CHLOROFORM SOLUBLE
CONSTITUENTS
OF MAPLE SYRUP

THE CHLOROFORM SOLUBLE CONSTITUENTS OF MAPLE SYRUP

by

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A Thesis

Presented to the Faculty of Graduate Studies and Research of McGill University, Montreal, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1937.

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I. Introduction

The fact that the sap of the maple tree could be used for the production of maple syrup was discovered by the Indians, prior to the arrival of the white race. Towards the end of the seventeenth century the French settlers devised better methods for concentrating the sap, and thus created an industry which at present has a value of approximately $4,000,000.00 annually.

The maple sugar industry is confined to Eastern Canada, and to a few states in North Eastern United States. The sap is obtained mainly from four species of maple:

(a) Acer saccharum, Marsh - hard, rock or sugar maple.
(b) Acer saccharinum, L. - soft or silver maple.
(c) Acer rubrum, L. - red maple.
(d) Acer nigrum, Michx. - black maple.

Acer saccharum is the species utilized predominantly in Canada for the production of maple syrup. The sap contains from 3 to 6 per cent sugar and is concentrated in large open evaporators to approximately 65 per cent total solids. The quality or grade of maple syrup is largely dependent on its flavour, and it is this property which is responsible for the high premium that is paid for maple products. The flavour is very characteristic, and though it varies to some extent, it is essentially a distinct odour and taste.

The definition of the word flavour given in the Oxford Dictionary is as follows: "The element in the taste of a substance which depends on the cooperation of the sense of smell." It is a fundamental fact that a substance that is odourous must be soluble in both lipoids and water in order to affect the sensory organs. Bearing the above points in mind, we note that a flavour and a perfume have at least two points in common:

(1) Volatility.

(2) Partial solubility in water.

Whereas our knowledge of essential oils and perfumes has made tremendous strides in the last decade, our knowledge of flavours is still rudimentary. The literature on the subject of natural flavours is very limited. The chemical nature of the constituents responsible for flavours in fruit, grain, vegetables, and meats is largely unknown. The commercial flavouring extracts on the market today are in practically all cases a "concentrate" of the material, rather than the pure constituent or constituents responsible for the flavour, for example, strawberry and celery extracts.

One of the chief difficulties underlying the isolation of natural flavours is the fact that there is no method for the exact estimation of the concentration of odour. Furthermore, it has been found that, generally speaking, flavours are extremely elusive, and are converted to odourless products.

A knowledge of the chemistry of maple flavour would be of distinct value to the maple industry. The mechanism of its formation, and the conditions governing the optimum production of maple flavour in syrup, would place the industry on a more scientific basis. Previous investigators have found that chloroform extracts the flavouring principle from maple syrup. The present investigation is primarily an attempt to fractionate the various constituents of the chloroform extract, without impairing the product or products responsible for maple flavour, and to determine the chemical nature of the various products with special reference to the flavouring principle.

II. Historical

Prior to Robison's (24) research, the references regarding the nature of maple flavour can be considered as merely conjectural. Due to the strongly aromatic odours pervading the air in the vicinity of a sugar camp, Wiley (29) considered that maple flavour was probably an ether or aldehyde of high boiling point. Sy (28) distilled maple syrup and found that the distillate was practically odourless. He concluded that the flavouring substance is only slightly volatile.

Robison (24) attempted to isolate maple flavour by extraction of maple sugar with organic solvents. The following solvents were tried; benzene, chloroform, carbon tetrachloride, ether, ethyl alcohol, acetone, ethyl acetate, and the last three were discarded because they dissolved too much sugar. He considered that benzene was the best solvent, the residue having an excellent maple odour. The material was largely a resinous solid, containing a small percentage of a yellow oil. He found that bases tend to precipitate the material and that acids gave colour reactions.

He also attempted to obtain a concentrated maple extract by: (1) precipitation of the sugar in maple syrup with calcium oxide, and (2) adsorption of the flavour on charcoal. The first method proved unsuccessful and in the second he found that although charcoal adsorbed the flavouring principle, he could not recover it unchanged.

Nelson's Investigation

Nelson (19) first attempted to isolate the flavouring principle by using the Sale and Wilson (26) patent for obtaining a pure maple concentrate. This method involves precipitation of the sucrose of maple syrup by the use of barium hydroxide. He concluded that the barium process must have caused some change in the flavouring principle.

He then attempted to adsorb the flavouring material with norite. One litre of syrup was diluted with one litre of water, was stirred for 20 minutes with 50 grams of norite, and was then filtered. The norite was washed with water, dried, and exhausted with ether in a Soxhlet extractor. After evaporation of the ether, a red oil remained, in which the maple flavour was indistinct. The syrup filtered from the norite, however, had lost its maple flavour. He concluded that the flavour, although adsorbed by the norite, had either not been recovered from it, or had become changed and lost its identity.

Direct extraction of syrup with ether yielded a residue which had an intense maple odour. He found that this odour was very sensitive to caustic alkali.

Ether extracts of two 38 litre lots of maple syrup were fractionated as follows:

(1) Acids were separated by treatment with dilute sodium bicarbonate solution.

(2) Sodium bisulfite fraction: The bisulfite fraction was acidified with dilute sulphuric acid, and extracted with ether. 127 milligrams of material were obtained from 9 gallons of syrup. Of this amount approximately half was soluble in petroleum ether, yielding in one case a crystalline product. This product gave positive tests for vanillin, but crystallized in plates (vanillin - needles) and melted at 74 to 76°C. The odour was similar to vanillin, but crystallographic examination showed that the substance was not vanillin.

(3) Ammonium hydroxide fraction: After extraction with sodium bisulphite the ether solution was extracted with dilute ammonia, pouring the resultant ammonia solutions into dilute sulphuric acid and extracting with ether. A reddish yellow residue weighing 0.65 gram, which had an intense maple odour, was obtained. The substance changed easily to a dark coloured resin, at the same time losing its character-

istic maple odour.

(4) The ether solution remaining after the above extractions was taken to dryness, and yielded a very small odourless residue which partially crystallized on standing.

Nelson concludes: "Maple flavour appears to depend to a great extent on an unstable phenolic substance or substances, the exact nature of which remains undetermined. It seems a reasonable hypothesis that it may be related to the vanillin-like substance associated with it."

Skazin's Work

Skazin (27) made the important observation that maple flavour is not present in the sap, but develops during the concentration at atmospheric pressure. By concentrating sap under reduced pressure, or by the freezing method, he obtained syrups which had an odour resembling watermelons. Maple flavour could be developed in these syrups by boiling at atmospheric pressure.

Extraction of maple syrup with various solvents yielded the following results.

Solvent	Parts per million	Colour	Maple Odour
Carbon tetrachloride	14	pale yellow	No
Petroleum ether	17	" "	No
Carbon disulphide	18	reddish yellow	Approximate
Benzene	33	" "	"
Ethyl ether	38	yellow	Yes
Chloroform	57	reddish brown	Yes

Skazin attempted to isolate maple flavour by direct extraction of maple syrup with chloroform. He fractionated the chloroform extract by Nelson's method, and obtained essentially the same three fractions.

(1) Sodium bisulphite fraction. Three gallons of syrup yielded an oily residue weighing 48 milligrams, which had a strong odour of vanillin. Extraction of this material with petroleum ether yielded a white crystalline product (needles) mixed with a small quantity of yellow oil, weight 13 milligrams. This fraction gave various colour reactions for vanillin, and melted at 77°C to 79°C. One microcombustion analysis gave results approximating the composition of

vanillin (H 4.16%, C 64.00%; Vanillin H 5.2%, C 63.2%).

(2) Ammonium hydroxide fraction. The residue was reddish yellow, resinous, and had an agreeable odour. Microcombustions yielded the following values. H 7.4%, C 66.6%.

(3) Final residue. This fraction was a dark brown resin insoluble in water, slightly soluble in ether, and easily soluble in 10 percent aqueous potassium hydroxide, alcohol, and chloroform.

Skazin summarizes his results as follows:

(3 gallons of syrup)	grams	per cent of total weight
1. Sodium bisulphite extract		
(a) soluble in petroleum ether	0.013	1.4
(b) insoluble in petroleum ether	0.035	3.6
2. Ammonium extract	0.062	6.4
3. Resin	0.660	67.7
4. Unknown parts (by difference)	0.204	20.9
	0.975	grams

He concludes: "Maple flavour appears to depend to a great extent on vanillin, or vanillin-like substances. Some other compound which constitutes the flavour is also present."

Labrie's Investigation(15)

(1) Extraction of Maple Sap and Syrup

Eighteen litres of maple syrup of 30 per cent solids ~~was~~ were treated with emulsin, and then extracted with ether. An amorphous brown residue which had an intense maple odour was obtained. Extraction of this residue with petroleum ether yielded a very small amount of a crystalline powder which was very unstable. The crystals melted at about 60°C. and gave the colour reactions of vanillin. Treatment of fresh sap in the same way yielded a resinous residue. By rapidly washing this with petroleum ether he obtained a crystalline product which he states was vanillic acid. A strong odour of guaiacol was noted in this residue. Labrie concluded that the aromatic material of maple syrup is an unstable complex product. The solid part is composed of vanillin and vanillic acid, and the oily part is guaiacol.

(2) The occurrence of enzymes and glucosides in the maple.

Labrie reports the presence of an enzyme in maple bark, which showed slight hydrolytic action on coniferin and amygdalin, and also a partially crystalline product which gave the colour reactions of coniferin. A search for these products, and also coniferyl alcohol and coniferylaldehyde in maple syrup proved unsuccessful.

(3) "Hadromal"

The last section of his thesis is devoted to an attempt to show that maple flavour is due to the formation of a substance called "Hadromal." This material was first obtained by Czapek (5) in 1899, by treating wood with stannous chloride and extracting with benzene. It is said to be a yellowish microcrystalline substance, melting at about 80°C., and giving most of the reactions of lignin. According to Hoffmeister (10) this compound is closely related to coniferylaldehyde, but this is disputed by Pauly and Feuerstein (21), and by Grafe (9), who maintain that it consists of a mixture of wood gum, tannin, pyrocatechol, and a small quantity of phenolic substances - principally vanillin.

Labrie prepared hadromal from maple wood, and obtained a yellow resinous product which had a maple odour. It gave somewhat the same absorption spectrum and the same products of destructive sublimation as did the ether extract of maple syrup, the products being vanillin, vanillic acid, and guaiacol. He synthesized a product, which he states had the properties of hadromal, by refluxing a 5 per cent solution of sucrose, vanillin, and guaiacol for one hour, in a slightly acid medium. The solution was then made alkaline with sodium carbonate, and refluxed for from 4 to 5 hours. Extraction with ether yielded a yellow amorphous powder with an odour resembling maple products.

He concluded that maple flavour is due to the formation of hadromal during the concentration of the sap.

Findlay's Work

Search for Precursors

1. Findlay (8) confirmed Skazin's observation, that the characteristic flavour of maple syrup and sugar is not present in the sap, but is developed during the boiling. An exhaustive search for the precursor of maple flavour in sap, maple wood and vacuum sugar (sugar obtained by concentration of the sap at reduced pressure) was undertaken. The possibility that the hydrolysis of a glucoside might give rise to the flavouring principle suggested itself, but no evidence for the presence of glucosides was obtained.

Extraction of vacuum sugar and sap with ethyl acetate removed the precursor of maple flavour. Fractionation of the ethyl acetate extract yielded (1) succinic acid, (2) an amorphous product (C 62.7, H 6.9, OCH_3 10.1 per cent), and (3) a dark, sharp smelling viscous oil. Findlay concludes: "An amorphous material obtained from the ethyl acetate extract was probably an altered form of the precursor. It had the properties and composition of lignin and was not directly convertible into the flavouring principle."

2. Study of the Flavour of Maple Syrup

Extraction of 20 gallons of syrup yielded a dark red residue which weighed 10.0 grams, and had an intense odour of maple. A comparison of a number of colour reactions of this material with those of ferulaldehyde showed a fair similarity. The residue was dissolved in a small volume of alcohol, and was poured into water. The water-insoluble material is referred to as the "maple polymer." It was an amorphous product melting over a wide range of temperature, had a faint maple odour, and was slightly soluble in dilute ammonium hydroxide.

Analysis of the maple polymer gave the following values: C 63.10, H 6.85, OCH_3 14.58.

Two amorphous derivatives of the maple polymer were prepared:

(1) 2,4-dinitrophenylhydrazone, m.p. 119-120°.

Analysis: C 48.6, H 4.9, N 11.5 per cent

(2) Diphloroglucide, m.p. above 250°.

Analysis: C 61.46, H 5.64, OCH_3 7.01 per cent

Findlay's Summary of the properties of flavour extracts

"It has been shown that the chief constituent of the flavouring matter as extracted by chloroform or ether is a phenolic polymer giving color reactions similar to coniferyl aldehyde. The formation of the hydrazone and diphloroglucide showed that there is present a small amount of an available carbonyl group, which from the analyses must be closely related to ferulaldehyde. The failure to obtain other characteristic condensation products in sufficient quantity for identification indicated that the carbonyl group is not very reactive, or is masked in some way."

He further states: "The exact nature and method of formation of maple flavour cannot be determined from data available at present, but the results of this investigation indicate that it is related to compounds involved in the formation of lignin."

He postulates: "It is possible that the precursor of the flavour is an unstable form of lignin which is converted by heat into an aromatic aldehyde related to ferulaldehyde, which polymerizes either in maple syrup itself or on isolation."

Review of the Previous Investigations into the Nature of Maple Flavour

In reviewing the work of previous investigators one point in particular must be borne in mind, namely, that Nelson was the only worker who has claimed to have retained an intense odour of maple while proceeding through two steps of fractionation. He states that maple flavour is not extracted by sodium bisulphite, but is by ammonium hydroxide, and can be recovered by acidifying. Skazin found it impossible to fractionate the chloroform extract by Nelson's method, without changing the constituent responsible for maple flavour. He neither confirms nor denies Nelson's statement regarding the extraction of maple flavour by ammonium hydroxide.

Findlay devoted a great deal of time to an attempt to isolate the precursor of maple flavour. Extraction of the precursor from maple sap and vacuum sugar by the use of ethyl acetate yielded a yellow oily product. Attempts to develop maple flavour in this material by heat proved unsuccessful. Fractionation yielded an amorphous lignin-like compound and a reddish oil. Since we do not know the constitution of the constituent responsible for maple flavour, and since the precursor is odourless, it is quite obvious that any attempt to decide which product is the responsible one is at best speculative. Findlay believed that the amorphous lignin-like compound was the precursor.

On a direct attempt to isolate maple flavour from syrup, Findlay obtained 10 grams of crude extract which were dissolved in alcohol, and poured into a large volume of water. The water-insoluble product is referred to as the maple polymer, and without establishing the purity of this material he determined the percentage carbon and hydrogen, and compared the values obtained with coniferylaldehyde. We can assume from this that he considers the chloroform extract to consist of only one substance. This assumption is hardly tenable in view of Nelson's and Skazin's work.

Similarly, Labrie states that he isolated various products but gives no indication as to a systematic procedure that may be followed in fractionating the various constituents. No quantitative data are presented in proof of the conclusions that the various products are present. Although he states that hadromal is responsible for maple flavour, no method is given whereby one could isolate this product.

It is unfortunate that the latter two workers have not followed Nelson's method in attempting to isolate maple flavour. For instance, the question as to whether maple flavour can be extracted by ammonium hydroxide, has not been satisfactorily confirmed.

In conclusion, it appears that although a great deal of valuable work has been done recently with regard to the development of maple flavour from maple sap (Skazin and Findlay), no results have been reported since Nelson's work which have furthered our knowledge as to a systematic procedure to be used for the isolation of maple flavour.

III. Experimental

Fractionation of The Chloroform Extract

The object of this investigation was (1) to develop a method for the isolation of maple flavour, (2) to determine its chemical nature, and (3) to determine the chemical nature of the other products present in the chloroform extract of maple syrup. The value of any method depended on (1) whether it fractionated the products, and (2) whether it left maple flavour unimpaired. Many methods could be found which satisfied the requirements of the first stipulation, but very few were satisfactory in regard to the second. The great instability of maple flavour has complicated this problem to a marked degree. Various methods have been utilised and the results leading up to the method finally adopted will be reviewed.

