NATURE
OF THE
RESINS OF JACK PINE

THE NATURE OF THE RESINS OF JACK PINE

by

John Bernard Phillips, B.Sc., M.Sc. (McGill)

A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

1930

ACKNOWLEDGMENT

The writer desires to express his sincere thanks to Dr. Harold Hibbert for his kind advice and aid during the prosecution of this research,

also

to the National Research Council of Canada for the granting of a Bursary and a Studentship, which made possible the carrying out of this research.

TABLE OF CONTENTS

General Introduction 1

Bibliography viii

PART I

METHODS OF EXTRACTION AND INITIAL SEPARATION OF THE CRUDE RESIN PRODUCT.

Introduction 1

Experimental methods 7

Discussion of experimental results 28

Bibliography 35

PART II

THE RESIN ACIDS PRESENT IN THE CRUDE RESIN PRODUCT.

Introduction 1

Discussion of experimental results 30

Experimental part 40

Bibliography 57

PART III

THE FATTY CONSTITUENTS PRESENT IN THE CRUDE RESIN PRODUCT.

Introduction 1

Discussion of experimental results 19

Experimental:

Sec.1. Methods for identification of individual constituents 24

Sec.2. Methods for general separation of the fatty products 34

Bibliography 58

TABLE OF CONTENTS
(continued)

PART IV

THE ESSENTIAL OILS AND UNSAPONIFIABLE MATTER PRESENT IN THE CRUDE RESIN PRODUCT.

Introdtction 1

Discussion of experimental results 14

Experimental part:

Essential oils 17

Unsaponifiable matter 21

Bibliography 31

PART V

GENERAL SUMMARY OF THE RESULTS OF THE INVESTIGATION. 1

Final review, 7

.....

THE NATURE OF THE RESINS OF JACK PINE

General Introduction

The question of the composition and properties of the resinous secretions of Jack Pine (Pinus Banksiana) has formed a subject of peculiar interest during the past few years. Comparatively little is known of the true chemical nature of the mixture of natural substances, generally referred to broadly as "resins", which is secreted in the inner cells of such coniferous woods. On the other hand, the oleoresins, particularly those forming the raw material of the Naval Stores industry, have been the subject of considerable investigation. The question of the chemical nature of the inner secretions of the conifers has thus a peculiar scientific interest.

Jack pine is so very widely distributed throughout Canada, as well as parts of the United States, that the matter of its economic utilization has assumed considerable importance. This species of pine is now looked upon as a very significant factor in the matter of the supply of raw material for the paper industry. There are serious limitations to its use in this respect, however, on account of its high resinous content. Jack Pine can be used in the soda and sulphate processes of manufacturing chemical pulp, where strongly alkaline cooking liquors are employed, but serious difficulties are met with when attempts are made to cook this wood by the sulphite (acid) process, or to use it in the form of ground-wood for newsprint.

The high resin content of the pulp leads to the deposition of tacky, pitch-like masses on the wire and felts of the paper-machines, making it necessary to shut down the latter. This so-called "pitch-trouble" is the cause of considerable losses both in operating time, and in the value of the finished material.

This trouble also occurs frequently even when the less-resinous spruce wood is used.

The latter is the basic raw material of the sulphite pulp industry in Canada, and the enormous annual consumption of this wood has raised the question of the ultimate depletion of the supply. The abundance with which Jack Pine is distributed naturally gives the latter an important position as an alternative source of supply, provided the wood can be cooked successfully by the sulphite method. The latter problem is concerned solely with the resinous content of this particular wood, and consequently the question of the exact nature of these resins is of particular interest industrially. A thorough chemical investigation seems to be a logical preliminary step toward solution of difficulties in its utilization as an equivalent of spruce. It is quite possible, also, that the immense natural store of resin material from Jack pine may some day find important commercial applications. At the present time, the by-products of the sulphate and soda pulp processes, such as pine-oil, liquid rosin, wood turpentines, etc., are being utilized to a remarkable extent, particularly in Europe.

Very few investigations of importance have been carried out on the conifers of North America. Chief among these researches are those of Schorger[1], Bates[2], and Barnes[3]. Schorger examined the woods of several different species, and also ascertained the composition of the oleoresins, needle-oils, etc. His results are of great scientific interest. The work of Bates, on the waste wood of the longleaf pine (P. Palustris) indicated the possibility of obtaining large quantities of rosin, pine oil, turpentine, wood-pulp, etc., and several methods of treating this wood industrially, were examined, leading to important results as regards the chemical utilisation of this pine waste.

Various conifers have been examined by Barnes (loc. cit.) as regards their content of resins, cellulose, lignin, etc.

The commercial products obtainable from the waste material of American conifers, have been reviewed by Greenwood[4].

Botanical: Resins of all kinds are very widely distributed in the vegetable kingdom. They are not confined only to the intercellular "resin-canals", but are also present in the cell content, and in the cell wall itself. Resins are considered as belonging to the class of "extractives" from wood, because of the fact that they are readily removed by means of ordinary solvents. For this reason, they are not considered as forming part of the plant proper, but are merely extraneous constituents of the wood complex. Some investigators[5] have endeavoured to trace the existence of some kind of chemical union between the

lignin, resins and tannins of wood, but there is no definite chemical evidence of this so far.

A distinction is often made between what are termed "physiological" (or "natural") and "pathological" resins. The former term refers to those secretions of the normal, living tissues of the inner wood, which remain in the wood; the term "pathological", on the other hand, has reference to the exudations which follow soon after a wounding of the outer parts of the tree; this latter class includes the oleoresins. These two classes of "resins" obviously may not necessarily have the same composition, even for the same species. The inner secretions are much more complicated than the oleoresins, and have not been investigated to the same extent.

The present investigation is concerned solely with the so-called "physiological" resins of Jack Pine.

Natural resins are never homogeneous, and, especially in the case of the secretions of the inner wood, may be extremely complex. When such secretions are extracted from the woody tissues, by organic solvents for instance, a tarry mixture of materials is obtained, the complete investigation of which presents a problem not unlike the separation and identification of the constituents of coal tar, for example. The investigation of these secretions is always hampered to a very great extent on account of the relatively small proportions in which some of the constituents are present in the wood.

These natural products are, in general, extremely sensitive

to both physical and chemical reagents, and their examination requires the exercise of unusual precautions if the individual substances are to be isolated and identified in the same state as that in which they are normally present in the living tree.

A thorough review of the enormous mass of literature on the general subject of resins brought to light certain important facts with regard to "physiological" resins:

(1) The necessity for a critical examination of the methods used in the isolation in the unchanged state of the natural resins, and the selection of a suitable procedure for accomplishing this,

(2) The requirement of entirely new and revised technique in the qualitative and quantitative separation, classification and identification of the individual substances present in the crude resin mixture.

In order to facilitate a clearer oversight of the work actually accomplished in the present investigation, it was deemed advisable to divide the thesis into five main sections:

PART I: This gives a critical review of methods previously employed for the isolation of the crude resinous materials from the wood, and of the modifications found necessary, in the present instance, for the isolation of the four important groups of substances present, namely, (a) the resin acids, (b) the fatty constituents, (c) the essential oil, (d) the unsaponifiable matter.

PART II: This deals with the previous literature on the subject of the "RESIN ACIDS" of the conifers, and with the

methods used in the present investigation for the isolation and identification of the substances of this type.

PART III: This is concerned with a discussion of previous work relating to the "FATTY CONSTITUENTS" of wood, in general, and particularly with the few investigations that have been carried out on the fats present in the internal secretions of the conifers. The new methods used in the present work on Jack Pine for separating and identifying these products are described.

PART IV: This section comprises a brief review of the "ESSENTIAL OILS AND UNSAPONIFIABLE MATTER" of resins in general, and contains a detailed account of an improved method for the investigation of these constituents in Jack Pine.

PART V: This is devoted to a resumé of the results obtained in the present investigation, and their bearing on a future program of work concerned with the application of such results to the industrial side of the manufacture of pulp and paper.

******

Wood Used in the Present Investigation

(a) The "green" wood (Jack Pine) referred to in this work, was obtained in the form of four-foot, unbarked logs, from the Abitibi Power and Paper Company, Ltd., Iroquois Falls, Ontario. These logs were barked immediately before cutting up for extraction. The logs were from the trunks of trees varying from 125-140 years old. The wood was stored in a refrigerating room until used.

(b) The "seasoned" wood (Jack Pine), in this work, was obtained from the Howard Smith Paper Mills, Cornwall, Ontario, and was grown in the Eastern Townships of the Province of Quebec. This log was from the trunk wood of a tree 135 years old, and had been left exposed on the wood-pile out-of-doors for about three years. It was not extracted until one year later, and, in the meantime, was stored in a refrigerating room.

BIBLIOGRAPHY.

1. Schorger, "A Contribution to the Chemistry of American Conifers", Trans. Wis. Acad. Sc. Arts, Lett., XIX, part II, pp.728-766.

2. Bates, "Chemical Utilization of Southern Pine Waste," Pulp & Pap. Mag. Can., Jan.15, 1914, p.33; Feb. 1st, 1914, p.64; Paper, Vol.13, no.23, p.19; no.24, p.108; no.25, p. 19; (1914).

3. Barnes, Chem. & Met. Eng., 28, 503 (1923); Pulp & Pap. Mag. Can., 21, 1257 (1923).

4. Greenwood, Pap. Trade Jour.84, no.5,p.60; no.6, p.52; no.7, p.43, (1927).

5. Tschirch,"Die Harze und die Harzbehalter", p. 194 etc.
Fuchs,"Die Chemie des Lignins", Chap. XI, etc.
von Euler, Cell.chem. 2, 123,(1921); 3, 1, (1922).
Friederich & Salzberger, Monatsh. 53-54,989-1001,(1929).

PART I

METHODS FOR ISOLATION OF THE CRUDE RESINS AND FATS AND INITIAL SEPARATION OF GROUPS OF THE CONSTITUENTS.

PART I

Methods for Isolation of the Crude Resins and Fats and Initial Separation of Groups of the Constituents

Introduction

Resins and fats can be readily removed from woody tissues by means of various organic solvents, but in most cases, other extraneous products are removed as well. In order to avoid this, ether has been used by many workers, as it does not remove lignin, sugars, and other non-resinous materials. However, certain resinous products (particularly a number of resinous oxidation products) are difficultly soluble or are insoluble in ether, so that the extraction is always incomplete. Benzene[1] has been found to remove small amounts of lignin, and acetone probably behaves similarly. Wahlberg[2] tried various extraction solvents and finally chose benzene extraction, this being followed by a second treatment with alcohol. He found that the latter removed some lignin. A. G. von Euler[3] showed that the crude material removed from spruce by extraction with a hot alcohol-benzene mixture contained from 4.6 to 12.6% lignin.

Evidently not all solvents are applicable to a quantitative determination. Ether is very suitable for isolating constituents as they exist in the wood and it also has a low boiling-point, while alcohol and benzene also have specific advantages. The composition and amount of the extracted substances thus depend on the particular solvent used. This must be borne in mind in comparing the previous results of

different workers. A discussion of the factors affecting the choice of solvent has been given by Sieber[5]. Aqueous solutions of sodium hydroxide or carbonate remove acidic substances chiefly, and are of no use for quantitative work. The use of two successive solvents has been found to give consistent results generally, but requires double the time. A mixture of equal volumes of alcohol and benzene was employed in the present work, as it seemed to be the most efficient and convenient form of solvent for this kind of resinous material.

The time necessary for complete extraction depends a great deal on the apparatus used. In the present case, eight hours' extraction was found sufficient to remove practically all of the resins, only verysmall portions being extracted after that. These latter probably consist of lignin. Wahlberg[2] found 8 to 10 hours sufficient, the solvent used being benzene, but followed this treatment with an alcohol extraction. He found that small amounts of material could still be removed after extracting for one week, and noted that the alcohol removed much lignin.

The amounts of resins removed from certain species of pine and spruce, by a large number of workers using various solvents, are reported in detail by Sieber[6] The amount extracted depends, of course, on the origin of the wood, its freshness, conditions of exposure, and other factors. The percentages of resins in specimens of Jack pine removed by ether followed by an extraction with alcohol have been

reported by F. Barnes[7] as follows:

	% ether extract	% alc. extract	Total extract
Jack pine, seasoned, large tree	2.70%	0.99%	3.69%
" " green, small tree	3.24	1.71	4.95
" " river, seasoned	2.37	0.77	3.14

Similar figures were obtained by Johnsen and Hovey[8], using the same solvents.

Separation of the Pine Resin into its Constituents

The separation of the crude resin mixture into its components presents a difficult problem. The methods used will depend on the degree of accuracy required, and as to whether the materials thus separated are to be examined further, - in other words, an individual plan is necessary to suit the particular conditions. There has been no systematic procedure developed as yet for the complete separation of such resin mixtures and the identification of the individual constituents. There exists a well-known general method for the analysis of mixtures embracing rosin, fat and unsaponifiable matter and serving for the estimation of these materials[9] and adapted by Sieber[10] to the proximate analysis of wood-resin mixtures. This method involves a preliminary saponification of the crude resin mixture, by means of N/2 alcoholic potash, followed

by removal of the unsaponifiable products by petroleum ether extraction. The mixture of acids obtained from the alkaline solution is then separated quantitatively by Wolff and Scholze's[11] modification of Twitchell's[12] esterification method, into resin acids and fatty esters. The unsaponifiable products are obtained by evaporation of the petroleum ether extract.

This method is suitable for analytical purposes, and is comparatively simple, but it does not permit of the isolation or estimation of any esters, glycerides, essential oil, lignin, etc., which may be present. Saponification at an elevated temperature must necessarily affect the natural resin acids present, and perhaps also the liquid fats. It was evidently not intended for use where a further investigation of the individual constituents was planned.

A second scheme of analysis for conifer resin exudations was devised by Tschirch[13]. This is satisfactory with respect to some of the constituents, but not when applied to wood-resins, because Tschirch did not take into account the presence of fatty substances. Also the isolation of the resin acids by his method is a difficult and tedious matter. Tschirch first treats the crude resin with sufficient ether to dissolve all the soluble material, and discards any insoluble. This ethereal solution is washed well with water, to remove tannins, carbohydrates, etc. Successive treatments with certain alkaline solutions, $(NH_4)_2CO_3$, Na_2CO_3, etc., remove all of the acidic substances from the ethereal extract. These alkaline extracts,

when acidified, yielded only solid resinic acids, which were then purified.

The residual ethereal solution, containing only neutral substances, was evaporated, and the volatile oil present removed by steam-distillation. The residue was found to be a very inert, waxy material, which Tschirch termed "Resene".

This entire method is very tedious, and there is nothing to prove that the various alkaline extracts employed removed structurally different acidic substances as claimed by Tschirch. The purity of most of his products is open to serious question, and Dupont[14] considers that this author has merely encumbered the literature with a long series of meaningless names and has added nothing new to the chemistry of the resin acids.

Notwithstanding this serious objection, and the fact that no regard was paid to the presence of fatty acids, glycerides, esters, etc., the method of Tschirch enables an estimation to be made of the essential oil, total acids, and mixed neutral substances and, on this basis, there is the possibility of building up a systematic procedure for isolating these groups and investigating their individual constituents.

Both the method suggested by Sieber[10] and the general plan of Tschirch were investigated in detail in the present research and found to be unsatisfactory, and it was necessary to devise a new method, as outlined and discussed below.

New Method for the Isolation and Identification of the Constituents of Jack Pine Resins

The method developed for the complete separation of the crude resin mixture obtained by alcohol-benzene extraction and identification of the individual constituents includes some of the best features of the processes already mentioned, and is intended to serve as a basis for a thorough scientific investigation of these substances. The numerous details of this plan are given later in the different experimental parts, dealing with the initial separation of the groups, and examination of the resin acids, fats, unsaponifiable, and essential oil, each of which is taken up separately.

Experimental

Preparation of the Wood for Extraction

Each log was first subjected to a critical microscopic examination and identified by its botanical features, as Pinus Banksiana (Jack Pine)[15], before being used in the investigation.

The "green" pine logs (Pinus Banksiana) were stored in a cold room until required. The bark was removed just before the wood was used. The log was then cut into very small shavings, by means of a mechanical planer, and these were kept in closed boxes, stored in the cold room, until they were put in the extractor.

It is not advisable to grind the wood, on account of the heating effect and too much exposure of the resins to air. When chips are used, there is the possibility of poor penetration of the solvent. The best results can be obtained by using small shavings.

Determination of Moisture, etc.

The "loss in weight at 105°" was determined on separate samples, representative of the whole batch, by drying in the oven at 105° for four hours. This was done at the same time as the moist wood was weighed, just before loading it into the extractor. Yields of resin were calculated always on the "bone-dry weight" of wood, thus determined.

Extraction of the Wood with Benzene-Alcohol

The extraction of the wood was carried out in a large

bronze extractor, working automatically on the Soxhlet principle. About 500-600 grams, bone-dry weight, of wood, could be extracted at one time, using eight liters of solvent. The same solvent could be used for several batches of wood, but in the present work, owing to the possibility of transformations occurring with some of the dissolved substances, under the influence of heat, it was thought advisable never to use the same lot of solvent on more than three lots of wood.

Extraction for 8 or 9 hours in this apparatus was found sufficient to remove all resins and fats. The solvent used was made up of equal volumes of benzene and 95% alcohol.

Recovery of the Crude Resins and Fats

The alcohol-benzene extract was distilled, under slightly reduced pressure, from a water-bath, using a stream of CO_2 gas to assist the distillation and to prevent bumping. Most of the solvent could be recovered in this way. The solution was kept shielded from light.

Removing the last traces of solvent from the viscous mixture was extremely difficult. If the mixture were washed into a dish and dried at 60° in the vacuum-oven it was found that there were considerable losses of volatile fats and terpenes, due to foaming and distillation. Heating on a water-bath and then transferring to a desiccator, under vacuum, also led to losses in the same way, and possibly caused some oxidation of the material. The only effective way was to

dry the resins directly in the distilling flask by heating on a water-bath and connecting the flask with a good water-pump until the weight was approximately constant. The product contains volatile substances which may be removed with the last traces of solvent if the drying is carried too far.

The final residues were fairly viscous, dark brown material, very sticky, and having the odour peculiar to resinous pine wood. These products were kept in desiccators under vacuum, in the dark, until used.

TABLE I

Summary of Results obtained in the Various Extractions of Jack Pine (Pinus Banksiana) with alcohol-benzene

Extr'n No.	Kind of wood	Time since tree felled	Wood, % loss at 105° (4 hrs.)	Bone dry wt. of wood	Weight crude resins	% crude resins	% ether-soluble resins
1	Green Jack Pine	10 days	38.9%	557 gms.	26.5 gms.	4.76	3.37
2	do.	13 "	28.3%	749 "	18.7 "	(losses in drying products)	
3	do	15 "	9.1%	541 "	25.4 "	4.70	3.75
4	do	19 "	6.8%	575 "	26.0 "	4.52	3.51
5	do	74 "	6.9%	1107 "	50.6 "	4.57	3.12
6	do	80 "	7.3%	1079 "	56.4 "	5.22	3.12
7	do	81 "	7.3%	2402 "	64.6 "	(losses in drying products)	
8	do	89 "	7.3%	317 "	17.7 "	5.58	4.71
9	do	90 "	19.6%	2543 "	113.3 "	4.45	3.12
10	do	92 "	18.7%	1218 "	51.7 "	4.25	3.95
11	do	94 "	18.7%	766 "	28.5 "	3.72	3.26
12	do	16 mos.	11.3%	1720 "	76.8	4.46	
13	seasoned Jack Pine	5 yrs.	13.2%	13.4 kgs.	564.0	4.21	3.31

Preliminary Separation of the Total Crude Resinous Product

Method A (Saponification)[10]

A weighed quantity of the crude resin and fat mixture was saponified by heating with N/2 alcoholic potash for one hour, on a water-bath, under a reflux condenser. Approximately double the estimated theoretical amount of potash was used in each case, the theoretical being based on the neutralization value of the acids obtained later. The mixture was poured into a separatory funnel, rinsing with a little hot alcohol, and sufficient water added to reduce the alcohol strength to 50%. This latter precaution is very necessary in order to avoid the formation of troublesome emulsions at a later stage. The mixture was then extracted with about half the volume of petroleum ether (b.p. 30-50°) to remove the unsaponifiable. Usually four or five such treatments were sufficient. The total petroleum ether extract was then shaken with a small amount of 1% KOH solution, to which an equal volume of alcohol had been added, and this was repeated a second time; these washings were added to the main alkaline solution. The washing of the petroleum ether solution was continued with small portions of 10% aqueous alcohol until neutral to phenol-phthalein. The extract was then dried over calcium chloride, the solvent removed and the waxy residue left dried at 65° in the vacuum oven, to constant weight.

The alkaline solution containing the potassium salts was acidified with dilute hydrochloric acid, and extracted with

ethyl ether. In some cases, a little insoluble material, dark brown in colour, separated, and was removed. This latter was probably lignin or some oxidation product. The ethereal extract, containing the mixed acids, was washed with water, and dried over sodium sulphate. Evaporation yielded the acids as a sticky, brown mass and the product was dried to constant weight under suction on the water-bath.

Two methods for separating the above mixture quantitatively into resin acids and fatty acids, were carefully investigated, in order to decide which was the more suitable.

(a) Twitchell's method[12] for separation of fatty acids from resin acids

The dried mixture of acids was dissolved in ten times its weight of absolute ethyl alcohol, the mixture left standing over fused sodium sulphate over night, then filtered quickly into a dry distilling flask, immersed in cold water. A stream of dry hydrochloric acid gas was passed in slowly, under gentle suction and continued until the solution became saturated. This may require several hours when the volume is very large. The solution was left standing for at least three hours, to complete the esterification, and then poured into five times its volume of distilled water. The mixture was boiled until the aqueous layer was almost clear. (It is advisable to pass a stream of CO_2 gas to prevent bumping.) The oily esters and acids seperated as the upper layer. It was found advisable to siphon off the lower aqueous portions

and extract this with ether, this ethereal solution then being used to dissolve the main lot of resin acids and fatty esters. The ethereal extract was washed once with water and then the resin acids were separated from the fatty esters by shaking with a solution of nine volumes of 1% aqueous potassium hydroxide to one of alcohol. The ethereal extract was agitated several times with portions of this alkaline solution, until nothing further appeared to be removed. The alkaline extracts were then carefully washed with ether, thus removing an appreciable amount of the fatty esters and these were returned to the main ethereal solution. This latter was washed with water until neutral, dried over calcium chloride, the ether removed, and the residual fatty esters dried at 60° under suction to constant weight, leaving a brown oil.

The alkaline extract was acidified with hydrochloric acid, the resin acids recovered by ether extraction; the ether extract treated as above, and the resin acids obtained as a dark red, resin-like mass.

(b) Wolff and Scholze's method[11] for separation of fatty acids from resin acids

The dried mixture of resin and fatty acids was dissolved in five times its weight of absolute ethyl alcohol. To this was added 50% by volume of an esterification mixture, consisting of one part of concentrated sulphuric acid to four parts of absolute ethyl alcohol. The solution was brought quickly to the boiling point on the water bath, under reflux, and

kept at this temperature for three to five minutes only. After cooling rapidly, it was transferred to a separatory funnel, diluted with about four volumes of 10% aqueous sodium chloride solution, and extracted with ether. The total ethereal extract was washed with a little sodium chloride solution, and then treated with successive portions of 1% aqueous potassium hydroxide, to remove the resin acids completely from the fatty esters. The alkaline extract was washed with ether, and the washings returned to the main ethereal solution. The combined extract was then acidified with hydrochloric acid and treated with ether. A small amount of insoluble material generally separating at this stage was removed by filtration. Treatment of the ethereal solution and removal of the ether as described left a residue consisting of the resin acids.

The original ethereal solution, containing the fatty esters, was washed with water until neutral, treated as already described (p. 13) and the esters recovered.

In order to obtain the fatty acids from these esters, the latter were saponified for one-half to one hour, with N/2 alcoholic potash, on the water bath. The potassium soaps were salted out by adding a saturated sodium chloride solution, hydrochloric acid added, and the free acids taken up by ether. Treatment of this ethereal extract in the same way (p. 13) yielded a very viscous, dark brown mass of fatty acids.

TABLE II

Summary of Analytical Results, by Method A, in the Initial Separation of Crude Products from Various Extractions of Jack Pine Wood

Total crude resins, etc. from Extraction No.	Wt. of crude material used	Saponifiable products weighed as "total acids" and including lignin, insoluble, etc.		Unsaponifiable Matter and Essential Oil		Water-soluble material (by difference)	Separate Analysis of Saponifiable products (including lignin, etc.)*		
							Resin acids	Fatty acids	Lignin
		Wt.	%	Wt.	%	%	%	%	%
1	(a) 4.4813	3.4710	77.50	0.2220	4.95				
	(b) 3.9596	3.1340	79.30	0.1860	4.70				
	mean %		78.40		4.83	16.8	54.5	30.2	15.1
2	18.3576	14.4912	79.0	0.8064	4.39	16.6	41.6	32.9	21.7
3	(a) 2.0585	1.6926	82.2	0.1082	5.26				
	(b) 2.0230	1.6258	80.4	0.0870	4.30				
	mean %		81.3		4.78	13.9	42.1	42.3	7.80
4	(a) 3.1481	2.4464	77.7	0.1670	5.94				
	(b) 2.4150	1.8940	78.5	0.1200	4.97				
	mean %		78.1		5.45	16.4	45.4	38.4	7.50
11	28.5	22.60	79.3	1.50	5.26	15.4	53.2	23.8	6.2

Preliminary Separation of the Total Crude Resinous Product

The following method follows exactly the procedure as outlined by Tschiroh in connection with his numerous investigations.

Method B (Tschiroh's[13])

The crude resin mixture obtained by the alcohol-benzene solvent extraction was boiled with several portions of ethyl ether, under a reflux condenser, until nothing further was extracted. The hard residue was broken up when dry, and again boiled with ether. This was repeated until all the soluble matter had been extracted*.

The ethereal extract was washed with water, to remove tannins, dyestuffs, etc., which were shown to be present by qualitative tests.

The washed ethereal solution was then treated to remove the acidic constituents by extracting it exhaustively with 1% aqueous ammonium carbonate solution until no further acids were removed. This usually required a very long time.

The residual ether solution was then washed with water and subjected to a similar exhaustive series of extractions with 1% aqueous sodium carbonate solution, thus removing

---

*Tschiroh usually found the insoluble matter to consist only of small amounts of impurities; this does not apply to the resins in the present investigation.

considerable additional amounts of acidic substances. When nothing further could be removed, the ether solution remaining was washed again with water*.

The acids remaining in the ether solution were generally removed by a final series of washings with 1% aqueous potassium hydroxide. Tschirch also followed this by treatment with 10% caustic in certain cases where all the acids had not been removed by the previous alkaline solutions.

The residual ethereal solution, now containing only neutral substances, was evaporated to remove the solvent, and the residue subjected to steam-distillation, leaving the "unsaponifiable matter."**

---

* Tschirch frequently reported that resin substances were precipitated from the ether solution when too much of the aqueous extracting agent was used at this stage.

** Tschirch generally found that it was extremely difficult to remove all of the essential oil in this way, and the steam-distillations were continued for several weeks in some cases. He also often added alkali to the distillation-mixture, to saponify any esters present, but usually did not find any. The non-volatile, inert substance remaining in the flask he called the "resene", and purified it by reprecipitating from ether solution by addition of alkali or alcohol.

The volatile essential oil was purified by re-distillation and its physical properties noted.

The acids contained in the four alkaline extracts mentioned above were precipitated from these solutions by addition of HCl and separated by filtration. Each product was examined separately.

---

* Various means were employed by Tschiroh in this connection, such as fractional crystallization and precipitation, separation by the lead salts, solubility in ligroin, distillation under reduced pressure, purification by animal charcoal, etc. Treating four such lots of acids in this way is obviously a lengthy process. Tschiroh generally ended up with products of doubtful purity, and there seemed to be no object in using various strengths of alkali to remove acidic components.

TABLE III

Summary of Analytical Results, by Method B, in the Separation of the Total Crude Product from Various Extractions of Jack Pine Wood

Crude Material Used		Section a	Section b						Section c		
From Extr'n No	Wt.	Product insoluble in ether	Product removed by 1% ammonium carbonate		Product removed by 1% sodium carbonate		Product removed by 1% potassium hydroxide		Product removed by 10% potassium hydroxide		Glycerides, unsap. matter and volatile essential oil (by diff.)	Total water soluble
		Wt. %	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt. %	%
3	20.4 g	2.7 13.2	4.60	23.5	7.00	34.3	3.70	18.1	-	-	1.47 7.2	10.9
6	17.70	2.0 11.3	2.20	12.4	7.70	43.5	2.00	11.3	0.55	3.1	2.50 14.10	4.3
10	51.7	3.7 7.2	8.00	15.5	27.2	52.6	5.70	11.0	-	-	3.73 7.22	6.4

TABLE IIIa

Individual Analysis of Section b, Table III, for Extraction No.8 only.

Separation into Fatty Acids and Resin Acids (Method, p.12)

Product	Fatty Acids		Resin Acids		Total Wt. of Acids
	Wt.	%	Wt.	%	
Acids removed by 1% Amm. Carb.	0.82 g.	40.5	1.20	59.5	2.02 g.
" " " 1% Sod. Carb.	2.92	34.1	5.65	65.9	8.57 g.
" " " 1% Pot. Hydroxide	0.68	53.1	0.60	46.8	1.28 g.
" " " 10% " "	0.27	62.8	0.16	37.2	0.43 g.
Total	4.69	38.2	7.61	61.8	12.30 g.

Preliminary Separation of the Total Crude Resinous Product

Method C (used in the present investigation)

(a) Initial separation: The crude resins, immediately after final drying, were divided into two weighed portions and these examined separately, in order to provide a check on the results, and to avoid the possibility of total loss by any accident during the investigation. The crude products were treated (about six times) with large volumes of ether under a reflux, at the boiling point, to remove completely the resins, fats, etc. The insoluble cake left behind was broken up, and washed well with ether, then with distilled water, the ether washings being added to the main ether extract (A) and the aqueous washings(C) retained. Finally, the cake was dissolved in 1% aqueous KOH, reprecipitated by HCl, and the gelatinous colloidal material extracted with ether, to remove the resins, the ether extracts also being added to A. This re-precipitation was carried out three times, after which the ether removed nothing further. The "insoluble" material left was filtered off, and put aside for further examination.

