I. SYNTHESIS OF SPECIFICALLY SUBSTITUTED D-MANNITOL DERIVATIVES.

II. A COLOR PRECURSOR ISOLATED FROM WESTERN HEMLOCK WOOD

by

ALEXANDER JAMES FINLAYSON

A thesis submitted in partial fulfilment of the Requirements for the Degree of Master of Science in the Department of Chemistry

We accept this thesis as conforming to the standard required from candidates for the degree of

MASTER OF SCIENCE

Members of the Department of Chemistry

The University of British Columbia

April, 1956.
ABSTRACT

D-Mannitol was simultaneously tritylated and acetylated to give 1,6-di-0-trityl-D-mannitol tetraacetate in 70% yield, using a procedure that gave a product completely free from triphenylcarbinol. Direct tritylation of D-mannitol yielded 1,6-di-0-trityl-D-mannitol which was crystallized, probably as a solvated molecule, from alcohol and water. Acetylation of this compound gave 1,6-di-0-trityl-D-mannitol tetraacetate in good yield.

Detritylation of 1,6-di-0-trityl-D-mannitol tetraacetate to D-mannitol-2,3,4,5-tetraacetate was effected by hydrogen bromide in glacial acetic acid, catalytic hydrogenolysis over platinum oxide, and refluxing with dilute acetic acid, and the latter convenient method was found to give the highest yield of pure product.

A sirupy di-0-methyl-D-mannitol was prepared from D-mannitol-2,3,4,5-tetraacetate by methylation followed by deacetylation on an ion exchange column. The structure of the compound has not been established.

A nearly colorless, amorphous solid was isolated in 2.7% yield by 50% aqueous ethanol extraction of the finely-divided wood of a Western Hemlock tree (Tsuga heterophylla) which had previously been exhaustively extracted with benzene. Treatment of this material with
concentrated hydrochloric acid in methanol yielded a purple solid whose red-violet methanol solution was stable to ordinary light.

The paper chromatographic behaviour and color reactions compared with a red rose petal extract and the methoxyl content indicated that the purple solid was probably an anthocyanidin containing methoxyl groups.

Acetylation of the original solid extract with pyridine and acetic anhydride gave a dextrorotatory yellow sirup soluble in chloroform.
ACKNOWLEDGEMENTS

The writer wishes to express his sincere thanks to Dr. L. D. Hayward for his encouragement and guidance throughout the course of this investigation.

The writer also wishes to express his thanks to the Powell River Company for the award of a scholarship, and to the Sugar Research Foundation Incorporated for financial assistance.
# TABLE OF CONTENTS

## PART I

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>HISTORICAL INTRODUCTION</td>
<td>4</td>
</tr>
<tr>
<td>DISCUSSION OF RESULTS</td>
<td></td>
</tr>
<tr>
<td>A. 1,6-Di-O-trityl-D-mannitol Tetraacetate</td>
<td>10</td>
</tr>
<tr>
<td>B. 1,6-Di-O-trityl-D-mannitol</td>
<td>10</td>
</tr>
<tr>
<td>C. Detritylation Procedures</td>
<td>11</td>
</tr>
<tr>
<td>D. D-Mannitol-2,3,4,5-tetraacetate</td>
<td>13</td>
</tr>
<tr>
<td>E. Di-O-methyl-D-mannitol</td>
<td>14</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td></td>
</tr>
<tr>
<td>A. Materials</td>
<td>18</td>
</tr>
<tr>
<td>B. Analytical Methods</td>
<td>20</td>
</tr>
<tr>
<td>C. D-Mannitol hexaacetate</td>
<td>21</td>
</tr>
<tr>
<td>D. 1,6-Di-O-trityl-D-mannitol Tetraacetate</td>
<td>21</td>
</tr>
<tr>
<td>E. 1,6-Di-O-trityl-D-mannitol</td>
<td>23</td>
</tr>
<tr>
<td>F. Acetylation of 1,6-di-O-trityl-D-mannitol</td>
<td>25</td>
</tr>
<tr>
<td>G. Detritylation Procedures</td>
<td>25</td>
</tr>
<tr>
<td>H. Acetylation of D-Mannitol-2,3,4,5-tetraacetate</td>
<td>28</td>
</tr>
<tr>
<td>I. Methylation of D-Mannitol-2,3,4,5-tetraacetate</td>
<td>29</td>
</tr>
<tr>
<td>J. Deacetylation of the Di-O-methyl-D-mannitol</td>
<td>30</td>
</tr>
<tr>
<td>K. Di-O-methyl-dimethylene-D-mannitol</td>
<td>31</td>
</tr>
<tr>
<td>CLAIMS TO ORIGINAL RESEARCH</td>
<td>32</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>33</td>
</tr>
</tbody>
</table>

## PART II

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>35</td>
</tr>
<tr>
<td>HISTORICAL INTRODUCTION</td>
<td>38</td>
</tr>
<tr>
<td>DISCUSSION OF RESULTS</td>
<td>45</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td></td>
</tr>
<tr>
<td>A. Preparation of the Wood</td>
<td>51</td>
</tr>
<tr>
<td>B. Extraction of the Wood with Benzene</td>
<td>52</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (cont.)

EXPERIMENTAL (cont.)

C. Extraction of the Wood with Acetone............. 52
D. Extraction of the Wood with Aqueous Ethanol... 53
E. Acid Treatment of the Ethanol Extract........... 54
F. Extraction of the Pigment from Rose Petals.... 55
G. Chromatography of the Pigments.................. 56
H. Tests of Link and Robinson....................... 57
I. Acetylation of the Ethanol Extract............... 61

CLAIMS TO ORIGINAL RESEARCH......................... 62

BIBLIOGRAPHY........................................... 63

LIST OF TABLES

TABLE I Solubility Characteristics of the Ethanol Extract........ 54
TABLE II Chromatography of the Crude Rose Extract... 56
TABLE III Chromatography of the Purified Rose Extract........ 57
TABLE IV Anthocyanidin Tests........................ 58
TABLE V Robinson's Tests............................. 60
INTRODUCTION

It has long been known that the several hydroxyl groups present in a carbohydrate molecule possess different reactivities (26). Where these different are known it is possible by choice of appropriate reagents and conditions to synthesize carbohydrate derivatives with substituent groups located at specified positions on the carbon chain. The purpose of this research was to study the specificity of some selective substitution and elimination reactions in the mannitol molecule and the following series of synthetic steps was carried out:

\[
\begin{align*}
\text{D-mannitol (I)} & \\
\text{1,6-di-O-trityl*-D-mannitol tetraacetate (II)} & \\
\text{D-mannitol-2,3,4,5-tetraacetate (III)} & \\
\text{1,6-di-O-methyl-D-mannitol tetraacetate (IV)} & \\
\text{1,6-di-O-methyl-D-mannitol (?) (V)} & \\
\end{align*}
\]

Isolation of crystalline 1,6-di-O-methyl-D-mannitol, which has been previously prepared by another method (30) would show that D-mannitol-2,3,4,5-tetraacetate resulted from the detritylation of 1,6-di-O-trityl-D-mannitol tetraacetate and that the acetyl groups in D-mannitol-2,3,4,5-tetraacetate did not undergo migration in a methylation reaction using the Purdie method (23). If a di-O-methyl

* Trityl is used as an abbreviated form for triphenylmethyl.
derivative other than $V$ was obtained some group migration would be indicated.
HISTORICAL INTRODUCTION

Trityl ethers have been valuable in carbohydrate synthetic work because of their specificity of substitution and stability. They are formed by the reaction of triphenyl-carbinol with an alcohol in the presence of an acid catalyst (25) or from trityl chloride by reaction with an alcohol (7). In compounds containing several hydroxyl groups, both primary and secondary, it is often convenient to specifically substitute the primary hydroxyls while another reaction is undertaken with the secondary hydroxyls to substitute them. Subsequent removal of the groups on the primary hydroxyls leaves these positions free to undergo further reaction. The trityl ethers serve this purpose. Experience has shown that in compounds containing both primary and secondary hydroxyl groups, the primary hydroxyls react preferentially with the tritylating agent (Helferich's Rule) (10).

Although tritylation was considered to substitute only the primary hydroxyls in carbohydrate molecules, tritylation does occur at secondary hydroxyls but only at a much slower rate. Hockett and coworkers (15) investigated quantitatively the tritylation of the following compounds:
All three compounds gave crystalline trityl ethers, however a considerable difference in velocity of tritylation was found. Using a four-fold excess of trityl chloride and following the reaction polarimetrically, it was found that compounds VI and VII were half-tritylated after 21.6 and 57.9 hours respectively, while compound VIII was half-tritylated only after 2500 hours. These results show the marked difference in tritylation reaction rates between the primary and secondary hydroxyl groups in carbohydrates.
Hockett and Hudson (16) prepared two ditrityl ethers of \(\alpha\)-methyl-D-xylopyranoside showing that more than one secondary hydroxyl group in a molecule is substitutable. The relative rates of tritylation in primary hydroxyls and secondary hydroxyls have not been studied in the hexitol series.

In their studies of the reactions between benzene and carbon tetrachloride, Friedel and Crafts (7) prepared the first trityl ethers. Helferich (14) first studied the tritylation of the free monosaccharides and prepared the mon\(\alpha\)-trityl derivatives of D-glucose and D-galactose. He noted that these compounds reduced Fehling's solution and when acetylated gave a crystalline tetraacetate derivative which, when treated with phosphorus pentabromide yielded 1,6-dideoxy-dibromo-2,3,4-tri-\(O\)-acetyl-D-glucose or D-galactose. He concluded that he had tritylated glucose in the six position. The 6-trityl-D-glucose and galactose were probably glasses since their melting points (13) were not sharply defined.

