SOME EXTRACTIVES FROM BLACK SPRUCE (PICEA MARIANA) BARK

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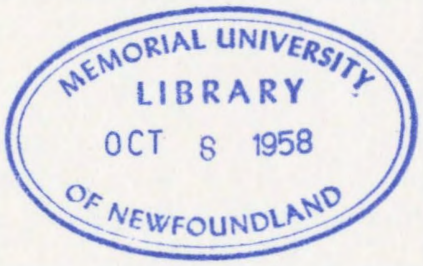

SOME EXTRACTIVES FROM BLACK SPRUCE (PICEA MARIANA) BARK

A Thesis

by

© Vernon W. Hiscock, B.A.

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Abstract

THESIS

Some Extractives of Black Spruce (Picea Mariana) Bark. (V. W. Hiscock)

Bark is a great waste product and comparatively little work has been done to investigate the constituents of the different varieties o f it. A review of the components reportedly found in various barks shows that there are four main classes. These are cellulose, cork, lignin, and minor constituents. These minor chemicals are reported and a short review of the utilization of waste bark is included. Minor chemicals include fats, waxes, fatty acids, alcohols, sterols, resin acids, phlobaphenes, and polysaccharides.

Minor chemicals found in black spruce wood are reported and a section is devoted to methods that have been used for extraction of minor chemicals from barks. Many workers have begun extraction with alcohol but since this solvent extracts, among other things, tannins, petroleum ether was used first, followed by diethyl ether.

The petroleum ether extract has been found to contain steroid type compounds. The crude extract could not be further separated easily using solvents, so chromatography was used. This is an excellent method for separating substances of closely related structure. A volatile oil as well as the steroids is reported.

The Ether extract was found to contain flavone type compounds, one of which is Quercetin (3, 5, 7, 3', 4', -pentahydroxyflavone). This appears as a glycoside which acetylates quite readily to produce the penta-acetate. Quercetin and its methoxy derivative (3, 5, 7, 3', 4', -pentamethoxyflavone) were prepared.

Paper chromatography and spectrographic analysis were used to identify the quercetin compounds.

The petroleum ether extract is 5.6% of the weight of the dry bark, and the ether extract about 4%. Difficulty was experienced in attempting to obtain, by crystallization, pure substances. It was found almost impossible to reproduce, exactly, results of any one run. This is because the whole bark was extracted and also because some changes occur in the bark during storage.

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Some Extractives From Black Spruce Bark (Picea Mariana)

Chemicals Isolated From Barks And Review of Bark as a Waste Product

Bark represents a great waste in industry. Generally it makes up 10-15% of the trees harvested. In Canada in 1951 the pulp and paper industry alone used 15,053,910 cords of unpeeled wood[1] which was practically all spruce. It has been estimated that spruce wood affords about 250 pounds of bark per cord - this means that the yield of bark for the year 1951 was about 2,000,000 tons.

Many mills burn this bark to produce steam, others are forced to burn it just to get rid of it while still others dump it into rivers. The heating value of dry bark can be considered equal to that of wood but bark which is peeled from pulp wood is usually river driven and thus quite wet; unless space and equipment for drying is available the wet bark is still a problem.

Attempts to make structural boards and hardboards from the bark have in many cases produced results which were disappointing.[2] It has been found that generally the boards do not have the strength equal to that of those made from wood chips. Douglas fir bark is used to make Silvacon products by Weyerhauser Timber Co. (Wash. U.S.A.). These products, consisting of ground fibers, are finding use as soil conditioners, in cork products, rubber products, and in flooring adhesives, to mention only a few. Conifer barks contain resins and fats which act as emulsifying agents in alkaline solution; and these barks are used successfully to remove printing inks in the recovery of paper.

Bark offers yet other opportunities for chemical investigation. Within the wood cell cavities are found "minor chemicals" comprising from 1 to 2% up to 25% or so, of the weight of the bark. In contrast to wood substance which does not vary much in composition from species to species, the minor chemicals may be expected to include compounds of the most diverse chemical character. Purves, Bishop and Harwood[3] report 20% extractives (i.e. substances which can be isolated by extraction with solvents) from white spruce bark.

Comparatively little research has been done on bark extractives, and this field offers great opportunity for study.

Between 1924-1934 Zellner et al. produced the following table[5] of extractives from barks. (23 species including red spruce).

Table 1

Group	Class of Substance	Soluble in	Insoluble in
1	Volatile terpenes, aldehydes, aliphatic oils, fats, waxes, higher alcohols, resins, and plant sterols.	Petroleum Ether, Ethyl Ether, Alcohol	Water
2	Amorphous resin acids, substances in 1, but more hyrdoxylated.	Ethyl ether, Alcohol	Water Petroleum Ether
3	Phlobaphenes and some glycosides	Alcohol	Water, Petroleum Ether, Ether
4	Tannins, simple sugars, glycosides	Alcohol Water	Petroleum Ether, Ether
5	Polysaccharides, gums & pectins	Water	Petroleum Ether, Ethyl Alcohol

To follow up this table and to enlarge upon it the following is included. Zellner's work reports also the barks used and the substances isolated from each bark. His work divides the composition of barks as follows:-

1) Lignin
2) Cork (suberin)
3) Carbohydrates-cellulose and hemicelluloses
4) Extraneous substances (In table below)

Table 2

Bark	Substance found
Alder	Resin acids, ceryl alcohol, almulin
Ash	Pectin, hemicelluloses (mannose, galactose arabinose) phytosterols, tannins, fats carotenoid pigment, ceryl alcohol
Birch	Betulinol, gallic acid, pentosan, tannin, waxes, alkaloids, volatile acids, suberin
Chestnut	Phlobaphenes, the glucoside esculin, fatty acids, sitosterol, resins, invert sugar
Fir	Pectin, tannin, suberin
Larch	Starch, pentosans, suberin
Oak	Tannin, cork (suberin)
Pine	Pectin, suberin, lignoceric acid, palmitic acid, caproic, oleic and other acids, dihydrositosterol, carotene
Spruce	Ceryl alcohol, palmitic and stearic acids, tannins, invert sugar

There are in bark, substances which appear to be lignin, and some of it is like wood lignin - the phenolic polymer which is thought to bind the cellulose fibers together in the cell wall. However, in many studies of bark it has been found difficult to differentiate between lignin and other bark substances. Bark lignin has been found to be more complex than wood lignin.