The combined ethereal extract (A) was filtered from suspended solids and the clear solution (B) extracted repeatedly with small portions of distilled water, and the aqueous extracts added to the water-washings (C) obtained previously from the insoluble matter. This solution (C) containing all the water-soluble constituents of the crude resins, was

put aside for subsequent examination.

The ethereal solution (B) containing the pure resins and fats, was then exhaustively extracted with 5% aqueous sodium carbonate, to remove all the free acidic substances. The quantity of aqueous alkali that could be used for each shaking was limited, as substances tended to precipitate from the ether solution if too much water were present. More ether had to be added later to make up for the large amount removed in the extractions. This treatment required considerable time. A mechanical shaker and sealed container ere used throughout this work.

The sodium carbonate extracts were united, and allowed to stand over night. A dark mass collected at the surface of the solution. The main portion of the liquid was removed by siphoning and the residual material then treated with an equal volume of ether, to remove the neutral substances remaining suspended in the soda extract as a result of emulsification. The ether washings were added to the main ethereal solution of the resins, and the aqueous layer returned to the main sodium carbonate extract.

The ethereal solution was washed with water until neutral and the ether removed leaving a dark brown, viscous mass. This was subjected to steam-distillation to remove the volatile oil (see Part IV). In the earlier work, solid KOH was added to the distillation-mixture, to saponify the glycerides, etc. present; but later it was found more satisfactory not to depend

on complete saponification under these conditions, but to saponify the residue at a later stage with alcoholic potash. This avoids considerable trouble with emulsions.

The volatile oil was separated from the aqueous layer, and the latter extracted with ether to recover the rest of the oil. The remaining aqueous solution contained some glycerol produced in the steam-distillation, so it was kept aside to be added later to the aqueous residues from the saponification of these glycerides.

The residue in the flask after the steam-distillation was exhaustively extracted with ether, and the aqueous layer also kept aside for examination for glycerol. This ethereal extract, when evaporated, yielded a viscous, brown material. This was saponified for one hour with alcoholic potash, on the water-bath. Treatment for as long as five hours did not produce an increased saponification. The products were recovered in the usual way (p. 14), the acids isolated being quite oily in character. The aqueous residues were retained, together with the previous ones, to be evaporated later and examined for glycerol. The unsaponifiable matter was recovered from the ethereal layer, and corresponds with the "resene" of Tschirch's, although less simple in character. The further investigation is described in another chapter. The subsequent examination of the acids mentioned in this paragraph, as obtained from the esters and glycerides, showed them to consist entirely of the aliphatic type and are discussed in the chapter

dealing with the fatty constituents.

The sodium carbonate extract of the total resins, obtained as already described (p. 21) was treated with glacial acetic acid, and the mixture allowed to stand over night. The oily, fatty acids and some of the resin acids separated as an upper layer. It was found most efficient to siphon off the lower portion and extract this with ether repeatedly, using this extract to dissolve the mixture in the upper layer. Prolonged mechanical shaking was necessary to remove all the ether-soluble acids (Extract D). A large volume of a reddish-brown aqueous solution finally remained, from which nothing further could be removed by ether. This probably contained water-soluble oxidation or decomposition products formed during the lengthy treatment of separation. In addition, there were always obtained at this stage, varying quantities of a light brown solid material, insoluble in ether. Since this had originally dissolved in the ether during the preliminary treatment, it apparently consisted of oxidized acidic substances, probably of the resin acids, that had become insoluble. It was added to the crude acids mixture.

The ethereal solution (D) of the total acids was washed with water, and dried over fused sodium sulphate. Evaporation left the acids as a dark brown mass. The separation of this mixture into resin acids and fatty ethyl esters was effected by the method of Wolff and Scholze[11] already described (p. 13) The method was modified in that the resin acids were precipitated

from the alkaline solution by means of acetic acid, in order to avoid the isomerizing effect on these substances now known to be caused by hydrochloric acid[16]. The methods used in the present research, in determining the composition and properties of these fats and resin acids, are discussed in detail in the chapters dealing with these substances.

DIAGRAMMATIC REPRESENTATION
of
METHOD "C"
for
SEPARATION OF THE CRUDE RESIN PRODUCT INTO GROUPS OF SUBSTANCES.

(See pp.20-24 for description)

Jack Pine wood

alc.benz.extraction

Crude resin product

(See PART I)

ether extraction

(soluble)
Total ether-soluble resin products.

(insoluble)
Lignin, etc.
(See PART I)

water

(water-soluble)
Tannins, etc.
(See PART I)

(water-insoluble)
Crude fatty and resin acids, glycerides, esters, unsaponifiable, and essential oils, - in ether solution.

5% Na_2CO_3 extraction.

(soluble)
Crude Fatty and Resin Acids

Esterification

(not esterified)
Crude resin acids.
(See PART II)

(esterified)
Crude fatty ethyl esters.
(See PART III)

(insoluble)
Glycerides, esters, unsaponifiable, and essential oil.

Steam distillation.

(non-volatile)
Glycerides, esters, unsaponifiable matter.

(volatile)
Essential oil
(See PART IV)

Saponification

(saponified)
Fatty acids.
(See PART III)

(not saponified)
Unsaponifiable matter.
(See PART IV).

Summary of Analytical Results, by Method C (the method finally used in the present investigation), in the Initial Separation of Crude Products from Various Extractions of Jack Pine Wood

(a) For the purposes of this investigation, the crude products obtained in Extractions Nos. 5, 6 7 and 9, Table I ("green" wood), were combined, and the results of the analysis of the combined products are shown below as "Extraction 9x".

(b) The analysis of the crude product from "seasoned" wood (Extraction No.13, Table I) was carried out on two separate lots (a) and (b) of the same product, and the results are recorded separately below, together with the mean values, as Extraction No.13

	Extn No.	Wt. crude resins grams	lignin etc. insoluble in ether		Total "free" acids, from 5% Na_2CO_3 extraction		Glycerides (calculated as triolein)		Unsaponifiable only		Ess. oil.	Water-soluble material (by diff.)		Analysis of total "free" acids only	
			Wt.	%	Wt.	%	Wt. (acid portion only)	Glycerides % approx.	Wt.	%	Wt.	%	%	Resinic acids	Fatty acids
Green Wood	9x	275.1	25.30	9.2	181.5	66.0	19.00	7.6	12.42	4.5	4.10	1.5	8.3	81.6%	18.3%
Seasoned Wood	13	(a) 235.0	16.20	6.9	146.7	62.4	10.00	4.7	18.10	7.71	3.25	1.38	13.2	87.5	12.5
	13	(b) 250.0	20.25	8.1	156.6	62.6	14.50	6.4	19.90	7.96	5.30	2.12	13.8	86.1	13.9
	Mean	%		7.5		62.5		5.6		7.84		1.75	13.5	86.8	13.2

TABLE V

A Summary of Analyses of Crude Products from Extractions of Jack Pine Wood ("Green" and "Seasoned")

Crude product of Extrn No. (See Table I)	Kind of Wood	Water-soluble tannins, carbohydrates, etc. %	Ether-insoluble, lignin, etc.) %	Resin Acids %	Fatty acids %	Glycerides and esters %	Volatile essential oil (averages) %	Polymerized terpenes, etc. (averages) %	Phytosterol (averages) %	Resene (averages) %
1	Green	16.8	11.9	42.7	23.7			2.75	0.70	1.39
2	"	16.6	17.1	32.8	26.0			2.49	0.63	1.26
3	"	13.9	6.4	34.3	34.5		4.30	0.82	0.31	0.27
4	"	16.4	5.9	38.0	30.0			3.10	0.79	1.57
8	"	4.3	11.3	43.5	26.9	6.15	1.50	2.26	0.85	0.74
5,6,7,9	"	8.3	9.2	53.5	10.9	7.6	1.50	2.62	1.00	0.87
10	"	6.4	7.15	54.3	22.7	6.2	4.30	0.82	0.31	0.27
11	"	15.4	4.91	51.5	23.0	-	0.60	3.05	1.00	0.61
13	Seasoned	13.8	7.5	54.2	8.3	5.54	1.75	6.58	0.54	0.73

Notes: (1) The analytical results reported in Tables I to IV (pp.10-26) are tabulated above, on the basis of the weight of total crude product as 100%.

(a) Where the products of two or more extractions were combined for analysis, the "average" percentages of the constituents for each extraction have been reported above (see Part IV, p.29).

DISCUSSION OF THE THREE METHODS (A, B and C) USED FOR THE INITIAL SEPARATION OF THE CRUDE RESIN MIXTURE

Method A: Hot Saponification, etc.

Some of the advantages and disadvantages of this method have been mentioned already, in the Introduction (p.4)

In the case of the present investigation, the analytical results were found to check very well (Table II, p.15). Appreciable losses in water-soluble material were noticeable during the procedure. These may be due to hydrolysis of the glycerides and esters, and also of the lignin which is present in amounts from about 4-12% of the crude product. There may also be some decomposition of the fatty and resinic acids, during the hot saponification, yielding water-soluble products. In every case, the percentage of water-soluble material has been calculated by difference. The percentages of fatty acids and resin acids, respectively, have been based on the total weight of the recovered products, the latter being taken as 100%.

A disadvantage of this method is that the lignin, insoluble, etc., is not removed before the chemical separation of the true resin products is made. The insoluble material separating out at various stages of the process, adds greatly to the difficulties in this method.

The hot saponification treatment does not permit of the isolation of the esters and glycerides as such; these are included with the total free acids, by this method.

It is highly probable that any volatile essential oil originally present is completely polymerized during the saponification at an elevated temperature. Only traces of it were found in the "unsaponifiable portion" isolated by this method, as in the case of the crude resinous product from Extraction No.3 (see Table I, p.10). Another portion of the same crude product (Extraction No.3) was investigated by Method B (Tschirch's), and the essential oil recovered in this case (without the preliminary saponification treatment) was found to be 4.3% of the crude product.

Oxidation of the unsaturated fats and the resin acids, as well as isomerisation of the latter, under the influence of heat, probably occur to some extent during the preliminary hot saponification.

Discussion of the Two Methods Used in Separating the Total Crude Acids into (1) Fatty Acids, and (2) Resin Acids.

Both methods are based on the ease of esterification of the fatty acids, as compared with the resin acids. The latter are very difficult to esterify.

(a) Twitchell's Method[12]

The separation by this method is a lengthy process. It involves saturation of the alcoholic solution of the acids with HCl gas (which may require several hours), and this probably causes isomerisation of the resin acids, to an

appreciable extent, even though the treatment is carried out at room temperature. This method has the advantage of not requiring the application of heat. The modification proposed by Wolff and Scholze[11], and used in the present investigation, has been found to be more simple and convenient than the original method of Twitchell.

(b) Wolff and Scholze's[11] Method

This procedure involves boiling the mixture of crude acids for about 3-5 minutes, with diethyl sulphate, in dilute alcoholic solution, followed by rapid cooling. The heating in this case is probably not sufficient to cause any appreciable change in the resin acids or the fats.

Sandquist[17] made some slight changes, as regards quantities, etc., in the above method, in order to adapt it to the examination of mixtures such as were obtained in the present investigation, and his experimental methods were followed exactly in this investigation.

Certain other methods for separating resin acids from fatty acids have been proposed by various authors[15], but these are either slight modifications of the original Twitchell method, without any particular advantages, or are unsuitable for use where comparatively large quantities of material are to be treated.

Method B (Tschirch's)

It has been thought advisable, on account of the numerous specific details pertaining to this method, to discuss the advantages and disadvantages of the method in the Experimental Part (pp.16-18) with several explanatory foot-notes.

The method is a very tedious one as there are too many alkaline extracts to be treated, without any apparent advantage to be gained.

The analytical results obtained by this method, in the present investigation, are given on p. 19, Table III.

The four acidic products, separated by extraction of the crude product of Extraction No 8, with the four different strengths of alkali were examined, individually, for fats and resin acids (see Table IIIa, p.19a) in order to determine the composition of the acidic products.* In each of the four products, appreciable percentages of fatty acids were found, as shown in Table IIIa, and the solid resin acids could not be isolated until the oily fats had been removed. It is evident that there are limitations to the use of Tschirch's method in the examination of these wood-resins, and a further separation into (a) fatty acids, and (b) resin acids, must be made.

The use of alkaline solutions of 1% strength necessitates the use of very large volumes of solutions, and there seems to be no good reason for using such dilute solutions. The final extraction with 10% KOH (after the exhaustive treatment with

*Tschirch obtained only resin acids from similar products which he isolated in his investigations.

1% KOH), as used by Tschirch, was found to remove still more acidic products, but this is probably due merely to partial saponification of glycerides and esters present, on account of the use of such strong alkali. It is possible that 10% KOH may also resinify, to some extent, other substances present in the crude resins mixture.

Method C (finally employed in the present investigation)

By this method, the total insoluble material (lignin, etc.) is removed completely before any separation of the resins is attempted. The process of dissolving the residue insoluble in ether in 1% KOH and reprecipitating by acid, followed by shaking with ether, and repeating the process three times, is quite laborious, but is quicker and more efficient than merely refluxing the residue with ether for a long time. The latter procedure does not permit of removing the entire resinous and fatty materials.

Removal of free acidic components by extracting the ethereal solution with 5% Na_2CO_3 leaves the glycerides and esters behind unchanged and these can be examined independently, when separated from the unsaponifiable. The use of this strength of alkali results in smaller volumes of extract to be treated than when 1% strength is used, and the tedious series of extractions is completed in less time. No trouble is experienced with regard to emulsions in using the 5% carbonate

solution, although considerable difficulty is experienced later in the treatment of the unsaponifiable matter and glycerides. It is necessary to keep the alcoholic content of the solution as near 50% as possible, to avoid this trouble. The use of saturated sodium chloride solution was found very effective, except in the later stages when most of the salt had been removed by washing. Addition of small amounts of alcohol, or of sodium sulphate, were found to be of some help in breaking up emulsions. In many cases, however, it was necessary to acidify the ether-soap-unsaponifiable emulsion, and then extract with a little alkali, to get the soaps again into solution and free from the ether-soluble components.

With regard to the treatment of the resinic acid constituents by this method of separation, these were always precipitated by means of glacial acetic acid thus avoiding the possibility of isomerisation of the resinic acids under the influence of HCl, etc. Wolff and Scholze's[11] method was used for separating them from the fatty constituents, because it was considered as less likely to involve chemical transformation of the products (see p.24).

By this new method (C), the products are isolated in groups, of related substances, in a form suitable for further scientific investigation. Only the details of the method concerned with the initial separation of the crude product into the above groups have been discussed in the present part (I) of the thesis; the details of the subsequent examination of these

groups, and identification of the individual constituents, are described in the following separate sections dealing with the particular products, viz., Part II, Resinic Acids; Part III, Fatty Constituents; Part IV, Essential Oil and Unsaponifiable Matter.

In addition to Tables I, II, III, IIIa, and IV, summarizing the individual analytical results obtained in the initial separation of the crude products from all the extractions, Table V (p.27) gives a complete summary of entire analytical data, and includes, for completeness, certain figures from the experimental data contained in two later Sections (III and IV).

BIBLIOGRAPHY

of

PART I

only.

1. Klason, Cell.chem 4, 83 (1923).

2. Pfkt, 20, 1136, (1922).

3. Cell.chem, 4, 1, (1923).

4. R.Sieber, "Harz der Nadelholzer und die Entharzung von Zellstoffen", 2nd.ed. (see p.133-134).

5. Sieber; see ref. 4.

6. Sieber, ref.4, pp.1-39.

7. Chem. & Met.Eng. 28, 504 (1923).

8. See ref. 7.

9. Allen's Comm.Org.Annal. Vol.II,p.92-101: vol.IV p.226-234.

10. See ref.4, p.136-137.

11. Chem.Ztg. 38, 369-370, 382-3, 430, (1914).

12. J.S.C.I. 10, 804 (1891)

13. "Harze und die Harzbehalter", p.60-62, 546 etc.

14. Bull.Soc.Chim, 35, 1209-70, (1924).

15. Penhallow,"North American Gymnosperms",P.Banksiana,pp.296,299,321. Sargent,"Man.of trees of North America",P.Divaricata, p.27.

16. Dupont, Bull. Soc.Chim, 35, 1236 (1924).

17. See ref.4, p.137.

18. Ulzer & Defris, Zeit.anal.Chem. p.24 (1897). Gladding, Chem.News, 45, 159 (1882); Fahrion, Chemische Revue, 18, p.239.

PART II

THE RESIN ACIDS PRESENT IN THE CRUDE RESINOUS PRODUCT.

PART II

An Introduction to the Study of the Resin Acids of the Conifers

The study of these resin acids is inseparably linked with the investigation of colophony (rosin) and of various oleoresins, which has been carried out by a vast number of workers for over a century. The rosin industry was already in existence in Carolina at the end of the seventeenth century. The discovery of various uses for rosin naturally led to chemical investigations of its properties, and gave the first impetus to the enormous amount of research that has been in progress since that time. This research has been fostered, particularly in France, by the careful development along scientific lines of the naval stores industry, as well as to a lesser extent in the United States. Similar development is now in progress in Russia, the Scandinavian countries, and other parts of Europe, indicating the importance that the industry has assumed.

The attention of previous investigators has been directed, for the most part, to the so-called oleoresins, that is, the "pathological" resins which serve as the raw material of the naval stores industry and yielding rosin (or colophony) together with turpentine as the ultimate products of the industry. The resins and similar substances actually contained in the cells of the inner wood,

ahd often referred to as "physiological resins", as distinct from "pathological", have received comparatively little attention. Researches on these internal secretions of the conifers have been few in number, and always restricted in scope.

Certain conifers, when wounded externally by boring, scarifying, or "letting", excrete gradually a viscous material which is referred to under the general name of "oleoresin". It is always a mixture of a non-volatile solid material (chiefly resin acids) with a volatile, liquid essential oil; this mixture is secreted by certain cells in the outer wood (sapwood) as a direct result of wounding, and on this account, has been referred to as "pathological resin". The oleoresins of the pines have acquired particular importance because they form the basic raw material of the naval stores and resinous wood distillation industries. In France, the oleoresin of the Bordeaux pine (Pinus Maritima) is the basis of these industries, while in the United States, the chief pines used in this connection are the Longleaf (P.Palustris) and Cuban or Slash pine (P. Heterophylla). Many other pines, however, find similar use, both in America and in Europe.

The crude secretion on the outside of the tree is called the "gemme" in France. After fusion and a preliminary purification, it is called "turpentine". The solid part, removed by mere pressure, without heating, is the

"galipot" of France, and has been the subject of intense investigation.

This French turpentine when subjected to steam-distillation, yields a mixture of volatile terpenic hydrocarbons, called "essence of turpentine" The non-volatile residue remaining in the retorts, is the"colophony", or rosin, of commerce. It is obviously a transformation product produced by the action of heat on the solids (galipot) originally present in the oleoresin (gemme), a treatment which has been shown to bring about fundamental changes in its chemical character.

These products, from different species of conifers, present a wide variation in properties and composition. The essential oil is generally of the same type for a particular variety of conifer, a property which has been used in certain cases, where botanical distinction was difficult, as a means of identification. The terpene, α-pinene, $C_{10}H_{16}$, is peculiar to the pine family, but the rotatory power varies with each species. Both dextro- and levo-pinene are generally present, sometimes accompanied by nopinene (β-pinene). Other terpenes, and even sesquiterpenes, have been found in different species. In P. Sabiniana (Digger Pine)[1], however, the volatile oil is made up almost entirely of the aliphatic hydrocarbon n-heptane. Schorger[2] points out that, although there are over 90 species of conifers in North America, their oleoresinous products have been examined in only very few

cases. This is all the more true as regards the internal resin secretions of these conifers.

The solids contained in the various oleoresins are generally considered as being polyterpenic acids, all possessing the common formula $C_{20}H_{30}O_2$. Considerable work has been and is being done in an endeavour to trace the relationship[2a] between the terpenes and the solid acids in resins, and a great deal of evidence lends support to the theory that such a relationship actually exists. Dupont[3], in this connection, considers that in view of the extensive isomeric relationships known to exist among the terpenes, it would be natural to expect a still more extensive group of isomers in the resin acids since these acids are more complex than the terpenes. This view is supported to a surprising degree by many independent investigators in the discovery of the existence of a considerable number of such products obtained from various sources. The elucidation of their identity was hindered greatly by the failure to recognize the existence of such isomerism in the products, and for nearly a century there was recorded in the literature a vast number of different and apparently contradictory results, and from which data nothing of a conclusive nature could be drawn. Dupont contends that even though there are numerous resin acids present in the conifers there yet exists a remarkably close relationship between all of them, since they nearly all lead ultimately, by isomerisation, to a common product, which is a well-known

member of the abietic group, namely, levo-alpha-abietic acid.

The vastness of the work carried out, covering a period of over a century and still in progress, in the investigation of these resin acids, would render futile any attempt to give here a complete record of the chemistry of these substances and a discussion of the way in which the vast number of researches have been prosecuted. Moreover, this work has been reviewed more or less thoroughly by certain authorities on the subject[3a], and many bibliographies given[4]. Nevertheless, a brief account of the chemistry of certain acids, pertinent to the present research, as well as a short discussion of the difficulties, which for such a long time retarded the accomplishment of their identification, is necessary for a proper understanding of what follows.

As far back as 1806, Braconnot[5] prepared certain "soaps" from rosin, and thus established its acidic properties. Two years later, Gay-Lussac and Thenard[6], considering it to be a single pure substance, assigned to it the formula, $C_{30}H_{50}O_4$. Other investigators of this period, including Sausaure[7], Thomson[8], and Ure[9], noted the variation in composition which took place on fusion, and suggested different formulae. It was now apparent that rosin is not a simple substance.

The first crystalline product obtained from rosin was

isolated by Riess[10], in 1824, by the addition of mineral acids to its alcoholic solution.

Baup[11], in 1826, was able to obtain from the colophony of P. Abies, a crystalline acid, in the form of 4-edged tablets, which he named "abietic acid", while, from French colophony (P. Maritima) he isolated an entirely different acid, in 3-edged tablets, which he called "pinic acid". This was the beginning of a nomenclature which has presented one of the greatest examples of confusion ever known in organic chemistry. For the sake of clarity, this nomenclature has been omitted, as far as possible, in the present writing, except in those isolated cases where a definite chemical identity, or a well-defined group of substances, is concerned. As will be noticed, this nomenclature has been greatly abridged as the result of more recent work.

Following Baup, Unverdorben[12] prepared various salts of rosin acids. He assumed from his results that there were two distinct acids present (a) Pinic Acid, amorphous, and (b) Sylvic Acid, crystalline, as well as neutral substances, not isolated. The acids could be separated by their different solubilities in 72% alcohol. He examined several kinds of Colophony, and the wide disagreements in his results made it evident that every rosin was not the same, but that each conifer yielded a product peculiar to itself.

Cailliot[13], in 1830, isolated an acid which he also called "abietic", although it was not identical with the one obtained by Baup.

Tromsdorff[14] prepared the so-called "sylvic acid", and from analysis of its copper salt, arrived at the formula $(C_{20}H_{30}O_2)_2$. This formula was disputed by Blanchet and Sell[15], but was later confirmed by the careful analytical work carried out by Liebig[16] and by Rose[17]. The latter prepared "pinic acid", analyzed the lead salt, and found the same formula as for sylvic acid. He drew attention to the fact that they had the same percentage composition and also found the amorphous part of rosin possessed the same formula as the crystalline. Rosin, in alcoholic solution, was shown to undergo profound changes in composition and crystallizability, if left standing exposed to the air for any length of time.

Laurent[18] prepared both pinic and sylvic acids, arriving at the formula $C_{40}H_{60}O_4$ for each.

Cailliot[19] discovered an acid in Strassburg turpentine which he identified as abietic acid, while Bordeaux colophony yielded an entirely different acid, "pimaric acid" $C_{40}H_{60}O_4$, the name recalling its origin (P. Maritima)

Laurent[20] obtained a third acid, by distillation of pimaric acid, and called it "pyromaric" He stated that it was identical with sylvic acid, and assumed that under influence of heat pimaric acid is transformed into pinic

and sylvic acids. Pimaric acid was very unstable under the influence of light, heat, and air, while the distillation product, pyromaric acid, was quite stable under these conditions.

These various names assigned by the different workers were the cause of much confusion, so that efforts were made now to straighten out the matter.

Siewert[21], in 1859, prepared the acids already described, examined the melting-points of the purified products, and also subjected them to crystallographic investigation for the first time. He also noted their optical rotations and was able to differentiate clearly the different products, all of which on careful analysis proved to be isomers, $C_{40}H_{60}O_4$ (or $C_{20}H_{30}O_2$). He drew a sharp distinction between pimaric and sylvic acids.

Maly[22], in 1861, prepared a new acid from American rosin, which he also called "abietic acid". He obtained the same acid from P. Abies and P. Larix and assigned to it the formula $C_{44}H_{64}O_5$. Maly further demonstrated that there was a difference when an acid was precipitated by sulphuric acid, or merely by water, and showed that this brought his results in agreement with those of Siewert, above. He also prepared and examined several salts of his abietic acid. He made an important observation in that he found that no crystals of abietic acid could be obtained

from a concentrated alcoholic solution, even after several months, while it was quite easy to obtain crystals from a solution to which a little water had been added. He also remarked that on pines, spruces and balsams, the viscous, transparent resin drops would be transformed into crystalline masses in rainy weather. He thus concluded that rosin was composed largely of abietic anhydride, an assumption which is held tenaciously even today by not a few authorities. In the presence of water, the acid would be formed. Maly attributed the confusing results so far, to impure products, saying that those acids are probably all one and the same, but mixtures of anhydride and acid are present.

The next work of importance was carried out by Fluckiger[23] in 1867. He observed that an acid solution was formed when water was added to rosin, also an increase in weight. He strongly supported the anhydride theory of Maly's. He also showed that abietic acid could be prepared in two ways, (a) by recrystallization from a 70% alcohol solution, and (b) by introduction of a stream of HCl gas into a dilute alcoholic solution of rosin. He confirmed the presence of pimaric acid in Bordeaux Galipot, and concluded that abietic and pimaric are two distinct acids.

Duvernoy[24] (1868) studied pimaric acid, and some of its salts; he confirmed the formula $C_{40}H_{60}O_4$ Upon distilling it, he obtained an acid like sylvic acid, and confirmed the views of Laurent as regards transformation by

heat. This work was also supported by that of Dieterich[25], Liebermann[26], and Haller[27]. Maly[28], in the light of subsequent work admitted the existence of pimaric acid in French rosin and also the presence of "a small amount" of an acid, $C_{20}H_{30}O_2$, in American rosin. Dieterich's[25] results provided evidence tending to disprove Maly's anhydride theory, however, and Dieterich attributed the discordance in Maly's figures to the presence of impurities in his products.

Liebermann[26], and Haller[27] proved definitely that sylvic, pimaric and abietic acids were all isomeric. The melting-points of the purified products were never sharp, but always indicated a transformation near the melting-point.

Ducommun[28] was able to confirm the isomerism of the acids, and established that they were not identical.

The situation was now considerably clarified, and it was above all evident that a distinction must be made in the different kinds of colophony, from various sources, each having its own characteristics. It was now recognized that there were at least two different kinds of conifer resin acids, (a) abietic or sylvic acid, from American colophony, and (b) pimaric acid, from French galipot. Difference of opinion existed concerning the percentage composition, and whether they were identical or merely isomeric. The work of the investigators mentioned above[25, 26, 27, 28] was generally accepted as establishing their isomerism.

The subsequent discoveries of isomorphous isomers and stereo-isomers of these apparently pure substances, again raised serious questions, which occupied the attention of a large number of workers from 1860 onwards.

Cailliot[29] had previously (in 1674) been able to make an apparent separation of pimaric acid into simpler substances: (a) dextro-pimaric acid, difficultly soluble in alcohol; (b) pyropimaric acid, levorotatory, easily soluble in alcohol; (c) a transition product between the above. These all had distinctive crystalline forms. Levo-pimaric acid appeared to be absent.

Vesterberg[30] continued the work, and by preparing highly purified sodium salts, was able to isolate and identify, on only one occasion, the levo-pimaric acid. He showed that pimaric acid could yield three distinct sodium salts. He also isolated dextro-pimaric acid, and mentioned a third acid, weakly levorotatory, very unstable, and not isolated in pure form. Vesterberg claimed that all previous pimaric acids were simply mixtures of these three.

It must be mentioned that the dextro- and levo-pimaric acids isolated so far were chemical isomers of the common formula $C_{20}H_{30}O_2$, but were not optical antipodes, as the names would imply.

An extensive investigation was carried out by Maeb[31], in 1894; he obtained a very pure specimen of abietic acid, from American colophony, by 30 recrystallisations from methyl al-

cohol. He obtained an identical product by Fluckiger's[23] HCl method. He showed that discrepancies in carbon and hydrogen values in previous work were due to the degree of purity, and that fractional crystallization should have been carried further in preparing the products. He also claimed to show that pimaric acid was neither identical nor isomeric with abietic, but was a homologue of it. His analytical figures supported this clearly.

Rimbach[32] subsequently prepared dextropimaric acid from American colophony; abietic (or sylvic) acid had already been obtained from French and other resins, so that the old restriction as regards the distinctive source of these two acids was no longer tenable. The work of Vesterberg and of Mach were important advances, but there still remained considerable confusion as regards the identity of the resin acids. No one had yet attempted a thorough investigation regarding their actual constitution, although certain hypotheses had been advanced[33].

About 1900, a vast amount of work was done by Tschirch[34] and a large number of co-workers, on a great variety of resins. Certain parts of this work have value, from a chemical and botanical standpoint; but, as regards the resin acids in particular, Tschirch seems to have been greatly misled. His methods for obtaining these acids were faulty, and the chemical evidence in many cases is practically of no value as indicating the individuality of the substance. Tschirch claims to have isolated about fifty new acids, from only a few resins, and gave each one a name recalling its origin. Dupont[35] is of the

opinion that the methods used by Tschirch in isolating resin acids, and the purity of the ultimate products (frequently non-crystalline) are such as to render his work of negligible value as concerns resin acids, and that he has merely succeeded in encumbering the literature with a considerable nomenclature, without chemical significance. His work on these resin acids served to confuse greatly a question upon which chemists were slowly beginning to obtain some light.