By means of the relative specificity of the trityl group for the primary hydroxyls Helferich (12) was able to develop the synthesis of some disaccharides which previously had been very difficult. He synthesized gentiobiose, which is 6-(\(\beta\)-D-glucopyranosyl)-D-glucopyranose, by the following reactions:

\[
\begin{align*}
\alpha-D-glucose & \quad \rightarrow \quad \alpha-D-glucose \\
2,3,4,6-tetra-\(O\)-acetyl-\(\alpha\)-D-glucosyl bromide & \quad \rightarrow \quad 6-0-trityl-2,3,4-tri-\(O\)-benzoyl-\(\alpha\)-D-glucosyl fluoride \\
& \quad \downarrow \quad 2,3,4-tri-\(O\)benzoyl-\(\alpha\)-D-glucosyl fluoride \\
& \quad \downarrow \quad 6-(\text{tetraacetyl-}\(\beta\)-glucopyranosyl)-2,3,5-tribenzoyl-\(\alpha\)-D-glucosyl fluoride \\
& \quad \downarrow \quad 6-(\beta-D-glucopyranosyl)-\(\alpha\)-D-glucopyranose (gentiobiose).
\end{align*}
\]
By the tritylation reaction it was possible to synthesize a molecule with the C-6 position unsubstituted, thus opening the way to 1-α-6' and 1-β-6' disaccharide syntheses (10).

The first preparations of the trityl ethers of the polyols were made by Valentin (28). By reacting one mole of hexitol with two moles of trityl chloride he prepared the ditrityl derivatives of the common hexitols except dulcitol. He presumed the low solubility of dulcitol in pyridine to be the reason for his failure to prepare 1,6-di-O-trityl-dulcitol. Wolfrom (31) however, found that 1,6-ditrityl-dulcitol crystallized as a dipyridinium addition compound from the reaction mixture and when this addition compound was recrystallized from ethanol, the acidity of the addition compound was sufficient to de-tritylate it leaving triphenylcarbinol and dulcitol. The addition compound was decomposed by bases without detritylation to give the 1,6-di-O-trityl-dulcitol.

Triphenylcarbinol as a tertiary alcohol has the tendency to react by splitting out the entire hydroxyl group together with its binding electrons, leaving a fairly stable trityl carbonium ion (8). This carbonium ion is stable in acid solution. The proton provided by the acid adds to the oxygen atom of the triphenylcarbinol or trityl ether.

\[
\begin{align*}
\text{Ph}_3\text{C}-\text{OH} + H^+ & \rightarrow \text{Ph}_3\text{C}^+ + H_2O \\
\text{Ph}_3\text{C}-\text{Cl} + H^+ & \rightarrow \text{Ph}_3\text{C}^+ + \text{HCl} \\
\text{Ph}_3\text{C}^+ + \text{OR}^- & \rightarrow \text{Ph}_3\text{C}-\text{OR}
\end{align*}
\]

This mechanism shows why trityl ethers are easily hydrolyzed by dilute acids and are quite stable to alkaline conditions, since alkali causes little or no formation of
the trityl carbonium ion (10).

Cleavage of trityl ethers is easily accomplished by dilute acids which shifts the above equilibrium to the left. This fact is of value in syntheses since a molecule substituted with both trityl groups and acetyl groups upon treatment with dilute acid would have the trityl groups selectively removed and leave the acetyl groups intact and thus form a specifically substituted molecule.

Isomerization of a polyol derivative by acyl migration has been observed many times (26). Fischer (6) studied acylation experiments on substituted glycerides and found that in an α,β-diglyceride (IX) migration took place to form an α,α'-diglyceride (X) during an acylation

\[
\begin{align*}
\text{CH}_2\text{OR'}^+ \\
\text{CH}_2\text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{OR'}^+ \\
\text{CH}_2\text{OH}
\end{align*}
\]

Fischer proposed the formation of a cyclic orthoester (XII) as an intermediate in this migration

\[
\begin{align*}
\text{CH}_2\text{O-C}^\circ\text{CH}_3 \\
\text{CH}_2\text{O-C}^\circ\text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{O-C}^\circ\text{CH}_3 \\
\text{CH}_2\text{O-C}^\circ\text{CH}_3
\end{align*}
\]

In methylations of acylated carbohydrates using the Purdie technique (23) acyl migration has frequently been
observed. Haworth (9) showed acetyl migration occurred in the methylation of methyl-2,3,4-tri-O-acetyl-α-D-glucopyranoside, yielding methyl-2-O-methyl-3,4,6-tri-O-acetyl glucopyranoside. Helferich (11) noted that in the methylation of the corresponding tribenzoyl derivative no migration of the benzoate ester group occurred. In the majority of cases examined, the movement of the ester group was toward the primary hydroxyl. Helferich and Klein (12) found that alkali present in soft glass was sufficient to cause isomerization of 1,2,3,4-tetra-O-acetyl-α-D-glucose to the corresponding 1,2,3,6 tetraacetate. The proximity (9) of the groups in positions four and six in the cyclic sugar molecule appeared to enhance the formation of a strainless ring, thus making migration feasible.

Although Fischer (6) studied acyl migration between the adjacent positions in glycerol derivatives, no work has been done on acyl migration in partially acetylated hexitol molecules during a methylation reaction.

In 1946 Wiggins (30) prepared 1,6-di-O-methyl-D-mannitol using the following series of reactions:

D-mannitol
↓
triacetone-D-mannitol
↓
3,4-monoacetone-D-mannitol
↓
1,6-di-O-tosyl-3,4-monoacetone-D-mannitol
↓
1,6-di-O-tosyl-2,5-di-O-acetyl-3,4-monoacetone-D-mannitol
↓
1,2,5,6-dianhydro-3,4-monoacetone-D-mannitol
↓
1,6-di-O-methyl-3,4-monoacetone-D-mannitol
↓
1,6-di-O-dimethyl-D-mannitol
DISCUSSION

A. 1,6-Di-O-trityl-D-mannitol Tetraacetate

The simultaneous tritylation and acetylation of D-mannitol proceeded smoothly in this synthesis. The modification of Jeanloz (18) of the acetylation procedure gave 1,6-di-O-trityl-D-mannitol tetraacetate completely free from triphenylcarbinol. The older acetylation procedure required pouring of the reaction mixture into a large volume of ice and water. In cases where no solid contaminants or only water soluble solid contaminants were present this method offered no difficulties. However, in the case of simultaneous tritylation and acetylation this procedure resulted in the precipitation of unreacted trityl chloride as triphenylcarbinol, and since 1,6-di-O-trityl-D-mannitol tetraacetate and triphenylcarbinol have very nearly the same solubilities in recrystallization solvents, the separation of these two compounds presented a problem. The Jeanloz method (18) avoided this difficulty and 1,6-di-O-trityl-D-mannitol tetraacetate was recovered almost uncontaminated from the reaction mixture.

B. 1,6-Di-O-trityl-D-mannitol

1,6-Di-O-trityl-D-mannitol has not previously been reported crystalline. It was obtained here in solid form, presumably as a crystalline solvate since solid material was obtainable only from an alcohol-water solvent pair. Examination under the microscope clearly revealed a needle-like crystal form, although the melting point range was 73 - 78°C. Wolfrom (31) reported that sometimes trityl ethers form complexes
with such solvents as alcohol or pyridine. Several preparations of 1,6-di-O-trityl-D-mannitol were made and the trityl group analysis was the same for each preparation after a recrystallization from alcohol-water.

Further purification of this compound was made by chromatographic adsorption on an alumina column (32). Several exploratory columns were run to check the adsorbability of the alumina toward the 1,6-di-O-trityl-mannitol, and it was found that, using concentrated sulfuric acid as a streak reagent, a high concentration of trityl group-containing material remained at the top of the column even after washing with a considerable volume of chloroform. According to Zeile and Kruckenberg (32), chloroform removes triphenylcarbinol from an alumina column.

Since the compound was purified chromatographically and could be converted in good yield to 1,6-di-O-trityl-D-mannitol tetraacetate by acetylation, it appeared that 1,6-di-O-trityl-D-mannitol had been prepared, possibly in a solvated form, and any monosubstitution or trisubstitution products as well as triphenylcarbinol were eliminated in the purification. Valentin (28) reported a melting point of 98 - 103°C for 1,6-di-O-trityl-D-mannitol but his product was subsequently shown to be amorphous (22).

C. Detritylation Procedures

Trityl ethers of carbohydrates are extremely sensitive to acid and even small amounts are sufficient to cause detritylation (10). They are cleaved to triphenylcarbinol and an alcohol by acids at room temperature, a reaction applied to carbohydrate detritylations by several authors (10).
C. (1) Hydrogen Bromide in Glacial Acetic Acid

Trityl ether cleavage by hydrogen bromide was achieved and the insoluble trityl bromide removed. Wolfrom (31) reported that, in some cases, excess hydrogen bromide present in the reaction mixture brominated the detritylated product to form the corresponding deoxybromide compound.

\[
\text{TrOR} + 2\text{HBr} \rightarrow \text{TrBr} + \text{RBr} + \text{H}_2\text{O}
\]

In the present case it appeared possible that the hydrogen bromide brominated the D-mannitol-2,3,4,5-tetraacetate. The sirup from this reaction did not crystallize after many trials; a mixture containing 1,6-dideoxy-dibromo-D-mannitol tetraacetate and trityl bromide or triphenylcarbinol would be difficult to crystallize and halogen analysis would not be conclusive.