The maple syrup used in this investigation was of high quality, medium color, and good flavour.

Method

Two gallons of maple syrup, one and a half gallons of water and 1500 cc. of chloroform were transferred to a five gallon glass bottle placed in a motor-driven butter churn. The churn was rotated for four hours,and the chloroform layer was drawn off by the use of a suction pump. An additional 1500 cc. of chloroform were added, and the contents again rotated for four hours, The two chloroform extracts were combined, and concentrated at atmospheric pressure to a volume of approximately 200 cc. The chloroform concentrate was stored in the ice-box until required. On completing the extraction of the required number of gallons of syrup, the total chloroform concentrate was taken to dryness at reduced pressure in an all-glass Claissen distilling flask. The residue was a resinous red product, which had an intense odour of maple.

Fraction A. - The Alcohol Insoluble Fraction

After many preliminary experiments on the solubility of this residue, it was found that a large percentage of the material was only slightly soluble in ice-cold alcohol. Therefore the chloroform residue was dissolved in alcohol, using 3 cc. of alcohol for each gallon of syrup, and placed in the ice-box overnight when a yellow oily solid was deposited. The alcoholic layer was decanted, and the solid material washed with 5 cc. portions of ice-cold alcohol. The insoluble product (Fraction A) was equivalent to 50% of the total weight of the chloroform extract for the year 1935 and was absent in the chloroform extract for the year 1936. Examination of Fraction A has shown it to be largely a glycerol ester (fat). Nelson (19) states: "The residue from the Vermont sirup contained a small quantity of a saponifiable oil. This was not found in the corresponding residue from the Michigan sirup, and it was probably an oil used in the kettles to stop foaming."

The results obtained in this study cannot bear out Nelson's conclusions. It hardly appears likely that the maple syrup producers would use a fat in 1935 and discontinue using it for the sugar season 1936. It must be remembered that trials were run on 1, 2, 4, etc. gallons of the 1935 syrup, and in every case the fat was present. Furthermore the syrup came from individual farmers in the Province of Quebec.

In any event, by the use of cold alcohol the chloroform extract of 1935 was fractionated into (1) an oily substance which was odourless, and (2) the maple fraction. The particular advantage of this step was the elimination of the oil, thus rendering the maple fraction more suitable for further work, viz. as a substance which could be dried and powdered.

Fraction B. - The Sodium Bisulphite Fraction

After removal of Fraction A, the red alcoholic solution was taken to dryness in vacuo, in an all-glass Claissen distilling flask, care being taken to prevent oxidation by passing in a slow stream of nitrogen (passed through alkaline pyrogallol). The residue was a scarlet red oily amorphous product which had an intense odour of maple. Previous workers state that sodium bisulphite forms an additive product with a fraction of this material. This point was investigated and their conclusion confirmed.

The method used in obtaining the bisulphite fraction will be briefly outlined.

The scarlet powder was dissolved in chloroform, freshly prepared saturated sodium bisulphite was added, the flask was partially immersed in an ice-bath, and the contents were stirred for six hours with a motor driven stirrer. The bisulphite layer was transferred to a glass-stoppered flask, an additional 25 cc. of sodium bisulphite were added to the chloroform, and the contents again stirred for 3 hours. The combined bisulphite fractions were washed with chloroform, transferred to a glass-stoppered bottle and stored in the ice-box (Fraction B.)

Fraction C. - The Chloroform Extract minus Fractions A+B

The red chloroform solution was dried over anhydrous sodium sulphate, and taken to dryness in vacuo. A viscous red oil was deposited which on further evacuation increased in volume to completely fill the flask. This material was readily pulverized into a scarlet-red amorphous powder, which had an intense maple odour. This powder represented approximately 40 per cent of the total weight of the chloroform extract for the sugar season 1935 and 80 per cent for the year 1936.

A preliminary examination of this material indicated the presence of phenols, acids, carbonyl compounds, and neutral products. Steps were taken to fractionate these products without impairing maple flavour. Various methods have been tried.

Method I. - Distillation of Fraction C.

25 milligrams of the material were transferred to a micro sublimation apparatus. A red viscous oil distilled at a temperature of approximately 100°C, vacuum 0.025 mm. The volatile oil had a pleasant sweet odour; the non-volatile material was odourless. Apparently maple flavour is sensitive to heat and this method is not satisfactory for its isolation. It was also found that steam distillation destroyed

the flavour. The distillation of the constituents of the chloroform extract will be dealt with at greater length elsewhere.

Method 2. - Use of Organic Solvents.

Skazin extracted maple syrup quantitatively with various solvents and found that maple flavour is definitely soluble in both ethyl ether and chloroform. However, he states that chloroform extracted 57 parts per million whereas ethyl ether extracted only 38 parts per million. Furthermore petroleum ether extracted 15 parts per million without dissolving the constituent responsible for maple flavour.

Bearing in mind Skazin's results, it follows that since chloroform extracts almost twice as much material as does ethyl ether, and since both solvents extract maple flavour, it should be possible to obtain a strong maple concentrate by extracting the chloroform residue with ethyl ether.

The amorphous maple fraction was mixed with an equal volume of sand, then transferred to an extraction thimble, placed in a Soxhlet extractor, and exhaustively extracted successively with (1) petroleum ether, (B.P. 30-40°), (2) ethyl ether, and finally chloroform.

Petroleum ether extract

The residue consisted largely of a yellow oil containing a small percentage of white crystals. This fraction contains only 5 per cent of the weight of the maple fraction, and has merely a trace of maple odour. For the year 1935 it was found that the yellow oil was largely saponifiable and belonged to Fraction A. The crystals were acidic in nature, but the quantities obtained were so small that no attempted isolation has been made.

Ethyl ether extract

After the extraction with petroleum ether the material in the thimble still had an excellent maple odour. On extracting this with ethyl ether and taking the ether to dryness, the residue had merely a trace of odour; the ether insoluble fraction was odourless. Only by the most rigid purification of the ether could maple flavour be transferred from the amorphous powder to the ether fraction. Purification was obtained by shaking one litre of ether with 200 cc. of 40 per cent sulphuric acid, and then refluxing the ether over sodium, and distilling just before use. It appears very likely that the peroxides in ether must have destroyed the flavour. Nelson reported that ether diminished the strength of maple flavour but did not determine whether this was due to oxidation. Confirmation that the peroxides were responsible was obtained by adding a few drops of hydrogen peroxide to a strong maple flavour extract. In a few minutes the odour was destroyed.

Using purified ethyl ether, a solution with a deep yellow colour was obtained, and a reddish oil was deposited on the sides of the flask. Fresh ether was used daily and extraction was continued for six days without exhausting the ether soluble constituents. The ether extracts were immediately taken to dryness in vacuo, and the residue dissolved in chloroform, in which solvent maple flavour shows a greater stability.

The chloroform extract was taken to dryness in vacuo, and the residue was a reddish oily product which had an intense odour of maple. On looking at the residue under a microscope a small percentage of white crystals were noted. This material gave the following tests:

Fehling's solution - reduced
Ammoniacal silver nitrate - reduced
Folin-Denis (phenols) - positive
Millon reagent (phenols) - positive
Liebermann reagent (phenols) positive
2,4-dinitrophenylhydrazine (C=O) red precipitate (amorphous)

Xanthate and Acetic anhydride tests(alcohols) -	negative
Basic lead acetate -	grey precipitate
Calcium hydroxide -	grey precipitate
Ferric chloride -	brown coloration followed by dark brown precipitate
Phloroglucinol hydrochloride -	red colour
Concentrated sulphuric acid -	dark red colour
Bromine water -	precipitate

Table I

Summary of the Action of Organic Solvents on Fraction C (30 gallons of syrup)

Solvent	Physical form	Odour	Weight	Percent of total weight
			grams	
Petroleum ether	Yellow oil, white crystals	nil	0.5	5
Ethyl ether	Mixture of red oil and amorphous solid	excellent maple	4.4	45
Chloroform	Dark red amorphous solid (m.p. 60-90°)	nil	4.8	50

By removal of Fractions A and B, and extraction of the residue with ethyl ether, the constituent responsible for maple flavour was concentrated into 20 per cent of the total weight of the chloroform extract for the sugar season 1935 and into 40 per cent for the year 1936. Attempts to fractionate this material

(Fraction D) into its various constituents, without destroying maple flavour, were undertaken.

Method 3. Fractionation of Fraction D by the Use of Solvents.

Further fractionation of this material was attempted by the use of various solvents, but in every case it was found impossible to get a clear cut separation of the substances. A partial separation could be effected by the use of either methyl alcohol, or carbon tetrachloride which were immiscible with the red oil. However, on taking the extracts to dryness the residues were all oily and had faint maple odours. The constituent responsible for maple was present in all the fractions. Fractional solubility in ether yielded an amorphous odourless product, and an oily product which had a fair maple odour. Other solvents tried were carbon disulphide, benzene, amyl alcohol, dioxane, ethylene glycol, pyridine, ethyl acetate, and acetone. In all cases a definite overlapping of the substances occurred, which could readily be shown by testing qualitatively with any of the reagents which had been found to give positive reactions for the ether extract, for example, Millon's reagent. Furthermore, in spite of rigidly purifying all the solvents used, it was found that the substance responsible for maple flavour tended to change and become odourless. It has been found impossible by this method to ascertain definitely which product in the ether extract (Fraction D) is responsible for maple flavour.

Method 4. Fractionation of Fraction D by the Use of Alkalies.

Nelson states that maple flavour is extracted by ammonium hydroxide and can be recovered by acidification of the alkaline solution. Since solvents were ineffective in fractionating the ether extract, an attempt was made to do so by the use of alkalies.

Three grams of fresh ether extract which had an excellent maple odour were dissolved in 100 cc. of chloroform, and extracted successively with (1) 5 per cent solution of sodium bicarbonate, (2) 1:10 ammonium hydroxide solution, and finally, with 2 per cent sodium hydroxide solution.

Sodium Bicarbonate Fraction

The chloroform solution (red colour) was extracted 25 times with 20 cc. portions of 5 per cent sodium bicarbonate solution. The colour of the chloroform layer changed from a red to a blood-red colour. The alkaline solution was washed with chloroform, acidified with dilute hydrochloric acid, and extracted with chloroform, which was dried over anhydrous sodium sulphate and taken to dryness. The residue was a red resinous product having a slightly rancid odour; weight 0.20 gram.

The Ammonium Hydroxide Fraction

The chloroform solution (blood-red colour) was then extracted 20 times with 40 cc. portions of 1:10 sodium hydroxide solution. Difficulty was experienced in breaking the emulsion that formed. The chloroform layer changed from a blood red to an orange colour, resembling a suspension of antimony sulphide. The combined alkaline solutions were washed with chloroform, acidified and extracted with chloroform. The chloroform extract was dried over sodium sulphate, and taken to dryness. The residue weighed 0.31 gram, and was a light red amorphous solid.

The Sodium Hydroxide Fraction

The material extracted by sodium hydroxide was an amorphous brown odourless solid, which weighed 1.6 grams.

The Neutral Fraction

This fraction weighed 0.70 gram. It was a reddish oily product having a faint pleasant odour.

Table II

Summary of the Effect of Alkalies on the Ether Extract (Fraction D)

3.0 grams (equivalent to 20 gallons of maple syrup)

Treatment	Weight of material obtained grams	Physical State	Colour	Odour	Per cent of total weight
5% Sodium bicarbonate	0.20	resinous	red	slightly rancid	7
1:10 ammonium hydroxide	0.30	amorphous solid	brownish red	nil	10
2% sodium hydroxide	1.60	amorphous solid	brown	nil	57
Neutral fraction	0.70	mixture of a solid and an oil	light red	faint pleasant odour	25

A simpler experiment was planned to ascertain definitely (1) whether maple flavour could be extracted by ammonium hydroxide, and (2) whether maple flavour if not extracted was left unchanged in the chloroform layer.

10 cc. of the original chloroform extract of maple syrup were transferred to each of four separatory funnels. Separatory funnels Nos. 1 and 2 were kept as blanks, No. 3 was extracted once with 1:10 ammonium hydroxide solution, and No. 4 once with 5 per cent potassium carbonate solution.

The results are summarized below:

	Treatment	Colour of the residue	Odour
(1)	Blank	Light red	Strong maple
(2)	Blank	Light red	Strong maple
(3)	Ammonium hydroxide (neutral fraction)	Deep red	Sweet odour
(4)	Ammonium hydroxide extract	Light yellow	Vanillin
(5)	Potassium carbonate (neutral fraction)	Deep red	Sweet odour
(6)	Potassium carbonate extract	Light yellow	Vanillin

Both aqueous ammonium hydroxide and potassium carbonate convert maple flavour to a compound which has a sweet odour, and also change the color of the chloroform extract from a light red to a scarlet red colour. In contrast to Nelson's results, in which he found that ammonium hydroxide extracted nearly all the substances present in the ether extract, we find that only 17 per cent is extracted. This is in agreement with Skazin's work. Furthermore, it was found impossible to confirm Nelson's conclusions regarding the extraction of maple flavour by ammonium hydroxide. A possible reason for the marked discrepancy in the results may be found in the nature of the syrups. Nelson worked on Michigan and Vermont syrups, whereas this work was done on Quebec syrup. It has been found in this study, that marked differences occur not only in the relative quantities of the products present in the chloroform extract from year to year but also in their chemical nature, for example, Fraction A (totally absent in 1936), and Fraction B (dealt with elsewhere.

From the above results it becomes apparent that in the isolation of maple flavour the use of alkalies must be avoided. The question as to which fraction of the ether extract is responsible for maple flavour is still undecided. However, by a study of the ether insoluble fraction, which was odourless, and relating these products to substances present in the ether extract, some indirect evidence was obtained.

The Ether-insoluble fraction (See Table I, page 26)

This fraction is a dark brown amorphous powder (5.2 grams from 30 gallons of syrup). It is odourless, melts between 60° and 90°, and is completely acidic in nature. This material was fractionated by the use of barium hydroxide into (1) material precipitated by barium hydroxide, and (2) material extracted from the chloroform solution by barium hydroxide but not precipitated. These two fractions were then separated into their acidic and phenolic constituents. The nature of these products will be dealt with at length in the second part of this thesis, under the section dealing with the lignin-like substances. It is sufficient at this stage to indicate that the ether-insoluble fraction is a mixture of four or more high molecular weight compounds. A comparison of these products with the acidic products obtained from the ether-soluble fraction definitely showed a close relationship, and this suggests that some product in the ether extract, other than the acidic substances, is responsible for maple flavour. This point will be discussed at greater length elsewhere.

The Neutral Fraction (Table II, page 30)

This fraction consists of a red oily viscous product which has a sweet pleasant odour. As yet no method has been found to isolate this fraction without changing maple flavour. Indirect evidence as stated previously would indicate that maple flavour is due to a neutral compound, and therefore some product in this fraction, although in an altered form, is responsible for maple flavour. A preliminary examination showed the presence of at least three products, namely, (1) A small percentage of white crystals, (2) an amorphous solid, and (3) a reddish viscous oil. Fractionation of these products by the use of solvents rendered the material odourless. Any attempt, from this point on, to decide which constituent was responsible for maple flavour was quite speculative.

