A mechanically driven stirrer and a glass

FIGURE 12

ESTERIFICATION APPARATUS



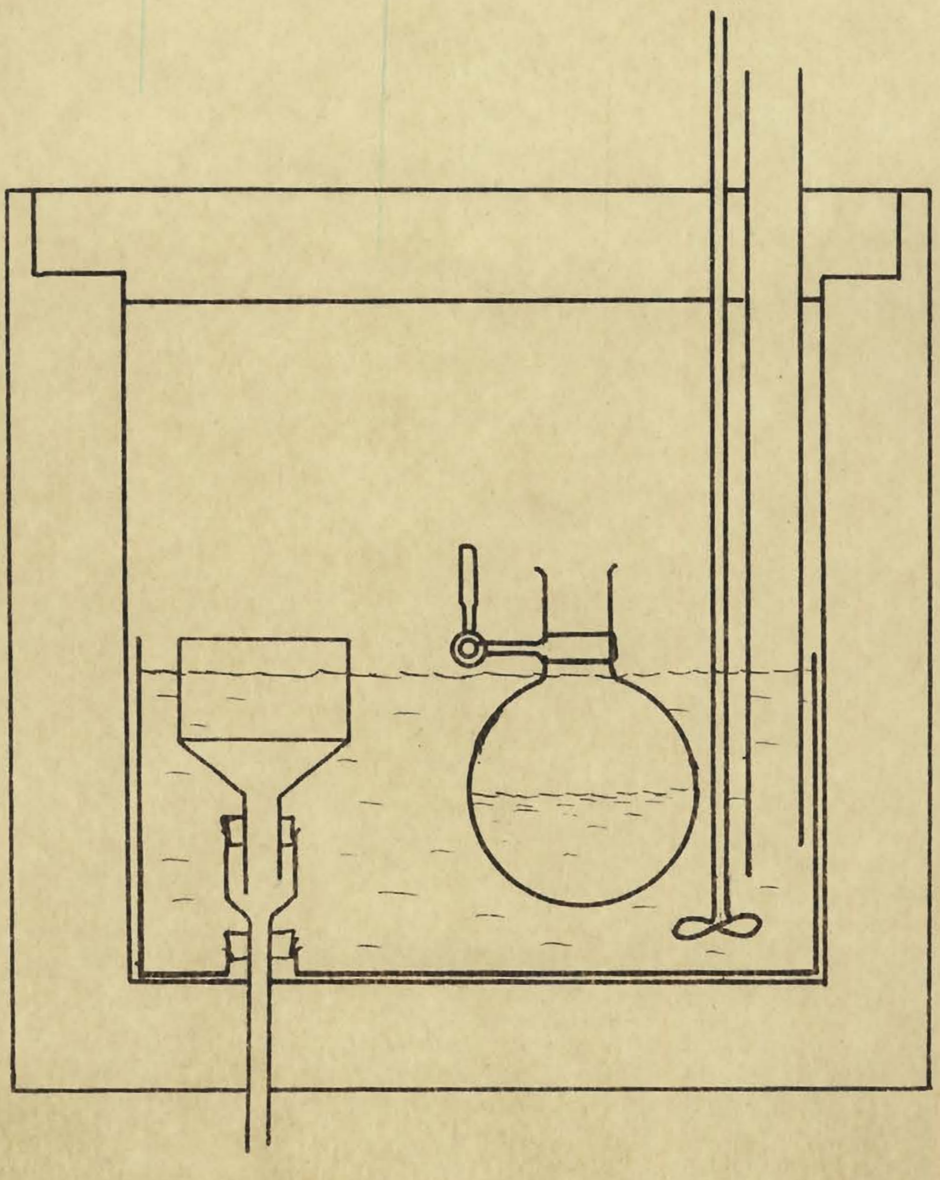
tube for the addition of solid carbon dioxide passed through the lid into the tank. The stem of the Büchner funnel located in one end of the tank passed through the bottom of the tank and connected by means of a glass tube to the filter flask located beneath the box. The seam between the funnel stem and tank was made water-tight with Litharge cement. A 500-ml. Florence flask was suspended in the other end of the tank from a metal bar so placed that when the bar was rotated the flask was lifted from the tank and inverted over the funnel.

Procedure:- The tank was filled with ethanol and the Florence flask containing a solution of tall oil in an organic solvent was immersed in the bath. Solid carbon dioxide was added to the ethanol with mechanical stirring until the desired temperature was reached. The temperature was maintained by small additions of solid carbon dioxide. The temperature of the cold bath was measured with a copper-constantan thermocouple.

After four or five hours of refrigeration the solids were collected on the filter. The filter paper was removed from the funnel and the crystallized material washed from the paper and funnel with hot solvent. Solvent was removed from the crystallized material and the mother liquors by distillation followed by heating at 50°C. in the vacuum drying oven.

FIGURE 13

LOW TEMPERATURE CRYSTALLIZATION TANK



Crystallization from petroleum ether I:- A solution of 45 g. of tall oil in 450 ml. of petroleum ether, b.p. 30-60°C., was refrigerated at 0°C. for four hours and filtered. Precipitate weighing 3.27 g. was removed. The mother liquors were returned to the tank at -20°C. for four more hours and another 3.78 g. of precipitate removed. Precipitates weighing 1.45 g., 1.38 g. and 0.70 g. were removed by refrigeration, for four hours in each case, at -40°C., - 60°C., and -70°C. respectively.

Crystallization from petroleum ether II:- A solution of 50 g. of tall oil in 250 ml. of petroleum ether, b.p. 30-60°C., was refrigerated for five hours at 0°C., and 3.50 g. of precipitate removed. The mother liquor was refrigerated at -30°C. for five hours and another 4.69 g. was crystallized at -70°C. for five hours.

Crystallization from petroleum ether III:- Fractions were removed from a solution of 50 g. of tall oil in 100 ml. of petroleum ether, b.p. 30°-60° C., by refrigeration for five hours at 0°C., -30°C., and -70°C. Only a trace of material crystallized at 0°C., but 8.39 g. and 3.41 g. were removed at -30°C. and -70°C.

Crystallization from ethanol:- A solution of 50 g. of tall oil in 250 ml. of 95% ethanol was refrigerated at -70°C. for five hours, yielding 17.22 g. of crystallized solids and a soluble oil. The solids in 172 ml. of ethanol were refrigerated for five hours at 0° C. and -30°C. Two fractions insoluble at 0° C. and weighing 4.20 g. and 3.53 g. were removed from 6.82 g. of an oil soluble at -30°C.

<u>Crystallization from acetone</u>:- A solution of 50 g. of tall oil in 250 ml. of acetone was refrigerated at -70°C. for five hours, yielding 18.45 g. of crystallized solids and oil, soluble at -70° C. The crystallized solids in 92.5 ml. of acetone were separated into two fractions crystallizing at 0° C. and -30° C. and a fraction soluble at -30° C.

Analysis of the fractions:- The sodium soaps of the fractions insoluble ethanol at 0°C. and -30° C. and of the fraction insoluble in acetone at 0° C. were insoluble in water. When suspensions of these soaps were shaken with ether, stable emulsions were formed. The standard methods of analysis could not be used for the examination of these fractions. The alkaline suspension was treated with excess calcium chloride and the calcium chloride and the calcium soaps coagulated by heating and subsequent cooling. The soaps were collected on a wire screen and extracted with ethyl ether in a Soxlet apparatus. Neutral material was recovered from the ether extract. Because of mechanical losses incurred during these manipulations it was not possible to determine the fatty acid and resin acid contents.

Isolation of N-tetracosanic acid:- The fatty acid methyl esters (4.4 g.) prepared from the fraction insoluble in two volumes of petroleum ether at -30° C. were twice crystallized from methanol. The small white platelets melted at 55-56°C. alone, and at 55-56.5° C. in admixture with the ester prepared from fraction 9, molecular distillation II. A sample of the ester weighing 0.611 g. was saponified, consuming 3.40 ml. of 0.5065N alkalie. The observed molecular weight was 384; the molecular weight calculated for $C_{25}H_{50}O_2$ is 382.6. The acid isolated from the saponification mixture melted at 81-82°C.

The extracted calcium soaps prepared from the fraction insoluble in acetone at 0°C. (about 3 g.) were boiled with 25 ml. of 10% aqueous sulphuric acid, cooled, and extracted with ether. The residue from the ether extraction weighed 1.89 g. after crystallization from acetone and melted at 81-82°C. alone or in admixture with N-tetracosanic acid isolated from the saponification mixture.

<u>Isolation of N-tetracosanol</u>:- A 0.0921 g. sample of the neutral material prepared from the fraction insoluble in acetone at 0°C. was warmed to 60°C. with 40 ml. of a 0.5% solution of digitonin in ethanol. The precipitate was removed by filtration and the filtrate evaporated to dryness. The residue was extracted with diethyl ether, the ether evaporated and the residue crystallized from methanol. White platelets (0.03 g.) melting at 76-76.5°C. were obtained. This material with 2.5 ml. of pyridine and 1.0 ml. of acetic anhydride was boiled under reflux for one hour, then poured into 10 ml. of water. The insoluble product was twice crystallized from a minimum amount

of methanol to yield 0.01 g. of white platelets melting at 56°C.

Sterol-Oxalic Acid Addition Compounds

Preparation of anhydrous oxalic acid:- Anhydrous oxalic acid was prepared by the method of Organic Syntheses (127). Oxalic acid dihydrate was powdered in a mortar to pass a 40-mesh screen. The powder was spread in a layer 3 to 4 mm. deep on a glass tray and heated in an oven for two hours at 99-100°C. The anhydrous acid was quickly ground in a mortar and stored in a desiccator over phosphorous pentoxide.

Preparation of addition compounds:- Mixtures of tall oil, solvent, and anhydrous oxalic acid in various proportions were boiled under reflux for two hours, allowed to cool to room temperature and chilled overnight in an ice chest. The product was collected on a sintered glass Gooch crucible, washed with a minimum amount of cold ethylene dichloride, dried at 50°C. in the vacuum drying oven and weighed.

From 135 g. of tall oil, 320 ml. of ethylene dichloride and 3.0 g. of oxalic acid was obtained 13.5 g. of crystalline addition product.

Chromatography of product:- The crystalline addition compound was twice recrystallized from ethanol. The product melted at 90°-117° C. A solution of 1.0 g. of the product in 200 ml. of petroleum ether, b.p. 30° - 60°C., was applied to a column of activated alumina, 14 mm. in diameter and 70 cm. high. The column was then washed with a succession of solvents, the eluates collected and evaporated. When sufficient material was available the residues were crystallized from methanol and their melting points measured. The fractions obtained are listed in Table LII.

Fraction 38 (0.20 g.) was dissolved in 4 ml. of pyridine and 1.1 ml. of acetic anhydride. The mixture was boiled under reflux for five minutes and poured into 20 ml. of ice water. The product was collected on the filter, washed with dilute hydrochloric acid and then with water. The product, after crystallization from methanol, melted at 127-128°C.