C. (2) Hydrogenation in Glacial Acetic Acid over a Platinum Oxide Catalyst.

This method gave crystalline D-mannitol-2,3,4,5-tetraacetate in low yield. Tricyclohexylmethane was recovered from the reaction indicating that complete reduction of the benzene nuclei had taken place.

The conditions in this reaction were difficult to control and a crystalline product was only once obtained. Two reasons for incomplete reaction are suggested.

(a) Susceptibility of the catalyst to poisoning: Unless extreme care was exercised in purifying the 1,6-di-O-trityl-D-mannitol tetraacetate the catalyst was poisoned and the starting material was recovered unchanged.
(b) The possibility that the reduction of the benzene nuclei had occurred before the ether cleavage thus inhibiting the reaction (21).

C. (3) **Dilute Acetic Acid**

This method gave the most consistent results, highest yields, and easiest purification of product. The addition of excess water precipitated the triphenylcarbinol almost quantitatively and the mannitol tetraacetate remained in solution. The melting point \((158 - 162^\circ C.)\) of the precipitate was almost that for pure triphenylcarbinol indicating that very little hexitol material had co-precipitated.

The acetic acid method was used almost exclusively after several trials with methods (1) and (2). The use of mineral acid in chloroform was not considered since the solubility of trityl chloride, triphenylcarbinol, and D-mannitol-2,3,4,5-tetraacetate in chloroform was high and an initial separation of the end products would be difficult.

Chloroform petroleum-ether formed a good recrystallization solvent since any triphenylcarbinol, which was not removed by the initial water precipitation, remained in solution and was not precipitated by the addition of petroleum-ether.

D. **D-Mannitol-2,3,4,5-Tetraacetate**

D-Mannitol-2,3,4,5-tetraacetate was reported crystalline by Micheel (21). He assumed that detritylation of 1,6-di-O-trityl-D-mannitol tetraacetate would give D-mannitol-2,3,4,5-tetraacetate because detritylation of 6-trityl-1,2,3,4-tetra-O-acetyl-D-glucopyranose gave the corresponding crystalline 1,2,3,4-tetraacetate.
The physical constants and acetyl analysis of the compound in this laboratory agreed with the prepared data given by Micheel (21) and acetylation of our D-mannitol-2,3,4,5-tetraacetate gave D-mannitol-hexaacetate in good yield. An attempted tritylation of the tetraacetate to give 1,6-ditrityl-D-mannitol tetraacetate yielded only triphenylcarbinol and a sirup.

D-Mannitol-2,3,4,5-tetraacetate was slow to crystallize, and even when seeded required one to two weeks at 0°C. for complete crystallization (m.p. of pure compound 123°C).

A literature search revealed that of the nine possible isomeric D-mannitol-tetraacetates only three have been prepared: D-mannitol-1,3,4,6-tetraacetate, m.p., 107°C. (3). D-mannitol-1,2,3,4-tetraacetate, m.p., 92°C. (29). D-mannitol-2,3,4,5-tetraacetate, m.p., 123°C. (21).

E. Di-O-methyl-D-mannitol

Methylation of the D-mannitol-2,3,4,5-tetraacetate followed by deacetylation gave a syrupy di-O-methyl hexitol derivative. The solubility and methoxyl value reported by Wiggins (30) for 1,6-di-O-methyl-D-mannitol agreed well with the compound prepared here. The dimethyl-mannitol was a sirup and it was investigated by means of paper chromatography to show one or more constituents were present. It is known that separation of individual hexitols on paper chromatograms is difficult, since they all have approximately the same Rf values (19). Finding a satisfactory spray reagent also proved difficult since sodium metaperiodate in acidic potassium permanganate; alkaline potassium permanganate; ammonical
silver nitrate gave no reaction and neutral sodium metaperiodate followed by dilute ethylene glycol and aqueous potassium iodide was so sensitive that no definite conclusions could be drawn.

In an attempt to get a crystalline derivative the dimethyl mannitol was treated with formaldehyde to obtain a dimethylene compound. 1,6-Dimethyl-2,4;3,5-dimethylene-D-mannitol was also reported by Wiggins (30). Seed crystals of this compound and of 1,6-di-O-methyl-D-mannitol have been obtained recently from Dr. Wiggins but their use had not yet yielded any crystalline products.

Acetyl migration has been reported (9) from the four position to the six position in partially acetylated D-glucopyranose derivatives during the course of methyl iodide-silver-oxide methylation. According to Haworth (9) the methylation itself was not the determining factor in acetyl migration but rather the alkaline conditions produced by the silver oxide. In the present case, if migration occurred even to a small degree, a mixture of dimethyl compounds would result which would make crystallization difficult.

Isolation, in good yield, of crystalline 1,6-di-O-methyl-D-mannitol would prove that the mannitol tetraacetate obtained was actually the 2,3,4,5-tetraacetate isomer, since methylation would substitute only the primary hydroxyls of the molecule. Furthermore, it would show in this case at least that acetyl migration did not occur during a methylation using the Purdie reagents. This was partially borne out in the first methylation which, after proceeding for three hours followed by the usual treatment of the reaction mixture, yielded crystals of the starting material. Although not all the
starting material was recovered it indicated that, in this case at least, migration did not readily take place, since some of the compound must have been methylated.

If acetyl migration had taken place then after deacetylation a mixture of dimethyl mannitols was obtained. Separation of this mixture into two or more components would show that acetyl migration had occurred.

The following table gives a list of the nine isomeric dimethyl-D-mannitols and the results of their reaction with periodic acid. Except for case 5 and case 6 the behavior of each of the dimethyl-D-mannitols toward periodic acid would be unique. From a consideration of the structures involved the four compounds 1,2,7,9 would be the most probable components of a mixture resulting from acetyl migration and these are easily distinguishable by their reaction towards periodate.

If migration occurred here it would be of interest to prepare other D-mannitol tetraacetates and study acyl migration under methylation conditions. Since acyl migration during methylation is known to occur (9) in partially acetylated ring forms of sugars it would be of interest to study the relative ease of migration occurring in these forms with that occurring in the straight chain hexitol molecules.

Since trityl chloride has been shown to react with both primary hydroxyls and secondary hydroxyls in a polyhydroxy compound containing these groups, the study of the relative tritylation rates of the primary and secondary hydroxyls in a hexitol molecule could be made.
<table>
<thead>
<tr>
<th>Case</th>
<th>Position of -OCH₃ groups</th>
<th>Moles Periodate consumed</th>
<th>Oxidation Products (moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1,6</td>
<td>3</td>
<td>HCHO: 2, HCOOH: 2, CH₂OMe: 2, CHO: 1, OHC-CH₂OMe and HO₂CH: MeOCH-CHO</td>
</tr>
<tr>
<td>2</td>
<td>2,6</td>
<td>2</td>
<td>HCHO: 1, HCOOH: 1, CH₂OMe: 1, CHO: 1, HCOOMe &amp; CH₂OMe</td>
</tr>
<tr>
<td>3</td>
<td>3,6</td>
<td>2</td>
<td>HCHO: 1, HCOOH: 1, CH₂OMe: 1, CHO: 1, CH₂OH: MeOCH</td>
</tr>
<tr>
<td>4</td>
<td>1,2</td>
<td>3</td>
<td>HCHO: 1, HCOOH: 1, CH₂OMe: 1, CHO: 1, MeOCH</td>
</tr>
<tr>
<td>5</td>
<td>1,3</td>
<td>2</td>
<td>HCHO: 1, HCOOH: 1, CH₂OMe: 1, CHO: 1, MeOCH</td>
</tr>
<tr>
<td>6</td>
<td>2,3</td>
<td>2</td>
<td>HCHO: 1, HCOOH: 1, MeOCH: 1</td>
</tr>
<tr>
<td>7</td>
<td>2,5</td>
<td>1</td>
<td>CH₂OH: 2MeOCH</td>
</tr>
<tr>
<td>8</td>
<td>3,5</td>
<td>1</td>
<td>HCOOH: MeOCH, HCOOMe-CH₂OH</td>
</tr>
<tr>
<td>9</td>
<td>3,4</td>
<td>2</td>
<td>2 MeOCH, CHO</td>
</tr>
</tbody>
</table>

Theoretical Products of the Periodate Oxidate of D-Mannitol Dimethyl Ethers
EXPERIMENTAL

All melting points were determined in an apparatus previously calibrated with a series of pure compounds. In view of the sensitive nature of the carbohydrate material all evaporations were carried out at reduced pressure and temperatures not exceeding 50°C.

A. Materials

Benzene

Absolute benzene was prepared by the method of Fieser (5) and stored over sodium.

Pyridine

Pyridine was stored over solid potassium hydroxide for several weeks and distilled from barium oxide immediately before use.

Methanol

Absolute methanol was prepared by two successive distillations of the commercial absolute grade material from magnesium turnings.

Acetic Anhydride

Analytical reagent grade acetic anhydride supplied by British Drug Houses was distilled before use.

Toluene

Absolute toluene was prepared by the method of Fieser (5) and stored over sodium.

Acetyl Chloride

Reagent grade acetyl chloride as supplied by Merck and Company and used without further purification.
Methyl Iodide

Methyl iodide was obtained from British Drug Houses and distilled before use.