A careful examination, made by the writer, of the methods used, and the results obtained by Tschirch, has led to the conclusion that although a vast amount of work was performed by this chemist it is so inconclusive, and at times uncertain, that nothing was really added to the knowledge of these resin acids. In fact, Dupont does not appear to be exaggerating when he says that greater confusion resulted.

Tschirch examined various kinds of colophony, and isolated a number of acidic substances. From American colophony[36] he obtained three acids which he called α-, β-, and γ-abietic acids, and, although a little uncertain about their composition, inclined towards the formula $C_{20}H_{30}O_2$. He considered them to be isomers, and said that all previously reported abietic or sylvic acids were merely mixtures of these.

A careful investigation by Paul Levy[37] in 1905, of abietic acid, its esters and salts, definitely established its identity and the formula $C_{20}H_{30}O_2$. Levy did not rely merely on carbon-hydrogen analyses, which have always been uncertain in

the case of these unstable substances, but based his conclusions on determinations of molecular weight by the ebullioscopic method, and by alkalimetry. The discrepancies in the figures reported by Maeh[31] was evidently due to impurities caused by oxidation taking place during the lengthy series of recrystallisations.

Much of the recent progress in the chemistry of the resin acids has been due to the work carried out by J. Kohler[38], and by Klason and Kohler[39], from 1906 to 1911. There was considerable uncertainty as to the actual existence of the levo-pimaric acid, which Vesterberg[30] claimed to have obtained in a simple experiment and by mere chance. Kohler, however, devoted particular attention to this question, and was finally rewarded by discovering this acid present in large amount in the winter resin of Picea Excelsa, the Red Spruce of the Swiss Alps. This is a rare resin, and the whole work was indeed a great accomplishment, because in addition to isolating the levopimaric acid, Kohler found other products, which he identified with the so-called "sapinic" or "native" acids postulated by him, all of which enabled him to draw important conclusions regarding the formation of these resinic acids from their unstable intermediates, which latter have never been isolated, and are not definitely known.

This winter resin is contained in natural pockets in the bark. From some pockets, liquid products were obtained, together with some crystalline material, and Kohler took great

pains to gather the substance in its primitive state, without exposure to light or air. He examined these natural substances, and advanced certain hypotheses with regard to the transformations of these substances[40]. In certain isolated cases, the resin pockets were found in the upper parts of the tree, and in these, the substance was almost entirely the pure levopimáric acid, identical with that described by Vesterberg[30]

Kohler found the levo-acid to be very unstable and readily oxidizable. Heat transforms it to a colophenic acid (levo-abietic acid) and to an inactive acid not isomorphous with the latter. The melting-point, about 157°, was very indefinite on account of the isomerization by heat.

The "sapinic" acids which accompanied the above, are even much more sensitive to heat and oxidation. Kohler proposed a classification[41] of the resin acids of the conifers, and considered, in the light of his investigations, that these consisted of

(a) Natural resin acids ("native" acids), also called "terebenthic acids" by Dupont,

(b) Colophenic acids, which are transformation products of the above, under the influence of heat or of mineral acids.

The natural resin acids (a) are made up of:

(1) Pimaric acids, dextro- and levo-, yielding insoluble sodium salts,

(a) Sapinic acids; at least two, never isolated pure, very unstable, and yielding very soluble sodium salts.

The colophenic acids are characterized by their yielding gelatinous ammonium salts, while those of group (a) are said to yield crystalline ammonium salts. Kohler considers that at least three forms of colophenic acids exist, namely, α, or levo-form, β, or dextro-form, and a third inactive form. The acid now commonly referred to as "abietic acid" belongs to the colophenic group. The latter are not present in natural resins, but are transformation products.

In view of the ready transformations noted by Kohler, it is not surprising that levopimaric acid, the more unstable of the two, had never been isolated previously, except on the one occasion reported by Vesterberg.

Dupont[42], in his work on the "galipot" of P. Maritima, has been able to confirm the work of Kohler on the resin of Picea Excelsa.

Kohler[40] also was able to throw much light on the relationship existing between the so-called abietic acid, and the natural resin acids. The idea of separating this abietic acid into simpler constituents originated with Kohler (and Klason). He showed that abietic acid was a product of the isomerization of pimaric by heat; also, that abietic was not confined to American colophony, but was always found as a product of isomerization of natural resinic acids. The wide divergence in the properties reported for abietic acid indicated that it could not be a pure substance:

Melting-point, from 120° to 182°.
Optical rotation, from 66° to 94°.

Klason and Kohler[43] then attempted the separation of this mixture by a long series of recrystallizations, and finally arrived at a product with a m.p. 171-172°, α = -94°. Their material had vanished at this stage.

Later (1917), Schulz[44] showed that an alcoholic solution of American rosin, originally levorotatory, became dextrorotatory when treated with varying amounts of hydrochloric acid. This isomerized product, when recrystallized, was not homogeneous, since the values for rotation and melting-point increased with the number of recrystallizations. Schulz ultimately arrived at constant values of -96° for α , and a constant m.p. of 171°, and thus appears to have obtained one pure constituent from the mixture known as abietic acid. The product was found to be identical with the one obtained by Kohler starting with levopimaric acid. The isomerization of these natural acids under the influence of heat or of mineral acid, was thus established. These facts explained much of the conflicting data that had hitherto been recorded.

In recent years (1919 to date) intensive research on the oleoresin of P. Maritima has been carried out by Dupont and co-workers[42], which has helped considerably in clearing up many points in connection with the inter-relations of the resinic acids. Dupont has made extensive use of both physical and chemical methods for the isolation of products of a high degree of purity. He obtained his acids chiefly by recrystallization of the "galipot" from alcoholic solutions, converting

them into the sodium salts, and then separating these by their different solubilities. The presence of the dextro- and levo-forms of pimaric acid was established, as well as of certain other very unstable acids (sapinic), in addition to intermediate transformation products. The mother liquors always yielded abietic acid finally. By employing the physico-chemical technique of Darmois[45], it was shown that pimaric acid is made up of two substances only, the dextro- and the levo-pimarics, in the proportion of 37% and 63% respectively. The extreme instability of the latter was confirmed.

The sapinic acids were found to constitute the greater part of the "galipot", and were very unstable, also yielding very soluble sodium salts. They have never been obtained in their original state. Dupont found evidence of at least two forms of these acids.

Dupont also found that dextropimaric acid could not be readily isomerized by heat or HCl, while the levo-acid was readily isomerized. He found that isomerization of these two acids took place in two successive stages, an unstable intermediate acid being formed in each case, and the ultimate product being one form of abietic acid. The same was true of the sapinic acids, but the change was extremely rapid for these. Dupont confirmed his results for other oleoresins[42] in addition to that from P. Maritima. He proved that the various "galipots" differ greatly in their composition. He considers that certain of the terebenthic acids may be common to a large number of

resins, but that the actual number of these acids is probably small, while their relative proportions in these various resins differ widely. Dupont found that the colophonies, as well as the natural (terebenthic) acids, all yielded, by isomerization, the same form of abietic acid. He further states that all the terebenthic acids exhibit similar crystalline form, and are extremely difficult to separate. If not quite pure, the product always seems crystallographically homogeneous. The same is largely true of abietic acid. Dufour[46] claims to have identified many of the acids previously mentioned in the literature under a wide variety of names with abietic acid by their crystallographic date, thus greatly simplifying the literature. They are not, of course, chemically identical, but possibly isomorphous mixed crystals. Dupont states that if the terebenthic acids have their own crystallographic individuality, all the acids derived from them, the mixture of which constitutes colophony, are isomorphous and isomeric substances. Of these he has been able to distinguish at least two chemical individuals. It is evident from the above that ordinary abietic acid can no longer be considered a definite chemical substance.

The elucidation of the chemical constitution of the resinic acids has been a very slow process, having been greatly hindered by the publication of much contradictory data, a very small portion of which has been referred to, briefly, above. It has also been obscured by the question of whether

the abietic acids existed, particularly in rosin, in the form of anhydrides or as free acids. This question was first raised by Maly[22], in 1861; he adduced evidence to show that rosin consisted almost entirely of abietic anhydride, and this was supported later by Fluckiger[23], but denied by Dieterich[25]. A violent controversy existed for many years concerning the work and opinions of Dieterich[25], Fahrion[47], Henriques[48], Tschirch[49], Schick[50], and others, in this matter, and on the constitution of abietic acid. The validity of certain constants for the substance, such as the Saponification number, Ester number, Iodine value, Acetyl index, etc., was brought into question. An "ester number" was always found for the acid, and could not be interpreted. It was sometimes attributed to the anhydride supposed to be present, and Henriques[48] even claimed to have proven the existence of a lactone, and later[51] a lactonic acid, when the anhydride theory was disproved. All seemed agreed on the absence of any ester, however. Fahrion[52] (1903-15) also investigated the changes which took place in the resin acids on heating, or when exposed to air or oxygen. He worked only with American rosin, and claimed to have obtained but one acid, "sylvic acid", $C_{20}H_{30}O_2$. His theory for its formation was that it proceeded from the oxidation of terpenes:

$$2C_{10}H_{16} + 3\,O \longrightarrow C_{20}H_{30}O_2 + H_2O$$

This is practically the same theory as advanced by others many years before[53]. He questioned the purity of the products used

by all previous workers, and showed that if allowance were made for a definite amount of oxygen, due to oxidation in the prolonged recrystallisation process carried out by Nash[31], the results of the latter would be in complete agreement with the formula $C_{20}H_{30}O_2$. Fahrion[52] also proved the presence of oxidized resin acids and of unsaponifiable matter (resene) in sylvic (abietic) acid; also the amorphous nature of the product after heating, and the resulting rise in molecular weight. The oxidation products were found to be insoluble in petroleum ether. He drew an analogy between these changes and the autoxidation of linseed oil, and supported his "autoxidation theory" for abietic acid with much experimental data as regards increase in weight, characteristic reactions for peroxides, etc., but does not appear to have isolated any of the transformation products. He claimed that his acid (sylvic) contained two double bonds, and hence was very susceptible towards autoxidation, the products later rearranging to soluble hydroxy acids. He considered that the formation of the neutral, unsaponifiable "resene" found in most resins was the result of a secondary oxidation process. He also admitted the presence of a small amount of anhydride in rosin. Tschirch[54] is extremely critical of the autoxidation theory of Fahrion, and considers his results as due to the use of impure substances. Tschirch[55] has recently (1926) commenced an investigation of autoxidation, particularly of turpentine oils.

The relationship of abietic acid to its so-called

anhydride, and the structure of these substances, has occupied the attention of several investigators recently. Knecht and E. Hibbert[56] secured considerable experimental data to support their view that rosin consists almost entirely of the anhydride. They showed that the loss in weight on heating abietic acid for a long time at 180°, corresponded with the theoretical value required for anhydride formation. However, Schwalbe[57] refutes this by his experimental evidence showing that, as low as 120°, colophony commences to show signs of decomposition, with loss of CO_2. Hence, mere reduction in weight does not necessarily mean removal of H_2O in this case. Dupont[42] also points out that colophony can be converted into crystalline abietic acid by two perfectly dry methods (a) by heating in a sealed tube at about 130° (Labatut[58]) and (b) by the action of dry HCl gas on fused rosin, at 150°. Dupont also claims that the formation of crystalline sodium acid abietate, prepared according to his directions[59], from colophony, is contrary to the anhydride theory[42] The various terebenthic acids do not form this salt ($3C_{20}H_{30}O_2.C_{20}H_{29}O_2Na$). Colophony, in alcoholic solution when boiled with aqueous soda also does not y ield this salt, but if the initial solution is isomerized by adding HCl this liberating abietic acid, a yield of 75% of the salt can be obtained readily, and then yield an abietic acid of fairly high purity[59]. Dupont considers that the anhydride, if present, certainly could not exist after the addition of so much aqueous soda solution. This is quite true, but it must be pointed out that Dupont and his co-workers place what seems to be too much reliance on the insolubility of such a salt in aqueous and alcoholic solutions,

or mixtures of the two solvents; also on the conditions of crystalline salt formation in abietic acid solutions since a variation in concentration often has been found to lead to gelatinous precipitations, where crystalline formations could be obtained under other conditions[59]. They have even advanced this as a possible method for the quantitative estimation of abietic acid in rosin[59].

Dupont[60] discards the idea of an anhydride in rosin, and is of the opinion that, considering (a) the original acids of the "galipot", (b) the intermediate transformation products, and (c) the ultimate colophenic acids, - there are ten possible acids in colophony. The intermediate non-crystallizable, inactive and soluble products usually predominate. The resulting complex mixture, which he considers to be merely a solid solution of a number of isomorphous acids, (analogous to the glasses), does not readily crystallize at ordinary temperatures, but if the transformation to the ultimate form is completed by isomerisation under the influence of heat or chemical reagents, a crystalline product is obtained, the purity of which depends on the conditions. Hence, from colophony, which undergoes almost complete transformations during the period of its formation, a yield of 80-90% of abietic acid is obtained.

Dupont[61] further supports the view held by Fahrion[52] on autoxidation of these acids.

At the present time, there are investigations in progress on the question of the existence of abietic anhydride, and of the composition of the crystalline product usually called "abietic

acid". The careful work of Knecht and Hibbert[56], Steele[62], and others, the results of which clearly supported the anhydride theory, cannot be ignored. Schorger[63] has produced evidence to show that the anhydride cannot exist in colophony. However, in 1926, Fonrobert and Pallauf[64] brought forward evidence proving that the product of vacuum distillation of abietic acid was a crystalline anhydride. Dupont[65] and his associates in 1928 carried out careful investigation of this subject and concluded that the disagreements in analytical figures and values for the various constants obtained previously could be traced to the presence of a small percentage of "water of hydration", or "crystallization", not merely adsorbed or occluded. The formula for this hydrate appeared to be $4C_{20}H_{30}O_2 \cdot H_2O$. Fusion in vacuo caused loss of this water, giving rise to the crystalline "anhydrous acid", with different properties. The loss of water was equivalent to 2% of the whole, and took place at the beginning of the distillation, indicating a loose chemical bond; anhydride formation corresponding to a 3% loss. He also prepared a "pyroabietic acid".

The work of Fonrobert and Pallauf was repeated by Nagel[66], and he agrees with their views; he found the "anhydride" to be even more resistant to hydration than they had reported.

Fonrobert and Greth[67] prepared the pyroabietic acid of Dupont[65], carrying out some additional work and providing some additional evidence for the structure as abietic anhydride, as distinct from "water-free abietic acid".

An examination of the evidence on both sides makes it apparent that the question of the existence of abietic anhydride or

of the free acids, as the major constituent of rosin and similar mixtures, is still an open one, as is also the matter of the relationship existing between the acid and the anhydride. Considerable attention has been devoted in recent years to the structure of the acid, and even if these other questions have not been settled, the matter of the chemical constitution is on a much firmer basis due to the very careful research that has been carried out.

The old idea held by Tschirch[68] that abietic acid was a diphenolic substance, was not supported by the careful experiments of Fahrion[69] on various phenolic substances. The latter came to the conclusion that the acid had no phenolic properties, but was a true carboxyllic acid.

Certain formulae, proposed by Bischoff and Nastvogel[70], and by Easterfield and Bagley[71], were looked upon favourably for many years, but there was insufficient evidence to establish their identity.

Levy[72], in 1916, summarized the evidence to date, and showed that the structural formula of Easterfield and Bagley[71], if brought into agreement with the gross formula $C_{20}H_{30}O_2$, explained the facts in a very complete way. A brief summary of the data quoted by Levy, is given below:

1. Abietic acid readily gives acid and neutral salts, also esters, and an acid chloride; hence it must be a true carboxyllic acid.

2. It is unsaturated; the Iodine Number has always been found to vary greatly with the conditions, but points to the presence of two ethylenic linkages. This was finally proved by Levy[73], and by Ruzicka and Meyer[74], in preparing the tetrahydroxy-and tetrahydro-abietic acids.

3. Abietic acid, when distilled with iron filings, yields the hydroaromatic hydrocarbon "abietene",[75]

$$C_{20}H_{30}O_2 \rightarrow C_{19}H_{30} + CO_2.$$

This is also obtained by treating the acid with hydriodic acid. Abietene, distilled with sulfur, gives rise to the phenanthrene derivative, "retene", $C_{18}H_{18}$. Hence it is concluded that abietic acid has a hydrogenated phenanthrene skeleton. Fuming nitric acid acting on abietic acid has been found to give dinitro-abietic acid, which can be reduced to a diamino acid. It is therefore assumed that there are closed chains in abietic acid, not necessarily of the aromatic type. Oxidation by nitric acid has been found to produce some hexahydrophthalic acid, indicating the presence of a very stable hexahydrobenzene nucleus.

4. Pinene and abietic acid are supposed to be closely related, not only because both are generally found together, but also on account of the fact that terebic acid is one of the oxidation products of either substance, by action of nitric acid.

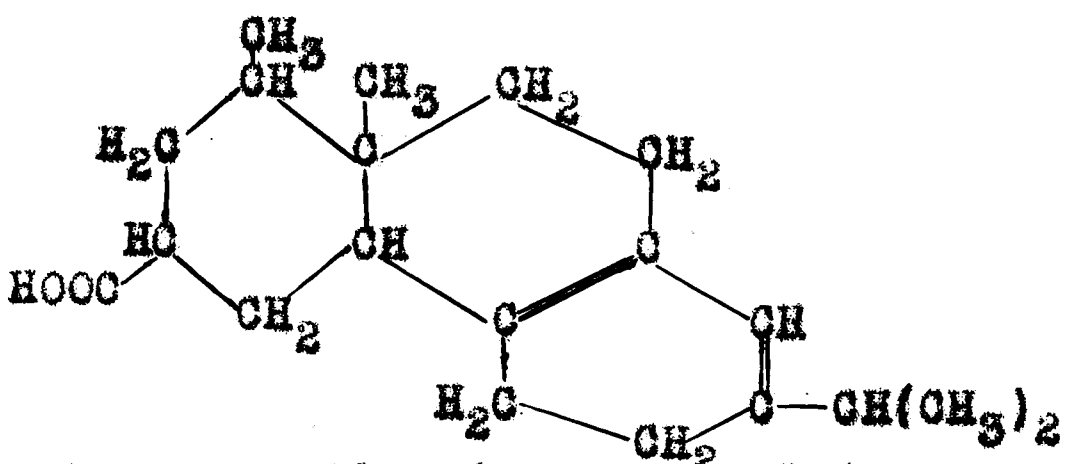
5. The carboxyl group is considered to be attached to a tertiary carbon atom, on account of the impossibility of esterifying abietic acid by the usual method using HCl or H_2SO_4 and alcohol.

6. The presence of an isopropyl group is assumed, from the fact that Levy[76] obtained isobutyric acid by vigorous permanganate oxidation.

7. The presence of a methyl group on a tetrasubstituted carbon atom of a hexagonal nucleus was indicated by the formation of retene by distillation of either abietic or dihydroabietic acids. The presence of an ethylenic CH_2 group was thus excluded.

These facts are accounted for almost completely by Easterfield & Bagley's formula[74], put forward in 1906, except that they based it on a formula $C_{19}H_{28}O_2$. The same skeleton structure made to agree with the formula $C_{20}H_{30}O_2$ by addition of a CH_2 group was considered formerly as being the true one for abietic acid. However, the positions of the double bonds, and of the substituent groups, were not definitely established, and it remained for

Ruzicka[77] and his co-workers to finally clear up the problem of the constitution of this acid. Based on his results of hydrogenation, permanganate oxidation, and other methods, he was able to put forward a formula that seems to have received unanimous acceptance:-



Ruzicka has recently given a complete account[78] of his work, as well as of that of various other investigators of the subject, and no attempt is made to describe it here. The whole subject has also been reviewed by Levy[79], and by Dupont[80], on the same occasion, and the matter of the constitution of this versatile chemical substance is now considered as having been settled.

The isomeric forms of this product have not been explained however, although this matter is receiving attention. Grunn[81] has put forward certain structural formulae which explain most of the above facts, and has also attempted to account for the assumed relationship to pinene, nopinene, and isomeric terpenes. However, his views are not acceptable in the light of the findings of Ruzicka[77]. It is apparent that a large number of structural formulae are possible for the above, which may have some bearing on the fact that so many isomers are known.

Ruzicka[82] has recently established the presence of two

double bonds in d-pimaric acid. Levy[83] has also provided similar evidence. Other important work on the constitution of abietic acid and its isomers, during the past few months has been reported by Dupont, Dubourg and Rouin[84], who claim to have isolated two sapinic acids. T. Tsukamoto[85] by the dry distillation of abietic acid and of Japanese rosin, obtained several different derivatives, including anthracene, phenanthrene, and retene, as well as aliphatic hydrocarbons from the side chains. This should have an important bearing on the possible isomeric forms assumed by the acids. The oleoresins of Russian and Finnish pines have been thoroughly examined lately[86,87,88]. The question of the degree of unsaturation of the resin acids has been taken up by Margosches, K. Fuchs, and W. Ruzicka[89](1929), and from their work and that of others, they are able to confirm the opinions of L. Ruzicka, mentioned above, as regards the structure of abietic acid. Aschan[90] and co-workers have provided valuable data on the subject and have isolated an isomeric synthetic acid, pinisosylvic acid. Aschan has published a classification of the resin acids[91], an extension of that previously proposed by Klason and Kohler[41].

Other recent work of interest on the subject has been carried out by Dupont[92], in support of his theory of the origin of these resinic acids, and the nature of their atmospheric oxidation products and their catalytic autoxidation[93]. Various nitrogen derivatives of the resinic acids have been prepared and examined by Dubourg[94], and the chemistry of this branch of the subject reviewed. An extensive research on the chemistry of retene is being carried out in Dupont's laboratory by L. M. Cheung[95].

In the above review, it is impossible to even mention every piece of work that has been carried out and has a bearing on the subject. The recent contributions to the literature made by Ruzicka[78], Levy[79], Dupont[80], Rouin[96], and Nagel[97], summarizing their work on the resinic acids, constitute a very adequate and accessible record of this field of chemical research up to the present time.

In the present investigation, it was first necessary to find a method for freeing the crystalline acids from the amorphous material (oxidation products) accompanying them, and the various methods investigated in attempting this are first discussed, followed by a description of the examination of the purified crystalline acids, and the identification of the individual constituents.

DISCUSSION OF EXPERIMENTAL RESULTS OBTAINED IN THE PRESENT INVESTIGATION.

The experimental results obtained in the course of a thorough examination of the crude mixture of resin acids obtained from Jack pine (P. Banksiana) indicate clearly that, in this mixture, there are three groups of acids:

Group 1:

"AMORPHOUS ACIDS", distinguished by their solubility in low-boiling petroleum ether, and their ready solubility in alcohol.

They are always found present with the "crystalline acids" (Group 2) but can be seperated by repeated recrystallization from alcohol, in which the amorphous acids are much more soluble than the crystalline products. These amorphous acidic substances probably belong to the "sapinic" group of the so-called "natural resin acids"(page 15). The latter have never been isolated in the pure state since they are extremely unstable,[41,42] readily changing into colophenic acids under the influence of heat and chemical reagents.

They are also characterized by yielding verysoluble sodium salts as distinguished from the less soluble salts of the "crystalline" acids. This offers another means for separating these substances.(See experimental part.)

Group 2:

"CRYSTALLINE ACIDS", comprising members of both the "natural" and the "colophenic" types. (Page 15.)

The pimaric acids come under the heading of "natural" acids, while the abietic acids are in the"colophenic"class.[41,42] Both of these two types of acids were isolated, together with the

sapinic acids, when the crude "resin acid" mixture was treated exhaustively with petroleum ether. The sapinic acids were recovered as a yellow scum from the semi-crystalline mixture, and the remaining crystalline products then separated into (1.) the "natural" acid component (pimaric) and (2.) the "colophenic" component (abietic acid), by means of the crystalline ammonium salt of the former, and the crystalline acid sodium salt of the latter. The colophenic (isomerised) acids do not yield crystalline ammonium salts,[102] while acid salts, such as acid sodium abietate, cannot be obtained in crystalline form from the "natural" acids.[59]

Group 3:

AMORPHOUS TRANSFORMATION PRODUCTS DERIVED FROM GROUPS 1 and 2.

These products are insoluble in petroleum ether (see Kohler[103] and Fahrion[52]). When subjected to higher temperature, as in the distillation of the free acids or of the esters, in the present investigation, crystalline acid derivatives were obtained. These crystalline products may or may not be identical with some of the acids present in the original resins; the so-called "natural" acids generally give rise to "colophenic" acids when heated[41,42] but dextro-pimaric acid (a "natural" acid) is exceptional, in that it can be distilled, in vacuo, without undergoing change[42]

Discussion of results obtained in the separation of Groups 1, 2, and 3.

The difficulties encountered during the earlier stages of the work, in attempting to isolate the crystalline acids from the crude mixture, can be understood when consideration is given to the fact that the original crude resin acids consist of such a complicated mixture embracing groups 1, 2, and 3.

The earlier experimental methods employed (See Experimental Part, a to i) to isolate the crystalline products, were not successful in some cases, and, in other cases, for example, (vacuum distillation of the free acids or the esters) yielded mostly transformation products. Fractional crystallization, fractional precipitation, purification by means of animal charcoal, separation by means of the phenylhydrazide or methylamide, were of no use, on account of the presence of such large percentages of impure, amorphous material. Tschirch[104] reported having considerable difficulty in purifying the acidic products which he obtained, by fractional crystallization, etc., and frequently ended up with insufficient material for investigation. This difficulty is present to an even greater degree in the case of the more complex mixtures from the wood-resins obtained in the present investigation.

Methods (c) and (e), involving, respectively, the use of hydrochloric acid gas, and the action of heat under pressure, were found to be of no use on account of the tarry nature of the substances formed. These are probably transformation products due to

isomerisation under the influence of heat and mineral acid (see Dupont[42] and Kohler[41]

Method (g): in the separation by means of the lead salts, the analytical results show that the products (soluble and insoluble salts) are impure mixtures, and that no actual separation has been effected. The acids recovered from those salts could not be isolated in crystalline state.

Methods (h) and (i). Vacuum distillation of the crude mixture of acids, or of the esterified product involves considerable losses of material, due to decomposition at high temperatures which must be employed so that re-fractionation of the small yields of the distillates is almost out of the question. The acids thus obtained are chiefly isomerisation products formed by the action of heat,[41,42] and contain considerable amounts of amorphous substances. The ordinary methods for esterification of abietic acid[105] are suitable only for the relatively pure acid. The dark, tarry products, in the present investigation, had to be esterified under different conditions in order to yield a product that could be isolated in comperatively pure form. The method used (Johnston[101]) was one intended particularly for the purification of dark coloured rosin.

Method (j). Exhaustive extraction by means of petroleum ether, was ultimately found to be the only procedure by which the pure resin acids could be isolated, in unchanged condition, from the tarry mixtures. Amorphous, yellow substances (probably "sapinic"

acids) were also found with the crystalline acids, by this method, and these had to be removed by a lengthy series of recrystallizations from alcoholic solutions. The sapinic acids are very soluble in alcohol.[106]

The residue insoluble in petroleum ether, when dried, formed a dark brown mass, resembling very impure rosin. This material probably consists chiefly of amorphous transformation products (colophenic acids) derived from the "natural" acids.[42]

An examination of the constants (see Table VI p.56) for the four resinic acid products isolated,

Resinic acid (A) by vacuum distillation,("green" wood products)
Resinic acid (B) by esterification, ("green" wood products)
Resinic acid (C) by petroleum ether extraction("green" wood products)
Resinic acid (D) by petroleum ether extraction(seasoned wood product)

shows that, as already mentioned certain of these constants are of little or no value in characterizing the individual acids present since they always depend on the experimental conditions.(see page 20.) The "Iodine number" and the "Saponification and Ester numbers" reported in the literature, for abietic acid and its isomers, vary over a considerable range. These very sensitive acidic substances readily change under the influence of heat, and of chemical reagents generally. The values obtained for the products in the present investigation agree with some of those that have been reported previously.[107]

"ACID NUMBER". The neutralization value ("Acid number") is a direct measure of the true acidity of the pure substances, in the

present case, because it was determined by neutralisation of the free acid, in the cold, with very dilute($\frac{N}{20}$) alcoholic potash. A micro-burette was used, for greater accuracy. The determination of the neutralization value, under careful conditions, offers probably the best means of ascertaining the molecular weight of the acid very accurately.[108]

The molecular weight,(302.8) determined in this way, for the abid (C) obtained by petroleum ether extraction and re-cryetallised twenty times, agrees with the theoretical value (302.2) for $C_{20}\ H_{30}\ O_2$.

The acids (A) and (B) were found to have a slightly higher molecular weight, 309.0; this may be due to slight oxidation having occurred during the esterification and distillation of these products. Ultimate analyses (for carbon and hydrogen) are not of any particular value in the case of these substances, on account of the difficulty in obtaining absolutely pure products. The confusion resulting from interpretations of such analytical data, in the past, has been mentioned already (pp. 7-14).

The melting point is of no significance here, since each of the acid products examined is obviously a mixture of isomers and these acids are known to undergo isomerisation near the melting point:[109] Knecht[110] reported a melting range (149-161°), for his acids from American rosin, and attributed the indefiniteness to the formation of abietic anhydrides. These temperatures correspond almost exactly with the range observed (150-162°) in the case of the acids in the present investigation. The melting

point is of use in characterizing the individual components, only after employing some suitable procedure for separating the mixture into simpler constituents. The products may have all the external appearances of being chemically pure, as in the case of the above substances, and yet may be a complex mixture of isomorphous isomers.[42]

The complete identification of any of the individual acids comprising such a complex mixture, is obviously a difficult task, and requires considerable time, as well as a large amount of working material. In the case of the acids from certain oleoresins, this has been accomplished to some extent.[111] Some of the products have been mentioned frequently in the introductory remarks(p.p. 11-19).