Chromatographic Separation of Tall Oil Alcohols

Molecular distillation of the neutral material:- A solution of 300 g. of tall oil soap in 3 l. of 5% aqueous methanol was exhaustively extracted with diethyl ether and the ether extract washed with 5% sodium carbonate solution and then with water. After removal of the ether the neutral material weighing 42.4 g. was dissolved

Table LII

Chromatography of Addition Compounds

Fraction	Eluting Solvent	Weight in mg.	m.p.
1	1:3 benzene-petroleum ether	1.0	
2		12.1	72.5-73°
3		12.3	70 - 70.5°
4		5.4	
5		1.6	
6		0.7	
7		0.9	
8		0.9	
9	2:1 benzene-petroleum ether	0.4	
10		3.9	82 - 84.5°
11		3.0	
12		2.1	
13		1.1	
14		0.7	
15		0.9	
16		1.1	
21	0.1% ethanol in benzene	2.5	87.5-89.5
22		4.1	120 - 140
23		6.1	152 - 160
24		8.4	156 - 159
25		8.8	158 - 163
26	0.5% ethanol in benzene	7.5	155 - 159
27		6.9	150 - 153
28		8.6	141 - 144
29		11.8	136 - 138
30		3.8	134 - 136
31	1% ethanol in benzene	22.2	134 - 135
32		17.0	123 - 125
33		2.2	
34		1.9	
35		1.3	
36		1.0	

(Continued)

Table LII (Continued)

Chromatography of Addition Compounds

Fraction	Eluting Solvent	Weight in mg.	m.p.
17		1.7	
18	benzene	1.0	
19		1.2	
20		2.4	
37		1.3	
38	ether	374.4	137 - 138
39	alcohol	190.9	137.5-138
41		2.5	

in 300 g. of triölein. The solution was distilled at 4 to 10 microns pressure in the molecular still using 10°C., temperature increments and thirty minute cycles.

<u>Synthesis of azobenzene-p-carboxylic acid</u>:- A mixture of 125 ml. of nitrobenzene and a solution of 75 g. of ammonium chloride in 2,500 ml. of water in a large earthenware crock was vigorously stirred with a wooden paddle, and 186 g. of zinc dust added in small portions over a period of about five minutes. When the temperature of the mixture reached 65°C., enough ice was added to reduce the temperature to 50°C. Twenty minutes after addition of the zinc was started, the solution was filtered through a large Büchner funnel and the zinc oxide residues were washed with 1,500 ml. of boiling water. The filtrate and washings were returned to the crock and cooled by the addition of ice to a temperature of -2°C. and an additional kilogram of ice added.

To this cold suspension of β-phenyhydroxylamine a mixture of 375 ml. of concentrated sulphuric acid and sufficient ice added to bring the temperature down to -5°C. An ice-cold solution of 85 g. of sodium dichromate dihydrate in 300 ml. of water was poured rapidly into the stirred mixture. After a few minutes the crude nitrosobenzene was collected on a Büchner funnel and washed with about a litre of water.

The crude nitrosobenzene was distilled with steam and the distillate was collected in a receiver cooled by ice. The nitrosobenzene was ground in a mortar, transferred to a Büchner funnel and washed with water until the washings were no longer brown. The product, weighing 62 g. was crystallized once from ethanol, pressed between filter papers and air dried.

To 13.7 g. of p-aminobenzoic acid in 30 ml. of glacial acetic acid was added a solution of 10.7 g. of nitrosobenzene in 100 ml. of ethanol, and the mixture heated with stirring on the steam cone for fifteen minutes. The red-black solution was cooled, about 50 ml. of water added, and the brown precipitate collected, washed with 50% ethanol and recrystallized from ethanol. Azobenzene-p-carboxylic acid, m.p. 237-238°C., was obtained in a yield of 18.4 g.

Nitrosobenzene was synthesized by the method of Organic Syntheses (128). The condensation of nitrosobenzene with p-aminobenzoic acid has not been previously described.

Preparation of the azo ester:- To 0.6 g. of azobenzene-p-carboxylic acid was added 3 ml. of thionyl chloride and the mixture boiled under reflux for fifteen minutes. Excess thionyl chloride was removed by distillation. To the acyl chloride residue was added 1.0 g. of neutral fraction in 3 ml. of anhydrous pyridine. The mixture was heated at 100°C. for one hour, taken up in diethyl ether and washed with 2% sodium hydroxide solution, 2% hydrochloric acid, and finally with distilled water. The red ether solution was dried with anhydrous sodium sulphate and the ether removed by distillation. The residue was dissolved in 35 ml. of dry benzene and 35 ml. of dry petroleum ether, b.p. 30°-60°C., added. This solution was then applied to the chromatographic column.

Preparation of the chromatogram:- Alumina (Marshall Chemical Co.), was heated at 250°C. until water ceased to be evolved, and for an additional hour. The activated adsorbant was cooled in a desiccator and stored in tightly stoppered flask.

The chromatographic column consisted of a glass tube 40 cm. long and 1.5 cm. in diameter, closed at the bottom by a cork stopper carrying a stopcock and outlet tube. The stopcock was lubricated with a paste of bentonite clay and glycerol. The column was partially filled with petroleum ether and alumina added in a petroleum ether slurry. Solvent was permitted to drain slowly from the column and the adsorbant allowed to settle, with continous tapping of the tube, to a height of 30 cm.

The red solution from the esterification of 1.0 g. of neutral matter distillate was allowed to pass through the adsorbant bed at a rate of one to two drops per second, under the influence of a gentle vacuum of 600 to 700 mm. of mercury. A mixture of two parts of petroleum ether to one part of benzene was then passed through the column at a rate of about 100 ml. per hour until the fastest moving red band had almost reached the bottom of the column. This usually required three to four hours. Development was then stopped, the bulk of the solvent drained from the column and the alumina sectioned according to the zones of adsorbed material.

Preparation of chromatogram 5A:- In chromatogram 5A the procedure was varied slightly to permit better resolution of the bands of more highly adsorbed material at the top of the column. The ester prepared from fraction 5 (1.5 g.) was applied to the column and developed with a mixture of two parts of petroleum ether, b.p. 30°-60°C., and one part of benzene. Two series of red bands formed, one series occupying the lower half of the column and the other series occupying the upper half of the column. Development was continued until the upper series of bands occupied nearly the whole column. The lower bands were, of course, washed into the eluate. The column was then drained and sectioned.

Sectioning of the columns:- It was not found possible to extrude the alumina from the columns. An apparatus was, therefore, improvised. A strip of sheet metal about four inches in length was cut to a width of 14 mm. A longitudinal cut 7 mm. in length was made in one end and the two flaps thus formed were bent in opposite directions at right angles to the body of the strip. The other end of the strip was attached to a wooden stick and the corners of the two flaps trimmed to permit insertion of the assembly in the tube. The apparatus was similar to a carpenter's bit and was used in a similar manner. By inserting the apparatus in the tube with the flaps in contact with the alimina and twisting, a cross-section could be cut cleanly and removed from the body of the adsorbant.

Elution of the adsorbant sections:- A small Soxlet thimble containing the adsorbant section was suspended from a "cold finger" condensor, over boiling ethanol, in a manner such that the alumina was continously washed with hot reflux. Sections were extracted for three hours. The alumina retained a red coloration but more material could not be eluted, even by extraction for eight hours.

Examination of eluted material:- Solvent was removed from the extracts by distillation and the residues were gently boiled under reflux for two hours with 2 ml. of 0.5 N potassium hydroxide in ethanol, diluted with 10 ml. of water and free alcohol extracted with ether. Solid fractions were crystallized from methanol.

Acetate esters were prepared by refluxing a solution of the fraction in 2.5 ml. of pyridine with 1.0 ml. of acetic anhydride for fifteen minutes. The reaction mixture was poured into water and the ester collected on a filter, washed with water and crystallized from methanol.

Essential Oils from Coniferous Foliage

Plant Preparation of the Oil

Plant materials used:- Branches with foliage were selected to have stems less than 0.5 in. thick at their thickest point. Foliage of Pinus banksiana Lamb., P. resinosa Ait., P. sylvestris L., P. strobus L., Tsuga canadensis (L.) Carr., and Picea glauca Voss., was kindly supplied by the Ontario Forestry Station at Vivian Forest. Foliage of Thuja occidentalis (L.) Mill., Picea mariana (Mill.) B.S.P., and Abies balsamea (L.) Mill., was supplied by the Ontario Forestry Station at Midhurst. Foliage of Pinus contorta Dougl. was made available by the Forestry Division of British Columbia. Cones were supplied by the Ontario Forestry Station at Angus. Foliage was collected in late March and April.

Distillation of the foliage:- The still consisted of a cylindrical pot made of 3 in. fir, 6.5 ft. in diameter and 11 ft. deep, and provided with a tight fitting aluminum lid. Steam at 90 lbs. per sq. in. pressure was injected into the bottom of the still and after passing through the foliage was condensed in a water cooled copper coil. The oil collected on top of the aqueous distillate and was separated. The water was returned to the boiler.

The oils were weighed and the yields calculated as weight percent of green plant material as charged to the still. The oils were dried over anhydrous sodium sulphate and stored in dark bottles under nitrogen. Distillation of the foliage was conducted by Mr. J. N. Brown and supervised by Dr. T. F. West.

Laboratory Preparation of the Oil

Plant materials used:- Needles and small twigs from open grown trees, twenty to fifty years of age were collected and made available by the Division of Research, Department of Lands and Forests, Province of Ontario. Foliage was one year's growth, picked from the south side of the tree at head height. Black spruce was grown at Port Arthur in alkaline clay silt. Other species were grown on acid sandy soil at Angus.

Laboratory distillation of the foilage:- A sample of 400 g. to 1000 g. of foliage was weighed into a 6-l Florence flask and covered with five times its weight of water. An oil trap and condensor of the Clevenger type (129) was mounted in the neck of the flask. The flask was heated in a glycerol bath and distillation carried out at about two drops per second for twelve hours.

Physical properties of the oils:- Refractive index was determined with an Abbé refractometer at 20°C. Specific gravities were determined at 20°C. at referred to water at 4°C. by means of pycnometers. The densities of fractions obtained by fractional distillation of the oils were measured with a Fisher-Davidson gravitometer. Optical rotations were measured in a 1 dcm. tube by the light of a sodium vapour lamp.

Chemical properties of the oils:- Esters were determined by saponification. To a 1.5 g. sample of oil in a 100 ml. Kimble glass flask was added 10.0 ml. of 0.5N alcoholic potassium hydroxide solution and the mixture refluxed gently for one hour. Excess alkali was titrated with 0.5N hydrochloric acid using phenolphthalien indicator. A blank determination was also carried out.

$$\text{Ester number} = \frac{28.05\ a}{S}$$

$$\%\ \text{esters} = \frac{\text{Mx ester number}}{561.04}$$

a = ml. of 0.5000 N potassium hydroxide consumed in saponification.

S = sample weight in grams.

M = molecular weight of the ester

Alcohols were determined by acetylation. A mixture of 10 ml. of oil, 10 ml. of acetic anhydride, and 2 g. of anhydrous sodium acetate was refluxed gently for one hour. Water (50 ml) was then added and the mixture allowed to stand with occasional shaking for fifteen minutes. The mixture was then transferred to a separatory funnel, the water layer separated and the oil layer washed with three 100-ml. volumes of saturated brine. The oil was then dried with sodium sulphate and the ester content of the acetylated oil determined by saponification.

$$\text{Ester number after acetylation} = \frac{28.05\ a}{S}$$

$$\%\ \text{free alcohols in original oil} = \frac{dM}{561.04 - 0.42\ d}$$

a = ml. of 0.500 N potassium hydroxide consumed in the saponification.

S = sample weight in grams.

d = difference between ester number after acetylation and ester number.

M = molecular weight of the alcohol.