Triphenylcarbinol

A student preparation of triphenylcarbinol was purified by one recrystallization from carbon tetrachloride and one from 95% ethanol: m.p., 161.0 - 162.5°C.

D-Mannitol

D-Mannitol as supplied by the Matheson Chemical Company was recrystallized from aqueous ethanol: m.p., 165 - 166°C.

\[ [\alpha]_D^{24} = 0.195^\circ (C, 5.359; 1,1;H_2O) \]

The specific rotation was also observed in 5% aqueous ammonium molybdate solution (27).

\[ [\alpha]_D^{22} + 24.0^\circ (C, 5.093; 1,1;aq. (NH_4)_6Mo_7O_{24}) \]

Barium Methylate

A solution of barium methylate in absolute methanol was prepared by the method of Isbell (17). Titration of a 6.0 ml. aliquot with 0.572 N sulfuric acid showed the barium methylate concentration to be 0.381 normal.

Triphenylmethyl Chloride (1)

Dry, pure triphenylcarbinol (100.0 g.) was dissolved in dry, thiophene-free benzene (32 ml.), the solution was heated on a steam bath and, when hot, acetyl chloride (20.0 ml.) was added with stirring. After five minutes heating additional acetyl chloride (30.0 ml.) was added over a period of one-half hour. The reaction mixture was
refluxed a further half-hour after the final addition of acetyl chloride. The addition of two volumes of low-boiling petroleum-ether to the cooled reaction mixture caused the formation of a heavy white precipitate. The flask was placed in an ice bath for two hours to complete precipitation. The crystals were removed by filtration, washed with 100 ml. petroleum-ether, and dried in vacuo over calcium chloride, soda lime and paraffin shavings. Yield: 71.5 g. (68%), m.p., 110 - 111°C. (1).

The product was colorless but for a pale green luminescence and was used without further purification.

Trityl chloride prepared by this method was much superior to that commercially available. The commercial product even after several recrystallizations had a dark yellow color and melted at 106 - 108°C.

B. Analytical Methods

Acetyl analysis

Acetyl groups were determined by the method of Clark (4).

Methoxyl analysis

Methoxyl groups were determined by the modified Zeisel method as described by Clark (4).

Trityl analysis

Trityl groups (triphenylmethyl groups) were determined by the method of Valentin (28).

In order to determine the maximum solubility losses in a trityl determination by the use of excess reagents a sample of triphenylcarbinol was analyzed for trityl
Triphenylcarbinol (0.10285 g.) was dissolved in concentrated sulfuric acid (5.0 ml.) and the resulting orange solution was poured into cold water (50.0 ml.). The characteristic precipitate was collected on a sintered glass funnel, washed with cold water (175 ml.) to remove all traces of sulfuric acid and dried in vacuo over phosphorus pentoxide to constant weight; (0.10180 g.), m.p., 159 - 161°C.

It was concluded that approximately a one per cent loss might be expected in a trityl analysis.

C. D-Mannitol Hexaacetate

D-Mannitol hexaacetate was prepared by the method of Baer and Fischer (2) for use as a reference compound. The melting point after two recrystallizations from 95% ethanol was 122 - 123°C; yield, 89.5%.

Found: acetyl, 58.7%, 58.2%
Calculated for C\(_{18}\)H\(_{26}\)O\(_{12}\): acetyl, 59.4%

D. 1,6-Di-O-trityl-D-Mannitol Tetraacetate

After several preliminary experiments the following procedure adapted from Reynolds and Evans (24) and Jeanloz (18) was found to be satisfactory for the preparation of 1,6-di-O-trityl-D-mannitol-tetraacetate.

Dry D-mannitol (0.083 mole) and trityl chloride (0.166 mole) were dissolved in dried pyridine (150 ml.) containing Drierite (3.5 g.) as an internal desiccant. The resulting pale yellow mixture was heated to 40°C. and
stirred mechanically for 24 hr. Freshly distilled acetic anhydride (0.87 mole) was then added and stirring and heating were continued for an additional 48 hr. At this time the reaction mixture was dark yellow in color. The drierite was removed by filtration and excess absolute methanol was added slowly to the ice cold filtrate to decompose the excess acetic anhydride. Norite (1.0 g.) was then added and the mixture was allowed to stand for several hours at room temperature. Removal of the norite left a yellow solution. The methyl acetate, methanol, acetic acid were removed by distillation under reduced pressure and the pyridine was removed by azeotropic distillation with dry toluene also under reduced pressure. Several distillations with toluene were necessary for complete removal of all traces of pyridine. When about one-half of the volume of the solvent had been removed a heavy white precipitate formed which was filtered, washed with absolute methanol, and dried in vacuo over phosphorus pentoxide. Weight of dried product, 21.0 g.; m.p., 184 - 186°C. Upon further evaporation two more crops of crystals were collected weighing 24.0 g. and 3.0 g. respectively; m.p., 180 - 184°C. The mother liquor, being dark red and giving no further precipitation upon the addition of absolute methanol, was discarded. Total yield, 48.0 g. (70%). The crystals were very soluble in chloroform and insoluble in methanol, ethanol, and petroleum-ether. Two recrystallizations from chloroform-methanol gave a colorless
product melting at 184.5 - 186.0°C.

\[ [\alpha]_{D}^{21} = +48.4^\circ \text{ (C, } 4.878; \text{ 1, 1; CHCl}_3) \]

Recrystallization from chloroform-methanol gave almost quantitative recovery of the compound. Use of this solvent pair gave more satisfactory results than n-butanol which was used by Micheel (21).

Wolfrom (31) reported a melting point of 183 - 184°C.

and a specific rotation of +46.4° in chloroform for 1,6-ditrityl-D-mannitol-tetraacetate.

Found: trityl, 57.2%; acetyl, 20.25%.

Calculated for C\textsubscript{52}H\textsubscript{50}O\textsubscript{10}: trityl, 57.5%; acetyl, 20.6%.

From the observed constants it was concluded that the compound was 1,6-ditrityl-D-mannitol tetraacetate as reported by Micheel (21) and Wolfrom (31).

E. 1,6-Di-O-trityl-D-Mannitol

Dry D-mannitol (0.028 mole) and trityl chloride (0.056 mole) were dissolved in dried pyridine (75 ml.) containing Drierite (1.0 g.) as an internal desiccant. The reaction mixture was heated to 40°C. and stirred mechanically for 17 to 18 hr., after which the drierite was removed by filtration and the filtrate added dropwise to three liters of ice and water. A viscous, colorless sirup formed which after standing for two days at 0°C. hardened to a semicrystalline mass. This material was dried in vacuo over concentrated sulfuric acid and solid potassium hydroxide. Yield, 13.28g.; (72%). According to Valentin (28), ditrityl hexitols are soluble in hydroxylic solvents as well as chloroform and benzene. Hence, the
dried product was extracted three times at room temperature with an equal weight of absolute methanol, and the extracts were combined, concentrated, warmed and filtered through norite. Hot water was added to the filtrate to turbidity. After standing several days at room temperature, the solution deposited a semicrystalline product, m.p., 75 - 85°C. Two further precipitations from methanol-water yielded a colorless product which softened to a clear liquid between 73 and 78°C. Observation of this product under a microscope showed no definite crystal structure and it was assumed to be a glass.

Three hundred mg. of the 1,6-di-O-trityl-mannitol were dissolved in 20 ml. chloroform and placed on a 3.5 x 10.0 cm. alumina column which was then washed with 300 ml. chloroform to remove any triphenylcarbinol. The last two milliliters of chloroform eluate gave a negative trityl test. Evaporation of the chloroform eluate yielded only a small amount of solid material which had a melting point of 151 - 155°C. This was assumed to be triphenylcarbinol (21).

The column was extruded and extracted in a Soxhlet extractor with 95% ethanol containing 1.0% chloroform for 4 hr. Evaporation of the ethanol extract yielded 200 mg. of solid material which observation under a microscope proved crystalline. The product had the same melting point as before (73 - 78°C.) and was not altered by a recrystallization from methanol-water. Attempted recrystallizations from ethanol petroleum-ether and chloroform petroleum-ether
gave sirups in each case.

Found: trityl, 65.0%
Calculated for C_{44}H_{20}O_{6}: trityl, 72.8%
Calculated for C_{44}H_{20}O_{6}\cdot H_2O: trityl, 65.8%.

F. Acetylation of 1,6-di-O-trityl-D-Mannitol

1,6-Di-O-trityl-D-mannitol (100 mg.) was dissolved in dried pyridine (2.0 ml.) and freshly distilled acetic anhydride (1.5 ml.) was added. The resulting solution was heated to 60°C on a water bath and then allowed to stand at room temperature for 24 hr. when it was poured into an excess of ice and water. The crystalline product which separated was filtered, dried in vacuo over phosphorus pentoxide and recrystallized from chloroform-methanol solution. Yield, 0.082 g. (66%), m.p., 184.5 - 186°C.

A mixed melting point taken with an authentic sample of 1,6-di-O-trityl-D-mannitol tetraacetate showed no depression.

G. Detritylation Procedures Applied to 1,6-Ditrityl-D-Mannitol Tetraacetate

(1) Hydrogen Bromide in Glacial Acetic Acid (21)

1,6-Di-O-trityl-D-mannitol tetraacetate (2.0 g.) was dissolved in warm glacial acetic acid (25 ml.) (distilled from chromium trioxide). The solution was cooled to 5°C. and 3.0 ml. of a 30% solution of hydrogen bromide in glacial acetic acid was added. One minute later a precipitate formed which was immediately removed by suction filtration, washed with petroleum-ether and dried in vacuo over phosphorus pentoxide and paraffin shavings. The filtrate was immediately
evaporated to dryness under reduced pressure in order to insure complete removal of any excess hydrogen bromide.