Abietic acid has been estimated;[59] in the presence of other resinic acids, by means of the crystalline acid salt, $3C_{20}H_{30}O_2 \cdot C_{20}H_{29}O_2Na$, which it forms. The "natural" acids do not yield crystalline acid salts of this type.[59] In the case of the acids in the present investigation, the crystalline acid salt was obtained without difficulty and the percentage of abietic acid present calculated from the yield of the salt. The neutral sodium salt could not always be obtained in crystalline form. Dupont[59] says it is always gelatinous.

A crystalline ammonium salt was obtained, indicating the presence of "natural" acids (pimaric group). Acids of the other class (colophenic) have been found to yield only

gelatinous ammonium salts.

The formation of these two crystalline derivatives indicates the presence of acids of both the pimaric (natural) and abietic (colophenic) groups. The further separation of these derivatives into the individual isomeric components, optical antipodes, etc., is an extremely complicated matter, and beyond the scope of the present work.

It is noticeable that the acid products (A and B) which had been distilled in vacuo, or esterified, gave a higher yield of the acid sodium salt than the product D, which had been isolated by petroleum ether extraction. The latter probably contains a considerable percentage of the "natural" acids; this is confirmed by the high yield of crystalline ammonium salt. This acid product D was recrystallized only twice, while the others (A, B, C) had been purified to a much higher degree (see Table at end of Experimental part.) Abietic acid is formed from the "natural" acids during recrystallization from alcoholic solutions.

The crystalline ammonium salt was obtained from both the acids present in the "green" wood (C), and those of the "seasoned" wood (D). The yield is higher in the case of the latter, probably because it was not recrystallized as often as the former, hence more "natural" acids were present.

It would be possible to separate a mixture of two acids, such as pimaric and abietic, into the individual components, by fractional recrystallisation, but an extremely large number of recrystallisations would be required[42]. The crystalline acid (C) in the present investigation, melted at 142-156°, 150-158°, and 151-161°, after eight, fourteen and twenty recrystallisations,

respectively. The final yield of product was about 1-2% of the original crude acids. When each of the three lots of crystals above, were examined microscopically, a mixture of at least two crystal forms was always observed. The apparently-pure product consisted chiefly of needle-like crystals, with which was mixed a considerable percentage of the peculiar triangular crystals of abietic acid. It was impossible to separate these by mechanical means.

Pure specimens of certain acids of the pimaric and abietic groups have been reported by previous investigators[112] to have melting-points as follows:

Levo-pimaric acid	m.p 150-152°
Dextro " "	" 211-212°
Alepic Acid (Alpha)	" 148°
Abietic acid (alpha)	" 173-174°

The total crude acids obtained from "green" Jack Pine, were found to contain 66.3% "crystalline" acids, while those from the seasoned wood yielded 51.8%. These figures are only rough approximations on account of the question of the solubilities of the "natural" acids, in petroleum ether, and of completeness of extraction under the experimental conditions, as well as the changes which the natural substances undergo in the course of chemical treatment. Both pimaric and abietic acids are soluble in petroleum ether; the latter may be present originally in the wood, or it may be a transformation product of the "natural" acids after they have been isolated.

It is possible to obtain small amounts of crystalline resin

acids, probably in unchanged condition, (and accompanied by other substances), by extracting the wood with petroleum ether, alcohol, or benzene, etc., at comparatively low temperatures. The yield is extremely small and the extraction is far from quantitative. The product, in any case, would probably have to be subjected to chemical treatment of some kind, in order to isolate the resin acids, free from the other substances.

Extraction of the wood by means of 1% aqueous sodium hydroxide, at about 90-100°, was tried in the present investigation, in attempting to remove the crystalline resin acids in unchanged condition. The method was of little use, on account of the very large amounts of cellulose and lignin removed with the resinic substances. The acidic products finally obtained were found to contain considerably fatty acids, so that the resinic acids could not be obtained without resorting to a chemical process of separation, and this involves the possibility of transformations of the original products, to some extent, in the course of the chemical treatment. It does not seem possible to obtain the pure resinic acids from wood-resins ("physiological resins") without employing a chemical treatment of some kind.

EXPERIMENTAL PART.

Sec. 1. Attempts to separate crystalline products from the crude mixture of resin acids. p.41.

Sec. 2. Method for isolating the crystalline acids in unchanged chemical condition, p.49.

Sec. 3. Identification of individual resin acids by conversion into their crystalline salts, p.53.

EXPERIMENTAL

Section 1

Attempts to separate crystalline products from the crude mixture of Resin Acids.

(a) Method of fractional crystallization:-

The crude mixture of resin acids was dissolved by heating with various organic solvents, and the dark red solutions allowed to stand, after the sides of the dishes had been carefully scratched to assist formation of crystals. The following solvents were used: Ether, Benzene, Acetone, Chloroform, Glacial Acetic Acid, Ethyl Acetate, Methyl Alcohol, Ethyl Alcohol, and a mixture of the two latter. The solutions were kept in desiccators under vacuum, shielded from light, and such materials as calcium chloride, phosphorous pentoxide, paraffin, ligroin, etc., were put in the desiccators to absorb the solvent slowly. The desiccators were kept in the ice-chest, and also later at room temperature: Cooling to -15°C. had no effect. The solvent was frequently replenished, and the dish scratched vigorously, but the solutions did not yield crystalline material, for a very long time. The solution in ethyl alcohol, on becoming quite dry, finally yielded some small crystals, and these spread slowly throughout the mass. But it was found impossible to free these from the amorphous material by which they were surrounded. The crystals only formed after the mass had dried completely. They redissolved readily when solvent was added.

(b) Method of fractional participation from mixed solvents.

The acids were dissolved in benzene, some petroleum

ether added until turbidity appeared, the solution cleared again by warming, and the sides of the vessel well scratched. In other experiments water was added to an alcoholic solution, and the same treatment given. The precipitated colloidal fractions were removed by filtering under suction, in an atmosphere of carbon dioxide, but these yielded only oily residues, not crystallizable from alcohol, etc. The colloids generally settled to the bottom of the vessel, forming a dark scum.

(c) Action of heat under pressure:-

The crude mixture was heated in sealed tubes at 110°, for several days (Labatut[58]). No crystals separated and the products would not crystallize from solvents.

(d) Purification by animal charcoal:-

Alcoholic solutionsof the resin acids were boiled with animal charcoal, filtered hot, and left to crystallize. Some crystals formed after complete evaporation of the solvent, but the impurities had not been removed, and still surrounded the crystals.

(e) Use of hydrochloric acid gas:-

Hydrochloric acid gas was passed into an alcoholic solution of the crude acids[43]. When 95% alcohol was used, a precipitation was noticeable, but this was difficult to obtain with absolute alcohol. This precipitate yielded only a scummy residue when attemps were made to separate it.

(f) Separation by means of Phenylhydrazine and Methylamine:-

The crude acids were treated with phenylhydrazine in equivalent proportions, by heating at 100° for three hours[98]. Attempts to purify the viscous residue from ether, benzene, and

petroleum ether, and mixtures of these, were unsuccessful.

The formation of the methyl amide was also tried, by passing dry (CaO) methylamine gas into an absolute methyl alcohol solution of the acid[99]. The separation of the pure substance, could not be effected by organic solvents. Precipitation of the phenylhydrazide and methylamide from mixed solvents (benzene and petroleum ether) was ineffective, on account of the large amounts of impurities present.

(g) Lead salt separation (Tschirch[100]):-

A hot, dilute alcoholic solution of the crude resin acids was treated with an excess of hot, concentrated aqueous lead acetate solution, causing the precipitation of a yellow, gelatinous product. The mixture was allowed to stand and cool and then filtered. The solid matter was washed free from lead salts with warm water. It was then repeatedly extracted with hot alcohol, and the insoluble residue dried at 65° in vacuo. The alcoholic mother liquor yielded a similar yellow, amorphous solid on evaporation, and this was dried in the same way.

Analysis:-

Portion soluble in alcohol.	Portion insoluble in alcohol.
Sample, 4.476 mgs. Wt. $PbSO_4$, 2.111 " % Pb., 32.2%.	Sample, 5.205 mgs. Wt. $PbSO_4$, 2.546 " % Pb., 33.4%.
Sample, 5.227 mgs. Wt. $PbSO_4$, 2.582 " % Pb., 33.7%.	Sample, 3.709 mgs. Wt. $PbSO_4$, 1.788 " % Pb., 32.9%.

Theory for the lead salt of pure abietic acid $(C_{40}H_{58}O_4)Pb$;

Pb. equals 25.6%.

These lead salts were decomposed by the addition of dilute hydrochloric acid, and the free acids removed by shaking with ether. Removal of the solvent yielded brown, viscous residues from which no crystalline material could be obtained.

(h) Vacuum distillation of the crude acids:-

As only small quantities of material were available and the distillation temperatures quite high, specially designed pyrex flasks were made, ranging in capacity from 1 c.c. to 50 c.c., which were fitted with very long necks, to avoid decomposition of the rubber stoppers. The side-arm of each had to be placed almost directly at the top of the bulb, otherwise the rapid condensation prevented distillation at these extremely low pressures. The flasks had to be "lagged" well with asbestos; Wood's metal was used for a heating-bath, and it was necessary to keep the flask immersed up to the side-arm, thus making the boiling point temperature readings on the thermometer inside the small flask dependent, to some extent, on the bath temperature. Every precaution was taken to minimize the inaccuracy due to this, by keeping the bath at fixed temperatures for each fraction, etc., and, carefully observing what seemed to be the true boiling temperatures. As mixtures of substances were being dealt with, which could not be completely separated by fractional distillation, no correction of the boiling points was made. A Langmuir mercury vacuum pump was used in these experiments.

Distillation of the tarry mixtures was always an extremely slow procedure. Elevating the temperature helped somewhat, but seemed to cause decomposition of the material in the flask before

it had time to distil over. A pale yellow initial fraction, free from darker material, could be obtained by slow fractionation. Above 210°, very dark fractions were obtained, with an odor of burnt tar, and as the bath temperature rose to 270°, gaseous decomposition occurred, so the distillations were stopped usually about this temperature. The residue in the flask, upon cooling, formed a brittle, black mass, having the odor of wood tar.

The average results of several such experiments are tabulated below:-

Fraction	Boiling Temp.	Pressure	Distillate	Average % Yield of Original Crude
1	165-180°	0.01-0.05 m.m.	Amber-coloured rosin.	25%
2	180-210°	"	" Dark brown, brittle.	23%
3	210-250°	"	" Very dark; tarry odor.	10%
Residue, black tar,				40%

These distillates were then dissolved in alcohol and other solvents, as in the crystallization experiments already described. Nothing could be done with fraction 3 on account of its impure character. It evidently contained considerable amount of amorphous transformation products.

Fraction 2 showed signs of crystallizing on standing, but presented the same difficulties as regards removal of the crystals from the amorphous solid material in which they were embedded.

Fraction 1 readily yielded solid material when deposited from 95% alcohol, but a very large proportion of a brown scum,

very viscous in nature, surrounded the poorly formed crystals. This viscous material could not be removed even on porous tile. Repeated recrystallization from ethyl alcohol did not serve to separate the crystals from the scum. Finally, the addition of water to the alcoholic solutions appeared to have a beneficial effect, and a mass of fairly well-formed crystals resulted on standing, but still having much amorphous material adhering to them. The solids were filtered from the brown mother liquors, and redissolved in boiling alcohol. Water was added slowly until turbidity was just reached, the precipitate dissolved again by warming, and this solution left to stand. The sides of the dish were scratched, to assist crystallization. After one hour, small crops of rosette-shaped crystals began to form. These seemed to be centered on a nucleus of brown, amorphous material. Better crystals were obtained by allowing to stand at room temperature than when kept on ice. The yellow, viscous material could now be removed largely by allowing each mass of material to drain several hours, on porous tile, in the refrigerator, at first washing with a very small amount of cold alcohol. By repeating these operations several times, a crop of glistening, pure white crystals was obtained (acid A). These were dried for three or four days over sulfuric acid, and then for one day at 100° in vacuo, before being analyzed.

(1) <u>Purification through the ethyl ester</u>:-

This was based on the method of A. C. Johnston[101]:-

The dark coloured rosin mixture (150 parts by weight) was added to an alcoholic caustic soda solution made by

dissolving 20 parts NaOH in 25 parts of water, and diluting with 150 parts of 95% ethyl alcohol. The mixture was refluxed, in a small pyrex flask with high neck, suitable for subsequent vacuum distillation. When a clear solution had been obtained, 40 parts of diethyl sulphate (115% of theoretical) were added drop by drop, with stirring. The alcohol was then distilled off, and the last traces removed by a water pump. The residue was heated at 145° for two hours, stirring at intervals. An extremely viscous, dark brown mass was obtained.

This product was distilled in vacuum, and generally yielded a light brown, very viscous distillate, boiling between 155 and 180°, at 0.05 m.m. Three redistillations of the material yielded a pale yellow viscous, and odourless liquid. Some very dark fractions of the rosin (acids mixture) were found to yield slightly darker ester products, however. considerable amount of tarry residue always remained in the distilling flask.

Estfn. No	Colour of Orig. Acids	Press.	Boil. Temp.	Wt. Acids Used	Wt. of Ester	Yield %	n_D^{22}
1.	Dkst. brown	0.02-0.05 m.m.	158-163°	10.0 gms.	3.05	27.5%	1.5440
2.	Med. dark	" "	161-168°	7.5 "	2.75 "	33.5%	1.5395
3.	Very dark	" "	158-168°	7.5 "	2.35 "	28.7%	1.5520
4.	" "	" "	156-163°	8.0 "	4.30 "	49.1%	1.5425

(From dark rosin, Johnston[101] obtained ethyl abietate, b.p.195-200° at 4 m.m., and n_D^{22} = 1.520; it was found to be very difficultly saponified).

The above products were used for getting the pure acids, as follows:- the ester was saponified by means of alcoholic potash, on a boiling-water bath for about 28 hours; four times the

theoretical amount of KOH was used. The saponification mixture, after cooling, was extracted with ether to remove unchanged ester, and the ethereal extract washed with water, these washings being added to the main alkaline portion. The latter was acidified by addition of hydrochloric acid, a white colloid being precipitated, rapidly turning yellow. This was removed by ether extraction, the extract washed with water, and dried over fused sodium sulphate. Evaporation yielded a very viscous, yellow residue, which was found to crystallize readily from ethyl alcohol, accompanied by some yellow scum. Purification of this product in exactly the same manner as described already for the distillate from the crude resin acids yielded pure white crystals (acid B). These were dried over sulphuric acid and then at 100° in vacuo.

The other ethereal solution containing the unchanged ester, similarly yielded a brown oil, which was dried at 100° in vacuo, and weighed. The percentage saponified was calculated from this by difference, to avoid having to dry the original yield of resin acids for weighing.

Another lot of the ester was saponified, with isoamyl alcohol and potassium hydroxide, in order to get to a higher temperature and hence effect greater saponification. The mixture was heated for 28 hours, at 130-140°, then the products obtained as described above.

Prod.from Estfn.No	Wt.of Ester	Wt.of KOH	Time of Saponfn.	Wt.of unchgd.Ester	% unchanged Ester	% Saponfn.
2	2.7 gms.	1.84 gms (Ethyl alc)	28 hrs	0.7 gm.	25.4%	74.6%
4	4.3 "	3.40" (isoamyl alc)	28 hrs	1.24 "	28.8%	71.2%

The acids recovered after saponification using isoamyl alcohol, were found to be less pure and more difficult to obtain

crystalline, than when ethyl alcohol (lower temperature) had been used.

The following method was finally employed for the isolation of the crystalline acids.

(j) Recovery of the crystalline acids in unchanged chemical condition:-

The crude mixture of acids was treated with petroleum ether (b.p.30-50°) until no more could be extracted. At first, this was done by warming the mixture, with large volumes of petroleum ether, under a reflux. Then the residue was broken up, and triturated repeatedly with the solvent, and the extract decanted. This procedure was found very difficult and tedious, as the residue became gummy, and the extraction was not thorough. The viscous mass could not be treated in the ordinary manner in a Soxhlet. The only efficient method found was to grind the crude product with a large amount of pure quartz sand, and extract this mass in Soxhlet extractors, with petroleum ether.

The entire material was treated continuously in this way for 48 hours, then removed, dried, and thoroughly ground again, now forming a very finely powdered mass. This was again extracted for 24 hours. The whole process was repeated until no more material was removed by the petroleum ether. This required seven days extraction, using one large and two small Soxhlet extractors. The extract was removed at intervals, to avoid prolonged heating of the dissolved acids.

The petroleum ether extract was pale yellow, with green fluorescence. Evaporation of the solvent left a yellow, granular

residue (acid C). Where the yield was to be determined, this mass was broken up, dried over sulfuric acid in vacuum for two days, and then at 60° in vacuum, for 8 hours, and weighed.

Purification of the acid:-

The mass (C) was dissolved in hot alcohol, and left to crystallize. Only some crusts of solid material were obtained, accompanied by rosin-like masses. (The same result was obtained with acetone). These masses were left to drain on porous plates. Several such treatments with alcohol finally yielded large masses of solid, pale yellow in colour, but not definitely crystalline. When the alcoholic solutions were treated with water, however, as described previously, beautiful crystalline masses were obtained. There was sometimes a separation of a rosin-like mass at the bottom of the flask when the turbid aqueous alcoholic solution was boiled to redissolve the precipitate. The upper liquid was decanted from this, and left to stand. The rosin, when dissolved separately in alcohol and reprecipitated, yielded some crystalline material also, which was added to the main lot.

The crystalline masses obtained from the various solutions were washed once with alcohol, and then allowed to drain on porous tile after each recrystallization; this improved their colour considerably. There was a tendency to become yellow on the surface when they were exposed to air. The dishes were kept in desiccators under vacuum, away from light.

Another crop of crystals could be obtained from the mother-liquors. These comprised the more soluble acids.

The above treatments finally yielded a lot of beautiful,

needle-like crystals, (purified acid C), apparently free from impurities, but still having a very faint yellow colouration, even after twenty recrystallizations. These were dried as usual over sulfuric acid and then at 100° in vacuum.

The portion of the crude acids insoluble in petroleum ether (acids C'), was also investigated. It was removed from the sand by extraction with alcohol. It showed no signs whatever of crystallizing. The dark colour indicated the presence of much oxidised material.

When the crude acids (C') were heated in sealed tubes, as already described, no crystals were formed, and the product would not crystallize from alcohol, even after saturating with hydrochloric acid gas. On distilling at 0.05 m.m. pressure, a small amount of pale brown distillate came over, between 165 and 210°, and after repeated treatments by the methods already described, (p. 41), some crystalline product was obtained, which seemed identical with that obtained by distillation of the original crude acids (product 1).

Some of this insoluble material was also esterified[101], and yielded the products mentioned under Esterifications Nos 3 and 4, (page 47). The product from No 4 was saponified (page 48), and yielded some amorphous material, with a small quantity of crystalline substance, identical with the product of distillation mentioned above.

Approximate percentages of crystalline acids in the crude acids mixture:-

(a) Crude resin acids from Lots Nos 1 to 11 (part I, page 27), of resins from "green"wood: wt. = 98.8 grams. Refluxing and stirring with petroleum ether removed 33.3 grams crystalline acids (C).

Insoluble residue (C') = 65.5 grams. 8.0 grams of this residue gave 4.30 grams, ester (see Esterification No 4, page 47), = 49.1%, calculated as acids; 65.5 x 49.1% = 32.2 grams esterifiable acids in the residue; (33.3 + 32.2) = 65.5 grams, total crystalline acids; $\frac{65.5}{98.8}$ x 100% = 66.3% approximately of crystalline acids in the crude acids mixture.

(b) Crude resin acids from Lot No 13, (part I, page 27), of resins from "seasoned" wood:-

Weight = 239.8 g.; extracted in Soxhlet, with petroleum ether, for 7 days.

Weight of crystalline acids (D) obtained = 124.25 g.; = 51.8%, of the total crude acids.

Examination of Resin Acids A and B (together).

These acids were obtained by vacuum distillation,(A), and from the ethyl esters,(B).

Products of "green" wood.

		Details
Melting point	151-161°	Slow decomposition above 151°; completely liquid at 161°.
Iodine Number	90.9	0.1146 gm. equivalent to 8.33 c.c. Thios. (0.01250 gm. Iodine per c.c.);
Saponification Number:	239.0	0.1669 gm. equivalent to 1.37 c.c. HCl (0.5179N.);
Acid Number	181.5	0.0917 gm. neutralized by 5.78 c.c alc. KOH (0.05127N.), = 0.01665 KOH. calc'd for $C_{20}H_{30}O_2$, 0.01700 gm. KOH.
Ester Number	57.5	(239.0 - 181.5) = 57.5 ;
Molecular Weight	309.0	56,100 mg.KOH/181.5 = 309.0; Calc'd for $C_{20}H_{30}O_2$, mol. wt. = 302.2

Examination of Resin Acids C.

Acids obtained by petroleum ether extraction.

Products of "green" wood.

		Details
Melting Point	150-162°	Slow decomposition above 150°; completely liquid at 162°.
Iodine Number	129.2	0.1442 gm. equivalent to 14.91 c.c. Thios. (0.01250 gm. Iodine per c.c.)
Saponification Number	243.4	0.0847 gms. equivalent to 0.71 c.c. HCl (0.5179N.)
Acid Number	185.3	0.1420 gm. neut. by 9.13 c.c. alc. KOH (0.05127N.), = 0.02627 gm. KOH; Calc'd for $C_{20}H_{30}O_2$, 0.02633 gm. KOH.
Ester Number	58.1	(243.4 - 185.3) = 58.1 ;
Molecular Weight	302.8	56,100 mg. KOH/185.3 = 302.8; Calc'd for $C_{20}H_{30}O_2$, mol. wt. = 302.2

IDENTIFICATION OF INDIVIDUAL RESIN ACIDS BY CONVERSION INTO THEIR CRYSTALLINE SALTS

(1) Preparation of sodium acid abietate[59]:-

Material used for the experiment: the mixture of highly purified distilled acids (A and B) recovered from the saponification and acid number determinations (see page 52) on products from green wood, lots 1 to 11, (part I, page 27) was employed. Method: 0.1885 gm. of the dried material was dissolved in 5 c.c. alcohol, and 1.57 c.c. aqueous NaOH (0.1004N.) run into the hot solution slowly, with continual shaking. The sides of the vessel were scratched well, and the solution left in a desiccator over

calcium chloride. After most of the solvent had disappeared, some fine solid particles were precipitated; after further scratching, needle-like crystals began to form. The solid was filtered from the small amount of mother liquor, and washed with a little alcohol;

Wt. of crystalline product = 0.17 g.,

Yield = 88.6% (calc'd. on $3C_{20}H_{30}O_2 \cdot C_{20}H_{29}O_2Na$.)

This product was purified by recrystallization from alcohol, yielding colourless needles of acid sodium abietate, m.p.170-175°.

Similar treatment of the acids (D) removed by petroleum ether from lot 13 (part I, page 27), which had not been further purified beyond two recrystallizations from alcohol, resulted in only a minute quantity of crystalline salt, but considerable gelatinous product.

(2) Preparation of neutral sodium abietate:

Method: 0.1000 gram of the acids (A and B) above, was dissolved in alcohol, treated with aqueous NaOH until neutral, and the clear solution left to stand, after vigorous scratching of the sides. After some time, a very slight precipitation of flocculent material took place, but a crystalline product could not be obtained, even after prolonged treatment.

(3) Preparation of the ammonium salts:-

(a) Material used:- The highly-purified acids (c), originally obtained by petroleum ether extraction; ("green" wood product).

Method: Dissolved 0.1000 gm. acids in 5 c.c. alcohol, and added concentrated ammonia water slowly, until a slight excess was present. After standing several hours, a small amount

of crystalline material separated, as well as considerable gelatinous product. The mixture was allowed to stand in a vacuum desiccator for one week, during which time most of the solvent had evaporated. The precipitate was removed by filtration, and washed with alcohol until the gelatinous material had been removed. The crystalline residue was dried at 60° in vacuo, and weighed. It was further purified by repeated recrystallization from alcohol containing a few drops of ammonia, and finally obtained in the form of colourless needles.

Wt. of product obtained = 0.06 g.,

Yield =56.6%

M.P. of purified product: Decomp. = 115°; liquid at 161°;(see below).

(b) Material used: The acids (D), originally obtained by petroleum ether extraction; ("seasoned" wood product);

Method: Dissolved 0.5000 gm. in 25 c.c. alcohol, and treated the solution in the same manner as described under (a), above. Most of the precipitate was crystalline, in this case.

Wt. of product obtained =0.36 g.;

Yield = 68.2%;

M.P. of purified product: Decomp. = 115°, liquid at 161°.(Note: Klason & Kohler[39] reported that this salt decomposes readily with loss of ammonia).

TABLE VI

SUMMARY OF PROPERTIES OF THE PURIFIED RESIN ACIDS OF JACK PINE

Acids from	Reference letter used	Method of isolation	No. of times re-crys. from aq alc.	M.P.	Iod. No.	Sap No.	Acid No.	Est. Mol. Wt.	% abietic acids	% pimaric acids	Approx. % cryst. acids in the crude acids
"Green" wood Extr'ns Nos. 1 to 11	A, B,	(A) dist'd (B) ext'd	8	151-161°	90.9	139.0	151.5	57.5	309.0	88.6	-	66.3
do.	C	extr'd by pet. ether	20	150-162°	129.2	143.4	165.3	58.1	302.8	-	56.8	
"Seasoned" wood Extr'n No. 13	D	extr'd by pet. ether	2	140-152°	(Not purified further)					Traces	68.2	51.8

BIBLIOGRAPHY

of

PART II

only.

1. Schorger "Contribution to the Chemistry of American Conifers", Trans. Wis.Acad.Sc., Arts, Lett., XIX, part II; p.752.

2. See ref.1, p.729.

2a. Pugulevskii, J.Russ, Phys.Chem.Soc. 59, 299 (1927); C.A. 22, 1433.

3. Bull. Soc.Chim, 36, 1210 (1924)

3a. Vezes, Monit.Scient.Quesneville, 15, 41 (1901); 16, 339 and 426, (1902)
Tschirch, "Die Harze und die Harzbehalter" pp.623-725.
Dupont, Bull.Soc.Chim. 35, 1209-70 (1924).
Levy, Bull.Inst.Pin, 59, 122-124 (1929)
Ruzicka, Bull.Inst.Pin, 49 to 53 (1928); 59,124-137 (1929)
Nagel, Mettalborse, 19, 173-4 (1929); Zent.100 (I) 1750 (1929)

4. See Tschirch, Dupont, Ruzicka, Rouin in ref.3a. above.

5. Ann.de Chim. 68, 67 (1808).

6. Recherches phys.Chim II, 311 (1810).

7. Ann.de Chim. et phys. 13, 342 (1820).

8. Schweigger's jour., 31, 480 (1821).

9. Ann. de chim. et phys., ser.2, 23, 377 (1823).

10. Jahrsb. polytech. Inst.Wien. I, 435 (1824).

11. Ann. de chim.et phys. 31, 108 (1826).

12. Trommsdorffs Neues Jour.Pharm., VIII, 21 (1824).
Poggendorff Ann., VII, 311.

13. Thesis, Strassburg, 1830.

14. Lieb.Ann., 13, 169 (1835).

15. Journ.Pharm. 20, 229, (1834).

16. Lieb.Ann. 13, 174, (1835)

17. Lieb,Ann. 13, 174, (1835)

18. Ann.Chim. & Phys. (2), 65, 325 (1837).

19. Bull.Soc.Chim. 21, 387, (1874).

20. Ann. de Chim. et Phys., Ser.3, XXII, 460 (1848).

21. Zeits.ges. Naturwissenschaften, XIV, 311,,(1859)

22. Sitzungsb.Akad. Wiss.,Wien, XLIV, 121, 1861.

23. Journ.prakt. chem.,CI, 235 (1867).

24. Lieb.Ann., CXLVIII, 143, (1868).

25. Diss., Berne, 1883.

26. Ber., 17, p.1884 (1884)

27. Ber., 18, 2165, (1885)

28. Dissertation, Berne, 1885.

29. Bull.Soc.Chim. 21, 387 (1874).

30. Ber. 18, 3331 (1885); 19, 2167, (1886); 20,3248 (1887; 36, 4200 (1903); 38, 4125 (1905); 40, 120 (1907).

31. Monats., 14, 186 (1893); 15, 627, (1894).

32. Ber.Deut. Pharm. Ges., VI, 61 (1896)

33. Tschirch, "Harze und die Harzbehalter", p.684, etc.

34. Tschirch, "Die Harze und die Harzebehalter", 2 vols., Leipzig, 1906.

35. Bull. Soc.Chim., 35, 1217-8, (1924).

36. See ref.34, p.660.

37. Zeit,Ang.Chem. 1739-41, Nov.3,1905; Ber.39, 3043-6 (1906) 40, 3658-60 (1907); 42 4305-8, (1909.

38. Zent., 15 (5), 1598; Jour.Prakt.Chem., 85, 523 and 534 (1911).

39. Jour.Prakt.Chem. 73, 337-58, (1908).

40. Jour.Prakt Chem. 85, 523 (1911).

41. Jour.Prakt.Chem. 85, 534 (1911).
see also Aschan, Chem. Zeit 48, 149 (1924).

42. Bull.Soc.Chim. 35, 1209-70, (1924).

43. Jour.Prakt.Chem. 85, 534 (1911); see also refs.38 and 39.

44. Chem.Rew.16, 186 (1909); Chem. Zeit (1917).

45. Thesis, Paris, 1910; see Bull.Soc.Chim. 35, 1223 (1924).

46. Soc.Scien. Bordeaux, p.37 (1919); p.79, (1920).
See also Bull.Soc.Chim., 35, 1238 (1924).

47. Zeit.Ang.Chem.1197-1208, 1221-33, 1253, (1901).
239-241 (1904); 20, 356 (1907); Chem. Rev.18, 239 (1913);
Chem.Ums 26, 65 (1919); Bull.Soc.Chim. 30 (4), 1546.

48. Chem.Rev. Fett und Harzind, p.106 (1899); Chem. Ind. 6
106-11 (1903).

49. "Harz und die Harzbehalter" pp.639-648; 665-671.