Carbonyl compounds were determined by reaction with hydroxylamine. Hydroxylamine hydrochloride (35 g.), in 40 ml. of warm water was made up to 1000 ml. with 95% ethanol. A 0.1% solution of bromphenol blue (15 ml.) was added and the color of the reagent adjusted to a faint green by addition of a few drops of 0.5 N alcoholic potassium hydroxide.

This reagent, (35 ml), was added to an accurately weighed 0.5 g. sample of the oil and allowed to stand at room temperature in a stoppered flask for twenty-four hours. The liberated hydrochloric acid was then titrated with 0.5N alcoholic potassium hydroxide until the original greenish shade was restored.

$$\% \text{ carbonyl compound} = \frac{a\,M}{20\,S}$$

M = molecular weight of the carbonyl compound.

S = sample weight in grams.

These are essentially the procedures recommended by Guenther (130) for the determination of ester, alcohol, and aldehyde or ketone contents.

Chemical Composition of the Oils

Oil of Picea Mariana (Mill.) B.S.P.

Fractional Distillation

Oil of P. mariana (4,000 ml.) was distilled in four 500-ml. batches at 10 mm. pressure, through a 15 in. unjacketed column consisting of eight pear-shaped bulbs. The fractions of about 600 ml. each were obtained with boiling ranges 43-48°C., 45-95°C., and 95-100°C. The fraction boiling between 45°C. and 95°C. was redistilled. That portion boiling below 70°C. was combined with the lower boiling fraction and the residue combined with the fraction boiling up to 100° C. at 10 mm. The residue boiling above 100° C. at 10 mm. was distilled at 1 mm. and a sesquiterpene fraction, b.p. 80-110°C. at 1 mm. was removed. Recoveries are shown in Table LIII.

Table LIII

Preliminary Distillation of Black Spruce Oil

Fraction	Boiling Range °C.	% by Weight
A	43 - 70° (10 mm.)	48.0
B	70 - 100° (10 mm.)	37.5
C	80 - 100° (1 mm.)	6.5
Residue and loss		

Fraction A (1,000 ml. was carefully fractionated using a 6 ft., 25 mm. I.D. Podbielniak Hyper-Cal column at a reflux rate of 300-400 ml. per hour. Fractions 1 to 21, Table XXXVI were distilled at 48 mm. pressure and reflux ratio 15/1. Fraction 21 was solid. It was removed from the column by passing warm water through the condensors, and by heating locations where distillate began to crystallize with an infra-red lamp. Fractions 22 to 34, Table XXVI, were distilled at 20 mm. pressure and reflux ratio 20/1.

Fraction B, (1250 ml.) was combined with volatile material removed from the pot residue of the preeeding distillation and fractionally distilled through the 6 ft. Podbielniak column at 20 mm. pressure and reflux ratio 30/1. Fractions 35 to 46 were collected in this way. At this point solid borneol plugged the side arm of the still By reducing the reflux ratio to 10/1 the distillation of borneol in a less pure, liquid state was possible. After removal of about 50 ml. of borneol-rich fraction in this way the remainder of fraction B distilled with almost constant properties.

Fractions 48, 49 and 50, Table XXV were obtained from fraction C by repeated fractional distillation at 1 mm. pressure through the unpacked column employed in the preliminary distillation.

Identification of Constituents

Santene:- To an ice cold mixture of 2.5 g. of fraction

1 in 20 ml. of diethyl ether and 5.5 g. of sodium nitrite in 15 ml. of water, 3.9 ml. of concentrated hydrochloric acid was added over a period of fifteen minutes. The mixture was allowed to stand, with ice cooling, for one hour and the ether layer separated and dried with anhydrous sodium sulphate. The oily residue remaining after evaporation of the ether was pressed on a cold porous tile. The crude product, weighing 2.0 g. was crystallized from 5 ml. of methanol. The method of preparation was described by Müller (131). The product, obtained in a yield of 49% formed beautiful blue platelets from methanol and melted at 124 - 125° C. after four crystallizations. Aschan (132) records m.p. 124 - 125° C. The melting point was not depressed when the sample was mixed with santene nitrosite prepared from the fraction boiling at 37° - 38° C. at 20 mm. from oil of Abies siberica.

<u>Tricyclene</u>:- Fraction 3 was redistilled through 3 ft., 8 mm. I.D. Podbielniak Hyper-Cal column at 100 mm. pressure and 8 mm. pressure drop across the column. Two liquid fractions were collected followed by 1.0 ml. of a fraction, N^{20}_{D} 1,4620, d^{20}_{4} 0.874, α_{D} - 2.5°, b.p. 151.7°C./764 mm., from which solid crystallized. A waxy solid then began to distil. About three grams of volatile, crumbly crystals with the odour of camphene was obtained. The product was

sublimed, crystallized from methanol and resublimed from sodium. Purified tricyclene melted at 61 - 64° C. and boiled at 153° C.

Synthesis of tricyclene:- Synthetic tricyclene was prepared by the method of Aschan (133). Concentrated hydrochloric acid, (140 ml.), was allowed to drop on 25 g. of potassium permanganate and the chlorine evolved dried over sulphuric acid followed by phosphorous pentoxide. Dry chlorine was bubbled through 50 g. of α-pinene, b.p. 39.1 - 39.5° C./10 mm., which had been purified by distillation from metallic sodium. The reaction temperature was maintained at 0° - 10° C. The increase in weight of the reaction mixture was 22 g. The product was washed with 5% sodium carbonate solution and then with water. The liquid was dried over anhydrous calcium chloride and distilled at 10 mm. pressure. Below 120° C. two solid fractions were obtained which probably contained pinene hydrochloride. The fraction boiling between 120° and 140° C. was an oil which partially crystallized after long standing in the refrigerator. Supernatant oil was decanted off and the white crystals (6.0 g.) twice recrystallized from ethanol. White platelets (2.5 g.) of β-camphor dichloride, m.p. 173 - 174° C., were obtained. This compound in 25 ml. of anhydrous diethyl ether was refluxed for twenty-four hours with 3.0 g. of finely chipped sodium. The mixture was then distilled. Between bath temperatures of 150° - 200° C., about 3.0 g. of semi-solid distilled. After repeated crystallization from methanol and sublimation from sodium, tricyclene, m.p. 60 - 65° C. was obtained.

Infra-red absorption spectra:- Spectra of natural and synthetic compounds in 46.6 % solution and 41.4% solution in carbon disulphide respectively were determined between 3 - 4 μ and 7-13 μ in a 0.025 mm. cell in a Perkin-Elmer single beam, infra-red spectrometer.

dl - and l-α-Pinene:- Fractions 6 and 7 were oxidized by the method of Delepine (134). A mixture of 79 grams of potassium permanganate 16.5 g. of ammonium sulphate 50 ml. of water, 750 g. of crushed ice, and 34 g. of fractions 6 and 7 in a heavy glass bottle was shaken mechanically for four hours and refrigerated overnight. The precipitate of manganese dioxide was separated in the centrifuge and washed with four 50-ml. volumes of hot water. Concentrated ammonium hydroxide (5 ml.) was added to the combined liquors and wash waters and the latter extracted with four 25-ml volumes of chloroform. The aqueous layer was acidified with 25 ml. of concentrated sulphuric acid in 45 ml. of water and extracted with four 25-ml. volumes of chloroform. The chloroform extract was washed with water, dried over sodium sulphate and the chloroform removed under vacuum. The semi-solid mass (22 g.) was dissolved in 25 ml. of benzene and 25 ml. of petroleum ether, b.p. 30 - 60° C., added. The mixture was refrigerated and two crops of crystals removed.

After three crystallizations from 50 ml. of benzene and 25 ml. of petroleum ether, 5.6 g. of dl-pinonic acid, m.p. 103 - 104° C., $[\alpha]_D - 1.4^\circ$ (c = 6.77, chloroform), was obtained. The mother liquors from these crystallizations were concentrated and solvent removed in vacuum. The yellow paste was freed of oil on a porous tile leaving 4.1 g. of white powder. The powder was dissolved in 10 ml. of diethyl ether and the ether allowed to evaporate slowly. Three crops of crystals were removed, pressed between filter papers, and recrystallized from water. l-pinonic acid (1.5 g.), m.p. 68 - 69.5° C., $[\alpha]_D - 89.6^\circ$ (c = 2.66 in chloroform), was obtained. The literature records m.p. 103 - 104° C. for the racemic acid (135), and m.p. 67 - 69° C., $[\alpha]^{25}_D - 90^\circ 33'$ for active pinonic acid (136).

<u>l-Camphene</u>:- Fraction 21 was twice crystallized from methanol and sublimed to yield a soft waxy solid m.p. 47° - 48° C., $[\alpha]_D - 97.5^\circ$ (c = 10.56 in benzene). This material was hydrated by the method of Bertram and Walbaum, (137). Glacial acetic acid (25 ml.) and 1.0 ml. of 50% sulphuric acid were mixed and 18 ml. of this reagent added to 6.08 g. of the solid terpene. The mixture was heated at 55° - 60° C. for three hours and poured into water. The yellow oil which separated was washed with water, then mixed with a solution of 4 g. of potassium hydroxide in 20 ml. of ethanol and heated at 60° - 65° C. for fifteen minutes. The solution was poured into 100 ml. of water and the white solid

which separated was filtered off, washed with water and pressed between filter papers. The resulting light yellow powder (6.2 g.) was dissolved in 25 ml. of boiling petroleum ether, b.p. 60 - 68° C., filtered, and the solvent allowed to evaporate until crystals began to form. It was then refrigerated and the crystals which separated recrystallized from petroleum ether to yield snow-white platelets of isoborneol, m.p. 210.5° - 212° C. in a sealed tube, undepressed by mixing with an authentic sample.

<u>1-β-Pinene</u>:- A mixture of 10.0 g. of fractions 22 and 23 with 23.3 g. of potassium permanganate, 5 g. of ammonium sulphate, 50 ml. of water, and 300 g. of crushed ice was shaken for four hours. The solution, which had heated up to about 40°C., was refrigerated for one hour, then shaken for an additional two hours and allowed to stand at room temperature overnight. The manganese dioxide was separated in the centrifuge, washed with 200 ml. of warm water, and the combined supernatant liquid and washings concentrated under vacuum to about 100 ml. The concentrated liquors, when refrigerated for six hours, deposited 1.2 g. white powder crystals which formed beautiful transparent rectangular prisms from water. The combined mother liquors were extracted with three, 10-ml. volumes of chloroform, acidified with 3.5-ml. volumes of sulphuric acid and extracted with four, 10-ml.volumes of chloroform. After removal of the chloroform 1.3 g. of

neutral extract and 3.5 g. of acid extract was obtained. The latter red oil was treated with 0.72 g. of sodium hydroxide in 10 ml. of water, refrigerated and a crop of crystals removed. A part of the crystalline material (0.84 g.) in 15 ml. of water was acidified with 0.2 ml. of sulphuric acid and extracted with chloroform. The residue (0.2 g.) remaining after removal of the chloroform was dissolved in 2 ml. of benzene and 5 ml. of petroleum ether, b.p. 60-68° C. slowly added. Nopinic acid was obtained, melting at 126 - 127° C. alone or in admixture with an authentic specimen.

Myrcene:- Fractions 25 and 26 were distilled through a 3 ft., 8 mm. I.D. Podbielniak column at 100 mm. pressure, 9 mm. pressure drop across the column and reflux ratio 20/1. The fractions listed in Table LIV were collected.