Little is known about cork although it is a very common commodity and is so widely used. This is because cork is so resistant to water and other solvents which would ordinarily be used in investigating it. It is believed that all corks contain suberin, which may be formed in the physiological process occurring in the change from living to dead wood. Fir bark does provide cork but in layers too small for most purposes (it is used in the Silvacon process mentioned above). Practically all cork obtained from forest products comes from oak bark.

As for extractives there is no sharp distinction between the different components and thus separation is difficult. Also it has been found that an extract obtained with any solvent contains many substances of like structure, and these are difficult to separate by conventional methods. β-sitosterol, dihydrositosterol and a diterpene have all been reported from white spruce bark.[7] Also oleic, linoleic and linolenic acids[7] are also found in white spruce bark, but these are found in very low concentration as also are palmitic and stearic acids, reported from white spruce[8]. The concentration is low enough to discourage extractions from waste barks.

Tanins are found in many barks as shown above, even to the extent of up to 10-15%. This represents a proportion of the bark large

enough to invite and encourage investigation, Since most of the waste bark available in Canada is soggy with water due to river driving of the logs, this presents difficulties great enough to discourage large scale extractions, because much of the tannins have been leached out with water. On the other hand it is questionable if bush peeling and subsequent transportation of the dry bark would either fit into the logging methods or be done at a cost which could even be considered.

It is known that alkaloids are found in the barks of some trees but research in this field has not been great and few conclusions have been reached. It is a fact that the natives of South America tipped their spears and arrows with poisons made from the bark of trees found in their localities. These poisons are alkaloid compounds. The alkaloids commonly reported from barks are quinnine, brucine and strychnine.

Barks are so complex compared with woods, that it appears improbable that the whole bark from any source could be converted into a useful product. Work on cellulose, lignins and cork from barks has met with formidable difficulties. The pulping procedure used on wood does not work because it does not effectively remove lignin and poly-saccharides; so that the residue cannot be classed as α-cellulose.[10]

Most work on bark in the past has been with extractives and it is to be expected that research in the future will delve into convenient methods of separation of the components of bark.

Review of Black Spruce Wood[9]

This section will deal with the components of black spruce wood so that comparison with those of black spruce bark may be made. There are clearly some similarities although it may be stated here that bark is different from wood in the following:-

a) lower cellulose content in bark.

b) bark lignin contains less methoxy groups than wood lignins.

c) ash and nitrogen content are higher in bark.

Black spruce wood has been analysed for cellulose, lignin, fats, and polyoses, and the findings of three investigators are tabled here.

Table 3

	% Cellulose	% Lignin	% other Carbohydrates	% Wax/Fats
1	41.5	28.0	24.3	4.8
2	45.0	28.2	19.4	5.1
3	53.0	29.0	14.4	4.0

The polyoses reported in black spruce wood are:

mannan	on hydrolysis	mannose	10.3%
glucan	" "	glucose	0.8%
galactan	" "	galactose	1.8%
araban	" "	arabinose	0.5%
xylan	" "	xylose	7.6%
glucuronic acid			0.3%
pentosans			traces
fructan			none

Many of these have already been reported from white spruce bark,[7] but in much smaller amounts. It is reasonable to assume that black spruce bark will contain some, if not all, the polyoses found in the wood.

Pectins in traces were found in the young black spruce wood, but these were not detected in the older wood due to change to lignin type compounds.

Hagglund[9] established the percentage of native lignin extractable from spruce wood at 27% of the weight of the wood. A method of extracting this "native lignin" using methanol was published by Brauns[15]. Much of the lignin is firmly bound to the woody substance and cannot be extracted.

Among the minor components of black spruce wood are resins which make up about 2.3% of the weight of the wood. These are sometimes dissolved in turpentine which keeps the shole soft and mobile. The turpentine in black spruce wood consists of α-pinene and α-limonene. The resins also contain mixtures of sterols like β-sitosterol and dihydrositosterol already reported in white spruce bark.[7] Wise and Moore[16] have isolated 22,23-dihydrostigmasterol from black spruce wood.

When wood is pulped with alkali, fats are brought into solution as well as resins. After salting out the alkali, the fats are obtained as alkali salts (soaps). These soaps contain palmitic, oleic and linolenic acids.

Black spruce has been found to contain conidendrin, a lactone, by Erdtman[9]. He found it impossible to extract it with ether but easy with acetone. Pinoresinol has also been found by the same chemist. Cox[17] found 3, 5-dimethoxystilbene in resin from black spruce.

While tannins are important constituents of wood they are usually found in, and extracted from, the barks.

Methods of Obtaining Bark Extractives

In most work on barks the procedure has been exhaustive extraction using inert solvents such as water, ethyl ether, alcohol etc. The substances thus extracted were placed in one category and the residual bark in another. When thinking of bark analysis it is natural to start with the work of Zellner.

Zellner[5] exhaustively extracted 3-12 kilos of air-dried bark with boiling 95% ethanol to remove all the substances in groups 1-4 listed in Table 1 above. The solution was concentrated and the extract was poured into cold water when groups 1-3 precipitated out. Group 4 stayed in solution. This precipitate was dried and extracted with petroleum ether to separate group 1, and group 2 was taken out in ethyl ether leaving group 3 substances still undissolved. Substances in group 5 remained in the bark left after the successive extractions, and were dissolved out with water.

Richter[11] found that the black spruce bark yielded 3.1% of weight of bark in the petroleum ether-ether extract, 8.1% in the alcohol extract and 11.6% in the water extract, or 22.7% altogether. He observed that the solvent sequence could be reversed to yield the same total percentage of extractives. He noticed too that there was no sharp distinction between the groups.

Clotofski[12] and his collaborators found that the sequence alcohol, acetone, dioxane, methanol, extracted 9.2%, 7.6%, 12.8% and 12.2% respectively from beech bark, but further separation of these groups themselves into simpler groups could not be effected using solvents.