50. See ref.49, p.640

51. Chem.Ind. 6, 106-11 (1903)

52. See ref.47; also Chem.Ums.27, 158-160, 201-4 (1920);
28, 5-7, 20-1 (1921); C.A. 15, 1225.

53. Dieterich, Dissert, Berne, 1883; see ref.25; see also
ref.49, p.635.

54. See ref.49, p.18, 647-8.

55. Bolin and Tschirch; see abstract Pine Inst.Amer.Abs. 2
86-90 (1928)

56. Jour.Soc.Dyers Col., 35, 148 (1919); J.S.C.I. 1234 (1923).

57. Zeit.Ang.Chem. 1852 (1905); Chem.Rev.14, 174 (1908).

58. Soc.Scien. Bord. July 21, 1904.

59. Dupont, Desalbres and Bernette, Bull, Soc.Chim. 39, 488
(1926); Dupont, Bull Soc.Chim 35, 1236 (1924).

60. Bull Soc.Chim. 35, 1237 (1924); see also Vezes et Dupont,
"Resines et Terebenthines", pp. 417-8.

61. Vezes et Dupont "Resines et Terebenthines, p.427-8.

62. J.A.C.S. 44, 1333 (1922).

63. J.A.C.S. 45, 1339 (1923).

64. Farb.Ztg. 31, 1848 (1926)

65. Dupont, Dubourg, and Rouin, Chim. et Ind. 19, 4A, 549 (1928)
Pine Inst.Amer.Abs. 2, 105 (1928).

66. Chem.Ums. F.O.W.H., 36, 33-4 (1929); P.I.A.A. 3, 34 (1929),

67. Chem.Ums. F.O.W.H. 36, 93-7, 373-7 (1929); P.I.A.A. 3, 75
(1929); 4, 3, (1930).

68. "Harze und die Harzbehalter", pp.684-725.

69. See ref.68, p.646.

70. Ber.23, 1921 (1890)

71. J.C.S. 85, 1238-49.

72. Zeit.Anorg. Chem. 81, 145 (1916); Dupont et Vezes, "Resines et Terebenthines", pp.428-436.

73. Ber.42, 4305 (1909)

74. Helv.Chim. Acta V, 1761 (1922).

75. Easterfield and Bagley, J.C.S. 85, 1238.

76. Ber.42, 4305 (1909).

77. Helv.Chim.Acta 6, 1077 (1924); 8, 637 (1926).

78. Bull Inst.Pin 59, 112-22 (1929).

79. Bull.Inst,Pin 59, 122-4 (1929).

80. Bull Inst.Pin, 49 to 53 (1928); 59, 124-37 (1929).

81. Zeit.Deuts. Ol u.Fett Ind. 41, 48 (1921)

82. Rec.Trav.Chim. 47, 363 (1928); see also Tschugaeff and Tesaru, Ber.46, 1769 (1913).

83. Ber.61, 616 (1928).

84. Chim. et Ind. 19, 4A, 549 (1928); P.I.A.A. 2, 105 (1928).

85. J.Pharm. Soc. Japan, 48, 170 (1928); Chem.Zent. 100 (I) 1101, (1929).

86. Filipovich and Vuisotzkii, J.Chem.Ind. (Moscow) 4, 953 (1927). C.A. 22, 4579; P.I.A.A. 3, 3, (1929). P.Bobrov, C.A. 22, 1485 (1928); C.A. 24, 738 (1930).

87. Balas; see C.A. 23, 1644 (1929); P.I.A.A. 3, 74 (1929); Coll.Czech.Chem.Comm. 1, 401, (1929).

88. Nordstrom, J. prakt, Chem. 121, 204 (1929).

89. Chem.Ums. F.O.W.H. 36, 115 (1929).

90. Bull Soc.Chim. 30 (4) 1545; Ber.54, 867 (1921); Fenno.Chim. I 15-27 (1929); P.I.A.A. 4, 42 (1930).

91. Chem.Ztg. 48, 149 (1924).

92. Bull.Soc.Chim, 35, 1257-62 (1924).

93. Bull.Inst.Pin. 52, 205 (1928); P.I.A.A. 2, 167 (1928)
Comp.Rend.189, 763-5 (1929); P.I.A.A. 4, 4-5 (1930).

94. Bull.Inst.Pin, 59, 138 (1929); see also Levy,Ber.62B 2497 (1929).

96. BullInst.Pin, 49 to 53,(1928); 59, 124 (1929).

95. Bull.Inst.Pin. 60, 159; 61, 183, 215 (1929);P.I.A.A. 3 166 (1929).

97. Mettallborse 19, 173 (1929); Chem.Zent.100 (I) 1750 (1929).

98.Haworth, J.C.S. 89 (1926).

99. Haworth J.C.S. 2351,2432 (1927).

100. "Harze und die Harzbehalter p.653.

101. Johnston, J.Ind.Eng.Chem. 21 (7) p.688 (1929).

102. Kohler, Jour.prakt.Chem. 85, 534 (1911);
Vesterberg; see ref.30; Dupont, Bull.Soc.Chim. 35
1215 (1924).

103. Journ.prakt.Chem. 85, 534 (1911).

104. "Harze und die Harzbehalter" pp.61, 651.etc.

105. Levy, Zeit.Ang.Chem. 1739 (1905).
Schulz.Chem;Rev.19, 291 (1912).
Kesler, Lowy & Faragher J.A.C.S. 49, 2898 (1927).

106. Kohler, Jour.prak.Chem. 85, 534 (1911).

107. Vezes et Dupont, "Resines et Terebenthines", pp.425-431.

108. Levy, Zeit.Ang.Chem. 1739 (1905); Ber.39, 3043 (1906).

109. See ref.106; also Dupont Bull.Soc.Chim. 35, 1212 (1924).

110. J.S.C.I. p.1234 (1923).

111. Review by Dupont, Bull.Soc.Chim. 35, 1209-70 (1924).

112. Dupont, Bull.Soc.Chim. 35. 1215-21, (1924).

PART III

THE FATTY CONSTITUENTS OF THE CRUDE RESINOUS PRODUCT.

PART III

(A) Introduction.

The Fatty Constituents of Conifer Resins.

The looseness with which the term "resin" has been applied in the past, and even at the present time, is undoubtedly responsible in a great measure for the general lack of appreciation of their actual composition, insofar as the natural resinic products are concerned. Every natural so-called resin is a mixture, chemically, and a more restricted use of terms must henceforth be adhered to, if confusion is to be avoided. The composition of a certain "resin" may be comparatively simple, that is, as regards the groups of organic substances of which it is made up; on the contrary it may be very complex. For example, the "resin" which exudes from certain conifers after wounding, consists, in general, of a group of volatile terpenes, and certain solid acids. Another "resin", colophony, or rosin, consists almost entirely of certain solid, acidic substances while the extreme complexity of another class of so-called "resins", secreted in the inner, woody portion of conifers, is only now beginning to be realized.

Even the limited knowledge that actually exists of the complicated phytochemical changes occurring in the cell-wall and the cell proper, of growing plants, is sufficient to warrant

the assumption that a large variety of chemical substances must be present in such tissues, and quite a number of these have already been isolated. It may be said without hesitation, that there is but one efficient method known, by which such substances can be removed from the woody tissues for examination, and that is by use of a suitable solvent, usually of the organic class. It is evident that selective removal of any one group of substances in this way, is an extremely difficult matter in those cases where various materials present may have almost identical properties as regards solubility in any one of these solvents that could be employed. Consequently, it is to be expected that when woody tissues are extracted with organic solvents, the resinous product will be nothing but a very complicated mixture of a large number of substances; it will also be found that a not inconsiderable portion of this mixture is made up of fatty materials.

The presence of considerable amounts of fat, in many kinds of seeds is, of course, well known,- for instance, flax seed, soya-bean, etc.; certain young plants also have a high content of fat. The existence of relatively large percentages of fats in the woody portions of aged trees, however, has never been fully appreciated until quite recently. This fatty substance plays a most important role in the growth of the tree. Czapek [1] considers the protoplasmic membrane of the cell is a compound colloidal system consisting of emulsified fats suspended in a hydrosol. It is generally considered that these fats constitute a reserve food supply for the growing plant. Czapek [2] describes the metabolism in plants as functioning through a mechanism of precipitation,

whereby substances to be stored in reserve, such as starch, fat and proteins, are deposited as insoluble solids, always on hand for use and ready to be dissolved as soon as required in the life process. Other substances which are formed at the same time, and of a useless and poisonous nature, are withdrawn from the sphere of the reactions normally taking place in the living protoplasm; oxalic acid, resinous substances and essential oils, are mentioned by Czapek as examples of the latter class.

It has been found that the average melting temperature of fats in seeds and in plants, is remarkably adapted to the surroundings, as regards climate.[3] Also, there has been found to exist a certain balance between the proportion of carbohydrates stored, and the content of fat. The latter seems to be at its maximum during the winter months according to the evidence obtained by phytochemists,[4] due to a conversion of starchy material into fats. Examination of various stems shows that, in the xylem, or woody part, there is a gradual change, during the winter, of the starch grains into tiny oil droplets. The formation of much liquid fat (olein,etc.,) results from this process. Under the microscope, the progress of this change can be followed by simple application of the iodine test for starch, and the reaction of fats with a reagent such asosmic acid.[5]

The above seems to be particularly true as regards the conifers. In certain other trees, including same hard-woods, Fischer [6] reported that very little change occurs in the carbohydrate content during the winter, and that these species show only a slight tendency towards the storage of fats.

It might be anticipated that conifers indigenous to the more northerly latitudes, would, from ecological considerations of their environment, show a higher degree of fat production than other types. This seems to be the case generally, from data gathered so far. [7] A preponderance of fat has been noted in the sapwood, where the above changes are still taking place, while the mature heart-wood shows a higher secretion of resin. [8] There appears to be no definite distinction to be made yet, however, since Wahlberg [9] found, in pines and spruces, that there was a very irregular distribution of resins and fats from layer to layer in the tree, and that there was no regular variation with height. The distribution of these substances around the circumference was also found to show no regularity. Mayr [10] had previously found that there was, in general, a concentration of resinous and fatty material on the south side of each tree.

While the study of natural fats, in general, constitutes one of the most highly developed fields of chemical research to-day, it cannot be said that this is true as regards the fats known to exist in various woods. In fact, the subject has received practically no attention heretofore, and only a few scattered observations were recorded up to comparatively few years ago. Hawley & Wise[11] mention two instances of earlier work: Metzger[12] examined the oil in the heart and sap-wood of oak, and found it to be a mixture of olein, palmitin and stearin. Weichman[13] reported a semi-drying oil from basswood, but did not define it further.

Investigation in this field was neglected no doubt for very good reasons: it did not seem feasible to make use of such oils commercially, in the quantities available from treating large amounts of the wood; and, in addition, extremely small proportionspresent in the wood cells, and the admixture with various other extraneous components, probably rendered very difficult the isolation of the fats in sufficient quantities for investigation. It may not be surprising, then, that by far the greater part of the knowledge accumulated to date concerning these wood-fats, has been gathered from a few examinations that have been carried on certain by-products of industries using such woods, these by-products having found important commercial applications within the last few years. On account of the vigorous treatment to which the fats have been subjected in the manufacturing processes, it is not to be expected that the results of the examinations would indicate accurately the nature of these fats in the original wood; nevertheless, they have afforded some interesting and valuable data on a subject which has otherwise been neglected.

Since about 1905, there have been a few investigations conducted on a material called "Liquid Rosin" (Tallöl, in Sweden). This is also referred to as "Sulfate Resin", and usually consists of the material obtained by drawing off the upper layer after acidifying Sulfate pulp liquors, or distilling the product. It is a mixture of acidic substances derived from resins and fats in the wood, with some of the

terpenic substances as well. A dark oil ("pine oil") can also be distilled from the mixture. Another sort of "pine oil" is obtained by destructive distillation of pine stumps and other waste; this generally is the product called "pine oil" in America. The recovery of the oily by-products from the spent liquors of the alkaline pulping processes has not been developed in America as it has been in Europe. These materials have been found to have a very large number of commercial uses. The terpenic substances and the resin acids (rosin) available from this source have been reviewed thoroughly by certain authors,[14,15,16], and the fatty products that have been identified so far will be referred to below.

A brief examination of Swedish "pine oil" (the by-product or distillation by-products from sulfate pulp waste liquors) was made in 1905, by E. Larsson,[17] followed by Fabrion[18], in 1909; these were only of a superficial nature. In 1911 - 1913, Bergstrom[19] carried out the first investigation of interest; he established that the acidic constituents were made up of approximately equal proportions of rosin acids and the fatty acids. Some neutral substances were also present, including terpenes, but these were not investigated The fatty acids consisted of palmitic acid, in small amount, and there were also indications of the presence of unsaturated acids of the oleic series. When superheated, considerable sebacic acid resulted. A phytosterol, m.p. 133.5^{0}, was also present in small quantity in the soapy mixture, but not in the distilled pine oil.

A black residue in the alkaline mixture, when separated and acidified, was found to yield lignin. The heartwood was found to be rich in rosin, while the sapwood was high in fats. The fat content was found to be highest in the winter.

Sandquist[20] found that "pine oil" from different mills varied widely in composition, and published considerable data concerning the relative amounts of various groups present in it. He obtained much stearic acid upon hydrogenation of the fats, so assumes the presence of the oleic group. He was unable to find any palmitic acid. He mentions the presence of a substance which he considers to be a dibasic acid, of high molecular weight, probably over 800, and containing but little oxygen. It melts at 72-73°. Sandquist considers this to be a lactonic acid, for reasons which he does not discuss. He also obtained a substance of the nature of the phytosterols, and melting at 136-137°. Certain other substances were noted also, in the crude oil.

Sandquist[20], as well as von Euler[21] have tried to devise a method for separating on a commercial scale the fats from the rosin, etc., in the crude oil. They were not successful in this.

Dittmer[22] investigated Swedish "talldl", obtaining certain fractions containing chiefly resin acids, fatty acids, oxidation products, and unsaponifiable. The latter contained a phytosterol, m.p. 136°, and some unidentified hydrocarbons resembling rosin oils. The Iodine Number of the fatty portion indicated partial polymerization. Hydrogenation yielded only stearic acid, while no saturated acids could be detected in the original oil. The

presence of oleic, linolic and linolenic acids was established by means of their oxidation and bromination products.

E. Pyhala[23] conducted a similar investigation, and in the particular oil examined, he found evidence of a still greater variety of fats. The acids which he claims to have identified were oleic, linolic, linolenic, ricinoleic; he stated that much smaller amounts of erucic, brassidic and palmitic were also present. Sugars were detected in the oil. Rosin and other substances were also present.

The distillation product from Swedish Tallöl was investigated by M. de Keghel[24] recently, who found resins, fatty acids, and some unsaponifiable matter present. The fatty acids were examined further, and found to contain mostly liquid acids, (oleic and linolenic) and some lauric, myristic and palmitic acids. A phytosterol was also found. The "unsaponifiable" consisted of "rosin oil" mainly, with a small amount of soft, waxy substance. Hydrogenation yielded a solid acid, m.p.67-68°, probably stearic acid.

It may be pointed out that the fatty acids from formic to myristic, which may be present in such by-product materials, are in all probability only fission products, formed during the cooking process from higher, unsaturated acids originally present. Palmitic acid also can result from oleic acid.[25] (The presence of lower fatty acids of the above class was noted during permanganate oxidation of liquid fats, in the present research.)

Hasselstroem[26] devoted his attention entirely to the study of the fatty acids in the pine oil of Swedish origin. The ethyl esters were distilled at 11 m.m. pressure, and fractionated. The fractions were saponified, and the acids examined. Palmitic acid was found in large quantities. Oleic and linolenic acids were also identified; the latter one was present in very small amounts. Evidence of another acid, unsaturated, and boiling at 165-170° at 11 m.m., was also found, but its amount was less than 1% of the whole. A solid acid, not very soluble in methyl alcohol, may be identical with the high molecular lactonic acid reported by Sandquist.[20] Hasselstroem thinks that the palmitic acid present may be due to some chemical change during the process of cooking; other authors could not find it in their working material. He draws attention to the fact that it has been shown that oleic acid subjected to caustic potash fusion yields palmitic acid almost quantitatively, so that its presence here may be due to the alkaline cooking treatment at high temperature. He also considers that the solid acid reported by Sandquist[20] and still unidentified, may have originated during the many distillations involved. The presence of linolic acid was merely assumed from the fact that capric acid was among the fission products of oxidation.

As regards the preceding data, it must be pointed out that a mixture of spruce and pine is generally employed in kraft and soda cooks in Sweden and Finland so that the above results cannot in consequence be applied specifically to either class of wood.

Hasselstroem mentioned that, to the best of his knowledge, the fatty materials described above came from the black liquors of various sulfate cooks made with a mixture of approximately 75% spruce, and 25% pine. The raw material was obtained from various pulp mills in Finland.

Since pine is generally rich in fats, while spruce is not, the above data can probably be applied for the most part to the pine used (pinus Sylvestris), but it is not necessarily specific. The cork cells of the bark, forming the highly suberized phellem, are no doubt rich in fatty substances. A certain amount of bark always finds its way into any kraft (sulfate) cook, and this probably adds to the complexity of the fatty by-products, which thus do not represent the condition of the fats in the inner woody tissues; these latter tissues alone are made use of in other pulping processes (sulfite, groundwood, etc.). It is evident, however, that the above investigations have been of great value in helping to establish the nature of these fats.

Various investigations[27] have been carried out on the fats and waxes of smaller plants of industrial importance, such as cotton, flax, etc. The seeds of certain members of the pinus family have been examined in a cursory way, as regards their fat content. The seed-kernels of P. Gerardiana[28] have been found to contain a considerable percentage of fats, mostly olein and linolein, with only about 6% of saturated acids. The kernels of P. Pinea[29] were found to contain acids consisting of 95% oleic, with only 5% of stearic. The fats contained in the seeds of certain Japanese conifers[30] have been found to contain high percentages

of unsaturated acidic material, not however, clearly defined. The predominance of liquid, unsaturated fats thus seems to be general in these naturally-occurring products.

The numerous isomeric forms that these unsaturated acids can assume obviously add to the difficulties involved in their investigation. Several such isomers are very well known.[31] The complexity involved can be appreciated to some extent from recent work of Boeseken and co-workers,[32] who have been able to prepare several of the isomeric forms of linoleic and related acids, from natural sources. The presence of both α and β forms of linolenic acid was found in oils from the seeds of the "Abietinea" group, including pine and spruce, by Eibner and Reitter[33]; these oils belong to the linseed oil class. The presence of a large number of mixed glycerides of these isomers was also indicated.

The only examinations of any consequence carried out on the fats of woody stems of conifers in their original state, appear to have been made by Schwalbe,[34] Schulz,[35] and Sieber.[36] These examinations were not by any means of an extensive character but were sufficient to show that the fats present were largely composed of unsaturated glycerides, that is, olein and its two higher homologues, the latter being present only in small amounts. The wood in question was presumably Pinus Sylvestris, extensively used in the European pulp industry. Their investigations were limited on account of the small amounts available.

The nature of the changes occurring in these "drying" and

"semi-drying" oils, through autoxidation and polymerization, forms an extensive field of study in itself, as indicated by the vast literature on the subject.

The variation of resin and fat content of certain coniferous woods with age has received attention from a small group of workers and a certain amount of data is available, from which some general conclusions can be drawn. It may be said however, that the results do not always agree, and this may be due to differences in experimental conditions; in any case, the results await further confirmation before the conclusions can be regarded as facts.

In this connection, it was found by Richter[37] that with increased seasoning of wood there was a decline in the amount of ether-extractable material, and an increase in the alcohol extract, but this latter increase did not equal the loss in the former extract; hence, no regular ratio was maintained. It is generally considered that ether removes the non-oxidized resins and fats while a subsequent treatment with alcohol takes out the oxidized substances. This is not strictly true by any means.

Richter also found that the acid and saponification numbers of the extracted material increased with time, and the ester number decreased; he stated that oxidation was the cause of these changes. Schwalbe[38] also found the acid value to increase with seasoning of the wood; the resins of the old wood also showed much lower iodine values than in the case of the fresh wood, indicating oxidation or polymerization.

Mahlberg[39] conducted an extensive investigation on the variation of resin content with time, and methods of extraction. He tried various organic solvents, and found disadvantages with each. He reported that 8 to 10 hours extraction was sufficient to remove practically all resinous matter, but small amounts of substance could still be removed even after a week's treatment. He found that drying at 105° caused changes in the wood, and decreased the benzene extract without affecting the alcohol extract. He reported very little change noticeable in resin content after chips had been stored 10 days. He did not investigate the nature of the resins. He denied that resins were concentrated on the south side of the tree, as Mayr[40] had reported.

Sieber[41] quoted the following data, for wood in the form of small chips:

Number of days stored.	Solvent	Extract % of wood.	Resin % of extract.	Fat % of extract.
85	Ether	6.9	67.2	32.8
127	"	2.5	87.9	12.1

These figures are not of much value because ether removes only a part of the resins and fats, and is a poor solvent for the oxidized portion. After 85 or 127 days, the latter must be considerable. The results of Schwalbe and Schulz[42] are in accord with the above figures.

It has been found that the finer the state of division of the wood, the more rapidly these changes in solubility occur. Aschan[43] consequently considers that it is purely a matter of oxidation. Considerable data are quoted by Sieber[44] on the subject, but

no definite conclusions can be drawn yet.

The solubility of the extracted material in petroleum ether is considered a criterion of the progress of oxidation, and this has been discussed by Sieber,[45] who quoted the following:

Resins	Ratio between ether and alcohol extract	% of ether-extract soluble in pet. ether
Fresh	17.7 : 1	94.5%
Old	4.7 : 1	13.4%

These data are similar to those reported by Kohler,[46] and Nordens-Kjold.[47]

It must be noted that, also here, ether is the extraction solvent. It should be evident how necessary it is to take into account the solvent used, especially when comparing the data of different investigators. This has been the cause of confusion in such work. The matter of suitable solvents as regards quantitative determinations, has been discussed already in the present thesis. (See part I.)

The data collected by Sieber and by several other investigators, have been reviewed at length in his recent book.[48] An inspection of this shows convincingly the difficulty involved in the task of interpreting these results, confused by differences in experimental conditions, choice of solvents, etc.; Sieber quotes the conclusions arrived at by the various authors and gives a general discussion. However, it is quite apparent that in the present state of knowledge on the subject of those conifers resins, and the changes which they undergo, it is purely a matter of each individual drawing his own conclusions.

Sieber[49] has devoted a chapter to the discussion of evidence

concerning the changes occurring in the wood and the "resins" with seasoning; the most important data have been discussed already in this review.

It seems evident that there is an appreciable drop in the resin and fat content as wood becomes seasoned; but the extent of this, of course, will depend almost entirely on whether the wood has been chipped, ground to sawdust, or left in the form of logs or smaller pieces. It also will depend on whether the logs were rafted, and barked, and the length of time in the water; the time of exposure to air, the season of the year, and the average temperature of the region. These factors have to be considered in any discussion of the subject.

The changes which occur in the resin and fat substance in such wood are still comparatively unknown. Assumptions have been made by analogy with the quick-drying oils, as regards autoxidation and polymerization.[50] The presence of the terpenic substances and unsaponifiable matter has served to complicate the question as well. It is also noticeable that conclusions are very frequently based on fluctuations in the acid number, iodine number, saponification number, solubility in petroleum ether, etc., and it is a moot question just how reliable these criteria are under the circumstances. Oxidized material is invariably present here. In addition, the constants are generally referred to certain fractions, such as "ether-resins", "alcohol-resins", "petroleum ether-soluble", etc., which also places serious limitations on their actual value, and emphasizes the uncertainty which exists as regards the composition of the crude products, and the changes which they undergo.

Methods used in the present investigation.

The fatty constituents of the resins examined in the present investigation were found to exist, as already mentioned (part 1) partly in the form of free fatty acids and partly in combined state, as glycerides or other esters. A systematic plan was developed for the investigation of each of these two groups, since it was found possible to isolate these separately.

(a) Procedure for examination of the glycerides and esters.

The portion of the resins containing the glycerides, esters, unsaponifiable matter, etc., when saponified (part 1), yielded a mixture of acids. This mixture was separated by either of the methods described in part I and found to consist only of fatty acids, no resin acids being present. The ethyl esters were either distilled fractionally, and the products examined by method (b) below, or the acids were treated as follows:

Any volatile acids present were removed by distillation with boiling water, and identified in the distillate. The non-volatile acids were then separated, by means of the solubility of their lead salts in ether or alcohol, into (1) "solid", and (2) "liquid", fatty acids. The "solid" acids were divided into "pure fatty acids" and "oxidized or polymerized fatty acids" by petroleum ether extraction. The pure solid acids were recrystallized fractionally, while the liquid acids were identified by their bromination derivatives and permanganate oxidation products. Constants such as iodine number, saponification number, refractive index, etc., were also noted.

In some cases, glycerol was estimated by evaporating the aqueous residues from the initial saponification, and extracting the residue with anhydrous acetone, to separate the glycerol from the salts.

(b) Procedure for examination of fatty acids present in free state.

The "free fatty acids" present in the resins were always converted into the ethyl esters, in the process of separating them from the resin acids.(see part I) This mixture of ethyl esters was subjected to fractional distillation, at extremely low pressures, using a Langmuir mercury-vapour pump. Three definite fractions were usually obtained, each one probably a mixture of homologues. The various constants of these ester fractions were determined. The acids recovered from the "saponification-number" experiments thus comprised three groups, as well as water-soluble acids which might be present in the aqueous liquors, and which were examined qualitatively. The three acid fractions were investigated separately; their constants determined, distillation products examined, and their bromine and oxidation derivatives prepared. The last fraction was separated into solid and liquid acids by means of the lead salts, and these products examined.

In order to simplify as much as possible the actual details of the above work, which are given in the experimental part that follows, the description has been taken up in two separate parts, (1) Specific methods for identification, and (2) General methods of investigation. It has been thought advisable to give first of all, (section 1) full details of particular procedures made use of throughout the general investigation,

and then merely refer to these in the description of the systematic examination of the fats (section 2), with a report of analytical results, properties, etc., where necessary.

At the end of the experimental part, the complete analytical results have been summarised in a table, for comparison. (See Table 18, p. 57).

THE FATTY CONSTITUENTS OF THE CRUDE RESIN PRODUCT

DISCUSSION OF EXPERIMENTAL RESULTS

The products identified embrace (a) volatile acids (butyric), (b) liquid unsaturated acids (oleic, linolic and linolenic), and (c) solid acids (presumably saturated, - stearic, and a lactonic(?) acid).

Volatile acids:

The procedure used in the present investigation, namely, distillation with boiling water, is based on the standard method[51] for determination of total volatile acids. The identification of butyric acid, as the only volatile constituent, was made only by a qualitative test[52], on account of the small amount obtained. The distillation method employed probably involves a certain amount of oxidation of the non-volatile products.

Separation of solid and liquid acids:

The separation of the solid and liquid acids by means of the relative solubilities of the lead salts in ether[53], is more difficult to carry out than by the lead salt-alcohol method[54]. The latter is undoubtedly the more convenient to use, and is said to be just as accurate[55], although the separation by either method is probably not strictly quantitative. The "solid acid" portion was always accompanied by some of the liquid acids, and vice versa, in the case of the products in the present investigation.

Identification of "liquid acids" by bromination:

The products were always brominated in the form of the acids, and not as the esters; the brominated acid products are easier to identify and have been better studied.

The new method of Steele and Washburn[56], for determination

of hexabromides (of linolenic acid) was found to be better than the older method of Hübner[57], although it is longer, and requires very careful preparation of the reagents. Both methods were intended for estimation of the hexabromides only. The isolation of the lower bromination products (dibromide and tetrabromide) in fairly pure state, was found to be very difficult and tedious. There is no satisfactory method for the complete separation of the various bromination products from each other. The solubilities in petroleum ether, etc., as tabulated by Lewkowitsch[58] are only relative, and a lengthy series of recrystallizations is possible only when a fairly large amount of material is available.

The hexabromide fractions obtained from the fats of Jack Pine melted at 172-173°, 177-178°, and 180-183°.

The tetrabromide fractions had very indefinite melting points and showed signs of decomposition between 95° and 116°. The dibromide was a dark brown, oily product. Linolic acid is said to yield, in some cases, an oily tetrabromide[59] in addition to the crystalline one.

The melting points for linolenic hexabromide, and linolic tetrabromide, given by some previous investigators[60], are as follows:- Hexabromides, 181-182°, 180-181° and 177° respectively.

Tetrabromides, 113-114° and 114-115°.

The hexabromides obtained in the present investigation, establish the presence of linolenic acid. The quantitative analytical results obtained indicate that this acid is present in very small amounts (less than 1% of the total fats).

The bromination products were readily soluble in chloroform, which indicates the absence of bromides of any acids higher than linolenic[61].

In addition to the evidence supplied by their bromination products, indicating the presence of oleic and linolic acids, the fact that dihydroxy-stearic acid, and sativic (tetrahydroxy stearic) acid, were obtained, by permanganate oxidation, also serves to definitely establish the presence of the two former acids. Both α-sativic (m.p.155°), and β-sativic acid (m.p.173°) were isolated. These two products are assumed to correspond with two different forms of linolic acid[62].

No liquid unsaturated acids other than the above three (oleic, linolic and linolenic), could be detected in the purified fractions obtained by distillation of the esters, or in the intermediate fractions.

It is possible to estimate the amounts of these unsaturated acids present, from (a) their bromination products, and (b) from the degree of unsaturation of the mixture of them, although there are limitations as regards the use of these methods, and the degree of accuracy to be expected. The difficulties mentioned above in connection with the isolation of the pure dibromides and tetrabromides and the losses occurringduring the process of purification, made it impossible to use this method, except with respect to the hexabromide of linolenic acid. The method of Steele and Washburn[56] gives very accurate quantitative results, when applied to the estimation of the latter acid, and was used for this purpose in the present investigation.