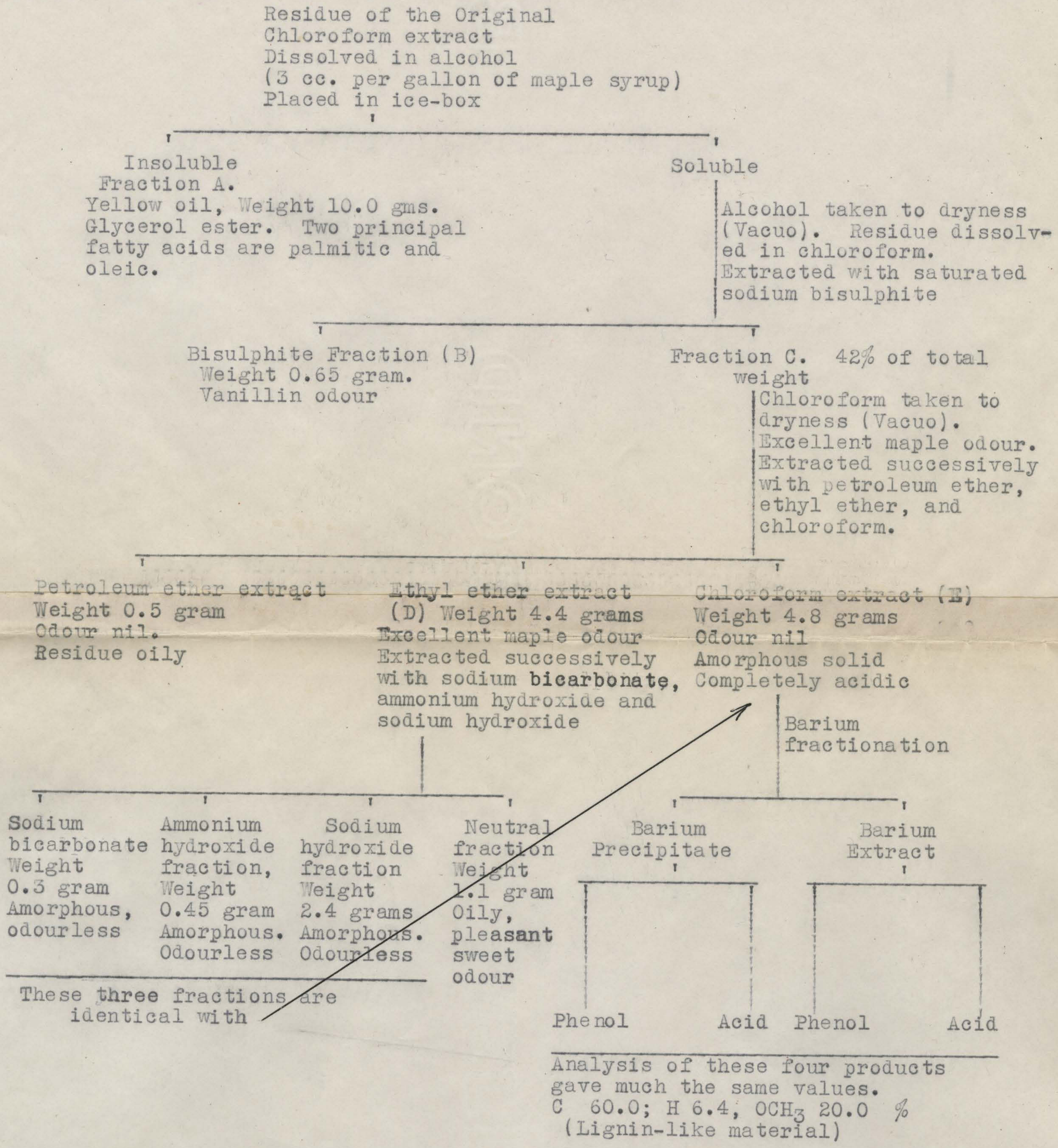
At the conclusion of this phase of the work, it was felt that some new method must be obtained for the isolation of maple flavour. Organic solvents were ineffective, and alkalies changed the flavour. The problem is greatly complicated by the fact that although a large percentage of acidic material is present (See Table II, page 30), this must be fractionated without recourse to alkalies. Furthermore, maple flavour is quite unstable, and repeated fractionation reduces the strength of the odour, for example, the use of ether. A further difficulty is the fact that maple flavour must always be kept in solution, preferably chloroform, since in the dry state it becomes odourless within a couple of days.

A summary of the separation of Fractions A and B, and of Methods 2 and 4 is given in the flow sheet on the following page.

TABLE III

Flow Sheet.

Fractionation of the Chloroform Soluble Constituents of Maple Syrup by the Use of Organic Solvents (Sugar Season 1935). (30 gallons of maple syrup).



Residue of the Original
Chloroform extract
Dissolved in alcohol
(3 cc. per gallon of maple syrup)
Placed in ice-box

Insoluble
Fraction A.
Yellow oil, Weight 10.0 gms.
Glycerol ester. Two principal fatty acids are palmitic and oleic.

Soluble

Alcohol taken to dryness (Vacuo). Residue dissolved in chloroform. Extracted with saturated sodium bisulphite

Bisulphite Fraction (B)
Weight 0.65 gram.
Vanillin odour

Fraction C. 42% of total weight

Chloroform taken to dryness (Vacuo).
Excellent maple odour.
Extracted successively with petroleum ether, ethyl ether, and chloroform.

Petroleum ether extract
Weight 0.5 gram
Odour nil.
Residue oily

Ethyl ether extract
(D) Weight 4.4 grams
Excellent maple odour
Extracted successively with sodium bicarbonate, ammonium hydroxide and sodium hydroxide

Chloroform extract (E)
Weight 4.8 grams
Odour nil
Amorphous solid
Completely acidic

Barium fractionation

Sodium bicarbonate
Weight 0.3 gram
Amorphous, odourless

Ammonium hydroxide fraction,
Weight 0.45 gram
Amorphous. Odourless

Sodium hydroxide fraction
Weight 2.4 grams
Amorphous. Odourless

Neutral fraction
Weight 1.1 gram
Oily, pleasant sweet odour

These three fractions are identical with

Barium Precipitate

Phenol

Acid

Barium Extract

Phenol

Acid

Analysis of these four products gave much the same values.
C 60.0; H 6.4, OCH_3 20.0 %
(Lignin-like material)

Conclusion:- Although maple flavour is changed by this method, indirect evidence suggests that the neutral fraction is responsible for maple flavour.

Method 5. The Use of Water as a Solvent for Maple Flavour.

During this investigation it was often noted that the chloroform soluble constituents of maple syrup were slightly soluble in water. It is a known fact that substances which are odourous must be partially soluble in water; and with these points in mind, an attempt was made to extract maple flavour by the use of water. It was found that the aqueous extract had both an excellent maple odour and taste. The method used for the extraction of maple flavour was as follows.

The procedure outlined for the separation of Fractions A and B was carried out as usual. The pulverized amorphous maple fraction was then transferred to a 500 cc. Erlenmeyer, 250 cc. of freshly boiled and cooled distilled water were added, and the contents stirred vigorously for two hours in an atmosphere of nitrogen, using a motor driven stirrer. The aqueous solution (light yellow colour) was transferred to a glass stoppered bottle, the contents saturated with nitrogen, and then stored in the ice-box. Fresh water was used every three hours and extraction continued, using in all approximately 60 cc. of water for each gallon of maple syrup. To facilitate complete extraction, the water-insoluble material was dried in vacuo, over concentrated sulphuric acid and solid sodium hydroxide in an atmosphere of nitrogen, repowdered, and again extracted, using an additional 50 cc. of water for each gallon of syrup.

The combined aqueous extract was concentrated in vacuo to a small volume in an atmosphere of nitrogen. Although the temperature of the bath never exceeded 45°C. the intensity of both the maple odour and taste was diminished. The aqueous concentrate was transferred to an all glass continuous extractor, and exhaustively extracted with chloroform, the solvent dried over sodium sulphate, and taken to dryness in vacuo. The residue consisted of an oily yellow resinous product which had both an excellent maple odour and taste.

The water soluble material gave the following tests.

Folin Denis (phenols)	-	positive
Millon's Reagent (phenols)	-	positive
Ammoniacal silver nitrate	-	reduced
Fehling's solution	-	reduced
Schiffs' Test (aldehydes)	-	nil
Sodium nitroprusside test (methyl ketones)	-	nil
Phloroglucinol hydrochloride	-	red colour

Bromine water - yellow precipitate

Basic lead acetate - grey precipitate

Ferric chloride - red precipitate

2.4-dinitrophenyl hydrazine (C=O group) - red precipitate

Xanthate test (alcohols) - nil

These tests give results identical to those obtained for the ether extract (See page 25), and this suggests that the aqueous extract is composed of the same constituents. However, it was thought that probably the relative proportions of these products might show a great difference. Steps were taken to determine this point.

Determination of the Percentage Phenolic Material in the Aqueous Extract

100 milligrams of the aqueous residue were dissolved in 5 cc. of alcohol, fresh bromine water was added, and a light coloured voluminous product was precipitated. The precipitate weighed 9 milligrams, and this indicates that only a small fraction of the water-soluble material is phenolic in nature. This confirms previous work, in so far that it suggests that maple flavour is not due to a phenolic substance.

Use of Alkalies in the Fractionation of the Aqueous Extract.

Attempts to fractionate the ether extract by the use of solvents or alkalies without destroying maple flavour had proved unsatisfactory (Methods 3 and 4). Nevertheless some method must be devised for the separation of the acidic and the neutral products. Experiments were conducted to find a method for fixing the acidic substances in the aqueous solution in the form of their salts, by the use of mild alkalies without impairing maple flavour. Then by extraction with chloroform the neutral fraction might be isolated.

Experiment I - Use of Potassium Bicarbonate

0.5 gram of potassium bicarbonate was added to 75 cc. of the aqueous concentrate. The solution was transferred to an all glass continuous extraction apparatus, and exhaustively extracted with chloroform. It was then acidified with dilute hydrochloric acid and again extracted. The two chloroform extracts were dried over sodium sulphate and taken to dryness in vacuo, thus yielding

		Weight grams	Odour
(1)	Fraction not fixed by potassium bicarbonate	0.20	maple
(2)	Acid fraction	0.015	slight rancid

The acid fraction consisted of a resinous red substance which had a faint rancid odour. The fraction not fixed by potassium bicarbonate consisted of an oily light grey solid. It gave a positive test for a carbonyl group, reduced Fehling's solution, and gave positive tests for phenols.

Experiment II - Use of Ammonium Hydroxide

An attempt was made to fix the phenolic as well as the carboxyllic substances by carefully neutralizing 75 cc. of the aqueous concentrate with dilute ammonium hydroxide, using phenolphthalein as an indicator. The following two fractions were obtained.

		Weight	Odour
(1)	Fraction not fixed by ammonium hydroxide	0.24 gram	pleasant,sweet.
(2)	Fraction fixed by ammonium hydroxide	0.021 "	slight rancid.

The acidic fraction appeared identical with the one obtained by the use of potassium bicarbonate. However, the other fraction, although having the same appearance, had a sweet rather than a maple odour. This experiment confirms previous results, in that it indicates that ammonium hydroxide does not combine with maple flavour. The non-carboxyllic fraction had a sweet pleasant odour, and throughout this investigation it has been found that this odour is closely related to maple odour, and is probably the result of some slight change in its constitution. Examination of this fraction indicated the presence of phenolic material.

Experiment III - Use of Barium Hydroxide

The Sale and Wilson Patent (26) is based on the assumption that barium hydroxide will precipitate the sucrose in maple syrup without impairing maple flavour. With this point in mind, 75 cc. of the aqueous concentrate were made alkaline with N/10 barium hydroxide. It was hoped that barium hydroxide would combine with both the acidic and the phenolic material, thus making it possible to obtain the true neutral fraction. The following two fractions were obtained.

	Weight grams	Odour
(1) Fraction not fixed by barium hydroxide	0.24	sweet
(2) Fraction fixed by barium hydroxide	0.03	slight rancid

The fraction not fixed by barium hydroxide gave a positive Millon and Folin-Denis test for phenols. The two fractions appeared identical with those obtained by the use of potassium bicarbonate and ammonium hydroxide. In all three cases the alkalies combine with a red resinous acid, which comprises approximately 10 per cent of the weight of the aqueous extract.

Furthermore all three alkalies are ineffective in fractionating the phenolic from the neutral substances.

Experiment IV - Use of N/10 Sodium Hydroxide

Although past experience has shown that maple flavour is extremely sensitive to alkalies, a final attempt was made in that direction. The aqueous concentrate obtained from 20 gallons of syrup was carefully titrated potentiometrically with N/10 sodium hydroxide to a pH of 8.1. The pH of the original concentrate was 6.4 and it required 8.0 cc. of N/10 alkali to neutralize the extract. The alkaline concentrate was then transferred to an all glass continuous extractor, and the extraction of the substances not fixed by sodium hydroxide was carried to completion. Carbon dioxide was then passed through the aqueous solution and the phenolic fraction was obtained. Finally the aqueous concentrate was acidified with dilute hydrochloric acid and the true acid fraction was extracted.

Table IV

Fractionation of the Aqueous Extract by the Use of N/10 Sodium Hydroxide (20 gallons of maple syrup)

Fraction	Weight grams	Odour
Neutral	0.60	Sweet burnt odour
Phenolic	0.07	nil
Carboxyllic	0.05	Slight rancid

The Neutral Fraction

This is the fraction not fixed in aqueous solution by sodium hydroxide at a pH of 8.1. All attempts to obtain this fraction free from acidic substances without impairing or destroying maple flavour failed, but it has the sweet odour that has been found to be closely associated with the true maple odour, and there can be little doubt that the neutral fraction contains the substance responsible for maple flavour. It corresponds very closely in appearance and properties to the neutral fraction obtained from the ether extract. (See page 33). It reduces Fehling's solution, contains a carbonyl group, and gives a negative test for phenols.

Fractionation of the Neutral Constituents by means of Vacuum Distillation

A preliminary experiment indicated that appreciable quantities of the neutral material were volatile at a vacuum of 0.03 millimetres. The total neutral fraction (0.4 gram) was transferred to a micro sublimation apparatus.

The following fractions were obtained.

Fraction (1) - $<140^\circ$C. Oily yellow product which had a sweet burnt odour. On looking at this material under the microscope a small percentage of white crystals were noted. The oily material failed to crystallise after storing it in the ice-box for one month, but during this time the sweet odour changed to a distinctly maple odour.

Fraction (2) - < 215°C. Light orange, odourless, amorphous solid.

Fraction (3) - Non-volatile fraction. This product was decomposed by the heat, because it is now a dark red amorphous solid, whereas before distillation the material was all light colored. It is readily soluble in chloroform and alcohol. This material is equivalent to 50 per cent of the weight of the Neutral Fraction.

It becomes evident from the above experiments that if it were possible to separate the neutral from the acidic products without impairing maple flavour, the problem of fractionating the neutral constituents without destroying the flavour would still require solution. Various tests were made on the three neutral fractions and the most promising methods found for the fractionation of the neutral constituents without recourse to distillation are tabulated below.

Reagent	Fraction (1) ($<140^{\circ}$C.)	Fraction (2) ($<215^{\circ}$C)	Fraction (3) (non-volatile)
Ferric chloride	nil	dark colour & precipitate forms	nil
Lead acetate	nil	Heavy white precipitate	Heavy dark brown precipitate
Solubility in cold concentrated ethyl alcohol	White crystals only slightly soluble	Very soluble	Very soluble

In the development of a future method for the separation of the neutral constituents steps should be taken (1) to eliminate the crystalline product by the use of alcohol, and (2) to precipitate Fraction (2) and (3) with lead acetate. Findlay found that lead acetate precipitates a fraction of the chloroform extract but did not state whether the flavour was precipitated or remained in solution. This point was investigated and it was found that lead acetate neither precipitated nor impaired maple flavour.

Summary

Although it was found impossible to isolate pure maple flavour from the aqueous extract, several important facts were established, namely:

(1) The substance responsible for maple flavour is not fixed by sodium hydroxide in aqueous solution at a pH of 8.1.

(2) The aqueous extract consists of approximately 10% carboxyl~~l~~ic material, 13% phenolic material, and the remaining 77% of neutral substances.

(3) The neutral fraction has the sweet odour which has been found to be closely related to maple flavour. It consists of at least four substances: (i) white crystals, (ii) yellow oil, (iii) light orange solid, and (iv) non-volatile material. One of these substances is undoubtedly related to maple flavour.