Kurth[13] used the following procedure for examining the extractives of balsam bark:-

1) Volatility with steam
2) Solubility in ethyl ether
3) Solubility in alcohol
4) Solubility in water

Group 1 contained volatile oils, acids and hydrocarbons such as α-pinene and β-pinene.

Group 2 contained fats, fatty acids, resenes, sterols, waxes and non volatile hydrocarbons.

Group 3 contained many removed in group 2, plus tannins.

Group 4 contained the soluble carbohydrates, cycloses, and salts.

At Memorial the first extraction was made with petroleum ether and not with alcohol. This was done so that tannins and sugars, glucosides etc., in which we were not immediately interested, would be kept back for later extractions. It was hoped to obtain in the first extractions with petroleum ether terpenes, oils, fats and waxes, higher alcohols and plant steroids if any were present. The extract after recovery was molasses-brown in colour and quite viscous. Attempts to isolate simpler substances using solvents had to be abandoned, as will be described later in the experimental section. The first step was steam distillation of the crude extract, which left a light brown residue which gave a strongly positive reaction to the Liebermann-Burchard test showing the presence of steroid compounds. To carry out this test the substance is dissolved in chloroform (a few drops) and mixed with an equal volume of acetic anhydride. Next a drop or two of concentrated

sulphuric acid is allowed to run down the side of the test tube held in a slanting position. It is usual for a pink colour to appear in the mixture which almost immediately turns some shade of green depending on the concentration. In one case only, the change was to distinct blue first, and then to green. This test was used extensively on various fractions of the petroleum ether extract. It is specific to $\Delta 5$ unsaturated sterols.

Exhaustive extraction with petroleum ether was followed by extraction with ethyl ether. This treatment is intended to take out any substances not extracted by the first solvent, while still keeping back the tannins, etc. It was necessary to use strictly dry ether to do this. The ether extract after recovery of solvent is best powdered with a spatula whence it is a light yellow brown solid. Up to now only two extractions with ether have been made. This is because very much interest was aroused in the detecting in this extract of a flavone type compound. More ether extractions will follow.

It is planned to follow the ether extractions with further extractions using acetone, alcohol and finally water. Right now considerable quantities of petroleum ether extract are on hand to be studied and the bark which has been fully treated with this solvent has been stored to await ether treatment. Another sample of bark which has been given complete petroleum ether extraction and two ether extractions is still in the extractor for further treatment with ether.

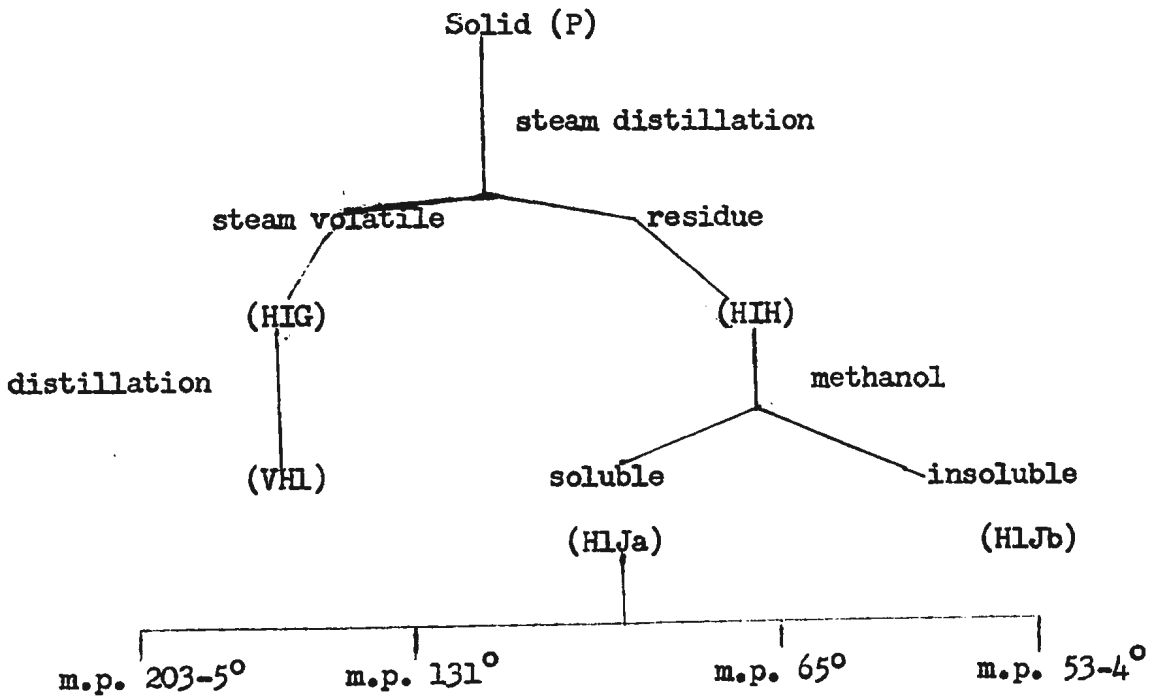
Petroleum Ether Extract

Exhaustive extraction with petroleum ether afforded about 5.6% of extract, somewhat higher than the percentage reported by previous workers for extractions with hydrocarbon solvents.

Solvent removal afforded an amorphous solid (P) which was steam distilled and thus separated into two fractions, one steam volatile (HIG) and the other not (HIH). These and subsequent separations are illustrated in table 4.

Table 4

Schematic Representation of Petroleum Ether Extract



The steam volatile fraction contained cyclopentane which obviously came from the hydrocarbon solvent, and a pale yellow oil with a terpene-like odour. This second compound also gives a positive Liebermann-Burchard reaction.

The residue HIH was insoluble in 10% aqueous sodium carbonate and 2N hydrochloric acid. It was separated into a methanol-soluble

(H1Ja) and a methanol-insoluble (H1Jb) fraction. H1Jb gives a positive Liebermann-Burchard reaction and chromatographic separation shows it to be a mixture.