The precipitate weighed 0.95 g. (62%), melted at 151 - 152°C. and gave a positive test for the trityl group. It was assumed to be trityl bromide (21).

The filtrate, after evaporation, yielded a light yellow sirup which was dried and then dissolved in 95% ethanol containing a trace of acetic acid. Filtration through norite yielded a colorless solution. The solvent was again removed and the sirup dried in vacuo. The sirup was very soluble in methanol, ethanol; relatively soluble in chloroform and insoluble in ether and petroleum-ether. Several attempts to crystallize this sirup from various solvents were unsuccessful.

(2) Catalytic Detritylation using Hydrogen and Platinum Oxide Catalyst (21)

1,6-Di-O-trityl-D-mannitol tetraacetate (2.0 g.) was dissolved in warm glacial acetic acid (40 ml.) (distilled from chromium trioxide), platinum oxide (0.20 g.) was added and the mixture was hydrogenated in a low pressure Parr Hydrogenator. After 28 hr. heating and shaking, the pressure became constant, the used catalyst was filtered off and the filtrate evaporated under reduced pressure. When one-half the volume of the solvent had been removed, an oil separated which crystallized when cooled to 10°C. and seeded with a crystal of tricyclohexylmethane. Yield, 0.30 g.;
Further evaporation of the mother liquor yielded a colorless sirup which was dissolved in ethanol, containing a trace of acetic acid, and petroleum-ether added to turbidity. After standing several days at 0°C., crystals appeared which were removed on a sintered glass disc, washed thoroughly with petroleum-ether, and dried. Two more recrystallizations from ethanol petroleum-ether gave crystals; m.p., 122 - 123°C. Another recrystallization failed to alter the melting point. Yield, 0.055 g. (7.0%).

3. Detritylation using Dilute Acetic Acid (31)

1,6-Di-O-trityl-D-Mannitol tetraacetate (4.1 g.) was dissolved in glacial acetic acid (25 ml.) and the solution was heated to reflux temperature. Distilled water (4.25 ml.) was then added and refluxing was continued for one hour when, after cooling the reaction mixture a further 12.5 ml. of water was added. The precipitate of triphenylcarbinol which appeared immediately, was filtered off, dried in vacuo over phosphorus pentoxide, and weighed. Yield, 2.40 g. (94%); m.p., 158 - 162°C. After a recrystallization from 95% ethanol the carbinol had a melting point of 161 - 162°C. and no depression was observed when a mixed melting point was taken with an authentic sample.

The mother liquor was evaporated to dryness under reduced pressure and the resulting sirup was taken up in 95% ethanol and petroleum-ether was added to turbidity. After standing at 0°C. for one week, crystals
separated which after two further recrystallizations from the above solvent pair had a melting point of 122 - 123°C. Yield, 0.35 g. Partial evaporation of the solvent from the mother liquor and further addition of petroleum-ether yielded a second crop of crystals after fourteen days at 0°C. These crystals melted at 122 - 123°C. after two recrystallizations from ethanol petroleum-ether. Yield, 0.10 g. The total yield of D-mannitol-tetraacetate was 0.45 g. (26%). Further evaporation of the mother liquors and subsequent treatment with petroleum-ether failed to yield further amounts of crystalline material.

Found: acetyl, 45.8%

Calculated for C_{14}H_{22}O_{10} : acetyl, 46.2%.

A mixed melting point taken with a sample of D-mannitol hexaacetate showed a considerable depression (m.p., 105 - 115°C.)

H. Acetylation of D-Mannitol-2,3,4,5-tetraacetate

Dry D-mannitol-2,3,4,5-tetraacetate (0.10 g.) was dissolved in dried pyridine (4.0 ml.) and freshly distilled acetic anhydride (4.0 ml.) was added. The colorless solution was allowed to stand at room temperature for two days and then poured into 100 ml. of ice and water. The heavy, colorless crystalline precipitate which separated was filtered, air dried, and recrystallized from 95% ethanol. Yield, 0.106 g. (85%), m.p., 122 - 124°C.

A second recrystallization did not alter the melting point.
When mixed with a sample of D-mannitol hexaacetate, no depression in melting point was observed.

I. Methylation of D-Mannitol-2,3,4,5-tetraacetate

D-Mannitol-2,3,4,5-tetraacetate (0.350 g.) was added to dry acetone (5.0 ml.) containing methyl iodide (7.0 ml.), dry silver oxide (4.0 g.), and drierite (4.0 g.), and the mixture was refluxed for 20 hr. The reaction mixture was then cooled to room temperature and the solids removed by filtration. The solid material was exhaustively extracted with acetone and the chloroform and the combined extracts added to the first filtrate. Evaporation of the colorless solution under reduced pressure left a clear, colorless sirup which, when dried in vacuo, gave a low methoxyl analysis.

Found: OCH₃, 12.2%, 11.8%;
Calculated for C₁₆H₂₆O₁₀ : OCH₃, 16.3%.

The methylation was repeated using the above method except that the acetone was omitted, the methyl iodide acted as the solvent. From this methylation a clear, colorless sirup was obtained; yield, 0.290 g.

Found: OCH₃, 15.4%, 15.5%;
Calculated for C₁₆H₂₆O₁₀ : OCH₃, 16.3%

Several attempts to crystallize this sirup were unsuccessful. This compound has not been previously reported in the literature.
J. Deacetylation of the Di-O-methyl-D-mannitol tetraacetate

(1) Method of Isbell (17)

The sirup (0.274 g.) from the above methylation was dissolved in absolute methanol (10.0 ml.) containing 0.38N barium methylate (1.0 ml.) and the solution was kept at 0°C. for 24 hr. The excess barium methylate was decomposed by the addition of the stoichiometric amount of 2.00N sulfuric acid which at the same time precipitated the excess barium ion as the sulfate. The precipitate was removed by filtration and the filtrate was evaporated to dryness leaving a colorless sirup, yield, 0.150 g. The dried sirup was extracted several times with hot ethyl acetate and the combined extracts were again evaporated to dryness leaving a colorless sirup.

(2) Method of McKeown and Hayward (20)

This method made use of an anion exchange resin, Dowex 1, for deacetylation.

The sirup (0.285 g.) from the methylation dissolved in absolute methanol (5.0 ml.) was run on the resin in the column and washed on with a further 6.0 ml. of absolute methanol. The compound was left in contact with the resin overnight and then eluted with 200 ml. 90% methanol. The eluate when evaporated, left a clear colorless sirup.

The dried sirup was extracted with hot ethyl acetate (150 ml.). Evaporation of an aliquot of the
ethyl acetate extract showed it contained 0.125 g. of a dimethyl-D-mannitol, yield, 78%.

Found: $\text{-OCH}_3$, 29.2%

Calculated for $\text{C}_8\text{H}_{18}\text{O}_6$: $\text{-OCH}_3$, 29.5%.

Di-O-methyl-dimethylene-D-mannitol

The di-O-methyl-mannitol sirup (0.06 g.) was mixed with paraformaldehyde (0.2 g.) and the mixture was stirred with concentrated sulfuric acid (0.5 ml.) until it had cooled to room temperature. The pale yellow sirup was shaken with chloroform (25 ml.) overnight. One more extraction was made and the chloroform extracts were combined, neutralized with dilute ammonium hydroxide, washed with water, and dried over anhydrous magnesium sulfate. Evaporation of the chloroform extract left a colorless sirup; weight, 0.043 g., yield, 64%.
CLAIMS TO ORIGINAL RESEARCH

1. A simultaneous tritylation and acetylation procedure has been developed for D-mannitol which gives 1,6-di-O-trityl-D-mannitol tetraacetate in good yield and almost completely free from triphenylcarbinol.

2. Direct tritylation of D-mannitol yielded 1,6-di-O-trityl-D-mannitol which was obtained in crystalline form for the first time.

3. The aqueous-acetic acid detritylation technique has been extended to the hexitol series of compounds.

4. A sirupy dimethyl-D-mannitol has been prepared using a new synthetic route. Crystallization of this compound would show the stability of acyl groups towards migration in partially acetylated hexitol molecules during a methylation reaction using silver oxide and methyl iodide. If 1,6-dimethyl-D-mannitol was isolated it would show that tritylation is specific for the primary hydroxyls in D-mannitol in this reaction.
BIBLIOGRAPHY


(20) McKeown, G.G. and Hayward, L.D. Private communication.


(22) Muller, A. Ber. 65 : 1051. 1932.


(29) von Vargha, L. Ber. 66 : 1394. 1933.


PART II

A COLOR PRECURSOR

ISOLATED FROM WESTERN HEMLOCK WOOD
INTRODUCTION

In the groundwood pulping of Western Hemlock (Tsuga heterophylla) a pink color develops during the grinding operation which must be removed before the pulp is of satisfactory brightness for commercial use. This is usually done with a bleaching agent. The color has been attributed, with very little experimental evidence, to the formation of an anthocyanin salt, long known to be responsible for some of the colors appearing in flowers and fruits (11). In many plants these compounds have a colorless precursor, rather loosely defined as a leucoanthocyanin (17). This thesis describes for the first time the isolation and steps toward the identification of a color precursor from Western Hemlock wood.