(4) The most promising methods found for the fractionation of the neutral products were (i) use of cold alcohol, and (ii) the use of lead acetate.

(5) The necessity for the absolute avoidance of all alkalies is stressed. Maple flavour is unstable at a pH of 8.1.

(6) In spite of concentrating the dilute aqueous extract, in vacuo, and in an atmosphere of nitrogen, the intensity of both the maple odour and taste diminished.

(7) The weight of the three main fractions, obtained by this method, for the sugar season 1936 was as follows:

	Weight (30 gallons of maple syrup)
Bisulphite fraction	4 grams
Aqueous fraction (maple)	1 gram
Aqueous insoluble fraction	6 grams

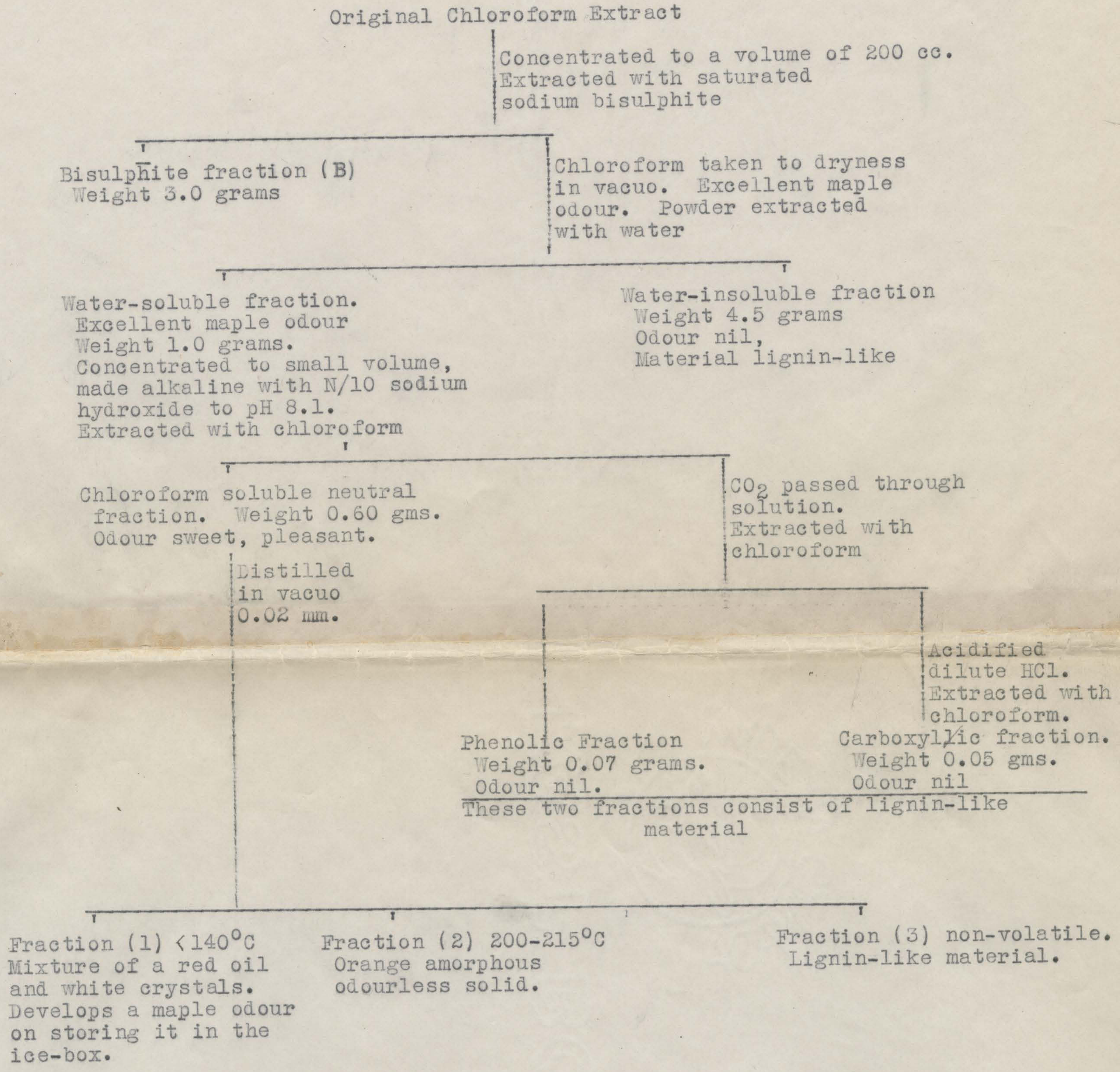
A study of the aqueous insoluble fraction indicated the presence of the same high molecular weight acids and phenols as had been isolated by the previous method (see page 32). These products will be dealt with under the section dealing with lignin-like compounds.

A schematic outline of Method 5 is given on the following page.

Table V.

Flow Sheet (Method 5)

Fractionation of the Chloroform Soluble Constituents of Maple Syrup by the Use of Water (Sugar Season 1936). (20 gallons of maple syrup)



Original Chloroform Extract

Concentrated to a volume of 200 cc.
Extracted with saturated
sodium bisulphite

Bisulphite fraction (B)
Weight 3.0 grams

Chloroform taken to dryness
in vacuo. Excellent maple
odour. Powder extracted
with water

Water-soluble fraction.
Excellent maple odour
Weight 1.0 grams.
Concentrated to small volume,
made alkaline with N/10 sodium
hydroxide to pH 8.1.
Extracted with chloroform

Water-insoluble fraction
Weight 4.5 grams
Odour nil,
Material lignin-like

Chloroform soluble neutral
fraction. Weight 0.60 gms.
Odour sweet, pleasant.

Distilled
in vacuo
0.02 mm.

CO_2 passed through
solution.
Extracted with
chloroform

Acidified
dilute HCl.
Extracted with
chloroform.

Phenolic Fraction
Weight 0.07 grams.
Odour nil.

Carboxylic fraction.
Weight 0.05 gms.
Odour nil

These two fractions consist of lignin-like material

Fraction (1) < 140°C
Mixture of a red oil
and white crystals.
Develops a maple odour
on storing it in the
ice-box.

Fraction (2) 200-215°C
Orange amorphous
odourless solid.

Fraction (3) non-volatile.
Lignin-like material.

Method 6. The Final Method Used in This Investigation for the Fractionation of the Chloroform-soluble Constituents of maple syrup 1936. (50 gallons of maple syrup)

Only approximately 3 per cent of the acidic constituents of the chloroform extract are dissolved by extraction with water (Method 5), whereas the constituent responsible for maple flavour is completely soluble. All the constituents are very soluble in alcohol. Experiments were outlined to determine whether the acidic products could be precipitated, by dilution of the alcoholic extract with water, while leaving maple flavour in solution. This method would have the advantages of Method 5, without the necessity of prolonged extraction, concentration, etc. It was found that at a concentration of 35 per cent alcohol, approximately 85 per cent of the acidic products were precipitated. Maple flavour remained unimpaired in the solution. This observation and also the use of lead acetate was incorporated in the final method used in attempting to isolate maple flavour.

The extraction of maple syrup with chloroform, and the concentration of the chloroform extract was carried out as previously outlined. Since Fraction A was absent in the maple syrup for the year 1936, the first step in the fractionation was the use of sodium bisulphite. This was carried out as previously outlined.

The Use of Lead Acetate

After the removal of the bisulphite fraction, the chloroform extract was diluted with chloroform to a volume of 500 cc. and vigorously shaken with 2 per cent lead acetate solution (1000 cc.). This step must be performed in a dark coloured bottle as the moist lead precipitate darkens when exposed to light. The weight of the lead precipitate was 2.4 grams. The chloroform (700 cc.) was then dried over anhydrous sodium sulphate, transferred to a dark colored bottle, and stored in the ice-box. On taking a few cc. of the chloroform extract to dryness, an excellent maple odour was noted.

The Use of Other Precipitating Agents

In an attempt to precipitate additional constituents from the chloroform extract, experiments were conducted using copper chloride, barium chloride, ferric chloride, copper sulphate, mercuric sulphate, potassium platinic chloride, cinchonine sulphate, tannic acid, phosphotungstic acid, and picric acid. In every case no precipitate developed. On using basic lead acetate, calcium hydroxide, and barium hydroxide heavy precipitates formed. The basic compounds destroyed maple flavour so their use was discarded.

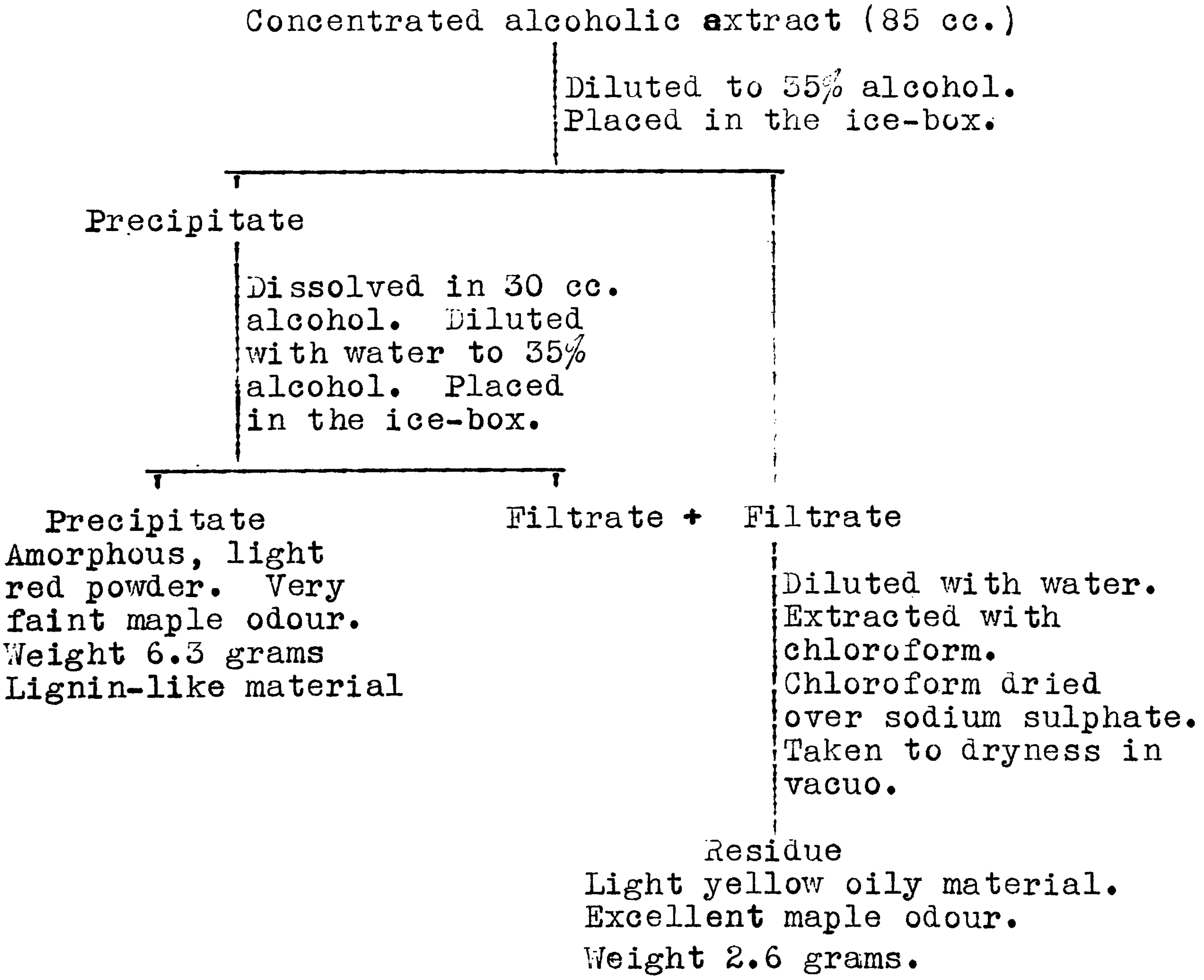
The Use of Absolute Alcohol

The chloroform solution was taken to dryness in vacuo, and the residue dissolved in 85 cc. of absolute alcohol (wine-red colour). On placing the alcoholic solution in the ice-box overnight a greyish product was precipitated. The solution was filtered and the material insoluble in cold alcohol was recrystallized - weight 0.090 gram. Approximately 50 per cent of this material sublimed at a temperature of 120°C, vacuum 0.03 mm. It is an odourless neutral product, and is undoubtedly the white crystalline substance which was present in the yellow oil (see page 42). Since this product is odourless, and was present in such small quantities, no attempted identification has been made.

at what temperature?

Precipitation of the Lignin-Like Material by the Use of 35 Per Cent Alcohol

Recently boiled and cooled distilled water was slowly added with constant agitation to the alcoholic extract, until a concentration of 35 per cent alcohol was attained. A fast flow of nitrogen (passed through alkaline pyrogallol) was passed into the solution throughout this operation. The 35 per cent alcoholic solution was well stoppered and placed in the ice-box for 24 hours. A heavy red resinous precipitate settled, which was redissolved in 30 cc. of alcohol, and again precipitated with water. The two filtrates were combined, diluted with water, extracted with chloroform, the solvent dried over sodium sulphate and taken to dryness in vacuo. An oily yellow residue, which had an excellent maple odour, was obtained. These steps are illustrated as follows:



Concentrated alcoholic axtract (85 cc.)

Diluted to 35% alcohol.
Placed in the ice-box.

Precipitate

Dissolved in 30 cc.
alcohol. Diluted
with water to 35%
alcohol. Placed
in the ice-box.

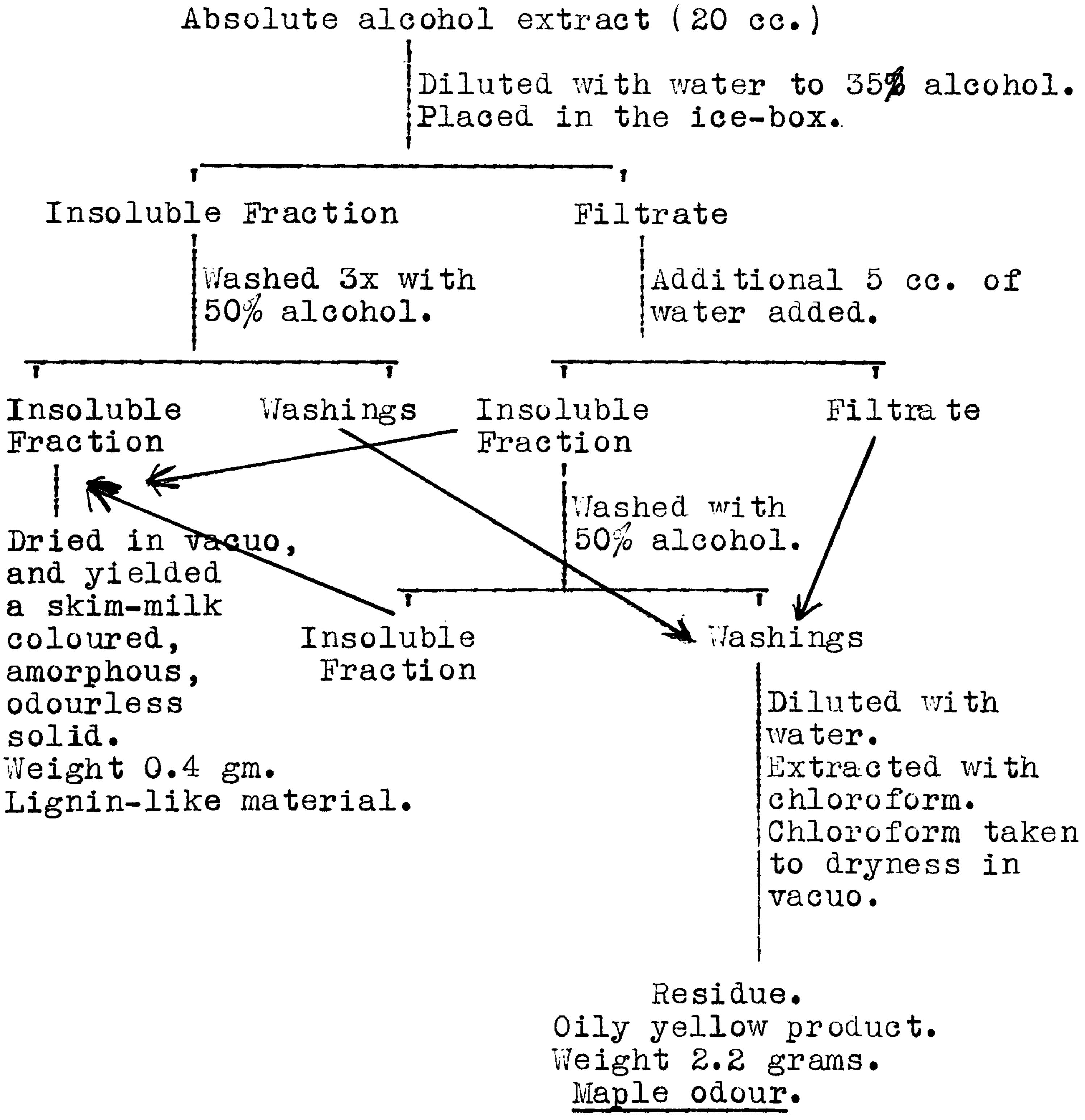
Precipitate
Amorphous, light
red powder. Very
faint maple odour.
Weight 6.3 grams
Lignin-like material

Filtrate + Filtrate

Diluted with water.
Extracted with
chloroform.
Chloroform dried
over sodium sulphate.
Taken to dryness in
vacuo.