The methanol-soluble fraction (H1Ja) was recrystallized from boiling methanol to give a sharp melting cream-coloured solid in small yield, which analysed for $C_{28}H_{54}O_3$, and had a melting point of 53-54°C. The formula indicates a long hydrocarbon chain, and this accounts for the observed properties (negative Liebermann-Burchard reaction, no reaction with aqueous potassium permanganate or ferric chloride, and absence of active hydrogen). This substance was also obtained on chromatography.

Chromatography of (H1Ja) also afforded the following compounds:-

(1) A solid, melting point 203-205°C., which analysed for $C_{22}H_{38}O_2$. It is probably steroidal in character since it contains no nitrogen and gives a positive Liebermann-Burchard reaction. It contains no double bond as indicated by the lack of action with tetranitromethane.

(2) A compound, crystallizing as colorless plates, melting point 131°C. It gave a characteristic Liebermann-Burchard reaction and analysed for $C_{27}H_{46}O_2$.

(3) A solid, melting at 65°C., which gave a positive Liebermann-Burchard reaction.

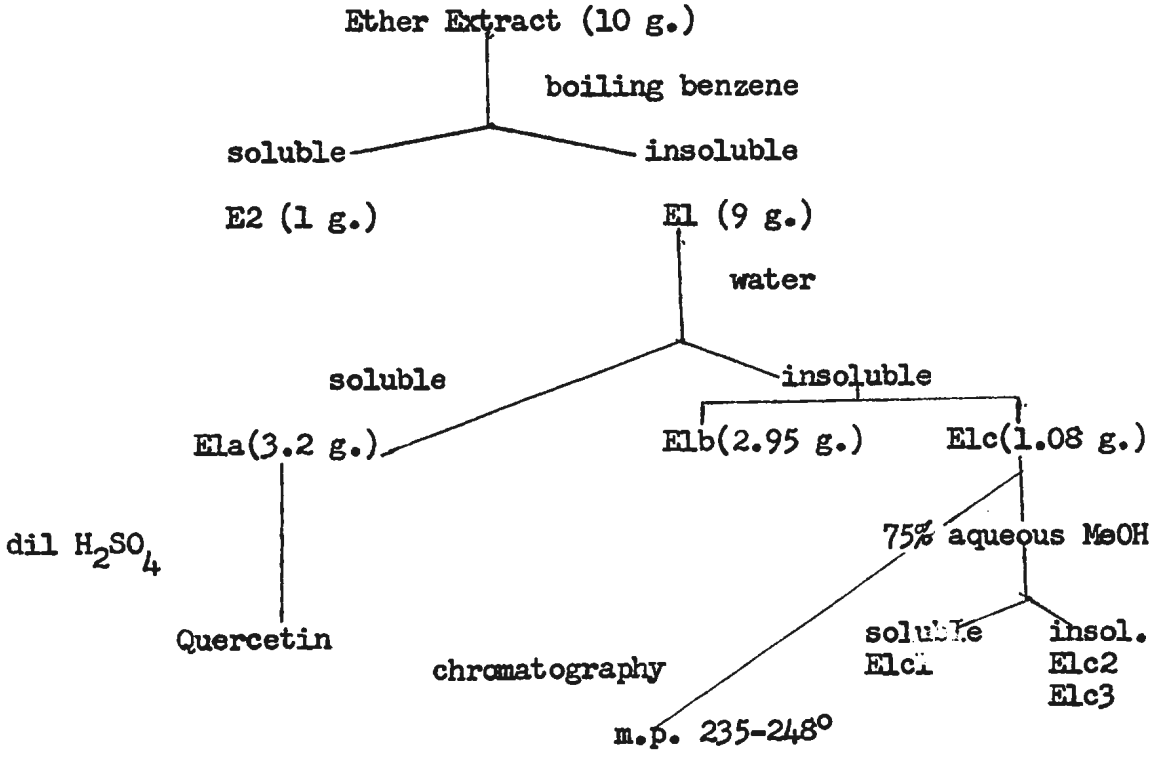
Ether Extractives

The ether soluble fraction, after removal of the solvent, was treated with benzene to ensure removal of any remaining petroleum ether-soluble material (E2). The benzene-insoluble fraction thus obtained (E1) was separated into a water soluble fraction (E1a) and a

water-insoluble fraction which on further treatment with water could be separated into a non-flavonoid fraction (E1b) and a flavonoid fraction (E1c). Table 5 illustrates this separation. The quantities shown in brackets indicate the actual amounts obtained in a typical separation.

Table 5

Schematic Representation of Ether Extract



The solid E1a could not be crystallized directly from aqueous ethanol and could not be sublimed under vacuum. On hydrolysis with dilute sulphuric acid E1a afforded Quercetin (3,5,7,3',4'-pentahydroxyflavone), which was identified by its melting point, by its pentaacetate, and by the light absorption properties of the acetate and the parent compound. (See table 6). Additional evidence for the structure of quercetin was provided by elemental analyses of the

two compounds and by the preparation of the methoxy derivative.

Paper chromatography of Ela in various solvents confirmed the presence of quercetin but also indicated the presence of a persistent impurity. Separation of Ela by chromatography on silica gel did not yield a completely homogeneous substance as shown by paper chromatography. However, some of the fractions thus obtained (i.e. by chromatography on silica gel) on treatment with phenylhydrazine and acetic acid yielded glucosazone.

The evidence therefore suggests that Ela consists of a mixture of quercetin and at least one or more glucosides of this substance.

The Elb fraction is mostly non-flavonoid in character (as indicated by colour reactions), dark brown in appearance and very hydrophobic. It probably consists of tannins, which are extracted by traces of water or ethanol present in the ether used. The fraction has not been further investigated.

The other water-insoluble fraction (Elc), after removal of Elb using boiling water, is a yellow solid. This solid does not move on paper when treated with 60% isopropanol thus showing the absence of any appreciable amounts of quercetin. The spectrum, however, closely resembles that of quercetin (See table 6). The most promising method of separation appears to be treatment with 75% aqueous methanol followed by chromatography on magnesol or silica gel, which afforded these compounds:-

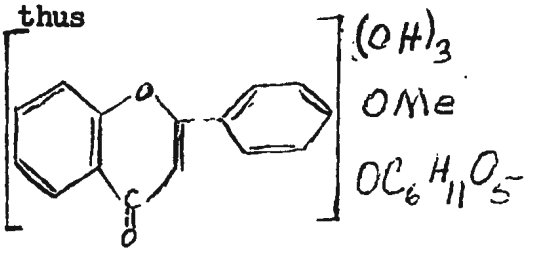
(1) A brown solid (red solution in alcohol), melting range 235-248°C. The spectrum (table 6) indicates a quercetin-type molecule and this substance may contain the 3-galactoside of quercetin or other similar

compound.