Table LIV

Redistillation of Myrcene

Fraction	Volume	N^{20}_{D}	d^{20}_{4}	α^{26}_{D}	b.p.
i	5.2 ml.	1.4788	0.867	-18.13°	164.9°/757 mm.
ii	5.0	1.4783	0.863	-16.79	165.4
iii	4.9	1.4738	0.825	- 7.43	167.2
iv	6.1	1.4710	0.800	+ 0.83	167.5
v	3.2	1.4723	0.814	- 0.80	
vi	5.1	1.4711	0.810	- 0.89	167.8/739 mm.
vii	4.0	1.4715	0.834	+ 6.54	168.8
viii	1.5	1.4721	0.857	+11.62	169.0

follow page 158

Extensive polymerization took place in the pot during distillation.

Powdered maleic anhydride (2.0 g.) and 2.7 g. of fraction vi were warmed in a test tube until the anhydride began to melt, then shaken to mix the two components. A violent reaction ensued with great evolution of heat. The resulting oil was distilled at 10 mm. pressure. Below 190° 1.02 g. of terpenes and unchanged maleic anhydride were removed. The viscous oil (3.21 g.) boiling at 190° - 210° at 10 mm. formed a waxy solid when refrigerated. This material was dissolved in 50 ml. of petroleum ether, b.p. 30°-60°C. and refrigerated, White leafy crystals m.p. 34-35°C. were obtained after two crystallizations.

The adduct (1.4 g.) was warmed with 1.4 g. of potassium hydroxide in 5 ml. of water, acidified with sulphuric acid and filtered. The solid formed granular crystals from acetonitrile, m.p. 120-122°C. after three crystallizations. Diels and Alder (108) described the preparation of these compounds and reported melting points of 122-123°C. and 34-35°C. for cis-4-isohexenyl-Δ^4-tetrahydrophthalic acid, and for the corresponding acid anhydride, respectively.

d-Δ^3-Carene:- Fractions 27 and 28 were combined and redistilled through the 3 ft. 8 mm. I.D. Podbielniak column The fractions obtained are listed in Table LV.

Table LV

Redistillation of Δ^3-Carene

Fraction	Volume	N_D^{20}	d_4^{20}	α_D^{25}	b.p./762 mm.
i	4.5 ml.	1.4720	0.820	$+$ 4.19	166.8°
ii	5.0	1.4725	0.854	$+$10.72	169.3
iii	9.0	1.4726	0.862	$+$12.17	171.9
iv	9.0	1.4729	0.866	$+$12.81	172.3

Simmonsen (103) reported N_D^{30} 1,468, d_{30}^{30} 0.8586, $[\alpha]_D + 7.69$, b.p. 168 - 169° C./705 mm. The refractive index of fraction iv at 30°C. was 1.468; the boiling point corrected to 705 mm. was 169° C.

Fraction iv (5 g.) in 2 ml of glacial acetic acid and 4 g. of amyl nitrite was cooled in a freezing mixture and 3.5 ml. of concentrated nitric acid added dropwise. After fifteen minutes, 10 ml. of methanol was added and the solution refrigerated overnight. Crude nitrosate (1.0 g.) was separated, dissolved in chloroform and precipitated by addition of methanol. After three precipitations it melted with decomposition at 147.5° C.

A sample of turpentine oil of Pinus longifolia Roxb. was fractionally distilled and an authentic sample of d-Δ^3-carene nitrosate prepared from the fraction b.p. 102-103° C./ 100 mm., N_D^{20} 1.4729, d_4^{20} 0.860. The melting point of a mixture of the two nitrosites was 147° C.

dl- and l-Limonene:- To a well cooled solution of 10 g. of fraction 33 in 10 ml. of iso amyl alcohol and 20 ml. of absolute ether, bromine was added dropwise until a persistant coloration was obtained; about 7.5 ml. of bromine was required. Most of the ether was allowed to evaporate and the remaining solution refrigerated. The product (9.0 g.) was collected on a Buchner and washed with iso amyl alcohol. After recrystallization from 40 ml. of ethyl acetate it melted at 123.5° - 125°C. alone or in admixture with an authentic sample.

Terpinolene:- Fractions 39 and 40 were combined and distilled from metallic sodium. The distillate (4.35 g.) when brominated in the same way as was limonene, yielded 1.2 g. of crystalline bromides which, after crystallization from ethyl acetate, melted at 116-118°C. A mixture with dl - limonene tetrabromide melted about 100°C.; the melting point of a mixture with terpinolene tetrabromide was undepressed.

l-Borneol:- The solid which separated from fraction 46 after crystallization from petroleum ether, b.p. 60-68°C., melted at 204-205°C. in a sealed capillary, $[\alpha]_D$ -31.5° (c = 9.832 in toluene.). This solid (0.6 g.) in 3 ml. of pyridine was boiled for five minutes with 0.75 g. of p-nitrobenzoyl chloride, poured into water, and the product recrystallized from ethanol. l-Bornyl p-nitrobenzoate (1.2 g.) m.p. 136-137° C. was obtained after one crystallization.

Borneol melts at 203-204° C. and forms a p-nitrobenzoate which melts at 136° C. according to Hückel (138).

<u>l-Bornyl Acetate:</u>- Two samples, one collected early, and the other towards the end of the distillation of fraction 47, were made to crystallize at dry ice temperatures. Their melting points, measured with an immersed thermometer were 22.4° and 23.4° C. respectively. Pickard and Kenyon reported m.p. 27.4° C. for l-bornyl acetate with α_D -44°. Other properties of the fraction are in close agreement with those recorded by these investigators, (106).

A solution of 20 g. of ester and 12 g. potassium hydroxide in 60 ml. of ethanol was boiled under reflux for fifteen min., and then poured into 300 ml. of water. The l-borneol which separated (12.5 g. after one crystallization) melted at 207.5 - 208° C. α_D -31.83° (c = 10.62 in toluene): p-nitrobenzoate m.p. 136-137° C. alone, or in admixture with the ester described in the preceding section. The aqueous liquors were extracted with ether, treated with carbon dioxide to remove free alkali, and evaporated to dryness. The residue was extracted with 50 ml. of boiling ethanol. After removal of the alcohol the residue, weighing 7.5 g., was boiled for one hour with 10 ml. of thionyl chloride, diluted with benzene and filtered. The filtrate was cautiously treated with 11 g. of aniline in 20 ml. of benzene, and the benzene solution washed with dilute acid, dilute base, and finally with water.

The mass remaining after evaporation of the benzene was leached with boiling water. This extract, after decolorization with charcoal and cooling, separated 0.5 g. of acetanilide melting at 113-115°C.

Camphor:- Fraction 42 (10 g.) in 50 ml. of ethanol was allowed to stand at room temperature for three weeks in the presence of 5 g. of semicarbazide hydrochloride and 5 g. of sodium acetate. After distillation of the solvent and removal of the unchanged terpenes in a current of steam, the residue, weighing 2.1 g. after crystallization from ethanol, was leached with boiling acetonitrile. The insoluble fraction yielded 0.9 g. of semicarbazone melting at 234-235°C. Steam distillation of this material from 10 g. of oxalic acid in 10 ml. of water regenerated a ketone melting at 171-174° C., $\alpha_D + 6^{\circ}$ (c = 1.07 in ethanol). The 2,4-dinitrophenyl hydrazone, prepared by refluxing the compound for 30 min., with 2, 4-dinitrophenylhydrazine in ethanol containing 5% of sulphuric acid, formed orange needles from ethanol, melting at 164-166° C. alone, or in admixture with a known sample of dl-camphor 2,4-dinitrophenyl hydrazone.

Terpene alcohol:- A mixture of fractions 43, 44 and 45 (27.7 g.) with 18.0 g. of tri-n-butyl borate in a 100-ml. distilling flask was heated at 100° C. and 30 mm. pressure for six hours. Butyl alcohol (7.2 g.), b.p. 49°/30 mm., distilled from the reaction mixture. A distillate of 12.1 g. of colorless liquid boiling at 57°C. was then removed at 1 mm.

was mixed with 50 ml. of 10% sodium hydroxide solution and distilled with steam. The colorless oil was separated from the condensate and distilled at 3 mm. pressure. An alcohol b.p. 65° C/3 mm., N_D^{20} 1.4842, d_4^{20} 0.955, α_D $+13.2$, was obtained in a yield of 12.3 g.

<u>Sesquiterpene</u>:- Fraction 48 (10 g.) in 20 ml. of dry diethyl ether was saturated with dry hydrogen chloride at -30° C. The ether was allowed to evaporate and the product pressed on a cold porous tile. The product (7.6 g.), after crystallization from ethanol, melted at 118-119° C., $[\alpha]_D$ -37.9° (c = 5.50 in chloroform). The yield was 56% of theoretical. An authentic specimen prepared from the fraction of Cubeb oil (120) boiling at 130-135°C./10 mm. had $[\alpha]_D$ -38.0°. The melting points of the mixture was 118-119° C.

<u>Oil of Tsuga Canadensis (L.) Carr</u>.

Fractional Distillation

By simple distillation at 10 mm. pressure through a 15 in. column consisting of eight pear-shaped bulbs, the oil was separated into a hydrocarbon fraction boiling below 70° C., a fraction containing oxygen compounds, b.p. 70 - 105°C. at 10 mm., and a high boiling fraction distilling below 140° C. at 0.4 mm., comprising 48.5%, 46.0% and 3.9% of the original oil, respectively. The hydrocarbons were distilled through a 6-ft., 25-mm. I.D. Podbielniak column at reflux ratio 20/1 and pressure drop across the column 15 mm.

Fractions 1 to 26, Table XXXIX, were distilled at 50 mm. pressure, fractions 27 to 50 at 20 mm. pressure. After collection of fraction 35 volatile material was removed from the pot residue, combined with the oxygen compounds, and the resulting mixture fractionated through the 6-ft. Podbielniak column at 20 mm. pressure, 15 mm. pressure drop across the column and reflux ratio 20/1. Fractions 51 to 59 were obtained from the pot residue by simple distillation at 10 mm. pressure. Non-volatile residues and loss amounted to 2.2% and 3.6% of the original oil respectively.

The high boiling fraction was distilled at 4 mm. pressure, 3 mm. pressure drop and reflux ratio 15/1 through a 1-ft., 25-mm. I.D. Podbielniak column.

Identification of Constituents

Tricyclene:- Fraction 2 was added to 100 ml. of 10% aqueous potassium permanganate and the mixture heated to 65° C., with frequent shaking until evolution of carbon dioxide ceased. The solution was cooled and the solid tricyclene removed, sublimed, twice crystallized from methanol and sublimed from sodium. The purified product melted at 65° C. alone or in admixture with an authentic sample.

dl- and d-α-Pinene:- Fractions 6 and 7 (34 g.) were oxidized with neutral permanganate by the method of Délépine (134). Crude acids were obtained in a yield of 21.5 g. After four crystallizations from benzene - hexane mixtures, dl-pinonic acid, m.p. 103.5 - 105° C. undepressed by an authentic sample was obtained in a yield of 9.6 g.