Residue
Light yellow oily material.
Excellent maple odour.
Weight 2.6 grams.

Further precipitation of the acidic constituents was attained as follows. The maple oily residue (2.6 grams) was dissolved in 20 cc. of alcohol. This material was quite soluble in 50 per cent alcohol, but deposited a resinous product at a concentration of 35 per cent alcohol. These steps are best illustrated as follows:



Absolute alcohol extract (20 cc.)

Diluted with water to 35% alcohol.
Placed in the ice-box.

Insoluble Fraction

Washed 3x with
50% alcohol.

Filtrate

Additional 5 cc. of
water added.

Insoluble
Fraction

Washings

Insoluble
Fraction

Filtrate

Dried in vacuo,
and yielded
a skim-milk
coloured,
amorphous,
odourless
solid.
Weight 0.4 gm.
Lignin-like material.

Washed with
50% alcohol.

Insoluble
Fraction

Washings

Diluted with
water.
Extracted with
chloroform.
Chloroform taken
to dryness in
vacuo.

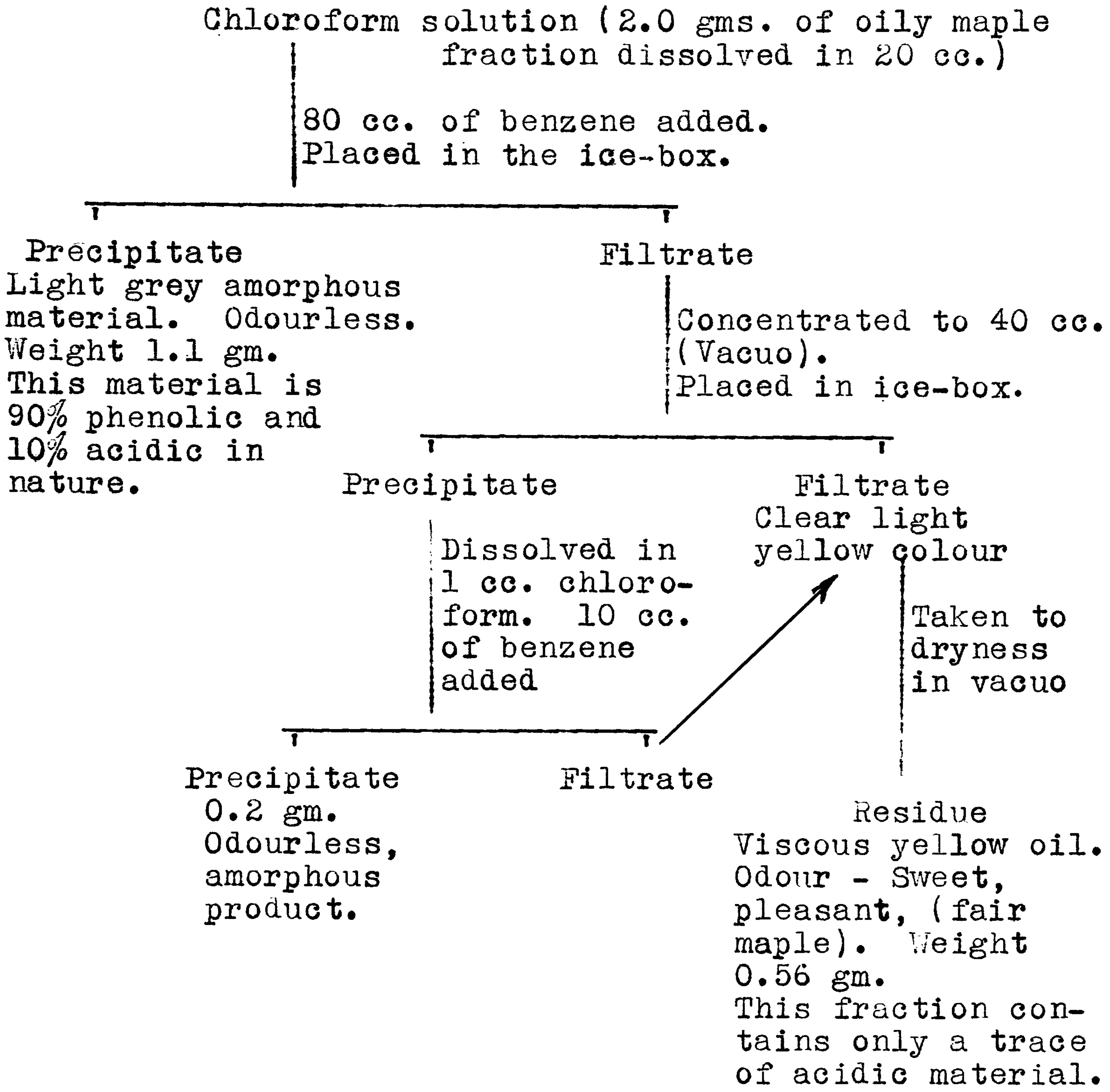
Residue.
Oily yellow product.
Weight 2.2 grams.
Maple odour.

Attempts to concentrate the maple fraction further, by taking the residue up in a smaller volume of alcohol and again precipitating acidic substances by the addition of water proved ineffective. The residue is an oily yellow solid, identical in appearance with the extract obtained by the aqueous method (See page 37). It has an excellent maple odour. A quantitative determination of the percentage acidic substances in this material gave a value of approximately 60 per cent.

Use of Benzene or Ether

Previous work has shown that the acidic substances are only slightly soluble in benzene and ether, whereas maple flavour is soluble in both these solvents. The maple residue was dissolved in 20 cc. of chloroform to which 80 cc. of benzene were added with constant shaking. A heavy grey suspension formed and slowly settled on the sides of the flask.

The benzene-chloroform solution (light yellow colour) was decanted, and concentrated in vacuo to a volume of 40 cc. and again placed in the ice-box. The method is illustrated as follows:



Chloroform solution (2.0 gms. of oily maple fraction dissolved in 20 cc.)

80 cc. of benzene added. Placed in the ice-box.

Precipitate
Light grey amorphous material. Odourless. Weight 1.1 gm. This material is 90% phenolic and 10% acidic in nature.

Filtrate

Concentrated to 40 cc. (Vacuo). Placed in ice-box.

Precipitate

Dissolved in 1 cc. chloroform. 10 cc. of benzene added

Precipitate
0.2 gm. Odourless, amorphous product.

Filtrate

Filtrate
Clear light yellow colour

Taken to dryness in vacuo

Residue
Viscous yellow oil. Odour - Sweet, pleasant, (fair maple). Weight 0.56 gm. This fraction contains only a trace of acidic material.

The problem of eliminating the last traces of acidic material from the neutral fraction without recourse to alkalies has yet to be solved. In the work here outlined this separation was effected by the use of N/10 sodium hydroxide (2 cc.). The neutral material will be dealt with under the section dealing with "The Maple Fraction."

Discussion

The method is quite simple, and starting with a new batch of syrup there can be no doubt that an excellent maple oily fraction which contains only a small percentage of acidic material can be obtained. Care must be taken to carry out the various operations in an inert atmosphere, rigidly purify all the solvents, and to distil the solvents under reduced pressure. In the work here outlined more than two months elapsed from the beginning to the last step of the method, and consequently the substance responsible for maple flavour was subjected to a much greater chance of change than if all the operations had been carried out within a week. Also, it is believed that in future work ether would probably be more suitable than benzene for the final precipitation of acidic substances.

A schematic outline of Method 6 is given on the following page.

Table VI.

Final Method Used for the Fractionation of the Chloroform Soluble Constituents (1936)
(50 gallons of maple syrup)
Method 6.

Original Chloroform Extract

Extracted with saturated $NaHSO_3$

Bisulphite Fraction (B)
Weight 6.5 gms.

Chloroform layer diluted to 500 cc. Shaken with lead acetate (5% solution).

Lead acetate precipitate
Weight 2.4 gms.
Amorphous, odourless material.

Chloroform layer taken to dryness (vacuo). Excellent maple odour. Dissolved in 80 cc. alcohol. Placed in ice-box.

Precipitate
Weight 0.09 gm.
Mixture of white crystalline product and a yellow oil.
Odour - nil.

Alcoholic solution diluted with water to 35% alcohol.

Precipitate.
Weight 6.7 grams.
Odour - very faint maple
Mixture of high molecular weight acids and phenols.
(Lignin-like)

Alcoholic solution taken to dryness. Excellent maple odour. Weight 2.2 gms. Residue taken up in 10 cc. chloroform and 90 cc. benzene added.

Precipitate.
Weight 1.1 gm. Odourless.
Mixture of 90% phenolic and 10% carboxylic material. (Lignin-like)

Solution taken to dryness. Viscous yellow oil. Weight 0.6 gms. Maple odour. Distilled in vacuo 0.02 mm.

Fraction (1) 100°C.
Weight 0.3 gram.
Reddish oil.
Sweet odour.
<u>Develops maple odour on standing in the ice-box.</u>

Fraction (2)
Non-volatile.
Lignin-like material.

IV. The Chemical Nature of the Various Products Isolated from the Chloroform Extract of Maple Syrup

Fraction A. The Alcohol-Insoluble Fraction

This fraction constituted approximately 50 per cent of the total weight of the chloroform extract of maple syrup for the sugar season 1935, and was found to be totally absent for the year 1936. It is a light yellow oil which solidifies when left in the ice-box. The elimination of this oil from the chloroform extract was essential, as it made it possible to obtain the remaining material in the dry amorphous form.

Although Fraction A was odourless, steps were taken to determine its chemical nature. It was thought that perhaps the oil might be related to maple flavour. This work was done previous to the arrival of the 1936 syrup, and in view of its total absence for that year, it is obvious that the oil can bear no relation to the flavouring substance. However, the results obtained are briefly outlined.

On standing at room temperature, the oil deposited a large crop of crystals (m.p. 54-57°C.) which on examination proved to be crystallised fat.

The oil had the following constants:

Refractive index (20°C.)	-	1.457
Acid number	-	nil
Saponification No. (oil)	-	270
Iodine No. (Hübl) oil	-	41-43

Separation of Saponifiable from Non-Saponifiable Fraction

15 grams of oil were saponified by the A.O.A.C. method (20).

(a) The Unsaponifiable Fraction

This fraction weighed 0.28 gram. It consisted of a viscous yellow oil which had a strong shellac-like penetrating odour. Both the Liebermann-Birchard and Whitby tests for sterols gave negative results.

(b) The Saponifiable Fraction

The alkaline solution was acidified with dilute sulphuric acid and heated to boiling. The fatty acids formed a white solid cake when placed in the ice-box. This cake was washed twice with 100 cc. portions of boiling water, the solution being cooled each time in the electric refrigerator, and the fatty acids allowed to solidify. The saturated and unsaturated acids were separated by the Lead Salt-Ether Method, with the following results:

Fraction	Weight grams	Iodine Value (Hübl)	Mol Weight (titration)
(i) Saturated fatty acids	6.4	6 - 7	261
(ii) Unsaturated fatty acids	5.6	88 - 90	275

The Unsaturated Fatty Acids

Both the Iodine Value and Molecular Weight obtained for this fraction correspond closely to the values required for oleic acid:

Oleic Acid - $C_{18}H_{34}O_2$ -	Calculated	Iodine Value	90
		Molecular Weight	282
	Found	Iodine Value	89
		Molecular Weight	275

The unsaturated acid was a light yellow oil. Bromination of this oil in glacial acetic acid yielded no crystalline bromo derivatives, thus indicating the absence of more highly unsaturated fatty acids.

The Saturated Fatty Acids

The saturated fatty acids were fractionated by a method recommended by Rosenthaler (25),which involves fractional crystallization from alcohol. The melting point and molecular weight of the various fractions is given in the following table.

Fraction		Weight grams	Melting Point	Molecular Weight (titration method)
Fraction 1		1.05	62-64°C.	261
2		1.40	60-61	256
3		1.20	57-61	254
4	lost			

The corresponding values for palmitic acid are: Melting Point 62.6o; Molecular weight 256. Although Fraction 1 was recrystallized from alcohol, no higher melting product was isolated.

Unfortunately Fraction 4, which contained the fatty acids soluble in 50 cc. of ethyl alcohol at 0°C.,was lost. Possibly a small fraction of myristic acid may be present. The principal saturated fatty acid present in the oil is palmitic acid. A very small percentage of stearic and myristic acids may be present.

The Water-Soluble Volatile Acids

The combined washings from the insoluble fatty acids were made alkaline, concentrated on the water-bath to a volume of 50 cc., reacidified with dilute sulphuric acid, and subjected to steam distillation. The distillate required 59.5 cc. of N/10 alkali for neutralization. Calculated as butyric acid (1 cc. N/10 alkali is equivalent to 0.0088 mgm.) this corresponds to 0.52 gram. The percentage soluble volatile fatty acids in the oil is 3.4.

(c) Detection of Glycerol

After steam distillation, the aqueous solution was taken to dryness on the steam bath, and tested for glycerol. A positive acrolein test was obtained on heating the residue with potassium bisulphate. Three colour tests recommended by Morrow (18) for glycerol gave positive tests.

(i) Potassium bromide + salicylic acid - red colour
(ii) Resorcinol + glycerol - deep red colour
(iii) Thymol + glycerol - wine-red changing to blue.

(d) The White Crystalline Product (m.p. 55-57°C.)

The oil was stored for two months at room temperature and during this time deposited a large crop of white crystals. Seven to ten recrystallizations from ethyl-ether were sufficient to free the crystals completely from the yellow oil. The crystalline product (needles) melted at 54-57°, and felt waxy to the touch.

Analysis	C	76.2	H	12.4	per cent
Calculated for $C_{53}H_{102}O_6$	C	76.2	H	12.2	per cent.

This product was saponified, and the liberated acids were recrystallized from ethyl alcohol. A crystalline white acid was isolated which melted at 62°C, and had a molecular weight of 260 (titration). An unsaturated oil was also present. The presence of glycerol was detected by the tests previously mentioned. This product is undoubtedly crystallized fat, and by assuming the presence of two molecules of palmitic acid and one molecule of oleic acid combined in the form of a glycerol ester, we get a product which has an empirical formula of $C_{53}H_{102}O_6$. The theoretical saponification value for the above product is 202, and the value experimentally obtained was 215.