(2) A yellow solid melting at about 290°C. (Elc1), soluble in 75% aqueous methanol, which analysed for $C_{15}H_{12}O_7.3H_2O$. Colour reactions indicate that this substance is a flavone. The acetate melts at 208°C. and analysed for the pentaacetate.

(3) A yellow solid (Elc2), melting with decomposition in the range 270-280°C. It is insoluble in 76% aqueous methanol but crystallized from aqueous acetone and analysed for $C_{18}H_{22}O_{10}\cdot 3H_2O$. This substance is therefore probably a trimethylquercetin, since colour reaction and spectrum suggest a flavone-type compound.

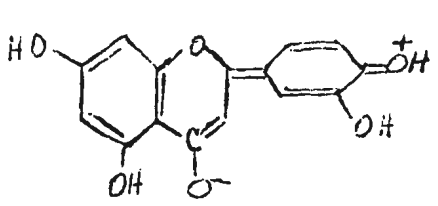
(4) A yellow solid (Elc3), melting point 270-280°C. (with decomposition, insoluble in 75% aqueous methanol and probably identical with Elc2, except that the compound analysed for $C_{22}H_{22}O_{12}$ which corresponds to one methyl group only, thus



The compound absorbs maximally at 252.5, 303, and 370 mμ, again indicating a flavone-type nucleus; this is also confirmed by colour reactions.

Ultraviolet Absorption Spectra

The ultraviolet absorption spectra of quercetin, its derivatives and related compounds are shown in Table 6. Quercetin absorbs maximally at 257.5 mμ and 382 mμ. The long wave absorption band is ascribed to transitions involving the whole molecule, that is transitions involving polar excited states of type 1, whilst the band at 257.5 mμ is ascribed



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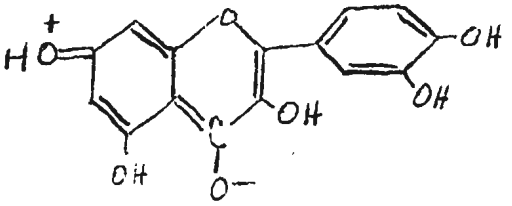

II

to benzoyl absorptions, that is transitions involving polar excited states of type II. Whilst a full account of the reasons for this assignment of absorption bands will be reported elsewhere, it may be stated that this assignment is based primarily on the impossibility of any other transition (such as secondary bands) accounting for the observed high-intensity absorptions.

A second indirect argument is provided by the study of acetylations. Acetylation usually nullifies the effect of an hydroxyl group; thus, for instance, the acetates of 5-hydroxy- and 7-hydroxyflavones both absorb maximally near 255 and 295 mμ, close to the absorption of flavone itself (Pillin D)[22], and 2-, 3-, 4- acetoxyacetophenone all absorb within 6 mu of the parent acetophenone[21]: Now, 5-hydroxy-4'-methoxy flavone absorbs maximally at 323 and 273 mμ which we can ascribe to transition involving polar excited states of type I and II respectively. Acetylation would be expected to leave the 323 mμ band relatively unchanged but to alter the 273 mμ band moving the maximal absorption to the 255 mμ region. The acetate in fact absorbs maximally as expected at 322 and 258 mμ.

Table 6

Absorption Spectra of Quercetin and Related Compounds in Absolute Alcohol

Wave-lengths and intensities of the main maxima

Compound	λ_{max} mμ	ϵ_{max}	λ_{max} mμ	ϵ_{max}
3,5,7,3',4'-pentahydroxyflavone (quercetin)	257.5	22,000	382	26,500
Elc	254	16,993	372	20,844
Ela	254	13,232	370	16,955
3,5,7,3',4'-pentaacetoxyflavone	255	16,000	290	15,500
VH3	255	15,261	296	14,666
Flavone	250	12,000	297.5	16,000
3,5,7,3',4'-pentamethoxyflavone	266	14,000	346	14,000
methoxy crystals reported above	266	14,391	346	14,146
4-hydroxy-2,6-dimethoxyacetophenone	278	6,500		
2,4,6-trimethoxyacetophenone	288	8,000		
4'-hydroxy-3,5,7,3'-tetramethoxy-flavone	263	16,500	345	22,000
5-hydroxy-3,7,3',4'-tetramethoxy-flavone	269	19,500	352	22,000

The pentaacetate of quercetin also absorbs as expected at 290 and 255 mμ near the absorption of flavone itself, thus providing additional evidence for the flavone-type structure of the above-described compounds.

Methylation of quercetin causes a hypsochromic shift of the long-wave absorption band accompanied by loss of intensity of absorption (see table 6), presumably because the replacement of the hydroxyl group by a methoxyl group gives rise to a steric effect which opposes the setting up of polar excited states for type 1. This type of steric effect has been discussed elsewhere[22]. The short-wave absorption band, however, which has been postulated to be due to transition involving polar excited states of type II, is not affected by this steric effect and because of the electron-releasing properties of the methyl group, this type of excited state will be favoured causing a bathochromic shift. An entirely similar phenomenon is observed between 4 -hydroxy-2,6-dimethoxy-acetophenone and 2,4,6-trimethoxyacetophenone which absorb maximally at 278 and 288 mμ respectively. 4'-hydroxy-3,5,7,3'-tetra-methoxyflavone as expected, exhibits spectra very similar to that of the pentamethoxy compound except that the intensity of the long-wave band in the 4'-hydroxy compound is increased somewhat compared to the other absorption band, presumably because a hydroxy group in the 4'-position favours the setting up of a positive charge there.

Conclusion

The chemical composition of barks varies from species to species and most data on bark extractives is sketchy; work by different people on the same bark shows great discrepancies. We know that bark is of heterogeneous composition and that many changes occur in the chemical composition as well as in the physiological composition as growth proceeds.