The more soluble material from the mother liquors was sublimed at 100° C. in high vacuum and the sublimate crystallized from benzene-hexane mixtures to give 0.2 g. of not quite pure d-pinonic acid, m.p. 66-70° C., $[\alpha]_D^{25}$ $+87.7°$ (c = 1.97 in chloroform).

l-Camphene:- The melting points of fractions 24, 25, and 26 were determined from the cooling curves measured with an immersed thermometer as the samples crystallized from the molten state. Hydration of 10 g. of fraction 26 with Bertram-Walbaum reagent followed by saponification yielded 11.1 g. of crude isoborneol (137). After crystallization from hexane it melted at 210.5-212° C. undepressed by an authentic sample. Details of this preparation were described in the section dealing with oil of Picea mariana.

l-β-Pinene:- Fraction 28 was oxidized with neutral permanganate exactly as described for fractions 22 and 23 of oil of Picea mariana. Treatment of the 3.1 g. of acidic oil obtained with 7 ml. of 10% sodium hydroxide resulted in the precipitation of a white powder. Pure sodium nopinate (0.2 g.) was obtained from this powder after three crystallizations from water. The free acid, extracted from an acidified solution of the salt melted at 127° C., undepressed by an authentic specimen.

<u>Myrcene</u>:- Fraction 30 (0.2 g.) was warmed with 2.7 g. of maleic anhydride and the product purified as previously described (108) Cis-4-isohexenyl-Δ^4-tetrahydrophthalic anhydride, m.p. 34-35°. was obtained in a yield of 2.2 g. The dicarboxylic acid, obtained by saponification of the adduct, melted at 120-122° C.

<u>d-α-Phellandrene</u>:- The maleic anhydride adduct was prepared by the method of Goodway and West (139). Fraction 32 (5 g.) in 10 ml. of ether was refluxed for thirty minutes with 2.5 g. of maleic anhydride. Ether was allowed to evaporate and adduct crystallized from methanol to give 2.45 g. of product. After crystallization from methanol, ethanol, hexane, and finally diethyl ether it melted at 124-125° C., $[\alpha]_D^{25}$ + 12.0 (c = 4.18 in chloroform). The melting point was not depressed by an authentic specimen prepared from Canada balsam turpentine. Goodway and West reported m.p. 126 °C., $[\alpha]_D^{25}$ - 8°54' (c = 12.59 in chloroform).

Fraction 32 (5 g.) was heated at 130° C.for fifteen hours with 10 g. of β-naphthol as described by Salfeld (140). The product was dissolved in 100 ml. of ether, washed with three 40-ml. volumes of 2N sodium hydroxide solution. Ether and unchanged terpenes were removed by distillation at 100° C. and 15 mm. pressure. The oily residue (2.68 g.) deposited crystals from hexane which, after two crystallizations from methanol, decolorization with carbon, and crystallization from hexane, melted at 138-139.5° C.

The adduct (0.27 g.) in 10 ml. of anhydrous ether was treated with 1 g. of chipped sodium, refluxed for thirty minutes, and the ether solution washed into 0.18 g. of p-nitrobenzoyl chloride in dry ether. The ether solution was washed with water and sodium carbonate solution. After removal of the ether, the pale yellow crystalline product (0.368 g.) was recrystallized from 30 ml. of ethanol. The ester melted at 166-168° C. Salfeld reported m.p. 139-140° C. for the adduct, and m.p. 164-165° C. for the p-nitrobenzoate ester (140).

<u>dl- and l-Limonene</u>:- A solution of 5 g. of fraction 35 in 5 ml. of isoamyl alcohol and 10 ml. of diethyl ether was treated with 4 ml. of bromine at 0°C. A solid bromide separated in a yield of 4.38 g. on evaporation of the ether. After crystallization, twice from ethyl acetate and twice from ethanol, 0.6 g. of dl-limonene tetrabromide m.p. 124-125° C. was obtained. The combined mother liquors were evaporated to 75 ml. and a crop of impure racemate removed. The mother liquors were then stored at Dry Ice temperatures for three days. l-Limonene tetrabromide separated in a yield of 0.8 g. and after three crystallizations from ethanol melted at 103-104° C., $[\alpha]_D^{25}$ -69.7° (c = 1.46 in chloroform.).

<u>Tertiary alcohol</u>:- A mixture of 14 g. of Fraction 39, and 10 g. of tri-n-butyl borate was heated at 100° and

30 mm. pressure for five hours, during which time 2.50 g. of n-butyl alcohol (N_D^{20} 1.3999, d_4^{20} 0.814) distilled over.

Unchanged terpenes and butyl borate were removed at 100° and 1 mm. pressure, leaving 8.0 g. of non-volatile residue. This material was steam-distilled from 1 g. of sodium hydroxide and the resulting oil layer redistilled at 10 mm. to give 4.35 g. of optically inactive alcohol, b.p. 10 mm. 189-192° C., N_D^{20} 1,4806, d_4^{20} , 0.942.

The alcoholate, prepared by refluxing 0.5 g. of the compound in 4 ml. of benzene with excess sodium for half an hour, was decanted into a solution of 0.6 g. of p-nitrobenzoyl chloride in benzene. The ester obtained by evaporating the benzene melted at 55.5-57°C. after crystallization from methanol.

1-Thujone:- A solution of 10 g. each of fraction 38, simicarbazide hydrochloride, and sodium acetate in 70 ml. of 70% ethanol was allowed to stand for three days at room temperature. Solvent and 2.9 g. of unchanged terpenes were removed in a current of steam, leaving 12.0 g. of mixed semicarbazones. The alcohol insoluble portion was crystallized from water to give 0.5 g. of white microcrystalline powder m.p. 251-253° which was not decomposed by the action of oxalic acid or 2N sulfuric acid at 100°.

From the fraction slightly soluble in ethanol was obtained 0.5 g. of a pure compound m.p. 193°C. $[\alpha] 27_D$ +/57.1 (c = 4.763 in methanol), which was probably l-thujone semicarbazone.

The fraction soluble in cold ethanol was crystallized from aqueous ethanol to give a partial separation of two compounds. One formed anisotropic needles m.p. 176° C. which may have been d-isothujone semicarbazone. The other was an isotropic powder m.p. about 128° C.

When the latter three compounds were steam distilled from an equal weight of oxalic acid, ketones were obtained with refractive indices $N20_D$ 1.4496, 1.4502 and 1.4509, respectively. The latter two ketones were combined and oxidized according to Tiemann and Semmler (141). A mixture of 0.5 g. of ketone and 35 ml. of cold 2% aqueous potassium permanganate was allowed to stand, with occasional shaking, at 0°C. for two hours. Manganese dioxide was separated and washed. Neutral materials were extracted from the supernatant liquid and washings with ether. The aqueous liquors were then acidified to pH 2 and extracted with ether. Evaporation of the ether gave 0.36g.of α-thujaketonic acid, m.p. 74.5-75.5°C. after two crystallizations from hexane and two crystallizations from water.

<u>l-Bornyl Acetate</u>:- Twenty grams of fraction 48 was boiled under reflux for 15 minutes with 60 ml. of 20%

alcoholic potassium hydroxide, and poured into 250 ml. of water. The precipitate was crystallized from hexane to give crude borneol in a yield of 59%. The aqueous liquors were treated with excess carbon dioxide and evaporated to dryness. 9.2 g. of organic salts were leached from the residue with boiling alcohol. A mixture of 4 g. of the salt, 10 ml. of aniline, and 3 ml. of 35% hydrochloric acid was heated at 150-160°C. for one hour, then at 200°C. until excess aniline ceased to distill. A benzene extract of the residue on cooling deposited 1.86 g. of acetanilide m.p. 114-115°.

The crude borneol, after sublimation melted at 206-208°C. $[\alpha]$ D -16.9° (c = 10.145 in toluene). The p-nitrobenzoyl ester of this substance was crystallized repeatedly from ethanol until melting point and rotation remained unchanged by further crystallization. It melted at 152-154° C., $[\alpha]$D -0.8 (c = 10.05 in chloroform).

The ester was refluxed for one hour with excess 0.5 N alcoholic potassium hydroxide, and poured into water. After sublimation the recovered borneol melted at 201-206°C. $[\alpha]$ D -0.2° (c = 8.18 in toluene).

<u>Sesquiterpenes</u>:- A mixture of 1.0 g. of fraction 81 and 0.10 g. of palladiumcharcoal was heated under carbon dioxide for 6.5 hours, during which time the temperature

raised from 200 to 243°. The reaction mixture was diluted with hexane, filtered and the filtrate extracted with 5% aqueous sodium hydroxide. The extracted filtrate was added to 10 ml. of 10% absolute ethanolic picric acid and refluxed for 15 minutes. On cooling 540 mg. of cadalene picrate separated, m.p. 116.5-117.5°, identified by comparison with an authentic sample. A solution of 270 mg. of fraction 8 in an equal volume of dry ether was treated with dry hydrogen chloride at -20°; 90 mg. of l-cadinene dihydrochloride m.p. 120-120.5°, $[\alpha]^{27}_{D}$ -39.3° (c = 6.37 in chloroform) was obtained.

Oil of Abies Balsamea (L.) Mill.

Fractional Distillation of the Oil

The oil (7,800 g.) was separated by simple distillation through a 15-in. unpacked column at 10 mm. pressure. A hydrocarbon fraction weighing 6382 g. and boiling below 70° C. at 10 mm., a fraction containing oxygen compounds weighing 1215 g. and boiling at 70° - 110° C. at 10 mm., and a sesquiterpene fraction boiling above 110° C. at 10 mm. and weighing 161.1 g. was obtained. A 1550 g. aliquot of the hydrocarbon fraction was distilled through a 6-ft., 25-mm. I.D. Podbielniak column at 20 mm. pressure, 20 mm. pressure drop across the column, and reflux ratio 20./1 to separate 509 g. of a fraction boiling below 166°C., a β-pinene fraction boiling at 166-167°C. and weighing 520 g. and a fraction weighing 520 g. and boiling above 167°C at atmospheric pressure. The fraction boiling below 166°C. was redistilled through 6-ft. Podbielniak column at 20 mm. pressure, 20 mm. pressure drop and reflux ratio 20/1. Fractions 1 to 13, Table XLII, were collected. Volatile material (157 g.) was removed from the pot residue and combined with the fraction boiling above 167°C. at atmospheric pressure. Fractions 15 to 40 were then obtained by distillation through the 6-ft Podbielniak column at 20 mm. pressure, 20 mm. pressure drop, and reflux ratio 20/1. The volatile residue from this distillation was combined with the oxygen compounds, and fractions 41 to 60 obtained by distillation through the 6-ft. Podbielniak column at 20 mm. pressure, 20 mm. pressure drop, and reflux ratio 20/1. Fraction 61 was obtained from the pot residue and combined with the sesquiterpene fraction. The sesquiterpenes were fractionated through a 1-ft., 25 mm. I.D. Podbielniak column at 10 mm. pressure, 3 mm. pressure drop, and reflux ratio 15/1.

Identification of Constituents

The oil was found to be qualitatively very similar in composition to oil of Picea mariana. With the exception of the sesquiterpenes all constituents were identified by methods described in the previous section dealing with oil of Picea mariana.

The identifications of all solid derivatives were confirmed by determination of their melting points in admixture with authentic samples.