For purposes of identification leucoanthocyanins are usually converted to their corresponding anthocyanidin salts by treatment with alcoholic mineral acid (8). With the isolation and identification of the anthocyanidin salt and the nature of its substituent groups the structure of the leuco-compound may be elucidated.

Anthocyanidins are flavylium salts and have the fundamental structure (I).
They may be considered to represent a chemical class of natural products (11). They are responsible for many of the colors appearing in the flowers, fruits, and leaves of plants and may exist free, esterified to an hydroxy acid, or as glycosides. The latter classification comprises the largest group of the anthocyanidins. Most of the anthocyanidins found in nature have the 3, 5, 7 positions hydroxylated and a sugar residue, if present, attached to the 3-hydroxyl or at the 3 and 5 positions if it is an anthocyanidin diglycoside. The hydroxyl or methoxyl content of ring C varies with the individual members of the group and hence the characteristics of the individual members differ: delphinidin (3',4',5',3,5,7-hexahydroxy flavylium chloride) is very soluble in water but malvidin (3',5'-dimethoxy-4',3,5,7-tetrahydroxyflavylium chloride) is almost insoluble in water.

Flavylium salts exist as ions and differ from true oxonium salts formed by ethers and γ-pyrones (18). They are considered by Shriner (19) to be carbonium salts and carbons 2, 3, and 4 represent the members of an allylic system.
Flavones form oxonium salts in acids but these are very unstable in the presence of water, unlike the anthocyanidins, whose acid salts are very stable. In fact, anthocyanins and anthocyanidins frequently occur as acid or basic salts in plants (11).

Anthocyanins are sensitive to pH changes: the acid salts are red, the neutral salts are purple, and the metallic salts with bases are blue (11).

The identification of the individual anthocyanidins before 1948 rested upon color reactions outlined by the Robinsons (15) and alkali cleavage to a polyhyroxy phenol and a substituted benzoic acid using methods outlined by Karrer (9). In 1949, Bate-Smith (1) developed an identification technique for anthocyanidin glycosides using paper chromatography with n-butanol, acetic acid, water as the solvent. In 1954 (2), he applied the Forestal solvent (acetic acid, hydrochloric acid, water) to the identification of the anthocyanidin salts obtained by acid hydrolysis of the anthocyanidin glycosides. Those salts fairly soluble in water gave excellent results and show strong adsorption when viewed under ultraviolet light (2).
The term leucoanthocyanin was proposed by Rosenheim (17) who, investigating an anthocyanin present in a species of grape, succeeded in isolating a water soluble colorless compound which he believed to be a precursor to the anthocyanin. This colorless compound was converted to the crystalline anthocyanidin by treatment with hydrochloric acid. He obtained the leucoanthocyanidin in crystalline condition. Rosenheim postulated that the leucoanthocyanin (III a) was an intermediate in the formation, by reduction, of an anthocyanidin from the corresponding flavonol (II).

Rosenheim suggested that the carbohydrate was attached to the 4-hydroxyl group in the pseudo base (IIIa) and must be removed by acid hydrolysis before anthocyanidin formation is possible and the presence of acid causes the liberated pseudo base (IIIa) to isomerize to the color base (IIIb).
Isomerization was assumed to take place by the migration of the hydroxyl from position 4 to position 1. The colorless pseudo-base and the pigment were present in the plant in equal quantities.

In 1933, the Robinsons (15) took up the work of Rosenheim and found colorless precursors in almost every type of plant material examined. They found that these leucoanthocyanins were stable to aqueous hydrochloric acid but methanolic hydrochloric acid brought about gradual formation of the anthocyanidin chloride. Upon this point the Robinsons (15) disagreed with Rosenheim's postulation of a colorless intermediate unstable to aqueous mineral acid. They found that alkyl substitution of the 4-position did not protect the leucoanthocyanin against the action of even weak mineral acid. To explain this discrepancy the Robinsons (15) suggested that the group (-CHOH:CHOH-) was present in the 3,4 position of the pyran ring and postulated the structure (IV) to be the colorless precursor of cyanidin chloride (V).

Most of the leucoanthocyanins investigated (16) yielded cyanidin chloride (V) after treatment with methanolic hydrochloric acid.
In a later publication, the Robinsons \((16)\) divided the leucoanthocyanins into three classes:

1. Those insoluble in water and the usual organic solvents and giving only colloidal suspension.
2. Those readily soluble in water and not extracted from water by means of ethyl acetate.
3. Those capable of extraction from aqueous solution by means of ethyl acetate.

Class (3) compounds were sugar-free and would be more currently termed leucoanthocyanidins.

The Robinsons \((16)\) isolated from *Peltogyne porphyro-cardia* a compound peltogynol for which they postulated the structure \((\text{VI})\).

\[
\begin{array}{c}
\text{OR} \\
\text{RO} \\
\text{OR} \\
\text{CH}_2
\end{array}
\]

\(\text{VI}\)

Oxidation of peltogynol gave a compound which had the anthocyanidin characteristics, but was not one of the known naturally occurring anthocyanidins; hence they did not consider peltogynol to be a true leucoanthocyanin. Noting that Rosenheim \((17)\) showed that color formation was independent of the presence or absence of oxygen, the Robinsons \((16)\) proposed the hypothesis that anthocyanidin formation from
the corresponding leucoanthocyanin was an oxidative process although they were unable to explain the formation of the pigment when oxygen was excluded from the system.

G. M. Robinson in a later paper (14) isolated cyanidin chloride from the gum of *Butea frondosa* and found that a preliminary oxidation of the leucoanthocyanin was necessary in order to obtain the anthocyanidin. If the gum was treated with alcoholic hydrochloric acid in the absence of oxygen, a red color developed which was apparently caused by a modification of cyanidin chloride. It was necessary to boil the solution of the gum with aqueous picric acid before pure cyanidin chloride could be obtained. The coloring material obtained without previous oxidation was not discussed.

In 1953, King and Bottomley (6) isolated a leucoanthocyanin, melacacidin, from *Acacia melanoxylon* which gave all the characteristic reactions when treated with mineral acid. Previous to this year, most of the work in the detection of leucoanthocyanins had been accomplished by their conversion to the corresponding anthocyanin or anthocyanidin with the identification of the latter by the color reactions and paper chromatography.

Although melacacidin itself is amorphous, King and coworkers (7) obtained a crystalline tetramethyl ether of melacacidin which was identified as 7:8:3':4'-tetramethoxyflavan-cis-3:4-diol (VII).
Starting from 7:8:3'4'-tetramethoxy-flavonol they synthesized the compound (VII) by hydrogenation over Raney nickel obtaining a racemic tetramethoxy flavan-cis-3:4-diol. King was able to identify the synthetic diol with the naturally occurring levorotatory diol by comparison of their infra-red spectra, chromatographic behaviour after treatment with alcoholic hydrochloric acid, and by comparison of some of their derivatives (8).

Melacacidin is the only fully characterized flavan-3:4-diol isolated from natural sources and the anthocyanidin derived from it has not been found in nature.

Bate-Smith (3) felt that since anthocyanidins have not been detected as a result of acid treatment of catechins (flavan-3-ol) the 3:4-diol structure was necessary for flavylium salt formation. Since anthocyanidin formation in this case was an oxidation, it may have occurred because of the presence of atmospheric oxygen or by a disproportionation in which an equivalent of the flavandiol was reduced to a lower oxidation state (6).
The formation of the flavan-3-ol structure (X) has been proposed by King and Bottomley (6) since it has been detected by paper chromatography among the products from the acid treatment of the cacao bean leucoanthocyanidin (4). Furthermore, this anthocyanidin formation proceeded in the absence of oxygen (17).

In 1953, Pigman and coworkers (13) isolated a leucoanthocyanin from black spruce inner bark. It was a water soluble solid which gave the characteristic anthocyanidin reactions when treated with alcoholic mineral acid. Hydrolysis of the leucoanthocyanin with mineral acid followed by treatment of the hydrolyzate with phenylhydrazine led to the recovery of glucose phenylosazone. They prepared the methyl and acetyl derivatives of the leucoanthocyanin and proposed the following structure (XI).
The conversion to the anthocyanidin could result from hydrolysis of the glycoside, and ring closure, with simultaneous dehydration.

A color precursor was found in all parts of Western Hemlock wood by Pigman et al.

Several different extraction methods were tested, but the material was not investigated further.
DISCUSSION OF RESULTS

Wood from a 68 yr. old Western Hemlock tree (Tsuga heterophylla), felled in the University forest, was used in this work. The wood sample was free of decay and the tree was described as suppressed.*

Preliminary treatment of the finely ground wood with methanol containing hydrochloric acid produced a red-violet color, in solution and on the surface of the wood itself, characteristic of an anthocyanin salt. Using the standard methanol-hydrochloric acid solution of Pigman (13), this reaction was used to follow the course of extraction of the color precursor from the sawdust.

The procedures of isolation and identification employed in the investigation are summarized in Figure 1.

Two methods of extraction were compared colorimetrically using the methanol-hydrochloric acid reaction. Preliminary extraction with benzene followed by 50% aqueous ethanol proved to be the most efficient method, although the acetone extraction gave a much larger total quantity of extract. The 50% aqueous ethanol extract (hereafter called the extract) was a nearly colorless amorphous solid, in class (1) of Robinson's classification for leucoanthocyanins, and gave

* We are indebted to the late Dr. D.N. Buckland of the Forest Pathology Laboratory, U.B.C., for the examination of the disc sample.
an intense red-violet color in methanol containing hydrochloric acid. Using the standard methanol-hydrochloric acid solution of Pigman (13) the color produced was stable to ordinary light for several weeks.