It appears that most work on bark has followed the methods used to investigate wood, and wood, as mentioned above is less complex than bark. It would perhaps be well to standardise samples of bark for analysis and even then the analyses could be confined to one section of the bark. In the case of black spruce bark, as with some others, the proportion of some constituents changes from season to season; therefore, the time of harvesting and type of handling should be considered also. General information on what substances occur in each stage in the life of the bark might disclose valuable evidence which would add to our knowledge of lignin and other complex substances.

Although a large percentage of the bark can be extracted with inert solvents, each extract contains very many substances and consequently the amounts of these substances are small. Thus the establishment of correct yields is difficult and data varies from report to report. It is suspected that even in the process of removing the different solvents changes occur in the composition of some constituents of the crude extracts. Repeat runs with identical conditions have produced extracts which appear to be different.

The possibility that the whole bark may be used to provide diverse products seems remote although it is quite conceivable that some one or two substances could be produced if economical methods for obtaining these are developed. The enormous volume of waste bark does throw a challenge to science to find uses for it and because of its complexity, the bark provides an excellent field for pure research.

Experimental

Analyses were carried out in the microanalytical laboratory (Mr. P. Oliver) of the department of Organic Chemistry, Imperial College, London, England, and in the microanalytical laboratory (Mr. A. Bernhardt) of the Max-Planck Institut fur Kohlenforschung, Mülheim, Ruhr, Germany.

All extractions were carried out in a copper extractor of cylindrical shape 1 ft. by 4 ft. A glass gauge was built into the side about two in. from the bottom; the cover was made from a brass plate ($\frac{1}{4}$ in. thick) which was bolted to the top of the cylinder with wing nuts. A hole in the top was fitted with a brass collar which in turn held a rubber gasket so that the end of the condenser would fit tightly. The extractor was heated with a hotplate. One large condenser (3 ft.) was used in the petroleum ether extractions but two smaller ones (24 in.) were connected to the top of this one for the ether extractions. The bark was contained in an aluminum cylinder which was 8 in. by 3 ft. This cylinder was supported on three legs 6 in. high and the bottom of it was perforated. A bottom of copper wire screens and glass wool was used to prevent channelling and to keep the bark particles from getting into the solvent. The solvent was kept at the boiling point for periods up to ten days.

Black spruce bark was ground (after drying at room temperature) to pieces about $\frac{1}{4}$ in. by $\frac{1}{4}$ in. in size. The chips (5 kg) were exhaustively extracted with petroleum ether, 12 l., 60-80°C. This produced, after four extractions of seven to ten days each, approximately 280g. of

crude extract, after removal of the solvent or 5.6% by weight of the bark. The extractions gave the following weights of extract:-

Extraction #1 118g.

#2 139g.

#3 20g. ----this extract was burned by accident.

#4 23g.

The solvent was removed by distillation over a boiling water bath until the extract became fairly thick whence the rest of the solvent was removed under slightly reduced pressure. From the first extraction approximately 56g. of crude extract was steam distilled. (Attempts to separate simpler substances from the extract met with failure - with the usual solvents all or almost all of the extract would dissolve.) The steam distillate contained a terpene type odour and the distillation was continued while this characteristic odour prevailed. Ten fractions (400 ml. each) were taken and then extracted with diethyl ether (3-70 ml. portions). These ether extracts were dried (anhydrous sodium sulphate) and the ether was recovered. Fraction 1, after recovery of the ether, was a relatively clear liquid (40 ml.). This was distilled and the information obtained is tabled herewith:-

	Bath Temp.°C.	Boiling Range °C.	Vol. of Fraction	Index of Refraction	Liebermann-Burchard test
1.	60-90	33-36	20 ml.	(ether)	---
2.	118-120	40-50	3 ml.	1.3962 n_D^{25}	neg.
3.	130-135	42-47	3 ml.	1.4000 n_D^{26}	neg.
4.	135-140	43-45	3 ml.	1.4052 n_D^{26}	neg.

5. A thick residue of a few drops which was later lost on attempted redistillation under reduced pressure.

Fractions 2, 3, and 4 were combined and redistilled. The results are as follows:-

	Bath Temp.	Boiling Range	Volume	Index of Refrac.
1.	110-120°C.	33-40° C.	0.5 ml.	1,3908 n_D^{25}
2.	123-125°C.	40-45° C.	3.0 ml.	1,4004 n_D^{24}
3.	125-155°C.	45-50° C.	2.0 ml.	1,4019 n_D^{24}
4.	Residue			

Fraction 2 from this second distillation was found to be free from nitrogen (sodium fusion) and gave a negative reaction with the Liebermann-Burchard test. The fraction (VHI) Anal.: Calc. for C_5H_{10} C, 85.6; H, 14.4%. Found: C, 84.44; H, 15.12%. (Heilbron *et al.*[18] give cyclopentane, b.p. 50°C., n_D^{20} 1.4039).

This same procedure was followed for the second and the third extracts but since very small volumes of residue were obtained no further work was done on them. The combined residues have been retained, labelled (HIG). They showed faint reaction with the Liebermann-Burchard test. The nonvolatile steam distillation residue was cream-brown and was retained, labelled (HIH).

A second extraction with petroleum ether yielded 139 g. of crude extract after removal of the solvent. This was combined with the unused part (62g.) from extract #1. The 200g. thus obtained was steam distilled and the four (500 ml.) fractions were treated as above. In each case after recovery of ether a thick oil was left. These oils were combined and retained as (HIG). This time no clear oil was obtained although the terpene type was detected.

The next work was done on the steam distillation residue (HIH). It was a fawn-coloured solid which was quite sticky. It reacted very strongly with the Liebermann-Burchard test, showed no nitrogen (sodium fusion), was insoluble in aqueous sodium carbonate solution (10%) and was insoluble in 2N hydrochloric acid. A portion (50g.) was treated with methanol and brought to the boiling point of the alcohol. Part of the solid remained undissolved - this was set aside labelled (HIJ1) and has not been further studied. On cooling, the methanol solution precipitated out a waxy cream-coloured solid, m.p. 57-60°C. Recrystallization from methanol gave a cream solid (HIJa) melting at 65-66°C.