Santene and tricyclene:- Fractions 1, 2 and 3, Table XLIII, were combined and redistilled at atmospheric pressure, 0.5 mm. pressure drop and reflux ratio 40/1 through the 1-ft. Podbielniak column, to separate two optically inactive compounds, b.p. 151.5-153.0., N^{20}_{D} 1.4664, d^{20}_{4} 0.862, and b.p. 151.5-153.0°C., N^{20}_{D} 1.4610, d^{20}_{4} 0.866. The former; when treated with nitrous acid according to Muller (131), gave santene nitrosite m.p. 123-125°C., in 30.9% yield. The latter compound was treated for one hour with 10 volumes of 10% aqueous potassium permanganate at 100°C. to remove unsaturated impurities. Unchanged tricyclene, recovered in a current of steam, melted at 65°C. after crystallization from methanol and sublimation from sodium.

dl-α-Pinene:- Oxidation with neutral potassium permanganate (134) of 39.5 g. of fractions 6 and 7 yielded 15.02 g. of crude acids from which, by repeated crystallization from benzene-hexane mixtures, dl-pinonic acid, m.p. 104-106°C., η^{25}_{D} - 0.18° (c = 10.88 in chloroform), was separated.

1-Camphene:- Fraction 13 was a solid, m.p. 43°C., b.p. 160.5° C., $[\alpha]$ D -91.3° (c = 10.33 in benzene). Hydration of 5.0 g. of this material by the method of Bertram and Walbaum (137) yielded 5.56 g. of isoborneol which, after crystallization from hexane, melted at 210-213° C. in a sealed capillary.

1-β-Pinene:- Fraction 18 (20.0 g.) in 200 ml. of acetone was cooled in a freezing mixture and 48 g. of powdered potassium permanganate added with mechanical stirring over sixteen hours. The manganese dioxide was separated, washed with acetone and extracted with six, 100-ml. volumes of boiling water. When concentrated under reduced pressure to a volume of 75 ml., the aqueous extracts deposited 2.60 g. of a sparingly soluble potassium salt. The salt was recrystallized from water, and the free acid extracted from an acidified aqueous solution with ether. By crystallization from benzene-hexane mixtures, small needles of 1-nopinic acid, m.p. 126-127°C., $[\alpha]^{25}_{D}$ -15.2° (c = 6.12 in ether) were obtained.

Myrcene:- Refractionation of fraction 22 gave a liquid, b.p. 167.5-168°C./751 mm., N^{20}_{D} 1.4720, d^{20}_{4} 0.820, α_D -4.60° which reacted with maleic anhydride to give, in 39% yield, an adduct m.p. 34-35°C., b.p. 145-155°C./0.4 mm. The dicarboxylic acid obtained by saponification of the adduct melted at 123-125°C.

<u>d-Δ^3-Carene</u>:- Fraction 29 (5 g.) was mixed with 4 g. of amyl nitrite and 2 ml. of glacial acetic acid, and to this mixture 3.5 ml. of concentrated nitric acid was added dropwise with ice cooling. After fifteen minutes the product was precipitated with 10 ml. of methanol. After several precipitations from warm chloroform with methanol, 0.45 g. of d-Δ^3-carene nitrosate, m.p. 146°C. with decomposition, was obtained.

<u>l-Limonene</u>:- Bromination of 10 g. of fraction 37 in isoamyl alcohol-ether solution yielded 7.2 g. of solid bromides, which were crystallized from ethanol to give 1.6 g. of dl-limonene tetrabromide. The mother liquors, after concentration and removal of a further quantity of racemate, deposited at -70°C., 150 mg. of l-limonene tetrabromide, m.p. 102-104°C.

<u>Examination of Fractions 43 and 44</u>:- Fraction 43 (10 g.) was allowed to stand for one week in an ethanol solution containing excess semicarbazide hydrochloride and sodium acetate. Nonketonic compounds (4.6 g.) were separated from the crude semicarbazones (2.9 g.) by steam distillation. The crude semicarbazones deposited 0.69 g.

of crystalline material, m.p. 231-233°C. from ethanol which when mixed with an equal weight of oxalic acid and steam distilled was decomposed to yield 0.02 g. of dl-camphor m.p, 178-179°C.; 2,4-dinitrophenyl hydrazone m.p. 167-169°C.

The nonketonic portion was treated with n-butyl borate as previously described, and liberated n-butyl alcohol and nonalcoholic compounds separated from the borate esters (0.65 mg.) by distillation. By bromination of this distillate 0.11 g. of terpinolene tetrabromide m.p. 115-116°C. was obtained.

Fraction 44 (15.5 g.) treated in an analogous manner gave 6.45 g. of crude semicarbazones yielding camphor with $[\alpha]_D$ -24.0° (c = 4.99 ethanol) on hydrolysis, and 10.2 g. of nonketonic compounds from which 4.5 g. of borate esters were prepared. The alcohol, regenerated by saponification of the combined borate esters of fractions 43 and 44, boiled at 82-83°C. (10 mm.), N_D^{20} 1.4743 and formed a p-nitrobenzoate (0.05 g. from 0.6 g.) m.p. 105-107°C., $[\alpha]_D$ $+21.02$ (c = 0.76, ethanol).

<u>Examinations of Fractions 45 and 46</u>:- Fraction 45 (10.4 g.) yielded 1.9 g. of semicarbazone and 8.7 g. of non-ketonic oil. From fraction 46 (13.7 g.), 4.10 g. of semicarbazone and 9.6 g. of nonketonic compounds were obtained. The semicarbazones did not crystallize well from

common solvents. Fraction 46 semicarbazones were crystallized from acetonitrile to give 0.43 g. of a crystalline compound m.p. 205-212°C. The acetonitrile insoluble semicarbazones were crystallized from nitromethane, and 0.36 g. of a compound m.p. 214-219° C. obtained. Too little material was available to allow crystallization to constant melting point. As the semicarbazones were not hydrolyzed by the action of 1N sulphuric acid or oxalic acid at 100°C., the ketone was not isolated or identified.

The combined alcohols of fractions 45 and 46 (9.66 g.) isolated by means of their borate esters, deposited 0.86 g. of solid borneol when cooled. The liquid portion, b.p. 91.5-96° C, (12 mm.), N_D^{20} 1.4848, d_4^{20} 0.952, α_D -12.34° yielded only derivatives of borneol when treated with p-nitrobenzoyl chloride, α-naphthyl isocyanate, or phenylisocyanate, and failed to form a solid hydrochloride. This fraction then consists of a mixture of borneol, and an unidentified tertiary alcohol.

Bornyl Acetate:- Forty grams of fraction 56 were saponified, and 33.5 g. of borneol, m.p. 206-207°C. $[\alpha]_D$ -39.02 (c = 10.89, toluene), and 15 g. of crude potassium salts obtained. A 20% aqueous solution of 5 g. of the salts was allowed to percolate through 50 gm. of Amberlite resin IR-120, (hydrogen cycle) and the eluate refluxed briefly to remove carbon dioxide. The Duclaux constants of this solution were 7.0, 7.3, and 7.7 indicating the absence of significant amounts of volatile acids other than acetic. From 4.0 g. of the salt, 0.5 g. of acetanilide m.p. 114-115°C. was prepared.

Dehydrogenation of the sesquiterpene fractions:- Only those fractions which appeared to contain a maximum concentration of one constituent were examined. Fractions 67, 69, 72, 73, 78, 80, and 81 were dehydrogenated as follows: A mixture of 1.0 g. of the oil and 0.10 g. of palladium-charcoal catalyst was heated under nitrogen, at 200°C. for two hours, then at 245°C. for six hours, The reaction mixture was diluted with hexane, filtered, and the filtrate extracted with 5% aqueous sodium hydroxide. Hexane was removed from the extracted filtrate and the residue in 10 ml. of ethanol was refluxed for fifteen minutes with 1.0 g. of picric acid. Cadalene-picric acid addition compound, m.p. 116-117°C. was obtained in a yield of 700 mg. from fraction 78, 296 mg. from fraction 73 and a few crystals from fraction 72. Fraction 80 gave a black product which apparently decomposed during recrystallization as only picric acid, m.p. 121°C. was recovered. No picrate forming substances were obtained from fractions 67, 69, or 81.

Preparation of palladium-charcoal calalyst:- The dehydrogenation catalyst was prepared by the method of Linstead and co-workers (114). A solution of 0.84 g. of palladium chloride and 0.50 ml. of concentrated hydrochloric acid in 5.0 ml. of water was cooled in an ice-salt mixture and 1.1 g. of Nu-char carbon, previously activated at 340°C., and 0.4 mm., and 5.0 ml. of 40% formaldehyde added with mechanical stirring. A solution of 5.0 g. of potassium hydroxide in 5.0 ml. of water was added dropwise and the temperature of the mixture then raised to 60°C. for fifteen minutes. The precipitate was washed with water, dilute acetic acid, and finally with water until free of chlorides, then dried at 100°C and stored in a desiccator.

<u>S-Guiaiazulene</u>:- A further quantity of dehydrogenation product from 1.02 g. of fraction 80 in six volumes of hexane was extracted with two 1-ml. volumes of 85% phosphoric acid at 0°C. as described by Sherndal (107). The phosphoric acid was exhaustively extracted with hexane, diluted with ice water, and extracted with ether. Steam distillation of the ether extract gave 23 mg. of an intensely blue oil which, when refluxed with 0.5 ml. of ethanol and 25 mg. of symtrinitrobenzene formed black needles,m.p.147°C.S-guiaiazulene was prepared by dehydrogenation of the fraction, b.p. 120-130°C./10 mm. of gurjun balsam oil.

<u>Sesquiterpene hydrochlorides</u>:- A solution of 2.4

g. of fraction 77 in 2.5 ml. of diethyl ether was saturated with dry hydrogen chloride at 0°C., and refrigerated overnight. After evaporation of the ether and removal of oily products on a cold porous tile, 1.1 g. of hydrochlorides remained. The hydrochloride mixture was separated by crystallization, from ethanol, and by mechanical means, into long needles of l-cadinene hydrochloride, m.p. 138-139°C., and small platelets of bisabolene hydrochloride, m.p. 80-81°C. The authentic sample of bisabolene was isolated from distilled lime oil residues kindly donated by Dr. E. Guenther.

Oil of Thuja Occidentalis L.

Fractional Distillation of the Oil

Oil of Thuja occidentalis (3,436 g.) was distilled through a 6-ft, 25-mm. I.D. Podbielniak column at 50 mm. pressure, 20 mm. pressure drop across the column, and reflux ratio 20/1. A hydrocarbon fraction boiling below 105°C. at 50 mm. pressure was separated from the pot residue and redistilled under the same conditions. Fractions 1 to 22, Table XLVII, were collected. Volatile material (67.77 g.) was then separated from 7.96 g. of non-volatile residue by simple distillation and combined with the pot residue from the preliminary distillation. The mixture was distilled through the 6-ft. Podbielniak column and the large fraction 23 boiling at a constant

105.5°C. at 50 mm. removed. Fractions 24 to 27 were distilled at 50 mm. The pressure was then reduced to 20 mm. and fractions 28 to 59 collected. After collection of fraction 59 the side arm of the column plugged with solid and it was not possible to melt the obstructions with an infra-red lamp. Distillation was continued until as much solid as possible had accumulated on the reflux condensors. Distillation was then interrupted and fraction 60 scraped from the condensors. Distillation was then resumed and fractions 61 to 69 collected at 20 mm. pressure, 20 mm. pressure drop and reflux ratio 20/1. Fractions 70 to 72 and the sesquiterpene fraction were separated from the pot residue by distillation through a 15-in. unpacked column at 10 mm. pressure and finally at 1 mm. pressure.

Identification of Constituents

<u>1-α-Thujene</u>:- Fraction 1 was redistilled through a 1-ft., 25-mm. Podbielniak column at atmospheric pressure and the fractions described in Table LVI collected.