It is possible to determine very accurately the percentages of oleic and linolic acids, respectively, in a mixture consisting of these two acids only, if the iodine number is known. In the

present case, the percentage of linolenic acid present in some of the products was known from the hexabromide determinations, so that it was possible to estimate the relative amounts of oleic and linolic acids from the unsaturation value of the mixture of the three acids[63]. The percentages reported under "approximate composition" of the various fractions, are only approximations, because they are based on the assumption that no impurities, such as oxidation or polymerization products, are present. The mixture probably contains small, unknown amounts of such impurities.

The two acidic products found present in the fats in very small amounts (pp. 42,46,49,53,56experimental part) could not be identified definitely. One of these is a colourless liquid (b.p. 55-60° @ 0.005 m.m.). The formula of its ethyl ester correspondswith $C_5H_9O_2$; the acid has a pleasant, aromatic odor, and it may possibly be derived from terpenes present in the wood, although it was obtained with the fatty acids in the chemical separation.

The other product is a white solid, m.p. 70-80°; by fractional recrystallization, a main fraction was obtained, m.p. 71-72°, as well as two very small fractions, m.p. 69-72° and 68.5-69.5° respectively. The melting-point of stearic acid is reported as 69.3° usually, and other values, up to 71.5°, have been given[64]. It is possible that stearic acid is present in the fats of Jack Pine, in very small percentage. The small yield of the above products rendered further investigation difficult.

With regard to the solid product, m.p. 71-72°, the isolation

of a similar white solid substance, m.p.72-73° by Sandquist[20] has been mentioned already (page 7). He considered it to be a lactonic acid, of molecular weight above 800. Hasselstroem[26] also obtained a similar acidic product. The acid isolated in the present investigation was obtained from two solid ester fractions, which were contaminated by some adhering liquid ester; these impure ester fractions had saponification values of 100.8 and 145.5 respectively; if the acid were dibasic, as Sandquist[20] reported, these values would correspond with molecular weights of 1112 and 772 for the ester, or 1054 and 714 for the acid. These figures are of the same order as reported by Sandquist, and the substances may be identical. There was not sufficient of the pure material obtained to enable a thorough examination to be made. It is present only to the extent of 2-3% in the total crude resinous material.

It is difficult to effect a separation of the fatty constituents by fractional distillation. The boiling points of the unsaturated esters are very close to each other, and constant boiling mixtures are eventually obtained. It is also difficult to separate the "liquid" from the "solid" esters by fractionation, so that the lead-salt alcohol method of separation was used on the mixtures obtained, in some cases. Repeated redistillation of the fractions causes considerable losses in material, on account of polymerization and oxidation of the unsaturated acids, even when very low pressures and temperatures are employed. In the case of each lot of crude esters, a considerable residue of dark, tarry material, of unknown composition, always remained after distillation. It is difficult to treat these unsaturated acids without causing a change to some extent, in the original material.

EXPERIMENTAL (Section 1.)

ANALYTICAL METHODS USED FOR IDENTIFICATION OF THE FATS PRESENT IN JACK PINE RESINS.

These embrace the following:

(a) IODINE NUMBER:

Approved method of Am.Chem. Soc. Committee[65] using Wijs Iodine Chloride solution.

(b) SAPONIFICATION NUMBER:

Approved method of American Chemical Soc. Comm.[66] ; except that Alkali Blue 6B was used together with phenolphthalein, as indicator, on account of the dark colours of the saponification mixtures.

The acids were recovered for further examination in each case, by extraction with ether, washing, drying over fused sodium sulphate, and completely evaporating the solvent, under vacuum, on the water bath.

(c) ACID NUMBER:

Official method, A.O.S.[67]; the acids were recovered as above.

(d) REFRACTIVE INDEX:

Abbé refractometer was employed, keeping each specific temperature constant for 10 minutes before taking final reading.

(e) DETERMINATION OF GLYCEROL:

The glycerol split off from the glycerides was contained in the aqueous residues from the saponification

of the mixture of glycerides, esters, and unsaponifiable matter (p.22,part I) and in certain aqueous washings and distillates, (see p.22,part I). The total aqueous extract was evaporated on a water-bath, under suction (water-pump), and the residue, containing considerable inorganic salts as well as the glycerol, dried thoroughly in the vacuum oven at 60°. This dried cake was powdered in a mortar, and extracted with anhydrous acetone[68] in a Soxhlet, for 10-12 hours. The acetone extract was evaporated down, and dried to constant weight at 60° in vacuo. This gave the weight of crude glycerol.

(This method, as well as all others for determining glycerol in aqueous extracts, is inaccurate on account of the volatility of the glycerol during evaporation of the extract. The results always give only an approximation of the amount of glycerol present.[69]

The presence of glycerol in the above extract can be shown by the acrolein[70] and the oxalic acid tests.[71]

(f) DETERMINATION OF VOLATILE FATTY ACIDS:

A weighed quantity of the fatty acid mixture, was mixed in a distilling flask, with about six times its weight of boiling water, and distilled over a small flame. The distillate after passage through a small filter was collected in a flask. Distillation was continued until neutral to phenol-phthalein. No solid acids were found on the filter, and the small amount of oil carried over was returned to the main residue. Water had to be added at intervals during the distillation, to replace the amount passing over. The total aqueous distillate

was then titrated with standard alkali, acidified with hydrochloric acid, extracted with ether, the ether removed. The residue was taken up with a little hot water, and baryta water run in slowly until neutral. After cooling, the insoluble barium salts were filtered off. The smallness of the amounts of these volatile acids obtainable prevented other than qualitative tests for the acids being used. For this purpose, the soluble and insoluble barium salts were decomposed separately by hydrochloric acid, neutralized by the potash, and each of the two solutions evaporated down until approximately 1.5% strength. These were then examined for fatty acids, including formic to capric, by the qualitative method of Dyer,[52] based on the relative solubilities of the iron and copper salts of these acids in water and organic solvents.

(g) SEPARATION OF THE SOLID FROM THE LIQUID FATTY ACIDS: (two methods were used.)

METHOD 1: LEAD SALT - ETHER METHOD: (Muter & de Koningh,[53] this method was used in part).

The weighed fatty acid mixture was dissolved in ten times its weight of alcohol, and alcoholic potash added, until faintly alkaline. This solution was poured slowly, with constant stirring, into a 1.5% boiling aqueous lead acetate solution, and the resulting mixture cooled rapidly to 0° with stirring. The clear liquid was removed by decantation, and the residue washed several times with boiling water. Ether was added to the residue (25 c.c. ether for each gram of original sample), and product well stirred. After standing for one hour, the mixture was filtered,

and the residue again treated with ether, using about one fifth of the previous amount. This was allowed to stand overnight, and then filtered.

The ethereal extracts, containing the soluble lead salts, were united, and the ether removed by evaporation. The residue was acidified by alcoholic hydrochloric acid,(90 pts.alc.,10 pts. HCl) and the liquid fatty acids recovered by ether extraction. The ethereal extract was washed well with water, dried over fused sodium sulphate, the ether removed, and the residue dried on the water-pump until constant weight.

The ether-insoluble residue, containing the insoluble lead salts of the solid acids, was decomposed by the addition of alcoholic hydrochloric acid, and the solid fatty acids recovered in the same way.

(g) METHOD 2: LEAD SALT - ALCOHOL METHOD:[54]

LEAD ACETATE SOLUTION:

10 grams of normal lead acetate was dissolved in 200 c.c. boiling 95% alcohol;

SOLUTION OF FATTY ACIDS:

About 25 grams (weighed accurately) of the acids was dissolved in 200 c.c. of 95% alcohol.

These two solutions,(or other solutions containing equivalent quantities), were heated to the boiling-point and then the one containing the acids added slowly to the lead acetate solution, with constant stirring. Sometimes a brown precipitate of solid fatty acid salts (A) came down while hot, and this was removed by filtration. The clear solution was allowed to stand overnight

in the refrigerator, then filtered from the solid fatty acid salts (A) and the latter washed with cold alcohol, the washings being added to the main portion of the filtrate.

The combined alcoholic filtrates were evaporated under reduced pressure, and after nearly all the alcohol had been removed, the residue was decomposed by the addition of alcoholic HCl, (10%). The liquid acids (B) seperating out were taken up in ether, and the extract washed with water , and dried over fused sodium sulphate. Evaporation and drying in vacuo to constant weight, yielded a brown, oily residue of liquid fatty acids.(B)

Similarly, by decomposing the insoluble lead salts of the solid fatty acids (A) and extracting with ether, etc., the solid fatty acids were obtained.

(h) SEPARATION OF OXIDIZED FATTY ACIDS FROM THE PURE SOLID ACIDS:

The fraction containing the "solid" fatty acids (Section (g) was carefully dried $\sim 60^{\circ}$, in vacuo, mixed with a large quantity of sand, and ground in a morter. This mass was extracted in a Soxhlet, with petroleum ether (b.p.30-50°) until no more was dissolved. Evaporation of the petroleum ether extract yielded the pure solid acids that were present, and these were subjected to fractional crystallization from alcohol.

The insoluble residue after treatment with petroleum ether, consists of oxidized and polymerized fatty substances of acidic nature![72] These were not investigated further.

(1) BROMINATION OF THE PURIFIED "LIQUID" FATTY ACIDS:

Various liquid acid fractions, obtained by saponification of purified low-pressure distillates of the mixture of ethyl esters, were subjected to bromination, in order to obtain solid derivatives for purposes of identification. Two main methods of bromination were tried.

METHOD 1: BROMINATION IN ETHER SOLUTION (based on Hibner[57]):-

The mixed fatty acids were dissolved in sufficient anhydrous ether to give a 10% solution, and this cooled to - 5° C. (About 1 c.c. of bromine is considered sufficient for each 2 gms. of acids used.) Half of the required quantity of bromine was added, from a capillary pipette, during a period of about 20 minutes. The remainder was then added during a further 10 minutes period. (The actual operating time is considered very important by Hibner). The temperature was kept at about 5° C. throughout, by using an ice-salt mixture. After bromination, the flask was allowed to stand at the same temperature, in the dark, for two hours. Any solid bromides separating were removed by decantation, centrifuging where necessary, - and washed with five portions (5 C.C. each) of previously dried and cooled ether. The precipitate recovered by centrifuging was then dried at 70° in vacuo, for four hours, and the hexabromides weighed.

The ethereal solution, together with the washings from the hexabromide were shaken in a separatory funnel with a little thiosulfate solution, to remove excess bromine, washed with water, and dried over calcium chloride. The filtered solution was evaporated, and the residue dissolved in boiling petroleum ether (b.p. 30-50°).

Oleic dibromide is very soluble in petroleum ether, linolic tetrabromide is less soluble, while linolenic hexabromide is only sparingly soluble in this solvent. The product separating on cooling the petroleum ether solution, as well as the material remaining in the mother liquor, was repeatedly fractionated by means of fresh petroleum ether, the products being examined for melting point. This was continued until comparatively pure fractions of the three kinds of bromides were obtained.

METHOD 2: BROMINATION IN CHLOROFORM SOLUTION:

Method, Steele and Meshburn.[56]

REAGENTS:

(a)Chloroform: washed well with water to remove all alcohol, dried over calcium chloride, and then redistilled. To the distillate is added 3 c.c. of absolute alcohol for every 100 c.c. of purified chloroform, and the solution kept in a stoppered brown bottle.

(b)Bromine: One volume of C.P. Bromine is added to two volumes of the above chloroform solution, a new solution being prepared after 24 hours.

(c)Ether: This is washed three times with ice-cold water, dried over calcium chloride, then distilled from metallic sodium.

BROMINATION PROCEDURE:

A 5% solution of the fatty acids was prepared using the chloroform prepared as above. The solution, in a centrifuging tube, of proper size, was kept at -5° C, and the bromine solution run in from a burette at the rate of one or two drops per second, until a permanent orange colour appeared. Then

0.5 c.c. additional of bromine solution was added rapidly, the solution shaken well, and allowed to stand at -5° for ten minutes, in the dark. Amylene was added drop by drop, with shaking, until thedeep colour was discharged, thus taking up the excess bromine.

The solvent was evaporated off completely on the water-pump, heating finally at 50-60° for 15 minutes. The oily residue was well rotated during this process, and then allowed to dry at 60° under reduced pressure for a further 15 minutes.

The tube was now placed in an ice-bath, and 40 c.c. of the specially purified ether, cooled, 0°C., was run in, and the mixture well stirred with a rod. After cooling again for two minutes, the product was centrifuged at 4000 r.p.m. for 5 minutes, cooled again to 0°, and the dark brown ether solution now poured off. In this way, the dibromide and tetrabromide products were removed. The washing and centrifuging process was repeated three times, using each time 40 c.c. of purified ether, and stirring well. Finally, the hexabromide residue was spread in a thin layer inside the tube, the residual ether removed on the water-pump, and the product dried at 70° under reduced pressure, until constant weight was attained. It was purified by recrystallisation from chloroform.

Evaporation of the ether wash liquors yielded the mixed dibromides and the tetrabromides. These were separated by treatment with petroleum ether and fractional crystallisation, exactly as already described.

(j) PREPARATION OF SOLID HYDROXY DERIVATIVES OF THE PURIFIED LIQUID FATTY ACIDS:[73]

The mixture of liquid acids (weight, 2.82 grams) was neutralized by the addition of 3.5 c.c. of aqueous potash, containing 1.25 grams of KOH. The potassium salts were dissolved in 190 c.c. water, and an equal volume of 1½% aqueous solution of potassium permanganate added at 20°, with constant stirring during a period of 15 minutes. After standing for 10 minutes, sulfurous acid was added until the solution became acid, and the manganese hydroxide, etc., had dissolved. The white solid precipitated out and consisting of dihydroxystearic and sativic acids, was removed by filtration, (A).

The filtrate was neutralized with caustic potash, evaporated to one-twelfth of its original volume, and acidified with sulfuric acid. The brown flocculent mass separating, was filtered, washed with water, allowed to dry in the air, and then washed well with ether to remove lower acids formed as secondary products of the oxidation process. The product was recrystallized from alcohol and then from water, in order to isolate the two hexahydroxy acids, linusic and isolinusic, if present.

The previous solid precipitate, (A) containing dihydroxy-stearic and tetrahydroxy-stearic (Sativic) acids, was freed from unchanged liquid acids by washing several times with small portions of cold ether. The dihydroxystearic acid was then removed in large measure by extracting the precipitate (B) with considerable volumes of ether, at room temperature, until about 320 c.c. of extract had been thus obtained. This solution was

evaporated, and the residue recrystallized from alcohol several times, yielding pure dihydroxy-stearic acid.

The portion of the original precipitate (B) insoluble in the ether, was extracted repeatedly with portions of hot 80% alcohol, until no more was removed. On cooling this extract, sativic acid was obtained as a white solid, and was purified by recrystallization first from alcohol and then from water.

EXPERIMENTAL (Section 2)

INVESTIGATION OF THE FATTY CONSTITUENTS OF JACK PINE

(A) Analysis of the Fatty Glycerides and Esters from "Green" Jack Pine

(Crude product of Extractions 5, 6, 7, 8, 9, 10, Part I)

The combined lots of fatty glycerides and esters, obtained as described on p. 22, Part I, were saponified in order to obtain the free acids (method, p. 22, Part I).

The mixture of acids was then treated by the method described on p. 13, Part I, in order to obtain the (a) fatty ethyl esters, and (b) resinic acids. Under (b), only traces of resinous matter (impurities) were found, so that all the acidic constituents here, were of the fatty type.

This mixture of fatty ethyl esters was subjected to fractional distillation, by the method already mentioned (Part II, p. 44). A Langmuir mercury vacuum pump was used. A current of carbon dioxide was passed through the mixture, by means of an extremely fine capillary.

The results are tabulated below.

TABLE 7

Distillation of Ethyl Esters of Glycerides of "Green" Jack Pine

I	II	III	IV	V	VI	VII
No. of fraction	Pressure - m.m. mercury	B.P.	Wt. of product grams	Yield %	Description	Temp. of constant boiling mixture of carefully refractionated material
1	0.001-0.03	60-89°	0.3	1.5	mobile yellow liq., pleasant odour	47-49° at 0.004 mm
2	"	142-150°	17.0	84.6	viscous yellow liq. fatty odour	135-137° " "
3	"	155-188°	0.5	2.5	partly solid, yellow; fatty odour	156-158° " "
Tarry residue			1.9			
Volatile decomp. products (by difference)			0.4	11.4	decomposed at 220°	
Total			20.1	100%		

The data shown in column VII of the previous table represent the temperatures of the constant-boiling mixtures obtained by repeated careful distillation of Fractions 1, 2 and 3, and of the intermediate fractions derived from them

Since the yields of Fractions 1 and 3 were too small to permit of investigation, they were added at a later stage to larger quantities of identical fractions obtained from other lots of the fats; their properties are reported on pages 42 and 44, respectively.

TABLE 8

Analysis of Constant-Boiling Fraction II (B.P. 135-137°)

		Details
Refractive Index	n_D^{10} = 1.4626 n_D^{20} = 1.4587 n_D^{30} = 1.4544	
Iodine Number	130.3	0.3626 gm. sample = 38.19 cc. Thios. 0.4423 " " = 46.15 " " Iodine nos. 130.7, 129.9; mean 130.3 (1 cc. Thios. = 0.012435 gm. Iodine)
Saponification Number	185.6	0.8325 gm. = 5.32 cc. HCl (0.5179N) 1.1495 " = 13.47 " " " Sapon. nos. 185.6, 185.5
Bromination of the acids:- Fibner method (see p. 29)		Products obtained: (a) Traces only of linolenic hexabromide, m.p. 172-173° (b) 0.04 gm. impure linolic tetrabromide, melting between 95° and 115° (c) Considerable amount of oily dibromide (or tetrabromide)
Approximate Composition (Fraction 2) Method 63	41.7% Ethyl Oleate 58.3% Ethyl Linolate Traces of Ethyl Linolenate	Calculation: x = % oleic ester in frac. II y = % linolic ester in frac II Iodine nos. 81.8, 165.0 respectively Linolenic acid present only in traces, as shown by presence of only traces of the hexabromide hence x + y = 100% (approx.) and 81.8x/100 + 165y/100 = 130.3 (Iodine % absorp. of mixture) from which x = 41.7% oleic ester y = 58.3% linolic "

TABLE 8 (continued)

Approximate Composition (continued) (Fraction 2)

This fraction, (II), comprises 84.6% of the total fatty esters (see Table 7; using this as a factor, then:

Ethyl Oleate = 35.3% approx. of total esters

Ethyl Linolate = 49.3% " "

Ethyl Linolenate = Traces only, of total esters

Oxidation by Potassium Permanganate

This was carried out on a small fraction, b.p. 130-140° at 0.01 mm. which could not be resolved further.

Saponification of the Ester Fraction

3.1000 gm. ester, equiv. to 20.63 cc. HCl (0.5179N); Sapon. No. = 195.0.

The acids recovered from this saponification (see method, p. 24), weight = 2.82 grams, were oxidized by alkaline permanganate (method, p. 32).

Products: Dihydroxystearic acid[74], m.p.128.5°; yield, 0.235 gm.
α-Sativic acid[62] (cryst. from water), m.p.155°
β-Sativic acid[63] (" " alcohol), m.p.173° yield 0.270 gm

The yields of products by this method are never quantitative (Lewkowitsch[75])

(B) Analysis of the "Free" Fatty Acids from "Green" Jack Pine

(Crude product of Extractions 5, 6, 7, 8, 9, 10, Part I)

The "free" fatty acids present in the crude extraction product were obtained in the form of their ethyl esters, in the separation (esterification) processes described on p.13 , Part I.

The mixture of ethyl esters was subjected to fractional distillation using the methods already mentioned (p.44).

The results are tabulated in Table 9 on the following page.

TABLE 9

Distillation of Ethyl Esters of "Free" Fatty Acids of "Green" Jack Pine

I	II	III	IV	V	VI	VII
No. of fraction	Pressure	B.P.	of product grams	Yield %	Description	Temp. of constant boiling mixture of carefully refractionated material
1	0.007-0.03 mm.	60-84°	2.10	5.4	mobile yellow liq. pleasant odour,	47-49° at 0.01 mm.
2	"	140-155°	18.20	46.9	viscous yellow liq., fatty odour,	135-138° " "
3	"	159-185°	9.60	24.6	partly solid; yellow, fatty odour,	156-160° " "
Tarry residue			7.90	20.3	(Decomposed at 217°)	
Vol. decomp. product (by difference)			1.10	2.8		
Total wt. used			38.90	100.0%		

Over page

The data shown in column VII represent the temperatures of the constant-boiling mixtures obtained by repeated careful distillation of Fractions 1, 2, and 3, and of the intermediate fractions derived from them.

The analysis of the above Fractions 1, 2, and 3 follows:

TABLE 10

Examination of Ester Fraction 1

		Details
Refractive Index	n_D^{20} = 1.4931	
	n_D^{30} = 1.4885	
Boiling Point		47-49° at 0.01 mm.; 180° at 1 atm., with decomposition
Iodine Number	30.3	0.1326 gm. equiv. to 3.21 cc. Thios (0.01253 gm. iodine) per cc. (Insufficient material for a duplicate analysis)
Saponification Number	182.0	0.2794 gm. equiv. to 1.75 cc. HCl (0.5179 N) (Insufficient material for a duplicate analysis)
Elementary Analysis	C = 57.31%	2.721 mg. spl. gave 5.712 mg. CO_2 = 57.25% C
	H = 9.34%	2.721 mg. spl. gave 2.306 mg. H_2O = 9.48% H
	O = 33.35%	3.550 mg. spl. gave 7.468 mg. CO_2 = 57.37% C
		3.550 mg. spl. gave 2.916 mg. H_2O = 9.19% H.
		Corresponds with $C_5H_9O_2$
Acids liberated on saponification (see method, p.24)		After three distillations, yielded a colourless, mobile liquid, b.p.55-60° at 0.005 mm. possessing a pleasant, ethereal odour. Polymerized when heated in air. Quantity of purified product too small for further investigation.

TABLE 11

Examination of Ester Fraction 2

		Details
Boiling-point	135-138°	at 0.01 mm.; easily decomposed by heating in air
Refractive Index	$n_D^{10} = 1.4702$ $n_D^{20} = 1.4655$ $n_D^{30} = 1.4616$	
Iodine Number	117.5	0.4267 gm. equiv. to 40.26 cc. Thios. (0.012435 gm. Iodine per cc.); 0.2530 gm. equiv. to 23.93 cc. Thios. Iodine Nos. = 117.3, 117.7; mean = 117.5
Saponification Number	173.5	1.2036 gm. equiv. to 7.17 cc. HCl (0.5179 N); 1.0522 gm. equiv. to 6.29 cc. HCl Saponif. nos. 173.3, 173.8; mean = 173.5
Bromination of the acids (Eibner's method, p. 29)		Products obtained: (a) Oily dibromide of oleic acid; (b) A white, solid fraction, m.p. 65-68°, (possibly a bromine-substitution product); (c) A tetrabromide fraction, m.p. 95-116°; (d) Two hexabromide fractions, m.p. 172-173° and 180-183°, respectively; present in traces only.
Approximate Composition (Ester Fraction 2)	57.1% Ethyl Oleate 42.9% Ethyl Linolate Traces of Ethyl Linolenate	
(method, see p37)		Calculated on the total fatty ethyl esters (see p40), the above represent: 26.8% ethyl oleate 20.2% ethyl linolate

TABLE 12

Examination of Fraction 3

		Details
Boiling Point	156-160°	At 0.01 mm.; decomposes when heated in air.
Melting Point	31-33°	
Refractive Index	$n_D^{30} = 1.5410$ $n_D^{40} = 1.5335$ $n_D^{50} = 1.5269$ $n_D^{70} = 1.5220$	
Iodine Number	120.5	0.1462 gm. equiv. to 14.09 cc. Thios. (0.01253 gm. Iodine per cc.) 0.1550 gm. equiv. to 14.88 cc. Thios. Iodine nos. 120.7, 120.2; mean 120.5
Saponification Number	100.8	3.1124 gm. equiv. to 10.80 cc. HCl (0.5179 N)

Acids liberated in Saponification

Recovered by method on p. 24
Wt. of acids = 2.94 gms. = 94.4% yield
(Further separated as described on the following page)

Isolation of the Liquid and Fatty Acids from Fraction 3

The acids (wt., 2.94 gms.) were separated by the lead salt-ether method (see p. 26), and recovered as

(a) "solid" fatty acids; wt. 0.21 gm. = 7.15%;

(b) "liquid" fatty acids, =92.85% (by difference)

The "liquid" acids (b) were brominated (method p. 29), and yielded mostly the oily dibromide of oleic acid, together with small fractions of tetrabromide and hexabromide, the same as had been obtained already from Fraction 2 (see p. 43).

A small fraction very similar to Fraction 3, but boiling at 145-190° (0.01 mm.) was also examined;

Saponification number: 145.5;

Wt. of acids recovered: 0.46 gm.;

"Solid" acids, wt., 0.10 gm. were obtained by recrystallization from alcohol;

Total weight of acids used: (2.94 + 0.46) = 3.40 gm.

" " solid acid recovered (0.21+ 0.10) = 0.31 gm.

= 9.1% (approx)

of Ester Fraction 3;

Fraction 3 = 24.6% of total esters mixture (see p. 40);

Hence, this solid acid = 2.24% (approx.) of the original mixture.

Purification of the Solid Acid Product from Fraction 3

The substance, after one recrystallization from alcohol, and drying at 60° in vacuo, was pure white, of waxy consistency,

and without crystalline form; m.p., 70-80°

A second recrystallization yielded a product of m.p. 71-72°, as well as two fractions from the mother liquors, m.p. 69-72° and 68.5-69.5° respectively.

These purified fractions were obtained in such extremely small quantities that further examination was not possible.

Intermediate Fractions of the Ethyl Esters

In addition to the three main fractions (constant-boiling mixtures) just described (see Tables 10, 11, & 12.) certain smaller intermediate fractions were obtained, having the following properties:-

TABLE 13

Fraction			Iodine No.	Saponif. No.	Bromides
B.p.60-120°	(0.01 mm.)	yellow liquid	81.7	234	Only oily dibromides
" 130-135		" "	89.7	127	
" 130-150°	"	" "	-	141.5	Oily dibromide and traces of hexa-bromides.
" 140-165°		very viscous yellow liquid	-	207.6	All three bromides

The values for the constants indicate that these fractions are mixtures of the main fractions; the absence of other unsaturated acids than oleic, linolic and linolenic is to be inferred from the fact that only the bromides of these three were obtained.

(C) Analysis of the Fatty Glycerides and Esters from "Seasoned" Jack Pine

(Crude product from Extraction 13, Part I)

The mixture of glycerides and esters (obtained as described on p.$_{22}$, Part I), was saponified in order to recover the fatty acids, the mother liquor (a) being retained for the glycerol analysis.

Since no resin acids had been found in previous examinations of this portion from other lots (see p.$_{34}$, Part III). the usual separation into (1) resin acids and (2) fatty ethyl esters (see p.$_{13}$, Part I) was omitted, in this case.

Determination of Glycerol: Glycerol was determined in the aqueous [illegible]ues from the above saponification (a) by the method described already (see p.$_{24}$, Part II)

Wt. of glycerol = 2.20 gms.; from 56.90 gms. original crude resins; = 0.39% yield.

The acids recovered from the above saponification (a) formed a dark brown, semi-liquid mass; wt. = 24.50 gms.

Analysis for Volatile Acids, (method p.25).

370 cc. aqueous distillate obtained in the distillation,

neutralized by 21.90 cc. of 0.1004 N.NaOH. When examined qualitatively for lower fatty acids (method of Dyer, p.26, Part III), negative tests were obtained for all except butyric acid, which was indicated by the following result: 2 cc. of the 1.5% solution of potassium salts of the volatile acids was treated with 1 cc. of dry ether and two drops of the ferric chloride reagent; after vigorous agitation, the ether layer was found to be clear and colourless, while an emulsion had formed in the aqueous layer which was coloured brown. Insoluble salt was deposited at the junction of the two layers. This test (ferric butyrate) indicates the presence of butyric acid, the other volatile acids having been shown to be absent

21.90 cc. of 0.1004 N.NaOH = 0.088 gm. NaOH

88/40 0.088 = 0.1936 " Butyric acid

On 24.50 gms. total acids = 0.79% Butyric acid.

Analyses for Non-Volatile Acids

The acids remaining after the above aqueous distillation were recovered by ether extraction, and dried to constant weight, in vacuo, at 65°.

These acids were then separated by the lead salt-alcohol method (see p.27, Part III) into (a) solid and (b) liquid fatty acids: -

Original wt. of acids used = 23.55 gms.

(a) solid acids obtained = 12.02 " = 51.0%

(b) liquid " " = 11.45 " = 48.7%

Losses (by difference) = 0.3%

100.0

(a) Examination of the Solid Acids Portion

The solid acids contained considerable oxidised material so were separated by the method described on p. 28 Part III, into

Solid (pure) acids, 4.90 gms. (= 40.8% of mixture)

Oxidised " 7.12 " (= 59.2% " ")

From the above data,

"Solid" acids in the original 23.55 gms. crude acids	= 51.0%	40.8%	= 20.8%
"Oxidised" acids ditto	= 51.0%	59.2%	= 30.2%

The "solid" acid fraction was contaminated by some of the liquid acids, however. (The separation by the lead salts is never complete.) The mixture was therefore dissolved in alcohol, and a small yield of yellow solid obtained. This was allowed to drain on porous tile, and recrystallised several times from alcohol. The final product was pure white, of waxy consistency, and melted at 71-72°; the small amount of it so obtained was examined together with the product (m.p. 71-72°) mentioned on p. 46 Part III, with which it seemed to be identical.

(b) Examination of the "Liquid" Acids Fraction

		Details
Iodine Number	119.4	0.6988 gr. equiv. to 66.73 cc. Thios. (0.01244 gm. Iodine per cc.) 0.4654 gm. equiv. to 44.85 cc. Thios. Iodine Nos. = 118.9; 119.9. Mean 119.4

Bromination

(Steele-Washburn method, see p30)

Wt. of acids	Wt. of hexabromide m.p.177-178°	% hexabromide	% linolenic acid (calc. from wt. of hexabromide
1.0534 gm.	0.0317 gm	3.01%	1.10%
1.0268 "	0.0290 "	2.82%	1.03%
		Mean =	1.07%

From the mother liquors, obtained (method, p30) a tetrabromide fraction, m.p. 108-115°, and considerable oily dibromide.