The solid HIJa (1 g.) was chromatographed (alumina-benzene) and nine fractions of 50 ml. each were collected. Recovery of the benzene left small amounts of solid in each case. These were treated with aqueous potassium permanganate and with the Liebermann-Burchard test, and the melting point was recorded. The following table gives the data:-

Solid left from	Liebermann-Burchard test	m.p. °C.	Reaction with $KMnO_4$
#1	positive	approx. 55	decolourized
2	negative	62-64	"
3	positive	approx. 35	"
4	negative	43-46	"
5	negative	63-64	"
6	"	65-66	"
7	"	64-66	"
8	"	63-65	"
9	"	63-64	"

Since chromatography showed this substance to be inhomogenous, a larger chromatogram was run on the steam distillation residue (HIH) itself. Accordingly a 10g. sample of the residue was dissolved in chloroform and a column was prepared as follows:- The column was $1\frac{1}{2}$ in. by 4 ft. and was fitted with a sintered glass filter and a tap at the bottom and a reservoir at the top. It was found most convenient to pour benzene into the column and to stir alumina into the liquid -- this prevented the formation of air spaces. The column was packed without pressure with alumina to a depth of about three feet. The solution of chloroform and steam distillation residue was poured carefully onto the top of the column when the benzene at the top was about $\frac{1}{4}$ in. deep. The solution was allowed to seep into the column until only $\frac{1}{4}$ in. of liquid remained above the alumina. Then elution with benzene commenced and at no time was the column allowed to go dry until 28 fractions (300-400 ml. each) had been taken. The table below gives the data.

Fraction	m.p.°C.	Liebermann-Burchard	With $FeCl_3$
1.	approx. 252	faint green	negative
2.	40-50	faint yellow	"
3.	40-50	faint yellow	"
4.	approx. 50	strong green	"
5.	approx. 50	strong green	"
6.	semi-liquid	strong green	"
7.	185-188	faint green	"
8.	semi-liquid	" "	"
9.	liquid	" "	"
10.	liquid	" "	"
11.	liquid	" "	"
12.	liquid	" "	"
13.	approx. 35	" "	"
14.	approx. 55	" "	"

Fraction	m.p.°C.	Liebermann-Burchard	$FeCl_3$
15.	approx. 55	faint green	negative
16.	approx. 55	" "	"
17.	approx. 55	" "	"
18.	277 charred	" "	"
19.	270-80 charred	" "	"
20.	285-90 charred	" "	"
21.	280 charred	" "	"
22.	55-60	" "	"
23.	approx. 110	strong green	"
24.	121-123	" "	"
25.	approx. 110	" "	"
26.	approx. 124	blue to green	"
27.	approx. 55	strong green	"
28.	approx. 55	" "	"

Fraction #7 contained the greatest amount of solid which was, like most others, cream-coloured. Some of the semi-liquids were darker, bordering on amber. Fraction #7 was treated with methanol and practically all dissolved when the solvent was heated almost to boiling. Several recrystallizations from aqueous ethanol afforded a white solid (VH2), m.p. 203-205°C. This substance gives a positive Liebermann-Burchard test, shows no nitrogen (sodium fusion), does not decolorize aqueous potassium permanganate, nor does it give any yellow colour with tetranitromethane. Anal.: Calc. for $C_{22}H_{38}O_2$: C, 79.04; H, 11.37; O, 9.58%. Found: C, 79.2; H, 11.7; O, 9.58%. Light absorption in ethanol: no maximum in the range 220-400 mμ.

Fraction #26 afforded on crystallization from ethanol colorless plates, m.p. 123-124°C. This solid gives a blue to green Liebermann-burchard test and has been retained, labelled #26. However, later a new petroleum ether extract was steam distilled and the residue was chromatographed as above. One of the fractions from this run crystallized from ethanol as plates, m.p. 131°C. This sample resembled

#26 in shape of crystals and in its reaction with the Liebermann-Burchard test. (VH4) Anal.: Calc. for $C_{26}H_{46}O_2$: C, 80.0; H, 11.79; O, 8.2%. Found: C, 79.6; H, 11.9; O, 8.5%. No molecular weight could be obtained because the sample decomposed in camphor.

One other attempt was made to isolate crystalline substances from the steam distillation residue using only solvents. A small amount (2 g.) of the residue was treated with boiling methanol and after several crystallizations a white solid, m.p. ~52°C. was obtained. This was chromatographed (benzene-alumina) and one fraction afforded a white solid melting sharply at 53°C. (VH5). Anal.: Calc. for $C_{28}H_{54}O_3$: C, 76.7; H, 12.3; O, 10.9%. M.Wt., 438: Found: C, 77.1; H, 12.2; O, 10.7%. M. Wt., 503.

A fourth extraction yielded 23 g. of extract. A quantity (10 g.) of this was chromatographed but the fractions, after removal of benzene, were not investigated because no appreciable separation had been effected. The rest of this extract was steam distilled and the characteristic terpene odour was detected; however, very small volumes of liquid remained after recovery of ether.

The steam distillation residue was chromatographed like the other residues and in this run only fractions #7 and #8 appeared to be worth experimenting with. They were combined and after recovery of benzene left a yellow solid. This gave, on crystallization from ethanol, a white waxy substance, m.p. 52-53°C. This does not give a positive Liebermann-Burchard test, does not decolorize $KMnO_4$, nor change the colour of $FeCl_3$ solution. It does contain active hydrogen as shown by reaction with Zerewittinov reagent. It was retained, labelled (L).

Extraction with diethyl ether

The bark (2 kg.) which had been extracted with petroleum ether was then extracted with diethyl ether. Three separate extractions were made using calcium chloride dried ether. Again about 12 l. of the solvent were used and three extractions produced a total of 83g. of crude extract. This represents 4.5 % of the weight of the bark.

A small chromatogram (alumina-benzene) was run on this extract but its solution in various solvents adhered to the column and could not be eluted. Steam distillation was tried also on a small quantity of the extract but nothing was obtained in the distillate. The residue was a light brown solid and was insoluble in benzene.