Since many flavonoid type compounds exist as glycosides in the plant (11), the ethanol extract was treated with alcoholic mineral acid to effect hydrolysis of the glycosidic bond. Refluxing the extract in methanol, 3N in hydrochloric acid, produced a red-violet solution which left a purple solid upon evaporation of the solvent. It was insoluble in water and gave a faint pink color with aqueous 1% hydrochloric acid solution which was completely extractable by n-amyl alcohol. Robinson (10) reported that the water, n-amyl alcohol distribution coefficient for hirsutidin, because of its low water solubility. If an anthocyanidin salt was produced by the action of methanolic hydrochloric acid on the extract, the tests outlined by Link (11) and those of the Robinsons (15) indicated a malvidin or hirsutidin type of anthocyanidin. The methoxyl value was sufficiently high to indicate the presence of ether groups. The test with ferric chloride was negative, indicating that the 1,2-dihydroxy grouping was absent (11). Cyanidin chloride (3',4',3,5,7-pentahydroxy flavylium chloride) gave a positive ferric chloride test because of the 3',4'-dihydroxy grouping but malvidin (3',5'-dimethoxy-4',3,5,7-
tetrahydroxy flavylium chloride) gave a negative ferric chloride test because the 1,2-dihydroxy grouping was absent.

Paper chromatographic behaviour of the purple substance using Forestal solvent gave a single spot of high $R_f$ value, whose colors in the visible and in the ultraviolet indicated the presence of an anthocyanidin. The high $R_f$ value may be explained by the low solubility of the purple material in water. An $R_f$ value for hirsutidin or malvidin in Forestal solvent has not been reported.

Flavonoid type compounds are yellow in both acid and alkali and give yellow spots on a chromatogram in Forestal solvent, whether viewed in the visible or in the ultraviolet. In this solvent isoflavones exhibited a pale-blue fluorescence under ultra-violet light, which darkened when exposed to ammonia vapors (2).

Refluxing the extract with a more concentrated solution of hydrochloric acid in methanol, approximately 6N, yielded a purple solid which gave similar reactions to those already described above. A purification procedure yielded a product of lowered methoxyl content, which could result from either the purification or from partial demethylation arising from the strong acid treatment.

Bate-Smith (2) used cyanidin chloride ($R_f$, 0.5) as a chromatographic standard in Forestal solvent. To obtain some of this compound, dark red rose petals were ex-
tracted by the method of Willstatter. Treatment of the rose extract with alcoholic mineral acid, to hydrolyze any glycosides present, followed by paper chromatography of the extract in Forestal solvent, gave four spots on the chromatogram, which by their behaviour in visible and ultra-violet light indicated that two were anthocyanidins, one was a flavone and one was an isoflavone.

The purification procedure of Geissmann (5) removed two of the flavonoid components since rechromatography of the purified rose extract showed two spots characteristic of anthocyanidins. A spot with $R_f$, 0.47 gave the characteristic color reactions of cyanidin chloride, both in the visible and ultra-violet. The color tests of Robinson on the rose extract gave evidence for the presence of cyanidin chloride. Robinson (16) reports that when two anthocyanidins are present in a plant, one was usually a methoxylated derivative of the other, hence in this case it was possible that the anthocyanidin spot with higher $R_f$ value was a methoxyl derivative of cyanidin chloride.

It would be of interest to reinvestigate the rose extract in view of the chromatographic evidence that several components were present. In one specie of rose the relative structures of the flavones and anthocyanidins could be compared and possibly the natural relationship between these structures could be elucidated.
Acetylation of the 50% ethanol extract in pyridine using acetic anhydride gave a 50% increase in weight. The acetylated product was completely soluble in chloroform, whereas the original product was not completely soluble in any solvent. It had a positive rotation in chloroform indicating the presence of asymmetric centers, either as sugar residues or as a 3;4-diol structure in the case of melacacidin. The acetylation procedure gave three possible modes of attack on the problem: a method by which a pure crystalline derivative may be obtained without the development of the flavylium salt, a method to isolate the pure flavylium salt after deacetylation, and a method to isolate the colorless precursor itself.
SAWDUST

- benzene extraction
  - sawdust & resinous extract (1.017g.)
  - 50% aq. ethanol
  - sawdust & extract (1.28g.)

- acetone extraction
  - sawdust & sirupy extract (5.048g.)
  - fractionation
  - resinous material & white amorph. powder
    - pos. Fehling's after treatment by acid.
    - Neg. anthocyanin tests.

HCl in methanol
6N
- purple solid (B) purified by p'tion from acetic acid -OCH₃: 11.0%
R_f: 0.95 color tests

HCl in methanol
3N
- purple solid (A) yellow sirup -OCH₃: 13.9%
α_d = +3.78°
R_f: 0.95 color tests

C₆H₅N₂, acetic anhydride

Figure 1.
EXPERIMENTAL

A. Preparation of the Wood

After felling, the tree was cut into three-foot lengths from which the length next to the butt log was used. The bark was removed manually and the log split into segments which were sawed into 1.5 inch lengths with a table saw, then split into sticks approximately one square cm. in cross-section, dried in air, and ground to a fine sawdust in a model no. 2 Wiley Mill (screen size, 2 mm.). The sawdust was stored out of direct contact with light in a 15-liter bottle. A sample of freshly ground sawdust was dried in vacuo over phosphorus pentoxide to constant weight and contained 22.35% moisture by weight.

When sawdust (5.0g.) was refluxed for fifteen minutes with methanol containing concentrated hydrochloric acid (4:1), the methanol turned red-violet and the wood itself attained a red-violet coloration. Removal of the sawdust by filtration and evaporation of the filtrate left a red-brown powder, insoluble in water but soluble in n-amyl alcohol. The course of extraction of the color precursor from the sawdust was followed using the standard methanol-hydrochloric acid solution of Pigman (13).
B. Extraction of the Sawdust with Benzene

In order to remove resinous material the sawdust was exhaustively extracted with benzene. Sawdust (251.0g.) was dried in vacuo over phosphorus pentoxide to 240.0g. and extracted in a Soxhlet extractor for 24 hr. with dry benzene. After extraction the sawdust was air dried until the odor of benzene was no longer apparent and then stored out of contact with light. Evaporation of the lemon yellow benzene extract left a brown resinous material (1.017g.). It was redissolved in benzene and petroleum-ether was added causing the precipitation of an amorphous white solid which was removed by centrifugation, washed with petroleum-ether, and dried in vacuo over soda lime and paraffin shavings. This material reduced boiling Fehling's solution, decolorized neutral potassium permanganate, and gave negative anthocyanin tests. Evaporation of the mother liquors gave a dark brown resinous material which also gave a negative anthocyanin test. The benzene extract was not investigated further.

6. Extraction of the Sawdust with Acetone

Air-dried sawdust (80.0g.) was packed into a glass tube (48.5 x 3.3 cm.) closed by a sintered glass disc and exhaustively extracted by allowing acetone to percolate through the column at a very slow rate. The
The amount of material extracted was determined by evaporation of an aliquot of the eluate at intervals. When the amount of material in the eluate became negligible the extraction with acetone was stopped although a sample of the sawdust from the column still gave a positive anthocyanin test. Evaporation of the acetone extract (1390 ml.) yielded a light brown sirupy material (5.408 g.) which gave a positive anthocyanin test.

D. Extraction of the Benzene-Extracted Sawdust with 50% Aqueous Ethanol.

Benzene-extracted sawdust (47.6 g.) was packed into a column (28.5 x 3.3 cm.) and exhaustively extracted with 50% by volume aqueous ethanol by the above procedure. When all the material soluble in 50% ethanol had been removed from the column the residual sawdust gave a very weak anthocyanin test. The total percolate (1025 ml.) was evaporated under reduced pressure and when its volume was halved a heavy, nearly colorless precipitate formed; the evaporation was continued and the last 100 ml. of solvent was evaporated using a lyophilizing unit leaving an almost colorless amorphous powder (1.282 g.). The dried 50% aqueous ethanol extract was used in all further tests; Table I gives the solubility characteristics of the dried material. A clear solution could not be obtained in any solvent hence an optical rotation was
not feasible at this point.

TABLE I

Solubility Characteristics of the 50% Aqueous Ethanol Extract of Western Hemlock Wood.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>very slightly soluble</td>
</tr>
<tr>
<td>methanol</td>
<td>soluble giving a colloidal suspension</td>
</tr>
<tr>
<td>ethanol (95%)</td>
<td>soluble giving a colloidal suspension</td>
</tr>
<tr>
<td>benzene</td>
<td>insoluble</td>
</tr>
<tr>
<td>chloroform</td>
<td>insoluble</td>
</tr>
<tr>
<td>3N aq. HCl</td>
<td>insoluble and gave no color reaction when warmed.</td>
</tr>
<tr>
<td>3N HCl in methanol</td>
<td>immediate solution giving a red-violet color. This test positive even if the acid was present in low concentrations.</td>
</tr>
</tbody>
</table>

The ethanol extract, at a concentration of 2 mg. per ml. in methanol containing concentrated hydrochloric acid (4:1) gave a color described as 7.5 RP^{3.5}/10 by the Munsell Book of Colors (12), approximately red-violet.