Fraction B. The Sodium Bisulphite Fraction

Both Nelson and Skazin extracted the ether or chloroform extract of maple syrup with saturated sodium bisulphite, decomposed the bisulphite fraction with sulphuric acid (1:4), extracted this with ether, and obtained an oily residue which had a strong odour of vanillin. By extraction of this residue with boiling petroleum ether, a small fraction of crystalline material was isolated, which gave the various colour reactions of vanillin. It melted at 74-76°C (Skazin) and 77-79°C (Nelson). Nelson made a crystallographic examination of these crystals and concluded that his product was neither vanillin nor its ethyl homologue. Although Skazin states that his crystalline product was contaminated by a yellow oil, he performed one micro analysis with the following results: C 64.0, H 4.2 (Vanillin C 63.2, H 5.2). He concluded that in all probability the product was vanillin. Labrie reported the presence of vanillin in the ether extract of maple syrup but gave neither the analysis nor melting point of the isolated product. He appears to have based his conclusions largely on colour reactions. Findlay attempted to duplicate the work of Nelson and Skazin but was unable to isolate the crystalline product.

It has been found in this investigation that a large variation occurs both in the chemical nature and in the relative quantities of the substances present in the bisulphite fraction, from one year to the next. This observation is in agreement with the results obtained by previous investigators. Using identically the same method, Nelson isolated a crystalline product from the Michigan syrup but was unable to do so from the Vermont syrup. His product was quite stable. Labrie and Skazin state that the isolated crystalline product was very unstable, readily changing to a yellow oil. In view of this irregularity, the bisulphite fractions obtained for each year will be dealt with independently.

The Sodium Bisulphite Fraction (1935)

The sodium bisulphite additive product was decomposed as follows:

The bisulphite solution and 200 cc. of chloroform were transferred to a 500 cc. separatory funnel and with constant agitation 10 cc. portions of 10 per cent sodium carbonate solution were slowly added. Caution must be exercised not to raise the pH above 7, since some of the liberated product, or products, are acidic in nature and are converted to their salts, which are then insoluble in chloroform.

The bisulphite layer was exhaustively extracted with chloroform, the solvent dried over sodium sulphate, and taken to dryness in vacuo. The residue was a yellow viscous oil, weight 0.65 gram (30 gallons of syrup). It had a strong odour resembling that of vanillin.

Fractionation of the bisulphite products

(1) Use of Solvents

50 cc. of petroleum ether (38-40°) were added to this residue, and the contents were refluxed on a water-bath for one hour. This operation was repeated six times. On cooling the extract to 0°C, a small quantity of material was precipitated, which on examination under the microscope proved to be a mixture of white needles contaminated with a yellow oil. The weight of the total residue from the petroleum ether extract was 40 mgms. (30 gallons of syrup). Attempts to purify this crystalline product by fractional crystallization from petroleum ether proved ineffective. Unfortunately the material obtained from the first lot of syrup was decomposed by exposure to air (1 day). Great care was required since this substance was very sensitive to both heat and oxidation, changing readily to a cherry red oil.

Starting with a fresh lot of syrup identical results were obtained. The petroleum ether extract was impure. Purification was attempted by the use of various solvents; water, alcohol, ether, benzene, etc., and also mixed solvents; ether-petroleum ether, benzene-petroleum ether, and chloroform-petroleum ether. However, in every case this method proved ineffective, since the crystalline product retained a small fraction of the yellow oil. Its isolation was complicated by its instability. A semi-pure product melted at 83-89°C.

(2) Use of Ammonium Hydroxide

0.2 gram of the original residue was taken up in 20 cc. of chloroform and extracted with ammonium hydroxide (1:10). The alkaline layer was washed exhaustively with chloroform, acidified, and extracted with chloroform, the solvent dried over sodium sulphate and taken to dryness in vacuo. The residue consisted of a crystalline deposit of silvery white needles, contaminated with only a trace of yellow oil. It had a strong vanillin odour; weight 14 mgms. This crystalline substance reduced Fehling's solution, gave a brown colour with ferric chloride, and decolourized bromine water. It melted at 90-92°C.* A 2.4-dinitrophenyl-

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* All melting points reported in this investigation are uncorrected.

hydrazine prepared by Allen's (1) method was a brick red product, m.p. 215-217°. The percentage methoxyl determined by Clark's (3) modification of the Zeisel method was 12.7 per cent.

Having found an effective method for the isolation of this product, it was believed that in the ensuing year (1936), a sufficient amount would be isolated for the determination of its empirical formula and for the preparation of various derivatives. Unfortunately this product was totally absent in the bisulphite fraction for the year 1936. Its instability excludes the possibility of it being vanillin.

Bisulphite Products for the Year 1936

A marked difference was immediately noted in the action of sodium bisulphite on the chloroform extract for the two years, namely, in 1935 no additive insoluble product was formed, whereas in 1936 a large red gummy product was precipitated. (Weight 4.0 gms. from 30 gallons of syrup.) The insoluble product was washed with water and dried. The washings were added to the bisulphite soluble layer, which was then washed with chloroform, and decomposed by the method described for the previous year. Thirty gallons of syrup yielded a yellow oily residue, weight 0.5 gram. This residue had a strong vanillin odour.

Fractionation of the Bisulphite Products

(1) Use of Petroleum Ether

Prolonged extraction of this material with boiling petroleum ether yielded a small, partially crystalline product (25 mgms. from 30 gallons of maple syrup). This method is unsuitable and was discarded. However, one important fact was noted, i.e. the stopper in the flask was accidentally loosened, and the petroleum ether evaporated spontaneously. The crystals were exposed to the air for over a week and they showed no tendency to decompose. The product was quite stable.

(2) Use of Ammonium Hydroxide

The bisulphite residue in 1935 was composed of (1) an acidic crystalline product, and (2) a neutral oil. By dissolving the residue in chloroform and extracting it with ammonium hydroxide, the two products were separated. Fractionation of the bisulphite residue (1936) was attempted by this method, but it was found ineffective because the total residue consisted only of phenolic substances. Both the crystalline product and the yellow oil, present in the bisulphite fraction in 1936, differed chemically from the products present in this fraction for the previous year.

(3) Use of Vacuum Distillation

After determining by preliminary experiments that at a vacuum of 0.03 mm. the white crystalline product sublimed unchanged, the total residue (0.4 gm.) was transferred to a micro-sublimation apparatus. Three fractions were obtained.

		Physical Form	Percent. of total weight
(1)	Temperature of oil bath 80°	White crystals combined with trace of yellow oil	10
(2)	Temperature of oil bath 120°	Yellow oil	30
(3)	Non-volatile fraction		60

The best yield of the crystalline product was obtained by first distilling or subliming all the products volatile at 150° (bath temperature). This eliminated the non-volatile product which constituted approximately 60 per cent of the total weight of the bisulphite fraction. The volatile material was then subjected to vacuum distillation (0.03 mm.) at a temperature of 80° (bath temperature). The sublimate was a light yellow crystalline substance, and in spite of resubliming this product three times the crystals still retained a tinge of the yellow colour. This substance was quite stable and had a vanillin odour. Attempts to decolorize the crystals by the use of activated charcoal and other adsorbents proved ineffective.

Finally, the crystals were almost completely freed of the yellow oil by fractional crystallization from a mixture of petroleum ether (38-50°) and chloroform. The sublimate was dissolved in chloroform (½ cc.), sufficient petroleum ether was added to just initiate the formation of crystals (turbidity), and the contents were then placed in the ice-box. This operation was repeated 10 to 15 times. The crystalline product (needles), which still had a very faint yellow colour, melted at 110-112°, weight 45 mgms.

Micro analysis C 57.3 H 5.5 CH3O or C2H5O 30.3 per cent

Calculated for
$C_9H_6O_4(CH_3O)(C_2H_5O)$ C 56.7 H 5.5 $CH_3O + C_2H_5O$ 29.8 per cent

Preparation of a 2.4-dinitrophenylhydrazone yielded a scarlet red product, which crystallized from alcohol in the form of long fine needles; m.p. 232-233°. The percentage methoxyl was 12.4%. (Calculated for one carbonyl group 17.5%; calculated for two carbonyl groups 12.3%.) The white crystalline product contains two carbonyl groups.

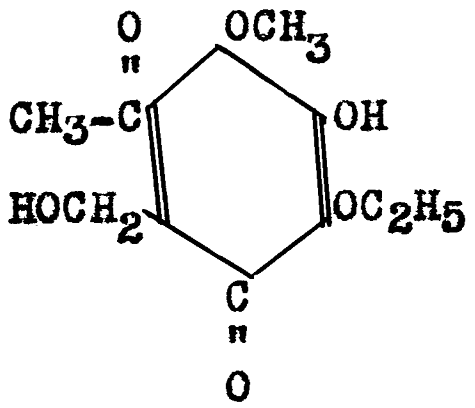
The following tests were made:

Fehling's solution	nil
Liebermann test (phenols)	nil
Millon's test (phenols)	nil
Xanthate test (primary and secondary alcohols)	positive
Sodium nitroprusside test (methyl ketones)	positive
Iodoform test (methyl ketones)	positive
Test for ethylenic linkage (few mgms. of the product were dissolved in chloroform. One drop of alcoholic bromine was added.)	not decolorized, and therefore negative
Test for esters (Feigl (7))	negative
Schiff's test (aldehydes)	negative

Although both the Liebermann and Millon tests gave negative results for phenols, this product is known to be phenolic in nature, as indicated by the second method used in attempting to isolate this product. This apparent discrepancy is readily explained by the fact that the Millon reagent does not react with phenols that are either di-meta- or di-ortho-substituted, and that the Liebermann reagent does not react with phenols that are para- substituted.

A careful search of the current literature, and also Beilstein,and Richter, for a compound of this type was unsuccessful, thus indicating that this product has not been previously described. Its empirical formula is $C_{12}H_{14}O_6$. Two of the oxygen atoms are combined in the form of carbonyl groups, one of which is a methyl ketone. Two more oxygens are accounted for, namely, one in the methoxyl group, and the second in the ethoxyl group. A fifth oxygen is present as an alcoholic hydroxyl, and the last is present in the phenolic hydroxyl group.

Obviously, in view of the limited amount of the product isolated (45 mgm.), it is impossible to establish definitely its chemical constitution. The presence of so many functional groups complicates the problem to a marked degree. An attempt to prepare a crystalline acetate failed. A possible structural formula for this product might be as follows:



$C_{12}H_{16}O_6$ (C 56.2, H 6.2)

This formula does not agree very closely with the combustion values obtained (C 57.3, H 5.5), and is presented only with the greatest of reservation.

Summary

A great deal of time and effort was expended in the attempt to isolate the crystalline product present in the bisulphite fraction. The methods that could be utilised for one year were inadequate the next. This fraction, again, illustrates the complexity that is attendant upon problems concerned with the isolation of natural products. One point has been definitely established, namely, vanillin was not present in the bisulphite fraction for either of the two years. The crystalline product, however, has a vanillin odour. A second point that can be definitely established is the fact that this fraction, although possibly contributing to the final maple odour, does not contain the constituent mainly responsible for both the maple odour and taste.

The Lignin-Like Fraction

Fractionation of the chloroform extract by the methods previously outlined has in all cases given rise to a large amorphous odourless fraction. (See pages 35, 47, 56). Further fractionation of this material has indicated that it is composed of at least four closely related high molecular weight compounds. These products correspond closely to the components of wood, collectively known as Lignin. They bear no relation to the substance responsible for maple flavour, but rather their presence complicates the problem to a marked degree.

They are acidic red amorphous products, m.p. 60-90^{o}, and are partially soluble in sodium bicarbonate or ammonium hydroxide, and completely soluble in dilute sodium hydroxide. They are very soluble in chloroform, alcohol, dioxane, acetone, fairly soluble in benzene, amyl alcohol, and pyridine, slightly soluble in ether, and almost insoluble in petroleum ether, carbon disulphide, carbon tetrachloride and water.

In view of the fact that both Findlay and Labrie concluded that an amorphous lignin-like compound was related to maple flavour, it was decided to make an exhaustive examination of this fraction. Three main methods of fractionation were tried.

(1) Use of alkalies.

(2) Use of various solvents.

(3) Use of barium hydroxide.

Fractionation of the Lignin-Like Products

I (a) Use of Alkalies

Three grams of the ether-insoluble fraction (See page 26) ~~were~~ was dissolved in 100 cc. of chloroform, and successively extracted with 5% sodium bicarbonate, 5% potassium carbonate, 5% ammonium hydroxide, and 2% sodium hydroxide. The four alkaline solutions were acidified, extracted with chloroform, the solvents dried over anhydrous sodium sulphate, and taken to dryness. A comparison of the four residues showed little difference other than shade of colour, and a very slight difference in melting, or rather, softening-point. All the products were amorphous. The results are given in the following table.

Table VII. Fractionation of the Lignin-Like Products by the Use of Alkalies

Fraction	Colour	Percent. of the total weight	Melting Point °C
Sodium bicarbonate	Light brown powder	12	50-60
Potassium carbonate	Dark red resinous	15	not definite
Ammonium hydroxide	Light red; turns granular when washed with ether	30	not definite
Sodium hydroxide	Dark brown powder	38	76-82

Fractionation of the acidic constituents by the use of alkalies was not very satisfactory. In spite of extracting the chloroform layer 25 to 30 times with ammonium hydroxide, the alkaline solution still continued to extract small quantities of the material. Furthermore, emulsions formed which broke only on long standing. Some other method was sought which would sharply fractionate these products.

I (b) Separation of Phenols from the Acids

One gram of the ether-insoluble fraction was dissolved in 100 cc. of N/10 sodium hydroxide. The phenolic substances were precipitated by passing carbon dioxide through the solution for 1 hour. The precipitate was a gummy red product which was found to be largely insoluble in both the alkaline solution and in chloroform. It was very soluble in ethyl alcohol. This method caused some chemical change in the molecule and was not satisfactory for the fractionation of the phenols from the acids.

2. Use of Solvents

Three grams of aqueous insoluble fraction (See page 47) were transferred to a Soxhlet thimble and exhaustively extracted, successively with (1) carbon disulphide, (2) benzene, and finally chloroform. Carbon disulphide extracted only 5% of the material, the residue consisting of an amorphous red odourless product.

Benzene Extract

Extraction was continued for three days without completely exhausting the benzene-soluble constituents. The benzene extract was taken to dryness, and yielded an amorphous red product, weight 1.4 gram. It was dissolved in 2% sodium hydroxide solution and the phenols were liberated by passing carbon dioxide through the solution. The results are best shown diagrammatically.

Benzene residue
dissolved in
2% sodium hydroxide.

Phenols liberated by passing
carbpn dioxide through the
alkaline solution.

Phenols	Acids
A skim-milk coloured product was first liberated, followed by a dark red product. Both phenols are very soluble in chloroform. This was the first indication that two phenols were present in the lignin-like fraction.	Amorphous light brown substance. m.p. 50-52°. This fraction is soluble in 5% sodium bicarbonate

Chloroform Extract

The chloroform extract had a dark brown colour. It was found that the chloroform residue was composed of approximately 75% dark brown phenolic material and 25% red acid material. The phenol softened at a temperature of 80-82° but had no definite melting point. The melting point of the acid was indefinite, ranging between 70° and 80°.

Discussion

It was found, by the use of solvents, that at least two phenols and two acids were present in the lignin-like fraction. Further attempts were made to obtain each of these products in the pure form. A method was finally developed which proved very suitable for the fractionation of these constituents. This method is given in detail.

3. Fractionation of the Lignin-Like Material by the Use of Barium Hydroxide.

4.6 grams of the aqueous insoluble material (See page 47) were dissolved in 300 cc. of chloroform, and with constant stirring, 300 cc. of N/2 barium hydroxide were added. The solution was stirred vigorously for 1 hour and allowed to stand for 24 hours. The contents had settled into:

(1) Aqueous layer - light red colour

(2) Precipitate - light yellow colour

(3) Chloroform layer - faint yellow colour.