The first extract was treated with benzene (almost up to the boiling point) and most of the extract was insoluble. On removal of the benzene soluble part (E2), a powdery brown-yellow solid remained (E1).

It appears that the ether extract undergoes some changes during the removal of the solvent. Accordingly, the solid which used to accumulate in the bottom of the flask during evaporation of the solvent was scraped out at regular intervals to try to prevent this change occurring. The extract thus obtained was a dry solid and easy to handle. It is light brown in colour. All attempts to separate any component using solvents met with failure. The extract is quite soluble in most solvents that were tried (ethanol, methanol, acetone, petroleum ether). After treatment with ethanol and cooling, the solid left, undergoes a change. A yeasty smell suggested the setting in of enzyme reactions. The extract contains no nitrogen nor sulphur

and is negative to the Liebermann-Burchard test. It decolorizes aqueous potassium permanganate solutions and gives a positive test with Fehling's solution. When treated with hydrochloric acid/ethanol, followed by a few slivers of magnesium, a wine coloration was produced suggesting the presence of flavone type compounds. All the ether extract was given the benzene treatment and the residual solid was studied in detail.

The residue (E1) was treated with boiling water for two or three minutes whence there was a three way separation. Part dissolves and the solution when cooled yields a yellow solid (E1a). The insoluble part contains two materials - one yellow, much like (E1a), will be referred to as (E1c); the other is a dark brown solid (E1b).

The solid E1a decomposed when crystallized from water or ethanol or mixtures of both. When left to dry on a suction filter this substance turned green and brittle. It could not be sublimed.
E1a (0.5 g.) was refluxed with 2% sulphuric acid for one hour. The residue was filtered and washed with aqueous 2% sodium carbonate solution and with water. A yellow solid remained which could not be sublimed. Recrystallization from methanol afforded yellow needles which decomposed at 285-295°C. (Mayer and Cook[14], page 188 give Quercetin m.p. 310°C.)

The solid E1a while still wet was removed from the suction filter and acetylated as follows:- The solid (1 g.) was treated with 10 ml. of acetic anhydride and sodium acetate (10 g.) and refluxed for one hour. This was almost completely acetylated and, decomposed with ice, yielded a white solid. This is microcrystalline and melted at 200°C. after crystallization from ethanol. When crystallized from methanol the

solid is cream coloured. A careful redetermination gave a melting point of 195.5°C. (VH3) Anal.: Calc. for $C_{25}H_{20}O_{12}$ (3,5,7,3',4' -pentacetoxyflavone); C, 58.6; H, 3.93; O, 37.47%; $(OAc)_5$, 42.02%. Found: C, 56.1; 56.4; 56.43; 56.6; H,4.1, 4.2, 4.15, 4.05; O, 37.1, 39.7, 39.42, 39.3%; $(OAc)_5$, 43.5%. Light absorption in ethanol, see table page 18.

Ela (1 g.) was treated with excess diazomethane followed by decomposition with KOH and a brown solid remained. This was chromatographed (alumina-benzene) and two fractions on recovery of benzene yielded oils which were not investigated. The top of the column contained a bright yellow substance which could not be moved with benzene or chloroform. The alumina which was yellow was extruded and soxhleted with chloroform. Recovery of the chloroform yielded a residue which crystallized from methanol in pale yellow crystals, m.p. 148°C. (Mayer and Cook[14] page 188 give pentamethoxy derivative of quercetin, m.p. 148°C.)

Paper Chromatography on Ela

Whatman #1 paper was used in strips 1 in. by 6 in. The solution of Ela in ethanol (0.01 ml.) was applied using a fine tipped glass dropper. The eluents used were 15% acetic acid, 60% acetic acid and 60% isopropyl[x] alcohol. The R_f values are given herewith.

	15% Acetic Acid	60% Acetic Acid	60$ isopropanol
Ela ...	0.035	0.59	0.67
Quercetin (19)	0.04	0.40	0.67

The solvent fronts were not visible under ordinary light but could be detected under ultraviolet light.

[x] 2-propanol

Ela was chromatographed also on a column of silica gel specially prepared by overnight soaking in water. Benzene was the eluent and each of three fractions yielded solids, m.p. 125°C. Each on treatment with phenylhydrazine and acetic acid produced a glucosazone. These formations melted or decomposed while being observed on a microscope slide. Besides the glucosazone all give another fuzzy type crystal like a lactosazone. Ela when observed under the microscope showed a mixture of needles and plates.

Treatment of the water insoluble part

The dark brown Elb mentioned above was not investigated. However, when it is prepared there comes down with it a yellow insoluble substance (Elc), which is very different from Ela just described. When the latter was chromatographed with 60% isopropanol, three zones are detectable beyond the original spot. With Elc under the same conditions there is practically no movement.

Elc was washed several times with hot water and left a product showing only one type of crystals, m.p. 250-260°C. with decomposition. Spectrographic investigation shows this to be 80-90% quercetin. See table page 18.

A chromatogram was prepared from waterglass according to the method of Martin and Synge[20]. The Elc was dissolved in methanol and eluted with benzene. Six fractions were taken in 50 ml. flasks. Only superficial observations were made as follows:-

Eluent	Fractions
Benzene	2 Yellow liquid turned suddenly red on removal of the solvent under reduced pressure, leaving a black solid. Spectrographic analysis showed no connection with quercetin molecule.
Benzene	3 Fluorescent liquid left only trace of solid.

Eluent	Fractions	
Ethanol	5	Red fraction on evaporation left a brown solid, p.m. 235-248°C. This when given spectrographic analysis shows a max. for the quercetin molecule.

Elc ($\frac{1}{2}$ g.) was hydrolysed with 2% sulphuric acid and the residue was filtered after cooling. The washings were neutralized with barium carbonate and evaporated to 10 ml. This liquid turns Fehling's solution and leaves a brown precipitate after boiling. The solution was treated with phenylhydrazine and acetic acid, whence some osazone formed; but only traces were of glucosazone form. Attempts to re-crystallize Elc from ethanol or methanol failed to produce crystals large enough to be seen with the naked eye. From these facts it must be assumed that quercetin occurs in the bark as a glycoside.

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