Approximate Composition of "Liquid Acids"

69.00% Oleic Acid - in the "liquid" acids;
29.93% Linolic Acid
1.07% Linolenic Acid

This fraction represents (approximately) 48.7% of the total acids, 24.50 grams, (see p47), so that the original acids mixture contains:

(approx.) 33.6% Oleic acid,
" 14.6% Linolic acid,
" 0.5% Linolenic acid,

together with the "volatile" and "solid" acids portions

TABLE 14

Distillation of Ethyl Esters of "Free" Fatty Acids from "Seasoned" Jack Pine

I	II	III	IV	V	VI	VII
No. of fraction	Pressure	B.P.	Wt. of product grams	Yield %	Description	Redistilled
1	0.007-0.01 mm	50-65°	2.00	5.2%	Nearly colourless liq., pleasant odour	Once
2	"	125-150°	16.70	43.5	Pale yellow, viscous; fatty odour	"
3	"	150-170°	9.95	25.8	Pale yellow, partly solid, fatty odour	"
Tarry residue			8.50	22.1	Decomp. at 220°	
Vol. decomp., loss (by difference)			1.30	3.4		
			38.45 g.	100.0%		

In order to avoid losses, and the effects of heat on the products, the above fractions were not distilled further.

TABLE 15

Examination of Fraction 1

		Details
Boiling Point	50-65°	at 0.01 mm.
Refractive Index	$n_D^{20} = 1.4940$	
	$n_D^{30} = 1.4892$	
Saponification Number	51.2	0.9242 g. equiv. to 1.65 cc. HCl (0.5184 N) 0.8517 " " 1.55 " " " Saponif. Nos. = 51.9, 50.4. Mean 51.2 The acid recovered (method, p.24) was investigated with the same product from a previous lot of fats (see p.42 , Part III).

TABLE 16

Examination of Fraction 2

		Details
Boiling Point	125-150°	at 0.01 mm.
Refractive Index	$n_D^{20} = 1.4732$ $n_D^{30} = 1.4692$	
Iodine Number	127.6	0.5848 g. equiv to 59.84 cc. Thios. 0.4016 " " " 41.31 cc. " Iodine Nos. = 127.5, 128.1. Mean 127.8 (Thios. = 0.01244 gm. Iodine, per cc.)
Saponification Number	157.0	2.8156 g. equiv. to 15.30 cc. HCl (0.5184N) 3.3648 " " " 18.11 " " " Saponif. Nos.=158.0, 156.0. Mean 157.0

Bromination of Acids

(Steele-Washburn method, see p.30)

Wt. of acids	Wt. of hexabromide m.p. 177-178°	% hexabromide	% linolenic acid (calc. from wt. of hexabromide
2.82 g.	0.0352 g.	1.25%	0.46%
2.82 g.	0.0202 g.	0.72%	0.36%
		Mean =	0.36%

Also obtained a tetrabromide fraction, melting between 106-115°, and considerable oily dibromide.

TABLE 16 (continued)

Examination of Fraction 2 (cont.)

	Details
Approximate Composition of Fraction 2 (see method on p. 37, Part III)	43.1% Ethyl Oleate 54.5% " Linolate 0.36% " Linolenate
	This fraction represents about 43.5% of the total ethyl esters (see p.52, Table 14); hence:
	(approx.) 19.6% Ethyl Oleate, " 23.7% " Linolate, " 0.16% " Linolenate,
	present in the total weight of esters.

TABLE 17

Examination of Fraction 3

		Details
Boiling Point	150-170°	at 0.001-0.01 mm.
Refractive Index	$n_D^{30} = 1.5481$ $n_D^{40} = 1.5355$	
Saponification Number	103.2	1.3328 g. equiv. to 4.72 cc. HCl (0.5154N 1.2188 g. " " 4.34 " " " Saponif. Nos. 103.0, 103.5. Mean = 103.2

Acids Liberated in Saponification

The acids recovered (method, p.24) consisted of a very viscous mass, which was crystallized from alcohol. The solid material so obtained was allowed to drain on porous tile, in the refrigerator, and recrystallized repeatedly from alcohol. A white, waxy product was finally obtained, m.p. 71-72°, in very small amount. This was added to the previous quantity of the same material (see p.46 , Part III), and examined with it, in one lot.

TABLE 18

THE FATTY CONSTITUENTS OF JACK PINE RESINS.

Complete Summary.

Kind of Wood.	Product Exam'd.	Unsatd. acids, approx. % of each acid.	Main ester fractions, %			Iodine Nos. of ester fractions			Sapon. Nos. of ester fractions			% Vol. acids	% Lig. ac.	% Sol. ac.	% Oxid acids
			I	II	III	I	II	III	I	II	III				
Green	Glycerides & esters	35.3% oleic 49.3 linolic, Traces, linolenic,	1.5	84.6	2.5		130.3			185.6					
"	Free fatty acids	26.8% oleic 20.2% linolic, Traces, linolenic,	5.4	46.9	24.6	30.3	117.5	120.5	182	173.5	100.8				
Seasoned	Glycerides & esters	33.6% oleic 14.6% linolic, 0.5% linolenic,					119.4					0.79	48.7	20.8	30.2
"	Free fatty acids	19.6% oleic 23.7% linolic, 0.16% linolenic,	5.2	43.5	25.8		127.8		51.2	157.0	103.2				

BIBLIOGRAPHY

of

PART III

only.

1. Chemical Phenomena in Life p.53.

2. See ref.1, p.70.

3. See ref. 1,p.68; also Lewis & Tuttle,"New Phytologist",22,no.5 p.226 (1923).

4. "Chemistry of Wood" Hawley & Wise p.115.
Lewis & Tuttle, "New Phytologist" 22.no.5,p.226,(1923).

5. Czapek, Vol.I, p.750.

6. Jahrb. wiss. Bot.22, 73, (1890).

7. Lewis & Tuttle,"The New Phytologist",22,no.5,226-32,(1923).
Sinnott, Bot. Gaz.,66, 162,(1918).

8. Bergstrom, Pap.fbkt. 9, 76 (1911); 11, 730 (1913).

9. Wahlberg, Pap.fbkt. 20, 1136 (1922).

10. "Das Harz der Nadelholzer" Berlin (1894).

11. See ref. 4, p.115.

12. Dissertation, Munich, 1896.

13. J.S.C.I. 665, (1895); Am.Chem. J. 17, 305 (1895).

14. Epple & Rubemann, Ber.59B, 294 (1926).
Carosselli and Schneider, Zeit Ang.Chem. 38. 470 (1925).

15. O.Czerny, see C.A. 20, 1320.
C.A.Lambert, J.I.E.C. 14, 491 (1922).

16. J.Lagerquist, see C.A.19, 2881; Dittmar, C.A. 20, 2072
R.H.Stevens, Chem. & Met.Eng. 26, 629 (1922).

17. Svensk Kemi Tids., 17, 148, 1905.

18. Zeit.Ang,Chem. 22, 582 (1909).

19. Papierfabr 10, 359 (1911).

20. Zeit Ang.Chem. 35, 531 (1922).

21. Arkiv.Kemi Min,Geol 8,No.4 (1921); C.A. 16, 2405.

22. Zeit.Ang.Chem. 39, 262 (1926).

23. Chem.Ums.Fett.O.W.H. 34, 145, 189, (1927); C.A. 21, pp.2072, 3472.

24. Rev.Chim.Ind. 35, 170, 202 (1926).

25. Varrentrapp, Ann. 35, 210 (1840); Edmed, J.C.S. 73, 627 (1898).

26. Paper Trade Jour. July 8, 1926, pp.60-64.

27. "Die Chemie der Zellulose" Kurt Hess (1928) pp.200-210.

28. Hardiker J.Indian Chem.Soc. 5, 63 (1928) C.A. 22, 4000.

29. Maranis, Arch.Pharm., 266, 121 (1928); C.A. 22, 4667.

30.Miura; see C.A. 22, 3287.

31. Lewkowitsch and Warburton, "Chemical Tech.and Anal.Oils, Fats, & Waxes", 6th.ed. vol.I, see pp.177-214.

32. Rec.Trav.Chim. 46, 619 (1927).

33. Chem.Ums.F.O.W.H. 33, 114 (1926).

34. Zeit.Ang.Chem. 29, 486 (1916); J.S.C.I. 36, 395 (1917) Forst. u.Jagdwesen, 47, 99 (1915); Chem.Ztg. 38, 926 (1914).

35. Zeit.Ang.Chem. 31 (I) 125 (1918); Chem.Zeit.42, 229 (1918). J.S.C.I. 37, 382A.

36. Zeit.Ang.Chem. 29, 429 (1916).

37. Wochbl Papierfabr, 44, 2486 (1913).

38. Schwalbe & Grimm, Wochbl, Papierfabr, 44, 3247 (1913).

39. Papierfabr, 20, 1136, (1922).

40. H.Mayr "Das Harz der Nadelholzer" (Berlin 1894).

41. "Harz der Nadeholzer" pp.23-36.

42. See ref.35 above.

43. Finska Kemi Medd. 5, 4 (1918); see also in Hagglund's "Holzchemie" p.160.

44. "Harz der Nadelholzer" pp.23-39.

45. See ref.44, p.38.

46. Arkiv. f. Kemi, 4, No.28 p.9 (1912).

47. Arkiv, f. Kemi, 4, No.28 p.9 (1912).

48. R.Sieber "Uber das Harz der Nadelholzer und die Entharzung von Zellstoffen" 2nd.ed. Berlin 1925.

49. See Sieber ref.48 above, pp.33-39.

50. Schwalbe & Schulz, Zeit.Ang.Chem., 31 (I) 125 (1918). Chem.Zeit 42, 229 (1918): Sieber. ref.49 above.

51. See Lewk, ref.31 above. p.540.

52. Dyer, J.Biol.Chem. 28, 445 (1917).

53. Muter and de Koningh "Analyst" (1889) p.61.

54. Twitchell, J.Ind.Eng.Chem. 13, 806. (1921).

55. Armstrong & Hilditch, J.S.C.I. 44 no.5 pp.43-7,T, (1925).

56. J.Ind,Eng.Chem. 12, 52 (1920).

57. Farb.Ztg. No.3 1912; see Lewk.ref.31. above p.585.

58. Lewk. ref. 31 above, p.580.

59. " " " " pp.202-203.

60. " " " " pp.202,580

61. " " " " p.580

62. " " " " p.237-8.

63. Lewk.ref.31 above p.574; Allen's Comm.Org.Anal. Vol. II p.519.

64. Lewk. ref.31 above. p.166

65. J.Ind.Eng.Chem. 18, 1349 (1926).

66. " " " " 18, 1350 (1926).

67. " " " " 18, 1348 (1926).

68. Lewk, ref.31 above. p.453.

69. Allen's Comm.Org.Anal. vol.II. p.658.

70. Lewk., ref.31 above. p. 253.

71. Lewk, ref.31 above. p.255; 453-7.

72. " " " " p.593.

73. " " " " p.575-6.

74. " " " " p.233.

75. " " " " p.578.

PART IV

THE ESSENTIAL OILS AND UNSAPONIFIABLE MATTER PRESENT IN THE CRUDE RESINOUS PRODUCT.

PART IV.

"ESSENTIAL OILS AND UNSAPONIFIABLE MATTER IN RESINS OF THE CONIFERS".

INTRODUCTION.

Resins and fatty oils of all kinds, whether of animal or vegetable origin, may be said, in general, to consist chiefly of acids, esters and glycerides. In addition, however, there are invariably present some hydrocarbons, alcohols and oxidation products thereof, as well as degradation products of the acids, esters and glycerides, and possibly polymerized substances from the above; all these can be considered under the broad heading of "essential oils and unsaponifiable matter".

These may have a tremendous importance in widely different fields. The essential oils of the genus Pinus form the basis of the turpentine industry all over the world; pinene, the chief constituent, is the keystone of one of the greatest branches of organic chemistry, - the study of the terpenes. Again, considering for the moment an entirely different field, it is found that certain other oils, particularly marine animal oils, contain large quantities of unsaponifiable matter which has very important industrial applications, as well as medical uses. The study of fish oils, including the liver oils of the elasmobranch, as regards their unsaponifiable constituents, has led to a vast accumulation of invaluable knowledge in the fields of organic and biological chemistry. The discovery of the very high hydrocarbon "squalene"[1] and the isolation of the sterol alcohol "ergosterol"[2], bearing the anti-rachitic vitamin, are outstanding examples in these fields.

It is evident from the above that the constituents under this heading deserve more consideration than might be imagined from the insignificant proportions in which they are present in some resins. The oleoresins, of course, usually contain a high percentage of volatile oils, and a rather low proportion of "unsaponifiable" products. Rosin is free from volatile oils, but contains unsaponifiable matter. Resins extracted from the woody tissue contain unsaponifiable constituents generally distinct from those extracted from rosin and usually referred to as "waxes", without regard to their chemical constitution, which is unknown. The percentage of "unsaponifiable" in such wood-resins is usually very small;[3] there are isolated cases, however, where it has been found to be quite large, [4,5] for unknown reasons.

The amounts of volatile oils present in resins of the inner wood are very small, and this is to be expected. The large amounts of turpentine oils are obtained from the oleoresinous exudation on the outside of certain trees. The viscous resins of the balsams, high in volatile matter, are also external secretions. The essential oils existing in the woody stems with the resins, have not been thoroughly investigated, on account of the extremely small amounts available with which to work. Schorger [6] states that there are approximately 92 species of conifers in North America, the oils and resins of which form a field of investigation which has been scarcely touched. The oils obtainable from the needles, oleoresin, wood and bark, respectively, usually differ materially, so that there are at least 368 oils possible, from the above, apart from any resin, fat, waxes, unsaponifiable, etc. Schorger [7] has published an excellent contribution to the chemistry

of American conifers, in which he furnishes much valuable data on oleoresins and volatile oils of several important species, together with analyses of the woods. Various other oleoresins have been investigated, but there is very little data on the oils in the wood.

Pinene is the chief constituent of the volatile oils of the genus Pinus, and is very widely distributed. Pinene was the first terpene investigated, and an extensive literature has been built up in its study.[7] Nevertheless, it cannot be said that the mechanism involved in its various transformations has been definitely established and many of its reactions are not yet understood.[7]

The various isomeric forms in which pinene can exist as such, as well as its transformations to different isomeric substances under certain conditions, give some indication of the complexity of this terpene, and the difficulty involved in the investigation of oils containing it in its various forms.

Essence of turpentine generally consists chiefly of α-pinene, but β-pinene (nopinene) and camphene are often found as well, in small amounts. Other terpenes[8] have been assumed to be present in certain oils of this class, and sesquiterpenes[9] have also been detected in a few instances. While terpenic hydrocarbons, chiefly pinenes, may be said to be invariably present in essential oils of the Coniferae, it is worthy of particular note that, in isolated cases, certain aliphatic hydrocarbons are also present; for example. Schorger[10] reports that the volatile oils of Pinus Sabiniana and Pinus Jeffreyi consist principally of

n-heptane, while Simonsen and Rau[11] detected n-undecane mixed with pinene and other terpenes, in the oleoresin of Picea Excelsa. Since these two hydrocarbons are common constituents of American petroleum, Simonsen[12] has been able to make some very plausible speculations concerning the vegetable origin of petroleum.

By means of special distillation apparatus, Dupont[13] has succeeded in effecting a separation of α-pinene from the inactive β-form (nopinene). The production of cymene and colophene by the action of phosphorus pentachloride on pinene, has been reported by L. Bert.[14] The transformations which pinene may undergo by treatment with mineral acids have also been discussed.[15]

In addition to its industrial importance as a constituent of commercial turpentine, pinene is the source of "synthetic camphor". Pinene also is a source of isoprene, which can be polymerized into an artificial rubber, although this has not been commercially feasible yet.

α-pinene is probably the most abundant of the two forms, but the presence of β-pinene as major constituent in several American conifer oils, was noted by Schorger.[16]

The composition of "wood turpentine", obtained by distillation of pine stumps, is not exactly the same as that of the essence of "gum turpentine" previously referred to; it contains more terpenes also oxyterpenes,[17a] and its properties are somewhat different.[17] It may be remarked, also, that "spruce turpen-

tine", a by-product of the sulphite pulp industry, consists largely of p-cymene.

The"needle-oils" of conifers differ essentially in composition from the oils present in the wood, bark or oleoresin of the same tree. These needle oils contain various terpenes, sesquiterpenes and esters such as bornyl acetate, which give the characteristic odour.[18] Pine needles have been found to contain waxes;[19] also, while the existence of such bodies in the wood-resins has never been shown, although often assumed. Comparisons of oils from different parts of the same tree, have brought out interesting results.[20] Hawley[21] found that turpentine oil from the sap-wood of freshly cut pine (longleaf) had almost identical properties with those from the oleoresin exudation.

Ordinary turpentine oil is a mixture of products from different sources, and shows a wide variation in properties; its optical rotation depends upon its source. The commercial oil from the longleaf pine is dextro-rotatory,while that from the slash pine is levo-rotatory. The French "essence" (from P. Maritima) is invariably levo-rotatory.[22]

The role played by the terpenes and the related resin acids in the life process of the plant has not yet been clearly explained; Czapek[23] believes that these substances are simply poisonous end-products of normal metabolism in the plant, and are excreted as such. Their origin and mode of formation are being carefully studied,[24] and the relationship of the terpenes to the acidic substances is being traced quantitatively.[25] The

essential oils of the numerous Russian conifers are being systematically investigated now, with regard to origin and chemical properties.[26]

Very interesting experiments have been carried out recently by E. Gerry,[27] on longleaf and slash pines. Pairs of trees were selected, adjacent to each other, which were identical in external appearances, size, age, etc. These were tapped for turpentine oleoresin, as usual, and records kept of the yields over a period of many years. It was remarkable that variations of over 100% in yield from two "matched" trees were recorded in some cases. The relationship as regards yield seemed to stay constant. Some trees tended to grow much wood after wounding, while others produced oleoresin at the expense of the wood.

It is thus evident that the yield of resinous substances for any species is not necessarily constant, and may vary greatly from tree to tree.

The method generally used in obtaining the essential oils for scientific investigation involves steam-distillation of the wood or needles; very often superheated steam has to be employed, and addition of alkali. In the investigation of the "wood-resins", however, as used in the present research, the essential oil is removed with the resin and fat by extraction with a suitable solvent, and later recovered from the mixture by steam-distillation. In either case, a certain amount of oxidation and polymerization must be expected, since these terpenes are so sensitive to heat and air. In the case of the oil in wood-resins, there is also

the possibility of a certain degree of transformation due to chemical reagents which must be employed in the lengthy process of separating the crude resin mixture into constituent groups. These are only some of the factors which require consideration in any plan for separating these products with the object of further investigation.

A thorough investigation of the composition and properties of the volatile oils contained in such wood-resins, is not possible to any extent approaching that attained in the examination of the oils from oleoresins, largely because of the matter of the supply of the oils in sufficient quantity for investigation. The yield of this internal secretion, even from highly resinous pines, is always very small, and such considerations as capacity of apparatus for treating enormous quantities of the wood, under proper conditions, and the amount of time involved in getting out the small quantities of material offer serious difficulties.

Sieber[28] reported that pine and spruce chips, distilled with steam at atmospheric pressure, gave only traces of turpentine oil. Ether and alcohol extracts of these woods usually gave negative results when tested qualitatively for turpentine. But if the chips were cooked with alkali, in an autoclave, some essential oil could be recovered from the relief gases. Stored pine chips yielded only 0.3%, calculated on the bone-dry weight of wood, while spruce yielded none. From very fresh wood, 0.81% of turpentine oil was thus obtained, in the case of pine; from spruce, only 0.11%. It is evident that some of the oil is lost by evaporation when the wood is stored in the form of chips; this

is probably true to a much lesser extent when the wood is left in log form. These terpenic substances have been thought to play some part in the formation of troublesome pitch-like deposits on the wires of paper machines;[29] but this has not been proven.

Bergstrom[30] removed the total resinous matter from pine and spruce trunk-woods, by extraction with ether and alcohol and obtained values in agreement with the above as follows:

Wood used	% based on B.D.wt. of wood.		% of turp. oil in total extract:
	Resin & fat	Turp. oil	
Pine	6.0	0.35	5.5
Spruce	2.3	0.05	2.1

Austerweil and Roth[31] reported a similar set of figures. In examinations of oil from pine and from spruce woods, Aschan[32] and Bergstrom[33] reported the presence of pinene, dipentene, and sylvestrene.

"Unsaponifiable Matter."

What has been said concerning the small amounts of volatile oil available, and consequent difficulties connected with their investigation, perhaps applies even more so as regards the non-volatile, unsaponifiable matter of the wood-resins. No attempt has been made heretofore to establish what this material actually consists of, although Tschirch[34] mentioned its inert properties, and gave ultimate analytical data. Tschirch was considering the "resene", as he named it. The term "unsaponifiable" has a very broad application. It may include hydrocarbons, resene,

oxidized and polymerized products, difficultly saponifiable esters or waxes, and free alcohols. The"resene" refers particularly to a certain material always found in resins, and characterized by its inertness towards reagents. Tschirch[34] reported that it does not show the usual reactions for carboxylic, aldehydic, ketonic or hydroxylic groups; neither has it an ester nor a lactone. He found it to consist of carbon, hydrogen and oxygen, in variable proportions. He considered it to be made up of oxyterpenes or oxypolyterpenes, but had no definite proof of this. He does not appear to have tried to remove any sterols assuming such are present.

The "resene" is obtained by removing everything else from the unsaponifiable, by chemical or physical means. The "resene" may be a viscous oil, or a waxy mass. It is never crystalline. It is soluble in most organic solvents. It is usually only a minor constituent of resins, except in certain cases[35] reported, as much as 20-25% of the resin appears to be made up of this material, for unknown reasons. Colophony usually contains 5-10%. Wood-resins contain it in very small amounts. It is extremely difficult to free it from essential oils completely. It is often precipitated from the ethereal solution of crude resins in extracting with aqueous alkaline solutions. It can be purified by adding dilute KOH to its ethereal solution, or by adding alcohol. "Resenes", phytosterols, and abietic acid all give very similar colour reactions, which may indicate some relationship.

The percentage of "resene" in various resins has been determined

by Tschirch[34], and by Herty and Dickson[35]. The latter reported from 4.1 to 7.4% in certain pines of America. They later commenced an investigation of the chemical nature of the resene of Pinus heterophylla (slash pine), but this work was abandoned[36]. There is no information in the literature on the actual composition of such "resenes". The unsaponifiable matter in oxidized turpentine oils has been investigated recently by Tschirch[37], who claims to have isolated some aldehydic substances.

Other possible constituents of the unsaponifiable portion of resins, are the higher alcohols. Tschirch[34] reported many cases where positive colour reactions for sterols could be obtained. He also mentions the presence of resin alcohols in certain oleo-resins[38], and these have been identified also by other investigators[39]. They generally resemble the phytosterols, and may be closely related to them.

Phytosterols have been found in the liquid resin by-product obtained in cooking sulphate pulp (see part III, pp.6-10).

Waxes:

Waxes or difficultly-saponifiable esters have not been shown to exist generally in the wood-resins of conifers. Tschirch[40] reported that amber was the only such resin containing these esters. His work did not include fatty constituents, nor take into account any esters of sterols with fatty acids.

The needle-oils of pines differ essentially from the wood-resins, and Kaufmann and Friedebach[41] claim to have isolated some higher waxes from these oils, and identified the constituents as cetyl, ceryl and myricyl alcohols, combined with oleic, palmitic, hydroxypalmitic and stearic acids. These waxes were

extremely difficult to saponify, and had to be heated with alcoholic potash in sealed tubes for four hours at 140°. The products were separated by a lengthy series of fractional precipitations from petroleum ether solutions. A substance resembling a sterol was also obtained. Similar waxes were reported previously by other workers[42].

Cutin:

Cutin-like substances were obtained in traces only, by Konig & Rump[43], from beech and fir. Cutin is normally a constituent of the epidermal layer in the bark, and is probably a mixture of waxes. Doré[44] reported it absent from pines which he examined, and does not consider it an important constituent of wood. Its presence is scarcely to be expected in wood-resins, and it probably is not present normally in the raw material used in pulping processes, since this wood is always barked previous to cooking.

The "unsaponifiable matter" of commercial rosin was examined by Knecht and Maurice[45], and yielded some interesting comparative results. They found no oxygenated substances. Some residual pinene was present; four "high molecular weight" hydrocarbons were isolated, comprising three diterpene isomers, $C_{20}H_{32}$, and colophene, $C_{19}H_{30}$. The latter could not be found in the original oleoresin although it is present in rosin oil. When "turpentine essence" is polymerized by heat or by sulphuric acid, the three hydrocarbons $C_{20}H_{32}$ are obtained. They are found to form substitution products when brominated, but do not yield any nitrosochlorides or nitrosites, nor do they combine

with hydrochloric acid gas.

The "unsaponifiable matter" of wood-resins probably contains similar substances[46], formed from the original terpenes by the action of heat, air, or the chemical reagents employed during the lengthy process of extracting the crude resins and later separating them into various groups of products.

The effect which the "unsaponifiable matter" of rosin has on the sizing of paper, has been discussed at length by A. Haug[47].

Sieber[48] has expressed the opinion that the "unsaponifiable matter" of wood-resins is probably made up of phytosterol-like substances and waxes. Up to the time of the present investigation, this had never been established. There is always some of this material in the resin-extract of paper-pulp, and Sieber thinks that the "unsaponifiable" may play an important role in regard to pulp and paper problems.

Methods for quantitatively estimating the unsaponifiable portion of resins and fats have been very carefully studied[49]. Such analytical methods are often of no use in the treatment of very large volumes of extracts for purposes of isolating the material for further investigation, but can be made use of in modified forms. The methods employed by Heilbron[50], Tsujimoto[51], André[52], and others, in investigating unsaponifiable matter of fish oils, might be of some use so far as the vegetable product is concerned. They generally used fractional distillation under low pressure; fractional crystallization; hydrogenation, and acetylation. Fractional distillation was practicable in this case, because large quantities were available for investigation.

Of all the constituents of resins, in general, the unsaponifiable portion is probably that of which least is known.

Discussion of experimental results obtained in the present investigation.

Essential Oil.

The constants of the two products show that there is no noticeable difference in the oil from the green wood and that from the seasoned wood, also that the amount of volatile oil obtainable in both cases is extremely small. No constant variation in quantity with the age of the wood, is shown. The quantity secreted probably varies from tree to tree (see Gerry.[27])

The specific gravity is higher than for most turpentine oils, but this is probably due to the presence of polymerised products along with the pure pinene. The material could not be purified completely by redistillation, on account of the small quantities obtained, so that no elementary analyses (for carbon and hydrogen) were made.

The optical rotation $[\alpha]_D^{24} = +39.0^{\circ}$, is of the same order as that for dextro-rotatory, American turpentine essence, rotation +35 to +50°. The fact that a pure nitrosochloride could not be obtained is no doubt due to the presence of substances having a high optical activity. Schorger[53] and Dupont[54] reported that this derivative could not be obtained from pinene specimens with this property.

The inconclusive results reported in the search for camphene, sylvestrene, dipentene, limonene, and β- pinene do not necessarily prove that they are absent since these reactions are extremely difficult to carry out, where such small amounts

of material are available.

II. Unsaponifiable Matter.

The two main methods of investigation employed, (a) fractional precipitation, and ultimate separation by means of the digitonide or the acetate and (b) fractional distillation, and purification by means of petroleum ether and alcohol, are both very tedious, but are probably the only effective means by which such a mixture can be investigated. Fractional distillation, however, causes a very high percentage of loss in the working material, on account of decomposition and polymerization of the product at the high temperature necessary to distil it.

The digitonin separation method is suitable for isolating small amounts of the pure phytosterol. The excess digitonin contaminates the "resene" and has to be removed from it by further treatment. This method is too tedious for general use. The acetate separation is also unsatisfactory on account of the difficulty in separating the acetate from the "resene" in the same solution, their relative solubilities being almost identical.

A thorough separation by means of fractional precipitation from alcohol followed by fractional recrystallization from this solvent, or acetone, without attempting to separate further by acetylation or use of digitonin, would seem to be the most efficient means of isolating the individual constituents.

The crystalline product actually isolated was shown to be one of the phystosterols, m.p. 132°; it is present in

extremely small amounts in the original crude resins. The amorphous, waxy material, melting over the range 62° to 68° could not be saponified even at 140° under high pressure, which proves that it is not a true wax. Its properties indicate that it is identical with the "resene "[34] usually found in this class of resinous material.

The third fraction obtained from the "unsaponifiable" has the properties of the polymerized terpenic substances, usually present in the residues after distillation of turpentine oil. It is not as hard as rosin, but otherwise resembles it in external appearances.

The high percentage (83.9%) of this material found to be present in the unsaponifiable matter from the seasoned wood as compared with an average of 60.3% from the green wood, indicates that extensive polymerization of the essential oil has evidently taken place during the seasoning of the wood.

The yields of pure sterol and "resene" from the seasoned wood were also found to be much smaller than in the case of the green-wood, this probably being due to various transformations having occurred during the time of seasoning.

EXPERIMENTAL

Examination of the Essential Oil and "Unsaponifiable Matter" in the resins extracted from Jack Pine (Pinus Banksiana).

I. Essential Oil:-

Material: This volatile oil was separated by steam-distillation in the initial separation of the crude resins mixture (p.21 part I) and when recovered from the ethereal solution, was usually in the form of a yellow oil, smelling of turpentine.

Purification:- When distilled on the Langmuir pump, it passed over between 55° and 64°, at 0.007 m.m., in the first distillation. Several redistillations finally yielded a colourless product, boiling at 59-63° at 0.01 m.m. This was almost odourless when freshly distilled. On account of the very small quantities obtained it was not possible to fractionate this product. All distillation residues were kept for examination. A polymerized residue always remained in the flask after distillation.

Boiling-point and refractive index indicated that the essential oil obtained from the "green" wood (Extractions 1 to 11, part I) was identical with that from the "seasoned" wood (Extraction 13, part I).