Table LVI

<u>Redistillation of α-Thujene.</u>

Fraction	b.p.	N_D^{20}	αD
i	96 - 133.0°	1.4265	+ 1.60
ii	138 - 147.5	1.4468	+ 1.78
iii	147.5 - 150.5	1.4523	- 2.63
iv	150.5 - 152.5	1.4559	-14.76
v	153.7	1.4581	-15.10

Fraction iv (0.5 g.) in 2 ml. of diethyl ether was saturated at 0°C. with dry hydrogen chloride, allowed to stand overnight, the ether removed by evaporation and the residue refrigerated. After removal of oily products on a cold porous tile, 0.09 g. of hydrochloride was obtained, m.p. 50-51°C. undepressed on mixing with a sample of terpinene hydrochloride.

A mixture of 1.0 g. of fraction iv and 2.35 g. of potassium permanganate in 100 ml. of water was allowed to stand with frequent shaking at 0°C. for twenty-four hours. The manganese dioxide was separated in the centrifuge and well washed with water. The aqueous liquors and washings were extracted with ether, acidified and again extracted with ether. The latter extract after removal of the ether yielded about a gram of yellow oil which failed to crystallize. This oil in 10 ml. of ethanol was added to a solution of 0.8 g. of semicarbazide hydrochloride and 0.8 g. of anhydrous sodium acetate in 1 ml. of water, and allowed to stand in an unstoppered flask for several days. The semicarbazone which separated crystallized from alcohol in short needles, m.p. 198 - 199°C., undepressed by an authentic sample of α-thujaketonic acid semicarbazone. Seyler (142) reported m.p. 198.5°C. for α-thujaketonic acid semicarbazone.

dl -α-Pinene:- Fraction 5 (17 g.) when oxidized with potassium permanganate by the method of Delepine (134) yielded 11.2 g. of crude acids. After two crystallizations from a benzene-hexane mixture, the product melted at 103-104°C. alone, or in admixture with a known specimen of dl-pinonic acid.

l-Camphene:- Fractions 9 (5 g.) in 15 ml. of a solution of 1 ml. of 50% sulphuric acid in 25 ml. of glacial acetic acid, was heated at 60°C. for three hours, poured into water, and the oily paste which separated saponified with 20% alcoholic potassium hydroxide. The resulting oily paste was triturated with cold hexane and pressed between filter papers to yield 2.1 g. of crude isoborneol. A part of this material was boiled for five minutes with an equal weight of p-nitrobenzoyl chloride in pyridine to yield isoborneol p-nitrobenzoate m.p. 131-133°C. Huckel reports m.p. 131-132°C. for dl-isoborneol p-nitrobenzoate (138).

d-Sabinene and l-β-pinene:- Fraction 11 (5.0 g.) in 10 ml. of diethyl ether was saturated with hydrogen chloride at 0°C., refrigerated overnight, freed of ether, and refrigerated for several days. Oils were removed from the partially crystalline product on a cold porous tile, leaving 1.6 g. of a hydrochloride, m.p. 50-51°C. after three crystallizations from ethanol. The melting point of a mixture of this derivative and the hydrochloride prepared from α-thujene, and with a sample of terpinene hydrochloride prepared from an authentic sample of sabinene isolated from oil of savin, was not depressed.

Fraction 11 (24.1 g.) was shaken for one hour with 57 g. of potassium permanganate and 12 g. of sodium hydroxide in 400 ml. of water containing 400 g. of crushed ice. The solution was freed from manganese dioxide in the centrifuge, once extraced with chloroform, concentrated to 300 ml. and refrigerated. The sparingly soluble sodium salt which separated (1.7 g.) was recrystallized from water. The free acid was extracted from an acidified aqueous solution of the salt with ether and crystallized from hexane. l-Nopinic acid was obtained melting at 125-126°C. alone, and at 126-127°C. in admixture with an authentic specimen.

The aqueous oxidation liquors were acidified and extracted with chloroform. The oil obtained after removal of the chloroform was treated with 5 ml. of 10% aqueous sodium hydroxide to yield a crystalline salt weighing 0.08 g. after two crystallizations from water. The free acid, isolated as before and crystallized from hexane and then from water melted at 56-57°C. alone, or in admixture with an authentic sample of sabinenic acid prepared from sabinene from oil of savin.

Myrcene:- Fraction 16 (2.7 g.) reacted violently when warmed with 2.0 g. of maleic anhydride. Crude adduct (2.6 g., b.p. 190-200°C. at 10 mm.) was separated by distillation and crystallization from hexane. The product melted at 35-36°C. alone, or in admixture with cis-4-isohexenyl-Δ^4-tetrahydrophthalic anhydride.

dl-Limonene:- About 7 ml. of bromine was added dropwise to an ice-cold solution of 10 g. of fraction 21 in 10 ml. of isoamyl alcohol and 20 ml. of ethyl ether. On evaporation of the ether, 8.7 g. of bromide separated and was recrystallized from ethanol. dl-Limonene tetrabromide, m.p. 125-126°C., undepressed by an authentic sample, was obtained.

l-Fenchone:- Fraction 23 was redistilled through the 6-ft. Podbielniak column at 50 mm. pressure and reflux ratio 20/1. The fractions listed in Table LVII were collected from the lower boiling part of the fraction. The higher boiling portion of the fraction consisted almost entirely of l-thujone.

Table LVII

Redistillation of Fenchone

Fraction	N_D^{20}	d_4^{20}	αD	b.p./50 mm.	% of original oil
23-1	1.4670	0.9141	-40.18°	101.8°	1.29
23-2	1.4633	0.9361	-53.06	103	2.55
23-3	1.4624	0.9387	-55.03	103	3.88
23-4	1.4620	0.9406	-55.66	103	5.15
23-5	1.4619	0.9406	-55.41	103	6.48
23-6	1.4594	0.9360	-48.51	104	8.14
23-7	1.4550	0.9249	-35.24	105.5	9.36
23-8	1.4535	0.9230	-31.80	106	10.55
23-9	1.4542	0.9230	-32.74	107	11.73

Fraction 23-5 (20 g.) was warmed to 90-100°C. with 60 ml. of concentrated nitric acid until brown fumes were no longer evolved, then washed with water, distilled in a current of steam and dried. Unchanged terpene (16.5 g.) was made to solidify in Dry Ice. The melting point, measured with an immersed thermometer, was 5.0°C. For pure fenchone, Huckel reported m.p. 5.5°C. (143).

A solution of 5.0 g. of this fraction, with 11 g. of hydroxylamine hydrochloride and 6 g. of potassium hydroxide in 80 ml. of ethanol, in 10 days at room temperature deposited 1.21 g. of beautifully crystalline oxime, m.p. 165-167°C., α_D - 44.0° (c = 3.03 in ethanol). Huckel and Sachs for d-fenchone-α-oxime reported m.p. 167°C., $[\alpha]_D$ + 46.5 (in 96% ethanol (144).

<u>l-Thujone and d-isothujone</u>:- Fraction 32 yielded a semicarbazone, m.p. 197-199°C., $[\alpha]_D$ + 53.7° (c = 1.02 in ethanol). This semicarbazone was mixed with an equal weight of oxalic acid and steam distilled to yield an oil with N_D^{20} 1.4490. This oil (0.4 g.) was shaken with 40 ml. of cold aqueous 2% potassium permanganate and allowed to stand overnight. The product was recovered in the same manner as was the oxidation product of α-thujene. α-Thujaketonic acid (0.19 g.), m.p. 74.5-75.5°C., $[\alpha]_D$ + 173.4° (c = 1.04 in ether), was obtained.

Fraction 53 semicarbazone melted at 170-175° C., $[\alpha]_D$ +180° (c = 2.49 in chloroform). The ketone, regenerated from the semicarbazone by the action of oxalic acid and steam as before had N_D^{20} 1.4494, d_4^{20} 0.916, α_D +66.3, and was oxidized by potassium permanganate to α-thujaketonic acid.

Short and Read reported, for l-thujone semicarbazone m.p. 186-188°C., α_D +42.0° (c = 1 in methyl alcohol), and for d-isothujone semicarbazone m.p. 172°C., α_D +222° (c = 1 in methyl alcohol), (145).

For optically pure l-thujone and d-isothujone they reported α_D -19°56', and α_D +72°28' respectively.

<u>l-Camphor</u>:- The solid from fraction 60 melted at

90-110°C., $[\alpha]_D$ -35.5° (c = 2.49 in chloroform). This solid (0.5 g.) was boiled under reflux for thirty minutes with 0.5 g. of 2, 4-dinitrophenylhydrazine in 50 ml. of ethanol containing 2 ml. of concentrated sulphuric acid. A 2,4-dinitrophenylhydrazone (0.37 g.) was obtained, m.p. 175-176°C., undepressed by l-camphor 2,4-dinitrophenylhydrazone.

<u>d-Terpinen-4-ol</u>:- Fraction 63 (14 g.) was heated at 100 °C. and 30 mm. pressure for five hours with 10 g. of tri-n-butyl borate, and volatile materials removed at 100° C. and 1 mm. pressure. The residue was decomposed by alkalie in a current of steam and the product redistilled to give 4.54 g. of regenerated alcohol, b.p. 91°C./10 mm. N_D^{20} 1.4784, d_4^{20} 0.936, α_D +21.20°.

A solution of this alcohol (0.5 g.) in 0.5 ml. of diethyl ether was saturated with dry hydrogen chloride at -5°C., allowed to stand overnight and ether removed in a stream of air. The resulting oily paste was pressed on a cold porous tile and the product twice crystallized from ethanol to yield a few milligrams of terpinene hydrochloride, m.p. 50-51°C. alone, or in admixture with an authentic sample.

A mixture of 3.75 g. of the alcohol and 2.55 g. of potassium permanganate in 200 ml. of cold water was shaken vigorously and refrigerated overnight. The oxidation liquors were freed of manganese dioxide, neutralized with carbon dioxide, extracted twice with 25-ml. volumes of ether, and finally evaporated to dryness. The residue was extracted with three, 5-ml. volumes of ethanol and the ethanol evaporated. This residue was exhaustively extracted with boiling benzene. On cooling, the glycerol separated from the benzene as a gelatinous precipitate, which was purified by repeated crystallization from chloroform. The product consisted of an isotropic powder, m.p. 65-70°C., and rectangular anisotropic needles which began to undergo a dehydration or transition about 100°C., and melted at 128°C.

Bornyl acetate:- Fraction 69 was saponified, and borneol and acetic acid were identified in the reaction mixture by the methods employed in the investigation of oil of Tsuga canadensis. l-Borneol, m.p. 204-206°C., p-nitrobenzoate, m.p. 135-136° C., $[\alpha]_D$ -37.8° (c = 4.53 in chloroform), and acetanilide, m.p. 114°C., were obtained.

Fractions 18 and 19:- Fraction 19 (1.0 g.) in 2 ml of ether was treated with dry hydrogen chloride to yield 0.13 g. of dl-limonene hydrochloride, m.p. 50°C.

Fraction 18 (1.0 g.), treated with hydrogen chloride in the same manner, gave 0.06 g. of a hydrochloride, m.p. 71.5 - 72°C.

Higher boiling compounds:- Fraction 48, Table XXXVI, was saponified, and the alcohol and potassium salt of the organic acid, isolated from the saponification liquors by the methods employed in the study of bornyl acetate from oil of Abies balsamea. Acetic acid was obtained with Ducleaux constants 6.8, 7.1 and 7.4 and yielding an s-benzyl thiuronium salt, m.p. 133°C. The alcohol portion (N^{20}_{D} 1.4748, d^{20}_{4} 0.935, α_D -49.62°) formed a p-nitrobenzoate (0.29 g. from 0.75 g.), m.p. 140-141.3°C. $[\alpha]_D$ -38.1° (c = 5.67 in chloroform).