E. Treatment of the 50% Ethanol Extract with Alcoholic Mineral Acid

The extract (0.100 g.) was refluxed with methanol containing concentrated hydrochloric acid (4:1) for 15 min. then cooled and the solvents removed under reduced pressure leaving a purple amorphous solid (A) which was dried in vacuo over phosphorus pentoxide. It was completely insoluble in water but gave a faint pink solution with 1% aqueous hydrochloric acid. It was soluble
in methanol, ethanol, n-amyl alcohol, and glacial acetic acid.

Found: -OCH₃; 13.9%.
Calc'd for C₁₅H₁₀₅Cl (OCH₃)₂ (malvidin chloride); 16.8%.
Calc'd for C₁₅H₉₀₅Cl (OCH₃)₂ (malvidin chloride monohexoside);
C₆H₁₀₀₅; 11.72%.

A sample of the extract (0.100 g.) was refluxed with methanol containing concentrated hydrochloric acid (2:1) for 30 min. then cooled and the solvent removed under reduced pressure leaving a purple amorphous powder (B) which, when dried, was similar to (A). (B) was dissolved in glacial acetic acid giving a red-purple solution and was completely precipitated by the addition of excess water. The precipitate was recovered at centrifuge, washed with water, and dried in vacuo over phosphorus pentoxide.

Found: -OCH₃; 11.0%.
Calc'd for C₁₅H₁₀₀₅Cl (OCH₃)₂ (malvidin chloride); 16.8%.

F. Extraction of Pigment from Rose Petals

Dark red rose petals (55.0 g.) previously dried in a vacuum desiccator over phosphorus pentoxide and magnesium perchlorate was extracted with 2% methanolic hydrochloric acid using the method of Willstätter (20). Part of this extract was evaporated to dryness under reduced pressure and dried in vacuo over phosphorus pentoxide leaving a dark red powder. This material was treated in the usual
manner to hydrolyze any glycosides present and the resulting red powder was dried \textit{in vacuo}.

G. Chromatography of the Compounds Resulting from the Alcoholic Acid Treatment of the Ethanol Extract and the Rose Extract.

Chromatograms were run on No. 1 Whatman paper using Forestal solvent (acetic acid:concentrated hydrochloric acid:water) (10:1:3) (2).

Chromatography of either (A) or (B) showed one spot on the chromatogram with $R_f$ value, 0.95. The spot was red-brown in visible light, blue-violet under the ultraviolet lamp, and became darker blue when exposed to concentrated ammonia vapors. A chromatograph of the crude rose petal extract under similar conditions gave four spots on the chromatogram as described in Table II.

\begin{center}
\textbf{TABLE II}
\end{center}

\begin{center}
Paper Chromatography of the Crude Rose Extracts
\end{center}

\begin{center}
\begin{tabular}{cccc}
\hline
$R_f$ & Color, visible & Color, U.V. & Color In U.V. & NH$_3$ \\
\hline
0.63  & red-brown & scarlet & blue & \\
0.48  & red-brown & scarlet & blue & \\
0.38  & yellow & yellow & golden-yellow & \\
0.32  & not visible & pale blue & darker blue & \\
\hline
\end{tabular}
\end{center}

The rose petal extract was purified using a method outlined by Geissmann (5). A 1% aqueous hydrochloric acid solution of the rose extract was shaken with one quart of its
volume of n-amyl alcohol containing 30% by volume aceto-
phenone. The pigment was quantitatively extracted by the
alcohol layer and the aqueous layer was discarded. Addition
of six volumes of benzene to the amyl alcohol layer caused
the precipitation of the pigment which was redissolved in
1% aqueous hydrochloric acid giving a violet-red solution.
Evaporation of the aqueous acid solution gave a dark red
powder which was chromatographed in Forestal solvent giving
two brown-red spots (Table III).

**TABLE III**

Paper Chromatography of the Purified Rose Extract

<table>
<thead>
<tr>
<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Color under U.V.</th>
<th>Color under U.V. NH&lt;sub&gt;3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.63</td>
<td>scarlet</td>
<td>blue</td>
</tr>
<tr>
<td>0.47</td>
<td>scarlet</td>
<td>blue</td>
</tr>
</tbody>
</table>

H. Tests Outlined by Link and Robinson Applied to the
Acid-treated Ethanol Extract and Rose Extract

The tests outlined by Link (11) are shown in Table IV:
other members of the anthocyanidin family are included for
comparison.
<table>
<thead>
<tr>
<th>Reaction</th>
<th>Pelargonidin</th>
<th>Cyanidin</th>
<th>Delphinidin</th>
<th>Peonidin</th>
<th>Malvidin and Hirsutidin</th>
<th>(A) or (B)</th>
<th>Rose Ext.</th>
</tr>
</thead>
<tbody>
<tr>
<td>color of aq. sol.</td>
<td>red</td>
<td>violet</td>
<td>red</td>
<td>blue-red</td>
<td>violet</td>
<td>red</td>
<td>pink</td>
</tr>
<tr>
<td>sol'nty in water</td>
<td>readily</td>
<td>slightly</td>
<td>very sol.</td>
<td>red</td>
<td>only sl.</td>
<td>sol.</td>
<td>very sl.</td>
</tr>
<tr>
<td>FeCl₃ rx.</td>
<td>not def.</td>
<td>int. blue</td>
<td>int. blue</td>
<td>not def.</td>
<td>no rx.</td>
<td>no rx.</td>
<td>int. blue</td>
</tr>
<tr>
<td>Fehling's Test</td>
<td>red. if warmed</td>
<td>reduces in cold</td>
<td>reduces in cold</td>
<td>red. if boiled</td>
<td>red. if boiled</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>color in Na₂CO₃</td>
<td>violet</td>
<td>then blue</td>
<td>violet</td>
<td>then blue</td>
<td>violet</td>
<td>then blue</td>
<td>green-blue</td>
</tr>
<tr>
<td>Behavior in aq. sol.</td>
<td>color fades</td>
<td>color fades</td>
<td>color fades</td>
<td>color fades</td>
<td>color fades</td>
<td>color fades</td>
<td>color fades</td>
</tr>
<tr>
<td>Behavior to NaOH</td>
<td>blue then green</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE IV
Anthocyanidin Tests.
Robinson (15), using the following tests, was able to distinguish between the main classes of anthocyanidins.

1. A portion of an aqueous hydrochloric acid solution was extracted with n-amyl alcohol and then sodium acetate was added followed by a drop of ferric chloride solution.

2. A portion was extracted by cyanidin reagent (1 part cyclohexanol, 5 parts toluene) and the upper layer was observed in a narrow tube.

3. A portion was shaken in air with one-half its volume of 10% aqueous sodium hydroxide then immediately acidified with concentrated hydrochloric acid and extracted with n-amyl alcohol.

4. A portion was shaken with a 5% solution of picric acid in a mixture of methyl amyl ether (1 part) and anisole (4 parts).

Using solutions of (A) or (B) and the rose extract in 1% aqueous hydrochloric acid the color reactions with the various reagents were compared with Robinson's results as shown in Table V.
### TABLE V

**Robinson's Tests**

<table>
<thead>
<tr>
<th>Test</th>
<th>Pelargonidin</th>
<th>Cyanidin</th>
<th>Malvidin</th>
<th>Delphinidin</th>
<th>(A) or (B)</th>
<th>Rose Ext.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a NaOAc</td>
<td>violet-red</td>
<td>red-violet</td>
<td>blue-red</td>
<td>blue</td>
<td>blue-red</td>
<td>red-violet</td>
</tr>
<tr>
<td>1b FeCl₃</td>
<td>no-change</td>
<td>intense blue</td>
<td>no change</td>
<td>intense blue</td>
<td>no change</td>
<td>intense blue</td>
</tr>
<tr>
<td>2</td>
<td>extracted</td>
<td>extracted</td>
<td>not extracted</td>
<td>not extracted</td>
<td>slightly extracted</td>
<td>extracted</td>
</tr>
<tr>
<td>3</td>
<td>stable</td>
<td>stable</td>
<td>destroyed</td>
<td>stable</td>
<td>stable</td>
<td>stable</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>not extracted</td>
<td></td>
</tr>
</tbody>
</table>
I. **Acetylation of the 50% Aqueous Ethanol Extract**

A sample of the 50% ethanol extract (0.10g.) was dissolved with difficulty in dried pyridine (5.0ml.) and acetic anhydride (5.0ml.). After the dark red solution had stood for two days at room temperature, it was poured into 100ml. crushed ice and water whereupon a heavy cream colored precipitate formed which did not crystallize after standing two weeks at 0°C. The supernatant liquid was poured off and the sirup was taken up in chloroform. The solution was washed with water and dried over anhydrous magnesium sulfate. Evaporation of the dried chloroform solution left a sirup (0.156g.), insoluble in water and petroleum-ether and soluble in chloroform and benzene.

\[ [\alpha]_D^{21} + 3.78^\circ (c, 1.560: 1, 0.5: \text{CHCl}_3). \]
1. The presence of an extractable color precursor in Western Hemlock wood was confirmed and an efficient procedure was developed for its isolation.

2. The near colorless, solid, 50% ethanol extract of Western Hemlock wood was shown to give, after treatment with alcoholic mineral acid, a purple solid which had the characteristic reactions of an anthocyanidin of the malvidin or hirsutidin type.

3. Acetylation of the dried extract yielded a dextro-rotatory yellow sirup soluble in chloroform and suitable for further purification.
BIBLIOGRAPHY

2. Bate-Smith, E.C. Symposium on "Recent Advances in the Chemistry of Naturally Occurring Pyrones and Related Compounds". University College, Dublin. 1955.