(A) Oil from Green wood.	(B) Oil from "seasoned" wood
B.P. 59-63° @ 0.01 m.m.	B.P. 59-62° @ 0.01 m.m.
B.P. 156-158° @ 1 atm.	B.P. 159-160° @ 1 atm.
Refr.Ind. $n_D^{10} = 1.4914$	Refr.Ind. $n_D^{10} = 1.4929$
$n_D^{20} = 1.4874$	$n_D^{20} = 1.4879$
$n_D^{30} = 1.4825$	$n_D^{30} = 1.4845$
Spec.Gr. @ 20° = 0.9289	Spec.Gr. @ 20° = 0.9330

Rotation of Product (A)

0.1242 gm. in 10 c.c. absolute alcohol.

mean reading = 0.485°

$$[\alpha]_D^{24} = +39.0^\circ \quad (c = 1.242)$$

Yields: from combined lots of crude resins:

TYPE OF WOOD	FROM EXTRACTION NO.	WT. TOTAL CRUDE RESINS	WT. OF ESS. OIL (NOT PURIFIED)	% ESSENTIAL OIL
"Green"	3 and 10	72.1 gm.	3.10 gms.	4.30%
"	5,6,7,8,9	292.8 "	4.40 "	1.50%
"Seasoned"	13	485.0 "	8.55 "	1.75 %

These data are included in Table V, Part I.

Identification of constituents of the essential oil.

Preparation of pinene nitrosochloride[55]

Equal parts of the essential oil, glacial acetic acid, and isoamyl nitrite were mixed together and the mixture saturated with dry HCl gas, the container being kept in an ice-salt bath during this treatment. The intense green colouration characteristic[66] of the formation of pinene nitrosochloride was observed. The solution became quite viscous, but no solid separated even after several hours in the refrigerator. On allowing it to evaporate in a dish a white solid was deposited. This was washed free of the viscous brown material by means of methyl alcohol, and recrystallized from chloroform. From 1.866 gms. of the essential oil only 0.030 gm. of this solid was obtained (yield = 1.8%). It began to show signs of decomposition at 103°, but did not have a definite melting point. (Pinene nitrosochloride[67] melts at 103° to 115°.

(b) Preparation of pinene nitrol-piperidine:[56]

The above solid product was warmed on the water bath with a little alcohol and piperidine for ½ hour. Some water was added and a white precipitate formed in very small amount. This was separated by centrifuging, and dried, but due to the small amount available no purification was possible. It had an indefinite melting point.

(c) Preparation of pinene hydrochloride:[57]

0.3 gm. of essential was dissolved in dry chloroform, and the solution saturated (very slowly) with dry HCl gas, keeping it at 0°. Then an equal volume of water was added, the mixture neutralized by sodium bicarbonate and distilled with steam. A very small amount of white solid distilled over with the chloroform, but not sufficient for examination. The residue in the flask was extracted with ether, and the ethereal extract evaporated. No solid separated from the oily residue after several days standing. The small amount of white solid distilling over with the chloroform, remained on the sides of the receiver. It had a distinct odour of pinene hydrochloride (synthetic camphor).

Tests for Camphene.

(1) After standing for several days at -20°C, no solid camphene separated from the sample of oil.

(2) Method of Balbiano and Paolini:[58]

No addition product was obtained.

Tests for Sylvestrene, Dipentene and Limonene:[59]

No solid dihydrochlorides could be isolated, even

after keeping on ice for several weeks. The products obtained consisted only of viscous, oily material.

Bromination in glacial acetic acid solution did not yield any solid tetrabromide. The solution was kept in the refrigerator for three weeks, and then evaporated to smaller volume but no solid product could be isolated.

β-pinene (nopinene)[60]

After oxidation with alkaline permanganate, and steam distillation, the filtered solution yielded a very small amount of white precipitate. This was removed and another small crop obtained from the mother liquors. These products were recrystallized from water, and dried at 70° in vacuo.

Unsaponifiable Matter

EXPERIMENTAL

The complete analytical results have been tabulated at the end of this section.

Method I:- Fractional precipitation.

Material used:- The "unsaponifiable matter" separated in the process described on p.22, part I, consisted of a light brown waxy mass, various specimens melting between 65 and 68°.

Procedure: It was dissolved and allowed to stand for several days at room temperature. A precipitate of very fine needles (a) separated out, often accompanied by some flocculent, white material; in some cases there was also a deposition of a brown, colloidal substance, which settled to the bottom as a scum.

The precipitate was filtered off, and a second lot of solid material (b) obtained by allowing the filtrate to stand in the ice-box over night, and filtering again.

The residual alcoholic solution was evaporated on the water bath under vacuum to about three quarters of its original volume, and, after allowing to stand for a few hours, the solid material was filtered (c). This evaporation of the mother liquor, and removal of the precipitated product (d & e) was repeated, after which the small amount of residual solution became very viscous. No more solid material could be obtained from it, even after allowing to stand in the ice-box several days, or diluting with more alcohol and cooling. After removal of the volatile solvent completely, under reduced pressure, a mass of polymerized terpenic material was obtained very similar to the residue from turpentine distillations.

The five precipitates obtained (a,b,c,d,e) above were subject to fractional crystallization from alcoholic solutions. The initial crop of material from each product was found to contain crystalline solids. The amorphous, waxy material accompanying the latter was found to dissolve more readily in the alcohol, than the crystals, so the solutions were warmed gently after precipitation and then filtered. The amorphous products were thus concentrated in the mother liquors. A long series of such treatments with all of the solutions of these materials, as well as all the mother liquors, finally yielded three products:-

(A) A pure white crystalline substance (needles);

(B) A yellow, amorphous product of waxy consistency;

(C) A dark brown mass of polymerized or oxidized terpenic substance.

Examination of product A

The crystalline substance was still contaminated by traces of amorphous material, but by employing several recrystallizations from alcohol, and finally drying at 100° in vacuo, it was obtained in pure form, melting at 131.5-132°, which corresponds with the melting point of one of the phytosterols.[61]

Another very pure specimen of this sterol was obtained by the digitonin separation method described below and melted at 132°.

It was further identified by yielding a crystalline acetate when treated with acetic anhydride by the usual method.[62] This acetate melted at 125-126°, corresponding with phytosterol acetate.[63]

The sterol also gave the characteristic colour reactions[64] of phytosterol, namely:

(1) Liebermann's reaction with acetic anhydride and concentrated sulfuric acid. The material gave a pink colouration rapidly changing to blue, and finally to greenish-blue.

(2) Hesse-Salkowski reactions: The substance treated with chloroform and sulfuric acid, produced a reddish-violet colour in the chloroform layer, while the acid layer became yellow, with green fluorescence.

Examination of product B;

This could not be completely purified by fractional crystallization, because it always separated as a gelatinous mass, still containing some of the crystalline product.

Two methods were tried in order to effect a complete separation: (1) Treatment with digitonin, to remove the sterol; (2) Acetylation, also to remove the sterol.

(1) Treatment with digitonin.

Method of Windaus:[65] (see also 66)

This method had to be slightly modified in the present case as unknown percentages of the sterol were present, and also excess digitonin had to be removed later.

Treatment with 1% alcoholic digitonin solution had to be repeated several times, until no more precipitation was obtained on standing. The product consisting of the complex $C_{27}H_{45}OH.\ C_{55}H_{94}O_{28}$ was washed with ether. The mother liquors (a) contained the amorphous unsaponifiable material, with the excess digitonin.

The solid complex was decomposed by treating with boiling

xylene for 10 hours the material being suspended in a small thimble immediately above the boiling liquid. The digitonin was then recovered unchanged from the thimble, while the pure sterol was obtained by distilling off the xylene in a current of steam, and recrystalkizing the residue several times from alcohol. The product was pure phytosterol, in the form of very find needles (m.p.132°).

The mother liquors (a) above, were evaporated on the water-bath, and the residue treated with boiling xylene exactly as described above. The excess digitonin remained behind in the thimble, and the xylene solution was steam-distilled, leaving a wax-like residue behind. This was reprecipitated from alcohol several times, and finally a pure white product ("resene") was obtained. It was of waxy consistency, and its melting point (different specimens) varied between 62° and 68°.

The above method is long and tedious, so an acetylation method was employed.

(2) <u>Acetylation</u>

0.5 gr. of the product)B) was heated with 25 c.c acetic anhydride, on the water-bath for two hours, and yielded a homogeneous solution. On cooling, a gelatinous precipitate settled out, and this was filtered off. A further amount of precipitate was obtained when the mother liquors were evaporated. These two substances were dissolved in boiling acetic anhydride; the products which separated on cooling were then purified by solution in hot alcohol from which they separated on cooling. They were then separated from the mother liquors by centrifuging

since filtration was almost impossible. Various fractions were obtained but great difficulty was experienced in separating the crystalline acetate from the amorphous material, as their solubilities seemed to be practically the same. Acetone was then tried, as a precipitating medium, but was equally ineffective. The acetate fraction had a constant melting point of 110°: (pure phystosterol acetates, m.p. 115° to 128°).[62-63]

The lower fractions melted at 62° to 66°. These were merged with the products (m.p.62-68°) from the digitonin separation.

The product (B) now completely free from the sterol, and melting between 62 and 68° was now heated with alcoholic potash in order to determine if it was a true wax, or not.

Procedure.

The sample of "resene" substance (B) purified as above, weighing 0.5-0.6 gram, was heated in a sealed Pyrex tube with 10 c.c. of saturated alcoholic sodium hydroxide, at 140° for 8 hours. The product when cool, was transferred to a separating funnel, some saturated aqueous sodium chloride solution added, and the mixture extracted with petroleum ether, until nothing more could be removed. (The aqueous layer contained some yellow solid suspended in it; this, when filtered off and examined, was found to consist of inorganic salts.)

The product removed by petroleum ether was recovered by evaporation of the solvent; it was dried on porous tile, and recrystallized twice from alcohol, then dried at 80° in vacuo. M.P. 65-66°. It seemed to be identical with the original material.

The aqueous layer, after extracting by petroleum ether, and filtering from the inorganic salts, was acidified with dilute hydrochloric acid. Only a very slight turbidity was noticeable. The acidified solution was extracted with ether, the ether solution dried over sodium sulphate, and evaporated. No acids were found and only a trace of residue remained. It was dark brown in colour, and probably consisted only of impurities.

Examination of the polymerized terpene.product (C)

This residue was a dark brown mass of polymerized terpenic substance, probably derived from the essential oil. It had a "softening range" of 9-25°C, and was extremely viscous at 0°, but did not solidify even at-15°. Prolonged distillation with steam yielded only traces of volatile turpentine oil.

Refractive index:- n_D^{10} = 1.5644: n_D^{20} = 1.5600; n_D^{30} = 1.5569

Method II:- Fractional distillation under reduced pressure:

Material: The material used was the crude "unsaponifiable matter" (m.p.65° to 68°) separated in the process described on p.22 part I. Only one lot of this material was subjected to fractional distillation; namely the one from extraction 13 (seasoned wood).

Procedure:

The technique employed in these distillations (using a Langmuir mercury vacuum pump) has been discussed already in connection with the resin acids (p.44part II) and the fatty esters (p.34part III)

19.10 grams yielded five initial fractions. The distillation was stopped when the temperature of the bath reached 320° because of decomposition of the residue in the flask.

Initial Fractions

Frac.	B.P.	Press.	Description	Wt.	%
1	60-100°	0.01 m.m	Nearly colorless liquid	0.60g.	3.1
2	170-177°	" "	Yellow viscous "	1.50	7.8
3	177-185°	" "	Pale Yellow Solid	4.20	22.0
4	185-220°	" "	Dark " "	6.05	31.6
5	220-270°	" "	" " viscous liquid	2.60	13.7
Residue in flask				3.65	19.2
Losses (by difference)				0.50	2.6
Original wt. of material used				19.10 g.	100.0%

Fractions 1 and 2 were purified by redistillation; the other fractions were not redistilled on account of the effect of the high temperature on these products.

Fraction 1: was redistilled @ 0.01 m.m.: b.p. 55-60°; the product was a colourless, mobile liquid possessing the odour of turpentine. Refractive index: n_D^{20} - 1.4875; Yield 0.5 gr.

(The above properties indicate that this fraction is merely a residue of essential oil, not removed completely in the steam-distillation (p.21 part I)

Fraction 2 was redistilled at 0.01 m.m.: b.p. 168-170°, yield 1.2 gr. of a viscous, green oil, which formed a gelatinous mass when cooled to 0°. (This fraction probably consists of polymerized terpenes resulting from action of heat on the essential oil; see p.6, part IV)

Fractions 3, 4, and 5: These were each fractionally crystallized by dissolving in hot petroleum ether (b.p. 30-50°). Fraction 5 dissolved completely, and yielded crystalline masses on cooling. Fractions 3 and 4 dissolved partly, but each left a brown, oily

portion (a) almost insoluble in petroleum ether, from which the solution was decanted. Continuation of this treatment gave rise to similar oily residues, which were united with (a) and dissolved in hot alcohol. After standing at 0° for some time, a small amount of white, amorphous product separated, and was removed by filtration. No further separation could be effected from the dark brown filtrate, so it was evaporated on the water-bath, under suction, and yielded a reddish-brown mass of polymerized terpenic substances, very similar to the residues obtained in the first method of separation (see p. 22 part IV.)

The products from the petroleum ether fractions, after removal of the above polymerized oil, were recrystallized several times from alcohol, as it was difficult to effect an appreciable fractionation with petroleum ether (B.P. 30-50) or with mixtures of the latter and higher-boiling fractions (from 70 to 110°). The purification with alcohol finally yielded two main fractions: (a) a crystalline product, m.p. 129-130°; and (b) an amorphous, wax-like substance, m.p. 62-69°; the product (a) was dried at 100° in vacuo, while (b) was dried at 50°, also in vacuo. The products were then weighed.

Further recrystallization of these main fractions from alcohol yielded two pure products, (a) m.p. 131-132°, and (b) m.p. 68-69°. (These correspond with the melting-points for the two similar products already obtained, see p. 22, part IV.)

Analytical results by the above methods (I and II) are tabulated in the following summary. It was necessary to group together the unsaponifiable products from different extractions due to the small amounts obtained.

In order to obtain the "average" percentages of the individual constituents, for each of the extractions involved, (as reported on table V, part I), the percentages reported in the following tables were used as factors in conjunction with the original percentage of total unsaponifiable matter (see pp.-1926 tables I to IV, part I) in the crude resins from the extraction involved.

UNSAPONIFIABLE MATTER

(Summary of Analytical Results)

Wood used	Unsapon. matter from Extr'n Nos.	Method used in Examination	Total weight of substances obtained after purif'n	Composition					
				Sterol		Resene		Polymerized terpenes	
				Wt. gms.	%	Wt. gms.	%	Wt. gms.	%
From "green" wood	1,2,4	(I) Fractional precip'n	1.867 gm	0.269	14.4	0.538	28.8	1.060	56.8
"	3,5 to 10	"	10.563 "	2.325	22.0	2.048	19.4	6.190	58.6
"	11	"	1.328 "	0.283	21.3	0.172	13.2	0.870	65.5
"			mean % →		19.2%		20.5%		60.3%
From "seasoned" wood	13	"	15.61	0.91	5.8	1.60	10.2	13.10	84.0
"	13	(II) Fractional distill'n	7.21	0.57	7.9	0.6	8.3	6.04	83.7
			mean % →		6.9%		9.3%		83.9%

BIBLIOGRAPHY

of

PART IV

only.

1. Tsujimoto J.Ind.Eng.Chem. 8, 889 (1916).

2. Bio.Jour. 21 (1) p.78,127,389 etc. (1927).

3. R.Sieber "Harz der Nadelholzer" pp.12-23.

4. Herty & Dickson, J.Ind.Eng.Chem. 4, 495 (1912).

5. Tschirch,"Harze und die Harzbehalter" pp.579,593.

6. Schorger, Trans. Wis.Acad.Sc.Arts Lett.XIX part II p.729.

7. Schorger "Contribution to the Chemistry of American Conifers" Trans,Wis.Acad.Sc.Arts.Lett. XIX, Part II, pp.728-766.

8. See ref.7, p.733.

9. See ref.7, p.733.

10. See ref.7, pp.739-752.

11. Ind.For.Records 9, Part IV, p.116 (1922).

12. See Hawley & Wise "Chemistry of Wood" p.111-114.

13. Bull.Soc.Chim. 34, 1431, 1667; 36, 1753;Comp.Rend. 176 1881, (1923).

14. Bull Soc.Chim. 33, 787 (1923).

15. J.C.S. 126, p.1088.

16. See ref. 7, p.733.

17. See ref.12, above p.109.

18. See ref. 7 above, pp.736-751.

19. Kaufmann & Friedebach, Ber.55B , 1509 (1922).

20. Filipovich, J.Chem.Ind. (Moscow) 4, 953 (1927).
C.A.22, 4579;
Pigulevskii, J.Russ,Phys Chem.Soc. 54, 259 (1922).
C.A. 18/1366; Semmler & Schiller, Ber.60B, 1591 (1927)
Schorger (see ref.7) p.733.

21. Forest Serv.Bull.105, 54 (1913); see also ref.12 p.109.

22. Herty, J.A.C.S. 30, 866 (1908).

23. "Chemical Phenomena in Life" p.70.

24. Bull.Soc.Chim. 36,1761-2; J.C.S. 126, 657; J.S.C.I. 43 526, (1924).

25. Pigulevskii, J.Russ, Phys,Chem. Soc.59, 299 (1927) C.A. 22, 1433.

26. See refs.20, 25 above. also C.A. 17,3231; 18, 1366, 19, 2222.

27. J.Plant Physiol 6, 1, (1929) P.I.A.A. 4, 6 (1930).

28. Sieber see ref.3 above p.39-40.

29. See ref.3 above (p.39).

30. See Hagglund "Holzchemie" p.157.

31. See Hagglund "Holzchemie" p.157.

32. Ber.39, 1447 (1906).

33. Jarnkont Ann. 1910; see also ref.30 above. p.158.

34. See ref.5 above. p.1080-82.

35. Tschirch, see ref.5 above p.593.
Herty & Dickson J.Ind.Eng.Chem. 4, 495 (1912).

36. Private communication from Dr.Herty.

37. Bolin & Tschirch; see abstract Pine Inst.Amer.abs. 2, 86-90 (1912).

38. Tschirch; see ref. 5 above, p.620.

39. Zinke, et al (Monat 42, 439 (1922); Monat 44, 371 (1924) C.A. 15, 1016, 1507; C.A. 16, 2323; C.A. 18, 2711.

40. See ref. 5 above pp.736-748.

41. Ber. 55B, 1509 (1922).

42. J.pharm.chim. 29 (6), 561; C.A. 3, 2445.

43. Zeit.Unters. Nahr.Genussm, 28 (1914) p.177: see also "Die Chemie der Zellulose" (Hess) p.200-210.

44. J.Ind.Eng.Chem. 11, 556 (1919).

45. Jour.Soc.Dyers Col. 41, 356 (1925).

46. Aschan see P.I.A.A. (1927) no.5, p.49.

47. Pap.Trade Jour. 76, 136, 185, 244 (1923); 77, 11 (1923).

48. Sieber; see ref. 3 p.54-55.

49. J.Ind.Eng.Chem,18, 1346 (1926);
Smith "Analyst" 53, 632 (1928);
Allen's Comm.Org.Anal. II and IV.
Lewkowitsch "Tech.& Anal.Oils,Fats,Waxes" vol.I

50. J.C.S. (1926) p.1630.

51. Chem.Ums. 29, 27, 43 (1922).

52. Ann.de Chim.. 7, 99 (1927)
Bull.Soc.Chim. (1929) p.501.

53. J.Ind.Eng.Chem. 6, 631 (1914).

54. Vezes et Dupont "Resines et Terebenthines" p.369.

55. Wallach Ann. 245, 251 (1888); Aschan Ber.39, 1445 (1906).

56. Wallach Ann. 245, 253 (1888)

57. Frankforter and Frary, J.A.C.S. 28, 1461 (1906);
Beilstein, V, (450-498) p.94.

58. Ber.36, 3575 (1903).

59. Aschan, Ber.39, 1447 (1906).

60. Wallach, Ann. 356, 228.

61. Allen's Comm.Org.Anal. vol.II p.770-771.

62. Allan and Moore J.S.C.I. 46, 433T (1927).

63. See ref. 61, p.773.

64. See ref. 61, pp.768-773.

65. Ber.42, 240 (1909).

66. Klostermann & Opitz, Zeit.Nahr.Genussm, 27, p.713
C.A. 8, 3246.

PART V

GENERAL SUMMARY.

PART V.

GENERAL SUMMARY.

PART I: Methods of extraction, and initial separation of the crude resinous product.

Alcohol-benzene extractions of "green" wood were made at intervals, over a period of 3 - 4 months (See Tables I - V). "Seasoned" wood, 5 years since felling, was also examined. The resin content in each case was noted. Certain methods of separating the crude resin product into simpler groups, were investigated and found unsatisfactory. A systematic procedure was developed for the efficient separation of this crude material into specific groups, suitable for further investigation, in a comparatively unchanged condition, and a discussion given (P. 32, Part I) of the advantages of this method. Each group of substances was examined separately later.

Table I (p.10, part I) shows that there is no marked variation in resin content in the "green" wood, when it is stored in the form of logs. Other investigators have found a pronounced drop in resin content when the wood is stored in the form of chips.

The average value of crude resin (by alcohol-benzene extraction) is 4.58%. The "ether-soluble" resins content is based on the weight of crude product less the amount of water-soluble constituents and of lignin. The average value of ether soluble resins in all the extractions is 3.52%.

The "seasoned" Jack Pine log examined (5 years since cutting) had as high a resin content as the "green" wood. This may have been a particularly resinous log; the resin content usually varies from tree to tree.

Table IV (p.26, part I) shows that the "green" wood has approximately the same percentage of total acids as the "seasoned" wood, but has more fatty acids present, and considerably less total unsaponifiable matter than the seasoned wood. The seasoned wood contains about 5% less fats, than the green wood, and a corresponding higher percentage of resin acids.

Table V (p.27, part I) also shows the higher content of fatty acids in the green wood; there are also more glycerides present. The percentages of polymerized terpenic substances (in the unsaponifiable matter) is quite high (6.5%)in the resins of seasoned wood, compared with those of green wood (0.8-3.1%). There is a decrease in the amount of phytosterol present, with seasoning, also (0.70% in green wood, 0.54% in seasoned wood); this is alsotrue of the resene (0.87% in green wood, 0.73% in seasoned wood). These are "average" percentages for all the extractions of "green wood".

PART II: RESIN ACIDS.

Various methods were investigated, for isolating the pure resin acids, the one finally chosen for effecting their removal from the impure acidic substances being based on the solubility of the pure acids in petroleum ether. This method removes the pure acids in comparatively unchanged condition.

A method of identifying certain of the individual acids was also evolved. This was based on the formation of crystalline ammonium salts by acids of the pimaric group, and of crystalline acid sodium salts, by acid of the abietic group.

Three main groups of acids were shown to be present:-

(1) Amorphous acids, of the "Sapinic" class;

(2) Crystalline acids, of the pimaric and abietic groups;

(3) Transformation (amorphous) products derived from the above two groups.

The "crystalline acids" were carefully purified and their constants examined. The molecular weight was determined by alkalimetry and found to correspond with the formula $C_{20} H_{30} O_2$. The acids after distillation in vacuo were found to have a molecular weight of 309.0, and a lower iodine number (90.9), possibly on account of a slight oxidation having taken place. Transformations near the melting point were observed, as is the usual case with these acids. The presence of the pimaric (natural) and the abietic (colophenic) types was established, by means of the crystalline salts (see above). Abietic acid was also identified by its crystal-form (triangular) but the other acids could not be identified in this way, on account of the imperfectly formed crystals.

It was found that when the acids were not very carefully purified (see Acid D, table VI), a higher percentage of "natural" acids was present (based on the yield of crystalline ammonium salts). Evidently abietic acid is formed to a great extent during the lengthy series of recrystallisations.

A higher percentage of crystalline acids was obtained from "green" wood (66.3%) than from seasoned wood (51.8%), probably due to transformations occurring in these products during the process of seasoning.

Other means of removing these resin acids from the wood in unchanged condition, were investigated (petroleum ether, 1% alkali extractions, etc.,) but on account of the large amounts of other material removed at the same time, it was not possible to isolate the crystalline products, by these methods, without employing some subsequent chemical separation process.

PART III: FATTY CONSTITUENTS.

The fatty constituents were examined by very carefully controlled and accurate methods, in order to prevent any changes in the material.

Various methods employed in fat analysis were examined, and the advantages and disadvantages of each have been discussed fully in part III. The fatty constituents were isolated free from the other components of the resins. It was also possible to examine the free fatty acids and the fatty glycerides separately.

The only volatile acid found was butyric, in traces only. The unsaturated acids, oleic, α-and β- linolic, and linolenic, were identified.

Two other acids were also found in very small amounts among the fatty acids: (1) a liquid product, b.p. 55-60° @ 0.01 m.m., which was examined as far as the quantity available would permit, and (2) a solid acid, amorphous, m.p.70-71°, which seems to be identical with the lactonic acid isolated by Sandquist. (see p.7, part III).

Another very small fraction, melting at 68-69°, possibly consists of stearic acid.

The bromination and oxidation derivatives of oleic and linolic acid were also prepared, and used in establishing the identity of these acids. Linolenic acid was identified by means of its hexabromide. No other unsaturated acids of this series were found to be present.

The composition of each fraction of the liquid (unsaturated) acids was estimated, by means of its bromination products and unsaturation value.

The glycerides and the free fatty acids of the green wood were examined separately. This was also done with the two similar groups from the seasoned wood.

A complete summary of the investigation of these four groups of fats has been given on Table 18, (page 57, part III). An inspection of this shows that seasoned wood contains much less linolic acid in the glycerides than does the green wood. This is probably due to oxidation or polymerization during the time of seasoning. The amounts of oleic acid present is approximately the same in each case; linolenic acid was never found in more than traces. The free fatty acids appear to have practically the same percentage composition (of oleic, linolic and linolenic acids) in both kinds of wood.

The amount of the total (unsaturated) fatty acids fraction is higher in the green wood glycerides (84.6%) and free acids (46.9%), than in the seasoned wood (43.5%), while the seasoned wood contained a percentage (30.2%) of oxidized fatty acids, indicating that a considerable portion of the liquid fatty acids

of the green wood become oxidized or polymerized during the process of seasoning.

PART IV: ESSENTIAL OIL.

Only α-pinene could be identified with any certainty in this oil. Various other terpenes may be present, but the test made for these gave negative or indefinite results, probably due to the fact that insufficient of the pure material was available with which to try the reactions. The pinene was found to have high optical activity, $[\alpha]_D^{24} = +39.0^0$, similar to various American turpentines.

The essential oil was found to be present in the wood in very small amounts, in every case. It forms from 1.5 to 4.3% of the total resinous material. No apparent variation in properties or in the percentage were noticeable in the oil from green and seasoned wood.

UNSAPONIFIABLE MATTER:

This was isolated from the rest of the resins and completely separated, by two different methods, into three distinct products; (a) phytosterol, (b) resene, (c) polymerized terpenic oils. No waxes were found present. The amounts of (a) and (b) were extremely small but appreciable amounts of the polymerized terpenic substances were present especially in the seasoned wood, where extensive polymerization of the essential oil has probably occurred during the period of storage. As already mentioned, the amounts of sterols and resene present in the seasoned wood were

appreciably less than in green wood, probably on account of similar transformations.

FINAL REVIEW.

The present thesis is concerned only with the devising of appropriate technique for the separation of the constituents of the crude resins of Jack Pine as they exist in the original wood, and with the identification of these individual constituents. The investigation includes not only "green" but also "seasoned" wood, since, in this way, it was hoped to throw new light on the factors relating to the changes, if any, in the chemical composition of the crude resins, which take place during the storage of Jack Pine logs.

In the above general summary, it has been pointed out that the main points of difference between the resinous content of "green" as compared with "seasoned" wood, are the following:

1. In "seasoned" wood, the glyceride fat content has decreased by about 5%, while the free fatty acid content also shows a decrease of about 5%.
2. The percentage of total resin acids is practically the same in each kind of wood, but the "seasoned" wood contains a higher percentage of amorphous, transformed acids than the "green" wood.
3. The percentage of the total unsaponifiable fraction is much greater in the "seasoned" wood, this being due to a marked increase in the amount of polymerized terpenic substances.

It will be noted that the differences in the character of the two classes of resins, from "seasoned" and "green" Jack Pine, respectively, are associated with the changes taking place in the character and amount of certain constituents, such as the unsaturated fats and fatty acids, on the one hand, and with the resin acids, on the other. The former contain unsaturated, readily oxidisable and polymerisable substances, such as oleic, linolic and linolenic acids, while the latter represent a group of products characterized by marked instability under the influence of light, heat and oxidising agents.

As regards the bearing which these changes might have on the matter of pulping such wood efficiently, the question as to whether any of such changes are facilitated by the action of solutions of sulfurous acid and bisulfites at an elevated temperature, cannot be answered until the necessary experiments involved in such a research have been carried out, but it seems quite possible that marked changes of one or more of the constituents may take place under such conditions. On the other hand, the opportunity afforded the resinous constituents in "green" wood during storage for a year or more on the wood pile, possibly provides a time-period and environment in which the more unstable constituents of the fats, fatty acids and resin acids are converted into more inert substances of such a character as to indicate a certain degree of inertness under the conditions involved in the manufacture of sulfite pulp.

The question of "pitch trouble" is evidently associated to a notable degree with the character of the pitch, in other words, its

"tackiness", - a factor previously pointed out by Campbell.

The solution of the problem would seem to lie in the prosecution of additional investigations, in which each of the individual constituents of Jack Pine resins indicated by the results of this investigation as possibly playing a definite role in "pitch trouble" is treated under pressure and at various temperatures with sulfurous acid alone, and also in the presence of the acid and bisulfites, together with wood chips and sulfite pulp. The possibility, of course, exists, that the effect of "pitch trouble" may be due to the combined action of several constituents, but this seems less likely.

Following the above work, the necessary conditions for the prevention of "pitch trouble" during an "industrial cook", e.g., hydrogen-ion control, addition of emulsifying and dispersing agents, etc., should then be investigated.