The chloroform was distilled off on the water bath and the contents were filtered; weight of precipitate 1.9 gram. The filtrate was exhaustively washed with chloroform, then acidified with hydrochloric acid (1:4), and extracted with chloroform. The chloroform extract was concentrated to a volume of 100 cc. and again shaken with 100 cc. of N/20 barium hydroxide. The weight of the second precipitate was 0.18 gram. The filtrate was acidified and extracted with chloroform (No precipitate developed on shaking a few cc. of this extract with N/20 barium hydroxide). The chloroform extract was then dried over sodium sulphate, and taken to dryness. The residue was a hard resinous product, which when washed with ether pulverized into a light skim-milk coloured powder. It had an odour faintly reminiscent of cocoa, weight 1.8 gms. (Barium aqueous fraction.) It was dissolved in 100 cc. of N/10 sodium hydroxide and the phenolic substances were liberated by the use of carbon dioxide.

Barium Aqueous Phenol

The phenolic substance was light grey in colour, weighed 0.90 grams, and softened at a temperature of 66-68°. It decomposed, giving off a gas at a temperature of 85°. A small portion of the material wad dissolved in chloroform and a few cc. of barium hydroxide were added. No precipitate formed, indicating that this fraction is free of substances precipitated by barium hydroxide. Another portion was dissolved in dilute alkali and carbon dioxide was passed through the solution. The aqueous layer retained part of the material but the greatest percentage was precipitated. The above procedure was repeated three times, with the result that a fraction was obtained free of carboxyllic acid material. Semi-micro combustions yielded the following values: C 60.6%, H 6.4%. The percentage methoxyl was 21.4 per cent.

Barium Aqueous Acid

The acidic fraction was a yellowish light red amorphous product which weighed 0.80 grams. It softened at 50-52°, but never really melted. To test whether this fraction was completely carboxyllic in nature it was dissolved in dilute sodium hydroxide and carbon dioxide was passed through the solution.

This operation was repeated three times before the acidic material was completely freed of phenolic substances. This fraction had a faint pleasant aromatic odour.

Analysis: C 62.6, H 6.5, CH_3O 21.4 per cent.

Barium Precipitated Fraction

The barium precipitate, obtained by the method previously described, was washed exhaustively with chloroform, and then suspended in dilute hydrochloric acid (1:4). The liberated acidic material was extracted with chloroform. The chloroform residue had a red, glossy appearance, resembling a varnish; m.p. 78-82°. This is the fraction largely responsible for the red colouring of the chloroform extract. It should be mentioned that this method is not very satisfactory since a considerable percentage of the material is insoluble in chloroform, indicating that either a change has taken place in the organic material, or that dilute hydrochloric acid does not readily hydrolyze the salt or complex.

The material readily hydrolyzed by hydrochloric acid was dissolved in dilute sodium hydroxide, and the phenolic substances were liberated by the use of carbon dioxide. This fraction contains at least three substances, as shown in the following table.

Table VIII

Products present in the Barium Precipitate

	Fraction	Weight grams	Colour	Analysis C %	H %	OCH3 %
(1)	Acid	0.4	light red	59.0	6.5	19.6
(2)	Phenol (readily decomposed by HCl)	0.8	light red	61.8	6.7	
(3)	Phenol (not readily decomposed by HCl)	0.6	very dark red	46.9	6.1	19.2

As yet no clear cut method has been developed for the separation of the two phenols present in this fraction. The combustion and methoxyl values for substances (1) and (2) are very similar to those obtained for the products in the Barium Aqueous Soluble fraction.

Substance (3) differs markedly in its analysis from the other four products. It is the substance largely responsible for the red colouration of the chloroform extract. This product is quite insoluble in ether, benzene, water, and 50% ethyl alcohol. It gives the typical tests for phenols, and contains a carbonyl group. It also gives a positive test for glucosides (reduction of Fehling's solution after, but not before, hydrolysis with dilute hydrochloric acid). Its empirical formula, which must be accepted with reservation, is $(C_4H_6O_3)_n$. A study of the chemical nature of this product would be of interest, but it was found impossible to obtain it in the crystalline or pure form, and since it cannot bear any relation to maple flavour, further work on this product was discontinued, and it is included under the lignin-like fraction.

A schematic outline of the fractionation of the lignin-like products by the use of barium hydroxide is given in the following table.

Table IX

Fractionation of the Lignin-Like Products by the Use of Barium Hydroxide

4.6 grams of lignin-like material (Method V)

Dissolved in 300 cc. of chloroform. 300 cc. of N/2 barium hydroxide added. Contents well shaken.

- Barium Precipitate
  - Washed with chloroform. Suspended in HCl (1:4). Extracted with chloroform. Chloroform solution extracted with N/10 alkali. CO_2 passed through the alkaline solution.
    - Barium precipitate. Phenol
      - Phenol (not readily decomposed by HCl.) Weight 0.6 gm.
      - Phenol (readily decomposed by HCl.) Weight 0.8 gm.
    - Barium precipitate. Acid. Weight 0.4 gm.
- Barium Aqueous Soluble
  - Acidified HCl (1:4). Extracted chloroform Chloroform layer extracted with N/10 alkali. CO_2 passed through alkaline solution
    - Barium aqueous phenol Weight 0.9 gm.
    - Barium aqueous acid Weight 0.8 gm.
- Neutral Products 0.1 gm. of oily material Heavy odour.

Fractionation of the Ether-Soluble Acidic Products by the Barium Hydroxide Method

2.0 grams of the ether soluble acidic material (See page 30) were treated with barium hydroxide by the procedure previously described. It was found that the ratio of barium aqueous soluble products to barium precipitated products was much higher for this fraction than for the ether-insoluble material. The three main products isolated were:

		M.P.	Weight grams
(1)	Barium aqueous acid	50-52°	0.6
(2)	Barium aqueous phenol	64-68°	0.8
(3)	Barium precipitate phenol	indefinite 90°	0.3

These products are identical in appearance, properties, and melting point to the analagous substances obtained from the ether insoluble or aqueous insoluble fraction. There can be no doubt that the lignin-like compounds are slightly soluble in ethyl ether.

Relation of the Lignin-Like Compounds to Lignin

A comparison of the analyses of four of the products isolated in this fraction with the composition of various proposed formulae for lignin shows a close relationship.

	C %	H %	OCH_3 %
Barium aqueous phenol	60.6	6.4	21.4
Barium aqueous acid	62.6	6.5	21.4
Barium precipitate phenol	59.0	6.5	19.6
Barium precipitate acid	61.8	6.7	
$C_{47}H_{60}O_{16}$ (2)	64.7	6.0	17.7
$C_{40}H_{48}O_{15}$ (11)	62.5	6.3	11.6
$C_{40}H_{44}O_{15}$ (22)	62.8	7.8	16.2
$C_{92}H_{108}O_{38}$ (13)	60.7	5.9	
Findlay's Lignin-Like Products			
(1) Lignin from maple wood	59.7	6.4	
(2) Lignin from vacuum sugar	60.6	6.1	16.3
(3) "Maple Polymer"	63.2	6.8	14.6

Furthermore, although lignin used in structural studies is usually prepared by chemical treatments which liberate it from combination in ligno-cellulose, there is a form of soluble or free lignin present in wood, and this may be obtained by extraction with organic solvents (Von Euler - 6). Recently, Klason (12) has demonstrated that lignin is present in pine sap, and that it forms up to 14% of the dry weight of the sap. Marion (16) extracted wheat straw with 1:1 methanol-benzene. The residue was extracted successively with petroleum ether, ethyl ether and chloroform, and he found that 94% of the chloroform extract consisted of lignin, and also reported a considerable percentage of lignin in the other fractions. Wislicenus (30) reported the presence of free lignin in the sap of the maple tree.

In view of the above investigations, it is not at all surprising to report the presence of lignin in the chloroform extract of maple syrup.

The Nature and Properties of the Lignin-Like Compounds

The four lignin-like compounds isolated under the conditions described for their fractionation, are amorphous powders varying in shade from a cream to a dark red colour. The acid fractions are soluble in dilute sodium bicarbonate and other alkalies. The phenolic fractions are insoluble in sodium bicarbonate, only slightly soluble in sodium carbonate or ammonia, and quite soluble in dilute sodium hydroxide. All the compounds reduce an ammoniacal solution of silver nitrate, but only the phenols reduce Fehling's solution. The barium aqueous soluble acid has a pleasant aromatic odour distinctly recalling vanillin in some cases. The phenols give positive tests with the Folin-Denis, Liebermann, and Millon's reagents. All the products give characteristic red colours with phloroglucinol hydrochloride.

The determination of the acid value of the carboxyllic acid lignin fraction was performed as follows. 0.131 gram of material was suspended in about 10 cc. of distilled water. 5 cc. of N/20 sodium hydroxide were added and the contents allowed to stand for a few hours. The alkaline solution was carefully titrated back to neutrality with N/20 acid, using phenolphthalein as an indicator. The determination is subject to considerable error as the end point was indistinct. 0.131 gram required 2.8 cc. of N/20 alkali. Hence the acid value is 930.

All the lignin-like compounds are unsaturated. They are readily oxidized with potassium permanganate, and are brominated with bromine water to yield amorphous yellow products of high melting points. The phenols are easily methylated with dimethyl sulphate, and are converted to neutral amorphous products.

The phenols gave positive tests for the presence of enolic hydrogen (17). A quantitative determination indicated approximately 43% enolizable hydrogen. The determination was performed as follows. 0.1 gram of material was dissolved in 10 cc. of alcohol. An excess of alcoholic bromine solution was added and after two minutes the excess bromine was destroyed by the addition of α-naphthol. Potassium iodide was then added and the liberated iodine titrated with N/20 sodium thiosulphate. 0.1 gram required 1.1 cc. of N/10 sodium thiosulphate. 100 mgm. of the phenolic material is equivalent to 14 mgm. of iodine. 1 molecule of the phenolic material reacts with one molecule of iodine. Assuming that the phenolic compound has a molecular weight of 800, the percentage enolic hydrogen is $\frac{14}{258} \times \frac{800}{100} = 43.4\%$.

Relation of the Lignin-Like Compounds to Maple Flavour

Judging from the various chemical properties, physical appearance, combustion values, and methoxyl content, there can be little doubt that these amorphous acidic products which constitute approximately 60-70% of the total weight of the chloroform extract (year 1936) are closely related to lignin. Bearing this in mind, the important question is whether these lignin-like compounds are in any way related to the substance responsible for maple flavour. Findlay concluded that the results of his investigation indicate that the formation of maple flavour is related to compounds involved in the formation of lignin. He further states: "The flavouring substance was a reddish brown phenolic material,which gave similar colour reactions to ferulaldehyde."

From the results presented in this investigation it is apparent that Findlay's conclusion is speculative. As stated previously (See page 16), according to Findlay's conclusion, we might assume that the chloroform extract consisted of only one substance. However, in this investigation it was found that the chloroform extract consists of at least ten different products. Furthermore, it was discovered that 60-70% of the chloroform extract consisted of at least 4 high molecular weight lignin-like compounds. Since Findlay found it impossible to fractionate the chloroform extract, he assumed that the total extract consisted of lignin-like material. This assumption is now definitely disproved.

Labrie (15) concluded by stating that the aroma of maple products is principally due to the formation of hadromal during the boiling process, from materials claimed to be in the sap, namely vanillin, guaiacol, and furfural (formed from sucrose during the boiling process). As stated previously (see page 11), the chemical nature of hadromal is very doubtful, but we may consider it as a phenolic lignin-like compound, and the arguments used to discuss Findlay's work are also applicable in this case. An attempt to synthesize hadromal by Labrie's method (See page 11) proved fruitless. Furthermore, a careful examination of the bisulphite fraction for both the years 1935 and 1936 has indicated the absence of vanillin, and Findlay states that vanillin is not present in the sap of the maple tree.

There is one point regarding maple flavour upon which the previous investigators, Nelson, Labrie, and Findlay are agreed, namely, that maple flavour is phenolic in nature. The evidence supporting this fact is of dubious value. Nelson's conclusion was based on the fact that he found that maple flavour could be extracted from its ether solution by ammonium hydroxide and could be recovered by acidification. None of the other workers attempted to verify this point. Findlay's evidence is based on the fact that the "maple polymer" is partially soluble in dilute ammonium hydroxide, or sodium carbonate, but he does not report whether maple flavour could be recovered by acidifying the alkaline solution. Since hadromal is a phenolic compound, it can be assumed that Labrie also believed that a phenol was responsible for maple flavour.

The evidence presented in this investigation definitely shows that maple flavour is not phenolic or lignin-like in nature. The point is discussed fully in Part III of this thesis. The four main methods used in attempting to isolate maple flavour indicate that the lignin-like compounds are incidental, and that a neutral product is responsible for maple flavour.

In Method II, the ether-soluble fraction had an excellent maple odour, whereas the ether-insoluble fraction was odourless. The ether-soluble fraction consisted of approximately 70% lignin-like material, whereas the ether-insoluble fraction was 100% lignin-like. It is obvious that there is some product in the ether-extract, other than the lignin-like products, which is responsible for maple flavour. In Method IV the aqueous extract, which had both an excellent maple odour and taste, contained only approximately 20% lignin-like material, 10% of which was phenolic. In Method V, successive fractions of lignin-like substances were precipitated from the alcoholic extract by the addition of water, while the substance responsible for maple flavour remained unimpaired in solution.

In conclusion, approximately 65% of the material in the chloroform extract of maple syrup (year 1936) consists of lignin-like compounds. These products bear no relation to the substance responsible for maple flavour.

The Maple Fraction

It has been shown in this investigation that the chloroform extract of maple syrup contains a mixture of at least ten substances, and it is a distinct advance to be able to state definitely which of these is the one responsible for maple flavour. This point is established, and the various steps leading up to this fact are dealt with in Part III of this thesis.

The substance responsible for maple flavour is a viscous yellowish red oil , refractive index (20°C) 1.555. In the method used for its isolation, the oil initially had a sweet pleasant odour, but when stored in the ice-box slowly developed one distinctly similar to maple odour. This oil (0.5 gm.) was used up in obtaining the following information regarding its chemical nature.

Analysis: C 70.2, H 9.3, CH_3O 10.2 per cent

Calculated for
$C_{17}H_{25}O_3(OCH_3)$ C 70.1, H 9.1, CH_3O 10.1 per cent

Various qualitative tests were made with the following results.

Alcoholic Ferric Chloride - slight darkening in colour

Millon's test (phenols) - nil

Liebermann test (phenols) - nil

Bromine water - decolorized

Neutral potassium permanganate - reduced

Fehling's solution - reduced

Phloroglucinol hydrochloride - red colour.

Xanthate test (alcohols) - nil

Acetic anhydride test (alcohols) - nil

Kurt Meyer Test (enolic H) - positive

Test for carbonyl group (2.4-dinitrophenylhydrazine) - red precipitate

Iodoform test (methyl ketones) - nil

Action of conc. sulphuric acid - material slowly changes to greenish colour and then goes black.

A qualitative test was made for the ester group by a method recommended by Feigl (7), which involves the combination of hydroxylamine with the esters of carboxyllic acids to give hydroxammic acids. This product combines with ferric chloride to give colored complex salts. The test yielded negative results.

From the above mentioned tests, and also its analysis, several facts regarding the chemical nature of this viscous oil can be enumerated.

1. Empirical formula $C_{18}H_{28}O_4$
2. It contains one methoxyl group.
3. It is readily oxidized.
4. It contains a carbonyl group.
5. It contains enolic hydrogen.

6. The other two oxygen atoms are not present in the form of phenolic, carboxyllic, ester, or alcoholic groups. This fact must be accepted with reservation, since steric hindrance may prevent a functional group from reacting.

Action of dilute N/10 Sodium Hydroxide on the Viscous oil.

The final trace of acidic material was eliminated from the maple fraction by extraction with N/10 alkali (See page 54). The material in the chloroform extract appeared to exhibit the property of retarded neutralization. 1 cc. of N/10 alkali was neutralized instantly, and then for a period of two days, 1 drop was neutralized approximately every 4 to 5 hours. The neutral fraction was then distilled (vacuo 0.02 mm.), and it was found that the volatile fraction now exhibited definite acidic properties, and could be readily extracted by N/10 sodium hydroxide. Also the non-volatile fraction was converted by the heat to an acidic product, which could readily be precipitated from an alkaline solution by the use of carbon dioxide. The properties and analysis of this non-volatile substance show that it is lignin-like in character (C 63.1, H 6.6, CH_3O, 23.1 per cent).