Fractions 48 and 49 and 50 were heated at 200-245° C., for eight hours with palladium-charcoal catalyst (114) and the products examined for picrate-forming substances by the methods used in the study of the sesquiterpenes of Abies balsamea. Fraction 48 (0.7 g.) yielded 0.08 g. of cadalene picrate, m.p. 115-117° . No aromatic products were obtained from fractions 49 and 50.

The Dextrorotatory Sesquiterpene

Preparation of 1-Cadinene

The fraction of Cubeb oil boiling at 120-140°C./10 mm. (50 g.) in 30 ml. of anhydrous ether was saturated with hydrogen chloride and stored in the Dry Ice chest overnight. The pasty solid remaining after removal of the ether was dissolved in ethyl acetate, an equal volume of ethanol added and the solution chilled. The long needles which separated were recrystallized from ethanol to give 10.0 g. of l-cadinene hydrochloride, m.p. 120.5-121°C.

This hydrochloride (10 g.) with 10 g. of anhydrous sodium acetate and 35 ml. of glacial acetic acid was heated at 90°C. for four hours, boiled under reflux for one hour, diluted with water and the regenerated hydrocarbon extracted with hexane, distilled, distilled from sodium, and again distilled to give a colorless liquid, b.p. 131.5-132.5°C. at 10 mm., N_D^{20} 1.5064, d_4^{20} 0.917, α_D -94.1°.

Henderson and Robertson (146) reported b.p. 134-136°C./11 mm., N_D^{20} 1.5079, d_4^{20} 0.9189, α_{5461} - 125.2°, for a sample of l-cadinene prepared by this procedure.

Preparation of the Dextrorotatory Sesquiterpene

The crude sesquiterpene fraction from oil of Picea mariana (fraction 48, Table XXXVI.), was redistilled through the 1-ft., 25-mm. I.D. Podbielniak column at 4 mm. pressure, 3 mm. pressure drop and reflux ratio 15/1. After removal of a lower boiling levorotatory fraction, the fractions listed in Table L were collected.

Infra-red Absorption Spectra

The infra-red absorption spectra were determined from 4,000 to 600 cm.$^{-1}$, with a Perkin-Elmer single beam infra-red spectrometer, using a sample thickness of 0.051 mm.

Oxidation with Perbenzoic Acid

Preparation of perbenzoic acid:- A solution of 150 g. of benzoyl peroxide in 600 ml. of chloroform was added, with shaking and cooling, to a solution of 15.6 g. of sodium in 300 ml. of methanol at -5°C. After five minutes the mixture was extracted with 1,500 ml. of water containing crushed ice, and the aqueous layer washed with cold chloroform. To the aqueous solution of sodium perbenzoate was added 675 ml. of cold I N. sulphuric acid. The perbenzoic acid was then extracted with 1,000 ml. of chloroform, the chloroform solution washed with water, dried with sodium sulphate, and stored in the refrigerator (147).

Reaction with the dextrorotatory sesquiterpene:- The hydrocarbon was oxidized by the method of Ruzicka and Sternback (148). A solution of 0.2496 g. of the sesquiterpene, 10 ml. of perbenzoic acid in chloroform (1.0 ml. of reagent $\equiv$ 6.44 ml. of 0.100 N sodium thiosulphate), and 75 ml. of cold chloroform were mixed and allowed to stand at 0°C. for ninety-six hours. Periodically, 10.0 ml. samples were withdrawn, added to a solution of 1.0 g. of sodium iodide and 5 ml. of glacial acetic acid in 50 ml. of water, and the mixture shaken vigorously and titrated 0.100 N sodium thiosulphate. A blank experiment was conducted at the same time. The difference in titration between the blank and the sample was equivalent to the oxygen consumed by the sesquiterpene.

Isomerization of the Sesquiterpenes

Dextrorotatory sesquiterpene:- A solution of 1.01 g. of the dextrorotatory sesquiterpene in 2.0 ml. of a 10% solution of sulphuric acid in ethanol was boiled under reflux for fifteen minutes, diluted with water and the oil separated. The oil was washed with sodium carbonate solution followed by water, dried over anhydrous sodium sulphate and distilled from sodium. The recovered oil had $\alpha_D + 5.6^{\circ}$. A solution of 0.8281 g. of the recovered oil was boiled an additional forty-five minutes with 1.7 ml. of 10% ethanolic sulphuric acid. The sesquiterpene recovered from this solution had α_D -38.75.

A fresh sample of dextrorotatory sesquiterpene (1.74 g.) was refluxed for four hours with 3.5 ml. of 10% ethanolic sulphuric acid. The recovered oil (1.625 g., α_D -47.5) was refluxed an additional three hours with 3.5 ml. of 10% ethanol sulphuric acid. The product had α_D - 41.93°. A solution of 0.614 g. of the oil in 2 ml. of ether was treated with hydrogen chloride to form 0.2327 g. of l-cadinene hydrochloride.

Another 1.768 g. of sesquiterpene in 4 ml. of ethanolic sulphuric acid was boiled for eighteen hours and then for twenty hours. The isomerides had α_D -27.00° and -8.5° respectively. From 1.0 g. of the latter product, only 0.0463 g. of l-cadinene hydrochloride was obtained.

<u>l.-Cadinene</u>:- The sample of l-cadinene regenerated from the hydrochloride was boiled with two parts of 10% ethanolic sulphuric acid and the product recovered as before. The optical rotation of the products of isomerization was -89.42° after one hours, -48.92° after an additional five hours, and -2.4 after a further twenty-four hours.

SUMMARY

1. The sterol contents of Canadian tall oils were three to four times greater than sterol contents reported for foreign oils. Sterol contents ranged from 3.92% to 12.61% for six Canadian oils.

2. Under the conditions of molecular distillation the fatty acids of tall oil were slightly more volatile than the resin acids and less volatile than the sterols, but no useful separation of these three classes of compounds could be achieved in a single distillation. The sterols and the resin acids distilled in a manner characteristic of single compounds, but the rates of elimination of the fatty acid fraction and the neutral fraction were characteristic of two component and five component mixtures, respectively. From the least volatile fatty acid distillates, N-tetracosanic acid was isolated.

3. Esterification of tall oil acids with tall oil sterols proceeds very slowly at temperatures below 130°C. The presence of other, more easily esterified alcohols was indicated, however.

4. Less material crystallized from solutions of tall oil at low temperatures than would be predicted from the solubility of the constituents individually. In all solvent systems, n-tetracosanic acid crystallized more

readily than other constituents. More substance crystallized from polar solvents than from non-polar solvents. The sterols crystallized preferentially from polar solvents at low temperatures.

5. The sterols of tall oil form sparingly soluble addition compounds with oxalic acid. The sterols may be separated conveniently by this means. The product of the process contains a small amount of 1-tetracosanol. The reaction is, therefore, not specific for sterols.

6. Chromatography of azobenzene-p-carboxylic acid esters of tall oil alcohols led to the appearance of a single alcohol in two separate bands. It was concluded that the azo compound existed in cis and trans modifications, and the chromatographic behavior of the esters was largely determined by the configuration of the azo group.

7. Oil of Picea mariana (Mill.) B.S.P. was shown to contain l-bornyl acetate, l-α-pinene, l-camphene, l-β-pinene, dl- and l-limonene, d-Δ^3-carene, myrcene, santene, terpinolene, tricyclene, l-borneol, dl-camphor, a compound related to l-cadinene, two unidentified alcohols and an unidentified ester.

8. In oil of Tsuga canadensis (L.) Carr the presence was demonstrated of l-bornyl acetate, d-α-pinene, l-camphene, l-limonene, l-β-pinene, myrcene, tricyclene, d-α-phellandrene, l-thujone, a tertiary alcohol, and a compound

related to l-cadinene.

9. Oil of Abies balsamea (L.) Mill. contained l-β-pinene, l-bornyl acetate, d-Δ^3-carene, l-limonene, l-α-pinene, l-camphene, myrcene, santene, tricyclene, l-camphor, l-borneol, bisabolene, terpinolene and compounds related to l-cadinene and guiaiazulene.

10. Oil of Thuja occidentalis L. contained l-thujone, d-isothujone, l-fenchone, l-bornyl acetate, dl-limonene, d-sabinene, l-β-pinene, d-terpinen-4-ol, dl-α-pinene, l-camphor, l-camphene, myrcene, l-α-thujene, and an unidentified ester.

11. The dextrorotatory sesquiterpene of Picea mariana was not identical with l-cadinene, but had the same configuration as l-cadinene about the asymmetric centers. When heated with acid it underwent a rapid inversion to a levorotatory compound which was probably l-cadinene. The sesquiterpene was doubly unsaturated. A structure was proposed.

CLAIMS TO ORIGINAL RESEARCH

1. The sterol contents of Canadian tall oils was shown to be much higher than those of foreign oils. No sterol content of Canadian tall oils have been reported previously.

2. A new gravimetric method was developed for the estimation of fatty acids and resin acids in tall oil. The precision of this method is approximately ten times that of the volumetric method of Hastings and Pollak.

3. The molecular distillation of tall oil was studied, and the elimination maxima of the fatty acids, the resin acids, and the sterols were measured in two distillations.

4. A study of the crystallization of constituents from solutions of tall oil at low temperature showed that the solubilities of constituents were greatly increased by the presence of other constituents. The behavior of tall oil solutions at low temperatures cannot be predicted from published values for the solubilities of its constituents.

5. n-Tetracosanic acid was isolated from a Canadian tall oil for the first time. The compound had been reported previously in European tall oils.

6. Tall oil sterols were shown to form a sparingly soluble addition compound with oxalic acid. Conditions for formation of this product from tall oil in maximum

yield were established. The presence of 1-tetracosanol in the product suggests that the reaction is not limited to sterols.

7. The essential oil of Picea mariana (Mill.) B.S.P. was investigated for the first time and 1-bornyl acetate, 1-α-pinene, 1-camphene, 1-β-pinene, dl- and 1-limonene, d-Δ^3-carene, myrcene, santene, tricyclene, terpinolene, 1-borneol and dl-camphor were identified.

8. The composition of the essential oil of Tsuga canadensis (L.) Carr was studied. 1-Camphene, 1-limonene, 1-β-pinene, myrcene, tricyclene, d-α-phellandrene and 1-thujone were identified in the oil for the first time.

9. The essential oil of Abies balsamae (L.) Mill. was examined. Compounds found which had not previously been identified in this oil were, 1-β-pinene, d-Δ^3-carene, 1-limonene, 1-camphene, myrcene, santene, tricyclene, 1-camphor, 1-borneol, terpinolene and bisabolene.

10. A reinvestigation of the essential oil of Thuja occidentalis L. led to the identification of dl-limonene, d-sabinene, 1-β-pinene, d-terpinen-4-ol, dl-α-pinene, 1-camphene, myrcene, and 1-α-thujene in this oil for the first time.

11. The first tricyclic terpene hydrocarbon to be found in nature, tricyclene, was identified in three of the oils.

12. A new sesquiterpene was isolated from oil of Picea mariana (Mill.) B.S.P., its relationship to 1-cadinene was studied, and a structure proposed.

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