The chemical nature of both the above-mentioned changes is unknown. In the case of the viscous oil this change may in some way be related to the enolizable hydrogen that is present in the molecule.

Determination of the Percentage Enolic Hydrogen in the Viscous Volatile Oil.

10 mgm. of the oil were dissolved in 2 cc. of alcohol, to which an excess of alcoholic bromine solution was added. After one minute, the excess bromine was destroyed by the addition of α-naphthol. Potassium iodide was added, and the liberated iodine was titrated against N/100 sodium thiosulphate.

10.2 mgm. of oil required 1 cc. of N/100 sodium thiosulphate.
1 cc. N/100 thiosulphate is equivalent to 1.27 mgm. of iodine.
One molecule of the oil reacts with one molecule of iodine.
($C_{18}H_{28}O_4$) 308 gms. reacts with 258 gm. of iodine.
1.27 gms. of iodine reacts with $\frac{1.27}{258} \times 308 = 1.52$ gm. of oil.
% enolic hydrogen is $\frac{1.52}{10.2} \times 100 = 15.0\%$.

Preparation of Derivatives

2,4-dinitrophenylhydrazone

0.04 gm. of the viscous oil was dissolved in a few cc. of alcohol, and a 2,4-dinitrophenylhydrazone was prepared according to Allen's directions (1). A brick red amorphous product was isolated, which weighed 8 mgm. By heating the alcoholic solution longer than the time specified in Allen's directions, a slightly better yield was obtained. All attempts to obtain this product in the crystalline form failed. Its melting point was indefinite (110-150°). Apparently this derivative is not a pure compound and the low yield that was obtained suggests that either the viscous oil is a mixture, or that the carbonyl group in the molecule is not very reactive.

The methoxyl content of this derivative was 5.0 per cent. This value does not agree with the value theoretically required for the compound $C_{18}H_{28}O_4$ (one carbonyl group requires 6.4%; two carbonyl groups require 4.6%).

Semicarbazone

0.04 gm. of semicarbazide hydrochloride was dissolved in a minimum quantity of water. 0.03 gm. of fused sodium acetate was dissolved in 95% alcohol along with 0.04 gm. of the oil, and the two solutions were mixed, well shaken, and allowed to stand for 24 hours. No precipitate developed on standing. The solution was diluted with water, and extracted with ether. The ethereal solution was taken to dryness, and the residue consisted of an oily yellow product which appeared to be identical with the original material.

Acetylation

0.020 gm. of the material was dissolved in a mixture of 2 cc. of acetic anhydride and 1 cc. of pyridine. The solution was allowed to stand at room temperature for three days, and was then poured on a small cake of ice. No insoluble product was present.

Oxidation of the Viscous Oil with Nitric Acid (Sp.Gr.1.14)

Meyer (17) states that the side chains of benzol homologues are often split off by the use of dilute nitric acid, and also that this method is useful in the oxidation of fatty substances. To isolate the oxidation product he recommends dilution with water, neutralization, and precipitation of the oxidised product in the form of its salt.

0.025 gm. of the viscous oil was dissolved in 15 cc. of nitric acid and the solution was slowly taken to dryness on the water-bath. The residue was dissolved in acetone (light yellow colour), and transferred to a micro-sublimation apparatus. A volatile product distilled at a temperature of 100°C (bath temperature), vacuum 0.05 mm. 5 mgms. of the volatile oil were dissolved in water, the solution made alkaline with ammonium hydroxide, and the excess ammonia was boiled off. A few drops of silver nitrate were added to the solution but no precipitate developed, although the solution slowly blackened. Since a silver salt could not be obtained, a single micro combustion was performed on the remaining material.

Analysis - C 84.0 H 14.5 per cent

Calculated for $(C_7H_{14})_n$ - C 85.5 H 14.4 per cent. Presumably the carboxyl group in the molecule was split off during the distillation. The product was obtained in very small quantities (6 mgm), and since the purity of this product is questionable, the results must be accepted with reservation.

Oxidation with Potassium Permanganate (neutral).

Excess 2% potassium permanganate was added to 20 mgm. of the viscous oil. The contents were heated on a water-bath for one hour, and then extracted with chloroform. The chloroform residue was an odourless oily product, which showed no tendency to crystallise. The material obtained was insufficient for analysis.

Reduction with Powdered Zinc

Meyer (17) recommends the use of zinc in the formation of oxygen free compounds. 0.04 gm. of the material was mixed with powdered zinc, and heated on a Woods metal bath at a temperature of 300-400°C for four hours. The mixture was then subjected to distillation at a vacuum of 0.04 mm. The volatile constituent was an odourless yellow viscous oil, which weighed less than 1 mgm. No analysis was performed on this product. Extraction of the non-volatile fraction with chloroform yielded a very small oily residue (1-2 mgm.). Apparently this method was too drastic and decomposed the compound.

Optical Activity of the Viscous Oil

10 mgm. of the oil were dissolved in 10 cc. of chloroform, and transferred to a 10 cm. polariscope tube. The chloroform solution did not rotate the plane of polarized light, and therefore the oil does not contain any asymmetric carbon atoms.

The Possible Chemical Nature of Maple Flavour

The determination of the chemical nature of any natural product resolves itself into two main parts, namely, (1) the isolation of the pure product, and (2) its identification, or if the product has not been previously reported, the determination of its chemical constitution. In this investigation it is still questionable whether the first part has been definitely accomplished. Although the author has used relatively large quantities of maple syrup (200 gallons) it is believed that in the future this problem will require still larger quantities of syrup. Fifty gallons of maple syrup yielded only 0.3 gm. of the maple fraction. The complexity that was attendant in the isolation of this material can be readily observed by reviewing Part III of this thesis. In fact, the major contribution of this effort is connected primarily with the methods of fractionation, rather than with any distinct contribution to the elucidation of the chemical nature of maple flavour.

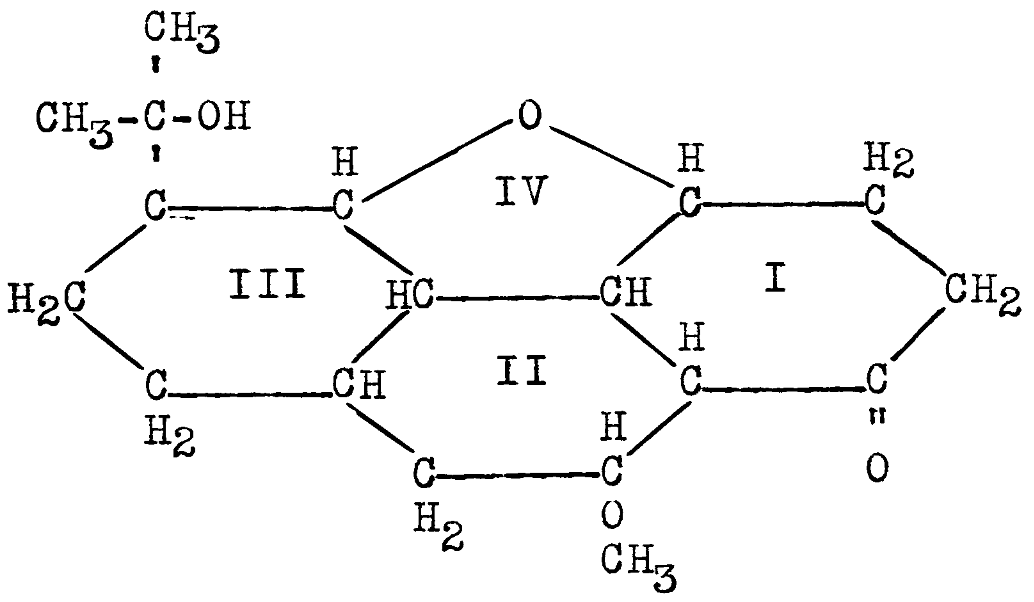
The major difficulties encountered in the determination of the chemical constitution of the viscous oil were (1) the limited quantity of material, (2) the lack of functional groups in the molecule, and (3) the failure to obtain either crystalline derivatives, or crystalline degradation products. At present the available data are wholly inadequate for the formulation of any definite chemical constitution for maple flavour. However, with one assumption, namely, that the viscous oil is a pure compound, we shall try and speculate as to the possible chemical nature of maple flavour.

This compound has the empirical formula of $C_{18}H_{28}O_4$. It contains one oxygen atom in the form of a methoxyl group. A second oxygen atom is present in the form of a slightly reactive carbonyl group. The compound can therefore be written as $C_{16}H_{25}(CH_3O)(C{=}O)O_2$. The parent member of the series is $C_{17}H_{28}(O_2)$, which corresponds to $C_nH_{2n-6}O_2$.

In recent times a large number of substances have been isolated from both vegetable and animal products, which have empirical formulae corresponding closely to the values given above. Many of these products contain the phenanthrene nucleus. By assuming an octahydrophenanthrene nucleus for the viscous oil, the formula could be written as $C_{14}H_{18}\cdot C_3H_{10}O_2$. The isopropyl group is very common to many naturally occurring phenanthrene compounds, e.g. abietic acid. This group would account for the remaining three carbon atoms, thus yielding $C_{14}H_{18}\cdot C_3H_7\cdot H_3O_2$.

The chemical form in which two of the oxygen atoms in the molecule are combined is unknown. Both are non-reactive. Knorr and Pschorr (14) found that the morphine alkaloids which contain a phenanthrene nucleus have one of their oxygen atoms combined in the form of an ether linkage. This type of linkage might easily account for one of the oxygen atoms in the viscous oil. The last one might be linked to the isopropyl radicle in the form of a tertiary alcohol which is prevented from reacting because of steric hindrance.

The structural formula for maple flavour could then be reconstructed as follows.



$C_{18}H_{28}O_4$

The isopropyl group may be anywhere in the molecule. The methoxyl group may be anywhere in the molecule.

The chemical properties of maple flavour will be discussed in relation to the above structural formula.

1. Enolization and Retarded Neutralization

Ring I contains the cyclohexanone group. Schmidt (26a) states that cyclohexanone is a tautomeric compound which exists either as a ketone or a hydrogenated phenol. It should, therefore, contain active hydrogen, and when purified cyclohexanone was tested for enolic hydrogen it gave a positive test. It also exhibited the property of retarded neutralization. Taking the foregoing into consideration, the assigned structural formula could explain both enolization and retarded neutralization in maple flavour.

2. Stability to Alkalies

Maple flavour is extremely sensitive to alkalies, and is converted, depending on the conditions, either to a sweet odour, or to an odourless compound. Ring IV (see structural formula) contains the furane group. This group is also present in morphine in exactly the same position. Pictet and Biddle (23) state that the ether linkage in the furane ring of morphine is readily broken by dilute acids such as sulphuric, hydrochloric, phosphoric, and oxalic; by dilute alkalies, and also by zinc chloride. The converted product (apomorphine) differs physiologically from morphine.

Quite conceivably analogous changes might take place in maple flavour when subjected to alkalies.

3. Reduction of Fehling's Solution

As mentioned above, the ether linkage in Ring IV is easily hydrolysed by alkalies. Also it is known that the hydrogen atom adjacent to the carbonyl group in Ring I is active and forms a hydrogenated phenol. If these two reactions were to take place simultaneously, Ring I would contain the hydroquinone group. This product is an active reducing agent and would readily reduce Fehling's solution.

4. Action of Concentrated Sulphuric Acid

On adding one cc. of concentrated sulphuric acid to 5 mgm. of the viscous oil, the solution slowly changed to a greenish colour, and finally became dark brown. These colour changes are quite similar to those obtained for concentrated sulphuric acid and morphine.

5. Refractive Index

The refractive index of the viscous oil is 1.555 (20°C.), and the value reported by Cook (4) for octahydrophenanthrene is $\frac{19.2}{D}$ 1.5527.

6. The Instability of Maple Flavour

The assigned structural formula for maple flavour could readily explain its great instability. The phenanthrene compounds readily isomerise, and it is this property which has greatly complicated the study of this group of compounds. A typical example is abietic acid, which appears to have a number of isomers, and whose properties vary according to the source and method of isolation. Isolation of this product by distillation in high vacuum yields a product m.p. 158°. By extraction with acetic acid, m.p. 161-5°; by extraction and crystallization direct from colophony, m.p. varying from 137 to 166°.

Discussion

In conclusion, although the structural formula postulated for maple flavour can explain most of the known chemical properties regarding maple flavour, it must be borne in mind that the chemical evidence for this structure is wholly inadequate. Until pure derivatives are prepared and various known degradation products are isolated, no definite structure can be assigned to the flavouring principle of maple syrup.

V. Summary and Conclusions

1. A systematic fractionation of the chloroform extract of maple syrup has shown the presence of at least ten substances. These products were separated into five main fractions: (1) alcohol-insoluble fraction, (2) sodium bisulphite extract, (3) lead acetate precipitate, (4) lignin-like fraction, and (5) the maple fraction. The first three and approximately 95% of the lignin-like fraction were eliminated from the chloroform extract without destroying the maple flavour.

2. The alcohol-insoluble fraction, which was equivalent to 50% of the total weight of the chloroform extract in 1935 and was totally absent in 1936, consisted of a saponifiable oil. A chemical examination showed the presence of glycerol, palmitic and oleic acids, and 3.4% water-soluble volatile acids.

3. The sodium bisulphite fraction was a mixture of at least three substances. It had an odour strongly resembling that of vanillin. The products that were present in this fraction in 1935 were totally different from those present in 1936. In 1935 an unstable crystalline product was isolated m.p. 90-92°. A 2,4-dinitrophenylhydrazone was prepared, m.p. 215-217°,

with a methoxyl content of 12.7 per cent. In 1936 a stable crystalline product was isolated m.p. 110-112°. It has an empirical formula of $C_{12}H_{14}O_6$, with a methoxyl content of 30.3%, which corresponds to one methoxyl and one ethoxyl group in the molecule. A scarlet red crystalline 2,4-dinitrophenylhydrazone was prepared m.p. 232-233°; the methoxyl content was 12.4% (calculated for two carbonyl groups in the molecule 12.3%). Neither of these products have been identified.

4. It was found impossible to confirm Nelson's results regarding the extraction of maple flavour by ammonium hydroxide. Maple flavour is extremely sensitive to alkalies, and its characteristic odour is readily destroyed by ammonium hydroxide.

5. All the results obtained in this investigation indicate that a neutral compound is responsible for maple flavour, and that it is not, as previously considered, phenolic in nature.

6. The lignin-like fraction, which was equivalent to 35% of the total weight of the chloroform extract in 1935 and to 60-70% in 1936, consisted of an amorphous odourless product. This product was a mixture of at least four closely related high molecular weight

compounds, which on analysis yielded approximately the same values; C 61.0, H 6.5, CH_3O 20.8 per cent. These products bear no relation to maple flavour.

7. The maple fraction consisted of a yellow viscous volatile oil, R.I. (20°C.) 1.555. In the method used for its isolation, it initially had a sweet pleasant odour, but when stored in the refrigerator slowly developed one similar to maple odour. Its empirical formula is $C_{18}H_{28}O_4$, and it contains one methoxyl, and one carbonyl group in the molecule. Although the available data are inadequate for the formulation of a definite structure, a postulated structural formula containing a phenanthrene nucleus accounts for many of the observed chemical properties, such as, (1) reduction of Fehling's solution, (2) enolization, (3) instability to alkalies, and (4) ready conversion to an odourless compound.

VI. Acknowledgments

The maple syrup for this investigation was supplied through a grant from the Quebec Department of Agriculture. This grant is gratefully acknowledged. The author wishes to express his sincere thanks to Dr. J. F. Snell, under whose supervision this work was carried out, for his continued interest and helpful suggestions during the course of this investigation; also to Dr. C. F. H. Allen of McGill University and Dr. W. D. McFarlane for their criticism and advice.

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